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A pilot study investigating the complexity of Fusarium wilt of bananas in West Sumatra, Indonesia

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Abstract. Fusarium wilt symptoms were observed on 15 different banana cultivars growing in commercial plantations and backyard holdings, in 6 districts in the Province of West Sumatra, Indonesia. Affected cultivars included the most popular dessert bananas Pisang buai (AAA), Pisang raja (AAB), Pisang raja serai (AAB), and Pisang ambon (AAA). Disease symptoms were also observed on the most popular cooking banana Pisang kepok (ABB), formerly considered to be resistant. However, no disease symptoms were observed on wild *Musa* species.

The 37 isolates of the pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*) recovered from the vascular tissue of the diseased bananas were assigned to the vegetative compatability groups (VCGs) 0120–01215, 0124, 01213–01216, 01218, and 01219. VCG 01216 was isolated from all districts, and from 14 of the 15 cultivars exhibiting wilt symptoms. However, the distribution of the other VCGs was more variable, reflecting the patterns of human migration within the different districts. Districts designated as expanding urban centres or as transmigration centres had a higher diversity of banana genotypes present, with a correspondingly higher diversity of VCGs isolated from the diseased plants. The traditional practice of transplanting banana suckers and rhizomes irrespective of the disease status of the parent plant, and the recent increase in the mobility of the Indonesian population, indicate that disease control strategies based on quarantine will not be effective. Accordingly, the selection of both popular dessert and cooking banana cultivars with resistance to VCG 01216 should be a priority for the control of Fusarium wilt in West Sumatra.

Additional keywords: Fusarium oxysporum f. sp. *cubense,* Panama disease, vegetative compatability groupings, Indonesia.

Introduction

South-East Asia is considered to be the centre of origin of the genus *Musa*, with up to 60 species identified (Simmonds 1962). Bananas have been cultivated as a traditional fruit for centuries by the people of this region. For example in the Indonesian archipelago approximately 200 banana cultivars are grown in virtually all areas, at different altitudes and in various soil types (Nasution 1992). Diploid, triploid, and tetraploid cultivars are grown in plantations, or more commonly in backyards (Subiyanto 1990). Cultivar selection usually depends on family requirements and personal preferences, with the transplanting of suckers being the most common method of plant propagation. The Fusarium wilt pathogen is readily spread in infected vegetative propagation material, and in soil adhering to tillage implements. Traditionally, growers recognise Fusarium wilt symptoms, which they call 'penyakit layu kuning' meaning yellow wilt disease. However, despite its economic impact, the complexity of Fusarium wilt in Indonesia remains unknown, and there are no regional strategies to manage this disease.

Fusarium wilt caused by *Fusarium oxysporum* Schlecht. f. sp. *cubense* (E. F. Smith) Snyder and Hansen (*Foc*) was first reported from Indonesia in 1916 from banana plantations on the island of Java (Stover 1962). Currently the

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disease is recognised as a major constraint to banana production in most parts of Indonesia including Java, Sumatra, and Sulawesi (Subiyanto 1990, Buddenhagen 1995). Losses in export plantations of the commercial dessert cultivar Cavendish in Southern Sumatra have exceeded 70% (Haryano 1997, pers. comm.), and in Northern Sumatra over 1000 ha of commercial Cavendish plantations were destroyed within 3 years of the first appearance of disease symptoms. To date, 21 vegetative compatability groupings of the pathogen *Foc* have been identified world-wide (Moore *et al.* 1995), with the greatest diversity being found among south-east Asian populations of the fungus. The only proven strategy to control Panama disease has been the implementation of local quarantine, and the replacement of susceptible commercial cultivars with resistant alternatives (Pegg *et al.* 1996). However, these successful control programs are restricted to countries where bananas are exotic fruits, where resistant cultivars are available, and where the provision of planting material is controlled. In these countries the level of diversity of the pathogen is limited, and the distribution of strains of the pathogen is generally known. This is not the case in Indonesia.

This pilot study was initiated to investigate the impact of traditional agricultural practices on the current distribution of *Foc* within districts in the Province of West Sumatra, Indonesia. The Province of West Sumatra includes established agricultural regions, developing urban centres, and designated transmigration centres. Government-sponsored industrialisation and transmigration programs are having a major impact on the movement of ethnic groups within the Indonesian archipelago. There are no quarantine policies restricting the movement of planting material within Indonesia. Although farmers recognise diseased parent plants, they continue to propagate from the symptomless suckers of their preferred traditional cultivars. These recent changes in migration patterns may therefore be responsible for the spread of different strains of the Fusarium wilt pathogen. The objectives of this study were to use vegetative compatability group (VCG) analysis and volatile odour production to determine the diversity of strains of the pathogen isolated from different banana cultivars showing symptoms of the disease. The ethnobotanic traditions operative within each district were compared with the patterns of diversity of pathogenic strains and cultivars, to investigate the feasability of implementing local quarantine as a disease control strategy.

Materials and methods

Survey and pathogen isolation protocols

The study was conducted in the Province of West Sumatra, Indonesia. Localities within 6 districts where Fusarium wilt was observed in a preliminary survey in 1992 were revisited. Symptomatic banana plants growing in plantations, backyard holdings, or in jungle vegetation alongside major roads were sampled. Banana cultivars were identified according to Simmonds (1960). Where possible, 3–5 affected banana plants of each cultivar from each location were sampled. Discoloured vascular strands were taken from the lower section of the pseudostem, 10–15 cm above the corm. The strands were placed between 2 sheets of sterile blotting paper and were dried for 3–5 days at ambient air temperature. Sampling was conducted over a 2-week period in October 1996, with the vascular strands being sent by airmail to the Indooroopilly laboratory of the Department of Primary Industries in Queensland, Australia. The incidence of Fusarium wilt and pathogen identification records for each cultivar sampled at each location were compared with published records for Indonesia, and south and south-east Asia.

Identification of the pathogen

Fungal cultures were isolated from the vascular strands by plating 2 or 3 pieces (0.5–1.0 cm length) onto potato dextrose agar amended with 50 µg/g streptomycin. Cultures were incubated at room temperature (25–30°C) for 3–5 days under a black light. Cultures exhibiting aerial mycelial growth and mauve-pink pigmentation in the agar were selected as wild types of the fungus *Fusarium oxysporum* (Booth 1971). One wild type culture was selected from colonies isolated from each of the banana cultivars showing disease symptoms, from each site surveyed. Each wild type culture was characterised on the basis of volatile odour production and their vegetative compatability group. Volatile odour production was assessed by growing monoconidial isolates of each culture in steamed rice in Erlenmeyer flasks at 25°C for 14 days (Moore *et al.* 1991). Cultures were designated as strong or weak producers (odouratum strains), or nil (inodouratum strains) according to the strength of the odour detected in the flask.

Mutants incapable of utilising nitrate (nit mutants) were generated for the vegetative compatability tests, by growing 5 monoconidial isolates of each culture on potassium chlorate agar (Correll *et al.* 1987). Hyphal tips were subcultured from the nit mutant colony sectors and stored on minimal media with nitrate supplied as the sole nitrogen source (MM). Each nit mutant was tested for the ability to produce functional heterokaryons with reference testers, by placing paired unknown and tester inoculum blocks onto MM and incubating them for 5 days at 25°C. Five mutant replicates of each unknown isolate were tested against each of the 8 reference tester strains. The testers used represented the VCGs 0120, 0124, 0125, 01213, 01215, 01216, 01218, and 01219. Isolates capable of forming functional heterokaryons were assigned to the grouping of the compatible tester strain.

Results

The distribution and disease status of banana cultivars in West Sumatra

Six of the 14 districts in the Province of West Sumatra were surveyed during this study. The districts, which supply much of the local and export demand for West Sumatra, were Bukit Tinggi, Agam, Tanah Datar, Solok, Sijunjung, and Padang (Fig. 1).

Pisang buai (AAA, Cavendish subgroup: Robusta), Pisang ambon (AAA, Gros Michel), Pisang raja serai/sereh (AAB, Silk), Pisang kepok (ABB cooking type), and Pisang raja (AAB cooking type) are the most important commercial cultivars grown in the 6 districts surveyed. Large commercial plantations of the cooking cultivar Pisang kepok have been traditionally grown in the districts of Bukit Tinggi and Solok, whereas the districts of Agam and Tanah Datar are recognised production centres for the dessert cultivar Pisang buai. Banana production in Sijunjung and in the developing

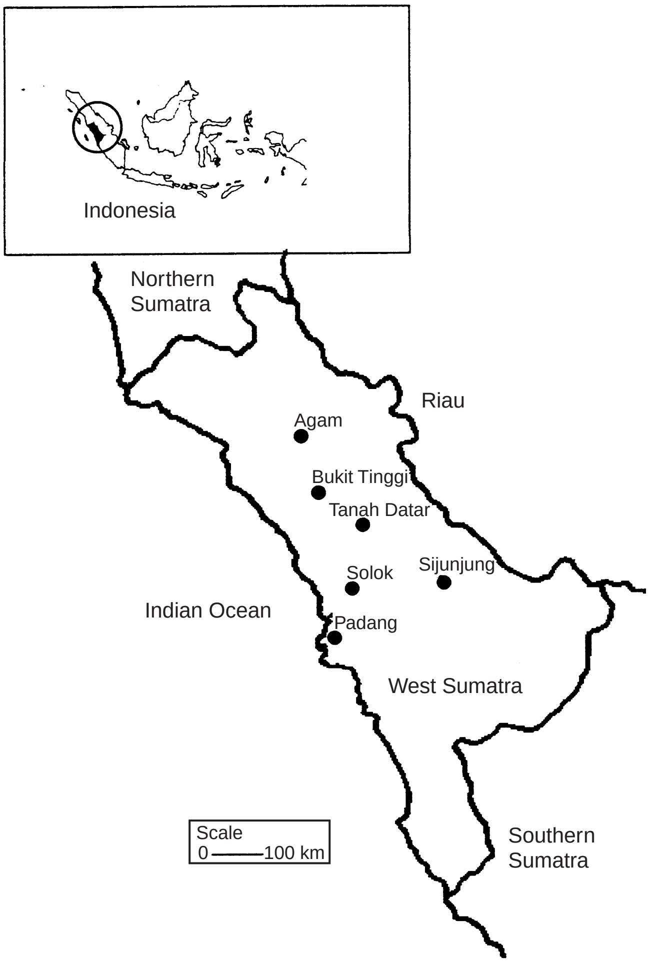

Fig. 1. Location of the 6 districts surveyed for the incidence of Panama disease in the province of West Sumatra, Indonesia. The number of sites surveyed within each district varied from 6 to 14, with an average of 10. The inset indicates the location of the province of West Sumatra within the Indonesian archipelago

urban trading centre of Padang is restricted to subsistence backyard or smaller plantations of 20–50 plants.

In this study, wilt symptoms characteristic of Panama disease were observed on 15 of the 19 banana cultivars, occurring in 37 of the 56 sites surveyed (Table 1). Of the 15 cultivars 3 were cooking types, and the remainder were dessert types. On average 10 locations per district (range 6–14) were surveyed. Commonly, only 1 cultivar at each site was diseased, and cultivars showing symptoms at one location were unaffected at other locations. Cultivars without disease symptoms growing within 10 m of an affected cultivar were recorded. Of the 4 cultivars recorded as having no disease symptoms (Pisang raja siam ABB, Pisang rotan AA, Pisang lilin AA, Pisang batu ABB), all were restricted to small plantings in 1 location within 1 district only. No disease symptoms were recorded on any of the wild *Musa* species observed during this survey.

Table 1. Distribution of banana cultivars showing Fusarium wilt symptoms and the vegetative compatability groupings (VCG) of *Fusarium oxysporum* f. sp. *cubense*, in the six West Sumatran districts surveyed in this study

Within each district an average of 10 (range 6–14) locations were surveyed. Question mark indicates cultivars whose genome has yet to be confirmed. VCGs listed with a slash indicate cross-compatability with two Nit mutant tester strains

District	Affected cultivar	Cultivar genome	VCG
Bukit Tinggi	Pisang ambon	AAA	01216
Agam	Pisang ambon kuning	AAA	01216
	Pisang buai	AAA	01213
	Pisang raja	AAB	01216
	Pisang raja kinalun	?	01216
Solok	Pisang kalek air	AAB	01216
	Pisang raja serai	AAB	01216, 01219
	Pisang kepok	ABB	0124
	Pisang manis	AA	01216
	Pisang mas	AA	01216
Tanah Datar	Pisang buai	AAA	01213, 01213/16, 01218
	Pisang udang	AAA	01216
	Pisang raja serai	AAB	01218
	Pisang kepok	ABB	01215
	Pisang lidi	AA	01218
Padang	Pisang sirandah	AAA	0120
	Pisang raja serai	AAB	01218
	Pisang kepok	ABB	0120
	Pisang raja siam	ABB	0120/15
	Pisang jantan	?	01213/16
Sijunjung	Pisang ambon	AAA	01216
	Pisang buai	AAA	01216
	Pisang sirandah	AAA	01216
	Pisang raja serai	AAB	01213/16, 01216
	Pisang kepok	ABB	01216, 01219
	Pisang mas	AA	01216

Since the preliminary survey conducted in 1992, >60% of some 1000 plants derived from tissue culture in 2 Pisang ambon kuning (AAA) plantations in the district of Agam have succumbed to Panama disease. At most other sites the expression of disease was more localised. In the district of Solok some 30 ha of Pisang kepok (ABB) exhibited disease symptoms whereas no symptoms were evident in larger plantations of this cultivar in the district of Bukit Tinggi, only 100 km away. In both districts the source of the planting material had been suckers or corms. Pisang buai (AAA) plantations in Bukit Tinggi also appeared free of Panama disease in contrast to several smaller plantations of this cultivar in the district of Tanah Datar (only 50 km away), which were severely diseased. Whilst the expression of external symptoms on all cultivars was similar, the colour of diseased vascular strands in affected Pisang kepok cultivars differed. The infected vascular strands dissected from most cultivars were thick, and dark brown to black in colour. In contrast infected Pisang kepok strands were thin, and light yellow in colour.

Identification and distribution of the Foc isolates

Wild type cultures were isolated from each of the 37 diseased banana cultivars sampled. The majority of the 37 isolates tested formed strong heterokaryons when grown with a compatible tester. Two isolates formed only weak heterokaryons with tester 01215 (one cross-compatible with 0120), but the production of strong volatile odours supported their designation as putative race 4 types. Of the remaining 35 cultures, 30 were volatile odour producers conforming to the VCGs 0120, 01213, 01216, and 01219, characteristic of *Foc* race 4 (Moore *et al.* 1991). Only 5 of the isolates did not produce an odour and were grouped as VCGs 0124 and 01218, characteristic of *Foc* race 1. Cavendish cultivars (AAA) are considered to be resistant to *Foc* race 1 (Hwang 1991). However, in this study, the Cavendish type Pisang buai from Tanah Datar was infected with an isolate assigned to VCG 01218. Within Indonesia the cooking cultivar Pisang kepok has always been considered resistant to Fusarium wilt. However, the results of this study indicate that this cultivar is susceptible to 4 of the putative race 4 VCGs (0120, 01215, 01216, and 01219), and to one of the putative race 1 VCGs (0124). Several instances of cross-compatability with the testers were observed, with isolates from Pisang buai (AAA) from Tanah Datar, Pisang jantan (unknown genotype) from Padang, and Pisang raja serai (AAB) from Sijunjung being compatible with both VCGs 01213 and 01216. An isolate from Pisang raja siam (ABB) from Padang was cross-compatible with VCGs 0120 and 01215.

Of all the *Foc* populations identified, the VCG complex 01213–01216 was the most widespread, being found in all the districts surveyed. The distribution of the other VCGs varied considerably both within and between the 6 districts. When plotted as a function of the frequency of VCGs isolated and the frequency of banana cultivars exhibiting disease symptoms (Fig. 2), differences between these districts become evident. Bukit Tinggi not only had the lowest incidence of Fusarium wilt symptoms, but also had the lowest number of banana cultivars planted (5), and only one VCG (01216) was recovered. In contrast, the transmigration zone of Sijunjung, the developing urban area of Padang, and the rural commercial area of Tanar Datar had a higher cultivar diversity, a higher disease incidence, and a greater VCG diversity. These results suggest that the ethnobotanical traditions operative within the different districts are key factors in determining the distribution of Fusarium wilt in West Sumatra.

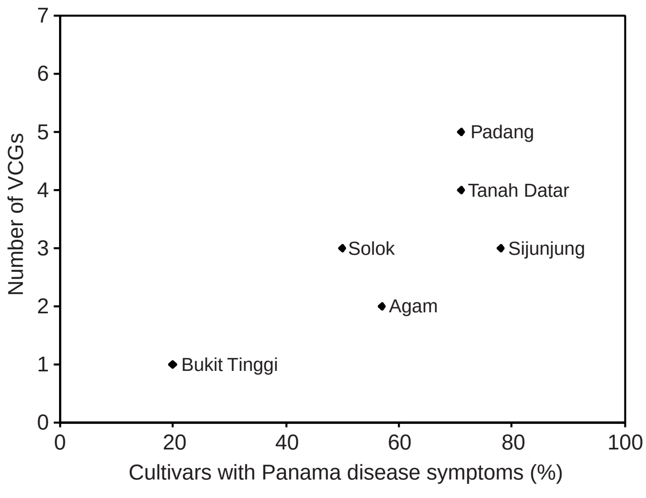

Fig. 2. Diversity of banana cultivars with Panama disease plotted against the diversity of *Foc* vegetative compatability groupings (VCG) isolated from each of the 6 districts surveyed. The percentage of diseased cultivars was derived by calculating the number of cultivars showing Panama disease symptoms, as a ratio of the total number of cultivars observed within a 10-m radius of all sites surveyed within each district. Of the 19 cultivars recorded, 15 showed typical Panama disease symptoms.

Discussion

Fusarium wilt of bananas has not previously been considered to be widespread in Indonesia (Stover 1990). During this survey disease symptoms were commonly observed in backyard plantings, indicative of the lack of awareness of farmers about the dissemination of the pathogen in infected planting material and on tillage implements. Of the 15 VCGs isolated from the Asian region (Moore *et al.* 1995), 10 have been confirmed as being present in Indonesia (Bentley *et al.* 1998), with 7 of these isolated during this survey of West Sumatra. VCG 01216 was the most common, isolated from all districts and recovered from 14 of the 15 different banana cultivars showing disease symptoms. Populations in the VCG complex 01213–01216 referred to as Tropical race 4 are recorded as affecting the widest range of banana cultivars (Ploetz and Pegg 1997). However, perhaps with the exception of VCG 01216, the most important agent for the spread of the different strains of *Foc* in West Sumatra is human activity.

The 6 districts surveyed can be categorised as a developing rural export area (Agam), developed agricultural export area (Bukit Tinggi), developing rural and trading areas (Solok and Tanah Datar), developed urban area (Padang), and a developing transmigration area (the resettlement of residents from overpopulated islands to less populated regions, such as Sijunjung). Given the long history of banana cultivation by the Indonesian people, the greatest diversity of banana cultivars and of *Foc* strains would be expected to occur in regions experiencing population immigration from diverse regions. The designated transmigration and urban areas do indeed have the highest diversity (Fig. 2). These results imply that the imposition of quarantine restrictions within the province of West Sumatra for the control of Fusarium wilt is not feasible. However, at the district level, there is evidence that local planting selection preferences have effectively excluded the introduction of pathogenic strains other than VCG 01216. Bukit Tinggi is an established agricultural region, renowned for quality banana production. Accordingly there is no incentive for growers to go beyond the local region when selecting new planting material. In the absence of a tissue-culture program, the effective voluntary quarantine status of this district could be utilised as a site for the provision of disease-tested planting material to other regions.

The results presented here indicate that the interactions between the banana cultivar hosts, the pathogenic strains of the fungus, and the environment in Indonesia are potentially very complex. Within West Sumatra local quarantine may not be a feasible strategy for the control of Fusarium wilt. However, recently the International Musa Testing Program established several trial sites in Indonesia, consisting of a number of known banana genotypes with plantings replicated within each trial site. Data from these sites should improve our knowledge of the Fusarium wilt host–pathogen systems operative within Indonesia, and expedite the development of effective disease control strategies.

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