Brown rot and die-back disease induced by a cucurlionid (*Lixus camerunus* Klobe) in amaranth (*Amaranthus hybridus* L.) in Nigeria

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SUMMARY

Studies were conducted in the field, screenhouse and laboratory to investigate the role of *Gasteroclisus rhomboidalis* Boh and *Lixus camerunus* Klobe in the transmission and induction of brown stem rot and die-back in *Amaranthus hybridus* L. in Nigeria. Field studies indicated a positive relationship between insect population and the incidence of disease. The screenhouse investigation also confirmed field observation but only implicated *L. camerunus* as the major vector of the disease. The transmission and infection were found to be related to oviposition damage on healthy *A. hybridus* stem by *L. camerunus*.

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RÉSUMÉ

ANNO-NYAKO, F. O., ADEBANJO, A. & AGUNLOYE, O.: *Les maladies "brown rot" et "die-back" provoqués par cucurlionid* (Lixus camerunus *Klobe) en l' amaranth* (Amaranthus hybridus *L.*) *au Nigeria.* Des études ont été conduites au champs, à la serre et au laboratoire dans le but d'enquêter sur la rôle de *Gasteroclisus rhomboidalis* Boh et *Lixus camerunus* Klobe dans la transmission et la provocation des maladies "brown stem rot" et "die-back" en *Amaranthus hybridus* L. au Nigeria. Des études conduites au champs indiquaient un rapport positif entre la population d'insecte et l'incidence de deux maladies. L'étude conduite à la serre a aussi collaborée des observations du champs mais c'était seulment *L. camerunus* qui a été impliqué comme un vecteur important de deux maladies. Le transmission et l'infestation ont des rapports étroites avec les dégats causés par *L. camerunus* à l'oviposition sur la tige *d' A. hybridus* saine.

Introduction

Amaranthus is a major vegetable cultivated and consumed in the tropical region of the world. It forms a large percentage of the daily intake of leafy vegetable (Anon., 1977), particularly along the west coast of Africa (Grubben, 1975). In western Nigeria, this vegetable is popular for its leaves which are used in making soup or, as spinach.

Insect-plant relationship has been explored. These include physiological reactions, diseases caused by the presence of insects or their toxic products, as well as those caused by mechanical injury (Carter, 1973).

Relatively few fungal diseases depend on insects for dissemination (Carter, 1973). Fungal diseases induced by insects usually occur through feeding and oviposition wounds and most of the insects involved are gross and mandibulate feeders (Carter, 1973). The fungal diseases transmitted are usually those whose spores are produced in millions in liquid matrices and which easily adhere to insect bodies (Westcott, 1960). They have a better chance of reaching suitable infection courts where infection occurs immediately (Carter, 1973).

Few reports on insect-transmitted fungal diseases on plants in general and on vegetables in particular have been reported. Austwick (1958) estimated 45 different fungi among 34 plant species

associated with insect transmission involving over 100 species of insects. The incidence of fungal diseases has also been reported on amaranth in Nigeria (Irvine, 1969; Odebunmi-Oshikanlu, 1977; Ikediugwu, 1981) and the Republic of Benin (Grubben, 1976). Adebanjo & Dede (1985) reported the incidence of the disease on okra in Nigeria. There have been no reports of the association of insects with the pathogen to date on amaranth.

Experience in Ibadan and other parts of Nigeria has shown that *Gasteroclisus rhomboidalis* and *Lixus camerunus* (Coleoptera: Cucurlionidae) are major insects on amaranth, attacking the stem and roots. Apart from the mild defoliating activities of the insects, female oviposit into the stem, where eggs develop into larvae which bore their way by feeding through to the roots. It has also been observed that the brown stem rot and die-back (BSRDB) of amaranth caused by *Choanephora cucurbitarum* (Berk. & Rav.) Thaxt. is a major destructive fungal disease on amaranth. The disease is characterized by dark brown water-soaked lesions on the stem accompanied by progressive rotting of infected regions and shoot die-back.

The study was carried out to investigate the role of *G. rhomboidalis* and *L. camerunus* in the transmission of BSRDB on *Amaranthus hybridus*.

Materials and methods

All experimental studies were carried out at the National Horticultural Research Institute (NTHORT), Ibadan, Nigeria, during 1984 growing seasons (from May to October).

Field study

Eight plots of 3 m × 2 m each with 2 m between each plot were prepared by ploughing, harrowing and bedding with a tractor. Seeds of *A. hybridus* obtained from NTHORT, Ibadan, Nigeria, were drilled along rows of 1m apart on each plot with a plot containing three rows. Germination occurred within 4 -5 days and plants were thinned down to 20 stands per plot. Plots were then divided into two, each comprising of four sub-plots. One set was maintained under insecticide regime of primiphos-methyl applied at 1kg ha^{-1} a.i. (Agunloye & Osisanya, 1985) fortnightly, while the other set was left unsprayed as control.

The population of *G. rhomboidalis* and *L. camerunus* were determined by counting the number found on 10 plants selected at random from each plot.

The percentage of infected plants based on the number of plants exhibiting BSRDB symptoms were also recorded. These observations were carried out weekly starting from 13 days after germination (DAG). A correlation test was done on the relationship between the individual species population per plant and the percentage infection of BSRDB. Based on the positive relationship observed, means of insect population density and percentage diseased plants were calculated for the two species.

Screenhouse study

Seeds of *A. hybridus* were planted in polyethylene bags of 5-litre capacity filled with loamy soil in the screenhouse. Plants were thinned down to one per bag after germination and 24 such bags were used for the study. At 14 DAG, the stems and leaves of plants were covered with muslin cloth. Females of *G. rhomboidalis* from infected fields were introduced to a set of eight covered plants (one per plant). This procedure was repeated for *L. camerunus* and the remaining set of eight were left as controls. Observations were recorded on the number of oviposition points on plants and the number of plants showing BSRDB symptoms.

Laboratory study

Five female insects of each species from infested amaranth fields were brought to the laboratory and dissected. Isolations were made from their mouth parts, appendages, wings, abdomen and guts using potato carrot agar (PCA). Five plates of each body part were incubated at room temperature for 3 days. Spores of the pathogen were obtained within 24 h from plated materials and plates were observed using the binocular micro-

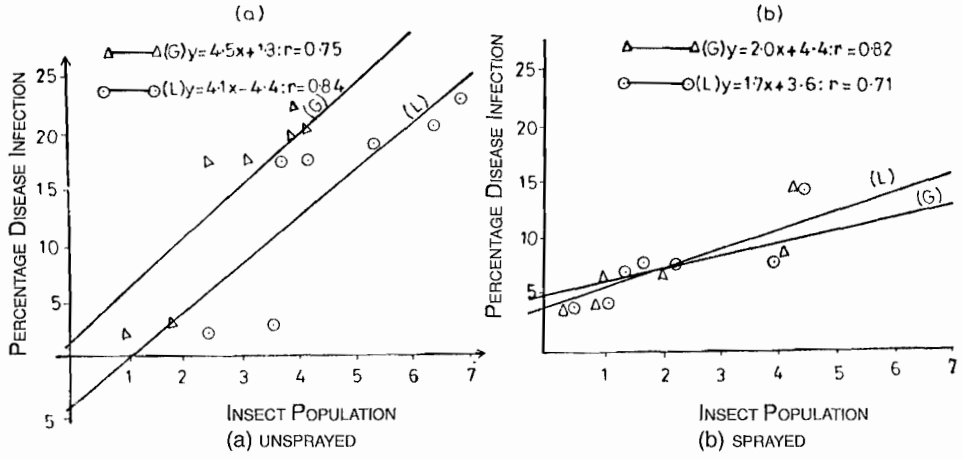

Fig. 1. Percentage disease infection in relation to insect population densities for *L. camerunus* and *G. rhomboidalis* on unsprayed (a), and sprayed (b), plots respectively.

scope and the plates with spores were recorded. The pathogen was then sub-cultured and pure spore suspensions were used in the pathogenicity test.

Pathogenicity test

Pure spore suspension of a 72 h old culture of the fungus growing at incubation temperature of 28 °C were communited in a Waring blender with 100 ml sterile distilled water (SDW). The inoculum was adjusted to give a concentration of about 25700 spores ml^{-1}. Four 3-week-old *A. hybridus* plants were wounded by slightly pricking the stem surface with sterile needle over an area of 1-2 mm diameter. They were inoculated with 1 ml spore suspension on the wounded site and such plants were placed in humidity chamber set at 70-90 per cent r. h. and incubated for 5 days.

Isolates were considered pathogenic if they induced characteristic symptoms on test plants. The fungus was re-isolated from lesioned amaranth tissues by plating tissue segments in PCA. Plants of the same age as used above inoculated with sterile (pure) PCA served as controls.

Results

Field study

The changes in the population density of the two insect species on the field were observed to affect the percentage infection by BSRDB on *A. hybridus*. For the unsprayed plots, the correlation relationship was higher between *L. camerunus* population and percentage infection by BSRDB (r=0.84, P = 0.01) than between *G. rhomboidalis* population and percentage infection by BSRDB (r=0.75, P=0.05) (Fig. 1a). Insecticidally-treated plots showed averagely lower insect population with correspondingly lower percentage infection (Fig. 1b) for the two insect species.

Although the mean population density of *L. camerunus* was higher than that of *G. rhomboidalis* on the field under unsprayed conditions, the percentage diseased plants were not different from each other (Table 1). However, with insecticide application, the mean population density of *G. rhomboidalis* was appreciably reduced from 2.69 to 1.96 while that of *L. camerunus* was reduced from 4.39 to 2.03 with no significant difference between percentage diseased plants (Table 1).

Screenhouse study

In the screenhouse, *G. rhomboidalis* produced fewer number of oviposition holes (6.83) per plant than *L. camerunus* (8.16) (Table 1). However, 87.5 per cent of plants infested with *L. camerunus* showed BSRDB symptoms, while those infested with *G. rhomboidalis* showed no apparent symptoms on plants.

TABLE 1

Mean Population Densities and Oviposition Points in Relation to Percentage Disease Infection by Gasteroclisus rhomboidalis *Boh and* Lixus camerunus *Klobe under Sprayed and Unsprayed Regimes*

Insect	*Sprayed plots*		*Unsprayed plots*		*Insect inoculated*	*No. of plants showing BSRDB symptoms*
	Mean population density	*Mean percent diseased plants*	*Mean population density*	*Mean percent diseased plants*	*Mean number of oviposition points*	
G. rhomboidalis	1.96 ±1.55	8.10 ±4.11	2.69 ±1.10	15.29 ±8.88	6.83 ±2.85	0/24
L. camerunus	2.03 ±1.46	7.95 ±3.85	4.39 ±1.72	15.35 ±9.02	8.16 ±3.30	21/24

Laboratory study

Spores of the fungus contaminated with *Xanthomonas* sp. were only obtained from the appendages and abdominal parts of plates containing *L. camerunus* and virtually no spores were recorded from plates containing parts of *G. rhomboidalis*. All plates with abdominal sections of *L. camerunus* had spores while 20 per cent of plates containing appendages yielded spores.

Discussion

The obvious changes in percentage infection in relation to insect population show the importance of these weevils in the transmission of BSRDB. The higher correlation coefficient of *L. camerunus* population density with percentage disease infection compared with *G. rhomboidalis* may be an indication of the efficiency of *L. camerunus* as a vector of BSRDB on the field. The screenhouse results also clearly implicate *L. camerunus* as the most efficient vector of the pathogen during oviposition after acquiring inoculum from infected plants.

That the pathogen was isolated only from the appendages and abdominal parts and not the gut of the insects signify that it is carried externally. The detection of saprophytic *Xanthomonas* sp. in the medium as a secondary organism could be expected.

Attempts have been made to control the fungus on the plants using fungicide (Anon., 1984). The control of *L. camerunus* using appropriate insecticide before the insect reaches reproductive stage may, therefore, be a reasonable strategy for the management of this insect and BSRDB on *Amaranthus* in southern Nigeria.

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