Fungi colonizing microsclerotia of *Verticillium dahliae* in urban environments

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Fungi that colonize microsclerotia of the plant pathogen *Verticillium dahliae* were surveyed from a number of natural habitats in order to identify potential hyperparasites of the pathogen in urban environments. Microsclerotia of *V. dahliae* were buried in soil at the field sites and the fungi that colonized the microsclerotia were isolated on a variety of media and identified to species based on morphology and DNA (internal transcribed spacers of nuclear rDNA) sequences. Twenty-seven fungal species among 70 isolates were identified, including known mycoparasites of *V. dahliae* and other fungi. Thirteen of the 27 species were found across multiple field sites, whereas fourteen species were limited to a single location. Species appearing at 3 or more different locations included *Alternaria alternata*, *Trichoderma koningii*, *Fusarium solani*, *Trichoderma hamatum*, *Trichoderma harzianum*, *Zygorhynchus moelleri*, and *Clonostachys rosea*. Chi-square analysis showed that three species, *A. alternata*, *T. koningii*, and *Fusarium oxysporum*, occurred at frequencies significantly greater ($p<0.05$) than expected, suggesting that they have a higher affinity for microsclerotia of *V. dahliae* than other fungi. This study provides a list of potential mycoparasites of microsclerotia of the pathogen *V. dahliae* in urban environments.

Key words: biocontrol, colonization, molecular identification, mycoparasites, *Verticillium dahliae*.

Introduction

Verticillium dahliae Klebahn is an economically important plant pathogen. Unlike many other pathogenic fungi, it does not confine its attack to one host or a few closely related hosts. Instead, *V. dahliae* is a common causal agent of vascular wilt diseases encompassing a large number of widely unrelated plant species, many of which are crop plants of economic importance. Known important agronomic and horticultural host plants include cotton, dahlia, mint species, tomato, potato, eggplant, olive, pistachio, horseradish, stone fruits, brussel sprouts, groundnut, red pepper, strawberry, soybean and others (Suwa *et al.*, 1987; Farr *et al.*, 1989). Additionally, *V.*

dahliae causes vascular diseases on a number of woody plants in urban environments, but the disease is rarely observed in natural forests (Sinclair *et al.*, 1987). Practical control of *Verticillium dahliae* is difficult due to the pathogen's widespread geographic distribution throughout temperate and subtropical regions, its wide host range, and the indefinite longevity of its resting structure microsclerotia. Ongoing research on biological control has investigated likely candidates of mycoparasites to control *V. dahliae* in field crops (Marois *et al.*, 1984; Fravel *et al.*, 1987; Spink and Rowe, 1989; Ordentlich *et al.*, 1990; Keinath *et al.*, 1991). In order to study the potential of biological control of Verticillium wilt of urban landscape plants, this study was undertaken to survey and examine the diversity of fungi that colonize microsclerotia of *V. dahliae*, and compile a list of fungal species possessing the ability to colonize microsclerotia of the pathogen in urban environments.

Materials and Methods

Inoculum Preparation

Two sets of microsclerotia of *V. dahliae* were produced using isolates 9-6, 9-10, 9-14, 90-1, 90-2, and 90-5 from the culture collection at the Illinois Natural History Survey (Chen, 1994). A first set of microsclerotia was produced on Difco potato dextrose agar (PDA) overlain with sterile cellophane. After 5 weeks of incubation in the dark at 20-24 C, microsclerotia (adhering mycelia and cellophane fragments) were rubbed off the medium and dispersed in deionized water with a blender. The resulting mixture was poured through nested 1 mm, 125 μm, and 38 μm mesh sieves as outlined by Francl *et al.* (1988). The material left in the 125 μm and 38 μm sieves was then rinsed in place with additional water and air-dried. Microsclerotia were collected from the sieves and stored dry for later exposure in soil. A second quantity of inoculum was produced utilizing sterilized diced potatoes. The fungi produced prolific microsclerotia after 5 weeks in the dark at 20-24 C. Microsclerotia were harvested and separated from the plant tissue via the blending and sieving procedures outlined above. This second set of inoculum was used to better mimic conditions in nature where microsclerotia are produced on or in plant tissue, and to reduce any undesirable effects of the synthetic medium PDA on morphological or physiological properties of the microsclerotia.

Exposure and isolation procedures

Microsclerotia were placed in small nylon pouches (~ 2 cm^2) constructed of 41 μm porosity SpectraMesh (Spectrum, Houston, Texas). The pouches were sealed with a staple, and fishing line was attached to facilitate retrieval.

The pouches were submerged in soil at a range of depths from 0.5 to 30 cm and in a variety of orientations from horizontal to vertical. The amount of time that the pouches remained in the soil was from as little as 14 days to as much as 6 months. Upon careful retrieval at the field sites, the pouches were lightly brushed off to remove adhering soil colloids, and immediately placed in sterile petri dishes to prevent contamination. The Petri dishes were placed in an insulated container for transport back to the laboratory.

Aggregates of microsclerotia were aseptically removed from the pouches and plated on 4 isolation media: full-strength Difco PDA, half-strength PDA amended with 7.5 g Difco agar and lactic acid (APDA), Difco cornmeal agar, and Difco water agar. Isolation of fungal species was achieved either by hyphal tip excision under a dissecting microscope, or in most cases by selection of colonies originated from single spores following serial dilution in sterile water.

Identification techniques

Identification of fungal species was carried out, for the most part, via standard taxonomic procedures from squash mounts in an azure-A lactophenol preparation or lactofuchsin. Additionally, the use of slide cultures as outlined by Hawksworth (1974) was also implemented to more accurately observe the sporangial wall of *Mucorales* and conidiogenesis of hyphomycetes. After adequate growth was observed in a slide culture, hyphae and conidiophores adhering to the slide and coverslip were used to produce slide mounts in Azure-A stain, and measurements were taken on a Leitz compound microscope fitted with an ocular micrometer. For identification, *Trichoderma* and *Mucor* species were grown on Difco malt extract agar, and *Fusarium* species on a non-commercial preparation of PDA made from diced potatoes (Toussoun and Nelson, 1968). Following identification, [herbarium] specimens were dried down and deposited with label data, including GPS readings from their sites of isolation, in the Illinois Natural History Survey mycological collections (ILLS).

PCR and DNA sequencing

In some cases, DNA sequences of the internal transcribed spacer region (ITS) of nuclear ribosomal DNA were used to augment morphological observation in determining species identities. DNA extraction was accomplished using one of two protocols. In one protocol, fungal mycelium was harvested from a liquid medium (10 g malt extract, 10 g yeast extract per liter deionized water) and was ground to a fine powder in liquid nitrogen. DNA was isolated from mycelium powder using the method of Lee and Taylor (1990). In the other protocol fungi were grown on plates of APDA, and DNA

was isolated using the *fast*DNA Kit (Bio 101 Inc. Vista, CA) as described by Chen *et al.* (1996). The ITS region was amplified using primers ITS1 and ITS4 (White *et al.*, 1990). Amplification mixtures consisted of 1 µM of each primer, 50 µM of dNTP, 1.75 mM $MgCl_2$, 3 units *Taq* DNA polymerase (Life Technologies, Rockville, Maryland), 5 to 10 ng of DNA templates in 100 µl of PCR buffer. The temperature cycles for PCR were 94 C, 2 min pre-denaturation, followed by 30 cycles of 94 C, 45 sec denaturation; 50 C, 1 min annealing; 72 C, 2 min extension, and a final 3 min 72 C extension. The amplified ITS region was digested with enzymes *Hae* III, *Mbo* I, *Taq* I, and *Hinf* I (Life Technologies, Rockville, MD) to determine related species and help select representative isolates for sequencing. The restriction digests were 10.0 µl PCR products, 1.5 µl 10× buffer, and 1 unit of restriction enzyme in a total of 15 µl volume. Restriction digested fragments were separated by electrophoresis in a 3% agarose gel (1% regular agarose and 2% NuSieve GTG agarose) stained with ethidium bromide and visualized under UV light.

The entire ITS region (ITS1, ITS2 and 5.8s gene) of the nuclear ribosomal DNA of selected isolates was amplified by PCR and the nucleotide sequences were determined. PCR products were gel-purified in a 1% TAE agarose gel and the DNA band excised and purified using an Elu-Quik purification kit (Schleicher and Schuell, Keen, New Hampshire). Sequencing reactions were performed using the ABI Prism Big Dye™ terminator cycle sequencing kit following the manufacturer's protocol. Sequences were determined at the University of Illinois Biotechnology Center, and were analyzed and edited using the Sequencher ™ software program (Gene Code, Ann Arbor, Michigan). All sequences were confirmed by sequencing both strands. BLAST searches (Altschul *et al.*, 1990) were carried out on the GenBank, and the United States Department of Agriculture ARS Culture Collection (Microbial Properties Research Unit) at Peoria, Illinois for most similar sequences. The combination of morphological observations and DNA sequences were used to determine the species identities of the selected isolates.

Field Site Information

Field sites spanning three counties in east-central Illinois were comprised of natural habitats of mostly urban environments including agricultural land and natural areas managed by the University of Illinois at Urbana-Champaign, local compost piles, a municipal reclamation area for natural waste, and residential vegetable gardens (Table 1). Soil type and slope were determined from the county Soil Surveys, pH was measured using a soil probe pH meter (AMI Medical Electronics), and GPS readings were taken with a Magellen GPS unit. In most cases, submersion of microsclerotia was carried out on

Table 1. List of east-central Illinois field sites along with soil type, slope, pH, location, and brief description.

Code[a]	Field Site	Soil type	slope	pH	GPS utm zone 16	description
A	Shurts Ave. compost	NA	2-7%	4.7	397791mE 4438150mN	urban compost pile
B	Urbana Natural Waste Area	Loam	n/a	5.4	399086mE 4441917mN	urban landfill
C	University of IL South Farms	Loam	2-5%	5.6	394092mE 4436312mN	agronomic research site
D	Lake Park Estates	Loam	level	5.3	393944mE 4435676mN	rural compost site
E	Winfield Village Gardens	Silt Loam	level	6.0	393963mE 4436082mN	suburban vegetable garden
F1	Robert Allerton Park Site #1	Silt Loam	2-5%	5.7	359118mE 4427394mN	deciduous oak forest
F2	Robert Allerton Park Site #2	Silt Loam	2-5%	5.9	359094mE 4427394mN	deciduous maple forest
G1	Brownfield Woods Site #1	Silt Loam	0-3%	5.7	400860mE 4444264mN	deciduous upland forest
G2	Brownfield Woods Site #2	Silt Loam	1-5%	6.0	400884mE 4444221mN	virgin deciduous forest
G3	Brownfield Woods Site #3	Silt Loam	4-11%	4.9	400888mE 4444179mN	low-lying forest floodplain
G4	Brownfield Woods Site #4	Silt Loam	1-5%	5.8	400774mE 4444160mN	deciduous upland forest
G5	Brownfield Woods Site #5	Silt Loam	1-5%	5.5	400800mE 4444128mN	deciduous upland forest
H1	Hart Memorial Woods Site #1	Silt Loam	2-5%	5.4	384557mE 4453994mN	150-yr old upland woods
H2	Hart Memorial Woods Site #2	Silt Loam	1-5%	5.2	384653mE 4453992mN	mesic woods dense understory
I1	Trelease Woods Site #1	Silt Loam	level	6.1	402532mE 4442786mN	mature deciduous forest
I2	Trelease Woods Site #2	Silt Loam	0-3%	4.9	402579mE 4442798mN	mature deciduous forest
J1	Phillips Tract Site #1	Clay Loam	level	5.4	401997mE 4442701mN	restored tallgrass prairie
J2	Phillips Tract Site #2	Silt Loam	1-5%	5.1	402274mE 4443137mN	woodlots next to a river
K1	Rutan Research Area Site #1	Silt Loam	1-5%	6.2	422755mE 4436435mN	grassy ag. drainage basin
K2	Rutan Research Area Site #2	Silt Loam	16-35%	6.0	422692mE 4436237mN	open upland woodlot
L1	Richter Research Area Site #1	Silt Loam	35-75%	6.5	430940mE 4438109mN	steeply sloped woodland
L2	Richter Research Area Site #2	Silt Loam	1-5%	6.2	431051mE 4438635mN	successional woodland
M1	CCDC Collins Woods Site #1	Clay Loam	level	6.0	411714mE 4443646mN	forest river oxbow bottomland
M2	CCDC Collins Woods Site #2	Silt Loam	2-5%	5.5	411780mE 4443631mN	successional oak forest

[a] Codes for the field sites are used in Table 3

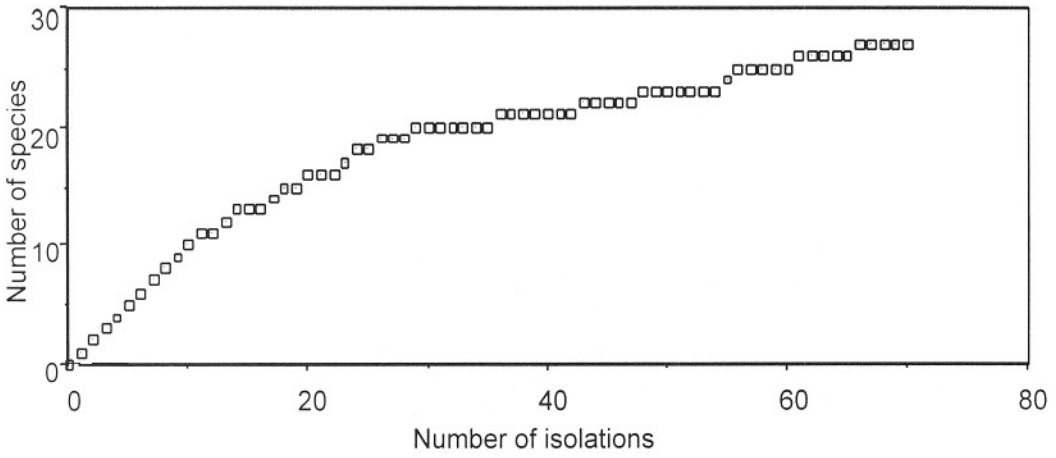

Fig. 1. Scatterplot showing the relationship of number of isolates and number of species observed.

location. However, in cases where this was not feasible due to the nature of the site, 4-gallon soil samples were collected, potted, and maintained in the greenhouse under conditions mimicking nature. The greenhouse samples included Urbana Waste Reclamation Area, Shurts Avenue, Winfield Village, South Farms, Lake Park Estates, and Allerton Park.

Results

Known saprobes, plant and animal pathogens, mycorrhizal fungi, and mycoparasites, encompassing zygomycetes, ascomycetes, and mitosporic taxa were recovered from *V. dahliae* microsclerotia buried at 24 field sites. Twenty-seven species were identified among 70 isolates. One to seven dried specimens for each species were deposited at ILLS (Table 2).

The distribution of fungal species across the field sites varied. Some species (e.g., *Alternaria alternata*, *T. koningii*) seemed almost ubiquitous, appearing at several sites often separated by great distance and possessing quite different local conditions (i.e. pH, biome, soil type). Other species were only encountered once (Table 3). Initially, the number of species identified increased proportionally to the number of isolations made. However, after approximately 30 isolations species began to noticeably recur. The scatterplot of species observed against number of isolations illustrates that after approximately 60 isolations, continued and increasing repetition can be anticipated, although species previously not encountered still intermittently occur (Fig. 1). This indicates that the majority of species present at these sites capable of colonizing microsclerotia in a competitive natural environment have been isolated.

Table 2. List of species isolated from microsclerotia of *Verticillium dahliae*, their herbarium accession numbers and GenBank accession numbers.

Species	Herbarium Accession number(s)	GenBank accession number
Alternaria alternata (Fr. : Fr.) Keissler	ILLS 54695 - 54701	
Aspergillus flavus Link : Fr.	ILLS 54703	AF176658
Aspergillus fumigatus Fresenius	ILLS 54704	AF176662
Aspergillus niger van Tiegham	ILLS 54705 - 54707	
Botryotrichum piluliferum Sacc. and Marchal	ILLS 54708 - 54709	
Clonostachys rosea (Link : Fr.) Schroers, Samuels, Seifert and W.L. Gams	ILLS 54710 - 54713	
Diheterospora chlamydosporia (Goddard) Barron and Onions	ILLS 54714	
Fusarium culmorum (W.G. Smith) Sacc.	ILLS 54715 - 54716	AF176657
Fusarium oxysporum Schlecht : Fr.	ILLS 54717 - 54721	AF176656
Fusarium solani (Mart.) Sacc.	ILLS 54722 - 54725	AF161222
Humicola fuscoatra Traaen	ILLS 54726 - 54727	
Humicola grisea Traaen	ILLS 54728	
Mariannaea elegans var. *punicea* (Corda) Samson	ILLS 54729	
Mucor hiemalis forma *luteus* (Wehmer) Linnemann	ILLS 54730 - 54731	
Mucor racemosus Fresenius	ILLS 74732	AF176659
Neosartorya fischeri (Wehmer) Malloch and Cain [a]	ILLS 54702	AF176661
Penicillium pinophilum Hedgcock	ILLS 54734 - 54735	AF176660
Talaromyces flavus (Klöcker) Stolk and Samson var. *flavus*	ILLS 54736 - 54738	
Talaromyces macrosporus (Stolk and Samson) Frisvad *et al.*	ILLS 54737	
Talaromyces wortmanii (Klocker) Benjamin	ILLS 54739	
Trichoderma hamatum (Bon.) Bain.	ILLS 54740 - 54742	
Trichoderma harzianum Rifai	ILLS 54743 - 54746	
Trichoderma koningii Oudem.	ILLS 54747 - 54752	
Trichoderma longibrachiatum Rifai	ILLS 54753	
Trichoderma spp.	ILLS 54755	
Trichoderma pseudokoningii Rifai	ILLS 54754	
Zygorhynchus moelleri Vuillemin	ILLS 54756 - 54758	

[a]anamorph: *Aspergillus fischeri*

PCR and DNA Sequencing

To verify species identification, isolates belonging to the three genera *Fusarium*, *Aspergillus*, and *Penicillium* were selected and subjected to molecular analysis. Initially a restriction digest was run on 18 isolates with four enzymes (*Hae* III, *Mbo* I, *Taq* I, and *Hinf* I) and yielded banding patterns that were used to determine isolates of the same species (Fig. 2). Based on the restriction banding patterns, in conjunction with morphological observations, it was determined that there were 7 distinct species present. Using this

Table 3. Frequency and distribution of species isolated from microsclerotia of *Verticillium dahliae*.

Species	Field sites[a]																								
	A	B	C	D	E	F1	F2	G1	G2	G3	G4	G5	H1	H2	I1	I2	J1	J2	K1	K2	L1	L2	M1	M2	Total
Alternaria alternata		1										2		1	1	1					1				7[b]
Aspergillus flavus										1															1
Aspergillus fumigatus				1																					1
Aspergillus niger		2		1																					3
Botryotrichum piluliferum								1	1																2
Clonostachys rosea	1					1					1											1			4
Diheterospora chlamydosporia									1																1
Fusarium culmorum																			3						3
Fusarium oxysporum													1	1					2	2					6[b]
Fusarium solani				1	1								1				1								4
Humicola fuscoatra		1							1																2
Humicola grisea					1																				1
Mariannaea elegans var. *punicea*																		2							2
Mucor hiemalis forma *luteus*														1										2	3
Mucor racemosus				1																					1
Neosartorya fischeri [b]		1	1																						2
Penicillium pinophilum					1															1					2
Talaromyces flavus var. *flavus*		2																							2
Talaromyces flavus var.*acrosporus*		1																							1
Talaromyces wortmanii										1															1
Hyopcrea gelatinosa [c]											1														1
Trichoderma koningii			1				1								1		1	1	1						6[b]
Trichoderma hamatum	1												1										1		3
Trichoderma harzianum						2	1									1				1					5
Trichoderma longibrachiatum		1																							1
Trichoderma pseudokoningii																						1			1
Zygorhynchus moelleri																			1		2		1		4
Total	2	9	2	4	3	3	2	1	3	2	2	2	3	3	2	2	2	3	7	4	3	2	2	2	70

[a]See Table 1 for field site codes.

[b]Species occurred at a frequency significantly ($p<0.05$) greater than expected based on χ^2 test.

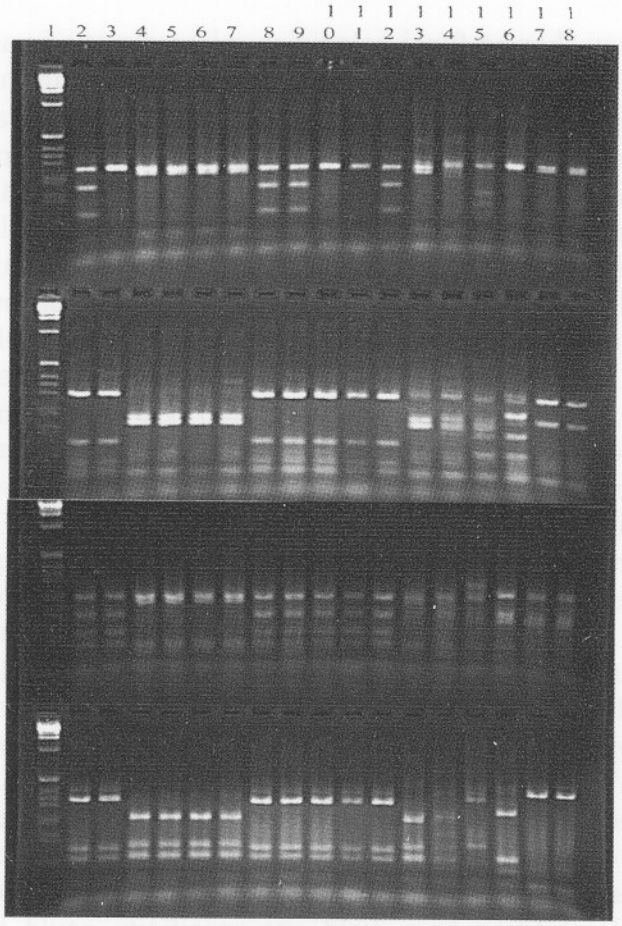

Fig. 2. Restriction banding patterns of PCR-amplified ITS region of DNA from 17 fungal isolates. Lane 1: 1 Kb DNA Ladder; lanes 2, 8, 9, and 12: isolates rn2c1, hw1a1, hw2d3 and rn2c2 respectively of *Fusarium oxysporum*; lanes 3, 10, and 11: isolates rn1c3, rn1d1 and rn1c2 of *Fusarium culmorum*; lanes 4, 5, 6 and 7: isolates hw1c2, pt1c1, lp1c1 and wv1b2 of *Fusarium solani*; lanes 17 and 18: isolates rn2d1 and wv1a2 of *Penicillium pinophilum*; lanes 13 and 14: isolates lp1a2 and lp1a1 of *Aspergillus fumigatus*; lane 15: isolate sf1a1 of *Neosartorya fischeri*; lane 16: isolate bf3a1 of *Aspergillus flavus*.

information, a representative of each group was selected for sequencing of the ITS region. Additionally, one *Mucor* isolate was also analyzed via sequencing of the ITS region. The sequencing reactions yielded 500-600 unambiguous bases. The final edited sequences of the entire PCR products were deposited in GenBank, and their accession numbers are listed in Table 2. BLAST searches using these sequences as queries against GenBank and the USDA database at Peoria, IL confirmed the species identification with only one incidence of ambiguity on *Aspergillus fumigatus* which is discussed below.

Discussion

This survey showed fungi colonizing microsclerotia in a wide variety of habitats with silty clay and loam soils, pH values ranging from as acidic as 4.7 to nearly neutral at 6.5 and with slopes varying from level to as steep as 75%. These fungi were inhabiting a wide range of biomes, including virgin upland forests, tallgrass prairies, wooded floodplains, agronomic fields, rural compost sites, urban waste reclamation areas, and suburban vegetable gardens. It is unknown whether any of these factors played a role in limiting the recurrence of certain species, but it is notable that some species were encountered multiple times, often across widely varying sites. Of the 27 species isolated, *Alternaria alternata* was encountered the most, occurring seven times. *Trichoderma koningii* and *Fusarium oxysporum* occurred six times and *Trichoderma harzianum* occurred 5 times. Three other species were encountered four times, four species were encountered three times, six species were encountered twice, and the remaining ten species were encountered only once (Table 3), and are probably opportunistic. Chi-square analysis was run on the frequency data with a null hypothesis that the number of times a species was encountered equaled the probability of the mean. Using our data, it took an occurrence frequency of 6 times or greater to exceed the critical value ($p<0.05$) of 3.841 given by the χ^2 distribution tables (df = 1), and reject the null hypothesis. Therefore, *T. koningii*, *Fusarium oxysporum* and *Alternaria alternata* that were encountered more than five times occurred at a rate significantly ($p<0.05$) greater than the expected frequency of 2.59. These species may possess a greater affinity for microsclerotia of *Verticillium dahliae* than other species present at field sites, or their geographic distribution was significantly greater across our selected field sites. Independence from field site specificity could be an important characteristic when choosing candidates for biocontrol. For example, *Alternaria alternata*, *Fusarium solani*, *Trichoderma koningii*, and *T. harzianum* were present at sites with varying slope, a diversity of flora, acidic to neutral soils, and different soil types, while *Diheterospora chlamydosporia* and *Mariannaea elegans* were only encountered at a single site. This has important implications regarding the selection and suitability of a strain or species to serve as a biocontrol agent in the various urban environments.

The fact that some of the fungal species isolated in this study are known mycoparasites of *Verticillium dahliae* (e.g., *Clonostachys rosea*, *Trichoderma harzianum*,) lends credence to speculation that other species present may also be parasites of *Verticllium dahliae* and agents that warrant further research for biocontrol. However, it should be noted that this study did not set out to prove parasitism, and that colonization does not imply mycoparasitism. The presence of the colonizer may have been due to chance growth over the surface of the

microsclerotia, or a symbiotic, fungiculous, or other relationship. However, it is worth noting that *V. dahliae* has been observed inhibiting fungal growth of other species *in vitro*, and is known to produce antibiotics, detoxify antifungal compounds, and can itself act as a destructive mycoparasite (Barron and Fletcher, 1970; Domsch *et al.*, 1980). Therefore, any colonizer must, at least, possess the traits needed to overcome such defenses.

Following retrieval from soil, microsclerotia were not surface sterilized to ensure that surface dwelling fungal species degrading/attacking the microsclerotia in an extracellular fashion were not excluded from the survey. Taxonomically, there was some discrepancy with the isolate (lp1a2) believed to be *Aspergillus fumigatus*. A BLAST search of GenBank using the sequence (AF176662) of this isolate resulted in the 2 closest matches being a Japanese isolate of *A. fumigatus* (AF078889) and another *A. fumigatus* specimen (AF138288). These matches were followed somewhat closely by *Neosartorya fischeri* (NFU18355). However, a search of the USDA's database produced *N. fischeri* as the closer match. Sequences from the extype and other isolates of *A. fumigatus* were present in the USDA's database, but were reported as being a "significantly worse match" than sequences on file for *Neosartorya fischeri*. Dr. Stephen Peterson, curator for the USDA's *Aspergillus* and *Penicillium* collections, speculated via correspondence that the isolate in question "may be a representative of an undescribed species in the *Neosartorya* clade". Morphology, including densely crowded compact columnar heads, echinulate conidia, and a lack of cleistothecia, fit more with the descriptions of *Aspergillus fumigatus* than *Neosartorya. fischeri* according to Raper and Fennell (1965). For this reason, coupled with the GenBank result, the isolate was tentatively identified as *Aspergillus fumigatus*.

Many research efforts have examined the use of mycoparasites for biological control of *Verticillium* and other sclerotium-forming fungi. Some of the fungal species encountered in this study (e.g. *Trichoderma harzianum*, *Clonostachys rosea*, *Talaromyces flavus*) have previously been studied as antagonists of *Verticillium dahliae* (Marois *et al.*, 1984; Fravel *et al.*, 1987; Spink and Rowe, 1989; Ordentlich *et al.*, 1990; Keinath *et al.*, 1991). Other species, such as *Alternaria alternata*, *Diheterospora chlamydosporia*, *Fusarium oxysporum* and *Humicola fuscoatra*, are notable for parasitizing oospores of *Phytophthora sojae* (Sneh *et al.*, 1977), sclerotia of *Aspergillus flavus* (Wicklow *et al.*, 1998), rotifers (Barron, 1985), and nematode cysts/eggs (Carris and Glawe, 1989). Additionally, *Humicola fuscoatra* produces the antifungal compound monorden (Wicklow *et al.*, 1998), and *Talaromyces wortmanii* produces the antifungal metabolite wortmannin (Brian *et al.*, 1957).

Future research will utilize bioassays and pathogenicity tests to determine the suitability of the isolates as biocontrol agents.

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