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Fusarium species associated with vanilla stem rot in Indonesia

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Abstract. Indonesia is one of the world's leading producers of vanilla, an important cash crop for smallholders. Stem rot disease is a major constraint to vanilla production in Indonesia and has caused significant economic losses over the last decade. Previous reports of vanilla stem rots in the Asia-Pacific region include those caused by *Fusarium*, *Colletotrichum* and *Phytophthora* species. In this paper, we report *Fusarium* species associated with the disease. Seven major vanilla-producing provinces were surveyed for disease incidence and 850 samples were collected. Isolates were recovered from diseased stem tissues using a selective medium. Pure cultures on carnation leaf-piece agar and potato dextrose agar were identified based on morphological criteria. Some ambiguous species were verified based on DNA sequences of the translation elongation factor gene. A total of 542 *Fusarium* isolates were recovered, comprising 12 species, namely *F. decemcellulare*, *F. fujikuroi*, *F. graminearum*, *F. mangiferae*, *F. napiforme*, *F. oxysporum*, *F. polyphialidicum*, *F. proliferatum*, *F. pseudocircinatum*, *F. semitectum*, *F. solani* and *F. subglutinans*. *F. oxysporum* was the most commonly isolated species from all areas surveyed, followed by *F. solani* and *F. semitectum*. *F. oxysporum*, *F. solani* and *F. semitectum* were tested for pathogenicity to vanilla but only *F. oxysporum* was shown to be pathogenic. The vanilla stem rot pathogen in Indonesia is verified to be *F. oxysporum* f. sp. *vanillae*.

Introduction

Vanilla planifolia is a high value crop (Elizabeth 2002), which is cultivated for the production of vanillin, one of the most valuable flavouring commodities in the food and beverage industry worldwide (Westcott *et al*. 1993; Muheim and Lerch 1999). The crop is cultivated in several countries including Indonesia, one of the leading producers and exporters together with Madagascar (Dignum *et al*. 2001; Roling *et al*. 2001).

Various fungal diseases have been reported on *V. planifolia* such as root rot, leaf rot, stem blight, brown spots and anthracnose (Thomas and Suseela 2001). Several viral diseases have also been reported (Wisler *et al*. 1987; Pearson *et al*. 1993; Farreyrol *et al*. 2001). However, the main constraint to increasing vanilla production in Indonesia is the devastating disease known as vanilla stem rot caused by *Fusarium oxysporum* f. sp. *vanillae* (Tombe *et al*.1997; Thomas *et al*. 2002).

In Indonesia, vanilla stem rot was first reported in Central Java in 1960 and the pathogen identified as *F. oxysporum* f. sp. *vanillae* (Tombe *et al*. 1993). The disease has been reported to cause substantial crop losses of up to 80% by farmers (Lestari *et al*. 2001). The disease is non-seasonal and has spread extensively throughout vanilla production areas, the most serious losses occurring in Java, Bali, North Sumatera and North Sulawesi (Tombe *et al*. 1992).

In a preliminary survey of fungi associated with vanilla stem rot conducted in North Sulawesi in 2002, it was found that *Fusarium* species, including *F. oxysporum* f. sp. *vanillae*, were the dominant species isolated from diseased stems (Liew *et al*. 2004). The role of *Fusarium* species associated with vanilla stem rot other than *F. oxysporum* f. sp. *vanillae* has not been investigated.

Fusarium species are economically important pathogens of plants. Many species are also endophytic or saprophytic colonisers. As pathogens, *Fusarium* species cause a wide range of diseases on agricultural, horticultural and forest crops (Burgess *et al*. 1994; Moore *et al*. 2001; Ploetz 2001; Summerell *et al*. 2003). More than 81 economically important plants are affected by at least one disease caused by *Fusarium* (Leslie and Summerell 2006). *Fusarium* species, however, are often present as endophytes in many crops in agricultural ecosystems (Burgess 1981; Leslie *et al*. 1990; Kuldau and Yates 2000). They can occupy the internal plant tissue without causing any symptoms, but may induce disease symptoms when the plants are subjected to drought or other stress factors (Burgess 1981).

The preliminary survey of *Fusarium* species associated with the disease in North Sulawesi was extended to six other vanilla-producing provinces in order to gain a wider understanding of the role of *Fusarium* species associated with vanilla stem rot in

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Indonesia. Representative isolates were tested for pathogenicity. We report the outcome of these studies here and discuss the implications for disease management.

Materials and methods

Sample collection

Seven provinces, representing the major vanilla-growing regions, namely Bali, Central Java, Jogjakarta, Lampung, North Sulawesi, West Java and West Nusa Tenggara were surveyed. Samples from North Sulawesi were collected in 2002, whereas samples from the other provinces were collected in 2006. Samples were collected from one to three regions per province and one to three sites per region. Regions and sites were selected arbitrarily based on logistics and disease presence and a total of 23 sites in 15 regions were surveyed (Table 1). The numbers of disease samples collected at each site reflected the size of the production area. Samples were collected only from stems showing characteristic symptoms, namely discolouration and rotting of stem tissue (Fig. 1). Stem samples were 100–150 mm long and included the margin of necrotic and healthy tissues.

Isolation and purification of Fusarium isolates

Stem samples were rinsed in tap water and surface sterilised by brief flaming after swabbing with 70% ethanol. Two small stem segments (~5 mm thick) were removed aseptically from the margin of necrotic and healthy tissues and plated on a *Fusarium* selective medium, peptone pentachloronitrobenzene agar, (PPA) (Burgess *et al*. 1994). PPA plates were incubated for 5–7 days and grown under conditions described by Burgess *et al*. (1994). Filamentous fungi growing from the tissue pieces were subcultured onto carnation leaf-piece agar (CLA) for identification. CLA plates were grown under alternating light/dark incubation for 7 days as described by Burgess *et al*. (1994). Each isolate was purified by single-spore transfer (Burgess *et al*. 1994) to CLA and potato dextrose agar (PDA) for morphological identification.

Morphology and molecular identification

Pure cultures of *Fusarium* on CLA and PDA were identified to species level based on morphological criteria as outlined by Burgess *et al*. (1994) and Leslie and Summerell (2006). The identification of morphologically ambiguous *Fusarium* species was verified based on DNA sequencing of the translation elongation factor gene (EF-1α) and compared with the GenBank database, using BLAST.

DNA extraction

Isolates were cultured on PDA plates for 10–14 days to allow mycelia to cover the surface of the medium. Mycelia were then harvested and placed in a sterile 1.5-mL Eppendorf tube. DNA was extracted using the FastDNA Kit (Qbiogene, Inc., Seven Hills, NSW, Australia) according to the manufacturer's instructions. The integrity and concentration of genomic DNA was estimated using gel electrophoresis.

PCR amplification and DNA sequencing

The EF-1α gene was amplified using primers EF-1 and EF-2 as described by O'Donnell *et al*. (1998). PCR amplification of the EF-1α gene was carried out in a 25-μL reaction volume containing 1× PCR reaction buffer, 2.5 mM of $MgCl_2$ (Bioline, Alexandria, NSW, Australia), 1 Unit Taq (AmpliTaq Gold, Applied Biosystems, Mulgrave, Vic., Australia), 1.0 mM dNTP mix (Astral Scientific, Gymea, NSW, Australia), 0.25 μM of each of the EF-1 and EF-2 primers (Sigma Aldrich, Castle Hill, NSW, Australia) and ~50–100 ng of

Table 1. Location of sampling sites

Province	Region	Site	GPS coordinates
West Java	Sukabumi	Cikembar	06°56.613′S, 106°46.363′E
	Sumedang	Tanjungkerta	06°45.671′S, 107°52.482′E
	Bogor	Cimanggu	06°34.650′S, 106°47.267′E
		Pasirgaok	06°32.429′S, 106°43.336′E
Central Java	Temanggung	Disbun	Not available
Jogjakarta	Kulonprogo	Samigalo	Not available
		Nangwulan	Not available
Bali	Jembrana	Pekutatan 1	08°24.674′S, 114°49.768′E
		Pekutatan 2	08°25.512′S, 114°50.515′E
	Tabanan	Selemadeg Barat	08°29.143′S, 114°59.227′E
West Nusa Tenggara	Lombok barat	Gangga Selelos	08°24.121′S, 116°14.355′E
		Gangga Bentek	08°24.120′S, 116°14.165′E
	Mataram	Lingsar batukumbung	08°34.172′S, 116°11.960′E
		Lingsar batumekar	08°23.295′S, 116°12.242′E
Lampung	Lampung Selatan	Natar	05°18.978′S, 105°10.503′E
	Tanggamus	Gisting	05°25.514′S, 104°42.821′E
	Lampung Timur	Bandar Sribhawono 1	05°17.016′S, 105°40.670′E
		Bandar Sribhawono 2	05°17.328′S, 105°39.833′E
North Sulawesi	Manado	Mapanget	01°30.929′S, 124°55.403′E
	Minahasa	Warembungan	01°25.091′S, 124°48.656′E
	Minahasa selatan	Pondos-wakan	01°06.815′S, 124°30.845′E
		Langsot	01°13.102′S, 124°44.503′E
		Rasi	01°02.844′S, 124°46.776′E

Fig. 1. (*a*) Healthy vanilla vine, (*b*, *c*) symptoms of vanilla stem rot.

genomic DNA. Amplifications were performed in a Corbett DNA thermocycler (Corbett Research, Mortlake, NSW, Australia). The cycling profile consisted of an initial denaturation of 1 min at 97°C, 35 cycles of denaturation at 96°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

The PCR product was cleaned using ExoSAP (ExoSAP-IT; Amersham Biosciences, Arlington Heights, IL) to remove excess primer and dNTP. The ExoSAP protocol consisted of adding 2 µL ExoSAP into 5 µL of the PCR product and incubating in a DNA thermocycler for 15 min at 37°C followed by 15 min at 80°C. After incubating, the total mixture was then diluted in 12 µL sterile deionised water. Following the ExoSAP protocol, the sequencing reaction was performed in a 20-µL reaction volume containing 0.34 µL sterile water, 3.5 µL 5× dilution buffer, 1 µL BigDye terminator ver. 3.1 (PE Applied Biosystems, Foster City, CA), 3.2 pmol primer and 15 µL of purified PCR product. The sequencing product was then transferred to a 1.5-mL Eppendorf tube and purified using ethanol precipitation by adding 5 µL of 125 mM EDTA and 60 µL of 100% ethanol. The mixture was then centrifuged for 20 min at room temperature, followed by supernatant removal. The 1.5-mL tube was again added with 250 µL of 70% ethanol and centrifuged for 10 min at room temperature, followed by supernatant removal. The 1.5-mL tube was dried on a heating block at 90°C for 1 min. The purified sequencing product was sent to the DNA sequencing facility at the Ramaciotti centre for gene function analysis at The University of New South Wales, Sydney, Australia.

Pathogenicity test

The three most commonly isolated *Fusarium* species, *F. oxysporum*, *F. solani*, and *F. semitectum*, were tested for pathogenicity. These species were selected because they were isolated from all provinces sampled, contributed more than 90% of the total *Fusarium* isolates, and each species comprised more than 10% of the total *Fusarium* isolates. Seven isolates of each species were selected at random to represent each of the seven provinces. Two additional *F. oxysporum* isolates from East Kalimantan and South Sulawesi obtained from Dr Mesak Tombe (Indonesian Agency for Agricultural Research and Development) were included in the test. A randomised complete block experimental design was used with five replications of each isolate. The inoculum for the experiment was generated by incorporating colonised millet seed into sterile soil. Four-hundred grams of millet seed was soaked in 1-L volumetric flasks containing 500 mL water for 2 days. Afterwards excess water was drained. Non-absorbent cotton wool was used to plug the flasks, capped with aluminium foil, autoclaved for 20 min at 121°C and allowed to cool overnight. The following day the millet seed was inoculated with a spore suspension from cultures which had been initiated from single spores and grown on CLA for 12 days. The spore suspensions were harvested by scraping mycelium, microconidia and sporodochia from the surface of the agar of each Petri dish into a test tube of sterile distilled water (10 mL). The spore suspension was homogenised using a vortex mixer before being added to the flasks containing the sterile millet seed under aseptic conditions. A control mix was also made by adding 10 mL of sterile deionised water only to the millet seed. The flasks were shaken to disperse the spore suspension through the seeds and were then incubated at room temperature. Every 4 days the flasks were shaken to ensure thorough and even colonisation of the millet seed. After 15 days' incubation, inoculum was mixed with sterile soil, at 20% inoculum per 300 g soil and placed in a 120 by 100-mm pot. A vanilla stem cutting (two nodes and two internodes per cutting) was planted in each pot. Pots were placed on a bench in a glasshouse and watered daily.

Disease assessment and statistical analyses

The vanilla cuttings were observed daily for the appearance of typical stem rot symptoms, such as discolouration, water-soaking and necrosis. Lesions that developed on symptomatic plants were excised, rinsed in sterile distilled water and surface sterilised by quick flaming with 70% ethanol before plating on PPA. The plates were incubated as described above and colonies developing from the stem sections were purified and identified as described above. Reisolation of the fungus used to inoculate the soil was taken as confirmation of Koch's Postulates. The recovery of a pathogenic isolate was scored as 1, whereas non-recovery of any isolate was scored as 0. Since the data of pathogenicity tests were dichotomous, the Cochran test, a nonparametric statistical analysis, was used to determine treatment differences within isolates (Conover 1999). Statistical analyses were performed using SPSS ver.13.0 (SPSS Inc., Chicago, IL, US).

Results

Fusarium *species associated with vanilla stem rot*

A total of 542 *Fusarium* isolates were recovered during the surveys in 2002 and 2006, consisting of 12 *Fusarium* species. The 12 *Fusarium* species were *F. decemcellulare*, *F. fujikuroi*, *F. graminearum*, *F. mangiferae*, *F. napiforme*, *F. oxysporum*, *F. polyphialidicum*, *F. proliferatum*, *F. pseudocircinatum*, *F. semitectum*, *F. solani* and *F. subglutinans*. Of these, *F. oxysporum*, *F. solani*, *and F. semitectum* were the three most prevalent species associated with the disease, accounting for 92.44% of all isolates recovered. *F. oxysporum* contributed 55.72% of total isolates and was the dominant species, followed by *F. solani* 25.65% and *F. semitectum* 11.07% (Table 2). Species which were verified based on DNA sequencing of the EF-1α gene are listed in Table 2.

F. oxysporum was the dominant species recovered in each province, except West Nusa Tenggara where *F. solani* was the dominant species (Table 2). The highest recovery of *F. oxysporum* isolates was from the Lampung samples, accounting for 98 (32.45%) of the total *F. oxysporum* isolates. *F. solani* was the second most dominant species after *F. oxysporum*. The Bali samples contributed 50 (35.97%) of the total of *F. solani* isolates, the highest recovery compared with other provinces. *F. semitectum* was the third most dominant species after *F. oxysporum* and *F. solani*. Lampung province contributed 20 isolates (33.33%) of total isolates.

F. oxysporum, *F. solani* and *F. semitectum* were isolated from samples from all seven provinces (Table 2), whereas *F. graminearum and F. subglutinans* were only recovered from samples from North Sulawesi.

Table 2. *Fusarium* species and number of isolates obtained during the survey in 2002 and 2006

Fusarium species	Province[A]							Total number of isolates	Frequency of isolation (%)
	A	B	C	D	E	F	G		
F. decemcellulare[D]	–	1	–	–	–	2	–	3	0.55
F. fujikuroi[B,C]	–	–	–	3	–	–	–	3	0.55
F. graminearum[D]	–	–	–	–	2	–	–	2	0.38
F. mangiferae[B,C]	1	–	–	–	–	1	1	3	0.55
F. napiforme[C]	–	–	–	1	–	–	–	1	0.18
F. oxysporum[B,C]	70	34	17	98	23	36	24	302	55.72
F. polyphialidicum[B,C]	–	–	–	1	–	–	–	1	0.18
F. proliferatum[C]	1	–	–	6	1	–	–	8	1.48
F. pseudocircinatum[B,C]	–	10	3	–	–	–	2	15	2.77
F. semitectum[C]	7	19	1	20	4	8	1	60	11.07
F. solani[C]	50	2	11	17	16	12	31	139	25.65
F. subglutinans[D]	–	–	–	–	5	–	–	5	0.92
Total	129	66	32	146	51	59	59	542	

[A]A = Bali; B = Central Java; C = Jogjakarta; D = Lampung; E = North Sulawesi; F = West Java; G = West Nusa Tenggara.
[B]*Fusarium* species verified using DNA sequences of EF-1α based on BLAST analysis at similarity levels ranging from 89 to 99.68%. GenBank accession numbers: GQ425225 (*F. oxysporum*); GQ425226 (*F. fujikuroi*); GQ425229 (*F. polyphialidicum*); GQ425230 (*F. pseudocircinatum*) and GQ425231 (*F. mangiferae*).
[C]*Fusarium* species stored at the Royal Botanic Gardens Sydney culture collection: *F. oxysporum* (RBG5370–RBG5382), *F. fujikuroi* (RBG5383), *F. mangiferae* (RBG5384), *F. napiforme* (RBG5385), *F. poliphialidicum* (RBG5386), *F. proliferatum* (RBG5387), *F. pseudocircinatum* (RBG5388), *F. semitectum* (RBG5389), *F. solani* (RBG5390).
[D]*Fusarium* species non-viable after storage.

Pathogenicity test

The first sign of discolouration was detected on day 4 after inoculation with *F. oxysporum* (Fig. 2). All isolates of *F. oxysporum* induced discolouration and necrosis symptoms in every replicate. In contrast, none of the isolates of *F. solani* produced symptom. One isolate of *F. semitectum* from North Sulawesi caused discolouration in one replicate. However, *F. semitectum* was not reisolated indicating that this species was not the cause of the discolouration. No symptoms were recorded in the negative controls at the completion of the pathogenicity test (60 days).

Each of the *F. oxysporum* isolates from Jogjakarta, Lampung and East Kalimantan were successfully reisolated from cuttings in all five replicates. In contrast, *F. oxysporum* isolates from Bali, Central Java, North Sulawesi, West Java and West Nusa Tenggara were recovered from cuttings in only four of the five replicates. *F. oxysporum* from South Sulawesi was reisolated from three replicates. There was no significant difference in pathogenicity between isolates of *F. oxysporum* tested based on Cochran's test at $P = 0.05$.

Discussion

Twelve *Fusarium* species were recovered from disease specimens from seven provinces in Indonesia. The three most prevalent species were *F. oxysporum*, *F. semitectum* and *F. solani*. These three species accounted for 92.44% of the total *Fusarium* isolates. Of these, *F. oxysporum* was the dominant species, contributing 55.72% of the 542 isolates recovered, followed by *F. solani* and *F. semitectum*.

This finding in regards to *F. oxysporum* is congruent with the study reported by Tombe *et al*. (1993). They revealed that 100 *Fusarium* isolates from Bali, Central Java, North Sulawesi and West Java, collected from root, stem, leaf and bean of vanilla affected by stem rot were identified morphologically as *F. oxysporum*. However, no mention of other *Fusarium* species was made.

All isolates of *F. oxysporum* tested were pathogenic to vanilla according to Koch's Postulates. Hence, the *forma specialis* designation of *F. oxysporum* isolated from diseased, viz. *F. oxysporum* f. sp *vanillae*, is verified. *F. solani* and *F. semitectum* were not pathogenic to vanilla. Although one cutting inoculated with *F. semitectum* from North Sulawesi developed discolouration, the pathogen was not reisolated. Our findings concur with previous studies reported by Tombe *et al*. (1993) that *F. oxysporum* f. sp. *vanillae* is the sole cause of stem rot of vanilla in Indonesia. However, *F. solani* was also reported to cause root rot of vanilla in Puerto Rico (Alconero and Santiago 1969). In their report, both species were attributed to be the cause of root rot but the cause of stem rot was unclear. *F. solani* is also reported as an endophyte associated with root of other plant species (Maciá-Vicente *et al*. 2008), in addition to being a ubiquitous soil fungus (Leslie and Summerell 2006).

F. semitectum has been associated with various diseases such as canker of walnut, pod and seed rot of beans, reduced seed germination and seedling growth of sorghum, corky dry rot of melons, bananas and other fruits, and storage rot problems of mushrooms (Leslie and Summerell 2006). However, the role of *F. semitectum* in the study reported here revealed that *F. semitectum* is either an endophytic or saprophytic coloniser of vanilla stem rot as it was not pathogenic. *F. semitectum* was also reported by Wang *et al*. (2007) as a stem endophyte in four native *Gossypium* species in Australia. This is in general agreement with the ability of *F. semitectum* to colonise aerial plant parts as a

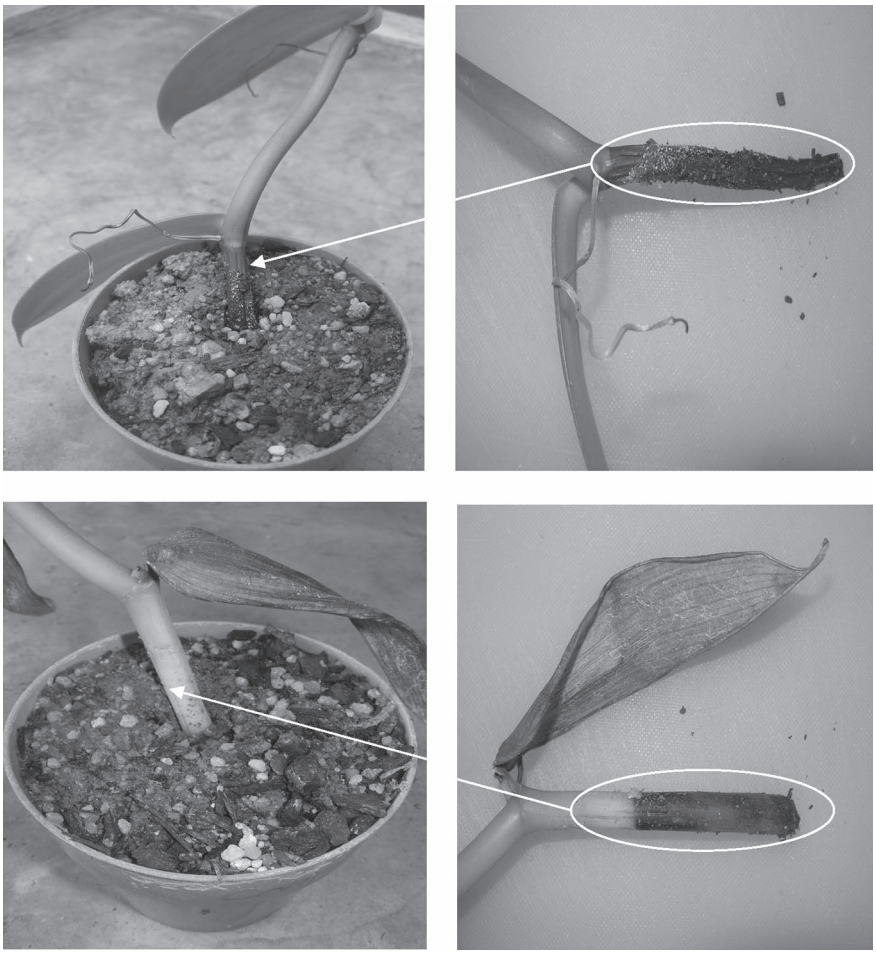

Fig. 2. Symptoms of discolouration and necrosis on vanilla cuttings inoculated with *Fusarium oxysporum* f. sp. *vanillae*.

secondary invader (Burgess 1981) and that it is recovered commonly from tropical regions (Burgess *et al*. 1994; Phan 2006; Walsh 2007).

The less common *Fusarium* species recovered in this study namely *F. proliferatum*, *F. subglutinans*, *F. decemcellulare*, *F. fujikuroi F. mangiferae*, and *F. graminearum* have been reported to be pathogenic to at least one crop species (Leslie and Summerell 2006). The remaining species, *F. pseuodocircinatum*, *F. napiforme* and *F. polyphialidicum* have not been reported to cause disease. As the recovery of these less common *Fusarium* species was lower than 3%, these species are considered as saprophytic or endophytic colonisers of vanilla in Indonesia.

This study has documented the association of a wide range of *Fusarium* species with vanilla stem rot in Indonesia. The recovery of *F. oxysporum* f. sp. *vanillae*, the pathogen, from all sampling provinces indicates that the pathogen is widespread throughout vanilla- growing areas in Indonesia. The most promising strategy in controlling disease caused by *F. oxysporum* is the use of resistant cultivars (Louvet and Toutain 1981; Fouché and Jouve 1999; DiPietro *et al*. 2003; Fravel *et al*. 2003; Belabid *et al*. 2004; El Hadrami *et al*. 2005). Breeding for resistance to the pathogen has been attempted by crossing *V. planifolia* with a non-commercial species, *V. phaeantha*. Some progenies resistant to the disease were produced but fruit quality was poor. Other progenies had poor growth in general or were susceptible to the disease (Theis and Jimenez 1957). Resistant clones to the pathogen were also generated in Indonesia using colchicine (Lestari *et al*. 2001).The clones were tested in field trials, and four clones were shown to be resistant to the pathogen. These need to be evaluated under a range of climatic conditions.

Biological control is another area of disease management that has been researched extensively. Tombe *et al*. (1997) used *Pseudomonas fluorescens* as an antagonistic agent against the pathogen within the soil and found that they were effective in suppressing the activities of *F. oxysporum* f. sp. *vanillae*.

Fungicides have also been evaluated to control stem and root rot of vanilla (Fouché and Jouve 1999). Tombe and Sitepu (1986) reported that a combination of phytosanitation and spraying and drenching with 0.25% Bavistin 50 WP at a 15–20-day interval was effective in reducing the disease.

Other cultural control methods such as the application of mulching and removing entire sections of infected stem have been successfully used to manage the disease (E. Liew

unpubl. data). In addition, the use of clove leaves, *Syzygium aromaticum*, as mulch in Indonesia has also been introduced to control the disease. Tombe *et al*. (1997) demonstrated that the addition of cloves leaves suppressed activities of *F. oxysporum* f. sp. *vanillae*, probably due to the leaching of inhibitory compounds, e.g. eugenol, from the leaves. Removal of infected tissue reduces potential sources of pathogen inoculum. The removal of infected sections does not result in vine death as the vines form aerial roots and grow epiphytically on trees.

Non-pathogenic species of Fusarium have been reported to control Fusarium wilt on various crops (Alabouvette *et al*. 1998). For examples, non-pathogenic *F. oxysporum* was reported to control Fusarium wilt on watermelon (Larkin *et al*. 1996) and to reduce severity of vanilla stem rot (Tombe *et al*. 1997). Also, non-pathogenic strains of *F. oxysporum* and *F. solani* were effective against three races of *F. oxysporum* f. sp. *lycopersici* causing Fusarium wilt of tomato (Larkin and Fravel 1998, 2002).

Endophytic isolate of *F. oxysporum* has been reported to induce host systemic resistance against *Radopholus similis* on banana (Vu *et al*. 2006) and suppress *R. similis* reproduction within host roots (Athman *et al*. 2007). The potential role of endophytic or non-pathogenic *Fusarium* species in the control of vanilla stem rot in Indonesia is worthy of further study.

The findings include valuable information on the occurrence of recently recognised *Fusarium* species in Indonesia. Indeed nine species listed in Table 2 except for *F. fujikuroi*, *F. oxysporum* and *F. solani* have been recorded for the first time in Indonesia. The data on *Fusarium* species associated with vanilla, in particular on the occurrence of non-pathogenic and endophytic *Fusarium* species will contribute to future work on disease management. The findings reported here will contribute to a better understanding of the aetiology of this disease in Indonesia, as well as the ecology of *F. oxysporum* f. sp. *vanillae* and the development of integrated disease management strategies.

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