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Diversity, Pathogenicity, and Management of Verticillium Species

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Key Words

taxonomy, species concept, host range expansion, host colonization, seed transmission, host resistance

Abstract

The genus *Verticillium* encompasses phytopathogenic species that cause vascular wilts of plants. In this review, we focus on *Verticillium dahliae*, placing emphasis on the controversy surrounding the elevation of a long-spored variant as a new species, recent advances in the analysis of compatible and incompatible interactions, highlighted by the use of strains expressing fluorescent proteins, and the genetic diversity among *Verticillium* spp. A synthesis of the approaches to explore genetic diversity, gene flow, and the potential for cryptic recombination is provided. Control of Verticillium wilt has relied on a panoply of chemical and nonchemical strategies, but is beset with environmental or site-specific efficacy problems. Host resistance remains the most logical choice, but is unavailable in most crops. The genetic basis of resistance to Verticillium wilt is unknown in most crops, as are the subcellular signaling mechanisms associated with *Ve*-mediated, race-specific resistance. Increased understanding in each of these areas promises to facilitate management of Verticillium wilts across a broad range of crops.

INTRODUCTION

The genus *Verticillium* encompasses a cosmopolitan group of ascomycete fungi, including several phytopathogenic species that cause vascular wilts of plants. The two most notorious species are *V. dahliae* and *V. albo-atrum*, which cause billions of dollars in annual crop losses worldwide (109). Yield losses in potato crops may reach 50%, but are more commonly in the range of 10–15% (114, 124, 125), whereas in lettuce, losses can easily reach 100% (150). The soil habitat of these species, the ability of their survival structures to persist for years, and their capacity to infect a bewildering array of hosts make them chronic economic problems in crop production. Four other plant-pathogenic species historically associated with the genus *Verticillium* are *V. tricorpus*, *V. nigrescens*, *V. nubilum*, and *V. theobromae* (13). The recent assignment of *V. nigrescens* and *V. theobromae* to the genera *Gibellulopsis* and *Musicillium*, respectively (170), reduced the number of plant pathogenic species in the genus *Verticillium* to four. In addition, both the entomopathogenic *V. lecanii* and *V. fungicola*, a pathogen of agaric basidiomycetes, were assigned to the genus *Lecanillium* (169).

A variant of *V. dahliae* from horseradish, first described by Stark (13) produces microsclerotia like *V. dahliae* but also conidia significantly longer than the typical *V. dahliae* strains, and thus was named *V. dahliae* var. *longisporum*. This morphological difference and other characteristics were considered sufficient to elevate such strains into a new species, *V. longisporum* (76). Erecting this new species has been controversial and much effort over the past decade has focused on resolving the taxonomic, phylogenetic, and evolutionary status of the long-spored crucifer strains. This is the subject of the first part of this review. In the context of this review, the taxonomic status of *Verticillium* spp. is discussed relative to the morphological and phylogenetic species concepts, as described in numerous reviews (11, 86, 153, 154), and does not include the biological species concept because it does not apply to this genus. *Verticillium* spp. have no described sexual stage.

Of the four remaining species in the genus, *V. dahliae* is the suggested type species of the genus (56) and also the more ubiquitous member of the genus. It is the primary causal agent of Verticillium wilt in temperate and subtropical climates (17, 69, 109) and is the focus of this review. *V. dahliae* has great genetic plasticity and is able to infect more than 200 plant species (1), including high-value annual and perennial crop plants, as well as landscape, fruit, and ornamental trees and shrubs (17, 109). The list of the hosts infected by *V. dahliae* is continually expanding as disease outbreaks on new hosts are identified (16, 43). One example of host range expansion occurred in lettuce in coastal California where entire crops have been lost to Verticillium wilt (150, 161). The population biology of *V. dahliae* remains the least understood aspect of this ubiquitous phytopathogen. Whereas strains of *V. albo-atrum* were divided into two groups based on their virulence and aggressiveness to lucerne (*a.k.a.* alfalfa, *Medicago sativa*), *V. dahliae* is divided based on vegetative compatibility into six groups (16, 71). Nevertheless, vegetative compatibility groups (VCGs) do not describe the overall genetic diversity among strains, gene flow, or the potential for recombination. They will, however, aid in the deployment of resistant cultivars, preventing pathogen introductions and exploring the evolution of an agronomically important group of phytopathogens.

The availability of cultivars tolerant to Verticillium wilt has reduced the disease to a minor nuisance in some crops such as cotton. When this type of resistance is compromised, Verticillium wilt is likely to re-emerge as a significant production problem for such crops. Several potato cultivars with improved resistance to *V. dahliae* are available, in addition to wild and cultivated accessions of *Solanum tuberosum* and hybrids of *Solanum* spp. (32, 70). On tomato, resistance to race 1 was overcome within a few years after its introduction (109). Race 2 steadily supplanted race 1 in various regions of the world because of the extensive use of race 1-resistant

cultivars (41). Two races were also described in lettuce (65, 162), and germplasm with resistance to race 1 was identified (65). However, there remains no source of resistance to race 2 in either tomato or lettuce. More importantly, resistance in most crops is either scarce or unavailable, making Verticillium wilt a significant chronic problem in the production of these crops. Substantial economic losses caused by Verticillium wilt are expected in the absence of fumigation for high-value crops such as strawberry, potato, cotton, tomato, and ornamentals (44, 125). For the few crops in which resistance exists, the nature of resistance has received little scrutiny. In this review, we also cover studies exploring the *V. dahliae*-lettuce interaction at the microscopic level, providing a unique description of the infection process from seedling to crop maturity. We further define host resistance in a new light based on studies using a fluorescently tagged strain (163).

VERTICILLIUM TAXONOMY

Nees von Essenbeck erected the genus *Verticillium* in 1816 (69, 109) based on its unique, branched conidiophores, which form whorls capped with flask-shaped and pointed phialides carrying terminal conidia. Although a few species of the genus *Verticillium* have been associated with ascomycetous teleomorphs (109, 170), *V. dahliae*, *V. albo-atrum*, and *V. tricorpus* are solely anamorphic with no evidence of sexual recombination or a meiosporic stage. In 1879, Reinke and Berthold first described wilt on potato (*Solanum tuberosum*), and named the causal agent *V. albo-atrum* (69, 109, 120). It was not until 1913 that a second, morphologically distinct species causing wilt on dahlia (*Asteraceae* family) was described by Klebahn, and named *V. dahliae* (13, 69, 109, 120). *Verticillium* spp. by convention, are identified based on the type of resting structures produced, namely: pigmented resting mycelium, pigmented microsclerotia, and chlamydospores. The two most distinctive features separating *V. dahliae* and *V. albo-atrum* are: (*a*) the production of melanized microsclerotia as survival structures by *V. dahliae*, in contrast to *V. albo-atrum*, which produces melanized hyphae but no microsclerotia (13, 69, 109, 120), and (*b*) although *V. albo-atrum* fails to grow in culture or wilt plants at 30°C, *V. dahliae* grows and infects unhindered at 30°C (69, 109, 120). Even though this information existed in the literature, the taxonomic debate relative to the distinctiveness of these two species continued until the late 1970s when *V. dahliae* was universally accepted as a species separate from *V. albo-atrum* (53, 109). Subsequent phylogenetic studies have clearly identified *V. albo-atrum* and *V. dahliae* as distinct taxa (9, 13, 26, 117).

Based on host specificity, two clear subspecific groups in *V. albo-atrum* are recognized. Strains from alfalfa that cause severe symptoms on this host and also on numerous other hosts, and strains from hosts other than alfalfa that do not infect alfalfa or do so poorly (13, 31). This grouping is strongly supported both by molecular markers (13) and VCG data (31, 61). A number of strains morphologically described as *V. albo-atrum* cluster separately based on the internal transcribed spacer region (ITS) rDNA regions (99, 119), and thus were designated as *V. albo-atrum* Grp2. All other strains of *V. albo-atrum* are referred to as Grp1 (13). The resting structures and molecular markers such as random amplification of polymorphic DNA (RAPDs) and ITS sequences all distinguish these two groups (96, 119). Despite recognizing that the differences are significant enough to elevate Grp2 strains to a separate species, they are currently only recognized as a distinct operational taxonomic unit (96).

Unlike *V. dahliae* and *V. albo-atrum*, *V. tricorpus* is a successful soil saprophyte, which may not be impeded in its germination and growth by the absence of a potential host (69). Chlamydospores, microsclerotia, and melanized hyphae serve as survival structures for *V. tricorpus*. Because it is considered a weak pathogen on many hosts, research has focused on the potential for *V. tricorpus* to reduce the impact of Verticillium wilt induced by *V. dahliae* or *V. albo-atrum*. Co-inoculations of *V. tricorpus* with *V. dahliae* in lettuce and artichoke, and

V. dahliae or *V. albo-atrum* in potato have resulted in a lower severity of Verticillium wilt or potato early dying symptoms, compared with plants inoculated with either of the latter two species separately (116, 121).

CONTEMPORARY TAXONOMIC CONTROVERSY

A variant strain of *V. dahliae* from horseradish was described in 1961 by Stark (13). This variant produced microsclerotia but also conidia that were significantly longer than the typical *V. dahliae* strains, and thus was named *V. dahliae* var. *longisporum*. Similar strains causing Verticillium wilt have since been isolated from cauliflower, oilseed rape, horseradish, and other crops in North America and Europe (17, 18, 29, 49, 73, 76, 147, 172). These long-spored strains produced conidia 7–9 μm in length, nearly twice the size of typical *V. dahliae* conidia (76). These long-spored strains actually produce a continuous distribution of size classes (147) with the shorter conidia identical to *V. dahliae* in DNA content, whereas the longer conidia contain *approximately* 1.75-fold nuclear DNA content relative to most short-spored *V. dahliae* (76). These long-spored strains of *V. dahliae* were therefore described as being "near-diploid" (76), and subsequently described as amphihaploid (29). However, a strain from Brussels sprouts in the United Kingdom has been reported with short spores but higher nuclear DNA content (76), as well as long-spored strains with lower DNA content (147), calling into question the relationship between conidia length and DNA content.

Working exclusively with long-spored strains from oilseed rape in Europe, Karapapa et al. (76) considered the molecular and other morphological differences with typical *V. dahliae* strains distinctive enough to elevate the long-spored, oilseed rape strains to a new species, *V. longisporum*. It was further suggested that the new species was host-specific and borne out of an interspecific hybridization between *V. dahliae* and a lucerne form of *V. albo-atrum* (76). The elevation of this new species has been questioned because the studies by Karapapa et al. (76) were limited to long-spored strains from European oilseed rape and dismissed the type specimen used by Stark (146) for the initial description of *V. dahliae* var. *longisporum* as a recombinant (13, 17, 26, 29). Erecting this new species appears to be contrary to the morphological and phylogenetic species concepts and has generated focused research efforts to resolve the taxonomic, phylogenetic, and evolutionary status of strains assigned to this species.

A feature used to distinguish the crucifer strain from the short-spored *V. dahliae* strain is the in vitro morphological differences between their respective microsclerotia (73, 76). Karapapa et al. (76) found the microsclerotia produced in cultures of *V. dahliae* to be compact, whereas those of the long-spored strains were more diffuse and elongated and contained dark hyphae. However, microsclerotia recovered from infected crucifer crop tissues or from soil have not revealed consistent morphological differences among microsclerotia from long-spored strains and those of typical *V. dahliae* strains. The in vitro variability may be an artifact of the culture medium rather than a taxonomic feature distinguising *Verticillium* species (62).

There has been speculation that the amphihaploid crucifer strains of *V. dahliae* may have arisen from hybridization between two or more strains of *V. dahliae* or interspecific hybridization between *V. dahliae* and *V. albo-atrum* (28, 29). Interspecific hybridization has been documented in certain fungi. For example, interspecific hybridization of *Botrytis aclada* and *B. byssoidea* gave rise to the allopolyploid *B. alli* (145). Furthermore, strains of *Verticillium* spp. carrying auxotrophic markers have been used to obtain recombinant prototrophic strains in laboratory studies, supporting parasexualism and inter- and intraspecific hybridization in *Verticillium* spp. (24, 63, 109). The high level of genetic diversity observed in *Verticillium* spp. (10, 78) could also be explained by the occasional generation of hybrid strains, which subsequently undergo recombination and haploidization. The

diploid state may be stabilized, or haploidization may occur through repeated chromosomal nondisjunction during mitosis (135).

In the parasexual cycle, fungal hyphae of the same or different species may undergo anastomosis to produce a heterokaryon (132), which serves as the premise for vegetative compatibility and relies on the matching of specific loci in both individuals (92). Subsequently, the fusion of two haploid nuclei may occur in compatible interactions, resulting in the formation of a diploid nucleus. It is probable that one or multiple hybridization event(s) led to the formation of the amphihaploid crucifer strains, but when or where these occurred is unknown. Hybridizations possibly occurred in a cruciferous host plant, and subsequently the hybrid gained a selective advantage. Unlike the model described for endophytic hybrid *Neotyphodium* spp. (138), however, it is unlikely that *Verticillium* hybrid strains were saved by vertical transmission because the major route of transmission of crucifer strains is horizontal through the production of resting structures released to the soil.

Although *V. dahliae* has consistently been described as one of the parents of the long-spored *V. dahliae* strains, the identity of the second parent has proven elusive, with *V. dahliae*, a *V. albo-atrum*-like species (29, 49, 76), or some unknown *Verticillium* spp. as proposed donors (13, 26). Karapapa & Typas (77) also suggested a potential for horizontal transfer of an intronic region with the small subunit of the rDNA (SSU rDNA), from either another fungus or even a plant host. This horizontal transfer of group I introns in fungi is a viable possibility for such organisms (123). However, whether horizontal transfer of an entire genome is possible in the context of long-spored crucifer strains remains to be determined. Another possible hypothesis is that the amphihaploid strains are actually ancestral. Perhaps *V. dahliae* and *V. albo-atrum* evolved by haploidization and subsequent fixation of their genomes in a sympatric manner, leading to their evolution as two separate species. Clewes & Barbara (25) proposed that the *V. dahliae* parent and the other parent could infect one host, whether susceptible, or slightly resistant, leading to hybridization. If the long-spored strains are the ancestral form, this may explain their relatively narrower host range compared with *V. dahliae* (17, 149) and potentially answer questions raised by Clewes & Barbara (25) regarding the nature of the host where the hybridization may have occurred. If hybridization has led to the long-spored strains, why the literature seems to refer to one or two hybridization events and one set of parents is unclear. Although proposing a restricted number of hybridizations is perhaps the most parsimonious way to conceptualize the origin of long-spored strains, it is only one of many plausible explanations for such an evolutionary event. This is especially true given the cosmopolitan distribution of *Verticillium* spp. and *Brassica* spp., whether domesticated or wild, which opens the opportunity for potentially infinite possibilities for hybridization to occur, if indeed it occurred. Hybridizations among potential progenitors could occur repeatedly in multiple crucifers or other hosts, and could include multiple *Verticillium* spp. or other fungal parents.

The genetic distinctiveness of the long-spored amphihaploid strains is now well accepted. It is less certain whether these differences from short-spored *V. dahliae* strains are significant enough to warrant erection of a separate species, especially when both produce microsclerotia, which is the primary basis for the identification of *V. dahliae*. Molecular approaches including amplified fragment length polymorphism (AFLP) markers subdivided the long-spored strains into two groups, α and β. Limited variation exists in either group (29, 171). Whereas the α group was homogeneous, the β group could be further subdivided on the basis of ITS rDNA sequence variation and mitochondrial DNA type (29). A third group possessing solely short conidia clustered with *V. dahliae* (29).

Several studies using RAPD or AFLP markers, as well as ribosomal DNA sequences contend that because the long-spored crucifer strains cluster separately from *V. dahliae*, the

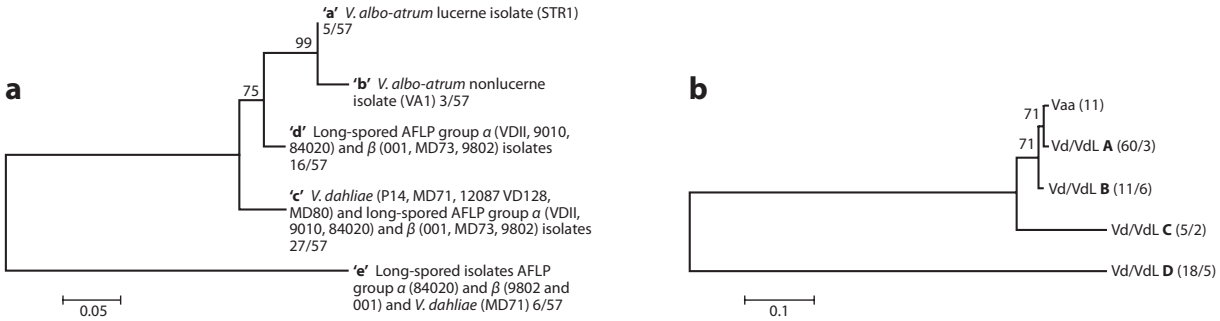

Figure 1

Two consensus neighbor-joining (NJ) dendograms showing the relationship among β-tubulin sequences of *Verticillium dahliae* (Vd), *V. albo-atrum* (Vaa), and long-spored crucifer strains (VdL). (*a*) Dendogram reprinted with permission from Blackwell Publishing, Ltd. (26). Numbers adjacent to entries indicate number of strains associated with cluster out of a total of 57 (*b*) Dendogram including 121 sequences available in Genbank, also comprising all sequences deposited by Clewes et al. Numbers adjacent to entries indicate the number of individuals associated with cluster. Where two numbers are present, the first number pertains to Vd and the second to VdL. Numeric values above branches indicate bootstrap support (1000 replications). Capital letters (A–D) identify the various clades comprising Vd and VdL.

crucifer strains ought to be raised to the level of a distinct species (49, 76, 107, 172). The decision on where to split well-delineated groups into distinct species is subjective (154). Taylor et al. (154) indicated that finding as few as one locus showing fixation may be indicative of genetic isolation, not necessarily speciation. Because crucifers are not the only hosts for the long-spored strains (17, 149), it is possible that this isolation is driven by the infected hosts. Additionally, relying on a DNA fingerprinting-like technique potentially generates polymorphic loci that may not be sequenced. The use of such techniques only allows the grouping of strains rather than the establishment of taxonomic status and therefore, is not compatible with the phylogenetic species concept.

We conducted independent comparisons of intronic sequences in the β-tubulin gene from *V. dahliae*, *V. albo-atrum*, and the long-spored strains from crucifers. The sequences from Clewes et al. (26) and others were retrieved from GenBank. It was apparent from these analyses that long-spored strains cluster with *V. dahliae* (**Figure 1**). A comparison of a further truncated c. 90 bp region used by Clewes et al. (26) to build a neighbor joining dendogram exhibited the same clustering (**Figure 1**). Regardless of the copy number per genome and hypothetical progenitor(s), all sequences obtained from long-spored crucifer strains were present in the same clades as *V. dahliae* strains. We note that by clustering with the established species the long-spored strains would then, at least by looking at this one locus, not be considered a distinct species, as is clear from **Figure 1**. Because divergence at one locus is only representative of the evolutionary history of that locus, not the species as a whole, Fahleson et al. (49) and Pantou et al. (107) used multi-gene sequences from nuclear and mitochondrial regions to estimate the phylogenetic relatedness of various species of *Verticillium*. We compared the aligned sequences generated by the latter two studies and found no more than three single nucleotide polymorphisms (SNPs) separating long-spored crucifer strains of *V. dahliae* or *V. albo-atrum*. Larger differences were found between this group and other *Verticillium* spp. in both studies. Furthermore, the observed DNA polymorphisms were not consistently associated with either *Verticillium* species, in some cases the long-spored strains shared SNPs with *V. dahliae*, whereas in others they shared them with *V. albo-atrum*. If such minimal differences consistently separate the long-spored crucifer strains from other *Verticillium* species across multiple unlinked genes, then a new species

level designation would be conceivable. However, inconsistencies in current data and a lack of resolution call into question a change in classification. Moreover, in contrast with the conclusions of Pantou et al. (107), the ITS rDNA sequence unequivocally grouped the long-spored strains from crucifers with *V. dahliae*. The conclusion of Pantou et al. (107) that molecular and immunochemical data justify the recognition of *V. longisporum* as a separate species, and it being closer to *V. albo-atrum* than to *V. dahliae* is unsupported. This strengthens the argument extended by the authors of this review and others (13, 26) that *V. longisporum* should not be recognized as a separate species.

Karapapa et al. (76) considered the new species *V. longisporum* to be host specific. *V. dahliae* generally lacks host specificity, although some strains are more aggressive on certain hosts than others (17, 117) or classified as distinct pathotypes (87, 110). Usually, strains of *V. dahliae* are considered to be host adapted rather than host specific because strains have the potential to infect a wide range of hosts but often seem to be most aggressive [disease severity on individual hosts (5)] towards hosts from which they originated (42, 117, 125). Although the existence of host-adapted strains of *V. dahliae* are reported for some plants, such as horseradish (22, 45) and mint (42), it seems likely that cross pathogenicity and the ability of certain strains from an established host to adapt to a new host under selection pressure is advantageous to the fungus and possibly explains the sudden appearance of Verticillium wilt in previous nonhosts such as lettuce (150). Extensive analysis of the host range of the cauliflower strain on a range of crops showed that it was not host specific (149), but was particularly aggressive on crucifer crops.

A NOVEL LOOK AT THE VERTICILLIUM DISEASE CYCLE

Recent advances in fungal transformation techniques and the transgenic expression of fluorescent proteins, like the green fluorescent protein (GFP) from *Aequoria victoria*, have enabled an unprecedented view of plant-fungal interactions, especially when combined with the use of confocal microscopy (101a). In this context, we provide a historical framework on the examination of the disease cycle, and interject some of the recent findings from studies that have employed GFP-labeled strains.

For *V. dahliae*, the disease cycle begins with dormant microsclerotia, which are distributed in the soil or embedded within plant debris (8, 37, 89). In the absence of a host, microsclerotia can remain viable for up to 14 years (165), whereas the survival of melanized mycelium of *V. albo-atrum* is limited to 2–5 years (81, 139). Upon germination, microsclerotia produce one to several hyphae that extend toward host roots (52). There is no evidence of any host-specific requirements or relationships that limit the initial colonization of roots during germination (47, 52, 90, 101, 136). Colonization of the root surface was often observed at or near root tips, or following root hairs to the root surface, with hyphae growing between epidermal cells (48, 52, 58, 163, 172).

Reports vary regarding preferential colonization sites that lead to successful vascular infections. Most studies of *V. dahliae* using immunoenzymatic and histological staining techniques observed colonization of the root cap and within the zone of root elongation on cotton (58), potato (20, 112), and sainfoin (68). Conversely, Gerik & Huisman (59), using immunoenzymatic techniques, observed *V. dahliae* colonization at distances greater than 1 mm from the root tip on cotton. Colonization of the root cap and within the root elongation zone did not lead to successful vascular infections in potato (112). The colonizing hyphae of a GFP-expressing strain of *V. dahliae* and a long-spored crucifer strain were absent from the root tips of oilseed rape (*Brassica napus*) in a gnotobiotic (aseptic) system, but were found intermingled with developing root hairs and surface regions along main and lateral roots. Most studies are in agreement that wounded root tissues (whether by biotic or abiotic means) and areas of lateral root emergence are not predisposed to colonization by *V. dahliae* (20, 48, 59, 112, 163).

Although *V. dahliae* can successfully penetrate the root epidermis of most plants and usually reach cortical tissues, this does not guarantee vascular infection. Most infections fail to reach vascular tissues of the plant (58, 59, 112). Gerik & Huisman (59) estimated that only 0.02% of root infections by *V. dahliae* on cotton led to systemic infection of the plant. Similarly, a single-site root inoculation study with microsclerotia on eggplant found that a small number of microsclerotia was sufficient to infect most roots, but only 1 out of 205 inoculations led to a systemically infected plant (14). On lettuce inoculated with conidia of *V. dahliae*, colonies were distributed at random, but with time only those that initiated at the root cap or zone of root elongation led to vascular infections (163). No epiphytic colonies were observed along potato roots growing in soil infested with microsclerotia of *V. dahliae*. Instead, only the root tip was colonized (20). En masse, these findings suggest that successful vascular infection of the host is probably more dependent on the initial colonization site, rather than the frequency of root colonization.

Vallad & Subbarao (163) observed that root colonization of lettuce by *V. dahlia*e was spatially and temporally dynamic. Successful vascular infections at the root cap on lettuce were associated with sparse growth of hyphae directly towards vascular tissues with little hindrance, whereas infections originating from the zone of root elongation developed more extensive inter- and intracellular colonies before invading vascular tissues. Colonies that developed along regions above the root tip, at root hairs, root eruption zones, or within damaged regions of the root were numerous, but failed to persist. Furthermore, two colonization fronts developed over time in association with successful vascular colonization. The first front enveloped the epidermis and cortical tissues leading to the collapse of the root tip, whereas the second front consisted of hyphae progressing acropetally through the vascular tissues far from the infection site. Expanded colonization of the root tip may be necessary to provide additional nourishment for the acropetal colonization of vascular tissues. This spatial and temporal colonization pattern would be difficult to observe without synchronized inoculation with conidia because microsclerotia would be distributed randomly throughout the soil profile and could germinate asynchronously until exhausted (51).

In many hosts of *Verticillium* spp., once vascular tissues are infected, conidia are produced within the xylem vessels and move acropetally with the transpiration stream. Conidia often become trapped at pit border members between vessels, where they germinate, penetrate through the member to the neighboring vessel, and produce more conidia to repeat the process (58, 112, 134). Eventually, the fungus emerges from the xylem vessels to colonize neighboring vascular and cortical tissues, corresponding with the development of disease symptoms. Although the roles of conidia in vascular colonization are evident, questions abound on their overall role in the disease cycle. Conidia are short-lived in the soil, although they may still survive for several months (136). *V. dahliae* produces conidiophores and conidia on the root surface at sites of successful systemic infection, and also following the death of the plant (163). Thus, conidia may accelerate secondary infections and colonization of multiple crops, especially where they are grown year round in the same location.

Various structural barriers that restrict invading hyphae of *Verticillium* spp. have been described in plant roots. The formation of a dark gum was observed to prevent hyphae from entering the root cortex of cotton (57). Whereas in potato, lignified cell wall appositions and lignitubers surrounded the invading hyphae at attempted penetration sites (111, 112). Differences in the colonization pattern among resistant and susceptible crop cultivars have also been observed. In resistant tomato cultivars, a coating response developed in colonized xylem vessels that apparently restricted the lateral movement of incompatible *V. dahliae* strains into neighboring vessels (60), and limited the fungus from reaching foliar tissues by reduced cyclical movement of conidia through xylem

vessels (66). Resistance in lettuce appears to rely on preventing colonization of the taproot and possibly even eliminating the fungus through the turnover of infected lateral roots (163).

Successful vascular infection of lettuce occured in as little as 3–5 days after inoculation, whereas foliar symptoms required an additional 8–10 weeks to develop. The appearance of foliar symptoms coincided with the transition from vegetative to reproductive growth, which signals harvest maturity in commercial head type varieties (150). Foliar symptoms develop as mycelia ascend the crown in a few vessels and reach basal leaves. This is followed by acute wilting, which begins as mycelia erupt from the xylem vessels and colonize cortical tissues in the crown, coinciding with the sudden collapse of the head. The rapid collapse of lettuce does not involve the production or movement of conidia through xylem vessels at this point. However, as mycelia progress further into the stem, small isolated colonies and conidia are observed in the xylem vessels. These microcolonies and conidia could also be observed in the developing inflorescence and flower heads of early maturing lettuce lines and in susceptible head type lettuce cultivars that managed to produce an inflorescence.

Closer inspection of achenes from lettuce infected with a strain of *V. dahliae* labeled with GFP revealed that internal colonization was limited to the maternal tissues, including the endosperm, but never in the tissues of the embryo (**Figure 2**). No microsclerotia were ever observed within the achene. However, massive quantities of conidia and conidiophores were present on external and internal tissues of the pappus, which are easily disrupted mechanically, and could accelerate the movement of *V. dahliae* by airborne dispersal in lettuce seed production areas (163).

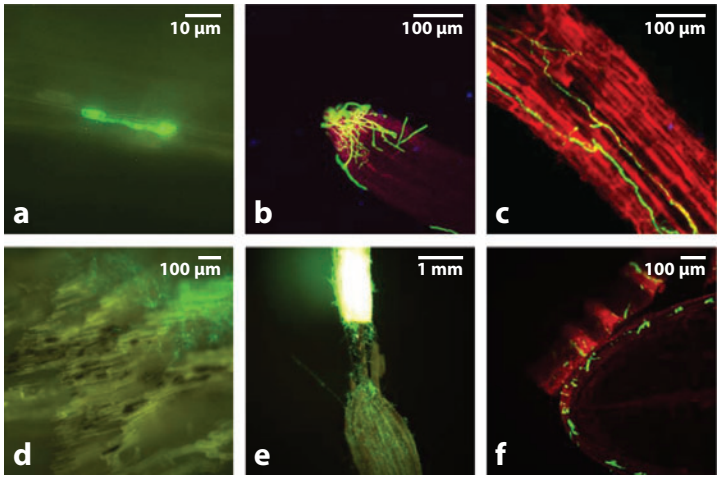

Figure 2

The Verticillium wilt disease cycle on lettuce as observed using a GFP-tagged strain of *V. dahliae*. (*a*) Conidium with a single germ tube and appressorium developing at the junction of root epidermal cells 48 hrs after inoculation. (*b*) Tip of a lateral root colonized by *V. dahliae* with simple conidiophores protruding from root surface, 12 days after inoculation. (*c*) A longitudinally dissected lettuce root exhibiting advanced colonization of cortical and vascular tissues, 2 weeks after inoculation. (*d*) A longitudinal section of a lettuce taproot showing the massive eruption of mycelia from infected xylem vessels into the surrounding cortical tissues, 10 weeks after inoculation. (*e*) A dehiscent capitulum collected after flowering, ~16 weeks after inoculation, containing several ripened achenes with pappi covered with sporulating conidiophores of *V. dahliae*. (*f*) Cross-section of a mature achene with hyphae ramified through all maternal tissues, but not into the embryo. Images captured using epifluorescence and confocal laser scanning microscopy. Scale bar = 10 µm for (*a*), 100 µm for (*b–d* and *f*), and 1 mm for (*e*). All images are reprinted with permission from APS Press, St. Paul, MN.

SEED TRANSMISSION AND COLONIZATION OF WEED HOSTS

Seed transmission of *V. dahliae* has been documented in numerous crops in addition to lettuce (161), such as cotton (4), eggplant and tomato (75), safflower (137), sunflower (84), and spinach (143); however, its role in epidemiology has been long debated. Rudolph (126) and Rudolph & Harrison (127) argued that seed transmission was of little consequence in the epidemiology of Verticillium wilt of tomato and cotton, respectively, as a result of the rare incidence of seed infection observed in field experiments. Seed to seedling transmission of *V. dahliae* has also been demonstrated for a number of crops (43, 130, 161). Schippers & Schermer (133) observed that under field conditions, seed to seedling transmission of *V. dahliae* was not significant. However, seed transmission may play a more important role in the epidemiology of Verticillium wilt by

facilitating the spread of the pathogen to new production areas. du Toit and colleagues (43) tested 75 commercial lots of spinach seed produced in the U.S. and Europe and found that 68 were infested with species of *Verticillium*, demonstrating the potential for international movement.

Of equal concern is the potential of seed transmission among weed hosts of *Verticillium* spp. A broad host range gives *Verticillium* strains the potential to persist, not only on susceptible agronomic hosts, but also on weed species present in production areas; of which some are nonsymptomatic hosts (93, 109, 161). Several weed species were found to harbor *V. dahliae* in the lettuce production areas of California, from which several strains were found to be particularly aggressive on lettuce (161). Evans (46) suggested that seedborne transmission of *V. dahliae* in weed species of *Xanthium* was responsible for spreading Verticillium wilt throughout the cotton production area of New South Wales, Australia.

POPULATION BIOLOGY OF *VERTICILLIUM* SPP.

The population biology of *V. dahliae* has been primarily addressed on the basis of VCGs (92) and several molecular markers including RAPDs (17, 87), restriction fragment length polymorphisms (RFLPs) (21, 103, 104), amplified fragment length polymorphisms (AFLPs) (27, 29), and specific primers (110).

There are six main VCGs in *V. dahliae* (16, 71). VCG3 and VCG6 (16) are comprised of only one strain each, but VCGs 1, 2, and 4 are cosmopolitan and associated with a wide host range. All VCGs exhibit broad virulence, but some show differential aggressiveness (35, 42, 71, 72, 105, 125, 160). VCG4 was subdivided into VCG4A and VCG4B; with the former more prevalent and damaging on potato (72, 148). VCG1A was commonly associated with severe Verticillium wilt in cotton (33, 88), olive (36), and woody ornamentals (23).

Similarly VCG2 is also subdivided into two subgroups, of which VCG2A is significantly more aggressive than VCG2B (159). Although VCGs are informative, they fail to describe the overall genetic diversity among strains and are seldom useful in exploring gene flow and the potential for sexual recombination in *V. dahliae*. Moreover, VCG analysis is impossible in amphihaploid *Verticillium dahliae* strains from crucifers, as the duplication of loci prevents the generation of *nit* mutants (17).

Molecular genotyping approaches, based on RFLP and AFLP analyses, have been applied for the study of *Verticillium* spp. populations. Dobinson et al. (39, 40) applied RFLP to study the genetic diversity of *V. dahliae* in solanaceous plants (potato, tomato, and eggplant in particular) in North America and discovered that VCG4A strains from potato belonged to at least two distinct genotypes with potential gene flow with the potato seed. Although RFLP subgroupings have been associated with geographical origin of strains (104), host (104), pathotype (87, 110), and VCG (39), other studies found no correlation between biological properties (VCG, virulence on specific host), geographic origin and molecular data (17, 40). Using a single RAPD primer, Korolev et al. (88) clearly differentiated defoliating (D) and nondefoliating (ND) strains. Strains that showed a particular molecular haplotype were distributed over different geographic locations in both countries (88). Other studies employed AFLPs (27, 29, 50) to measure the genetic diversity among strains within species of *Verticillium* and relationships among species. Fahleson et al. (50) primarily analyzed the relationship of *Verticillium* spp. and the long-spored crucifer strains. Greater than 85% of the variability occurred at the level of individual populations, and ~14.5% of variability could be attributed to differentiation by geographic origin. Collins et al. (29) found that groups α and β of the long-spored *Verticillium* strains from crucifers formed two clusters showing more than 60% similarity. Only three AFLP bands were shared by both short- and long-spored strains, whereas 39% of the bands were shared with *V. dahliae* strains isolated from noncrucifer hosts (29).

The genetic diversity of populations of *Verticillium* spp. has also been studied using sequences of the intergenic spacer region of the ribosomal DNA (IGS rDNA), which evolves rapidly. Conversely, the internal transcribed spacer (ITS rDNA) and six nuclear and one mitochondrial genes failed to differentiate the strains from *Verticillium* spp. (9, 117). Pramateftaki et al. (115) identified the region between 350 and 700 bp of the IGS sequence of *Verticillium* as particularly rich in mutations. Populations from various geographic locations in coastal California collected over 12 years (1995–2007) were not significantly differentiated using IGS rDNA sequences. This indicated either a persistent gene flow among locations or multiple introductions from a restricted pool of sources with a similarly high genetic diversity. Migration analyses clearly indicated that *V. dahliae* genotypes can be exchanged on a national or international scale with planting material (106, 110, 158).

MANAGEMENT OF *VERTICILLIUM* SPP.

Because of its inaccessibility during infection, long-term persistence in the field, broad host range, and scarcity of resistance in host germplasm, control of Verticillium wilt has relied heavily on soil fumigation (44, 109), but is contingent on the economic returns from the crop. Soil fumigation with methyl bromide plus chloropicrin has become an indispensable tool for the past four decades because of its broad-spectrum biocidal activity but was primarily developed to manage Verticillium wilt in strawberries (44, 166, 167). The environmental problems associated with methyl bromide and the efficacy of alternative fumigants have been reviewed extensively elsewhere (2, 44, 97) and hence, this section will focus on nonchemical approaches to control *Verticillium* spp.

For monocyclic pathogens in general, and *Verticillium* spp. in particular, the principal goal of management is to reduce the primary inoculum. Although the most desirable approach is pathogen avoidance, it is also the most difficult to achieve. Because *Verticillium* spp. are cosmopolitan, virgin lands to grow crops such as potato in North America are virtually impossible to find. Quarantine restrictions to prevent the movement of contaminated plant or seed material are commonly employed for pathogen avoidance. With the exception of a quarantine imposed in Mexico on spinach seed (113), no regulations govern the international and interstate (within the United States) movement of seed or vegetative material infested by *Verticillium* spp. (A. O. Charkowski, personal communication). Such free movement of infested plant material could potentially facilitate significant geneflow as demonstrated with the spinach seed imported into central coastal California (**Figure 3**) (10) and other hosts (41, 71, 106, 158).

Crop rotation that is intuitive for the management of other pathosystems is largely ineffective for Verticillium wilt because of the wide host range of *Verticillium* spp. (17, 109, 149). A detailed analysis of the host range suggests that there is negligible host specificity in *V. dahliae* (149). However, strains from cauliflower (*Brassica oleraceae* var. botrytis) were only weakly virulent on broccoli (*Brassica oleracea* Italica group) and Brussels sprouts (*Brassica oleracea* var. gemmifera). In several studies, broccoli not only significantly reduced resident soil microsclerotia, but also wilt incidence and severity in subsequent susceptible crops, preventing the build up of soil inoculum (91, 140, 151, 152, 168). Unlike soil fumigants, rotations with broccoli did not eradicate the pathogen, but maintained soil microsclerotia below the threshold at which crop losses accrue, despite the cultivation of susceptible crops such as strawberry or cauliflower (140, 152). Empirical data evaluating the mechanisms of broccoli-induced *V. dahliae* suppression are only now becoming available. Although bacterial populations, especially actinomycetes (syn. actinobacteria), increased by as much as three orders of magnitude following the incorporation of broccoli residue, identifying an actual cause and effect relationship has proven difficult (K. V. Subbarao, unpublished data). Instead, the

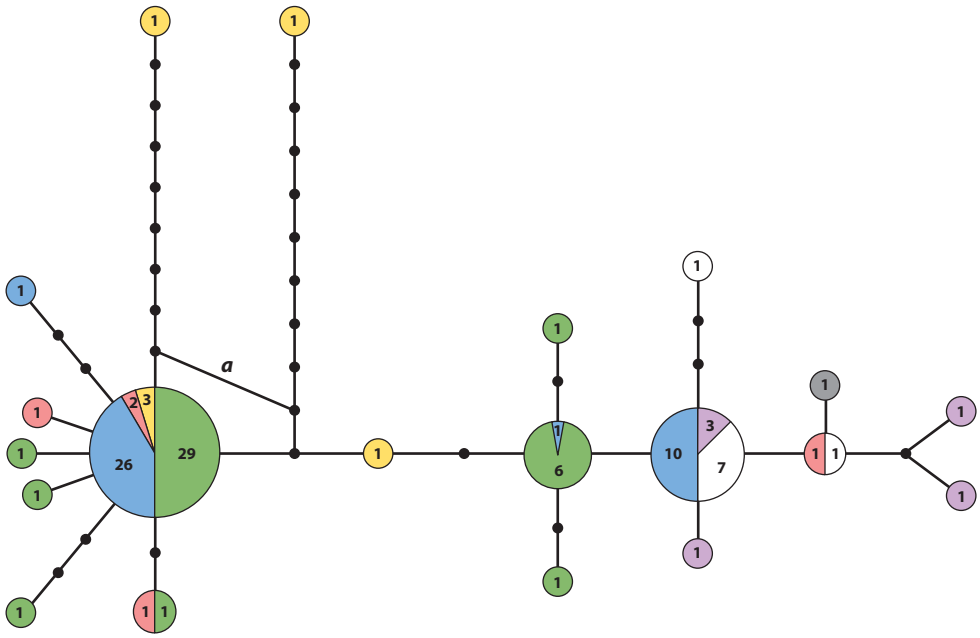

Figure 3

Statistical parsimony network of haplotypes of the complete sequence of the IGS rDNA from 86 *Verticillium dahliae* strains from artichoke ($n = 6$, yellow), bell pepper ($n = 6$, fuchsia), chili pepper ($n = 9$, white), lettuce ($n = 41$, green), potato ($n = 1$, black), spinach ($n = 38$, blue), and strawberry ($n = 5$, red). Another 20 strains clustered individually and could not be associated with this network because of their distinct haplotypes. Haplotypes at interior nodes would be considered ancestral, from which haplotypes at the tips have evolved more recently. Small circles are putative haplotypes not found in the studied samples. The size of colored circles is proportional to the number of individuals that form each haplotype. Numbers in circles indicate the number of individuals from each host forming each haplotype. The lowercase a above a branch indicates a possible recombination.

ontogenic changes in the type and levels of glucosinolates, structural components such as lignin, and phenolic compounds have been offered as possible explanations as to why broccoli is resistant to *V. dahliae* relative to cauliflower.

Colonization patterns of *V. dahliae* in cauliflower and broccoli were compared using immunohistochemical staining (140) and a GFP-tagged *V. dahliae* strain from cauliflower (102). Minimal differences in the colonization of cortical tissues were observed between broccoli and cauliflower (140) (**Figure 4**), but the vascular tissue in broccoli was uncompromised in contrast to the extensive colonization in cauliflower (102) (**Figure 4**). The type of glucosinolates and the range of their catabolic products have been associated with the suppressive effects of crucifer crops in general (98). However, it is clear from the high susceptibility of many crucifer crops to *V. dahliae*, including strains from crucifer crops, that not all glucosinolates and their catabolic products are involved in pathogen suppression. However, *V. dahliae* suppression by broccoli remains effective long after the volatilization of the isothiocyanates and is independent of glucosinolate content (91), suggesting that other biological factors are involved. Interestingly, preserved soil samples from field plots infested with a long-spored crucifer strain and planted to broccoli in the mid-1990s inhibit weed growth and have remained microsclerotia-free. In contrast, soils planted to cauliflower support abundant weed growth and *V. dahliae* microsclerotia have increased over time (K. V. Subbarao, unpublished data).

A related area that has been thoroughly researched is the employment of cyanogenic green manure crops for pathogen suppression. Davis et al. (34) determined that incorporating sudangrass and corn residues increased potato yields in fields infested by *V. dahliae*. The release and accumulation of hydrogen cyanide in amended soils is believed to be responsible for the pathogen suppression in these crops (98).

Implementing more intricate crop rotation strategies requires knowledge of the preexisting genotypic diversity of the pathogen in the field (105). Planting potato in fields previously planted with mint requires the identification of the VCGs composing the population of *V. dahliae* in mint fields. As described in the population biology section of this review, mint is host to VCG2B, which is not highly aggressive on potato (42, 74). If the *V. dahliae* population in mint fields was comprised predominantly of VCG4A, which is highly aggressive on potato, and VCB4B, which is less aggressive on potato, then planting a moderately resistant potato cultivar (7, 12, 108) would reduce the incidence and severity of Verticillium wilt on potato (D. A. Johnson, personal communication).

Evaluation of organic amendments for the management of *V. dahliae* has been explored intensively over the past decade (91). Criteria used for product evaluation have included ability to enhance crop growth; the potential for use at the field scale; whether environmentally safe; availability and effectiveness of byproducts from the meat, fishery, paper, and other industries; lower cost; and consistency between batches; (6, 30). The products fall into two broad categories, those that contain proteins and those that contain volatile fatty acids.

Both categories of products were effective in reducing *V. dahliae* microsclerotia in soil and wilt on potato, but the mechanisms by which they were suppressive are different. Two mechanisms govern efficacy of products rich in proteins. First, microbial degradation of proteins in the above products releases NH_4^+ ions into soil and as soil pH rises, accumulation of NH_4^+ ions increases. Soil pH at or above 8.0 initiates the conversion of NH_4^+ into NH_3 with the equilibrium (pK_a) between $NH_4^+ \leftrightarrow NH_3$ occurring at pH 9.3. Whereas NH_4^+ is nontoxic even at high concentrations, NH_3 is highly toxic (91). This mechanism becomes progressively less effective with increasing levels of soil organic matter as NH_4^+ is rapidly converted into nitrate (NO_3^-), which is pathogen nonsuppressive (156).

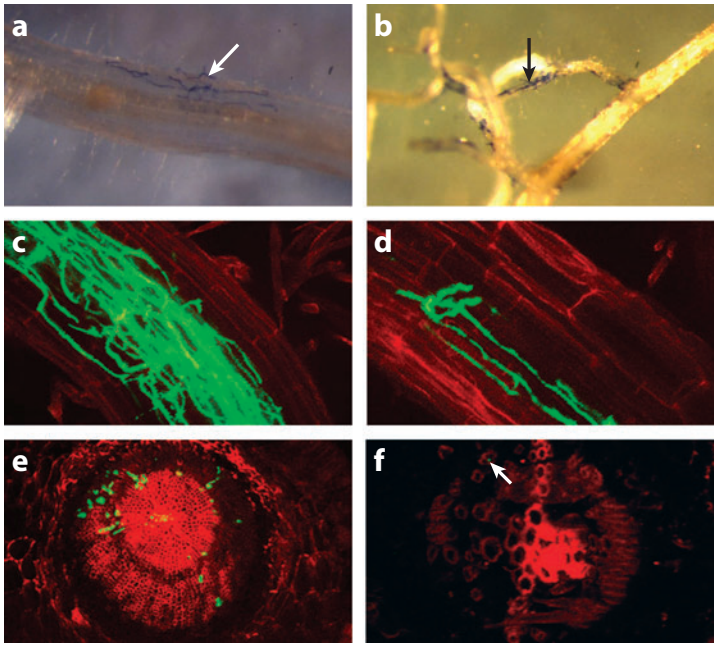

Figure 4

Colonization of cauliflower (*a*, *c*, and *e*) and broccoli (*b*, *d*, and *f*) by the cauliflower strain (Bob70) of *Verticillium dahliae*. In (*a*) and (*b*) hyphae stained immunohistochemically were observed under a stereomicroscope (140). (c–*f*) plants were inoculated with a green-fluorescence-protein-transformed Bob70 strain and viewed using confocal microscopy (102). (a–*d*) exhibit the colonization of cortical tissues in both cauliflower and broccoli. (*e*) and (*f*) contrast the elevated colonization of cauliflower (*e*) relative to broccoli (*f*) root vascular tissues. Only incipient infections are observed in broccoli, which subsides five weeks postinoculation. Images (*a*) and (*b*) are reprinted with permission from APS Press, St. Paul, MN.

The second mechanism by which organic amendments suppress pathogens is when soil pH is lowered upon coversion of NH_4^+ to nitrite (NO_2^-). When the pH drops below 5.5, NO_2^- is converted to HNO_2 (nitrous acid) (pK_a occurs at pH 3.3). Nitrous acid is approximately 300–500 times more toxic to microsclerotia than NH_3 (155) and is also toxic to many plant pathogens. The formation of HNO_2 is

most influenced by soil buffering capacity (156) as nitrification lowers soil pH only in poorly buffered soils. Both ammonia and nitrous acid were highly effective at reducing the microsclerotia in acidic soils (155). Because of the site-specific nature of soil pH and organic matter, efficacy of these products in pathogen suppression is inconsistent. Mechanisms by which certain manures suppress *V. dahliae* are based on the release of volatile fatty acids. Although acetic acid was the major component of the volatile fatty acids released, others such as propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids also contributed to pathogen suppression (155). Because only acidic forms of the volatile fatty acid molecules were toxic, disease control occurs only in acidic soils (30). Volatile fatty acids are metabolized by soil microbes and this rapid degradation may also explain their site-specific efficacy.

Reports on the biological control of *Verticillium* spp. abound in the literature (54, 55, 100, 144), but few have developed into products with field-level efficacy. Successful biocontrol agents at the field scale need to satisfy all of the following criteria: (*a*) reduce microsclerotia in the soil bank; (*b*) reduce incidence of Verticillium wilt on the crop; (*c*) improve yields of infected plants to levels comparable with healthy plants or crops grown in fumigated soils. Although several biological agents have been identified for potential activity against *V. dahliae* in recent years (6, 157), none have met the above criteria for the successful development of an applied field product.

Development of plant resistance remains the preferred strategy for the control of Verticillium wilt. Genetic resistance to Verticillium wilt was described in alfalfa, cotton, potato, tomato, strawberry, sunflower, oilseed rape, lettuce (65, 109, 129), and other crops. Characterization of resistance at the genetic and physiological levels is best characterized in tomato, potato, and cotton.

Genetic analyses of Verticillium wilt resistance in tomato were determined by Schaible et al. (131), demonstrating that a single dominant allele conferred wilt resistance against *V. albo-atrum* and was designated as *Ve*. Subsequent analyses highlighted the race-specific nature of *Ve*-mediated resistance (3, 120). This early work led to the differentiation of *V. dahliae* strains into race 1 and race 2 based on their respective avirulence or virulence on tomato with *Ve*-mediated resistance (15). Race 2 strains are widely distributed, vary in aggressiveness (109), and have rapidly supplanted race 1 strains in many tomato production areas (41).

Ve-mediated resistance was transfered to tomato cultivars soon after its discovery. The locus was positionally cloned and sequenced, revealing two inverted open reading frames (ORFs), designated *Ve1* and *Ve2* (79). The two ORFs encode leucine-rich repeat (LRR) proteins and share 84% identity at the amino acid level (79). Functional complementation analyses were carried out in a highly susceptible potato cultivar, and both *Ve1* and *Ve2* conferred resistance to *V. albo-atrum*, providing confirmation of function in another solanaceous host. Unlike many other R proteins, both Ve proteins possess a cytoplasmic C-terminus (carboxyl) similar to sequences that stimulate receptor-mediated endocytosis in mammalian cell surface receptors (80). The plasma membrane-bound receptors may bind a ligand from race 1 of *V. dahliae* or *V. albo-atrum* to initiate signaling. However, analysis of GFP-tagged Ve2 indicated that this protein localized exclusively to the endoplasmic reticulum (ER) and that this localization was dependent on a dilysine motif present at its C-terminus (128). The subcellular localization of Ve1 has not been examined, but it lacks the dilysine motif (128). Potentially, this differential localization may regulate signal perception and transduction because the dimerization of surface receptors may be required for downstream signaling.

At present, little is known about *Ve*-mediated signal perception and the components involved in downstream signaling, although insights into these processes may be obtained from comparisons with related R proteins. The tomato receptors for the fungal elicitor ethylene-inducing xylanase (EIX) possess a structure similar to the Ve proteins, including amino acid

sequences that may specify receptor-mediated endocytosis (122). Silencing of *LeEIX1* and *LeEIX2* prevented the binding of the EIX elicitor to the cells, and the presence of wild-type, mammalian-like endocytosis signal in LeEIX2 was required to mediate the hypersensitive response (122). By analogy, the Ve proteins may bind a *Verticillium* spp.-associated effector, and subsequently transmit a signal via endocytosis. Downstream signaling of both Ve1 and Ve2-mediated resistance in tomato is apparently mediated in part by *SUN1*, a homolog of the *Arabidopsis* gene for enhanced disease susceptibility (*EDS1*), although the functions of EDS and SUN1 are not characterized (67).

In related crops, such as the solanaceous tomato and potato, association mapping with a candidate gene may prove useful for the identification of loci conferring wilt resistance or susceptibility. Simko et al. (141) used the tomato *Ve1* to identify homologs (*StVe1*) in diploid and tetraploid potato. Analyses of the homologs derived from the resistance quantitative trait locus revealed 11 members of a putatively related R gene family, each encoding LRRs. A particular locus of a microsatellite marker that was closely linked to a *StVe1* homolog was absent in approximately 20% of the tetraploid genotypes. Absence of the marker locus was strongly associated with Verticillium wilt susceptibility and explained approximately 25% of the total phenotypic variance in disease resistance (141). An additional three chromosomes were significantly linked with wilt resistance in two diploid mapping populations of potato (142). Adding an additional layer of complexity, it is possible that different types of Verticillium wilt resistance are controlled by different genetic systems. For example, tolerance is a polygenic trait in diploid *S. chacoense*, whereas resistance to infection and colonization is apparently due to a major gene (95). Resistance in at least some cotton cultivars is quantitative and was associated with three loci on chromosome 11 (19).

Lettuce also presents an example of race-specific resistance (162). *V. dahliae* strains virulent on the cultivars La Brillante and Salinas were designated as race 2, whereas those capable of causing disease only on cv. Salinas were designated as race 1 (162). Although some lettuce cultivars, breeding lines, or accessions show some form of resistance or tolerance to race 2 of the pathogen (R. J. Hayes, personal communication), resistance to race 2 of the pathogen has not been identified in lettuce, and brings into question the durability of race 1 resistance (65). Race 1 resistance in lettuce is conditioned by a single dominant gene (64), but whether the same factor(s) control(s) race 1 resistance in both lettuce and tomato is unknown.

CONCLUDING REMARKS

Verticillium dahliae and *V. albo-atrum* are phytopathogenic species that differ markedly in host range and the types of survival structures produced. The contemporary controversy on the appropriate classification for the long-spored strains will continue to evoke debate. Currently available morphological and phylogenetic data suggest that the long-spored crucifer strains are not a species distinct from *V. dahliae*, although *V. longisporum* continues to appear in the literature. Because the taxonomy of *Verticillium* species has implications for the systematic study of pathogenic strains and their management, this is an area of research that deserves additional attention.

The newly available genome sequences of *V. dahliae*, *V. albo-atrum* (85, 164), and the limited set of sequences of long-spored crucifer strains have empowered studies to address some of the topics discussed in this review through comparative genomics. Comparative genomic analyses between those species may shed light on the hybrid hypothesis and the evolutionary history of *Verticillium* spp. In a previous study, a single chromosome difference between *V. albo-atrum* (n = 7) and *V. dahliae* (n = 6) was used to explain the stability of a potential hybrid through improper chromosomal pairing and a lack of nuclear division (29), but recent genome sequencing and a detailed physical map has revealed the presence of eight chromosomes in a lettuce strain of *V. dahliae*. Additionally, comparative genomics will reveal species-specific

and conserved gene sets. Some of these gene sets may provide insight into the genetics of microsclerotia production (*V. dahliae*) or dark resting mycelia (*V. albo-atrum*) as well as genes that may be unique to vascular wilt pathogens and those that specify host range differences. In spite of the economic importance of *Verticillium* spp., there have been few studies to elucidate the molecular basis of pathogenicity, although some targeted gene disruptions have provided insights into the genetics of signaling and development (38, 82, 83, 118). These types of studies, coupled with the availability of the genomic resources, will enable rapid identification and verification of genetic determinants of pathogenicity.

Historically, long distance migration has been largely overlooked as an avenue of pathogen spread for soilborne diseases. However, recent research suggests that the migration of *Verticillium* spp. is likely, and thus it is critical to assess the impact of migration on host range expansion and genetic diversity relative to endemic populations. Increased understanding of these relationships is likely to offer novel strategies to manage soilborne diseases, including avoidance.

The lack of population genetics studies in *Verticillium* spp. that address the diversity at the smallest geographic level (i.e., a plant, or a group of plants) makes it difficult to understand the distribution and relationship among strains within one field. Furthermore, there is a pressing need for evaluating the level and impact of gene flow from seed sources into production areas, and determining the evolution of individual *Verticillium* spp. Results from such analyses may lead to the formulation of effective regulatory policies at the national and regional levels.

Nonchemical approaches to the management of Verticillium wilt are limited by site specificity of available methods. An understanding of the modes of action of these methods likely will improve their range and efficacy. The genetic factors or physiological processes controlling resistance to Verticillium wilt resistance are unknown for most crops, as are the subcellular signaling mechanisms associated with the *Ve*-mediated, race-specific resistance. Understanding these areas may provide insight into resistance mechanisms across a broad range of crops, and facilitate the introgression of resistance. An important area of future research also involves examining *Verticillium* spp. for the determinants of race specificity. For example, do determinants of race specificity in *V. dahliae* directly or indirectly interact with Ve1 or Ve2 proteins? With the availability of the genomes of *V. dahliae* and *V. albo-atrum*, advances in these areas research are more plausible in the near future. The availability of these genomic resources, tools for facile genetic analyses, and the tools for the visualization of the fungus mark the beginning of a new era in *Verticillium* spp. research.

SUMMARY POINTS

1. Currently available evidence does not support classifying amphihaploid crucifer strains as *V. longisporum*. Rather, these strains are more similar to *V. dahliae*.
2. The use of GFP-tagged *Verticillium* spp. has provided unprecedented views of the disease cycle of *Verticillium* spp. in lettuce and other crops.
3. Innovative management options to reduce microsclerotial banks in the soil that are also environmentally benign are limited by site specificity. Improving their range and efficacy remain the goals of many research programs. Thus, plant resistance remains the preferred strategy for the control Verticillium wilt.

4. *Verticillium* spp. are cosmopolitan and evidence for gene flow is apparent in multiple crop production regions and involves *V. dahliae*, *V. albo-atrum* and the long-spored crucifer strains. However, migration analyses are lacking, primarily as a result of the choice of molecular and genetic markers previously used. Additionally, the evolutionary biology of these species has yet to be investigated.
5. Analyses of *V. dahliae* interactions with tomato and more recently, lettuce, have provided insight into race-specific resistance.

FUTURE ISSUES

1. What is the nature of the origin of interspecific hybrid strains? Comparative genomic analyses will shed light on the potential origin of these strains.
2. Does recombination among *Verticillium* spp. occur in nature? What is the relative impact of parasexuality? And is there evidence of cryptic sexual recombination in *Verticillium* spp.?
3. Are there biological and/or cultural approaches, yet unidentified, that provide significant management of Verticillium wilt and may potentially reduce the need for chemical fumigants?
4. What are the genetic determinants that govern race structure in *V. dahliae*? What effectors modulate pathogenesis in *Verticillium* spp.? What are the genetic determinants that underly features unique to *Verticillium* spp.? These questions are currently under investigation.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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