Are some endophytes of *Musa acuminata* latent pathogens?

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Fungi isolated as endophytes from wild banana (*Musa acuminata*) were tested in order to ascertain whether they are capable of causing disease symptoms in healthy banana leaves. The endophytes *Cladosporium musae*, *Colletotrichum gloeosporioides*, *Cordana musae*, *Deightoniella torulosa*, *Guignardia cocoicola*, *Periconiella musae* and *Pestalotiopsis* sp. were inoculated on banana leaves *in vitro* to test their pathogenicity. Only *Deightoniella torulosa* was able to cause leaf spots on banana leaves *in vitro*. This result confirms earlier reports that fungal pathogens may be latent in their host long before the outbreak of disease symptoms.

Key words: banana, *Deightoniella torulosa*, disease symptoms.

Introduction

Endophytes are fungi that colonize healthy plant tissues and either persist in a dormant phase or comprise more extensive, but symptomless infections (Petrini, 1991). There have been numerous studies on tropical endophytes (e.g. Kumaresan and Suryanarayanan, 2002; Toofanee and Dulymamode, 2002), but few studies have addressed the problem that some endophytes may be latent pathogens (Brown *et al*., 1998). Endophytes have also often been isolated by plant pathologists and described as weak pathogens of little consequence, except under unusual conditions in which the host plant is subject to physiological stress (Kulik, 1984). Latent infection of plants by pathogens has been long recognized (Gäumann, 1951). It is a situation in which a pathogen infects a host, but the plant does not show disease symptoms. The pathogen persists, and later produces signs or symptoms of disease, prompted by

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environmental or nutritional conditions, or by the state of maturity of the host or pathogen (Verhoeff, 1974; Agrios, 1988). Changes may be induced by an alteration of host physiology due to the activity of the fungus, or changes in the environment that increase the stress on the plant. Early observations with tropical fruits, particularly banana, indicated that *Colletotrichum* sp. survived latent periods as subcuticular hyphae, which remained quiescent until fruit ripening (Simmonds, 1963). The information on internal fungi from banana addresses specific latent pathogens such as *Colletotrichum gloeosporioides* or *Pyricularia grisea* on banana fruit in commercial hybrids (Meredith, 1963; Verhoeff, 1974). The purpose of this study was to identify other endophytic taxa that may exist as latent pathogens in *Musa* sp.

Materials and methods

Isolation of endophytes

Endophytes were isolated from leaves, petioles and pseudostems of healthy symptomless plants of *Musa acuminata* from five sites at Doi Suthep Pui National Park, Chiang Mai, Thailand (Photita *et al*., 2001). One hundred and fifty leaves were first washed in running water. Leaf disks (3 mm diam) were cut to include the vein (10 samples) and intervein tissues (10 samples) from each leaf sample, using a sterile cork borer. Ten segments (5 × 5 × 5 mm) were cut from each of the petiole, midrib and two pseudostem samples using a sterile razor blade. All leaf disks and segments were then surface sterilized to remove epiphytic fungi, in 75% ethanol for 1 minute followed by 1% sodium hypochlorite for 3 minutes and 95% ethanol for 0.5 minutes before drying between sterilized paper. This protocol was found to be the optimum triple sterilization procedure for isolating endophytes from *M. acuminata* following a pilot experiment (Photita *et al*., 1999). Five surface sterilized leaf disks or segments were evenly spaced in Petri-dishes (9 cm diam) containing 2% (w/v) malt extract agar (MEA) with added Rose Bengal (30 mg/l) to slow fungal growth, and streptomycin sulfate (50 mg/l) to suppress bacterial growth. Mycelium developed within two weeks and the fungi were then transferred to cornmeal agar slants. The fungi were identified following sporulation.

Pathogenicity tests

Representative isolates of endophytic fungi, especially genera and species reported as pathogens of banana (Brown *et al*., 1998), were tested in this study. Five replicates of the endophytic isolates of *Cladosporium musae*, *Colletotrichum gloeosporioides*, *Cordana musae*, *Deightoniella torulosa*,

Guignardia cocoicola, *Periconiella musae* and *Pestalotiopsis* sp. were tested for their ability to cause leaf spots on banana leaves. Fungal isolates were grown on potato dextrose agar (PDA) for one week.

To test for pathogenicity of the fungal isolates, large pieces of healthy leaves of *Musa* sp. (20 cm long) were freshly cut from plants and then surface sterilized with 95% ethanol. Each healthy leaf piece was incubated individually in sterilized plastic boxs, with an addition of sterilized tissue paper moistened with sterilized water (Fig. 1). Conidial suspensions were prepared by growing isolates for 1-2 weeks until sporulation occurred and the conidia were then scraped off into a 1.5 ml sterile plastic tube containing sterile distilled water.

Conidial suspensions were standardized to 5×10^6 conidia per ml by diluting culture filtrates with sterile distilled water. Conidial suspensions (20 µl) were sprayed on to healthy detached banana leaves. One subset of leaves was wounded by puncturing with a sterile needle before inoculation.

Fig. 1. Banana leaf incubating in moist chamber (lid removed).

Disks containing mycelia from the actively growing edge were cut using a sterile cork borer and placed on a subset of healthy wounded and unwounded detached banana leaves. In the control treatment, leaves were treated by using disks of sterile water agar (WA) or by spraying with sterile distilled water. A positive control was developed using *Deightoniella torulosa*, a pathogen isolated from banana leaf spot. The treated tissue was incubated for 1-2 weeks under high humidity (90%) at room temperature. If the endophyte was pathogenic the banana leaf became infected. Necrotic lesions were measured. To re-isolate pathogens at the end of the trials, tissue pieces were cut aseptically from symptomatic leaves, surface sterilized in 1% NaOCl for 1 minute and placed onto PDA. Plates were incubated for 1-2 weeks, and colonies were subcultured and identified.

Results

Sixty-one different fungal endophytes were isolated from healthy symptomless plants of *Musa* sp. in a previous study (Photita *et al*., 2001). Of these, *Cladosporium musae*, *Colletotrichum gloeosporioides*, *Cordana musae*, *Deightoniella torulosa*, *Guignardia cocoicola*, *Periconiella musae* and *Pestalotiopsis* sp. have previously been reported as banana pathogens. The endophytic isolates of these taxa, plus one pathogenic isolate of *Deightoniella torulosa* derived from leaf spots were tested for their pathogenicity against banana leaves.

The endophytic isolate of *Deightoniella torulosa* and the isolate of *Deightoniella torulosa* from a banana leaf spot (positive control) both caused leaf spots on banana leaves (Table 1). The leaf spot comprised a necrotic, pale brown region, surrounded by a chlorotic yellow halo.

Two methods were used to inoculate banana leaves with the test fungi. A spore suspension was sprayed onto leaves or an agar disk with mycelia was placed on the leaves. To increase the chance of infection, a subset of leaves were wounded by puncturing. The disease symptoms with *Deightoniella torulosa* were the same on the wounded and unwounded leaves, irrespective of the type of innoculation (Fig. 2). The purportedly pathogenic isolate of *Deightoniella torulosa* isolated from a banana leaf spot (positive control) caused a larger leaf spot than the endophytic isolates. The leaf spot comprised necrotic, pale brown regions, surrounded by a chlorotic yellow halo (Figs. 2, 3).

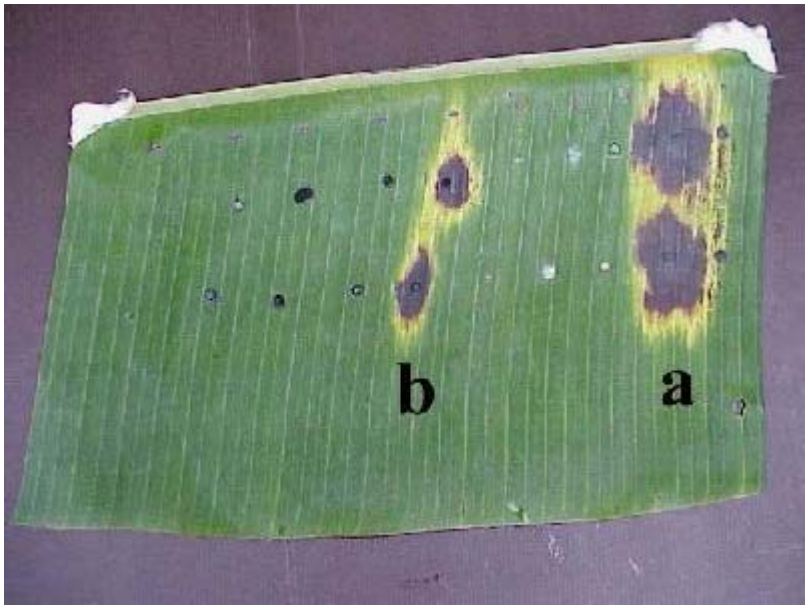

Fig. 2. Typical disease symptoms on detached banana leaves when isolates of *Deightoniella torulosa* were inoculated as a mycelial plug, **a.** pathogen: positive control, **b**. endophytic isolate. Other fungi did not produce symptoms.

Fig. 3. Disease symptoms on detached banana leaves when endophytic isolates of *Deightoniella torulosa* were inoculated as a conidial suspension, **a.** unwounded, **b**. wounded (other disks not causing infection). Other fungi did not produce symptoms.

Table 1. Pathogenicity of fungal taxa isolated as endophytes, and a pathogenic strain of *Deightoniella torulosa* inoculated onto healthy detached banana leaves, using four inoculation methods.

Taxa	Inoculation method[1]		Symptomatic disease severity[2]
	Inoculum	Wounding	
Cladosporium musae	Mycelia	-	0
	Mycelia	+	0
	Conidia	-	0
	Conidia	+	0
Colletotrichum gloeosporioides	Mycelia	-	0
	Mycelia	+	0
	Conidia	-	0
	Conidia	+	0
Cordana musae	Mycelia	-	0
	Mycelia	+	0
	Conidia	-	0
	Conidia	+	0
Deightoniella torulosa	Mycelia	-	3
	Mycelia	+	3
	Conidia	-	3
	Conidia	+	3
Guignardia cocoicola	Mycelia	-	0
	Mycelia	+	0
	Conidia	-	0
	Conidia	+	0
Periconiella musae	Mycelia	-	0
	Mycelia	+	0
	Conidia	-	0
	Conidia	+	0
Pestalotiopsis sp.	Mycelia	-	0
	Mycelia	+	0
	Conidia	-	0
	Conidia	+	0
Deightoniella torulosa (Pathogen on banana leaf/ positive control)	Mycelia	-	5
	Mycelia	+	5
	Conidia	-	5
	Conidia	+	5
Control (WA only)/sterile water		+	0
		-	0

[1]The four inoculation methods involved two types of inucola, mycelial plug, conidial suspensions, and unwounded and wounded detached leaves.
[2]Disease severity was based on symptom development on banana leaves using scale of 0 to 5; 0 = not necrotic, 5 = necrotic surrounded by a chlorotic yellow halo about 5 cm.

The other six species tested, *Cladosporium musae*, *Colletotrichum gloeosporioides*, *Cordana musae*, *Guignardia cocoicola*, *Periconiella musae* and *Pestalotiopsis* sp. did not produce any disease symptoms.

Discussion

Evidence that an endophytic stage may occur in the life cycle of some banana pathogens has been discussed by several authors (e.g. Sinclair and Cerkauskas, 1996; Brown *et al*., 1998). By definition an endophyte cannot be considered to cause disease (Sinclair and Cerkauskas, 1996), however, genera and species that can cause disease are regularly isolated and identified as endophytes. The distinction between a pathogen and endophyte is not always clear (Sinclair and Cerkauskas, 1996). The purpose of this study was to establish if any fungi regularly isolated as endophytes from banana have the ability to be pathogenic (i.e. a latent pathogen). Genera that include common pathogens and were isolated as endophytes here were *Cladosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Guignardia*, *Nigrospora*, *Phoma* and *Verticillium* (Photita *et al*., 2001). These genera also include species, many of which are the most serious pathogens of *Musa* species, e.g. *Fusarium oxysporum* and *Colletotrichum gloeosporioides* (Jeger *et al*., 1995). Pathogens previously recorded from leaves of *Musa* are listed in Table 2. Strains of four of the 15 species occurred endophytically in tissues of *Musa acuminata*.

Some pathogens have a latent phase within the host tissue and some saprobes can also be facultative parasites. It has been observed that certain endophytes become pathogenic when the host plant is stressed (Millar, 1980; Andrews *et al*., 1985). A latent phase represents a specific condition where the fungus can either develop symptoms or cause change in the physiology of host plant (Romero *et al*., 2001). Change in host susceptibility caused by excessive humidity, or poor nutrient supply, will induce the transition from one life mode to the other and determine the duration of the period during which an individual will remain in the same life mode (Fisher and Petrini, 1992).

The presence of *Deightoniella torulosa* in healthy banana plants confirms earlier reports (Kulik, 1984; Petrini *et al*., 1989; Sinclair and Cerkauskas, 1996; Romero *et al.*, 2001) that some fungal pathogens are latent in their host long before the outbreak of disease symptoms. This result suggests that an endophytic stage may also be important in the life cycles of some banana pathogens. Both *Cladosporium musae* and *Periconiella musae* are reported to cause a leaf speckle on banana leaves (Stover, 1972; Holliday, 1980; Farr *et al*., 1989; Jeger *et al*., 1995), but such symptoms did not develop in current experiments. *Cordana musae* can cause large pale brown leaf spots on banana, but these spots are often found in association with another fungus such as *Phyllachora musicola* or *Deightoniella torulosa* (Stover, 1972; Holliday, 1980; Farr *et al*., 1989; Jeger *et al*., 1995).

Table 2. Fungi previously recorded as pathogens from leaves of *Musa* sp. (Stover, 1972; Holliday, 1980; Farr *et al*., 1989; Jeger *et al*., 1995). *Species also isolated as endophytes.

Fungi	Disease
**Cladosporium musae*	*Cladosporium* speckle
**Colletotrichum gloeosporioides*	Anthracnose, leaf spot
Colletotrichum musae	Anthracnose, leaf spot
**Cordana musae*	Leaf blotch, Cordana leaf spot
Curvularia sp.	Leaf spot
**Deightoniella torulosa*	Black leaf spot
Drechslera gigantea	Eye spot
Guignardia musae	Leaf freckle
Helminthosporium sp.	Leaf spot
Mycosphaerella fijiensis	Black sigatoka, black leaf streak
Mycosphaerella musicola	Yellow sigatoka, leaf streak
Mycosphaerella musae	Leaf speckle
Phyllachora musicola	Black cross leaf spot
Phyllosticta musae	Leaf spot
Phyllosticta musicola	Leaf spot
Pyricularia grisea	Pitting

In this study a strain of *Deightoniella torulosa* isolated as an endophyte was shown to be capable of causing leaf spots on healthy banana leaves. *Deightoniella torulosa* was also recorded as a saprobe on dead banana leaves (Photita *et al*., 2003). It is also a weak parasite of the older foliage of banana and has been reported on young leaves of *Musa* seedlings (Stover, 1972). The leaf spot is more prevalent if plants are growing under poor conditions and humidity is high. Senescing or injured leaves are more prone to the disease (Jones, 2000).

The other endophytic isolates did not produce disease symptoms in the experiment. *Periconiella musae* is not regarded as a serious pathogen and has no effect on yield or growth of banana. It is a pathogen and in Australia symptoms are only seen in unsprayed plantations located in rainforest clearings in high-rainfall areas (Jones, 2000). The two *Pestalotiopsis* pathogens of *Musa* species are *P. palmarum* and *P. leprogena* and are not regarded as serious pathogens.

It is therefore quite feasible that saprobes, endophytes and pathogens of a plant may be the same strain/species. This has important implications for estimated global fungal diversity numbers, which rely heavily on host-fungus ratios (Hawksworth, 2001; Hyde *et al*., 2002). If saprobes have the potential for endophytic or pathogenic life modes, they are much more likely to be host-specific or host-recurrent due to evolutionary relationships developed with the host plants.

Evidence suggests that endophytes have evolved directly from plant pathogenic fungi (Carroll, 1988; Isaac, 1992). Many pathogens of economically important crops may be endophytic or latent in weeds. Many endophytes are sister species to virulent pathogens on the same or closely related host, e.g. *Acremonium coenophialum*, the important grass endophyte is very closely related to *Epichloë typhina*, a pathogen (Clay, 1988).

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