metabolic pathway enzyme phospholipase A_2 (bee venom PLA_2) each showing 96% inactivation at 1.6 μg/ml[2]. Hence, vidalols A and B could provide interesting lead molecules for the design of inhibitors of this important enzyme.

Line many ortho-catechols, vidalols A and B slowly oxidize, in air, to yield red solutions and amorphous powders consisting of their corresponding ortho-quinones **4** and **5**. Because of the difficulty in maintaining vidalols A and B pure during bioassays, we are not sure if the catechols or the more reactive ortho-quinones exhibit the antibiotic and enzyme-inhibitory properties reported here. Indeed, in a similar case with the metabolites from the brown alga *Stypopodium zonale*[12], it was observed that air oxidation yielded an ortho-quinone which reacted as an electrophile binding sulfhydryl groups to selectively inhibit the enzyme tubulin[13].

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The phytotoxins of *Mycosphaerella fijiensis*, the causative agent of Black Sigatoka disease of bananas and plantains

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Abstract. Black Sigatoka is the most costly to control disease of bananas and plantains in the world. Currently, a worldwide search is underway either to find or to produce cultivars that are disease-resistant or -tolerant. Phytotoxins isolated from the pathogen might facilitate the discovery of such cultivars. Several aromatic compounds from liquid cultures of *Mycosphaerella fijiensis*, the causal agent of Black Sigatoka disease of bananas and plantains, have been isolated. The most abundant and phytotoxic of these compounds is 2,4,8-trihydroxytetralone, which induces necrotic lesions at 5 μg/5 μl in less than 12 h on sensitive cultivars of bananas. This compound exhibits host-selectivity that mimics that of the pathogen. Other phytotoxins isolated from this fungus, in lesser amounts, were juglone, the novel compound 2-carboxy-3-hydroxycinnamic acid, isoochracinic acid and 4-hydroxyscytalone. Some of the phytotoxins isolated are melanin shunt pathway metabolites, which makes this fungus unique among plant pathogens.
Key words. Epidemic; juglone; 2,4,8-trihydroxytetralone; melanin shunt pathway; plant pathogen; Sigatoka.

Bananas and plantains are the primary food source for millions of people in many areas of the world, including Central Africa, Southeast Asia, Central and South America, and the Caribbean. People in these regions are generally faced with high population growth and recurring food shortages, conditions that augment the importance of high yield, low cost crops like bananas and plantains[1]. They yield a sweet, nutritious fruit and produce a starch that can be used to prepare a variety of staple foods. Bananas and plantains also provide more than just complex carbohydrates. They also yield a diverse array of useful secondary products such as fibers, wrappers, confectioneries, vegetables, catsup, beer, wine and vinegar[1,2].

Commercial cultivation of bananas in tropical America, the Philippines, and Caribbean and Pacific islands is a significant economic resource. In these regions they not only maintain their importance as a food staple, but also contribute to the GNP, provide employment and fiscal earnings, and generate foreign currency. Of all of the plants grown as food, bananas and plantains produce the highest yield for the lowest cost[3]. This vital resource is currently being threatened by a leaf disease known as Black Sigatoka, the most severe form of a disease complex involving three closely related fungal pathogens: *Mycosphaerella musicola* Leach ex Mulder, causative agent of Yellow Sigatoka, first identified in the Sigatoka district of Java in 1902; *M. fijiensis* Morelet, causative agent of Black leaf streak, described in Fiji in 1964; and *M. fijiensis* var. *difformis* Mulder and Stover, causative agent of Black Sigatoka discovered in Honduras in 1972[4]. As the Sigatoka diseases have progressed in time their virulence has increased. Their host range has broadened to include plantains, which are resistant to Yellow Sigatoka disease. Black Sigatoka has supplanted Yellow Sigatoka as the major threat to the cultivation and economic importance of bananas and plantains[5].

The Black Sigatoka epidemic in Honduras and several other countries in 1972–1973 resulted in enormous crop losses[6]. Crop yields for Horn-type plantains have been reduced by 50%[7]. Control costs for this disease totaled more than $ 17 million/year (US) in Costa Rica alone; no other disease or pest control program in that country can compare[6]. The cost of controlling Black Sigatoka disease in Central America, Colombia, and Mexico surpassed $ 350 million (US) in a recent eight year time span[6]. Large fruit producing companies must spray fungicide mixtures in 14 cycles annually to control Black Sigatoka, expending up to 30% of their gross incomes. Small land owners cannot control the disease in their fields, lacking both the equipment and the funds required for fungicide application. The impact of this plant disease on marginal farmers of the tropics has been enormous.

An alternative solution is the isolation and dissemination of banana and plantain cultivars resistant to the Sigatoka disease complex. Such a program could involve either breeding for resistance or the screening of existing cultivars for inherent resistance. Breeding bananas and plantains is a slow, tedious process, due to serious problems with polyploidy and poor seed yields and pollen infertility[7]. Furthermore, movement of the germplasm of bananas and plantains and different pathogenic biotypes in the screening process could inadvertently introduce new forms of the disease into disease-free areas. The isolation and identification of wild-type resistant cultivars circumvents these difficulties.

Unfortunately, finding such resistant cultivars can be an expensive, time-consuming process. Using traditional methodology, mature plants are challenged by fungal spores produced on naturally infected leaves according to fastidious inoculation schemes to artificially induce the disease. This process may require over twelve months to establish unequivocally the susceptibility or resistance of a particular cultivar to the disease[8]. Fortunately, a preliminary report in 1989 indicated that disease symptoms could be induced by the crude extract of the pathogen, suggesting the presence of one or more phytotoxins[9,10]. Because of the extreme importance of this disease we began a comprehensive investigation of the phytotoxins of *M. fijiensis*. It was hoped that phytotoxins isolated from the fungus could provide a rapid indication of cultivar susceptibility or resistance using a simple leaf assay. This report describes the isolation, characterization, and biological significance of the phytotoxins isolated to date.

Materials and methods

Fungal culture. The culture of *M. fijiensis* (IMI 105378) used in this study was originally isolated from *Musa sapientum* by R. Leach in 1964 in Fiji, and was supplied by Commonwealth Mycological Institute, London. *M. musicola* (357) was provided by R. A. Fullerton, DSIR, New Zealand; *M. fijiensis* var. *difformis* was isolated by G. C. Molina, FHIA, Honduras. All cultures were maintained at 26 °C on modified M-1-D medium to which 12 ml/l coconut milk was added[11].

Mass culture and extraction. The fungi were grown in 2-l Erlenmeyer flasks containing 800 ml modified M-1-D medium (autoclaved)[11]. The medium was inoculated with two mycelial disks (1 cm^2) cut from the edge of a vigorously growing fungal colony. Each flask was shaken at 140 rpm for 28 days at 26 °C with 12 h of light (50 $Em^{-2}s^{-1}$). After incubation, an equal volume of methanol was added to the fungal culture, which was left for 24 h at 4 °C. The culture was filtered through eight layers of cheesecloth and the volume of the filtrate was reduced by 2/3 by rotary evaporation at 40 °C. The concentrated filtrate was exhaustively extracted (3 times) with an equal volume of ethyl acetate. The three extracts were combined and reduced to a brown oil (ca 90 mg/l). For the investigation of fungal metabolites at timed intervals, cultures were grown for 10, 15, and 20 days following the protocol outlined above. The crude extract yields were ca 35, 75, and 86 mg/l, respectively.

Bioassay. A simple leaf puncture bioassay was used as a guide in the isolation of phytotoxins[12]. Five test varieties of bananas and plantains including Gran Nain (*Musa acuminata* Colla AAB), Horn plantain (*M. acuminata* x *M. balbisiana*), Saba (*M. balbisiana*, ABB), IV-9 (breeding line of FHIA), and Bocadillo (AA) were propagated through meristem culture to produce plantlets in test tubes. Plantlets were ready for testing at the 3–5-leaf stage when plants were approximately 4 inches tall. Test fractions were dissolved in 2% ethanol and applied to the nicked surface of the fully opened heart leaves in 5-µl droplets. The punctures were encircled with Vaseline petroleum jelly to maintain droplet integrity. Leaves were placed in sterile plastic chambers with moistened filter

Table 1. Comparison of molecular formulae and separation procedures of phytotoxins isolated from *M. fijiensis*.

Compound	Molecular formula	Final isolation	R_f-TLC[a]	% Yield[b]
1	$C_{10}H_{10}O_4$	RP-HPLC[c]	0.39	0.63
2	$C_{10}H_6O_3$	Flash silica	0.95	0.02
3	$C_{10}H_8O_5$	Sephadex LH-20	0.0	0.03
5	$C_{10}H_8O_5$	RP-Flash[d]	0.57	0.03
6	$C_{10}H_{10}O_4$	Sephadex LH-20	-	0.50

[a] Silica gel developed in chloroform-methanol, 10-1; [b] %yield = (mass of compound isolated/mass of total organic extract) ×100; [c] 25%–75% acetonitrile in water over 25 min; [d] 40%–70% MeOH in water over 20 min.

paper to provide humidity. Crude extracts were tested at 25, 50, and 100 µg per puncture wound; pure compounds were tested at 0.01, 0.1, 1.0, 5.0, and 10 µg per puncture. Necrotic tissue around the wound was measured at 24 and 48 h after treatment. Activity indexing utilized a 0–5 scale (+), ranging from no necrosis (0) to lesions 12 mm in diameter (5 +). Bioassays were repeated three times, with compounds applied to ten puncture sites during each replication. Ethanol-water (2%) was used as a control. Organic extracts of sterile (noninoculated) medium did not exhibit phytotoxicity.

Results and discussion

Isolation of phytotoxins. The ethyl acetate soluble extracts of the culture filtrate of *M. fijiensis* were chromatographed on Sephadex LH-20 [$CHCl_3$ – MeOH, 1:1 (v/v)]. Fractions 3, 4, and 5 were phytotoxic. Fraction 4, the most active fraction, was further resolved by reverse phase-HPLC on an Altex C-18 column using gradient elution with acetonitrile-water (25% MECN-75% MeCN, 25 min.). This step concentrated the primary phytotoxins in the sixth and eighth of eight fractions. Repeating the HPLC procedure on the sixth fraction yielded 2,4,8-trihydroxytetralone **1**. Flash silica gel chromatography of the eighth fraction yielded juglone **2** as the first eluant.

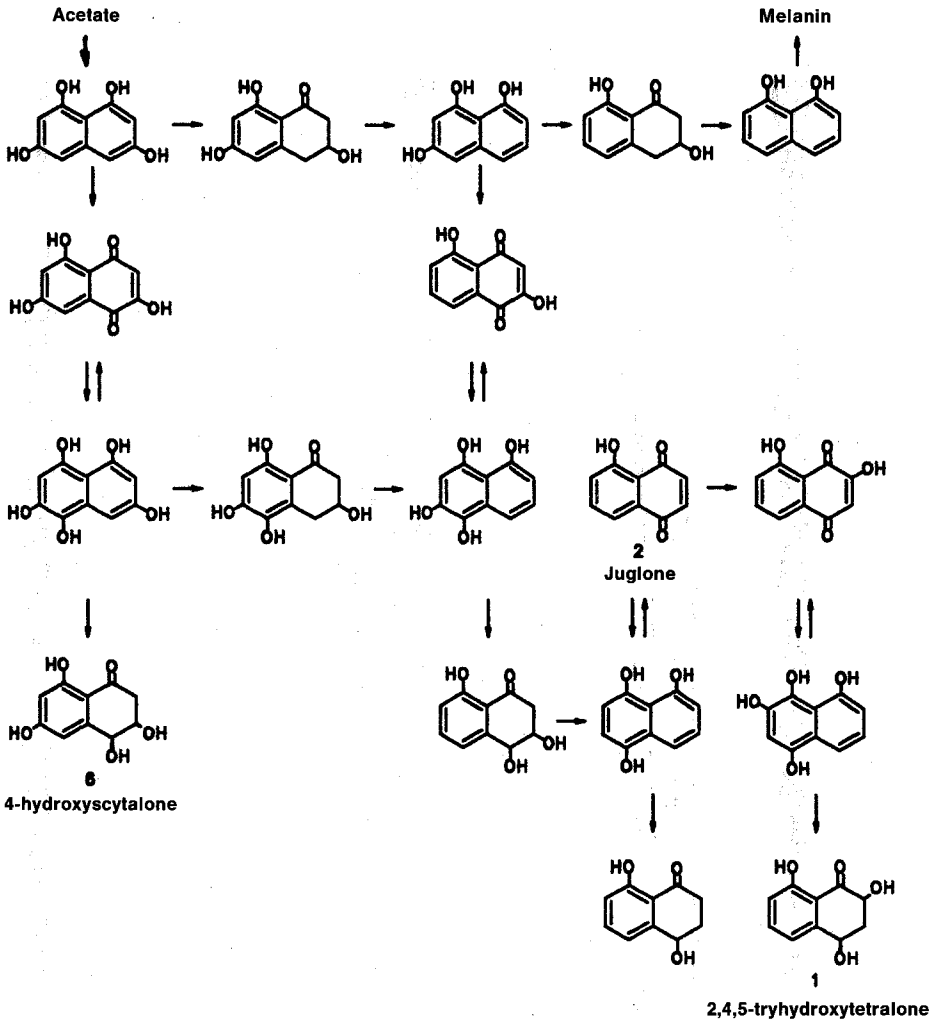
Fraction 3 was resolved into nine fractions by reverse phase HPLC using a MeOH-water gradient. The third fraction was the known phthalide isoochracinic acid **5**. Fraction 5 yielded 2-carboxy-3-hydroxycinnamic acid **3** after chromatography on the LH-20 column as described above. Fraction 6 was 3,4,6,8-tetrahydroxytetralone **6** (4-hydroxyscytalone). Table 1 summarizes the data for the isolation of compounds **1–6**.

Structural elucidation of phytotoxins. The molecular formula of compound **1** was determined to be $C_{10}H_{10}O_4$ by high-resolution mass spectrometry, indicating a compound with six sites of unsaturation. Phenolic and ketone functionalities were indicated by infrared absorptions at 3590 and 1640 cm^1. Both 2D COSY and 1 D decoupling experiments indicated a trisubstituted benzene ring with three adjacent protons and a cis-1,3-diol system. Detailed analysis of the mass spectral and ^1H NMR data resulted in the proposal of structure 1,2,4,8-trihydroxytetralone. Compound **1** was proposed as a melanin shunt-pathway product by Stipanovic[13], although it was only isolated when an appropriate precursor was supplied to the fungus *Verticillium dahliae* (fig. 1). The compound was also isolated by Fujimoto from *Penicillium diversum* var. *aureum*[14]. No biological activity has ever been ascribed to the compound.

Compound **2**, juglone, has been previously isolated from several plant sources including walnuts (*Juglans nigra*)[15] and species of *Penicillium*[14] and *Verticillium*[16]. It was readily identified by spectral data analysis: both spectroscopic and physical characteristics were identical to the authentic compound purchased from Aldrich Chemical Company.

Compound **3** has a molecular formula of $C_{10}H_8O_5$, as determined by chemical ionization mass-spectrometry, indicating seven sites of unsaturation. ^1H NMR analysis indicated three adjacent protons on a tri-substituted benzene ring, with the highest field aromatic proton, δ6.95 ppm, ortho to a hydroxyl group. Two mutually coupled (J = 16 Hz) olefinic absorptions at δ8.32 and δ6.18 ppm suggested a trans-α,β-unsaturated carboxylic acid derivative. This was a very polar compound which streaked on silica TLC plates unless 0.5% trifluoroacetic acid was added to the solvent system. The behavior suggested the presence of carboxylic acid functionality; infrared analysis supported this idea with broad OH stretch absorption from 3150 to 3550 cm^{-1} and carbonyl absorption at 1720 cm^{-1}. The compound was methylated by dropwise addition of freshly prepared diazomethane[17]. Examination of the ^1H NMR spectrum of the single product **4** indicated the addition of three methyl groups at δ3.94, δ3.83 and δ3.77 ppm, which suggested the presence of either three carboxylic acid moieties or two acids and a phenolic moiety in the original compound. Difference nOe experiments with the methylated product led to the proposed structure of the novel compound 2-carboxy-3-hydroxycinnamic acid.

Compound **5**, isoochracinic acid, $C_{10}H_8O_5$, is also a bicyclic compound with the same aromatic substitution pattern found in **1** and **2**. It contains both a fused-ring aromatic lactone and a carboxylic acid, as indicated by the infrared absorptions at 1723 and 1718 cm^{-1}. Isoochracinic acid has been isolated from *Hypoxylon coccineum* and *Alternaria kikuchiana*, the causative agent of black leaf spot of pear[18,19]. The biogenesis of isoochracinic acid has been of particular interest. It is one of the few naturally occurring phthalides. During this study we were able to establish **3** as a possible precursor of **5**. If left in contact with $CHCl_3$ – MeOH (1:1) for 24



h at 25 °C, cyclization would occur spontaneously, resulting in a racemic mixture of **5**.

Compound **6** has a molecular formula of $C_{10}H_{10}O_4$, with six sites of unsaturation. It has the same aromatic moiety as **1** and **2** but differs in the aliphatic portion. Examination of the spectral data indicates the structure shown, 4-hydroxyscytalone, which is also a known metabolite in the melanin shunt pathway[20].

2,4,8-trihydroxytetralone **1**. MS: m/z(%) 194(13.4), 176(5.6); HRMS: 194.0550 (observed), 194.0552 (actual); ^{1}H NMR (MeOH$-D_4$): 7.54 (t, J = 8.1 Hz), 7.21 (d, J = 8.1), 6.86 (d, J = 8.1), 4.89 (dd, J = 11.4, 5.1), 4.41 (dd, J = 13.3, 4.8), 2.63 (dt, J = 4.8, 11.4) and 1.98 (dd, J = 13.3, 5.1).

Juglone **2**. MS: m/z 174; ^{1}H NMR (MeOH$-D_4$): 7.68 (t, J = 7.5 Hz), 7.59 (d, J = 7.5), 7.31 (d, J = 7.5), 7.00 (s), 7.01 (s).

2-carboxy-3-hydroxycinnamic acid **3**. MS: m/z 208; ^{1}H NMR (MeOH$-D_4$): 8.32 (d, J = 16 Hz), 7.39 (t, J = 7.1 Hz), 7.09 (d, J = 7.1), 6.95 (J = 7.1).

2-carboxy-3-methoxycinnamic acid dimethyl ester **4**. HRMS: 250.0821 (observed), 250.0817 (actual); ^{1}H NMR ($CDCl_3$): 7.63 (d, J = 16 Hz), 7.38 (t, J = 8.0), 7.19 (d, J = 8.0), 6.94 (d, J = 8.0), 6.37 (d, J = 16).

Isoochracinic acid **5**. HRMS: 208.0364 (observed), 208.0368 (actual); ^{1}H NMR (MeOH$-D_4$): 7.52 (t, J = 7.5 Hz), 7.03 (d, J = 7.5), 6.86 (d, J = 7.5), 5.81 (dd, J = 6.0, 5.8), 2.85 (dd, J = 15.4, 5.8), 2.71 (dd, J = 15.4, 6.0).

4-hydroxyscytalone **6**. MS: m/z(%) 210(22.1), 191.9 (9.2), 137(39.1); ^{1}H NMR (MeOH$-D_4$): 6.73 (d, J = 2.5 Hz), 6.21 (d, J = 2.5), 4.30 (d, J = 3.0), 3.97 (ddd, J = 3.0, 4.8, 8.1), 2.96 (dd, J = 4.8, 17.5), 2.66 (dd, J = 8.1, 17.5).

Biological activity. Each of the phytotoxins isolated displayed phytotoxicity at one or more test concentrations on one or more cultivars of banana or plantain. Compound **1** exhibited host selectivity comparable to that of the fungal pathogen at the 5 µg/5 µl level. Variety IV-9 is resistant to Black Sigatoka disease and was insensitive to **1** up to the 10 µg/5 µl application rate in the leaf bioassay test. Saba, a tolerant banana cultivar, was only slightly reactive to **1**, unlike the extremely disease susceptible varieties Boca and Horn plantain which developed large

Table 2. Comparison of the reactions of various banana/plantain cultivars to the phytotoxins produced by *M. fijiensis**.

µg/puncture	Gran Nain	Horn plantain	Saba	Boca	IV-9
Compound **1**					
0.10 µg	–	–	–	–	–
1.00	–	+	–	–	–
5.00	+	++	–	+	–
10.00	++	+++	+	+++	–
Compound **2**					
0.01 µg	–	–	–	–	–
0.10	+	+	+	+	+
1.00	+++	+++	++	+++	+++
5.00	+++	++++	++	++++	++++
Compound **3**					
1.00 µg	–	–	–	–	–
5.00	+	+	–	+	–
10.00	+++	++++	+	++++	++++
20.00	++++	++++	++	++++	++++
Compound **5**					
1.00 µg	–	–	–	–	–
5.00	–	+	+	+	+
10.00	+	++	++	++	+
20.00	++	+++	+++	+++	++
Compound **6**					
1.00 µg	–	–	–	–	–
5.00	–	–	–	–	–
10.00	–	+	+	+	+
20.00	+	+	+	+	+

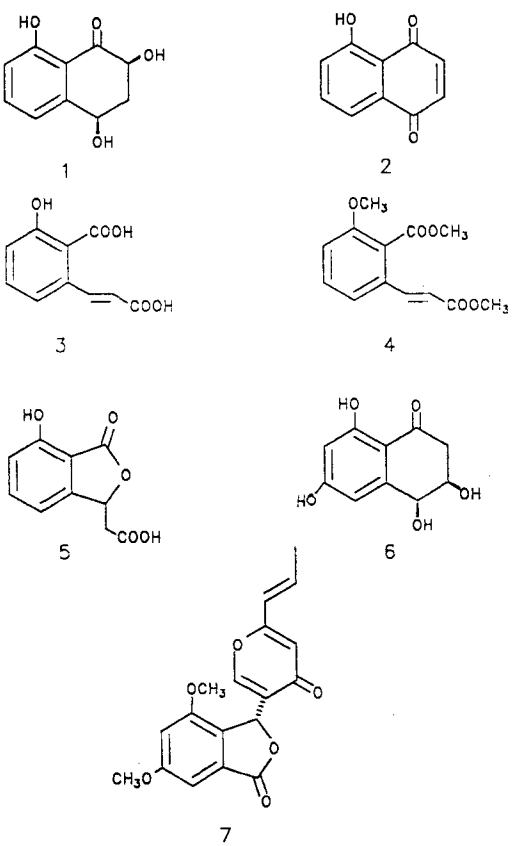
*Toxins were applied at the rate indicated in 5 µl of solution (2% EtOH in water) as described in materials and methods. Readings recorded above were after 48 h of treatment.

necrotic lesions after application of toxin **1**. Compound **1** shows definite potential as a screening tool for toxin sensitivity in tissue culture systems, especially with the recent development of such systems[21]. In repeated fermentations **1** was produced at 30 times the rate of the other toxins, suggesting that it is the principle phytotoxic agent.

Juglone **2** was more toxic than **1** (table 2), but was isolated at extremely low concentrations (table 1), and therefore may not play an important role in symptom induction. The novel phytotoxin 2-carboxy-3-hydroxy-cinnamic acid **3** demonstrated limited host selectivity at the 5 µg/5 µl level. Toxins **5** and **6** were considerably less active than the other phytotoxins isolated and displayed no host selectivity. Fijiensin **7**, previously reported as a phytotoxin from the fungus, exhibits a low level of bioactivity and no host-selectivity[10].

In this study we found no evidence of phytotoxins in the aqueous phase of the fungal culture extractions and no evidence of additional phytotoxins in the ethyl acetate extract. It would appear on the basis of yield, level of bioactivity, and host selectivity, that 2,4,8-trihydroxytetralone **1** is the most important phytotoxin produced by *M. fijiensis*.

Our study also involved analysis of toxin production at different times during the fermentation process. We were particularly interested in determining when **1** was produced in culture. It has been noted in field studies that a 3–4-week lag time occurs between inoculation of a host with the pathogen and onset of disease symptoms[9]. It would seem unlikely that a toxin that is capable of inducing disease symptoms in the lab in a matter of hours would require 3 weeks for symptom induction in the field. To this end, we grew the fungus for intervals of 10, 15, 20, and 28 days. The ethyl acetate soluble extract of



each culture was analyzed in precisely the same manner as before. No evidence of either **1** or **2** was found until the cultures had reached 28 days (or past 20 days), although isoochracinic acid **5** was found in all of the cultures. This interesting discovery correlates the observed lag time between inoculation and onset of disease symptoms in the field with the production of **1** in culture. Further investigation of the metabolites of the 10-, 15- and 20-day cultures is in progress. One extremely important aspect of this study concerns the determination of any morphological or chemical differences among the three fungi of the Sigatoka complex, *M. musicola*, *M. fijiensis*, and *M. fijiensis* var. *difformis*. Preliminary data show some interesting chemical trends. All three fungi were grown and processed as described above. *M. musicola*, which causes the least virulent Yellow Sigatoka, does not appear to produce **1** or **2**. *M. fijiensis* var. *difformis*, causal agent of the most severe aspect of Black Sigatoka, produces toxin **1** at 6–10 times the level of its less virulent counterpart *M. fijiensis*. Detailed comparisons of the three fungi will be presented at a later date.

It is interesting to note the biosynthetic origin of the major toxin, **1**, and related metabolites in the fungal extract. Examination of the literature [13, 20, 22, 23] indicated that 2,4,8-trihydroxytetralone **1**, juglone **2**, and 4-hydroxyscytalone **6** are actually melanin shunt-pathway metabolites, as shown in the figure. Wheeler studied the biosynthesis of melanin in 20 species of ascomycetous and imperfect fungi, and found that in all species tested except *Aspergillus niger*, the melanin precursor is 1,8-dihydroxynaphthalene, not indole, as was previously suspected [24]. In a series of elegant experiments using melanin-deficient mutants, these investigators carefully mapped not only the melanin pathway but also the major shunt-pathway metabolites [13, 20, 22, 23, 25]. Many of the compounds isolated from *M. fijiensis* may arise via these pathways.

Melanin occurs in the cell walls of many fungi, apparently protecting the cells from desiccation and ultraviolet radiation. It may also protect fungal cells from other microorganisms [16]. The production of melanin can also be an important determinant or pathogenicity: melanization of the fungal appressorium is a necessary prerequisite to invasion of rice by *Pyricularia oryzae* [26, 27], of cucumbers by *Colletotrichum lagenarium* [28], and of beans by *C. lindemuthianum* [29]. Application of the fungicide tricyclazole, a systemic inhibitor of melanin biosynthesis, can control these fungal infections by interfering with melanization of the fungal cells [16, 25, 26]. Examination of the biosynthetic melanin pathway, however, indicates an interesting departure from the norm. Tricyclazole inhibits pathogenicity by blocking melanization, which leads to accumulation of shunt metabolites in the fungal cells [25]. In *M. fijiensis*, it is the shunt-pathway metabolites that are phytotoxins. Application of tricyclazole could actually increase the virulence of the fungus by increasing the production of these phytotoxins.

We ground and extracted with EtOAC 500 g of infected banana leaf tissue in order to find evidence of any or all of these phytotoxins within the plant. Chromatography on an LH-20 column, as previously described, did not yield any fraction that possessed biological activity. Likewise, no evidence for the presence of any of these compounds could be found by TLC (table 1), or NMR. This is not surprising, considering the strong possibility that in the process of fungal infection, these phytotoxins could conceivably be oxidized, reduced, or derivatized into other compounds not having biological activity. Comparable studies using plant material in the very early stages of infection may prove more fruitful in discovering these substances in the tissue. Such information would be helpful in more firmly establishing their role in the infection process.

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Gel-electrophoretic description of European populations of *Terellia virens* (Loew) (Diptera, Tephritidae); implications for its use as an agent for the biological control of *Centaurea* spp. (Asteraceae) in North America

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Abstract. Allozyme frequencies of 15 enzyme loci, 14 of which were polymorphic, were used to characterize seven *Terellia virens* populations originating from three allopatrically distributed *Centaurea* species. The two populations whose origins were geographically furthest apart, from Israel (on *C. iberica*) and from Switzerland (on *C. vallesiaca*), showed relatively high values of genetic distance from the 5 populations sampled in Austria and Hungary (on *C. maculosa*) (Nei's D > 0.07). The latter five displayed a high degree of genetic similarity. No diagnostic (fixed) allelic differences were observed between these three groups of *T. virens* populations, but they could be well characterized by significant differences in allelic frequencies at 9 enzyme loci. Independently of this study, the populations from Switzerland (*C. vallesiaca*) and eastern Austria (*C. maculosa*) were selected as potential source populations for future introductions into North America for the biological control of introduced *C. maculosa* and *C. diffusa*. Based on the observed genetic differences and results from field experiments on the host specificity of these two potential source populations, it is argued that host specificity screening tests should be conducted separately for local (host plant) populations, as such populations might accept a different set of hosts. Biotype mismatch and the risk of spill-overs to native species could thus possibly be reduced.
Key words. Terellia; *Centaurea*; allozymes; host races; biological control.

Introduction

The seed head fly *Terellia virens* (Loew) (Dip.: Tephritidae), which has been reported to feed on 14 different *Centaurea* (Asteraceae) species[1], was studied at the European Station of the Commonwealth Institute of Biological Control (CIBC), Switzerland, between 1986–1989, in order to investigate its potential as a biological control agent for *Centaurea maculosa* Lam. and *C. diffusa* Lam. in North America[2].

In biological control projects against weeds, the studies predicting an agent's potential host range and its impact on weed density are of critical importance. Experimental investigations of the host range of a potential control agent to guarantee its relative 'safety' are especially important in classical biological control projects, which attempt to control an alien weed by introducing the weed's natural enemies from its area of origin[3]. However, dietary specialization is often a flexible attribute of a population in response to features of its particular community, rather than an attribute of a species throughout its geographical range[4]. The coupling of mating site, oviposition site and larval food resources is a property of many specialized herbivorous insects. In such a case, the switch to a new host plant may simultaneously channel the gene flow and completely change the food and habitat niche, possibly leading to the development of host races[5–12]. The formation of host races as a result of either allopatric or sympatric processes is considered to be a widespread phenomenon in tephritids[5], and recent studies reported significant differences in allele frequencies between fly populations reared from their sympatric host plant species[10–12].

Unruh and Goeden[8] also reported high levels of genetic and behavioural differentiation between *Carduus* and *Silybum* races of the weevil *Rhinocyllus conicus*, introduced into California for the biological control of composite thistles. They could further show, on the basis of allozyme frequency data, that the weevil populations recently found on two thistle species native to California probably all originate from one race only. Genetic evaluations of a widespread complex of single species populations should therefore be performed prior to host-screening tests in order to assess the degree to which a candidate