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## Advances in the Study of Anthracnose/Blotch Disease of Dioscorea alata in Nigeria

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### ABSTRACT

Water yam (Dioscorea alata L.) known to have high nutrient value, is becoming increasingly important in Nigeria as a source of carbohydrate food. However, production is hampered by a disease complex, anthracnose/blotch disease, especially in the rain-forest zone of Nigeria. The field appearance most characteristic of the disease is blackening of affected parts and scorching of leaves, especially under high humidity, followed by heavy defoliation of the vines. Tuber yield losses range up to 80%.

The disease may be reduced by planting with the first rains in the year, applying balanced fertilizer or farm yard manure, and staking vines immediately after sprouting.

Field and green-house screening of available cultivars have revealed resistant clones. Techniques for screening and production of enough inoculum of the Colletotrichum gloeosporioides for inoculation purposes have been developed. Completed work on the source of resistance is presented and trends of on-going projects on biochemical aspect of resistance are indicated.

Breeding problems as well as difficulties in using fungicides to control the disease are discussed. Suggestions for future work are presented.

### Introduction

Root and tuber crops have important roles in developing countries. Major production problems of yam are high labour requirements, high cost of staking, large quantities of planting material needed, and low yields per hectare. It is propagated vegetatively so diseases are easily transmitted.

Dioscorea alata, one of the common yam species grown in Nigeria, originated in the Burma-China area (Burkill 1951). It spread through New Zealand to West India. Some speculations is that Malay sailors may have brought this yam to the coast of Africa and it spread to Central and West Africa. There is also speculation that it first was in Sao Thome and Gambia before spreading to Nigeria (León, 1976).

However, D. alata failed, to become important in Nigeria because other native yams were already under cultivation-the Guinean yams (D. cayensis and D. rotundata). These Guinean yams were domesticated in 5 000 Bp (Ayensu and Coursey, 1972); while D. alata came in years latter. D. alata could not displace the na-

tive yams at that time probably because of the morphology of the tubers. Tuber skin of most clones is rough and covered with adventitious roots. It is not as popular as D. rotundata due to its peculiar taste and colour when peeled.

Currently, D. alata is becoming increasingly important in Nigeria as a carbohydrate source. It has high nutrient value, stores well, and has gained a place in the farming systems (Bell and Favier, 1980). It has been popularised in some States of Nigeria (Rivers and Cross River) where special preparations called "Ikwukwo" are made from freshly harvested tubers.

D. alata, generally known as the greater or 10-month yam (Martin, 1972) and commonly called the water yam in Nigeria, is grown in every part of the country and is affected by various diseases, the most serious of which is anthracnose/blotch disease. The disease may cause yield reduction of up to 80%. The terms "yam die back" (Anon, 1974); "leaf and stem blight" (Jackson and Newhook, 1978; Nwankiti and Arene, 1978); "Scorch" (Anon, 1974) and "anthracnose/blotch" (Nwankiti, 1982) are sometimes applied to the disease, while "lightning strike" is erroneously used by laymen.

Waitt (1963) and Coursey (1967) first reported the disease in Nigeria, although it probably existed at a much earlier date. The disease has been recorded from South Pacific (FA'Anunu, 1977); the Caribbean (Ferguson et al., 1970); Guyane (Fournet et al., 1975); New Zealand (Jackson and Newhook., 1978) and India (Prasad and Singh, 1960).

It appears the disease occurs in every part of Nigeria with varying intensities. Extreme temperatures of northern Nigeria are not favourable for development of the fungus. It becomes a serious problem in rain forest zones.

### Geographic Distribution

Geographic distribution of crops and plant diseases depends to a large extent upon climatic factors such as rainfall, humidity, and temperature. This is true of most diseases which affect the aerial parts of crops.

Chowdbury (1937) reported that conidia of Colletotrichum are sensitive to atmospheric humidity. Germination could be inhibited below 95 percent humidity. Singh and Prasad (1967) reported that optimum temperature for growth of mycelium of C. gloeosporioides was 26°C while for spore production of the same organism, both 24°C and 26°C had similar effects. Sporulation was good above 22°C but was reduced at 30°C and inhibited at 32°C. In Nigeria, the disease is important in the rain forest and less important in the transitional zone of savannah in the north and swamp forest in the south. These zones correspond to areas with mean annual temperatures of 22°C and 30°C and annual rainfall of about 2000 mm.

### The Disease

Available D. alata clones react in different ways when infected by the casual agents. These reactions led many workers to give different names to the disease.

Generally, infection starts from the lower leaf surface facing the soil. Movement of infection then progresses upwards along the vine. Percent of leaves infected from ground level up to 0.2 metres along the vine of a susceptible cultivar was significantly (P.01) higher than at 0.2-0.4 metres and at 0.4-0.6 metres along the vine.

This indicates the causal agents are probably soil-borne. In some stands, black lesions appear first on the lower part of the main vine just before the first mature leaves.

In severe cases, aerial parts of some susceptible clones may become chlorotic and stunted; while in others the young leaves at the initial infection tend to fold or twist and often become stunted; indicating some toxic effect. Production of toxic principles by Glomerella cingulata pathogenic to other crops has been reported (Koch and Schulz, 1979; Ebenebe, 1982).

Typical anthracnose lesions on leaves and vines of a susceptible clone predominate in an infested field. On most parts of infested leaves, the cuticle is sloughed-off leaving apparently superficial fruitification. This is an indication of hypostomatic development characteristic of C. gloeosporioides. Young stands are susceptible to the disease and early infection may result in premature death and severe yield losses. The scorch symptoms are noticeable 3 to 4 months after planting and becomes more severe under heavy rains, hence the erroneous use of the term "lightning disease." Scorched leaves show signs of being burned due to toxic effects of some chemicals. The degree of scorching varies with cultivars.

#### Techniques for Effective Isolation of the Causal Agent(s)

Isolation of one major causal agents, C. gloeosporioides, may be difficult.

The following isolation procedures may be helpful:

The organism may not be isolated from:

- a. Scorched leaves and stems. These are dead tissues.
- b. Chlorotic stands. Some stands turn yellow as they emerge from the soil. No necrotic areas are visible.
- c. Curled, or slightly folded or twisted leaves. These early symptoms on younger leaves hardly show any necrotic areas which may be useful for isolation studies.

Specimens most suitable for isolation studies are those with parts of their cuticle sloughed-off and specimens with small dark necrotic lesions which may be surrounded by a yellow halo.

Frequently, other organisms grow on the leaves in association with C. gloeosporioides, especially Botryodiplodia theobromae and Fusarium semitectum (Nwankiti, 1982). These organisms usually grow faster than C. gloeosporioides. Therefore, the following procedure may be necessary for isolation of C. gloeosporioides.

Infected leaf with typical anthracnose necrosis is placed on a piece of damp sterile filter paper in a Petri dish until parts of the acerruli sprout. These are then cut into small pieces, passed through 25% sodium hypochlorite solution for 1 minute, rinsed in distilled water and placed to dry for 4 hours in covered sterile Petri dishes and then placed in tap water agar. Hyphal tip transfers are then made from all colonies in tap water agar and incubated at 25°C. Subsequent transfers could be made on potato dextrose agar (PDA) or potato carrot agar (PCA). For faster sporulation, all cultures could be submitted to ultra violet/black light irradiation in plastic Petri dishes at 25°C.

## Green House Inoculation Procedures

Successful artificial inoculation of plants with C. gloeosporioides may be difficult. The following experiment describes a suggested procedure for artificial inoculation (Barrus, 1921).

A randomized complete block design experiment including four inoculation treatments with one highly susceptible cultivar was on the floor of a wall-watered greenhouse, (temperature range approximately 20° to 26°C). Treatments, including control, consisted of four plots, each plot consisting of two stands. Each plot was either inoculated with spore concentration of  $5.3 \times 10^6$  spores/ml or with a "blank" inoculum of sterile water as control. Two month old potted yam stands of cv "Obunaenyi" were inoculated using either dipping for 5 minutes; leaves of potted stands dusted and rubbed gently with carborundum before dipping for 5 minutes; foliar spray using a 450 ml plastic atomiser spray gun; with leaves dusted and rubbed with carborundum. Inoculum was prepared from pure cultures of C. gloeosporioides obtained from field infections. Each inoculated stand was covered with heavily moistened polythene bags for 4 days. Readings of the percent number of leaves successfully infected were taken 20 days after inoculation.

Anthracnose disease incidence in the greenhouse experiment was more obvious with the foliar sprays. Percent incidence obtained by wounding of leaves followed by foliar spray was significantly higher than any of the dipping methods. But typical anthracnose lesions were obtained with the foliar sprays, though wounding tended to present signs of scorching. The organism was recovered from all treatment except the control. D. alata leaves look glossy and smooth, especially the resistant ones. There are no hairs on the laminae. The leaves of some of the cultivars may contain a lot of waxy substances. It is very pertinent that from these properties of the leaves, the dipping method will be inadequate as shown by our results. The smooth nature of the leaf surface causes easy run-off of water which may result in retention of small amount of inoculum on the leaves. With the foliar spray, several small droplets of the inoculum are left and retained on the leaves, which becomes even more effective when leaves are wounded. This method may be suitable for screening studies.

## Cultural Management

### Time of Planting

Early planting of D. alata cultivars susceptible to anthracnose/blotch disease lowers incidence and severity of disease. Yield of yams planted in March was 65% higher than those planted in May. This early planting gave the crop time to grow vigorously, thereby permitting it to escape serious damage from the disease (Nwankiti, 1982).

### Effect of N Application

The quantity of N (ranging from 0 to 90 kg N/ha as Urea and 0 to 9.6 tons N/ha Farm Manure) applied as separate treatments, significantly lowered apparent infection rates of the anthracnose/blotch disease complex (Nwankiti 1982). Yield response to N applied was higher with increase in quantity of N. Application of N, therefore hastened the bulking rate of the tubers before damage by the disease.

### Time of Staking

Staking vines of D. alata clones immediately after they emerge from the soil reduces chances of the leaves and vines picking up spores of the causal organisms

of anthracnose/blotch disease from the soil. Infection is minimal and yield increases by 35.66% over the unstaked one in cv "Obunaenyi" (susceptible) and by 38.78% for the early staking over the unstaked one in cv "Um 680" (resistant). On the other hand leaf dry matter in cv "Obunaenyi" of the unstaked increased by 14.81% over the leaf dry matter of those early staked, while leaf dry matter in cv "UM 680" of the unstaked increased by 16.66% over the early staked ones.

#### Field and Green House Screening for Resistance

Fifty three available clones were subjected to natural infection in a field previously infested with the anthracnose/blotch disease. Eight clones were later selected on the basis of high yield and tolerance to the disease and used for greenhouse screening.

Inoculum for the greenhouse experiment was prepared by soaking rice grains and maize grains separately in tap water for 24 hours in erlenmeyer flasks, decanting off excess water, autoclaving the seeds for 15 minutes at 15 p.a. 1: and inoculating them with mycelial discs cut off from the margins of 10-day-old cultures of C. gloeosporioides on potato dextrose agar. Inoculated seeds were incubated for 17 days at 27° to 28°C in the dark. Sterile distilled water was added to the flasks which were shaken vigorously to dislodge conidia from the seeds. Spore concentration was adjusted to give a haemocytometer count of  $5.3 \times 10^6$  spores/ml.

For the screening, 2 month-old plants were used. These were rubbed gently with carborundum and then sprayed to wetness with the spore suspensions. They were then covered with wet polythene bags for 5 days.

These susceptible clones - "Um680," "Ominelu," and IITA "13" - were resistant, while "Nvulsogbe" was moderately resistant. These resistant clones had pink colouration on their vines and petioles.

#### Source of Resistance

Plant characters investigated to explain the resistance in D. alata included thickness of cuticle, position, and length of stomata and polyphenol content of leaf tissues. The resistant cultivar had thicker cuticle than the susceptible one. The stomata of the resistant cultivar was sunken below the epidermal layers while the stomata of the susceptible clone are even with or slightly raised above other epidermal cells (Nvankiti, 1982). Also resistant clone possessed stomata with longer pore lengths than those of the susceptible clone. Leaf extracts showed higher polyphenol activity against the organism in the resistant clone than in the susceptible clone; as was also shown by the presence of a chemical substance which accumulated around infected areas in the resistant clones. Further work is to investigate what the chemical is and its function.

It is also probable from our investigation that lack of moisture retention on the waxy leaves of the resistant cultivars reduces or prevents the germination of spores.

#### Chemical Control (Foliar Spray)

It may be difficult to control the disease by foliar sprays without proper timing for plant growth and timing for disease outbreak. Height of stakes may also be important. If the crop climbs too high on the stake, spraying becomes

difficult because the upper parts may not be reached. Another problem may be frequency and duration of sprays. Finally, the fungicide must kill the organisms without scorching the leaves.

### Breeding for Resistance

Yam improvement over the years has been by selection of "high quality" tubers by farmers planting new crops (Okoli, 1980). Currently there is renewed interest in the possibilities of improving yams by hybridization, especially in D. rondundata. This has been hampered by poor seed set, low seed viability, small size of tubers produced from true seeds, (Waite, 1961), and inconsistency in flowering habit. In D. alata flowering may further be hampered by the early devastation of the aerial part of the crop by anthracnose/blast disease. Among populations of primitive clones of D. alata, some bear only bulbils (cv "Ominaelu") and others only inflorescences. Some susceptible clones to the anthracnose/blast disease such as cv. "Nvulaogbe", "Obunaenyi", "Bindem", and "Agbo" have desirable characters including high yields. To tap this character, breeding for resistance may be possible if (1) the disease is controlled early (2) high level of vegetative growth necessary for full female expression (Degras, 1976) is realised since generally flowering may be triggered by a certain level of development of the vegetative organs, and (3) early staking of the vines which permits more light (necessary for flowering) to enter the canopy.

These and other cultural management procedures may be necessary to produce healthy crops of the susceptible clones before breeding studies are possible.

### Conclusions

The disease of D. alata, which is worldwide in distribution, is caused primarily by C. gloeosporioides penz, which produces the anthracnose symptoms. Toxic substances which may be produced by the C. gloeosporioides on the leaves or chemical substances leaching out to the leaf surfaces due to wounding of the plant tissues, may cause scorching of leaves. The disease must be effectively studied by:

- a. isolating the primary causal agent from infected leaves with peeled off epidermis.
- b. wounding healthy plants before inoculation under high relative humidity.
- c. producing high inoculum for screening purposes by inoculating rice-grains or maize grains with the C. gloeosporioides isolate.

Farmers and scientists may control the disease in the field and improve yields by:

- a. planting clones with the first rains in the year.
- b. applying balanced fertilizer.
- c. staking vines immediately after they emerge from the soil.
- d. using resistant D. alata clones.

It is important to investigate the cause of the curling of leaves in the field and the role of anthocyanin and phytoalexins in resistance. Breeding for resistance should be given a prominent place and causes of non-flowering among D. alata clones should be investigated. More work on cultural control should be initiated and ways of improving chemical control should be sought.

## Acknowledgements

We acknowledge members of the Board of Governors of the Institute for permission to present the paper and the IFS for some financial help.

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