Early screening for black leaf streak/black Sigatoka disease resistance under natural inoculation conditions

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Introduction

Black leaf streak/black Sigatoka (BLS) disease caused by *Mycosphaerella fijiensis* Morelet is considered as the major constraint to plantain production worldwide (Stover, 1983; Fouré, 1985). The disease reduces the functional leaf area and consequently the photosynthates which are needed for fruit filling. Plantain yield losses of 33% in the first and 76% in the second cropping cycle have been estimated at Onne, southeastern Nigeria (Mobambo, 1993; Mobambo *et al.*, 1993).

All known plantain cultivars collected from West and Central Africa, tropical America and Asia, are susceptible to BLS under Onne conditions (Mobambo *et al.*, 1994). Chemical control strategies exist, but are not feasible for the resource-poor farmers who grow the crop in Africa. The use of organic matter helps to enhance plantain growth and thus to reduce the BLS effects in backyard gardens (Mobambo and Naku, 1993; Mobambo *et al.*, in press). However, supply of organic matter on large scale plantations is still problematic (Nweke *et al.*, 1988; Ruhigwa *et al.*, 1994). Therefore, breeding for resistance seems the most appropriate way to control BLS.

The production of new *Musa* genotypes through breeding requires appropriate methods to evaluate host-plant response to BLS. Several artificial inoculation methods based on improved laboratory techniques have been described (Mourichon *et al.*, 1987; Fouré and Mouliom Pefoura, 1988). These methods are laborious and expensive because they require laboratory equipment and glasshouse facilities. Pasberg-Gauhl (1994) reported that *M. fijiensis* is a very slow growing fungus in culture and it is time consuming to produce adequate inoculum for artificial inoculations. Due to these limitations, and the problems associated with employing these methods in Africa, a simple and rapid evaluation method for determining the BLS resistance of a large number of plants is needed.

Screening is most reliable when it takes place under environmental conditions closely resembling those in which the crop is cultivated. It is also most efficient when carried out under conditions favouring full symptom expression as when there is an adequate disease pressure. Therefore, in this study we investigated the possibility of developing a rapid method to evaluate host response to BLS under natural conditions.

Materials and methods

The experiment was conducted at the International Institute of Tropical Agriculture's High Rainfall Station at Onne, southeastern Nigeria (04° 43'N, 07° 11'E) in the humid forest zone of the Niger Delta. The annual rainfall averages 2400mm in a monomodal distribution lasting from March to November. Annual mean temperature is 27°C. Relative humidity remains high throughout the year with mean values ranging from 62 to 97%. The soil at the experimental site is an ultisol derived from coastal sediments, it is well-drained, but poor in nutrients and highly acidic (Hulugalle *et al.*, 1990).

In vitro propagated plants of three plantain hybrids (TMPx 597-4, TMPx 548-4 and TMPx 548-9) were evaluated for reaction to BLS together with their female and male parents. These hybrids were obtained by crossing Obino l'Ewai (AAB 'Plantain', French type) (female parent) with the wild non-edible banana clone Calcutta 4 (*Musa acuminata* ssp. *burmannicoides*) (male parent) (Swennen and Vuylsteke, 1993).

Young tissue cultured derived plants were planted in polyethylene bags, filled with a mixture of top soil, palm fibre and poultry manure (7 : 2 : 1). After 8 weeks of acclimatization in the nursery, the plants were about 30 cm high. At this stage of development they were transferred to the field. The plants were set in a completely randomized design in a field established with the BLS susceptible Agbagba, (AAB 'Plantain', False Horn type) (Mobambo *et al.*, 1994). This guaranteed a high level of natural inoculum for the young plants. The experimental unit was five observable plants with four replicates. The plants in the polyethylene bags were watered when necessary. The experiment was conducted twice during the rainy season (May-August and August-November) of 1992.

The evaluation started when plants were 2 months old. Observations on leaves began three days from when they reached cigar leaf stage B (Ganry and Laville, 1983). On five subsequent leaves of each plant, the following parameters were measured (Meredith and Lawrence, 1970; Fouré, 1982, 1987).

- Incubation time (IT): days between cigar leaf stage B, when infection occurs, and appearance of first symptoms of the disease. The yellowish depigmentation on the lower leaf surface was considered as the first symptom (Fouré, 1982).
- Symptom evolution time (SET): days between the appearance of the first symptoms and the appearance of spots with dry centers (last symptom).
- Disease development time (DDT): days between cigar leaf stage B and the appearance of spots with dry centers.
- Youngest leaf spotted (YLS): number of the leaf bearing spots with dry centers counting down from the first open leaf.
- time of leaf (LTL): days between cigar leaf stage B and leaf death, either due to senescence or 100% of leaf area being spotted by BLS.

An ANOVA procedure, based on plot means (Kwanchai-Gomez and Gomez, 1984), was carried out for data analysis. Duncan's multiple range test at the 0.05 significance level was used to compare treatment means for each parameter.

Results and discussion

On young plants, differences between clones were significant for SET, DDT, YLS and LTL (Table 1). IT was very similar for all clones.

The male parent clone Calcutta 4 displayed a highly resistant response to BLS by stopping symptom development at the brown streak stage of the disease (stage 2) (Fouré, 1987). Similar results have been reported by Fouré *et al.* (1990) on the same banana clone Calcutta 4 under field conditions in Cameroon. Death of Calcutta 4 leaves after 81 days was due to leaf senescence and not to BLS disease.

BLS symptom development in the hybrids was slower than in Obino l'Ewai. In TMPx 548-4 and TMPx 548-9, the disease needed almost double the amount of time to develop the last symptom stage and 3-4 weeks longer to destroy the leaves. TMPx 548-4 and TMPx 548-9 were very similar in their host response to BLS, but both differed significantly from TMPx 597-4 which had a 9 day longer SET. Slower disease development on TMPx 548-4 and TMPx 548-9, prolongated the LTL by 2-3 weeks compared to TMPx 597-4. These results demonstrate the partial resistance of the hybrids and the susceptibility of the female plantain parent to BLS.

Table 1. *Host response to BLS of young plants of three plantain hybrids (TMPx) as compared to their plantain female parent Obino l'Ewai (AAB) and diploid banana male parent Calcutta 4 (AA) at Onne, southeastern Nigeria.*

Clone	Incubation time (days)	Symptom evolution time (days)	Disease development time (days)	Youngest leaf spotted	Life time of leaf (days)
Calcutta 4	10.2 a	-*	-*	-*	89.9 d
Obino l'Ewai	10.1 a	17.5 a	27.6 a	2.7 a	42.1 a
TMPx 597-4	10.2 a	24.8 b	35.0 b	3.4 b	52.1 b
TMPx 548-4	10.2 a	34.1 c	44.3 c	3.9 c	69.8 c
TMPx 548-9	10.2 a	33.6 c	43.8 c	3.9 c	65.1 c

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to Duncan's multiple range test.
* Calcutta 4 stopped symptom development of BLS at an early stage.

Table 2. *Host response to BLS of mature field established plants of three plantain hybrids (TMPx) as compared to their plantain female parent, Obino l'Ewai (AAB) and diploid banana male parent Calcutta 4 (AA) at Onne, southeastern Nigeria*

Clone	Incubation time (days)	Symptom evolution time (days)	Disease development time (days)	Youngest leaf spotted	Life time of leaf (days)
Calcutta 4*	14.2	-**	-**	-**	152.3
Obino l'Ewai	14.0 a	24.9 a	38.9 a	5.8 a	65.5 a
TMPx 597-4	14.0 a	52.0 b	66.0 b	9.0 b	94.2 b
TMPx 548-4	14.2 a	58.8 c	73.0 c	10.3 c	128.8 c
TMPx 548-9	10.1 a	61.0 c	71.1 c	10.7 c	127.8 c

Within columns, means followed by the same letter are not significantly different at the 0.05 probability level, according to Duncan's multiple range test.
* Data of Calcutta 4 are taken from another field trial and therefore not included at the statistical analysis
** Calcutta 4 stopped symptom development of BLS at an early stage.

No significant differences between clones were found for the IT. This indicates that IT is not a useful parameter to distinguish host-plant response to BLS. Similar observations have been reported from the evaluation of 110 different plantain cultivars at Onne Station (Mobambo *et al.*, 1994). On young plants, IT was 10 days which was 4 days faster than on field established mature plants. On Grande Naine (AAA 'Cavendish'), an IT of 10 days has been observed by Fouré and Mouliom Pefoura (1988) after artificial inoculation in a screenhouse. Since IT is the period from infection until the appearance of first symptoms, the 10-days IT on small plants confirms that, under natural inoculation conditions, leaves are infected by BLS at the cigar stage.

IT is the same for all clones and has already been reported as an unreliable parameter to differentiate host response to BLS of different clones under both field (Fouré, 1982, 1987; Fouré *et al.*, 1990; Mobambo *et al.*, 1994) and controlled conditions (Beveraggi *et al.*, 1993). Only SET, which is the interval from the first to the last symptom stage, allows susceptible and resistant *Musa* clones to be distinguished. However, sometimes it is very difficult to recognize the first symptom stage on the leaves. Therefore, the easiest parameter to evaluate host response to BLS in *Musa* germplasm is DDT. Only the dates of the leaf at the cigar stage and the last symptom stage, which are easy to recognize, need to be recorded.

Host-plant response to BLS changed on the five consecutive leaves observed for all the parameters studied. In all clones, IT, SET and LTL values increased from the first to the fifth evaluated leaf. These results suggest that field adaptation of young plants has an influence on host response to BLS. Both early and late symptom stages developed faster on earlier emerged leaves. Changes in plant physiology in different growth stages were found to have an effect on BLS development of 110 plantain cultivars at Onne (Mobambo *et al.*, 1994). Differences in host response between different clones are maintained on plants at a similar stage. Therefore, it is important to compare host response only on plants of a similar age and under similar climatic conditions.

In a field experiment (Mobambo, 1993) using the same clones, mature plants six months old (Table 2) were evaluated during the same season as young plants (Table 1). The similarity in significance levels between clones of young and mature plants indicates that the evaluation of host response to BLS in *Musa* germplasm on young plants is reliable and efficient. With young plants, the disease evaluation was also faster than with mature plants. Young plants showed the first symptoms of BLS much earlier than mature plants. In young plants, the disease needed only half the time to develop the last symptom stage, as compared to mature field plants. As suggested by Pasberg-Gauhl (1990), young, *in vitro* multiplied plants have potential for use in screening for BLS resistance.

Conclusion

Evaluation of young *Musa* plants for response to *M. fijiensis* with natural inoculum from an infected plantain field confirmed the resistance of hybrids and the susceptibility of the female plantain parent and results were comparable to those obtained on mature plants of the same clones. This method was also much faster in evaluating host response to the disease than field assessment. This is an advantage for early BLS screening, because it requires less that half the time to determine the host response to the disease compared to field trials. The early screening method is also cheaper, less labor intensive for maintenance purposes and it requires much less field space than field assessment. In view of the limitations of technology in African countries, the early screening method under natural infection conditions seems more convenient and cheaper than artificial inoculation methods. Highly susceptible plant material can be eliminated at this early screening stage, thus reducing the number (and cost) of mature plants to be tested later in the field.

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East Africa

Hot water treatment for banana planting material made easier

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Parasitic nematodes such as *Pratylenchus goodeyi*, *Radopholus similis*, *Helicotylenchus multicinctus* and the banana weevil, *Cosmopolites sordidus*, are obnoxious pests threatening banana cultivation in the African continent. Hot water treatment to pared suckers at 55°C for 15 to 25 min has been found effective in the elimination of these pests and is in practice in Australia and the Americas (Stover, 1972). Although this treatment is considered superior to nematicidal dips, the technique is quite difficult to manage because of the critical balance required between a temperature that is lethal to nematodes in the corm tissue and one that causes permanent damage to the plant (Inomoto and Monteiro, 1989; Gowen and Quénéhervé, 1990). In South Africa, the method is not recommended due to the cost and the equiment needed for such treatment (Jones and Milne, 1982). In the present study, an attempt has been made to simplify this effective technique using cheap material which would be easily available to resource-limited, small-scale farmers.

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Materials used in the study were paraffin wax with a melting point of 55-58°C, an iron plate (3 x 3 cm and weighing about 10 g) and a white pith block (3 cm^3). The pith block was fixed to the iron plate using a thin film of molten wax. This apparatus was allowed to sink in a water tank (half-empty oil drum) in which the banana material to be hot water treated had been placed. Wood was burnt underneath the water tank to raise the temperature of the water. The temperature of the water was recorded using a thermometer. When the temperature rose to 55°C, the wax film between the iron plate and the pith melted and the pith was released. This rose to the surface of the water where it floated. The burning firewood underneath the water tank was then removed. The temperature in the water tank was maintained at 55 ± 2°C for 15-20 min even after the removal of the firewood. The above process was repeated using church candle wax with similar results.

Materials used in this study are cheap, locally available, within the reach of the small-scale farmer, easily assembled and easy to use. The farmer knows that he must remove the fire once he sees the pith floating in the tank after its release from the iron plate. He need not use any thermometer to record the temperature. Both the pith and the iron plate are reusable. A small piece of wood could be used in place of the pith. Hence, with these advantages, it is felt that it would be a suitable technique for practice by small-scale farmers. This technique is recommended to extension services in countries where banana or plantain are grown by smallholders.

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East Africa

Backyard banana cultivation in Uganda

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Introduction

Banana plants are heavy feeders and as a result, their nutrient requirements cannot be supplied indefinitely by any soil. Thus the need to use fertilizers and manures (Purseglove, 1988). Declining soil fertility has been considered a primary production constraint for highland banana production (Rubaihayo, 1990; Sebasigari, 1991; Stover, 1991) and the cause of production decline after a few years of planting (Rubaihayo, 1991). Plots near homesteads, however, are known to have high growth status by colour of their leaves and fruit sizes which has been attributed to high organic matter and nutrient levels resulting from the disposal of household refuse in these plots. Swennen (1990) reported that soils from backyard gardens are rich in organic matter and nutrients from household refuse and that plants in these plots grow luxuriantly, become very large and produce heavy bunches. The objectives of the study reported in this paper was to access the causes of high growth and yield performance of banana plants in these gardens.

Materials and methods

Twelve farmers where household refuse was disposed of in plots near homesteads were selected from rapid rural appraisal survey sites (Rubaihayo, 1991). For each farmer, two plots, one far from the family house (distant plot) and another near the home where household refuse was utilized (home plot), both with about 25 stools, were demarcated. Three soil samples per plot per site were taken at a depth of 0-15 cm and analysed. Data on banana growth characters were collected at two months interval, while data on bunch characters were collected when the bunches matured and the total yield per hectare was estimated.

Results and discussion

Soil nutrient analysis results are presented in Table 1. Statistical analysis show than home plots had significantly higher potassium and calcium levels than distant plots. The results also indicated that levels of all parameters recorded in all plots were well above minimum levels necessary for normal growth.

Table 1. *Soil nutrient contents for near home and distant plots from homestead*

Plot	pH	OM (%)	AVP (ppm)	N (%)	K me/100g	Na me /100 g	Ca (ppm)
Distant	6.4±0.1	8.42±0.83	61.97±18.05	0.20±0.02	1.84±0.39	0.18±0.03	7.67±0.85
Home effect	7.0±0.1	10.32±0.94	106.45±22.08	0.24±0.21	4.23±0.65	0.16±0.01	10.03±1.54
Critical values	5.2	3.00	5.00	0.12	0.34	-	-
LSD (0.05)	-	2.10	48.88	0.06	1.29	0.06	1.76

OM: Organic matter - AVP: Available phosporus - ppm: parts per million - me: milliequivalent
LSD: Least significant difference

Table 2. *Mean bi-monthly growth changes and mean yield readings in home and distant plots*

Plot	Height (cm)	Girth at 100 cm	Leaves added	Bunch weight (kg)	Hands/ bunch	Fingers/ bunch	Estimated yield tonnes ha^{-1}
Distant	25.0±1.1	5.1±0.6	3.7±0.1	14.9±1.3	7.9±0.3	92.8±3.7	10.6±1.0
Home effect	63.4±2.9	12.8±0.8	4.6±0.2	20.5±2.3	9.1±0.5	121.6±7.4	17.6±1.7
LSD (0.05)	4.1	1.9	0.4	3.3	0.7	15.8	2.3
Cv (%)	13.56	30.42	15.11	27.02	11.22	21.41	23.97

Plant growth and yield statistics for the two plots are presented in Table 2. For the three growth parameters recorded, home plots had significantly higher values than the distant plots. Similar high growth status was earlier reported by Swennen (1990) and Stover (1991). The higher growth rates in the home plots were attributed to a better potassium status and higher soil moisture content. Higher levels of moisture were due to rain water from roofs running into home plots and the effect of refuse mulch in reducing evaporation. A similar positive trend was recorded for yield data in the home over distant plots. Bunch weight in home plots was an average 37.6% greater than distant plots. Yield expressed as tonnes ha^{-1} was estimated to be 66% greater in home plots.

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