***Verticillium* Systematics and Evolution: How Confusion Impedes *Verticillium* Wilt Management and How to Resolve It**

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ABSTRACT

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Verticillium wilts are important vascular wilt diseases that affect many crops and ornamentals in different regions of the world. *Verticillium* wilts are caused by members of the ascomycete genus *Verticillium*, a small group of 10 species that are related to the agents of anthracnose caused by *Colletotrichum* species. *Verticillium* has a long and complicated taxonomic history with controversies about species boundaries and long overlooked cryptic species, which confused and limited our knowledge of the biology of individual species. We first review the taxonomic history of *Verticillium*, provide an update and explanation of the current system of classification and compile host range and geographic distribution data for individual species from internal transcribed spacer (ITS) GenBank records. Using *Verticillium* as an example, we show that species names are a poor vehicle for archiving and retrieving information, and that species identifications should always be backed up by DNA sequence data and DNA extracts that are made publicly available. If such a system were made a prerequisite for publication, all species identifications could be evaluated retroactively, and our knowledge of the biology of individual species would be immune from taxonomic changes, controversy and misidentification. Adoption of this system would improve quarantine practices and the management of diseases caused by various plant pathogens.

Verticillium is a small genus of ascomycete plant pathogens that causes *Verticillium* wilt diseases, a type of vascular wilt. *Verticillium* wilts are among the most devastating fungal diseases worldwide and affect hundreds of different plant species including high value agricultural crops (62). *Verticillium* wilt is most important in temperate regions, occurs less frequently in the subtropics, and is rare in tropical regions of the world (32,34–36,41,62). Economic losses of 50% or higher commonly occur on high value crops, including cotton (22), lettuce (4,80), olive (49), and potato (71). In strawberry without soil fumigation, losses of up to 75% have been recorded (91). Many more important crops and ornamentals (62) are affected, but precise loss estimates are not readily available.

Verticillium species are soilborne and are able to survive in the soil by means of melanized resting structures without a host for at least 14 years (90). Resting structures are microsclerotia, chlamydospores, or resting mycelia, depending on the species (41).

Host infections are initiated upon germination of the resting structures and are followed by penetration into roots, slow progression through the vasculature, and entry into the shoots (21). Distinct external symptoms including wilting and tissue necrosis typically appear only near the end of the disease cycle and often coincide with host maturity (48). *Verticillium* management therefore relies on the elimination of resting structures. Soil fumigation is most effective, but methyl bromide, the most widely used soil fumigant, has been phased out (20), which has compounded the problem caused by *Verticillium* in agriculture. The critical use exemptions that have prolonged the use of methyl bromide in strawberry production in California will cease by 2015 unless renewed.

In addition to being plant pathogens, *Verticillium* species are also known to colonize plants without triggering symptoms (56,64), which is in agreement with an endophytic life style but few details are known. Human or animal infections have not been reported for *Verticillium*.

There are 10 *Verticillium* species (41), of which *V. dahliae* has the greatest economic impact and infects over 200 host species worldwide (62). The other species have narrower host ranges and more restricted global distributions (35,41), but *V. albo-atrum* (63), *V. alfalfae* (37), *V. longisporum* (5,19), *V. nonalfalfae* (18,65), *V. tricorpus* (63), and *V. zaregamsianum* (83) are known to cause significant losses. *Verticillium nubilum* is a minor patho-

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*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains six supplemental data files.

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gen and only causes disease on tomato and potato in pathogenicity tests (46). The agricultural importance of *V. isaacii* and *V. klebahnii* (41) is unknown, but they were isolated from lettuce and artichoke (41,64).

THE REDEFINED GENUS *VERTICILLIUM* SENSU STRICTO

Verticillium is among the older genera of filamentous fungi and was established in 1817 by Nees von Esenbeck (59) for *V. tenerum* from a stem of hollyhock (*Alcea rosea* L.) in Germany (Fig. 1) (3,73,84). The first plant-pathogenic *Verticillium* species, *V. albo-atrum*, was described in 1879 from diseased potato plants in Germany (67). Many more species were added over the years, and *Verticillium* grew into an ecologically diverse genus of around 190 species (97), which in addition to saprobes and plant pathogens, also included pathogens of animals and fungi. Because of this heterogeneity, *Verticillium* was first divided into different sections (24,25), and later DNA sequence data were used to identify distantly related species that were transferred to other genera (26,94–96), a process that is still underway (42). To avoid changing names of major *Verticillium* plant pathogens, the genus *Verticillium* was redefined with *V. dahliae* as the type species, a nomenclatural maneuver known as conservation (27). *Verticillium* species that belong to the same clade as *V. dahliae* are now referred to as *Verticillium sensu stricto* (s.s.), which excludes distantly related, residual *Verticillium* species awaiting transfer to other genera. Of the major *Verticillium* plant pathogens, only *Musicillium theobromae* formerly known as *V. theobromae*, is not part of *Verticillium* s.s. (98).

Verticillium s.s. is a member of the family *Plectosphaerellaceae* (98) in the subclass *Hypocreomycetidae* of the class *Sordario-*

mycetes, which is in the phylum *Ascomycota* (99). In addition to *M. theobromae*, the family *Plectosphaerellaceae* also harbor the generally minor plant pathogens *Gibellulopsis nigrescens* (33,98), *Lectera colletotrichoides* (13), and *Plectosphaerella cucumerina* (66). Related to the family *Plectosphaerellaceae* is the family *Glomerellaceae* (99), which contains *Colletotrichum*, the causal agents of anthracnose diseases (54). In this review, the name *Verticillium* is used in place of *Verticillium* s.s. unless indicated otherwise.

A HISTORY OF TAXONOMIC CONTROVERSIES AND SHIFTING SPECIES BOUNDARIES IN *VERTICILLIUM*

The history of *Verticillium* is rife with taxonomic controversies and shifting species boundaries that have profoundly confused our knowledge of the biology of individual species. *Verticillium dahliae* and *V. albo-atrum*, which historically are the two most important species, have been most affected. Due to taxonomic disagreements, *V. dahliae* was first confounded with *V. albo-atrum* and then with *V. longisporum*, and the two cryptic species *V. alfalfae* and *V. non-alfalfae* were recently described from *V. albo-atrum*. Thus, the meanings of the names *V. albo-atrum* and *V. dahliae* have changed over time and have depended on when and by whom they were used.

The longest-lasting controversy was about whether *V. dahliae* that was described in 1913 (51) should be a separate species from the earlier described *V. albo-atrum* (67). Much of the early literature does not raise any doubts about the status of *V. dahliae*, which was published with careful illustrations, descriptions, and comparisons to *V. albo-atrum* that differed most prominently in the morphology and the development of resting structures (51). These results were independently confirmed and illustrated from

FIGURE 1

Original illustration of *Verticillium tenerum* from 1817 by Nees von Esenbeck in 'Das System der Pilze und Schwämme', Plate 4, Illustration 55. Downloaded from Universitätsbibliothek Heidelberg at <http://digi.ub.uni-heidelberg.de/diglit/neesvonesenbeck1817/0473>.

Australia (55), Europe (88), and North America (7), and physiological differences between the two species were also detected. But Wollenweber (93) and Rudolph (72) disagreed, and their works were very influential (89), despite the fact that Wollenweber (93) only supported his arguments by illustrations of *V. dahliae*, and Rudolph (72) did not include any new illustrations at all. Over the years, there were further dueling publications for and against *V. dahliae* with proponents discovering additional physiological differences between the two species (45,69), and opponents emphasizing that resting structure morphology was not suitable for species separation (87,92). During this time, opinions about the status of *V. dahliae* were approximately evenly divided (69). The tide finally turned in favor of *V. dahliae* in the 1970s (89), as is illustrated by the ratios of *V. dahliae* to *V. albo-atrum* counts on Google Scholar (www.scholar.google.com) over 10-year periods from 1914, the year after publication of *V. dahliae*, to 2013 (Fig. 2; Supplemental data 1). Despite *V. dahliae* being much more widespread and more commonly isolated (86,93), *V. albo-atrum* counts on Google Scholar outnumbered *V. dahliae* counts by generally more than two to one until the beginning of the 1970s (Fig. 2). For the first time, *V. dahliae* counts were higher than the ones for *V. albo-atrum* between 1974 to 1983, and

between 2004 to 2013 *V. dahliae* counts outnumbered *V. albo-atrum* counts by more than four to one. The uptick of *V. dahliae* over *V. albo-atrum* counts in the beginning of the 1970s correlates with comprehensive reviews in favor of *V. dahliae* published in the 1960s (47,77). The two papers were relatively highly cited throughout the 1970s and up to this day (data not shown). The fact that none of the *V. dahliae* internal transcribed spacer (ITS) sequences in GenBank were mislabeled as *V. albo-atrum* (Fig. 3) shows that the controversy has been resolved.

Another taxonomic controversy affecting *V. dahliae* concerned the recognition of *V. longisporum* as a separate species instead of a variety of *V. dahliae* (50,78). The debate was short-lived (6,15), and the name *V. longisporum* is now widely accepted. There were 93 hits on Google Scholar for *V. longisporum* in 2012, as opposed to seven for *V. dahliae* var. *longisporum*, and none of the latter questioned the status of *V. longisporum* (data not shown).

PHYLOGENETIC SPECIES IN *VERTICILLIUM* ARE LINKED TO HERBARIUM TYPE SPECIMENS

The names of fungal species are permanently linked to type specimens through the rules of the International Code of Nomen-

FIGURE 2

Ratios of *Verticillium dahliae* to *Verticillium albo-atrum* counts on Google Scholar in 10-year windows from 1914 to 2013.

FIGURE 3

Cartoon of a phylogenetic tree with 574 *Verticillium* internal transcribed spacer (ITS) sequences from GenBank illustrating that all ITS sequences fall into 1 of 10 *Verticillium* species, with the exception of eight isolates of *V. longisporum* lineage A1/D3 that have *V. dahliae* ITS regions. Species are delimited by vertical bars and the numbers of ITS sequences in each species are given in parentheses following species names. Branch lengths are to scale and tree is unrooted. Supplemental data files provide details of ITS sequence retrieval, alignment, tree generation, DNA sequences, phylogenetic topology, and the alignment.

clature (ICN) for algae, fungi, and plants (57), but similar regulations for the definition of species themselves do not exist. The phylogenetic species concept is probably the most widely used framework for the definition of fungal species today. Recognition of phylogenetic species requires an understanding of evolutionary relationships that are described by a phylogenetic tree, and species status is assigned to individual groups in the tree (31,81). Since the phylogenetic groups are nested, and each group is part of a larger group, different criteria can be used to decide how many nested groups should constitute a species. The recognition criteria include phenotypic (31), genotypic or ecological characters, or genealogical concordance (81). Names are assigned to species-level phylogenetic groups by the type specimens that cluster within them. This is an intellectually satisfying approach since the criteria used to recognize species are known and the species are named in accordance with the nomenclatural rules of the ICN. Another advantage of the phylogenetic species concept is that all members of a species are more closely related to one another. Thus, members of a species are overall more similar to one another than to other species, both in terms of host range, fungicide resistance, reproduction system, and virulence, and other characters of importance to plant pathology. Substitutions at the DNA level are also more likely to be shared among members of the same species, which facilitates the design of species-specific diagnostic polymerase chain reaction (PCR) assays.

A recent revision of *Verticillium* recognized phylogenetic species that were named by integration of herbarium type specimens as described above (41). This became necessary because earlier studies showed that *V. albo-atrum* consisted of not one but two groups that were distantly related (6,58,68), the phylogenetic position of the hybrid *V. longisporum* was uncertain (14,15,50), and DNA sequence data and type specimens themselves were missing for some of the *Verticillium* species. Those concerns were addressed in studies of 257 isolates, which led to the recognition of five new species (41,43). The original description of *V. albo-atrum* (67) revealed that the name *V. albo-atrum* should be restricted to the lineage that is closely related to *V. tricorpus*, whereas the two groups closer to *V. dahliae* were named *V. alfalfae* and *V. nonalfalfae*, respectively. *Verticillium alfalfae* and *V. nonalfalfae* are each other's closest relatives and were delimited by differences in host preference, mating types (41), and vegetative compatibility groups (17). Similarly, *V. tricorpus* and the three cryptic species separated from it are closest relatives. These include *V. zaregamsianum* that is morphologically distinct and forms predominantly microsclerotia and yellow-pigmented hyphae. And *V. isaacii* and *V. klebahnii*, which share the same morphology as *V. tricorpus*, but collectively are about as diverse as the group formed by *V. alfalfae*, *V. dahliae*, and *V. nonalfalfae* at the loci

examined. Also, there is evidence for differences in pathogenicity between *V. isaacii*, *V. klebahnii*, and *V. tricorpus* (41,64,85). As opposed to other important plant pathogens including *Fusarium* (60), species complexes have not been described in *Verticillium*. In comparison to *Fusarium* species complexes, each *Verticillium* species corresponds to a single phylogenetic group with relatively little variation and generally few well-supported subspecific groupings (41,43).

The 10 *Verticillium* species are apparently sufficient to accommodate the currently known diversity, because phylogenetic analyses of the *Verticillium* ITS sequences from GenBank demonstrated that all sequences grouped with 1 of the 10 species (Fig. 3; Supplemental data 2, 3, 4, and 5). The exceptions were *V. dahliae* and the diploid hybrid *V. longisporum* as explained below.

THE DIPLOID HYBRID *V. LONGISPORUM* EVOLVED MULTIPLE TIMES

V. longisporum is a diploid hybrid and is an exception to the rule that a species should correspond to just one phylogenetic group. This is because the parents of *V. longisporum* represent four different lineages from three different species, which hybridized at least three different times, resulting in the three *V. longisporum* lineages that are known today (43) (Fig. 4). The parental species include *V. dahliae*, and two unknown species that have never been found and were provisionally named Species A1 and Species D1. Except for the ribosomal repeat region, *V. longisporum* isolates comprise two alleles for all genes examined (43,82). The naming of hybrids that evolved multiple times has not been settled in mycology (10). In plants, Brochmann et al. (11) used morphological differences as criteria to name different lineages of a hybrid.

The *V. longisporum* hybrid origin presents difficulties for the identification by PCR assay or DNA sequence data. The *V. longisporum* ITS region was inherited from Species A1 for two of the *V. longisporum* lineages, lineages A1/D1 and A1/D2, or from *V. dahliae* for the *V. longisporum* lineage A1/D3 (Fig. 4). Thus, PCR tests and DNA sequence comparisons based on the ITS region will falsely identify *V. longisporum* lineage A1/D3 as *V. dahliae*. Multiplex PCR assays are now available for the differentiation of *V. dahliae* from all lineages of *V. longisporum* (44).

PHYLOGENETIC RELATIONSHIPS IN *VERTICILLIUM* CORRELATE WITH YELLOW HYPHAL PIGMENTATION

Phylogenetic studies showed that *Verticillium* consisted of two major lineages (6,98) that correlate with yellow hyphal pigmentation (41). All species of the clade Flavexudans produce yellow

FIGURE 4

Evolutionary history of *Verticillium longisporum*.

V. longisporum evolved at least three different times by hybridization of species A1 with species D1, *V. dahliae* lineage D2 and *V. dahliae* lineage D3, resulting in *V. longisporum* lineages A1/D1, A1/D2, and A1/D3, respectively. *V. dahliae* isolates are in blue, species D1 in green, species A1 in orange, and *V. alfalfae* in gray. Red arrows indicate parents of *V. longisporum*, and connecting lines represent the three *V. longisporum* lineages. The *V. dahliae* lineage D2 is marked as *V. dahliae* D2 and comprises both *V. dahliae* isolates and D2 alleles of *V. longisporum* lineage A1/D2. The *V. dahliae* lineage D3 (*V. dahliae* D3) comprises only D3 alleles of *V. longisporum* lineage A1/D3. Most of the *V. dahliae* isolates in Inderbitzin et al. (43) belong to the clade marked *V. dahliae* main group. Tree is not to scale. Figure and legend from Inderbitzin et al. (44).

hyphal pigments, including *V. albo-atrum*, *V. isaacii*, *V. klebahnii*, *V. tricornis*, and *V. zaregamsianum*, whereas lineage Flavononexudans lacks yellow pigmentation and consists of species *V. alfalfae*, *V. dahliae*, *V. longisporum*, *V. nonalfalfae*, and *V. nubilum*. Plant pathogens are present in both lineages. The relationships of *Verticillium* species are generally well resolved, but further studies are needed to confirm the grouping of *V. nubilum* with the clade Flavononexudans, and Species A1, an unnamed *V. longisporum* ancestor, with *V. dahliae* and Species D1, another *V. longisporum* ancestor that has also not been recovered thus far (41,43).

HOST RANGES AND GEOGRAPHIC DISTRIBUTIONS OF VERTICILLIUM SPECIES

Verticillium species are able to infect a large number of plant species. *Verticillium dahliae* alone has been recorded from several hundred hosts (62). How many of these reports are true is difficult to verify because of taxonomic confusion of *V. dahliae* with *V. albo-atrum* and *V. longisporum* at different points in history. A compilation of host information from 574 *Verticillium* ITS GenBank records that were assigned to species using phylogenetic analyses (Fig. 3), and the host information in Inderbitzin et al. (41,43), confirmed that *V. dahliae* had the most hosts among *Verticillium* species with 74, which were from 14 orders of seed plants including *Asterales*, *Brassicales*, *Caryophyllales*, *Cucurbitales*, *Fabales*, *Lamiales*, *Magnoliales*, *Malpighiales*, *Malvales*, *Ranunculales*, *Rosales*, *Sapindales*, *Solanales*, and *Vitales* (Table 1). Most of the orders are part of the eudicots where they represent many of the major lineages, whereas *Magnoliales* belongs to the magnoliids (79). Among seed plants, the most prominent groups that according to this survey are not hosts of *V. dahliae* are gymnosperms and monocots. However, there are reports of *V. dahliae* from gymnosperm and monocot hosts, and even from horsetail (*Equisetum arvense*) (62). Thus, *V. dahliae* may be infecting most lineages of seed plants and potentially other plants including pteridophytes, which likely constitutes one of the broadest host ranges of any fungal plant pathogen.

Host counts were lower for the other *Verticillium* species, ranging from 1 to 10 hosts. *Verticillium longisporum* hosts are mainly in the family *Brassicaceae*, with sugar beet being the only exception. The single host of *V. alfalfae* was alfalfa, the one for *V. nubilum* was potato, and both hosts of *V. klebahnii* were in the family *Asteraceae*. The remaining species had a broader host range (Table 1).

Most records of *Verticillium* species are from agricultural crops or plants associated with agriculture or from ornamentals and little is known about host ranges in natural ecosystems. This is particularly striking for *V. dahliae* where none of the hosts in Table 1 could be confirmed to be collected from a natural habitat. Two strains of *V. albo-atrum* were isolated from *Petasites hybridus* in a nature reserve in the Netherlands, and from *Urtica dioica* in a mountainous region of Scotland (W. Gams, *personal communication*). One *V. nonalfalfae* strain was from *Ailanthus altissima* in the Tuscarora State Forest in south-central Pennsylvania (74).

One problem with using the ITS as a species marker in *Verticillium* is that *V. longisporum* lineage A1/D3 has a *V. dahliae* ITS region, and the two species can thus be confused. This is illustrated by the fact that in Figure 3 eight ITS sequences labeled *V. longisporum* clustered with *V. dahliae*. The potential for confusion between *V. dahliae* and *V. longisporum* may be greatest in the *Brassicaceae* that are the main hosts for *V. longisporum*. To evaluate whether some of the *V. dahliae* reports on *Brassicaceae* hosts may instead be *V. longisporum*, we consulted additional data from the literature including multigene phylogenies, conidial DNA content, and VCGs, which showed that all eight *V. dahliae* *Brassicaceae* hosts for which such data was available, could be

confirmed as *V. dahliae* hosts (Table 1). Outside of the *Brassicaceae*, it appears that due to the narrow host range of *V. longisporum*, confusion with *V. dahliae* based on ITS may be unlikely. However, when working with *V. dahliae*, other data in addition to the ITS region should be recorded in support of strain identification.

Mirroring the host range data, *V. dahliae* was most widely distributed among *Verticillium* species and occurred on all inhabited continents. *Verticillium klebahnii* and *V. nubilum* were the only two species that were confined to a single country, the United States and the United Kingdom, respectively (Table 1).

IDENTIFICATION OF VERTICILLIUM SPECIES

Our knowledge of the biology of *Verticillium* species hinges on correct, consistent, and verifiable identification of the strains collected from the environment and used in experiments. Whereas the future of identification may lay in DNA barcoding (53), *Verticillium* species are currently most often diagnosed by morphological examination using light microscopy or by sequencing of the ITS region followed by a blast search (1) at GenBank. Morphological identification is appropriate under certain situations, but the characters used for identification, including resting structures and yellow hyphal pigmentation, are unstable and may disappear in laboratory cultures, and some *Verticillium* species lack distinguishing morphological characteristics (41). The ITS region is the official barcoding region for fungi (75), and is also most widely used for DNA-based identification in *Verticillium*. Except for the hybrid *V. longisporum*, all *Verticillium* type specimens have unique ITS sequences (41). However, the ITS is similar for some groups of *Verticillium* species and may vary within species, which complicates species identification. This is illustrated by a nucleotide blast query (blastn) at GenBank with a *V. nonalfalfae* ITS sequence (GenBank Accession AB458830) that resulted in four equally close matches, three with *V. nonalfalfae* sequences (HE972032, HE972033, and FJ424082) and one with a *V. longisporum* sequence (GQ495792). A secondary barcode that is more variable than the ITS region (75) may be useful in *Verticillium*. A candidate is the *elongation factor 1-alpha* (*EF*), the most variable locus for *Verticillium* in Inderbitzin et al. (41).

Phylogenetic analyses are another way to compare query sequences with a reference data set for species identification, and appear to be better suited than blast for the *Verticillium* ITS region. In the ITS tree in Figure 3, all sequences, except the ones of the hybrid *V. longisporum*, fall into distinct groups, and each group contains a different type specimen. This suggests that all sequences in the tree represent one of the established *Verticillium* species. However, phylogenetic analyses based on multiple loci (81) are much more accurate for species identification, they provide more power for the detection of new species than single locus phylogenetic analyses, and are also able to detect hybrids. A reference data set for phylogenetic analyses is available at TreeBase (41) under Study 11756, strains representing type specimens are marked in bold in Figure 1 of Inderbitzin et al. (41). Other methods of DNA sequence comparison for species identification are available (16) and may become more popular in the future.

An issue that hampers DNA-based species identification is the high proportion of misidentified sequences in GenBank. For *Verticillium* species, we found that between 5 to 81% of GenBank ITS sequences represented incorrectly identified species (Fig. 5). Most commonly mislabeled were *V. tricornis* ITS sequences, whereas only 5% of the *V. dahliae* ITS sequences were misidentified. Since the *V. longisporum* lineage A1/D3 ITS region is indistinguishable from the one of *V. dahliae*, the proportion of mislabeled *V. dahliae* ITS sequences may be higher. Misidentified

TABLE 1
Substrates and geographic distribution of *Verticillium* species based on 821 GenBank ITS records^a

Species	Substrates	Countries	Plant hosts/ countries
<i>V. albo-atrum</i> Reinke & Berthold	Pestilence wort (<i>Petasites hybridus</i>), Potato (<i>Solanum tuberosum</i>), Soil, Stinging nettle (<i>Urtica dioica</i>)	Australia, Netherlands, Canada, UK, USA (WI)	3/5
<i>V. alfalfae</i> Inderb. et al.	Alfalfa (<i>Medicago sativa</i>)	Canada, Iran, Japan, UK, USA (CA, PA)	1/5
<i>V. dahliae</i> ^b Kleb.	African eggplant (<i>Solanum aethiopicum</i>), Alectryon (<i>Alectryon</i> sp.), Almond (<i>Prunus dulcis</i>), American black nightshade (<i>Solanum nigrum</i>), Anaheim pepper (<i>Capsicum annuum</i>), Apricot (<i>Prunus armeniaca</i>), Artichoke (<i>Cynara scolymus</i>), Aztec marigold (<i>Tagetes erecta</i>), Bell pepper (<i>Capsicum annuum</i>), Black raspberry (<i>Rubus</i> sp.), Black-eyed Susan (<i>Rudbeckia hirta</i>), <i>Brassica oleracea</i> var. <i>capitata</i> ^c , <i>Brassica oleracea</i> var. <i>italica</i> ^c , Broccoli (<i>Brassica oleracea</i> var. <i>botrytis</i>) ^c , Cabbage (<i>Brassica oleracea</i>) ^c , Celandine (<i>Chelidonium majus</i>), Chamomile (<i>Matricaria chamomilla</i>), Chicory (<i>Cichorium intybus</i>), Chili pepper (<i>Capsicum annuum</i>), Chinese cabbage (<i>Brassica oleracea</i>), Coleus (<i>Coleus verschaaffeltii</i>), Coneflower (<i>Echinacea purpurea</i>), Cosmos (<i>Cosmos bipinnatus</i>), Cota palaestina, Cotton (<i>Gossypium hirsutum</i>), Cyprus-vetch (<i>Lathyrus ochrus</i>), Eggplant (<i>Solanum melongena</i>), European smoketree (<i>Cotinus coggygria</i>), European yellow lupine (<i>Lupinus luteus</i>), Figmarigold (<i>Lampranthus</i> spp.), Flax (<i>Linum usitatissimum</i>), French marigold (<i>Tagetes patula</i>), Grapevine (<i>Vitis vinifera</i>), Green ash (<i>Fraxinus pennsylvanica</i>), Horseradish (<i>Armoracia rusticana</i>) ^c , Icelandic poppy (<i>Papaver nudicaule</i>), Italian cocklebur (<i>Xanthium italicum</i>), Jalapeno (<i>Capsicum annuum</i>), Japanese maple (<i>Acer palmatum</i>), <i>Lactuca sativa</i> var. <i>longifolia</i> , Lambsquarters (<i>Chenopodium album</i>), Lenspod whitetop (<i>Cardaria draba</i>) ^c , Lettuce (<i>Lactuca sativa</i>), Lupine (<i>Lupinus polyphyllus</i>), Nestegis sp., New Zealand spinach (<i>Tetragonia tetragonioides</i>), Norway maple (<i>Acer platanoides</i>), Oilseed rape (<i>Brassica napus</i> sp. <i>oleifera</i>) ^c , Okra (<i>Abelmoschus esculentus</i>), Old-man-in-the-Spring (<i>Senecio vulgaris</i>), Olive (<i>Olea europaea</i>), Opium poppy (<i>Papaver somniferum</i>), Orange coneflower (<i>Rudbeckia fulgida</i>), Paprika (<i>Capsicum annuum</i>), Peach (<i>Prunus persica</i>), Peanut (<i>Arachis hypogaea</i>), Peppermint (<i>Mentha x piperita</i>), Pistachio (<i>Pistacia vera</i>), Potato (<i>Solanum tuberosum</i>), Pumpkin (<i>Cucurbita pepo</i>), Purple coneflower (<i>Echinacea purpurea</i>), Radish (<i>Raphanus raphanistrum</i>), Rose (<i>Rosa</i> sp.), Scentless false mayweed (<i>Tripleurospermum perforatum</i>), Skullcap (<i>Scutellaria lateriflora</i>), Soil, Spinach (<i>Spinacia oleracea</i>), Stock (<i>Matthiola</i> Ait. f.) ^c , Strawberry (<i>Fragaria x ananassa</i>), Sunflower (<i>Helianthus annuus</i>), Tomato (<i>Solanum lycopersicum</i>), Tulip tree (<i>Liriodendron tulipifera</i>), Udo (<i>Aralia cordata</i>), Watermelon (<i>Citrullus lanatus</i>)	Australia, Austria, Belgium, Brazil, Canada, China, Denmark, France, Germany, Greece, Iran, Israel, Italy, Japan, Moldova, Netherlands, New Zealand, Russia, Spain, Tunesia, UK, Ukraine, USA (CA, IL, NY, OR, TX, WA, WI), Uzbekistan, Yugoslavia	74/25
<i>V. isaacii</i> Inderb. et al.	Artichoke (<i>Cynara scolymus</i>), Bear's breech (<i>Acanthus mollis</i> subsp. <i>mollis</i>), <i>Brassica</i> sp., Florist's daisy (<i>Chrysanthemum x morifolium</i>), Hairy nightshade (<i>Solanum physalifolium</i>), Lettuce (<i>Lactuca sativa</i>), Potato (<i>Solanum tuberosum</i>), Soil, Spinach (<i>Spinacia oleracea</i>), Tomato (<i>Solanum lycopersicum</i>)	Australia, Belgium, Canada, Israel, Italy, UK, USA (CA, WA)	9/7
<i>V. klebahnii</i> Inderb. et al.	Artichoke (<i>Cynara scolymus</i>), Lettuce (<i>Lactuca sativa</i>)	USA (CA, WA)	2/1
<i>V. longisporum</i> ^d Karapapa, Bainbr. & Heale	Birdrape (<i>Brassica rapa</i> var. <i>rapa</i>), Broccoli (<i>Brassica oleracea</i> var. <i>botrytis</i>), Cabbage (<i>Brassica oleracea</i>), Cauliflower (<i>Brassica oleracea</i> var. <i>botrytis</i>), Field mustard (<i>Brassica rapa</i> var. <i>rapa</i>), Horseradish (<i>Armoracia rusticana</i>), Oilseed rape (<i>Brassica napus</i> sp. <i>oleifera</i>), Sugar beet (<i>Beta vulgaris</i>), Turnip (<i>Brassica campestris</i> sp. <i>pekinensis</i>), Wild radish (<i>Raphanus raphanistrum</i>)	Belgium, France, Germany, Italy, Japan, Netherlands, Russia, Sweden, UK, USA (CA, IL)	10/10
<i>V. nonalfalfae</i> Inderb. et al.	Alfalfa (<i>Medicago sativa</i>), Cotton (<i>Gossypium hirsutum</i>), Hop (<i>Humulus lupulus</i>), Petunia (<i>Petunia</i> sp.), Potato (<i>Solanum tuberosum</i>), Soil, Spinach (<i>Spinacia oleracea</i>), Tomato (<i>Solanum lycopersicum</i>), Tree of heaven (<i>Ailanthus altissima</i>), Wild celery (<i>Apium graveolens</i>)	Belgium, Canada, China, Cuba, Japan, Middle Asia, Netherlands, Slovenia, UK, USA (IL, PA)	9/10
<i>V. nubilum</i> Pethybr.	Potato (<i>Solanum tuberosum</i>), Soil	UK	1/1
<i>V. tricorpus</i> I. Isaac	Carnation (<i>Dianthus caryophyllus</i>), Larkspur (<i>Delphinium</i> sp.), Lettuce (<i>Lactuca sativa</i>), Potato (<i>Solanum tuberosum</i>), Soil, Tomato (<i>Solanum lycopersicum</i>)	Japan, Netherlands, New Zealand, UK, USA (CA)	5/5
<i>V. zaregamsianum</i> Inderb. et al.	Tomato (<i>Solanum lycopersicum</i>), Potato (<i>Solanum tuberosum</i>), Lettuce (<i>Lactuca sativa</i>), Tenweeks stock (<i>Matthiola incana</i>)	Israel, Japan, Middle Asia	4/3

^a ITS records were grouped into phylogenetic species in Figure 3 and by Inderbitzin et al. (41,43) for the records not included in Figure 3. Inderbitzin et al. (41,43) provides hosts within each country.

^b Host and geographic information of the eight GenBank records labeled *V. longisporum* associated with the *V. dahliae* clade in Figure 3 were not considered.

^c Eight of ten *Brassicaceae* hosts (except Chinese cabbage and radish) of the *V. dahliae* clade not considering *V. longisporum* lineage A1/D3, were confirmed to be *V. dahliae* hosts with additional data as follows. Multigene DNA sequencing (42): horseradish, e.g., for *V. dahliae* strain PD727; cabbage, e.g., for *V. dahliae* strain PD584; oilseed rape, e.g., for *V. dahliae* strain PD717; stock, for *V. dahliae* strain PD680. *V. dahliae*-like conidial DNA content (14): broccoli, for *V. dahliae* strain Vd128. Formation of *nit* mutants (61) which are not inducible in *V. longisporum* (8): *Brassica oleracea* var. *capitata*, for *V. dahliae* strain VP63 (=414-1) which is VCG4B; *B. oleracea* var. *italica*, for *V. dahliae* strain VP19 (=449-2) which is VCG2B; Lenspod whitetop, *V. dahliae* for strain VP66 (=467-2) which is VCG4B.

^d Host and geographic information from records of *V. longisporum* lineage A1/D3 clustering in the *V. dahliae* clade were also included.

GenBank sequences may become less of a problem in the future, as the number of official barcode sequences (38) is expected to increase.

To facilitate identification of *Verticillium* species, a website, VertShield (S. Kang, B. Park, P. Inderbitzin, and K. V. Subbarao, *unpublished data*) is being developed. The website comprises a curated set of DNA sequences of strains representing type specimens and other correctly identified sequences and can be queried using blast. However, positive identification is dependent on a 100% match between query and database sequence. If a query sequence does not have a match in the database, phylogenetic analyses using multiple loci are still required to conclusively determine whether the query sequence is derived from a new or an existing species.

We suggest that identification of *Verticillium* species and *V. longisporum* lineages involve at least the following steps, which are summarized in Figure 6: morphological identification with the help of a dichotomous key (41), molecular identification using a diagnostic PCR assay (44,82), and a blast search against a reference set of ITS sequences, for instance at the VertShield website (S. Kang, B. Park, P. Inderbitzin, and K. V. Subbarao, *unpublished data*). Results should be reported and the rationale for selection of a name briefly discussed. Also, ITS sequences have to be submitted to GenBank, which ensures that phylogenetic analyses can be performed later if needed, and DNA extracts should be deposited in a public repository to permit additional DNA sequencing in the future.

ORIGINS OF INFORMATION CONFUSION IN *VERTICILLIUM* AND CONSEQUENCES FOR *VERTICILLIUM* WILT MANAGEMENT

Species names are carriers of information and are essential to the communication, storage, and retrieval of information (39). This system is critically important to biology including plant pathology (31,70), but is dependent on a stable taxonomic framework to prevent the confusion of information between species.

Verticillium has had a long history of taxonomic instability and information confusion. This included the controversy about the status of *V. dahliae* as an independent species from *V. albo-atrum*, which has affected disease management. For instance in Australia, *V. albo-atrum* was once considered a widespread and important pathogen on many different hosts, which turned out to be incorrect (89). Many of the herbarium specimens and cultures associated with literature records were found to belong to *V. dahliae* instead, and *V. albo-atrum* could only be confirmed from potato in three southern states. Most importantly, none of the records of *V. albo-atrum* from alfalfa (lucerne) or hops could be confirmed, which is significant because *V. albo-atrum* is more virulent on these hosts than *V. dahliae*. As a consequence, the status of *V. albo-atrum* was updated to a pathogen of major quarantine significance for Australia, and new control measures to exclude it were proposed (89). The measures appear to have been successful, as there are currently no alfalfa or hops strains from Australia in GenBank (Table 1). Another source of information confusion

FIGURE 5

Pie charts illustrating the proportions of correctly identified internal transcribed spacer (ITS) sequences in GenBank for select *Verticillium* species. Percentages of correctly identified sequences are given following each species name, preceding the numbers of correctly identified sequences and total number of sequences, which are in parentheses. Results were obtained by comparing species names on GenBank records with names inferred from phylogenetic analyses shown in Figure 3, and records in Inderbitzin et al. (41,43) were not considered.

in *Verticillium* are cryptic species that have only recently been formally named. These include *V. alfalfae* and *V. nonalfalfae* that were described for a lineage formerly in *V. albo-atrum* (41). This means that any record of *V. albo-atrum* may instead refer to *V. alfalfae* or *V. nonalfalfae*. *Verticillium albo-atrum* is morphologically very similar to *V. alfalfae* and *V. nonalfalfae*, and *V. alfalfae* and *V. nonalfalfae* are morphologically indistinguishable, so that reexamination of herbarium specimens cannot be used for reassessment of previous species identifications. Only DNA sequence data are reliable, but are unavailable for most of the *V. albo-atrum* literature. Thus, despite an over 130-year history as a plant pathogen, very little is known about the basic biology of *V. albo-atrum*, including host range and distribution, which certainly reduces the effectiveness of *V. albo-atrum* disease management. The problems are similar for *V. tricorpus* and the related cryptic species *V. isaacii* and *V. klebahnii* (41) but the three species are only minor pathogens. The practical importance of intraspecific diversity for disease management is illustrated by the bacterial citrus canker disease, where a newly discovered but less virulent pathovar triggered an unnecessary eradication effort to the cost of \$93 million (23,76).

STRAIN NAMES CANNOT BE CHECKED AND VERIFIED WITHOUT ADDITIONAL DATA

Information confusion between species is a serious problem in *Verticillium* and has diminished the usefulness and relevance of much biological data accumulated over the course of almost a century. Theoretically, information confusion can easily be pre-

vented by reevaluation of species names and the names of other taxa in the light of later knowledge. Examination of specimens kept in herbaria can serve this purpose if diagnostic morphological characters are available, as for separation of *V. albo-atrum* from *V. dahliae*, but cryptic species including *V. alfalfae* and *V. nonalfalfae* can only be distinguished using DNA. Compared with specimens and cultures, DNA has other advantages. DNA sequence data is accessible for anyone at repositories including GenBank and can be incorporated into phylogenetic analyses. Extracted DNA is stable and can be stored at room temperature at minimal cost and space requirements (2,9), is available for genome sequencing, and may one day be used to create living organisms, as was done in bacteria (29). Also, a new classification system based on DNA (40) will likely complement and then replace the current one in the future, and information linked to DNA in GenBank could then be automatically associated with taxonomic groupings. DNA samples are currently accepted for storage at the Centraalbureau voor Schimmelcultures (CBS), and by the members of the DNA Bank Network (28) that include the New York Botanical Garden (NYBG) (12).

Despite all the advantages in maximizing the value of biological information, DNA data for taxa name assessment are not available for all recent articles. For instance, of the 90 research publications in 2012 to 2013 that present new data on *V. albo-atrum*, only 56 of 90 (62%) articles link the strains used to DNA sequence data suitable for assessment of species identification, and an even lower 10 of 90 (11%) articles, if only articles are considered that do not include the *V. alfalfae* strain Ms.102 genome sequence (52) (Fig. 7). DNA data also illustrate that

FIGURE 6

Steps involved in the identification of *Verticillium* species. Implementing all steps will result in correct identification in most cases, and will establish a link between published research, DNA sequence data in GenBank, and a DNA sample, which ensures that the identification can be reevaluated in the future. GenBank submissions should at least note the date and location of collection, the substrate, and a reference to literature where the strain was published. For additional details, see text.

FIGURE 7

Pie chart illustrating that 62% of the 90 research articles on *V. albo-atrum* published in 2012 to 2013 include DNA data that can be used for retroactive species identification, either based on genome data (51%) by Klosterman et al. (52), or other suitable DNA data (11%). Only articles presenting new results with strains named *V. albo-atrum* were considered.

***V. albo-atrum* publications 2012-2013**

misidentification is an issue in *V. albo-atrum*, as of the 10 articles from the previous sentence where naming was verifiable, only two (20%) used the name *V. albo-atrum* correctly (Supplemental data 6). Thus, *V. albo-atrum* is a good illustration for the need to release DNA sequences and DNA extracts for strains used in research, which increases the impact and relevance of the associated data in the long term and maximizes the return on time and funding invested in research.

ADVANTAGES OF VERIFIABLE TAXON NAMES FOR DISEASE MANAGEMENT AND QUARANTINE REGULATIONS

Taxon names that can be verified and assessed retroactively increase the quality of biological information about species and other taxonomic units and thereby have a direct impact on practical aspects of plant pathology. If biological information is properly categorized according to the taxa from which it was derived, including *V. albo-atrum* and *V. dahliae* in Australia as described above (89), or the *V. dahliae* olive defoliating and nondefoliating pathotypes in Spain (30), quarantine regulations are more effective in excluding pathogens and are less likely to drive up economic costs by unnecessarily rejecting or delaying goods during inspection. Also, an inaccurate knowledge base about differences and similarities between species and other taxa may delay the realization of improvements to the currently used disease management strategies.

RECOMMENDATION

Because species names are an imperfect means to archive and retrieve information in mycology and plant pathology, we propose that releasing DNA sequence data and DNA extracts into the public domain for all strains used in research should be a prerequisite for publication of all manuscripts. This system provides the means for an independent assessment of taxon names in case of information confusion, resulting in improved disease management strategies, and also has the added benefit of preserving genetic resources. We believe that a major effort is warranted to establish a national DNA repository system dedicated to plant pathology, which would be much cheaper and logistically less challenging than a culture collection but would provide many of the same benefits.

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