*VII^th Symposium of the International Society for Tropical Root Crops,*
Gosier (Guadeloupe), 1-6 July 1985, Ed. INRA, Paris, 1988.

# USE OF A MICROWAVE TREATMENT TO ERADICATE SEED-BORNE PATHOGENS FOUND IN CASSAVA BOTANICAL SEED

*(Utilisation d'un traitement aux Micro-ondes pour éradiquer les agents pathogènes présents dans les graines de Manioc)*

J.C. LOZANO, R. LABERRY and A. BERMUDEZ *

* CIAT's Cassava Program
Apdo. Aero 6713, CALI, COLOMBIA

## SUMMARY

A high percentage of cassava seeds, collected from five different edapho-climatic zones, were found infected by several fungal and bacterial pathogens. The incidence and severity of seed infections were not correlated with the climatic conditions at harvest. These pathogens were eradicated from seeds by a microwave oven treatment (1400 W heating power, 2450 MHz) for 120 sec. The effectiveness of this treatment depends on reaching an optimum temperature of 77 C. Several factors, such as container capacity, water volume and seed number can affect the time required to reach 77 C. An arasan dust treatment after microwave exposure, reduces the rate of seed re-infection.

## RESUME

*Un pourcentage élevé de graines de manioc collectées dans cinq régions pédoclimatiques différentes, a présenté des infections dues à différents champignons et bactéries pathogènes. L'incidence et la gravité des infections des graines n'étaient pas corrélées avec les conditions climatiques prévalant au moment de la récolte. Les agents pathogènes ont été détruits dans les graines par un traitement au four à micro-ondes (1400 W, 2450 MHz) pendant 120 secondes. Ce traitement est efficace si l'on obtient une température optimale de 77°C. Différents facteurs tels que la capacité du conteneur, le volume d'eau et le nombre de graines peuvent modifier le temps nécessaire à l'obtention de la température de 77°C. Un traitement des graines par poudrage à l'arasan après l'exposition aux micro-ondes réduit leur taux de re-infection.*

Seeds of Cassava (*Manihot esculenta* Crantz) are commonly accepted by quarantine officials of many countries as free from pathological and entomological agents. The distribution of cassava seeds was thought to be the safest method for the interchange of genetic material among interested groups around the world. However few precautions are currently taken to insure the quality of the material distributed or to prevent dissemination of pathogens on or in seeds.

The causal agents of cassava bacterial blight (*Xanthosomonas campestris* pv. *manihotis*) and cassava anthracnose (*Colletotrichum* spp.), have been reported as seed-borne pathogens (3,4,8). The reported germination rate of cassava seed ranges from 17 to 80 per cent with seeds germinating sporadically over a period of 2 to 4 months (1,5,7). The purposes of this study were :

- 1) to evaluate the phytosanitary condition and quality of cassava seeds collected from different ecological regions under different climatic conditions ;

- and 2) to investigate the effect of microwave treatments on eradicating seed-borne pathogens and enhancing the germination rate of cassava seeds.

## MATERIALS AND METHODS

Mature cassava fruits were collected from 31 cultivars grown in five locations with varying ecological conditions (Table 1).

Fruits were collected during both wet and dry seasons to observe the effect of climatic conditions on the nature and severity of seed infection/infestation in each region.

Fruits were visually selected for absence of deformation, insect damage and rotting induced by pathogens. Selected fruits were sundried. After two day they normally dehisced by rupture of the fruit endocarp releasing the seeds. Seeds were collected in cloth bags, selected and stored at room temperature (approximately 23 C.). Seeds were first selected according to normal shape, color, and freedom from insect damage or rotting induced by pathogens. Selected seeds were then separated into two groups according to their density: seeds dense enough to sink in water were separated from those which floated. These two groups of seeds were then air dried and used for germination trials and for evaluation of their sanitary status.

Table 1. Climatological characteristics at sites where fruits of cassava were collected.

| Site | Altitude (Mosl) | Mean Temperature (C) | Rainfall[2] (mm) | Days to harvest |
|---|---|---|---|---|
| CIAT (IV)[1] | 1,000 | 27.8 | 760 | 330 |
| Media Luna (I) | 10 | 27.2 | 1,090 | 328 |
| Carimagua (II) | 200 | 26.2 | 2,560 | 360 |
| Caribia (IV) | 10 | 25.3 | 1,360 | 320 |
| Popayan (V) | 1,800 | 18.0 | 3,460 | 519 |

[1]CIAT identification zone (2,3).

[2]Total rainfall during the actual growing cycle

Seed germination was evaluated on : a) soaked paper towels or water-agar in Petri dishes ; b) sterile, sandy soil in cement seedling beds ; and c) ridges-rows under field conditions. Seeds in Petri dishes were incubated in growth chambers at 30C and those in seedling beds were maintained under glasshouse conditions at 24 + 5C with 80 per cent RH and 1200-1500 fc light during a 12 h photoperiod.

Seed infection and infestation were tested as follows : approximately 200 seeds were placed on either potato dextrose agar (PDA), PDA acidified with latic acid to pH 4.2 (acid-PDA), or 0,3 per cent tryptic soy agar media (6). Five seeds were placed in each Petri dish with the rescpective medium and incubated at 23C for at least 14 days. Microorganisms were isolated as they became noticeable on the growth media. Isolated fungal number of both bacterial and fungal species isolated per treatment was recorded at the end of the incubation period.

Seeds to be treated with microwaves were placed in Pyrex glass beakers containing water. The beaker with seeds placed centrally in the cavity (volume 39975 $cm^3$) of a microwave oven (Kenmore Model 99421, Sears Roebuck & Co., Chicago, Il 60684, U.S.A.) and exposed to full power (1400 W heating power, 2450 MHz) for various treatment times (Table 6). After treatment seeds were removed and allowed to cool and air dry before storage in paper bags at room temperature

(24°C, approximately). Some seeds were dusted with Arasan (*tetramethylthiuram disulfide*) before storage. Since a dry-heat treatment is reported to increase the germination of cassava seeds (2), some seeds were heat-treated in an oven at 60C for 14 days to compare to the microwave treatments.

Temperature appeared to be the most important factor for microbial control and to obtain an optimum level of seed germination. Therefore temperature variations during microwave treatment due to the following variables were investigated : a) size of the Pyrex beaker ; b) water volume per beaker ; c) time of microwave exposure ; d) water source ; and e) number of seeds per container. Water temperatures were taken immediately after all treatments.

## RESULTS

A high percentage of cassava fruits were found infected by pathogens in plantations located in each ecological zone (Table 2). Most infection was associated with fruit damage induced by the fruit fly (*Anastrepha* spp.) and infestations by mealybugs (*Phenacoccus* spp.) and thrips (*Franklíniella* and *Corynothrips* spp.). Wounds caused by these insects appeared to be the sites of infection for many pathogens, except *Xanthosomonas campestris* pv. *manihotis* and *Diplodia manihotis* which were able to infect uninjured plants under controlled conditions.

A variable percentage of the seeds collected from fruits floated on water. Most of the seeds with low density were collected from insect and pathogen-affected fruits. The weight of floating seeds was only a 50 per cent the weight of seeds that sank in water which ranged from 0.10 to 0.12 g per seed. Untreated seeds with high density had a germination rate of 45 per cent while seeds with low density had only a 6.5 per cent germination rate.

Species of eight different fungal genera were isolated from seeds. Species of four of these genera *Fusarium*, *Cladosporium*, *Colletotrichum*, and *Diplodia* are cassava pathogens capable of inducing damping off, root rots, and anthracnose (Table 3). Most fungal species were isolated both during the wet and dry seasons indicating that the sanitary condition of the seeds could be independent from the weather at the time the fruits were harvested (Table 4).

Fungi infecting or infesting the seeds were eradicated by the microwave treatment (Table 5). Apparently the Arasan dust treatment prevented reinfestation of the seeds after microwave treatments, since no microorganisms were isolated after storage for 15, 30 and 45 days. Nondusted seeds were found to be infested by common saprophytic species of *Aspergillus*, *Penicillium*, *Monilia*, etc. The dry-heat treatment did not eradicate *Fusarium* and *Colletotrichum*. Species of these two genera appeared six to ten days after treatment (Table 5).

Table 2. Sanitary condition of cassava fruits and density of seeds collected from 10- to 11- month old cassava plants in four edapho-climatic zones at the end of a 1- to 3- month dry season.

| Edapho-climatic zone | Sanitary condition of fruits (%)[1] | Density of botanical seeds(%)[2] |
|---|---|---|
| | Infected | Floating |
| CIAT | 73.0 | 81.0 |
| Media Luna | 73.0 | 57.0 |
| Carimagua | 15.0 | 81.0 |
| Caribia | 79.0 | 66.0 |
| Popayan | 99.5 | 100.0 |
| Average | 67.9 | 77.0 |

1/ The sanitary condition of the fruits was determined visually. Affected fruits refer to fruits with disease symptoms and insect infestations. Most of the problems caused by insect were due to fruit flies, mealybugs and thrips ; fruits were infected by *Fusarium* spp., *Cladosporium* spp. ; *Colletotrichum* spp., and *Xanthomonas campestris* pv. *manihotis*.

2/ Density selection of botanical seeds in water.

3 Data taken from 850 or more fruits or seeds/edapho-climatic zone.

Table 3. Percent infection of cassava seed collected in four edapho-climatic zones.

| Edapho-climatic zone | *Penicillium* spp. | *Neurospora* sp. | *Fusarium* spp. | *Cladosporium* sp. | *Aspergillus* spp. | *Rhizopus* sp. | *Colletotrichum* spp. | *Diplodia manihotis* |
|---|---|---|---|---|---|---|---|---|
| CIAT | 9 | 4 | 7 | 7 | 5 | 3 | 1 | 1 |
| Media Luna | 19 | 2 | 1 | 0 | 1 | 2 | 0 | 78 |
| Carimagua | 40 | 19 | 18 | 3 | 9 | 1 | 1 | 1 |
| Caribia | 27 | 14 | 5 | 0 | 1 | 6 | 0 | 53 |
| Popayan | 0 | 0 | 40 | 0 | 0 | 10 | 0 | 0 |

[1]Data taken from 950 seeds from each edapho climatic zone. Seeds were plated on acidified potato dextrose agar medium. Fungal species were isolated 6 days after incubation at 28C. *Xanthomonas campestris* pv. *manihotis* (causal agent of cassava bacterial blight (CBB))was isolated from seeds collected in Carimagua, Media Luna and Caribia, where the CBB is endemic, by using a tetrazodium chloride medium (TZC) (Kelman, 1956).

Table 4 Fungal species isolated from cassava seeds collected at CIAT headquarters during different dry and wet periods

| Date of collection | *Penicillium* spp. | *Neurospora* sp. | *Fusarium* spp. | *Rhizopus* sp. | *Aspergillus* spp. | *Cladosporium* sp. | *Diplodia manihotis* | *Colletotrichum* spp. |
|---|---|---|---|---|---|---|---|---|
| January/82 (dry) | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| February/82 (dry) | 3 | 0 | 11 | 5 | 0 | 33 | 0 | 1 |
| September/82 (dry) | 3 | 0 | 13 | 14 | 0 | 0 | 0 | 3 |
| October/82 (wet) | 5 | 18 | 0 | 15 | 4 | 0 | 0 | 0 |
| November/82 (wet) | 4 | 3 | 10 | 0 | 3 | 3 | 0 | 3 |
| March/82 (dry) | 0 | 0 | 7 | 0 | 7 | 0 | 4 | 0 |

[1]Data collected from more than 300 seeds per sample. Seeds were plated on acidified potato dextrose agar and incubated at 25° C. Organisms identified after 6 days of incubation

Table 5. Percentage of seeds of four clones affected by fungal microorganisms after a microwave or dry heat treatment or density selection.

| Treatments[2] | *Penicillium* spp. | | | | *Neurospora* spp. | | | | *Fusarium* spp. | | | | *Diplodia* manihotis | | | | *Colletotrichum* spp. | | | | *Aspergillus* spp. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D[1] | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| Microwave[a] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dry heat[b] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Selected seeds[c] | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 68 | 0 | 40 | 0 | 0 | 0 | 0 | 0 | 60 | 0 | 12 | 0 | 0 | 0 | 4 | 0 |
| Unselected seeds[d] | 0 | 4 | 0 | 0 | 0 | 0 | 8 | 0 | 76 | 12 | 56 | 4 | 0 | 0 | 4 | 0 | 65 | 0 | 18 | 0 | 0 | 4 | 7 | 8 |

[1]Identification : A=CM 340-30; B=SG 715; C=SG 821; D=SG 828 (nomenclature used in the CIAT Cassava Breeding Program (2,3).

[2]Treatments: a=120 seconds of microwave exposure (1400 w heating power, 2450 MHz) followed by an arasan dust; b=dry heat (60°C) for 14 days (CIAT Annual Report, 1982) followed by an arasan dust; c=selected seeds by the water-density system but not treated with arasan; d=unselected and untreated seeds. Data taken from 30 seeds per treatment/clone.

However, *X. campestris* pv. *manihotis* was eradicated by both the microwave and dry-heat treatments. High density seeds had fewer contaminated seeds than lots not selected on the basis of density (Table 5). These results have been confirmed using several thousand seeds from different cultivars.

A germination rate of over 90 per cent was obtained when seeds were microwave-treated for 120 sec in a 600 ml Pyrex beaker containing 300 ml of distilled water. Germination was at least 90 per cent on soaked paper towels, water, agar sterile sandy soil, and in ridge-rows under field conditions. The highest germination percentage was consistently obtained from those seeds which were treated for 120 sec microwave exposure (77C). The germination rate dropped to 55 per cent at 86C and to zero above 96C. From 77C to 56C, seed germination was high, but the control of seed-borne pathogens was not satisfactory (Table 6).

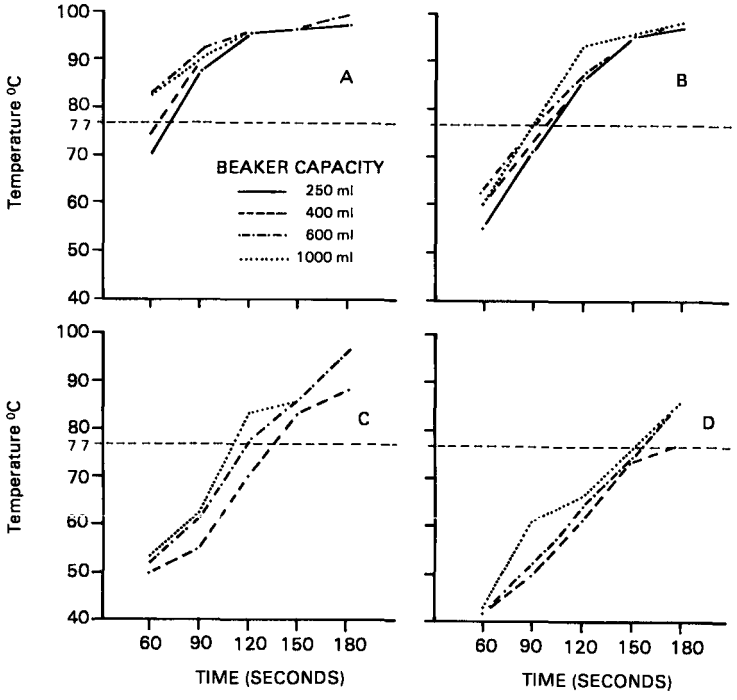


**Fig. 1. Effect of the beaker capacity and water volume on water temperature after periods (seconds) of microwave exposure. Water volume in each beaker used was: A=100ml; B=200ml; C=300ml; and D=400ml. The optimum temperature (77°C) is illustrated.**

Table 6. Effect of microwave exposure on botanical seed infections/infestations and germination as compared with dry heat (60°C for 14 days) treatment in an electric oven

| Time of exposure (seconds) | Temperature (C) | Percentage of infected/infested seed by[1] | | Germination[2] |
|---|---|---|---|---|
| | | Fungi | Bacteria | % |
| 0 | 28 | 80 | 80 | 65 |
| 30 | 38 | 12 | 90 | 60 |
| 60 | 52 | 8 | 56 | 65 |
| 90 | 61 | 6 | 28 | 68 |
| 120 | 77 | 0 | 0 | 95 |
| 150 | 86 | 0 | 0 | 55 |
| 180 | 96 | 0 | 0 | 0 |
| 240 | 98 | 0 | 0 | 0 |
| 14 days-dry-heat[3] | 60 | 60 | 0 | 90 |

[1] Average of 540 seeds/treatment. Visually selected and randomized seeds of clone CM 340-30 were used for each treatment

[2] Germination on sterile sandy soils

[3] Dry heat in oven at 60° C. For germination test all seeds were treated with Arasan (Thyram 75) after the microwave or dry heat treatments.

The optimum temperature of 77C obtained with the microwave treatment was modified significantly by the time of exposure, size of the container and quantity of water used (Figure 1). The quality of water (tap vs. distilled water) induced a variation of 1 to 5C, with the highest temperatures reached when tap water was used. The number of seeds/treatment did not induce significant changes in water temperature if the quantity of seeds was not higher than 100 units. When more than one beaker was introduced into the oven, the temperature obtained after 120 sec of microwave treatment dropped in relation to the number of beakers used ; for example, if two beakers were inside the oven, the temperature dropped to 71C ; if 3 to 53C ; etc., during a 120 seconds of microwave exposure).

## DISCUSSION

There are several seed-borne fungal or bacterial pathogens in most of the cassava growing areas which represent potential quarantine risk to cassava in areas where the infested or infected material is introduced. The dissemination of cassava seed-borne pathogens can be minimized by : a) collection of apparently healthy mature fruits and drying them before seed removal ; b) selection of seeds with normal size and shape ; c) discarding seeds which float in water ; d) treating high density seeds (approximately 100 seeds) in water (300 ml in a 600 ml Pyrex beaker) centrally in the cavity (volume 39975 $cm^3$) of a microwave oven at full power (1400 w heating power, 2450 MHz) for 120 sec ; and e) dusting microwave-treated seeds with Arasan (*tetramethyl-thiuran disulfide*) before packaging in sterile paper bags. Because optimum temperature obtained with the microwave treatment can be modified by several factors, developing the system according to the equipment available suggested.

## ACKNOWLEDGMENT

We wish to thank to Dr. C.H. HERSHEY, of the Cassava Program of CIAT, for his valuable suggestions.

## LITERATURE CITED

CAPINPIN, J.M., and BRUCE, U.C. 1956. Floral biology and cytology of *Manihot utilissima*. The Philippine Agriculturist. 39 : 306-316.

Centro International de Agricultura Tropical. 1981. Annual Report 1980. Cassava Program. CIAT, Cali, Colombia. 265 pp

Centro Internacional de Agricultura Tropical. 1983. Annual Report 1982. Cassava Program. CIAT, Cali, Colombia. In press.

ELANGO, F., and LOZANO, J.C. 1980. Transmission of *Xanthomonas manihotis* in seed of cassava (*Manihot esculenta*). Plant Disease 64 : 784-786.

HAHN, S.K., HOWLAND A.K., and TERRY, E.R. 1973. Cassava breeding at IITA. Proceedings of the Third International Symposium of Tropical Root and Tuber Crops pp.

MARTIN, J.K. 1975. Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7 : 401-402.

MARTIN, F.W. 1976. Cytogenetics and plant breeding of cassava. Plant Breeding Abstracts. 46 : 909-916.

PERSLEY, G.J. 1980. Studies on the survival and transmission of *Xanthomonas manihotis* on Cassava Seed Ann. Appl. Biol. 93 : 159-166.