Session VII

Management of biotics stresses for sustainable intensification of root and tuber crops

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Plastic barriers control Andean potato weevils (*Premnotrypes spp.*): large-scale testing of efficacy, economic and ecological evaluation and farmers' perception

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Abstract

Andean potato weevils (*Premnotrypes* spp.) are key potato pests in the Andes. Insecticides are the most widely used control method employed by farmers. Recently, plastic barriers at field borders proved to stop the migration of flightless Andean potato weevil (*Premnotrypes suturicallus*) adults to potato fields and have been equally effective compared to farmers' practice of using insecticides. The objectives of this study were to further validate the efficacy of this technology in two Andean villages with the participation of 40 farmers, to evaluate costs and environmental impact compared to insecticides, and to assess farmers´ perception and preparedness to use this technology. Plastic barriers effectively reduced Andean potato weevil damage by 65% and 70% in the two villages compared to farmers´ practice using 2 to 6 insecticide applications. Plastic barriers proved to be also effective to control other weevil species, such as *P. solaniperda, P. latithorax and P. vorax* in potato agroecologies of Peru, Bolivia and Ecuador. The use of plastic barriers resulted in an excellent investment with mean net benefits of US$147/ha and US$807/ha for farmers of the two villages. The environmental impact quotient (IEQ) was five times lower for the plastic barriers (32.86) compared to farmers' practice (191.52) indicating the overall benefits of the technology to reduce the risks of insecticide applications for farmers, consumers and the environment. Farmers' opinion was very positive with more than 90% of interviewed farmers considering plastic barriers as a very useful and easy-to-install control tool, and interested to further promote this technology among other farmers.

Keywords: Potato, Integrated Pest management, Andean potato weevil, *Premnotrypes suturicallus*, physical control.

Introduction

Andean potato weevils (*Premnotrypes* spp.) are native in the Andean region and the most important pests of potato (*Solanum* spp.) at altitudes between 2,800 and 4,750 m. The species are widespread from Argentina to Venezuela covering a mountainous territory of a length of 5,000 km. The most severe crop damage is caused by larvae which feed on tubers and cause tuber infestations and losses of up to 100%, if no control measures are applied. Main control measures used by farmers are the application of hazardous insecticides (Ewell *et al.* 1990, Orozco *et al.* 2009). Adults of Andean potato weevils are flightless and hence migrate from previous years potato fields to new potato production sites. Recently, a new technology using physical barriers around potato fields have been successfully tested to control Andean potato weevil *Premnotrypes suturicallus* Kuschel (Kroschel *et al.* 2009). The objective of this study was to validate the plastic barrier technology with a large number of farmers in two Andean villages in comparison to farmers' traditional practices of using several insecticide applications to control Andean potato weevil, and to study their economics and environmental impacts compared to insecticide applications. Additionally, we studied farmers' perception about the use of plastic barriers in one Andean village, which was involved in the evaluation and use of the plastic barriers for several years.

Materials and methods

Large scale validation of the plastic barrier technology

The study was carried out in the villages Ñuñunhuayo (3,800 m a.s.l., Department of Junin, Peru) and Aymara (3,900 m a;.s.l., Department of Huancavelica, Peru) with a participation of 20 farmers in each of the communities. Participating farmers established subplots in their potato fields of a size of 225 m^2 (15 x 15 m), which were surrounded by a plastic barrier at the day of planting. The plastic was fixed on wooden stakes and installed 10

cm below soil with a total height of 50 cm above soil as described in Kroschel *et al.* (2009). Except of the plastic to prepare the barriers all other inputs (seed, fertilizer) were provided by farmers. Further, farmers were equally responsible for all cultural labors (weeding, hilling). In the plastic barrier plots no insecticides were applied but fungicides Cymoxanil for controlling late blight (*Phytophtora infestans* Mont. De Bary), when considered appropriate. Potatoes grown outside of the plastic barriers received insecticide (carbofuran, fipronil) applications according to farmers' practice. At harvest, treatment efficacy was evaluated with farmers' participation by scoring tuber damage caused by Andean potato weevils. In the plastic barrier subplots as well as in the overall farmers plots 5 x 3 m subplots of potato rows with a total of 10 potato plants each (50 potato plants per subplot) were randomly selected and the number and proportion of healthy and damaged tubers per plant as well as the total tuber yield determined.

Validation of the plastic barrier technology in different potato agroecologies

Additional individual field studies, using the methodology as described above, were carried out in other potato growing regions of Peru as well as in Bolivia and Ecuador to test the plastic barrier technology in different potato agroecologies, where other Andean potato weevil species prevail: Puno, Peru (3,900 m a.s.l.): *Premnotrypes solaniperda* Kuschel*;* Huancavelica, Peru (3,800 m a.s.l.): *Premnotrypes suturicallus;* Carchi, Ecuador (8 field experiments at altitudes of about 3,000 m): *Premnotrypes vorax* (Hustache)*;* Cochabamba, Bolivia (3,400 m a.s.l.): *Premnotryopes latithorax* (Pierce).

Environmental impact of pesticides compared to the use of plastic barriers

The environmental impact for the use of pesticides was calculated by multiplying the Environmental Impact Quotient **(**EIQ) value for each insecticide (carbufuran, EIQ: 50.67; fipronil, EIQ: 90.92) and fungicide (cymoxanil, EIQ: 8.7) by the amount of pesticide used per hectare and the number of applications per season. EIQ values were taken from an updated table at Cornell University webpage: http://nysipm.cornell.edu/publications/eiq/files/EIQ_values.xls.

Farm survey on the adoption potential of plastic barriers

The adoption potential of plastic barriers was studied through 40 individual farm surveys (questionnaires) in the village of Ñuñunhuayo. Most of the farmers of this village were involved and could gain experiences with this technology over several years, in which this technology was tested on-farm. The survey comprised questions to learn about traditional potato crop management and farmers opinions about the use of plastic barriers as a new alternative control tool for Andean potato weevil.

Statistical analysis

Data for potato tuber infestation and tuber yield were subjected to analysis of variance (ANOVA). The Kruskal-Wallis test was applied to analyze statistical differences in potato tuber losses (SAS Institute 2003). For the calculation of the insecticide application costs per hectare we collected information throughout the cropping season on the quantity of product used per knapsack sprayer and area as well as on the number of applications. Plastic barriers' cost calculations are based on the price of the plastic per meter and the wooden sticks to fix the barriers. For the estimation of net profits we followed the methodology described by Ortiz *et al.* (1996).

Results

Large-scale validation of the plastic barrier technology

Efficacy of plastic barriers versus insecticide applications. In the 20 plastic barrier fields in Ñuñunhuayo, tuber infestation ranged from 0 to 27% with an average infestation of 6.7%. In contrast, farmers´ insecticide-treated fields showed a tuber infestation between 1.8 to 51.26% with an average of 19.6% (Table 1). These fields were treated 2-5 times with the hazardous insecticides Furadan, Carbofuran or Regent. Likewise, in Aymara, tuber damage at harvest ranged from 0.5% to 16% in the 20 plastic barrier fields with an average infestation of 5.5%. Here, farmers' insecticide-treated fields received 1-5 times insecticide applications and tuber damage ranged from 0.45 to 44.8% with an average of 18%.

Table 1. Mean potato tuber infestation by Andean potato weevil (*Premnotrypes suturicallus)*, total tuber yield, Andean potato weevil caused tuber loss and cost for insecticides and plastic barriers in plastic barrier and insecticide-treated farmers plots in the villages Ñuñunhuayo and Aymara, 2007 (N=20 for each treatment at each location).

Parameters	Ñuñunhuayo		Aymara	
	Plastic barrier	Insecticide-treated	Plastic barrier	Insecticide-treated
Mean proportion of infested tubers (%)[1]	6.66a (1.74)*[a]	19.61b (2.87)	5.48a (1.15)	18.03b (3.38)
Mean total tuber yield of (kg/ha)[1]	9,650.60a (763.37)	9,813.63a (639.21)	20,885.03a (2,896.15)	18,002.33[a] (2,926.80)
Mean proportion of damaged tuber weight (kg/ha)[1]	676.34 (200.46) a	1,850.31 (300.59) b	1,027.83 (270.31) a	3,813.21 (1,312.78) b
Mean technology costs (US$)	57.00	101.58 (6.42)	57	96.51 (8.17)

[1]Means followed by different letters within a row are significant different according to the LSD or Kruskal-Wallis test at P • 0.05. *Figures in brackets indicate Standard errors.

***Tuber yield in plastic barrier* versus *insecticide-treated plots*.** In the 20 plastic barrier fields in Ñuñunhuayo tuber yields varied from 4,950 kg to 17,033 kg/ha, with an average of 9,651 kg/ha. Losses caused by the Andean potato weevil through tuber damage ranged between 0 and 1,800 kg with an average damage of 676.34 kg/ha (Table 1).Yields in farmers' insecticide-treated plots ranged from 4,793 kg to 17,476 kg/ha, with an average tuber loss of 9,814 kg/ha. Here, tuber losses ranged between 320 to 5430 kg with an average of 1,850 kg/ha. In this community mainly bitter native potato varieties are planted, which are processed to freeze-dried potatoes called "chuño". Likewise, in Aymara, tuber yield in plastic barrier fields varied from 7,243 kg to 51,720 kg/ha, with an average of 20,885 kg/ha. Here, losses caused by Andean potato weevil ranged between 32.12 and 1,943.88 kg/ha with an average tuber loss of 1,027 kg/ha. In insecticide-treated fields, yield ranged from 7,130 kg to 57,880 kg, with an average of 18,002 kg/ha. The tuber yield loss caused by Andean potato weevil ranged in these fields between 44 and 25,108 kg/ha, with an average tuber loss of 3,813 kg/ha. In this village, commercial as well as native potato varieties are cultivated.

***Efficacy of the plastic barriers in different potato agroecologies*.** Preliminary on-farm experiments in different potato agroecologies in Peru (Puno and Huancavelica), Bolivia and Ecuador confirmed that plastic barriers also control other Andean potato weevil species like *P. solaniperda* in Puno*, P. latithorax* in Bolivia or *P. vorax* in Ecuador (Table 2).

Table 2. Potato tuber infestation (%) caused by different Andean potato weevil species (*Premnotrypes* ssp.) in plastic barrier and insecticide-treated fields in different potato agroecologies of Peru, Ecuador and Bolivia, 2008

Treatments	Peru		Bolivia	Ecuador
	Puno	Huancavelica	Cochabamba	Carchi
Species	*P. solaniperda*	*P. suturicallus*	*P. latithorax*	*P. vorax*
Plastic barrier	3.98	20.01	5.0	5.0
Insecticide-treated	10.9	40.86	18.0	5.0
Control	15.35	55.40	-	-

Economic benefits of plastic barriers versus insecticides

***Input costs for insecticides and plastic barriers*.** For farmers in Ñuñunhuayo costs for insecticide treatments varied from US$65 to US$162.5/ha, with an average of US$ 101.58/ha (Table 1). In Aymara, costs for insecticides ranged between US$65 to US$162.5/ha, with an average of US$96.50/ha. In contrast, the costs of plastic barriers (including wooden sticks) were US$57/ha.

***Net benefits for plastic barriers* versus *insecticide-treated plots*.** A net benefit for the use of plastic barriers of US$147.63/ha and US$807.31/ha was estimated for the potato production systems of Ñuñunhuayo and Aymara, respectively, based on mean tuber yield, mean tuber infestation at harvest, potato price with and without damage, proportion of damaged tuber weight, costs for plastic barriers and insecticide applications (Table 3).

Table 3. Estimated net benefits for plastic barriers and insecticide treatments to control Andean potato weevil (*Premnotrypes suturicallus*) in the villages Ñuñunhuayo and Aymara, Peru. Means derived from 20 on-farm experiments in each of the villages

Parameters	Ñuñunhuayo		Aymara	
	Plastic barrier	Insecticide-treated	Plastic barrier	Insecticide-treated
Mean total tuber yield of (kg/ha)	9,651	9,814	20,885	18,002
Mean proportion of infested tubers (%)	6.65	19.61	5.49	18.03
Price of healthy tubers (US$/kg)*	0.16	0.16	0.16	0.16
Price of infested tubers (US$/kg)**	0.05	0.05	0.05	0.05
Mean proportion of damaged tuber weight (kg/ha)	676	1,850	1,027.83	3,813
Mean technology costs (US$)	57.00	101.57	57.00	96.50
Total value of healthy tubers (US$/ha)	544.16	1,570.24	3,341.60	2,880.32
Total value of infested tubers (US$/ha)	33.80	92.50	51.39	190.65
Total weight of healthy tubers (kg/ha)	8,975	7,964	19,858	14,189
Value of healthy tubers (US$/ha)	1,436	1,274.24	3,177.28	2,270.24
Value of healthy and infested tubers (US$/ha)	1,469.80	1,366.74	3,228.67	2,460.86
Net production benefits (US$/ha)	1412.80	1,265.17	3,171.67	2,364.36
Net benefits from plastic barrier (US$/ha)	147.63		807.31	

*Considers a potato price of S/.0.50 (1$=3.10); **Considers a loss of value of infested tubers by 67% (Ortiz et al. 1996).

Environmental Impact of plastic barriers and insecticide applications

The Environmental Impact Quotient (EIQ) for the use of insecticides ranged between 32.4 and 486.4 with a mean of 144.8 per ha (Tables 4). Farmers used fungicides more frequently and hence also the EIQ for fungicides was higher in the insecticide-treated fields compared to the plastic barrier fields.

Table 4. Environmental Impact Quotient (EIQ/ha) for insecticide-treated and plastic barrier fields to control Andean potato weevil control. Ñuñunhuayo, 2007.

Treatments	EIQ for insecticides	EIQ for fungicides	EIQ: Total
Insecticide-treated	144.81 (24.48)* 32.43 – 486.43**	46.72 (3.72) 15.66 – 125.28	191.50 (30.10) 52.14 – 344.09
Plastic barrier	0	32.88 (2.42) 20.88 – 62.64	32.88 (2.42) 20.88 – 62.64

* Standard error; **minimum and maximum range

Adoption potential of plastic barriers

According to 97.5% of the farmers interviewed in Ñuñunhuayo, the Andean potato weevil is the main pest problem in potato production. Farmers (75%) are practicing fallow with periods of more than five years; a typical rotation (90% of farmers) is fallow-potato-oat (*Avena fatua* L.)-fallow. Almost all farmers apply highly toxic insecticides to control Andean potato weevil like carbofuran (92.5 %) with an average of three applications

during the potato cropping period. Most farmers (95%) valued plastic barriers as useful to reduce Andean potato weevil caused tuber damage (Table 5). Farmers (100%) consider installing barriers as easy and state that barriers do not interfere with cultural practices in potato (90%). Among main constraints plastic barriers may be affected by rain, sunlight, wind and animals. According to 82% of the farmers plastic is easy to find, but they would prefer to buy it in their own village. Ninety percent of farmers would accept a price of US$0.16/m and 65% of farmers would recommend this technology to other farmers.

Table 5. Farmers' opinion about the use and constraints of plastic barriers, Ñuñunhuayo (N=40)

Questions	Acceptation	%
What is your opinion about plastic barrier?	Useful; reduces Andean potato weevil damage	95
Any problem with the installation?	Easy to install	100
Any interference with cultural practices?	No	90
Any other constraints?	Rain, sunlight, wind and animals	30
Is it easy to find/buy plastic?	Yes	82
How much are you willing to pay for plastic?	Until US$ 0.16/m	90

Discussion

Large-scale validation of the plastic barrier technology

The large scale validation confirmed previous results that plastic barriers control Andean potato weevils effectively (Kroschel et al. 2009), and moreover clearly demonstrated its higher efficacy compared to the use and application of insecticides. In both villages Ñuñunhuayo and Aymará, plastic barriers reduced mean tuber infestation by 65% to 70% compared to insecticide applications directed by farmers. In this experiment, no control fields without any control could be set up; however, Ewell *et al.* (1990) for example reported a mean tuber infestation of 70% at harvest in 51 fields evaluated in the Rio Mantaro valley. According to Ortiz *et al.* (1996) an Andean potato weevil infestation of 10% already causes significant economic losses for a commercial potato producer. The preliminary evaluation of the plastic barrier technology in other potato agroecologies of Peru and the Andes (Bolivia, Ecuador) also successfully demonstrated the efficacy of barriers to hinder migration and reduce infestation of other Andean potato weevil species like *P. solaniperda* in Puno, *P. latithorax* in Bolivia or *P. vorax* in Ecuador (Belmont 2007).

Economic benefits of plastic barriers versus insecticides

The higher tuber infestation in insecticide-treated fields caused higher tuber yield losses compared to the use of plastic barriers. Further, the costs for insecticides were almost double as high as for the materials used to prepare the plastic barriers. We didn't consider labor costs for the insecticide applications, which also would include the transport of water, or the time for the installation and/or maintenance of the plastic barrier. The cost of insecticides also varied greatly with regard to the type of insecticide and the number of applications. However, high net benefits of US$147.63/ha and US$807.31/ha were estimated for the two villages Ñuñunhuayo and Aymará, when using plastic barriers.

Ecological assessment

Environmental Impact Quotient (EIQ). High EIQ values of a mean of 144.87/ha were determined for farmer's practice of using highly toxic insecticides to control Andean potato weevil. EIQ values are classified as low (0-20), medium (20.1-40) and high (more than 40) (Mazlan and Mumford 2005). The insecticides used by farmers in this study belong to the group of Carbamates (carbofuran) and Phenylpyrazole (fipronil), which have EIQ values of 50.7 and 90.92, respectively. Carbofuran is classified as a highly hazardous Ib pesticide, and fipronil belongs to class II, moderately hazardous pesticides (WHO 2005). The application of other highly hazardous insecticides to control Andean potato weevil is very common in the study region. Orozco *et al.* (2009) reported that farmers often also use metamidophos (Class Ib) in the Mantaro valley, which has an EIQ value of 36.8 (http://nysipm.cornell.edu/publications/eiq/files/EIQ_values.xls).

Adoption potential of plastic barriers. In the study village Andean potato weevils are the main biotic constraint in potato production to which farmers' response by using highly hazardous insecticides. Farmers

interviewed in the village Ñuñunhuayo generally had an overall good opinion about the use of plastic barriers, but first the following years will show and prove if they will adopt and continue using this technology on their own.

Conclusions

The application of insecticides is the only direct control method for Andean potato weevils that is employed by farmers. Earlier, integrated pest management recommendations suggested the use of various cultural practices (crop rotation, use of chicken after harvest to reduce the larvae population in soil, etc.) or baiting weevils with potato leaves treated with insecticides but which methods all do not reduce tuber damage in the short-term and hence are difficult for farmers to adopt. Based on an enhanced knowledge about the weevil migration and behavior, the plastic barrier technology was developed and successfully tested in two Andean villages over a period of fours years in more than 60 individual field experiments. The technology has shown not only to be more reliable than several insecticide applications but also to be more cost effective and environmental friendly. At present, the technology is being taken up by the national agricultural institutes in Peru, who will further distribute and promote the use of plastic barriers.

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How do insecticides affect potato yield and ecosystem resilience to manage potato pests? An ecological assessment from the central highlands of Peru

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Abstract

In potato (*Solanum* sp.) production in the Andes, farmers mainly apply insecticides to control Andean potato weevils (*Premnotrypes* sp.). The objective of our research was to make an insect inventory in potato agroecosystems and to explore the effects of insecticide applications on potato pests and natural enemies in an altitude gradient between 2800 to 3850 m in the central highlands of Peru. Potato fields were equally divided into insecticide-treated (farmers' practice) and insecticide-free plots. Active and passive evaluation methods were used to monitor arthropods. Phytophagous insects were the most numerous functional group constituting 75.4% of total arthropods. Predatory insects and parasitoids represented 23% and 1.4% of the total insect population, respectively. Of yet 36 identified phytophagous insects, 23 species are phytophagous on potato. Of 23 predator species, ground-dwelling predators were most numerous (21.7%); plant inhabiting predatory species only represented 1.3% of the total insect population. Although a total of 16 parasitoids were identified, parasitism of potato tuber moths was low (0.4%). Insecticide treatments reduced about 50% of the arthropod population compared to non-treated fields; however, no significant differences were found in predator and parasitoid species abundance and diversity. In non-insecticide treated fields, very striking were higher infestations with flea beetles (*Epitrix yanazara*) with up to 70% of potato foliar damage causing yield losses of up to 72%. Without insecticides, tuber damage by Andean potato weevils and potato tuber moths ranged between 74% to 95% and 5% to 35%, respectively. Insecticide applications were not always effective to control Andean potato weevil and tuber infestations ranged between 0 to 70%.

Keywords: Andean potato weevil, Premnotrypes suturicallus, potato tuber moth, Phthorimaea operculella, flea beetle, Epritrix yanazara, carabidae, natural enemies, Andean region.

Introduction

Potato (*Solanum tuberosum* L.) production in the Peruvian Andean highlands at altitudes between 2800 to 4200 m is severely constrained by many pest problems. Farmers mainly respond with the application of hazardous class Ia and Ib (aldicarb, carbofuran, methomyl, metamidophos) insecticides to control the most important pests which are Andean potato weevils (Orozco *et al.*, 2009). Andean potato weevils belong to the genera *Premmnotrypes, Rhigopsidius* and *Phyrdenus.* With 11 species the genus *Premnotrypes* is most important and most widely distributed. *P. suturicallus* Kuschel occurs mainly in the central highlands, *P. vorax* (Hustache) in the northern and *P. latithorax* (Pierce) in the southern highlands of Peru (Kaya *et al.*, 2009). Other important pests are the common potato tuber moth *Phthorimaea operculella* (Zeller) and the Andean potato tuber moth *Symmetrischema tangolias* (Gyen.), both native to South America, but which are mainly controlled in potato stores (Keller, 2003).

For Peru, several beneficial insect predators and parasitoids that could play an important role in the integrated pest management of potato pests have been reported. These include predators of the families Carabidae, Coccinelidae, Nabidae, Lygaeidae, Chrysopidae, and Syrphidae (Cisneros, 1995). The carabids *Harpalus turmalinus* Van Emden and *Notiobia schmusei* Van Emden have been reported as predators of Andean potato weevils (Cisneros, 1995; Loza, 1999); recently, Kroschel et al. (2009) reported of high numbers of carabids of the genera *Blennidus*, *Metius, Pelmatellus, Incagonum, and Notiobia peruviana* (Dej.) in the central highlands of Peru that greatly affect Andean potato weevil. *Apanteles subandinus* Blanchard, *Dolichogenidea gelechiidivoris* (Marsh), *Copidosoma koehleri* Blanchard, and *Incamyia cuzcensis* Townsend are parasitoids of the potato tuber moths; *Gonia peruviana* Townsend as well as *Patelloa robusta* (Wied.) are larval parasitoids of cutworms (Cisneros, 1995).

Depending on the complexity and species richness, agroecosystems can have a good potential to provide a high level of natural biological control, and hence ecosystem complexity can increase ecosystem resilience to pest outbreaks (Risch et al., 1987). However, various insecticides especially pyrethroids, organophosphates and carbamates are well known to be highly toxic to a wide range of natural enemies (Devine and Furlong, 2007) and hence may negatively interfere with natural control in agroecosystems. The objective of our research was to make an insect inventory in potato agroecosystems of the central highlands of Peru and to explore the effects of insecticide applications on potato pests and natural enemies in different potato agroecosystems in an altitude gradient.

Materials and methods

Study areas

The study was conducted in Huasahuasi (province of Tarma) and the Rio Mantaro valley (province of Concepcion, Jauja and Huancayo). In Huasahuasi, the main potato-cropping season lasts from June to December, in which about 90% of the fields are cultivated with potato. With some exceptions of irrigated potato, the Rio Mantaro valley has one potato-cropping season from November to June, in which period our study was conducted. At each location 5 fields were selected; in Huasahuasi, fields were located at an altitude between 2800 to 3600 m and in the Rio Mantaro valley between 3250 to 3850 m.

Experimental design

In each region and altitude, each of the experimental fields had a size of approximately 1300 m^2, which were divided into two equal plot sizes. One plot served as control (insecticide-free) while the other plot received 3 to 5 insecticide applications with aldicarb, carbofuran and fipronil according to farmers' pest management practice. Fungicides (Propineb plus Cimoxanil, dimethomorph, chlorothalonil, and mancozeb) were applied to control late blight (*Phythopthora infestans* (Mont) De Bary), if required. All fields were planted with potato (var. Yungay). In the Mantaro valley, the two plots were separated by a 3 m strip of oat (*Avena sativa* L.). Each experimental site was considered as one individual experiment.

Inventory and assessments of potato pests and natural enemies in insecticide-treated and non-treated field plots

Direct assessments of pests and natural enemies on potato plants. For direct assessments, ten plants were randomly selected in each field plot by crossing the field in a zig-zag course. The presence of potato pests and their natural enemies was assessed by shaking potato plants and collecting falling insects in 1 m^2 sheets of plastic placed under the plants, direct counts on the plant and using sweeping net sampling methods. Direct counting comprised the evaluation of the whole plant for pest damage (e.g., number of potato tuber moth mines; percentage of leaves damaged by flea beetles, *Epitrix* spp., or other type of damage). All instars of pests were collected and reared in the lab until the adult stage. The sweeping net was applied five times (repetitions) along three meters of 10 consecutive potato plants by making ten strokes. The samples of each repetition were placed in a small container and processed in the laboratory.

Evaluation of natural enemies of the potato tuber moths. Leaf and stem infestations by the potato tuber moth species *Phthorimaea operculella* and *Symmetrischema tangolias* are generally very low and do not allow collecting immature stages in larger quantities to study larvae parasitism throughout the season. Hence, plants were artificially infested with eggs and neonate larvae derived from CIP's insect rearing. In each field, at 10 randomly selected points three consecutive plants were infested either with 30 neonate larvae or 30 eggs (laid on board cards and pinned) of the *P. operculella* or *S. tangolias* in four development stages of the potato crop (emergence, hilling, flowering and pre-harvest). In addition, eggs were placed on the ground in Petri dishes covered by a fine wire-mesh. In total, 48,000 larvae and 113,000 eggs of each moth species were exposed to monitor parasitism. Infested plants were marked, removed and processed after three weeks of exposure to assess larvae infestation. Larvae were placed onto potato tubers (var. Peruanita) until hatching of either adults of moths or parasitoids.

Evaluation of ground-dwelling insects. Pitfall traps were used to sample and monitor ground-dwelling insects, consisting of phytophagous insects (e.g., Andean potato weevil) and predators (e.g., carabids). Apart, also a large number of saprophagous insects, spiders and some parasitoids were found, of which only spiders (Aranea) were considered in the present evaluation. The traps consisted of 1-l plastic pots with a funnel on top.

Inside the pot, a smaller jar (0.25 l) was placed to take out collected insects of the trap. The pitfall traps were buried into the soil and slightly covered with a wooden plate (18 x 18 cm) to protect traps from rainfall, but leaving sufficient space for insects to enter. In all treatments, ten traps were installed at planting inside each of the experimental plots, at field borders and in the middle of the fields and monitored throughout the experiment. The captured insects were stored dry in Petri dishes and sealed with Parafilm® until processing and taxonomic identification.

Taxonomic identification of insect species. Taxonomic identification was performed through comparisons with CIPs' existing collections, with available taxonomic keys or by various experts for each insect group. Reference specimens have been pinned and stored in CIP's Entomological Museum at the La Molina Experimental Station, Lima, Peru.

Evaluation of potato damage and yield

In each experimental plot, five subplots (five repetitions) of an area of 5 x 5 m (25 m^2) were randomly selected to evaluate tuber damage and yield. Tubers were counted, graded into three categories according to size, weight and evaluated for damage by Andean potato weevil, the two potato tuber moth species, *Epitrix* spp. and cutworm determining for each pest species infestation rate and intensity, respectively. Since potato tuber moth infestation cannot be finally determined at harvest, a sample of 100 tubers per subplot was stored in paper bags to evaluate infestation again one month after harvest.

Statistical analysis

The potato pest and natural enemy occurrence and infestation affected by the insecticide treatments were compared among individual experimental sites. High variability existed between plots and sites and the data showed no homogeneity and normal distribution. Hence, the Friedman non-parametric statistical test was applied to detect differences in insect population, damage of plants and tubers, and tuber yield between treatments at a significant level of $P \leq 0.05$, using R program (R, 2008). The effect of the insecticide applications on the community of arthropods was assessed by analyzing the communal variables abundance, dominance, and diversity (Shannon) (Southwood, 2000).

Results

Arthropod inventory

A total number of 27,308 individual insects from 9 orders and 56 families were collected from direct plant evaluation, sweeping net and pitfall traps comprising 20,581 phytophagous, 6,302 predators and 425 parasitoids in the study regions of Huasahuasi and the Rio Mantaro valley (Table 1). With 15,474 insects, pitfall traps proved being the most effective method for collecting phytophagous insects and predators. Only 15 pollinators were collected using sweeping net and pitfall traps (data not shown). However, we found a high number of saprophagous and omnivorous insects (13 families of Coleoptera and 23 families of Diptera, mainly). Further, also spiders (Aranea) with the families Lyniphiidae, Clubionidae and Lycosidae were very abundant in pitfall traps (Table 1). At present, we were able to identify 36 phytophagous insects (Table 2), 21 predators and 15 parasitoids to the genus or species level (Table 3), however, the taxonomic identification of many collected insects is still pending.

Table 1. Insect numbers of three functional groups sampled with different evaluation methods in insecticide-treated (I) and non-treated plots (C) in potato cropping systems of Huasahuasi and the Rio Mantaro valley, Peru

Functional group	Methods of evaluation	Huasahuasi			Mantaro valley		
		I	C	% of reduction	I	C	% of reduction
Phytophagous	Plant evaluation	193	615	69	1989	4133	52
	Sweeping net	25	21	-19	1331	2655	50
	Pitfall trap	1350	2844	53	1468	3957	63
Parasitoids	Plant evaluation + sweeping net	35	45	22	120	225	47
Predators	Plant evaluation	68	131	48	55	112	51
	Plant evaluation[1]	26	50	48	35	84	58
	Sweeping net	0	0	0	40	41	2
	Pitfall trap[2]	1654	2654	38	871	752	-16
	Pitfall trap[1]	335	453	26	162	197	18
TOTAL		**3686**	**6813**	**46**	**6071**	**12156**	**50**

[1]Araneae, [2] Mainly Carabidae and Staphylinidae

Phytophagous insects

The group of phytophagous insects includes 23 species that feed on potato; of those, 9 species can cause severe damage to potato reaching pest status. Most important is the Andean potato weevil *Premnotrypes suturicallus* in all cropping regions, especially above an altitude of 3000 m. In both study regions, 8 species of the family Curculionidae were identified yet, of which the Andean potato weevil was most abundant (about 95% of all Curculionidae collected). Other weevil species were *Amitrus alutaceus*, *Puranius* sp., *Cryptochrynchine* sp., *Adioristus* sp., *Cylydrorhinus* and many un-identified species, which feed on plants (roots and foliage) other than potato. The Chrysomelidae *Diabrotica* sp. and *Epitrix yanazara* occurred in the Rio Mantaro valley in higher numbers. The two potato tuber moth species *Phthorimaea operculella* and *Symmetrischema tangolias* as well as the cutworms *Agrotis ypsilon* and *Copitarsia decolora* are also wide-spread in both regions and altitudes. In total we collected 14 cutworm species of which five are identified yet. The aphids *Myzus persicae* and *Macrosiphum euphorbiae* occurred in all regions, but with a higher abundance in the region of Huashuasi. Although the leafminer fly is generally an important pest of potato at the coast of Peru, in the highlands it only causes feeding punctures on potato leaves; it is however an important pest of faba bean (*Vicia faba* L.) and therefore very common in the study region.

Parasitoids

Generally, the abundance of parasitoids was very low in all regions and altitudes although artificial infestation of potato plants with eggs and neonate larvae of *P. operculella* and *S. tangolias* were used, but which resulted only in a low parasitism percentage between 0.30 and 0.50% in all study areas, although 13 and 9 species were identified in Huasahuasi and the Rio Mantaro valley, respectively. Among the yet identified species are the Braconidae *Dolichogenidea gelechiidivoris* and the Encyrtidae *Copidosoma koehleri,* which both parasitize potato tuber moths, as well as the Tachinidae *Incamyia cuzcensis* (Table 3). *Halticoptera arduine* is a wide-spread parasitoid of leafminer flies in Peru.

Table 2. Phytophagous insects sampled with different evaluation methods in potato cropping systems at different altitudes in Huasahuasi and the Rio Mantaro valley, Peru

Functional group	Family	Species	Huasahuasi (m a.s.l.)			Mantaro (m a.s.l.)	
			3550	3350	2800	3850	3300
Phytophagous	Chrysomelidae	*Calligrapha curvilinea* Stal					*
		Diabrotica* sp. cerca *nigropuncta		**		**	**
		***Diabrotica* sp.**		*			*
		***Epitrix yanazara* Bechyne**	*	*	**	***	****
		Phyllotreta sp.				*	*
	Curculionidae	*Adioristus* sp.	*	*	*	*	**
		Amitrus alutaceus Schoenherr	*	*	*	*	*
		Cryptorhynchine sp.	*	*	*	*	*
		Cylidrorhinus sp (three species)	***	***	***	**	*
		***Premnotrypes fractirostris* Marshall**					*
		***Premnotrypes pusillus* Kuschel**				*	*
		***Premnotrypes suturicallus* Kuschel**	****	***	**	****	***
		Puranius sp. (two species)	**	**	*	*	*
	Meloide	***Epicauta latitarsis* Haag**				*	*
		***Epicauta willei* Denier**				*	*
	Melyridae	***Astylus luteicauda* Champ**				*	**
	Scarabaeidae	*Lygirus mainom* Erichson		*	*	*	*
	Agromyzidae	*Amauromyza* sp.				*	*
		***Liriomyza huidobrensis* Blanchard**	*	*		*	**
		***Phytoliriomyza papae* Spencer**	*	*	*	*	***
	Anthomyiidae	*Delia platura (Meigen)*	**	**	*	*	**
	Cecidomyiidae	***Prodiplosis longifila* Gagné**			*	*	*
	Aphididae	***Macrosiphum euphorbiae* (Thomas)**	**	*		*	*
		Myzus persicae (Sulzer)	***	**	*	*	**
	Cicadellidae	*Agallia* sp.	*	*	*	*	*
	Lygaeidae	***Nysius* sp.**	*			*	*
	Membracidae	*Heranice miltoglypta* (Fairmaire)	*				
	Psyllidae	***Russelliana solanicola* Tuthill**	*			1	***
	Gelechiidae	***Phthorimaea operculella* (Zeller)**	*	*	**	*	*
		***Symmetrischema tangolias* (Gyen)**	*	*	**	*	*
	Noctuidae	***Agrotis ypsilon* (Hufnagel)**	*	*	**	*	*
		***Copitarsia decolora* (Guenée)**	*	**	*	*	*
		***Copitarsia incomoda* (Walker)**			*		
		***Peridroma clerica* (Butler)**			*		*
		***Scania* sp.**		*		*	
	Plutellidae	*Plutella xylostela* (L.)		*			

**** Very abundant > 500; *** abundant [100-500]; ** moderate [10-100]; * scarce <10. Species in bold are phytopagous on potato

Predators

In the group of predators, only ground-dwelling predators of the family Carabidae and Staphylinidae occured in higher numbers; in Huasahuasi and the Rio Mantaro valley we found 16 and 18 species of carabids, respectively, but only some of the species are identified yet (Table 3). In Huasahuasi, *Pelmatellus* sp. was the most abundant species (74% of all species collected). In contrast, *Blennidus* sp. was more common in the Rio Mantaro valley (79% of all species collected). Other carabids were *Incagonum* sp. (near *chilense*), *Metius* sp., *Notiobia peruviana*, and *Pelmatellus columbianus*. Plant inhabiting predators, e.g. of the family Coccinellidae, Syrphidae or Lygaeidae, were very rare or even absent at specific locations or altitudes.

Table 3. Parasitoids and predators sampled with different evaluation methods in potato cropping systems at different altitudes in Huasahuasi and Rio Mantaro valley, Peru

Functional group	Family	Species	Huasahuasi (m a.s.l.)			Mantaro (m a.s.l.)	
			3550	3350	2800	3850	3300
Parasitoids	Tachinidae	*Eucelatoria* sp.	*	*			
		Incamia cuzcensis Townsend	*	*			
		Incamia sp.	**	**	**	*	*
		Leucostoma sp.					*
		near *Phasmonfrontina* sp.	*	*			
		Peleteria sp.		*		*	
		Prosopochaeta anomala Aldrich	*	*		*	*
		Trichophoropsis sp.	*				
	Braconidae	*Aphidius* sp. (two species)	*	*	*	*	*
		Dolichogenidea gelechiidivoris (Marsh)	*	* ▲	* ▲	*	*
	Cynipidae	*Ganaspidium sp.*	*				
	Encyrtidae	*Copidosoma koehleri* (Blanchard)		* ▲	*		*
	Ichneumonidae	*Enicospilus sp.*	*	*			
		Thymebatis sp. (two species)	**	**		*	*
	Pteromalidae	*Halticoptera arduine* Walker				*	*
Predators	Carabidae[1]	*Blennidus* sp. (two species)	*	*		***	*
		Incagonum sp. (near *chilense*)	**	**	*	*	***
		Metius sp. (five species)	*	**	*	**	**
		Notiobia (Anisotarsus) peruviana (Dej.)	**	**	*	*	**
		Notiobia (Anisotarsus) sp.		*			
		Pelmatellus columbianus (Reiche)	**	**	*	**	*
		Pelmatellus sp.	***	****	**	**	*
	Coccinellidae	*Eriopis conexa conexa* Mulsant					*
		Eriopis sp.	*	*		*	*
		Hippodamia convergens Guérin-Méneville				*	*
	Staphylinidae	*Oligota* sp.	*	*		*	*
		Paederus irritans	*	*	*	*	
		Paederus sp.	**	**	*	*	*
	Syrphidae	*Platycheirus saltana*	*				*
		Scaeva prob. *punctata* Shannon	*				
		Toxomerus prob. *mutum*					*
		Toxomerus sp.					*
	Lygaeidae	*Geocoris punctipes* (Say)	*				
	Nabidae	*Nabis punctipennis* Blanchard					*
	Hemerobiidae	*Hemerobius bolivari* Banks	*	*	*		
		Hemerobius tolimensis Banks	*	*		*	

**** Very abundant > 500; *** abundant [100-500]; ** moderate [10-100]; * scarce <10. ▲ found with artificial infestation.

[1] May include herbiovorous species

Insecticide effects on potato pests and natural enemies

Insecticide effects on potato pests

Compared to non-treated fields, the total number of phytophagous insects collected by three different evaluation methods (direct plant evaluation, sweeping net and pitfall traps) was reduced by 55% in insecticide-treated plots (Table 1). This means that insecticides controlled more than half of the pest population. The species number of the family Curculionidae was not affected by the insecticide treatments although abundance was reduced by 70% and 61% for Huasahuasi and Mantaro valley, respectively. Significant differences were observed in the dominance and diversity of species (Table 4A).

Insecticide treatments most heavily affected the Andean potato weevil population (Fig. 1). In the Rio Mantaro valley, the Andean potato weevil abundance was significantly higher at higher altitudes of 3800 m; here, three insecticide applications were needed to reduce the weevil population significantly. In Huasahuasi, treatment effects were similar (data not shown).

Table 4. Abundance and species diversity of ground-dwelling insects in insecticide-treated (I) and non-treated (C) fields as monitored in pitfall traps. Huasahuasi: Total of 5 fields at elevations between 2800 m and 3600 m; Rio Mantaro valley: Total of 5 fields at elevations between 3300 and 38050 m.

Biodiversity indices	Huasahuasi		Mantaro Valley	
	I	C	I	C
A Curculionidae				
Taxa S	18	12	15	13
Abundance	714	2210	1250	3779
Dominance	0.2979	0.4756 *	0.7938	0.9175 *
Shannon H	1.48	1.015 *	0.487	0.2475 *
B Carabidae				
Taxa S	16	17	18	16
Abundance	945	1795	731	629
Dominance	0.4389	0.4509	0.1645	0.1833
Shannon H	1.312	1.267	2.073	2.006
C Staphylinidae				
Taxa S	9	8	8	8
Abundance	686	837	93	73
Dominance	0.1993	0.249 *	0.3299	0.3898
Shannon H	1.715	1.549 *	1.422	1.279
D Araneae				
Taxa Family	7	11	12	11
Abundance	320	411	152	133
Dominance	0.7827	0.7815	0.2371	0.232
Shannon H	0.5071	0.5319	1.69	1.732

* $P < 0.05$

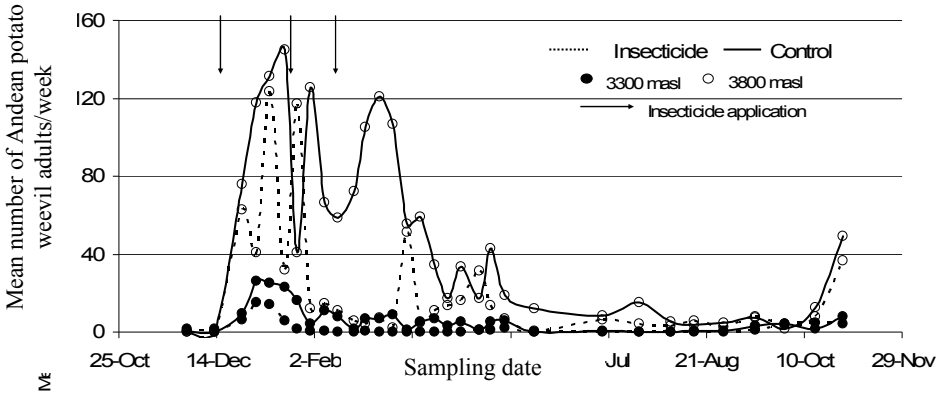

Figure 1. Effects of insecticide applications on Andean potato weevil (*Premnotrypes suturicallus*) population dynamics as monitored by pitfall traps in the Rio Mantaro valley at altitudes of 3300 m and 3800 m, Peru. At 3300 m: mean of three fields; at 3800 m: mean of two fields for each evaluation date

The non-application of insecticides caused a strong increase of the population of flea beetles (*Epitrix yanazara*) and of a stem borer fly (probably *Phytoliriomyza papae*) especially in the Rio Mantaro valley at altitudes of 3300 m. The flea beetle adult population increased up to 7 times (from 22 to 158 adults/plant) intensifying significantly the damage to the potato foliage of up to 70% (Fig. 2). Larvae of stem borer flies caused a plant infestation of up to 50% (data not shown).

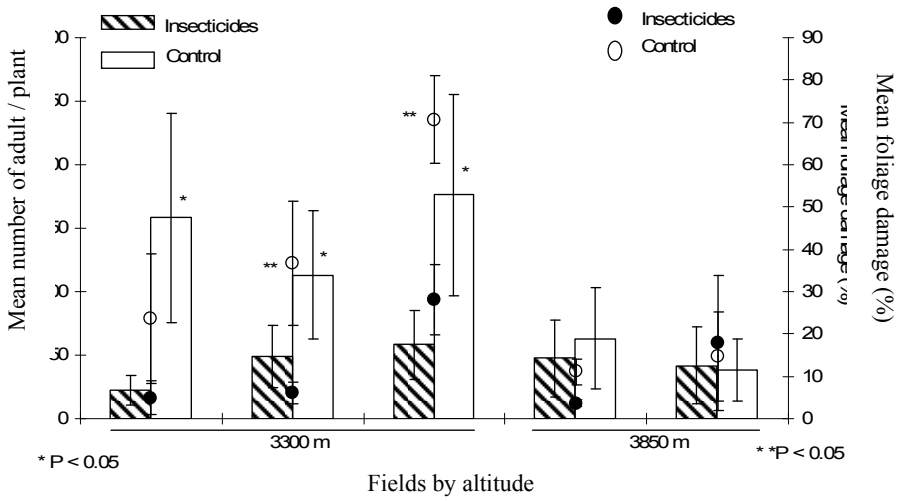

Figure 2. Flea beetle (*Epitrix yanazara*) adult population and mean potato foliage infestation in five insecticide-treated and non-treated fields at two altitudes of the Rio Mantaro valley, Peru

Effects on parasitoids and predators

The abundance of parasitoids (total of 424 individuals) and plant inhabiting predators (total of 336 individuals) was very low throughout the potato growing season in both study regions. Although a mean overall reduction of both functional groups by 35% and 49.5% could be found in insecticide-treated field plots, no significant differences could be established (Table 1). Ground-dwelling carabids, rove beetles (Staphylinidae) and spiders (Aranea) were found in higher numbers in Huashuasi in non-insecticide treated fields, but significant differences with regard to family or species number, abundance, dominance or diversity were not found (Table 4B, C, D).

Treatment effects on potato damage and yield

In Huasahuasi at altitudes of 2800 m, potato tubers were severely infested by the potato tuber moth, both in insecticide-treated and non-treated fields with 25% and 40% of tubers infested, respectively (Fig. 3A). At altitudes above 3350 m, Andean potato weevil infestation ranged between 60 to 70% in the non-treated control fields. The application of insecticides controlled weevil infestation almost by 100%. Without insecticide treatments, a total yield reduction of 40% was determined at lower altitudes of 2800 m, above 3350 m yield reduction ranged between 6 and 26% (Fig. 3B), however, differences were statistically not significant. In the Rio Mantaro valley, tuber infestation by the potato tuber moth complex was less than 5% (Fig. 3C). Andean potato weevil infestation differed greatly between experimental sites at an altitude of 3300 m. In two of the experimental sites Andean potato tuber infestation was very low (<5%) in the non-treated fields, but reached 90% at the third site. At 3850 m, both fields were highly infested by Andean potato weevil resulting in a tuber infestation of 80 and 100% in the non-treated control plots. Interestingly, also the insecticide-treated plots had a very high Andean potato weevil infestation of 20 and 76%, respectively. In three of the sites, total potato yields were significantly different between insecticide-treated and non-treated plots, which can be explained by the high flea beetle infestation on potato leaves especially at one site at 3300 m. In this cases, the total potato yield was reduced by 72% (Fig. 3D).

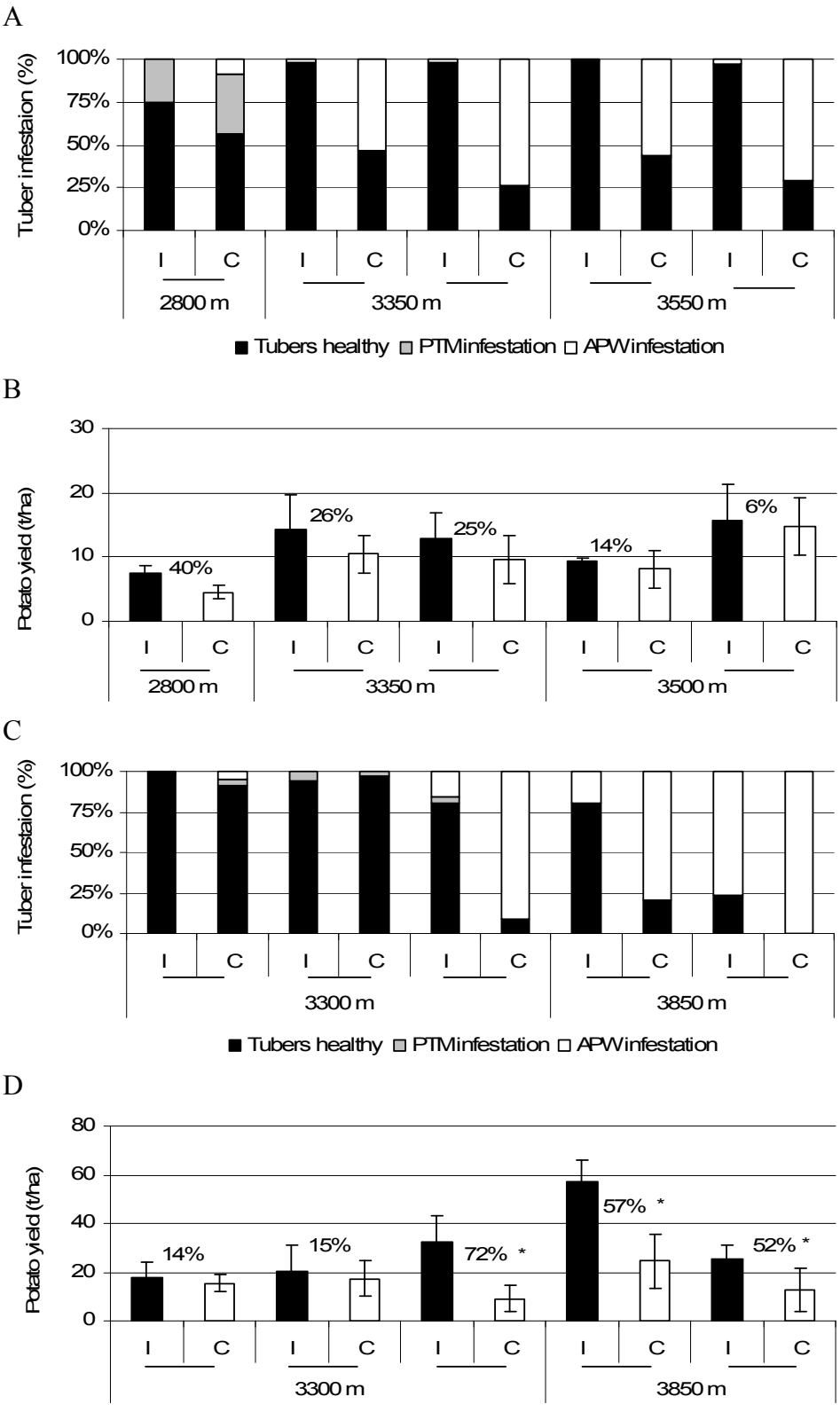

Figure 3. Tuber infestation by the potato tuber moth complex, *Phthorimaea operculella* and *Symmetrischem tangolias*, and the Andean potato weevil, *Premnotrypes suturicallus*, and total potato yield in insecticide-treated and non-treated fields in the potato growing regions of Huashuasi (A, B) and the Rio Mantaro Valley (C, D). *=P • 0.05

Discussion

Arthropod inventory and diversity

Our study presents the first detailed inventory on arthropods in potato agroecosystems of the high Andes at altitudes between 2800 and 3850 m. Phytophagous insects have been found being the most numerous functional group constituting of 75.4% of the total arthropods collected from direct plant evaluation, sweeping net and pitfall traps. In contrast, predatory insects and parasitoids represented 23% and 1.4% of the total insect population, respectively. Of all predators, ground-dwelling Carabidae and Staphylinidae were the most abundant families with 21.7%; instead predators inhabiting potato foliage like species of the families Coccinellidae, Syrphidae, Lygaeidae, Nabidae, and Hemerobiidae only represented 1.3% of the total insect population. Previously we reported of the high abundance and importance of carabids in Andean potato weevil natural control in the Rio Mantaro valley (Kroschel et al. 2009). The general very low abundance of parasitoids and plant inhabiting predators might be due to the low availability of hosts and preys (e.g., aphids) as a result of the high altitude and the harsh Andean climate. Moreover the landscape especially in the Rio Mantaro valley is poorly structured and consists mainly of introduced trees (*Eucalyptus globulus* Labill.) and bushes (Spanish broom, *Spartium junceum* L.) grown in hedgerows. We found that those hedgerows suppress the growth of annual flowering plants and hence are not ideal refugees for those species. Instead, flowering plants are mainly found as weeds along agricultural fields, which are more frequently inhabited by beneficial insects compared to hedgerows.

Effects of insecticides on potato pests and potato yield

The frequent insecticide applications are directed to control Andean potato weevil infestation. In the region of Huasahuasi insecticide efficiency reached almost 100% whereas in the Rio Mantaro valley a high weevil infestation was still found in insecticide-treated fields. Previously, we already reported that farmers' insecticide applications are often not very successful and that infestations of 10-30% are very common (Kroschel et al. 2009). The two potato tuber moth species *Phthorimaea operculella* and *Symmeytrischema tangolis* are important potato pests at lower altitudes up to 3400 m. The insecticide applications resulted in only minor reductions of tuber infestation. Generally, the infestation of potato leaves and stems is not very severe in the study areas, but an increase in tuber infestation is highly correlated to late potato harvest; main potato damage occurs during potato storage (Keller, (2003).

In non-insecticide-treated fields the flea beetle (*Epitrix yanazara*) population increased enormously in the Rio Mantaro valley; at some sites high tuber yield losses could be explained by the high flea beetle foliage infestation. Flea beetles overwinter as adults in the soil or under plant debris in undisturbed areas. Adults feed on potato foliage, producing numerous minute circular holes. Further, larvae feed on roots, and tubers causing superficial injury. *Epitrix* species are quite common in potato production in Peru; apart of *Epitrix yanazara,* Bravo et al. (1986) reported of *E. parvula* (Fab.), *E. subcrinita* Le Conte, *E. ubaquensis* (Harold) and *E. harilana rubia* Bech. & Bech. Our study revealed that flea beetles may become a very serious potato pest if alternative control measures for Andean potato weevil control would be applied. In our study we could not identify natural enemies of flea beetles. Ohashi and Urdampilleta (2003) identified predatory bugs in tobacco (*Nicotiana tabacum* L.) production in Argentina such as *Cosmoclopius nigroannulatus* and *C. poecilus, Apiomerus* sp., *Repipta flavicans* and *Zelus* sp. (Hemiptera: Reduviidae) as well as two species of *Campyloneuropsis* (Hemiptera: Miridae). Further it was reported that entomopathogenic nematodes (*Steinernema carpocapsae*) (Ambrosino, 2008) and fungi (*Beauveria bassiana*) (Rojas, 1982) can reduce flea beetle larvae infestation.

Another emerging pest is the stem borer fly *Phytoliriomyza papae*, which also occurred mainly in non-treated fields. Adult flies lay eggs in the leaf axils and hatching larvae bore into the stem where they destroy the vascular system.

In the region of Huashuasi, no statistically significant differences in total potato yields were found between insecticide-treated and non-treated fields. However, in the Rio Mantaro valley significant differences occurred, but which can be only partly attributed to the high flea beetles infestation. Generally, insecticide-treated potato plants looked healthier and greener than non-treated plants; hence yield stimulation may have also result directly from the insecticide application as it is described for carbofuran (Waibel, 1983).

Effects of insecticides on non-target organisms

Since decades the frequent use of insecticides is farmers' main practice to manage potato pests in the study area (Ewell et al. 1990). Considering all study sites, insecticide treatments reduced about 50% of the arthropods population compared to non-treated fields within one vegetation period. Since potato is the most widely grown crop especially at altitudes above 3800 m, the regular use of insecticides may have caused long-term negative effects on insect populations, especially where no effective recovery from unsprayed fields or hedgerows was given; however, no earlier reference base line studies exist that could give evidence for this assumption.

Long-term field studies in the United Kingdom in a range of arable crops found few adverse long-term effects of pesticides on non-target organisms including insects, spiders, earthworms and soil microbes (Young et al., 2001). Here, the application of broad-spectrum insecticides resulted in declines in the number of many non-target arthropods, but these usually recovered within the same growing season. Less temporary effects were seldom noted and affected only soil-dwelling collembolans (springtails). Numbers of these organisms remained comparatively low in treated plots up to two years after application (Devine and Furlong, 2007). Apart from the persistence of the insecticide, the degree to which affected populations can recover is also dependent upon the recruitment of new individuals from unaffected populations, which permit often the rapid recovery of species in insecticide-treated fields (Jepson and Thacker, 1990). Especially in small experimental areas the probability for a reinvasion increases compared to larger field sizes. In our study we used field sizes of 1300 m^2 divided into two equal plot sizes. Further, insecticide effects on natural enemies may be complex to interpret or fail to detect if only small areas within a field are monitored or if species exhibit either low numbers or spatial heterogeneity (Mead-Briggs, 1998). Such a distribution has been shown for most groups of beneficial arthropods including Carabidae, Staphylinidae, Araneae and parasitic wasps (Holland et al., 1999). The taxonomic level at which results are analyzed is also important because no effects may be detected at the family level but individual species may vary considerably in their response (Büchs et al., 1997).

The impact of insecticides is not limited to a decrease in number of individuals and taxa richness, but also changes species composition. In studies on the carabid community, species richness was a consistent indicator of change; dominance not (Teodorescu and Cogalniceanu, 2005; Dritschilo and Erwin, 1982). Also, precipitation or irrigation may play an important role on how insecticides are affecting insects. Residual toxicity tests suggest that post treatment irrigation reduces insecticide exposure to adult predators such as carabids. The insecticides bendiocarb or imidacloprid have an acute toxicity to the carabid *Harpalus pensilvanicus,* but applications followed by irrigation were found to have relatively little impact on season-long pitfall trap captures in golf courses roughs (Kunkel et al., 2001). In our study regions, potato is grown during the rainy season with not less than a mean precipitation of 750 mm during the potato vegetation period.

Apart from soil-dwelling predators, parasitoids and predators were too scarce in the study area to find significant differences in insecticide-treated and non-treated fields. However, several studies have shown that parasitic wasps are susceptible to insecticides. Gao et al. (2008) found that application of monocrotophos and methomyl in cotton (*Gossypium hirsutum* L.) affected the abundance of parasitoids, but not their diversity. Kao and Tzeng (1992) found a mortality of more than 90% of *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) adults (parasitoid of *Plutella xylostella* L.) after carbofuran and methomyl applications while effects to immature stages were less hazardous. In laboratory studies Zu-hua et al. (2004) confirmed that fipronil and methomyl caused over 93% mortality of *C. plutellae* within 24*.* Further, dimethoate and metamidophos were toxic to *Orgilus lepidus* Muesebeck and *Copidosoma desantisi* Annecke and Mynhardt*,* both parasitoids of *Phthorimaea operculella* in lab conditions (Keeratikasikorn and Hooper, 1981). According to other studies the effects on predators are mixed. Chlorpyriphos and cypermethrin affected predators like spiders, brown earwigs and carabid beetles in Australia (Curtis and Horne, 1995). The predator *Orius insidious* Say was significantly affected by the insecticides bifenthrin, ethyl-parathion, permethrin, and λ-cyhalothrin in corn (*Zea mays* L.) with more than 40% reduction compared to untreated controls (Al-Deeb et al., 2001). On the other hand, applications of dimethoate had less toxic effects on *Tachyporus* sp. in wheat in Germany and Great Britain (Holland et al., 2000). The pyrethroids fluvalinate and esfenvalerate did not significantly reduce hoverfly (Syrphidae) larvae in field plots but did affect ladybird larvae (*Adalia* spp.) and reduced the Coccinellidae population. By contrast, applications of carbamates such as pirimicarb had no effects on ladybirds' larvae but reduced the number of hoverflies significantly (Jansen, 2000).

Conclusion

Intensive potato production in the Central highlands in Peru is associated with the excessive use of highly toxic insecticide applications to control Andean potato weevil. These insecticide applications also control other pests such as flea beetles. The population of natural enemies is very low in the highlands and main predators are ground-dwelling carabids. Although the number of individual insects of all functional groups was reduced in insecticide-treated fields we could not found a significant direct reduction but assume that the long-term use of insecticides has contributed to a degradation of predators and parasitoids. Potato production with less use of insecticides could become possible by using plastic barriers installed at field borders, which showed a high efficacy to stop the migration of flightless Andean potato weevil adults to potato fields and thus to reduce Andean potato weevil tuber infestation effectively (Kroschel et al., 2009; Alcazar and Kroschel, 2009). No natural enemies of flea beetles could be identified in our study, which could provide effective natural control. However, compared to Andean potato weevils, flea beetle infestations on potato leaves could be more easily controlled by farmers applying economic damage thresholds and thus only applying insecticides, if needed. Augmentation strategies for parasitoids and predators could be applied to increase their population and hence natural control of potato pests.

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Impact of plant extracts and organic amendments on the growth of *Ralstonia solanacearum* and severity of potato bacterial wilt

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Abstract

Continuous potato (*Solanum tuberosum*) production in the tropics and subtropics is usually handicapped by bacterial wilt incited by *Ralstonia solanacearum.* Laboratory and screen house experiments were conducted to assess antibactericidal activities of plant extracts and organic amendments respectively on pathogen growth and bacterial wilt severity. Antibacterial activity of the extracts was determined by colonial growth on potato sucrose agar medium amended with various concentrations of the extracts. Potato (cv Cipira) tubers were planted Dschang and Foumbot in 10% organic amended soils and inoculated 30 days later with 10 ml of 10^7 cfu/ml of the bacterial suspension. Data on wilt severity and tuber production were recorded. Methanol leaf extracts of *Crotalaria falcata* and *Tephrosia vogelii* were more active (ED_{90} = 1.40–5.94 mg/ml) than the corresponding acetone extracts (ED_{90} = 47.60–77.14 mg/ml), while the reverse was observed for acetone leaf extracts of *Brassica integrifolia* and *Cissus aralioides*. All organic amendments significantly reduced bacterial wilt severity in both sites. Significant (P = 0.001) negative correlations were observed between bacterial wilt severity and tuber yields. Crotalaria and Tephrosia amendments were more effective in the reducing wilt severity and increasing tuber yield than Brassica or Cissus amendments. Results indicate a potential of plant extracts in bacterial wilt management and a necessity for an adoption of integrated *R solanacearum* management strategies through a judicious use of organic amendments in potato production.

Keywords: Potato, bacterial wilt, organic amendments, plant extract, *Ralstonia solanacearum.*

Introduction

Bacterial wilt caused by the soil borne vascular pathogen, *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*), is a devastating disease in both tropical and sub tropical regions, infecting over 200 plant families. Important host crops include the *Solanaceae* (e.g. potato, tomato, eggplant, garden huckleberry, tobacco), *Musaceae* (e.g. bananas), *Papilionaceae* (e.g. groundnuts) and *Zingiberaceae* (e.g. ginger) (Hayward, 1991). Thus, it is a major limitation to a sustainable production of a wide range of crops in Sub-Sahara Africa (Adipala *et al.,* 2001). Losses of up to 100% were reported for potato and tomato in the early 1970s in Uganda (Simbwa-Bunya, 1971).

In potato, bacterial wilt severity is often exacerbated by low fertility caused by intensive or continuous cultivation and use of infected seed tubers. Control has been difficult due to the high variability of the pathogen, its extremely wide host range, lack of possibility for chemical control and high ability of the pathogen to survive in diverse environments. The use of resistant varieties is the simplest and most effective method to control any disease. However, bacterial wilt resistance is overcome by the genetic diversity of the pathogen as well as genotype x environment interactions.

Incorporation of composts into soils is a fundamental cultural practice in crop production. Organic matter is usually incorporated into the soil for sustainable crop production, because it enhances soil fertility through modification of soil physical, chemical and biological properties (Islam and Toyota, 2004). Moreover, the use of organic amendments is a widespread means to control diseases caused by soil-borne plant pathogens (Huang and Huang, 1993). The effects of organic amendments on the severity of bacterial wilt have been reported in several countries, including Kenya (Muriithi and Irungu, 2004), Nigeria (Adebayo & Ekpo, 2001), Uganda (Lemaga *et al.*, 2001) and Taiwan (Akew *et al.*, 1996). However, few studies address the *in vitro* effects of plant extracts on bacterial growth and the impact of their amendments on bacterial wilt severity. This study was designed to investigate the effects of plant extracts and organic amendments respectively on the *in vivo* growth of *R. solanacearum* and the severity of bacterial wilt.

Materials and methods

Plant and bacterial materials. Fresh leaves of wild cabbage *(Brassica integrifolia* West) Rupr.), cissus (*Cissus aralioides* (Baker) Planch.)*,* crotalaria (*Crotalaria falcata* Vahl ex DC) and tephrosia (*Tephrosia vogelii* Hook. f.) were collected in Dschang, western highlands of Cameroon during September to November 2004. These plants are annual weeds that invade cultivated fields and also grow wild on fallow lands. Laboratory and greenhouse experiments were carried out to assess their antibacterial activities on potato bacterial wilt pathogen, *R. solanacearum*.

The bacterium, *R. solanacearum*, was isolated from infected potatoes in Dschang and cultured on potato sucrose agar (PSA). The strain belonged to race 3. The bacterial suspension was prepared in sterile distilled water after 48 h and adjusted to 10^7 colony forming units (cfu)/ml (OD=0.3 at 600 nm) using a Pye Unicam SP6-450 UV/VIS spectrophotometer.

Extraction and bioassay of the extracts. The collected plant leaves were dried at 30 °C for 7 days. The dried leaves were ground to powder and macerated at room temperature with methanol or acetone for 5 days. The combined methanol or acetone extracts were concentrated to dryness using a rotary vacuum evaporator at 65 °C for methanol and 56 °C for acetone. The yields of the extracts were expressed in g/100 g dry weight.

The antibacterial activity of the extracts was determined by colonial growth on potato sucrose agar (PSA) medium amended with various concentrations of the extracts. The required amount of extract was dissolved in 5% DMSO and mixed with the agar medium to produce concentrations of 1, 10, 100, 1000 mg/ml. Control plates contained the same treatment without any extract. Petri plates containing various concentrations of the extracts or control were streak inoculated with 0.1 ml of 10^3 cfu/ml of the bacterial suspension. There were five plates per treatment and the experiment was repeated twice. Inoculated plates were incubated at 30 °C in dark for 24 h. The number of colonies were counted for each plate and percent growth inhibition was determined using the formula: $(N_u - N_a)100/ N_u$, where N_u and N_a are the number of cfu on the unamended and amended PSA medium, respectively.

Effect of organic amendments on bacterial wilt severity. This trial was carried out at the screen house in Dschang (1400 m) and Foumbot (1000 m) during April to August 2005. The soil types of both sites were nitrosol (pH-H_2O = 6.8, 6.5% OM, 13-14% C/N, 23 % clay) for Dschang and andosol (pH-H_2O = 6.4, 12.9% OM, 12.1% C/N, 15 % clay) for Foumbot. Dschang and Foumbot are respectively major potato and tomato production regions of Cameroon. Experiments were designed in a randomized complete blocks with four replicates. A total of 100 pots each filled with 450g of soil were used, five pots for each treatment. Pots were amended by adding 50 g of fresh shoots of *B. integrifolia*, *C. aralioides, C. falcata* or *T. vogelii.* The amended combination was allowed to decompose for four weeks. Unamended pots served as controls and the trial was repeated once.

After amendment, potato (cv. Cipira) tubers, obtained from a certified commercial supplier, were planted one tuber per pot into each pot. Plants were inoculated 30 days after planting by pouring 30 ml of 10^7 cfu/ml round the base of each seedling. Each plant was watered daily with 30 ml of sterile distilled water.

Data on wilt severity and tuber production were collected. Wilt severity was assessed weekly using a 1-5 scale (Adebayo & Ekpo, 2001) in which 1 = no symptom, 2 = one leaf at least partially wilted, 3 = two or three leaves wilted, 4 = four or more leaves wilted and 5 = plant dead. Healthy tubers were collected and weighed 94 days after planting in both locations and yields were expressed in g/plant.

Data analysis. For *in vitro* tests, percent bacterial growth inhibition was transformed into probits and the values obtained were regressed on the logarithm of the concentration of the extracts. The equivalent concentrations for 90 % inhibition (EC_{90}) of bacterial growth were calculated for each extract as suggested by Finney (1971). All data were subjected to analyses of variance and Duncan's multiple range test (P = 0.05) was used to compare treatment means.

Results

In vitro experiments. The extraction of the various plant materials yielded 10.51 – 17.35 g/100g for methanol extracts and 9.13 – 14.93 g/100 g for acetone extracts. Consequently, for all plant materials used, methanol yielded relative higher extract than acetone. The colour of the extracts varied with the solvent used (Table 1).

Table 1. Yield and colour characteristics of plant extracts used for *in vitro* tests

Extract	Yield (g/100 g dry wt)		Colour	
	Methanol	Acetone	Methanol	Acetone
Crotalaria falcata	11.16	9.87	Green	Deep brown
Tephrosia vogelii	15.78	13.57	Green	Green
Brassica integrifolia	17.35	14.93	Brown	Deep brown
Cissus aralioides	10.51	9.13	Brown	Green

The extracts tested had adverse effects on the growth of the bacterium at different concentrations. Bacterial growth reduced with increase in concentration of each extract. Bactericidal activities of the extracts were rated in terms of EC_{90} values. The antibacterial activity of the extracts depended on the solvent used. Methanol extracts of Crotalaria and Tephrosia were more active (EC_{90} = 1.40 – 5.94) than the corresponding acetone extracts (EC_{90} = 47.60 – 77.14) while the reverse was observed for Brassica and Cissus extracts. The EC_{90} values for Crotalaria and Tephrosia methanol extracts were significantly (P = 0.01) lower than those of the Brassica and Cissus extracts tested, while the reverse trend was observed for acetone extracts. Based on EC_{90} values, Crotalaria and Tephrosia methanol extracts and Brassica acetone extracts were highly active on the bacterium (Table 2).

Table 2. Bactericidal activity (ED_{90}) of methanol and acetone plant extracts on *in vitro* growth of *R. solanacearum*

Extract	Methanol (mg/ml)	Acetone (mg/ml)
Crotalaria falcata	5.94 c	47.60 b
Tephrosia vogelii	1.40 c	77.14 a
Brassica integrifolia	62.87 b	6.12 d
Cissus aralioides	354.15 a	25.58 c

[z]Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05).

Effect of organic amendments on bacterial wilt severity. Bacterial wilt severity differed with location and soil treatments. All plant amendments significantly reduced wilt severity in both locations. Crotalaria and Tephrosia amendments were more effective in reducing disease severity than Brassica or Cissus amendments (Fig 1). The least wilt severities in both locations were obtained in pots amended with Crotalaria and Tephrosia leaves (Table 3).

Potato tuber yields were significantly higher in amended soils compared to the control. The yields were increased by 48-207% in Dschang and 206-745% in Foumbot. The increases were consistently higher in Foumbot than in Dschang. In both locations applications of Crotalaria or Tephrosia amendments consistently produced higher tuber yields compared to Brassica or Cissus amendments (Table 4). Tuber yields decreased with increase in wilt severity in both locations. Regression analyses showed significant (P = 0.001) negative correlations between bacterial wilt severity (x) and tuber yields (y). The regression equations were y = 383.11-62.20x (R^2 = 0.98) for Dschang and y = 344.55-60.17x (R^2 = 0.98) for Foumbot (Fig. 2).

Table 3. Severity of potato bacterial wilt as affected by organic amendments in two locations recorded 57 days after inoculation

Amendment	Dschang	Foumbot
Control	5.0 a[z]	5.0 a
Crotalaria falcata	2.4 cd	1.9 c
Tephrosia vogelii	2.2 d	1.2 c
Brassica integrifolia	4.0 b	4.4 a
Cissus aralioides	3.0 c	3.4 b

[z]Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05).

Table 4. Tuber yields (g/plant) of potato as affected by organic amendments in two locations recorded 64 days after inoculation

Amendment	Dschang	Foumbot
Control	82 c[z]	31 c
Crotalaria falcata	237 (189) a	242 (681) a
Tephrosia vogelii	252 (207) a	262 (745) a
Brassica integrifolia	121 (48) bc	95 (206) bc
Cissus aralioides	191 (133) ab	136 (338) b

[z]Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05). Values in parentheses are percent increases over the unamended control

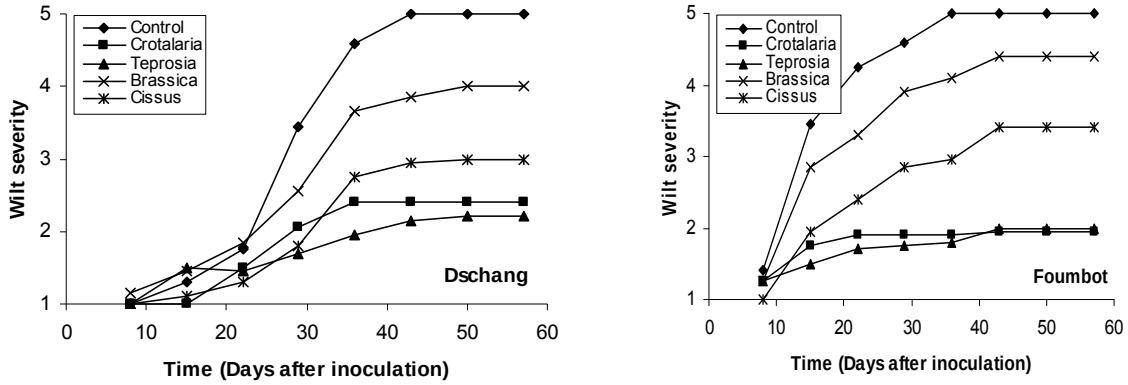

Figure 1. Effect of plant amendments on the progress of potato bacterial wilt severity in two location of Cameroon.

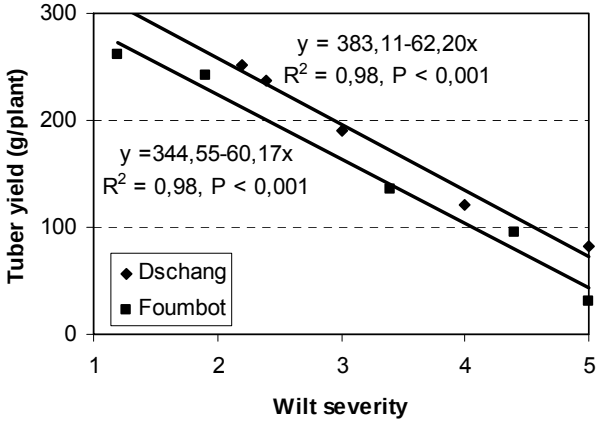

Figure 2. Relationship between bacterial wilt severity and potato tuber yield in two locations

Discussion

This study demonstrates the *in vitro* antibacterial effects of plant extracts and the *in vivo* effects of their amendments. Several studies have reported the *in vitro* antibacterial effects of some herbs (Satish *et al.,* 1999; Vudhivanich, 2003). As the bacterium is known to be transmitted through seeds, one important application of plant extracts is as a seed protectant.

Organic amendments significantly improved tuber yields in both sites, suggesting their importance in bacterial wilt management and plant production. In East Africa, some researchers (Lemaga *et al*., 2001; Muriithi and Irungu, 2004) also reported significant reductions of bacterial wilt incidence with improvements in potato yields following a combined application of organic and inorganic soil amendments. The effects of Crotalaria green manure on bacterial wilt control has also been reported (Akew *et al*., 1996; Adebayo and Ekpo, 2001).

The difference in wilt severity between both sites could be attributed to soil organic matter content as the organic matter content of the soil used in Foumbot was twice as high of that used in Dschang. Moffet *et al*. (1983) reported that bacterial wilt incidence reduces in soils with high organic matter content. There appears to be an indirect effect of organic amendment on pathogen population in the soil. Some researchers (Michel and Mew, 1998; Islam and Toyota, 2004) reported that organic amendments enhance antagonist activity in the soil while suppressing soil borne pathogens. Moreover, applications of these amendments increase soil pH and nitrate accumulation and reduce the C/N ratio that is necessary for microbial antagonists. Islam and Toyota (2004) did not observe any disease control in autoclaved amended soils and concluded that indigenous microorganisms in condusive soils rather than those in the compost play an important role in the suppressive effect.

In vitro results with methanol extracts were similar to screen house tests. Crotalaria and Tephrosia leaf extracts were more active *in vitro* than those of Brassica and Cissus and results of organic amendments followed a similarly trend. However, further studies are needed to confirm the use of *in vitro* tests in the screening of possible antibacterial organic amendments. This study shows that the four plant extracts used may be developed as effective antibacterial compounds. Moreover, Crotalaria or Tephrosia organic amendment induces a higher antibacterial activity on *R. solanacearum* than Brassica or Cissus amendment. Consequently, the former organic amendments could be envisaged as possible components of IPM tools for bacterial wilt.

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Using rhizobacteria to improve productivity of potato

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Abstract

Non-pathogenic root-colonizing bacteria that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as Plant Growth Promoting Rhizobacteria (PGPR). Over the last 20 years there have been numerous investigations of their use in potato, but often limited to in-vitro or pot trials testing few bacteria. In 2005/06 research activities on PGPR were implemented with the overall objective to develop a low cost 'bio-stimulant', which increases productivity and/or plant health of potato under low-input conditions. Therefore a total of 346 strains of 5 bacterial genera were isolated and identified from the rhizosphere of potato plants collected in different regions of the Central Andes. The bacteria were tested for their plant growth promoting abilities with low-cost in-vitro tests and best bacteria were further selected for pot trials, resulting in significant increases in tuber and plant growth of potato. PGPR probably influenced plant phytohormones and photosynthesis, which might explain gains in dry matter weight and earlier and more intense tuberization of inoculated plants.

Testing the PGPR in different production systems showed that in aeroponics inoculated plants produced more tubers. In farmer greenhouses PGPR increased yields of lettuce and chard by up to 30%. Results of field trials with potato were variable sometimes increasing yields by up to 60% but also showing no response in some cases.

In conclusion PGPR have capacities to promote plant growth. They are more effective in controlled and semi-controlled environments. The challenge is to identify bacterial strains and/or management options to use PGPR in potato-based rainfed production systems.

Keywords: Bio-stimulant, plant growth promoting bacteria, aeroponics, horticultural crops.

Introduction

Plant growth promoting rhizobacteria (PGPR) or yield improving bacteria (YIB) have been characterized as free-living soil microorganisms colonizing plant roots, exerting a beneficial effect(s) on plant development and/or suppressing plant pathogens (Kloepper and Schroth, 1978; Compant et al., 2005). There are multiple mechanisms by which the bacteria might induce better plant growth such as the production of phytohormones, the provision of nutrients (N-fixation, P solubilization), the control of plant pathogens or the induction of systemic resistance to diseases (Vessey, 2003). However, the exact mode of action is not yet completely defined and might be based on interactions among various factors including biotic (nutrient supply, competitiveness, rate of multiplication etc.) and/or abiotic ones (temperature, humidity, soil pH etc.) (Lucy et al, 2004). Although, their beneficial properties have long been recognized and have been demonstrated in various experiments, their use is restricted to few crops (sugarcane, sorghum etc.) in countries mainly in Latin America (Romero et al, 2003; Dalla Santa et al, 2004). The reasons are the great effort and investment in research and extension required to develop this technology, the variability of results depending on the crop, its management, climate, soil conditions and the availability of low-cost fertilizers (until recently).

However, bio-stimulants based on bacterial and/or fungal soil microorganisms could increase the capability of plants in low-input systems to adsorb nutrients, strengthening plant growth and resistance to abiotic and biotic stress improving eventually productivity and yields. In high-input systems with high fertilizer application their primary task would change to improve the fertilizer-use-efficiency of crops with the objective of reducing nutrient inputs but maintaining productivity. This would reduce production costs and hazardous environmental effects (Adesemoye et al., 2009).

The term bio-stimulants and not bio-fertilizer has been chosen for these kinds of products, as the microorganisms might facilitate the access to nutrients or their internal use by the plant but they themselves do not constitute any nutrient source.

Since 2005 work on the use of rhizobacteria has been implemented at the International Potato Center. In a first phase bacteria have been sampled, isolated and tested in-vitro for their ability to have plant growth promoting (PGP) characteristics. Then bacteria were selected and tested in greenhouse and field trials, studying their effect on plant growth and potato tuber yield but also investigating their mode of action or other characteristics which might influence their performance in the field or which might help to identify appropriate cropping management technologies to support their efficacy.

Investigative approach

Identifying and isolating potential plant growth promoting rhizobacteria (PGPR)

One of the major goals of this investigation was the development of a bio-stimulant for small-scale farmers in the Central Andean Highlands. Hence, in 2006 potato fields were sampled in the administrative regions of Huancavelica and Puno and in 2008 in Cajamarca, Junin, Huancavelica, Lima and Cusco (for more information see Calvo et al. in these proceedings). Potato roots and rhizosphere soil was brought to the laboratory in Lima and bacterial strains of different genera isolated, which had shown PGP characteristics in other studies. The genera were Azotobacter, Azospirillum, Actinomycetes, Bacillus and Pseudomonas. In total more than 340 different strains were isolated with standard techniques.

In vitro tests and first selections

The bacterial strains were used in different in-vitro tests: the production of indol acetic acid (IAA), the solubilization of phosphorus and the control of pathogenic fungi (*Rhizoctonia solani* and *Fusarium solani*). Other tests conducted with selected strains were the colonization of roots in agar-medium and sand and the production of siderophores.

Depending on the results of these tests the best bacteria were selected and further evaluated in pot trials (Table 1).

Table 1. Responses of rhizobacterial strains evaluated with different in-vitro test

Bacterial Genera	Total strains tested	Antagonistic to Rhizoctonia	Fusarium	Solubilization of P	IAA Test	Strains positive in 3 tests
Bacillus	63	43	39	25	36	20
Azotobacter	112	44	10	55	57	27
Actynomicetes	82	33	21	12	49	7
Azospirillum	58	17	10	16	30	3
Pseudomonas	68	23	44	47	29	21

Controlled conditions - greenhouse trials

In pot trials the bacteria were applied to potato plants, either to in-vitro plantlets or to mini-tubers, which were planted in a sterilized or non-sterilized soil-sand-moss mixture. With these trials the plant growth promoting capacity of the bacteria could be shown in controlled conditions (Table 2). Bacteria improved plant growth, tuber production and overall yield. They had a more pronounced effect in increasing tuber numbers rather then tuber mean weight. Other pot trials investigated the most effective concentration, times and number of applications as well as different carrier materials for the bacteria (water, talcum, manure etc.). Furthermore, effective bacteria were applied to other crops because farmer would prefer bio-stimulants with an ample spectrum of responsive crops (Table 3).

Table 2. Effect of *Bacillus* spp. strains on plant and tuber growth of the potato variety UNICA in pots, 2007

Strains	Tuber dry weight in g	Number of tubers/plant	Plant dry weight in g
Control	6.5 d*	4.3 de	12.9 d
FZB24	7.1 d	3.5 e	14.7 d
B1-40/06	12.7 b	5.5 cd	23.8 b
B1-26/06	13.0 b	6.5 bc	24.9 b
B1-6/06	10.0 c	3.8 e	19.9 c
B1-24/06	9.2 c	4.5 de	18.4 c
B1-36/06	13.6 b	8.0 a	24.9 b
B1-32/06	12.8 b	7.5 ab	23.8 b
B1-33/06	10.0 c	4.8 de	18.3 c
B1-29/06	18.2 a	8.5 a	29.8 a

*= means followed by the same letter within a column are not significantly different at 0.05 probability according to DMRT

Table 3. The effect of bacterial strains of Bacillus, Azotobacter and Actinomyctes on dry matter weights of different crops grown in pots, Lima 2008

Treatments	Plant dry weight in g				
	Lettuce	Chard	Maize	Spinach	Radish
Control	1.98 b*	0.74 b	5.00 b	2.43 b	1.98 b
B1-22/06	2.97 a	1.61 a	7.88 a	3.81 a	2.97 a
A1-30/06	2.73 a	1.41 a	7.62 a	3.27 a	2.73 a
A3-33/06	2.58 a	1.44 a	5.70 b	3.35 a	2.58 a

*= means followed by the same letter within a column are not significantly different at 0.05 probability according to DMRT

Controlled and semicontrolled conditions - aeroponic systems, farmers' greenhouse, irrigated horticulture production

Aeroponics is the process of growing plants in an air or mist environment without the use of soil or an aggregate medium, i.e. aeroponics is conducted without a growing medium (Stoner and Clawson 1997). The basic principle is to grow plants in a closed or semi-closed environment by spraying the plant's roots with a nutrient rich solution. Ideally, the environment is kept free from pests and disease so that the plants may grow healthier and quicker than plants grown in a medium. This technique is being adapted by CIP to develop an economic system for the production of pre-basic seed. Beneficial microorganisms can have the dual task of stimulating plant growth and tuber production and protect the plants against pathogens entering the system. First trials produced promising results increasing the number of tuber per plant by 40 to 100% (Figure 1). Trials are ongoing to further fine tune this technology and identify the best suited bacterial strains.

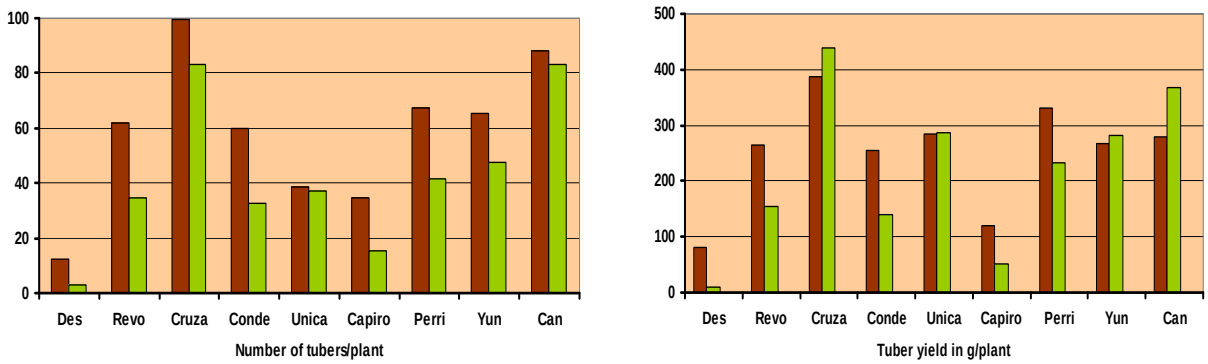

Figure 1. Tuber numbers and tuber yield/per plant produced by different potato cultivars in aeroponic system inoculated with a mixture of three bacterial strains, Huancayo 2007/08

Selected bacteria were also tested in rustic greenhouses managed by farmers. They were applied to a variety of crops and could improve plant weight by up to 40%. Likewise tests with bacteria and horticultural crops in irrigated plots showed an increase in plant weight and commercial yield. (Table 4).

Table 4. Yields of different horticultural crops inoculated with different bacterial strains in farmer managed greenhouses in Puno and irrigated peri-urban production plots in Huachipa-Lima, 2008

Bacterial strains	Puno			Huachipa	
	Fresh weight in kg/m^2				
	Lettuce	Chard	Spinach	Culantro	Beterraga
Control	3.28	3.28	3.63		
A1-30/06	3.70	5.25	4.13		
Control				3.28	19.44
A1-22/06				4.20	26.67
Control				3.28	19.44
A1-17/06				2.53	30.00
Control		4.69		3.28	19.44
B1-22/06		6.63		3.20	23.33
Control				3.28	19.44
B1-27/06				3.33	23.33
Control	3.17	3.01			
B1-15/06	3.53	3.65			
Control	2.02	2.58	3.88		
A3-15/06	2.70	2.90	5.00		
Control				3.28	19.44
A3-25/06				3.40	30.00
Control				3.28	19.44
A3-27/06				3.00	30.00

Field trials

The most promising bacterial strains from the greenhouse trials were selected for field trials with potatoes under irrigated and rainfed conditions. The bacteria were tested at various sites and several seasons. Results were somewhat ambiguous showing increased tuber production and yields in some seasons and sites but not in others. Yield gains could be considerable, especially in low input conditions, but so far no replicable stable response could be achieved – a necessity for a product offered to small-scale farmers. Further investigations will concentrate to a greater extent on agronomic practices which could improve and stabilize the bacterial effect on crop yield and also study the potential of these microorganisms to control pathogens, which would not only improve yield levels but also tuber quality.

Conclusions

A methodology was developed to screen rhizobacteria for their plant growth promoting effect. Results show that a great variety of bacterial strains have this ability in controlled and semi controlled conditions. In field conditions, however, a huge number of other factors interfere and\or interact with the applied bacteria and reduce or impede the beneficial relationship between plant and that specific organism. Nevertheless, the potential of PGPR to improve crop production in controlled and semi-controlled conditions opens a set of options for their practical use, for example, in the production of pre-basic potato seed (in aeroponics or seed beds) or the production of (horticultural) crops in greenhouses or intensively managed farmers plots. A second field for their use, the control of plant pathogens, has not yet been studied by this working group but might also offer interesting applications, especially for the control of soil borne diseases. A remaining challenge will also be the low-cost production of bacterial inoculum, which might be crucial for the wide-scale diffusion of such a technology.

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Pesticide use practices and awareness among potato growers in Nepal

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Abstract

Pests are serious problems in many major food and industrial crops grown in the Asia Pacific region, causing annual yield losses estimated at 30 to 60 %. Consequently, many developing countries in the region are heavily depending on the use of pesticides. Increased use of pesticides, however, has caused considerable concern about their effects on health, the natural environment and the quality of agricultural products. Many older, non-patented, more toxic, environmentally persistent and inexpensive chemicals are being used intensively in Nepal. Usage of pesticides in Nepalese agriculture is regulated by Act and Law; however, law enforcement is almost absent in major vegetable growing areas. Given the limited or poor literacy skills of Nepalese farmers and widespread use of pesticides, it is expected that occupational exposure to pesticides is likely to be high. This study was carried out to assess farmers' understanding of pesticide safety labels, pesticide handling and spraying practices that might potentially expose them to chemical hazards. Data was based on random sample of 471 pesticide practitioners (mainly potato farmers, but also field workers, extension officers, and pesticide dealers) across Nepal's' major potato production zones using structured interviews. This paper presents social characteristics, understanding of labels and pictograms on pesticide packages, source, preparation, and storage of pesticides, disposal of pesticide containers, practitioners' preventative measures, and understanding of WHO classes of pesticides among farmers, technicians, pesticide dealers and cooperatives.

Keywords: pesticides, pictograms, FAO class, cultivation, preventive measures, Nepal.

Introduction

Pesticides play a major role in pest management in agriculture and, pesticide sales have soared since the 1970s globally. Many older, non-patented, more toxic, environmentally persistent and inexpensive chemicals are used intensively in developing nations (Ecobichon, 2001). The Food and Agriculture Organization (FAO), estimates that up to 50% of the annual crop production in developing countries may be lost due to pests and diseases. Consequently, many of these countries depend heavily on the use of pesticides to increase agricultural production. Although developing countries currently account for about 20% only of the global pesticide market, pesticide use is expected to increase more drastically in the coming years then in industrialized countries, where minimal market growth is expected. While developing countries have benefited from pesticide use, increasing dependence on these substances and adverse effects on human health and the environment has caused considerable concerns; especially since more persistent and hazardous pesticides are commonly used, often with little or no education, monitoring or regulatory control.

Pesticide use in Nepal started in the early 1950s especially with the use of DDT for malaria eradication (Manandhar, 2005). This was subsequently followed by use of other organochlorines (BHC, dieldrin, and chlordane), organophosphates (Ethyl parathion, methyl parathion, malathion, and oxydemeton methyl), carbamates and synthetic pyrethroids. In Nepal, insecticide use increased rapidly over the last 10 years from 29.8 mt in 1998 to 102.8 mt in 2003 (PPD, 2003, PRMS, 2006). Since the 1960s, Nepal's government had given major emphasis to import and supply chemical pesticides to increase agricultural production and as a result, pesticides started to be used indiscriminately and widely throughout the country. Total amount of pesticides used annually in Nepal is 128.697 mt (active ingredient) that includes 46.553 mt of insecticides, 74.368 mt of fungicides, 5.701 mt of herbicides, 1.808 of roddenticides, 0.057mt of bio-pesticides, and 0.238 mt of acaricides for agriculture as well as 2.556 mt of pesticides for the public health sector (PPD, 2008). The national mean pesticide consumption of Nepal was 142g /ha in recent past, which seems low compared to pesticide consumption of other Asian countries; however, use of pesticides is not uniform in Nepal. Most pesticides are used in rice (40-50%), pulses (14-20%), cotton (13-15%) and vegetables and fruits (10-15%) (Manandhar, 2005). Moreover, pesticides are used

by vegetable farmers in the periphery of urban and sub-urban areas where they have access to vegetable markets (NARC, 2005).

A number of 306 commercial products grouped under 71 common names of pesticides have been registered in Nepal: insecticides (40); fungicides (18); herbicides (5); rodenticides (3); acaricides (1) and others (4) (NARC, 2005). Illegal trade and use of pesticide has been an issue for journalists and highlighted by media now and then. Till now, 14 pesticides (POPs) have been banned in Nepal, including DDT, BHC, aldrine, dialdrin, endrin, chlordane, lindane, heptachlor, toxaphene, mirex, phosphamidon, organomercury compounds, monocrotophos and methyl parathion (PPD, 2008). At present, commonly found pesticides in markets are organophosphates, synthetic pyrethroids and one organochlorine i.e. Thiodan (Manandhar, 2005).

Nepal government has passed Plant protection Act 1972; Plant protection Rules 1975; Pesticide Act in 1991; Pesticide rules 1993; Environmental Protection Act 1996; Environmental protection Rules in order to mange the discriminate use of pesticides (Palikhe, 1998). However, there is no comprehensive record indicating the volumes of pesticides used.

Due to lack of training and education programs for safe use from industries or government, Nepalese farmers are not much aware about the risks and rarely follow proper safety methods when using pesticides. Pesticides are applied at higher doses than needed (Manandhar, 2005), causing waste of pesticides and reduced farmers' profits. Generally, farmers make decision for applying pesticides once they notice pests in the field, irrespectively of damage level. Pesticide use is not static due to many factors such as availability of alternatives, market prices, effectiveness and pesticide availability in markets (Manandhar, 2005). Earlier studies have not explored sufficiently the recent use pattern of pesticides and its market system in totality. Available information does not provide information about the real status of pesticide use in Nepal. Regular monitoring on different issues of pesticide could be helpful to update the changing situation of pesticide use. Besides, Nepal is a member of World Trade Organization that requires authentic data of pesticide use for the export of agricultural products.

The objective of this study was to determine the potential health risks for farmers and the environment due to increasing pesticide use. The study focused on farmers' understanding of pesticide labels, farmers' awareness about the risks arising from pesticide use, and in how far appropriate safety measure are taken up by farmers. The results are used to quantify the environmental and health impacts of pesticides in agricultural production in Nepal.

Materials and methods

A standardized questionnaire was used to gather the information about the chemical pesticides used and farmers' awareness about its risk. The questions focused on pesticide handling, including pesticide application practice, storage, and disposal of pesticide containers, and farmer's understanding of pesticide labels and safety measures adopted. Farmers were also asked if they read pesticide labels and which other sources of information they used for appropriate handling of pesticides. Pictograms generally included in pesticide labels were shown on one sheet where farmers noted their understanding of the pictorial warnings. Personal figures, like farmers' sex, age, educational level, land tenure situation, years of farming and pesticide use experience were included.

The field survey was carried out during September 2008 to May 2009 in twenty vegetable (especially potato) growing districts of all development regions of Nepal. The districts covered high hills (3), mid-hill (11), and plain (6) agro-ecological zones (Fig). The potato growing areas of Chitwan, Dang, Banke, Bardiya, Kailali and Nawalparasi represent plains, the districts Arghakhanchi Dadeldhura, Kaski, Kavrepalanchowk, Kathmandu, Lalitpur, Bhaktapur, Parvat, Salyan, Dahding and Makawanpur represent mid-hills, and the districts Solukhambu, Jumla, and Sindhupalchowk represent high hills of Nepal. In total, more than 500 farmers were interviewed. The sample size varied between 5 and 58 potato growers in each districts.

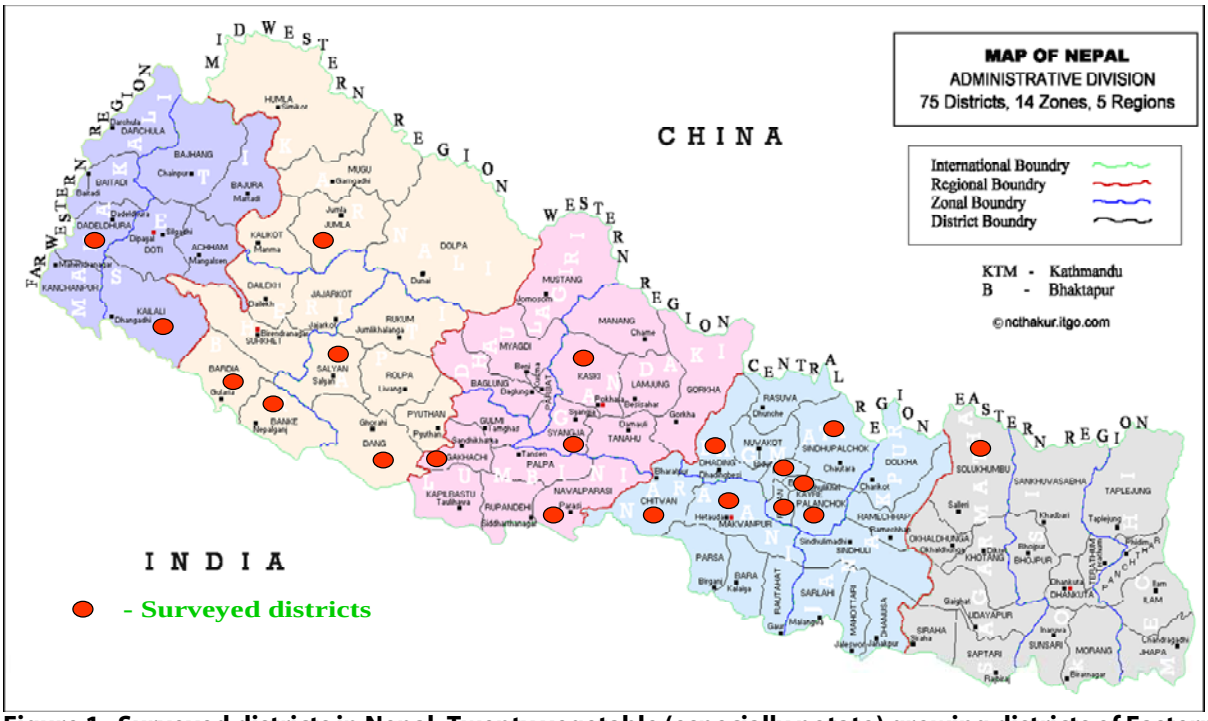

Figure 1. Surveyed districts in Nepal. Twenty vegetable (especially potato) growing districts of Eastern, Central, Mid-western and Far-western development region of Nepal were selected for survey covering high hill, mid-hill, and plain agro-ecological zones

The study was conducted by personal interviews using a (semi-)structured questionnaire. The questionnaire was pre-tested with farmers in Bhaktapur. Interviews were carried out by entomologists of NARC assisted by Plant Protection Officers of the Department of Agriculture (DOA) in each district and in some cases additionally by research assistants and junior technicians. Orientation meetings were organized for the survey team members before interviews. For verifying farmers understanding of pictograms and their knowledge about color codes and WHO classes on pesticide labels, respondents were asked to note their explications on a questionnaire sheet that presented the warning symbols. In case that the farmer was illiterate the interviewer noted the farmer's answers on the sheet. The team members also conducted observational studies on farmers' attitudes and practices for verifying the outcome of the questionnaire during field visits. Altogether a total of 504 pesticide practitioners, categorized as farmers that are non members of co-operatives (464), extension officers (15, from Kabre only), pesticide dealers (9, Kabre only) and members of co-operatives (15, Arghakhanchi) were interviewed.

All farmers in Solukhambu (5) and Sindhupalchowk (28) (high hills) had never used pesticides and were not included in the analysis. The 15 famer organized in co-operatives came all from the district Arghakhanchi and were also excluded from the category of farmers for the analysis. Comparison between the groups of pesticide practitioners is limited by the fact that subjects in the groups of 'extension officers', 'pesticide dealers' and 'farmers organized in co-operatives' is low and all subjects within each of these groups were derived from one district only. This paper therefore focuses more on the attitudes of farmers which are not organized in co-operatives. For verifying differences in response between categories of pesticide practitioners and differences within the group of farmers which are not organized in co-operatives (simple random sample) due to personal variables, i.e. sex, age, education level, farming experience, etc., data were submitted to ANOVA (ordinal data) or evaluated by Chi^2-test (dichotomous data). For analysis the software package SPSS-10 was used.

Results

Demography and social characterization of respondents

Majority of farmers (62.4%) interviewed were of the middle-age class, i.e. between 25 and 50 years old. The proportion of women (28%) interviewed was lower, which might reflect the involvement of women in pesticide applications since women were interviewed only when they apply pesticides. Educational levels of respondent were variable (Table 1). About 1.7% of farmers were illiterate, and another 21% did not complete more than 5 years of formal education, while over 50% had obtained 12 years of formal education with about 30% holding a B.A to M.A. level. Education levels were generally higher in Nepal's mid-hill regions then in high hills or plains and were also higher in the Western and Central Development region then in the Mid and Far Western region. Most farmers were land owners but some farmers rent additional land (13%) while 3.4% only have been using solely land in rent. Most farmers have been involved in agricultures for more than 10 years (>68%) but pesticides are used since fewer years. Only 32.7% of the farmers have been using pesticides for more than 10 years. Most farmers interviewed apply pesticides both in their own family land as well as in other's taken in rent; or apply pesticides as a hired labor for others.

Table 1. Social characterization of respondents

Types and characteristics	Number	Percentage
Occupational Categories		
Farmer	461	91.7
Agriculture Technician	15	3.2
Pesticide dealer	9	1.9
Representatives of farmer's co-operatives	15	3.2
Sex		
Female	134	28.5
Male	337	71.5
Age (years)		
Up to 25	87	18.5
25-50	294	62.4
More than 50	89	18.9
Education level		
Illiterate	8	1.7
Literate (below 5 class)	101	21.4
5-10 class	104	22.1
SLC-IA level	119	25.3
BA-MA level	139	29.5

Types and characteristics	Number	Percentage
Land tenure		
Own land	393	83.4
Land in rent	16	3.4
Both type of land ownership	62	13.2
Farming experience		
1-5 years	67	14.2
6-10 years	88	18.7
11-15 years	83	17.6
16-20 years	60	12.7
>20 years	173	36.7
Experience of pesticide use (years)		
1-5 years	190	40.3
6-10 years	120	25.5
>10 years	153	32.5
Work force		
Household labour	137	29.1
Wage labour	25	5.3
Both	307	65.2

Knowledge on pesticide categories and labels

Approximately 50% of the farmers do read pesticide labels before using them. The reading practice was significantly more frequent with increasing level of education (strong correlation), and significantly different between farmers' age groups (younger farmer read more frequently label than older farmers), farmers' farming experience (less experienced farmers read more frequently then farmers with longer farming experience) as well as between farmers in the three agroecological zones of Nepal (farmers in high hills and mid-hills read more frequently the label than in the plains (

). Most frequent answers why farmers do not read the labels were "because they are illiterate" (16%), that "they trust pesticide dealers" and follow their advice rather than to read and follow written instruction (18%), "trust in

pesticides without reading the label" (3.3%), "rely on neighbors" (9.4%), or simply "don't see the need to read the label" (8.2%); however, about 44% of farmer did not reply to this question at all.

Table 2. Percentages of farmers who do not read labels by personal and regional categories

Variables	Total number of respondents	Do not read (%)		Chi square
Education				
Illiterate	8	0.63	$p < 0.001$	113.5
literate (below 5 class)	96	0.92		
5-10 class	100	0.7		
SLC - IA Level	103	0.43		
BA - MA level	117	0.24		
Sex				
male	308	0.54	$p = 0.211$	1.57
Female	116	0.6		
Age (years)				
upto 25 years	85	0.44	$p < 0.001$	17.49
25 - 50 years	250	0.53		
> 50 years	88	0.74		
Farming experience				
1 - 5 years	64	0.41	$p < 0.001$	24.25
6 - 10 years	85	0.41		
11 - 15 years	80	0.55		
16 - 20 years	57	0.6		
> 20 years	138	0.7		
Pesticide use experience				
1 - 5 years	173	0.49	$p = 0.066$	5.6
6 - 10 years	114	0.6		
> 10 years	137	0.61		
Ecological zone				
Plains	144	0.66	$p = 0.005$	10.24
mid hills	270	0.5		
high hills	10	0.4		
Development region				
Far Western	32	0.69	$p = 0.233$	4.28
Mid Western	77	0.61		
Western	114	0.54		
Central	201	0.52		
Total (farmers)	424	0.55	$p < 0.001$	36.8
Extension officers	15	0.13		
Pesticide dealers	9	0		
Farmers in co-operatives	15	0		

All farmers organized in co-operatives (only from one district) and all pesticide dealers said that they always read the labels. From a total of 15 extension officers interviewed two responded that they do not read pesticide labels. As a major source for information about pesticide use and the risks, farmers rely on pesticide dealers (60.6%), extension officers (42%), neighbors (20%) and others (2%).

Among the farmers (all non-members of co-operatives) 82.4% indicated that they are not familiar with the FAO color-coding scheme; however, 8.6% only were able to correctly understand the meaning of all color classes

(16.4 and 10.6% understood correctly the meaning of two red color codes for extremely hazardous (Ia) and highly toxic (Ib) pesticides). All farmers which were organized in co-operatives (from one district only) were familiar with the color codes and interpreted them correctly. Three pesticide dealers (33%) and one extension officer (6.7%) were unfamiliar with the codes and not able to interpret them.

Similarly, the majority of farmers did not have a clear understanding of the pictograms' meanings (

).

Table 3. Frequencies for correct understanding of pictograms on pesticide labels

Pictograms	Meaning	*N*		%	
		Yes	*No*	*Yes*	*No*
Activity pictograms					
	Handle carefully - liquid product	42	429	8.9	91.1
	Handle carefully - powder or granulated product	29	442	6.2	93.8
	Application - use a hydraulic spray atomizer	265	206	56.3	43.7
Advisory pictograms					
	Use protective gloves	375	96	79.6	20.4
	Wash after use	352	119	74.7	25.3
	Wear a mask	367	104	77.9	22.1
	Wear a water proof apron	197	275	41.8	58.4
	Use a face shield	40	431	8.5	91.5
	Wear spactacles	342	129	72.6	27.4
	Wear boots	312	159	66.2	33.8
	Wear a pesticide respirator	50	421	10.6	89.4
	Wear protective clothing	18	453	3.8	96.2
Environmental hazard					
	Dangerous/harmful for livestock and poultry	154	317	32.7	67.3
	Dangerous/harmful for wild animals and birds	96	375	20.4	79.6
	Dangerous/harmful for fish/Do not contaminate water	153	318	32.5	67.5
Children hazard warning					
	Keep locked away and out of reach of children	192	279	40.8	59.2

All 15 farmers organized in co-operatives interpreted all 16 pictograms correctly; however, among other farmers 14.8% did not understand the meaning of any pictogram, 48% understood the meaning of at least 6 pictograms, while more then 10 were identified correctly by 13.9% of the farmers. Few farmers were able to understand the handling pictograms for liquid (8.9%) and granulated (6.2%) products, while frequently more farmers understood the pictogram for using a hydraulic sprayer (56.3%).

Likewise, understanding of advisory and warning pictograms was low; relatively frequently the signs for using protective gloves, washing after use, wear mask, wear spectacles were understood (all >72%), while understanding of the need for wearing boots (66%) and a waterproof apron (42%) was moderate, for wearing a respirator (10.6%) and a face shields (8.5%) extremely low, and for wearing protective clothes almost nil (3.8%). Pictograms for environmental hazard were also poorly understood; while the danger sign for livestock and poultry intoxication and danger of water and fish contamination was understood by about 33%, lower numbers of farmers were able to identify correctly the hazard sign related to wildlife (20%). Less than 41% of farmers identified correctly the hazard warning related to children.

None of respondents perfectly followed the recommended safety measures. However, majority of farmers (62.6%) used to wear a piece of cloths or cover mouth and nose, which is considered an important and easy to use protection measure, during applying pesticides and take a bath afterwards (41%). Some farmers only wear gloves (29.5%), an apron (27.8%), or a hat (22%). Wearing shoes during pesticide application was reported from 16.3% only (most farmers spray pesticides barefooted or wearing sandals). Spraying according to the wind for avoiding direct contact with pesticides consider 26% of the farmers only. Very few farmer (1.5%) use other than above mentioned protective measures

Farmers' pesticide handling practices

Nepalese farmers are using various types and wide range of chemical insecticides (organochlorines, organophosphates and synthetic pyrethroids). Among them, Endosulfan (Thiodan) is an insecticide that is still being used by a big group of farmers (52.2%). In addition, Malathion and Mancozeb (DM-45) have been found widely (36.7%). More than 28% of the farmers still use highly hazardous pesticides of the WHO class Ia and Ib (**Error! Not a valid bookmark self-reference.**). One farmer mentioned that he is still using DDT, but which could not be verified.

Table 4. Types of pesticides used

Trade name	Pesticide group	WHO classification	Numbers of respondents	Percentage
Insecticides				
Malathion	Organophosphates	III	182	38.6
Dimethoate	Organophosphates	II	51	10.8
Endosulfan	Organochlorine	II	246	52.2
Nuvan	Organophosphates	Ib	88	18.7
Methyle parathion	Organophosphates	Ia	47	10.0
DDT	Organochlorine	Ia	1	0.2
Chloripyriphos	Organophosphates		1	0.2
Cypermethrin	Synthetic pyrathroid	II	14	3.0
Fenfen	Organophosphates	II	1	0.2
Current	Organophosphates	Ib	1	0.2
Metasystox	Organophosphates	Ia	4	0.8
Super D			1	0.2

Trade name	Pesticide group	WHO classification	Numbers of respondents	Percentage
Fungicides				
Keronoxyl			20	4.2
Copperoxychloride	Copperoxychloride		1	0.2
Bavistin	Carbendazim	NH	8	1.7
Blitox	Copperoxychloride		3	0.6
DM-45	Mencozeb	U	173	36.7
Copperoxide	Copperoxychloride		27	5.7
Hinosan	Carbendazim		1	0.2
Benomyl		U	15	3.2
Endofil-45	Mencozeb		1	0.2
Dhanucup			1	0.2
Curex			1	0.2
Sixer			1	0.2
Carbedigm	Carbendazim		1	0.2
Indofil	Mencozeb		1	0.2

Most farmers (57.7%) prepare pesticides in the field just before its application. Pesticides are stored mostly in a separate store within the house along with agricultural tools (40.6%), which is followed by storing them outside the house (37.8%); however, 2.1% store pesticides in the bedroom and 1.5% in the kitchen. Farmers have different practices for disposing the empty pesticide containers. Disposing of empty containers in a pit has been most frequently reported (46.9%) followed by leaving them in the crop field (22.3%), burning (16.8%). 5% of the

farmer reported that they use empty pesticide container for home purpose rather than wasting or burning them (Table 5).

Table 5. Pesticide preparation place, storage and disposal practice

	Number	Percentage
Pesticide preparation place		
In home	88	18.7
In the field	272	57.7
Nearby water source	101	21.4
Pesticides storage place		
In bed room	10	2.1
In kitchen	7	1.5
Normal store room	75	15.9
Separate store room	191	40.6
Store out side house	178	37.8
Disposal of empty pesticide containers		
Home use	24	5.1
Disposing in pit	221	46.9
Throwing in sewage canal	37	7.9
Throwing in stream or canal	56	11.9
Burning	79	16.8
Throwing in crop field	105	22.3
Throwing in forest	41	8.7

Discussion

Similar studies have been carried out by various researchers (Giri *et al*., 2006; Giri, 1995; Ghimire and Katiwada, 2001; Maharjan *et al*., 2004) in Nepal. Giri *et al.* (2006) carried out a study in eastern and central mid hills and eastern, central, mid- and far western plains of Nepal and found that most vegetable growers were rarely using any safety measure during spraying of pesticide as found in the present study. Giri *et al.* (2006) reported that farmers avoid spraying pesticides in bright sunshine or under windy conditions (Maharjan *et al*., 2004) as a common measure preventing hazardous effect of pesticide. A second adopted safety practice by farmers was covering the face with cloth during spraying as it was reported by most farmers in the present study. Even if farmers are aware that the use of pesticides is unsafe, they are not conscious about all the risks (e.g., many farmers mentioned that they did not know that skin contact with pesticides might be hazardous (see also Giri, 1995)) and that farmers in Nepal have not adopted adequate safety measures for applying pesticides (Baker and Gyawali, 1994; Klarman, 1987; Dahal, 1995; Giri *et al*., 2006, this study). Chemical pesticides are commonly known as *"Kit Nasak Aushadi"* (insect destroying medicine), and they are handled carelessly. Farmers even use the broom to apply pesticides (Dahal, 1995).

Given the increasing trend of pesticide use in Nepal there is an urgent need for awareness and training activities which could enhance the adoption of safety measures. Today, little is known about the health impacts of chemical pesticides on farmers; however, some studies (Atreya, 2008) showed that increasing pesticide use affects farmers' health in Nepal. Nepal's authorities have realized that the use of pesticides has huge detrimental

effects within the country. For sustainable agricultural production it is important to reduce farmers' dependency on chemical pesticides and shift to integrated pest management practices and use of safer alternatives.

Giri et al. (2006) and Maharjan *et al.* (2004) have reported that vegetable growers of different districts and development regions have been using a long range of pesticides, using them with minimum protective measures. This study shows that the situation has not been changed yet; it seems that pesticide use by vegetable growers of Nepal is increasing while still some hazardous pesticides (WHO class IA and IB) are in use. Potato growers of high hills generally do not use chemical pesticides except in Jumla but where the use of chemical pesticides is also still low. The chemical pesticides commonly used are insecticides (organophosphates, pyrethroids, and organochlorins) and fungicides (mancozeb, carbendazim and copperoxychlroride).

Pesticide label reading practice of Nepalese farmers is very poor due to use of foreign language, unclear instruction of the label as well as carelessness of the users. This study revealed no differences in reading practices between man and women; however, Atreya (2007) showed that gender-specific difference on pesticide use knowledge and adoption of safety measures exist that need to be addressed in any awareness and training program. Most farmers are also unfamiliar with the color signs, which are specifically included in pesticide labels for users who are illiterate or unfamiliar with the language used on labels. Studies carried out by Eve (1995) have shown that reading and writing ability is high considering the geographical and resource constraints encountered by those providing education. It seems that technical language used for instructions discourages farmers to read pesticide labels. Giri et al. (2006) have also reported that a big segment of vegetable growers of Nepal were not aware about pesticide labels and it's expiring date. Similar trends were seen in this study too. Ghimire and Katiwada (2001) reported that farmers of Chitwan (Tandi) have very little or no knowledge of safe use of chemical pesticides in vegetable production. They are not aware of waiting period, environmental and health hazards. Pesticide use in commercial farming and fresh vegetables is excessively uncontrolled and without consideration of health of consumers.

Conclusions

Awareness on the correct use of pesticides by vegetable growers of Nepal is low and should be improved through adequate training programs and the provision of safer alternatives to chemical pesticides. Farmers from co-operatives showed a relatively good knowledge about pesticide use and safer pesticide application practices compared to farmer who are not organized in farmers groups. Other studies showed that farmers are worried about negative health impacts of pesticides and are willing to pay for safer alternatives (Atreya, 2008). Although integrated pest management has been developed for rice production in Nepal alternative control measures for other crops have been rarely developed and provided to farmers. It is recommended to strengthen research efforts for developing integrated pest management strategies especially for vegetable crops, including potato.

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Control of potato late blight with foliar application of phosphonate

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Abstract

Phosphonate, also known as phosphites, represents a class of fungicides that has activity against a number of fungi and has been associated with induced resistance. The efficacy of foliar applications of phosphonate against *Phytophthora infestans* was evaluated in potato field trials in 2007 and 2008 in Peruvian highlands. Potato varieties with horizontal resistance were used in theses trials because previous studies had shown that phosphonate works better when there is a measurable background level of host resistance. Phosphonate treatments alone or together with contact fungicide gave results similar to spray regimes involving both contact and systemic conventional fungicides. Phosphonate treatments gave sufficient control, even though they received fewer sprays than did treatments based on conventional practices. Based on an analysis of marginal rates of return, phosphonate appeared to be economically advantages over other treatments. Application of the Environmental Impact Quotient (EIQ) demonstrated reduced health and environmental risks of this class of disease control product.

Keywords: Phosphite, Environmental Quotient, horizontal potato varieties.

Introduction

One of the main problems of growing potato worldwide is the economic losses which occur due to late blight, which is caused by oomycete *Phytophthora infestans.* This pathogen can destroy all potato plants in few weeks under wet conditions. In recent years, highly aggressive strains of the pathogen —many insensitive to some popular synthetic fungicides—have surfaced and created new challenges for potato producers, making disease management efforts increasingly difficult (Powelson, 1998; Levy, 1983)

Several nonchemical options are available for managing this disease, including cultural practices, varietal resistance, and alternative sprays that inhibit disease development. Phosphonate (or as salts known as phosphites) in general can stimulate plant defense responses and is also active against oomycetes *in vivo* (Guest and Grant, 1991). In Argentina, phosphites applied to seed tubers of potato cultivars Shepody and Kennebec gave high levels of protection against *P. infestans*, intermediate protection against *F. solani* and low against *R. solani* (Lobato et al, 2008). These compounds pose a very low risk to human health and environment and therefore represent a potential alternative for use within an integrated crop management, especially potato varieties with some moderate resistance to *P. infestans.*

Materials and methods

Two field experiments were carried out between 2007 and 2009 in Huasahuasi (masl, latitude), Junin, Peru. This place is one of the most important areas of continuous potato production in the Peruvian Central highlands and a location with high disease pressure. Previous studies indicated that the current *P. infestans* population in this place is dominated by the EC-1 clonal lineage and causes important economic losses to farmers (Bustamante, et al, 2008; Otazu, personnel communication).

Potato genotypes and fungicide treatments.

Three potato varieties (Amarilis, Serranita and Chucmarina) and one elite clone from CIP's breeding programme (CIP 386549.9) catalogued as horizontally resistant to late blight were used in the experiments. Fungicide treatments consisted of applications of phosphonate (T1), alternation of phosphonate and contact fungicide

(T2), local farmers' strategy (T3) and a control treatment without fungicide application (T4). Here local farmers' strategy refers to use of systemic and contact fungicides as farmers customarily do it, including the mixture of more than 2 active ingredients in the same application. In 2007, treatments T1 and T2 were based on a calendar of sprays every 9 days and in 2008 these treatments were based on sprays after each 30 mm of accumulated rainfall. The minimum number of days between sprays in both years was set at 5 days. All sprays programs were continued until plants reached senescence. All pesticides were applied with a backpack sprayer with hollow-cone nozzle and application volumes were standardized by applying until runoff. Backpack sprayer operating pressure was uniformly adjusted to 2 bars.

Experimental design and agronomic practices

The experiments were carried out in a strip-plot design with two major factors (fungicides and potato varieties) with 3 repetitions. The experimental units consisted of 30 plants in 2007 and 100 plants in 2008. Planting density was 0.9 m between rows and 0.35 – 0.40 m between plants. Fertilizer application was 140 kg N ha^{-1}, 120 Kg P ha^{-1} and 80 kg K ha^{-1}. Nematicide was applied at planting and insecticides were applied when necessary.

Evaluation of late blight in the field

The percentage of foliar infection was estimated visually at the plot level every 7 days for 9 – 11 times after plants had reached a minimum size of 15 to 25 cm and until plants reached senescence. The area under the disease progress curve (AUDPC) was calculated for each plot from the estimates of foliar infection using the midpoint method (Campbell and Madden, 1990). To help standardize AUDPC values across years, AUDPC values were transformed into the relative AUDPC (rAUDPC) as described by Fry (1978).

Evaluation of potato yield

Tubers were harvested at maturity between 110 -120 days after planting. Tubers were separated in commercial (> 40 g) and non-commercial (<40 g) size and weighed for each plot.

Statistical analysis

Data from each year were analyzed independently in order to explore two-way interactions between potato varieties and efficacy of phosphonate treatments. Statistical analyses were done using SAS 9.1 statistical software (SAS Institute Inc., Cary, NC). The benefits of alternative treatments were analyzed by partial budgeting as reported previously (CIMMYT 1988) and by using the Environmental Impact Quotient (EIQ), which was calculated to compare spraying programs as reported by Kovach (1992).

Results and discussion

Disease was severe each year as evidenced by the statistically high rAUDPC values for control treatments (Table 1). The farmer's strategy resulted in significantly lower rAUDPC values each year, but yields were more similar among phosphonate and farmer treatments. EIQ values were many times higher in farmers' treatment in 2007-2009 and this tendency was even more marked in 2008-2009.

There was also a marked difference in resistance among varieties, with Amarilis being the most susceptible both years (Table 2). We explored the variety by treatment interaction graphically (Figures 1 and 2). In the first year there was no clear interaction but in the second year, phosphonate appeared to work very poorly for cultivar Amarilis which only had adequate control with the farmer's strategy.

The Farmers' strategy involves expensive systemic fungicides and this lead to much high costs (data not shown). An analysis of partial budgets (currently underway) will potentially demonstrate that there are economic benefits to the use of phosphonate.

In conclusion, it appears that phosphonate has potential to manage late blight of potato but more information is needed about the potential variety specificity and whether augmentation with fungicides may be needed under some conditions.

Table 1. Effect of treatments on control potato late blight (*Phytophthora* infestans) and yield during two cropping seasons on farms in Huasahuasi, Peru

Season	Treatment	Sprays	rAUDPC	Commercial yield	EIQ
2007 -2008	Control (T4)	0	0.202 c	10.14 b	0.0
	Phosphonate (T1)	7	0.058 bc	17.22 a	12.93
	Phosphonate + Fungicide (T2)	7	0.076 b	12.32 b	40.54
	Farmers' strategy (T3)	7	0.054 a	17.29 a	107.87
2008 -2009	Control (T4)	0	0.317 c	55.53 b	0.0
	Phosphonate (T1)	9	0.120 b	77.36 ab	16.62
	Phosphonate + Fungicide (T2)	9	0.090 b	81.86 ab	43.88
	Farmers' strategy (T4)	7	0.000 a	108.10 a	255.44

Table 2. Effect of potato variety on severity of late blight and production of commercial tubers during two cropping seasons on farms in Huasahuasi, Peru

	2007-2008		2008 - 2009	
Variety/Clone	rAUDPC	Yield	rAUDPC	Yield
Amarilis	0.17 a	19.72 a	0.30 a	46.091 c
Serranita	0.06 b	13.099 ab	0.10 b	74.968 b
CIP 386549.9	0.05 b	10.173 b	-	-
Chucmarina	-	-	0.00 c	121.074 a

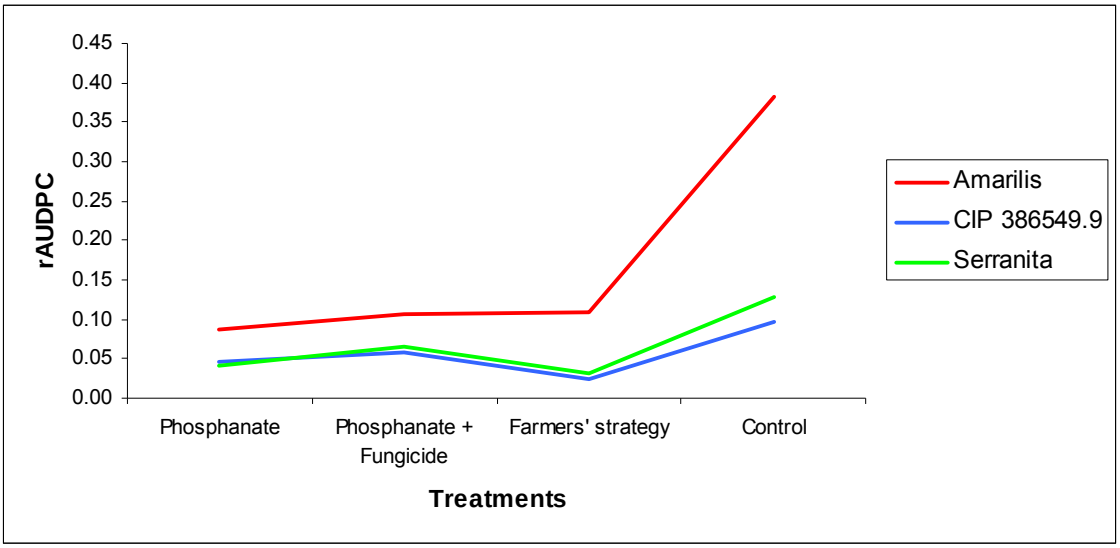

Figure 1. Resistance to Late blight in potato varieties during 2007 – 2008 growing season

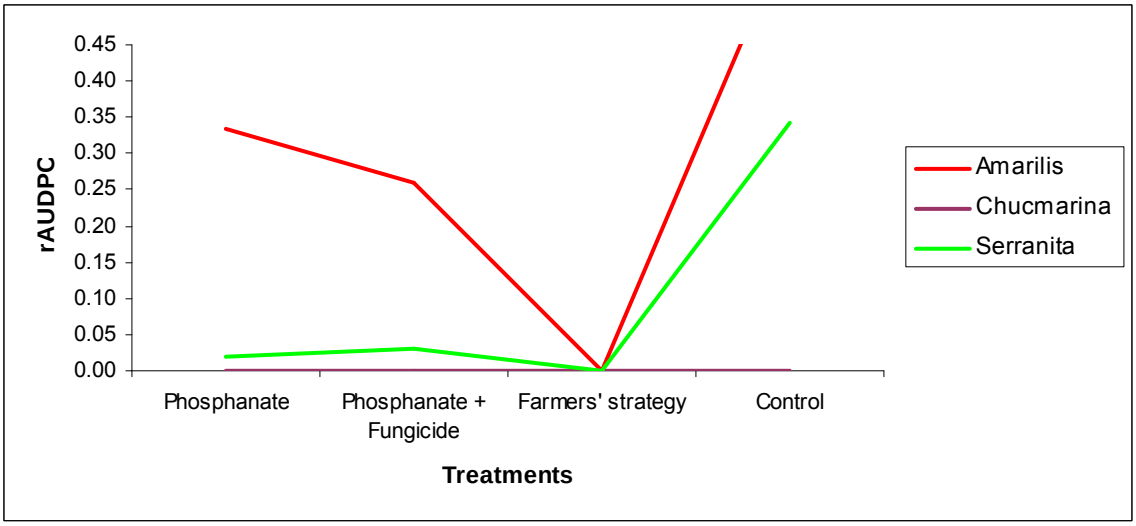

Figure 2. Resistance to Late blight in potato varieties during 2008 -2009 growing season

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Early maturing late blight resistant potato for cereal based system of Indo-Gangetic Plains

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Abstract

The lack of early bulking potato varieties and high late blight (LB) pressure due to mild temperatures and cool foggy weather limits potato production in the sub-tropical lowlands of Indo-Gangetic Plains (IGP). Potato's main niche is between two rice crops. The present study aimed to identify early maturing LB resistant potato varieties to plant after harvesting summer/fall rice and planting spring rice to enhance farm income. The field experiments were conducted in Bangladesh during 2007-08 and 2008-09 crop seasons at three locations. Visual observations of foliage infection with LB were recorded at weekly intervals starting from 27 days after planting (DAP). The crop was dehaulmed at 75 and 90 DAP to assess maturity periods. Late blight appeared early and spread fast in susceptible clones (391004.19, 391046.14, 396004.33, 3960.31.11) and the control variety Diamant. The susceptible clones and Diamant were devastated before senescence. Four clones (393077.15, 393085.5, 393371.58, 395011.2) out of 12 clone were found moderately resistant (MR) to LB. Average tuber yield of Diamant was recorded <9 t/ha compared to > 20t/ha of promising MR clones when harvested at 75 and 90 DAP. Two year field evaluation data concludes that two CIP clones:393371.58 and 395011.2 would be acceptable to farmers as they out-yielded most liked variety Diamant, have MR to LB and standard tuber characteristics and can fit effectively in cereal based systems in IGP enable farmers to generate greater income and enhance productivity of the systems.

Keywords: Early maturing, sub-tropical lowlands, late blight, clone.

Introduction

The rice-wheat is the dominant cropping system in the sub-tropical IGP, but there are many other important cropping systems practiced by farmers for sustainable livelihoods. Fertile land and enhanced irrigation facilities have provided farmers with opportunities for crop diversification and intensification. The kharif (wet season) rice-potato-boro (summer season) rice is the emerging cropping system in Eastern IGP (Bangladesh and West Bengal, India). The role of potato and wheat in rice-based cropping systems by latitude is presented in Figure-1 (Graham et.al 2007)**.** In Bangladesh, potato is the third largest food crop next to rice and wheat and is highly profitable compared to cereals. Nearly 9.2 million tons potatoes were produced from 0.5 million ha in Bangladesh in 2007-08 crop season (Hossain et.al 2008). The non-availability of 75 days late blight resistant varieties for rice based cropping system is major constraints to enhance productivity and profitability of cropping system. Late blight (Phytophthora infestans) is a most damaging disease for winter potatoes in IGP. The losses can go over 60% if crop is infected at early growth stage. The disease generally appears at the first week of January when the day temperature range 14-19^0 c and night temperature 9-14^{0}c ccompanied with foggy weather and heavy deposition of dew drops (Dey et.al 2008). The indiscriminate use of metalaxyl containing fungicides has developed metalaxyl resistant strain. The varieties Raja, Dheera and K.Jyoti having moderate resistant to late blight have also become susceptible. The Tuber Crops Research Center, Bangladesh and International Potato Center (CIP) have been working jointly in Bangladesh to test LB CIP advanced clones in sub-tropical lowlands under short day conditions and to identify promising 75 day clones to release varieties that can produce higher tuber yields than present varieties Cardinal and Diamant and fit in the existing rice-rice systems.

More than 0.15 million ha area can be brought under rice-potato-rice system by providing promising LB resistant 75 day potato to resource poor farmers.

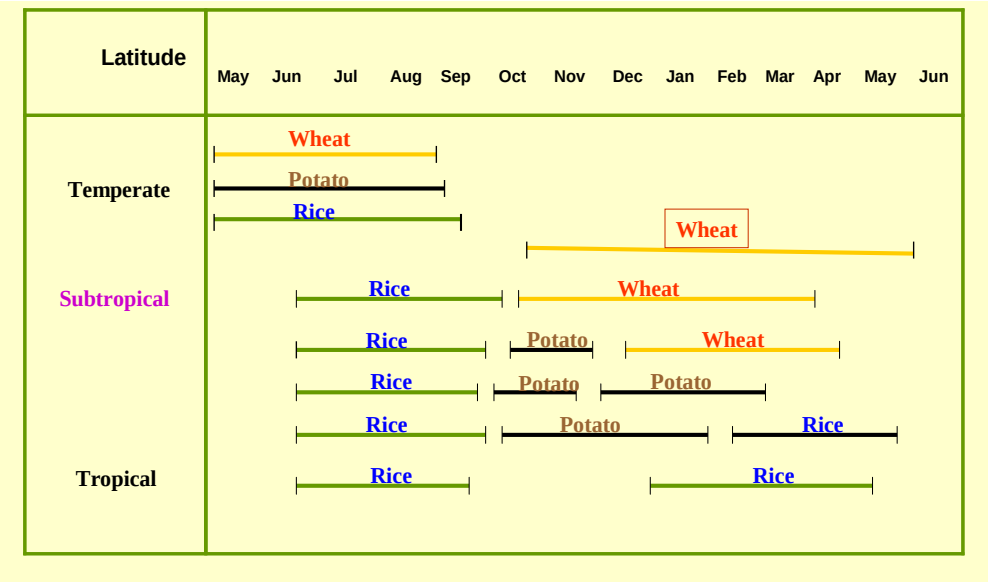

Figure 1. Agro-ecological description of the role of potato in wheat/rice based cropping system by latitude

Material and methods

Ten and twelve CIP advanced clones were evaluated at three locations (Bogra, Munshigonj and Jamalpur) conducive to LB in sub-tropical tropical lowlands in winter season under short day conditions in 2007-08 and 2008-09, respectively. For comparison, the dominant variety Diamant was planted. The uniform size of tubers of each clone/variety was planted in replicated (3 replication) plots of 2.4x3m size at 60x25 cm spacing in RCBD. The crop was panted in last week of November and dehaulmed at 75 and 90 days after planting (DAP) to evaluate for maturity. Recommended doses of fertilizers and intercultural operations were applied. No fungicide was applied at any stage to protect crop from LB. The percentage of leaf infection recorded at 10 days interval from 50 days after planting in 2007-08 and at weekly intervals after 27 days after planting in 2008-9 crop seasons. The tuber yield, average tuber weight and tuber characteristics were recorded at harvesting time. The data were analyzed statistically.

Results and discussion

The percentage infection of late blight recoded in 2007-08 and 2008-09 crop seasons is presented in Table 1 and 2. No CIP clone or Diamant exhibited complete resistance to late blight. Some clones found resistant in first year showed late blight infection in second year. The late blight appeared first at Bogra site during both years indicating that environmental condition of this location is more congenial to late blight spread than Munshigonj and Jamalapur. Second year (2008-09), the late blight appeared early and spread fast in susceptible clones (391004.19, 391046.14, 396004.33, 3960.31.11) and in variety Diamant (Table-2). Five CIP clones and Diamant were completely devastated at 63 days after planting before natural senescence. Four clones (393077.15, 393085.5, 393371.58, 395011.2) were found moderately resistant (MR) to LB.

The CIP clones performed better than variety Diamant for tuber yield (Table-3). Average tuber yield of Diamant was recorded <9 t/ha compared to > 20t/ha of promising moderately resistant clones when harvested at 75 and 90 DAP. The Diamant and some clones infected severely at early stage and therefore could not produce good yield. The mean tuber yield of two CIP clones:393371.58 and 395011.2 was recorded 21.7 and 21.0 t/ha, respectively, greater than other clones and Diamant when harvested at 75 days after planting (Table-3). CIP

clone 393085.5 gave maximum tuber weight (50g) followed by 393077.15 (47g), 393371.58 (42g) and 395011.2 and 396244.12 (41 g each) when harvested at 75 DAP (Table-4). The tuber weight of clones and variety Diamant infected by late blight at early stage was recorded significantly lower than moderately LB resistant clones. The clones giving maximum average tuber weight and yield at 75 DAP are only to be selected for further testing at farmer fields.

The tuber skin colour, tuber shape, eye depth and flesh colour of CIP clones and variety Diamant recorded at harvest are given in Table-5. The tuber characters play a vital role in selecting a variety because of consumers' liking. The oblong, oval or round yellow skin tubers having fleet eyes are most liked in Bangladesh. The pure red tubers are also acceptable. Two year field evaluation data concludes that two CIP clones (393371.58 and 395011.2) will be commercially acceptable as they out-yielded dominant variety Diamant, have MR to LB and standard tuber characteristics and can fit successfully in rice based systems in Indo-Gangetic Plains to generate greater income and enhance food security.

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Table 1: Performance of CIP clones and Diamant variety to late blight resistant in Bangladesh during 2007-08

CIP clones/varieties	Percentage of leaf infection at different duration														
	50DAP			*60 DAP*			*70 DAP*			*80 DAP*			*90 DAP*		
	Locations			Locations			Locations			Locations			Locations		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
LB1 (391004.19)	10	0	0	22	0	0	50	6	0	72	57	8	78	95	18
LB2 (391046.14)	4	0	0	35	0	0	82	10	0	92	53	5	95	99	13
LB3 (391058.18)	1	0	0	2	0	0	4	0	0	8	2	0	13	20	7
LB4 (393077.15)	3	0	0	5	0	0	6	2	0	8	7	0	12	38	0
LB 5 (393085.5)	2	0	0	4	0	0	5	0	0	4	2	0	8	12	0
LB6 (393280.64)	-	0	0	-	0	0	-	0	0	-	3	0	-	38	0
LB7 (393371.58)	1	0	0	1	0	0	2	0	0	4	2	0	5	12	0
LB 8 (395011.2)	1	0	0	2	0	0	3	1	0	4	8	0	5	38	0
LB11 (396031.11)	-	0	-	-	0	-	-	1	-	-	7	-	-	42	-
LB12 (396031.119)	8	0	-	25	0	-	68	5	-	78	32	-	82	87	-
LB14 (396244.12)	4	0	0	7	0	0	12	2	0	17	12	0	28	43	0
Diamant	10	0	0	53	0	0	83	16	0	96	72	0	96	83	4
$LSD_{0.05}$	3	0	0	5	0	0	9	3	0	5	13	3	4	19	3

DAP- Days After Planting, Locations: a- Bogra, b-Jamalpur, c-Munshigonj. LB are TCRC number

Table 2. Performance of CIP clones to late blight resistant at three locations in Bangladesh during 2008-09

CIP clones/varieties	% Infection of foliage by late blight																							
	27 DAP			*34 DAP*			*41 DAP*			*49 DAP*			*56 DAP*			*63 DAP*			*70 DAP*			*77 DAP*		
	Locations			Locations			Locations			Locations			Locations			Locations			Locations			Locations		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
LB1 (391004.19)	10	0	0	22	0	0	50	6	0	72	57	8	78	95	18	83	100	21	97	100	25	100	100	25
LB2 (391046.14)	4	0	0	35	0	0	82	10	0	92	53	5	95	99	13	99	100	16	100	100	20	100	100	20
LB3 (391058.18)	1	0	0	2	0	0	4	0	0	8	2	0	13	20	7	20	83	17	90	100	23	100	100	27
LB4 (393077.15)	3	0	0	5	0	0	6	2	0	8	7	0	12	38	0	13	63	0	30	91	4	32	98	5
LB 5 (393085.5)	2	0	0	4	0	0	5	0	0	4	2	0	8	12	0	9	56	0	30	85	0	34	95	0
LB6 (393280.64)	-	0	0	-	0	0	-	0	0	-	3	0	-	38	0	-	70	0	-	92	0	-	97	0
LB7 (393371.58)	1	0	0	1	0	0	2	0	0	4	2	0	5	12	0	6	53	0	32	85	2	38	96	2
LB 8 (395011.2)	1	0	0	2	0	0	3	1	0	4	8	0	5	38	0	6	63	1	37	87	5	38	97	5
LB9 (396004.33)	7	0	0	18	0	0	53	16	0	70	60	7	73	98	15	73	100	22	98	100	28	100	100	28
LB11 (396031.11)	-	0	-	-	0	-	-	1	-	-	7	-	-	42	-	-	73	-	-	98	-	-	100	-
LB12 (396031.11)	8	0	-	25	0	-	68	5	-	78	32	-	82	87	-	82	100	-	97	100	-	100	100	-
LB14 (396244.12)	4	0	0	7	0	0	12	2	0	17	12	0	28	43	0	30	100	5	76	93	12	94	100	12
Diamant	10	0	0	53	0	0	83	16	0	96	72	0	96	83	4	99	100	13	100	100	18	100	100	18
$LSD_{0.05}$	3	0	0	5	0	0	9	3	0	5	13	3	4	19	3	6	10	4	5	8	7	4	2	6

DAP- Days After Planting, Locations: a- Bogra, b-Jamalpur, c-Munshigonj. LB are TCRC number

Table 3. Performance of CIP clones and variety for tuber yield during 2007-08 and 2008- 09

CIP clones/variety	Tuber yield (t/ha)											
	75 DAP (2008-09)				90 DAP (200708, 2008-09)							
	I	II	III	Mean	I		II		III		Mean	
					a	b	a	b	a	b	a	b
LB1 (391004.19)	9.3	5.1	18.7	11.0	38.2	10.9	11.9	5.2	16.3	22.6	22.1	12.9
LB2 (391046.14)	6.5	2.3	14.4	7.7	37.0	9.8	12.3	3.5	25.9	17.2	25.1	10.2
LB3 (391058.18)	20.1	14.2	16.9	17.1	33.1	20.4	7.6	15.2	-	21.7	20.4	19.1
LB4 (393077.15)	17.9	13.1	22.7	17.9	34.0	22.9	24.1	16.8	22.9	31.4	27.0	23.7
LB 5 (393085.5)	25.6	18.2	13.8	19.2	-	33.7	18.2	22.3	-	18.1	18.2	24.7
LB6 (393280.64)	16.6	-	16.6	16.6	34.2	22.8	20.8	-	25.5	23.0	26.8	23.8
LB7 (393371.58)	24.7	17.7	22.7	21.7	49.7	33.5	22.2	18.7	-	28.7	36.0	27.0
LB8 (395011.2)	25.5	21.4	16.1	21.0	40.8	33.6	22.2	25.7	31.9	21.4	31.6	26.9
LB9 (396004.33)	10.8	3.9	9.6	8.1	-	29.0	-	4.2	-	12.8	-	15.8
LB11 (396031.11)	16.4	-	-	16.4	28.6	19.8	16.8	-	-	-	22.7	19.8
LB12 (396031.11)	10.9	3.5	-	7.2	32.9	12.9	15.4	5.3	-	-	24.2	12.9
LB14 (396244.12)	20.5	12.0	18.8	17.1	43.1	22.7	18,8	13.5	39.4	26.9	33.8	22.7
Diamant	5.5	3.2	14.0	7.6	38.3	8.1	11.7	5.6	16.3	17.8	22.1	8.1
$LSD_{0.05}$	5.4	3.3	4.6	-	3.9	5.8	4.0	3.9	2.3	5.9	-	-

CIP numbers in parentheses; LB are TCRC number; DAP-days after planting; I-Jamalpur; II-Bogra; III-Munshigonj;a=2007-08; b= 2008-09

Table 4. Performance of CIP clones and variety for average tuber weight during 2007-08 and 2008- 09

CIP clones/variety	Average tuber weight (g)											
	75 DAP (2008-09)				90 DAP (200708, 2008-09)							
	I	II	III	Mean	I		II		III		Mean	
					a	b	a	b	a	b	a	b
LB1 (391004.19)	14	12	31	19	102	17	32	13	34	37	56	22
LB2 (391046.14)	13	5	28	15	81	18	27	7	52	40	53	22
LB3 (391058.18)	35	31	52	39	92	41	23	34	-	63	58	46
LB4 (393077.15)	41	43	56	47	54	46	38	56	51	75	48	59
LB 5 (393085.5)	56	50	46	50	-	66	38	55	-	53	38	58
LB6 (393280.64)	32	-	26	29	61	37	38	-	47	36	49	41
LB7 (393371.58)	47	44	34	42	89	60	42	49	-	43	66	51
LB 8 (395011.2)	40	48	37	41	63	45	38	51	62	47	54	36
LB9 (396004.33)	28	16	36	27	-	65	-	17	-	42	56	43
LB11 (396031.11)	27	-	-	27	70	32	41	-	-	-	41	45
LB12 (396031.11)	17	19	-	18	66	20	31	22	-	-	46	29
LB14 (396244.12)	40	40	43	41	98	45	45	43	88	60	77	45
Diamant	13	14	40	23	79	16	30	22	42	45	50	28
$LSD_{0.05}$	9	12	11	-	18	18	10	13	10	11	-	-

CIP numbers in parentheses; LB are TCRC number; DAP-days after planting; I-Jamalpur; II-Bogra; III-Munshigonj;a=2007-08; b= 2008-09

Table 5. Tuber characters of CIP clones and Diamant evaluated for late blight resistance

CIP clones/variety	Tuber characters			
	Skin Colour	Tuber shape	Eye Depth	Flesh Colour
LB1 (391004.19)	Yellow	Oblong	Shallow	Yellow
LB2 (391046.14)	Yellow	Oblong	Shallow	Yellow
LB3 (391058.18)	Yellow	Oblong-round	Shallow	Yellow
LB4 (393077.15)	Yellow, picked eyes	Round	Medium	Light Yellow
LB 5 (393085.5)	Dull brown, russetting	Oval to flat	Shallow	Yellow
LB6 (393280.64)	Red	Round	Medium	Yellow
LB7 (393371.58)	Yellow, picked eyes	Round	Shallow	Light Yellow
LB 8 (395011.2)	Yellow	Oval	Shallow	Yellow
LB9 (396004.33)	Yellow	Round	Shallow	Yellow
LB11 (396031.11)	Yellow	Round	Shallow	Yellow
LB12 (396031.11)	Yellow	Oval	Shallow	Yellow
LB14 (396244.12)	Yellow	Oval to oblong	Shallow	Yellow
Diamant	Yellow	Oblong	Shallow	Yellow

Performance of CIP-derived population B3 potato (*Solanum tuberosum* L.) clones under Malawi conditions

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Abstract

From 2007 to 2009, experiments were conducted at multiple locations in the central and southern regions of Malawi to evaluate the performance of ten potato advanced clones bred at the International Potato Center in Peru for durable resistance to late blight (Population B3 clones). Two local cultivars were used as control treatments. Main objective of the experiments was to select high yielding potato clones resistant to late blight disease and suitable for boiled, French fries and Crisps products. Experiments were laid out in Randomized Complete Block Design with three replications. Data collected included late blight damage on crop, number of plants harvested per plot, number and weight of marketable and unmarketable tubers, quality of boiled and processing products after harvests. Analysis of variance was performed and treatment means were compared using the Duncan Multiple Range test.

Results showed that there were significant differences among potato genotypes with respect to total and marketable tuber yields and resistance to late blight ($p<0.05$). In 2008-2009 rain fed crop, all clones from CIP's population B3 germplasm were more resistant to late blight than local cultivars. In the winter crop of 2008, commercial tuber yields at Bembeke site ranged from 19.9 tonnes/ha for variety Lady Rosetta to 31.7 tonnes/ha for CIP-396036.201. High yielding B3 clones with resistance to late blight and good cooking and processing qualities included CIP-395015.6, CIP-396027.205, CIP-396035.107, and CIP-396036.201.

Keywords: Potato clones, late blight, tuber yield, processing qualities, Malawi.

Introduction

Chronic poverty is recognized as the major underlying cause of food insecurity in Malawi, with over 86% of those below the poverty line living in rural areas (MEPD, 2005). For over five years, the Government of Malawi pursued a policy of crop diversification to reduce their dependency on maize and tobacco. Root and tuber crops (cassava, sweet potatoes and potatoes) have been at the forefront of the diversification effort (FAO/WFP, 2005). Potato has emerged as an important food and cash crop in Malawi. The annual national production reported by FEWSNET (Food Early Warning System Network) for 2006 was 527,830 tons produced on about 40,601 ha of land, with potato ranking fourth nationally in terms of production compared to other major food crops. In the major growing high land Districts of Dedza and Ntcheu, potato is the second major food crop after maize. Potato is generally consumed in three forms: French fries, boiled and crisps (Demo *et al.*, 2007a, 2007b). Almost all potato growers are small scale farmers. 90% of them apply fertilizer on the potato crop. Late blight (*Phytophthora infestans*) disease is one of the major production constraints (CIP, 2008). The disease is typically combated with fungicides and non-resistant materials are sprayed four to seven times a season depending on the intensity of the attack. Small scale resource poor farmers in Malawi hardly own a sprayer or buy fungicide required for control of the disease. This implies that there is a very high risk of crop failure due to late blight during rain fed production season if susceptible varieties are planted. To increase productivity and reduce risk of crop failure caused by late blight, high yielding pro-poor varieties that can be grown without or with minimum spray of fungicide needed to be developed. Taking advantage of the potato genetic materials bred by the International Potato Center (CIP) for durable resistance to late blight (Population B3 potato clones), the objective of the study was to evaluate and select from germplasm introduced from CIP high yielding materials with resistance to late blight, good cooking and/or processing qualities.

Materials and methods

Between 2007 and 2009, ten potato genotypes from CIP's Population B3 clones were evaluated under field conditions at three different locations together with two checks made of one local cultivar (Rosita) and one imported variety (Lady Rosetta). The three locations were Bembeke research sub-station in Dedza District, Tsangano research sub-station in Ntcheu District in Central Region, and Njuli-farm situated at 25 km from Blantyre city in the Southern Region. The trials were conducted during dry winter (April-September) and wet summer (December-April) seasons of 2007/2008 and 2008/2009. The dry winter season trials of 2007 at Bembeke and Njuli-farm were planted with mini-tubers of very small size with diameter between 10 and 20mm that were produced under screen house conditions. For all trials, the Randomized Complete Block Design was used with three replications. Twenty tubers per genotype were planted per experimental unit that consisted of one ridge of 5 m long during the wet season of 2007/2008. The planting spacing was 80cm between ridges and 25 cm between plants within ridge. With increase availability of planting materials, starting from the dry season of 2008, 48 tubers were planted per experimental unit which consisted of 4 ridges of 4 m long each. The planting spacing remained unchanged. Data were collected on the two middle ridges (net plot). At planting, 600 kg of the fertilizer NPK 8-18-15 +6S was applied to supply 48 kg N, 108 kg P2O5, 90 kg K2O and 36 Kg S per hectare. At hilling up which took place 3 to 4 weeks after planting, additional nitrogen was applied at the rate of 60 kg N per hectare through CAN (Calcium Ammonium Nitrate) fertilizer. Trials were kept weed free from planting to harvest. Data were recorded on number of plants emerged from the ground at 4 weeks after planting, % of crop foliage damaged by late blight, tuber yield and yield parameters. After harvest, sample tubers were used to assess qualities of boiled and process product (crisps). Analysis of variance was conducted and where significant differences were detected among treatment means, the Duncan Multiple Range test was used to separate them.

Results and discussion

Tuber yield and yield parameters

Total tuber yield, total number of tubers per plant and average weight of individual tubers obtained from the first trials grown during the dry season of 2007 showed significant differences ($P<0.05$). Total tuber yield ranged from 20.2 tons/ha at Njuli-farm to 10.9 tons/ha at Bembeke. This relatively low yield could be explained by the fact that seed tubers planted were of screen house mini-tubers. Top yielding clones at Bembeke were equally high performers at Njuli-farm. The second field evaluation was conducted at the above two sites during the summer rain fed season in the period February –June 2008. It should be noted that the crop received rain fall for only 7 weeks out of a normal crop cycle of 12 to 14 weeks. Supplementary water supply to the crop was by irrigation after stoppage of rains. At Bembeke, total tuber yields ranged from 24.8 tons/ha (clone number 395111.2) to 13.7 tons/ha (clone 393075.54). The imported variety Lady Rosetta produced total tuber yield of 16.9 tons/ha (Table 1). Marketable tuber yield ranged from 18.0 tons/ha (clone 395111.2) to 7.8 tons/ha (clone 395011.2). The top performing clone at Bembeke was 395011.2. At Njuli-farm, tuber yields were generally higher than those recorded at Bembeke (Table1). Total tuber yields ranged from 39.5 tons/ha (clone 395112.19) to 21.4 tons/ha (clone 396027.205). On the basis of marketable yield, 2 of the 10 tested CIP's clones statistically out yielded the currently grown variety Lady Rosetta.

Trial conducted during the dry winter season of 2008**.** Results from this trial showed that all clones evaluated had marketable tuber yields ranging from 19.9 Mt/ha with Lady Rosetta to 28.1 Mt/ha with the B3 clone 395011.2. Best clones in terms of palatability taste were 393075.5, 395111.13, 391691.96, 3966027.205, and 395015.6. These clones were classified as having good taste of boiled product using a scoring scale of 1 (very poor taste) to 5 (very good taste).

Evaluation of Population B3 potato clones during the rainy season from December 2008 and March 2009**.** At Bembeke as well as at Tsangano, significant ($P<0.05$) yield differences were recorded with total tuber yield ranging from 33.6 tons/ha (clone 395015.6) to 18.1 tons/ha for 396033.102 (Table 1). In terms of marketable yield, five CIP derived clones out yielded the two checks Lady Rosetta and Rosita. At Tsangano, all CIP clones and Lady Rosetta out yielded the widely grown local cultivar Rosita. The cultivar Rosita generally produces a large proportion of sub-standard tuber size that contributes to lower the yield of marketable size tubers (tubers with diameter ≥ 35mm). Crisps processing evaluation was done by a potato processing factory and clones/varieties with good crisping qualities were identified.

Table 1. Total and marketable tuber yield of CIP-derived potato clones with genetic traits for durable resistance to late blight evaluated under field conditions at three different sites (Njuli-Farm, Bembeke and Tsangano) in Malawi during the summer rain fed seasons of 2008 (February-June) and 2009 (December 2008-March 2009)

Clones identities	Total and marketable tuber yields (Tons/ha)					
	2008		2008/2009			
	Njuli-Farm	Bembeke	Bembeke		Tsangano	
	Total yield	Total yield	Total yield	Marketable yield	Total yield	Marketable yield
395015.6	34.4ab	21.3 abcd	33.6 a	28.5 a	29.3 a	25.5 a
395111.13	32.7abc	18.9 abcd	19.6 de	17.4 c	19.3 ab	17.2 ab
395011.2	31.4 bc	24.8 a	28.5 abc	24.4 ab	21.1 ab	18.4 ab
396036.201	29.0 bcd	17.7 abcd	26.9 abc	24.4 ab	26.4 ab	22.0 a
391691.96	27.8 bcd	16.8 abcd	32.5 ab	27.8 a	19.6 ab	16.3 ab
396033.102	25.9 cd	15.5 abcd	18.1 e	15.7 c	17.9 ab	15.2 ab
396027.205	21.4 de	19.4 abcd	23.3 cde	21.2 bc	28.3 a	26.3 a
396035.107	-	-	33.3 a	30.7 a	28.0 a	25.2 a
395112.19	39.5a	15.6 abcd	-	-	-	-
393075.54	28.9 bcd	13.7 cd	-	-	-	-
Lady Rosetta (check)	**25.9 cd**	**16.9 abcd**	**23.3 cde**	**20.4 bc**	**16.5 ab**	**14.2 ab**
Rosita (check)	-	-	**26.1 bcd**	**18.2 bc**	**14.0 b**	**6.6 b**
CV (%)	13.36	20.60	13.82	15.09	29.87	35.27

Means in the same column with the same letter are not significantly different at 5% level of significance
DAP= Days after planting

Level of resistance of B3 clones to late blight disease

One late blight reading was done on the trial at Bembeke during summer 2007/2008. Results presented in Table 2 revealed that the variety Lady Rosetta suffered a severe late blight attack with 87.5% of leaves damaged by the disease. Only one late blight reading was made because the disease started late in the cropping cycle and rains stopped prematurely. All CIP's B3 clones showed good resistance to Late blight with levels of damage on crop leaves between 0.2% and 17.5 % (Table 1). It should be noted that weather conditions were not very favourable for late blight development during the entire vegetative period of the crop. While it was certain that Lady Rosetta was very susceptible to late blight disease, further evaluations were necessary. Results obtained at both Bembeke and Tsangano during 2008/2009 season showed that CIP-derived clones were significantly ($P<0.05$) more resistant to late blight than both the dominant local cultivar Rosita and the variety Lady Rosetta (Table 2). The disease caused 48.33% to 100% damage to the variety Lady Rosetta at 60 and 70 days after planting (DAP) at Bembeke. A similar level of damage was recorded on this variety at Tsangano. At Bembeke, the cultivar Rosita had a damage level of 0.0% and 63.3% at 60 and 82 DAP, respectively (Table 2). Over the two sites and scoring dates, damage level with CIP's B3 clones was between 0% and 26.7% in the period from 60 to 82 DAP. (Table 2).

Conclusions

The introduced CIP-derived potato clones developed for durable resistance to late blight showed higher field resistance to late blight compared to the local cultivar Rosita and the imported variety Lady Rosetta. Top five High yielding B3 clones that combined resistance to late blight, good cooking and good processing qualities were CIP-395015.6, CIP-396027.205, CIP-396035.107, CIP-396036.201 and 391691.96. These clones will undergo on-farm trials in collaboration with farmers prior to eventual release.

Table 2. Proportion (%) of crop foliage damaged by late blight disease for different CIP-derived potato clones with genetic traits for durable resistance to late blight evaluated under field conditions at two sites (Bembeke and Tsangano) in Malawi during the summer rain fed seasons of 2008 (February-June) and 2009 (December 2008-March 2009)

Clones identities	Proportion (%) of crop foliage damaged by late blight disease					
	2008	2008/2009				
	Bembeke	Bembeke			Tsangano	
	75 DAP	60 DAP	70 DAP	82 DAP	60 DAP	75 DAP
395015.6	17.5 c	0.0 b	2.3 cd	14.7 cde	0.0 b	6.7 c
395111.13	0.2 c	0.0 b	0.7 d	20.0 cd	3.0 b	1.7 c
395011.2	4.0 c	3.3 b	4.0 cd	10.0 cde	0.0	2.3 c
396036.201	2.5 c	1.0 b	1.7 d	13.3 cde	1.0 b	4.0 c
391691.96	1.5 c	0.0 b	0.0 d	0.0 e	0.0 b	1.7 c
396033.102	0.5 c	0.0 b	11.7 bc	26.7 c	0.0 b	1.3 c
396027.205	1.0 c	7.3 b	6.7 bcd	16.7 cde	0.0 b	3.3 c
396035.107	-	0.0 b	0.0 d	3.3 de	0.0 b	2.3 c
395112.19	0.5 c	-	-	-	-	-
393075.54	2.5 c	-	-	-	-	-
Lady Rosetta (check)	**87.5 a**	**48.3 a**	**100.0 a**	**100.0 a**	**26.7 a**	**100.0 a**
Rosita (check)	**-**	**0.0 b**	**13.7 b**	**63.3 b**	**2.0 b**	**63.3 b**

Means in the same column with the same letter are not significantly different at 5% level of significance.
DAP= Days after planting

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Phenology modeling and regional risk assessments for *Tecia solanivora*

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Abstract

The Guatemalan potato tuber moth, *Tecia solanivora* (Lepidoptera: Gelechiidae) is a serious pest in potato (*Solanum tuberosum*) production as larvae feed on potato tubers in field and storage and destroy them completely. Native in Guatemala, *T. solanivora* spread within the last 25 years to Venezuela, Colombia, Ecuador and Tenerife, Canary Islands, Spain. Exact understanding of pest ecology, specifically its temperature-dependent development, is helpful for targeting control efforts to the most affected and threatened regions. Life-tables describing development time and mortality of immature life stages, sex ratio, longevity of adults and oviposition of *T. solanivora* at constant temperatures ranging from 9.9 to 29.9°C were established. Functions were fitted to the observed data points and compiled to a temperature based phenology model. The model was validated with development data collected under fluctuating temperature regimes before it was linked to geographic information systems in order to produce maps indicating the risk of *T. solanivora* establishment and population growth potential in the Andean region. The indices chosen for mapping were the generation index (number of generations per year) and the activity index (potential population growth within one year). The maps were compared with available data on the distribution of *T. solanivora* in Colombia, Ecuador and Venezuela. The maps indicate that multiplication of *T. solanivora* is possible in various potato growing zones of all South American countries and that there is a high risk of further distribution of *T. solanivora* to Peru.

Keywords: potato production, pest management, Guatemalan potato tuber moth, insect modeling

Introduction

The Guatemalan potato tuber moth, *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae) is a potato pest whose larvae feed on potato (*Solanum tuberosum* L.) tubers during the cropping period as well as in storage making them unsuitable for consumption. Losses of up to 60% at harvest and of 100% during storage were observed in Ecuador (Suquillo 2004); for Colombia harvest losses of 10 to 50% were reported (Palacios *et al.* 1997). Originating in Guatemala, *T. solanivora* was unintentionally introduced into Venezuela in 1983 (Salasar & Escalante 1984) and into Colombia in 1985 (Durán 2001). In 1996 it reached Ecuador (Gallegos 1997) and in 1999 it was detected in Tenerife, Canary Islands (Trujillo *et al.* 2004).

Further distribution of the pest has to be prevented. In order to develop an appropriate strategy for managing the pest it is important to identify those potato growing areas that are most affected or threatened by *T. solanivora*. A *T. solanivora* phenology model based on temperature was developed and linked to Geographic information systems in order to produce risks maps indicating the pest's activity potential in the Andean region and South America in general.

Materials and Methods

Insect modeling and model validation

Development time and mortality of immature life stages, sex ratio and longevity of adults as well as fecundity of *T. solanivora* were determined at constant temperatures ranging between 9.9 and 29.9°C. Hereby, we followed the methods for life-table studies of the potato tuber moth *Phthorimaea operculella* (Zeller) as described by Sporleder et al. (2004). Functions were fitted to the distribution of development, median development rate, mortality and fecundity (Table 1).

Table 1. Functions and their estimated parameters fitted to *T. solanivora* development distribution, development rate, mortality of immature life stages and fecundity

Distribution of development and senescence time

	intercept (=a)							slope (=b)
temp.(°C) life stage	9.9	15.2	17.4	20.4	25.4	27.8	29.9	
eggs	-184.27	-132.78		-104.45	-84.22	-78.64	-86.96	49.37
larvae	-43.79	-33.18		-30.02	-27.15	-27.64		9.53
female pupae	-44.56	-33.92		-28.20	-23.94	-22.38	-23.99	10.50
female adults	-13.01	-10.27	-9.27	-10.68	-8.00	-7.82	-8.82	3.62

Development or senescence rate

life stage	P	To	Ha	Tl	Hl	Hh	Th
eggs	0.07349	289.006	18297.32	282.573	-206259	90647.2	303.131
larvae	0.03861	291.710	10260.36	283.148	-194683	161978.7	302.506
female pupae	0.104	298.303	12903.94	283.228	-57865.9	212441.4	303.810
female adults	0.06359	290.976	8850.484	282.974	-1629230	1138448	303.190

Mortality

life stage	Model	x0	Y0	a	b	C
eggs	y ~ y0 + a * exp(-0.5 * (log(x/x0)/b)^2)	64.57364	0.094914	30107.52	0.168595	
larvae	y ~ a * sqrt(x) + b * x + c			-2.67494	0.32969	5.76309
pupae	y ~ a * x^2 + b * x + c			0.00468	-0.17646	1.90151

Fecundity

	Model	x0	y0	a	B
total oviposition	y ~ y0 + a * exp(-0.5 * ((x - x0)/b)^2)	15.376	50.278	255.653	3.931
accumulated oviposition rate	y ~ pgamma(x, a, b)			2.542	5.624

For the variation of development time in eggs, larvae and senescence of female adults the logit model was used:

$$F(x) = \frac{1}{1 + \exp(-(a + b * \ln x)}.$$

For female pupae, we used a complementary log-log model:

$$F(x) = 1 - \exp\left(-\exp\left(a_i + b \ln x\right)\right).$$

For the development or senescence rate, the Sharpe & DeMichele model was applied (Sharpe and DeMichele, 1977).

A female ratio of 1:0.88 was established for all temperatures studied. The functions were compiled to an overall temperature-driven (computer-based) phenology model which uses rate summation and a cohort up-dating algorithm for simulating population growth. For considering temperature fluctuations within one day a cosine function was fitted in between the daily minimum and maximum temperatures and a 15 minute interval was used for calculating the population development as described by Sporleder *et al.* (2008). Development data collected under eleven different fluctuating temperatures were used for validating the overall model. Model

outputs were compared to observed development data and the deviation was found to be low enough to accept the model (Table 2).

Table 2. Deviation of simulated to observed values on *T. solanivora* development parameters

development parameters	deviation: simulated-observed values	
total immature development time (days)	-6.8	(±2.40)
mortality in immature life stages (%)	-16.5	(±3.06)
total oviposition per female	+33.5	(±4.76)

Values in parenthesis indicate the standard error.

Risk mapping

ILCYM 2.0 software (Sporleder *et al.* 2009) was used to generate *T. solanivora* risk maps based on the validated model. Spatial simulation was conducted using climatic data (WorldClim, http://www.worldclim.org) on a resolution of 2.5 min for South America or 30 seconds for Ecuador. The indices used for mapping were the Generation Index and the Activity Index.

Generation Index: The Generation index represents the estimated number of generations per year. The program calculates the mean duration of one generation for each day of the year (*Tx*; x = 1 to 365), which are summed up and divided by the number of days of the year for estimating the mean number of generations per year:

$$\text{Generation Index} = \frac{\sum 365 / Tx}{365}$$

where *Tx* = development time of eggs + larvae + pupae (days) + survival time of female adults (days) * 0.42 (normalized age of females when 50% of eggs are laid)

Activity Index: The Activity Index indicates the decimal power of the estimated population growth potential within a given year. It considers the development time, immature mortality and fecundity and is based on the finite rate of population increase modelled for each day of the year. The index is calculated by using the following formula:

$$\text{Activity Index} = \log \Pi \left(\exp[\ln(\text{fecundity}_i * \text{immature survival}_i/2)/T_i]\right) \quad i = 1, 2, \ldots 365$$

Results and discussion

The *Tecia solanivora* risk maps developed for Ecuador and South America with climatic data for the year 2000 indicate that the regions with the highest number of generations also have the highest activity and growth of the pest population (Fig. 1, 2). Colombia, Venezuela and Ecuador are already infested with *T. solanivora*. Five or more generations per year might develop in large parts of Venezuela and Colombia leading to a multiplication of the population of up to 10^{22} times within one year. *T. solanivora* infestation is possible in almost the complete potato growing area. In Ecuador the pest severity is less, only up to three generations might develop in the major potato growing zones in the northern and central provinces. Potentially, population increase might be in some small areas up to 10^{18} fold within one year; however, more than half of the central and northern potato growing zones are not at risk of *T. solanivora* because high altitudes and low temperatures render population growth. Literature data on the number of generations per year or the annual population growth are not available, therefore only figures about infestation levels from reports and literature can be used for validating (evaluating) the simulated resulting risk maps. In case of Venezuela, the whole Andean region is reported to be infested (Niño 2005), which coincides with the information of the maps. In Colombia, *T. solanivora* is distributed in the provinces Norte de Santander, Santander, Boyacá, Antioquia, Cundinamarca, Tolima and Nariño (Palacios *et al.* 1997), which are the same regions as indicated in the maps. In Ecuador, *T. solanivora* monitoring data also

confirm the simulation results for all potato growing zones with exception of the Pichincha region where an annual population growth of up to 10^{14} is predicted but no moths were detected during monitoring (PUCE-PROMSA 2004). This difference might be caused by high precipitation (1350 mm, Izobamba, INAMHI) as heavy rainfalls impede *T. solanivora* population growth (Barragán *et al.* 2004) and only temperature was considered for mapping.

Peru, Bolivia, Brazil, Argentina, Chile Uruguay and Paraguay are still *T. solanivora* free. The maps however show that the temperature conditions in these countries would favor the pests' population growth. Peru as neighboring country to *T. solanivora* infested Ecuador is especially at risk and preventive measures for early detection are taken since 1997 (Naccha and Villar 2005). Up until now *T. solanivora* was not introduced into Peru probably because southern Ecuador and northern Peru are no commercial potato growing zones and potato fields are small and sparse. Furthermore distribution occurred mainly through human influence; to Costa Rica, Venezuela and Colombia *T. solanivora* was introduced with infested seed tubers (Palacios *et al.* 1997). No potato trade from Ecuador to Peru was registered since 1994 because Peru most of the years does not import potatoes and furthermore potato prices in Ecuador are usually higher than in Peru since the dollarization of Ecuador in 2000 (FAOSTAT, 2009).

Conclusion

The fact that *T. solanivora* reached Tenerife, Canary Islands, shows that the pest might be introduced and also further distributed within Europe and other potato growing regions outside of Latin America. This should be reason for alert for all countries where potato cultivation plays a major role and where the maps indicate a high potential of population growth. Worldwide maps, which are still to be produced, will help to identify those zones in order to create awareness of the risk of *T. solanivora* introduction. Further factors important for the pests' population growth and infestation (e.g., rainfall, crop management etc.), which are not considered for risk mapping yet, have also to be taken in account to define those areas especially at risk.

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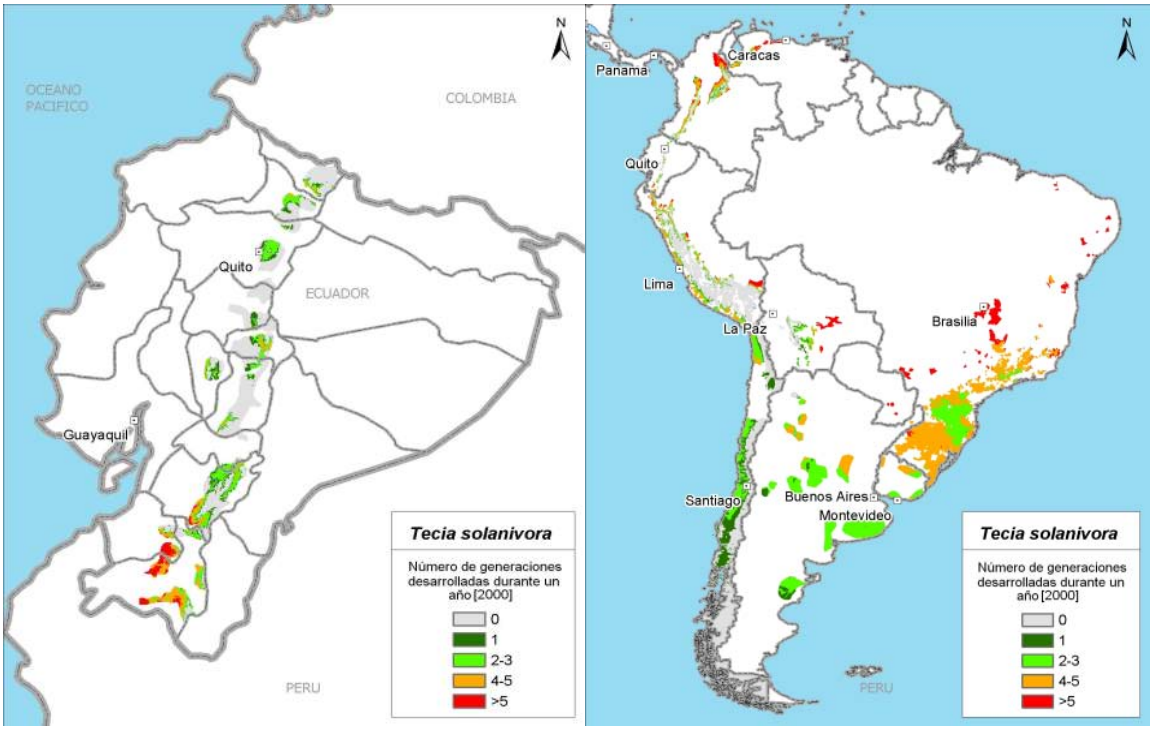

Figure 1. Simulated numbers of *Tecia solanivora* generations for the year 2000 in the potato growing zones of Ecuador (resolution: 30 sec.) and South America in general (resolution 2.5 min.)

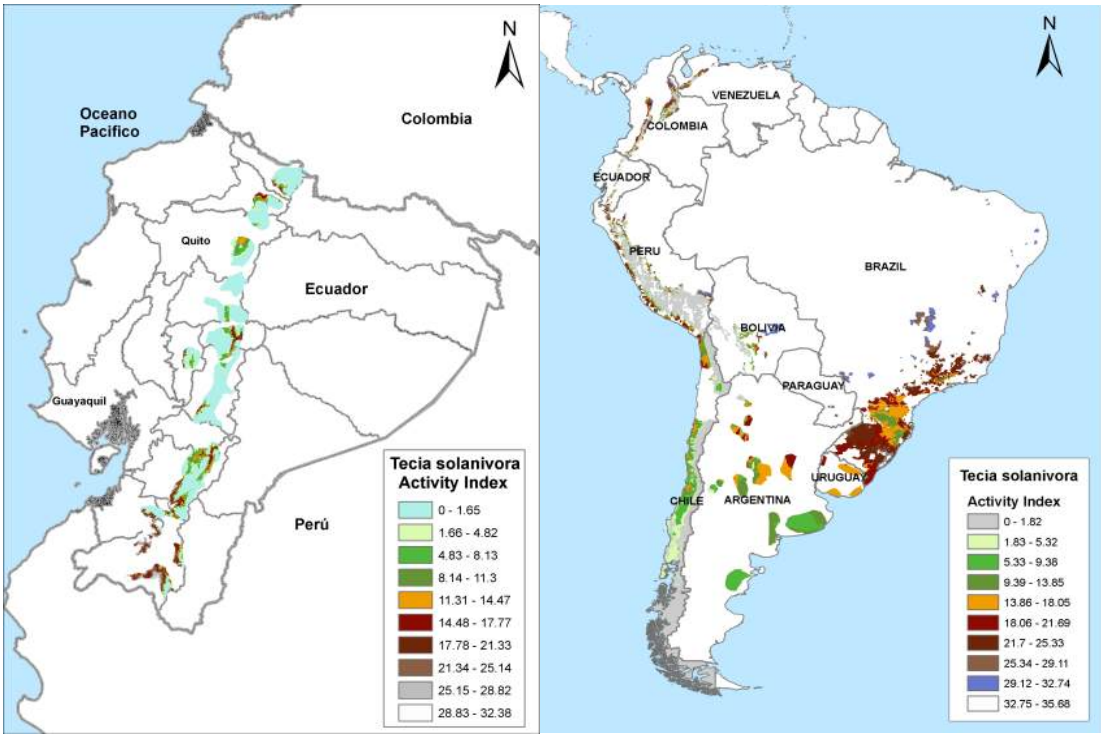

Figure 2. Estimated population growth (activity index) of *Tecia solanivora* within the year 2000 in the potato growing zones of Ecuador (resolution: 30 sec.) and South America (resolution: 2.5 min.)

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Performance of Orange-fleshed sweetpotato genotypes in different agro-ecological regions of India

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Abstract

Orange-fleshed sweetpotatoes (OFSPs) are gaining importance in India due to their additional nutritive value. It is necessary to evaluate and select promising OFSP genotypes in the varied agro-ecological conditions of India and analyse important nutrient characteristics before releasing them as varieties. In a preliminary effort, five OFSP genotypes introduced from CIP-Lima, Peru and CIP-Bhubaneswar, India were evaluated in the Initial Evaluation Trial at 12 locations under the ICAR sponsored All India Coordinated Project on Tuber Crops in the main cropping season *(kharif)* for two consecutive years. The pooled data on tuber yields indicated that CIP 440038 recorded good yields at locations such as in Port Blair (33t/ha) and Kalyani (28 t/ha). CIP SWA- 2 recorded a maximum of 36 t/ha at Faizabad. CIP 440127 recorded highest yield at Ranchi (33t/ha) followed by Coimbatore (28t/ha) location while CIP 187017-1 recorded a yield 31 t/ha at Raipur. OFSP genotypes were further analyzed for total carotenoids, β carotene and dry matter content for three seasons namely Summer (March-May), Kharif (June-Sep) and Rabi (Oct-Feb) in the five genotypes raised at CTCRI, Trivandrum. The highest total carotenoids (8 mg/100 g fr.wt.) and β carotene (5.5 mg/100 g fr.wt.) were observed in CIP 440127. There was hardly any variation for the biochemical characters in the genotypes in different seasons. Overall this preliminary study indicated that two OFSP genotypes viz., CIP 440127 and CIP SWA-2 showed considerably wider adaptability compared to other OFSPs with reasonable potential yields and also higher quantities of β-carotene and carotenoids.

Keywords: orange-fleshed sweet potato (OFSP), agro-ecological regions.

Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is a vegetatively and cross- pollinated crop. It is a short duration crop (3-4 months) cultivated for its edible tubers. It can produce highest energy (194Mj ha $^{-1}$ day^{-1}) and considerable yield with low inputs even in marginal lands (Woolfe,1992). The tubers and young leaves are used as a vegetable. The flesh colour of tubers of different sweet potato varieties vary from white to various shades of cream, yellow to dark orange colour depending on the carotenoid content with β- carotene as the major component. The leaves also possess high β- carotene content. The white- fleshed sweet potatoes are traditionally preferred by the local population all over India which however has no β- carotene content. The orange- fleshed sweet potato is an excellent source of β- carotene to control Vitamin A deficiency which affects millions of children in the developing countries. β- carotene is a precursor of vitamin A. The present study was under taken to asses the yield potential and carotene content of five orange-fleshed genotypes introduced from CIP, Lima, Peru and Bhubaneswar, India at different agro-climatic regions of India.

Materials and methods

The materials for the study comprised of five orange-fleshed genotypes CIP -187017-1, CIP-420027, CIP-440127, CIP-440038 and CIP-SWA -2 and the other orange- fleshed genotypes from the different co-ordinating centres. The trial was conducted at 12 centres on a Randomized Block Design in three replications for 2 consecutive years during the kharif season (June-September/October) under rainfed conditions following the recommended package of practices of each centre/location. The crop was harvested between 90 and 105 days after planting and the tuber yield was recorded. The biochemical analysis was carried out at CTCRI, Thiruvananthapuram for three seasons *ie* summer, kharif, and rabi. The tuber samples from each genotype raised at this location were analysed for carotenoids and dry matter content. The total carotene and β-carotene content were estimated

using the AOAC (1995) procedure. Dry matter content was determined by drying 100g of fresh tuber slices in an oven at 50^{0}C, till a constant weight was obtained. From the weight of dried sample the percentage of dry matter was calculated. The tuber yield data were pooled for two years for proper interpretation of the results obtained.

Results and discussion

The data on tuber yields are given in Table 1. Since most of the genotypes are not significantly varying in terms of yield across respective locations over two seasons, average yield was considered for each genotype across locations and seasons. The data indicated that the genotype CIP 440038 produced an yield of 21-28 t/ha at Thiruvananthapuram, Coimbatore, Kalyani, Raipur and Faizabad centres while CIP-440127 gave an yield of 28-33t/ha at three centers *viz*, Coimbatore, Kalyani and Ranchi. In CIP-187017-1, the yield 23 t/ha was observed at Kalyani and 31t/ha at Raipur. At Faizabad, CIP-SWA-2 recorded 36t/ha while at the other four centres (Thiruvananthapuram, Kalyani, Bhubaneswar and Jorhat) the yield was 16-18t/ha. The overall results showed that the genotypes CIP-440127 and CIP-SWA-2 had wider adaptability compared to the other genotypes. Grunberg et al,.(2005) reported that some high yielding genotypes had wider adaptability, while some genotypes had specific adaptation to medium to high yielding environments and low yielding environments. Haldavankar *et e*l (2009) also showed that some sweet potato cultivars had wider adaptability and produced stable yield at different environmental conditions in India.

Table 1. Tuber yield (t/ha) of CIP genotypes at different co-ordinating centres in India)

Centres/Locations	CIP -SWA- 2	CIP 187017-1	CIP 420027	CIP 440127	CIP 440038
Thiruvananthapuram, Kerala	16.17	6.04	2.16	15.04	20.82
Coimbatore, Tamil Nadu	13.69	20.94	2.42	27.90	21.16
Rajendra Nagar, A.P	8.52	6.65	10.42	10.91	12.04
Bhubaneswar Orissa	17.59	15.08	16.33	16.41	14.03
Kalyani,West Bengal	16.34	22.72	10.00	27.61	27.87
Ranchi, Jharkand	10.96	12.96	5.67	32.91	2.55
Dholi, Bihar	3.1	3.3	3.3	9.1	13.30
Port Blair, Andaman and Nicobar Islands	11.46	9.80	7.46	18.43	32.80
Raipur, chattisghar	-	31.02	27.49	-	27.99
Jorhat, Assam	17.66	14.57	8.58	-	-
Dapoli, Maharashtra	10.10	13.46	11.45	-	-
Faizabad, Uttar Pradesh (U.P)	35.94	-	27.67	-	26.83

The biochemical analysis of the orange-fleshed genotypes for the three seasons are given in Table 2. The data showed that the highest total carotenoids (7-8mg/100g.f.w.) and β-carotene (5-6mg/100g.f.w.) was observed in two genotypes CIP-440127 and CIP-SWA-2. The lowest total carotenoids (2mg/100g.f.w.) and β- carotene (1mg/100g.f.w.) was noticed in CIP-420027.The flesh colour and carotenoids were positively correlated. The depth of the orange flesh colour was mainly a function of the concentration of β-carotene (Simonne, *et al*; 1993) The genotypes included in the study showed different intensities of orange-flesh colour. The percentage of total carotenoids to β-carotene was 62-79%. Woolfe (1992) reported that the percentage of total carotenoids to β-carotene in the orange-fleshed sweet potato varied from 86-89%. Highest dry matter (28%) was found in CIP-SWA-2 followed by CIP-187017-1 (26%). The data for three seasons showed that there was not much difference in the total carotenoids and β-carotene between the seasons. Gruneberg, *et al.,* (2005) observed that the magnitude of genotypes and environment interaction for the nutritional traits in sweet potato was small. The genotype CIP-440127 and CIP-SWA-2 produced moderate yield with wider adaptability and possess moderate amount of total carotenoid and β- carotene content.

Table 2. Biochemical analysis of sweetpotato genotypes in different seasons

Genotype	Summer season				Kharif season				Rabi season			
	Total Carotenoids (mg/100g f.w.)	Carotene (mg/100g f.w.)	% of total carotenoids to carotene	DM (%)	Total Carot-noids (mg/100g f.w.)	Carotene (mg/100g f.w.)	% of total carotenoids to carotene	DM (%)	Total Carotenoids (mg/100g f.w.)	Carotene (mg/100g f.w.)	% of total carotenoids to carotene	DM (%)
CIP -187017-1	3.31	2.06	62.24	25.93	3.51	2.22	63.25	25.1	3.55	2.24	63.10	25.51
CIP- 420027	1.80	1.21	67.22	23.16	1.79	1.20	67.04	24.15	1.84	1.21	65.76	23.22
CIP- 440127	7.86	5.27	67.05	21.54	8.03	5.53	68.87	22.15	7.91	5.51	69.66	22.33
CIP- 440038	5.28	4.00	75.76	24.42	5.20	4.00	76.92	24.52	6.08	4.61	75.82	24.23
CIP- SWA -2	6.79	5.36	78.94	28.15	6.76	5.35	79.14	28.14	7.08	5.59	78.95	27.43

The people who are traditionally dependent on the consumption of white-fleshed local cultivars are unaware of the nutritive value of orange-fleshed sweet potato. Most of the consumers select varieties based on the best taste, flavour and texture, rather than those having a better nutrient profile (Chattopadhay et al, 2006). Introduction and evaluation of elite exotic genotypes with high β-carotene at different agro-climatic situations through the All India Co-ordinated Project enables to identify sweet potato genotypes having high yield and carotene content. The promotion of orange-fleshed genotypes in the house hold diets through nutrition programme could improve the vitamin A status.

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Understanding carotenoid losses in orange-fleshed sweet potato in drying and storage

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Abstract

Biofortified orange-fleshed sweet potato (OFSP) is being promoted to tackle vitamin A deficiency, a serious public health problem affecting children and pregnant/lactating women in sub-Saharan Africa. The aim of the study was to quantify and understand the factors influencing provitamin A losses in OFSP dried chips. Losses were determined after drying and storage. A preliminary pilot-scale study demonstrated that carotenoid levels were not significantly different after either solar or sun drying. Field conditions using locally-promoted varieties in Uganda and Mozambique showed losses associated with drying were less than 40%. Flour made from OFSP could therefore be a significant source of provitamin A. In contrast, storage of chips at room temperature in Uganda for four months resulted in high losses of pro-vitamin A (*ca*. 70% loss from the initial dried product). Low-cost pretreatments, such as blanching, antioxidants and salting, did not improve carotenoid retention during storage. To understand the cause of the losses, dried sweet potato chips were stored under controlled conditions of temperature (10; 20; 30; or 40ºC), aw (0.1; 0.3; 0.5 or 0.7) and oxygen (0 [under nitrogen]; 2.5; 10 or 21% [air]). Losses in provitamin A were the least during storage at the lowest temperature and oxygen level and at the highest humidity level. Enzymatic catabolism of β-carotene in the flour was considered unlikely because of low peroxidase activities at low water activities and the loss of peroxidase activity during storage.

Keywords: orange-fleshed sweet potato, carotenoid degradation, drying, storage.

Introduction

Carotenoids are organic pigments found in plants that play an important role as vitamin A precursors in the human diet. In contrast to most plant foods, 80% of the carotenoid content of orange-fleshed sweet potato (OFSP) is trans-β-carotene (Bechoff et al., 2009a; Bengsston, et al., 2008). Such varieties could contribute to tackling vitamin A deficiency, a main public health issue in the developing world (Bechoff et al., 2009a; Bengsston et al., 2008). Sweet potato is a very valuable crop widely consumed in sub-Saharan Africa (Woolfe 1992). Sun drying of sweet potatoes is a traditional practice. Roots are either sliced or crushed and then dried on rocks. These are stored in granaries; re-hydrated and boiled to be eaten like fresh roots, or milled into flour. There are efforts through the HarvestPlus Challenge Program to promote the use of orange-fleshed varieties with high β-carotene content. In order to tackle vitamin A deficiency in sub-Saharan African by increased consumption of OFSP, CIP and HarvestPlus Challenge Program have launched trials to incorporate OFSP in various recipes of African foodstuffs (Namanda et al., 2005; Owori et al., 2007). Food processing, drying and storage have been previously reported to have a major effect on pro-vitamin A retention because of the nature of unsaturated, unstable provitamin A carotenoids that are easily degraded by light, oxygen, ultra-violet and heating leading, to significant losses (Rodriguez Amaya 1997). It is therefore essential to optimise Orange Flesh Sweet potato (OFSP) processing in order to achieve products with high nutritional quality. Few data are found on carotenoid retention from sweet potato after drying and storage and the effect of direct sun drying compared to solar drying.

Materials and methods

Sweet potato varieties were obtained from partner farmers in Uganda and from Namulongue Agricultural Research Station of National Agricultural Research Organisation in Uganda and farmers partners with World

Vision International in Mozambique. For preliminary trials in France, roots were of an imported American variety (Rubina®, Agrexco).

Roots were chipped using hand rotary disc chipper or sliced by hand. Dryers used were hot air dryer and greenhouse fan-operated solar dryer in France; tunnel and tent dryers in Uganda and tunnel dryer, shade in Mozambique. Open air sun dryers were used as a comparison with other dryers in each location. Chips were dried up to 10% moisture in average. After drying, chips were stored using locally bought polythene or propylene bags or traditional bags made of jute.

Total carotenoids content was determined on sweet potatoes grown in Uganda and Mozambique by visible spectrophotometry (UV-visible spectrophotometer) using the method of Rodriguez-Amaya and Kimura (2004). For preliminary trials in France, the method of Dhuique-Mayer et al. (2005) was used. Trans-β-carotene content was measured by HPLC using the analysis method of Dhuique Mayer et al. (2005) on preliminary samples (OFSP from USA) and samples stored in controlled conditions. Samples were extracted in duplicate or triplicate. Readings were made at a wavelength of 450nm.

Losses (%) in total carotenoid (or trans-β-carotene) content were calculated following the equation (1):

$$\%loss = 1 - \frac{C}{C_0} \qquad (1)$$

Where: C: Carotenoid content after drying (or storage) expressed in µg.g^{-1} dry weight; C_0: Carotenoid content before drying (or storage) expressed in µg.g^{-1}.

Carotenoid degradation followed a first order degradation during storage (Equation 2).

$$\ln \frac{C}{C_0} = -kt \qquad (2)$$

Where: k: constant degradation (day^{-1}); t: storage time (days).

The carotenoid degradation at various temperatures was modelled using the Arrhenius equation (3).

$$k = k_\infty e^{-\frac{Ea}{RT}} \qquad (3)$$

Where: Ea: activation energy (kJ.mol^{-1}); R: gas constant = 8.314 J · K^{-1}.mol^{-1}. In order to validate the Arrhenius carotenoid degradation model in laboratory conditions, dried samples (Ejumula variety) were stored for 88 or 125 days at ambient room temperature (recorded temperature).

Statistical analyses on the analysis of variance were performed using SPSS 14.0 or 15.0. Significant differences per variety between samples ($p<0.05$) were given by Tukey test and are indicated by different letters in the same column (a, b, c).

Results and discussion

Preliminary trials

Losses in total carotenoid content and trans-β-carotene content using different dryers are reported in Table 1.

No significant difference was observed between drying by greenhouse solar dryer and direct sun in term of β-carotene and total carotenoids. Cross flow drying (hot air drying) significantly retained a higher content of β-carotenes and total carotenoids than sun-drying.

Field work in Uganda

On both varieties grown in Uganda (Ejumula and Kakamega analysed jointly), no significant difference was observed between retention of carotenoids in tent, tunnel or sun dryers with total carotenoid losses of 9.0; 9.2 and 8.7% respectively. This is contradictory to studies that reported that sun drying was more damaging than solar drying (Rodriguez Amaya 1997, Mulokozi and Svanberg 2003) but agreed with levels of loss in the recent study by Bengsston et al. (2008) working on Ugandan OFSP.

Table 1. Comparison of losses of total carotenoids and trans- -carotene after drying sweet potato by three different dryers

Treatment	Drying time (h)	Spectrophotometer Total carotenoids (ug/g)	Total carotenoid loss (%)	HPLC trans- β-carotene (ug/g)	trans- β-carotene loss (%)
Fresh	-	372±9a	-	293±13a	-
Hot air dried	2	324±17b	13	247±23b*	16
Solar dried	8	294±17bc	21	226±17bc*	23
Sun dried	8	250±8c	33	193±15c	34

Mean±standard deviation of duplicate* or triplicate extraction.

Carotenoid losses were high during storage as opposed to after drying (Table 2).

Table 2. Total carotenoids losses during the storage of OFSP dried chips at ambient temperature in polyethylene (PE) bags for 4 months (125 days)

Cultivar	Treatment	Dry matter content* (%)	Total carotenoid content ** ($\mu g.g^{-1}$ db)	Loss in storage (%)	Overall loss (%)
Ejumula	Sealed clear PE bag in black PE bag	88.4	64.2(1.0)b	67.9	79.9
	Black PE bag with simple knot	88.4	58.2(4.6)b	70.9	81.8
	Sealed clear PE bag	88.1	69.5(5.7)b	65.2	78.3
Kakamega	Sealed clear PE bag in black PE bag	88.8	18.0(0.5)b	65.7	77.2
	Black PE bag with simple knot	88.7	19.0(1.0)b	63.7	75.8
	Sealed clear PE bag	88.0	18.5(1.0)b	64.7	76.5

*Mean; standard deviation is not given because <1% on triplicate extraction
**Mean (standard deviation) on triplicate extractions. Values in the same column (same cultivar) followed with different letters are significantly different; ANOVA two ways Tukey test.

Dried chips stored for 4 months had important losses for both varieties (Ejumula and Kakamega) with an average of 67%. Samples of dried sweet potato stored in clear polythene bags placed under the window did not demonstrate any difference in terms of loss in carotenoids as compared with opaque (black bag) that was either sealed or tied with simple knot. This agreed with results from Vasquez-Caicedo et al. (2007) about the impact of packaging and storage on pro-vitamin A retention of mango puree.

Field work in Mozambique

Shade drying significantly retained more total carotenoids compared to sun and solar drying in Mozambique with 3.3%, 10.0% and 12.2% respectively for MGCL01 and Resisto varieties analysed jointly.

Carotenoid losses were high during storage in accordance with results in Uganda (Table 3).

Table 3. Total carotenoids losses after drying in Mozambique on two varieties in dry weather

	thin	thick	slices
MGCL01	86.6%	88.2%	89.7%
Resisto	76.8%	79.3%	81.9%

Chipping did not have an effect but variety had (ANOVA; $p<0.05$).

Effect of pre-treatment

The effect of pre-drying treatment by different chemicals was tested (Figure 1).

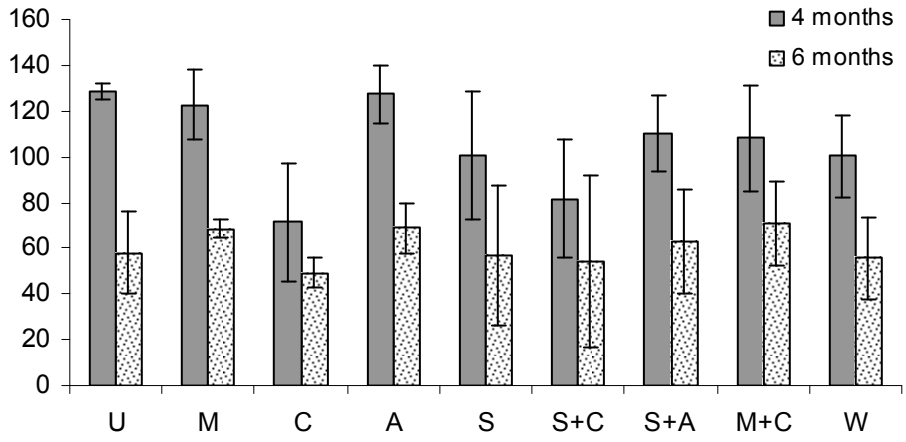


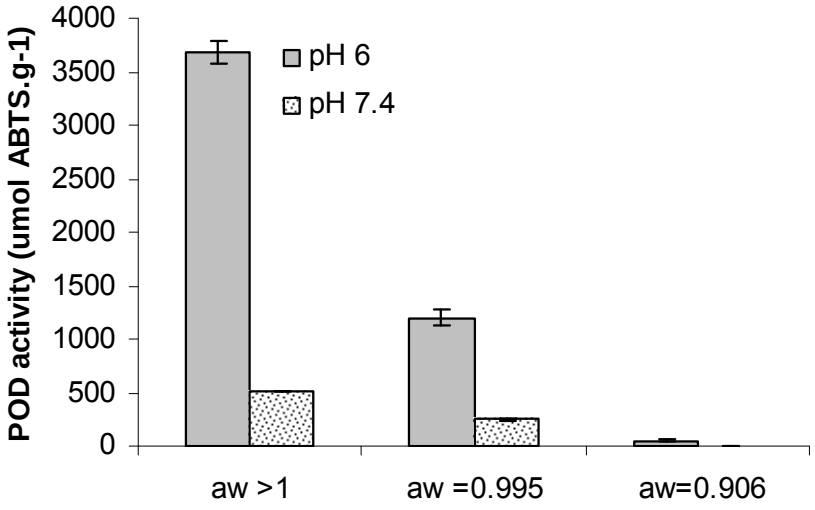
Figure 1. Total carotenoids losses after pre-treatment on dried and stored Ejumula variety for 4 and 6 months

Where: U: untreated; M: 0.5% sodium metabisulphite; C: 0.5% citric acid; A: 1% ascorbic acid; S: 1% salt; W: deionised water.

Pre-treatment did not reduce carotenoid loss. After four months of storage, deionised water treated samples had lower total carotenoid content than untreated that had lower carotenoid content than untreated ones. After six months of storage, there was no difference between samples.

Effect of enzymes

The effect of enzymatic activity (peroxidase) on carotenoid degradation was tested (Figure 2).



*mean of 3 measurements (error bars refer to standard deviation)

Figure 2. Peroxidase activity related to water activity (adjusted with glycerol concentration). Peroxidase activity (µg ABTS.g^{-1} sweet potato on a fresh basis*)

Peroxidase activity decreased with water activity (Figure 2) and with storage time (data not shown). Globally the effect of peroxidase on carotenoid degradation was unlikely.

Storage study under controlled conditions

The effect of temperature, water activity (aw) and oxygen was measured on Ejumula chips stored under controlled conditions of temperature (10; 20; 30; or 40ºC), aw (0.1; 0.3; 0.5 or 0.7) and oxygen (0 [under nitrogen]; 2.5; 10 or 21% [air]) (Figure 3).

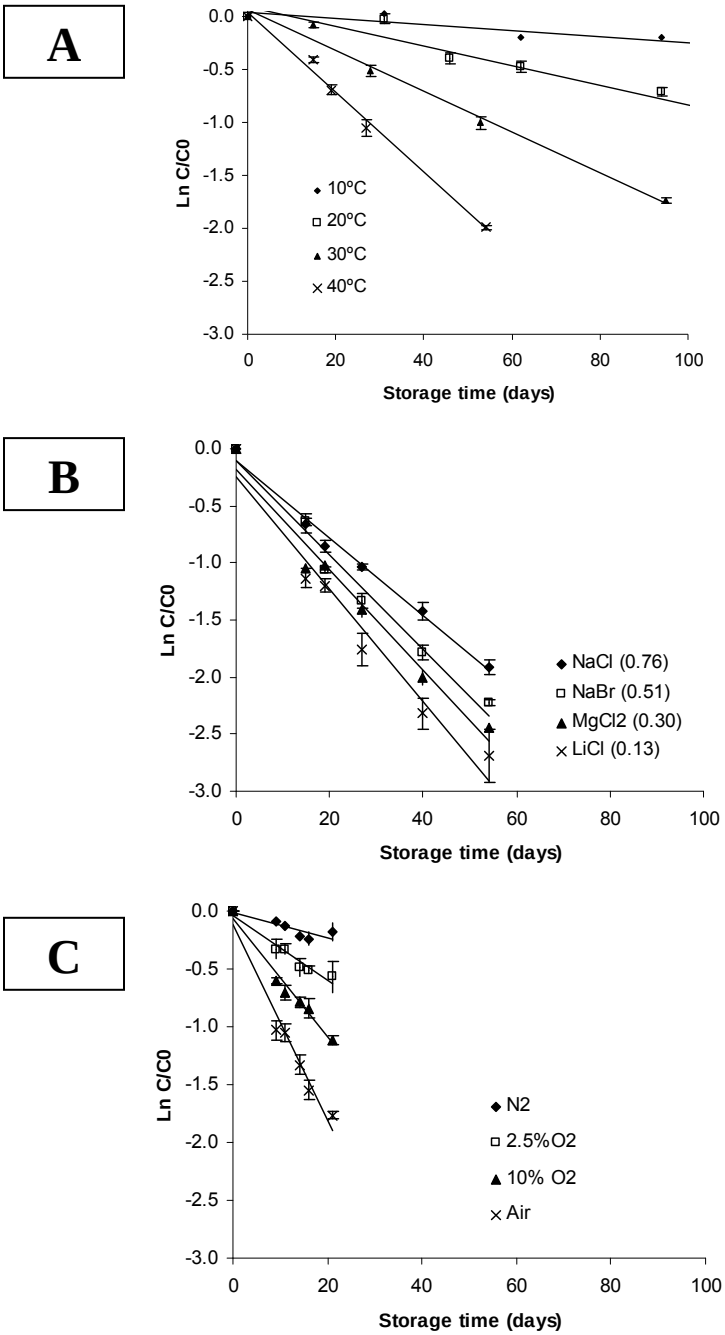


Figure 3. Trans-β-carotene degradation kinetics influenced by temperature between 10-40°C (A); and water activity (B) and oxygen (C) at 40°C on a fresh matter basis

Losses in provitamin A were the least during storage at the lowest temperature and oxygen level and at the highest humidity level.

Carotenoid degradation influenced by temperature was validated using the Arrhenius model (Table 4)

Table 4. Validation of Arrhenius model for a sample of dried Ejumula sweet potato chips stored under ambient anisotherm conditions during 88 days in the UK[a] and during 125 days in Uganda[b] on a fresh weight basis. Oxygen level 21% (air).

			Initial	Final		Difference (%)
		Storage time (days)	(µg.g^{-1})[c]	Experimental[c] (µg.g^{-1})	Predicted by Arrhenius model (µg.g^{-1})	
Sample stored in UK[a]	Trans- -carotene	88	181.2 (5.9)	74.6 (5.1)	72.0	3.5
	Total carotenoids		250.3 (1.1)	94.4 (1.1)	90.3	4.3
Sample stored in Uganda[b]	Total carotenoids	125	219.6 (1.6)	51.4 (1.5)	46.6	9.3

[a] This present study (Calculated a_w: 0.460 (0.012) from chips dry matter (90.4 (0.3) g/100g)
[b] Bechoff et al. (2009b) (a_w from BET model: a_w: 0.400 (0.255); range: [0.22-0.58] from chips dry matter 90.5 (3.5)g/100g; range ([88-92.9g/100g]). [c] Mean of triplicate (standard deviation)

.

For the total carotenoids and trans β-carotene under anisothermic conditions, the difference between the experimental value and value predicted by the model was 4.3% and 3.5% respectively. The robustness of the model was further tested by using it to predict the carotenoid content a a dried sweet potato sample (Ejumula) that had been stored in Uganda at ambient temperature in LPDE bags (permeable to oxygen) for 125 days in Uganda (Bechoff et al., 2009b). Similarly, the model was also accurate in it predictions where for total carotenoids under anisothermic conditions with a difference between the experimental value and model of 9.3%. Therefore it can be concluded that the model developed under samples stored under controlled laboratory conditions was robust enough to apply to samples stored under field conditions in Uganda and elsewhere.

Conclusions

The major conclusions from this work were:

- There were few carotenoid losses after drying of OFSP
- There were high carotenoid losses after storage of OFSP
- The chip size and pre-treatment failed to reduce carotenoid degradation in storage.
- The enzymatic degradation was unlikely because peroxidase activity was low or negligible after storage and at low water activities.
- Oxygen and temperature strongly influenced carotenoid degradation.
- In conclusion, there was no low-cost solution to preserve provitamin A. Further work should focus on special packaging (*eg.* vacuum) and low temperature for storage

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Molecular studies on virus interactions in sweetpotato

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Sweetpotato (*Ipomoea batatas*) is an important subsistence and famine reserve crop grown in developing countries. The most severe disease (SPVD) and yield losses are caused by a synergistic virus interaction between *Sweet potato chlorotic stunt virus* (SPCSV; *Closteroviridae*) and *Sweet potato feathery mottle virus* (SPFMV; Potyviridae). RNA interference (RNAi) is a conserved eukaryotic mechanism used by plants to counteract viral infections via virus-derived short RNA sequences known as small interfering RNA (siRNA); consequently viruses encode proteins able to block RNAi, known as RNAi suppressor proteins (RSP). Our data show that transformation of an SPFMV-resistant sweetpotato variety with SPCSV-encoded RSP proteins (RNase3 and p22) broke down resistance to SPFMV, leading to high accumulation of SPFMV and severe disease symptoms similar to SPVD. Interestingly RNase3-transgenic sweetpotatoes also accumulated higher titers and displayed enhanced symptoms of unrelated RNA viruses and two previously non-characterized DNA viruses. These viruses have been previously shown to synergize with SPCSV. Although siRNA-binding has been reported as a relatively common mechanism for suppression of RNAi by RSPs, SPCSV-RNase3 RSP function depended on its endonuclease activity. We show that siRNAs and total small RNA isolated from virus-infected sweetpotato plants were cleaved *in vitro* by RNase3, suggesting a novel viral mechanism for suppression of RNAi by cleavage of small RNA. Because some SPCSV isolates causing synergistic interactions do not encode p22, results implicate RNase3 as a sufficient factor for the development of SPVD and suggest a target for engineering virus resistance in sweetpotato.

Keywords: Plant virus, RNA silencing, suppression of RNAi, viral sinergismo.

Sweetpotato (*Ipomoea batatas*) is an important crop used in developing countries as a famine reserve food. More than 20 viruses are known to infect this crop worldwide (Valverde *et al.*, 2007) but many sweetpotato cultivars are highly resistant to single virus infection or are able to recover from initial single-infection during plant growth (Gibson *et al.*, 1998; Karyeija *et al.*, 2000; Untiveros et al., 2007). However, mixed virus infections can develop into severe symptoms and cause significant yield losses (Gibson *et al*, 1998). *Sweet potato chlorotic stunt virus* (SPCSV, *Closteroviridae*) has been identified as a critical component in these synergistic interactions. SPCSV infection renders sweetpotato susceptible to accumulation of several unrelated viruses while SPCSV titres remain little affected (Karyeija *et al.*, 2000; Mukasa *et al.*, 2006; Untiveros *et al.*, 2007). Indeed, the most severe disease affecting sweetpotatoes and thereby food security is caused by a virus complex that can reduce yields by 90% (Gibson *et al.*, 1998). This sweet potato virus disease (SPVD) develops in plants infected with SPCSV and *Sweet potato feathery mottle virus* (SPFMV, *Potyviridae*). SPVD is characterized by severe leaf malformation, chlorosis, and stunting of the plants. The synergistic effect of SPCSV on several unrelated viruses is observed independently of the strain of SPCSV (Untiveros *et al.*, 2007; Cuellar *et al.*, 2008) and indicates a general loss of resistance to viruses in sweetpotato in the presence of SPCSV.

Viruses are inducers and targets of RNA interference (RNAi), a fundamental antiviral defense mechanism in eukaryotic organisms (Haasnoot *et al.*, 2007) and essential for virus resistance and recovery from virus disease in plants (Covey *et al.*, 1997, Ratcliff *et al.*, 1997). RNAi is a cell surveillance system to recognize double-stranded RNA (dsRNA) and specifically eliminate by cleavage RNAs homologous to the inducer RNA (Fire *et al.*, 1998; Hammond *et al.*, 2000). Cleavage of dsRNA is carried out by Dicer, which is a class 3 RNase III endonuclease (Bernstein *et al.*, 2001). Plants encode 4 Dicer-like (DCL) enzymes that recognize and cleave long dsRNA molecules to 21-, 22-, and 24-bp fragments that act as small interfering RNA (siRNA). siRNA are key for the efficiency and specificity of the RNAi response (Hamilton and Baulcombe, 1999; Dunoyer *et al.*, 2007; Ding and Voinnet 2007).

Viruses express a wide range of dedicated RNAi-suppressor proteins (RSP) to interfere with the different steps of the RNAi pathway (Li and Ding 2006). It is therefore conceivable that in mixed viral infections, the presence of several RSPs might help to overcome RNAi, generating a synergism that allows at least one of the co-infecting

viruses to accumulate at higher titers than observed in single virus infections (Anandalakshmi *et al.*, 1998; Pruss *et al.*, 1997). However, so far this has been shown only for the N-proximal part (P1/HC-Pro) of the potyviral polyprotein that is known as the potent and sufficient mediator of synergism in transgenic plants infected with unrelated viruses (Pruss *et al.*, 1997). The central part of HC-Pro that mediates viral synergism is involved in suppression of RNAi (Shi et al., 1997; Kasschau and Carrington 2001). Given that RNase III endonucleases are key enzymes involved in RNAi, the presence of a homologous enzyme encoded by SPCSV prompted us to its characterization.

We report here that SPCSV, the causal agent of several virus synergistic interactions encodes one RSP protein (RNase3) that is sufficient to replicate SPCSV synergistic interactions with heterologous viruses. Our data suggest that SPCSV RNase3 is a novel suppressor of a basic antiviral defense system of sweetpotato. In addition, some SPCSV isolates encode one more RSP protein (Cuellar *et al.*, 2008) functionally different to RNase3, and may therefore have a further role in SPCSV synergistic virus interactions.

Materials and methods

Transgenic sweetpotato lines expressing SPCSV RSP

Pathogen-free in vitro plants of the SPFMV-resistant Peruvian sweet potato landrace 'Huachano' (accession no. CIP420065) were obtained from the germplasm collection of the International Potato Center (CIP). The *RNase3* and the *p22* gene of SPCSV-Ug were placed in the binary vector pKOH200, as described (Karim *et al.*, 2007), and used to transform sweetpotato leaf explants with *Agrobacterium. tumefaciens* strain EHA105. Plants were transformed and regenerated following a somatic embryogenesis protocol (Kreuze *et al.*, 2008). Independent transgenic lines were analyzed for transgene-protein expression and used as rootstocks for grafting different viruses.

Plant inoculation and virus detection

Sweetpotato plants were graft-inoculated with the East African strains of SPFMV-Piu, SPMMV and SPCSV-m2–47 (Untiveros *et al.*, 2007; Kreuze *et al.*, 2008), two recently characterized members of the *Caulimoviridae:* 'Sweetpotato cavemovirus A' (previously known as SPCaLV), 'Sweetpotato cavemovirus B' (previously known as C-9) and an isolate of Sweetpotato leaf curl virus (SPLCV-54) using scions from the virus propagation host, *Ipomoea setosa* in an insect-proof greenhouse at CIP. Viruses were detected from approximately 150 mg of the tissue sampled from the youngest fully opened leaves by double antibody sandwich ELISA (DAS-ELISA) (Cuellar *et al.*, 2008) and NCM-ELISA. Circular DNA viruses (SPLCV-54, 'Sweetpotato cavemovirus A' and 'Sweetpotato cavemovirus B' were amplified using phi29 polymerase (BioLabs) from small molecular weight DNA obtained after passing total plant DNA through a plasmid miniprep kit (Promega). Identification was done by sequence analysis of PCR-amplified regions or full virus genomes. DNA virus detection was carried out by PCR using a direct extraction protocol (Wang *et al.*, 1993).

Western blot analysis

Proteins were isolated from 200 mg sweet potato leaf tissue, separated in a denaturing 12% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (Hybond-P) by electroblotting. RNase3 protein was detected with a specific rabbit antiserum raised against SPCSV RNase3, as described (Kreuze *et al.*, 2005). Anti-rabbit monoclonal mouse antibodies conjugated with horseradish peroxidase (Amersham), the Supersignal West Pico chemiluminescent substrate (Pierce Biotechnology), and exposure to X-ray film were used to detect signals by the ECL method according to the manufacturer's instructions (Amersham).

Agroinfiltration assay

The cloning strategy and vector plasmids used in the agroinfiltration assays in this study have been described (Kreuze *et al.*, 2005). Sequences of the *RNase3* genes of SPCSV-Ug, SPCSV-M2–47, and SPCSV-Is have been described and encode the most different RNase3 protein sequences currently known (amino acid sequence identity 80–97%) (Cuellar *et al.*, 2008). In the mutated RNase3 of SPCSV-Ug (designated as RNase3-Ala), 2 substitutions (E37A and D44A) were made in the highly conserved RNase III signature motif required for the dsRNA endonuclease activity of RNase III enzymes. pA-GUS expresses the ß-glucuronidase (GUS) gene with a plant intron to prevent GUS expression in *Agrobacterium*. pBIN35S-GFP expressed the ''cycle 3'' GFP gene. Constructs were verified by sequencing. Agroinfiltration was done as described using different *A. tumefaciens*

cultures that were combined before infiltration (Kreuze *et al.*, 2005). For co-infiltration treatments that included fewer constructs than others, the missing volume was replaced by the *Agrobacterium* strain expressing GUS. Infiltrations were carried out on the *N. benthamiana* line 16c which constitutively express the jellyfish (*Aequoria victoria*) GFP (Brigneti *et al.*, 1998) in a controlled growth chamber. Infiltrated tissues were monitored daily for GFP fluorescence using a hand-held UV lamp.

Nucleic acids isolation and northern blot hybridization

For amplification using phi29 total DNA was extracted from 500 mg of infected leaf tissue using the CTAB protocol. For PCR detection of SPLCV-54, a quick DNA extraction from 200 mg of leaf tissue was tested using NaOH (Wang *et al.*, 1993) and PCR using primers targeting the REP gene. Total RNA was isolated from 400 mg fresh leaf material using TRIzol (Invitrogen) following the manufacturer's instructions. Low molecular weight (LMW) RNA was obtained by LiCl precipitation and used to detect siRNA, whereas high molecular weight (HMW) RNA was used to detect *gfp* mRNA accumulation as described (Hamilton and Baulcombe 1999; Kreuze *et al.*, 2005). A probe complementary to *gfp* was prepared and labeled with [alpha-32P]UTP (Amersham) by in vitro transcription of *gfp* cloned into pCR-Blunt (Invitrogen) behind the T7 promoter. After hybridization and washing, membranes were exposed to X-ray film (Kodak) for 4, 16, and 48 h and developed using an X-Omat 1000 automated developer (Kodak).

RNA cleavage assays with RNase3

SPCSV RNase3 and RNase3-Ala proteins were overexpressed and purified from *E. coli* BL21(DE3)-RIL cells. Purification of the 6x-His-tagged proteins was accomplished using Ni-NTA agarose columns according to the manufacturer's instructions (Expressionist, Qiagen). Synthetic siRNA oligonucleotides were labeled by phosphorylation with [alpha-32P]-ATP using T4 polynucleotide kinase (Fermentas). And used as substrates in a reaction mix containing RNase3, RNase3-Ala, or *E. coli* RNase III (New England Biolabs). The reaction products were separated by electrophoresis as above and visualized using a PhosphorImager (Fuji FLA-5010). For testing cleavage of siRNA isolated from sweetpotato plants, LMW-RNA was isolated using TRIzol (Invitrogen) and LiCl precipitation, 5ug was heated to 95 °C for 10 min, and then let to cool for 2 h at room temperature. The LMW-RNA samples were treated with RNase3 and RNase3-Ala in the presence of 10 mM MgCl2 for 2 hours and separated by electrophoresis as above gel stained with ethidium bromide, and visualized using an Epichemi3-Darkroom (UVP Bioimaging System) gel documentation equipment. SPFMV-specific siRNA was detected by hybridization using a radioactive probe. To reveal the proportions of SPFMV-derived double-stranded and singlestranded siRNA in SPFMV-infected sweet potato, small RNAs of 20–30 nucleotides in size were isolated by polyacrylamide gel purification, and sequenced on the Illumina Genome Analyzer at Fasteris Life Sciences SA according to the service provider's recommendations. The resulting sequences 21–24 nucleotides in length (~95% of all sequences) were mapped to the complete sequence of SPFMV-Piu (FJ155666) using the program MAQ (http://maq.sourceforge.net). Sequences were sorted according to size and polarity and the exact number of putative double stranded siRNA could then be analyzed for each size class.

Results and discussion

RNase3 is the second viral protein directly implicated in viral synergism in plants. The first was the P1/HC-Pro polyprotein of an unrelated virus family (*Potyviridae*) (Pruss *et al.*, 1997; Shi *et al.*, 1997) whose ability to mediate synergism suggested interference with RNAi. Suppression of RNAi was subsequently shown for HC-Pro (Kasschau and Carrington 1998; Brigneti *et al.*, 1998; Anandalakshmi *et al.*, 1998) and many other RSPs from a wide range of plant and animal viruses (Li and Ding 2006), but no other RSPs besides P1/HC-Pro were reported as causal agents of synergistic viral diseases. Our data show that RNase3 also mediates synergism with viruses of other taxa, several of them known to synergize with SPCSV (Mukasa *et al.*, 2006; Untiveros *et al.*, 2007). These data suggest that SPCSV encodes a suppressor of a basal antiviral defense system of sweetpotato that normally protects the plants. Transgenic 'Huachano' plants expressing SPCSV RNase3 following inoculation with different viruses accumulated high titers of them and developed more severe symptoms in comparison to the non-transgenic plants (Fig. 1). In particular, disease symptoms and SPFMV titers in RNase3-transgenic plants were similar to non-transgenic plants co-infected with SPCSV and SPFMV. Therefore, RNase3 alone was sufficient to predispose the plant to SPVD, increase titres and induce more severe symptoms of RNA and DNA viruses.

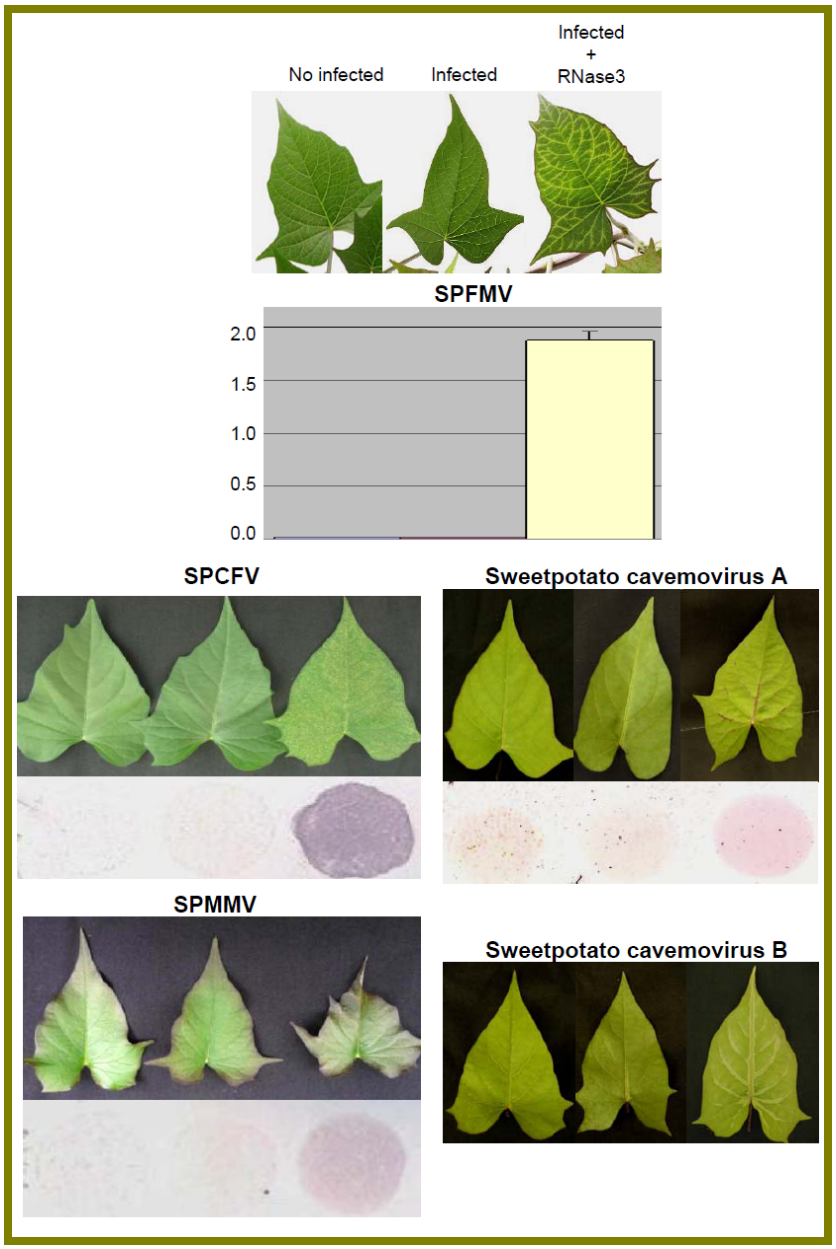

Figure 1. Systemic infection of non-transgenic (NT) and RNase3-transgenic sweet potato cv. 'Huachano' with *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato chlorotic fleck virus* (SPCFV), *Sweet potato mild mottle virus* (SPMMV), 'Sweet potato cavemovirus A' and 'Sweet potato cavemovirus B'. For each virus a 'no infected' (*left*), an 'infected' (*middle*) and an 'infected + RNase3' (*right*) leaf is shown. Viruses are barely detectable in the leaves of the inoculated NT plants (results shown from DAS-ELISA for SPFMV or NCM-ELISA for other viruses) and no symptoms are observed. However, in RNase3-transgenic plants, virus concentrations are elevated and readily detected; in all RNase3-transgenic plants virus symptoms are more severe. We are currently developing an antiserum for 'Sweet potato cavemovirus B' detection.

RNase3 is one of two reported RSP found in SPCSV, the other is p22 (Kreuze *et al.*, 2005). However, recent studies have revealed that many SPCSV isolates lack the *p22* gene but still synergize with SPFMV and with other unrelated RNA viruses (Untiveros *et al.*, 2007; Cuellar *et al.*, 2008), indicating that p22 is dispensable for synergy between SPCSV and other viruses but not ruling out a role for p22 in other virus interactions.

Viral RSPs may bind siRNA (Ye *et al*., 2003), affect their methylation status and stability (Vogler *et al*., 2007), and interfere with formation of the effector complexes required for RNAi (Ding and Voinnet 2007; Li and Ding 2006). These modes of action are known or suggested for RSPs encoded by many viral taxa (Li and Ding 2006), including those related to the sweetpotato viruses that cause synergistic diseases in co-infection with SPCSV (Mukasa *et al*., 2006, Untiveros *et al*., 2007). However, the viral RNase3 enzyme of SPCSV is the first viral RSP found to destroy siRNAs. This was confirmed by agroinfiltration in leaves of transgenic *N. benthamiana* 16c expressing GFP (Brigneti *et al*., 1998) were the induced RNAi of GFP mRNA was suppresses by the expression of RNase3. In addition, two point mutations introduced into the endonuclease signature motif of RNase3, known to abolish its RNase III activity also affected its RSP activity (Fig. 2). Therefore, RNase3 endonuclease activity is required for its RSP function.

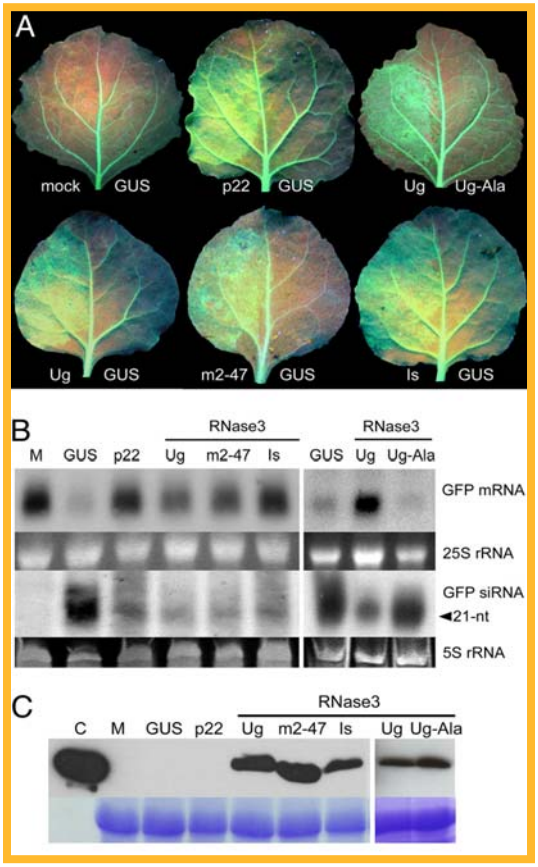

Figure 2. RNase3 from SPCSV suppresses RNA silencing. (*A*) *N. benthamiana* leaves were mock-infiltrated with buffer or with an *A. tumefaciens* strain expressing *gfp* and a strain expressing *p22* of a Ugandan isolate of SPCSV (Ug) or *RNase3* from isolates Ug, M2–47 (Peru) or Is (Israel). GUS indicates that leaves were infiltrated with a negative control. Ug-Ala indicates the RNase3-Ala mutant defective for endonuclease activity. The transgenic *N. benthamiana* plants (line 16c) constitutively express *gfp* (green fluorescence in veins of the leaf at the upper left corner and the right sides of the leaves). Leaves were photographed and analyzed 3 days post-infiltration. (*B*) Northern blot of *gfp* mRNA and siRNA in the leaf tissues illustrated in *A*. M=16c line mock-infiltrated with buffer. *Upper* shows the accumulation of *gfp* mRNA in the respective infiltrated regions. *Lower* shows accumulation of *gfp*-derived siRNA. Ethidium bromide-stained gels of rRNA were used as loading controls. (*C*) Western blot of the RNase3 protein in the infiltrated tissues. C= purified recombinant RNase3 protein. *(Lower)* Coomassie blue–stained gel.

Following this observation we tested whether RNase3 could target and modify the double-stranded siRNA essential for RNAi (Ding and Voinnet 2007). We observed that synthetic siRNA were all cleaved to products of ~14 bp by RNase3 (Fig. 3A), which are inefficient triggers of RNAi (Elbashir *et al* 2001; Paddison *et al*., 2002; Yang *et al*., 2002). RNase3 (but no RNase3-Ala) was also able to cleave 21–25 nt siRNA isolated from SPFMV-affected sweetpotato plants however detection with an SPFMV-specific probe revealed that the amounts of virus-specific siRNAs following treatment with RNase3 were only slightly less than with RNase3-Ala. These data indicated that RNase3 can act on the double-stranded forms of host-derived siRNA and/or miRNA, and that SPFMV derived double-stranded siRNA might be in a minority in the pool of total SPFMV-derived siRNA (Fig 3B).

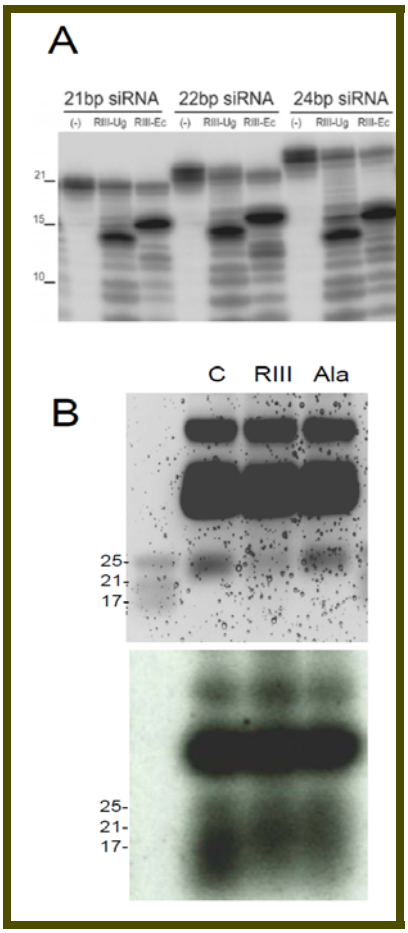

Figure 3. RNase3 of SPCSV cleaves synthetic siRNAs. ^{32}P-labeled double-stranded siRNA of the indicated sizes were incubated for 1 h with purified recombinant RNase3 proteins of SPCSV (RIII-Ug) or *E. coli* (RIII-Ec). Numbers at the left of the figure indicate the sizes in nucleotides. Samples were analyzed by electrophoresis in a TBE-UREA. (*B*) RNase3 [RIII (wt)] cleaved siRNAs isolated from an SPFMV-infected sweetpotato plant as revealed by comparison to the same amount of siRNA that was not treated (C) or was treated with mutant RNase3-Ala (Ala). The small RNAs were analyzed by 4% Agarose gel electrophoresis and stained with ethidium bromide (B-*Up*). Subsequently, RNA was transferred to Hybond NX membrane by capillary blotting and detected by Northern blot hybridization using an SPFMV-specific radioactive RNA probe (B-*Bottom*)

The latter was confirmed by calculating that only 3.95% of the total siRNA derived from SPFMV may form double-stranded siRNA. In addition, these results suggest that RNase3 may target a specific dsRNA host component in a manner which other viruses are unable to do and which releases a key obstacle that prevents other viruses from accumulating in higher titers. It is possible that after reaching a certain level thanks to the effect of SPCSV or RNase3 these co-infecting viruses can suppress silencing with its own RSS proteins. Identification of the specific target of RNase3 remains as an interesting topic for further study.

The results of this study provide a mechanistic understanding of synergism that is addressed to an important disease of a subsistence crop in developing countries. Identification of a viral class 1 RNase III enzyme as a key factor behind severe virus diseases and yield losses suggests possibilities for disease control. This is important because extensive screening of sweetpotato germplasm for sources of resistance, and conventional approaches of engineered, pathogen-derived resistance used in sweetpotato varieties have rendered little progress possible toward durable resistance to, for example, SPVD (Kreuze *et al.*, 2008). Our preliminary results indicate that an additional RSP encoded in some African isolates of SPCSV may also have an effect on virus accumulation (Fig. 4). The possibility that p22 may modify the outcome of SPCSV synergistic virus interactions in a different way as RNase3 remains to be studied.

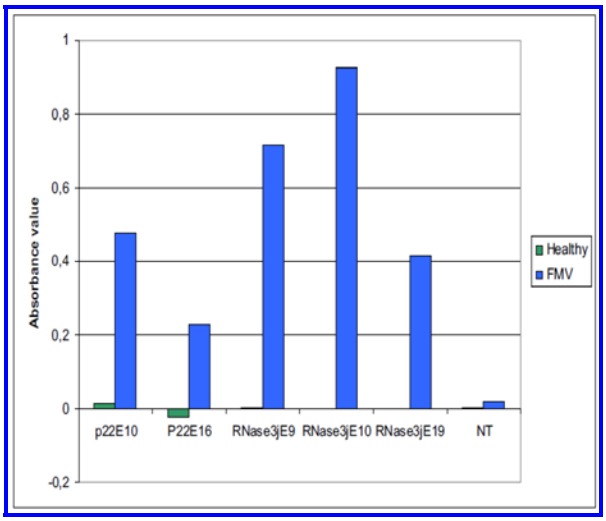

Figure 4. Relative amounts of *Sweet potato feathery mottle virus* (FMV) coat protein antigen in the systemically infected leaves of p22-transgenic (lines E10 and E16), RNase3-transgenic (lines E9, E10, E19) and non-transgenic (NT) sweet potato cv. 'Huachano' 3 weeks post-inoculation as detected by double antibody sandwich ELISA. Only RNase3-transgenic plants developed the severe symptoms of sweet potato virus disease (SPVD) following infection with SPFMV. The p22-transgenic and NT plants remained symptomless or expressed mild mottling 3 weeks post-inoculation

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The introduction of orange fleshed sweetpotato on the agricultural farming system of Central Mozambique: the opportunity cost of growing this nutritious crop

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Abstract

Recently, many efforts have been made in Mozambique to disseminate orange fleshed sweetpotato (OFSP) that is an efficacious and effective source of pro-vitamin A. It has been demonstrated that the consumption of OFSP during the harvesting period can reduce vitamin A deficiency (VAD) by 15%. However, little is known about the effect that the massive adoption of OFSP can produce in the overall agricultural system. This paper analyzes the effect that growing OFSP has on the Mozambican agricultural system, paying attention to potential impacts on farm income and household food security among small farmers. Using an optimization model, this paper performs an ex-ante analysis of the introduction of OFSP within representative multi-enterprise farms of Mozambique. Based on information coming from 84 small farmers in Central Mozambique the model analyzed the adoption and consumption of this nutritious crop under different scenarios and evaluated the effect produced on farm revenues, returns to production inputs and the status of household food security including the intake of nutritious crops. Results show that under current conditions, small farmers holding less than 1 ha of land would not adopt OFSP unless they get the planting material for free, reducing the intake of vitamin A rich crops. On the other hand, medium scale farmers holding between 3 and 5 hectares of land would adopt OFSP and will increase farm revenues and the returns to land and labor. These farmers would increase the consumption of vitamin A rich foods and have sweetpotato as an alternative source of energy crops.

Keywords: Sweetpotato, Optimization, Nutrition, Mozambique, Vitamin A.

Introduction

Sweetpotato is considered a secondary crop in Mozambique and is usually introduced into the agricultural system after the major staples, maize, rice, cassava and beans. Few regions in Mozambique manage sweetpotato as a cash crop and the investment to grow this rustic crop has remained low. However, the importance of sweetpotato as a food security crop and most recently as a source of important micronutrients has been largely recognized (Low et al 2007)

Recently, there have been many efforts in Mozambique trying to disseminate orange fleshed sweetpotato (OFSP) that is an efficacious and effective source of pro-vitamin A. In this country the levels of vitamin A deficiency (VAD) among children between 6 and 59 months old reaches 71% (Aguayo et al 2005) and it has been demonstrated that the consumption of OFSP during the harvesting period can reduce VAD by 15% (Low et al 2007). In addition, the different extension approaches used to disseminate OFSP have also encouraged the commercialization of the OFSP roots and the processing of these roots into many alternatives (OVATA 2007, Low et al 2005).

Currently, the dissemination of OFSP among small farmers in Central Mozambique is scaling up and the expectation of the adoption of OFSP planting material and the consolidation of OFSP as a major source of farm income have raised considerably. However, whether the adoption of this nutritious crop would be sustainable and how this adoption process would affect the whole agricultural system in Mozambique remains unknown.

Previous work have highlighted the importance of considering the various factors underlying technology adoption decisions that range from input availability to more contextual factors issues like access to markets, agro-ecological conditions and infrastructure (Feder & Umali 1993, White et al 2005). In the case of

sweetpotatoes in Mozambique, little is known about the potential effect of increasing the sweetpotato acreage under the current agricultural system, dominated by small scale agriculture in marginal lands.

This objective of this paper is to analyze the effect that growing OFSP have on the agricultural system of the Central Mozambique, paying attention to factors like farm income, household food security, nutrition status and the use of the limited labor resources. We use an optimization model and conduct an ex-ante analysis of the introduction of OFSP within a multi-enterprise farm. Based on information coming from 84 small farmers in Mozambique the model analyzed the different scenarios related to the adoption of OFSP planting material. Results show that under current conditions, small farmers holding around only 1 ha of land would not adopt OFSP unless they get the planting material for free and that medium scale farmers holding between 3 and 5 hectares of land would adopt OFSP and will increase farm revenues and the returns to land and labor.

The paper follows with a brief description of the production of sweetpotato in Mozambique and a summary of the current efforts to disseminate OFSP in this area. Then the model formulation and the model parameters are discussed. In the next section model results are presented and discussed while the last section is devoted to draw some conclusions of the analysis.

The production of sweetpotato and the introduction of OFSP in Mozambique

Sweetpotato is considered a secondary crop in Mozambique, but it is however planted by a large number of small households in Mozambique. During the 2001-2002 growing season Sweetpotatoes were in the seventh place of acreage among the crops grown in Mozambique with 96,515 hectares and in the sixth place regarding the number of households growing it (1'084,447) (TIA 2002).

Since 1999 there has been many efforts to introduce orange fleshed sweetpotato (OFSP) a promising food with high levels of pro-vitamin A carotenoids in many of its varieties (100-1600 ug Retinol Activity Equivalents (RAE) per 100 g for varieties in use in Africa) (van Jaarsveld et al 2005; Hagenimana et al. 1999). OFSP has proved to be and efficacious alternative to poor Southern Africa countries like Mozambique (Low et al 2007) where the Vitamin A deficiency is estimated in 71% in children of 6-59 months old (Aguayo et al 2005). Since the seminal work conducted by the Southern Africa Roots and Tubers Network (SARRNET) and more recent experiences like the Towards a Sustainable Nutrition Improvement (TSNI) project and the Reaching End Users (REU) project, the dissemination of OFSP have been scaling up in Central Mozambique and most of these activities have been using integrated approaches (seed systems, marketing and nutrition/demand creation components)in order to increase the likelihood of adoption of OFSP and to mainly increase the vitamin A intake among young children and pregnant women, the most vulnerable population regarding vitamin A deficiency.

Currently OFSP is mainly grown during the main agricultural season (November-June), but in certain areas sweetpotatoes can be grown in lowlands after the harvest of the rice production, taking advantage of the moisture that remains on these lowlands for a longer period of time. Although most of the farmers are familiar with this crop, maintaining the OFSP and other sweetpotato vines from one season to another has been the most difficult task that has limited the expansion of this crop. In addition, there have been massive free-vine distributions associated with post-war and emergency programs that have also reduce the incentives among farmers for keeping and multiplying the sweetpotato vines and increase the acreage of this crop (Rohrbach & Kiale 2007)

In the Zambezia province there are mainly two areas where sweetpotato are usually grown. The northern Zambezia where the precipitation level is high (1735 - 1997 ml) sweetpotato is grown in larger areas and can get yields around 10t/ha (CIP 2007). The southern Zambezia is typically considered a drought prone area due to the low levels of precipitation (less than 1000 ml) and the yields have been also remain at low levels and below 6t/ha (Low et al 2005). Under these production conditions, the efforts to disseminate OFSP in the Zambezia province have put a lot of emphasis in disseminated well adapted OFSP varieties and have insisted in the need to improve farmers' knowledge in keeping the vines from one season to another. The current REU project started the dissemination of OFSP planting material among around 10,000 households (World Vision 2007) and it is expected that by the need of 2008 having reached around 15,000 households in the Zambezia province.

Analytical methods

Data

This study uses data from 84 farm households in the Zambezia province that was recorded in quarterly visits. This data contains detailed information about all plots grown by these households during the 2006-2007 and 2007-2008 growing seasons. This information includes plot area, crop yields, crop prices, inputs used and input prices of all cropping systems grown. Likewise, monthly labor calendars were prepared in addition to calorie production of each cropping system as a mean to meet food security requirements.

In our sample we found mainly two groups of farmers. The small farmers hold no more than one hectare of land where that have to grow different cropping systems. These cropping systems include: 1) Rice, 2) Maize, 3) Cassava, 4) OFSP, 5) Maize/cassava, 6) Maize/cowpea, 7) Cassava/pigeonpeas and 8) Pigeonpeas/Groundnuts. The medium scale farmers are managing between 3 and 5 has of land for establishing the different cropping systems. The main cropping systems among this group of farmers are: 1) Rice, 2) Maize, 3) Cassava, 4) Sweetpotatoes (including OFSP) 5) Sweetpotato/Cassava, 6) Maize/Pigeon/Millet, 7) Maize/Pigeonpeas/Cowpea, and 8)Pigeonpeas/groundnuts.

In the Southern region, small and medium scale farmers are equally important. In our sample of 36 households we found 18 households with 1 hectare or less total land. In the northern region, medium scale farmers are the predominant sub-group in this area. Out of the 48 households included in our sample, 36 are medium scale producers while only 12 are growing one hectare or less as total farm acreage.

With the farm observations recorded we constructed detailed cropping system budgets consisting of all inputs and outputs related to household farms for the small and the medium scale farmers. The first step was to estimate gross revenues per hectare for each cropping system, including all crop yields and market prices (the price at which interviewed farmers sold different crops) The second step is to calculate the production cost associated to each crop and to each cropping system. An important part of this production cost is the total labor used for each farm activity. This labor has been value at the lowest labor market price found among the 84 selected farmers. This price is set at 5 Mt per a period of 4 hours of work (half of a typical working day). Table 1 summarizes the production costs of the different cropping systems and the net revenues as well.

Monocrops have the highest yield per crop comparing to the production of the same crops but under intercropping. As expected, nutrient competition in an intercropping reduces the potential yield of each crop, but on the other side, intercropping allow farmer to reduce some production costs like weeding that are done at once for all crops in the system. All cropping systems produce positive net revenues (subtracting production cost from gross revenues) but clearly the production of rice produces the highest per hectare net revenues. Maize/cassava and maize/pigeonpea/millet intercropping also provide high net revenues

Table 1. Average crop yields, crop price, gross revenues and production cost of different cropping systems in the Zambezia province, Mozambique

Cropping system	Crop yield (Kg/ha)	Price (Mtn/Kg)	Gross revenue (Mtn/ha)	Production cost (Mtn)
Monocrops				
Rice	2150	4	8600	2845
Maize	1980	3	5960	3970
Cassava	4000	1	4000	2775
OFSP	5000	1	5000	2970
Intercropping				
1) Maize	1730	3	8190	4325
Cassava	3000	1		
2) Maize	1730	3	7230	4690
Cowpea	680	3	7950	4000
3) Maize	1730	3		
Pigeonpea	720	3	3520	2480
Millet	600	1		
4) Pigeonpea	720	3		
Groundnuts	680	2		

In addition to crop revenues and production costs, we estimated the labor requirements and potential calories deducted from the total production of each cropping system. As indicated in Table 2, each system has different labor requeriments and this requirement happens at different periods of time. Likewise, the potential quantity of calories derived from the production of staple crops varies according to the crop and the cropping systems. Our data shows that although maize is a crop that produces low revenues, it is the crop that can provide the higher quantities of calories required by the family. Rice is the more profitable crop in Zambezia, however its contribution to the food security through energy calories is relatively low. Cassava and sweetpotatoes are also key crops in the household food security as they make available large quantities of calories.

Table 2. Average labor requirement and potential production of calories in different cropping systems. Zambezia province, Mozambique

Cropping system	Labor requirement (Man-days)	Potential quantity of calories (Kcal per kg)
Monocrops		
Rice	478	439.40
Maize	194	3450.00
Cassava	271	1548.88
OFSP	294	706.80
Intercropping		
1) Maize	265	439.40
Cassava		1548.88
2) Maize	258	3450.00
Cowpea		371.20
3) Maize	288	3450.00
Pigeonpea		1124.09
Millet		1124.09
4) Pigeonpeag	200	1124.09
Groundnuts		1410.00

A multi-enterprise optimization model

The use of farm household models has a long history in the agricultural economics literature (Barnum & Squire 1979, McGregor et al 2001). Usually these models have been developed to assess farm interventions, including technology diffusion, and to estimate the impact that these intervention have had on the whole household under various farm production environments. To evaluate the effect of the introduction of OFSP in the agricultural system of small scale farmers in Central Mozambique, two representative farms are employed. The first representative farm is a very small household with less than 1.2 ha of total land available and with limited labor resources that has as a major objective to maximize farm revenues but guaranteeing household food security. This household food security can easily be represented by the availability of the household to produce enough food to meet their required daily calorie intake. The Second type of households represents a medium scale producers that holds between 3 and 5 has of total land but that also face limited labor resources and has also to satisfy first the household food requirements and then sell the surplus in local markets.

The production function of the model is as follows:

$$\text{Max} \ \sum (R_i - C_i)$$

Where R_i is the Revenues provided by the cropping system I and Ci is the total production cost of the cropping system i

Subject to

Farm size constraint: A <=1.2 ha (Small farm only)

Labor constraint: L< 75 days or 150 half days (equivalent to the full time of 3 household members)

Food Security constraints: $\sum Kcal_i$ >= 4'822,560 Kcal (The sum of the quantities of Kcal produced by all cropping systems should exceed the minimum calorie intake required by the household of 7 members during one year)

Non-negative constraints: all cropping system area >= 0

As explained before, cropping systems revenues are estimated average crop yields found on the 84 selected households and crop market prices reported by farmers either for selling their crops produced or for buying the crop for home consumption. Labor is also valued as it lowest market price (5 Mt per half of a working day or 10 Mt for a full working day).

We estimated two base models of two representative farms that produce a combination of cropping systems suitable for the agro-ecological conditions of each specific area. Then we estimate some alternative scenarios and compare the farm outcomes across the scenarios. For the Southern Zambezia we analyze two alternative cases 1) The sweetpotato vines are given for free (no seed cost) and 2) The price of OFSP increases by 32%. In the Northern Zambezia we included one alternative scenario: the OFSP price increases by 27%. In all the scenarios we estimated the optimal area used for different cropping systems, the maximum potential profits, the proportion of calories produced by each system and farms returns per unit of labor and per unit of land (1ha)

Model results

The basic scenarios show how it would be the land allocation across different available cropping systems if farmers seek to maximize profits, subject to the production conditions and calorie intake requirements that the representative households face. As showed in Table 3a, the representative small scale farmer would choose to grow Rice as a monoculture and to grow the intercropping of Maize and cassava and cassava and pigeonpea. She/he would plant a total of 1.2 hectares, would make a total of 4,856 Mtn (186.8 US$), would produce most of the calories needed from the cassava/pigeonpea system (62%) and would have as returns to half of a working day a total of 13.7 Mtn (0.53 US$). An optimizing farmer would not plant OFSP under current production and market conditions. The model has also estimated that the opportunity cost of producing one hectare of OFSP is 1067 Mtn (42.7 US$). It means that if the representative small scale farmer decides to grow OFSP, the farm profits would be reduced by this 1067 Mtn.

Table 3a. Results of basic scenario on the representative small scale farmer

	Rice	Maize/ Cassava	Cassava/ Pigeonpea	Total farm
Area	0.46	0.20	0.54	1.20
Net revenues	2095	457	2303	4856
Kcal produced	8%	30%	62%	4822560
Returns to:				
Land (1 ha)	4554	2285	4265	4018
Labor (1/2)	14.9	15.0	13.2	13.7

Table 3b shows the results for a representative medium scale farmer in the Zambezia province. This farmers making optimal decision would allocate her/his land to the production of rice, and the intercropping of cassava and OFSP, maize, cowpea and millet, and groundnuts and pigeonpea. This farmer would plant a total of 3.06 hectares and would make a total of 11,616 Mtn (446.8 US$). This farmer would need to use a total of 1090 half days of their available labor and would produce a total of 2637 Mtn (105.5 US$) per hectare of land cultivated and 13.2 Mtn (0.53 US$) per half day of working of her/his available labor. In this case a representative farmer would find it profitable to plant OFSP jointly with cassava and will devote an important proportion of her land to produce it. It is also important to notice and in this case the food security constraint is not binding as the optimal solution would easily exceed the production of calories needed by the household.

Table 3b Results of basic scenario on the representative medium scale farmers

	Rice	OFSP/ Cassava	Maize/ Cowpea / pigeonpea	Groundnut/ pigeonpea	Total farm
Area	0.36	0.65	1.71	0.34	3.09
Net revenues	2255	667	7981	721	11616
Kcal produced	7%	43%	132%	14%	4822560
Returns to:					
Land (1 ha)	6264	1026	4667	2121	3759
Labor (1/2)	17.0	10.0	10.2	15.1	13.2

In order to evaluate the sensitivity of our base scenarios results we evaluated two alternatives scenarios for the small scale representative farmer and one scenario for the medium scale representative farmer. For the small scale representative farmer we first analyze the case when the growing farmer receive the OFSP planting material for free, which has been a common practice in Mozambique during various extension programs. Secondly, we evaluate the effect of increasing the price of OFSP by 32% of its current level of 1 Mtn per kilogram. We select this level of increase because after many model runs it turned out to be an inflexion point in the small scale representative farmer.

It turns out that the free vines possibility reduces considerably the production cost of the intercropping Rice/OFSP and increases significantly the net revenue of this cropping system. As this happens the first reaction of the representative small scale farmer is to reduce the production of rice as monoculture and combine the rice production with the OFSP production which is usually established after the rice harvesting and taking advantage of the residual moisture of the lowlands where rice is usually grown. By doing this representative farmer significantly increases farm revenues and the returns to land and labor.

The other scenario where the price of OFSP price is increased by 32% has a similar effect on farm outcomes as it also increases the rice/OFSP intercropping net revenues and therefore makes this option more attractive for the optimizing representative small farmer. Of course the changes in the farm profits and returns to land and labor are slightly lower as the cropping system revenues are increased at a lower level

As the representative medium scale producer would have already decided to grow OFSP, the effect of this change would only make OFSP more profitable and the representative farmer would increase it production by adding the cassava/sweetpotato intercropping. As a consequence the farm revenues and the returns to land and labor would increase even more.

Concluding remarks

Farmers' decisions among small scale growers in poor countries are made considering many factors. Maximization of farm revenues is always an objective of these farmers, but household food security is another component of this decision. We used an optimization model were combined farmers desire to maximize farm revenues with the minimum quantity of calories that should be produced in order to meet household requirement of food energy intake. We also include household production constraints related to labor demand and availability of land for agricultural production.

Analyzing a representative small farmer and a representative medium scale farmer in the Zambezia province, Mozambique, provides interesting insights of the impact of introducing orange fleshed sweetpotato (OFSP) in the Mozambican farming system. Small scale farmer would not adopt OFSP under current conditions. Giving land limitation, farmers would devote their resources to produce other crops (rice, maize, cassava, pigeonpeas). Labor availability is not really a constraint as farmer has no enough land to employ all its labor resources. On the other hand farmers has to carefully produce crops in the small piece of land available in order to provide the required quantities of calories needed by the household. Only when the vines of sweetpotato are subsidized, OFSP becomes an attractive crop (reducing its production cost) and making it another source of calories for the household.

Medium scale producers face different conditions. This type of farmers faces no restriction on the land available and the binding constraint is the availability of labor. Then famers would allocate these resources to the enterprise that provides the greatest return to labor. OFSP under current production and market productions is one of the crops that meet this requirement suggesting that medium scale producers in Zambezia, Mozambique would adopt this crop. For the medium scale producers the food security constraint it is not binding reflecting the different sources that a household of this type has for producing the required calories.

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Fast analysis of maca bioactive compounds for ecotype characterization and export quality control

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Abstract

Maca (*Lepidium meyenii Walp.*) storage hypocotyls have been used since pre-Columbian times by Andean dwellers due to their high nutritional value and energizing properties, which appear to counteract some of the negative physiological effects of high altitudes. Maca has been propelled to the status of highly successful Peruvian export through reports of its energizing/invigorating properties and its effectiveness against benign prostate hyperplasia. Although studies reporting these effects have been performed using hexane, chloroform, alcohol or aqueous extracts, none have used pure compounds and one cannot pinpoint the biologically active compounds in the plant tissue. Analytical methods are needed which can provide in a reliable, quick and simple fashion the amounts of potential bioactive compounds in export material and also, since maca is exported as the dry hypocotyls, of the quality of the postharvest treatment used to naturally dry the plant tissue. We propose two, possible methods, for the sequential evaluation of bioactive marker compounds in maca. A headspace capture system coupled to a 10 min GC-MS run for the fast analysis of benzylamines, aldehydes, alcohols, isothiocyanates and nitriles and a solid phase extraction method using ion exchange and reverse phase cartridges sequentially followed by LC-MS analysis for total glucosinolates and fatty acid benzylamides. Both methods also allow the direct comparison of maca ecotypes in order to target them in the export market for their various purported physiological effects.

Introduction

Maca (*Lepidium meyenii*, Walpers or *L. peruvianum*, Chacon), an annual herbaceous plant of the Brassicaceae family, is a native of the Andean high plateaus. It is the only reported species of the genus *Lepidium* which contains a fused hypocotyl and tap root forming a fleshy underground storage organ suited to the dry, windy and cold high-altitude environment (NRC, 1989). The plant has been cultivated and used for food and medicinal purposes since pre-Columbian times and has gained attention in recent years due to reports on medicinal properties which make it a good candidate for the nutraceutics market (Canales et al., 2000, Dini et al., 1994). Maca production has grown from 800 to well over 2,000 metric tons in the past 7 years and the export of dried maca powder and derived products reached US$ 5 million in 2008 (Prompex 2008).

Maca, however, presents a variety of chemical phenotypes regarding the major natural products that the plant accumulates in its tissues. These variants can be grouped into three major categories, red, yellow and black, based on hypocotyl and stem coloration. These, in turn, have a number of ecotypes which are characteristic of geographical regions where the plant is grown in Peru. Preliminary evidence indicates differences in the levels of major natural products characteristic of the species and in the reported biological effects or medical target for which these different types can be used. Gonzales and collaborators (Gonzales et al., 2005, Chung et al., 2005) report for example that black maca is useful in stimulating sperm count while red maca is useful against benign prostate hyperplasia.

Given the difficulties in obtaining phytosanitary certificates for the export of fresh tubers, the majority of the exported material is shipped in the form of powder obtained from naturally air-dried hypocotyls or from of lyophilized fresh material. The difference in composition between these two products is, as expected, fairly large. Powdered maca for export is usually obtained from roots that have been dried in the open air on site. The process takes usually one month and involves exposure to the extreme temperature cycles and strong light conditions typical at high altitudes. A significant degree of variation in the environmental conditions used for drying mean that one can expect also variations in the amounts of various hydrolytic products of the major maca storage compounds present in the tubers.

Any method aimed at targeting maca for particular medicinal or nutraceutic uses will have to deal with both chemical phenotypes and hydrolytic byproducts. In this progress report we propose a fast two-stage strategy for chemical phenotype profiling of maca and for quality control of naturally dried hypocotyls aimed for the export market.

Materials and Methods

Biological material

Fresh maca *(Lepidium meyenii,* Walpers) was bought in Lima markets. The hypocotyls were lyophilized for 3 days, pulverized in a blender and sieved (denominated the MFL fraction). Dry maca was bought in markets in the city of Huancayo dried in the traditional way (by direct exposure in the field for a month), pulverized in a ball mill and sieved (denominated the MSM fraction), both were stored at -20ºC until used.

Synthetic material

Macamides were synthesized by reaction of benzylamine or 4-methoxy-benzylamine (1.5 mmol) with the respective fatty acid (caprilic, palmitic, stearic, oleic, linoleic and linolenic) (1 mmol) promoted by dicyclohexylcarbodiimide (DCC) (1.0mmol) catalyzed with pyrrolidinepyridine (PPy) (0.5mmol). The amides obtained were purified by chromatography on silica gel 60 and characterized using a Bruker UltraShield 300 MHz NMR spectrometer, a Hewlett Packard 5971A GC-MS with electron impact ionization and a Perkin Elmer 1600 Series FT-IR spectrometer. The spectral data of the synthetic amides are listed in the appendix.

Extraction procedures

1g of maca was extracted in 20ml of 70% MeOH at 70ºC under nitrogen for 1h under constant agitation. The extract was vacuum-filtered through Whatman GF/A (1.6µm) glass microfiber filters and stored under nitrogen at -20ºC until used.

Analytical procedures

Liquid chromatographic analyses

Macamides. Five ml of stock solution were diluted with 2ml of water and loaded onto a Merck LiChrolut RP-18 500mg SPE column preconditioned with 5ml MeOH, 5ml water and 5ml of 50% MeOH. Washed with 0.5ml 50% MeOH and eluted with 2ml of 100% MeOH.

Macamides in the preparation were analyzed in a Merck-Hitachi LaChrom D-7000 HPLC with a Merck-Hitachi L-7450-A diode array UV detector and a LiChrospher 100 RP-18 column (250 x 4mm, 5µm particles). Temperature was set at 40ºC and flow at 1ml/min. Solvent A: ACN with 0.005% TFA. Solvent B: H_2O with 5% of MeOH and 0.005% TFA. Gradient: 4min at 65%A, 65%-85%A in 2min, 85%-100% in 20min, 5min at 100%A. Injection: 20µl. Detection: 210nm.

Amines. Amines were analysed by conversion to *o*-phthaldehyde (OPA) derivatives by adding 200 µl of maca stock solution to 250 µl of borate buffer 0.4M, pH 9.5, and 50 µl of OPA reagent, mixed on a vortex and allowed to react for 3 minutes at room temperature. After derivatization 50µl samples were analysed immediately by HPLC with diode-array UV detection, using a LiChrospher 100 RP-18 column (125 x 4mm, 5µm particles). Temperature 30ºC, flow: 1ml/min. UV detection at 340 nm. Solvent A: MeOH, solvent B: Na acetate buffer 20mM, pH = 6.0. Gradient: 9min 40%-90%A and then 4 min 100% A.

Glucosinolates. Glucosinolates were analyzed as desulfoglucosinolates following the procedure described by Thies (Thies1988) with some modifications. In brief, 5ml of stock with 1ml of water were loaded onto a Varian BondElut strong anion exchanger (SAX) SPE cartridge (500mg) pre-conditioned with 60% MeOH. After washing with 2ml of 60%MeOH and 3ml of water, 1 ml of MES 0.02M, pH 5.2 and 150 µl of sulfatase (E.C. 3.1.6.1, Sigma) were added and allowed to react overnight. Desulfoglucosinolates were collected the following day by sequential elution with 800 µl of EtOH 60% and 800 µl of MilliQ water and collected in the same vial. HPLC analyses were carried out by HPLC with diode array UV detection using a LiChrospher 100 RP-18 (250 mm x 4mm, 5µm particles) column. Temperature 30ºC, flow 1ml/min. UV detection at 254nm. Solvent A: ACN, solvent B: water. Gradient: 6min 2%-5%A, 2min 5% - 7%, 10min 7% - 21%, 5min 21% - 29%, 2min 29% - 100% .

Gas chromatographic analyses. The analysis of isothiocyanates, nitriles, aldehydes, alcohols and amines was performed by solid phase microextraction (SPME) using PDMS coated fibers (100 µm) exposed 20 min at room temperature to powdered dried maca or to crushed fresh maca tuber cubes (0.1 g) placed within 4 ml glass vials closed with Teflon/silicone septum caps.

SPME fibers were desorbed and analysed in a Perkin Elmer AutoSystem gas chromatograph with flame ionization detection using a J&W Scientific, DB-225 (50% cyanopropylphenyl/methylpolysiloxane) column (30m x 0.32mm, 0.25µm film thickness). Injector temperature 250ºC, detector temperature 250ºC, He flow 1ml/min. Temperature program: 1min at 70ºC, 70ºC-220ºC in 40ºC/min and 220ºC for 5min.

Colorimetric procedures. Phenolic compounds were analyzed using the Folin reagent as modified by Kursar and Coley (2003). The unretained fraction from SPE in RP-18 of the maca extract was used for this analysis. Thirty µl of sample in 50%MeOH were added to 220 µl of MilliQ water followed by 500 µl of Folin-Ciocalteu reagent, and the mixture was allowed to react at room temperature for 6 min. 500 µl of Na_2CO_3 0.5M were then added and incubated for 2 h at 30ºC in water bath. UV absorption was read at 725nm and 760nm.

Results and discussion

General considerations

Our analytical approach focused on two different sets of analytes. The first procedure was aimed at determining the content of the most likely candidate constitutive markers for chemical phenotype in maca. These included glucosinolates, amides, total phenolics and free amines present in the extracts (Li & Ammerman, 2001, Piacente et al., 2002, Muhamad et al., 2002, Tellez et al., 2002, Zhao et al., 2005, McCollom et al., 2005). These were analysed by solid phase extraction and liquid chromatography. These compounds can be considered "standard" indicators of the putative pharmacological potency of the plant material and their abundance is a result of environmental conditions during growth and the plant genotype.

In addition to the previous method, a fast SPME-GC approach was developed to evaluate volatiles in fresh and dried plant material. Most volatiles arise as a result of hydrolysis and further metabolic events during processing of the plant material. Figure 1 shows some of the expected products of thioglucohydrolases, nitrile lyases and amidohydrolases among others (Winkler et al., 2006).

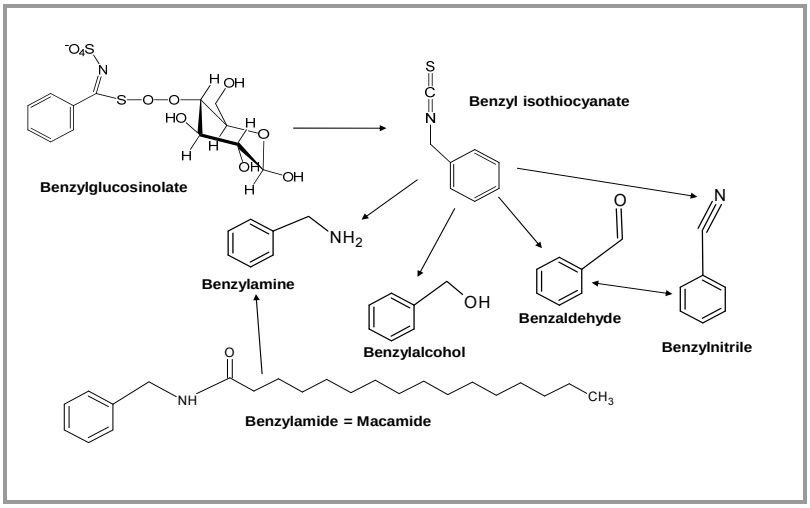

Figure 1. Volatile hydrolytic products of maca glucosinolates and amides. Putative interconversion between nitrile and aldehyde may be promoted by a nitrile lyase.

Analysis of glucosinolate, amide, amine and total phenolic content

The analysis of constitutive secondary metabolites of fresh and dried maca consisted of an extraction in 70%MeOH at 70ºC for 1 h, followed by analysis of aliquots in parallel (Figure 2). Amines were analyzed directly from an aliquot of the crude extract by derivatizing it with *o*-phthaldialdehyde reagent followed by HPLC with UV or fluorescence detection. Amides were separated from another aliquot by retention on a reverse-phase SPE cartridge at low solvent strength (50% MeOH). The unretained fraction contained most phenolic components and could be quantified colorimetrically. Amides were eluted with 100% methanol and analyzed by HPLC as described in Materials and Methods. A third aliquot of the extract was loaded onto an anion exchanger (SAX) SPE cartridge for glucosinolate analysis. Glucosinolates retained in the cartridge were treated overnight with sulfatase and the resulting desulfoglucosinolates, eluted sequentially with methanol and water and analyzed by HPLC as described in Materials and Methods.

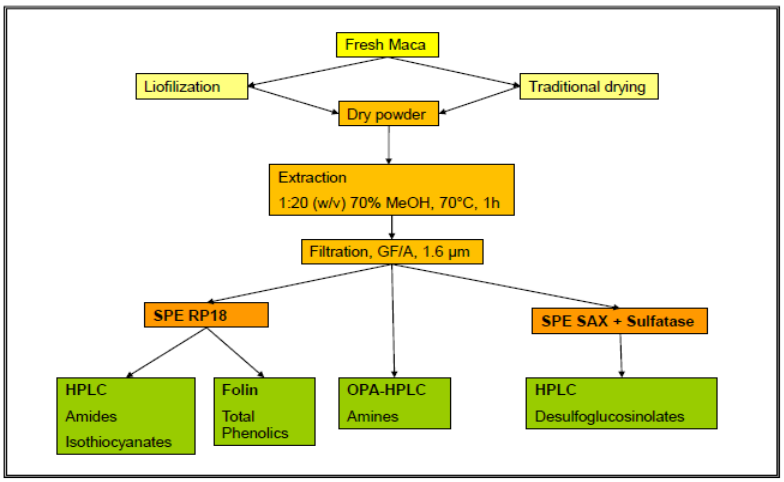

Figure 2. Flow chart describing the processing and analysis of maca constitutive components by liquid chromatography and colorimetric methods

Results using the previously mentioned procedure confirmed previous reports (Li & Ammerman, 2001) of significant loss of glucosinolates during the open air drying process. Figure 3 shows HPLC elution profiles of desulfoglucosinolates from naturally air dried maca and fresh maca dried by freeze-drying in the laboratory. Approximately 80% of the glucosinolates present in fresh maca were converted into other compounds by tissue disruption, hydrolysis and photochemical degradation due to exposure to sunlight at high altitudes and extreme climatic conditions present in the traditional drying process.

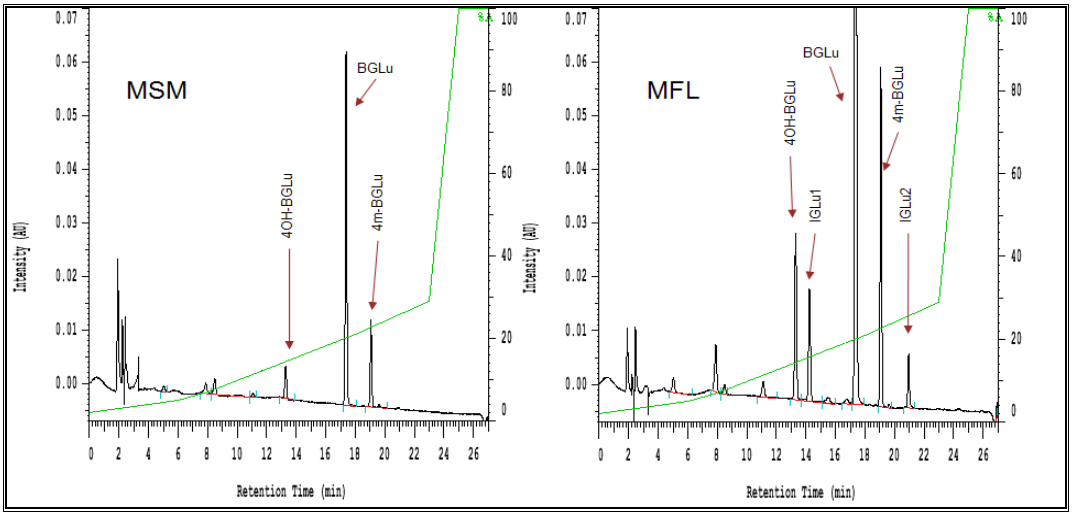

Figure 3. Glucosinolate content, determined as desulfoglucosinolates, decreases significantly in open-air dried material. The figure shows HPLC elution profiles of desulfoglucosinolates determined in naturally dried maca (MSM) and fresh lyophilized maca (MFL). BGLu, benzyl glucosinolate, 4m-BGLu, 4-methoxybenzyl glucosinolate, 4OH-BGLu, 4-hydroxybenzyl glucosinolate. IGLu 1 and 2 are indolic glucosinolates of unknown structure.

Twelve benzylalkamides (macamides) were synthesized and characterized for usechromatographic standards and for use in animal trials. The compounds prepared are listed in Figure 4. For the purpose of this report, only a liquid chromatographic method was used for the analysis of these compounds. An alternative GC-MS method is being evaluated. The HPLC separation of the macamides was down according to McCollom and collaborators (2005). However, modifications were introduced that improved the quality of the separation, given the large number of amides that were to be evaluated and the low levels we had previously determined in lyophilized plant tissues. The inclusion of 5% MeOH in the water solvent eliminated overlapping problems between longer chain macamides. Also, the method started at a lower acetonitrile concentration (see Materials and Methods) as we also included short-chain macamides (C8) and benzylisothiocyanate in the analytical run. These last two are good indicators of sample processing problems. The HPLC resolution of the amide standards and of potentially contaminating fatty acids is shown in Figure 5.

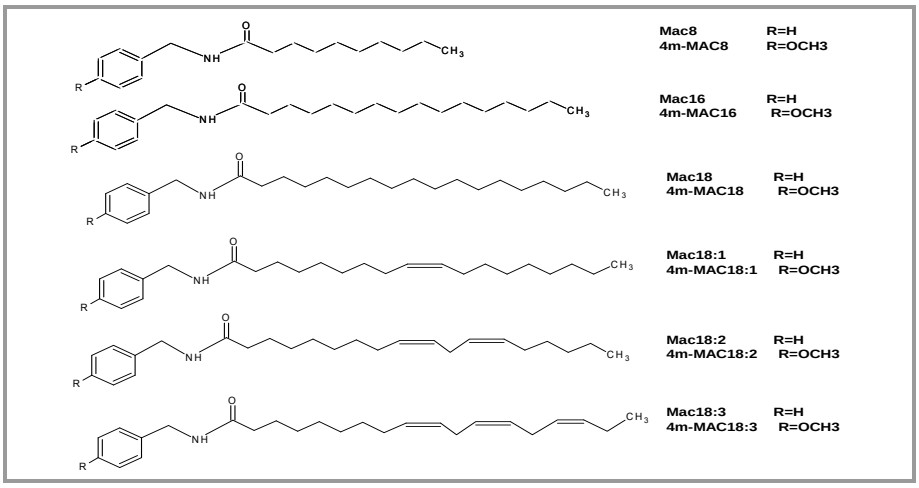

Figure 4. Amide standards synthesized for this study.

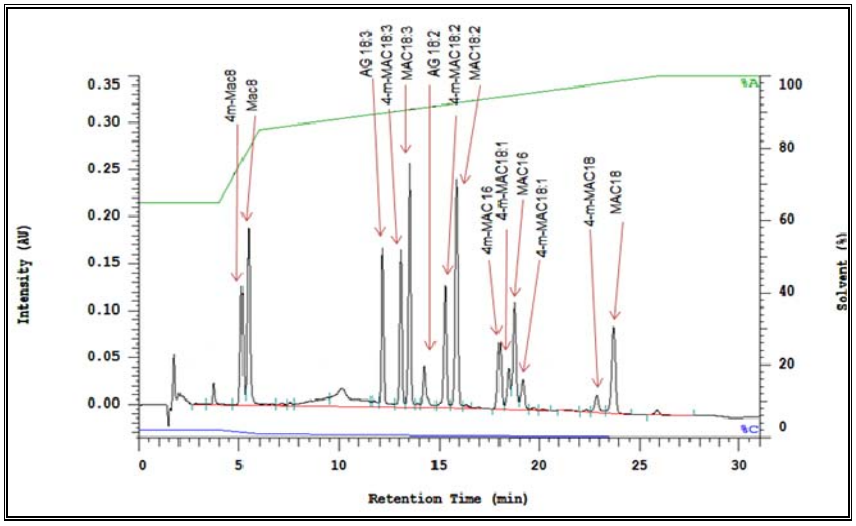

Figure 5. HPLC chromatogram of synthetic benzylalkamide (macamide) standards which can be found in maca hypocotyls. Abbreviations for the macamides are explained in Figure 4. AG 18:3 and AG 18:2 are free linolenic and linoleic acid respectively.

Analysis of amide contents in open-air dried maca (MSM) and of maca lyophilized in the laboratory (MFL) showed that the naturally dried material contained at least one order of magnitude more macamides than the laboratory dried material (Figure 6). This is a surprising find since we expected to find higher levels of "constitutive" macamides in the material dried under laboratory conditions. We speculate that macamide formation is also taking place during tissue destruction through the release of free fatty acids from membranes and storage lipids and of amines from the vacuole and through aminoacid decarboxylation. Formation could possibly occur through action of a free fatty acid (FFA) amide hydrolase working in the reverse direction in the presence of reversed amide to fatty acid ratios during the hydrolytic process, which is also accompanied by oxidation and photooxidation. It was interesting to notice that free fatty acid levels were also higher in the open-air dried material confirming lipid hydrolysis. At present we are evaluating additional marker compounds for these processes to follow their evolution during the open-air drying procedure.

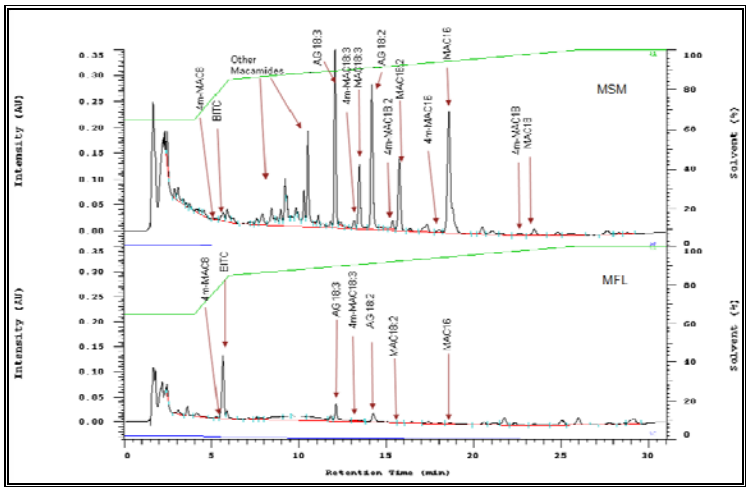

Figure 6. Reverse-phase HPLC chromatogram of the equivalent of 1 µg (dry wt.) ground dry maca hypocotyls (MSM) and of fresh lyophilized maca (MFL).

Figure 7 shows that the levels of free benzylamine are also 7-times higher in open-air dried material reinforcing our hypothesis that a number of the purported pharmacologically active principles in maca may arise during tissue maceration through open-air drying at high-altitude.

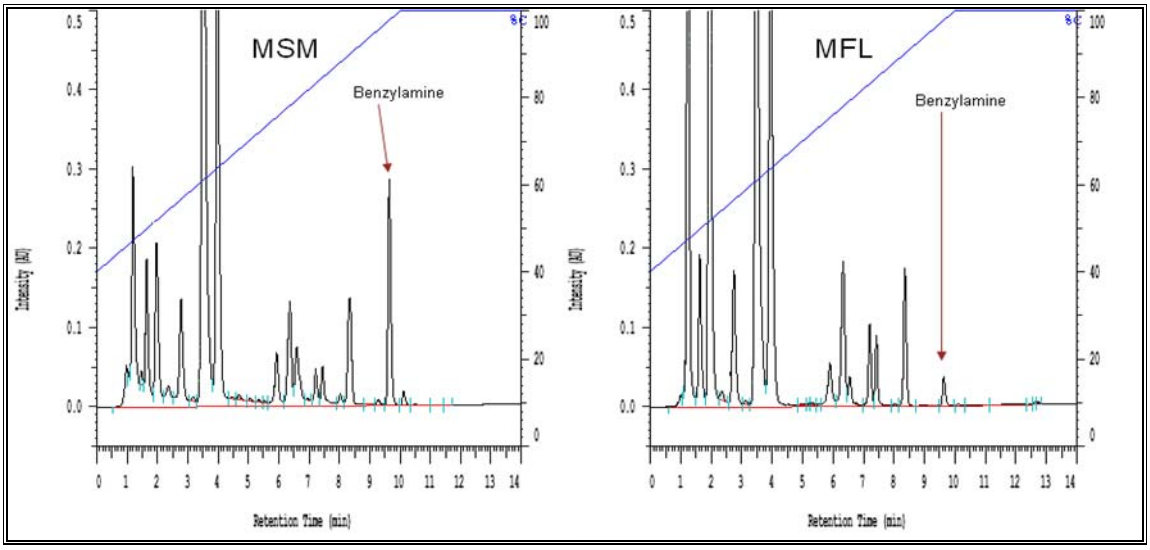

Figure 7. Free benzylamine contents in open-air dried (MSM) and freeze-dried maca (MFL) determined as OPA derivatives in HPLC.

Volatile hydrolytic products of the open-air drying process. To complete this analysis, observation of other possible metabolic products was necessary. Many of these were volatile compounds arising through the processes described in Figure 1. Active headspace sampling using pumps and adsorbing matrices, such a Porapak Q or Carbograph 5 were discarded early due to expense and complication involved in analysis by thermal desorption. Instead a SPME-based headspace technique was used. Figure 8 summarizes the SPME

strategy and Figure 9 shows a GC-SPME head-space profile of maca of crushed fresh maca tubers. We expect to use this technique to further characterize the hydrolytic procedures taking place during open-air drying.

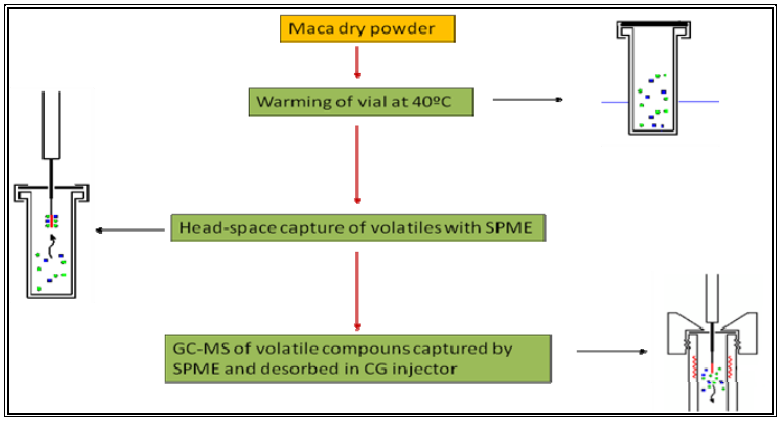

Figure 8. Analysis of volatiles in maca powders by SPME and GC-MS.

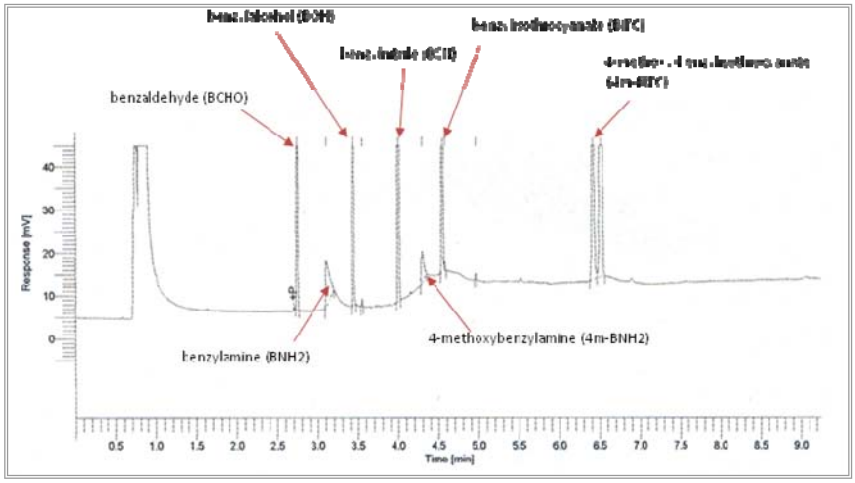

Figure 9. Hydrolysis products detected by headspace-SPME sampling after crushing 0.2 g of fresh maca tuber and sampling for 30 min at room temperature.

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Appendix

Spectroscopic characterization of synthetic amides

MAC 8: N-benzyloctanamide (N-benzylamide of caprylic acid). White crystalline solid. Molecular formula: $C_{15}H_{23}NO$ Mol. wt.: 233.35 **NMR:** δ = 2.202 (t, J=7.65Hz, 2H, H=2), δ =1.670 (m, J=7.50Hz, 2H, H=3), δ = 1.281 (s, br, 8H, H=4-7), δ =0.872 (t, J=6.75Hz, 3H , H=8), δ = 4.437 (d, J= 5.70Hz, 2H ,H= 1`), $\delta$ = 7.306 (m, 5H, H=3`- 7`), δ =5.856 (s, 1H, N-H). **FT-IR:** 3291.67cm^{-1} N-H, 1632.36 cm^{-1} C=O, 1553.99 cm^{-1} N-C amide, 1452.55 cm^{-1} N-C=O, 725.25 cm^{-1} amide and 695.65 cm^{-1} monosubstituted aromatic ring,2953.46cm^{-1} C-H, 2928.15cm^{-1} C-H, 2915.13cm^{-1} C-H, 2850.05cm^{-1} C-H. **EI-MS:** [M+]=233,[$C_9H_{11}NO$+]=149, [C_7H_8N+]= 106, [C_7H_7+] = 91.

MAC 16: N-benzylhexadecanamide (N-benzylamide of palmitic acid).White crystalline solid. Molecular formula: $C_{23}H_{39}NO$. Mol. wt.: 345.56. **NMR:** δ = 2.213 (t, J=7.65Hz, 2H, H=2), δ = 1.65 (t, J=7.05Hz, 2H, H=3) δ = 1.253 (s, br, 24H, H=4-15) δ = 0.88 (t, J=6.75Hz, 3H, H=16) δ = 4.437 (d, J= 7.65Hz, 2H, H=1`) $\delta$ = 7.292 (m, 5H, H=3`-7`) δ = 5.852 (s, 1H, N-H). **FT-IR:** 3291.55 cm^{-1} N-H, 1631.36 cm^{-1} C=O amide, 1553.54 cm^{-1} N-C amide, 1452.94 cm^{-1} N-C=O, 724.05 cm^{-1} and 695.78 cm^{-1} monosubstituted aromatic ring, 2953.33 cm^{-1} C-H, 2916.15 cm^{-1} C-H, 2847.57 cm^{-1} C-H . **EI-MS:** [M+]=345, ,[$C_9H_{11}NO$+]=149, [C_7H_8N+]= 106, [C_7H_7+] = 91.

MAC 18: N-benzyloctadecanamide (N-benzylamide of stearic acid). White crystalline solid. Molecular formula: $C_{25}H_{43}NO$. Mol. wt.: 373.62. **NMR:** δ = 2.203 (t, J= 7.5Hz, 2H , H=2), δ = 1.647 (m, J= 6.90Hz, 2H, H=3) δ = 1.255 (s,br 28H, H=4-17), δ = 0.881 (t, J=6.60Hz, 3H, H=18), δ = 4.440 (d, J=5.70Hz, 2H, H=1`), $\delta$ = 7.308 (m, 5H , H=3`-7`), δ = 5.843 (s, 1H, N-H). **FT-IR:** 3293.37 cm^{-1} N-H amide, 1631.95 cm^{-1} C=O amide, 1553.55 cm^{-1} N-C amide, 1462.12 cm^{-1} N-C=O, 727.82 cm^{-1} and 696.81 cm^{-1} monosubstituted aromatic ring,2951.92 cm^{-1} C-H, 2916.38 cm^{-1} C-H, 2847.63 cm^{-1} C-H. **EI-MS:** [M+]= 373, ,[$C_9H_{11}NO$+]=149, [C_7H_8N+]= 106, [C_7H_7+] = 91.

MAC 18:1: N-benzyl-(9Z)octadecenamide (N-benzylamide of oleic acid). Molecular formula: $C_{25}H_{41}NO$. Mol. wt.: 371.60. Liquid at room temperature. White crystalline solid at -20°C. **NMR:** δ = 2,211 (t, J=7,65 Hz, 2H, H=2), δ = 1,659 (m, J= 6,45Hz, 2H, H=3), δ = 1,284 (s, br, 8H, H=4-7), δ = 2,047 (m, J=3,90Hz, 4H, H=8,11) δ = 5,342 (m, J=2,00 Hz, 2H, H=9,10), δ = 1,300 (s,br, 12H, H=12-17), δ = 0,879 (t, J= 6,60 Hz, 3H, H=18), δ = 4,133 (q), J= 7,10Hz, 2H, H=1`), δ = 7,319 (m), 5H, H=3`-7`) δ = 5,65 (s, 1H, N-H). **FT-IR:** 3298.94 cm^{-1} N-H amide, 1639.56 cm^{-1} C=O amide, 3002.10 cm^{-1} C=C-H, 1553.61 cm^{-1} N-C amide, 1463.98 cm^{-1} N-C=O, 725.25 cm^{-1} and 695.65 cm^{-1} monosubstituted aromatic ring, 2919.45 cm^{-1} C-H, 2849.40 C-H. **EI-MS:** [M+]371, [$C_9H_{11}NO$+]=149, [C_7H_8N+]= 106, [C_7H_7+] = 91.

MAC 18:2: N-benzyl-(9Z,12Z)octadecadienamide (acid N-benzylamide of linoleic acid). Molecular formula: $C_{25}H_{39}NO$. Mol. wt.: 369.58. Colorless oil. **NMR:** δ = 2,211 (t, J=7,65 Hz, 2H, H=2), δ = 1,658 (m, J= 7,35 Hz, 2H, H=3), δ = 1,312 (s, br), 14H, H=4-7, 15-17), δ = 2,036 (m, J=6,45 Hz, 4H, H=8,14) δ = 5,360 (m, J=2,70 Hz, 4H, H=9,10,12,13), δ = 2,770 (t, J=5,85 Hz, 2H , H=11), δ = 0,889 (t, J= 6,70 Hz, 3H ,H=18), δ = 4,457 (d, J=5,70 Hz, 2H , H=1`), δ =7,319 (m), 5H, H=3`-7`), δ =5,691 (m, 1H, N-H). **FT-IR:** 3285.69 cm^{-1} N-H amide, 1645.62 cm^{-1} C=O amide, 1549.64 cm^{-1} N-C amide, 1454.07 cm^{-1} N-C=O, 725.74 cm^{-1} y 697.13 cm^{-1} monosubstituted aromatic ring, 3064.23 cm^{-1} y 3008.10 cm^{-1} C=C-H, 2953.68 cm^{-1} C-H, 2926.35 cm^{-1} C-H, 2854.34 cm^{-1} C-H. **EI-MS: [M+]=** 368, ,[$C_9H_{11}NO$+]=149, [C_7H_8N+]= 106, [C_7H_7+] = 91.

MAC 18:3: N-benzyl-(9Z,12Z,15Z)octadecatrienamide (N- benzylamide of linolenic acid). Molecular formula: $C_{25}H_{37}NO$. Mol. wt. 367.57. Colorless oil. **NMR:** δ = 2,208(t, J=7,65Hz, 2H, H=2), δ = 1,657 (m, J=7,20Hz, 2H, H=3) δ = 1,310 (s, br, 8H, H=4-7) δ = 2,077 (m, J=7,05Hz, 4H, H=8,17), δ = 5,361 (m, J=3Hz , 6H, H=9,10,12,13,15,16) δ = 2,805 (t, J=5,85Hz, 4H, H=11, 14), δ = 0,974 (t, J=7,5Hz ,3H, H=18), δ = 4,453 (d), J=5,70, 2H, H=1`), δ = 7,334 (m, 5H , H=3`-7`), δ = 5.715 (s, 1H, N-H). **FT-IR:** 3301.84 cm^{-1} N-H amide, 1639.13 cm^{-1} C=O amide, 1551.37 cm^{-1} N-C amide, 1453.88 cm^{-1} N-C=O, 3005.85 cm^{-1} amide, 720.74 cm^{-1} y 696.02 cm^{-1} monosubstituted aromatic ring, , C=C-H, 2962.02 cm^{-1} C-H, 2918.27 cm^{-1} C-H, 2848.44 cm^{-1} C-H. **EI-MS: [M+]**=367, ,[$C_9H_{11}NO$+]=149, [C_7H_8N+]= 106, [C_7H_7+] = 91.

4-m-MAC 8: 4´-methoxy-N-benzyloctanamide (4´-methoxy-N-benzylamide of caprylic acid). Molecular formula: $C_{16}H_{25}NO_2$. Mol. wt.: 263.38. White crystalline solid. **NMR:** δ = 2.182 (t, J=7.65Hz,2H, H=2), δ = 1.634 (m, J=7.50Hz, 2H, H=3), δ = 1.274 (s,br, 8H, H=4-7), δ = 0.868 (t, J= 6.75Hz, 3H, H=8), δ = 4.366 (d, J= 6.60 Hz, 2H, H=1`) δ = 6.86 (d, J=6.90 Hz, 1H, H=3`) δ = 7.209, (d), J= 8.70Hz,1H, 4`,6`), δ = 6.86 (d), J=6.90 Hz, 1H, H=7`) δ = 3.789 (s, 3H, O-Me), δ = 5.755 (s, 1H, N-H). **FT-IR:** 3289.51 cm^{-1} N-H amide, 1631.67cm^{-1} C=O amide, 1553.93 cm^{-1} N-C amide, 1463.84 cm^{-1} N-C=O amide, 1514.79 cm^{-1} C=C-O methoxy group, 1254.11 cm^{-1} y 1031.86 cm^{-1} C-O methoxy group, 815.80 - 810.71 cm^{-1} disubstituted aromatic ring in *para* position,2916.06cm^{-1} C-H ,2953.75cm^{-1} C-H, 2928.95cm^{-1} C-H, 2850.19cm^{-1} C-H. **EI-MS:** [M+]=263, [$C_{10}H_{13}NO_2$+]= 179, [$C_8H_{10}NO$+]=136, [C_8H_9O+] = 121

4´-m-MAC 16: 4´-methoxy-N-benzylhexadecanamide (4´-methoxy–N-benzylamide of palmitic acid). Molecular formula: $C_{24}H_{41}NO_2$. Mol. wt.: 375.59. White crystalline solid. **NMR:** δ = 2.187 (t, J=7.50 , 2H, H=2), δ = 1.680 (m , 2H, H=3), δ = 1.246 (s, br, 24H, H=4-15) δ = 0.872 (t, J=6.45Hz, 3H, H=16), δ = 4.367 (d, J=5.10Hz, 2H, H=1`), δ = 6.866 (d), J=8.40Hz, 1H, H=3`,7`) δ = 7.207 (d), J=8.40Hz 1H, H=6`, 7`) δ = 3.787 (s, 3H, OMe), δ = 5.789 (s,1H, N-H). **FT-IR:** 3299.90 cm^{-1} N-H amide, 1639.54 cm^{-1} C=O amide, 1551.58 cm^{-1} N-C amide, 1462.63 cm^{-1} N-C=O amide, 808.23 cm^{-1} disubstituted aromatic ring in *para* position, 2954.05 cm^{-1} C-H, 2917.06 cm^{-1} C-H, 2848.74 cm^{-1} C-H , 1515.43 cm^{-1} C=C-O methoxy group, 1260.74 cm^{-1} y 1032.26 cm^{-1} C-O methoxy group. **EI-MS:** [M+]= 375, [$C_{10}H_{13}NO_2$+]= 179, [$C_8H_{10}NO$+]=136, [C_8H_9O+] = 121

4´-m-MAC 18:4´-methoxy-N-benzyloctadecanamide (4´-methoxy –N-benzylamide of stearic acid). Molecular formula: $C_{26}H_{45}NO_2$. Mol. wt.: 403.64. White crystalline solid. **NMR:** δ = 2.192 (t, J=7.65Hz, 2H, H=2), δ = 1.637 (m, J=7.05Hz, 2H, H=3), δ = 1.250 (s,br, 28H, H=4-17), δ = 0.876 (s, J=6.60Hz, 3H, H=18) δ = 4.378 (d), J=5.40Hz, 2H, H=1`), δ = 6.875 (d), J=8.70Hz, 1H, 3`, 7`), δ = 7.216 (d), J=8.40Hz, 1H, H=4`, 6`), δ = 3.796 (s, 3H, OMe), δ = 5.697 (s), 1H, N-H). **FT-IR:** 3291.52 cm^{-1} N-H amide, 1630.05 cm^{-1} C=O amide, 1554.01 cm^{-1} N-C amide, 1462.30 cm^{-1} N-C=O amide, 815.80 – 807.68 cm^{-1} disubstituted aromatic ring in *para* position, 1514.73 cm^{-1} C=C-O methoxy group, 1254.39 cm^{-1} y 1029.54 cm^{-1} C-O methoxy group, 2917.05 cm^{-1} C-H, 2847.86 cm^{-1} C-H. **EI-MS:** [M+]= 402, [$C_{10}H_{13}NO_2$+]= 179, [$C_8H_{10}NO$+]=136, [C_8H_9O+] = 121

4´-m-MAC 18:1: 4´-methoxy-N-benzyl-(9Z)octadecenamide (4´- methoxy –N-benzylamide of oleic acid). Molecular formula: $C_{26}H_{43}NO_2$. Mol. wt.: 401.63. Liquid at room temperature. White crystalline solid at -20°C. **NMR:** δ = 2,189 (t, J=5,85, 2HH=2), δ = 1,645 (m, J= 7,00Hz, 2H, H=3), δ = 1,293 (s, br, 12H, H=4-7, 12-17)) δ = 1,996 (m, J= 3,90Hz, 4H, H=8, 11) δ = 5,342 (m, J=2,0Hz, 2H, H=9,10) δ = 0,879 (t, J=6,75Hz, 3H, H=18), δ = 4,384 (d, J= 5,70 Hz, 2H, H=1`), δ = 6,88 (d, J= 6,6Hz, 2H, H=3`,7`) δ = 7,214(d), J=4,50Hz, 2H, 4`, 6`), δ = 3,800 (s, 3H, OMe), δ = 5,608 (s, 1H, N-H). **FT-IR:** 3298.94 cm^{-1} N-H amide, 1639.56 cm^{-1} C=O amide, 1553.61 cm^{-1} N-C amide, 1463.98 cm^{-1} N-C=O amide, 810.11 cm^{-1} disubstituted aromatic ring in *para* position, 1514.79 cm^{-1} C=C-O methoxy group**,** 1255.11 cm^{-1} y 1031.97 cm^{-1} C-O methoxy group, 3002.10 cm^{-1} C=C-H, 2919.45 cm^{-1} C-H, 2849.40 C-H. **EI-MS:** [M+]= 400, [$C_{10}H_{13}NO_2$+]= 179, [C_8H_{10}NO+]=136, [C_8H_9O+] = 121

4´-m-MAC 18:2: 4´-methoxy-N-benzyl-(9Z,12Z)octadecadienamide (4´-methoxy-N-benzylamide of linoleic acid). Molecular formula: $C_{26}H_{41}NO_2$. Mol. wt.: 399.61. Colorless oil. NMR: δ = 2,19 (t, J= 7,65 Hz, 2H, H=2), δ = 1,629 (m, 2H, H=3), δ = 1,307 (s,br, 14H, H=4-7, 15-17), δ = 2,035 (m, J= 6,45Hz, 4H, H=8,14), δ = 5,349 (m), J=2,70 Hz, 4H, H=9,10,12,13), δ = 2,768 (t, J=5,70 Hz, 2H, H=11), δ = 0,888 (t, J= 6,75 Hz, 3H, H=18), δ = 4,384 (d, J=5,70 , 2H, H= 1´), δ = 6,873 (d), J=8,70 Hz, 2H, H=3`,7`) δ = 7,221(d), J= 8,70, 2H, 4`, 6`), δ = 3,800 (s, 3H, H=OMe) δ = 5,615 (m, 1H, N-H). FT-IR: 3289.51 cm^{-1} N-H amide, 1553.93 cm^{-1} N-C amide, 1631.67 cm^{-1} C=O amide, 1463.84 cm^{-1} N-C=O amide, 815.80 - 810.71 cm^{-1} disubstituted aromatic ring in *para* position**,** 1514.79 cm^{-1} C=C-O methoxy group, 1254.11 cm^{-1} y 1031.86 cm^{-1} C-O methoxy group, 3006.43 cm^{-1} C=C-H, 2916.06 cm^{-1} C-H ,2953.75 cm^{-1} C-H, 2928.95 cm^{-1} C-H, 2850.19cm^{-1} C-H. **EI-MS:** [M+]= 398, [$C_{10}H_{13}NO_2$+]= 179, [C_8H_{10}NO+]=136, [C_8H_9O+] = 121

4´-m-MAC 18:3: 4´-methoxy-N-benzyl-(9Z,12Z,15Z)octadecatrienamide (4´- methoxy –N-benzylamide of linolenic acid). Molecular formula: $C_{26}H_{39}NO_2$. Mol. wt.: 397.59. Colorless oil. NMR: δ = 2,187 (t, J= 7,50Hz, 2H, H=2) δ = 1,644 (m), J=7,20Hz ,2H, H=3), δ = 1,306 (s,br, 8H, H=4-7), δ = 2,054 (m, J=6,15Hz, 4H, H=8,17), δ = 5,369 (m, J=2,70Hz , 6H, H=9,10,12,13,15,16), δ = 2,804 (t, J=5,70 Hz, 4H, H=11,14), δ = 0,973 (t, J=7,50 Hz, 3H, H=18), δ = 4,381(d, J=5,70 Hz, 2H, H=1`), δ = 6,871(d), J=6,6 Hz, 2H, H=3`,7`), δ = 7,219 (d), J= 8,40Hz, 2H, 4`, 6`), δ = 3,798 (s, 3H, OMe), δ = 5,627 (s, N-H). FT-IR: 3300.51 cm^{-1} N-H amide, 1639.31 cm^{-1} C=O amide, 1552.42 cm^{-1} N-C amide, 1460.04 cm^{-1} N-C=O amide, 810.09 cm^{-1} disubstituted aromatic ring in *para* position, 1513.82 cm^{-1} C=C-O methoxy group, 1254.69 cm^{-1} y 1031.81 cm^{-1} C-O methoxy group, 3005.83 cm^{-1} C=C-H, 2916.51 cm^{-1} C-H , 2848.08 cm^{-1} C-H. **EI-MS:** [M+]= 396, [$C_{10}H_{13}NO_2$+]= 179, [C_8H_{10}NO+]=136, [C_8H_9O+] = 121

Crop protection by volatile organic compounds from mashua: what we can learn from ancient agricultural techniques

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Abstract

Mashua (*Tropaeolum tuberosum*) is a perennial plant which has been grown in the Andes since prehispanic times. Despite the fact that its tubers are highly nutritious and that they can be used to cure a number of kidney ailments, mashua is limited to subsistence agriculture systems. This is probably due to its strong and pungent flavor (a result of its high levels of benzylglucosinolates) and to its reputed anti-aphrodisiac properties. Because mashua has a high resistance to bacterial, fungal, nematode and insect pests, it has been used traditionally as a companion crop to repel pests in plants that are of higher economical value, such as potato or olluco. It has been proposed that the high levels of isothiocyanates and of other volatile compounds produced by the glucosinolate pathway are at least partially responsible for this protective activity. In order to study the biochemical basis of the protection obtained from mashua plants, we analyzed volatile emission by mashua in the field and also evaluated differential volatile emission patterns (BVOC profiling) during tissue damage of 119 accessions of Peruvian mashuas grown by CIP's genebank close to Huancayo at 3700 masl, Peru. Volatiles were absorbed onto Porapak Q cartridges by active sampling using a portable air pump, were eluted from the matrix with acetonitrile and analyzed by gas chromatography on a DB-225 column with FID or MS detection. This method allowed quantification of effective field concentrations of protective volatiles and also provided a means to evaluate mashua germplasm entries based on their differential BVOC phenotype.

Introduction

Mashua (*Tropaeolum tuberosum*) constitutes the fourth most important root crop in the Andean region, after potato, oca and olluco (National Research Council, 1989). Mashua is easy to grow, produces high yields and is extremely resistant to both cold weather and pests (National Research Council, 1989, Pissard et al, 2008). Its tubers are highly nutritious; as they have a high content of carbohydrates and vitamin C (Grau et al. 2003) and it has also been reported that mashua has a higher antioxidant capacity and a higher phenolic, carotenoid and antocyanin content than other tubers (Campos et al., 2006). In addition to this, mashua has been used traditionally in the Andes to cure a variety of kidney, liver and urinary disorders and it has been recognized as an antiaphrodisiac since prehispanic times (Johns et al., 1982). Despite all the benefits it seems to offer, currently mashua is limited to subsistence agriculture systems. This is probably due to its sharp flavor, which has been attributed to its high levels of glucosinolates.

Although mashua is not exactly a commercially important tuber, it does have an important economical value: since it has a high resistance to bacterial, fungal, nematode and insect pests, it has been used traditionally as a companion crop to protect other crops such as potato or olluco from their main pests (National Research Council, 1989, Figure 1). It has been proposed that the high levels of glucosinolates in mashua are at least partially responsible for this protective activity.

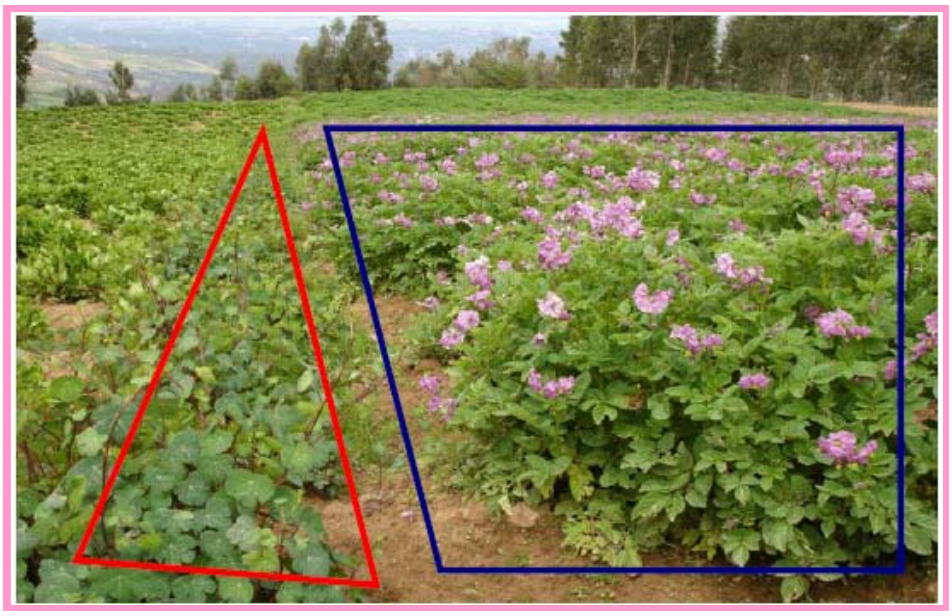


Figure 1. Potato-mashua co-cultivation system in the Peruvian Andes. Mashua (in red) is cultivated in alternated rows with potato (in blue) to protect the latter from its main pathogens

Glucosinolates are natural products that contain nitrogen and sulfur and are synthesized by plants from aminoacids (Halkier et al., 2006). Glucosinolates themselves are non-toxic, but they can be hydrolyzed to a series of compounds, such as isothiocyanates and nitriles that do have different biological effects and play an important role in plant defense mechanisms (Bennet and Wallsgrove, 1994). Glucosinolate producing plants also express thioglucosidases called myrosinases. Upon tissue damage, glucosinolates come in contact with myrosinases and are hydrolized to unstable aglycones, which rearrange to form different compounds, some of which can be toxic to insects and microorganisms (Wittstock and Halkier, 2002, Mutlib et al., 2002, Rask et al., 2000, Figure 2).

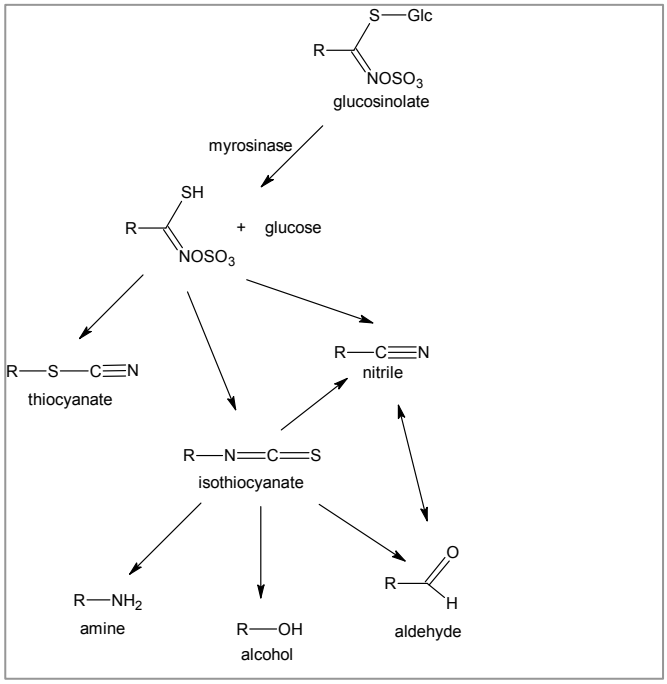


Figure 2. Products obtained from glucosinolate hydrolysis. Upon plant damage, glucosinolates are degraded to a variety of hydrolysis products. The initial step involves catalytic hydrolysis by myrosinase. R indicates a variable side chain and Glc a glucose residue

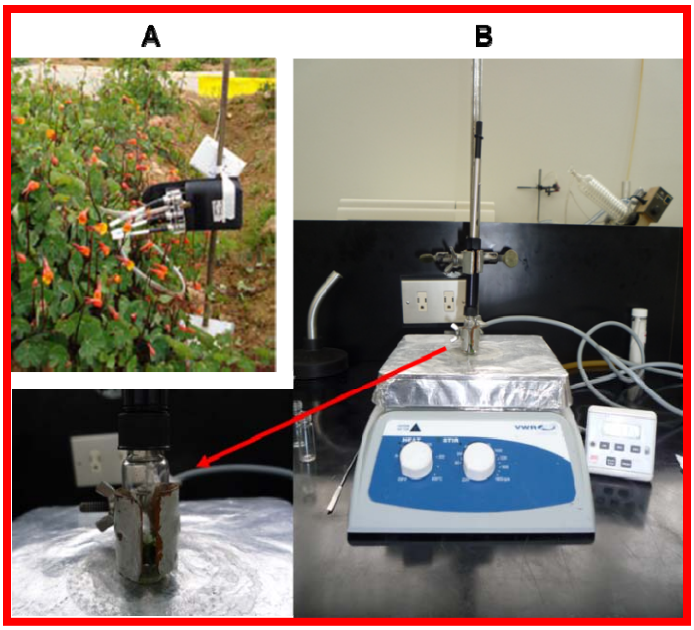
The predominant glucosinolates present in mashua are benzyl, 4-methoxybenzyl hydroxybenzyl and m-methoxybenzyl (Valer, 2001, Ortega et al., 2006) and the relative content of each of these compounds varies in the different mashua accessions that are known to date (Ortega et al., 2006). The present investigation was directed towards studying the biochemical basis of mashua´s protective activity and also towards developing a method that could allow for an evaluation of mashua germplasm entries based on their emission of volatile compounds that result from the glucosinolate pathway.

Materials and methods

Inhibition assays

Cells of *Candida albicans*, strain SC537, were provided by the European Saccharomyces Cerevisiae Archive for Functional Analysis (EUROSCARF), Frankfurt, Germany. Cells were grown to log phase in Nutrient Broth, at 30°C. Stock isothiocyanate dilutions were made in ethanol, with the following concentrations: 0.01, 0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 and 2.5 µg/ml. 195 µL of dilute *C. albicans* stock were incubated for 25 hours with 5 µL of each isothiocyanate dilution in sterile plastic 96-well microtiter plates at 30°C. Following incubation, turbidity was measured at 490 nm in a ELx800 Series Universal Microplate Reader (Bio-Tek Instruments, Inc.). IC_{50} values were obtained for each of the following isothiocyanates: allyl (AITC), n-propyl (n-PITC), phenyl (PITC), 4-methoxy-phenyl, benzyl (BITC), 4-methoxy-benzyl (4MBITC) and 2-phenylethyl (2PEITC).

Field measurements of volatile emissions by active sampling



Sampling was carried out in a mashua field maintained by CIP in La Libertad, Junin, close to the city of Huancayo, at 3700 masl. Volatiles present in the field were absorbed onto glass cartridges filled with 200 mg of Porapak Q 50/80 (Supelco, Inc.) by active sampling using a portable air pump (AirLite Sampler Model 110-100, SKC, Inc.) (Figure 3A). The flow rate used was 300 mL/min and 6 L of air were captured in each case. The mashua accessions studied were DP 0224 (black mashua), M5 COL 2B, ARB 5371 and S 64. Volatiles were then eluted from the matrix with 1 mL of acetonitrile and analyzed in the PUCP laboratories, in Lima, using a Perkin Elmer Autosystem gas chromatographer equipped with a DB-225 column (30 m x 0.32 mm x 0.25 µm) and a FID detector. The temperature program used was 70°C for 1 min, followed by 70-200°C at 40°C /min and 200°C up to 6.5 min.

Figure 3. Sampling systems for mashua volatile emissions. A: Active sampling in the field using a portable air pump and Porapak Q cartridges. B: Sampling in the PUCP laboratories in Lima by headspace solid-phase microextraction (HS-SPME).

Headspace solid-phase microextraction (HS-SPME) assays

The analyses were performed in a Perkin Elmer Autosystem gas chromatographer equipped with a DB-225 column (30 m x 0.32 mm x 0.25 µm) and a FID detector. The GC temperature program used was 40°C for 4 min, followed by 40-200°C at 40°C /min and 200°C for 7 min. HS-SPME assays were performed using a manual fiber holder and a 100 µm PDMS coated fiber (Supelco Inc.). Plant material for these assays was obtained from mashua accessions DP 2124 (black mashua) and ARB 5240. These plants had been transferred to Lima and kept in pots

for 4 months. Samples were prepared by crushing approximately 0.1g of leaf or tuber material in a 4 mL screw top vial. The vial was sealed with a cap and septum and the SPME fiber was inserted through the septum and exposed for 15 minutes (Figure 3B). The compounds absorbed in the fiber were then desorbed by insertion into the GC inlet for 4 minutes at 200°C.

Results and discussion

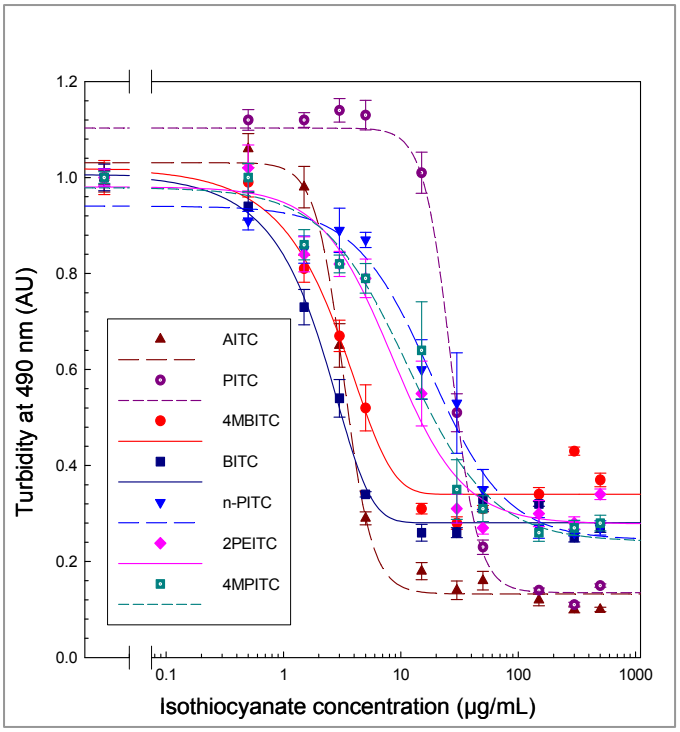


Figure 4. Antimicrobial activity of isothiocyanates. IC_{50} values were obtained using a micro titer plate based bioassay

It has long been known that isothiocyanates display antimicrobial activity. However, this issue was revisited in order to identify structural features that could be relevant for such activity. Inhibition assays performed with different isothiocyanates showed that benzyl and 4-methoxy-benzyl isothiocyanate had the lowest IC_{50} values against *C. albicans* (Figure 4).

Given the fact that benzyl and 4-methoxy-benzyl glucosinolate appear to be among the predominant glucosinolates found in mashua (Valer, 2001, Table 1), these results suggest that benzyl and 4-methoxy-benzyl isothiocyanate released by mashua in the field might be responsible for the protection provided by this plant to neighboring potato plants. A detailed assessment of the biocidal activity of these two isothiocyanates, as well as of compounds derived from isothiocyanate degradation, against the main natural potato pathogens *Phytophthora infestans, Pectobacterium carotovorum (*previously, *Erwinia carotovora)* and *Ralstonia solanacearu)* remains to be performed.

Table 1. Predominant glucosinolates present in different mashua organs of mashua accession ARB 5240. Content values are reported in µmoles per gram of dry matter (Valer, 2001). Abbreviations are as follows: 4HBGLS: 4-hydroxy-benzyl glucosinolate, MHBGLS: methoxy-hydroxy-benzyl glucosinolate, 4MBGLS: 4-methoxy-benzyl glucosinolate, BGLS: benzyl glucosinolate.

	4HBGLS	MHBGLS	4MBGLS	BGLS
Leaves	0.6	0.07	17.9	0.15
Stems	0.9	0.17	46.3	1.01
Tubers	2.4	0.45	117.1	1.16
Roots	0.3	0.63	62.9	0.43

Once we the putative defensive compounds in mashua had been identified, efforts were directed to the analysis of volatile emissions by mashua in the field. Surprisingly, the levels of isothiocyanates found in the collected samples were relatively low and the predominant emissions appeared to be benzaldehyde and benzylalcohol (Figure 5). It is possible that benzaldehyde results from further processing of benzyl isothiocyanate and benzyl

nitrile emitted by mashua (Mutlib et al., 2002). It has been observed that isothiocyanates and nitriles captured onto Porapak degrade rapidly with time (data not shown). In addition to this observation, isothiocyanates are less volatile than their corresponding aldehydes. Taking all of this into account, and considering that benzaldehyde has also been reported as being effective as a fumigant for crops (Lee et al., 2001), we propose that benzaldehyde, and not benzyl or 4-methoxy-benzyl isothiocyanates could be the main compound responsible for the protective activity of mashua, at least for aerial plant parts. We cannot discard that some amount of the benzaldehyde detected in the field could potentially originate from other plant sources such as the neighboring oca plants which, at the time of our analyses, were flowering or other nearby trees. However, the results observed from samples taken in the greenhouse confirm that mashua does emit high levels of benzaldehyde, especially when plants are in the flowering stage (Figure 5). The only plant species in the greenhouse at the time of the present investigation was mashua. More studies need to be done in which air sampling is not limited to mashua plants but is also extended to other species present in the field. We do not discard either that isothiocyanates and nitriles could still important defensive compounds in other plant organs such as tubers.

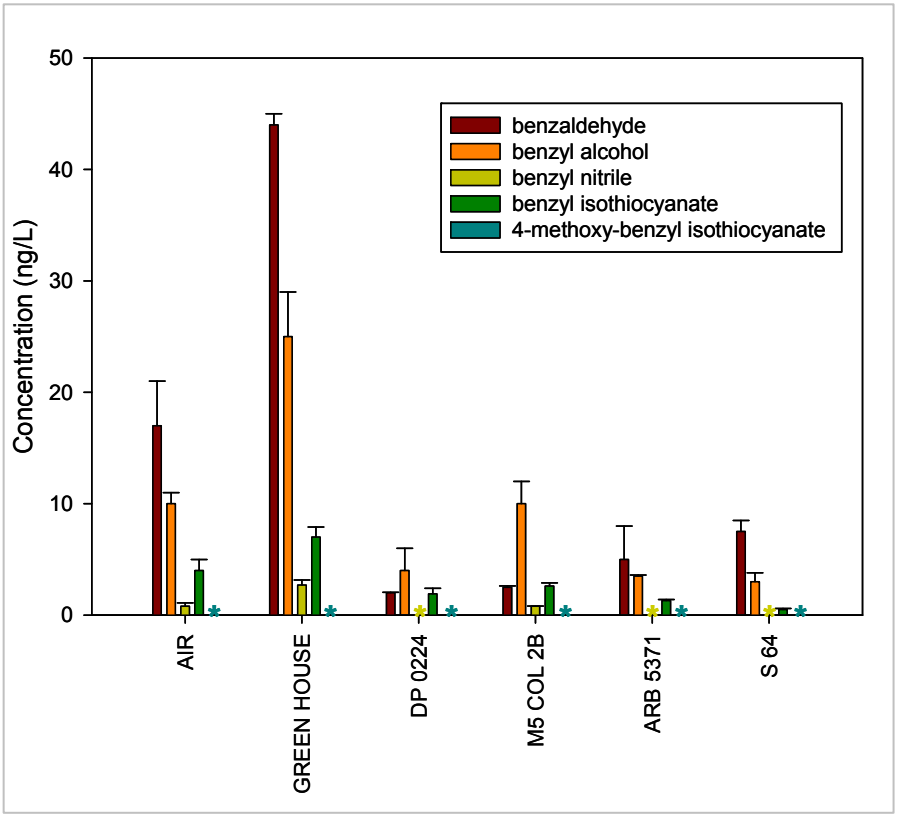


Figure 5. Volatile compounds detected in samples that were collected in the field in March, 2008. Concentration values are averages of three determinations using active sampling using a portable air pump and analysis by GC. Four different mashua accessions were studied: DP 0224 (black mashua), M5 COL 2B, ARB 5371 and S 64. Air samples from the field (AIR) and from a greenhouse were only mashua was kept were also taken Volatile compounds analyzed were: benzaldehyde (red), benzyl-alcohol (orange), benzyl-nitrile (yellow), benzyl-isothiocyanate (green) and 4-methoxy-benzyl-isothiocyanate (blue-green). The symbol (*) represents absence or very low levels of the compound, which could not be quantified.

During our studies, it could also be observed that the volatile emission patterns of different mashua accessions varied significantly (Figure 5). Therefore, active sampling in Porapak Q cartridges was also used to obtain preliminary volatile profiles of different accessions of Peruvian mashuas grown by CIP's genebank close to Huancayo (data not shown). For these analyses, volatile samples were not taken directly in the field. Instead, we used leaf material, obtained from the source plants and subjected it to mechanical damage prior to the absorption of volatiles onto the Porapak Q cartridges.

HS-SPME analyses of leaf material did not show significant levels of benzyl nitrile or benzaldehyde emission, although they did show low levels of isothiocyanate emission (Figure 6A). This could be due to the fact that the mashua plants used for the analyses had experienced a significant change in environmental conditions and they had already adapted to such conditions, as they had been kept in pots in Lima for a period of 4 months. Tuber material did show high levels of isothiocyanate and nitrile emission under the same experimental conditions (Figure 6B). However, no significant levels of benzaldehyde emission from could be observed from tubers. Since it has been proposed that benzaldehyde could be obtained upon isothiocyanate and nitrile further processing, we cannot discard that the absence of benzaldehyde in the tubers volatile profile is a result of the short emission time used in these assays (15 min). A more detailed study of the kinetics of volatile emissions from both leaf and tuber material is yet to be performed. Another possible explanation for the absence of benzaldehyde and, especially, of benzyl alcohol in the SPME fiber is that the fiber was coated with PDMS, which is a matrix of low polarity. The analyses will be repeated using a Carboxen/PDMS fiber (Supelco, Inc.), which should improve the absorption of the compounds with higher polarity emitted by mashua.

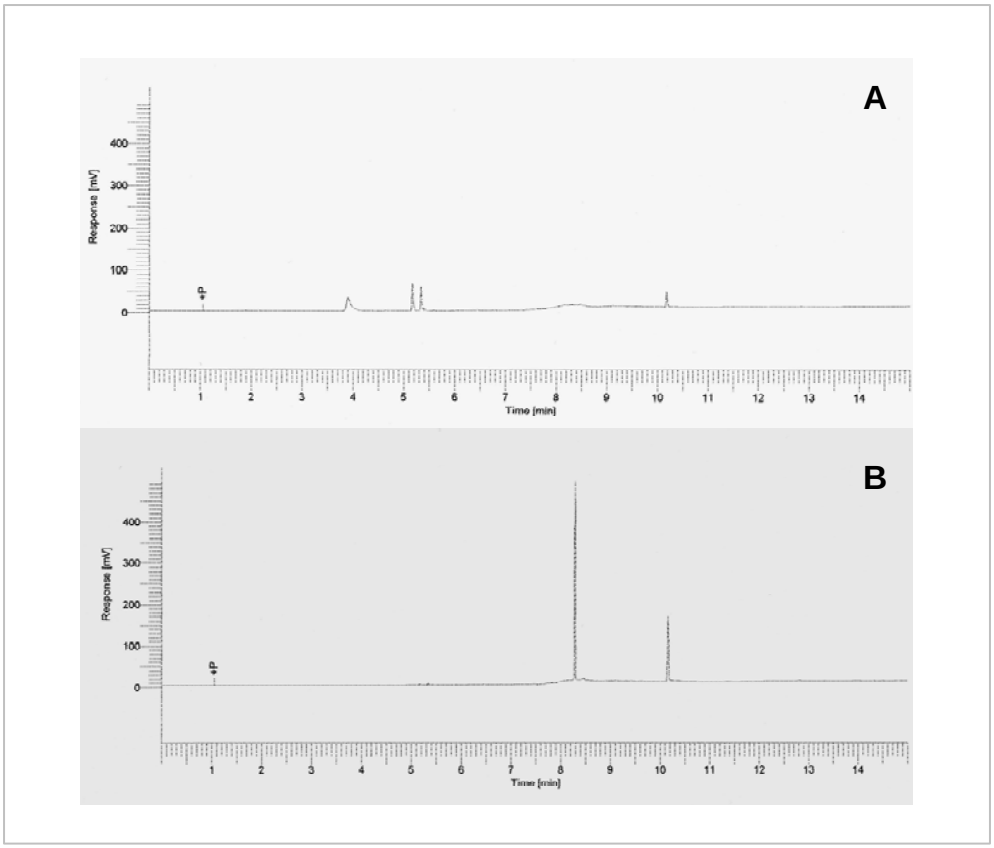


Figure 6. Volatile profiles of leaf and tuber material from mashua obtained by HS-SPME. The chromatograms show leaf (A) and tuber (B) emissions captured on a PDMS coated fiber. Compound identification was performed by comparing retention times with standards analyzed under the same chromatographic conditions.

Conclusions

Mashua has long been used in the Andes as a companion crop for its ability to repel pests from other commercially important plants such as potato and olluco. This protective activity has been traditionally assigned to the high levels of isothiocyanates and nitriles present in mashua. Our analyses of volatile compounds emitted by this plant in the field suggest that protection may also result from benzaldehyde and benzyl alcohol emissions. Benzaldehyde is also known as a defensive compound in plants and, since it has a higher vapor pressure than the aromatic isothiocyanates, it could play an important role in the protection of

mashua, especially of its aerial parts. We have also performed a preliminary evaluation of emission patterns during tissue damage of several mashua accessions grown by CIP's genebank close to Huancayo. Our results indicate that analyzing differential BVOC phenotypes in mashua is a potential tool for the evaluation of mashua germplasm entries.

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Microbiotic biodiversity and their functionality in roots and rhizosphere of potato plants

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Abstract

Soil microbiotic biodiversity plays an important role and fulfills diverse functions for the growth and development of plants and crops. Its beneficial effects include among others the control of soil pathogens, the supply and utilization of nutrients and/or soil moisture. In 2008 rhizosphere populations of bacteria and fungi of 12 different potato fields in 4 provinces in the Andean highlands of Peru were analyzed and possible PGPR (promoting growth rhizobacteria) genera were isolated from the root surface, the root interior (endophytic) and the rhizosphere. Total bacteria counts in the rhizosphere seemed to be influenced by altitude and soil electric conductivity. The rhizosphere populations of microorganism seems to be adapted to this micro-enviorenment so, even though the external conditions are extreme the rhizosphere acts like a support zone for the microbial development. We found the active presence of bacteria form the Azospirillum, Azotobacter, Pseudomonas and Actinomycetes genera even in low pH and temperature conditions and different inputs levels. We isolated a total of 62 *Azotobacter* strains, 45 Actinomycetes, 68 *Pseudomona*s and 55 *Azospirillum* from the surface of roots, their interior or the rhizosphere soil. The active presence of total bacteria and potential PGPR genera in the potato rhizosphere represents an important part to understand the influence of microorganism in this microenvironment and the possible relation with the plant development.

Keywords: biodiversity, plant growth promoting rhizobacteria, microorganism, soil.

Introduction

Soils sustain an immense diversity of microbes, which to a large extent still remain unexplored. Soil bacteria and fungi play important roles in various biogeochemical cycles being responsible for the cycling of organic compounds (Wall and Virginia, 1999). Many activities as inorganic fertilizers, use of pesticides and environmental pollution can potentially affect soil microbial diversity; but little is known how changes in microbial diversity influence below-ground and above-ground ecosystems.

The soil ecosystem is complex containing many microhabitats suitable for microbial growth. The rhizosphere microhabitat encompasses the millimeters of soil surrounding a plant root where complex biological and ecological processes occur, making it a specific zone for microbial growth (Bais et al., 2006). The physicochemical properties of this zone are mainly determined by the effect of plant root exudates, which create different growing conditions for microorganisms in comparison with the root-free soil; in rhizosphere soil, for example, a two-fold increase of bacterial populations over bulk soil can be observed (Kirk et al., 2004). The magnitude of the rhizosphere effect depends mainly on the nature and amount of root exudates which appear to be related to plant age as well as species on one hand and edaphic and climatic factors on the other (Pandey and Palni, 2007).

The root-soil interface is an important zone for root-microbe communication and exchange of metabolites. Positive interactions include symbiotic associations with epiphytes and mycorrhizal fungi, and root colonization by bacterial biocontrol agents and plant growth promoting rhizobacteria (PGPR) (Bais et al., 2006). Beneficial microorganisms in the rhizosphere have been studied in the past years to understand their role and the mechanisms involved in root-microorganism interactions.

However, microbial diversity and abundance in the rhizosphere not only depend on the interactions between plant root and bacteria, but also on a variety of other abiotic and biotic factors (Garbeva et al., 2004). Some of these factors influencing the survival and activity of bacteria in the rhizosphere are physical (texture, temperature and humidity), while others are chemical, such as pH, nutrient availability, organic matter content and, above all, interactions with other rhizosphere microorganisms (Barriuso et al., 2008). For bacterial strains

(including PGPR) to colonize plant roots and/or the soil rhizosphere, they have to compete with other microorganisms for best growth conditions to maintain minimum populations able to exert their biological functions. PGPR exert beneficial effects on plant development by direct or indirect mechanisms, such as the production plant hormones, nutrient supply, control of pathogens etc. (Vessey, 2003; Compant et al., 2005). They belong to different bacterial genera, such as Azospirillum, Azotobacter, Bacillus and others, with different demands on growth and environmental conditions.

Growing interest in microbial ecology reflects the importance of microorganisms in ecosystems. Their ecosystem functions encompass among others the decomposition of organic matter, the supply of plant nutrients, the surpressiveness of plant pathogens, the support of the buffering capacity of soils to stress events. Rhizobacteria have an even more prominent status, as they exert direct and immediate effects on plant growth. Making use of the beneficial capacities of these bacteria could increase crop productivity and resource use efficiency. Hence, a more profound knowledge of their ecosystem functions, their natural diversity and abundance as well as the factors influencing these parameters would improve the selection process for effective PGPR, adapted to and competitive in field conditions.

The objective of the present study was to investigate the diversity and abundance of bacterial strains of different PGPR-genera in various agro-ecological zones of potato-based cropping systems of the Peruvian Andes and to establish biotic or abiotic factors influencing their presence and development.

Materials and methods

In February 2008 potato (*Solanum tuberosum*) rhizosphere soil was collected from twelve fields in 4 different provinces in Andean regions of Peru (Table 1). Each sample (1 kg) was obtained by bulking three subsamples of rhizosphere soil and potato plant roots from individual fields. They were transported to the laboratory and processed within 24 h to minimize changes in the microbial community compositions.

For the rhizosphere analysis 10g of soil were placed in a sterile bottle with 90ml of peptone water (0.1%) (dilution 10^{-1}). For the isolation of bacteria from the root surface and the root interior 1g of potato roots were cleaned in each case with sterile water. The roots for the isolation of edaphytic bacteria were further ***sterilized*** with sodium hipocloride (1%) and ethanol (70%). ***Ten-fold*** serial ***dilutions*** were made for the three samples (rhizosphere, root surface and root interior). For total bacteria counts, plate count agar incubated for 48 hours at 28°C was used (APHA-AWWA, 1998). For total fungal populations potato dextrose agar was used (PDA) and plates were incubated for 6 days at 25°C. The quantification of *Azotobacter* spp. populations was done according to Zapater (1975) and Zúñiga and Gutiérrez-Correa (1982), using 3 tubes per dilution that contained mineral medium incubated at 28°C for 7 days. Characteristic surface veil, turbidity and color change were observed for positive responses. For Actinomycetes counts starch-casein agar was used; plates were incubated at 28°C for 10 days (APHA - AWWA, 1998). *Azospirillum* spp. populations were count using 3 tubes per dilution that contained nitrogen free mineral medium incubated at 28°C for 7 days. Finally the quantification of *Pseudomonas* spp. was conducted using 3 tubes per dilution with asparagine broth; incubation lasted for two days at 30°C. After the quantification of the populations of each bacteria genera, positive tubes and colonies were transferred to selective agar plates in order to identify the characteristic colonies and isolate them. All isolates were confirmed using a Gram stain.

The collected soil samples were also analyzed for their physico-chemical characteristics using standard techniques at the soil laboratory of the National Agricultural University La Molina, Lima, Peru.

Results and discussion

The fields sampled for microbial populations were located in different agro-ecological zones of the Peruvian Andes. Hence, the physicochemical characteristics of the 12 locations indicate a variety of different soil conditions, for example pH values range between 4.1 and 7.6 while soil organic matter varies between 2.5% and 9.5% (Table 2). Some general patterns are that high altitude fields have very low pH, a high organic matter but low clay content, while lower fields are acidic to neutral with lower contents of organic matter but often a greater percentage of clay.

Table1. Potato rhizosphere samples from 12 fields in the Peruvian highlands

Samples	Province - district	Site	Altitude	Geographical Location	Management	Variety
M1	Huancavelica - Tayacaja - Pazos	San Jose de Aymara	4030	L.S. 12º 14′ 17.34″ L.W. 75º 03′ 46.14″	Low input	Land race
M2	Huancavelica - Tayacaja - Pazos	Mullaca	3420	L.S. 12º16'47.9' L.W.75º28'46.2"	Low input	Land race
M3	Huancavelica – Tayacaja - Pazos	Vista Alegre	3840	L.S.12º16′15.24″ L.W.75º1′12.36″	Low input	Land race
M4	Junín – Huancayo - Pucara	Patala	4132	L.S. 12º 12′ 39.78″ L.W. 75º 04′ 24.3″	Low input	Land race
M5	Junín – Huancayo – El Tambo	La Victoria	3200	L.S. 12º03′21″ L.W.75º12′67″	Low input	Improved
M6	Junín – Huancayo - Pucara	Marcavalle	3362	L.S. 12º13′ 0″ L.W. 75º8′ 0″	Low input	Land race
M7	Huánuco - Huanuco-Churubamba	Paccha	3 400	L.S. 9º43′5″ L.W.76º41′15″	High input	Improved
M8	Huánuco- Huanuco-Churubamba	Huayllacan	3 700	L.S. 9º41′15″ L.W. 76º41′15″	High input	Land race
M9	Huanuco-Huanuco-Churubamba	Mision Punta	3500	L.W. 9º42'11.27" L.S. 76º31'51.6"	High input	Improved
M10	Huanuco- Huanuco-Churubamba	Mataos	3 000	L.W. 9º44'3.77" L.S. 76º31'51.6"	High input	Improved
M11	Cajamarca – Cajamarca-Encañada	Pampa Culebra	3098	L.S. 7º08′0″ L.W. 78º20′0″	High input	Improved
M12	Cajamarca-Cajamarca-Baños del Inca	Puylucana	2800	L.S. 7º 05′ 0″ L.W. 78º 27′0″	High input	Improved

In all rhizosphere samples total bacteria had greater populations than fungi, with 10^5 and 10^6 cfu/g soil compared to fungal population of 10^2 and 10^4 cfu/g soil (Figure 1). These results were consistent with the behavior of microbial populations in soil reported by Alexander (1994). The bacterial population of the rhizosphere soil were negatively correlated (r=-0.60) with the altitude of the potato fields. The fields at Aymara, for example, at 4,030m above sea level (asl), had the lowest populations, while at Pucara or Cajamarca situated about 700 to 1,000 m lower than Aymara, bacterial populations were clearly higher. This relationship between altitude and bacterial populations was also observed in rhizosphere soil of the Himalayan region (Pandey 2004). Altitude in this respect represents the effect of several

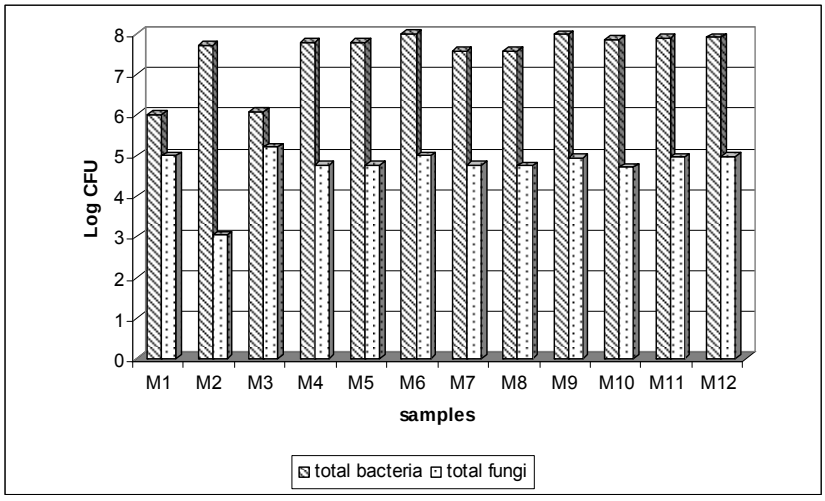

Figure 1. Total bacterial and fungal populations from 12 rhizosphere samples of potato plants

abiotic factors, such as rainfall, temperature but also biotic ones as for example, potato variety or crop management. However, no significant correlation between temperature or rainfall and bacterial or fungal rhizosphere populations could be found, indicating a rather complex relationship then a dependence on single factors. Only electric conductivity, also related to altitude, showed a negative correlation with bacterial populations (r = -0.53), Usually fungal population are more competitive in acid soils (Alexander, 1994), however it seems that the specific conditions of the rhizosphere alter this relationship as no direct correlation between soil pH and fungal populations could be established (Marschner et al., 2004).

Table 2. Physico-chemical characteristics of 12 potato fields soils

Parameters	Samples											
	M1	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**M8**	**M9**	**M10**	**M11**	**M12**
pH	4.1	7.5	5.5	4.2	7.2	7.4	5.0	4.7	4.5	5.1	5.5	7.6
C.E.(dS/m)[a]	1.1	0.9	0.7	0.8	0.4	0.8	0.3	0.3	0.3	0.2	0.4	0.2
$CaCO_3$ (%)	0	19.5	0	0	0	0	0	0	0	0	0	2.2
M.O. (%)	9.5	3.1	2.5	9.5	2.9	6.3	3.42	6.56	4.71	3.05	3.6	3.4
P (ppm)[b]	52	62	39	9	30	49	44	59	36	50	44	48
K (ppm)[b]	520	341	150	295	195	365	459	304	356	296	363	383
Sand (%)	58	58	32	68	24	28	40	56	52	52	38	44
Silt (%)	32	32	36	24	44	60	42	34	40	34	49	32
Clay (%)	10	10	32	8	32	12	18	10	8	14	13	24
Texture type	sandy loam	sandy loam	clay loam	clay loam	clay loam	silt loam	loam	sandy loam	loam	loam	Loam	loam
CIC (me/100g)[c]	23.0	11.7	13.1	25.1	15.2	8.6	11.2	16.3	16.8	11.5	15.2	22.4
Ca^{+2}	1.65	10.18	5.12	1.62	11.97	5.54	4.02	3.39	1.68	2.97	4.34	18.57
Mg^{+2}	0.67	0.85	1.67	0.35	2.72	2.35	1.35	1.07	0.63	1.37	1.32	1.62
K^+	0.71	0.55	0.36	0.41	0.4	0.61	0.89	0.54	0.62	0.62	0.69	0.82
Na^+	0.09	0.1	0.12	0.11	0.11	0.1	0.1	0.12	0.17	0.12	0.15	0.15
$Al^{+3}+ H^+$	2.9	0	0.3	2.6	0	0	0.4	0.9	1.7	0.6	0.5	0
N (%)	0.58	0.17	0.14	0.52	0.15	0.36	0.26	0.45	0.39	0.21	0.19	0.26

[a]Electric conductivity in deciSiemens/meter. [b]parts per million. [c]Cationic Exchange capacity in miliequivalent per 100g of soil

Of the total bacterial rhizosphere population several genera of bacteria were isolated which potentially possess plant growth promoting capacities, such as *Pseudomonas, Azospirillum*, *Azotobacter* and *Actinomycetes*. A total of 62 *Azotobacter* strains, 45 *Actinomycetes*, 68 *Pseudomonas* and 55 *Azospirillum* were obtained from the surface of roots, their interior or the rhizosphere soil (Figure 2). No correlation could be found between the diversity of these genera (number of strains) and their abundance (strength of population), i.e. bacterial strains, although viable and colonizing plant roots, might be dominated and limited in growth by other bacteria or different microorganisms occurring in less diversity but with a stronger competitiveness due to the prevailing growth conditions in the rhizosphere (Ramos et al., 2000).

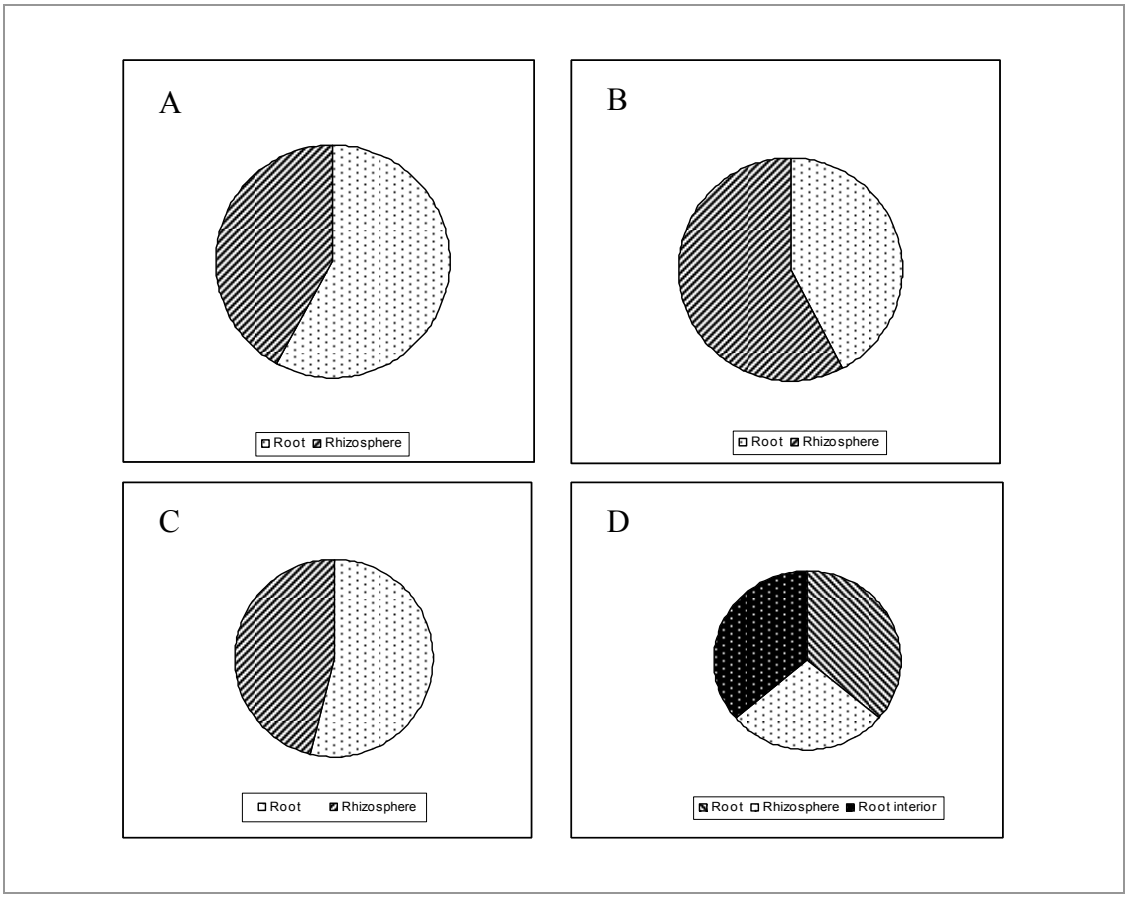

Figure 2. Isolated strains of different PGPR genera from distinct micro-environments (root surface, rhizosphere and root interior). A. *Azotobacter* spp. B. *Actinomycetes*. C. *Pseudomonas* spp. D. *Azospirillum* spp.

Azospirillum strains were found mainly in the root interior and root surface, as this genus is more associated with the root environment than soil rhizosphere (Bashan, 1995). *Pseudomonas* and *Azotobacter* strains were more associated with the root surface than rhizosphere, while *Actinomycetes* were rather found in the rhizosphere than on the root surface.

Pseudomonas spp. showed the greatest diversity of strains from plant rhizosphere or roots, compared with the other genera, confirming previous results from Breza-Boruta (2003) who observed the predominant role of fluorescent *Pseudomonas* among other rhizobacteria in potato. 72% of the *Pseudomonas* isolates originated from fields managed with low inputs (none or little inorganic fertilizer applied); thereby showing a positive correlation ($r = 0.59$) with soil total nitrogen content indicating a relationship to soil organic matter contents and crop management (amounts and types of applied nitrogen fertilizer, fallow periods etc.), confirming, for example, studies which found that organic nitrogen mainly from manure stimulated root colonization of *Pseudomonas* and that ecological farming systems favored the abundance and diversity these bacterial strains (Hoeflich et al., 2000; Breza-Boruta and Paluszak, 2003).

Mean maximum and minimum temperatures showed a positive correlation with *Azospirillum* diversity ($r_{max}=0.87$; $r_{min}=0.57$), i.e. growth and competitiveness of these bacterial strains were affected by low temperatures. A result which concurs with conclusions by Harris et al. (1989), that *Azospirillum* populations and diversity in winter crops are low due to their poor establishment and survival in low temperatures.

The amount of rainfall at the different locations had a positive effect on the abundance of *Actinomycetes* populations ($r=0.61$) and with increasing altitude the number of *Azotobacter* strains increased ($r=0.62$). No correlation could be established between abundance of *Actinomycetes* and *Azotobacter* and soil pH, although in bulk soil these two genera are sensitive to variations in soil pH, generally preferring alkaline conditions (Hervé

et al., 1994). Apparently the special conditions of the rhizosphere environment compensated the pH effect of the soil and facilitated bacterial development even in strongly acid soils.

The diversity and abundance of rhizosphere populations of bacteria and fungi are to some extent directly influenced by environmental and physicocemical factors, such as soil pH, rainfall temperature etc. However, the plant creates specific conditions within the soil surronding its roots, altering the pH, supplying nutrients and other compounds, which seem to modify adverse soil-environmental conditions and might have a similar or stronger influence on bacterial development than these 'external' biotic or abiotic conditions. Therefore, correlations could be established between bacterial development and temperature or rainfall; factors, which can only be slightly influenced by the plant, while, for example, the effect of soil pH was not significant because this factor is rather controlled by root activity.

The potato rhizosphere habours a great variety of bacterial strains which possess the potential to increase plant growth and crop yield (Oswald et al., 2009). The advantage of the isolation of indigineous populations are that they are well adapted to the local agro-ecological conditions and may have a better competitiveness as introduced organisms. But their high diversity and abundance also indicates that the potato crop uses these beneficial microorganisms in natural conditions. Hence, a PGPR atrificially applied to a crop not only has to be competitive in colonizing the plant root but also has to be more effective in supporting plant growth than the average of naturally occuring PGPRs. Given the diversity of bacterial strains with a PGP-potential encountered in the potato rhizosphere, the isolation and selection of such strains of excellence should be possible and could generate a significant impact especially in low-input agricultural systems.

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Assessing Potato Yellow Vein Virus (PYVV) infection using remotely sensed data and multifractal analysis

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Abstract

Potato Yellow Vein Virus (PYVV) reduces potato production in South America. Visual crop monitoring is a standard practice but the disease is generally detected after significant damage has occurred to photosynthetic tissues. Thus, a method for monitoring crop condition at different spatial scales to detect the disease before yields are severely affected is needed. Remotely sensed multispectral reflectance, based on the reflectivity and propagation of light inside plant tissues, was tested for the detection of PYVV infection in potato plants grown indoors. Visual assessment of symptoms in both virus-infected and healthy virus-free plants was compared to monitoring based on spectroradiometry and multispectral photographic images of the plants. Observed disturbances in light reflection by vascular tissues in infected plants, as well as spectral Vegetation Indices such as NDVI, SAVI, and IPVI, provided early detection of viral infection, long before symptoms of chlorosis were visually detected. To improve the earliness of detection, the raw remote sensing data from two subsequent experiments was further processed by multifractal analysis, a mathematical tool that addresses the scale dependency and variability of geophysical variables. Results showed that early diagnosis of PYVV infection was improved, providing the earliest detection of infection ever reported. For the first data set, the infection was evidenced 12 days after inoculation (23 days before the visual assessment did see the symptoms). For the second data set, our analysis denoted the disease 4 days after inoculation (33 days prior to the appearance of symptoms).

Introduction

Potato Yellow Vein Virus (PYVV), a Crinivirus of the Closteroviridae family (Salazar, 1998; Salazar *et al.*, 2000), is considered a threat to potato production in South America (Saldarriaga *et al.*, 1988; Salazar, 1998). PYVV is transmitted semi-persistently by 1) the whitefly *Trialeurodes vaporariorum* Westwood (Diptera, Sternorrhyncha, Aleyrodidae) (Díaz *et al.*, 1990; Tamayo and Navarro, 1984; Salazar, 1998; Salazar *et al.,* 2000), 2) tuber seed (Díaz *et al.*, 1990; Salazar, 1998) and 3) by aboveground and underground stem-grafts (Salazar, 1998). The virus does not affect the size and morphology of plants; the typical yellowing of the veins appears between 30 and 40 days after infection, when stems and leaves are already formed (Saldarriaga *et al.*, 1988). Research carried out on sugar beet (*Beta vulgaris*) also infected with a closteroviridae, the Beet Yellow Virus (BYV), showed damage in the photosynthetic mechanism of plants resulting in the reduction of net photosynthesis (Clover *et al.*, 1999). One effect of the virus was the reduction of stomatal conductance, and a 50% reduction of the splitting of water in isolated chloroplasts (Spikes and Stout, 1955) with thylakoidal membrane damage (Baker and Horton, 1988; Salazar, 1998). Similar effects have been observed with PYVV in potatoes by Saldarriaga *et al.* (1988). Unpublished research at CIP shows that PYVV does infect the phloem cells and prevents the flow of carbohydrates, reducing yields drastically.

PYVV infections are not evenly distributed across the field but they may occur in patches in current-season infections. A most widely used practice in the control of PYVV infection is the visual determination and manual elimination or roguing of infected plants and the spraying of pesticides over the whole stand at different times during the cultivation cycle, for the control of vectors. However, the use of pesticides increases costs and pesticide residue levels in agricultural products and soils, and contributes to ground water contamination.

Furthermore, indiscriminate use of pesticides could eliminate natural biological control agents, thus increasing pest-insect populations, such as *Trialeurodes vaporariorum*. For effective disease control, early identification of infection patches in the field is required, allowing for the eradication of infected plants and avoiding the spread of disease (Agrios, 1997).

The multispectral analysis is based on the reflectivity and propagation of solar radiation from and within plant canopies and tissues, where a fraction is absorbed and other reflected in all directions (Gilabert *et al.*, 1997). Reflectivity is linked to the biochemical and structural components of the plant, such as chlorophyll, water, proteins and cell wall materials (Gamon, 1992; Ritchie, 2003), which are affected by diseases, resulting in differences in the spectral signature of healthy and stressed plants.

The present work was aimed at 1) assessing the usefulness of remotely sensed multispectral reflectance imagery and spectroradiometry to determine PYVV infection in potatoes, before symptoms become visually perceptible, and 2) assessing the usefulness of applying multifractal analysis and the wavelet transform to remotely sensed multispectral imagery and spectral-radiometrical data, to enhance their precision and earliness in detecting PYVV infection in plants. Multifractal theory permits the characterization of complex phenomena for both temporal and spatial variations. An important property of the multifractal parameters is that they are scale invariant (Schertzer and Lovejoy, 1989), which means that the information they provide are constant across different scales, allowing for a valid extrapolation up and down scales. This property is particularly relevant in the work herewith reported, as it confers robustness and consistency to the observed results. Thus, it was hypothesized that applying multifractal analysis to remotely sensed multispectral data would improve the diagnostic capabilities of multispectral reflectance imagery and spectroradiometry to determine PYVV infection in potatoes.

Materials and methods

Plant material and treatments

In the first stage of research, three experiments conducted under a split-plot in time design with inoculated and control treatments in the main plot, and time measurements as sub-plots, were carried out indoors in Lima, Peru, from July to November 2004. A total of 27 potato plants, cv. Costanera, six infected with PYVV and 3 virus-free as control, comprised each repetition. In the second stage of research, two indoors experiments were carried out in Lima, Peru, from May to September 2007. Twenty plants of potato cv. Yungay per experiment, divided in Control and virus-inoculated PYVV plants comprised each repetition.

In both stages (2004 and 2007), plants were initially germinated in a nursery and then transplanted into individual 6 litres pots containing a mixture of compost (10%), vegetal soil (70%) and perlite stone (20%) as substrate. Then, the PYVV infection was induced through lateral grafting in PYVVi plants some 2 weeks after emergence. The environmental conditions (temperature, relative humidity, light) and management were similar for infected and control plants. The pots were located under an insects-free net house and irrigated to maintain the soil at field capacity.

Data collection

In both 2004 and 2007, a comparative visual assessment of both PYVV-infected plants (PYVVi) and healthy virus-free plants was continuously conducted to monitor the development of disease symptoms in the former. At the same time, multispectral photographic images of the same plants were recorded during their growth and development cycle, using an Agricultural Digital Camera (ADC Dycam, Inc., red and near infrared sensors, in the first stage; Tetracam Inc., red, green and NIR sensors, second stage). The camera was placed 0.60 m above the photographed plants. Pictures were taken at the same time of day every 4-5 days. The images were analyzed through the freeware software Briv32 (Band Rationing Image Viewer, Tetracam Inc. USA) to obtain the spectral vegetation indices (SVI): the normalized difference vegetation index (NDVI = $(R_{NIR}-R_{red})/(R_{NIR}+R_{red})$, R=reflectance, ranging from –1 to +1) that track changes in chlorophyll concentration (Dobrowski *et al.*, 2005); the soil adjusted vegetation index (SAVI = $[(R_{NIR}-R_{red})/(R_{NIR}+R_{red}+L)*(1+L)]$, where L ranges from 0 to 1), proposed as a soil-line vegetation index to reduce the background contribution (Huete, 1988); and the infrared percentage vegetation index (IPVI = $R_{NIR}/(R_{NIR}+R_{red})$), a ratio-based index that holds a limited range with no negative values (0<IPVI<1), whose main disadvantage is its sensitivity to atmospheric noise (Crippen, 1990). These SVIs had proved to be good indicators of infection by PYVV in potato (Chávez *et al.*, 2009a).

Light reflectance from the individual plants was recorded during approximately 40 days after the virus inoculation, until the disease's symptoms were visually perceptible. During the first stage, measurements of light reflectance were taken weekly using a Li-Cor 1800 spectroradiometer (Li-Cor Inc., Nebraska, USA) covering the 350–850 nm wavelength region. The aperture angle of the fore optics of the spectroradiometer was 180° that became 60º because of its built-in cosine corrector, so the instantaneous field of view (IFOV) was 0.2m diameter (distance from canopy was 0.2m). Measurements were taken from nadir. In the second stage, a computer assisted ASD Fieldspec Pro spectroradiometer (Analytical Spectral Devices Inc., Colorado, U.S.A.), covering the 325–1075 nm wavelength region, was used for data recording. 2007 comprised a first experiment with an aperture angle of the fore optics of 25º, and a second one using a collimator to reduce the aperture angle to 1º. Measurements were taken from nadir at a distance of 30 cm from the plant canopy, resulting in a IFOV of 13cm and 0.52cm diameter, respectively, first and second experiment. A white teflon and a Spectralon® panel were used in 2004 an 2007, respectively, for converting the reflected radiation into relative reflectance values. In the 2007's experiments, measurements were taken every 2 - 3 days with the sun at 30º of zenith angle throughout the observational period.

Data processing

The reflectance spectra data obtained in 2004 were assessed by dividing the spectrum measured into the main four sectors (blue, green, red and near infrared) for calculating the percentage of reflectance against time. First- and second-order derivatives were also calculated from the spectroradiometric measurements through the IDL 6.1 software (Research Systems Inc.), as well as for the values of the vegetation indices calculated from the spectral photography. A similar assessment of reflectance spectra was carried out with the data from the second stage (2007) to contrast the information it provided against the results of the wavelet-multifractal analysis of the same data.

A software that describe the canonical method of wavelet multifractal modulus maximum, originally implemented by McAteer *et al.* (2007) to run it in IDL6.2 for Linux, was modified to analyze our reflectance spectrum data. The methodology described by Arneodo *et al.* (1988) was followed and adapted to run in IDL6.3 for Windows. The reflectance data obtained in 2007 experiments were processed with the Continuous Wavelet Transform (CWT) and the wavelet transform modulus maxima (WTMM) method using as mother wavelet analyser the second derivative of the Gaussian function (Mexican hat). For more details on the subject, see the papers by Arneodo *et al.* (1995), McAteer *et al.*, (2007) and Chávez *et al.* (2009b).

Data pre-processing for multifractal analysis

The pre-processing consisted of two steps. In the first one a background correction was required to account for changes in the signal due to both measurement errors and natural changes in the atmosphere while measuring all the plants within a sampling date. The background correction was performed by adjusting the measured response (G) of individual plant signals to a reference response through fitting a linear regression (Equation 1; Yarlequé, 2009). In the second step, the anomalies over moving averages of 41 wavelengths (see equations 2 and 3) were calculated. Anomalies reduce the signal to noise ratio thus making small fluctuations in the physiology of the plant more perceptible by the analyses.

Background correction: $$S(t_i) = A.G(t_i) + B \qquad (1)$$

Where, $A = \frac{(G_{max} - G_{min})}{(G_{Total\ max} - G_{Total\ min})}$. In this equation the numerator is measured from every plant, while the denominator is the difference between maximum and minimum values of recorded reflectance among all the plants (Ctrl and PYVVi) in one determined date; and G and S are the measured and estimated passive reflectance according to t wavelength (nm), respectively. Finally, $B = G_{min} - A.G_{Total\ min} = G_{max} - A.G_{Total\ max}$

Moving average: $$\hat{S}(t_i) = \sum_{k=i-20}^{i+20} \frac{S(t_k)}{41}, \quad , \qquad (2)$$

Anomalies: $$S'(ti) = S(ti) - \hat{S}(ti) . \qquad (3)$$

Statistical analyses

Differences between means of infected and healthy plants were revealed by split plot in time design with repeated measurement analyses and independent samples test, using the SAS software package (SAS, North Carolina, U.S.A.). The GLM method described by Wolfinger and Chang (1998) was used for the repeated measurement analyses.

Results and discussion

Reflectance measurements

In the 2004 experiments, a distinct reflectance pattern from infected plants was evident 23 days after inoculation (dai) (Figure 1) through changes in reflectance in the blue region (450–495 nm) ($P<0.01$). Reflectance in the green region (495–570 nm) provided the same response at a similar time after infection, but it seems to be less reliable ($P<0.05$). Responses in the red region (620–750 nm) presented more noise and no differences between infected and healthy plants were detected ($P>0.05$). As to the NIR region (> 750 nm) differences in reflectance could be detected as early as 11 dai ($P<0.05$). However, the responses in the NIR were highly variable in time, making it an unreliable indicator of the presence of symptoms.

In 2007, it was impossible to determine the infection through the direct observation of the reflectance data obtained through a 25º solid-angle sensor's aperture. However, when the data was pre-processed prior to multifractal analysis, differences between treatments were detected ($P<0.05$) from the 12th dai (i.e. 23 days before symptoms became visible (Figure 2) as visual symptoms of viral infection in PYVVi plants were noticed from the 36th dai onwards). From the 21st to the 26th dai, the singularity spectrums of both treatments were transiently similar, suggesting a recovery of the PYVV infected plants, but differences again became significant from 28th dai onwards.

The second experiment of 2007 was designed to test whether a higher precision of the IFOV would reveal changes directly from recorded data, thus the sensor aperture of fore-optics of the spectroradiometer was adjusted to 1º by means of a collimator. In this instance, the multispectral reflectance revealed that PYVVi and Ctrl plants originated very distinct spectra that were easily noticeable, well before symptoms of PYVV infection were visually observed, which occurred 37 days after infection. Moreover, the multifractal analysis of both raw and pre-processed data strongly enhanced the evidence of such spectral differences making them noticeable even earlier than in the previous experiment, the 4th dai –i.e. 33 days before the symptoms were visible (Figure 3). As observed before, an apparent unexpected transient recovery of PYVVi plants occurred from the 10th to the 15th dai.

Earliness of PYVVi diagnosis was sharper and faster in the second experiment 2007 as a consequence of differences in the solid angle of sensor aperture producing a different size of their IFOV. As described above, the first experiment had a fore-optic aperture of 25º that provided an IFOV of 13cm of diameter, whereas in the second experiment the aperture was 1º and the IFOV diameter was 0,52cm. The smaller sensed area gave a sharper measurement as the incoming scattering was close to zero. In contrast, in the first experiment, the 25º of fore-optic aperture permitted a higher scattering influence from the surrounding area, producing a less sharp measurement.

Visible and Near-Infrared reflectance

Dividing the spectra into main regions for the 2007 experiments, a distinct reflectance pattern from infected plants was evident for several wavelengths at 25 dai (25º of sensor aperture) ($P<0.05$), and 23 dai (1º of sensor aperture) ($P<0.05$). The blue region (450-495 nm) showed the most robust statistical response. Reflectance in the green region (495–570 nm) seemed to be a less reliable indicator. Responses in the red region (620–750 nm) presented more noise. Near-infrared reflectance were highly variable in time (data not shown), then NIR would be an unreliable indicator of the infection, confirming the results obtained by the multifractal analysis described in the section above. Our results suggest that multifractal analysis of the entire visible region or the complete reflectance spectrum (visible and NIR) is required to obtain reliable information about the PYVV infection in potato plants. They also confirmed the accuracy of this methodology as obtained by Chávez *et al.* (2009) in which telltale anomalies in reflectance were detected some 14 days before symptoms were visible. In our previous work we used a sensor with an aperture of 180º -that became 60º due to its built-in cosine corrector-

without significant changes in the results-, so it would indicate that with sensor aperture ≥ 25º there might be no substantial changes in the measure.

Spectral vegetation indices

Dobrowsky *et al.*, (2005) pointed out that the majority of vegetation indices (SVI) are not sensitive to rapid changes in plant photosynthetic status caused by environmental stressors, due to most SVI have not direct link to photosynthetic functioning. Nevertheless, our results did show (Figure 4) a good diagnosis capability of NDVI, SAVI and IPVI for viral infection in potato plants, suggesting that damage to the photosynthetic tissues produced by the virus infection are rapidly reflected in the components of the SVI. In fact, the SVI's calculated from the multispectral images captured by the 3 bands (red, green and NIR) agricultural camera did show differences around 5 days before the diagnosis obtained in the previous work, which used a 2 bands (red and NIR) camera.

Recovery period

The recovery of infected plants could be explained by the adaptive defense mechanisms of plants specially targeted on avoiding viral infections, called RNA silencing. The plant perceives information from the infecting virus genome and produces a specific defensive response for that genome (Argerter, 1999). The silencing RNA mechanism mediates the post- transcriptional repression of the target gene expression and represses the proliferation and expression of different invading nucleic acids, such as those carried by viruses, viroids, transposons or transgenes, as well as regulates the gene expression (Baulcombe, 2004). The silencing response may not be limited to the plant cell the virus is actively infecting but extends into newly dividing cells at the plant's growing points, and enables plant cells far removed from the initial infection site to be prepared when viruses get to them (Argerter, 1999). Young plants can exert more resistance to viral infection and endogenously expressed viral transcripts (Siddiqui, 2007), so it could explain the temporary recovery of PYVVi plants, since plants at this stage were juveniles. Later on, the barrier of silencing RNA was overwhelmed by the infection and symptoms developed. Ding (2000) and Baulcombe (2008, personal communication), explained that the recovery is cyclical, but, after a period the virus accumulates to higher levels and the disease overcomes the plant.

Conclusions

Early diagnosis of PYVV infection in potato plants was improved by applying multifractal analysis to the primary remotely sensed multispectral reflectance data recorded in 2007, allowing for the earliest detection of infection ever reported: 12 dai –i.e. 23 days prior to the appearance of visual symptoms –and 4 dai –i.e. 33 days before visual symptoms appeared- (1º of sensor aperture).

Multifractal analysis of pre-processed data was very effective and sharper than multifractal analysis of raw data obtained through a fore optic sensor aperture of 25º. It appears that pre-processing is necessary when the solid angle aperture sensor is 25º, but it is not imperative when the solid angle aperture sensor is 1º, due to the precision of focus. So, pre-processing (background correction and the use of anomalies) increases the signal to noise ratio thus increasing the likelihood of earlier detection of differences

The main advantages of multifractal analysis lay on the earliness of diagnosis of PYVV infection in plants and the statistical accuracy of results. This is due to the amplification of differences, which is an intrinsic feature of the methodology. The sensitivity with this processing methodology is such that differences prior to the fast and interim recovery period can be detected.

The spectral vegetation indices NDVI, SAVI and IPVI confirmed their usefulness in accurately evidencing the infection caused by PYVV, and the use of a three bands camera improved the earliness of prediction in 5 days compared to the diagnosis based on the use of a two bands camera as reported before. Nonetheless, with SVI's the differences are evident after the recovery period.

The methodology based on dividing the multispectral reflectance spectrum into the main wavelength regions (blue, green, read, NIR), has confirmed its accuracy in diagnosing the PYVV infection, 25 and 23 days after the virus inoculation.

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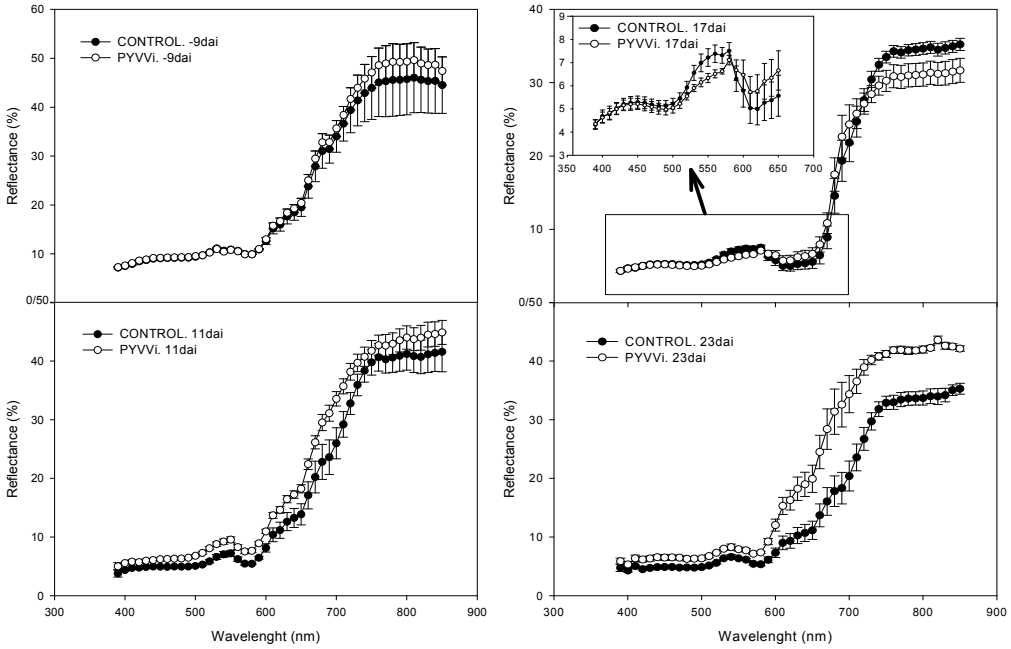

Figure 1. Reflectance of potato plants, 2004, Reflectance differences between treatments were observed from 11 dai onwards

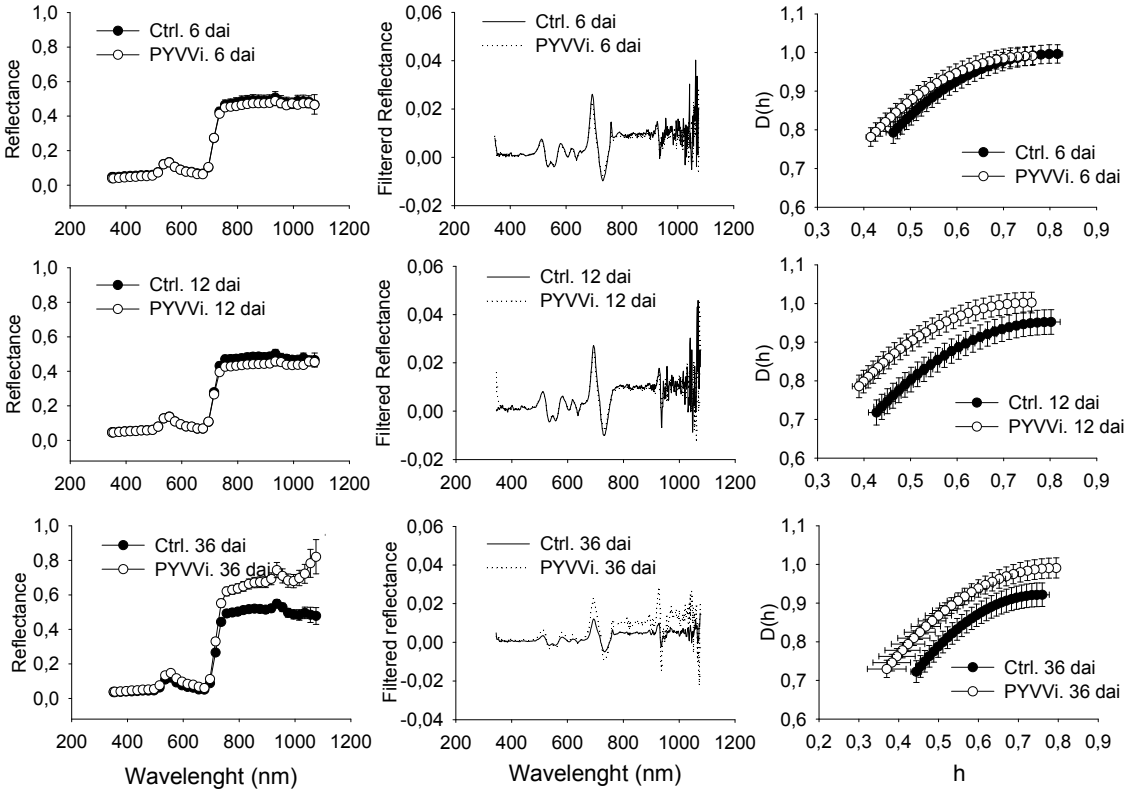

Figure 2. First 2007 experiment, 25º of sensor aperture. Passive reflectance of plants (*left*), the same reflectance after background correction, moving average and anomalies (*centre*), and their correspondent singularity multifractal spectra (*right*).

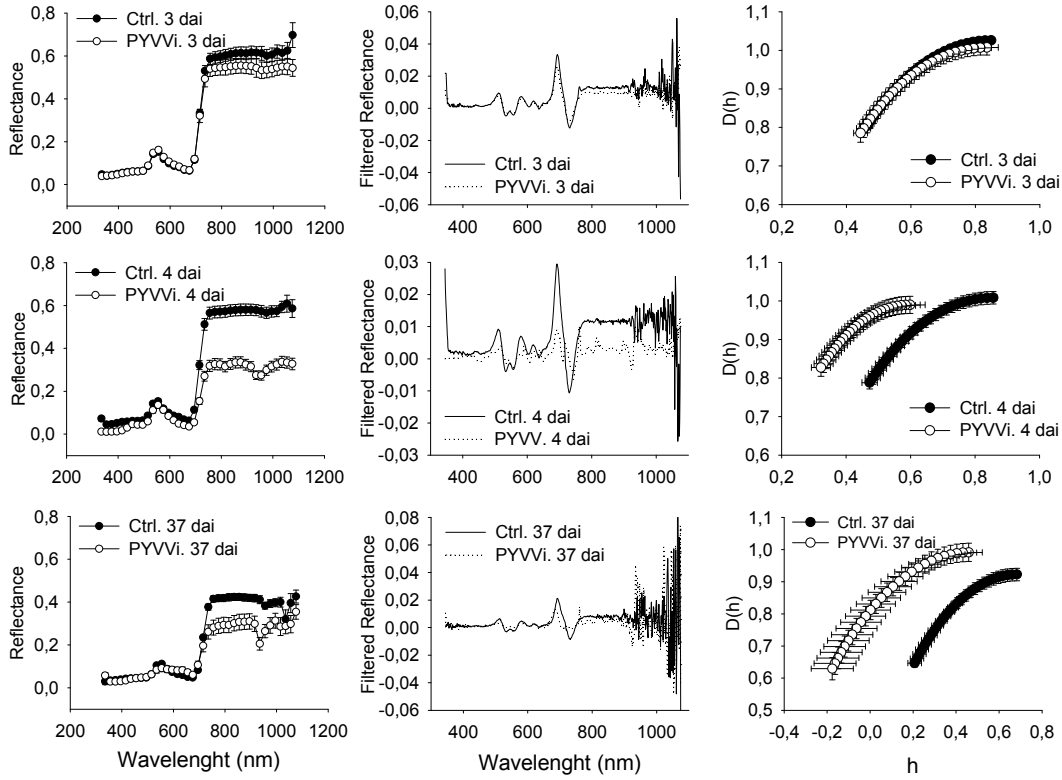

Figure 3. Second 2007 experiment, 1º of sensor aperture. Passive reflectance of plants (*left*), the same reflectance after background correction, moving average and anomalies (*centre*), and their correspondent singularity multifractal spectra (*right*). Multifractal spectrum reveals changes at the 4th dai.

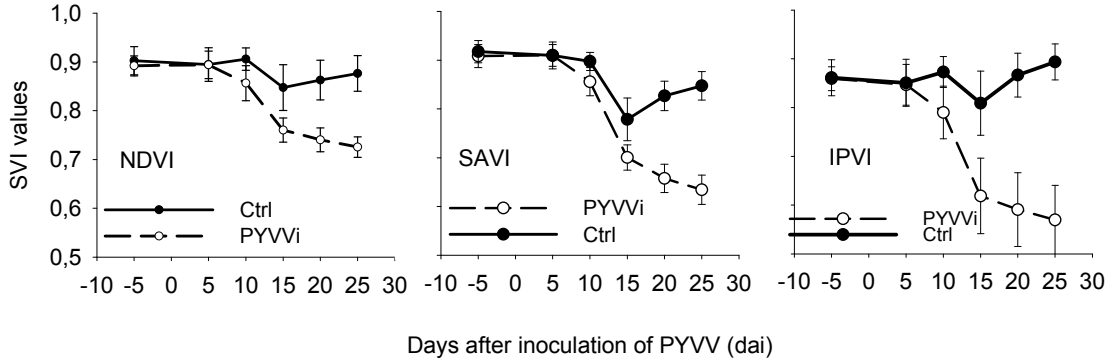

Figure 4. Spectral Vegetation Indices of the second experiment, 2007

Muña (sp. *Minthostachis mollis*) essential oil, as a natural alternative to control potato sprouting tested under different storage conditions.

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Abstract

Preferences of modern consumers for healthy food are challenging researchers to seek natural options to control potato sprouting. Early Incas used muña plant (sp. *Minthostachis mollis*) as an insect deterrent in potato storage due to its high content of terpenoids in its essential oil. Sprout suppressing property of muña essential oil, was tested during 4 months at room temperature and cold storage conditions (8°C/HR85%) at two different concentrations (50 and 100ppm), as compared to commercial rate of CIPC and non-treated control on quick sprouting native potato variety Amarilla Tumbay. Muña oil and CIPC treatments were applied with a hand spray, oil treatments were repeated every 15 and 30 days at both storage conditions. Effectiveness of muña oil to suppress sprouting (length and weight of sprouts) was not significantly different from CIPC when applied every 15 days, either at both concentrations, and treated potatoes stored at 8°C for 4 months. It was observed that suppressing effect of muña oil was lost when potatoes were treated every 30 days and then stored at room temperature or at cold storage conditions, because sprout suppressing property of muña oil is due to burning of tender sprout tips tissue. Therefore, it appears that after 15 days the tissue development of sprouts, become thicker and more resistant to the burning effect of muña oil and or can recover quickly to resume growth. No off odors were detected by a taste panel in boiled potatoes treated with muña oil.

Keywords: sprout suppressant, essential oil, storage, postharvest.

Introduction

Sprouting control is a constant concern during potato storage (sp. *Solanum tuberosum* L.), therefore CIPC (chlorpropham) is commonly used by the potato processing industry as sprout inhibitor since 1952 (Lewis, et al. 1997). However, in recent years studies related to excess in maximal residue limits (Noël, et al. 2002; Noël, et al. 2003) and possible side effects on human health are raising public concern on the use of chemicals in the food processing industry. Although no direct toxic side effects have been detected in humans because of the use of CIPC, a number of studies have been conducted to test natural products to control potato sprouting in storage (Kleinkopf, et al. 2003; Elsadr and Waterer, 2005) that can result in new options for organic production and organic postharvest management. In this regard Frazier, et al. (2004) found that both peppermint and spearmint oils were equally effective sprout suppressants, but peppermint oil caused fewer problems with culinary and palatability concerns. As compared to CIPC effect that has a direct effect on cell division, volatile oils and hydrogen peroxide are more correctly called sprout suppressants, as they physically damage burning developing sprouts with a high concentration of the product (Frazier, et al. 2004). In Perú, muña (sp. *Minthostachis mollis*) is a wild Andean herb that grows between 2500 to 3500 m of altitude, it has been utilized mainly as a medicine plant, but also in potato storage as an insect deterrent since pre-Inka times (Fournet et al 1996; Morris, 1985) because of its high content of terpenoids (Fuertes and Munguia 2001). Its potential as sprout suppressant has been reported by Aliaga y Feldheim (1985) in lab, but no storage trials have been conducted under controlled conditions. This comparative study evaluated the performance of muña natural oil as a natural alternative to CIPC to control sprouting in treated potato tubers kept at room temperature and cold storage for 4 months.

Materials and methods

This study was conducted in the storage facilities at the International Potato Center headquarters in Lima. A commercial concentrated emulsion formulation product of sprout inhibitor CIPC 300 g/L was utilized at the

recommended rate (60cc/ton) and two concentrations of muña essential oil (50 y 100 ppm) were applied to two lots of 150 kg of quick sprouting native variety Amarilla Tumbay (*Solanum goniocalyx*). Then each lot was grouped following a RCB design with 3 reps in 42 carton boxes, each containing 8 net-bags with 5 tuber samples. Both lots were placed under two different conditions, one lot was placed for 4 months at cold storage at 8°C/HR85% and the other lot was kept at room temperature (21°C/ HR78%) for the same period of time, in each lot there was an untreated control. The muña essential oil was applied prior to sprout development in two ways (spray and wick application) and two application frequencies were tested (every 15 and 30 days). The application schedule of all sprouting control treatments started on Nov 5th 2007 and finished on March 2008, considering a 10 days elapse to apply sequentially the treatments and to avoid sample congestion at the evaluation time. The evaluation of samples consisted in randomly sampling a net-bag from the box and registering tuber data: initial weight, weight loss, specific gravity, as well as length and weight of sprouts. Analysis of variance and mean comparison tests were used, as well as the area under the curve technique (Shaner and Finney, 1977) was used to calculate and estimate the sprout growth development. Statistical computation of data was done utilizing Proc GLM (SAS Institute Inc. 1989). Treated potato tubers were boiled and tested for odd odors by a taste panel.

Results and discussion

Analysis of variance conducted for specific gravity, as well as length and weight of sprouts showed significant differences ($p<0.001$) in response to the tested treatments. The muña essential oil was an efficient sprout suppressant as observed when evaluating length and weight of sprouts, its effect was not significantly different from CIPC (Table 1). The suppressant effect was observed during 4 months with both tested oil concentrations, but only when the oil application was carried out every 15 days and the treated potato tubers were stored at cold storage conditions (Figure 1). A similar result was obtained by Elsadr and Waterer (2005) who found that purified plant extracts (diallyl disulphide and carvone) completely suppressed sprouting for 14 days. When muña oil was applied at both concentrations using a saturated wick (MuñaW_50ppm and MuñaW_100ppm) to mimic a fumigation application, the sprout development continued during the same cold storage period at a rate not significantly different from the untreated control, even though the treated tubers were kept at low temperature.

Table 1. Sprout length (cm) in cold room storage at 8°C by product applied every 15 days for 4 months

	Frequency of application (15 days)								
	0	15	30	45	60	75	90	105	120
CIPC	0	0 c	0 b	0 b	0 c	0 c	0 d	0 c	0 C
MuñaS_50ppm	0	0 c	0 b	0 b	0 c	0 c	0 d	0 c	0 C
MuñaS_100ppm	0	0 c	0 b	0 b	0 c	0 c	0 d	0 c	0 C
MuñaW_50ppm	0	1,2 b	1,9 a	2,4 a	3 a	3,4 a	3,5 ab	4,3 a	4,4 ab
MuñaW_100ppm	0	1,7 a	2,1 a	2,4 a	3,6 a	3,8 a	3 bc	4,7 a	4,8 a
Control	0	1,7 ab	1,9 a	2,4 a	2,7 ab	2,8 b	2,8 c	3,3 b	3,8 b
DMS	n.s.	0,49	0,3	0,28	0,6	0,56	0,59	0,56	0,61

Common values followed by common letters don not differ significantly

The progression of the sprout growth on treated tubers with muña oil at 50 ppm sprayed every 15 days during 120 days and maintained under cold room conditions as compared to CIPC and the untreated control is shown in Figure 1. The effect of muña oil at 100 ppm was also successful under the same conditions of application and storage, although the application of muña at both concentrations with wick failed to control sprout development. CIPC was applied once at the beginning of the storage period.

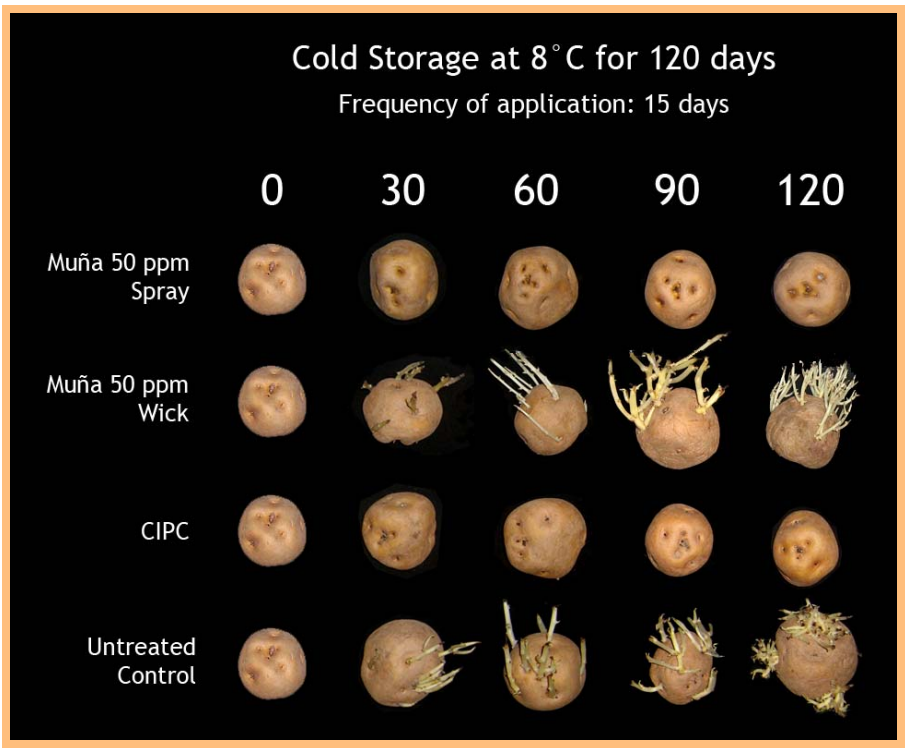

Figure 1. Sprout growth on treated tubers with muña oil at 50ppm every 15 days as compared to CIPC and the untreated control, stored under cold room conditions. (8°C/HR85%) for 120 days

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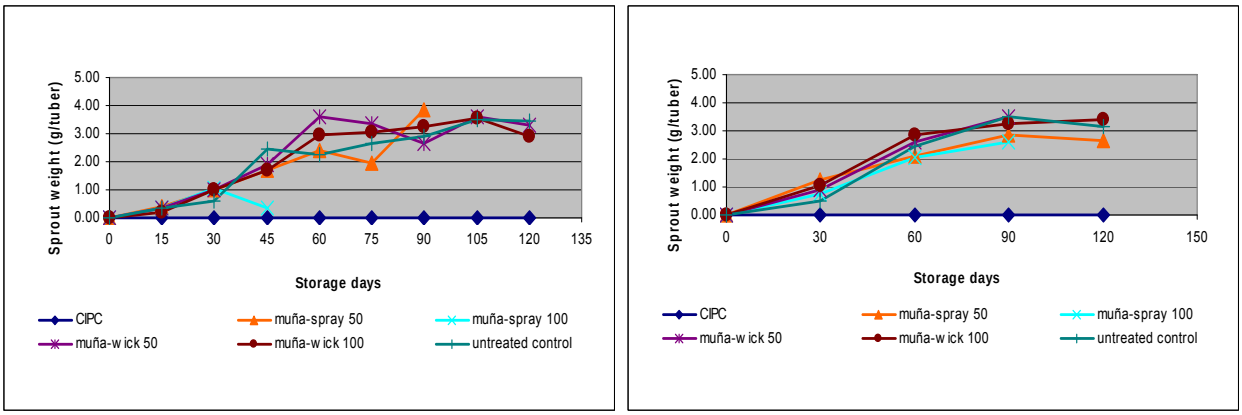
It was observed that the suppressant effect was lost when the frequency of application was every 30 days and the storage of treated potato tubers was done either in cold room (Table 2) or at room temperature (Figure 2).

Table 2. Sprout length (cm) in cold room at 8°C by product applied every 30 days for 4 months

	Frequency of application (30 days)								
	0	30		60		90		120	
CIPC	0	0	c	0	c	0	d	0	d
MuñaS_50ppm	0	1,9	b	1	b	2,2	c	3,2	b
MuñaS_100ppm	0	2,1	ab	0,1	c	0,5	d	2,1	c
MuñaW_50ppm	0	2,4	a	2,9	a	3,1	b	4,2	a
MuñaW_100ppm	0	2,1	ab	2,8	a	3,6	ab	3,5	ab
Control	0	2	ab	2,5	a	2,9	bc	3,7	ab
DMS	n.s.	0,43		0,72		0,8		0,8	

Common values followed by common letters don not differ significantly



15 days 30 days

Figure 2. Variation in sprout weight (g/tuber) when tubers were treated with CIPC every 15 and 30 days with muña essential oil at two concentrations (50 and 100ppm) and in two ways (spray and wick application) and kept at room temperature, as compared to the untreated control.

These results indicate that a longer period between oil applications allowed sprouts tissues to grow stronger and become less sensitive, or to recover after being sprayed with oil. At the end it appears that more mature tissues of sprouts are less sensitive to the burning effect of the essential oil, as compared to tender tissues of sprouts that were impaired to grow because of the burning effect when muña oil was sprayed at a higher frequency of 15 days. This finding is coincident with Frazier et al. (2004) who mention that alternative natural sprout suppressants are most effective when applied at "peeping" (initial status of sprout growth) or before sprouts are one-eighth of an inch long, otherwise application may result in sprout suppression failure. In a comparative prospective study run at the University of Idaho (N. Olsen, pers.comm.), to evaluate the effects of muña oil, clove oil and CIPC on potato sprout growth, the obtained results suggest that muña oil can become an effective replacement of clove oil to control potato sprouting in storage.

Although the evaluation of storage diseases was not part of the study, it was observed that treated tubers kept in cold room did not show any development of dry rot (*spp. Fusarium*) or decay, further studies are needed to determine how, when and how much of these 'natural' products should be used to control sprouting, and determine its effect on dry and soft rot.

It was observed in the potato lot stored at room temperature condition that the performance of muña essential oil as sprouting suppressant was negatively affected, may be because of the volatilization of the terpenoids components due to the higher temperature. The inhibitory effect of CIPC was also effective at room temperature during the storage period of the trial.

In the same lot at room temperature it was necessary to eliminate a number of treated tuber samples that decayed due to bacterial soft rot and water rot. The application of muña essential oil as fumigant (wick application) was ineffective as sprouting suppressant with both concentrations and under both storage conditions, since in all cases it was detected sprout development as well as weight loss. The taste panel that evaluated boiled samples of potato tubers treated with muña essential oil did not reported any odd odors.

Conclusions

This storage trial shows that muña essential oil has an effective potential as potato sprouting suppressant, but it has to be repeatedly sprayed every 15 days, and the treated potato tubers has to be stored under cold storage conditions. Although commercial feasibility of a sprouting control agent derived from muña essential oil are still to be developed, it is necessary further studies to investigate new ways to extend the sprouting suppressant effect of muña oil in potato tubers for fresh consumption, as well as other application treatments to optimize the

efficiency such as fumigant. Therefore, another trial need to be carried out to mimic a commercial context to test if the suppressant effect of 15 days is enough to control sprouting when native potato tubers treated with muña oil are marketed for fresh consumption in quality demanding markets. Improved and native potato cultivars need to be assessed on an individual basis for proper timing and frequency of application of muña oil, since response and sprouting differ by variety. These results suggest also that muña oil treatments could be used to suppress the sprouting of seed potatoes, without compromising the subsequent field performance of the seed. It is expected that the sprout suppression effect provided by the muña oil method does not stress the potatoes and thereby cause negative effects on internal processing quality of the tubers. However, further studies may need to be carried out to demonstrate that this method does not cause the potatoes to respond with increased sugar levels or cause the processed product to darken in color and become unacceptable for marketing.

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Potato Yellow Vein Virus: A model for emerging potato diseases and climate change

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Abstract

The emergence and management of plant viruses transmitted by insect vectors is an important problem, particularly in developing countries, where phytosanitary capacity may not be adequate. Key factors responsible for the emergence of new plant diseases include: the intensification of agricultural trade (globalization); changes in cropping systems and climate change. Potato yellow vein virus (PYVV, genus *Crinivirus*) is a threat to potato cultivation in the Andean region because of its potential for wide and rapid dissemination through infected planting material and a ubiquitous insect vector: the whitefly species *Trialeurodes vaporariorum*. PYVV, has spread in the last 7-10 years throughout the Andean region of northern in Peru. CIP is developing a risk prediction model of *T. vaporariorum* spread and indirectly PYVV (a model system for emerging diseases) using Geographic Information System (GIS) technology. The model predicts that PYVV could spread further south with apparent risk to southern Peru, Bolivia and Chile, based on the potential distribution of its vector. There is also some evidence of spread by seed since there are places where the disease is present but not the vector. We conclude that the model may be used to forecast potential occurrence of the vector and hence also PYVV under a given temperature and rainfall regime and be used to plan potential control measures. Species distribution modeling offers the possibility of predicting the potential distribution of the vector under different climate change scenarios and could estimate the potential risk of dispersion of the disease.

Keywords: Whitefly transmission, modeling distribution with GIS, ecological niche, maximum entropy, niche-based model, species distribution model.

Introduction

The potato (*Solanum tuberosum* L.) is one of the most important crops around the world. This crop is affected by disease and pest like nematodes, insects, fungi, bacteria and viruses. An important potato disease is caused by Potato Yellow Vein Virus (PYVV, a tentative member in genus *Crinivirus*, family *Closteroviridae*) (Salazar *et al,* 2000). Its origin has been traced to Northern Ecuador and Southern Colombia (Alba, 1952; Tamayo and Navarro, 1984) and was later reported in Venezuela in 1977 and in Peru in 1996 (Salazar et al, 1998). Currently, this disease is present in Colombia, Ecuador, Venezuela and Peru (Alba, 1952; Tamayo and Navarro, 1984; Salazar, 1996) and causes around 50% yield reduction (Salazar, 1996).

Since then the virus spread via infected seed tubers throughout the Central Andes, particularly to the most important potato-producing areas of Northern Peru (SENASA, 2008) and Venezuela. The virus does not affect the size and morphology of plants; the typical yellowing of the veins appears between 30 and 40 days after infection, when stems and leaves are already formed (Saldarriaga et al. 1988).

A most widely used practice in the control of PYVV infection is the manual elimination or rouging of infected plants and the spraying of pesticides over the whole parcel at different times during the vegetative cycle, for the control of its vector. However, the use of pesticides increases costs and pesticide residue levels in agricultural products and soils contributes to ground water contamination (Bravo et al. 2004).

Furthermore, indiscriminate use of pesticides could eliminate natural biological control agents, thus increasing pest-insect populations, such as *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), which is the PYVV.

The whitefly transmitt the virus in a semi-persistent manner (Salazar et al, 2000; Diaz et al, 1990; Tamayo and Navarro 1984), other types of transmission are through tuber-seed, and underground stem-grafts (Alba, 1952, Salazar, 1998).

During the "El Niño" phenomenon (1997-1998), this vector and other important insects increase their populations consequently; the transmission of important viral disease is increased. Nowadays, the current climate changes could affect the *T. vaporariorum* population because temperature affects its development rate and consequently its damage on crops and diseases dissemination.

Differences in the environmental characteristics of areas occupied by the insect can be examined by modeling species distributions, a technique that integrates locality data, GIS data, and modeling algorithms (Anderson et al*.,* 2002; Anderson and Martinez-Meyer, 2004; Elith et al*.*, 2006; Phillips et al., 2006). The resulting distribution model describes the common environmental and climatic characteristics of the known range of a given species or group of populations (Peterson, 2003; Soberón and Peterson, 2004). This approach has been used to predict species distributions (Illoldi-Rangel et al., 2004); to predict the potential geographic range of invading species (Peterson, 2003; Mau-Crimmins et al., 2006) and to predict changes in the distributions of fauna and flora associated with projected models of climate change (Peterson et al., 2002; Siqueira and Peterson, 2003; Oberhauser and Peterson, 2003; Thomas et al., 2004).

In crop species, GIS-based analyses have been used to predict yields of different cultivars in various geographic areas (Jeutong et al., 2000; Caldiz et al., 2002), to explore the distributions of wild relatives of crop species (Greene et al., 1999a, b; Hijmans and Spooner, 2001; Jarvis et al., 2004), and to model future distributions of crop pests and diseases (Bernardi, 2001).

CIP is interested in predicting and managing risk posed by emerging and re-emerging viruses and the effect of climate change. The models are important analytical tools for predicting, evaluating and understanding the dynamics of pest populations in ecosystems under a variety of environmental conditions and management practices. The aim of this work was: i) to generate data for vector and virus distribution in Peru, Ecuador and Colombia; ii) to estimate potential adaptability of the vector *T. vaporariorum* in the Andean region (using new modeling); and iii) to generate evidence of the potential distribution of the vector under climate change.

Materials and Methods

Insect distribution

Two hundred and ninety nine distinct potato fields were sampled in the Andean region of Ecuador, Peru and Colombia during 2007 year, to obtain a spatially referenced data set for vector and virus occurrence. Visual inspection of presence/absence of *T. vaporariorum* was assessed in each potato field. Potato leaf samples were collected in the field and tested by nucleic acid spot hybridization (NASH) to confirm the presence of PYVV. Position data for *T. vaporariorum* populations was recorded using a Garmin GPS 76s (Garmin International Inc., Olathe, Kansas, USA).

Climate scenarios

55 GIS data layers were used for either current [representing the year 2000] or future scenarios [representing the year 2050] from the WorldClim Global Climate GIS database (2.5 minute resolution) (Hijmans et al., 2005). These data included 36 monthly layers (maximum temperature, minimum temperature and rainfall) and 19 bioclimatic variables (annual mean temperature, mean diurnal temperature range, isothermality, temperature seasonality, maximum temperature of warmest month, minimum temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest period, precipitation of driest period, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter).

Insect distribution modeling

Environmental conditions were related to the insect occurrence data using MAXENT 3.3.1 (Phillips et al., 2006). MAXENT is a recent implementation of a statistical approach called maximum entropy that characterizes probability distributions from incomplete information (Phillips et al., 2006).

Insect distributions were predicted using the locality data in Table 1. The Maxent algorithm was run using the default parameters including a maximum of 500 iterations with a convergence threshold of 0.00001. During model development, 50% of the localities were used for model training, while 50% of the localities were held back to test model accuracy.

Cumulative probability distributions ranging from 0 to 100 were generated for *T. vaporariorum* populations that represent a relative measure of the probability of occurrence for the modeled insect.

Insect distribution modeling for future scenarios

The model trained on the set of environmental layers was projected by applying it to a set of environmental layers under changing climate conditions. The CCM3-2050 model of NCAR (National Center for Atmospheric Research, Boulder, Colorado) from the WorldClim Global Climate GIS database (Hijmans et al., 2005) was used.

Results and discussion

A preliminary full model based on 66 observations with presence of *T. vaporariorum* points yielded a very widespread and diffuse damage probability surface (Fig. 1), probably because of the many different climate types that comply with the basic parameters represented in the points analyzed.

Based on known occurrences of cultivated *T. vaporariorum* populations, distribution maps predicting the possible areas where this insect might occur were generated (Fig. 1). Predictions were highly significant based on a binomial probability distribution test calculated from the held back test localities (Area Under curve (AUC): training data 0.994 and p values < 0.001).

The minimum temperature for January, isothermality (the mean diurnal range divided by the Annual Temperature Range) and total rainfall for October and July estimate an overall relative contribution of 85.75% of the environmental variables to the Maxent model. The observation that *T. vaporariorum* adapts well to elevations above 1000 masl poses an important question. This species is widely adapted in a region extending from more than 11ºN in Colombia to 17ºS Peru. Therefore, the altitude limit does not seem to be related to temperature, since temperature varies widely over this latitude range. However, the annual temperature range increases with increasing distance from the equator.

Three particular regions highlighted in the map (Fig. 1, left), the northern highland of Colombia- Venezuela and the northern highland Peru-Ecuador, are areas where *T. vaporariorum* has caused only direct feeding damage to different crops (Anderson and Morales, 2005). The three areas (the central highlands of Bolivia, northern valley of Chile and the west part of Brazil) are shown on the map (Fig. 1 left) as a potential target for *T. vaporariorum*. These observations are interesting because the highland climate is quite similar to the climate found on the presence of *T. vaporariorum*. Even though, future conditions varied strongly between models. This preliminary map (Fig. 1, right) has resulted in major outbreaks in the highlands of Ecuador and Peru.

The results obtained in this study also suggest that countries, such as Bolivia, Chile and Brazil, should be particularly alert to the possible introduction of *T. vaporariorum* in vegetative material, considering the existence of environmental conditions suitable for the adaptation of this whitefly species.

On the other hand, PYVV has been detected in northern of highland areas of Peru, Ecuador and Colombia affecting annual field crops of potato (Salazar et al, 1996).

There is also some evidence of spread by seed since there are places where the disease is present but not the vector.

The southern of Peru, located over 1000 masl, is a marginal area for *T. vaporariorum*. Nevertheless, a prolonged dry period in the July-December of 2008 resulted in major outbreaks of *T. vaporariorum* in potato and ornamental plants, causing considerable direct damage on the plants and an indirect risk in transmitting PYVV (Anderson and Morales, 2005). This observation suggests that once *T. vaporariorum* reaches high populations in a region due to the presence of suitable reproductive hosts, pesticide abuse (overuse causing elimination of natural enemies and development of pesticide-resistant *T. vaporariorum*) and favorable climatic conditions (dry and temperature weather), the probability of PYVV outbreaks increases.

Table 1. Distribution of *Trialeurodes vaporariorum* using the locality data

Country	Place	COD__CULT	ZONE_UTM	LON_DEG	LAT_DEG	ALTITUDE	PYVV	T_VAPORA	S_FENOLO
Peru	Yungay	3	18	-77.680	-9.173	3047		x	Flowering
Peru	Yungay	1	18	-77.669	-9.168	3034	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3031	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3027	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3025	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3024	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3024	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3022	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3022	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3023	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3025	x	x	Tuberization
Peru	Yungay	1	18	-77.669	-9.168	3023	x	x	Vegetative development
Peru	Yungay	1	18	-77.669	-9.168	3022	x	x	Vegetative development
Peru	Yungay	1	18	-77.669	-9.168	3022	x	x	Vegetative development
Peru	Shancayan	2	18	-77.672	-9.515	3045		x	Tuberization
Peru	Chiquian (Obraje)	1	18	-77.158	-10.137	3141	x	x	Vegetative development
Peru	Chiquian (Obraje)	1	18	-77.158	-10.137	3142	x	x	Vegetative development
Peru	Chiquian (Obraje)	1	18	-77.158	-10.137	3146	x	x	Vegetative development
Peru	Chiquian (Obraje)	1	18	-77.158	-10.137	3145	x	x	Vegetative development
Peru	Chiquian (Obraje)	1	18	-77.158	-10.137	3134	x	x	Vegetative development
Peru	Chiquian (Obraje)	1	18	-77.158	-10.137	3140	x	x	Vegetative development
Peru	Chiquian (fuera de Chiquian)	1	18	-77.161	-10.149	3480	x	x	Vegetative development
Peru	Chiquian (fuera de Chiquian)	1	18	-77.161	-10.149	3485	x	x	Vegetative development
Peru	Namora(Cau-cau)	1	17	-78.288	-7.207	2876	x	x	Vegetative development
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2882	x	x	Tuberization
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2883	x	x	Tuberization
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2887	x	x	Tuberization
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2882	x	x	Tuberization
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2886	x	x	Tuberization
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2885	x	x	Tuberization
Peru	Namora(Cau-cau)	1	17	-78.287	-7.206	2885	x	x	Tuberization
Peru	Malcas	2	17	-78.137	-7.511	2880	x	x	Vegetative development
Peru	Malcas	2	17	-78.143	-7.509	2052	x	x	Vegetative development
Peru	Malcas	2	17	-78.143	-7.509	2049	x	x	Vegetative development
Peru	Malcas	2	17	-78.143	-7.509	2050	x	x	Vegetative development
Peru	Malcas	2	17	-78.143	-7.509	2053	x	x	Vegetative development
Peru	Huarimba (entrada Cajabamba)	2	17	-78.104	-7.602	2296	x	x	Vegetative development
Peru	Huarimba (entrada Cajabamba)	2	17	-78.104	-7.602	2296	x	x	Vegetative development
Peru	Huarimba (entrada Cajabamba)	2	17	-78.104	-7.602	2299	x	x	Vegetative development
Peru	Huarimba (entrada Cajabamba)	2	17	-78.103	-7.602	2298	x	x	Vegetative development
Peru	Huarimba (entrada Cajabamba)	2	17	-78.103	-7.602	2297	x	x	Vegetative development
Peru	La Punta (Panao)	1	18	-76.045	-9.851	2322		x	Vegetative development
Peru	La Punta (Panao)	1	18	-76.046	-9.850	2327		x	Vegetative development
Peru	La Punta (Panao)	1	18	-76.047	-9.850	2327		x	Vegetative development
Peru	La Punta (Panao)	1	18	-76.047	-9.850	2327		x	Vegetative development
Peru	La Punta (Panao)	1	18	-76.047	-9.850	2328		x	Vegetative development
Peru	Huanin	1	18	-76.029	-9.903	2704		x	Vegetative development
Peru	Huanin	1	18	-76.029	-9.903	2714		x	Vegetative development
Peru	Huanin	1	18	-76.029	-9.903	2711		x	Vegetative development
Peru	Huanin	1	18	-76.029	-9.903	2704		x	Vegetative development
Peru	Huanin	1	18	-76.029	-9.903	2703		x	Vegetative development
Peru	Quicacan I	1	18	-76.236	-10.017	1992		x	Vegetative development
Peru	Quicacan I	1	18	-76.236	-10.017	1990		x	Vegetative development
Peru	Quicacan I	1	18	-76.236	-10.017	1990		x	Vegetative development
Peru	Quicacan I	1	18	-76.236	-10.017	1996		x	Vegetative development
Peru	Quicacan I	1	18	-76.236	-10.017	1997		x	Vegetative development
Peru	Quicacan I	1	18	-76.237	-10.018	2002		x	Vegetative development
Peru	Quicacan II	1	18	-76.223	-9.965	2014		x	Vegetative development
Peru	Quicacan II	1	18	-76.223	-10.056	2014		x	Vegetative development
Peru	Quicacan II	1	18	-76.223	-10.056	2011		x	Vegetative development
Colombia	Zaragosa	2		-77.4507	1.1553	1970		X	Vegetative development
Ecuador	Píllaro	1	17S	-78.536	-1.089	3016	x	x	Vegetative development
Ecuador	Píllaro	1	17S	-78.536	-1.088	3018	x	x	Vegetative development
Ecuador	Píllaro	1	17S	-78.536	-1.089	3019	x	x	Vegetative development
Ecuador	Imbarugua	2	17N	-78.107	0.392	2244		x	Maduration
Ecuador	Imbarugua	2	17N	-78.107	0.392	2244		x	Maduration

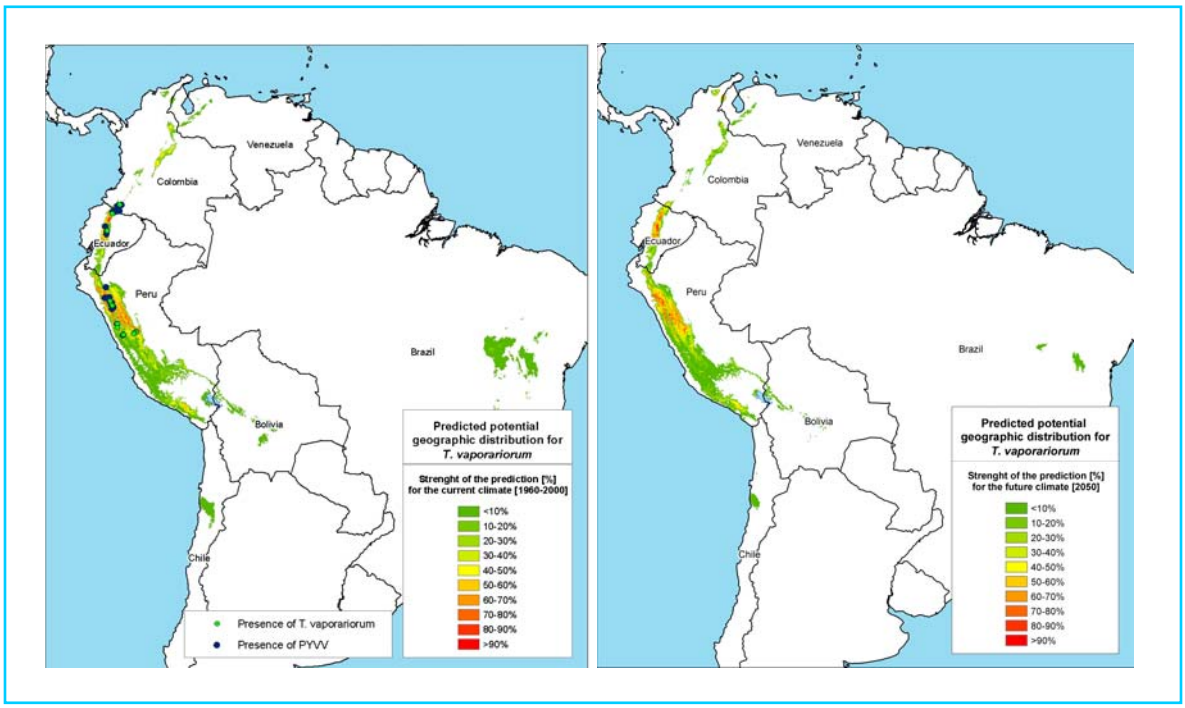

Figure 1. Maps showing predicting potential geographic distribution of *Trialeurodes vaporariorum* using all occurrence records [66] under different climate scenarios: current climate is represented by the period 1960-2000 (left) and future climate is represented by year 2050 (right).

Conclusions

The climate probability model described here can be used to identify regions of Latin America prone to *T. vaporariorum*/PYVV crinivirus attack. Regions highlighted in the map (Colombia, Venezuela and the northern highland Peru-Ecuador) are areas where *T. vaporariorum* has caused only direct feeding damage to different crops (Anderson and Morales, 2005). The central highland of Bolivia, northern valley of Chile and the west part of Brazil are potential areas for *T. vaporariorum* adaptation. These observations are interesting because the highland climate is quite similar to that climate where *T. vaporariorum* was found to be present. Even though, future conditions varied strongly between models. This preliminary map has resulted in major outbreaks in the highlands of Ecuador and Peru.

The investigation conduced in Latin America will be repeated in other continents affected by *T. vaporariorum*, primarily in Africa and Europe in order to further validate the applicability of this model to other parts of the world.

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Monitoring changes in *Phytophthora* populations in developing countries and the Phytophthora.exe database

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Abstract

Phytophtora infestans is a pathogen that is constantly adapting to the changing environment, increasing its genetic diversity through mutation and because of this, late blight epidemics have become more difficult to manage worldwide. Microsatellites or single sequence repeats (SSR) have proven to be a useful technique to characterize and monitor changes in *P. infestans* populations. This molecular tool is faster and cheaper than previously-used methods, easy to compare across laboratories and therefore could facilitate integration of the various genetic data sets available for this important pathogen. Standardization of microsatellite and other data has recently been facilitated by the development of data-entry software called Phytophthora.exe, which was developed as part of the Eucablight project in Europe. The International Potato Center (CIP) and some partners have embarked on an effort to promote the use of microsatellite markers and Phytophthora.exe in developing countries. To this end, data from Peru and Ecuador have been entered in the global database housed in Denmark and plans are underway to enter data from a number of other locations in South America, Africa and Asia. Potential uses of the microsatellite markers and the global database are discussed.

Keywords: Phytophthora, microsatellites, database.

Introduction

Phytophthora infestans, the oomycete pathogen that causes late blight on potato, tomato and other solanaceous crops occurs around the world. Late blight is considered the most devastating disease of potatoes and one of the most serious of all plant diseases. In central Mexico and South America, *P. infestans* is a pathogen of many different wild solanum species (Chacon, *et al.*, 2006; Garry, *et al.*, 2005; Fry and Smart, 1999). In Canada and the United States, *P. infestans* has been reported to infect hairy nightshade (*Solanum sarachiodes*), bittersweet (*S. dulcamara*) and Petunia (*Petunia hybride*). While in Europe *Solanum nigrum* was found to be a host of the new diverse population of *P. infestans.* (Fry, 2008).

Crop losses and cost of fungicides to control late blight together are estimated in the billions of dollars (US) per year. In most potato-growing areas, frequent fungicide applications are the main method of disease control and this can cause damage to human health and the environment. For this reason, the avoidance of primary inoculum sources and the use of cultivar resistance are increasingly important to minimize costs and environmental impact. However, changes in the population structure of *P. infestans* discovered since the early 1990s make those strategies for late blight management less effective in controlling the disease. These new strains have increased the severity of late blight on potato, because genetic variation, adaptive abilities, aggressiveness and virulence have increased. In addition, in areas where both mating types A1 and A2 are present, oospores as a persistent infectious survival structure constitute another constraint (Turkensteen *et al.,* 2003). To combat these strains it is necessary to use more resistant potato cultivars and to use fungicides more effectively. Potato cultivars with desirable market quality that show high levels of resistance are being developed, particularly in developing countries. New methods of breeding include the use of wild potatoes as resistant sources. The effectiveness of such control strategies will be influenced by changes in the pathogen population and it is thus important to understand population change on local and wider geographical scales. For this reason, accurate and up to date information on fungicide resistance, sources of primary inoculum, and aggressiveness of the pathogen is critical.

To characterize *P. infestans* populations, phenotypic and genotypic markers have been developed, and have contributed to our understanding of the population genetics of *P. infestans*. Mating types (A1 and A2) (Judelson, 1996) provided the first indication of major changes in *P. infestans* populations. Allozyme markers for Glucose-6-

phosphate isomerase (*Gpi*) and Peptidase (*Pep*) loci (Tooley *et al*., 1985) provided the first molecular evidence of diploidy in *P. infestans*. Nuclear DNA fingerprinting has allowed much greater resolution of population structures. A total of 30 markers were identified by the restriction fragment polymorphism (RFLP) (Goodwin *et al*., 1992); mitochondrial DNA haplotypes (Griffith and Shaw, 1998) enable the tracking of specific lineages; neutral nuclear markers (AFLP) (Vos *et al*., 1995) and more recently the approach using simple sequence repeats (SSR) or microsatellites (Knapova and Gisi, 2002; Lees *et al*., 2006) have provided even greater resolution. In a recent review of genetic markers for *P. infestans*, SSR were proposed as a powerful choice because they are highly specific, single locus, codominant, highly polymorphic, highly reproducible and less amount of pathogen DNA is needed (Cooke and Lees, 2004).

One way that researchers and extension workers may enhance their capacity to help farmers deal with the "new" potato late blight is by improved collaboration and exchange of knowledge and information. To this aim, several initiatives were funded. One such initiative was a concerted knowledge sharing effort in Europe called EUCABLIGHT, a Late Blight Network for Europe (www.eucablight.org). An important component of the information sharing of this initiative is a common database of markers (fingerprints) of the pathogen throughout Europe. A program called Phytophthora.exe was developed to ensure standardization of data and facilitate data input. Phytophthora.exe also allows for automatic uploading to a centralized database on the pathogen and thereby makes more informed estimates of risk of introduction of new strains.

In order to standardize methods to get a uniform and accurate data set, microsatellite markers were used at CIP to characterize *Phytophthora* Peruvian populations. Furthermore, to monitor changes of pathogen population, all existing data about Peruvian and Ecuadorian *P. infestans* (and *P. andina*) isolates were uploaded to the EUCABLIGHT database using the program Phytophthora.exe version 2.0 developed for South America.

Materials and methods

Phytophthora isolates

A sample of twenty-two representative isolates of *Phytophthora infestans* from the CIP collection, that previously were characterized using RFLP markers, were examined using SSR. These isolates were collected from potato and wild potatoes from 1998 to 2000 in different potato areas in Peru (Garry, *et al*., 2005; Perez *et al*., 2001). Another fourteen isolates collected in 2005 from tree tomato (*Solanum betaceum*) were also examined. Some Ecuadorian isolates of *P. infestans* and *P. andina* with known SSR loci were used as markers to determine the size of the alleles at different SSR loci (R. Oliva, personal communication).

Genotypic characterization using SSR markers

Isolates were screened with eight microsatellite (SSR) markers developed specifically for *P. infestans* (Knapova *et al*., 2001; Less *et al*., 2006). PCR amplifications were performed in a 10 µl volume containing 5 ng of genomic DNA, 1 µl of 10x reaction buffer B (Promega), 0.01 mM of each dNTP, 0.2 µM each of forward and reverse primers (Table 1), and 0.7 U of *Taq* polymerase (Promega). PCR was performed in a MJ Research cycler under conditions indicated by Knapova *et al*. (2001) and Lees *et al*. (2006) for each marker. Microsatellite alleles were separated by running the reactions on a 6% denaturing acrylamide gel. Before loading, the mixture was denaturated by heating at 94°C for 5 min. A non-radioactive detection method was used and fingerprints were scored visually after silver staining. Cluster analysis was conducted using the unweighted pair-group method with arithmetic mean using the software program NTSYS-pc version 1.70 (Exeter Software, Setauket, NY).

Table 1. The eight microsatellites markers selected for this study

Marker	Repeat	SSR Primer sequence [a]	Size range (bp)	Annealing temp (°C)
4B	$(TC)_{34}$	F:AAAATAAAGCCTTTGGTTCA R:GCAAGCGAGGTTTGTAGATT	205-217	58
G11	$(TC)_{27}$	F: TGCTATTTATCAAGCGTGGG R: ACAATCTGCAGCCGTAAGA	142-166	56
1F	$(TC)_{?}$	F: GAGAGTGAATGAGAGCGAG R:ACAATCTGCAGCCGTAAGAG	94-166	59
Pi63	$(GAG)_{8}$	F:ATGACGAAGATGAAAGTGAGG R: ATTCATTATTGGCAATGTTGG	148-160	58
Pi66	$(GT)_{7}$	F: ACCGACAGCTTCTGAAACC R:AAAATAAGAAGAGATTCGTGCC	153-155	58
Pi89	$(AT)_{9}$	F: GAGAACGCACAATGTAAGGC R: ACATAAATACACGCTGAACGG	179-185	58
2D	$(TC)_{9}$	F: AATTGAGTGAATGCGTCACC R: TTTCCTGCTATCCTCAGCAC	155	58
D13	$(CT)_{27}$	F: TGCCCCCTGCTCACTC R: GCTCGAATTCATTTTACAGA	108-142	50

[a] F, forward primer; R, reverse primer

Data input, storage and management using Phytophthora.exe

A complete Version 2.0 of Phytopthora.exe for South America was installed on a PC in both CIP-Lima and CIP-Quito with instructions from the EUCABLIGHT homepage (http://www.eucablight.org). The user manual can be downloaded from the same webpage. The data entry tool Phytophthora.exe provides a user-friendly interface that facilitates data entry and its submission to the EUCABLIGHT database (Hansen *et al.*, 2006).

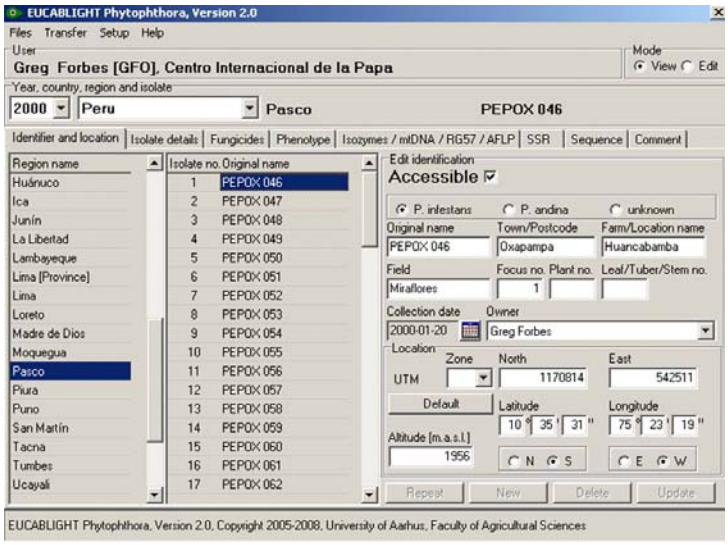


Figure 1. Computer screen showing a data entry page from Phytophthora.exe

All existing data of Peruvian and Ecuadorian *Phytophthora infestans* isolates were entered and stored. Information about country and year of collection for each isolate were required and "regionID" and "isolatedID" were also created (Fig. 1). Further information was filled in the 50 database fields for phenotypic (mating type, fungicide resistant, virulence), genotypic (SSR alleles, RFLP, mt DNA, allozymes) and cropping (cultivar, location, altitude) data. Data were uploaded to the central EUCABLIGHT database in Denmark, where they can be accessed in summarized form via the Internet. All primary data in the Eucablight database is owned by the supplier.

Results and discussion

The use of SSR markers to characterize Phytophthora isolates

From the eight selected microsatellite marker loci (Table 1), three (4B, G11, and 1F) were highly polymorphic and three (Pi63, Pi66 and Pi89) moderately polymorphic for the sample that was evaluated. The other two loci (2D and D13) were not polymorphic for these *P. infestans* isolates but they allow discrimination between *Phytophthora* species. The microsatellite analysis showed that isolates with identical RFLP genotype generally share identical alleles for each SSR marker. An exception was for the RFLP genotype US-1 which had different SSR alleles combinations. All the Peruvian isolates from tree tomato (*S. betaceum*) clustered together and they were genetically more closely related to Ecuadorian isolates from the Anarrichomenum complex (EC-2.1) than to Ecuadorian isolates from *S. betaceum* of *P. andina* (Fig. 2).

The congruence of results from both RFLP and SSR analyses, and the fact that SSR is cheaper and less time-consuming than RFLP, makes SSR markers an ideal tool for efficient analysis of *P. infestans* populations.

Analysis of data using Phytophthora.exe software

The database and Phytophthora.exe are ready to be used by countries outside Europe after minor adaptations. Phytophthora.exe is currently used at CIP, both in Peru and Ecuador. There are also plans to use this software in other countries of South America, Africa and Indonesia.

Phytophthora.exe is a user-friendly tool for the entry of primary data because minimal typing is necessary and uploaded data can be easily exported into common databases for analysis or for the exchange of data among partners. Furthermore, the strict input process in Phytophthora.exe helps avoid errors. Processed data can be seen online in graphs and tables (Fig. 3). Stored data from a number of locations will allow us to monitor changes in the pathogen population and to design effective strategies for late blight management according population structure. For example in Peru *Phytophthora* isolates are resistance to metalaxyl (Fig. 3), therefore, farmers should be aware of the inefficacy of products that include this component. This database has many practical applications like mapping pathogen diversity, in late blight simulation and forecasting. For instance, at the level of South America, the *P. infestans* A1 mating type is present in Ecuador, Peru, Colombia and Chile, while the A2 mating is present in Argentina, Bolivia and Brazil. The risk of presence of both mating types and therefore of sexual recombination, is highest in the area where these two populations meet. Similarly, the tracking of virulence and aggressiveness in *P. infestans* populations is also important. Late blight researchers in developing countries would benefit from greater information sharing and a globalization of the tools developed in EUCABLIGHT, particularly Phytophthora.exe

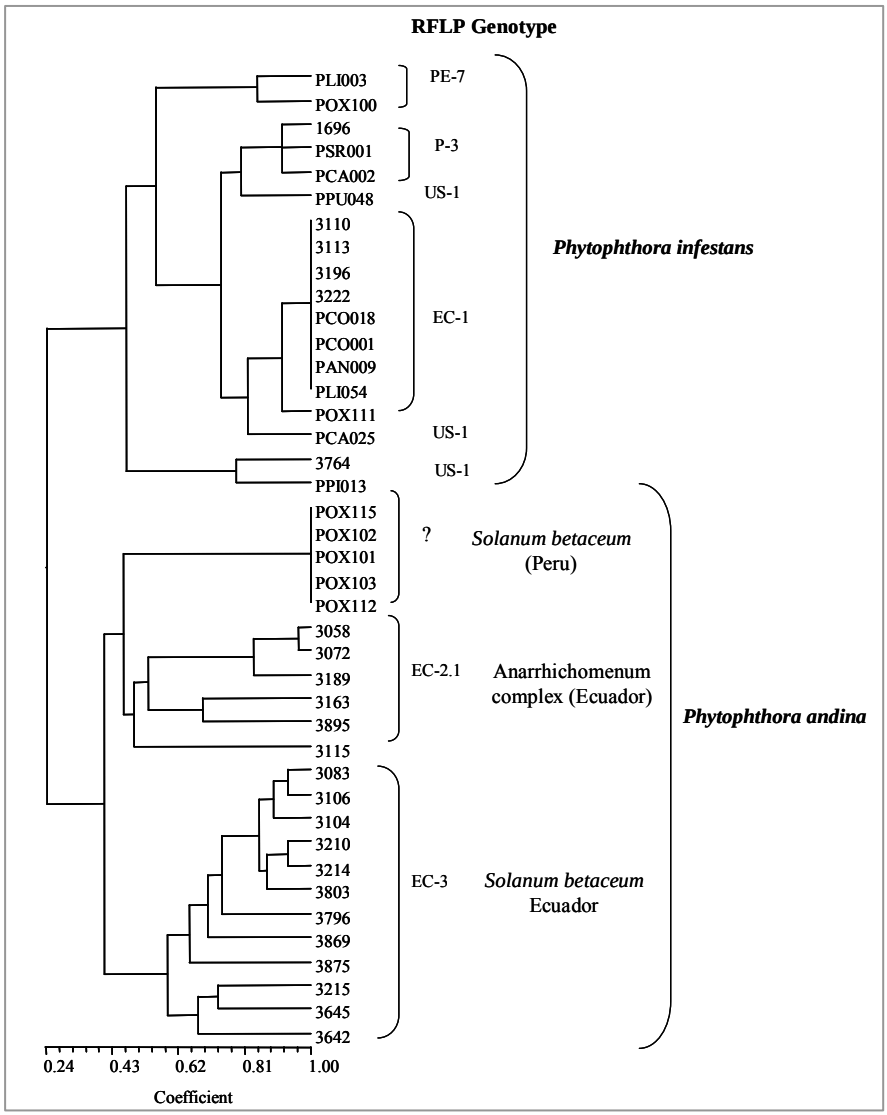
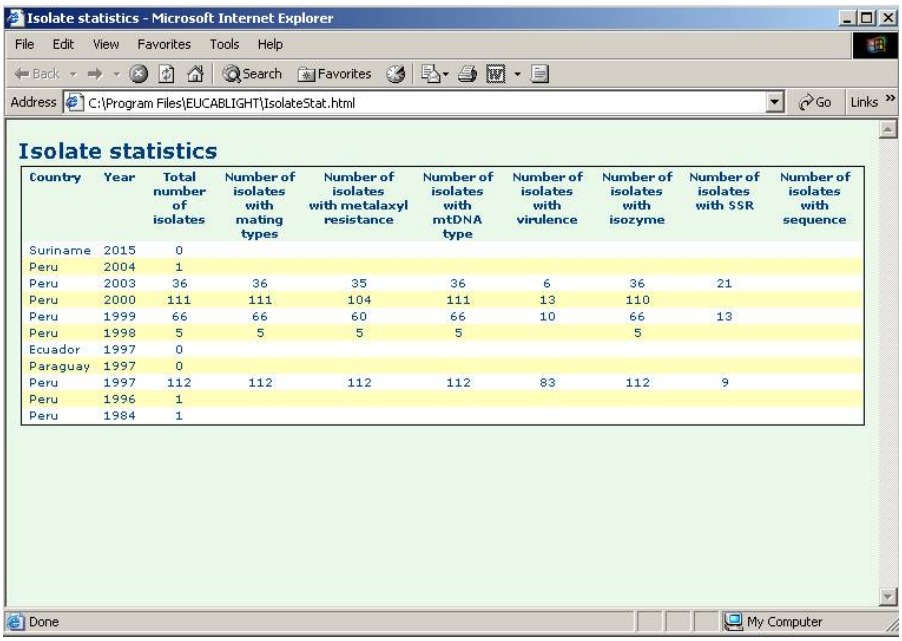


Figure 2. A phenogram derived from microsatellite markers, of isolates of Phytophthora *infestans* and *Phytophthora andina.* Phenogram based on the Dice similarity coefficient



Isolate statistics

Country	Year	Total number of isolates	Number of isolates with mating types	Number of isolates with metalaxyl resistance	Number of isolates with mtDNA type	Number of isolates with virulence	Number of isolates with isozyme	Number of isolates with SSR	Number of isolates with sequence
Suriname	2015	0							
Peru	2004	1							
Peru	2003	36	36	35	36	6	36	21	
Peru	2000	111	111	104	111	13	110		
Peru	1999	66	66	60	66	10	66	13	
Peru	1998	5	5	5	5		5		
Ecuador	1997	0							
Paraguay	1997	0							
Peru	1997	112	112	112	112	83	112	9	
Peru	1996	1							
Peru	1984	1							

Figure 3. Summary table for selected isolates from Ecuador, Peru and Paraguay from the Eucablight central database in Denmark (www.eucablight.org)

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Effect of suppressive composts and initial seed tuber infection on *Rhizoctonia Solani* in organic potato production

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Abstract

The suppressive effects of green yard composts to control *R. solani* in potatoes and the effect of initial infection of the seed tubers were tested in field trials under organic management at the University Kassel (Germany) in the years 2006-08 and at two farm sites in northern Germany in 2008. Compost directly applied at the seed tuber area at 5 t DM ha^{-1}, significantly reduced the infestation of harvested potatoes with black scurf and tuber malformations and plus dry core tubers by an average of 33% and 41%, respectively while marketable yields and tuber numbers were increased by 5 to 25%. The rate of initial black scurf infection of the seed tubers also affected tuber number, health and quality significantly. Compared to healthy seed tubers initial black scurf sclerotia infestation of 2-5 and >10 % of tuber surface lead in untreated plots to a decrease in marketable yields by 14-19 and 44-66 %, a increase of black scurf severity by 8-40 and 34-86 % and also increased amount of malformed and dry core tubers by 32-57 and 109-214 %.

Keywords: *Rhizoctonia solani,* organic potatoes, compost, initial seed tuber infection**.**

Introduction

As the *Rhizoctonia* pathogen is as well soil as seed tuber borne the primary means to reduce black scurf in potatoes are the use of healthy seed tubers and field hygiene. *Rhizoctonia*-infested seed tubers are of importance in disseminating the disease and adding to the pool of soil-borne inoculum (Jeger et al., 1996). While conventional seed tubers are usually treated with fungicides, in organic systems until now there exist no reliable measures for the control of seed borne inoculum. Especially the production of organic certified healthy seed tubers poses serious problems. Since the use of organic seed potatoes has become compulsory in organic potato production black scurf is increasing in importance. To reduce soil borne infection it is important to ensure good soil conditions with a high microbial activity with a high antagonistic potential. Soil organic matter greatly increases soil microbial activity and diversity which, in turn, are related to soil and seed borne disease suppression (Lumdsen et al., 1983; Fließbach & Mäder, 2000). Such organic matter could be supplied by high quality suppressive biogenic waste composts. Suppressiveness of composts is closely related to colonisation by disease suppressive micro-organisms during curing e.g. *Bacillus spp., Enterobacte spp., Flavobacterium balustinum 299, Pseudomonas spp*., and other bacterial genera and *Streptomyces spp*. as well as fungal species including *Penicillium spp, Gliocladium virens,* several *Trichoderma spp*. and others (Chung & Hoitink, 1990; Hoitink et al., 1996). Compost application leads to sustained increase in microbial activity and the establishment of microbial populations with antagonistic features (Hoitink & Boehm 1999) and promising results have been obtained with suppressive composts against several soil-borne pathogens. Tsror et al. (2001) and Lootsma (1997) already demonstrated that it might be possible to control R. solani with composts in practice. However, we have shown that good effects are generally dependent on the amount of applied compost material (Bruns & Schüler 2002). To reduce the total amount of compost needed, our study aimed at testing if the targeted application of limited amounts of compost near the seed tubers can reduce the total amount needed.

In this study, we conducted three field trials to test the effects of targeted compost application within the row during planting of potatoes on plant and tuber infection with *R. solani*. The following questions were addressed in this study: (i.) is it possible to achieve disease control with compost applications of only 5 t DM * ha^{-1} in field grown potatoes and can this be enhanced by application technology? (ii.) How effective are composts when used with tubers varying in initial black scurf infestation?

Materials and methods

Two-factorial field trials were performed as split-plot design with four replications (at the experimental farm of University of Kassel in Witzenhausen on a silty loam with 74 soil points (according to the German system scale 0-100) in the years 2006-2008. In 2008 the trial was conducted additionally at two On-Farm fields in Northern Germany, in Barnstedt on a sandy soil (25 soil points) and Sudwalde on a sandy loam soil (50 soil points).

Factor A: Compost application vs. contol. In all years, a 5 month old compost made of organic household waste-/ yard waste (60/40), composted according to the requirements in EEC regulation No 2092/91 (Annex II) was used. Control plots without compost received an N,P,K –nutrient-equivalent to the household/yard waste compost nutrient load (Table 1). Composts were applied at 5t DM ha^{-1}, directly to the seed tuber area by using a modified fertiliser application machine (Universal Kastenstreuer, UKS 150, Rauch, Sinzheim

Table 1. Properties of composts used in the field trials

	2006	2007	2008
DM (%)	68.97	79.12	70.19
NO3-N mg * kg TM^{-1}	392.01	433.02	387.55
NH4-N mg * kg TM^{-1}	97.96	107.18	88.8
pH	7.65	7.65	7.73
P mg *kg DM^{-1}	2060	1700	1811
K mg *kg DM^{-1}	14400	12000	13578
N t (%)	1.81	1.71	1.48
C t (%)	20.97	17.69	20.43
C / N	11.57 :1	10.34 :1	13.82 :1

Factor B: Infection severity of seed tubers. Seed tubers (variety Nicola same seed source in the respective years) naturally infested with black scurf were planted of three infection severities ≤1%, 2-5% and high infection > 10% (not in 2008) of the seed tuber surface area.

Assessments

All assessments of symptoms of *Rhizoctonia solani* were performed according to the EPPO – standard PP 1/32 (2) (EPPO, 2000). Tuber symptoms were assessed on 100 marketable tubers per plot. Black scurf severity was assessed as mean percentage infestation of tuber surface. Percent incidence of tubers with dry core and malformations were also recorded. Marketable yield consisted of tubers of marketable size without malformations and dry core and with black scurf infestation of less than 15 %.

Data analysis

Statistical analysis was based on the SPSS GLM procedure (version 13). All data of *R. solani* symptoms were calculated in percentage values and arc sine transformed before analysing. The Kolmogorov-Smirnov test was conducted to analyse the normal distribution. Data were analysed by using fixed effect models per compost treatment, initial seed tuber infection and harvest date. Due to the split plot design the interaction between replication and compost treatment was used as random effect. The Bonferroni - Holm Test was conducted to separate means with a confidence level of 95 %.

Results and discussion

Both, initial infection and compost amendments significantly affected final infection levels of harvested tubers in all years and in 2008 at all sites (Tab. 2 and 3). Compost directly applied at the seed tuber area at 5 t*DM ha^{-1}, reduced the infestation of harvested potatoes with black scurf by a mean of 2 % or relatively by 33 % and the

rate of tubers with deformations and dry core by 7.8% (relatively -41 %), on average, at final harvest (Tab. 2). Application of the household/yard waste compost increased marketable yields between 2.4 and 6.1 % t*ha^{-1} (average of + 4.1 t*ha^{-1} or + 20% (Tab. 3). Marketable yields were mainly increased, due to increased tuber numbers (+ 3.94*m$^{2\ -1}$) and less tubers with malformations and dry core symptoms (Tab. 2 and 3).

In terms of farmers' income this would mean an increase of the proceeds of up to 2000 /ha. Preliminary data on microbial activity indicate that the main impact of compost application is in the phase of potato emergence till end of flowering (Schulte-Geldermann et.al.2008). Probably, there is also a strong interaction between quality of organic matter of the composts and soil microbial life. However, these measurements were just on quantities and not on qualitative microbial activity; therefore it is not clear if the reduction of *R. solani* was caused by specific antagonism or due to the higher microbial activity. Nevertheless, the results confirm several studies that documented the suppressive effect of high quality compost amendments against soil borne diseases like *Rhizoctonia, Pythium* and *Fusarium* in potting mixtures and under field conditions (Hoitink & Fahy, 1986; Termorshuizen et al., 2006; Hoitink et al., 1996; Bruns & Schüler, 2002; Tsror et al., 2001). However, compost application amount in organic agriculture is limited at 5t DM ha^{-1} year^{-1} and the studies reporting successes in suppressive effects relatively high amounts of compost were used. By that reason effectiveness of compost application placements targeted close to the seed tubers was compared with broadcast application in 2006 (data not shown). The targeted application (ridge) of compost was considerably superior in reduction of *R. solani* symptoms especially in the amount of tuber malformations (Schulte-Geldermann, 2008). Therefore, there is a need to develop a targeted application system to achieve reliable control of *R. solani* with the limited compost amount of 5t DM ha^{-1}.

Table 2. Impact of initial seed tuber infection and compost treatment on the R.solani symptoms black scurf infestation , dry core and malformed tubers of harvest tuber in three consecutive years (2006-2008) at the research station Eichenberg and at two On-Farm sites in Northern Germany (Barnstedt and Sudwalde) in 2008

Year	Site / soil type	Initial seed tuber infection	Mean black scurf infestation (% of tuber surface)			Dry core and malformations (% of tubers)		
			Compost	Control	*P • 0.05*	Compost	Control	*P • 0.05*
2006	Eichenberg / silty loam	•1 %	3.36	5.95	*a*	7.75	15.75	*a*
		2 -5 %	5.8	8.51	*b*	14.75	20.75	*b*
		> 10%	6.57	10.43	*b*	14.75	32.25	*b*
		P • 0.05	*a*	*b*		*a*	*b*	
2007	Eichenberg / silty loam	•1 %	1.4	2.72	*a*	5.74	9.86	*a*
		2 -5 %	2.61	3.82	*b*	9.36	13.57	*a*
		> 10%	3.28	5.05	*c*	13.79	20.47	*b*
		P • 0.05	*a*	*b*		*a*	*b*	
2008	Barnstedt / sandy	•1 %	0.93	2.28	*a*	5.27	10.48	*a*
		2 -5 %	4.38	5.63	*b*	10.46	19.27	*b*
		P • 0.05	*a*	*b*		*a*	*b*	
	Sudwalde / sandy loam	•1 %	5.70	7.60	*a*	13.82	21.06	*a*
		2 -5 %	7.11	9.85	*b*	18.77	26.73	*b*
		P • 0.05	*a*	*b*		*a*	*b*	
	Eichenberg / silty loam	•1 %	1.37	3.13	*a*	5.71	14.58	*a*
		2 -5 %	5.16	6.59	*b*	11.24	19.73	*b*
		P • 0.05	*a*	*b*		*a*	*b*	

*** Different letters indicate significant differences: vertically between different initial seed tuber infection severities and horizontally between compost treatment and control, respectively.**

The effect of initial seed tuber infection on yield and disease was clearly demonstrated in our trials. Compared to relatively healthy seed tubers (≤1 % infestation of tuber surface) initial black scurf sclerotia infestation of 2-5 and >10 % of tuber surface in untreated plots lead to a decrease in marketable yields by 14-19 and 44-66 % as well as

tuber numbers by 3-14 and 7-24% , an increase of black scurf severity by 8-40 and 34-86 %, respectively. Also the amount of malformed and dry core affected tubers was increased by 32-57 and 109-214 %, respectively.

Similar results of the impact of seed tuber health were also investigated by Karalus et al. (2003) underlines the importance of using healthy seed tubers particularly in organic potato production because of the lack of reliable control measures of seed borne inoculum. Especially the production of organic certified healthy seed tubers poses serious problems. Since the use of organic seed potatoes has become compulsory in organic potato production black scurf is increasing in importance.

In 2008 the On-Farm site Sudwalde showed significant higher *R. solani* infection levels than the two other sites. This was mainly due to due to waterlogging and a high amount of fresh straw residues at that site, most probably releasing high concentrations of free nutrients (glucose) which represses enzymes produced by *Trichoderma spp*. required for parasitism and eradication of sclerotia of plant pathogens such as *R. solani* (Nelson et al, 1983, Hoitink and Boehm, 1999)

Table 3. Impact of initial seed tuber infection and compost treatment on the marketable yield (30-60mm, - black scurf >15% , - malformed and dry core tubers) and tuber number per area (m2) of harvest tuber in three consecutive years (2006-2008) at the research station Eichenberg and at two On-Farm sites in Northern Germany (Barnstedt and Sudwalde) in 2008

Year	Site / soil type	Initial seed tuber infection	Marketable yield (t*ha^{-1})			Tuber no.*m^{-2}		
			Compost	Control	*P • 0.05*	Compost	Control	*P • 0.05*
2006	Eichenberg / silty loam	•1 %	25.26	21.55	*a*	56.86	51.93	*a*
		2 -5 %	22.72	18.59	*b*	54.64	49.99	*b*
		> 10%	20.34	14.33	*c*	51.64	48.08	*c*
		P • 0.05	*a*	*b*		*a*	*b*	
2007	Eichenberg / silty loam	•1 %	20.88	16.8	*a*	40.60	36.61	*a*
		2 -5 %	16.45	14.05	*b*	35.57	31.51	*b*
		> 10%	13.94	11.44	*c*	30.72	27.87	*c*
		P • 0.05	*a*	*b*		*a*	*b*	
2008	Barnstedt / sandy	•1 %	28.38	25.85	*a*	53.41	49.33	*a*
		2 -5 %	25.94	21.44	*b*	51.45	47.18	*b*
		P • 0.05	*a*	*b*		*a*	*b*	
	Sudwalde / sandy loam	•1 %	31.02	25.13	*a*	60.74 a	53.44 b	
		2 -5 %	25.97	20.51	*b*	53.42 b	51.39 c	
		P • 0.05	*a*	*b*				
	Eichenberg / silty loam	•1 %	31.36	27.32	*a*	53.90	51.92	*a*
		2 -5 %	26.67	21.86	*b*	49.67	46.14	*b*
		P • 0.05	*a*	*b*		*a*	*b*	

* Different letters indicate significant differences: vertically between different initial seed tuber infection severities and horizontally between compost treatment and control, respectively.

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Relationship between potato late blight development and weather variables in the highlands of Southwestern Uganda

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Abstract

Field trails were conducted from 2002 to 2004 at Kalengyere Research Station, 2450 m above seal level, in southwestern Uganda to determine the effect of continuous potato planting on late blight (LB) epidemic behaviour over a cropping season. The experimental treatments consisted of three potato varieties and either three or four planting dates during the first or second rainy season, respectively per year. Ten potted, LB-infected potato plants were evenly distributed along alleys as infection sources. The date of LB onset per plot was recorded. Disease severity (%) was assessed every 3-10 days until 85-90 days after planting. No artificial disease control was used. Data revealed that the days from planting to disease onset was influenced more by the planting date than by potato variety. Disease appeared earlier during the first (Feb.-June) than the second (Sept.-Dec.) season. The days from planting to disease onset was significantly ($P<0.05$) related to accumulated hours with RH≤90% and accumulated rain days but computed from either 1st March or 1st September for the first or second rainy season, respectively. The point of inflexion of the disease progress curve was related to days to disease onset by a simple linear function. The function predicting the point of inflexion was able to predict disease onset in >70% of the studied epidemics. The study revealed that potato planted by 1st September can escape severe LB epidemics than one planted by 1st March, probably requiring no fungicides for disease control.

Keywords: Disease progress curves, logistic function, inflexion point, Kalengyere.

Introduction

The highlands in East Africa and perhaps elsewhere are cool, generally wet and humid, favouring growth of potato nearly all the year round. In the highlands of Uganda, can be planted in upper slopes, cool valley bottoms or reclaimed swamps but in different months per year (Low, 2000). This practice provides the crucial ware potato all year round, but also provides a bridge of late blight infection between the two main rainy seasons per year predisposing main season potato crops to spontaneous and severe late epidemic outbreaks. Relationships between late blight epidemic onset and weather factors, host resistance, fungicide use and basic cultural practices in Sub-Saharan Africa have been adequately studied (Olanya *et al.*, 2001). Such studies would allow designing fungicide deployment according to the nature of disease epidemic (Paveley *et al.*, 2000). Locally developed, zone-specific late blight prediction models can be useful guides in developing decision support tool (DST) for optimising fungicide use in potato production and disease management without compromising fresh tuber yields. They can be as simple as reference tables, a list of rules, flow charts and diagrams or as complex as computer generated empirical or simulated models. Late blight onset forecasts that would form a foundation for DST and a vital component of integrated late blight management (Forbes *et al.*, 2002; Dowley and Bourke, 2004) have been glaringly missing for tropical highland potato farming systems. An experiment was consequently designed with an objective to develop late blight onset prediction model befitting continuous potato planting commonly practised in highland tropics in relation to weather variables. This was envisaged as a basic decision tool for optimising fungicide deployment in potato crop and late blight epidemic development.

Materials and methods

Three *Solanum* potato varieties: Victoria (CIP 381381.20), NAKPOT4 (CIP 387121.4) and NAKPOT5 (CIP 381471.18) were planted at 21-day intervals during the first (Feb-July) and second (Aug.-Dec) rainy seasons in 2002, 2003

and 2004 at Kalengyere Research Station in Uganda. This site is located at 01°13.2′S, 020°47.8′E and 2,450 m above sea level on 134 ha of land. The area receives 1000-1500 mm rainfall annually and the mean monthly temperature is 16°C. The soil is volcanic Andosol, light and friable with pH ranging between 4.5 and 6.9 (Sendiwanyo, 1992). The climate, chemical and physical attributes of the sites make it ideal for potato cultivation throughout the year, hypothetically creating continuous presence of late blight inoculum. The crop rotation cycle at the research station takes 2.5-3 years involving potato, legume, cereal and 1.5to 2 year fallow but this does not totally void the land of volunteer potato; a good source of late blight inoculum for new epidemics.

The planting dates during the first rainy season were 1st March, 22nd March and 12th April. During the second season, the planting dates were 1st September, 22nd September, 13th October and 3rd November. By combining potato variety, planting date and seasons over three years, 60 disease epidemics were studied. For each planting date trial set, neither artificial disease inoculation nor disease control was done. Late blight infection was from naturally occurring inoculum. Experimental seed potato was obtained from disease-indexed lots of the national potato programme.

Experimental design and crop management

A split plot design was used. The date of planting constituted the main plot and potato varieties the sub-plot in order to reduce inter-plot effects. Each sub-plot was 4.5 m by 2.8 m and consisted of four rows spaced 70 cm apart. In each row, 15 fully sprouted seed tubers were planted at 30 cm apart within a row resulting in 47,620 seed sets ha^{-1}. Open alleys of 1.5 m and 2.0 m wide, were left between adjacent sub-plots and main plots, respectively. Compound N:P:K 17:17:17 fertilizer (YARA East Africa Ltd, Nairobi, Kenya) was applied at 100 Kg ha^{-1} as a single-dose side-band at planting. For each experimental set, three replications were used. The trials were kept free of weeds by hand-weeding. Insect pests were controlled with Agro-thoate 40 EC (TransAgro, Neukirchen-Vluyn, Germany) at 1.19 Kg a.i. ha^{-1} applied with a back-pack sprayer at 2-bar pressure. The crop was dehaulmed at full maturity, circa 90 days after planting (DAP) and harvested three weeks later.

Data collection

Percent crop emergence was recorded every three days from 12 DAP, as a ratio of number of hills where potato plant shoots had emerged to seed tubers planted. From 80-100% crop emergence, each potato plant was visually examined every 2-3 days to identify first late blight symptoms, determine the date of disease onset and initial disease severity ($\boldsymbol{Y_o}$). Disease severity was subjectively recorded as percent leaf area affected (PLAA) from the middle-rows at sub-plot level every 3-10 days until the crop approached senescence to avoid confounding crop aging with disease attack. Hourly temperature (°C) and relative humidity (%) were measured using an electronic, Hobo®, data logger placed within the crop canopy and downloaded with BoxCar Pro Version 3.51 computer programme (Onset Computer Corp, USA). Daily rainfall was recorded using a standard rain gauge installed within the Research Station.

Data analysis

Temperature and relative humidity (RH) data were processed with MS Excel (Microsoft Corporation, USA) and Polux (International Potato Centre, Lima, Peru) computer programs. The number of hours when the RH (%) and temperature (°C) exceeded 90% or 16°C, respectively, these being threshold values to support LB infection and epidemic development, were computed (Harrison, 1992; Forbes *et al.,* 2002). Also computed was number of hours when RH≥87% as a way of adjusting the leaf-wetness proxy.

Temporal characteristics of late blight epidemics were studied using disease progress curves and parametised with logistic function in FITCURVE alogarithm of Genstat 6.1 Release (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The logistic function is expressed as,

$$Y_L = A+C/(1+\exp(-B*(x-M)) \text{ or } A+C/(1+e^{-b(x-M)}) \quad \ldots\ldots 1$$

where; Y_L is disease severity (%), '***A***' is the lower asymptote, ***'B'*** is the rate constant of the disease progress curve at the point of inflexion or point of maximum rate of disease increase (***'M'***). The parameter **'C'** is the curve constant related to residual disease. The upper asymptote is **(A+C),** ***'x'*** is the exploratory variable (Payne, 2002; Mead *et al.,* 2003).

Accumulated hours when temperature and RH exceeded threshold values from either 1^{st} March, or 1^{st} September as reference planting dates or from the actual date of planting per trial set were computed, tabulated and used in disease onset prediction modelling. Similarly treated were daily rainfall and rain days. The number of days from planting to 80-100% crop emergence and late blight onset per crop cycle were computed.

Data queries were performed in MS-Excel and summaries generated where necessary. Correlation analysis was performed between number of days from planting to disease onset with the weather variables previously described. Multiple regression models were fitted to identify key weather variables that possibly influence the onset of late blight epidemics in a given crop cycle. Season, planting date and potato variety were included as fixed effects in regression models. The significance of fixed effects and their interactions were tested with analysis of variance. Differences among significant main effects and interactions were compared with standard error of mean. The multiple linear regression model for days from planting to disease onset was hypothesised as;

$$\mathbf{Y_d = Y_r + S+P+V+T_{16}+R_{(87,90)}+R_f+R_d+e} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\mathbf{2}$$

where $\mathbf{Y_d}$ is days from planting to disease onset, $\mathbf{Y_{r,}}$ **S, V, P** and **V** are year, season, planting date and potato variety terms, $\mathbf{T_{16}}$ is accumulated hours of temperature ≤16°C, $\mathbf{R_{(87,90),}}$ is accumulated hours with relative humidity (RH) equal to or greater than either 87 or 90% , $\mathbf{R_f}$ is accumulated rainfall (mm), $\mathbf{R_d}$ is accumulated rain days and **e** is the error term.

Least squares regression technique was used in selecting the most appropriate regression models. Models with high reliability and few variables were preferred (Mead *et al.*, 2003; Stern *et al.*, 2004). Functional relationships were explored between late blight onset and other disease progress curve parameters fitted with the logistic function with Genstat (6.1 Release) statistical computer package following pertinent procedures.

Results

Comparison of late blight onset between the first and second rainy seasons indicated that, disease appeared earlier during the first than the second rainy season on the potato varieties used in this study (Table 1). Among the early-planted potato, late blight appeared 37-40 and 50-55 DAP during the first and second seasons, respectively (Table 1). Late blight during both seasons appeared significantly (P≤0.05) earlier in late- than early-planted potato (Table 1). Late blight also generally appeared significantly earlier on potato var. Victoria and averagely two days later on both vars. NAKPOT4 and NAKPOT5 more so among early planted potato in every crop cycle over seasons across the years (Table 1).

Table 1. Variability in days from planting to late blight onset across seasons among potato varieties at Kalengyere Research Station from 2002 to 2004

Season†	Planting date	Number of days from planting to late blight disease onset			
		Victoria	NAKPOT5	NAKPOT4	Mean
2002A	30 March	39.9±1.1	40.8±1.1	40.5±1.1	40.2±0.1
	18 April	31.9±1.1	33.8±1.1	33.5±1.1	33.1±0.1
2002B	1 September	52.0±1.0	54.0±1.0	53.6±1.0	53.3±0.0
	22 September	45.0±1.0	46.9±1.0	46.6±1.0	46.2±0.0
	13 October	34.6±1.1	36.5±1.1	36.2±1.1	35.8±0.1
	3 November	25.7±1.2	27.6±1.2	27.3±1.2	26.9±0.1
2003A	1 March	40.0±1.0	41.9±1.0	41.6±1.0	41.2±0.0
	22 March	33.0+1.0	34.9±1.0	34.4±1.0	34.1±0.0
	12 April	22.5±1.1	24.5±1.1	24.1±1.1	23.7±0.1
2003B	1 September	53.1±1.0	55.0±1.0	54.7±1.0	54.3±0.0
	22 September	46.1±1.0	48.0±1.0	47.7±1.0	47.2±0.0
	13 October	35.7±1.0	37.6±1.0	37.3±1.0	36.8±0.0
	3 November	26.8±1.2	28.7±1.2	28.4±1.2	27.9±0.1
2004A	1 March	38.5±1.0	40.3±1.0	40.1±1.0	39.7±0.0
	22 March	31.5±1.0	33.4±1.0	33.1±1.0	32.6±0.0
	12 April	21.1±1.1	23.0±1.1	22.6±1.1	22.3±0.1
2004B	1 September	51.6±1.0	53.5±1.0	53.2±1.0	52.8±0.0
	22 September	44.6±1.0	46.5±1.0	46.2±1.0	45.7±0.0
	13 October	34.2±1.0	36.1±1.0	35.7±1.0	35.3±0.0
	3 November	25.3±1.2	27.2±1.2	26.9±1.2	26.4±0.1

†Season A starts from last week February and ends in July while season B begins in the last week of August and ends in January of another year

Relation between late blight onset and local weather variables

There was generally high and negative correlation between number of days from planting to late blight onset and accumulated hours with relative humidity ≥87% or RH≥90%, accumulated hours with temperature ≤16^{0}C, accumulated rain days and accumulated rainfall all computed from either 1st March or 1st September per crop cycle (Table 2). Combined data across each season over planting dates had low correlation coefficients albeit negative (Table 2). Accumulated hours with RH≥87% and RH ≥90%, were collinear however, the later had a higher predictive index (0.67) than the former (0.53) and consequently removed from further analysis. Year ($\mathbf{Y}_r$), seasons (**S**), planting date (**P**) and potato variety (**V**) were included as fixed effects, and $\mathbf{T_{16}}$, $\mathbf{R_{90}}$, $\mathbf{R_f}$, $\mathbf{R_d}$ as quantitative predictors in equation 3

$$\mathbf{Y_d = Y_r + S + P + V + T_{16} + R_{90} + R_f + R_d + e} \quad \ldots\ldots 3$$

The resultant model was highly significant (P<0.001) and explained 98.8% of the observed variation (Table 3). However, stepwise regression, removed the year (Y_r) and potato variety (P) terms with accumulated rainfall (R_f) and accumulated hours with temperature ≤ 16^{0}C (Table 3). The final model comprised of seasons (S) and planting (P) with accumulated hours with RH≥90% and accumulated rain days as variable predictors. The model accounted for 98.9% of the observed variability (Table 3).

Table 2. Correlation coefficients between days from planting to late blight onset and key weather variables at Kalengyere Research Station from 2002 to 2004

Weather variable	Correlation coefficients for days from planting to late blight onset						
	2002A	2002B	2003A	2003B	2004A	2004B	Combined
Accumulated hours RH≥87%	-0.746	-0.949	-0.536	-0.951	-0.809	-0.950	-0.549
Accumulated hours RH≥90%	-0.786	-0.948	-0.537	-0.953	-0.810	-0.950	-0.543
Accumulated rainfall (mm)	-0.860	-0.910	-0.551	-0.933	-0.873	-0.943	-0.287
Accumulated rain days	-0.614	-0.951	-0.541	-0.957	-0.813	-0.949	-0.336
Accumulated hours with temperature≥ 16 ^{0}C	-0.623	-0.948	-0.473	-0.945	-0.811	-0.949	-0.235

A scatter plot of fitted against observed values had a significant (P≤0.05) unit (1) gradient and R^2 was 96.8%. A plot of residuals against fitted values showed a random scatter, a non-significant (P≤0.05) intercept and R^2 was $1x10^{-6}$. This showed uniformity of residual variances and normality of the data suggesting that the fitted model provided a good description of the biological data (Table 3). Models for LB onset prediction were consequently fitted for each season and date of planting as a fixed effect.

Table 3. Analysis of variance from stepwise multiple regression model predicting number of days from planting to late blight onset in potato at Kalengyere from 2002 to 2004

Source of variability	d.f.	Sum of squares	Mean squares	Variance ratio	F prob.	R^2
Year	2	260.7	130.3	117.3	0.061	
Season	1	449.6	449.6	404.7	<.001	
Planting date	3	4244.3	1414.8	1273.5	<.001	
Variety	2	59.6	29.8	26.8	<.001	
Accumulated hours temperature ≤16°C	1	22.6	22.6	20.4	<.001	
Accumulated rainfall (mm)	1	174.4	174.4	157.0	<.001	
Accumulated rain days	1	111.2	111.2	100.1	<.001	
Accumulated hours with RH≥90%	1	11.5	11.5	10.4	0.002	
Residual	41	45.5	1.1			**98.8**
Dropping						
Accumulated rainfall (mm)	-1	-1.2	1.2	1.1	0.302	
Variety	-2	-3.3	1.6	1.5	0.241	
Accumulated hours temperature ≤•16°C	-1	-2.9	2.9	2.6	0.112	
Year	-1	-3.9	3.9	3.5	0.345	
Final model (Residuals)	53	5379.5	101.5			**98.9**

The difference in R^2 between common and separate gradients and y-intercepts for planting dates in each season was 0.01. Thus, for consistency in disease onset prediction for a given crop cycle in a season, a separate y-intercepts and gradients model procedure would adequately predict disease onset without serious effect on model output (Mead *et al.*, 2003). Consequently, in both seasons, separate models per planting date were used to estimate partial regression coefficients for late blight onset prediction (Table 4).

Model parameter estimates indicated that the y-intercepts were not significant (P≤0.05). The rest of the partial regression coefficients for the predictors (X_i) were significant (P≤0.05), except for the last-planted potato crop during both rainy seasons (Table 4).

Table 4. Partial regression coefficients for predicting the number of days from planting to potato late blight onset during the first and second cropping seasons at Kalengyere Research Station, from 2002 to 2004

Rainy season	Parameter	Model partial coefficients[†]			
		1 March	22 March	12 April	-
First	Intercept	$-10.5^{0.636}$	$6.5^{0.66}$	$-14.3^{0.878}$	-
	Accumulated rain days (X_1)	$3.2^{0.01}$	$2.5^{<.001}$	$1.1^{0.8}$	-
	Accumulated hours-RH≥90% (X_2)	$-0.034^{0.05}$	$-0.062^{0.039}$	$-0.0002^{0.9}$	-
		1 Sept.	22 Sept.	13 Oct.	3 Nov.
Second	Intercept	$6.5^{0.558}$	$7.7^{0.43}$	$5.1^{0.962}$	$4.9^{0.808}$
	Accumulated rain days (X_1)	$1.38^{<.001}$	$0.82^{<.001}$	$0.69^{<.001}$	$0.48^{0.077}$
	Accumulated hours-RH ≥90% (X_2)	$0.014^{0.05}$	$0.012^{0.02}$	$0.006^{0.303}$	$-0.002^{0.722}$

[†]Superscripts on each partial coefficient are levels of significance

Relationship between late blight onset and point of inflexion of the disease progress curve

There was a high and positive correlation coefficient between days from planting to late blight onset and the point of inflexion (r = 0.822). The point of inflexion (days) of the disease progress curve can thus be estimated from late blight disease onset predictions.

A general linear regression model estimating the point of inflexion from the number of days from planting to disease onset with year, season, planting date and variety as factor variables was highly significant (P < 0.001) explaining 80.9% of the observed variability. However, stepwise regression showed that the point of inflexion was best fitted with a simple, highly significant (P < 0.001) linear function with days from planting date to disease onset as a lone predictor.

The model had a significant (P = 0.013) y-intercept (±2.15) and highly significant (P<0.001) slope (±0.105) and accounted for 88.8% of the observed variability (Figure 1). A plot of residuals against fitted values indicated a random scatter alluding to uniformity of residual variance. The plot observed against fitted values was a straight line with an intercept not significantly (P≤0.05) different from zero and a unit (1) gradient indicating a good fit of the model to the biological data.

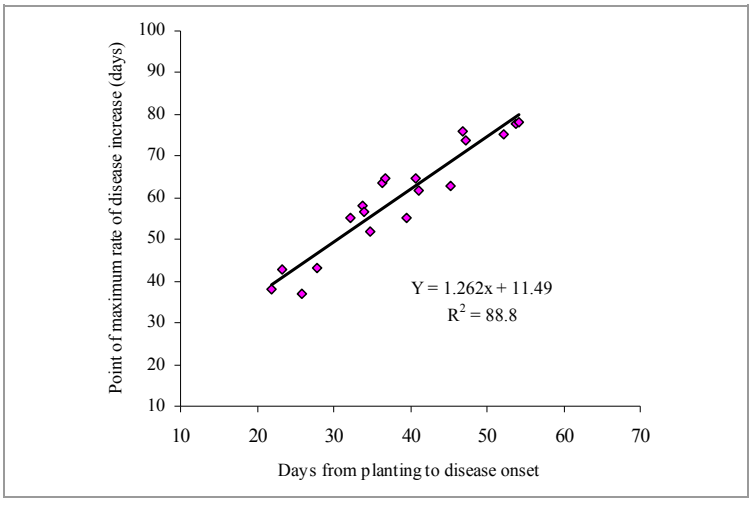

Figure 1. Relationship between days from planting to late blight onset and days to reach inflexion of the disease progress curve at Kalengyere Station from 2002 to 2004

Assessment of predictive precision of the model in estimating the inflexion of the DPC indicated that among 53 of the 60 epidemics whose parameters could be estimated with the logistic function, the model accurately estimated the inflexion in 24 (40%) of the epidemics, overestimated it in 15 epidemics (25%) and underestimated in 14 (23.3%) of the epidemics. However differences in inflexion estimate did not exceed twice the standard error of mean. The model was able to estimate the point of inflexion or some days before it in 71.7% of the studied epidemics.

Discussion

Late blight onset assessment among selected potato cultivars in natural field conditions in seasonal, continuous potato planting in the highlands of south western Uganda indicated that the date of planting had a strong influence on days from planting to disease onset than potato variety. However, LB appeared earlier during the first than second rainy season because the dry spell between the second and first rainy season between two successive years is shorter than between the first and second season in the same year. This offers more potato crop overlap, with more fields infested with late blight thus offering high inoculum loads early in the season. The number of days from planting to LB onset in a given potato crop decreased with delay in planting during both seasons. By the last planting date, disease was detected within 21-28 DAP and occasionally simultaneously among varieties irrespective of the previously observed resistance to LB in early-planted potato. Early disease onset among late-planted potato during each season may due to highly favourable weather for LB epidemic development soon after crop emergence coupled with probable high *P. infestans* inoculum loads.

Knowledge of disease onset is a crucial step in developing integrated disease management of potato late blight. There were high correlation coefficients between days from planting to LB onset and accumulated hours with, RH ≥ 90%, temperature ≤16°C, accumulated rainfall (mm) and accumulated rain days as predictors for LB onset. However, variables computed from 1st March or 1st September as seasonal reference planting dates but not from the actual date of planting per crop cycle best described the data. This approach is appropriate because fits the farming calendar and integrates previous weather events which have a strong cumulative influence on the plant growth, LB behaviour and future disease epidemic development (Harrison, 1992; Forbes, 2003; Andrade-Piedra *et al.*, 2005b). Pooled data across seasons had low correlation coefficients for days from planting to disease onset. The two seasons therefore should be analyzed separately and models developed for more homogenous environments if they are to adequately predict disease onset than developing universal functions which may suffer from loss of precision in natural systems (Andrade Piedra *et al.,* 2005a).

Stepwise multiple regressions with the measured weather variables and experimental fixed effects indicated that the days from planting to late blight onset on a given potato crop was quantitatively related to accumulated hours with RH≥90% and accumulated rain days both computed from either 1st March or 1st September for the first or second rainy season, respectively. Estimated model gradients were significant (P≤0.05), except for late-planted potato. This indicates a probable loss of predictive accuracy in April- and November-planted potato, possibly due to uniformity in weather conditions favouring disease infection or very high viable disease inoculum. The y-intercepts of the models though not significant (P≤0.05) decreased with delay, and probably had little or no effect on the model output since any of the quantitative variables in optimal potato growing conditions may not have a zero-value in an entire crop cycle.

The point of inflexion of the disease progress curve is a key parameter of disease development. Early attainment of the inflexion in a young potato would lead to serious disease attack in a crop with limited foliage resulting in high yield losses unless control measures are instituted. Late attainment of the point of inflexion irrespective of the disease severity may not grossly affect tuber yield. The duration from planting to attaining the inflexion (***"Y"***) was linearly related to days from planting to disease onset ***'x'*** by ***Y = 1.262x + 11.49***. The exploratory values of ***'x'*** can be determined from the previous weather-dependant disease onset prediction models. The significant (P = 0.013) y-intercept in this model indicates that the value of ***x*** can never be zero. The estimated date of disease onset and point of inflexion can thus be used to institute, defer or completely ignore disease control depending on the stage of crop growth. This would permit use of control measures when they have highest benefit. .

Conclusion and recommendations

This study demonstrated that late blight onset in a given potato crop in the highlands of Southwestern Uganda can be predicted from accumulated hours with relative humidity ≤90% and accumulated rain days. However, data must be computed from beginning of the rainy season in a farming calendar of this agro-ecosystem here taken as 1st March and 1st September for the first and second rainy seasons, respectively. Prediction of disease onset was linked to a model predicting the date of attaining inflexion of the disease progress curve. These functions can be iteratively used in designing a disease escape strategy or deployment of the crucial fungicide sprays that are vital in successful late blight control. Estimates of the point of inflexion would also prevent using fungicide when the crop is approaching maturity and further disease increase will have little or no effect on gross tuber yield. The LB onset prediction model however, suffered from loss of precision among late-season planted potato where disease tended to appear soon after crop emergence probably due to high and viable

inoculum loads. Variation in inoculum density over a rainy season was not included in this study and would thus be an important component in future late blight epidemiological studies for tropical highland potato cropping system. Finally, these models were developed with empirical data over three years and were not tested on independent data. It is crucial that they are tested in independent experiments in this and similar agro-ecological zones.

Acknowledgement

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Molecular characterization of *Colletotrichum gloeosporioides* responsible for anthracnose of yam and cassava in Nigeria, and development a diagnostic PCR assay

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Anthracnose, caused by *Colletotrichum gloeosporioides* Penz, is one the major diseases of yam and cassava in West Africa. Thirteen isolates, ten from yam and three from cassava, isolated in Nigeria were classified based on their symptoms and morphological characters into two groups: spot (S) and blight (B). Both S and B isolates infect yam, but only B isolates infect cassava. All these isolates were analyzed by nucleotide sequencing of nuclear ribosomal internal transcribed spacer (ITS) sequence (ITS-1, 5.8S and ITS-2), actin and histone genes. Phylogenetic analysis of the three gene sequence data grouped these 13 isolates into two major clades. Both S and B isolates distributed between the two clades, with all the three cassava infecting isolates formed a separate sub-clade. Further studies are underway to assess the implication of S and B isolates on host resistance.

A set of primers was designed from the nucleotide sequences of rDNA ITS region for specific detection of yam and cassava infecting *C. gloeosporioides* isolates. A simple method for extracting DNA from mycelia, leaf, stem and roots was established for *C. gloeosporioides* diagnostic PCR, which was validated by testing 36 *C. gloeosporioides* isolates from yam and 85 isolates from cassava. Specific product (500 bp) was obtained from all the yam and cassava isolates but not in *C. gloeosporioides* isolate from soybean indicating the high-specificity of the primers. This assay can is useful in diagnosis of *C. gloeosporioides* of yam and cassava.

Introduction

Nigeria rank first for production of yam in Africa and for cassava in World (FAO, 2007). These serve as staple food for millions of people in Nigeria. These crops are infected by several pathogens i.e. virus, fungus and bacteria. Among fungal *Colletotrichum gloeosporioides* are most important. This pathogen may once it appears in the field can destroy all susceptible variety and gave a blighted appearance to the field in few days.

A combination of techniques (culture on agar plates, colony morphology, growth on selective media, and various biochemical reactions) is used for the identification of fungi and are used to detect specific fungi or its strains (Jyan et al., 2002). The accuracy and reliability of the conventional methods depend largely on the experience and skill of the person making the diagnosis. This approach is time consuming (several days to weeks) and impractical to analyze large number of samples. Further, such methods are unsuitable for reliable identification of new strains or species that may not show distinctive phenotypic or physiological features. With the advent of biotechnology, molecular methods, such as polymerase chain reaction (PCR), are fast establishing as a choice method for routine identification of fungal pathogens (Henson and French, 1993). These so called modern methods are rapid, highly specific and can be used to detect minute quantities of fungal DNA from environmental samples before symptoms occur, therefore, allowing implementation of early control methods. PCR technology can also provide very accurate quantitative data supplying the necessary additional information required for control and quarantine decisions and for assessing how effective fungal agents are in the case of biological control (Atkins and Clark, 2004). These methods exploit genes such as rDNA, tubulin, the most and know to be conserved in a wide array of fungal species.

We design the primer based on rDNA region for *gloeosporioides* specific for diagnostic purposes and for Phylogenetic analysis. The method was tested for 121 strains of yam and cassava and 21 strains of other fungi to show the specificity of primer. The primer only amplified a specific band with yam and cassava strain.

Materials and methods

The *C. gloeosporioides* isolates are of dioscorea and cassava were received from Pathology and Germplasm Health Unit. The fungi cultured on potatao dextrose agar (PDA) at 28 °C. The 5 mm diameter for each isolate

incubated in rotary shaker at 100 rpm at 28°C. The mycelial mass of each isolate was harvested by filteration through sterile filter paper and used immediately for DNA extractions.

Genomic DNA extractions

Total genomic DNA extraction protocol developed in our lab and this was used in all tests performed. The 100 mg mycelia was grinded with mortar and pestle and 1.5 ml of extraction buffer {(100 mM Tris (pH 8), 10 mM EDTA (pH 8), SDS 1%, PVP 2% and 1 M NaCl. Autoclave and add 1% β-mercaptoethanol just before use} added containing proteinase K incubated to at 65 °C for 30 minutes in water bath. Bring the tubes out and cool it to room temperature for 3-5 minutes and centrifuge for 13,000 rpm for 10 minutes. Transfer the supernatant to new tubes and add 200 µl of PEG. It was observed that incubating the tubes at 4°C for 10 minutes improve the yield. Centrifuge at 13,000 rpm in microcentrifuge and transfer the supernatant to new fresh tubes. Add 2/3 volume isopropanol and incubate the tube at -20°C for 1 hour to precipitate DNA. Centrifuge at 13,000 rpm for 10 min. at 4 °C, remove supernatant, and wash the DNA pellet with 70% Ethanol, air dry, and resuspend in 50-100 µl of TE (10 mM Tris (pH 8) and 1 mM EDTA (pH 8) buffer. (It was observed that centrifugation at room temperature doesn't effect the quality of DNA). Remove RNA by adding 1 µl of RNase (10 mg/ml) incubated at 37°C for 30 minutes, and stored at -20°C until use.

PCR amplification and sequencing of ITS, Actin and Histone region

The 10 type isolate of yam and 3 type isolate of cassava were characterized by nucleotide sequence analysis. For this, the 18S ribosomal RNA gene partial, ITS1 1, 5.8S ribosomal RNA gene, ITS 2 complete and 28S ribosomal RNA partial gene sequence, regions were amplified with the universal primers ITS1 and ITS4 (White et al., 1990). Histone H3 is used for phylogenetic analysis with primers CylH3F and CylH3R (Crous et al., 2004) and actin region was amplified using primers Act512 and Act 783R (Carbone and Kohn, 1999). The PCR reaction (25 µL final volume) reaction mixture consisted of 5.0 µL 5x PCR buffer (Promega, Madison, WI, USA), 25 mM $MgCl_2$, 10 mM dNTPs, 10 pmol of each primer, 0.5 units of Taq polymerase (Promega) and 4.0 to 10 ng of template DNA. PCR conditions for the ITS were, after the initial denaturation at 94 °C for 5 min, 35 PCR cycles were performed with 30 sec at 94 °C, 1min at 55 °C and 1.5 min at 72 °C. After the last cycle, the temperature was maintained at 72 °C for 8 min and for actin and Histone annealing temperature used 58 °C for 30 sec and extension for 1 minute. DNA amplification was performed in a MJ Research Thermal Cycler. PCR products were visualized by electrophoresis in 1.5% (wt/vol) agarose gels run 1 X Tris-acetate-EDTA (TAE) buffer stained with ethidium bromide and visualized under UV light. DNA sequencing was performed with two primers (ITS1 and ITS4) in both directions to ensure that there was no misreading. PCR products were purified and sequenced by (BangloGenei, India). Alignment and edition were carried out with the BioEdit Program v 7.0.5 (Hall, 2005) and visually corrected. Sequences were then compared against those available in the GenBank database.

Diagnostics PCR with Strain-Specific primer

The primer YA(I)R (5'-GTTACTACGCAAAGGAGG-3') specific for *gloeosporioides* for yam and cassava was used in conjunction with the conserved primer ITS1 (White et al., 1990) for rDNA amplification. The PCR reaction and condition are same as explained above PCR products were visualized by electrophoresis in 1.5% (wt/vol) agarose gels run 1 X Tris-acetate-EDTA (TAE) buffer stained with ethidium bromide and visualized under UV light.

Results

PCR amplification and sequencing of ITS region

The DNA of fungal isolates 13 from yam and 3 from cassava were used in PCR with the Universal primers ITS1 and ITS4 for the amplification of the rDNA region comprising the two non-coding internal transcribed spacers ITS1 and ITS2 and the 5.8S rRNA gene, partial 18S and 28S. All isolates amplified a PCR product of approximately 550 bp. Sequences were determined of each isolate, and blast analyses were carried out for 13 isolates, previously identified by morphological and biochemical study. The results showed a 99-100% homology with DNA sequences from other *C. gloeosporioides* strains deposited in the GeneBank.

PCR amplification and sequencing of Actin and Histone region

The DNA of fungal isolates 13 from yam and 3 from cassava were used in PCR with the primers Act512 and Act783 for the amplification of the actin region and primers CylH3F and CylH3R for the amplification of Histone3 region. All isolates amplified a PCR product of approximately 270 bp with actin and 390 bp with histone. Sequences were determined of each isolate, and blast analyses were carried out for 13 isolates. Since there are no more sequences deposited in NCBI for *C. gloeosporioides* based on Histone and Actin there was no homology determined.

Diagnostics PCR with Strain-Specific primer

With PCR testing of gloeosporioides from fungal strains yam and cassava with primer pairs YA(I)R and ITS1, 500 bp band was successfully detected in all strains. Among the samples tested. To show the specificity of primer other fungal and bacterial strains were tested and no band was detected.

Discussions

The purpose of this research was to perform phylogenetic analysis of the C. gloeosporioides and develop data on the number of nucleotide differences between blight and spot causing strains to yam and cassava and to determine whether spot and blight represent different species. The another aim of this research was to use PCR-based methods to identify rapidly and accurately the species of *gloeosporioides* responsible for anthracnose in yam and cassava in Nigeria.

The size of the amplification product 500 bp confirmed with specific designed primer. PCR with ITS universal primers amplification of the 18S-5.8S-ITS-28S region of DNA, subsequent sequence analysis of the rDNA product revealed clearly the existence of two strains causing anthracnose lesions on yam. PCR amplification of the three target genes, rDNA ITS resulted in DNA bands of expected size from all the isolates ~550bp, actin 270 bp and histone 390 bp. The amplified fragments were sequenced and data was analyzed using bioinformatics packages, BioEdit and MEGA. The 13 isolates clustered into two major groups in a phylogenetic tree inferred from rDNA sequence information, with the isolates from cassava forming a sub-group in one of the major clades along with one of Yam strain (YA08-10) (Figure 1) suggesting that yam and cassava strain are genetically very close. Phylogenetic analysis revealed the composition of subclades by blight and spot isolates from Nigeria. This may be a consequence of intermatings between isolates introduced from distant regions and the resident isolates of the respective regions.

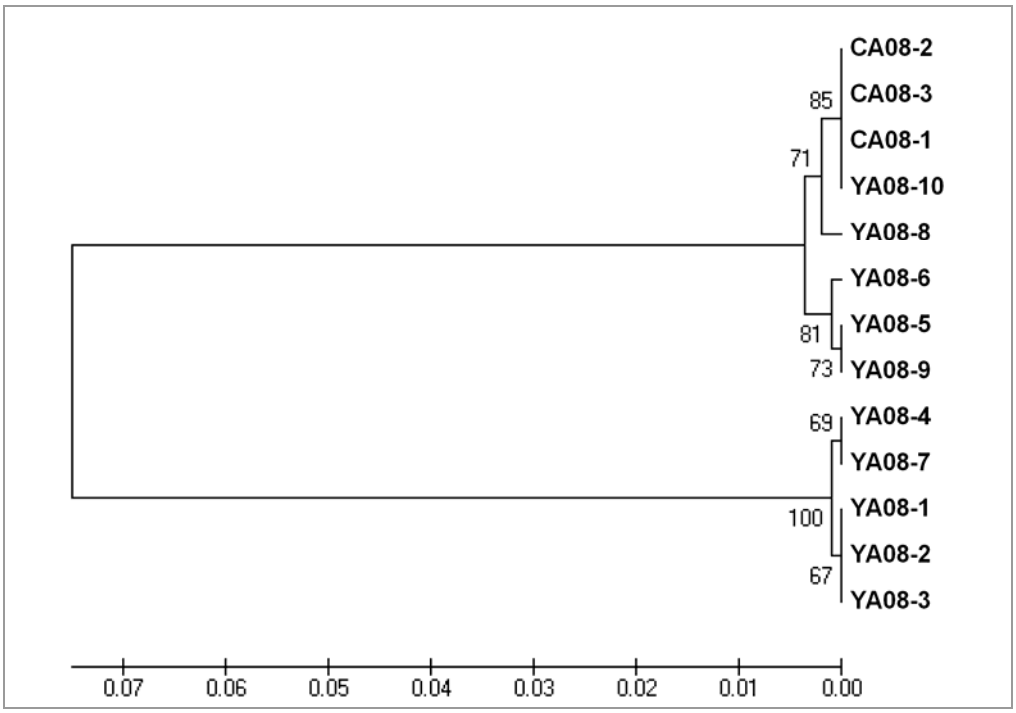

Figure 1

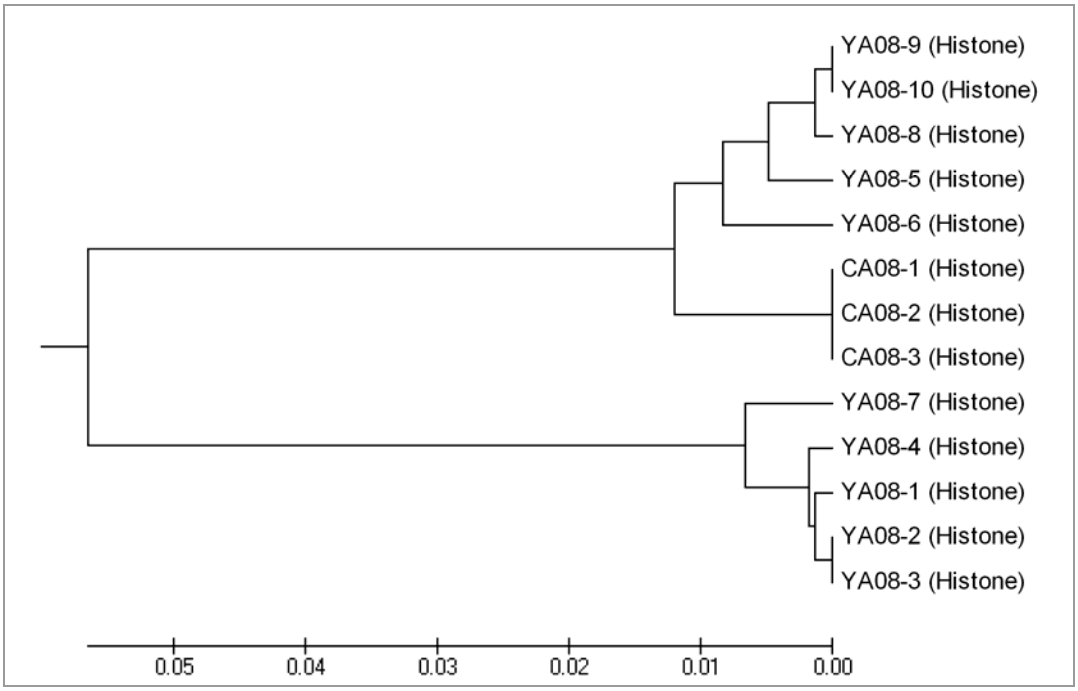

Figure 2

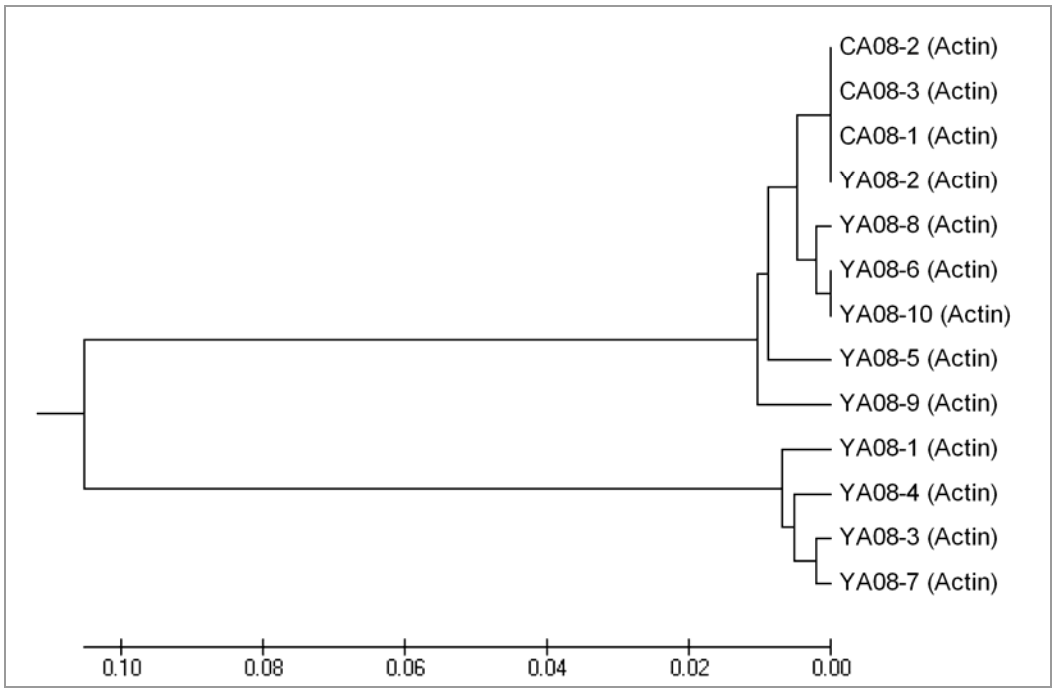

Figure 3

PCR with primers Histone and Actin amplified and subsequent sequence analysis of the product revealed clearly the existence of two strains causing anthracnose lesions on yam as showed with ITS analysis. The amplified fragments of actin and Histone gene were sequenced and data was analyzed. The 13 isolates clustered into two major groups in a phylogenetic tree inferred from above sequence information, with the isolates from cassava forming a sub-group in one of the major clades alone with Histone analysis (Figure 2) while along with one of Yam strain (YA08-2) with Actin sequence analysis (Figure 3) suggesting that yam and cassava strain are genetically very close. The ITS region has already shown before to be useful for *Colletotrichum* species identification (Press et al, 2002 and Chen et al., 2006). The rDNA sequence can discriminate at the level of orders and kingdoms. At the species level, spacers of the rDNA are widely used for phylogeny studies, as they usually vary between species within a genus, but show little or no intraspecific variation (Kim and Lee, 2001). The high copy number 18S-5.8S-ITS-28S rDNA region allows the amplification of very small amounts of DNA with PCR. The designed primer was tested for 121 strains of yam and cassava which includes spot and blight symptom and 21 strains of other fungi and bacteria. In all strains of yam and fungi a specific band of 450 bp amplified whereas no band for other than *gloeosporioides* fungi tested. This technique will facilitate routine work in phytopathogen diagnostics. To detect the pathogen it is essential to diagnose correct and rapid identification of the agents causing the diseases, and to be able to provide the crop producers with this response so that they can take the necessary steps towards the control of these diseases and reduce crop yield losses.

This study provides genetic relatedness of yam and cassava strain and opportunities for the detection of minute quantities of pathogen and a rapid PCR-based detection method was developed for detection and identification of *C. gloeosporioides* directly from total DNA extracts of fungal cultures and infected plants.

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Efficacy of botanicals and *Bacillus thuringiensis* to control potato tuber moth, *Phthorimaea operculella* (Zeller), in potato stores in Nepal

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Abstract

In Nepal potato is an important food security and a primary cash crop for many small-scale farmers. Potato production has steadily increased since the last decades; however, pests are major constraints limiting the yield potential and causing potato yield losses of up to 80%. Among limiting biotic factors, the potato tuber moth (*Phthorimaea operculella*) has become the major pests in potato cropping in Nepal. Current farmers' control practices rely on the use of highly toxic pesticides applied with little or no protective measures, causing substantial adverse impacts on human health and the environment. The objective of our study was to test the efficacy of two botanicals, crushed leaves and stems of *Artemisia* sp. and an extract of *Acorus calamus* rhizomes, in comparison to a commercial product of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) in rural potato stores in the mid-hills of Nepal and Bhutan. Potato tuber moth damage to tubers was evaluated in monthly intervals for a period of 4-5 month. Proportions of damaged tubers were highly related to the density of mines per tuber. Further, the distribution of damage in potato heaps was slightly aggregated. Our results showed that all treatments significantly reduced tuber damage. The rhizome powder of *Acorus calamus* provided best protection followed by *Btk* and *Artemisia*. Damage in potato heaps treated with *Artemisia* increased gradually while *Acorus calamus* and *Btk* delayed infection increase for about 1-2 month. None of the products provided satisfying protection after 3-4 month storage period at severe potato tuber moth pressure but might be efficient, when storage condition do not allow a reinfestation by moths.

Keywords: Farmers' Storage, botanicals, formulations, eco-regions, potato tuber moth, Nepal.

Introduction

Potato (*Solanum tuberosum* L., Solanaceae) is an important food and cash crop of Nepal. The crop is grown in various cultivation systems year-round and from high hills to plain areas throughout the country. Threat of insect pests is increasing with intensification of cultivation. The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera; Gelechiidae), is one of the major pests attacking potato. The pest, which is native to South Americas, is today cosmopolitan in distribution. In Nepal, it was reported from Kathmandu (Shankhu), Kavrepalanchowk (Banepa, Nala, Panchkahal), Nuwakot (Trisuli), Dhading (some places), Makawanpur (Palung) and Bara (Parawanipur) districts (Joshi, 1989, 1994). The larvae mine into potato leaves and tubers. Most important is the tuber damage in potato storage that leads to tuber weight losses and rotting (due to infection of fungi or bacteria). In storage, up to 90% weight loss is possible (Joshi, 1989) while infestation of tubers might reach 100% (Lal, 1987, 1998; CIP, 1988; Palacios and Cisneros, 1996). At present, many Nepalese farmers use broad-spectrum chemical pesticides, including organochlorines, to manage potato tuber moth in the field and storage, often with unsatisfying results. Considering the negative health and environmental impacts arising from the unwise use of chemical pesticides in such situations, it is crucial to develop alternative control measures for managing potato tuber moth.

Botanical insecticides have long been used in traditional pest management before the invention of chemical pesticides. They reputedly pose little threat to the environment and to human health and could still play an important role in modern pest management system. In Nepal, 324 plant species are recognized having insecticidal properties (Neupane, 2000). Still many farmers continue to control potato tuber moth by using indigenous control practices like the use of plant material. Kennedy (1984), Pradhan (1988), and Rivera and Retamazo (2000) reported some plants and weeds like Muna (*Minthostachys* sp.), Eucalyptus (*Eucalyptus globulus*), Chilca (*Baccharis* sp.), curry plants, Indian pivets, *Lantana camara*, Pangam leaves, *Chenopodium botrys*, *Mentha arvensis* and *Artemisia vulgaris* (Asteraceae), *Lycopersicon hirsutum* among others, are effective

in controlling potato tuber moth. Currently, many farmers cover their potato with *Artemisia* spp. for protecting them from potato tuber moth attack. Kroschel and Koch (1996) reported also of a significant reduction of tuber infestation by covering potatoes with leaves of the trees *Schinus molle* and *Eucalyptus* sp.

In the present study, two plants, sweet flag (*Acorus calamus)* and *Artemisia* sp. have been evaluated against potato tuber moth in rustic potato storerooms. *Artemisia* spp. is an herbal plant naturally distributed in mid hills of Nepal in wood edges, prairie restorations, herbal gardens, and miscellaneous waste places. It usually occurs in disturbed areas, but has displayed a capacity to invade more natural sites. For protecting the potato tubers, fresh or dried plant parts are used which act as repellent. Sweet flag (Acoraceae), native to India, central Asia, and Eastern Europe is found today in many temperate and sub-temperate areas of the globe. In Nepal, the herb is available up to 2000 meter altitude. Habitats include sedge meadows that are prone to flooding, edges of small lakes and ponds, marshes, swamps, seeps and springs, and wetland restorations. The plant contains β-asarone in stolons which is considered the main substance that acts as insecticide. Content of β-asarone in the plant varies according maturity and altitude where it has grown (Paneru et al. 1997). The two plants were tested along with a bio-pesticide based on *Bacillus thuringiesis* var. *kustaki* (*Btk*) formulated in talcum for comparison. *Btk* formulated in talcum powder or in quartz-rich fine sand and dusted over stored potato has been reported to provide good protection against the potato tuber moth (Lacey and Kroschel 2009, Kroschel and Koch 1996, Raman et al. 1987).

Materials and methods

Experimental sites

The experiments were conducted in 11 farmer's rustic potato storerooms in five different commercial potato growing areas of the central mid-hills of Nepal and Bhutan (Table 1).

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Table 5. Experimental locations in Nepal and Bhutan

Country	District	Sites	Altitude (msl)	No. of experiment	Used in analysis
Nepal	Sindhupalchowk	Thumpakar	2513	2	1, 2
	Makwanpur	Palung	1798	3	Excluded*
	Kavrepalanchowk	Banepa	1454	1	Excluded*
		Dhulikhel	1600	1	Excluded*
		Nala	1588	4	3, 9, 10, 11
Bhutan	Trashigang	Yangneer	1861	1	13
	Mongar	Drametse	1867	1	Excluded*

*Storages were excluded from the analysis because damage pressure by the potato tuber moth was low

In addition, *Acorus calamus* along with the *Btk*-talcum product was tested in two farmers' storerooms in Eastern Bhutan. *Artemisia* was not tested in these sites because the plant was not available during the time of experimentation.

Description of treatments and experimental processes

Artemisia: Mature leaves with stem were collected, shadow dried for 6 days and cut into pieces of 1 cm size in order to make it ready for application. The material was mixed with non-infected potato tubers at the rate of 20 g/kg potatoes.

Sweet flag (*Acorus calamus*): stolen were collected; shadow dried and crushed to a fine dust by using an electrical crusher. The material was spread over the stored potatoes at the rate of 5 g/kg potatoes.

Btk: Commercially available *Btk* (Z-52-strain, Biolep, India) was first mix at a rate of 35 g per liter water. Talcum powder was added to the solution at a 1:1 (w/w) ratio and mixed thoroughly. The paste was poured on the

plastic sheet and air dried in the shadow. After drying, the mixed material was crushed into fine powder using a kitchen role. The final product was applied at a rate of 6 g per kg potato tubers. Batches of 15 kg tubers were shaken together with the *Btk* powder in plastic bags until the tubers were uniformly covered with the dust formulation.

A total amount of 60 kg potatoes were used for each treatment and the control (without any protection). Potatoes for each treatment were piled in one corner of the farmers' storeroom exposed to naturally occurring potato tuber moth attack. Damage was evaluated over a period of about 5 month (from 2nd July to 19th November, 2008). In an interval of about one month 100 tubers were removed from each treatment and brought to the laboratory for assessing total numbers of infested tubers (without typical symptoms of potato tuber moth attack) and number of mines per tubers (sampling was destructive). For logistic reasons the evaluation interval was not always exactly one month.

Statistical analysis

The model of Nachman (1981, 1984) was fitted to the data from each sampled unit for determine the relationship between mean infestation (i.e. the mean number of mines per tuber) and the proportion of tubers damaged. The model is:

$$P_a = 1 - \exp\left(-a\, m^b\right)$$

in which P_a in the proportion of damaged tubers and m is the mean number of mines per tuber in the sample unit. For determining the relationship between the variance, s^2, and the mean, m, of mines per tuber Taylors' power law (1961) $(s^2 = a\, m^b)$ was used. The parameters a and b in the equation were estimated by linear regression after logarithmic linearization of the equation.

Data on damage levels (proportion of damaged tubers per sampling unit) were submitted to 3-way ANOVA; including the factors 'treatment', 'storage' and 'evaluation month' in the model. Since the data include one value only for each factor combination main effects only were evaluated (interaction between factors was not evaluated). Post analysis differences between groups (main effects only) were analyzed using Tukey-test ($p < 0.05$). In some storages damage levels of potato tuber moth were very low and populations did not develop during the course of experiments; these storages were excluded before analysis (Table 1). For multiple comparison chi-square test was applied to all pairs of samples (treatment × storage × sampling date) testing if the proportions of damaged tuber were equal. The test statistic was:

$$\chi^2 = \sum \frac{(O_i - n_i p)^2}{n_i pq}, \text{ for } i = 1, 2$$

where O_i was the observed frequency of damaged tubers for the i^{th} sample, n_i was the samples size for the i^{th} sample, p was $\Sigma\, O_i / \Sigma n_i$, and q was $1 - p$. *Chi*-square values were evaluated using 1 *df*, and $p = 0.05$.

Results and discussion

Mean infestation versus proportion of damaged tubers

The Nachman model described well the relationship between the frequency of damaged tubers and the damage density (mean number of mines per tuber) (Figure 1a).

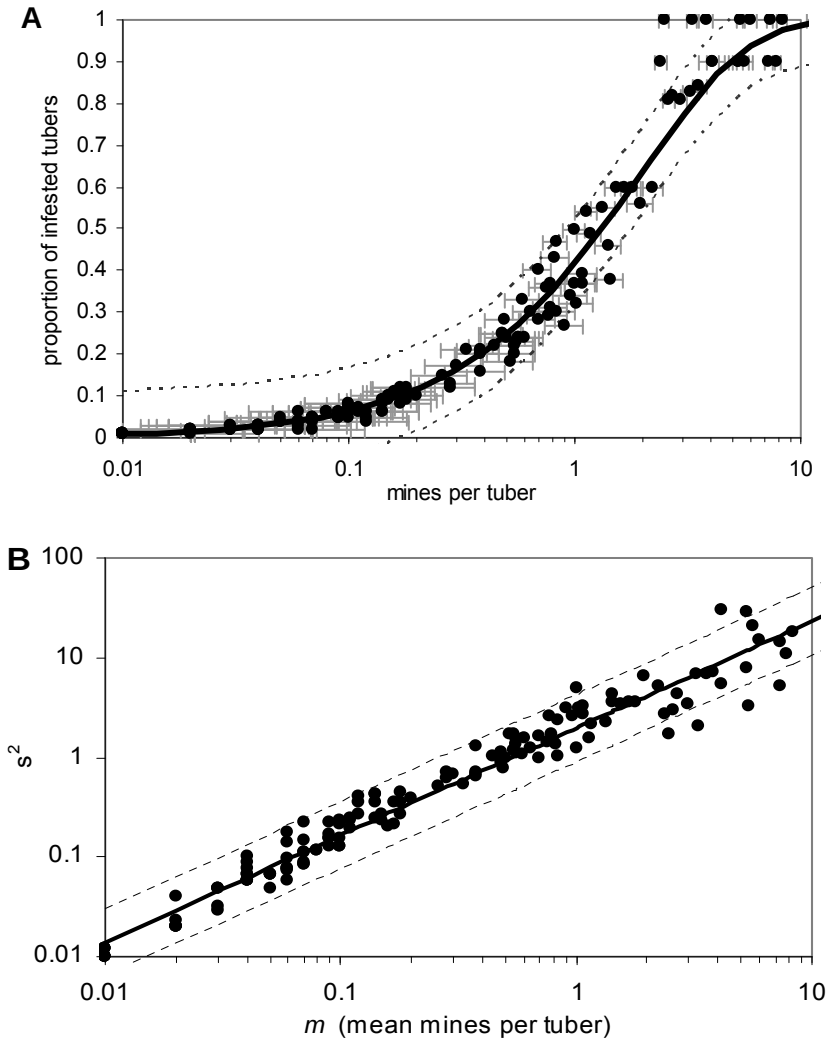

Figure 1.(A) Relationship between proportion of damaged tubers (P_a) and mean number of mines per tuber (m). Points are observed data, bars indicate the standard variation of the mean (m), bolt line is the Nachman function fitted to the data, and scattered line indicates 95% confidence bands. (B) Exponential response of the variance (s^2) to the mean number of mines per tuber (m). The function has the form $\log(s^2) = 1.074 \log(m) + 0.2931$, R2 = 0.9615.

The values of the parameters a and b (equation 1) were 0.578 and 1.001, respectively ($R^2 = 0.9673$). Similar results were obtained by Roux *et al.* (1992) who fitted the same model to data collected in Tunisian potato storerooms infested by potato tuber moth (he obtained parameter values of $a = 0.687$, and $b = 0.932$ with an regression coefficient of $R^2 = 0.994$). This substantiates the strong relationship between the infestation density and proportion of damaged tubers and that the Nachman model is highly suitable for describing this relationship. It implies that one of the two variables alone, frequency of damaged tubers or mines per tuber, can be use to describe potato tuber moth damage levels in potato storerooms.

Application of Taylors power law ($s^2 = a\, m^b$) showed that variance of the number of mines per tuber highly depends on the mean infestation (Figure 1b). The coefficients obtained ($a = 1.964$ and $b = 1.074$, $R^2 = 0.9615$) were quite similar to the coefficients obtained by Roux *et al.* (1992) ($a = 1.786$ and $b = 1.1$, $R^2 = 0.99$) that indicates a slight aggregation of the damage. Roux *et al.* (1992) also showed that the distribution of damage does not follow the Poisson distribution.

Efficiency of treatments

The potato tuber moth infestation density was very variable among the potato storerooms. Results from some storerooms were not included in the analysis because potato tuber moth was absent or did not develop substantial populations in these storages. Finally, results from 7 storerooms (6 in Nepal, 1 in Bhutan) were evaluated (Table 1). ANOVA, applied to the proportions of damaged tubers (number of mines were not further analyzed because the exact number could not be assessed well from heavily infested rotting tubers) revealed significant differences among the storerooms ($F = 8.4$, $df = 6, 97$, $p < 0.001$), while infestation levels significantly changed (increased) during the course of experiments ($F = 18.1$, $df = 4, 96$, $p < 0.001$). The effects of treatments were significant ($F = 19.8$, $df = 3, 96$, $p < 0.001$). The separation by using the Tukey-test ($p < 0.05$) showed that all three treatments, *Acorus calamus*, *Artemisia* and *Btk* significantly reduced damage levels compared to the control. *Acorus calamus* provided best protection and reduced overall mean infestation to 24%, while overall infestation in *Artemisia* and *Btk* treated potato piles was 39.5% and 36.6%, respectively, compared to an overall infestation of 69.7% in controls. However, since the variation in proportions of damaged tuber is not expected to follow the Poisson distribution and due to the fact that the increase of damage did not follow the same trend in all plots (interaction between the main factors could not be analyzed), the results from the ANOVA might be misleading. Therefore all pairs of samples were additionally compared by using the chi^2-test and separated into equal groups based on this analysis (**Table 6**). The results show that *Acorus calamus* in all storages provided the best protection and in some cases significantly higher then *Artemisia* and *Btk*. Overall damage levels in tubers increased with time of storage. In controls proportions of infected tubers increased gradually from almost zero at the beginning of the experiment to 100% after about 4 month in most storerooms. In *Artemisia* treated piles increased damage was already visible at the beginning of the experiment but at a lower rate than in the controls. In contrast *Acorus calamus* and *Btk* delayed the increase of infection for about 1-2 month; after that damage level increases as well in these treatments. Therefore, none of the treatments provided full protection of tubers, but might be used with satisfying results when potatoes are stored for short periods (2-3 month) only. In these experiments, the potato tuber moth density increase in each storeroom was most probably due to the development of potato tuber moth in the control tubers, and therefore the protective capacity of the treatments, especially of *Acorus calamus* and *Btk*, might be more satisfying even after a longer period of storage if all stored tubers are treated with the product. Kroschel and Koch (1996) showed that *Btk* mixed with fine sand was efficient for a storage period of more than 3 months. In areas where the potato tuber moth occurs, good storage practice is necessary to prevent an influx of moths and hence infestation of young unprotected potato sprouts. Without these additional methods, managing the potato tuber moth effectively with *Btk* or other biological methods in potato stores over a longer period time (four to five months) will be impossible (Kroschel and Sporleder 2006).

Niroula and Vaidya (2004) tested powder of sweet flag rhizomes (*Acorus calamus*) at three concentrations (0.05%, 0.5% and 5% w/w) against potato tuber moth and found 56.7%, 66.7% and 70% adult mortality after 168 hours of treatment application, respectively. Pradhan (1988) tested indigenous weeds against potato tuber moth under farmer's storage condition and recommended chopped and dried leaves of *Eucalyptus* sp. (Masala), *Artemisia vulgaris* (Titepati) or *Chenopodium botrys* at a rate of 300-330 g/crate with 8 kg of potato. Further, a dust preparation of *Acorus calamus* rhizomes applied at 10 g/kg potato was efficient to manage potato tuber moth similar to applications of the botanical Marauta (*Chenopodium* sp.). Tiwari (2006) reported of the high efficacy of *Btk* for controlling potato tuber moth.

Table 6. Proportion of damaged tubers in seven farmer's rustic potato storerooms after treatment with *Acorus calamus*, *Artemisia* sp and a *Btk*-powder compared with a control

Storage	Time (days)	Control		*Acorus calamus*		*Artemisia*		*Btk*	
1	0	0	a						
	32	86	h	8	bc	36	e	47	ef
	76	71	g	24	de	72	g	28	de
	101	90	h	56	f	77	gh	49	ef
2	0	0	a						
	13	0	a	1	ab	1	ab	1	ab
	44	35	de	6	bc	4	b	10	bc
	76	100	-	14	cd	8	bc	10	bc
	110	100	-	2	ab	50	f	100	
	140	100	-	90	h	90	h	90	h
3	0	1	ab						
	13	38	ef	1	ab	10	bc	2	ab
	44	100	-	3	ab	12	c	5	bc
	76	100	-	0	a	0	a	0	a
	110	100	-	100		100		100	
9	0	33	de						
	7	23	d	2	ab	60	fg	20	cd
	44	90	h	29	de	84	h	82	gh
	76	90	h	20	cd	90	h	90	h
	110	100	-	81	gh	100		100	
	140	100	-	81	gh	0		100	
10	0	6	bc						
	7	24	de	2	ab	21	cd	13	cd
	44	55	f	2	ab	5	bc	15	cd
	76	28	de	2	ab	2	ab	7	bc
	110	100	-	30	de	30	de	25	de
	140	37	ef	24	de	22	cd	27	de
11	0	6	bc						
	7	22	cd	3	ab	37	ef	9	bc
	44	100	-	10	bc	43	ef	11	bc
	76	100	-	60	fg	4	b	10	bc
	110	100	-	18	cd	38	ef	39	ef
	140	100	-	6	bc	32	de	34	de
13	60	12	c						
	91	18	cd	8	bcd	0	a	2	ab

Figures with identical letters are not significant different according to the pair wise evaluation using the Chi^2 test ($P < 0.05$).

Conclusions and recommendations

Acorus calamus stolon dust at 5 g/kg of potato tubers showed high efficacy to protect potato tubers against potato tuber moth for about three to four month in farmer's rustic potato stores. Further studies should address the preparation technique and dosage for using of *A. calamus.* However, its protective capacity is comparable with an already recommended bio-pesticide based on *Btk* in a powder formulation. *Btk* is commercially available but need to be mixed with talcum before application. *Artemisia* leaves chopped pieces (20 g /kg potato) were also effective in reducing potato tuber moth damage levels and therefore this traditional control technique also

warrants its application. Therefore, several alternative control methods are available for protecting stored potato from potato tuber moth damage which could be used by farmers in Nepal. Training and regular technical support through the governmental extension services is important to introduce these technologies along with an integrated pest management program to tackle the high risks of pesticide poisoning in resource-poor farm households in Nepal.

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Temperature-dependent development of three parasitoids of the leafminer fly *Liriomyza huidobrensis*

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Abstract

Leafminers flies (Diptera: Agromyzidae) are important pests in Peruvian highlands and coastal vegetable cropping systems, where more than 60 hymenoptera parasitoids constitute a natural source of biological control. The parasitoid *Chrysocharis flacilla* (Eulophidae) has the highest abundance in the southern part of the Peruvian coast while *Phaedrotoma scabriventris* (Braconidae) is the major parasitoid in the highlands. *Halticoptera arduine* (Pteromalidae) occurs in both agroecologies at high numbers. Temperature-dependent development of the three parasitoids was assessed to understand the comparative advantage of each species as biocontrol agent in different agro-climates. Biological development parameters were studied at five constant temperatures (10, 15, 20, 25, 30°C) in their main host *Liriomyza huidobrensis.* At 10ºC no development occurred in all three species. Above 15ºC, development time decreased with increasing temperature in all parasitoid species. *H. arduine* was the less tolerant to high temperature (30°C). Fertility of *P. scabriventris* decreased with increasing temperatures. In contrast, the fertility of *C. flacilla* increased with increasing temperature, but no progenies were observed at 10ºC. *H. arduine* did not follow a clear trend with respect to temperature. The temperature strongly influenced the proportion of males and females in the progenies off all species. The analyses of developmental time, fertility and progeny sex ratios suggest that *P. scabriventris, H. arduine* and *C. flacilla* have their optimum temperature between 15 to 20ºC, at 20ºC, and between 25 to 30ºC, respectively. The results clearly demonstrate the adaptation of the three species to different agro-climates and indicate their potential as biocontrol agents under specific temperature conditions.

Keywords: Biological control, integrated pest management, Halticoptera arduine, Phaedrotoma scabriventris, Chrysocharis flacilla.

Introduction

The pea leafminer *Liromyza huidobrensis* Blanchard (Diptera: Agromyzidae) is a highly polyphagous leafminer capable of inflicting severe damage to field and horticultural crops. Originating in the New World, it has been globally distributed with the worldwide trade of vegetables and ornamentals (Shepard *et al.*, 1998; Rauf *et al.*, 2000). The leafminer fly has developed resistance to insecticides and biological control using parasitoids is suggested as an important management option (Johnson *et al.,* 1980; Parrella *et al.,* 1984).

Liriomyza species are known to have many natural enemies, particularly in the regions of origin in the New World (Murphy and LaSalle, 1999; Waterhouse and Norris, 1987). Noyes (2004) listed over 300 species of agromyzid parasitoids, and over 80 species that are known to attack various *Liriomyza* species. Mujica and Kroschel (2008) observed in the central coast of Peru a rich complex of 63 species of parasitoids, with the endoparasitoids *Halticoptera arduine* (Walter) (Pteromalidae) (48.2%) and *Chrysocharis flacilla* Walker (Eulophidae) (19.5%) as the most important species. On the other hand, in the central highlands of Peru at altitudes of about 3300 m, *Phaedrotoma scabriventris* Nixon (Braconidae) has been described as an important parasitoid reaching a mean parasitism rate of 32.6% of *L. huidobrensis* in faba bean (*Vicia faba* L.) (unpublished data). All three parasitoids are also reported as primary parasitoids of agromyzid leafminers in a wide range of leafminer fly host plants in the Neotropics. Further, they are adapted to different agroecological zones between 0 to 4000 m asl and are important parasitoids of *L. huidobrensis* in natural, urban and agricultural systems (Hansson 1987, De Santis 1983, Neder de Roman and Arce de Hamity 1986, Salvo et al. 2005, Mujica and Kroschel 2007).

The high abundance of the three parasitoids *H. arduine*, *C. flacilla* and *P. scabriventris* in different agroecologies of Peru as well as their wide host plant and leafminer fly adaptation indicate their high potential to be used as

biocontrol agents in classical biocontrol programs of *L. huidobrensis* in other parts of the world (Mujica and Kroschel 2008). To better understand their optimum temperature requirements and their potential use in different agro-climates the objectives of this comparative study was to investigate the temperature-dependent development and reproduction potential of the three species in a wide range of temperatures.

Materials and methods

Insect origin and rearing conditions.- Laboratory cultures of the parasitoids *H. arduine* and *C. flacilla* were established from individuals reared from *L. huidobrensis* infested potato (*Solanum tuberosum* L.) leaves collected in the Cañete valley, Lima at an altitude of 50 m. *Phaedrotoma scabriventris* was obtained from *L huidobrensis* infested faba beans (*Vicia faba* L.) in Huancayo, Junin, at 3300 m. Rearing of the parasitoids was carried out using the host *L. huidobrensis* and faba bean as the host plant, respectively. For culture maintenance and experimental studies, late second to early third instar larvae of *L. huidobrensis* and 30-day old faba bean plants were used. Leafminer fly and parasitoid cultures were maintained at a temperature of 20ºC at a photoregime of 12:12 (L:D).

Influence of temperature on immature development.- Six *L. huidobrensis*-infested faba bean plants were placed in a wooden rearing cage (45 x 30 x 25 cm) and exposed to 50 mated parasitoid females for 12 hours at 20ºC. Afterwards, leaves were cut at the petiole base and placed in 1-liter plastic containers, which were transferred to incubators of five constant temperatures (10, 15, 20, 25 and 30ºC). Leaves were preserved until leafminer larvae emerged and pupated. Leafminer pupae were monitored daily until the emergence of adults. For *H. arduine* supplementary experiments were carried out at 18ºC.

Influence of temperature on progeny development (fertility).- For these experiments 1-liter transparent plastic cylinders (15 cm high, 7 cm wide) were used, which had a 5 cm diameter hole closed with nylon gauze on top for ventilation. Four faba bean leaves containing about 40-50 *L. huidobrensis* late second to early third-instar larvae were placed in one 50 ml glass vial filled with water and covered with the plastic cylinders. A set of 20 pairs of newly emerged male and female parasitoids of each species were transferred individually into the plastic cylinders, which were stored in incubators at temperatures of 10, 15, 20, 25 and 30ºC. The parasitoids had access to a honey solution, which was dropped onto the nylon gauze. After 24 hours, leaves were replaced by new *L. huidobrensis* infested leaves, which procedure was repeated daily until the female parasitoids had died. Removed leaves were stored in Petri dishes (9 cm diameter) at 20ºC until leafminer fly pupation. Then, pupae were removed from leaves and after hatching number and sex of parasitoid species recorded. Female parasitoids that died before day 1 or did not produce any offspring were excluded from the analysis.

Statistical analysis.- Data from immature and progeny development were analyzed with one-way analysis of variance, and means were separated with Kruskal-Wallis test at $P \leq 0.05$ or $P \leq 0.02$. Linear regression analysis was applied to determine temperature-dependent development rate (D), where D=1/d, with d being the time in days for parasitoids to complete development until the adult stage. Also, linear regression analysis calculated the lower threshold temperature for development (T_{min}).

Results

Influence of temperature on immature development.- At 10ºC no development occurred in all three parasitoids. Above 15ºC, the development time decreased with increasing temperature in all parasitoid species (Table 1). *H. arduine* successfully completed development in the temperature range of 15 to 25ºC. This species was least tolerant to high temperatures and no development occurred at 30ºC. Across all temperatures tested, development at 25ºC was significantly faster ($P \leq 0.001$). For *P. scabriventris,* developmental time ranged between 12 days at 30ºC to 31.9 days at 15ºC ($P \leq 0.001$). The total developmental time for *C. flacilla* ranged between 22.3 days at 30ºC to 29.73 days at 15ºC. A mean decrease of 18.1, 6.5 and 2.5 days in the developmental time for each 5ºC increase in temperature was calculated for *H. arduine P. scabriventris* and *C. flacilla,* respectively. The theoretical development threshold (T_{min}) was lowest for *P. scabriventris* (6.18ºC) and highest for *C. flacilla* (9.63ºC) (Table 2).

Table 1. Total development time (in days, from egg to adult) of *Halticoptera arduine*, *Phaedrotoma scabriventris* and *Chrysocharis flacilla* in larvae of *Liriomyza huidobrensis* at different constant temperatures

Temperature (°C)	*H. arduine*		*P. scabriventris*		*C. flacilla*	
	n	Mean[a] ± SE	n	Mean ± SE	n	Mean ± SE
10		All died[b]		All died	7	All died
15	10	55.90 a ± 0.48	50	31.92 a ± 0.27	26	29.73 a ± 1.36
18	32	31.66 b ± 0.38		-----[c]		-----
20	30	30.30 b ± 0.31	50	21.60 b ± 0.21	31	27.68 a ± 0.65
25	37	19.81 c ± 0.13	50	16.46 c ± 0.23	50	25.18 b ± 0.47
30		All died	50	12.04 d ± 0.19	50	22.34 c ± 0.43

[a] Means in the same column followed by same letter are not significantly different at P • 0.05
[b] All died prior to adult emergence
[c] No data available

Table 2. Estimated parameters of the linear model fitted to median development rate (1/day) for immature life-stages of *Halticoptera arduine*, *Phaedrotoma scabriventris* and *Chrysocharis flacilla*

Parasitoid species	Regression equation	R^2	T_{min} (°C)
H. arduine	Y = 0.0031x - 0.028	0.9742	9.03
P. scabriventris	Y = 0.0034x - 0.021	0.9884	6.18
C. flacilla	Y = 0.0074x - 0.071	0.7102	9.63

Influence of temperature on progeny and sex ratio.- The fertility of the three parasitoid species was significantly affected by temperature (Table 3). The parasitoid *H. arduine* showed a high variability in the progeny development with regard to temperature. Lowest development was observed at 10° and 30° C with 40.3 and 31.8 progenies per female, respectively. In contrast, most progenies developed at 15° and 30° C, with 60.4 and 75.6 progenies per female.

P. scabriventris developed lowest numbers of progenies at 10°C (36.2 progenies female) and highest at 15°C (151.2 progenies per female); at temperatures above 15° C the progeny development decreased gradually, but did not differ significantly at 20°C (122.9±4 progenies per female). At 25° C and 30°C, 85.2 and 81.4 progeny/female were produced, respectively. Compared to *P. scabriventris, C. flacilla* had a lower rate of progenies at all temperatures. At 10°C, no progenies developed, but with increasing temperature from 15° to 30°C progeny production increased significantly from 13.2 to 47.5 progenies per female.

Generally in all three species, the progeny production per female and day increased with increasing temperature. The optimal temperature for progeny production, defined as the temperature at which the fertility rate is highest, was 30°C for *P. scabriventris* and *C. flacilla* with 6.0 and 3.2 progenies per female respectively and 25°C for *H. arduine* with 1.9 progenies. In the case of age-specific fertility, most offspring were produced during the first eight days at 20, 25 and 30°C for all three parasitoid species. The highest peak of progeny development was reached at 30°C for *P. scabriventris* (12.4 progenies at day 3), at 25°C for *H. arduine* (6.5 progenies at day 2), and at 20°C for *C. flacilla* (13.8 progenies at day 1). As the temperature decreased the number of days to reach the peak of progeny development increased for *P. scabriventris* and *H. arduine*. However, this trend was not observed in *C. flacilla,* in which species the progeny emergency peak occurred at all temperatures between the first and second day.

Table 3. Fertility, progeny rate, peak of progeny production and sex ratio of *Halticoptera arduine*, *Phaedrotoma scabriventris* and *Chrysocharis flacilla* at five constant temperatures

Species /Temperatures	n	Fertility (progenies/female)	Progeny rate (progenies/female/day)	Peak of progeny production		Sex ratio (Female: Male)
				Progeny	Day	
H. arduine						
10	10	40.3 b ± 9.5	0.4	2.7	39	0.18:1
15	10	60.4 a ± 12.7	0.7	3.9	19	0.05:1
20	10	39.4 b ± 8.0	0.9	4.3	8	0.95:1
25	10	75.6 a ± 13.4	1.9	6.5	7	0.08:1
30	10	31.8 c + 5.9	1.6	4.7	7	0.21:1
P. scabriventris						
10	30	36.2 c ± 5.5	0.4	1.6	20	0.66:1
15	30	151.2 a± 14.6	3.4	9.1	10	0.76:1
20	30	122.9 a ± 3.4	4.6	7.9	6	1:1
25	30	85.2 b ± 4.4	4.3	8.1	5	1.18:1
30	30	81.4 b ± 7.3	6.0	12.4	3	1.31:1
C. flacilla						
10	32	------[b]	------	-----	-----	-----
15	33	13.18 b ± 1.5	0.3	2.0	1	434:1
20	31	36.51 a ± 3.1	1.2	13.8	1	188.7:1
25	35	43.34 a ± 4.6	2.3	6.8	1	59.7:1
30	31	47.52 a ± 8.5	3.2	10.0	2	7.7:1

[a] Means in the same column followed by same letter are not significantly different at P• 0.05
[b] No offspring developed

In all three parasitoids, the sex ratio was highly affected by temperature, but most in *C. flacilla and H. arduine* (Table 3, Fig. 1). For *P. scabriventris,* female progeny increased with increasing temperature with a female:male sex ration of 0.66:1 at 10ºC and 1.31:1 at 30ºC, respectively. A balanced sex ratio of 1:1 was registered at 20ºC. In all temperatures, *C. flacilla* has a much higher proportion of females; interestingly, at low temperatures at 15ºC a female:male proportion of 434:1 was recorded. In *H. arduine*, male progenies dominated in all temperatures. Only at 20ºC, an almost balanced female:male sex ratio of 0.96:1 was observed.

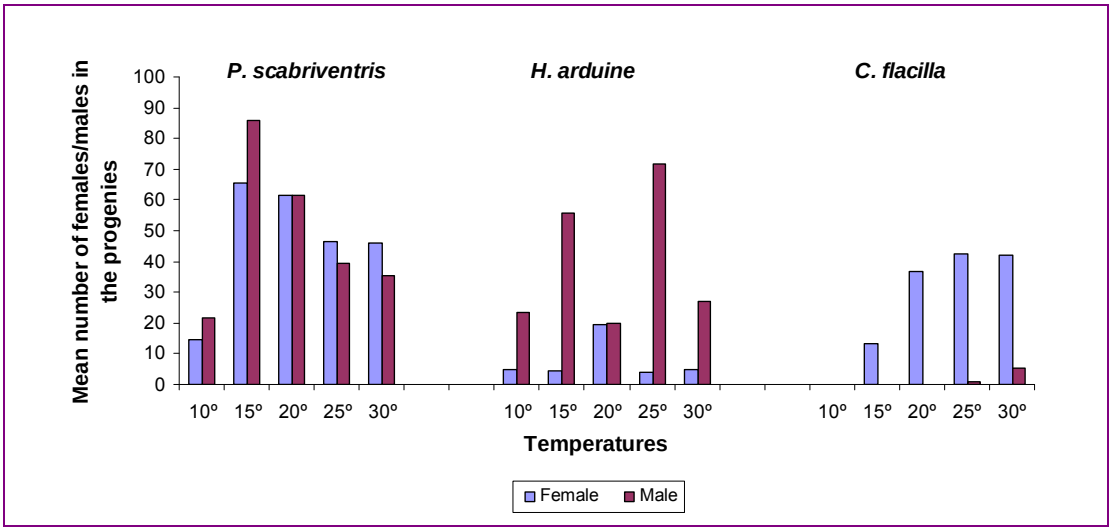

Figure1. Mean number of males and females in the progenies of the parasitoids *Phaedrotoma scabriventris, Halticoptera arduine* and *Chrysocharis flacilla* developed at different constant temperatures

Discussion

An overall good understanding of the temperature requirements of natural enemies is important for classical biocontrol programs in order to select the best potential candidates for the target regions under consideration. *Halticoptera arduine*, *Phaedrotoma scabriventris* and *Chrysocharis flacilla* are important leafminer fly parasitoids and are predominating in different agroecologies of Peru and South America.

Our temperature studies were carried out between 10 to 30ºC, and not surprisingly the developmental time of all three parasitoid species decreased with increasing temperature. This is a common phenomenon in insects and not only in parasitoids that immature development time is directly dependent on temperature, with much shorter durations at higher temperatures (Jervis *et al*., 2005). Similar trends have been observed in other leafminer fly parasitoids like *Chrysocharis pentheus* (Walker) (Hondo *et al.* 2006), *C. pubicornis* (Zeltersted) (Baeza *et al*. 2007), *C. parski* (Crawford) (Christie and Parrella, 1987), *Diglyphus isaea* (Walker) (Bazzocchi *et al.* 2003) and *Ganaspidium utilis* Beardsley (Lopez *et al*., 2005).

A theoretical minimum threshold of development of 6.18ºC could be established for *P. scabriventris,* which is among the parasitoids investigated in the study the most tolerant species to low temperature. This is also reflected in its native distribution and higher abundance and efficacy at higher altitudes in the Andes compared to *H. arduine* and *C. flacilla*. In contrast, our studies showed that the development time of *C. flacilla* is less dependent on temperature.

A short development time relative to its host is considered a desirable attribute for the selection of parasitoids (Jervis *et al*., 2005). For its immature development *L. huidobrensis* needs 43.6, 22.5 and 16.1 days at 15ºC, 20ºC and 25ºC on beans (*Phaseolus vulgaris* L.); and at 30ºC no adults develop from pupae (Lanzoni *et al.* 2002). Thus, our study could illustrate that *P. scabriventris* needs much shorter periods of development especially at low temperatures (15ºC: 31.9 days; 20ºC: 21.6 days) to complete its development compared to the leafminer fly.

Fertility varied between the species and *P. scabriventris* and *H. arduine* produced progenies in the temperature range from 10 to 30ºC; in contrast *C. flacilla* developed no progenies at 10ºC. For *P. scabriventris* and *H. arduine,* the optimum temperature for oviposition is between 15 and 20°C, and for *C. flacilla* between 20 to 30ºC. This difference among species is a common pattern in the relationship between temperature and the fertility of parasitoids (van Lenteran *et al.,* 1987), and is consistent with the general assumption that insects cannot mature their eggs or are unable to oviposit outside their tolerable temperature range (Greenfield and Karandinos, 1976).

From our results we can derive that the maximum tolerable temperature for *C. flacilla* is even above the temperatures tested in our study.

For *H. arduine* and *P. scabriventris* we found a female:male sex ratio of 1:1 at 20°C. The proportion of females of *H. arduine* was significantly lower at temperatures below and above the optimum temperature of 20°C. Similar responses to temperature were observed for *Opius* dissitus Muesebeck (Bordat *et al.,* 1995). Instead, Lopez *et al.* (2004) found that *Halticoptera circulus* (Walker) produced higher male progenies at 26ºC. The parasitoid *P. scabriventris* produced more females at temperatures higher than 25ºC. According to Denlinger and Yocum (1998) this behavior is a response to extreme temperature conditions; the stress for females is higher and mating may fail which would lead to a smaller female population in the next generation. The majority of parasitoids of the order Hymenoptera have a facultative parthenogenesis of an arrenotokia type (De Bach, 1985) as it is the case in *H. arduine* and *P. scabriventris*. Progenies composed of male recombinants allow for a better phenotypic adaptation to changing environmental conditions (Moreno, 1982). In *C. flacilla,* the female proportion was about 330% to 60% higher at temperature of 15 to 25ºC. Thus, one female of *C. flacilla* can produce more female progenies than the other two parasitoids at similar temperatures. In this respect, Abe and Tahara (2003) pointed out that parasitoids of an thelytokus type have advantages over the arrhenotokia type in mass rearing, because they do not "waste" expensive hosts for the production of males.

Duale (2005) considered that survival, growth and population development of parasitoids depend not only on the oviposition rate or fecundity, but to a greater extent on the appropriate environment, which mostly limits and affects the geographical distribution of parasitoids. Viable parasitoids of *H. arduine, P. scabriventris* and *C. flacilla* did not further develop in leafminer fly larvae at 10ºC, and in the case of *H. arduine* also not at 30ºC. Taking into account the strong temperature-dependent sex ratio in our parasitoids studied, the optimal temperatures for development of *P. scabriventris, H. arduine* and *C. flacilla* are between 15-20ºC, 20ºC and 25ºC.

Conclusion

The present study identified the optimal temperature range for the development and fertility of the leafminer fly parasitoids *H. arduine, P. scabriventris* and *C. flacilla*. The analysis of complementary life-table parameters such as net reproductive rate, intrinsic rate of increase, mean generation time, etc. will follow and allow to model the potential population growth and development in different agro-climates.

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Estimating potential impact of potato late blight resistant varieties in China with GIS

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Summary

Late blight of potato caused by *Phytophthora infestans* is known to be an important limiting factor in China, but disease pressure is highly variable and little is known of its variability in the different Chinese agro-ecosystems. Severity of late blight was estimated by linking a disease forecast model, SIMCAST, to a climate database in a Geographic Information System (GIS). The disease forecast model indirectly estimated late blight severity by determining how many sprays were needed during a growing season. Planting periods were established for different agroecosystems in the north for the spring season and in the rest of China for the fall and winter seasons. The model was run with parameters for susceptible and resistant varieties to analyse the potential impact of host resistance on fungicide use. The results indicated that late blight is least severe in the north and that in other regions late blight pressure is stronger for the fall than the winter. In all seasons there was a clear east-to-west gradient with little late blight pressure in the western area of the country and very high pressure in the central and eastern regions. Based on fungicide reduction, host resistance resulted in a simulated savings of more than US$742 million in spring-fall and US$1.1 billion in spring-winter.

Keywords: Potato late blight, Forecast model, Geographic Information System, SIMCAST.

Introduction

The International Potato Center (CIP) and partners have been involved in the development, diffusion and promotion of potato cultivars resistant to late blight (LB) for more than two decades. Although many of these varieties have been adopted to varying degrees in developing countries, estimating their impact is difficult for many reasons: i) LB resistance is quantitative, and the level of resistance of a given material is often not known; ii) LB affects farmers in many ways, including income lost to fungicides, direct losses to production and human health risks; iii) LB is strongly weather driven, and disease intensity is highly variable; iv) the actual degree of adoption of resistant varieties is often not well known for a particular country or area. For these reasons, the impact potential of host resistance is highly context-specific.

A potential solution to the problem of estimating the impacts of host resistance on late blight severity is agroecological zoning with a geographic information system (GIS). Zoning that is specific to a particular technology or production constraint can give realistic estimates of intensity and variation of the factor under study (Corbett, 1998; Wood & Pardey, 1998). This type of zoning has recently been facilitated by strong technological progress in computer hardware and GIS software, which has made the manipulation of large geo-referenced databases relatively straightforward. The availability of geo-referenced weather data makes the application of GIS based zoning possible for many areas of the world.

In this paper we describe the use of the late blight forecast model SIMCAST (Grünwald et al., 2002) linked to GIS to estimate the number of fungicide sprays needed for effective management of potato late blight. By running the model for both susceptible and resistant cultivars, the potential economic and environmental impact of host resistance can be explored geographically.

Materials and methods

The model

The LB model used in this study was SIMCAST developed at Cornell University. The model uses as daily variables average temperatures during hours of high relative humidity (Humtm), number of hours per day of high relative

humidity (Humhr), and accumulated daily precipitation. To determine the first and second variable, SIMCAST uses a threshold of 90% of relative humidity; adjusted for the height of the sensing device. If a weather sensor is located inside the plant canopy, the threshold is 90%, and if it is outside, the threshold is 85% (Andrade-Piedra et al., 2005). In this research the 85% threshold was used because the weather data used in this study were taken from airports.

To predict the need of a fungicide application, the model considers a threshold of either accumulated blight units (BU) or fungicide units (FU) – whichever is reached first. The BU are determined based on Humhr and Humtm, providing an index of the disease severity; while FU are based on precipitation and represent fungicide washing or chemical degradation of the active ingredient.

Weather data

Weather stations with daily data for China were downloaded from the National Oceanic and Atmospheric Administration (http://www.noaa.gov/) for a period of 11 years (1994 to 2004). The selected variables were minimum temperature (Tmin, C°), maximum temperature (Tmax, C°), dew point (Tdew. C°) and precipitation (Pp, mm). The selection of weather stations was done in the next order. First, weather stations, which had more than 80% of data per variable per year, were selected. Second, daily averages data for the 11 years per variable were calculated. Finally weather stations which had daily missing data were eliminated. As a result, 465 weather stations with complete data were selected. From these, 89% had average data from 7 to 11 years, 8% for 4 to 6 years and 4 % for 1 to 3 years. The data of 465 weather stations were used to build daily weather surfaces for each variable.

Estimating hourly temperatura and relative humidity

Hourly temperature data were obtained using the methodology developed by Cesaraccio et. al. (2001), which uses daily values for minimum temperature, maximum temperature, minimum temperature of next day, sunrise and sunset. Sunrise and sunset times were obtained using the weather station latitude and the day number of the year. Daily dew point data and calculated hourly temperature (T) data were used to estimate hourly relative humidity (RH) (Chow et al., 1994). Hourly RH is the quotient obtained between the real vapor pressure and saturation vapor pressure. To calculate the real vapor pressure, daily dew point was used. To calculate the saturation vapor pressure hourly T was used.

Validation of methods used to estimate hourly temperature and relative humidity

Estimation processes were validated with a USA hourly weather database (US-EPA, 1997), using 48 weather stations located in potato-growing areas (Hijmans, 2001), and the hourly variables Tmin, RH, Tdew and Pp. Of the 48 weather stations, 46 had data for six years (1990 to 1995), and two had data for only two years. A total of 282 datasets were used to validate estimations of hourly T and RH data from Tmin, Tmax, and Tdew daily data.

The validation process was done in two steps. First, hourly data of T, RH and Pp were used to calculate the *observed simcast input data* (HumTm, HumHr and Pp). Subsequently, the model was run to obtain the number of observed applications for the 282 datasets. Second, hourly data of T, Tdew and Pp were used to calculate daily data of Tmin, Tmax, Tdew and Pp. Hourly T was obtained using daily Tmin and Tmax; while hourly RH was obtained from hourly T and daily Tdew. The hourly data of T and RH, and the daily PP were used to calculate the *estimated simcast input data*. The model was then run to obtain the number of estimated fungicide applications for the 282 datasets. This was done for both *observed* and *estimated input data.* Finally, the number of applications from *estimated input* was regressed on applications derived from *observed* input, using a simple linear regression. The slope was statistically compared to 1 using a T test, with the intercept set to 0. Goodness of fit to the model was evaluated with R^2.

Weather surfaces for China

The ANUSPLIN suite of programs (Hutchinson, 2006) was used to construct average daily weather surfaces for each variable (Tmin, Tmax, Tdew y Pp) on a grid cell size of 6-arc minutes in both latitude and longitude. A digital elevation model (DEM) was a prerequisite in the interpolation process; thus a DEM developed by USGS at 30-arc seconds resolution (approx, 1 km) was clipped and fitted to the resolutions mentioned above (Hutchinson, 2006). The latitude and longitude data used in ANUSPLIN were obtained from NOAA; while the altitude of the weather stations was obtained from the fitted DEM.

Weather surfaces and SimCast

Once the daily weather surfaces were created (Tmin, Tmax, Tdew and Pp), the method to obtain *estimated SimCast input data* was applied in each cell. Next the model was run according the potato cropping seasons.

Determining growing planting date and season length

In China, potato is cultivated across a wide range of agro-ecological zones (Gitomer, 1996). In the south, climate allows for several planting seasons, while in the north only one season is possible, which is established in the spring. In this paper, planting in the rest of China is assumed to take place in the fall and winter. Each season was considered to last 120 days, 20 days pre-emergence and 100 days after emergence. Simulation was initiated at emergence and run for a period of 100 days.

Economic and environmental analisys

Host resistance can potentially result in a savings by reducing the use of fungicide. To capture this potential in a geographical context, resistance was geographically linked to a potato production area to make an economic and environmental analysis. In China the average production of the years 1998, 1999, 2000 and 2005 (http://www.cast.net.cn/SINOPOTATO/statistics/2000-e.htm) was used. To make an economic and environmental analysis, basic criteria about fungicide use were considered. We assumed that all applications were made with the fungicide clorothalonil, which is the product originally used to develop SIMCAST. Clorothalonil is similar in efficacy to Mancozeb, which is the most widely used fungicide in most developing countries. Labor costs were estimated at $5 per ha. Two kg of Clorotalonil were used per ha per application, and fungicide cost was estimated at $22.

The quantification of environmental impact (EI) was obtained with a formula proposed by Gallivan et al., (2001). The EI was calculated by multiplying the amount of pesticide used (kilograms of active ingredient) by the environment impact quotient (EIQ), a score for the potential risk to farmworkers, consumers and the environment.

The EIQ for Clorothalonil was considered 40.1 (http://nysipm.cornell.edu/publications/eiq/files/EIQ_values.xls).

Results

Comparison of predicted and observed values

For the 48 weather stations used in this analysis, the prediction of LB severity using estimated hourly data from daily variables (Tmin, Tmax, Tdew) to obtain SIMCAST variables was similar to the observed hourly data (Figure 1). This analysis served to validate the use of daily weather from China as input data for SIMCAST. The slope of the simple regression line comparing observed and estimated values was not significantly different from 1 in any of the years tested; R^2 values ranged from 0.85 to 0.94.

Late blight distribution in China

The principal potato production areas in China are located in the north (Nei Mongol, Gansu), northeastern (Heilongijiang), central (Sichuan, Chongquing, Shanxii, Shaanxii, Hubei, Shandong, Hunan) and south central regions (Yunnan).

With the use of susceptible or resistant varieties in different growing seasons, there was a clear east-to-west gradient with little late blight pressure in the western area of the country and very high pressure in the central and eastern areas. Nevertheless, disease severity was reduced in all regions when the model was run with parameters for a resistant.

In the north, where only one crop is established in the spring, disease severity is low, and there is no disease in Gansu or Nei Mongol West. However, disease severity increased from Nei Mongol east to Heilongjiang. In the other potato-growing areas, the severity of the disease varied according to the growing seasons (winter or fall) and spatial scenarios (central to south central; and central east to central south).

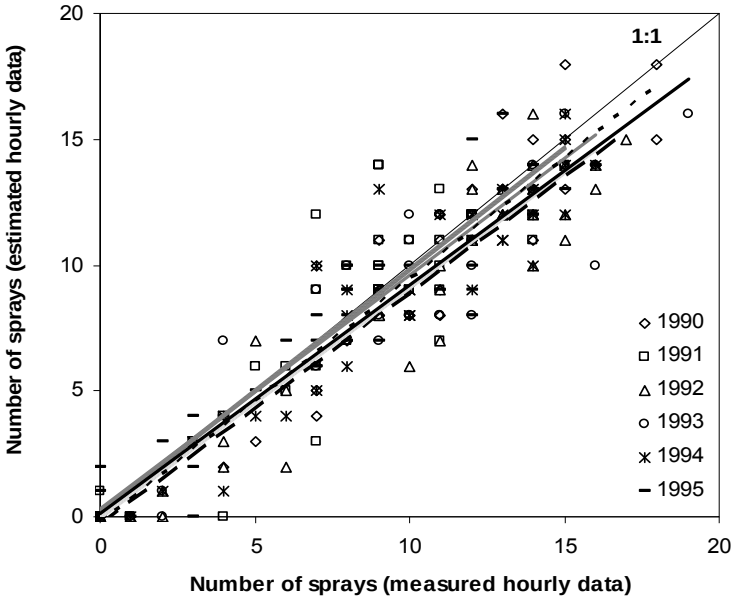

Figure 2. Observed and estimated fungicide applications needed to control LB obtained with the SIMCAST model using weather data of 48 locations from the US over 6 years. Observed applications were obtained with *measured* hourly data and estimated applications were obtained with *estimated* hourly data (from daily data) as described by Cesaraccio et al [1]. *R2* values for the linear regressions of each year averaged 0.92 with a minimum of 0.85 and a maximum of 0.94

In the central to south central region, estimated severity levels were higher in fall than in winter. In winter, the regions of highest severity included Guizhou, southeast Sichuan to southwest Chongquing and southern Yunnan. In fall, high severity reached a broad extension, including the provinces of Guizhou, Yunnan, Sichuan, Chongquing, Shanxii, Shaanxii, Hubei, Shandong, and Hunan. In the central east to central south region, where potato production is low, disease severity was higher in both seasons. The province of Guangdong was an exception because the severity level in winter was higher than in fall.

Economic and environmental analysis

Economic and environmental analyses were both based on the quantities of fungicide estimated necessary to control late blight. The economic aspect was determined by monetary cost and savings accrued due to use of a resistant cultivar, while the environmental aspect was determined by the percentage increment or reduction in the use of the chemical product due to use of a resistant cultivar. The economic and environmental aspects were analyzed in two ways: comparing the two growing seasons in the south and assessing the benefits of resistance.

Using a susceptible cultivar, the cost of controlling late blight nationwide was estimated at USD$1.09 billion in winter, and US$2.2 billion in fall. For example, in Guizhou, the estimated cost to control late blight was US$280 million in winter and US$388 million in fall. In other provinces —such as Xizang, Yunnan, Shaanxi, Shanxi, and Hubei— where the potato crop is smaller, the rate of fungicide application for the fall crop was generally twice that of the winter crop.

The economic and environmental impacts of the use of a resistant host were evaluated for a single season in the north (spring) and over two seasons in the rest of the country (winter and fall). The economic impact in the north was most significant, especially in the northeastern province of Heilongjiang, where savings due to the use of a resistant cultivar was US$54 million, and fungicide application was reduced by 50% (Figures 2A, 2B, 3A and 3B).

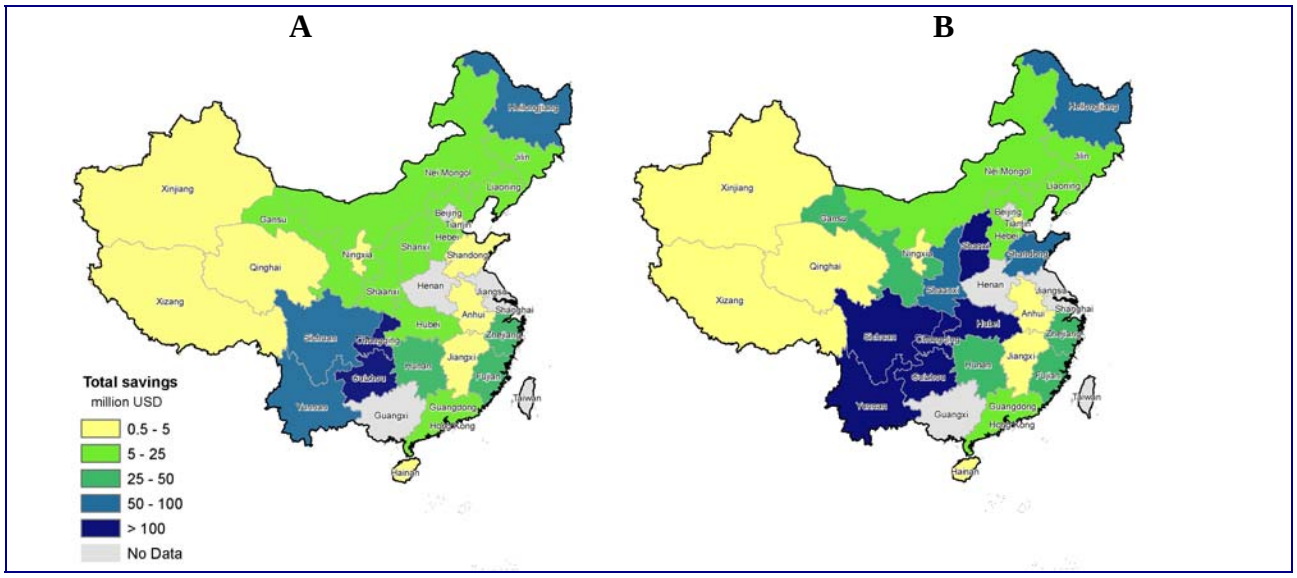

Figure 2. Total estimated savings in USD (millons) resulting from the use of a cultivar resistant to *Phytophthora infestans* in China in the winter (A) and fall (B) planting seasons

For the winter crop in other areas of China, the impact was large in Guizhou and Chongquing, where fungicide applications were reduced by 28% and 20%, respectively, resulting in monetary savings in each province of approximately $USD100 million. In the provinces of Jiangxi and Guandong, where potato is a less important crop, the use of a host-resistant cultivar resulted in a 50% reduction of fungicide applications (Figure 2A and 2B).

For the fall crop, the impact was large in Guizhou, Chongquing, Sichuan, Yunnan, Hubei and Shanxi, with reductions of fungicide use of 52%, 42%, 50%, 59%, 25%, and 10% respectively, and savings of $US100 million in each province. In the provinces of Anhui and Zhejian, areas of relatively minor potato production, the use of a host-resistant cultivar resulted in the reduction of fungicide application by 50% (Figures 2A, 2B, 3A and 3B).

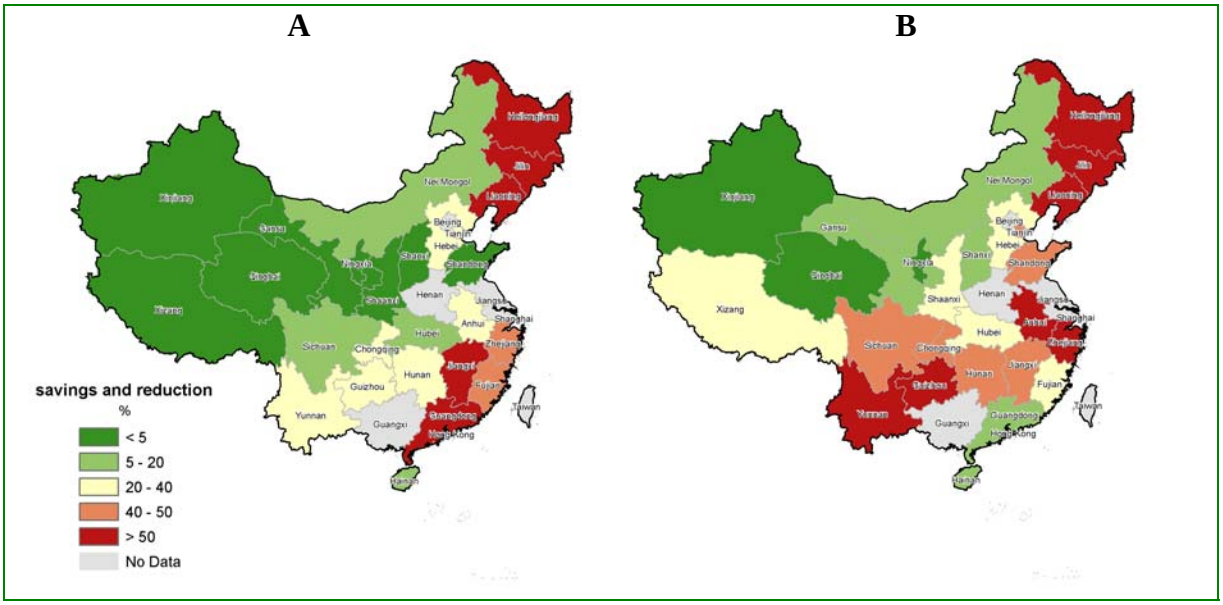

Figure 3. Percent reduction in the use of a fungicide due to the use of a cultivar resistant to *Phytophthora infestans* in China in the winter (A) and fall (B) planting dates

Table 1. Projected impact resulting reduced fungicide if a resistant cultivar is used to manage potato late blight in China

Type of savings	Spring and winter	Spring and fall
Fungicide (mt)	30,281	50,928
Value of fungicide saved (million USD)	76	127
Value of labor saved (million USD)	666	1,120
Total savings (millions USD)	742	1,247
Reduction of the fungicide use	32%	42%

Discussion

The success of a new method to create input hourly data for the SIMCAST disease forecast model was demonstrated. Previous studies have been done calculating hourly data from monthly data, while the approach described herein allowed us to use daily temperatures to obtain hourly data. As with the previous publication (Hijmans et al., 2000) where use of input data derived from monthly observed data gave acceptable results, our use of input data derived from daily observed data also gave good results (Figure 1). In fact, the R2 values from our regression analyses are slightly higher than that reported by Hijmans et al., (2000), which is perhaps to be expected as we estimated from data of higher (daily) temporal resolution.

Hijmans et al., (2000) provided a partial validation of the approach by comparing their estimates for number of sprays to a database of survey data which included this variable. Unfortunately, we do not possess such data to evaluate our results in China. Based on what workers in China have told us, the absolute number of sprays estimated by the model (not shown) may be high. This could possibly be explained by a number of factors, including, but not limited to, the real initiation date for disease (we assumed emergence), the actual level of resistance of the susceptible cultivar, and the aggressiveness of the local pathogen population. However, since the primary objective of our study was to make comparisons (either geographic, seasonal, or for host resistance level), we feel that the results we presented demonstrate valid tendencies. We propose that the results can be useful for priority setting for research and development aimed at LB remediation.

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Molecular variability of sweetpotato feathery mottle virus and other potyviruses infecting sweetpotato in Peru

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Abstract

Several potyviruses are found infecting sweetpotato (*Ipomoea batatas*) in Peru, of which sweetpotato feathery mottle virus (SPFMV, genus *Potyvirus*) is the most common. However, sequence data for these viruses are not available from Peru. In this study, the 3'-terminal ~1,800 nucleotide sequences of 17 potyvirus samples collected from the six main sweetpotato-producing areas of Peru over the past 20 years were determined and analyzed. Results of sequence comparisons and phylogenetic analysis showed that three of the four recognized SPFMV strain groups, including the East African strain, are established in Peru as well as two other potyviruses: sweetpotato virus G (SPVG) and sweetpotato virus 2 (SPV2). The analysis further revealed that SPFMV, SPVG and SPV2 are related and form an *Ipomoea*-specific phylogenetic lineage within the genus *Potyvirus* and identified for the first time recombination events between viruses from different strain groups of SPFMV.

Keywords: sweetpotato virus II, ipomoea vein mosaic virus, Sweetpotato virus Y.

Introduction

Sweetpotato virus disease (SPVD) is probably the most devastating disease constraint of sweetpotato [*Ipomoea batatas* (L.) Lam] worldwide [9]. It is caused by co-infection of the aphid-transmitted sweetpotato feathery mottle virus (SPFMV, family *Potyviridae*, genus *Potyvirus*) and the whitefly transmitted sweetpotato chlorotic stunt virus (SPCSV; genus *Crinivirus*; family *Closteroviridae*) [17, 20, 45].]. Single infections of SPFMV usually show mild or no symptoms, and no appreciable yield reduction can be observed [12, 20, 33]. However, co-infection with SPCSV causes SPVD, which is characterized by very severe symptoms such as general chlorosis, stunting, leaf strapping, leaf crinkling and even plant death [17, 23, 45], and yield losses ranging from 70 to100% [20, 33, 38, 40]. Molecular studies have shown that co-infection of SPCSV enhances SPFMV RNA viral titers by at least 600-fold, whereas SPCSV titers remain equal or are reduced as compared to single infection [23, 24, 38]. The severity of SPVD, and the degree of SPFMV titer increase, depends on the strain of SPFMV involved in the double infection [20, 24]. Besides SPFMV, several other potyviruses, as well as other, unrelated viruses, can cause synergistic diseases when co-infecting with SPCSV [24, 38, 51], although the importance of these interactions for yield losses in the field are not well known.

SPFMV is one of the most widespread viruses infecting sweet potato [36]. The CP genomic region of SPFMV has been used in previous studies to establish phylogenetic relationships among SPFMV samples. It can be divided into four phylogenetic lineages [5, 21, 25, 38, 49, 50]: East Africa (EA), constituted by East African samples; Russet Crack (RC), comprising samples from Australia, Africa, Asia and North America; Ordinary (O) containing samples from Japan, China, Korea, Niger, Nigeria and Argentina; and Common (C) including samples from USA, China, Australia, East Africa and Argentina. Unlike the geographically unrestricted C, RC and O strains, the EA strain has almost exclusively been detected in the East African countries, the possible exceptions being two sequences available from the GenBank from Spain and Portugal [53] (Table 1). SPFMV has been reported from sweet potato in Peruvian fields since 1987 [30], but it was only after 1998 that the prevalence of SPVD emerged, possibly due to the increase of whitefly populations during the exceptionally strong El Niño phenomenon of that year [20]. Although the impact of SPVD on the yield of Peruvian sweet potato cultivars has been assessed [20], no molecular studies have been carried out concerning variability of its two causal agents. Cedano et al. [10] reported that some SPFMV isolates differed in the severity of symptoms induced in *Ipomoea nil*. SPFMV-C1, was collected during the 1980s [39] and shown to be closely related to the C strain [22, 42], whereas three other isolates, M2-41, M2-44 and C-18, were obtained from SPVD-infected sweet potato plants and differed in their symptomatology and serological reaction with monoclonal and polyclonal antibodies [20].

Table 1. SPFMV isolates and samples used in this study

Isolate/ sample	Strain	Origin/ collection date	Acc number		Isolate/ sample	Strain	Origin/ collection date	Acc number
Fe	EA[a]	Ferreñafe, Peru/2006	EU021070		Bkb 1	EA	Tanzania	AJ781781
C14	EA[a]	Cañete, Peru/2006	EU021071		Bkb 2	EA	Tanzania	AJ781782
Ch2	EA[a]	Chimbote, Peru/2006	EU021067		Mis1	EA	Tanzania	AJ781783
M2-44	EA[a]	Cañete, Peru/2003	EU021069		Tz1	EA	Tanzania	AJ539131
Piu	EA[a]	Piura, Peru/2006	EU021072		Tz2	EA	Tanzania	AJ539132
SP-33	EA[a]	Huaral, Peru/1987	EU021068		Tar1	EA	Tanzania	AJ781784
Fio	RC[a]	Cañete, Peru/2005	EU021065		Tar2	EA	Tanzania	AJ781785
KmtMil	RC[a]	Cañete, Peru/2005	EU021066		Kby 1	EA	Uganda	AJ781791
M2-41	RC[a]	Cañete, Peru/1999	EU021064		Kby 2	EA	Uganda	AJ781792
C1	C[a]	Lima, Peru/1987	EU021057		Mbl2	EA	Uganda	AJ781788
Ch4	C[a]	Chimbote, Peru/2006	EU021062		Bny	EA	Uganda	AJ539130
C18	C[a]	Cañete, Peru/1999	EU021059		Nak	EA	Uganda	AJ781790
M2-63	C[a]	Cañete, Peru/1999	EU021060		Apa	EA	Uganda	AJ781787
C21	C[a]	Cañete, Peru/1999	EU021061		Mpg2	EA	Uganda	AJ781789
SR	C[a]	San Ramón, Peru/2005	EU021063		Rak6e	EA	Uganda	AJ010706
YV	C[a]	USA	EU021058		85-7S	EA	Kenya	AY459593
Aus5c	C	Australia	AJ781779		54-9S	EA	Kenya	AY459592
Aus4c	C	Australia	AJ781778		Canar3	EA	Spain	AY459600
C	C	USA	S43450		Port	EA	Portugal	AY459599
SOR	C	Uganda	AJ539129		Zam 1	EA	Zambia	AY523552S4
25-4[a]	C	Kenya	AY523543		Unj1	EA	Tanzania	AJ781786
51-9S	C	Kenya	AY459591		O	O	Japan	D16664
Nam 12	C	Uganda	AY459596		TZ4	O	Tanzania	AY459598
Aus6	RC	Australia	AJ781777		Strain 5	O	Argentina	U96624
Aus5	RC	Australia	AJ781776		Nig 3	O	Nigeria	AJ010705
Aus2	RC	Australia	AJ781775		Arua10	O	Uganda	AY459595
Eg1	RC	Egypt	AJ515378		CH	O	China	Z98942
Eg9	RC	Egypt	AJ515379		Bau	O	Nigeria	AJ010699
S	RC	Japan	D86371		115-1S	O	Kenya	AY523538S3
Bag	EA	Tanzania	AJ781780					

[a] Sequence determined in this study

Studies on other potyviruses infecting sweet potato are less abundant. These include sweet potato latent virus (SPLV), found in Asia, Africa and Peru [20, 29], sweet potato mild speckling virus (SPMSV) from Argentina and Peru [15, 20], sweet potato virus G (SPVG), identified in China, Egypt, Ethiopia, Europe and the United States [3, 6, 12, 13, 21, 47] and a potyvirus first reported as sweet potato virus II [43], and later named ipomoea vein mosaic virus [47], sweet potato virus Y [4] or sweet potato virus 2 (SPV2) [49], has been identified from the United States [47], Africa, Taiwan, China, Portugal [49] and Australia [6]. Since the proposal to refer to this new potyvirus

species as SPV2 has been favorably considered by the International Committee on the Taxonomy of Viruses, this name is used here. SPLV and SPMSV have been reported in Peru at low frequency [20].

Understanding the molecular variation of viruses is essential to design knowledge-based strategies to control them. In the present study, we determine the nucleotide sequence of the region encompassing the 3'-terminal ~1,800 nucleotides (nts) of 17 potyvirus samples mostly collected from SPVD-affected plants from the major sweetpotato-producing areas in Peru. Most of the viruses were identified as SPFMV, but we also report for the first time the occurrence of SPV2 and SPVG in Peru and South America. Phylogenetic analysis of SPFMV sequences indicates a variable population of SPFMV in Peru, including EA, C and RC strain groups, and provides evidence for the existence of recombinants between strains.

Materials and methods

Virus-infected plant samples and virus isolates

One symptomless sweetpotato plant and 14 with SPVD-like symptoms were collected at random from six main sweetpotato-producing areas in Peru (Fig. 1). Plants with SPVD-like symptoms were found in all locations except for the Chira Valley in Piura, where only symptomless plants were collected. Details of the samples and isolates, their names, province of origin and year of collection are shown in Table 1 (Fig. 4 for SPV2 and SPVG). Stem cuttings of collected plants were maintained in an insect-proof greenhouse at CIP headquarters, Lima, Peru, for at least 3 weeks before analysis. Asymptomatic plants were grafted onto the indicator plant *I. setosa*, which was observed for symptom development and analyzed by serological means. The presence and identity of sweetpotato viruses were confirmed using antisera included in the NCM-ELISA sweetpotato virus detection kit from CIP, Lima, Peru [51], according to the manufacturer's protocol. A number of SPFMV isolates kept in desiccated *I. nil* leaves for as long as 20 years, as well as the Peruvian isolate of SPFMV, C1 [39, 42], and the North American isolate, YV [35], maintained in *Nicotiana benthamiana* and *I. nil*, respectively, as part of the CIP virus collection, were also included in the study. Isolation of these viruses was done by three consecutive single-lesion transfers on *Chenopodium amaranticolor* [10, 30, 35, 39].

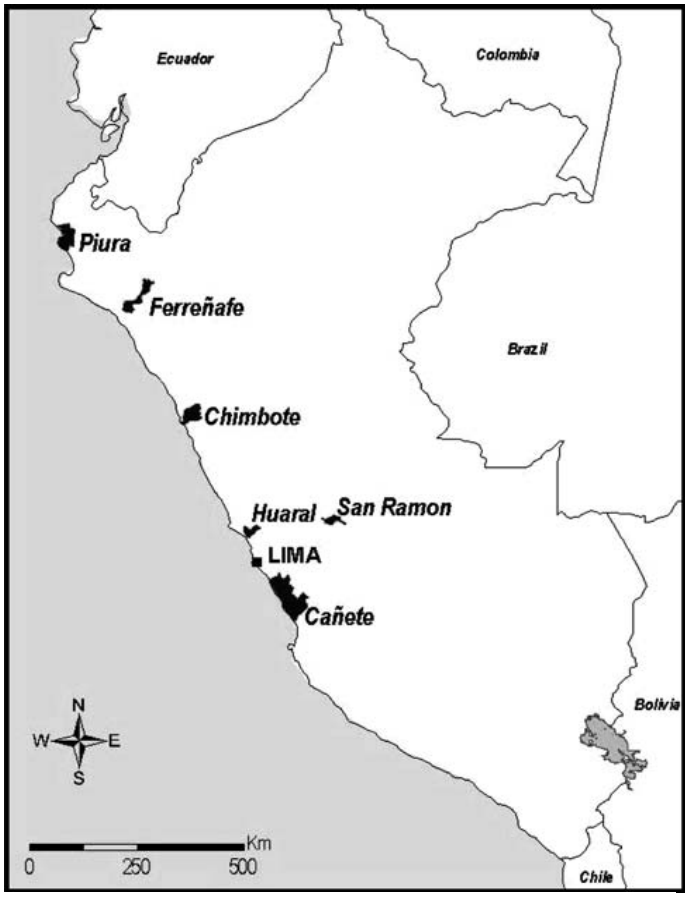

Figure 1. Locations of the sweet potato fields surveyed in Peru

RNA extraction, RT-PCR and cloning

Total RNAs were extracted from approximately 0.2 g leaves of SPVD-infected sweetpotato or SPFMV-infected indicator plants using TRIZOL reagent (Invitrogen, CA, USA) according to the manufacturer's procedure. For lyophilized samples, the initial amount of tissue was 0.02 g. Although this provided sufficient amounts of total RNA, a further purification of high-molecular-weight RNA with 4M LiCl4 significantly improved the subsequent RT-PCR reaction. The integrity of the isolated RNA was visually verified after electrophoresis in a standard formaldehyde agarose gel and staining with ethidium bromide. Reverse transcription was performed on extracted RNA using AMV reverse transcriptase (Promega, WI, USA) according to the manufacturer's

recommendations, with the primer FMV10820 (Table 2), corresponding to the last 20 nts of the virus genome excluding the poly A tail of the 'SPFMV' subgroup (see Discussion) of potyviruses [50]. A fragment comprising the 3'-terminal ~1,800 nts of the potyvirus genome including the 3'-terminal part of the NIb gene, the complete CP gene and the 30 UTR was amplified by PCR using the potyvirus-specific forward primer PVD-2 (Table 2) [16] and the reverse primer FMV 10820. The PCR cycling conditions were set as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 15 s, 52°C for 20 s and 72°C for 90 s, and then one final elongation at 72°C for 10 min. PCR products were separated on 1% agarose gels and fragments of interest recovered by using the Wizard SV gel extraction kit (Promega) according to the manufacturer's recommendation. The eluted DNA was ligated into plasmid vector pCR 2.1 (Invitrogen) according to the manufacturers' instructions and cloned in Escherichia coli strain DH5*a*.

Table 2. List of synthetic oligonucleotides primers used in this work

Virus	Primer name	Sequences	Reference
SPFMV, SPV2, SPVG (cloning)	FMV 10820	5'-GGCTCGATCACGAACCAA-3'	Tairo et al. (50)
	PVD-2	5'-GGBAAYAAYAGYGGDCARCC-3'	Gibbs and Mackenzie (16)
SPFMV (sequencing)	FMV 9675 F	5'-AGATGCIGGWGCRRACCCWCCAG-3'	This study
	FMV 9675 R	5'-CTGGWGGGTYYGCWCCNGCATCT-3'	This study
	FMV 10244 F	5'-CATGCAGTGCCTACTTTTAGGC-3'	This study
	FMV 10244 R	5'-GCCTAAAAGTAGGCACTGCATG-3'	This study
SPVG (sequencing)	SPVG – F	5'-GGATGAAACCTGGGCAAACAC-3'	This study
	SPVG – R	5'-CGATACACCACACCATGAGACC-3'	This study
SPV2 (sequencing)	SPV2 – F	5'-GGCAGCTTCAAAGAGTTAGGGC-3'	This study
	SPV2 – R	5'-TGTGTGTTATCTGGAGACGTGGC-3'	This study

DNA sequencing, sequence analysis and phylogenetic relationships

Plasmids containing the amplified viral sequences were sequenced in both directions (Macrogen, Seoul, Korea). Internal primers were designed for sequencing as shown in Table 2. At least two individual clones per sample were sequenced, and if inconsistencies were detected, then further clones were sequenced. In one case in which an unexpected gap was identified, the fragment was reamplified from a new RNA extraction and re-sequenced for confirmation. Sequences were assembled using ContigExpress, included in the Vector NTI software package (Invitrogen). The alignments and phylogenetic analyses were performed with the MEGA 4 software package [27] and included a number of sequences downloaded from the GenBank database (Table 1, Fig.4). Distances were calculated using the Kimura 2-parameter model, and trees were assembled using neighbour joining with 1,000 bootstrap replicates.

Recombinant analysis

Putative recombination events were predicted by the Recombination Detection Program (RDP) 2.0 [31]. For further confirmation, sequence alignments were cut at the predicted recombination junctions, and phylogenetic analysis of the aligned sequences corresponding to each side of the junction was performed. Viruses grouping in different strain groups with strong (>90%) bootstrap support in the different phylogenic trees were considered true recombinants.

Results

Nucleotide (nt) sequences encompassing the 3'-terminal part of the NIb gene, the complete CP gene and the 3'UTR of 14 Peruvian samples infected with SPFMV, the SPFMV isolate C1 from Peru, and the isolate YV from USA were determined in this study, as well as those of one sample of SPV2 and SPVG. RNA was also obtained from 12 desiccated leaf samples of SPFMV-infected plants collected in Peru as far back as 20 years ago. Although this yielded apparently intact RNA as assessed by gel electrophoresis, RT-PCR amplicons of the correct size were obtained from only three of these samples, and only one of these amplified products could be cloned (SP-33) and proved to be an SPFMV sequence.

Analyses of SPFMV sequences

Analysis of SPFMV included more than 40 sequences taken from the GenBank database in addition to the ones determined in this study. The deduced CP sequences of most SPFMV-infected samples were 315 aa in length with 155 aa showing variability (36.5%), 60 of which were located in the N-terminal region. With the exception of the type isolate C, all isolates belonging genetically to strain group C lacked two amino acid residues at positions 62 and 63, as reported previously by Tairo et al. [50]. In addition, however, a deletion of 14 aa was found in the CP N-terminal region (at aa position 42–55) of the Peruvian EA isolate M2-44. Nucleotide and amino acid sequence identities for different regions are presented in Fig. 2.

a NIb C-terminal (last 208 aa)

	C	RC	O	EA
C	93.2 ± 0. 6 **95.9 ± 0. 9**			
RC	75.9 ± 1.7 **85.3 ± 2.2**	98.4 ± 0.3 **98.9 ± 0.4**		
O	75.5 ± 1.7 **84 ± 2.3**	87.5 ± 1.2 **92.3 ± 1.6**	95.4 ± 0.9 **96.6 ± 1.2**	
EA	75.3 ± 1.7 **84.6 ± 2.3**	87.6 ± 1.2 **92.9 ± 1.5**	88.5 ± 1.2 **94.2 ± 1.3**	96.5 ± 0.3 **98.3 ± 0.3**

b CP

	C	RC	O	EA
C	96.1 ± 0.3 **96.4 ± 0.5**			
RC	77.5 ± 1.1 **83.3 ± 1.9**	98.5 ± 0.2 **98.5 ± 0.4**		
O	76.8 ± 1.1 **81.4 ± 1.9**	91 ± 0.7 **95.1 ± 0.9**	95.4 ± 0.3 **96.2 ± 0.6**	
EA	77.7 ± 1.1 **82.7 ± 1.9**	93.2 ± 0.6 **96 ± 0.9**	92.3 ± 0.6 **94.8 ± 0.9**	96.3 ± 0.3 **97.1 ± 0.5**

c CP N-terminal

	C	RC	O	EA
C	93.5 ± 0.8 **91.4 ± 1.5**			
RC	62.7 ± 3 **64.3 ± 5.2**	97 ± 0.6 **95.4 ± 1.4**		
O	59.1 ± 3 **57.9 ± 5.3**	83.6 ± 2 **86.1 ± 3.2**	93.5 ± 0.8 **92.1 ± 1.7**	
EA	60.8 ± 2.9 **62.1 ± 5.3**	87.5 ± 1.8 **87 ± 2.9**	86.1 ± 1.8 **85.4 ± 3.2**	94.3 ± 0.7 **92 ± 1.6**

d 3'UTR

	C	RC	O	EA
C	92.9 ± 0.9			
RC	85.2 ± 2.	99 ± 0.3		
O	84.4 ± 2	97 ± 0.7	96.7 ± 0.7	
EA	87.47 ± 2	98 ± 0.5	97.4 ± 0.5	98.1 ± 0.4

Figure 2. Mean nucleotide and amino acid (*bold*) inter- and intra-group identities (%) calculated for the C-terminal 208 aa of the NIb (a); the entire coat protein, CP (b); the N-terminal aa of the CP (c) and 3'UTR (d). Recombinant sequences were excluded

Phylogenetic analysis of the aligned sequences split the SPFMV samples into four strain groups: C, RC, O and EA. Peruvian SPFMVs were found to correspond to groups EA, RC and C (Figs. 3, 4). Visual inspection of the alignments of the complete ~1,800 nt fragments suggested that some SPFMV samples might be the result of recombination between strain groups; i.e. in isolates C and YV the CP encoding region and 3'UTR seemed to be derived both from a C strain and a non-C strain virus, respectively, and in both of the Egyptian samples, Eg1 and Eg9, the central part of the NIb gene and the remaining 3' part of the genome appeared to originate from viruses of strain groups EA and RC, respectively. To confirm this, recombination analysis was performed using the various algorithms of the RDP2.0 program, which all predicted, with high, but varying probability, the suspected recombination events in the strains in question. The algorithm Max-Chi Squared [32] provided by the RDP program predicted the following breakpoints, in accordance with the suspected points identified in the sequence alignments: the first one, located at nt position 9,597 (strain S [44]; nt 570 in our fragment) within the 3'-terminal part of the NIb gene was shared by SPFMV Eg-9 and SPFMV Eg-1, whereas the second one, located at nt position 10,500 (nt 1473 in our fragment) within the 3' terminal region of the CP was present in SPFMV-YV and -C (Fig. 3a). Further confirmation of recombination was obtained by construction of phylogenetic trees using the segments corresponding to each side of the predicted recombination breakpoints (Fig. 3b–e). The topologies of the four trees produced were similar, distinguishing the four strain groups, except for the tree created using the sequences encompassing nts 1473-3'end, in which only two well-supported clades could be discriminated; one comprising viruses of the O, RC and EA strains, and one comprising C strain viruses (Fig. 3c). The conflicting affinity of the Egyptian viruses between the trees produced using the different genome regions clearly confirms the predicted recombination events; i.e. Eg1 and Eg9 are members of strain group RC when alignments comprising the nt positions 570-3'-end are analyzed (Fig. 3e), but belong to strain group EA on the basis of the nt positions 0–570 (Fig. 3d). Similarly, isolates C and YV belong to the strain group C when the region encompassing nts 0–1452 is analyzed (Fig. 3b) but group together with RC, EA and O when 30 nts are analyzed (Fig. 3c).

Analyses of SPV2 and SPVG sequences

A Blast search indicated that sequences closely related to SPVG and SPV2 had been amplified from two plants from Huaral showing typical symptoms of SPVD. Infection by these two viruses was confirmed by NCM-ELISA. Phylogenetic analysis of the SPV2 sequence showed it was closely related to sample Thomas 16A from South Africa, and confirmed the four genetically distinct lineages suggested previously by Ateka et al. [6, Fig. 4). The SPVG sequence determined here, however, was quite distinct from other reported SPVG samples, suggesting that SPVGHua2 might represent a novel strain. Thus, SPVG also can be divided into at least three genetically distinct lineages represented by the CH2, Hua2 and the remaining samples, respectively (Fig. 4). Inclusion of additional potyvirus CP sequences into the analysis further revealed that SPV2, SPVG, SPFMV and a potyvirus isolated from sweetpotato in Zimbabwe are related, forming a well-supported separate phylogenetic lineage within the genus Potyvirus (Fig. 4).

Discussion

Our study, the first attempt to classify Peruvian sweetpotato potyviruses at the molecular level, demonstrated the presence of SPFMV strains C, RC and EA, as well as SPV2 and SPVG in the main sweetpotato-producing regions. This is the first time that SPFMV of strain group EA has been reported from the Americas and SPV2 or SPVG from South America. Two of the isolates, C1 and SP-33, corresponding to strain group C and EA, respectively, were collected in 1987, indicating that these viruses had been present in Peru before SPVD occurred at a high incidence in 1997.

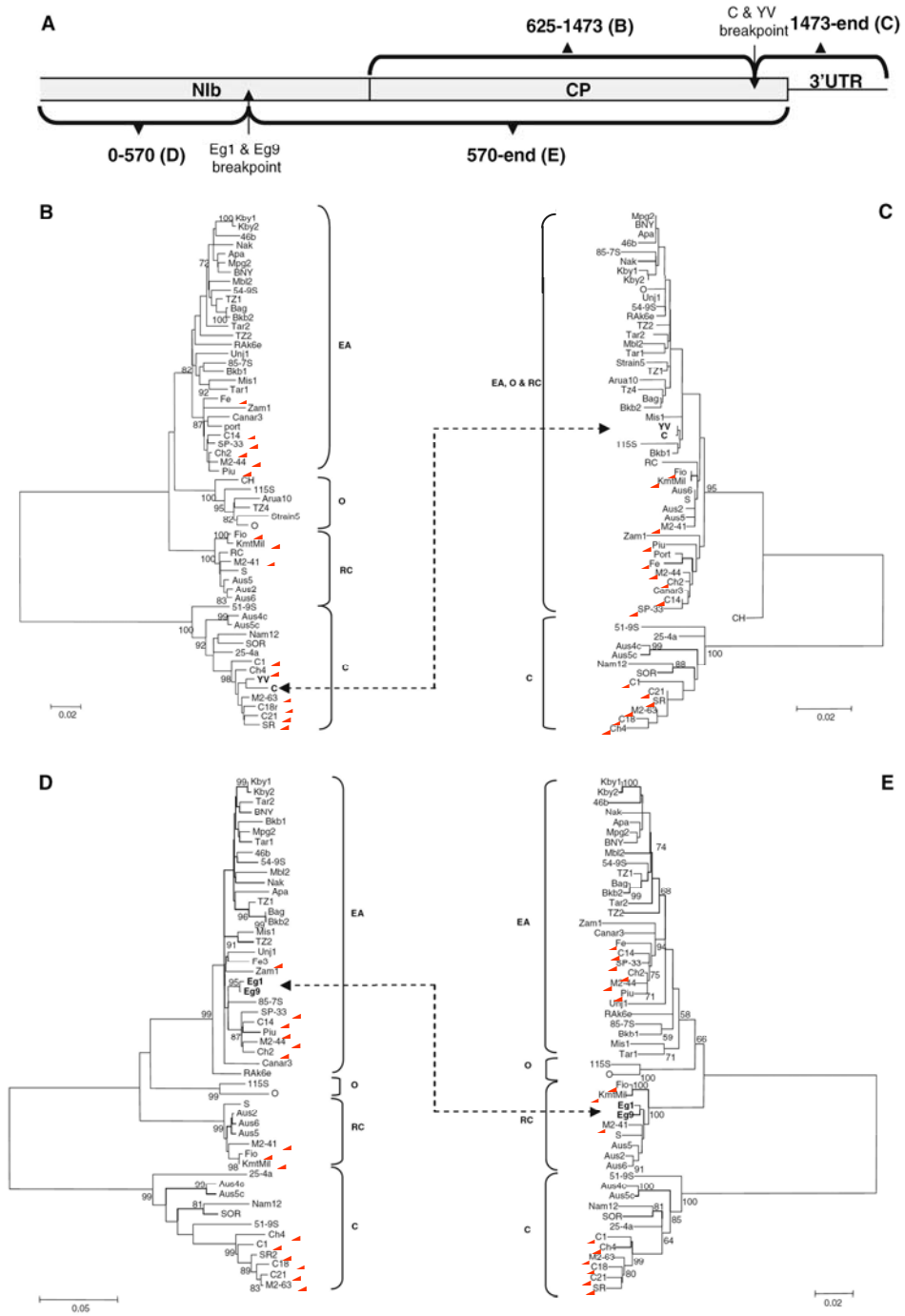

Figure 3. Phylogenetic trees calculated from alignments of nucleotide sequences on either side of recombination breakpoints identified with the RDP program. **A,** schematic representation of the 3' region of SPFMV used for analysis indicating the regions used and the corresponding trees matching to predicted recombination breakpoints in isolates C and YV, and Eg1 and Eg9. **B–E,** phylogenetic trees of SPFMV sequences encompassing the regions indicated in **A**, *arrowheads* indicate the conflicting groupings of sequences in which recombination events were predicted. The *scale bar* in each figure indicates Kimura nucleotide distances. In **B** the sequences corresponding to the NIb genes were not included because they are not available for isolate C. Similarly, the sequence of the 3'UTR is not included in the analysis in (**E**) as this region is not available for the isolates Eg1 and Eg9. The percentage of bootstrap support out of 1,000 replicates is given at each of the major nodes in the trees. Red arrowheads indicate the samples/isolates analyzed in this study.

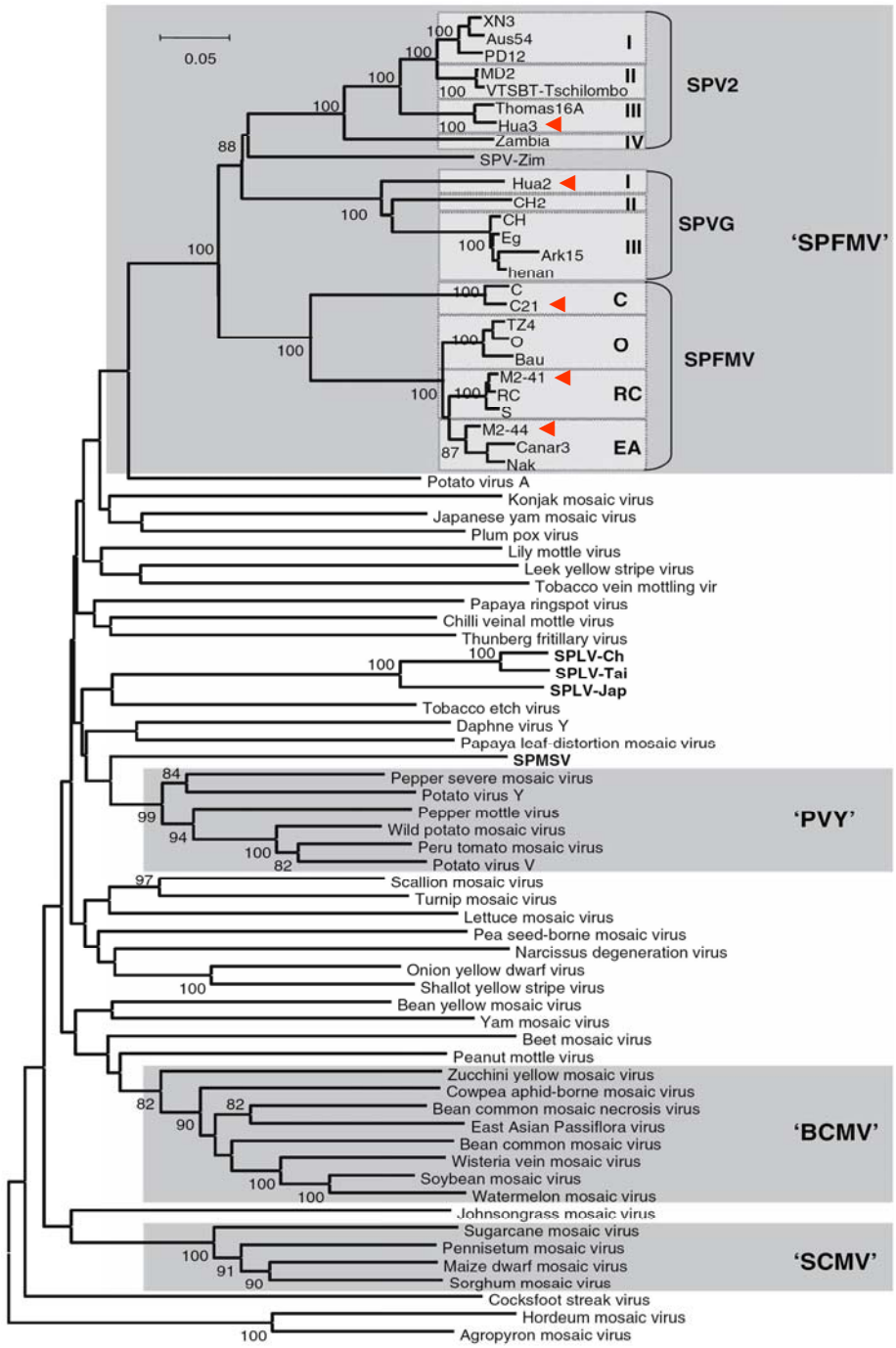

Figure 4. Phylogentic tree of CP nucleotide sequences of potyviruses. The *Ipomoea*-specific 'SPFMV' subgroup, as well as previously identified subgroups are shaded in *grey*. Sweet potato-infecting viruses are in *bold*, proposed virus strain groups are shaded in *light grey* with roman numerals, except for SPFMV, which is according to (25). The *scale bar* indicates Kimura nucleotide distance. The percentage of bootstrap support out of 1,000 replicates is given at the major nodes in the trees where they exceeded 80%. GenBank accession numbers: *SPFMV* sweet potato feathery mottle virus (see Table 1); SPV-Zim: (AF016366); *SPLV* sweet potato latent virus (Ch: X84011, Jap: E15420, Tai: X84012); *SPMSV* sweet potato mild speckling (U61228); *SPVG* sweet potato virus G (Ark15: Ref 3, CH2: X76944, CH: Z83314, Eg: AJ515380, Henan: DQ399861, Hua2: EU218528); *SPV2* sweet potato virus 2 (Aus54: AM050884, Hua3: EU218529, MD2: AY459606, PD12: AY459607, Thomas16A: AY459608, VTSBT-Tschilombo: AY459609, XN3: AY459611, Zambia: AY459610); agropyron mosaic virus

(NC_005903); bean common mosaic virus (NC_003397); bean common mosaic necrosis virus (NC_004047); bean yellow mosaic virus (NC_003492); beet mosaic virus (NC_005304); chilli veinal mottle virus (NC_005778); cocksfoot streak virus (NC_003742); cowpea aphid-borne mosaic virus (NC_004013); daphne virus Y (NC_008028); East Asian passiflora virus (NC_007728); hordeum mosaic virus (NC_005904); Japanese yam mosaic virus (AB027007); johnsongrass mosaic virus (NC_003606); konjac mosaic virus (NC_007913); leek yellow stripe virus (NC_004011); lettuce mosaic virus (NC_003605); lily mottle virus (NC_005288); maize dwarf mosaic virus (NC_003377); narcissus degeneration virus (NC_008824); onion yellow dwarf virus (NC_005029); papaya leaf-distortion mosaic virus (NC_005028); papaya ringspot virus (NC_001785); pea seed-borne mosaic virus (NC_001671); peanut mottle virus (NC_002600); pennisetum mosaic virus (NC_007147); pepper mottle virus (NC_001517); pepper severe mosaic virus (NC_008393); Peru tomato mosaic virus (NC_004573); plum pox virus (NC_001445); potato virus A (NC004039); potato virus V (NC_004010); potato virus Y (NC_001616); scallion mosaic virus (NC_003399); shallot yellow stripe virus (NC_007433); sorghum mosaic virus (NC_004035); soybean mosaic virus (NC_002634); sugarcane mosaic virus (NC_003398); Thunberg fritillary virus (NC_007180); tobacco etch virus (NC_001555); tobacco vein mottling virus (NC_001768); turnip mosaic virus (NC_002509); watermelon mosaic virus (NC_006262); wild potato mosaic virus (NC_004426); wisteria vein mosaic virus (NC_007216); yam mosaic virus (NC_004752); and zucchini yellow mosaic virus (NC_003224). Red arrowheads indicate the samples/isolates analyzed in this study.

Comparison of the SPFMV sequences with those available from the database enabled us to identify novel variations amongst SPFMV strains. The CP aa sequence of the EA sample M2-44, which, coincidentally, was reported as one of the most detrimental isolates identified in Peru [20], lacks 14 amino acids. Although deletions seem rare in the CP of SPFMV, deletions of 12 aa in the CP region are common in isolates of yam mosaic virus [2]. Besides the characteristic 42-nt (14-aa) deletion found in the C terminus of the CP of strain M2-44, we also obtained the first evidence for recombinants of SPFMV. Four samples containing recombinant segments were detected visually, and their occurrence was confirmed using specialized software and phylogenetic analysis (Fig. 3). The 3'-terminal sequences of the recombinant isolate YV were amplified and cloned from two individual RNA extractions to be sure that the recombination event detected was not an artifact of the PCR reaction. The fact that the same recombination is found in isolate C from the USA, which was cloned and sequenced using separate primers by a different laboratory, corroborates that this is unlikely to be a PCR artifact. The tentative recombinant viruses Eg1 and Eg9 [21] share the same recombination breakpoint in the NIb-encoding region and originate from the same geographic region in Egypt. It is therefore likely that they share a common evolutionary ancestor. The same can be argued for the two recombinant North American strains of SPFMV identified in this study. Recombination in evolutionary history of potyviruses is not a novelty and has been reported for a number of potyvirus species, such as *Yam mosaic virus* [8], *Yam mild mosaic virus* [7], *Potato virus Y* (PVY [18]), *Plum pox virus* [19], *Turnip mosaic virus* [41], *Lettuce mosaic virus* [26] and *Sugarcane mosaic virus* (SCMV [54]), but also between isolates of closely related species such as *Bean common mosaic virus* (BCMV), and *Soybean mosaic virus* [14], *Bean common mosaic necrosis virus* and BCMV [28] and those of other related viruses [52]. These reports are evidence for an important role for recombination in the evolution of potyviruses, although their frequency may vary significantly between virus species. Such recombination events may lead to more virulent virus strains [18, 54] and even new species [14, 52] or genera [52].

In a previous study by Gutiérrez et al. [20], the SPFMV samples M2-44, M2-41 and C18, here shown to correspond to strains EA, RC and C, respectively, were compared and found to vary in reaction to different antisera, as well as the severity of symptoms induced in *I. nil* and sweetpotato. In both hosts, the EA isolate produced the most severe symptoms, whereas the RC strain produced the mildest symptoms. However, Moyer et al. [37] reported that isolate C (strain group C) caused milder symptoms than isolate RC (strain group RC), and in Japan an RC isolate (SPFMV-S) was reported to be the most severe [34]. Although an EA strain group isolate was not included in these studies, the contradictory results obtained for severity of C and RC isolates in the study of Gutiérrez et al. [20], and that of Moyer et al. [37], suggest that symptom severity may not necessarily be a characteristic of the strains, but rather that of individual isolates. Similarly, the ability to infect *N. benthamiana* appears to be an isolate-specific rather than a strain-specific characteristic, as only some isolates from different strain groups (EA and C) are able to infect this host [20, 37]. Nevertheless, the isolates belonging to strain group C are genetically distinct from the other strain groups of SPFMV. Therefore, biological differences are expected. It has been suggested that strain group C may represent a separate virus species [50]. Our extended analysis agrees with the percentage of CP nt and aa sequence identities previously reported for samples/isolates of strain group C (Fig. 2b: 75.6–78.7% nt, 79.5–85.2 aa) [25, 50]. However, these identities do not provide a clue for classifying the C strain as another viral species, because they are very close to the CP nt and aa sequence identities of 76–77 and 82%, respectively, used currently as threshold values for potyvirus species demarcation [1]. Similarly, the partial NIb sequences analyzed in this study are at the edge of the recommended species demarcation criterion of 75%

identity (Fig. 2a). In contrast, the variability found in the 3'UTR (80.0–85.4% identity, Fig. 2c) appears well above that recommended to distinguish different potyvirus species (76% identity [1]). The definitive reclassification of SPFMV-C as a new species is something that can only be resolved with the help of the entire genome sequence of a C isolate. Preliminary data from the Peruvian C1 isolate indeed indicate that variability in other parts of the genome significantly exceeds those found for strains of the same species (our unpublished data). This and the fact that polyclonal or monoclonal antibodies to SPFMV may not recognize all isolates of strain group C [50] underlines the need to develop appropriate diagnostic methods for detection of these viruses. A PCR-RFLP-based method described by Tairo et al. [49] may be too expensive for routine detection purposes, especially in developing countries, which often lack facilities for using molecular techniques.

The identification of two of the potyvirus-specific amplicons as pertaining to SPV2 and SPVG presents the first report of the occurrence of these viruses in Peru and South America. Hence, all currently recognized sweetpotato potyviruses are endemic to Peru. In routine testing from CIP's germplasm collection and from seed production in Huaral and Can˜ete using specific antibodies, SPVG is frequently detected (c. 30% of symptomatic samples) and appears to be more prevalent than SPV2 (c. 3%). Despite this, SPFMV is by far the most common virus found (c. 90% of symptomatic samples), and consequently, the other potyviruses may contribute little to yield losses caused by SPVD. Although it has been shown that the titers of all these viruses increase upon co-infection with SPCSV, titers of SPFMV increase more than those of the other potyviruses [24, 47, 51]. These higher replication rates upon co-infection with SPCSV may provide a possible explanation for the prevalence of SPFMV over the other potyviruses, which are out-competed. This also implies that if cultivars with resistance to only SPFMV were deployed, the other potyviruses could rapidly replace it, causing similar synergistic virus diseases. Because available antisera to SPFMV show a weak serological cross-reaction in NCM-ELISA with SPVG as well as SPV2, infection with these viruses may previously in many cases have been attributed to SPFMV, and their prevalence worldwide may be greater than previously known. The availability of SPVG- and SPV2-specific antibodies is now facilitating the detection of both viruses in samples from different countries, discriminating them from the presence of SPFMV.

Phylogenetic analysis of various samples of the three viruses identified in this study, together with a representative repertoire of other potyviruses, enabled us to show that these viruses form a well-supported phylogenetic subgroup within the genus *Potyvirus* (Fig. 4), together with an unknown virus reported from Zimbabwe [11]. Besides notable sequence similarity (including identical last 20 nts), this 'SPFMV' subgroup distinguishes itself by having a narrow host range, mainly confined to the Convolvulaceae, indicating a likely common evolutionary ancestor adapted to this family of hosts. On the other hand, SPLV and SPMSV, the two sweetpotato-infecting potyviruses not belonging to the 'SPFMV' subgroup, are phylogenetically distantly related and have broader host ranges including Chenopodiaceae and Solanaceae [29]. Other potyvirus subgroups also have certain host specificities (Fig. 4), such as the 'SCMV', 'BCMV' and 'PVY' subgroups predominantly infecting gramineous, leguminous, and solanaceous plants, respectively [46, 48], suggesting a significant role for virus host co-evolution in potyvirus speciation. Further sequencing of SPV2, SPVG and SPFMV-C genomes may enable us to shed some light onto the specific characteristics required for adaptation to convolvulaceous hosts, although the identification of a highly conserved region in the P1 protein of SPFMV and the ipomovirus sweetpotato mild mottle virus [52] already alludes to an important role for that protein in host specificity.

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Bemisia afer sensu lato, a vector of Sweet potato chlorotic stunt virus[1]

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Abstract

Bemisia tabaci biotype B is considered the primary vector of *Sweet potato chlorotic stunt virus* (SPCSV, *Crinivirus*). However, *Trialeurodes abutiloneus* also has been shown to transmit SPCSV. Mixed infection of SPCSV with the aphid-transmitted *Sweet potato feathery mottle virus* (SPFMV, *Potyvirus*) causes sweetpotato virus disease (SPVD), the major virus disease affecting this crop. High populations levels of *Bemisia afer* sensu lato are seasonally associated with sweetpotato in Peru during times of low *B. tabaci* incidence. The transmission of SPCSV (in single and double infection with SPFMV) by laboratory-reared *B. afer* sensu lato and *B. tabaci* biotype B was investigated. For SPCSV transmission efficiency, individual adult insects were allowed 48h for acquisition and inoculation access periods at both 20°C and 25°C. SPCSV was transmitted by both whiteflies, with similar transmission efficiency when the virus was acquired from plants singly infected by SPCSV or doubly infected with SPCSV and SPFMV, at 20°C and 25°C. We conclude that *B. afer* sensu lato is a new vector of SPCSV. This finding may have important epidemiological significance for the spread of SPCSV and SPVD.

Keywords: *Ipomoea batatas*, semi-persistent, whitefly transmission.

Sweet potato chlorotic stunt virus (SPCSV) is a crinivirus (Family *Closteroviridae*) transmitted by *Bemisia tabaci* (Gennadius) and *Trialeurodes abutiloneus* Haldeman (Hemiptera: Aleyrodidae) (21) in a semi-persistent manner (4,10,28,34,40,44,45). SPCSV is the most important virus affecting sweetpotato (*Ipomoea batatas* (L.) Lam.) due to its ability to mediate severe synergistic diseases with several other sweetpotato-infecting viruses belonging to different genera (14,15,26,39). SPCSV, together with the aphid-borne potyvirus, *Sweet potato feathery mottle virus* (SPFMV; genus *Potyvirus*, family *Potyviridae*), are the causal agents of sweetpotato virus disease (SPVD), the main viral disease affecting this crop in different regions of the world (11,14,27,38). Sweetpotato yield reductions caused by SPCSV are ca. 30%, but can exceed 50% when interacting with other viruses (11, 17).

Bemisia afer (Priesner & Hosny) (3,6) sensu lato occurs in Africa, Australia, the Mediterranean coast of Europe, southern England, and South America (3,18,20). This whitefly species was first reported in the Americas in Peru, on sweetpotato in 2000 (3). *Bemisia afer* infests plants in the families *Anacardiaceae*, *Annonaceae, Apocynaceae, Bignoniaceae, Bixaceae, Bombacaceae, Burseraceae, Celastraceae, Caprifoliaceae, Combretaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Labiateae, Liliaceae, Loganiaceae, Lythraceae, Malvaceae, Moraceae, Myrtaceae, Papaveraceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae*, *Salicaceae, Sapindaceae, Solanaceae and Urticaceae* (3,5,16,20,24).

Bemisia afer and SPCSV have been previously reported in Peru, Uganda, Kenya, Tanzania, Madagascar, Nigeria, Egypt, and Spain (3,11,17). Although criniviruses are unique among whitefly-vectored viruses in that members of different genera of whiteflies can transmit them, *B. tabaci* biotype B is the only *Bemisia* species identified as a vector of SPCSV to date. The possibility that *B. afer* sensu lato is a vector for this virus is particularly relevant because this species of whitefly colonizes sweetpotato at high levels. The importance of *B. afer* sensu lato as a vector of sweetpotato viruses (or any other plant viruses) has never been documented.

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In this study, we describe for the first time, transmission of SPCSV by *B. afer* sensu lato, the effect of temperature on the transmission of SPCSV in single and double infections with SPFMV, and the epidemiological implications associated with efficient transmission of SPCSV by both *B. afer* sensu lato and *B. tabaci* biotype B.

Materials and methods

Plant material and virus isolates. Virus-free sweetpotato plants cv. Costanero, determined by indexing them through grafting to *I. setosa* Ker with subsequent serological test, were provided by the International Potato Center (CIP, Lima, Peru). Virus-free plants of *I. nil* (L.) Roth were grown from botanical seed, as no sweetpotato virus reported so far is seed-transmitted. In addition, random samples were tested by ELISA on nitrocellulose membranes (NCM-ELISA) (11) to confirm freedom of SPCSV and SPFMV. Hereafter, virus-free plants are referred to as "healthy plants".

SPCSV isolate M2-47, belonging to the East African (EA) strain (11), was obtained from infected sweetpotato plants collected in the Valley of Cañete, Peru, using *B. tabaci* biotype B as vector on *I. nil*.

The russet crack strain of SPFMV (USA isolate) was obtained from CIP virus collection and maintained in *I. nil* by mechanical inoculation.

Healthy sweetpotato plants were side-graft-inoculated with SPCSV (single infection) and with both SPCSV and SPFMV (double infection) to yield virus sources for transmission studies.

Whitefly colonies and species identification. CIP colonies of *B. afer* sensu lato and *B. tabaci* biotype B were established from the pupal stage obtained from sweetpotato plants growing in the Valley of Cañete, Lima, Peru. The whiteflies were mass-reared on healthy sweetpotato plants cv. Costanero. The third generation was used to get whiteflies colonies of the same age. Identification of the whitefly species was confirmed morphologically from the puparia as indicated in Fig. 1 (20,30) in two different laboratories: the International Center for Tropical Agriculture (CIAT), Colombia and The Food and Environment Research Agency (Fera), U.K. The two whitefly species were also differentiated by amplification and sequencing of a fragment of the mitochondrial 16S rDNA gene, obtained by polymerase chain reaction (PCR) at CIAT-Colombia. Universal primers 4118 (CCGGTCTGAACTCAGATCACGY) and 4119 (CGCCTGTTTAACAAAAACAT), constructed for the mitochondrial 16S rDNA gene of *Drosophila yakuba,* were used in the PCR reactions according to Xiong and Kocher (46) and modified by Calvert et al. (8).

For *B. afer* sensu lato, the mitochondrial 16S rDNA fragment obtained by PCR was sequenced and compared with that published in the GenBank database for *B. leakii* Peal (AF247531), *B. hancocki* Corbett (AF247532), *B. tabaci* biotypes B, Q and S (AF246636, AF246647 and AF247527, respectively), *B. tabaci* biotypes/collection (AF110722, AF110714, AF110713, AF110715, AF110716, AF110717, AF110719, AF110721), *T. vaporariorum* Westwood (AF110723), *Aleuroplatus coronata* Quaintance (EU471164) and those unpublished provided by CIAT for *B. tabaci* biotype A, *B. tuberculata* Bondar, *T. vaporariorum*, *T. variabilis* Quaintance, and *Aleurotrachelus socialis* Bondar (8). The alignments and phylogenetic analysis were performed with the MEGA 4 software package (37). Distances were calculated using the Kimura 2-parameter model, and a tree was assembled using neighbour joining with 2,000 bootstrap replicates.

Virus transmission. Completely randomized experimental design was set up as a 2^3 factorial (2 whitefly species, 2 temperatures and 2 virus sources) in 4 replications with 25 plants per experimental unit (Table 1). Adult whiteflies from the third generation of *B. afer* sensu lato and *B. tabaci* biotype B were used as vectors in the virus transmission efficiency tests. Individual whitefly adults were allowed an acquisition access period (AAP) of 48 h on sweetpotato plants infected with SPCSV and with both SPCSV and SPFMV. Single viruliferous whiteflies were then placed on individual healthy *I. nil* plants (test plants) for an inoculation access period (IAP) of 48 h (Table 1). Acquisition and inoculation periods were carried out at both 20°C and 25°C in a growth chamber (temperature controlled, with 3,500 lx / 12h of fluorescent and bulb lights).

Five infected plants with high levels of SPCSV (from single and double infection with SPFMV), as determined by NCM-ELISA (11), were selected as virus sources. At the end of each IAP, whiteflies were removed manually and plants were sprayed with 1% buprofezin. Inoculated *I. nil* plants were maintained in an insect-proof greenhouse at 10,000 to 15,000 lx light intensity for evaluation. Number of infected plants was recorded and transmission efficiency was analyzed by the test of equal or given proportions using the R Statistical program (31). For

interaction studies, normality of data was corrected through angular transformation of them which allowed doing analysis of variance.

Virus detection. Test plants were evaluated beginning two weeks after inoculation and monitored for 60 days. SPCSV infection was initially detected by symptom expression in the inoculated plants at 10 to 20 days after inoculation. All symptomatic and non-symptomatic plants were tested by NCM-ELISA (11) and/or by reverse transcription-PCR (RT-PCR) periodically throughout the 60 days. Primers CP1 (CGTCTAGATTGTTAGAAA) and CP3 (AACGCGGAAGTGTAAGGTAT) were used in the RT-PCR reactions according to Alicai et al. (2). Total nucleic acids were extracted from plants prior to RT-PCR using Plant RNA Purification Reagent (Invitrogen, CA, USA).

Results

Identification of whiteflies species. Both species of *Bemisia* were identified by the morphological characters observed on puparia, according to the key features shown in Fig. 1. Pupae of *B. afer* sensu lato are larger and are transparent or slightly yellowish compared to those of *B. tabaci*. Important diagnostic characters of *B. afer* sensu lato include short caudal setae, vasiform orifice subequal in length to caudal furrow, lingual head long and narrow, operculum triangular (Fig. 1). CIP colony was confirmed as *B. afer* sensu lato by whitefly taxonomists P. Hernandez at CIAT and C. Malumphy at Fera. A 529 bp PCR product amplified from the mitochondrial 16S rDNA was obtained from *B. afer* samples. Phylogenetic analysis of the *B. afer* sensu lato nucleotide sequence (GenBank accession FJ969473) indicated a relatively close relationship to *B. tuberculata, B. leakii,* and *B. hancocki* (84.1%, 82.8% and 81.9% identity, respectively) (Fig. 2).

Virus transmission. All plants exhibiting symptoms of SPCSV tested positive for SPCSV by NCM-ELISA and RT-PCR, and all symptomless plants tested negative. SPCSV was transmitted by both *B. afer* sensu lato and *B. tabaci* biotype B, with similar transmission efficiency (11.2 to 13% vs. 7 to 8%, respectively) when the virus was acquired from plants singly infected by SPCSV (Table 1 and 2). Similarly, when the SPCSV was acquired from plants doubly infected with SPCSV and SPFMV, *B. afer* sensu lato and *B. tabaci* biotype B did not differ significantly in their transmission of SPCSV (Table 2). No statistically significant differences in transmission of SPCSV were also observed in singly or doubly infected plants at 20°C and 25°C by both whitefly species (Table 2). However, the number of SPCSV-infected plants was higher when the virus was transmitted by *B. afer* sensu lato from singly infected plants than from doubly infected plants. The contrary occurred in the transmission of the virus by *B. tabaci* biotype B.

Interaction between whiteflies species and virus sources was moderately significant (p-value 0.09) in spite of a relatively high variation coefficient of 43%.

Discussion

Knowing the existence of new virus vectors is important for plant disease management. This study provides experimental data showing that in addition to *B. tabaci* and *T. abutiloneus*, SPCSV can also be transmitted by the whitefly species *B. afer* sensu lato. To our knowledge, this is the first time that *B. afer* sensu lato has been reported as a virus vector. Previous studies by Maruthi et al. (22) reported that *B. afer* sensu lato was not able to transmit the ipomovirus *Cassava brown streak virus*, even though the disease seemed associated with this species in the field. In the study presented herein, transmission of SPCSV was achieved by both *B. afer* sensu lato and *B. tabaci* biotype B. Similar transmission rates were also obtained by Wintermantel and Wisler (42) with the closely related crinivirus *Tomato chlorosis virus* (ToCV) and individual whiteflies of *B. tabaci* biotype B and *T. abutiloneus*. They observed that the transmission efficiency of ToCV increased with increasing numbers of whiteflies per plant, and that only the most efficient vectors were capable of a high transmission rate using single whiteflies. Ng and Falk (28) and Ng et al. (29) demonstrated that whitefly transmission of the crinivirus *Lettuce infectious yellows virus* is influenced by the virus concentration in the source plant and the number of individuals used to inoculate test plants. Taken together, this suggests that *B. afer* sensu lato is a relatively efficient vector of SPCSV. Obviously, the number of whitefly individuals found on plants under field conditions greatly exceeds the highest numbers of whitefly individuals tested under experimental conditions.

It seems possible that whiteflies might acquire the virus more readily from hosts other than sweetpotato (33). Acquisition and inoculation periods of 48 hours, fairly typical for criniviruses, have been shown to work well for

transmission of SPCSV (34,40). In this study, the transmission of SPCSV by single whiteflies of both *B. afer* sensu lato and *B. tabaci* biotype B was low from infected sweetpotato plants. It was reported that transmission efficiency of sweetpotato viruses increases when a larger number of whiteflies (more than 15) (12,32,33) are used per plant and when infected plants other than sweetpotato are used as the virus source.

Distinct isolates of SPCSV have been reported from different geographical regions (1, 9,13,36). These isolates form two groups according to their serological and molecular relationship: the East African (EA) and the non-EA strains. *B. tabaci* biotype B is able to transmit SPCSV isolates from EA (this study) and non-EA (34) with similar efficiency. In both cases, transmissions experiments were carried out using similar conditions: virus was acquired from SPVD-infected (SPCSV+SPFMV) sweetpotato by single whiteflies, and AAP and IAP of 48 h. In our studies, we found no significant differences in transmission efficiencies of SPCSV by *B. afer* sensu lato and *B. tabaci* biotype B at 20°C or 25°C, when acquired from singly infected or doubly infected plants. These results were similar to those obtained by Valverde et al. (40) who reported similar transmission rates for a non-EA isolate of SPCSV with *B. tabaci* biotype B from either single or double infections of SPCSV and SPFMV. It is known from previous reports (9,14,15,26) that SPCSV titers may significantly decrease in double infections with SPFMV. We did not estimate the SPCSV titer in the source plants in single infection and double infection with SPFMV. However, we observed that the number of SPCSV-infected plants was lower when the virus was transmitted by *B. afer* (but not by *B. tabaci* biotype B) from doubly infected plants than from singly infected plants. This is interesting and it seems to be in line with the previous reports, suggesting the SPCSV titer is somewhat lower in double infections.

Most whitefly-transmitted viruses are transmitted by a single genus of whitefly. *Tomato chlorosis virus* (ToCV), a crinivirus closely related to SPCSV (43) is the best-known exception to this. ToCV has the ability to be transmitted by four whitefly vectors in two genera: *B. tabaci* biotypes A and B, *T. abutiloneus,* and *T. vaporariorum* (41,42,45), although efficiency and persistence differ among the vectors (42). In the present study, SPCSV was shown to be transmissible by vectors within both the *Bemisia* and *Trialeurodes* genera (34,40). Taking our findings into account, SPCSV, like ToCV, shares the distinction of transmissibility by three different whitefly species (*B. tabaci* biotype B, *B. afer* sensu lato, and *T. abutiloneus*), and to the best of our knowledge is only crinivirus transmitted by a *Bemisia* species other than *B. tabaci*. Although we obtained a similar rate of transmission of SPCSV with both species of *Bemisia* (6.1% to 13.1%), it was higher than that reported for *T. abutiloneus* (3.2%) (34). Like other criniviruses, SPCSV is not transmitted mechanically (7); therefore, it is dependent on whiteflies for plant-to-plant dissemination in the field.

The presence of *B. tabaci* biotype B, *B. afer* sensu lato, and *T. vaporariorum* on sweetpotato, has been observed in the Peruvian coast. As attempts to transmit SPCSV with *T. vaporariorum* have been unsuccessful (unpublished data), *B. afer* sensu lato and *B. tabaci* biotype B are likely to be the predominant vectors of SPCSV in Peru. Temperature is one of the main environmental factors affecting whitefly population dynamics (23). In the Cañete Valley, *B. afer* sensu lato predominates in sweetpotato fields after September accounting for 99% of the whiteflies in November and December (25). Before September, *B. tabaci* biotype B is the whitefly species predominates in sweetpotato. This shift in whitefly populations' structure parallels changes in seasonal temperatures. It suggests that in the winter and spring seasons (cooler temperatures), *B. afer* becomes the primary vector for SPCSV in the Cañete Valley, whereas *B. tabaci* predominates in the summer and fall (25) seasons when temperatures are warmer, thus facilitating the dissemination of SPCSV, and subsequently SPVD all year round.

Bemisia hancocki has been synonymized with *B. afer* (6), but there remains doubt concerning this synonymy (19). The phylogenetic analysis of the nucleotide sequence indicated that *B. afer* sensu lato is different but closely related to *B. hancocki, B. leakii* and *B. tuberculata* (81.9 to 84.1% nucleotide identity). This finding is not surprising since the *B. leakii* group is a taxonomically unresolved complex that contains at least three described species, *B. leakii*, *B. afer*, and *B. hancocki* (35). The group *B. leakii* is probably more confused than the *B. tabaci* group (35). *Bemisia leakii* group has been reported in India, Fiji, Thaiti, Papua New Guinea, American Samoa, Marshall Islands, Nauru, Palau, Tonga, and Vanuatu; *B. hancocki* in Africa and southern Europe; and *B. tuberculata* in Ecuador, Peru, Colombia, Venezuela, Brazil, Nicaragua, Puerto Rico, Costa Rica, and Dominican Republic. *Bemisia tuberculata* seems to be the vector of the begomoviruses causing cassava mosaic disease and the agent of the cassava frog skin disease (5). This suggests that species other than *B. tabaci* can transmit begomoviruses.

Bemisia afer sensu lato is a new vector of SPCSV, the most important virus component of SPVD, and transmission rates seems to be sufficient to allow for disease spread. Since *B. afer* sensu lato outnumbers *B. tabaci* biotype B

during cooler season in Peruvian sweetpotato fields, it is likely the primary vector of SPCSV during those periods. *Bemisia tabaci* biotype B is likely the predominant vector during the warmer seasons when it becomes the predominant species. This observation has important epidemiological consequences for the management of sweetpotato virus diseases in Peru and other areas where *B. afer* sensu lato is present in sweetpotato crops. Because *B. afer* group exhibits considerable puparial morphological plasticity, we are referring to this species as *B. afer* sensu lato, as it may not be conspecific with the *B. afer* found in Europe, Africa and Australia.

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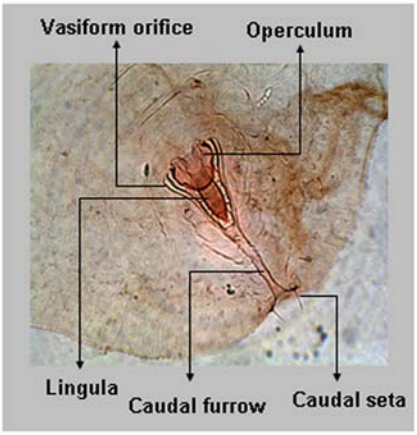
Table 1. Transmission efficiency of *Sweet potato chlorotic stunt virus* (SPCSV) with individual adults of *Bemisia afer* sensu lato and *B. tabaci* biotype B at two temperatures, using sweetpotato (*Ipomoea batatas*) plants cv. Costanero infected with SPCSV (single infection) and with SPCSV and *Sweet potato feathery mottle virus* (SPFMV) (double infection) as virus source plant and *Ipomoea nil* as test plant

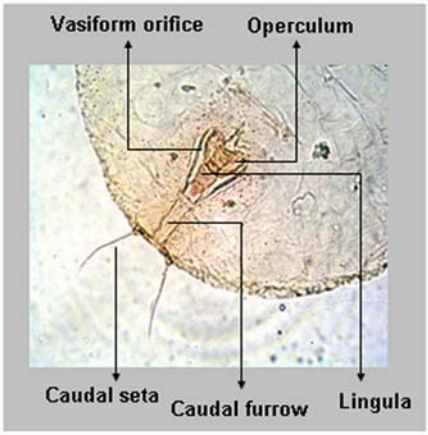
Whitefly species	Virus source	Temperature (°C)													
		20							25						
		Replications				Effi-ciency (%)	STD	SE	Replications				Effi-ciency (%)	STD	SE
		1	2	3	4				1	2	3	4			
B. afer sensu lato	SPCSV	3/23[a]	2/25	2/25	4/25	11.2[b]	0.96	0.24	4/25	2/25	2/25	5/25	13	1.5	0.38
	SPCSV+SPFMV	1/25	3/24	2/25	4/25	10.1	1.29	0.32	1/25	2/25	3/24	1/25	6.1	0.96	0.24
B. tabaci biotype B	SPCSV	4/25	2/25	0/25	1/25	7	1.71	0.46	3/25	2/25	0/25	3/25	8	1.41	0.35
	SPCSV+SPFMV	2/25	4/25	4/24	3/25	13.1	0.96	0.24	2/24	6/25	0/25	4/25	12.1	2.58	0.65

[a] Number of infected plants/number of total inoculated plant (experimental unit) per each replication.
[b] Percentage of total infected plants from total inoculated plants

Table 2. Statistical analysis by the test of equal or given proportion (n= 98-100) of the transmission of *Sweet potato chlorotic stunt virus* (SPCSV) from two virus sources by two whitefly species at two temperatures, using the R Statistical program (31)

Comparison of virus source (SPCSV vs. SPCSV+SPFMV)				
	at 20°C		**at 25°C**	
	χ^2	**p-value**	**χ^2**	**p-value**
SPCSV vs. SPCSV+SPFMV for *B. afer* sensu lato	6e-04	0.98	1.33	0.25
SPCSV vs. SPCSV+SPFMV for *B. tabaci* biotype B	1.47	0.23	0.53	0.46
Comparison of vector species (*B. afer* sensu lato vs. *B. tabaci* biotype B)				
	at 20°C		**at 25°C**	
	χ^2	**p-value**	**χ^2**	**p-value**
B. afer vs. *B. tabaci* from SPCSV+SPFMV source	0.19	0.66	0.93	0.33
B. afer vs. *B. tabaci* from SPCSV source	0.62	0.46	0.85	0.36
Comparison of temperatures (20°C vs. 25°C)				
	***B. afer* sensu lato**		***B. tabaci* biotype B**	
	χ^2	**p-value**	**χ^2**	**p-value**
20°C vs. 25°C with SPCSV+SPFMV source	0.26	0.61	0.00	1.00
20°C vs. 25°C with SPCSV source	0.03	0.87	0.00	1.00





Morphological character	*Bemisia afer*	*Bemisia tabaci* biotype B
Caudal setae	Always less than half the length of the vasiform orifice and often minute; highly variable among individuals	Always stout and usually as long or longer than vasiform orifice. Little variation among individuals
Vasiform orifice	Usually shorter than length of the caudal furrow, with sides often distinctly concave. The surface of vasiforme orifice underneath lingula is reticuled. The vasiform orifice is concave	Slightly longer than the length of the caudal furrow, with straight sides. The surface of vasiforme orifice underneath lingula in *B. tabaci* is not reticulated compared with *B. afer*. The orifice is triangular in shape.
Lingula	Long and narrow	Shorter and slightly wider than in *B. afer*
Caudal furrow	Similar in length to the vasiform orifice	Slightly shorter than the length of the vasiform orifice
Operculum	The operculum is triangular in shape	The operculum is oval in shape

Figure 1. Comparison of some morphological characters of *Bemisia tabaci* biotype B and *Bemisia afer* sensu lato puparia, used for taxonomic identification. Top, photographs of the posterior of the puparia. Bottom, descriptions of morphological characters of the puparia.

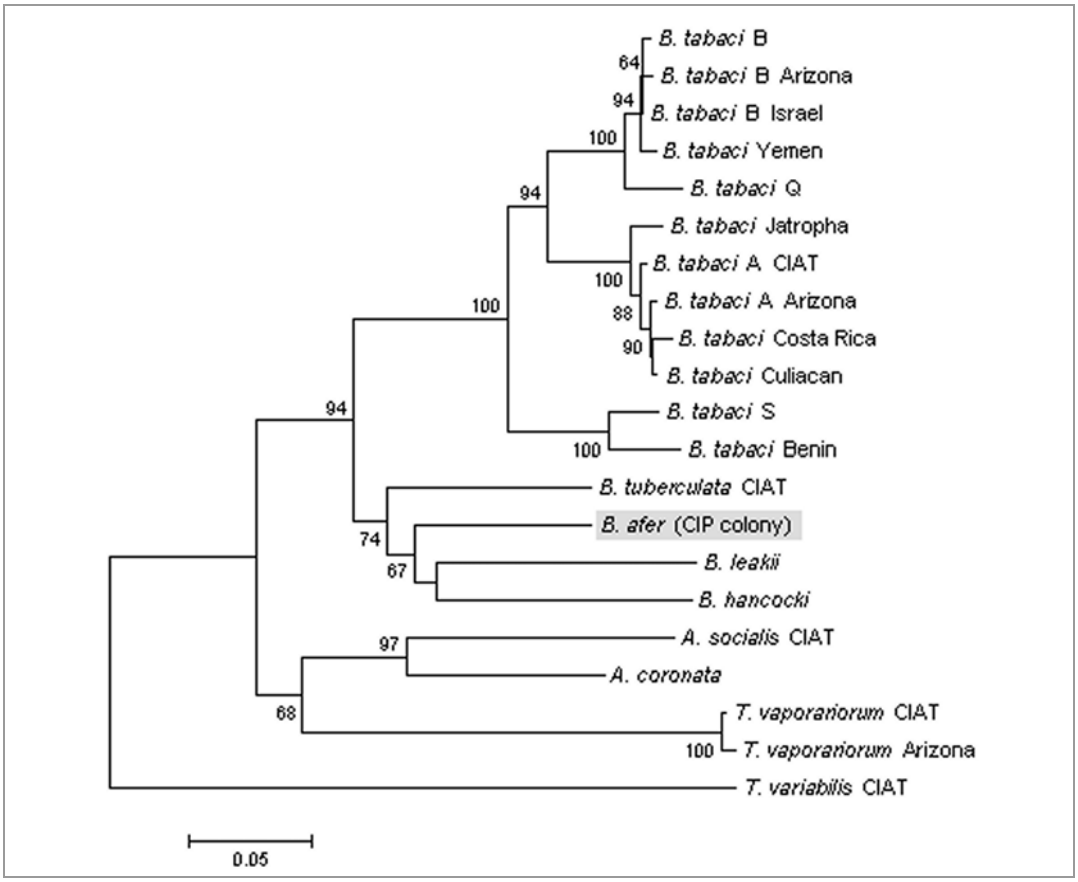


Figure 2. Phylogenetic tree calculated from alignments of nucleotides sequences on the mitochondrial 16S rDNA gene of *B. afer* sensu lato (GenBank accession FJ969473) with other whitefly species. The percentage of bootstrap support out of 2,000 replicates is given at each of the major nodes in the tree. The scale bar indicates Kimura nucleotide distances.

Effect of time of herbicide application and sweetpotato morpho-types on the effectiveness of herbicide on weeds

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Abstract

A field experiment was conducted at the National Root Crops Research Institute, Umudike in 2008 cropping season, to determine the time of herbicide application for weed control in sweetpotato production and also to determine the effect of sweetpotato morpho-types in weed control in sweet potato production. The treatments consisted of four times of herbicide application, namely: At planting, 14 days after planting, 21 days after planting, 28 days after planting, two sweetpotato morpho-types which include TIS 87/0087 (spread) TIS 8164 (erect), manual weeding and unweeded plot. The plot size was 6mx5m, weed control ratings in the plots were taken at 8 WAP on a scale of 0-10, where 0-4= poor weed species control: 4.1-7.9 = satisfactory control: 8.0-10.0 = excellent weed species control. Data collected were subjected to analysis of variance using GLM procedure of SAS and significant difference among means variable were tested, using fisher's least significant difference (LSD) at 5% alpha level. Application of herbicide at 14 days after planting significantly ($P<0.05$) controlled broad leaved weed species and also recorded the lowest weed density, compared to the other time of herbicide application. Combination of sweetpotato morpho-types TIS 87/0087 (spread) with time of application at 14 days after planting gave the highest significant total root yield (16.84 t/ha), compared to other treatment combinations. Application of herbicide at 14 DAP gave the highest total root yield (10.17 t/ha) which did not significantly differ with the yield (8.76 t/ha) obtained when manual weeding was employed and also had a comparative advantage over others as income was ₦128, 033.40.

Keywords: Time of herbicide Application, Sweetpotato morpho-types, Weeds.

Introduction

Sweetpotato *(Ipomoea batatas* [L.] Lam is a creeper of the convolvulaceae family; it is originated from Central America and is a widely grown important, staple food in most parts of tropical and sub-tropical region of the word. It ranks 7th among the world's major food crops. The crop has ceased to be a "back yard crop or "gap filler," survey reports in Nigeria show that production, Marketing and utilization have expanded in the last decade beyond its traditional central and riverine areas (Agboola, 1979) to almost all ecological zones in the country (Tewe *et al,* 2001).

Thus Nigeria production output has put Nigeria as the number one producer of sweet potato in Africa with annual output of 3.46 million metric tons (FAO, 2006). Globally, Nigeria is the second largest producer with China leading (106,197 million metric tons). The crop is grown for both human and animal consumption. Household income is supplemented by sales of the root tubers in local markets and urban dwellers. Its importance in starch, alcohol, livestock, pharmaceutical and textile industries cannot be over emphasized (Wolfe, 1992). The orange fleshed varieties with high β-carotene content have become very important in combating vitamin A deficiency especially in children. Despite the high agronomic potentials of sweet potato being a short duration crop 3-4 months that could be cropped more than once in the year, its production is fraught with a number of production constraints. Notable among them is weed competition.

Weed competition has been identified as a major production constraint in sweet potato production in Nigeria (Unamma, 1984). Yield losses caused by Un-controlled weed growth have been estimated at between 42 and 65% in Nigeria (Unamma, 1984). Weed competition with sweet potato may be reduced through many interventions such as timely herbicide application and use of sweet potato morpho-types.

The objectives of this study is to determine time of herbicide application for weed control in sweet potato production and also to assess the potentials of sweet potato morpho-types in checking weeds under sweet potato production.

Materials and methods

The experiment was conducted at the research farm of the National Root crops Research Institutes, Umudike, Nigeria ($05^{0}.29^{0}$N, $07^{0}.33$'E and 122m above sea level), in the 2008 cropping season. The soil was sandy loam, the plot size was 6mx5m. Each treatment was replicated three times using a randomized complete block design. The land was cleared, ploughed, harrowed and ridged before planting. Sweetpotato varieties 87/0087 and 8164 were planted during the second week of June, 2008 spaced 100cm x 30cm along the crest of the ridges at a density of 33,333 plants ha^{-1} , compound fertilizer (15:15:15 NPK) was applied to all the plots at 4 weeks after planting (WAP) at the rate of 400kg/ha. Application of herbicides was done four times namely; At planting, 14 days after planting (DAP), 21 days after planting (DAP) and 28 days after planting and two sweetpotato morpho-types TIS 87/0087 (spread) and TIS 8164 (erect), manual weeding at 4+6+8 weeks after planting (WAP) and unweeded plot. A mixture of Atrazine/metolchlor (primextra gold),fluazifopbutyl (fusilade) at 1.5 + 1.0kg ai /ha. At 8 weeks after planting, plots were visually scored for weed control by two independent assessors on a rating scale of 0-10, with 0, representing no weed control and 10 indicating complete control of weeds. Seven and half was regarded as an acceptable level of weed control. Sweetpotato yield was assessed at harvest (4 months after planting). Data collected were subjected to analysis of variance using the GLM procedure of SAS and significant differences among means were separated using Fisher's least significant difference (F-LSD) at 5% level of probability. Economic assessment of the weed control treatment was conducted using labour productivity index, available yield indexes as well as return per naira investment (Ezedinma *et al,* 2006).

Result and discussion

The results of the effects of sweetpotato morpho-types and time of herbicide application on weed types, weed density and rating are presented in Table1. Sweetpotato morpho-types showed no significant ($P>0.05$) effects on broad leaved weed, sedges, weed density and weed rating. However significant ($P<0.05$) effect was observed on gasses. TIS 87/0087 (Spread) suppressed grasses more than TIS 8164 (erect). This results indicated that the sweetpotato canopy of TIS 87/0087 emerge may have a smothering effect on grass weeds. Akobundu (1980) reported that sweetpotato (spreading type) is a weed suppressant in intercropping system. Sweetpotato morpho-types did not show any significant ($P>0.05$) effect on weed rating. However, the two varieties gave ratings greater than 7.5. Effect of time of herbicide application on weed rating showed significant difference. All the treatments performed better (7.5-9.0) than unweeded (4.8). Herbicide application at 14 DAP which is a candidate for recommendation also sustained a weed rating of 8.63 which did not differ significantly with the weed rating of 9.9 of manual weeding. Sweetpotato morpho-types and time of herbicide application interaction significantly ($P<0.05$) influenced total root yield (t/ha) (Table 2). Application of herbicide at 14 DAP gave the highest total root yield (10.17 t/ha) which did not significantly differ with the yield (8.76 t/ha) obtained when manual weeding was employed. Effect of sweetpotato morpho-type showed that TIS 87/0087 significantly ($p<0.05$) gave better total root yield (8.05 t/ha) than TIS 8164 (erect). TIS 87/0087 (spread) in combination with time of herbicide application of 14 DAP gave the highest significant total root yield (16.84 t/ha) compared to other treatment combinations.

Summary of economics of time herbicides application and sweetpotato mropho-types (Table 3), showed that TIS 87/0087 spread type combine with time of application at 14 days after planting (DAP) gave the highest return per naira investment (₦2.19), also had a comparative advantages over other as in income was ₦128, 033.40.

Table 1. Effect of time of herbicides application and Sweetpotato morpho-types on weed types control, weed rating and density in 2008 at Umudike

Variety	Weed type Broad leaves	(No/m^2) Grasses	Sedges	Weed density(No/m^2)	Weed rating at 8WAP
TIS 87/0087	75.40	17.60	6.90	96.00	7.7
TIS 8164	78.70	17.72	5.80	103.00	8.0
$LSD_{0.05}$	27.77ns	7.30	4.61ns	41.8ns	0.88ns
Time of herbicide application					
At planting	88.50	32.70	6.40	107	8.0
14 DAP	44.00	14.80	6.40	65.00	8.63
21 DAP	75.80	14.10	12.80	98.00	8.3
28 DAP	113.90	1.30	1.90	125.00	7.5
Manual weeding	2.90	2.90	1.50	8.00	9.9
Unweeded	137.40	40.70	9.00	194.00	4.8
$LSD_{0.05}$	48.09*	29.96*	7.98ns	72.4*	1.53*

Table2. Effect of time of herbicides application and Sweetpotato morpho-types on total root yield (t/ha) in 2008 at Umudike

Variety	Time of herbicides		Application 21 DAP	28 DAP	Manual weeding	Unweeded	Variety mean
	At planting	14 DAP					
TIS 87/0087	4.41	16.84	10.08	4.70	10.77	1.52	8.05
TIS 8164	3.96	3.49	5.67	9.04	6.74	6.26	5.86
Time of application							
Mean	4.18	10.17	7.87	6.87	8.76	3.89	
$LSD_{0.05}$							
Variety	0.97*						
Time of application	1.68*						
Variety xTime of application	2.37*						

Table 3. Summary of economics of time of herbicide application and sweetpotato morpho-types at Umudike

Treatments	Yield (t/ha)	Total costs #/ha	Gross return #/ha	Net return (#/ha)	Return/ # investment	Labour productivity t/md
TIS 87/0087x@planting	4.41	107726.6	61740	-45986.6	-1.74	0.036
TIS 87/0087x 14DAP	16.84	107726.6	235760	128033.4	2.19	0.138
TIS 87/0087x21 DAP	10.08	107726.6	141120	33393.4	1.34	0.082
TIS 87/0087x 28 DAP	4.70	107726.6	65800	-41926.6	-1.64	0.038
TIS 87/0087 x weed free	10.77	112296.6	150780	38483.4	1.34	0.062
TIS 87/0087x unweeded	1.52	101696.6	21280	-86446.6	-4.78	0.013
TIS 8164x14 DAP	3.49	107726.6	48860	-58866.6	-2.20	0.029
TIS 8164x 21 DAP	5.67	107726.6	79380	-28346.6	-1.36	0.046
TIS 8164x 28 DAP	9.04	107726.6	126560	18833.4	1.17	0.074
TIS 8164x weed free	6.74	115696.6	94360	-21336.6	-1.23	0.035
TIS 8164 x unweeded	6.26	101696.6	87640	-14056.6	-1.16	0.052
TIS 8164 x @planting	3.96	107726.6	55440	-52286.6	-1.94	0.032

Conclusion

Sweetpotato morpho-type, TIS 87/0087 spread combine with time of application at 14 days after planting (DAP) controlled grass weed and broad leaved weed species effectively and also gave the farmer the highest total root yield with an income of #128,033.40 when compared other treatments.

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Organic management of corm borer *Haplosonyx chalybaeus* (Hope) - A serious insect pest of Colocasia in north eastern India

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Abstract

The Corm borer *Haplosonyx chalybaeus (*Hope*)* (Coleoptera: Chrysomelidae) is a major insect pest of colocasia (*Colocasia esculenta*) in North Eastern Region of India. *H. chalybaeus* adults cause 50-70% damage to foliage and 70-90% to corms. Adult beetles hide in the leaf sheath during night and 6-7 beetles were recorded per plant. Tribal farmers in the region control the insect by hand-picking the adults. *Beauveria bassiana* found to infect *H. chalybaeus* at a dose of $1x10^9$ spores/ml and result in mortality of both adults and grubs upto 95.4 and 81.2% respectively *in vitro* and 72.6 and 50.0% in field conditions.Adults of *H. chalybaeus* were infected by entomopathogenic nematode *Heterorhabditis indica* and *Steinernema carpocapsae*, death occurs on 5th day after inoculation and emergence of nematodes after 7 days. *H. indica* is more effective than *S. carpocapsae* in killing the grubs of corm borer. *S. carpocapsae* killed 50% of the grubs within 72 hr whereas, *H. indica* produced a mortality rate of 95% within the same duration and the harvest rate from the dead cadavers was also more. The lethal doses of *H. indica* for the corm borer was 25 *Ijs* (Infective juveniles) for the second instar, 40 for the 3rd, 400 for the 4th and 5th instar grubs. The restricted movement of the grubs of *H. chalybaeus* in taro corms and the moist protected environment within corm suggest that these conditions might be conducive to the use of *S. carpocapsae* and *H. indica* for the management of *H. chalybaeus*.

Keywords: Colocasia esculenta, Corm borer, Haplosonyx chalybaeus, Beauveria bassiana, Heterorhabditis indica and Steinernema carpocapsae.

Introduction

Colocasia (*Colocasia esculenta*) is grown extensively in the North Eastern Hill (NEH) Region of India in plains and hill slopes as a rainfed crop during April to September. Many insect pests infest colocasia, while the corm borer *Haplosonyx chalybaeus* (Coleoptera: Chrysomelidae) is a regular and endemic pest causing 50-70% 50-70% damage to foliage and 70-90% to corms, which ultimately result in serious economic losses to the farmers of the region. *H. chalybaeus* is a new record from the NEH region and was not reported from other parts of India.

Organic farming was introduced in several states of NEH region by the respective state governments in the region. In view of these policy changes in the agriculture sector, a need has arisen to manage the insect pests in organic manner so as to prevent the accumulation of pesticide residues and production of clean and synthetic pesticide residues and production of clean and safe corms or any other agricultural produce. Hence, a study was undertaken to understand the biology of the *H. chalybaeus* on colocasia and the management of the borer with the use of entomopathogenic fungus *Beauveria bassiana* and entomopathogenic nematodes (EPN) *Heterorhabditis indica* and *Steinernema carpocapsae*.

Materials and methods

The adults and grubs of *H. chalybaeus* infected by *B. bassiana* were collected from field and incubated for the isolation of indigenous strains of the fungus. Both these stages were bioassayed to test the efficiency of *B. bassiana*, which was mass multiplied on cowpea grains (Sharma et al., 1999). Different doses of the *B. bassiana* were used to test the mortality of the *H. chalybaeus* viz. 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1} and for each dose 5 replications and 30 insects were used.

A mixed culture of EPNs isolated from other insect pests like rice leaf folder *Cnaphalocrocis medinalis* and maize cob borer *Steinachroia elongella* were used for the study. These EPNs were cultured on wax moth, *Galleria mellonella*, which was further, reared on artificial diet. Different cultures of the EPNs *S. carpocapsae* and *H. indica* were also tested for their efficacy on the mortality of the adult and grubs of the corm borer. Petridish assay was used for the bio-efficacy. Whatman No. 1 filter paper was kept in the lid of a 10 cm petri-dish. The infective juveniles (*ijs*) spread evenly over the filter paper in 1 ml of distilled water. One grub or adult was added and covered with inverted petri-dish lid and kept in a plastic bag to conserve moisture and incubated at 22^{0}C. The mortality was recorded at 24 h intervals.

Results and discussion

Haplosonyx is a genus earlier known as *Aplosonyx*. The adults of *H. chalybaeus* are bright metallic with blue and pink colour (Fig 1). The two colours were found both in male and female beetles. The head and thorax of *H. chalybaeus* are orange coloured and the abdomen is in blue to deep pink to purple in colour. However, the ventral side is yellowish to orange in colour. Sexual dimorphism is present. The *H. chalybaeus* infest both the leaf sheath and the corm. The adult beetles feed on the leaves by making cicular holes of different sizes generally half to one-inch size. Similar damage pattern was reported in *Aplosonyx amorphophallus* a pest on amorphophallus in Indonesia (Mohammedsaid, 2008). The holes are more on the edges of the leaf lamina than inside. The damaged plants wither, wilt and become yellow and emit foul smell. A maximum of 70-80 grubs were found per plant. The beetles during night efficiently hide and survive in the leaf sheaths at ground level. 2-3 beetles were found in each leaf sheath and 6-7 beetles/plant. The water present in the leaf sheath was also part of the nutritional source for these beetles. The local farmers collect the beetles and destroy them mechanically is one of the important management strategy for *H. chalybaeus*.

Figure 1. Adult *Haplosonyx chalybaeus* feeding colocasia leaf

H. chalybeaus was found to be highly susceptible to *B. bassiana*. The fungus at a dose of 1x10^{9} spores ml^{-1} was found to infect and result in mortality of both adults and grubs upto 95.4 and 81.2% respectively in vitro and 72.6 and 50.0% in field conditions. The beetles that were fed with taro leaves sprayed with *B. bassiana* died within 8-10 days and the grubs were killed within 3-4 days. In both the cases, about 90% mortality of the pest was recorded.

All the adult beeltes of *H. chalybaeus* were infected by the two strains of EPN *Heterorhabditis indica* and *Steinernema carpocapsae*, death occurs on 5th DAI and immergence of nematodes after 7 days. The entomopathogenic nematode *H. indica* is more effective than *S. carpocapsae* in killing the grubs of taro corm borer. *S. carpocapsae* killed 50% of the grubs within 72 hr whereas the *H. indica* produced a mortality rate of 95% within the same duration and the harvest rate from the dead cadavers was also more. The lethal doses of *H. indica* for the corm borer was 25 *Ijs* (Infective juveniles) for the second instars, 40 for the 3rd, 400 for the 4th and 5th instar grubs. These rhabditoid nematodes, found typically in soil, have been utilized successfully as microbial insecticides in control programmes for a variety of cryptic pests including the possibility to use against sweet potato weevil *C. formicarius*. The restricted movement of the grubs of *H. chalybaeus* in taro corms and the moist protected environment within corm suggest that these conditions might be conducive to the use of *S. carpocapsae* and *H. indica* for the management of the taro corm borer.

The predictable climatic conditions, the availability of high moisture with low temperatures during the cultivation of colocasia in the NEH region are suggested to be suitable for effective use of *B. bassiana* and *H. indica* together with the resistant or tolerant varieties could successfully manage the *H. chalybaeus*.

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ILCYM- Insect life cycle modeling: software for developing temperature-based insect phenology models with applications for regional and global pest risk assessments and mapping

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Abstract

Phenology models for insect pest species based on temperature are important analytical tools for predicting, evaluating, and understanding the dynamics of populations in ecosystems under a variety of environmental conditions, and more recently are also used in phytosanitary risk assessments. CIP had developed a temperature-driven, process-based full life-cycle phenology model for the potato tuber moth that predicted well life-table parameters for the pest in different agroecological zones. The model was further linked with geographic information systems (GIS) and atmospheric temperature databases, allowing simulation of pest risk indices on a worldwide scale. The approach used for developing and using a pest model can be principally adapted for other insect species. CIP further developed ILCYM, an open-source software package, which facilitates the development of phenology models on the basis of specific temperature-controlled experimental data by determining functions for temperature-driven processes in an insect species, i.e. development, mortality, reproduction, etc. compiling them into an overall pest model. A cohort up-dating algorithm and rate summation approach is used for simulating multidimensional age and stage structured populations. Tools for model validation are implemented. Further, ILCYM allows linking the models to a GIS environment; three generic risk indices (Establishment Index, Generation Index, and Activity Index) can be visualized. Life-table parameters can be forecasted over time for single locations. The paper presents the new up-dated version of ILCYM (version 2) and discusses how it might be used for supporting country-specific pest risk assessments, global climate change adaptation planning and improving pest management strategies.

Keywords: Insect Pests, Insect Pest Management, Temperature-dependency, Simulation Models, Computer-aided Decision Support, Potato Tuber Moth

Introduction

The distribution of insects and other poikilothermic animals is largely determined by climate. Insects cannot internally regulate their own temperature (exothermic organisms) and their development depends on the temperature to which they are exposed to in the environment. They require a certain amount of heat to develop from one developmental stage to another in their life-cycle (Uvarov 1931, Andrewartha and Birch 1955). Because of yearly variations in weather, calendar dates are faulty parameters for predicting pest population growth, and outbreaks and making management decisions. However, measuring the amount of heat accumulated over time provides a valuable physiological time scale that is biologically highly accurate. Phenology models are predicting the time of events in an organisms' development. Such models for insect pests based on temperature are important analytical tools for evaluating, understanding and predicting the dynamics of pest populations in ecosystems under a variety of environmental conditions, and more recently are also used in phytosanitary risk assessments (Baker 1991, Jarvis and Baker 2001a, b).

CIP has developed a temperature-driven phenology model for the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechidae) that predicted well life-table parameters for different agroecological zones (Sporleder *et al.* 2004); validated through field and laboratory data (Keller 2003). It was used to predict the establishment risk and potential pest activity in specific agroecologies according to temperature records (Kroschel and Sporleder 2006). Linked with geographic information systems (GIS) and atmospheric temperature the model allows simulating these risk indices on a worldwide scale or using it to predict future changes of these indices due to global warming (Sporleder *et al.* 2007, Sporleder *et al.* 2008). Possible applications of these models are manifold.

The approach used to develop and implement the *P. operculella* model can be principally used for other insect species. Therefore, CIP developed Insect Life Cycle Modeling software (ILCYM – version 2.0) to facilitate the development of further pest insect phenology models and provide analytical tools for studying pest population ecology. ILCYM software consists of two main modules: The first module (which can be called the "model builder") facilitates researchers to develop insect pest phenology models based on temperature experiment data of a specific pest. This module also provides tools to analyze insect life-table and to validate developed models. The second module implements the phenology model in a GIS environment that allows for spatial simulation of pest activities ("pest risk mapping").

The purpose of ILCYM

The objective of ILCYM software is to provide an open-source computer aided tool, especially for researchers in developing countries (p.e. entomologists, ecologists, researchers that are involved in integrated pest management (IPM) of agricultural and forest insect pests) that facilitate the development of generic phenology models based on temperature using advanced modeling techniques without having the mathematical knowledge, being experts in the field, or spending the time that is normally required to develop, implement and program a phenology model. We believe that the application of phenology models will lead to a better understanding of pests' biology and ecology and in the long-term also support decision making in pest management programs. Such models might provide important information on quantitative pest population biology and "Pest risk mapping" provides important information in pest risk analysis (PRA). The present paper explains in briefly how the developed modeling approach works and how to handle and use the software package. Because ILCYM interactively leads the user through the steps of developing a PPM, or to conduct spatial simulations with a developed pest model, it is especially helpful for users who don't want to learn programming languages before starting pest population modeling, and users who do not want to study mathematics before starting modeling.

The model builder

The model builder compiles full life cycle phenology models for insects species based on statistical analysis of experimental data, i.e. life-table or cohort-based development data, assessed over a range of constant temperatures. Different types of data might be analyzed; *a)* life-table data in which development time, survival, and reproduction are monitored for a group of individuals of the species of interest from egg to egg (i.e. fresh-laid eggs of the same age until the last egg of the offspring from these individuals is oviposited) - this data represent the full life history for a group of individuals of the population at a given temperature, or *b)* the data can be based on cohort studies. In the latter, a group of individuals of the same stage and age is monitored from the beginning to the end of a given life stage, i.e. fresh eggs to emerging larvae, or neonate larvae to pupation, etc. These experiments need to be conducted over a range of constant temperatures covering the temperature in which the insect species might develop; p.e. from 10°C to 30°C in 5°C intervals. If the female rate in the specie under study varies either with temperature to which the parent generation was exposed to or changes with female age, all offspring-eggs need to be reared to the adults' stage for determination of sex.

If such data are generated, ILCYM provides a set of functions (predefined functions) that can be fitted to the data for describing variation in development times between the individuals of the population, temperature-dependency of median development times and survival in a given immature life stage, temperature-dependent adult senescence, and reproduction. The reproduction model is compiled assembling different functions (p.e. temperature-dependent total reproduction per female, age-dependent relative oviposition frequency) depending on how female rates are influenced by temperature and female age. The "model builder" interactively facilitates choosing the best-fitting functions for describing these temperature-driven processes in the insect specie's development. These functions are model components, henceforth called "sub models" of the overall generic phenology model, or "modules" (the term "modules" is used because a sub-model can be replaced by another function that might describe better the specific process in the system) which are automatically implemented in the overall pest phenology model. The ILCYM "model builder" focuses strongly on "parameter determination" for models. The program produces outputs (statistics and graphs) that help to find the best parameters for a specific species on a statistical basis; parameters are stored in a database so that a model can be concluded part-by-part until its finalization.

Frequency distributions of insect development times are usually skewed toward the longer times and it is assumed that the intrinsic distributions of development times of an insect at different temperatures are of the same shape, i.e. the distributions at different temperatures will fall on top of each other when 'normalized' by a selected value such as the mean or median of each distribution ('same shape property', for further information on this theory see (Sharpe *et al.* 1977b, Curry *et al.* 1978a). In ILCYM three different probability density functions (PDF) of the general linear model type (GLM) are fitted to observed accumulated development frequencies in a parallel line approach using ln-development times as the explanatory variable. Fitted models (GLM) are the normal distribution, the logit model, and the complementary log-log (CLL) model having the following linearized forms:

Normal distribution model: $$F(x) = \Phi\left(a_i + b \ln x\right) \quad [1]$$

Logit model: $$F(x) = \frac{1}{1 + \exp\left(-\left(a_i + b \ln x\right)\right)} \quad [2]$$

CLL model: $$F(x) = 1 - \exp\left(-\exp\left(a_i + b \ln x\right)\right) \quad [3]$$

where *F(x)* is the probability to complete development at time *x*, and a_i and *b* are the parameters to be estimated; *b* is the commune slope of the regression model representing the dispersion of development time in the life stage and a_i's are the intercepts corresponding with temperatures *i*. ILCYM selects the best-fitting model by examining well known goodness of fit indicators, Akaike's Information Criterion (AIC) (Akaike 1973) and the Model Selection Criterion (MSC) (Scientist 1995). Median development times (T_{50}'s) for each constant temperature, *i*, are calculated depending on the selected GLM using the following formulae:

If the normal distribution or logit model was selected:

$$T_{50i} = \exp\left(\frac{-a_i}{b}\right) \quad [4]$$

If the CLL model was selected:

$$T_{50i} = \exp\left(\frac{-\ln\left(-\ln\left[0.5\right]\right) - a_i}{b}\right) \quad [5]$$

Confidence intervals (95%) for the ln(T_{50}) values are calculated by using the following formula:

$$CI_{95i} = \ln T_{50i} \pm t_{95\%} - \frac{1}{b}\sqrt{parameter} \quad [6]$$

in which $t_{95\%}$ is the value of the *t*-distribution corresponding to significance levels of 0.05 and the corresponding number of degrees of freedom, *b* is the parameter (slope) derived from the selected GLM (equations 1-3), and *parameter* is the number of parameters (a_i's and *b*) estimated in the model.

Temperature-dependent development of insects from one stage to another does not follow a linear relationship and hence linear models are often not satisfactory to be used in phenology models, especially when the phenology model is likely to be applied for regions where the temperature fluctuates frequently below or above the temperature where development is in the linear range. Therefore, for describing the relationship between temperature and the development rate in a given insect life stage ILCYM provides a set of functions, including the modified version of the Sharpe & DeMichele model (Sharpe and DeMichele 1977b, Schoolfield *et al.* 1981) and its derivates (Ikemoto 2005b), Logan's models (Logan *et al.* 1976, Logan *et al.* 1979, Logan 1988), Stinner *et al.* model (Stinner *et al.* 1975), and other models (Taylor 1981, Lamb *et al.* 1984, Lamb 1992, Lactin *et al.* 1995, Brière *et al.* 1999) that have been employed successfully for many insect species for this purpose. Survivorship in immature life stages is calculated from the relative frequency of survivors. Different functions, including polynomial models, can be fitted by regression to describe the relation between mortality rate and temperature for each life stage. Users might choose the most appropriate model to be implemented in the overall model by evaluating goodness-of-fit criterions and residuals. Daily reproduction is modeled in ILCYM assembling two or more functions. Mean survival time of adults is generally determined for both sexes separately. As for immature

life stages, the inverse of the female life span (days^{-1}) is plotted against temperature and a model similar to the functions used for describing temperature-dependent development employed to describe adult senescence. Senescence rate summation is then used to express the physiological age of females. Polynomial regression models are applied to find the relation between total oviposition per female and temperature. The cumulative proportion of eggs produced is plotted against female age expressed as normalized time, i.e. age in days divided by the median survival time, and different non-linear models can be fitted to the data to describe age-specific fecundity frequencies.

Once all functions required for describing the specie's full life cycle are elected ILCYM compiles the overall model automatically. Therefore users do not need the knowledge for programming complex insect life cycle models. An example for an overall model, which was used for modeling potato tuber moth populations, is given in Figure 3. ILCYM uses a rate summation and cohort up-dating approach based on scheme proposed by Curry *et al.* (1978b) that was further described by Wagner *et al.* (1985) and Logan (1988). In published articles there is not so much discussion on including temperature-induced mortality in immature life stages and recruitment. Both are necessary for more realistic simulation and both are included in ILCYM (for further information on these merits see the chapters about "immature mortality" and "recruitment model"). The population is structured in different life stages, which are represented in a so called "box car train (i.e. eggs [E], larvae [L], pupae [P], and adults [Af = adult females]) and into groups of individuals of the same age (i.e. cohorts), which are represented in a "box car" within each life stage (p.e. E_0, E_1, E_2… , E_x; see Figure 3). Users can run simulations for one or multiple generations either in a deterministic or stochastic approach depending on the simulation purpose. Deterministic simulation is more useful for spatial simulations since it requires less computer time. For deterministic simulation, each "box car" contains three numbers, i.e. the age of the individuals in this group in days, the physiological age determined through development rate accumulation and the number of individuals in this age-group. When running the model each "box car" is up-dated in daily intervals, i.e. the value 1 is added to the age in days, the development rate calculated for the last 24-hour interval is added to the physiological age (rate summation) and the number of individuals in the cohort is minimized by the simulated daily mortality and the proportion which develops into the next life stage according to the temperature-dependent mortality function and age-dependent development distribution function, respectively.

For stochastic simulation, the model simulates development, mortality and reproduction of 100 individual. For each individual, simulation started from the egg stage (fresh egg). Development rates are accumulated as in deterministic modeling. Equation 1, 2 or 3, depending on the selected model, is used to calculate the probability that the individual develop from one stage to the next according to its physiological age. A random value between 0 and 1 is generated for each individual and used to determine the day when this particular insect develops to the next stage; i.e. the day when *F(x)* exceeded the random value. A second random value between 0 and 1 is generated to simulate survival of the individual; i.e. when the random value exceeded the value calculated by the mortality function included for describing temperature-dependent mortality the insects is considered to survive. This process is repeated for each immature life stage. The sex of an emerging adult is determined through generation of a further random value between 0 and 1; in case of a fixed female rate of 0.5 a random value <0.5 produced a female and a value >0.5 produced a male. The senescence rate of females is accumulated (=physiological age) and the relative oviposition frequency calculated for each day post emergence using the model determined to describe age-dependent oviposition frequencies. The resulting value is multiplied by the number of total oviposition resulting from the model selected describing temperature-depending total oviposition per female (no random variable is included as a stochastic component for variability in oviposition numbers per female). Adults died in the simulation when the physiological age exceeded a value that marks the end of the oviposition period.

For both, deterministic or stochastic simulation, a 15 min time step length is implemented in ILCYM. Model calculations are based on daily maximum and minimum air temperatures. Temperature in each 15 min time step is calculated by using a cosine function for half-day temperature predictions. The equation for the first half-day is:

$$T_i = \frac{(Max - Min)}{2} \times \cos\left(\frac{\pi \times (i + 0.5)}{48}\right) + \frac{(Min + Max)}{2} \qquad [7]$$

in which T_i is the temperature (in ºC) of time step i (i = 1, 2, 3, …48), and *Min* and *Max* are daily minimum and maximum temperatures. The calculation is then repeated to obtain T_i for the second half-day employing the minimum temperature, *Min*, of the following day in the equation.

Both deterministic and stochastic simulation over one generation produces a life-table for a given temperature (user input). Life-table parameters, i.e. net reproduction rate (R_0), mean generation time (T), intrinsic rate of natural increase (r_m), finite rate of increase (λ), and doubling time (Dt), can be calculated in ILCYM from modeling outputs as described in standard biological texts (Begon *et al.* 1990, Southwood and Henderson 2000). ILCYM determines the intrinsic rate of increase, r_m, employing the equation of Lotka (Birch 1948):

$$1 = \sum \exp(r_m \cdot x) \cdot l_x \cdot m_x \qquad [8]$$

in which x = age in days (including immature stages), l_x = age-specific survival (including immature mortality) and m_x = age-specific number of female offspring.

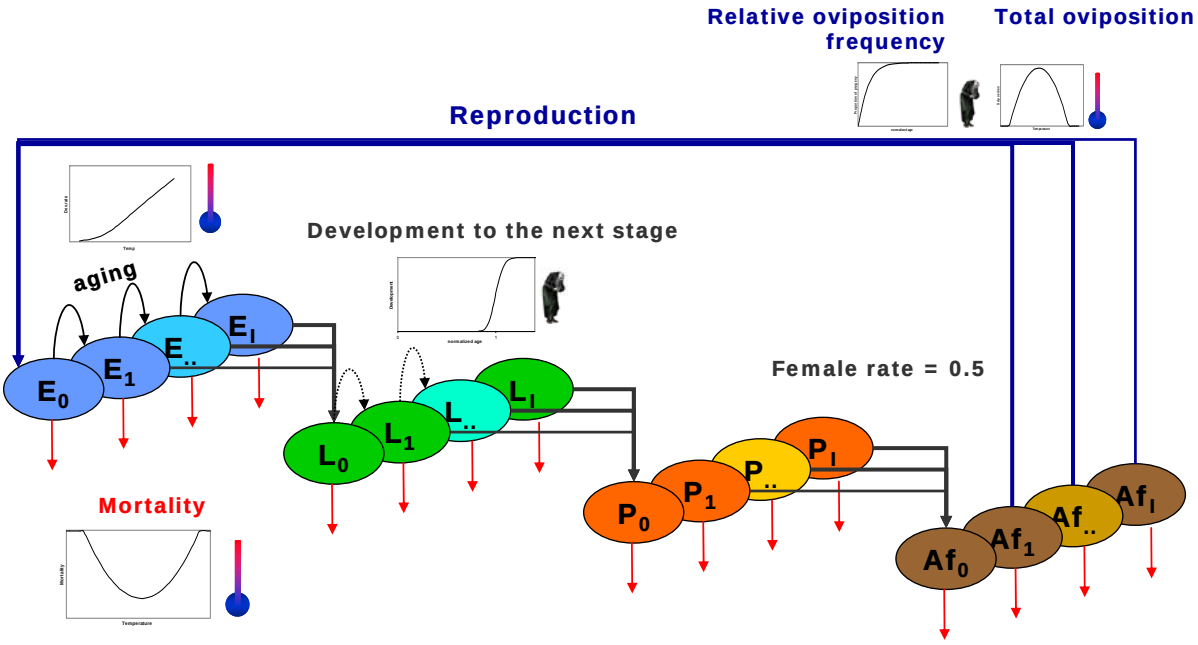

Figure 3. An schematic picture of an ILCYM temperature-based phenology/population model using a rate summation and cohort up-dating approach. See text for verbal description of the model.

Model validation

ILCYM provide several tools for validating developed phenology models. Repetitions of stochastic life table simulations for a given temperature allow determining confidence intervals for life table parameter means. Life tables of the same species established under fluctuating temperature can be used for comparing results (life table parameters) from real and simulated life tables. Individual functions (sub-models) used in the overall model can be examined and evaluated.

The environment for pest risk assessments

Once a full life-cycle pest model for a pest under study is completed the model can be used to simulate the species population growth potential spatially or for a given location over time using the GIS environment implemented in ILCYM. Three spatially referenced pest risk indices displaying the risk of establishment, numbers of generations per year, and an activity index can be computed spatially (see example in Figure 4). The simulation is based on daily maximum and minimum temperatures as inputs. For global or regional scale

simulations data based on WorldClim are preinstalled in ILCYM's pest risk modeling module. WorldClim is a set of global climate layers (grids) with a spatial resolution of 30 arc seconds (~1 km^2, downloadable at http://www.worldclim.org) and described in Hijmans *et al.* (2005). WordClim provides monthly aggregated climate variables. Because this aggregation might raises substantial problems of temporal scale, daily maximum and minimum temperatures are interpolated for each grid before simulation. For predicting the species' responses to climate change, similar maps can be generated for a scenarios of climate change using an atmospheric general circulation model (GCM) which is included in ILCYM. The latter data are described by Govindasamy *et al.* (2003) and forecast global climate for the year 2050. Specific spots (grids) in the map might be selected and the pest species population growth parameters can be simulated over time. For regional forecast at higher spatial resolution or higher data accuracy users are able load own temperature data, p.e. real data measured by meteorological stations for simulations over time or data sets from local meteorological Institutes for a given area for spatial simulations.

The approach for "risk mapping", i.e simulating the risk of pest establishment and expansion, in ILCYM differs from the 'match climate' approach in which climate match functions seek out the potential exploitation of a non-indigenous invasive species to new areas by comparing the long-term meteorological data for each of a selected location where the species is absent with the location of origin or locations where the species prevails. In IlCYM the risk maps are simulated using the pests' process-based phenology model that describes the basic physiological principals of insect species' growth, i.e. its development, survival and reproduction.

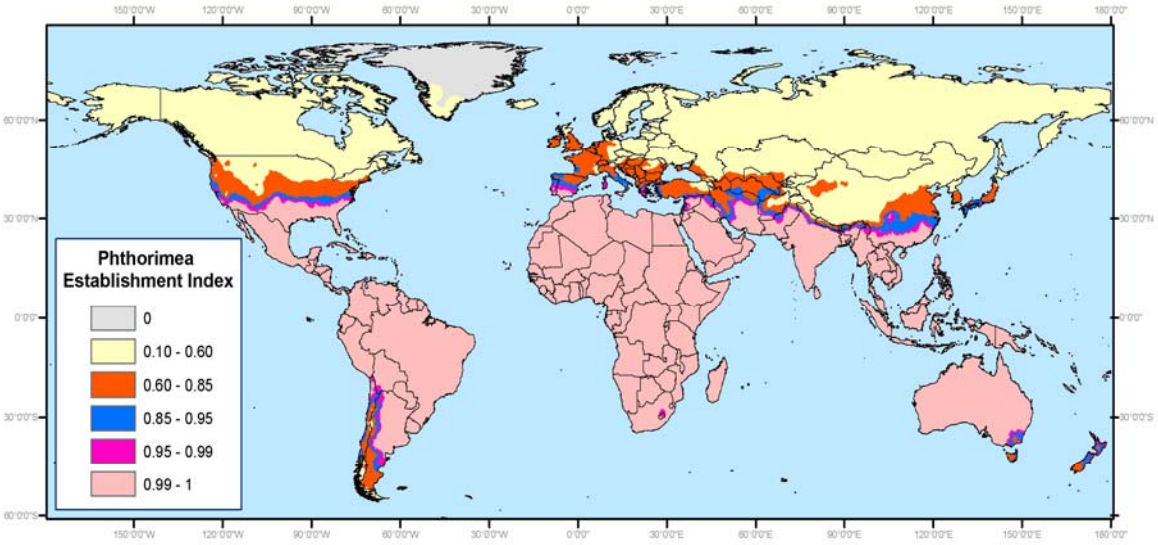

Figure 4. Survival index of *P. operculella* simulated globally using ILCYM. The index is 1 where all immature life-stages develop and survive throughout the year.

Conclusion

ILCYM provides advanced insect modeling techniques and analysis tool that can be used efficiently by NARS scientists which are not experts in this field. The program interactively leads the user through the steps of developing a pest phenology/population model and aids conducting spatial simulations. Users do not need to learn programming languages or other specific mathematical knowledge to develop and design the model (abstraction); however, ILCYM restricts the modeler to certain predefined modeling designs and might not provide solutions for every problem.

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Soluble chitosan applied as potato seed tuber treatment and foliar sprays under subtropical and temperate conditions - effect on yield parameters and disease incidence

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Abstract

Field plot trials were conducted with soluble chitosan in subtropical conditions (Remedios, Central Cuba) and temperate conditions (Rostock, Northern Germany, Baltic coast) in the years 2003 – 2005. Chitosan was applied as seed tuber treatment, foliar spray and combination of seed tuber and foliar treatment. In Remedios infection with early blight (*Alternaria solani*) was assessed, in Rostock infection with late blight (*Phytophthora infestans*).Under subtropical conditions chitosan application improved yield parameters compared to the untreated control and also to fungicide treatments, even where early blight incidence was not reduced. In temperate conditions chitosan application could reduce late blight incidence compared to the untreated control, depending on concentration and application mode. The combination of seed tuber treatment and foliar application had the most stable effects over the three trial years. Seed treatment and foliar treatment alone were less reliable in their effects on disease incidence. Decreased late blight scores did not result in significantly improved tuber yields, which were comparable to the untreated control.

Keywords: chitosan, plant growth promoter, elicitor, potato, late blight, early blight.

Introduction

Soluble chitosan is a natural product from the chitin of crab shells of polymeric structure, made water soluble by alkaline or enzymatic deacetylisation. Chitosan has been shown to trigger physiological mechanisms in plants, which are considered to be connected with disease resistance (Roby *et al.* 1987, Hadwinger *et al.* 1994, Tiuterev 1996, Kauss 1997, Hadwiger 1999, Stange & McDonald 1999, O'Herlihy et al. 2003). After artificial inoculation with *Pytophthora infestans* zoospores the progress of late blight symptoms was significantly slowed in chitosan-treated plants (Kowalski et al. 2005). Chitinase activity elicited by chitosan is considered similar to that triggered by fungal infection (Chen *et al.* 1999 ct. in O'Herlihy *et al.* 2003). Foliar chitosan application in greenhouse plants of potato significantly decreased water loss after cutting (Kowalski et al. 2006b) and significantly decreased intercellular peroxidase activity as a marker for oxidative stress, while the content of phenolic substances and proteins remained (Kowalski et al. 2005).

Positive Effects of chitosan treatments yield and plant health have been described for tomato (Benhamou & Theriault 1992, Benhamou *et al.* 1994) cucumber (El Ghaouth 1994), wheat (Vander 1998), potato (Kowalski *et al.* 2006a, Kowalski *et al.* 2006b) and discussed as an alternative to conventional chemical plant protection (Godenrath 2000).

However, practical application of chitosan in agriculture is still very much at the beginning. The higher dependency of plant growth promoting and elicitor effects on environmental conditions such as climatic influences (Schliephake und Trautz 2001), infection pressure, and application mode may be the cause for varied performance in dependence of trial site and year under field conditions.

This paper describes the effect of soluble chitosan on potato analysing parameters of plant grow and yield of potato and incidence of late blight (*Phytophthora infestans*), early blight (*Alternaria solani*) and tuber diseases in field trials under subtropical and temperate conditions.

Materials and methods

The chitosan used throughout the trials was a soluble powder preparation (ChitoPlant, ChiPro GmbH Bremen, chitosan content 99,9 %).

Field trials under subtropical conditions. See table 1. In all field trials minitubers of cv Désirèe derived from virusfree microplants acclimatised in the greenhouse were used. Minitubers were planted in randomised plots with three replicates per treatment in the field in December on a ferralitic soil under irrigation (every third day) with 120 kg ha^{-1} N. Plot size was 10 m^2, with 25 x 90 cm spacing; sample size was 15 plants per replicate. Size of the minitubers planted was calibrated to between 21 and 30 mm diameter; treatments to break dormancy were not necessary, as minitubers sprouted within three days after coming out of cold storage. The trial site was the experimental station of Remedios, province Villa Clara (central Cuba), 22° 29' 41N / 79° 32' 45W situated 26 m above sea level; in the potato growing season December – March the average maximum temperatures are 27°C, minimum temperatures 20°C, relative humidity 80 %, with an average of 8 hours sunshine per day.

Table 1. Seed tuber and weekly foliar treatment with soluble chitosan in the field plot trial in Remedios, Villa Clara Province, Central Cuba

Soluble chitosan treatment				
Seed tuber	Foliar	Trial year		
		2001/02	2002/03	2003/04
0	0	cv Désirèe	cv Désirèe	cv Désirèe
0	1,0 g l^{-1}	cv Désirèe	cv Désirèe	cv Désirèe
1.0 g l^{-1}	0	cv Désirèe	cv Désirèe	cv Désirèe
1.0 g l^{-1}	1,0 g l^{-1}	cv Désirèe	cv Désirèe	cv Désirèe

Weekly foliar sprays with soluble Chitosan commenced two weeks after emergence and were continued until day 60 after planting. Untreated plants and plants treated chemically (Fungicide regime following instructions of Sanidad Vegetal Nacional: copper oxicloride 2nd week after emergence, 3rd and 10th week Maneb, 4th week Zineb, 5th week Macozeb, 6th and 11th week Ridomil MZ, 7th week Tilt CE 25, 8th week benomyle (Fundazole), 9th week Silvacur combi) served as control.

Incidence of early blight (*Alternaria* spp.) was assessed 60 days after planting. Tubers were harvested 80 days after planting, 10 plants per repetition, counted, weighed and evaluated for tuber diseases common scab (*Streptomyces scabies, Fusarium spp.*, *Rizoctonia solani*, *Sclerotium rolfsi*, *Phoma spp.*), counting the numbers of tubers affected.

Evaluation of plant growth under subtropical conditions. Parameters of field growth (fresh weight, dry weight, shoot length and water loss 1 hour after cutting the stems) were recorded 45 days after planting.

A ranking system was used as described by Kowalski et al. (1999a) with the following modifications*:* The treatment with the significantly highest fresh and dry weight and shoot length respectively was ranked 1, the next lower 2 and so on. The treatment with the lowest water loss were ranked 1, the next higher 2 and so on.

Dry matter contents (DM %) of the control and those treatments not significantly differing from the control were assigned rank 2; treatments with lower DM % received rank 3, with higher DM % rank 1.

The sum of all ranks allocated to the treatment (Sum of ranks) was calculated, the lowest Sum of ranks representing highest plant quality.

Field trials under temperate conditions. See Table 2. In all field trials certified seed tubers of cv Adretta were planted in randomised plots with four replicates per treatment in the at the end of April on a sandy loam with 80 kg ha^{-1} N. Plot size was 10 m^2, with 30 x 75 cm spacing; sample size was 20 plants per replicate for evaluation of late blight. Size of the minitubers planted was calibrated to between 35 and 55 mm diameter. The trial site was the experimental station of Rostock university, 53°55'5"N / 12°16'41"E situated 0 m above sea level; in the potato growing season Mai– August the average maximum temperatures are 20.0 °C, minimum temperatures

11.2°C, relative humidity 81 %, with an average of 8.2 hours sunshine per day. In 2005 cv Alegria and cv Meridian were also planted.

Weekly foliar sprays with soluble Chitosan commenced three weeks after emergence and were continued until 50 % of the foliage was affected by late blight. Untreated plants and plants treated chemically (Akrobat, Shirlan) served as control.

Incidence of late blight was assessed at three successive evaluation dates in July, estimating percentage of leaf area affected using an evaluation key adapted from James, 1971.

Tubers were harvested 80 days after planting, 10 plants per repetition, counted, weighed and evaluated for common scab. Common scab was evaluated with a six scale key of lesion depth and diameter (0 = no scab, 1 = scab not raised, diameter < 10 mm, 2 = scab not raised, diameter >10 mm, 3 = scab raised, diameter < 10 mm, 4 = scab raised, diameter >10 mm, 5 - pithy scab). Each evaluation score was multiplied with the corresponding number of tubers of the sample, and the valued summed.

Table 2: Seed tuber and weekly foliar treatment with soluble chitosan in the field plot trial in Rostock, Northern Germany

Soluble chitosan treatment				
Seed tuber	Foliar	Trial year		
		2003	2004	2005
0	0	cv Adretta	cv Adretta	cv Adretta, Alegria, Meridian
0	0.1 g l^{-1}	cv Adretta	cv Adretta	cv Adretta
0	1.0 g l^{-1}	-	cv Adretta	cv Adretta
0.1 g l^{-1}	0	cv Adretta	cv Adretta	cv Adretta
0.1 g l^{-1}	0.1 g l^{-1}	cv Adretta	cv Adretta	cv Adretta, Alegria, Meridian
1.0 g l^{-1}	0	cv Adretta	cv Adretta	cv Adretta
1.0 g l^{-1}	1.0 g l^{-1}	cv Adretta	cv Adretta	cv Adretta, Alegria, Meridian
5.0 g l^{-1}	0	cv Adretta	cv Adretta	cv Adretta
5.0 g l^{-1}	5.0 g l^{-1}	cv Adretta	cv Adretta	cv Adretta, Alegria, Meridian

Statistical Analysis. For statistical analysis the SPSS statistical package was used. Analysis of variance (oneway anova) was carried out, using the Tukey test at $P<0.05$ to compare means.

Factor analysis was carried out using Varimax Rotation.

Results

Subtropical conditions 2003 – 2005 (Table 3). All chitosan treatments of cv. Désirèe, including seed tuber treatment alone, showed improved plant development, as expressed by lower Sums of Ranks, compared to the untreated control, and also to the chemically treated control plants. Foliar fresh and dry weight were significantly increased; in the trial years 2004 and 2005 dry matter content was significantly increased and waterloss after cutting decreased significantly, also in comparison with chemical treatment. Of the chitosan treatments foliar treatment and combined foliar + seed tuber treatment had better plant development than seed tuber treatment alone.

Table 3. Effect of application of soluble chitosan to seed tuber and/or as foliar sprays under subtropical conditions on morphological and physiological parameters, tuber number and yield and disease incidence of potato *Solanum tuberosum* L. c.v. Désirèe (mean values followed by differing letters differ significantly at $P < 0.05$)

Field trial year/ parameters	Control		Fungicide		Chitosan treatment 1.0 g l-1					
					Seed tuber		Foliar		Seed + foliar	
2003										
Fresh weight g	96.7 a	4	119.25 b	3	133.1 bc	2	146.5 c	1	153.8 c	1
Dry weight g	16.5 a	3	18.5 a	3	18.5 a	3	20.4 ab	2	22.2 b	1
Stem length cm	27.0 a	3	28.5 ab	2	30.7 ab	2	35.0 b	1	33.0 b	1
Dry matter %	17.1 a	2	15.5 a	2	14.0 a	2	13.9 a	2	14.4 a	2
Waterloss %	8.7 a	1	7.4 a	1	7.4 a	1	6.6 a	1	4.7 a	1
Sum of ranks		13		11		10		7		6
Tuber number/ plant	3.2 a		4.7 b		3.9 ab		4.1 b		4.3 b	
Tuber yield g/plant	430.0 a		670.0 c		451.3 a		500.0 ab		530.0 b	
Alternaria leaf spot %	34.0 a		34.7 a		33.8 a		39.0 a		33.8 a	
Tuber disease % *	2.0 b		0.7 a		1.5 ab		1.5 ab		1.6 ab	
2004										
Fresh weight g	98.8 a	3	109.7 a	3	164.5 b	2	194.2 c	1	218.2 c	1
Dry weight g	22.0 a	3	26.6 a	3	42.3 b	2	77.1 c	1	84.9 c	1
Stem length cm	30.3 a	2	32.0 a	2	32.1 a	2	35.7 b	1	35.8 b	1
Dry matter %	22.3 a	2	24.3 ab	2	25.4 b	1	39.4 c	1	38.9 c	1
Waterloss %	11.5 b	2	10.6 b	2	10.7 b	2	7.3 a	1	4.9 a	1
Sum of ranks		12		12		9		5		5
Tuber number/ plant	3.9 a		5.4 b		5.9 c		7.0 d		7.3 d	
Tuber yield g/plant	409.8 a		422.0 b		439.0 b		583.0 c		575.2 c	
Alternaria leaf spot %	71.5 c		12.2 b		58.5 b		63.0 bc		60.2 b	
Tuber disease % *	0.6 a		0.2 a		1.3 a		2.5 a		1.8 a	
2005										
Fresh weight g	156.0 a	4	162.1 b	3	165.5 c	2	168.0 d	1	169.3 d	1
Dry weight g	48.8 a	5	54.05 b	4	55.2 bc	3	57.3 cd	2	57.8 d	1
Stem length cm	31.4 a	3	31.3 a	3	34.9 b	2	36.3 c	1	37.0 c	1
Dry matter %	31.3 a	2	33.4 bc	1	33.3 bc	1	34.1 c	1	34.1 c	1
Waterloss %	10.9 b	2	13.0 c	3	6.7 a	1	7.0 a	1	6.8 a	1
Sum of ranks		16		14		9		6		5
Tuber number/ plant	6.1 a		6.7 b		7.1 c		7.6 d		7.8 d	
Tuber yield g/plant	543.2 a		570.2 b		578.5 bc		588.0 cd		594.0 d	
Alternaria leaf spot %	47.8 c		39.5 a		44.5 bc		45.0 bc		42.2 ab	
Tuber disease % *	1.4 b		0.1 a		0.6 ab		0.6 ab		0.4 ab	

** Fusarium spp., Sclerotium rolfsii, Rizoctonia solani, Phoma spp., Streptomyces scabies*

Tuber numbers were increased in all chitosan treatments compared to the untreated control, in 2004 and 2005 also compared to fungicide treatment. Tuber yields also were increased compared to the untreated control, for the foliar and combined seed tuber and foliar spray this was significant in all three trial years, for seed tuber treatment alone this was significant in 2004 and 2005.

Foliar treatment alone and combined seed tuber and foliar chitosan treatment showed the overall best plant growth and also the highest yields, compared to the untreated as well as fungicide treated plants.

Alternaria solani leafspots were reduced significantly in the seed tuber treatment in 2004 and the combined seed + foliar treatment 2004 and 2005 compared to the untreated control, but the effect of a chemical treatment was not achieved.

While in two trial years incidence of tuber disease (*Fusarium spp.*, *Rizoctonia solani*, *Sclerotium*, *Phoma spp*, *Streptomyces scabies*) was generally low, it was significantly lower in the chemical control, compared to the untreated control, while the chitosan treatments did not differ significantly from either.

Temperate conditions 2003 – 2005 (table 4). Combined seed tuber and foliar treatment with 1.0 und 5.0 g l^{-1} soluble chitosan led to significantly decreased late blight lesions in the trial years 2003 and 2005 in cv. Adretta; 2004 no differences between chitosan treatments and untreated control were apparent. Foliar treatment alone with 0.1 und 1.0 g l^{-1} decreased late blight incidence in the trial year 2005, but not in 2003 and 2004.

Table 4: Effect of application of soluble chitosan to seed tuber and/or as foliar sprays under temperate conditions on yield parameters and disease incidence of potato *Solanum tuberosum* L. c.v. Adretta

Field trial year/ parameters	Control	Fungicide	Chitosan treatment g l^{-1}							
			foliar		seed tuber			seed and foliar		
			0.1	1.0	0.1	1.0	5.0	0.1	1.0	5.0
2003										
Late blight 1	5.1 b	0.8 a	5.4 b	6.2 b	4.7 b	3.1 ab	3.5 ab	3.7 ab	1.5 ab	2.4 ab
Late blight 2	52.6 d	5.0 a	70.6 e	53.0 d	49.2 d	48.2 cd	47.8 cd	40.4 bcd	30.0 b	33.2 bc
Late blight 3	85.8 cd	10.0 a	90.1 d	88.8 d	87.9 cd	84.8 cd	84.2 cd	84.9 cd	77.5 bc	76.0 b
Late blight %	47.8 cd	5.3 a	55.4 d	49.3	47.3 cd	45.4 bc	45.2 bc	43.0 bc	36.3 b	37.2 b
Common scab	188.6 b	131.6 ab	121.0 a	111.0 a	187.5 b	156.2 ab	159.1 ab	121.7 a	124.7 a	134.9 ab
Tuber number/plant	9.2 a	10.2 a	9.6 a	8.8 a	9.4 a	8.6 a	9.1 a	9.7 a	9.4 a	9.7 a
Tuber yield g/plant	840.8 a	1000.0 b	847.1 a	859.6 a	813.9 a	839.4 a	820.0 a	835.9 a	835.5 a	892.0 a
2004										
Late blight 1	12.7 a	1.5 b	10.7 a	6.2 a	7.3 a	13.0 a	11.3 a	10.7 a	12.9 a	9.8 a
Late blight 2	57.3 a	9.0 b	61.3 a	52.2 a	54.1 a	59.0 a	60.6 a	55.8 a	55.5 a	53.8 a
Late blight 3	76.6 a	10.0 b	74.4 a	74.7 a	71.9 a	76.6 a	75.2 a	74.7 a	73.1 a	72.7 a
Late blight %	48.9 a	6.8 b	48.8 a	44.4 a	44.4 a	49.5 a	49.0 a	47.1 a	47.2 a	45.4 a
Common scab	170.2 a	132.0 a	149.0 a	157.1 a	128.2 a	120.2 a	138.5 a	139.4 a	144.9 a	150.1 a
Tuber number/plant	10.5 ab	11.5 ab	10.7 ab	11.9 b	11.4 b	11.7 b	9.5 a	10.9 ab	10.3 ab	11.1 ab
Tuber yield g/plant	721.9 a	953.5 b	745.3 a	760.9 a	764.1 a	774.7 a	707.0 a	797.8 a	765.8 a	842.5 ab
2005										
Late blight 1	18.3 d	1.6 a	9.9 bc	9.3 bc	14.3 cd	11.1 bcd	11.4 bcd	9.6 bc	5.0 b	6.8 bc
Late blight 2	39.0 d	10.0 a	22.0 bc	25.6 bcd	32.1 cd	31.8 cd	27.8 bcd	30.3 cd	16.6 b	14.7 ab
Late blight 3	56.0 e	10.0 a	45.1 cde	42.2 cd	54.2 de	53.3 de	45.8 cde	46.0 cde	37.5 bc	27.5 b
Late blight %	37.8 e	7.2 a	25.7 bcd	25.7 bcd	33.5 de	32.0 de	28.3 cde	28.6 cde	19.7 bc	16.3 b
Common scab	142.8 b	101.8 ab	133.0 ab	120.0 ab	80.1 ab	71.2 ab	63.5 a	94.2 ab	85.7 ab	114.5 ab
Tuber number/plant	11.5 a	12.0 a	10.8 a	11.1 a	10.9 a	10.9 a	10.6 a	10.8 a	10.5 a	10.2 a
Tuber yield g/plant	599.0 a	771.4 b	611.7 a	581.2 a	429.9 a	440.7 a	414.6 a	609.4 a	624.1 a	641.9 a

Common scab *Streptomyces scabies* symptoms were significantly decreased in the foliar and combined seed tuber + foliar treatments in the trial year 2003. In the following trial years tubers of all chitosan treatments had lower common scab scores than the untreated control, however the differences were not significant. Fungicide treatment also led to a decrease of common scab scores.

Tuberisation in chitosan treatments was the same as in the untreated controls; tuber yields in the combined seed tuber + foliar treatments were higher in all three trial years, however this was not significant.

Temperate conditions, three cultivars, trial year 2005 (table 5). During the season 2005 the varieties cv Alegria and Meridian were additionally trialled with the combined seed tuber + foliar treatment. Decreased late blight

affection was found for only one assessment date in cv. Alegria with 5 g l^{-1}; the average late blight score was lower, but not significantly. For cv Meridian significantly decreased blight scores were assessed with 1 g l^{-1} und 5 g l^{-1} chitosan compared to the untreated control; these values remained significantly higher than the fungicide treatment. Seed tuber + foliar treatment of 0.1 g l^{-1} did not reduce late blight in Alegria and Meridian.

Table 5. Influence of combined seed tuber and foliar application of soluble chitosan on yield parameters and disease incidence in two *Solanum tuberosum* varieties (cv Alegria and Meridian) under temperate conditions in the trial year 2005

cv	Control	Fungicide	Chitosan-treatment g l^{-1} seed tuber + foliar		
			0.1	1.0	5.0
Alegria					
Late blight 1	6.2 ab	2.2 a	10.2 b	7.6 ab	4.8 ab
Late blight 2	20.3 b	3.0 a	24.0 b	20.2 b	15.1 ab
Late blight 3	37.1 c	8.0 a	36.4 c	38.8 c	24.7 b
Late blight %	21.2 b	4.4 a	23.5 b	22.2 b	14.9 b
Common scab	76. b	10.6 a	54.6 ab	73.6 ab	51.ab
Tuber number/plant	11.9 a	11.0 a	11.3 a	9.0 a	11.0 a
Tuber yield g/plant	636.6 abc	922.8 d	671.0 abc	554.4 ab	667.3 abc
Meridian					
Late blight 1	5.1 ab	2.7 a	6.2 b	2.6 a	2.9 a
Late blight 2	21.6 c	10.5 a	21.4 c	17.8 bc	14.9 ab
Late blight 3	39.1 d	9.6 a	33.6 cd	28.6 bc	20.7 b
Late blight %	21.9 c	7.6 a	20.4 c	16.3 b	12.8 b
Common scab	80.6 ab	129.8 b	53.0 a	59.8 a	71.5 ab
Tuber number/plant	15.3 a	14.9 a	16.7 a	14.9 a	16.8 a
Tuber yield g/plant	731.8 ab	914.5 c	761.7 ab	716.2 a	828.7 abc

Chitosan treatments did not significantly influence common scab scores of the harvested tubers compared to the untreated control. In the fungicide control of cv. Alegria the common scab score was significantly decreased compared to the untreated control, but not in comparison with the chitosan treatments. In cv Meridian the common scab score of the fungicide control was increased, this was significant compared to the chitosan treatments 0.1 g l^{-1} und 1 g l^{-1}.

Tuber numbers and yields of cv. Alegria and Meridian were not significantly influenced by the chitosan treatments; only in the fungicide treatments were yields higher compared to the untreated control.

Discussion

Under subtropical conditions chitosan treatment led to an increase in yield parameters compared to the untreated control, and in two trial years also compared to fungicide treatment. This effect cannot be due to the reduction of disease incidence as *Alternaria* leaf spot incidence was reduced only by the combined treatment in 2004 and 2005. While the fungicide treatment did not reliably reduce early blight incidence, it also had higher yields than the untreated control. Plant growth promoting substances, including chitosan, have been shown to influence stress responses, such as peroxidase production (Kowalski et al. 2005). Foliar and stem fresh mass were increased with resulting increase in assimilating biomass. Decreased water loss after cutting in chitosan treatments indicates a generally enhanced stress resistance against the effect of high temperatures and temporary drought periods. Fungicides can cause similar metabolic changes (Wu and von Tiedemann 2002), however the fungicide treatment did not improve the plant growth parameters analysed.

Weekly foliar treatment and combined seed tuber- and foliar chitosan application showed the highest increase in yield parameters and is the treatment of choice, as in all three years yield parameters lay somewhat higher

than in foliar treatment alone, albeit not significantly so, and because it has been shown that seed treatment alone has already a positive effect (Fig. 1).

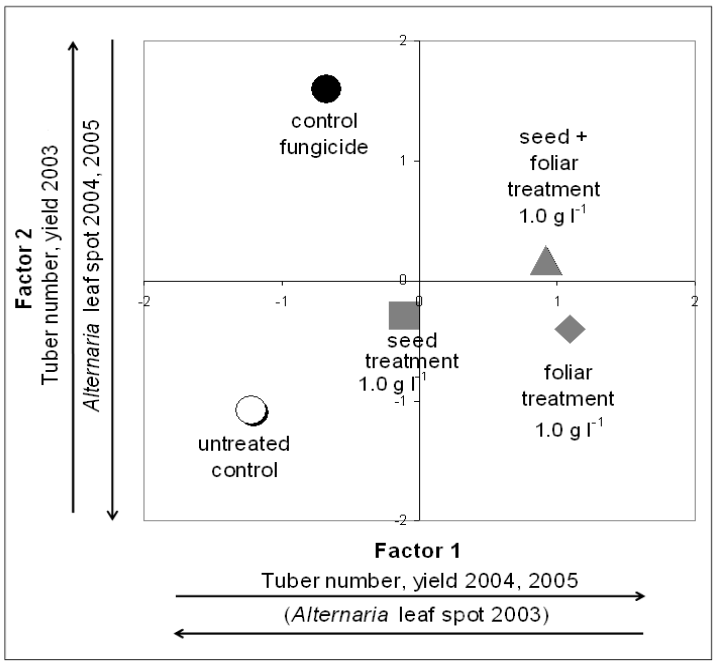

Figure 1. Factor analysis summarising three trial years (2003 - 2005). Yield parameters and early blight incidence in soluble chitosan treatments under subtropical conditions. Variation accounted for: 88,7 % (Factor 1 = 69,7 % Factor 2 = 19,0 %) .

The effect of chitosan under temperate conditions on yield and plant health depended on concentration and application mode (fig 2), and differed between cultivars (fig 3). Combined seed tuber and foliar treatment had the most reliable effect on plant foliar health considering all three trial years. However, slightly reduced late blight incidence did not result in a yield advantage under temperate conditions. Best control of late blight and highest yields were achieved with fungicides.

Multivariate (Factor) analysis over all trial years and with three varieties indicate that Chitosan may have slight effects on yield parameters also in temperate conditions; these mechanisms may be more strongly expressed under conditions of increased stress, as caused by the higher temperatures in subtropical regions.

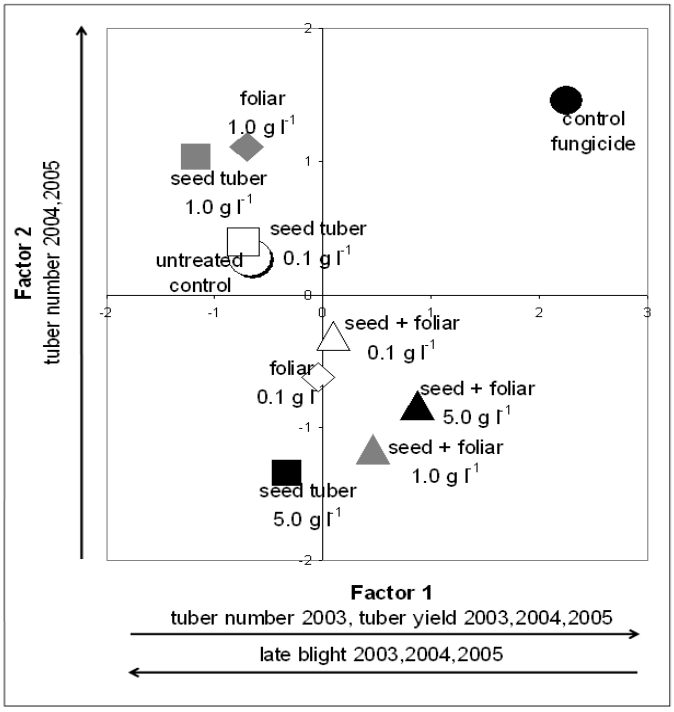

Figure 2. Factor analysis summarising results of the trial years 2003 – 2005 for yield parameters and late blight incidence under temperate conditions. Variation accounted for: 90,6 % (Factor 1 = 64,7 % Factor 2 = 14,2 % Factor 3 (common scab 2003) 11,8 %)

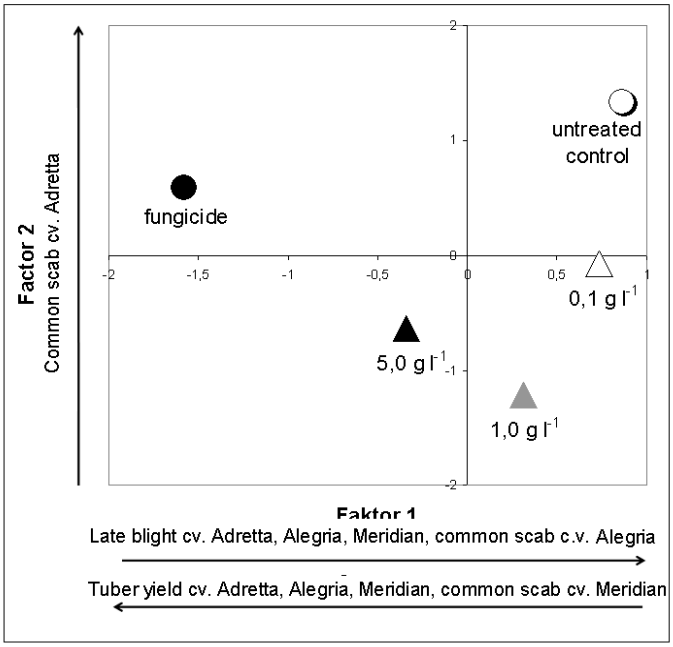

Figure 3. Factor analysis summarising results of three varieties in the trial year 2005 for yield parameters and disease incidence under temperate conditions. Variation accounted for: 93,3 % (Factor 1 = 62,5 % Factor 2 = 30,8 %)

Chitosan can be a useful additive to apply to potato as part of a strategy to reduce fungicide input, which also includes agronomic components and cultivars with improved horizontal resistances to major diseases, namely late and early blight (Darsow 2002 a,b, Darsow 2004). In such a strategy it is necessary to consider the interaction between plant growth promoting effects of chitosan and climatic conditions and varieties, and the dependency on the application mode (concentration, application to seed tuber and foliage). Good agricultural practice is the base for such a strategy involving chitosan or other substances with plant growth promoting and elicitor effects. The potential of chitosan to improve seed tuber quality (Kowalski *et al.* 2006b) can also contribute to the proposed composite strategy.

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