

Hidden Host Plant Associations of Soilborne Fungal Pathogens: An Ecological Perspective

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Accepted for publication 31 December 2012.

ABSTRACT

Malcolm, G. M., Kuldau, G. A., Gugino, B. K., and Jiménez-Gasco, M. M. 2013. Hidden host plant associations of soilborne fungal pathogens: An ecological perspective. *Phytopathology* 103:538-544.

Much of the current knowledge on population biology and ecology of soilborne fungal pathogens has been derived from research based on populations recovered from plants displaying disease symptoms or soil associated with symptomatic plants. Many soilborne fungal pathogens are known to cause disease on a large number of crop plants, including a variety of important agronomical, horticultural, ornamental, and forest plants species. For instance, the fungus *Verticillium dahliae* causes disease on >400 host plants. From a phytopathological perspective, plants on which disease symptoms have not been yet observed are considered to be nonhosts for *V. dahliae*. This term may be misleading because it does not provide information regarding the nature of the plant–fungus association; that is, a nonhost plant may harbor the fungus as an endophyte. Yet, there are numerous instances in the literature where *V. dahliae* has been isolated from asymptomatic plants; thus, these plants should be con-

sidered hosts. In this article, we synthesize scattered research that indicates that *V. dahliae*, aside from being a successful and significant vascular plant pathogen, may have a cryptic biology on numerous asymptomatic plants as an endophyte. Thus, we suggest here that these endophytic associations among *V. dahliae* and asymptomatic plants are not unusual relationships in nature. We propose to embrace the broader ecology of many fungi by differentiating between “symptomatic hosts” as those plants in which the infection and colonization by a fungus results in disease, and “asymptomatic hosts” as those plants that harbor the fungus endophytically and are different than true nonhosts that should be used for plant species that do not interact with the given fungus. In fact, if we broaden our definition of “host plant” to include asymptomatic plants that harbor the fungus as an endophyte, it is likely that the host ranges for some soilborne fungal pathogens are much larger than previously envisioned. By ignoring the potential for soilborne fungal pathogens to display endophytic relationships, we leave gaps in our knowledge about the population biology and ecology, persistence, and spread of these fungi in agroecosystems.

Endophytes are defined as organisms living inside plants that exhibit no visible symptoms as a result of this colonization (60) and generally include bacterial and fungal organisms. Endophytic relationships often go unnoticed due to the lack of symptomatology in the plant and are usually only discovered by examining internal tissues under a microscope, by aseptic isolation from plants, or from polymerase chain reaction (PCR) detection of microorganisms in DNA extracted from surface-disinfested plant tissues (67). Yet fungal endophytes, our focus in this article, are nearly ubiquitous across all groups of vascular plants, as documented by the extensive literature describing isolation and identification of these organisms (2,61). There is also a significant biological diversity among fungal endophytes, and it is not rare for some plant species to host hundreds of different fungal endophytic species (30,51,63), at least under tropical environments. Many of these endophytic fungi have been sought and characterized for their ability to produce biologically active secondary metabolites with potential uses in medicine, agriculture, and other areas (73).

Fungal endophytes are distinct from mycorrhizal fungi, which grow both inside and beyond the plant root system, whereas the growth of endophytes is limited to internal tissues of the plant. Defined in this way, endophytic fungi comprise a highly diverse group of species. Rodriguez et al. (63) categorized endophytic fungi into four classes: class 1, Clavicipitaceous; class 2, non-

Clavicipitaceous fungi colonizing the entire plant; class 3, non-Clavicipitaceous hyper-diverse fungi colonizing aerial plant tissues; and class 4, dark septate endophytes. The Clavicipitaceous endophytes of grasses (class 1) are the best-studied group of fungal endophytes, and fungi in this group belong to *Epichloë/Neotyphodium* and related genera. These latter fungi exist in a highly specialized, co-evolved symbiosis as obligate endophytes of grasses, and confer a number of benefits on their hosts, including drought tolerance, pest and pathogen resistance, and alleviation of phosphorous deficiency (50,65). These endophytes are best known for providing defense against herbivory associated with synthesis of various biologically active metabolites, such as alkaloids, which are also toxic to vertebrate herbivores (11,13,34,43,65,78). It appears, however, that most fungal endophytes are more generalists and not obligate symbionts in their growth (63). Importantly, many of these endophytes, such as those in the genera *Fusarium* and *Rhizoctonia*, for example, are mainly known as plant pathogens; or, in genus *Xylaria*, as decay organisms of plants (2,44,61). However, with much of the research focused on these organisms as plant pathogens, our comprehensive understanding of the role of these fungi in agroecosystems is incomplete.

Regarding plant disease in agricultural ecosystems, research on plant–fungus interactions has been essentially focused on plant pathogenicity, and studies on fungal endophytes have been largely limited to the development of biocontrol agents (1,3,27), which sometimes target fungi traditionally considered pathogens (66). An alternative that has been poorly addressed is that these organisms may have a dual role: a pathogenic lifestyle on certain plants and an endophytic one on others. The fact that plant pathogens can be endophytes on other plants has important implica-

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<http://dx.doi.org/10.1094/PHTO-08-12-0192-LE>
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tions, such as asymptomatic plants inadvertently serving as reservoirs of inoculum and potentially initiating epidemics in other crops (71), or even serving as sources of hidden diversity of plant-pathogenic species. Because most research conducted on soilborne fungi has focused on pathogens infecting important agricultural commodities, we still lack a thorough understanding of the true nature of the associations of these fungi with other plants and their environment. Certain questions remain largely unaddressed. What are the ecological roles of plant-pathogenic fungi in agroecosystems when not causing disease on host plants? What are host plants, anyway? Here, we aim to synthesize the literature that supports the concept that many plant-pathogenic fungi, with a specific emphasis on *Verticillium dahliae* as our case study, have a cryptic endophytic life in plants traditionally considered to be nonhosts. Our goal is to describe a novel, expanded view of the biology, spread, and persistence of this plant fungal pathogen–endophyte, and discuss some research needs to fill in the gaps in knowledge.

Redefining the concept of host plant for fungal pathogens.

The term “host” in plant pathology has been traditionally focused on the presence of disease symptoms as a consequence of fungal infection and colonization, modulated by the environment. However, from an ecological perspective, a host plant refers to any plant that harbors a fungus, regardless of the presence of disease symptoms. If a plant is colonized by a fungal pathogen but shows no disease symptoms, it may be most appropriate to describe that plant as a host rather than a nonhost to embrace an ecological framework (60). Such conflict was early addressed by Whetzel (74) by proposing the use of “suscept” to refer to a plant susceptible to disease. Other terms used to refer to plants that harbor a pathogenic organisms in the absence of disease symptoms include “symptomless carriers” (36) or “tolerant” hosts (10,62). In the rest of this article, we will examine reported instances of fungi (specifically *V. dahliae*) in endophytic relationships with plants that may also be pathogenic on a different plant species. Thus, we will differentiate between “symptomatic hosts” as plant species displaying visible disease symptoms as a consequence of fungal infection, versus “asymptomatic hosts,” where fungal infection results in an endophytic relationship (Fig. 1A). The term “asymptomatic host” differs from “tolerant or resistant” symptomatic host (62), where fungal colonization inside the plant may result in varying degrees of disease symptoms from no disease to a reduction in severity. The varying degrees of disease symptoms observed on symptomatic hosts may be due to (i) pathogenic variability as a pathogen attribute, (ii) different levels of resistance in the plant, or (iii) a consequence of differential interactions between plant and pathogen genotypes (Fig. 1A). For example, the defoliating pathotype of *V. dahliae* had increased quantum virulence on cotton and olive when compared with the nondefoliating pathotype, regardless of host genotype (31). Also, under controlled conditions, isolates of *V. dahliae* from artichoke caused disease on artichoke, cardoon, eggplant, or watermelon but isolates from watermelon, muskmelon, and eggplant showed differential pathogenicity, depending on the host–isolate combination (6). Symptomatic hosts of *V. dahliae* seem to be limited to dicotyledonous plants, whereas the current range of asymptomatic host plants of *V. dahliae* is unknown but appears to be associated with cereals or monocotyledonous crops in general (Table 1).

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Symptomatic host plants of *V. dahliae*. By far, most of the literature on *V. dahliae* is from a plant-pathogenic perspective, and this role has been extensively addressed in various excellent reviews (12,31,38,64). In brief, *V. dahliae* is an anamorphic Ascomycete mainly known as an important soilborne plant pathogen that survives in the soil for extended periods of time due to the production of microsclerotia (clusters of thick-walled, heavily melanized cells), the fungal resting structures (12,31,38,59). Disease is the consequence of extensive colonization of the plant’s vascular system, resulting in wilting and necrosis. There are >400 plants reported as hosts (sensu plant pathology), or symptomatic hosts (as defined herein) of *V. dahliae* (38,59), all of which are dicotyledonous species and are important agricultural, ornamental, or forest plants.

Isolates of *V. dahliae* are considered host-adapted rather than host-specific (i.e., they are pathogenic on many hosts but are more virulent to the host from which they were isolated) (6,8,23,33,39,41,64). *V. dahliae* populations have been studied extensively through vegetative compatibility tests, referring to the ability of isolates to undergo hyphal anastomosis and form a stable hetero-

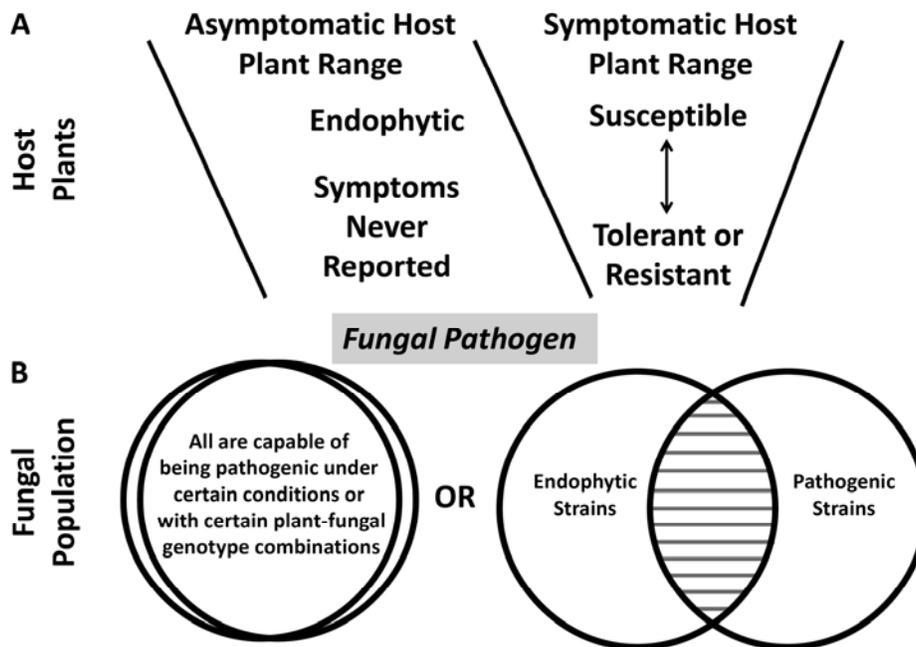


Fig. 1. A, Potential scenario for host plant range of a fungal pathogen and its expansion from a traditional plant pathology view (“symptomatic host plants”) to a broader ecological framework, including the potential for *Verticillium dahliae* as an endophyte on “asymptomatic host plants.” **B,** Potential fungal population structure options for a pathogen–endophyte, where the entire fungal population is capable of being pathogenic and disease symptoms depend on the plant genotype–fungus genotype–environment combinations, or a spectrum exists from endophytic to pathogenic strains.

karyon, and several vegetative compatibility groups (VCG) have been described (33,37,64). In potato, populations corresponding to VCG 4A are considered to be adapted to this crop (58,64) and are also the most virulent of the two main VCGs (VCG 4A and VCG 4B) found associated with potato crops in the United States (64). However, other VCGs (2A and 2B) are associated with potato in other countries (5). These types of findings extend beyond potato crops. For instance, in Israel, VCG 2B isolates that originated from cotton were highly virulent on cotton and were less virulent on eggplant, but VCG 2B isolates that originated from other plants were more virulent on eggplant than they were on cotton (41). All of these examples, taken together, indicate a certain degree of adaptation of specific populations (VCGs or specific genotypes) to particular plant hosts, even if, under controlled conditions, isolates are still pathogenic to plant species other than the one of origin (6,32,79).

Asymptomatic host plants of *V. dahliae*. In the scientific literature, observations of *V. dahliae* as an endophyte colonizing asymptomatic plant species have been documented. Many of these asymptomatic plants are cereal crops, used in crop rotations as part of management for Verticillium wilts, and weeds, which may be present in fields along with cultivated crops.

Associations between *V. dahliae* and monocot plants, although limited, result mostly from inoculation studies and not from field sampling (Table 1). In controlled experiments, Mol (55) inoculated several plant species with one isolate of *V. dahliae* from potato and did not observe any symptoms in barley and wheat. Significant amounts of microsclerotia, however, were observed in roots of barley compared with the limited amounts found in wheat roots. In greenhouse experiments using two *V. dahliae* isolates (one from potato and one from pea), Krikun and Bernier (42) inoculated several plant species, including the monocots wheat,

TABLE 1. Agricultural crops and common weed plant species found in endophytic relationships with *Verticillium dahliae*

Scientific name	Common name	Type of study ^a	Tissues and observations ^b	Reference
Agronomic crops				
<i>Allium cepa</i>	Onion	Root inoculation	Microsclerotia in roots	49
<i>Avena sativa</i>	Oat	Field sampling	Stem isolation	This study
		Field sampling	Microsclerotia in ground foliage and stubble	16
		Soil inoculation	Stem isolation	42
		Root inoculation	Microsclerotia in roots	29
<i>Hordeum vulgare</i>	Barley	Field sampling	Microsclerotia in ground foliage and stubble	16
		Soil and root inoculation	Microsclerotia in roots	55
		Soil inoculation	Stem isolation	26, 40
		Root inoculation	Microsclerotia in roots	29,46,54
<i>Secale cereale</i>	Rye	Root inoculation	Microsclerotia in roots	29
<i>Sorghum bicolor</i>	Sorghum	Soil inoculation	Stem isolation	26
<i>Sorghum vulgare</i> var. <i>sudanense</i>	Sudangrass	Field sampling	Microsclerotia on foliage and stubble isolation	16
		Soil inoculation	Stem isolation	26
<i>Tulipa</i> spp.	Tulips	Root inoculation	Microsclerotia in roots	49
<i>Triticum aestivum</i>	Wheat	Field sampling	Microsclerotia on foliage and stubble isolation	16
		Soil inoculation	Root colonization	45
		Soil inoculation	Stem isolation	26,42
		Root inoculation	Microsclerotia in roots	29,49,52,55
<i>Zea mays</i>	Corn	Soil inoculation	Colonization of roots	45
		Soil inoculation	Stem isolation	26
		Root inoculation	Microsclerotia in roots	29
Weeds				
<i>Amaranthus retroflexus</i>	Redroot pigweed	Soil inoculation	Root, stem, and petiole isolation	9
<i>Bidens pilosa</i>	Spanish needle	Field sampling	Stem isolation	72
<i>Calandrina ciliata</i>	Fringed redmaids	Field sampling	Stem isolation	72
<i>Chenopodium album</i>	Common lambsquarter	Soil inoculation	Root, stem, and petiole isolation	9,26
		Field sampling	Stem isolation	21,22
<i>Cichorium intybus</i>	Chicory	Soil inoculation	Root, stem, and petiole isolation	9
<i>Datura innoxia</i>	Thornapple	Soil inoculation	Stem isolation	26
<i>Convolvulus arvensis</i>	Field Bindweed	Field sampling	Stem isolation	21,22
<i>Erodium cicutarium</i>	Redstem filaree	Field sampling	Stem isolation	72
<i>Lamium amplexicaule</i>	Staggerweed	Field sampling	Stem isolation	72
<i>Malva neglecta</i>	Common mallow	Soil inoculation	Root, stem, and petiole isolation	9
<i>Malva pusilis</i>	Low mallow	Soil inoculation	Root, stem, and petiole isolation	9
<i>Malva sylvestris</i>	Zebra mallow	Field sampling	Stem isolation	72
<i>Marrubium vulgare</i>	White horehound	Soil inoculation	Stem isolation	26
<i>Medicago hispida</i>	Bur clover	Field sampling	Stem isolation	72
<i>Medicago lupulina</i>	Black medic	Soil inoculation	Root, stem, and petiole isolation	9
<i>Polygonum lapathifolium</i>	Curlytop knotweed	Field sampling	Stem isolation	21,22
<i>Portulaca oleracea</i>	Common purslane	Soil inoculation	Root, stem, and petiole isolation	9
<i>Salvia verbenaca</i>	Wild sage	Soil inoculation	Stem isolation	26
<i>Silybum marianum</i>	Milk thistle	Soil inoculation	Stem isolation	26
<i>Sinapis arvensis</i>	Charlock mustard	Field sampling	Stem isolation	21,22
<i>Solanum nigrum</i>	Black nightshade	Field sampling	Stem isolation	72
		Soil inoculation	Root, stem, and petiole isolation	9
<i>Tagetes elliptica</i>	Mint marigold	Field sampling	Stem isolation	72
<i>Tagetes foeniculacea</i>	Marigold	Field sampling	Stem isolation	72
<i>Tagetes multiflora</i>	Marigold	Field sampling	Stem isolation	72
<i>Thiaspis arvense</i>	Field pennycress	Soil inoculation	Root, stem, and petiole isolation	9

^a Summary of the type of study conducted on the endophytic colonization of *V. dahliae*. Field sampling: plants collected in the field to either look for fungal structures or to isolate the fungus from surface-disinfested plant tissue. Soil inoculation: plants grown in soil or potting mix inoculated with microsclerotia from particular fungal isolates. Field soil: plants grown in soil collected from fields known to be infested with *V. dahliae*. Root inoculation: plants inoculated by root dipping in a *V. dahliae* conidial suspension.

^b Summary of the tissues examined for endophytic colonization of *V. dahliae* and observations made by the authors of the study.

barley, and oat. Those authors were able to recover the fungus growing asymptotically in aboveground tissues but this depended on the plant species–*V. dahliae* isolate combination. For example, the potato isolate was not recovered from barley but was recovered from oat, whereas the pea isolate was obtained from barley but not from oat. Interestingly, these differential interactions may apply even to host genotypes within a given plant species because *V. dahliae* was only recovered from one wheat cultivar ('Glenlea') and not from another ('HY-320'). These results suggest that the endophytic relationship between monocots and *V. dahliae* may be highly specialized. Do these associations represent an initial stage of adaptation for pathogenicity to monocots, or are endophytic relationships with asymptomatic host plants stable? In 1986, a disease in barley was reportedly caused by *V. dahliae* (53,54). However, Verticillium wilts in monocots have not been reported since then, indicating that, although the potential exists, *V. dahliae* isolates pathogenic on monocots may be rare. Overall, and based on the above studies, we can conclude that (i) *V. dahliae* has the ability to colonize monocot plant species endophytically and (ii) the endophytic interaction seems to be highly specialized and may depend on specific plant genotype–fungus genotype interactions.

Another interesting group of plants that needs further consideration regarding endophytic relationships is weeds. Weeds are often incorporated into integrated disease management practices as reservoirs of pathogen inoculum; however, the potential of endophytic interactions between weeds and soilborne fungi serving as a pool of cryptic diversity has been somewhat ignored, likely due to their lack of agronomic value and to their pest status. In the case of weeds, numerous potential asymptomatic plant–fungus associations with *V. dahliae* in field studies have been reported (9,21,22,26,72) (Table 1). *V. dahliae* was isolated from lamb's quarters (*Chenopodium album*), common purslane (*Portulaca oleraceae*), and black nightshade (*Solanum nigrum*) in more than one of the above studies, which is suggestive that these weeds may commonly associate with the pathogen in many regions of the world. However, this tells us little about the frequency or the nature of the association in different weed populations. As a caveat, though, overlap exists between some of the weedy plant species described as symptomatic and as asymptomatic hosts for *V. dahliae*. For instance, *V. dahliae* was isolated from asymptomatic shepherd's purse plants (*Capsella bursa-pastoris*) (9) but wilting and chlorotic symptoms were observed on *C. bursa-pastoris* in a study examining weed hosts in Crete (48). Thus, it could be that some weed species are symptomatic hosts and their disease symptomology ranges from susceptible to resistant, whereas other weeds are true asymptomatic hosts. Unfortunately, in several studies, weeds were assessed as hosts for *V. dahliae* without distinguishing whether or not they showed disease symptoms, making it difficult to tease apart resistant symptomatic hosts from asymptomatic weed hosts (21,22,35,71,75,77). Regardless of symptomatology, it is likely many of these weeds go unnoticed as hosts for the pathogen. These types of results highlight the need to understand the interactions between environment, fungal pathogen population biology, and location, to assess whether *V. dahliae* from weed plants are endophytic or pathogenic in nature, and to truly understand the breadth of this pathogen's host range.

A case study in Pennsylvania: association between *V. dahliae* and oats in fields with a history of Verticillium wilt of potato. Although a relationship between *V. dahliae* and oat was observed earlier in laboratory experiments (42), we had a unique opportunity to characterize the frequency of this association under field conditions in a situation with natural levels of fungal inoculum.

From field collections during the 2009 and 2010 growing seasons, we sampled *V. dahliae* from asymptomatic oats and, in 2010, plants from a hay mix (timothy [*Phleum pratense*], alfalfa [*Medicago sativa*], red clover [*Trifolium pratense*], and hairy

chess [*Bromus commutatus*]) in different fields on a farm located in Schuylkill County, PA. These crops were grown in rotation with potato crops that had exhibited significant Verticillium wilt incidence and severity in previous years. *V. dahliae* was isolated using routine protocols that included a thorough surface disinfection of the lower portion of plant stems with 2.5% commercial bleach (6.15% NaOCl), followed by plating a 0.6-cm stem fragment on semiselective NP-10 medium and incubation at room temperature. We followed this with repeated subculturing of the fungus until a pure culture was obtained and identified by morphological characteristics (59). The 2-year crop rotation sequence at this farm was potato (year 1) followed by fall-seeded oat which then was under seeded with the hay mix (year 2), then repeated with potato. The fields sampled varied in the disease incidence and severity observed over multiple previous years of potato crops.

In 2009, 30% of oat plants sampled at inflorescence stage from a field with high disease incidence were colonized by *V. dahliae*, as shown by isolation of the fungus from oat stems. At this time, microsclerotia were not visible on the young green plant tissues. Comparatively, in 2010, only 5 to 8% of early-growth stage oat plants (≈15 to 20 cm tall; low Verticillium wilt disease incidence fields) were colonized by *V. dahliae* (Fig. 2). It is likely that the lower frequency of *V. dahliae* colonization of oat in 2010 was due to the observed differences in disease pressure in the fields in previous potato crops (which was probably associated with differences in soil inoculum densities) and to differences in plant phenological stages at the time of sampling. For instance, in a laboratory study, oat, wheat, and barley had a higher incidence of pathogen colonization in older versus younger plants (42). In addition to sampling oat plants for *V. dahliae* colonization, in 2010 we also sampled 20 plants each from the hay mix, which included timothy grass, alfalfa, red clover, and hairy chess, from a field with historic high disease incidence in potato crops. *V. dahliae* was not isolated from any of the plants in the hay mix sampled (Fig. 2). Until shown otherwise, the plants in the hay mix could be considered nonhosts of *V. dahliae*. Oat, rather than being considered a nonhost of *V. dahliae*, meets criteria for being an asymptomatic host for this fungal pathogenic species. To put this in perspective, growers in Pennsylvania are recommended to achieve a plant population of 1.2 to 1.5 million spring oat plants per acre (28). If 5% of 1.2 million spring oat plants were colonized by *V. dahliae*, that would correspond to 60,000 plants colonized per acre; and if 30% of spring oat plants were colonized, it would

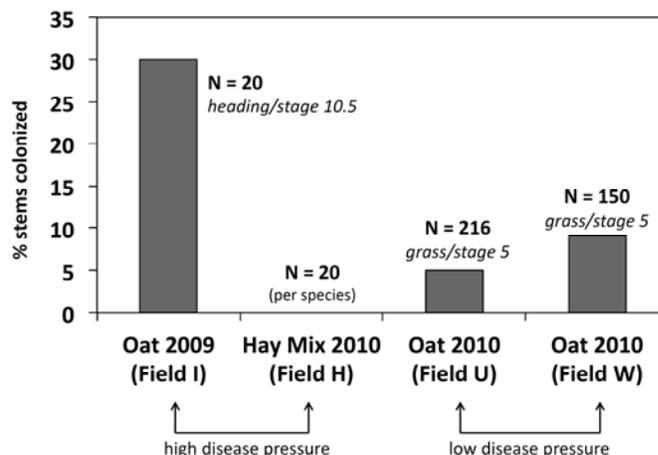


Fig. 2. Frequency of association between *Verticillium dahliae* and oat and hay mix (alfalfa, timothy, red clover, and hairy chess) plants sampled in fields previously planted to potato. Oat plants collected in 2009 were headed out in full flower, whereas oat plants collected in 2010 were in the last stages of tillering. Fields I and H had high incidence of Verticillium wilt in previous potato crops whereas fields U and W had low disease incidence.

translate into 360,000 plants of oat colonized by *V. dahliae* per acre.

Supporting the notion that *V. dahliae* is recovered from monocot crops in the field, Davis and colleagues (17) reported that oat, wheat, barley, sudangrass, and field corn were colonized by *V. dahliae* but to a lesser degree than a susceptible potato cultivar. This report was based on the number of fungal CFU produced in plant tissue (17). Unfortunately, the data did not reveal how frequent the association was between these crops and *V. dahliae* in the fields (i.e., percentage of plants colonized by the fungus in a given area).

Managing the disease with a broadened ecological perspective: Why asymptomatic hosts matter. Fungal inoculum produced on diseased plants can persist in soil for a long period of time. As a consequence, the long-term management of Verticillium wilt remains a challenge (12,31,38,64). Effective management of the disease has often required the use of soil fumigants with broad-spectrum biological activity, such as metam sodium or methyl bromide (64). Unfortunately, although crop cultivars with resistance to Verticillium wilt have been identified, their implementation has been limited in some cases because they often lack the very specific horticultural and processing characteristics sought by the industry. For example, for potato in the United States, mainly one cultivar, 'Russet Nugget', with resistance to Verticillium wilt has been released in the past 20 years (64). With the phase-out of soil fumigants and a lack of desirable cultivars with resistance to the disease, growers have been largely left with manipulating their crop rotations by selecting crop plants traditionally considered nonhosts as a way of managing this disease by reducing pathogen inoculum (14,19), but have had limited success (25,57,70,76).

Research addressing rotational crops and crop sequences, as well as the use of green manures to reduce Verticillium wilt of various crops has been extensive over the past two decades and has yielded inconsistent results, depending on fields or regions. Also, at least for potato, none of the studies characterized the population of *V. dahliae* established in those soils, and most of the work was conducted in experimental fields in Idaho, Maine, and Washington (15,16,18,19,25,46,47). For example, Davis et al. (15,16,18,19) repeatedly reported a reduction in Verticillium wilt incidence of potato grown in 2- to 3-year rotations that included monocotyledonous and green manure crops. Green manures generally included sudangrass (*Sorghum vulgare* var. *sudanense*), sweet corn (as either an annual rotation or a summer rotation, depending on the cropping region), and brassica crops (4,47,68). However, most experiments by Davis and co-authors were done in the same research experiment center in Southern Idaho, and none characterized the *V. dahliae* target population (15,18). A closer examination of the soil microbial community after two to three seasons of corn green manure concluded that, although Verticillium wilt was reduced in the subsequent potato crop, microsclerotia soil densities remained the same as before the incorporation of corn to soil, and an increase of *Fusarium* spp. was observed. The authors speculated on the effect of green manures in modifying soil microbial communities and their role in disease suppression (15). The same type of experiments done in Washington State concluded that 1-year rotations of sudangrass, green pea–sudangrass (same year), spring wheat, spring wheat–sudangrass (same year), sweet corn, and field corn had no effect on Verticillium wilt of potato grown subsequently, and no reduction of microsclerotia in soil was observed following the rotations (25).

Our overall conclusions from the above studies are twofold. First, rotation with monocotyledonous and green manure crops can potentially reduce Verticillium wilts; however, the inconsistency of results may possibly be due, in part, to the fact that many of these "nonhosts" may actually form endophytic associations with *V. dahliae* (Table 1). Second, in all studies, the compo-

sition of the target *V. dahliae* population was virtually unknown; therefore, the effectiveness of specific crop rotations for disease management may be highly dependent on the pathogen population structure. So long as we ignore the potential for *V. dahliae* to form associations with asymptomatic plants, and understand the role that population structure plays in asymptomatic interactions, we may never be able to incorporate rotations effectively into management of Verticillium wilts.

Implications and research needs. Understanding the full potential of *V. dahliae* to colonize host plants, from asymptomatic to symptomatic, will broaden our view of this pathogen (Fig. 1A). From an applied stand point, a better understanding of relationships among cereal crops and *V. dahliae* may allow us to use crop rotations more effectively in efforts to reduce soil inoculum levels as an efficient management practice. Perhaps some cereal crops, or even cultivars of a given species, are not as readily colonized by *V. dahliae*, or the fungus produces less inoculum or microsclerotia with a lower inoculum potential, when colonizing an asymptomatic host than a symptomatic one or when colonizing different asymptomatic host species. From a different applied stand point, further research on weed associations with *V. dahliae* might be quite important, especially in the context of sustainable and organic agriculture, where weed populations may not be as negligible as they can be in conventional agricultural systems. It may be that particular weed species might be more of a concern than other species as potential reservoirs and sources of inoculum for *V. dahliae*.

To our knowledge, few studies exist that analyze the population structure of *V. dahliae* in asymptomatic host plants, limited to VCG typing of isolates obtained from weed plants (21,22,40). Some interesting and relevant questions need to be addressed. Is there a separate portion of the population that colonizes asymptomatic hosts (i.e., endophytic) compared with symptomatic hosts (i.e., pathogenic) (Fig. 1B)? Or is the entire *V. dahliae* species capable of being an endophyte and a pathogen (Fig. 1B), depending on the plant species, under a certain set of environmental conditions in a particular location? The limited research available suggests that isolates of *V. dahliae* collected from asymptomatic plants are capable of colonizing symptomatic host plants and causing disease in controlled conditions (21,22,42). Although these studies imply that *V. dahliae* can be an endophyte in one situation and a pathogen in another, additional research, particularly under field conditions, is clearly needed. Another important question to consider is whether these asymptomatic plant hosts are adequate reservoirs for *V. dahliae* in the absence of a symptomatic plant host. For example, are the amounts of microsclerotia produced in asymptomatic hosts similar to those produced in symptomatic ones? In other words, are asymptomatic hosts as suitable for survival of *V. dahliae* as hosts exhibiting disease? Inoculations of potato and several cereal species showed that significantly lower numbers of microsclerotia were found in aerial parts and roots of cereal crops in comparison with the host potato plant, suggesting that these asymptomatic plants may not be hosts as adequate as susceptible potato in the short term but, over time, the fungal population can be maintained within the environment to some degree (17,20,49,52,55,56). However, we should be cautious; these are just a few studies conducted on a small set of asymptomatic host plants under laboratory conditions, making it hard to make extensive conclusions and justifying the need for more research.

More importantly, the significance of endophytic relationships between asymptomatic host plants and fungi otherwise known as plant pathogens may expand beyond *V. dahliae*. For example, the fungus *Fusarium oxysporum*, well known as a causal agent of vascular wilts on hundreds of important crops, is one of the most commonly encountered fungi in asymptomatic plants (44), yet this endophytic association has been poorly characterized. When analyzing endophytic *F. oxysporum* isolates associated with field-

grown tomato collected from a field in Pennsylvania, populations associated with four tomato cultivars were significantly different, although populations from surrounding soil were not, suggesting a potential effect of host genotype on the composition of endophytic populations (J. E. Demers and M. M. Jimenez-Gasco, unpublished). This suggests that the endophytic interaction between *F. oxysporum* (and possibly other plant-pathogenic fungi) and asymptomatic hosts may be more complex than previously envisioned.

Finally, as for many soilborne fungal pathogens, new *Verticillium* wilt diseases are being reported in crops that were once considered to be nonhosts or minor symptomatic hosts of *V. dahliae*, such as cauliflower, horseradish, lettuce, or pepper (7, 24,69). These examples, together with the earlier report of a disease caused by *V. dahliae* in barley (53,54), indicate that there may be more biological diversity in this species that our traditional phytopathogenic research focus has completely ignored and, therefore, is yet to be discovered. Do endophytic relationships offer opportunities for virulence evolution in plant-pathogenic fungi? More importantly, does this biological diversity hold keys to identifying mechanisms of pathogenesis in fungi? Addressing some of the issues we have raised in this article may help understand the complex plant–fungus relationships in agricultural systems that can potentially lead us to develop environmentally sustainable disease management practices.

ACKNOWLEDGMENTS

We thank the grower who graciously allowed us to sample potato and oat fields; E. Shin, S. Colihan, and J. Krockner for excellent technical support; and R. M. Jiménez-Díaz for critically reading the manuscript prior to submission.

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