Rapid assessment of *Musa* for reaction to Sigatoka disease

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Received: October 1993
Accepted: February 1995

Fruits, vol 50, n°1, 11-22

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ABSTRACT

A technique for the rapid assessment of *Musa* for reaction to Sigatoka disease (*Mycosphaerella musicola*) was devised. The youngest leaves of plants grown from *in vitro* plantlets were inoculated with mycelium fragments of *M musicola* when 19-22 cm in length. After incubation at 25°C under continuous mist for 7 d and then in a growth cabinet with a 16L/8D photoperiod for 23-34 d, plants were rated for disease reaction. Susceptibility was defined as the development of profuse, mature lesions; partial resistance as the development of a few, immature lesions and extreme resistance as the failure of any lesions to develop. Wild *Musa* species, banana and plantain cultivars and breeding lines were screened for disease resistance. The results are analysed in the light of known responses of germplasm to Sigatoka and current knowledge on *Musa-M musicola* interactions. Limitations of the methodology are discussed.

KEYWORDS

Australia, *Musa*, disease resistance, *Mycosphaerella musicola*, vitroplants, contamination, symptoms, variety trials.

Évaluation rapide de la réaction du genre *Musa* à la maladie de Sigatoka.

RÉSUMÉ

Une technique d'évaluation rapide de la réaction des bananiers à la maladie de Sigatoka (*Mycosphaerella musicola*) a été élaborée. Les plus jeunes feuilles de plants issus de culture *in vitro* ayant atteint 19 à 22 cm de long ont été inoculées avec des fragments de mycélium de *M Musicola*. Après 7 j d'incubation à 25°C en conditions d'humidité continue, puis 23 à 34 j de culture avec une photopériode de 16 h jour / 8 h nuit, la réaction des plants à la maladie a été évaluée. Les plants sensibles présentent de nombreuses lésions matures, les plants partiellement résistants ont quelques lésions immatures et les plants les plus résistants n'ont aucune lésion. Des espèces sauvages et des cultivars de bananiers et de plantains ont été analysés. Les résultats ont été confrontés aux connaissances concernant les réponses des variétés à la maladie et les interactions entre le genre *Musa* et *M musicola*. Les limites de la méthode ont été abordées.

MOTS CLÉS

Australie, *Musa*, résistance aux maladies, *Mycosphaerella musicola*, vitroplant, contamination, symptôme, essai de variété.

Evaluación precoz de la sensibilidad de los plátanos a la enfermedad de Sigatoka.

RESUMEN

Se elaboró una técnica rápida de evaluación de la reacción de los *Musa sp* a la enfermedad de Sigatoka (*Mycosphaerella*). Se inoculó un triturado miceliano de *M Musicola*. Tras 7 d de incubación a 25°C en condiciones de humedad continua, luego de 23 a 34 d de cultivo con un fotoperíodo de 16 horas de día y 8 horas de noche, se observó la reacción de las plantas. Se evalua la sensibilidad mediante el desarrollo de numerosas lesiones necróticas; la resistencia parcial corresponde a la aparición de algunas lesiones recientes y la resistencia total a la ausencia de síntomas. Se analizaron especies silvestres así como variedades de bananos y plátanos. Los resultados obtenidos en condiciones controladas fueron comparados con el comportamiento de los diferentes bananos en condiciones naturales. Se abordaron los límites de dicho método.

PALABRAS CLAVES

Australia, *Musa*, resistencia a la enfermedad, *Mycosphaerella musicola*, vitroplantas, contaminación, síntomas, ensayos de variedades.

introduction

Sigatoka, caused by *Mycosphaerella musicola* Leach, is the major leaf disease of banana in commercial production areas in Australia. It is very serious in North Queensland, where warm and wet environmental conditions in the summer months favour development on Williams (Cavendish subgroup), the main cultivar grown. The disease is kept in check by regular spray programmes with the protectant fungicides mancozeb and oil or chlorothalonil. The systemic fungicide propiconazole is applied when inoculum pressure is high, but its continuous use is discouraged because of fears of the evolution of fungicide-resistant strains of the pathogen.

Work on the effect of Sigatoka severity banana production and greenlife of bananas indicates that more than ten leaves at harvest are necessary to obtain optimum yields or marketable fruit (RAMSEY *et al*, 1990). Good control is therefore needed if the grower is to achieve maximum returns. In 1990, the cost of control was estimated to be A$820/ha/year or 14% of total production costs.

One of the main strategic goals of the Queensland Department of Primary Industries (QDPI) is to reduce amounts of chemicals used in agriculture, because of health and environmental concerns. To achieve this aim with banana, two approaches to the problem are being adopted. The first involves adapting prediction systems based on climatic data or disease-monitoring techniques that have been developed for the control of Sigatoka diseases overseas (GANRY and LAVILLE, 1983; WIELEMAKER, 1990) to Queensland conditions. This will lead to more timely applications of fungicides and reduce the total number of sprays necessary for adequate control. The second is to find an agronomically suitable banana cultivar that has good resistance to Sigatoka. If such a banana is found, the cost of control will be substantially reduced or even eliminated.

As a first step in a long-term project based on the second approach, germplasm was collected from field collections of banana cultivars in South East Asia and obtained from the Fundacíon Hondureña de Investigacíon Agrícola (FHIA) banana breeding program in Honduras. QDPI also has access to wild banana species and diploid banana cultivars collected in Papua New Guinea (PNG) by the International Board for Plant Genetic Resources (IBPGR) in 1988–1989.

Screening for resistance to Sigatoka in the field is time-consuming and expensive. One of the immediate requirements of the project was to devise a method whereby banana germplasm could be evaluated quickly and cheaply in the laboratory or greenhouse. *Musa* can now be routinely propagated in tissue culture (KRIKORIAN, 1989), with the possibility of providing small plants suitable for such work. Provision of a reliable supply of inoculum of the pathogen is, however, more difficult. It is impractical to collect conidia from diseased banana plants on a regular basis, and it is difficult to induce profuse sporulation in *in vitro* cultures of *Cercospora* and related genera (GOODE and BROWN, 1970; EL-GHOLL *et al*, 1982). Sporulating isolates of *Mycosphaerella* spp have been used in inoculation experiments (GOOS and TSCHRICH, 1963; MOURICHON *et al*, 1987), but the authors were unable to identify isolates of *M musicola* that would reliably and continually produce conidia. Mycelium of *M fijiensis*, the cause of black leaf streak disease of banana, has been successfully used as inoculum, although symptom development was reported to be slower than with conidia (MOURICHON *et al*, 1987; FULLERTON and OLSEN, 1991), and this approach was pursued in the present study.

This paper reports the development of a rapid screening technique to determine Sigatoka reaction using fragments of mycelium as inoculum and preliminary results from tests undertaken on selected *Musa* germplasm.

materials and methods

isolation and maintenance of pathogen

Necrotic banana leaves with mature lesions of Sigatoka disease were collected from plants at Maroochy Horticultural Research Station, Nambour, Queensland. Leaf samples were incubated under high humidity for 48 h and then

cut into 5 cm squares. After immersion in a 2% solution or sodium hypochlorite to sterilise surface contaminants, the leaf sections were rinsed in water and stapled to pieces of paper towel with the abaxial surface outwards. After soaking in water for a further 3 min, the paper towel pieces with attached leaf sections were pressed onto petri dish lids. The lids were placed on petri dishes containing 3% water agar. Single ascospores of *M musicola*, which were discharged from perithecia in mature lesions onto the agar surface within 2 h at 25°C, were transferred to 4% potato dextrose agar (PDA). Fragments of colonies which developed were used to initiate subcultures on PDA. Samples of fungal mycelium of each isolate were stored in liquid nitrogen (SMITH and ONIONS, 1983) and reconstituted when cultures being used to produce inoculum lost their virulence.

preparation of inoculum

Two, single spore isolates of *M musicola* from the same leaf section, designated A2 and A3, were routinely used to provide inoculum for experiments. Colonies were grown on PDA at 25-26°C, the optimum temperature for growth of *M musicola* in culture (MEREDITH, 1970), until at least 20 mm in diameter. Pink surface mycelium was removed from the underlying dark, compact, hyphal matrix of each colony and weighed. After maceration for 120 s in a small volume of sterile distilled water using a mortar and pestle, the suspension of hyphal fragments was further diluted with sterile distilled water. Aliquots of 1.0 ml of suspension containing a known weight of mycelium were measured into glass vials prior to inoculation.

growth and inoculation of banana plants

Tissue cultures of banana cultivars, breeding lines and wild *Musa* species originally derived from apical meristems or embryos were removed from culture jars and planted in small pots in pasteurised peat/sand (1:2) mix. Plantlets were incubated in the shade under high humidity for 7 d before pots were transferred to controlled temperature cabinets in a glasshouse. Here, plants were grown at 25-26°C under conditions of natural light for 2-4 months before inoculation. Active growth was sustained by replanting into larger pots when necessary and using granules of slow-release fertiliser.

Plants were selected for inoculation when the lamina of the youngest leaf was 19-22 cm in length. The youngest leaf was marked using a black felt-tipped pen for future identification and the dimensions of the leaf blade noted. In each experiment, leaves were inoculated with 0.05 g of mycelium in 1.0 ml of sterile distilled water. This amount of inoculum had been found in preliminary studies to give an acceptable level of infection in susceptible cultivars. The 1.0 ml aliquot of inoculum in the vial was usually applied to the underside of the leaf as a fine mist using a gas-powered spray can. Afterwards, 0.5-1.0 ml of sterile distilled water was used to flush out the vial and applied in the same way. Immediately following inoculation, plants were placed in a controlled temperature cabinet at 25-26°C under conditions of natural light and almost continual mist generated from a humidifier. Plants were usually transferred to artificially lit (200-500 $\mu mol/m^2/s$) growth cabinets on a 16L/8D photoperiod at 25-26°C after 7 d under mist.

experiments

In an initial experiment, the effect of the duration of misting on infection was determined by inoculating the lower leaf surfaces of 14 plants of cv Williams and transferring two plants after 1, 2, 3, 4, 5, 6 and 7 d to a similar, naturally lit cabinet at an identical temperature regime, but without mist. After 7 d, all plants were transferred to growth cabinets as previously described.

In another early experiment, the effect of inoculating the upper leaf surface was investigated by comparing disease development in six plants, three of which were inoculated on the upper surface and three on the lower surface. These plants were misted for the usual 7 d before transfer to growth cabinets.

In experiments to determine the reaction of cultivars, breeding lines and *Musa* species to *M musicola*, at least three plants of each accession were inoculated during the course of the study. In each experiment, plants of either Williams or Grande Naine, susceptible cultivars

in the Cavendish subgroup, were inoculated and used as controls.

reaction assessment

Leaves were examined at regular intervals for disease symptoms. Between 30 and 41 d after inoculation, when symptoms on susceptible control cultivars incubated at 25-26°C had fully developed, experiments were terminated and leaves assessed visually for disease severity as indicated in table I. Extremely resistant, partially resistant and susceptible reactions are illustrated in photo 1. Photographic records were kept of inoculated leaves when experiments were concluded. No information was recorded in experiments where control plants failed to develop a susceptible reaction. Initially, this occurred every 3-4 months when cultures lost virulence after continuous subculturing on PDA. Later, loss of virulence was anticipated. Fresh cultures of isolates were initiated and stock built up and used as inoculum before problems were encountered with old cultures.

results

effect of duration of mist on infection

No symptoms developed on plants of cv Williams that had been misted for 1 and 2 d. A few lesions developed on plants under mist for 3, 4 and 5 d. Numerous lesions were observed on plants transferred after 6 and 7 d.

effect of inoculating the upper leaf surface

No symptoms developed on plants of cv Williams inoculated on the upper leaf surface and misted for 7 d. Numerous lesions developed on those plants inoculated on the lower surface.

symptom development on cv Williams under mist for 7 d following inoculation of the lower leaf surface

Faint circular chlorotic spots about 1-2 mm in diameter appeared simultaneously on both surfaces of the inoculated leaf beginning 12-16 d after inoculation. These spots enlarged and a faint brown discolouration became noticeable when the diameter exceeded 3-5 mm. In some cases, the colour of the spot deepened to a reddish-brown. Later, the leaf tissue within and surrounding the spot appeared water-soaked and translucent. Guttation often occurred at this stage and a drop of liquid was noticeable on the spot at the beginning of the light cycle in the growth cabinet. This liquid dried during the light cycle to leave a shiny, white deposit. Death of the leaf tissue within the spot followed quickly, resulting in a dark brown, necrotic lesion. The colour of the lesion slowly changed to light brown and surrounding leaf tissue became translucent and died, thus enlarging the area of necrosis. The interior of the expanded lesion later became grey. A dark brown, well-defined border developed around the lesion. Surrounding leaf tissue, especially between the lesion and the leaf margin, often turned yellow. A large number of discrete and mature lesions had formed at 30 d after inoculation. Spermagonia and conidiophores with conidia were sometimes seen on grey tissue after 40 d.

The development of the lesion through these stages was not simultaneous. At any one time, there were spots and lesions in various stages of development. It was not uncommon for mature lesions and chlorotic spots to be present on the same leaf during symptom evolution.

reaction of *Musa* germplasm

The reaction of *Musa* species, breeding lines and cultivars to inoculation with *M musicola* is presented in table II. Reactions sometimes varied between plants of the same accession if more than one was included in each experiment and also between plants of the same accession in different experiments. The reactions recorded are the most susceptible observed for each accession.

discussion

Conditions of continuous mist at night alternated with periods (6-8 h) of lower humidity during the day have been shown to enhance penetration and disease development when conidia of *M musicola* have been used as inocu-

lum (GOOS and TSCHIRCH, 1963). A similar technique utilising fluctuating humidity has been used by MOURICHON *et al* (1987) working with both conidia and mycelium of *M fijiensis*. However, early work in this study showed that good symptom development occurred if plants remained under continuous mist for 7 d after inoculation and, as it was more convenient to permanently maintain this environment in the controlled temperature cabinet, it was adopted as standard procedure.

In other studies (GOOS and TSCHIRCH 1963; MOURICHON *et al*, 1987), workers inoculated only the lower surface of banana leaves because it has been reported to be more susceptible to infection than the upper leaf surface (STAHEL, 1937; SIMMONDS, 1939). Work reported here indicates that inoculation of the lower leaf surface is probably essential for good disease development as no symptoms appeared following inoculation of the upper leaf surface.

The stages of symptom development on young plants of cv Williams closely paralleled the susceptible reaction observed in naturally infected plants (MEREDITH, 1970). However, the speed of development of symptoms was quicker than has been recorded in the field. This may be because inoculated plants were held continuously at temperatures which were optimum for growth of the pathogen. In addition, streak and spot symptoms tended to be circular and not elongated as occurs on mature field inoculated plants. Round lesions are characteristically found on young plants and are associated with disease development in juvenile tissue (MEREDITH, 1970).

The reaction of *Musa* germplasm to inoculation with mycelium of *M musicola* was analysed visually after sufficient time had elapsed for the development of symptoms on susceptible control cultivars. It can be argued that this is a subjective rating, but it is considered a more reliable method than comparing the number of days after inoculation to the appearance of various stages of development of symptoms. Although time from infection to symptom expression is an established method of determining reaction in plants in the field to Sigatoka diseases, results of tests with young plants of the same cultivar have been variable (FOURÉ, 1990). This variability may be related to differences in the density of infection in different plants which is known to influence the incubation period and time from appearance of first symptoms to mature spot formation (MEREDITH, 1970). Other factors associated with plant physiology may also be involved. Observing disease levels over the whole leaf area after symptoms had had time to fully evolve on susceptible

Table I
Criteria used to assess *Musa* germplasm for reaction to *M musicola* 30-41 days after inoculation.

Reaction	Assessment
Necrotic fleck response or no visible symptoms. Spots fail to develop	Extremely resistant (ER)
Some necrotic lesions, but no fully developed mature spots	Partially resistant (PR)
Numerous mature spots	Susceptible (S)

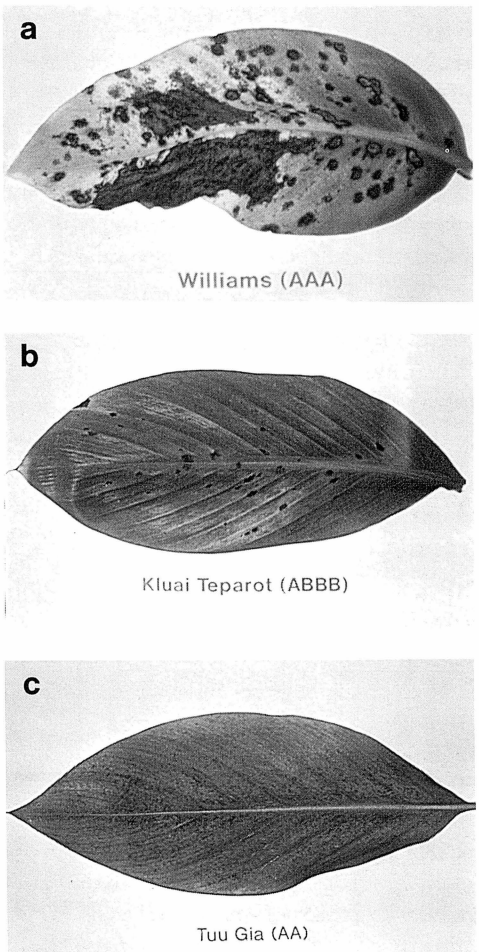

Photo 1
Sigatoka disease symptoms on leaves: between 30 and 41 d after inoculation, when symptoms on susceptible control cultivars incubated at 25-26°C had fully developed, leaves were assessed visually for disease severity.
a) Leaf of Williams (AAA genotype), susceptible cultivar in the Cavendish subgroup;
b) Leaf of Kluai Teparot (ABBB genotype), partially resistant to M musicola *cultivar;*
c) Leaf of Tuu Gia (AA genotype), highly resistant cultivar.

control cultivars was considered more appropriate for determining reaction. Even so, although key parameters such as size of inoculated leaf, inoculum weight and incubation conditions following inoculation were constant, the reaction of a number of cultivars was inconsistent. The unreliability of the method in some instances is a drawback, but this can be overcome to a certain extent if sufficient numbers of plants are tested; the most susceptible reaction becoming the documented response.

Clones rated as extremely resistant (table II) did not develop lesions during the course of experiments. Either no symptoms were observed or disease development ceased after a necrotic fleck (hypersensitive) response. The latter type of response is probably analogous to that of cv Yangambi Km 5 challenged by *M fijiensis* (MOURICHON *et al*, 1987). Here the reaction progresses no further than a small, rusty brown fleck.

The partially resistant response is expressed by the slow development of lesions so that when experiments were terminated, fewer, more immature lesions were visible as compared to the susceptible response. This type of resistance is possibly controlled by multigenic factors and may ultimately be of more value than resistance based on the hypersensitive response which, if controlled by a single gene, may be overcome by a simple mutation of the pathogen.

An analysis of the reactions of *Musa* species to *M musicola* shows that accessions of *M acuminata* spp *burmannica, M balbisiana, M boman, M lolodensis, M peekelii* spp *angustigemma* and *M schizocarpa* tested had high levels of resistance. However, all but the one accession of *M acuminata* spp *banksii* were susceptible (table II). In field screening trials in Honduras, VAKILI (1968) found that of 26 accessions of *M acuminata* spp *banksii* tested, 14 were susceptible, 7 were partially resistant and 5 were resistant to *M musicola*. The Madang and Samoa accessions of spp *banksii* have also been observed to be susceptible (SHEPHERD, 1990; VAKILI, 1968) thought that the high level of susceptibility could have been because it is the only subspecies of *M acuminata* that is indigenous to the island of New Guinea and some South Pacific Islands and may have been isolated from *M musicola* in its natural habitat. This may be true, as there is no firm evidence to suggest *M musicola* has ever been present in coastal areas of PNG (SHAW, 1984) where *M acuminata* spp *banksii* is found.

Ten *Musa* diploids have been suggested as being suitable as standards for use in determining if pathogenic variation of *M musicola* and *M fijiensis* occurs (FULLERTON and STOVER, 1990). These standard clones are being distributed by the International Network for the Improvement of Banana and Plantain (INIBAP) to interested parties so that reaction might be assessed at different locations around the world. Most of the set was tested in this work (table II).

The Calcutta IR 124 accession of *M acuminata* spp *burmannica* was recorded as extremely resistant, as reported earlier. Tuu Gia was also found to be highly resistant. Both of these results are in agreement with reactions indicated by FULLERTON and STOVER (1990). However, the Pahang IR 296 accession of *M acuminata* spp *malaccensis* was rated as only partially resistant in this work and not as highly resistant as proposed by FULLERTON and STOVER (1990). This species has been reported as having strong resistance to *M musicola* in Honduras (VAKILI, 1968) and Brazil (SHEPHERD, 1990). In Brazil, it is used as a source of resistance in the banana-breeding program (SHEPHERD, 1990). It is also highly resistant to *M fijiensis* in the field in the South Pacific (FULLERTON, 1990), but not always in glasshouse tests using young plants (FULLERTON and OLSEN, 1991). The Sigatoka resistant cv Buccaneer (AAAA), which was bred using *M acuminata* spp *malaccensis* (SHEPHERD, personal communication), was also only partially resistant in tests reported here (table II). It is possible that the resistance of *M acuminata* spp *malaccensis* to Sigatoka diseases is expressed more in mature leaf tissue than in juvenile tissue.

The reaction of the standard Pisang Mas cultivar, Figue Sucrée, was susceptible, which is in agreement with FULLERTON and STOVER (1990), but cv Pisang Berlin was rated as partially resistant and not susceptible. Surprisingly, cv Mambee Thu, which was suggested as being susceptible by FULLERTON and STOVER (1990), was found to be extremely resistant. NBA 14 (SF 215) was rated as extremely resis-

Table II
The reaction of young plants of selected *Musa* germplasm to inoculation with *M musicola*.

Musa germplasm	*Subgroup/ Breedinginformation*	*Additional information*	*QDPI tissue culture code*	*Number of plants inoculated*	*Reaction*
Wild species					
M acuminata ssp *banksii*		Collected at Erima, Madang, PNG	PNG 151	5	PR
M acuminata ssp *banksii*		Collected at Ombisusu, Oro, PNG	PNG 251	5	S
M acuminata ssp *banksii*		Collected at Ambogo, Oro, PNG	PNG 255	4	S
M acuminata ssp *banksii*		Collected at Kiunga, Western, PNG	PNG 269	3	S
M acuminata ssp *banksii*		Collected at Manus Island, PNG	PNG 292	3	S
M acuminata ssp *banksii* x *M schizocarpa*		Collected at Brahmin, Madang, PNG	PNG 180	5	PR
M acuminata ssp *burmannica* (Calcutta IR 124)		ex INIBAP (KUL BS249)	20.19	7	ER
M acuminata ssp *malaccensis* (Pahang IR 296)		ex INIBAP (KUL BS609)	20.22	6	PR
M balbisiana		Collected at Oro Bay, Oro, PNG	PNG 238	3	ER
M boman		Collected at Sosi, Weskepik, PNG	PNG 051	3	ER
M jackeyi		Native to North Queensland, Australia	00.06	3	ER
M lolodensis		Collected at Yawreng, East Sepik, PNG	PNG 364	5	ER
M maclayi ssp *ailuluai*		Collected at Sebutuia Bay, Fergusson I, PNG	PNG 340	3	ER
M maclayi ssp *maclayi*		Collected at Sebutuia Bay, Fergusson I, PNG	PNG 339	3	ER
M peekelii ssp *angustigemma*		Collected at Erima, Madang, PNG	PNG 150	3	ER
M peekelii ssp *angustigemma*		Collected at Yelso, Madang, PNG	PNG 158	3	ER
M schizocarpa		Collected at Ngaswampum, Marobe, PNG	PNG 042	8	ER
M schizocarpa		Collected at Gobari, Marobe, PNG	PNG 047	3	ER
Fe'i banana					
Wain		Collected at Bietata, Madang, PNG	PNG 177	3	ER
Skai		Collected at Ningerum, Western, PNG	PNG 274	3	ER
Menei		Collected on Manus Island, PNG	PNG 293	3	ER
Sar		Collected on Manus Island, PNG	PNG 294	4	ER
Utafun		Collected at Namasalang, New Ireland, PNG	PNG 311	5	ER
Asupina		Collected at Amaho, East Sepik, PNG	PNG 361	3	ER
AA genotype					
Sucrier	Pisang Mas	QDPI selection, Australia	20.01	6	ER
Amas	Pisang Mas	ex Philippines	20.06	8	S
Figue Sucrée	Pisang Mas	ex INIBAP (KUL BS107)	20.18	6	S
Pisang Mas	Pisang Mas	ex FHIA Collection, Honduras (AVP28)	BAN 52	5	S
Inarnibal	Pisang Lemak Manis	ex Davao Collection, Mindanao, Philippines	20.13	6	PR
Inarnibal	Pisang Lemak Manis	ex FHIA Collection, Honduras (II257)	20.46	3	ER
Lonsing		ex Davao Collection, Mindanao, Philippines	20.14	3	PR
Kluai Hom		ex Pakchong Collection, Thailand	BAN 10	4	S
Kluai Pa		ex Pakchong Collection, Thailand	20.11	6	PR

Table II (continued)

Musa germplasm	*Subgroup/Breeding information*	*Additional information*	*QDPI tissue culture code*	*Number of plants inoculated*	*Reaction*
Fa'i Kumakuma		ex Western Samoa	20.43	6	ER
Paka	D1	ex Zanzibar *via* Jamaica	BAN 8	5	ER
NBA 14 (SF215)		ex INIBAP (KUL BS267)	20.20	5	ER
Niyarma Yik (NBB19/SF248)		ex INIBAP (KUL BS269)	20.21	5	PR
Tuu Gia		ex INIBAP (KUL BS610)	20.23	7	ER
Pisang Berlin	Pisang Lemak Manis	ex INIBAP (KUL BS611)	20.24	20	PR
Mambee Thu		ex INIBAP (KUL BS612)	20.25	5	ER
Amau	Kokadja	ex FHIA Collection, Honduras (III154)	20.45	12	S
Gu Nin Chio		ex FHIA Collection, Honduras (II249)	BAN 47	4	ER
SH3142	Pisang Jari Buaya in ancestry	ex FHIA breeding program, Honduras	20.05	11	S
SH3362	SH3142 x SH3217	ex FHIA breeding program, Honduras	20.03	12	S
NBC 20		ex Biological Foundation Collection, Laloki, PNG	20.27	7	ER
Galum		Collected at Vunapalading, East New Britain, PNG	PNG 002	3	PR
Aivip		Collected at Vunalir, East New Britain, PNG	PNG 020	3	S
Taputaput		Collected at Rapitok No3, East New Britain, PNG	PNG 027	2	PR
Tapo		Collected at Vunapope, East New Britain, PNG	PNG 029	3	S
Waimara		Collected at Situm, Morobe, PNG	PNG 043	5	PR
Hung tu		Collected at Situm, Morobe, PNG	PNG 044	3	S
Yenai	Pisang Mas	Collected at Sosi, East Sepik, PNG	PNG 052	5	S
Somani		Collected at Mundiharanji, East Sepik, PNG	PNG 064	4	PR
Gorop		Collected at Vunapalading, East New Britain, PNG	PNG 103	6	S
Agul		Collected at Vunapalading, East New Britain, PNG	PNG 112	3	S
Tubanator		Collected at Kokopo, East New Britain, PNG	PNG 139	3	PR
Vudu Beo		Collected at Kokopo, East New Britain, PNG	PNG 141	4	PR
Luba		Collected at Kokopo, East New Britain, PNG	PNG 146	5	PR
To'o		Collected in Brahmin, Madang, PNG	PNG 179	5	PR
Meleng		Collected in Madang City, PNG	PNG 188	3	PR
Inori		Collected at Koipa, Oro, PNG	PNG 228	3	S
Meinje		Collected at Embi, Oro, PNG	PNG 237	7	PR
Maka		Collected at Pongani, Oro, PNG	PNG 243	3	PR
Porapora		Collected at Mendi, Southern Highlands	PNG 256	5	PR
Malka		Collected at Mendi, Southern Highlands	PNG 257	3	PR
Grupnai		Collected at Somogos, Western Highlands, PNG	PNG 278	3	S
Kwonta		Collected at Murua Gulf, PNG	PNG 286	3	PR
Tainga		Collected at Murua Gulf, PNG	PNG 288	3	S
Te'engi		Collected at Luwaita, East Sepik, PNG	PNG 358	3	PR
Tamat		Collected on Manus Island, PNG	PNG 296	6	S
Papat		Collected at Kavieng, New Ireland, PNG	PNG 297	3	S

Table II (continued)

Musa germplasm	*Subgroup/Breeding information*	*Additional information*	*QDPI tissue culture code*	*Number of plants inoculated*	*Reaction*
AAA genotype					
Gros Michel	Ambon	QDPI selection, Australia	30.01	4	S
Williams	Giant Cavendish	QDPI selection, Australia	30.31	202	S
Dwarf Parfitt	Extra Dwarf Cavendish	QDPI selection, Australia	BAN 57	3	S
Grande Naine	Cavendish	ex South Africa	BAN 105	34	S
Umalag	Cavendish	ex Philippines	BAN 111	7	S
Valery	Giant Cavendish	ex FHIA Collection, Honduras (AVF20)	BAN 85	6	S
Green Dacca	Green Red	QDPI selection, Australia	BAN 165	7	ER
Red Dacca	Red	QDPI selection, Australia	30.49	12	ER
Pisang Nangka		ex Cibinong Collection, Indonesia	30.37	7	S
Pisang Susu		ex Cibinong Collection, Indonesia	30.38	6	S
Kluai Khai Bonng		ex Pakchong Collection, Thailand	30.39	6	ER
Ga-o		ex Davao Collection, Mindanao, Philippines	30.41	8	S
Inzirabushera		ex East Africa	30.58	6	S
Yangambi km 5		ex INIBAP (ITC 1123)	BAN 106	4	ER
Pagatau		Collected at Korere, East New Britain, PNG	PNG 028	4	S
Kokopo 1		Collected at Kokopo, East New Britain, PNG	PNG 030	8	S
?		Collected at Vunapope, East New Britain, PNG	PNG 144	3	S
Tonton Kepa		Collected in Madang City, PNG	PNG 190	4	S
Mata Kun	Red ?	Collected at Mt Hagan, Western Highlands, PNG	PNG 205	4	ER
Agu		Collected at Yan, Western Highlands, PNG	PNG 210	6	S
AAAA genotype		ex Trinidad breeding program	40.10	12	PR
IC2	(Paka x Samoa) x Highgate	ex Jamaican breeding program (6-86)	40.15	3	ER
T6		ex Jamaican breeding program (61-882)	40.09	9	ER
T8		ex Jamaican breeding program (65-168-12)	40.12	5	PR
Buccaneer (T12)	SH3142 x Highgate	ex Jamaican breeding program (65-3405-1)	40.04	5	PR
Calypso (T13)					
SH3436		ex FHIA breeding program, Honduras	40.05	21	S
AAB genotype					
Lady Finger	Pome	QDPI selection, Australia	21.06	5	S
Santa Catarina Prata	Prata	ex Brazil *via* Hawaii	21.14	8	S
Pacific Plantain	Maia Maoli/Popoulu	QDPI selection, Australia	21.13	9	PR
Pisang Rajah	Pisang Raja	QDPI selection, Australia	21.17	8	S
Horn Plantain	Plantain	QDPI selection, Australia	21.12	7	ER
Dwarf French Plantain	Plantain	ex US Virgin Islands	21.24	3	ER
Pisang Lampening		ex Cibinong Collection, Indonesia	21.26	5	PR
Pisang Ramo		ex Cibinong Collection, Indonesia	21.28	7	S
Pandili		ex Davao Collection, Mindanao,Philippines	21.32	3	ER
Ternate		ex Davao Collection, Mindanao, Philippines	21.30	3	S
Sugar	*Silk*	QDPI selection, Australia	21.34	12	ER

Table II (continued)

Musa germplasm	*Subgroup/Breeding information*	*Additional information*	*QDPI tissue culture code*	*Nb plants inoculated*	*Reaction*
Tomnam (NBH10)		ex Biological Foundation Collection, Laloki, PNG	21.40	6	S
Mysore		ex India *via* Honduras	21.36	6	ER
Apindikay		Collected at Vunapalading, East New Britain, PNG	PNG 110	5	ER
Garunga		Collected at Warangor, East New Britain, PNG	PNG 136	5	PR
Midi		Collected at Dalam, Madang, PNG	PNG 167	6	S
Gamaha		Collected at Kufana, Eastern Highlands, PNG	PNG 192	4	PR
Kumunamba		Collected at Kingkio, Eastern Highlands, PNG	PNG 195	4	PR
Uzakan		Collected at Zabunka, Eastern Highlands, PNG	PNG 196	3	PR
Kanim		Collected at Kassam, Eastern Highlands, PNG	PNG 199	3	S
Terema		Collected at Yan, Western Highlands, PNG	PNG 208	3	S
Horul		Collected at Mendi, Southern Highlands, PNG	PNG 258	5	PR
Hogolo		Collected at Tari, Southern Highlands, PNG	PNG 264	4	PR
Tigua		Collected at Tari, Southern Highlands, PNG	PNG 265	7	PR
Kundaba		Collected at Hwanda, Southern Highlands, PNG	PNG 268	3	S
Kokor		Collected at Fanamapei, New Ireland, PNG	PNG 313	3	S
Yagoa		Collected at Esa'ala, Normanby I, PNG	PNG 328	3	PR
Boung Fu		Collected at Blackwater, West Sepik, PNG	PNG 347	3	PR
AAAB genotype					
SH3481	SH3142 x Prata ana	ex FHIA breeding program, Honduras	40.11	3	ER
ABB genotype					
Blue Java		QDPI selection, Australia	12.14	3	ER
Bluggoe		QDPI selection, Australia	12.01	4	ER
Ducasse	Pisang Awak	QDPI selection, Australia	12.03	3	ER
Kluai Namwa Khom	Pisang Awak (dwarf)	ex Pakchong Collection, Thailand	12.09	3	ER
Monthan		ex India *via* Hawaii	12.05	11	ER
Pisang Kosta Hijau		ex Cibinong Collection, W Java, Indonesia	12.06	5	ER
Pisang Gajih Merah		ex Purwodadi Collection, E Java, Indonesia	12.07	8	PR
Kluai Niu Mue Nang		ex Pakchong Collection, Thailand	12.08	3	ER
Pelipia		ex Davao Collection, Mindanao, Philippines	12.13	3	ER
Abuhon		ex Davao Collection, Mindanao, Philippines	12.11	4	ER
Siusak		ex Davao Collection, Mindanao, Philippines	12.12	3	ER
Cardaba		ex Davao Collection, Mindanao, Philippines	12.10	7	ER
Tukuru (No 2)		Collected at Kokopo, East New Britain, PNG	PNG 141	3	ER
?		Collected at Vunapope, East New Britain, PNG	PNG 143	3	ER
Kalapua (No 2)		Collected at Vunapope, East New Britain, PNG	PNG 145	4	ER
Yawa	Pisang Awak (dwarf?)	Collected at Omuru, Madang, PNG	PNG 155	6	ER
Dwarf Kalapua		Collected in Madang City, PNG	PNG 171	4	ER
ABBB genotype					
Kluai Teparot		ex Pakchong Collection, Thailand	13.01	3	PR
Rekua		ex Cook Islands, South Pacific	13.02	3	ER
Kandrian		ex Biological Foundation Collection, Laloki,	PNG 148	3	
AAT genotype		PNG Collected at Erima, Madang, PNG	PNG 186	1	ER
Sar	*M textilis* in ancestry?				ER

tant and Niyarma Yik (SF 248) as partially resistant. No reactions of these latter two diploids to *M musicola* were proposed by FULLERTON and STOVER (1990).

Differences in reaction could be due to pathogenic variability of the Queensland isolate of *M musicola* or an artifact of the screening technique. It is interesting to note that Gu Nin Chio (II-249), a "Sigatoka Resistance Differential Variety" proposed by VAKILI (1968) was resistant in these tests which is in agreement with VAKILI (1968), but Amau (III-154) was susceptible and not partially resistant as found by VAKILI (1968).

The Fe'i edible banana group with characteristic upright bunches is thought to have evolved from *M maclayi* (SIMMONDS, 1962) of which two subspecies have been identified in PNG (ARGENT, 1976). It is highly likely that *M jackeyi*, which is found in Australia, is also a member of the *M maclayi* complex (ARGENT, 1976). The Fe'i banana cultivars, *M maclayi* spp *ailulai*, *M maclayi* spp *maclayi* and *M jackeyi,* have all been shown to be extremely resistant to *M musicola* in these tests (table II).

The reaction of edible diploid cultivars (AA genotype) to *M musicola* was investigated by VAKILI (1968). He found that 86% of accessions from the island of New Guinea and the South Pacific region and 66% of accessions from South East Asia were susceptible. The results recorded here (table II) show that 48% of the diploid accessions collected in PNG by IBPGR and tested are susceptible to *M musicola*, 50% partially resistant and 2% extremely resistant.

It is of interest that the FHIA breeding diploids SH 3142 and SH 3362 and most accessions of cv Pisang Mas are susceptible to *M musicola* (table II), but resistant to *M fijiensis* in the field in the South Pacific (JONES, 1993). It does not always follow that *Musa* germplasm is more susceptible to *M fijiensis* than *M musicola.* However, one accession of Pisang Mas, cv Sucrier from Queensland, was extremely resistant to *M musicola* in tests (table II). This reaction is surprising as Sucrier is known by personal observation to be susceptible in the field in North Queensland.

Triploids of the AAA genotype were generally susceptible to *M musicola* in the tests (table II). Exceptions were cvs Red, Green Red, Kluai Khai Bonng and Yangambi Km 5, which expressed strong resistance. The reaction of Red and Green Red was unexpected as it is listed as being susceptible or partially resistant (VAKILI, 1968). Indeed, personal observations of Red in the field in North Queensland indicate that it is susceptible. This would suggest that with this clone, as with Sucrier, resistance is expressed in young plants and disappears as tissues mature. The reaction of one FHIA tetraploid cultivar of the AAAA genotype was also unexpected. SH 3436 was rated as susceptible, but is known to have resistance in the field.

Resistance levels were generally higher in triploids of the AAB genotype, a higher percentage of accessions being in the partially resistant and extremely resistant categories than in the AAA genotype.

All accessions of the ABB and ABBB genotypes tested were resistant to *M musicola* (table II). This is in agreement with field observations (CHEESMAN and WARDLAW, 1937).

conclusion

Results indicate that the technique described here for the rapid assessment of *Musa* germplasm for reaction to Sigatoka disease is useful and will detect most clones that have resistance. However, it may mislead on occasion. Useful resistance may be missed because it is not fully expressed in young plants. Conversely, some clones may show resistance as young plants, but this resistance may be lost in mature plants. More work needs to be undertaken to refine the methodology and also to distinguish more accurately the differing types of response of *Musa* to *M musicola.*

acknowledgments

I am very grateful for the technical help provided by Miss H Hayden and Miss J Morris. I also thank Dr MK Smith and Mrs S Hamill of the Horticulture Branch, QDPI for providing tissue cultures of cultivars used in this study. QDPI is grateful to Dr P Rowe of FHIA for supplying diploid and tetraploid *Musa* germ-

plasm, IBPGR for allowing access to PNG cultivars and INIBAP for providing standard diploids and other accessions. Some of the germplasm tested was obtained from *Musa* field collections in South East Asia in 1988 and QDPI is indebted to the National Centre for Research in Biotechnology and the National Biological Institute, Indonesia, Kasetsart University Research and Development Institute, Thailand, and the Bureau of Plant Industry, Ministry of Agriculture, Philippines, for allowing access to material. The work was financially supported by the Australian Centre for International Agricultural Research (ACIAR).

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