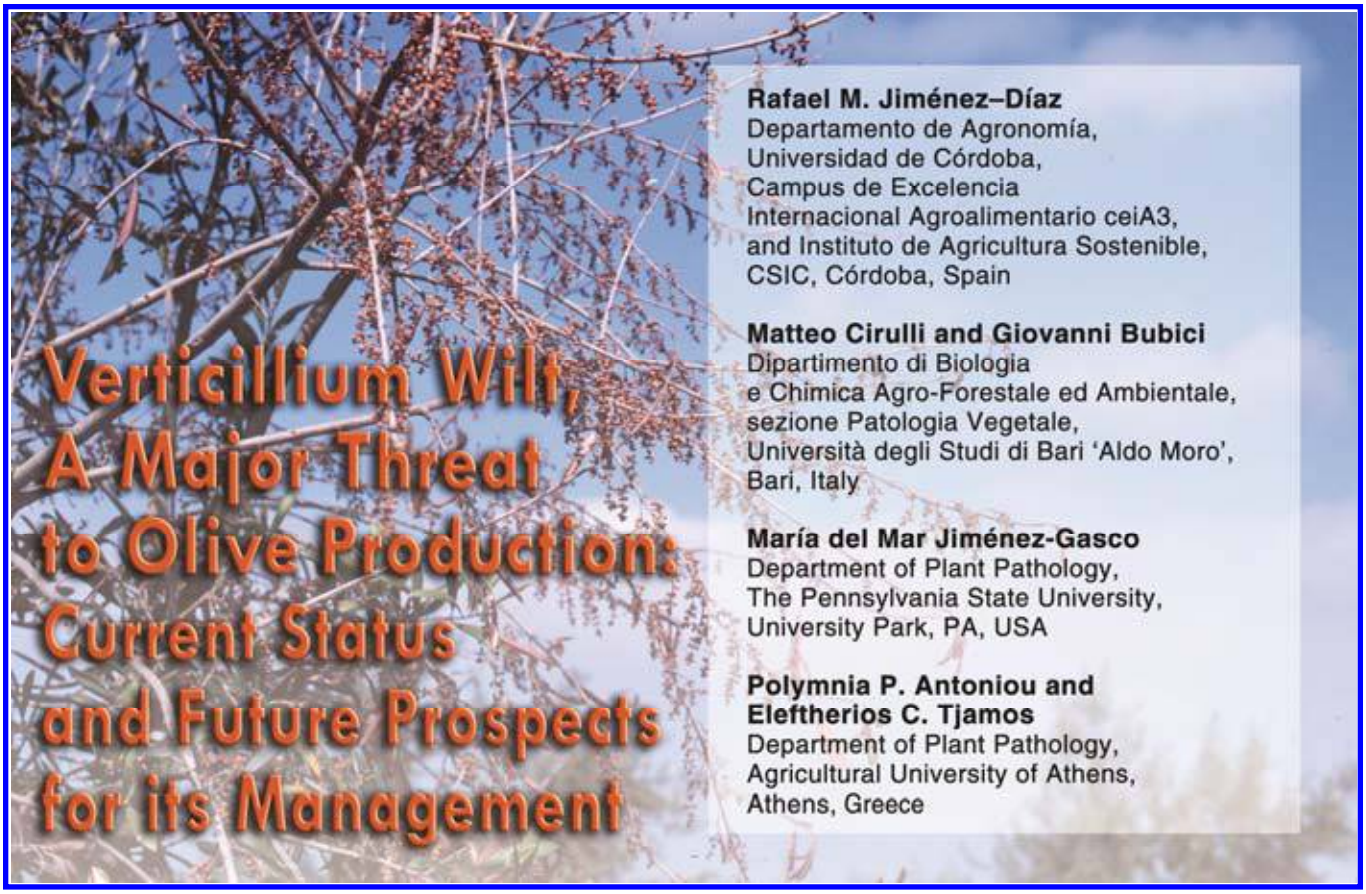

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Olive: An Ancient Crop Under a Major Health Threat

Olive (*Olea europaea* L. subsp. *europaea* var. *europaea*) (diploid, 2n = 46) is the only species producing edible fruits within the botanical family Oleaceae and is one of the most ancient cultivated plants (90,264). The genus *Olea* comprises some 35 species, including the wild form *O. europaea* subsp. *europaea* var. *sylvestris*. Olive was probably domesticated from the wild form somewhere in the Persian–Syrian region and was subsequently introduced throughout the Mediterranean Basin by ancient Mediterranean civilizations (51). Olive is a wind-pollinated, partially self-incompatible, woody, perennial tree producing ovoid-shaped, 1.5- to 3-cm-long drupe fruits that are used mainly for oil extraction but also for direct consumption after processing (183).

Approximately 10^7 ha of olive are cultivated in more than 20 countries worldwide that yield near 18.3×10^6 t (75). Of that, ≈95% is grown in the Mediterranean Basin. Spain is the leading olive-producing country with 25% of the world acreage and nearly 34% of the production. Italy ranks second with 12% of world acreage and approximately 20% of production, followed by Greece (8% acreage, 13% of production), Tunisia (12% acreage, 7.5% of production), Turkey (7.3% acreage, 7.1% of production), Syria (6.4% acreage, 4.8% of production), and Morocco (5.5% acreage, 4.2% of production) (75). In Spain, by far the largest olive producer, ≈65% of the 2.5×10^6 ha cultivated to olive are in Andalusia, at the southernmost part of the Iberian Peninsula, which contributes 85% of the total national production (42).

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Olive is cultivated under a wide range of soil and climatic conditions between latitudes 30° and 45° in both the northern and southern hemispheres. It shows tolerance to salinity and can endure drought, although olive yield is significantly increased by irrigation (133,134,252). Olive can be vegetatively propagated because of the numerous latent buds that occur on the wood (40). This has led to on-farm rooting of woody stems by farmers, a traditionally used practice for olive propagation. Modernization of olive cultivation during the last two decades has led to significant changes in cropping practices for increasing olive yield. These changes include the use of nursery-produced planting stocks of commercial cultivars, either own-rooted, micropropagated from axillary buds, or grafted onto rootstocks developed from olive seeds, for establishing high-tree-density, drip-irrigated orchards with reduced tillage and high inputs of fertilizers (190,252). The use of irrigation has led to traditionally managed dry-land groves with densities up to 100 trees/ha being replaced by intensively managed high-density orchards (400 to 600 trees/ha) (Fig. 1A), or even by the so-called hedgerow, super-intensive high-density orchards (up to 2,000 trees/ha) (Fig. 1B) (170). The expansion of olive cultivation and changes in cropping practices for increasing olive yields have occurred concomitantly with an increase in both incidence and severity of attacks of Verticillium wilt, making this disease a major threat to olive production in many olive-growing areas in Spain and elsewhere (Fig. 1C) (111,113).

In this article, we do not intend to review the literature on general aspects of *Verticillium* and Verticillium wilt diseases. For that, the reader is referred to excellent, recently published reviews (17,81,124). Rather, we discuss current prospects for the management of Verticillium wilt in olive based on critical assessment of available knowledge on the disease etiology, epidemiology, and disease control strategies and measures.

Verticillium Wilt on Olive: Importance and Distribution

Verticillium wilt of olive is caused by the soilborne fungus *Verticillium dahliae* Kleb. It is currently considered the main soilborne

disease threatening olive production worldwide. This disease was first described in Italy in 1946 (197), and shortly afterward it was reported in California (216) and Greece (260). Subsequently, new descriptions of disease occurrence have been reported from 1970 through 2005 in Turkey, France, Spain, Syria, Morocco, Jordan, Algeria, Israel, Iran, Malta, and Australia (26,113,133,169, 186,201,204,210). Because of the long history of olive cultivation in the Mediterranean Basin, it might be expected that Verticillium wilt–like disorders were described much earlier than the first reports of it in the twentieth century. However, no references to

Fig. 1. Modern olive cultivation in southern Spain: **A,** intensively managed, high-tree-density (600 trees/ha) orchard of 2.5-year-old 'Arbequina' olives; **B,** hedgerow orchard with 1,500 trees/ha; and **C,** Verticillium wilt attack in an orchard of 'Picual' olives (arrows indicate diseased trees).

symptoms similar to Verticillium wilt were found by authors who explored early records of disease management in the oldest agricultural systems (230). The earliest records of olive root diseases concerned the obvious signs of fungal root rots described by Hartig and Petri in Italy (i.e., *Armillaria mellea* Vahl, *Fomes fulvus* (Scop) Fr., *Stereum hirsutum* (Vill.) Fr.) (78,97). Perhaps an early observation of wilt in olive could be that of Ibn Al-Awam (6), a Spanish–Arab writer of the twelfth century, who mentioned olive trees in the Seville province of southern Spain with some twigs showing yellowing and partial defoliation from which the trees did not recover despite repeated watering.

Verticillium wilt is becoming an increasing concern in olive production because of the potential of the disease for rapid spread and increased severity associated with recent changes in cropping practices, as exemplified in the Spanish olive industry (111). In Spain, Verticillium wilt of olive was first observed in 1979 in experimental fields near Córdoba, Andalusia, southern Spain (41). However, the disease was already established in this region, as indicated by surveys carried out in old olive groves at Córdoba, Jaén, and Seville, the three main olive-growing provinces of Andalusia (30). Currently, Verticillium wilt is causing devastation in young and old olive orchards in that region and has spread to all other major olive-growing areas of Spain (111; R. M. Jiménez-Díaz, *unpublished*).

Estimates of the importance of Verticillium wilt attacks and associated yield losses vary according to countries. Thanassoulopoulos et al. (227) first reported 2 to 3% mean disease incidence, with 1% tree mortality, in a survey of 1.4×10^7 olive trees in Greece (roughly 1.5×10^5 ha). However, recent extensive olive orchard surveys in Greece by P. P. Antoniou and S. E. Tjamos (*unpublished*) demonstrated a dramatic impact of the disease fluctuating from 10 to 50% of the trees in young olive groves established in fields previously cultivated to cotton. In Spain, a disease prevalence of near 39% affected orchards was reported in old groves and newly established orchards at Córdoba, Jaén, and Seville provinces of Andalusia (30,200). Recently, inspections of 90 arbitrarily chosen orchards in these provinces indicated 71% disease prevalence with a mean incidence of 20% in the affected orchards (145). Verticillium wilt surveys in other olive-growing countries indicated 0.85 to 4.5% disease incidence over 6.5×10^6 olive trees inspected in Syria (3), 60% disease prevalence with 10 to 30% affected trees in Morocco (211), and 90% prevalence with 12% mean disease incidence in Algeria (26). In Italy, inspections of 1,390 young and old orchards in Bari, Brindisi, Foglia, Lecce, and Taranto provinces of the Apulia region revealed a range of 6.2 to 35.8% disease prevalence with a mean of 18.3% (173). A similar massive survey of 919 orchards in 14 provinces in Turkey indicated 35% disease prevalence with a mean incidence of 3.1% (61).

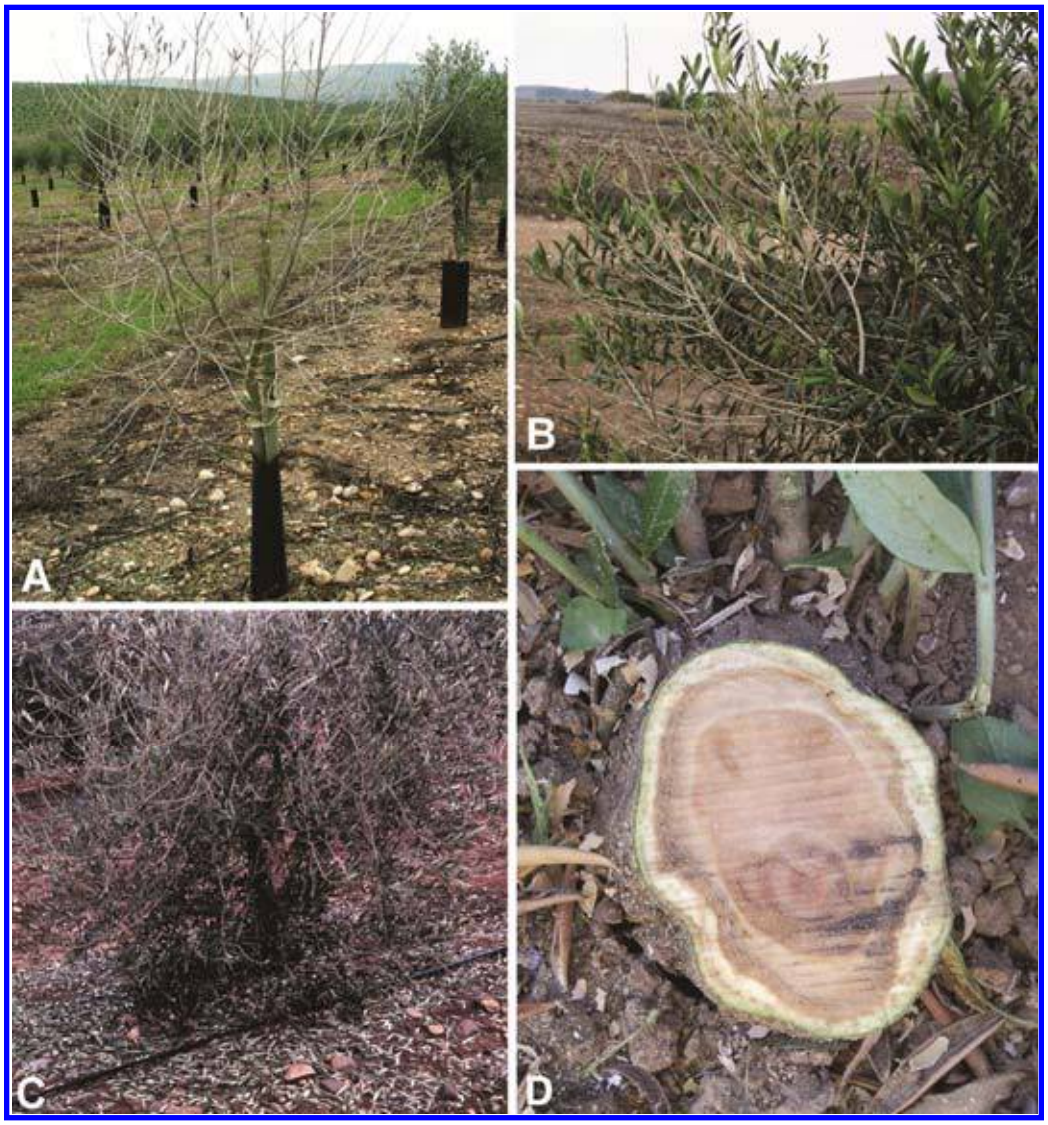

Fig. 2. Field symptoms of the defoliating Verticillium wilt syndrome in 'Arbequina' olives: **A,** a 2.5-year-old tree completely defoliated; **B,** defoliation of twigs and branches in sectorial pattern; **C,** extensive defoliation of 4-year-old tree (note abundant fallen green leaves on soil under the tree canopy); and **D,** dark brown xylem discoloration in transverse section of a trunk from a Verticillium wilt–affected olive tree.

Losses from Verticillium wilt include death of trees and reduction of fruit yield. Tree death occurs mainly in young orchards but also in adult trees (Figs. 2A and 3B). Reductions in fruit yield occur with nonlethal infections because drupes formed on infected olive branches shrivel, desiccate, and lose weight (Fig. 3D and F). Thanassoulopoulos et al. (227) estimated an annual harvest loss of 1.7×10^6 t associated with 2 to 3% disease, amounting to 1% of the total olive production in Greece. In Syria, Al-Ahmad and Mosli (3) estimated harvest losses associated with 0.9 to 4.5% disease incidence of 1 to 2.3% annually. The impact of *V. dahliae* infection on olive yield was further confirmed by Levin et al. (133,134) by careful assessments of yield loss per tree in 3- to 5-year-old irrigated trees. These authors estimated 87 and 73% yield reduction in affected 'Picual' olives the third and fifth year after planting, respectively (133), and 48.3 and 12% yield reduction in affected 'Barnea' olives the third and fourth year after planting, respectively (134). No indication was given of symptom severity in the individual trees, but a disease severity index (ranging from 2, all trees healthy, to 10, all trees dead) averaged 2.7 and 3.4 in 146 and 190 affected 'Picual' olives, respectively (133), and 4.2 and 2.4 in 459 and 482 affected 'Barnea' olives, respectively (134).

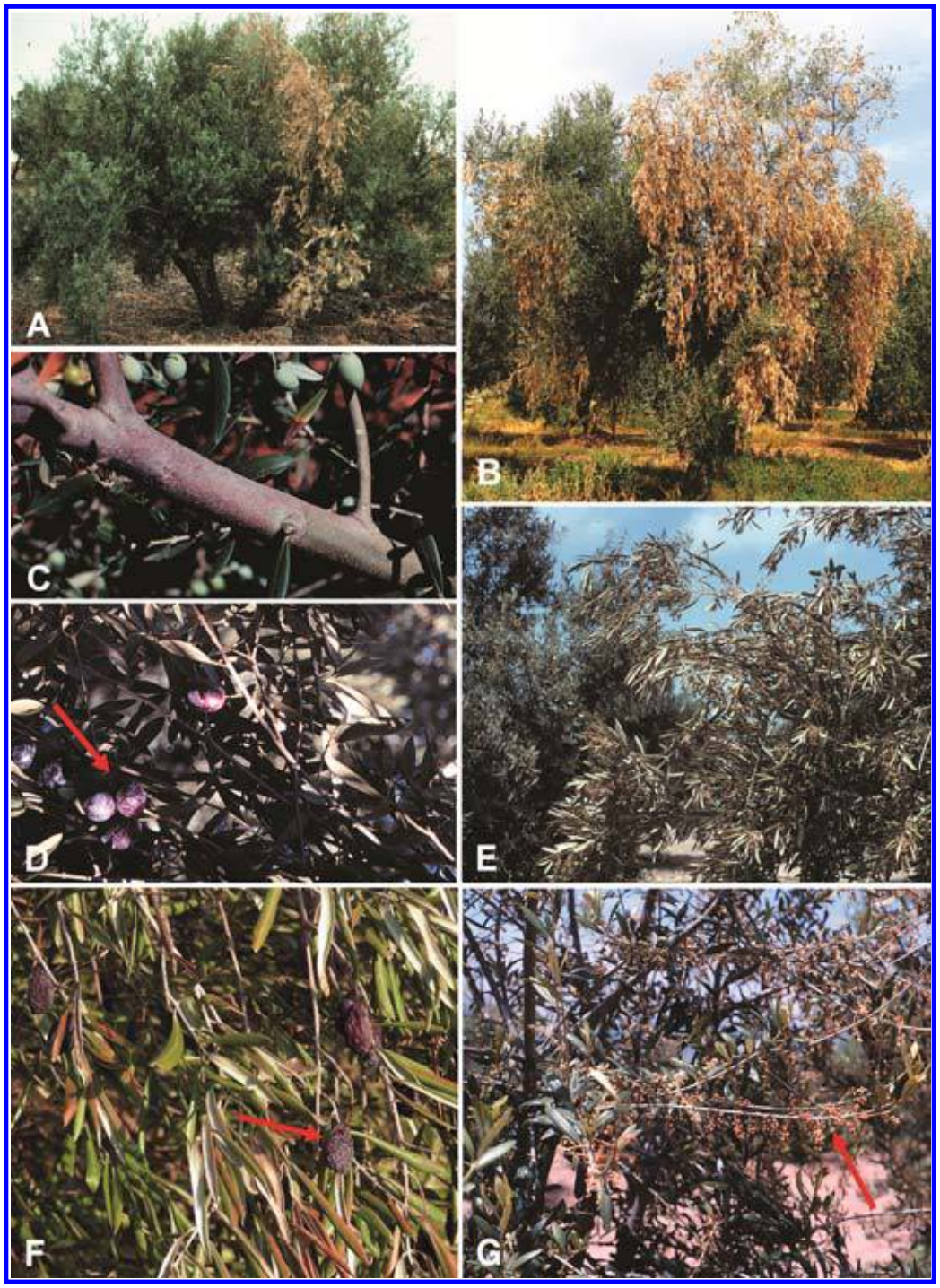

Fig. 3. Field symptoms of the nondefoliating Verticillium wilt syndrome in olives: **A,** a canopy sector of necrotic twigs and branches in a 'Picual' tree affected with the apoplexy symptoms complex (note necrotic leaves attached to necrotic twigs); **B,** severe Verticillium wilt in a 50-year-old tree of the susceptible 'Amfissis'; **C,** purplish-blue bark discoloration in a branch of Verticillium wilt–affected tree; **D,** ripened olives formed in healthy trees (arrow); **E,** leaf chlorosis in an olive tree affected with slow decline symptom complex of Verticillium wilt; **F,** shriveled and desiccated olives formed in Verticillium wilt–affected tree (arrow) (note chlorotic leaves rolled back toward the abaxial side); and **G,** flower mummification and necrosis of inflorescences (arrow) in an olive tree affected with slow decline.

Syndromes and Symptoms of the Disease

Verticillium wilt in olive comprises two main disease syndromes, namely defoliating (D) and non-defoliating (ND) (171). The D syndrome is characterized by early drop of asymptomatic, green leaves from individual twigs and branches that eventually give rise to complete defoliation and necrosis) (Fig. 2A). These symptoms can develop from late fall through late spring; they may appear in a sector of the tree canopy or affect the entire canopy and result in death of the tree (Fig. 2B). Conversely, the ND syndrome comprises two symptom complexes, designated apoplexy (or acute form of the disease) and slow decline (or chronic form) (30,227,260). Apoplexy develops mainly in late winter to early spring, and is characterizes by a quick dieback of olive twigs and branches where leaves turn light brown, roll back toward the abaxial side, and dry up (Fig. 3A). Necrotic leaves remain attached to the symptomatic shoots, although partial defoliation may occur in young trees. Usually, the bark of affected twigs and branches shows a purplish-blue discoloration (Fig. 3C). Apoplexy can kill young trees (Fig. 3B). Slow decline occurs in the spring, by the time of flowering, and proceeds slowly through early summer. Slow decline consists mainly of flower mummification and necrosis of inflorescences together with chlorosis and necrosis of leaves that develop on individual branches (Fig. 3E and G). Symptomatic leaves often fall off except those at the distal end of branches. Symptoms affecting inflorescences usually develop before leaf symptoms. The bark of affected branches may show a reddish discoloration. Fruits that eventually form on affected branches often shrivel, desiccate, and become mummified (Fig. 3F). As with other Verticillium wilt diseases, xylem browning can be seen on longitudinal and transverse sections of twigs and branches affected by either of the disease syndromes, which is useful for diagnostic purposes (Fig. 2D). However, xylem discoloration is occasionally absent in some diseased varieties.

Depending on the nature of the source of inoculum and means of dispersal of the pathogen (see below), affected trees may initially appear distributed randomly in an orchard, aggregated in patches, located at the entrance to the orchard, or along the borders of neighboring fields with crops like cotton that are susceptible to Verticillium wilt. Thereafter, the disease can progressively develop over larger areas in subsequent cropping seasons. Symptoms of Verticillium wilt may appear between the first and second year after planting, depending upon factors that influence disease development (i.e., environmental conditions, amount and virulence of inoculum in soil, susceptibility of olive cultivars, etc.; see below).

Verticillium dahliae on Olive: Taxonomy and Population Biology

V. dahliae is a worldwide-distributed, strictly asexually reproducing haploid fungus able to infect over 400 plant species, including annual, herbaceous crops and weeds, as well as fruit, landscape and ornamental trees, and shrubs (179). Multilocus phylogenetic analyses indicate that *V. dahliae* is phylogenetically placed within the Ascomycetes in the newly established family Plectosphaerellaceae of subclass Hypocreomycetidae, class Sordariomycetes (218,261,262). Recent studies indicated first that *V. dahliae* isolates from diverse geographic origin harbor only one (*MAT1-2*) of the two idiomorphs of the *MAT* gene that regulates sexual reproduction in Ascomycetes (248). Subsequently, *MAT1-1* was identified in a few *V. dahliae* isolates lacking *MAT1-2*, indicating that *V. dahliae* is potentially heterothallic but that sexual reproduction in the fungus is improbable because mating type distribution of isolates studied so far is biased toward *MAT1-2* (249). The genome sequence of this fungus is available at: http://www.broadinstitute.org/annotation/genome/verticillium_dahliae/MultiHome.html, and its complete mitochondrial genome was recently published (177).

V. dahliae produces hyaline, uninucleate (haploid, n = 6 or 7) conidia (2.5 to 8.0 × 1.4 to 3.2 µm) aggregated in moist droplets at the tip of flask-shaped, uninucleate phialides (16 to 35 × 1.0 to 2.5 µm) which are arranged in whorls (verticils) on unbranched, erect conidiophores (Fig. 4A) (178,215,246). This fungus is characterized by the production of dark, elongated to irregularly spherical microsclerotia (clusters of thick-walled, heavily melanized cells formed by lateral budding from hyphal cells (15 to 50 µm, occasionally up to 100 µm) that can remain viable in soil for up to 14 years (Fig. 4B) (215,255). *V. dahliae* can grow and infect at 30°C, but not at 33°C, with an optimum growth range at 21 to 27°C depending on the strain (19,62,107).

Population biology of *V. dahliae* from olive. Populations of *V. dahliae* are considered host-adapted rather than host-specific, i.e., they display cross pathogenicity but are more virulent (virulence is herein defined as the relative capacity of a pathogen strain to cause a given amount of symptoms on individual hosts or host genotypes) to the host from which they were isolated (28,29,66,108, 110,222,233). Under selection pressure, host adaptation in *V. dahliae* can give rise to host-specific pathotypes, such as the tomato, sweet pepper, and eggplant pathotypes differentiated among *V. dahliae* isolates from horticultural crops in Japan (103,247), or cultivar-specific races such as those identified in *V. dahliae* infecting lettuce, tomato, or sunflower following the use of highly resistant cultivars of each host (7,94,250).

Isolates of *V. dahliae* from other hosts are pathogenic to olive (50,204,216). However, the development of an olive-defoliating (D) pathotype exemplifies that host adaptation in *V. dahliae* may also result in quantum increments in virulence. *V. dahliae* isolates infecting olive can be classified into defoliating (D) or nondefoliating (ND) pathotypes based on their ability to induce the corresponding syndrome in the infected tree (171,194,207). D and ND pathotypes also occur in *V. dahliae* infecting upland cotton (*Gossypium hirsutum* L.). Isolates of the D or ND pathotype from cotton and olive induce the corresponding syndrome when used for artificial inoculations of the other host (161,162,192,202,207,208). Also, several studies have shown that D isolates from cotton or olive are consistently more virulent than ND isolates across olive cultivars (61,144,151,158,162,192,194). This increased virulence of D isolates on olive compared with that of ND isolates is expressed by significantly higher: (i) incidence and severity of symptoms at different inoculum densities of the pathogen (158,159,192,194,207); (ii) percentage of infected xylem vessels in roots and stem, as well as intensity of lumen occlusion by fungal structures (192); and (iii) fungal biomass in roots and stem estimated by the number of colony forming units observed from tissue macerates on agar media or *V. dahliae* DNA quantified by real-time polymerase chain reaction (qPCR) assays (148,158). Additionally, the inoculum density of D *V. dahliae* needed for the development of severe olive wilt is much lower than that needed of the ND pathotype. For example, López-Escudero and Blanco-López (142) found that 3.3 microsclerotia (ms) per gram of soil of the D pathotype were sufficient to cause 47% disease incidence in 9-month-old 'Picual' olive by 32 months after planting in infested soil, whereas no disease had developed at that time in soil infested with 10 ms g^{-1} of the ND pathotype (31). A similar relationship of inoculum density of *V. dahliae* pathotypes to disease was reported on upland cotton (20). Moreover, infections by the D pathotype can be lethal to olive, whereas ND-infected olive trees can eventually show complete remission from symptoms (see below; 133,140,161, 192,235,258).

The D pathotype was first recognized on cotton in California (206). Subsequently, this pathotype was reported on cotton in Peru (154), China (146,263), Spain (32), Iran (95,202), Tadjikistan (59,187), Greece (70), Turkey (87), and Israel (127). In olive, the D pathotype was first reported from California (207,208), and it is now widespread in the most important olive-growing areas of southern Spain (111), Iran (202), and the Aegean, Marmara, Mediterranean, and southeastern Anatolia regions of Turkey (60,61).

In addition to its now widespread distribution in North and South America, Greece and Spain in Europe, the Near East, and Asia, and its high virulence on cotton and olive (19,61,126,127, 144,206–208), isolates of the D pathotype also display great variation in virulence on other hosts. For example, the cotton-D T-1 strain of *V. dahliae* in California was reported causing defoliation

on okra and cotton, but it was non-defoliating, although highly virulent, on celery and safflower, mildly virulent on tomato, and nonpathogenic to cowpea, muskmelon, and watermelon (206). Comparatively, cotton D isolates from Israel were highly virulent and defoliating on cotton and okra, but ND and moderately virulent on safflower, sunflower, and watermelon, and mildly virulent on eggplant (127). Similarly, D isolates from cotton in Spain were highly virulent but ND on flax, and those from artichoke and cotton were moderately virulent and ND on artichoke (110; R. M. Jiménez-Díaz, *unpublished data*). Conversely, the D *V. dahliae* T-1 strain from California was nonpathogenic to artichoke (29). The *V. dahliae* D pathotype is considered indigenous to the southern United States and northern Mexico (24). However, such variation in virulence raises doubts about a single origin (and subsequent migration) of the D pathotype. Two competing hypotheses have been proposed for the origin of the D pathotype. Bell (23) suggested that the D pathotype arose once and was spread worldwide with contaminated cotton seed. Since the T-1 strain is not internally seedborne in cotton, such a mechanism of dissemination would implicate widespread use of poor quality seed carrying infested refuse (206). Nevertheless, the wide geographic range of the D pathotypes in cotton and cotton field soil (e.g., Iran and Tadjikistan [59,95,202]) is also consistent with differentiation from native populations rather that exotic introduction. Thus, the alternative hypothesis is that the D pathotype may have originated multiple times, as suggested by the marked variation in virulence profiles described above. In Spain, the D pathotype was first found to be restricted to cotton crops in a few locations of Seville Province, southwestern Andalusia (32). Subsequently, this pathotype became widespread in that province (19) and was found in neighboring olive-growing provinces (21). Now the D pathotype occurs throughout the entire olive-growing area in Andalusia; it was found infecting olive in 83.1% of 65 wilt-affected orchards in this region and accounted for 78% of 637 *V. dahliae* isolates sampled from

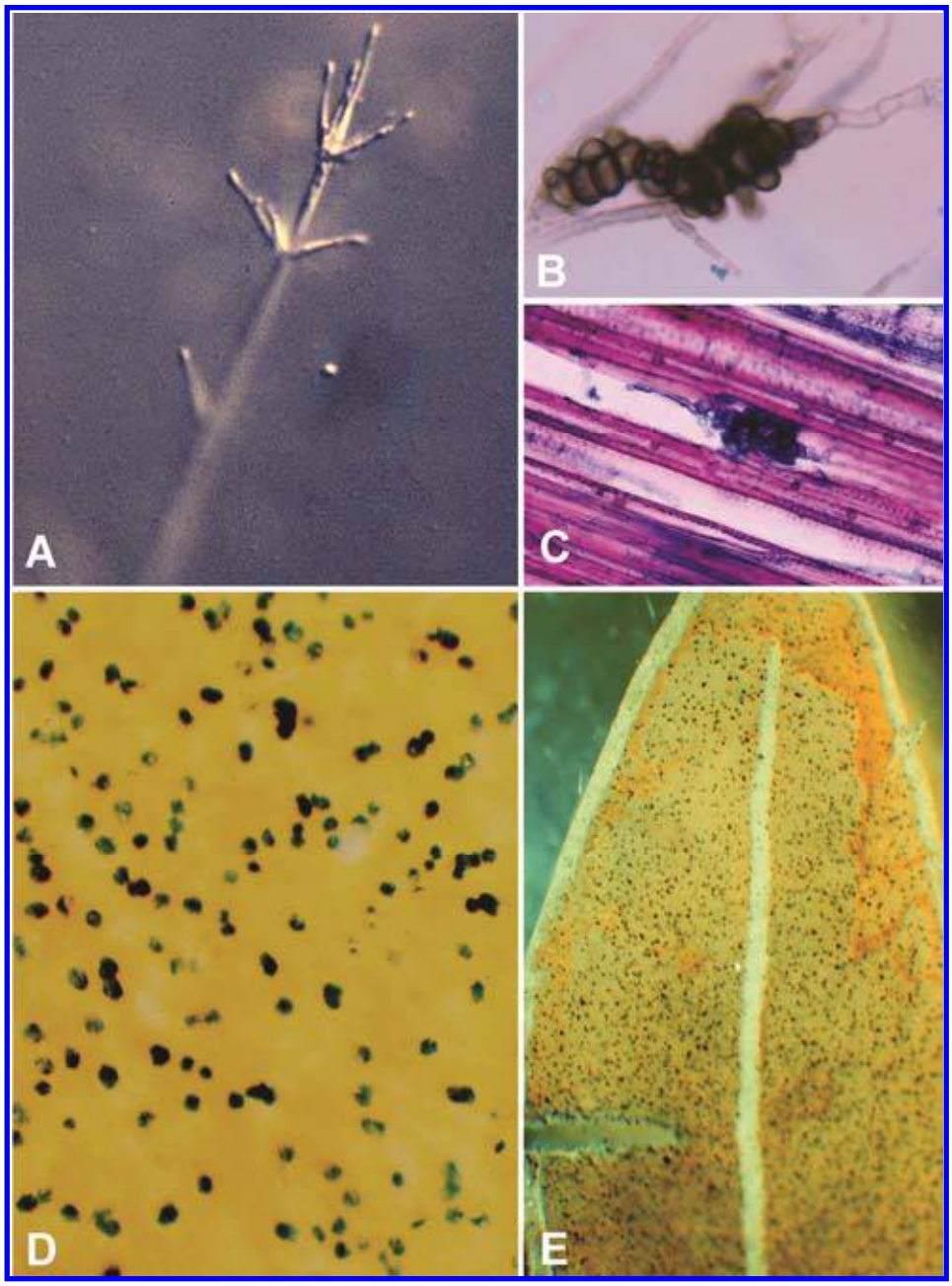

Fig. 4. Reproductive structures of *Verticillium dahliae*: **A,** conidiophore (note phialides arranged in whorls and conidia at the tip of phialides); **B,** elongated microsclerotia formed on water agar characteristic of the cotton- and olive-defoliating *V. dahliae* pathotype; **C,** microsclerotia formed within a xylem vessel of an infected olive plant; **D,** microsclerotia formed in olive leaves dropped from trees infected with defoliating *V. dahliae* following incubation under high moisture conditions; and **E,** an olive leaf showing a large number of microsclerotia.

those orchards (111). Whether such spread is a result of the independent development of new strains from native populations in the newly infested areas or migration of D strains from the original sites has not yet been determined. Comparatively, molecular evidence suggests that the *Ve* resistance-breaking race 2 of *V. dahliae* in tomato has evolved independently several times (65) and occurs worldwide (65,79,92,174,232); therefore, we consider the independent evolution of the D pathotype also to be possible.

The few examples referred to above of specific adaptation in *V. dahliae* pathotypes and pathogenic races, together with the quantum increment in virulence exemplified by the D pathotype and a continuum of virulence found to occur within populations of *V. dahliae* infecting cotton, potato, and tomato (12,92,116), suggest that a considerable genetic variation must occur in *V. dahliae*. Genetic diversity in asexually reproducing *V. dahliae* can arise through accumulation of mutations, leading to a clonal structure in pathogen populations (54,55). Alternatively, genetic variation in *V. dahliae* could result from cryptic sexual reproduction (14) or parasexual recombination following intraspecific hybridization as suggested for one subpopulation of *V. dahliae* (53). However, these conclusions must be confirmed by additional studies because of shortcomings in the previous studies. Understanding the nature and source of genetic diversity in populations of *V. dahliae* is important for disease management through risk assessment, as well as development and deployment of resistant cultivars, exploring the pathogen potential for evolving new strains with improved pathogenicity traits, and preventing their introduction into new areas.

Clonality in *V. dahliae* populations has been described mainly by vegetative compatibility groups (VCG) using spontaneous nitrate-nonutilizing (*nit*) mutants (54,55,110,123,195). Vegetative compatibility is determined by the *het* (heterokaryon incompatibility) loci-controlled ability of individual fungal strains to undergo hyphal anastomosis and form heterokaryons (132). Fungal isolates that are vegetatively compatible are placed in the same VCG (123,132). VCGs in *V. dahliae* are often thought of as genetically isolated populations, which may vary in ecology, physiology, and virulence (123,195). Six VCGs (VCG1 through VCG6) have been identified in *V. dahliae* worldwide, of which VCG1, VCG2, and VCG4 were further divided into subgroups A and B based on the frequency and vigor of complementation (24,45,116,219). Amplified fragment length polymorphism (AFLP) analysis of *V. dahliae* DNA demonstrated that isolates within a VCG subgroup are genetically similar to the extent that AFLP clustering of isolates correlates with VCG subgroups regardless of the host source and geographic origin (55).

Studies on the evolutionary relationships among *V. dahliae* VCGs using parsimony analysis of AFLP fingerprints together with sequences of six conserved DNA regions identified two main lineages (I, II). Lineage I included VCG1A, VCG1B, and one subgroup of VCG2B; and lineage II comprised two closely related subclades: subclade I (VCG2A and VCG4B) and subclade 2 (VCG4A, VCG6, and another subgroup of VCG2B). VCG subgroups were monophyletic except for VCG2B that occurred in lineages I and II. The use of additional markers, such as microsatellites and single nucleotide polymorphic markers, which provide better resolution, also detected polyphyly for VCG2A and VCG4B (27; M. M. Jiménez-Gasco, *unpublished*). While monophyly assumes that isolates within a VCG should be related by common descent, polyphyly (i.e., evolving independently more than once) indicates that the VCG trait may have evolved convergently rather than divergently as often assumed. Polyphyly of *V. dahliae* VCGs may be important for the management of Verticillium wilt of olive because it implies that new lineages can arise with isolates varying in ecological, physiological, and/or virulence traits.

In spite of their interest as genetic markers, VCGs do not fully describe the overall genetic diversity among strains of *V. dahliae*. In fact, VCG2B isolates from artichoke in east-central Spain were shown to be genetically heterogeneous through complementations with local and international reference VCG testers (110), and this genetic heterogeneity was further supported by high intra-VCG molecular variability shown by PCR markers as well as AFLPs and *V. dahliae*–specific DNA sequence analyses (53–56). Such genetic and molecular diversity may underlie the origin of *V. dahliae* races and pathotypes. For example, all D isolates from cotton and olive of different geographic origin are of VCG1A (24,55,60, 61,110,111,126,127). However, *V. dahliae* isolates from cotton or woody hosts, genetically close to VCG1A based on their assignment to VCG1B, were of the ND pathotype when tested on cotton (24,45,55; R. M. Jiménez-Díaz, *unpublished data*). In addition, geographically diverse D isolates in VCG1A (VCG1A/D) from cotton and olive were highly diverse when genotyped with AFLPs, DNA sequencing of conserved loci, and a 462-bp sequence characterized amplified region (SCAR) marker developed from a random amplified polymorphic DNA (RAPD) amplicon. This 462-bp PCR marker was developed using a set of closely related D isolates from cotton and olive in southern Spain (180); it was amplified from all D isolates from China and Spain (110,111,180) but was not found in VCG1A/D isolates from Greece, Israel, and Turkey (55,60,127). However, all VCG1A/D isolates from Greece, Israel, Spain, and Turkey displayed a 334-bp *V. dahliae*–specific PCR amplicon produced by primers derived from a *V. dahliae* genomic library (43). Also, analysis of molecular variance of AFLP data from VCG1A/D isolates indicated that there was a significant genetic difference between cotton and olive isolates from Spain and cotton isolates from Greece and Turkey (55). Overall, the genetic diversity within VCG1A/D isolates from diverse geographic origin reinforces the alternative hypothesis mentioned above in that the D pathotype may have originated independently in different geographic locations and multiple times and presumably from different hosts.

Several studies have shown that *V. dahliae* populations infecting olive in Mediterranean countries comprise limited VCG diversity. In southern Spain, VCG typing of 637 *V. dahliae* isolates from 433 olive trees in 65 arbitrarily chosen orchards at the five most important olive-growing provinces in Andalusia identified four VCGs: VCG1A, VCG2A, VCG2B, and VCG4B. However, only two of them, VCG1A and VCG2A, occurred in all provinces and jointly accounted for 97.9% of all isolates. VCG1A was the most prevalent VCG, comprising 78.1% of the tested isolates (111). These two VCGs occurred singly in an orchard in most cases, but in 13 orchards in association with either VCG2B or VCG4B. Therefore, the overall prevalence of VCG1A and VCG2A reached 83.1 and 33.8% of the 65 surveyed orchards, respectively. This limited VCG diversity and predominance of VCG1A among *V. dahliae* infecting olive in southern Spain is congruent with a clonal structure of the pathogen population within the region. Interestingly, a similar predominance of VCG1A and limited VCG diversity was reported for olive *V. dahliae* in Turkey, where VCG2A and VCG4B were found to be minor components of the pathogen population (60,61). Studies in other olive-growing countries in the Mediterranean Basin reported VCG2A or VCG2B and VCG4B as predominant groups in Greece (69) and Morocco (46), and VCG2A and VCG4B in Israel (245). Typing of individual *V. dahliae* isolates also indicated the occurrence of VCG2A in olive in Cyprus, Italy, and Syria (56). Also, Sanei et al. (202) found *V. dahliae* VCG1 (subgroup not determined), VCG2A, and VCG4B on olive in Iran, and Bellahcene et al. (25) reported a single VCG among a few isolates from olive in Algeria, France, and Syria, which was not further identified. The limited *V. dahliae* VCG diversity associated with a crop in an area, which has been reported in several crops and regions (110,116,125,126,219,220), may reflect the cropping history of *V. dahliae*–susceptible plants in soils and is consistent with the perception of *V. dahliae* isolates being primarily host-adapted. Interestingly, the described scenarios concerning VCG1A/D pathotype in Andalusia and Turkey coincide, in that the olive crops in the two countries are expanding to occupy soils previously cropped to cotton, which is as susceptible to VCG1A/D-pathotype as olive (19,60,61,110,126,127,194). This scenario of the relationship between prevalence of the D pathotype in neighboring cotton and olive crops within an agricultural area was earlier pointed out by Schnathorst and Sibbett in California (208).

While all VCG1A isolates from olive tested so far are of the D pathotype (61,111), olive isolates of VCG2A, VCG2B, and VCG4B were proven to be of the ND pathotype both by biological and molecular pathotyping (61,111). Overall, an isolate's VCG correlates to a limited extent with its virulence on olive. VCG1A/D isolates are significantly more virulent than those in VCG2A, VCG2B, and VCG4B (61,111; and R. M. Jiménez-Díaz, *unpublished*), but isolates of VCG2A and VCG4B were found to be similarly virulent on olive (61,245). Little information is available regarding intra-VCG variation in virulence on olive. Dervis et al. (61) reported a continuum of virulence among 13 VCG1A/D isolates from Turkey tested on three Turkish cultivars, with some of these isolates being as virulent as a single VCG1A isolate from Spain that was used for comparison. Conversely, all VCG1A isolates from olive in Spain tested so far are similarly virulent (109,111; R. M. Jiménez-Díaz, *unpublished*). However, a critical comparison on virulence range among VCG1A isolates from Spain and Turkey differing in the 462-bp PCR marker remains to be done.

Disease Cycle

The cycle of pathogenesis in Verticillium wilt of olive is characterized by the same aspects that are typical of other *V. dahliae*–induced diseases: (i) wide host range of the pathogen (as discussed above); (ii) the ability of the fungus to survive many years as dormant microsclerotia free in soil or within plant debris; and (iii) pathogen growth confined within the xylem during the pathogenic phase (102,179).

Pathogen survival and dispersal. *V. dahliae* exhibits little or no saprophytic activity in soil and survives by means of microsclerotia free in soil or embedded in colonized stem and root tissues (13,63,74). Microsclerotia can germinate multiple times in uncropped moist soil and give rise to mycelia and conidia. However, conidia or mycelia of the fungus in soil do not contribute to long-term survival of the pathogen, and germinated microsclerotia lose tolerance to desiccation (76,91,157). Direct assessment of *V. dahliae* in soil indicated that the number of microsclerotia is highest in the top 10 cm of soil and decreases to almost 0 at 40-cm depth (117). However, infectivity soil assays on tomato indicated that *V. dahliae* can occur in significant amounts up to 75-cm depth, the highest amount occurring in the top 30-cm depth (254).

Microsclerotia form in large numbers in host plants. For example, up to 90,000 microsclerotia per infected potato stem and 2×10^5 ms g^{-1} dry stem tissue have been reported (165,214); and monoculture of cotton 'Acala SJ-2' in California increased the inoculum density (ID) of *V. dahliae* by 13 to 15 ms g^{-1} dry soil per year (106). Microsclerotia in infested tissues are gradually released into the soil as host residues decompose, and the rate of decomposition determines the time course of increase in inoculum potential after cultivation of susceptible hosts. Microsclerotia form in colonized olive branches and leaves during senescence (112,240,258). *V. dahliae* formed more than 300 microsclerotia per leaf when partially disintegrated or green olive leaves fallen from Verticillium-affected trees were buried within the top 6 cm of moist soil under drip-irrigated trees (Fig. 4D and E) (112; D. Rodríguez-Jurado and R. M. Jiménez-Díaz, *unpublished*). This location of microsclerotia in soil is coincident with higher density of feeder roots (<0.5 mm diameter) in the root zones wetted by irrigation bulbs formed in the drip-irrigated olive trees (77). Since drip irrigation is designed to supply water to the root zone at high frequencies that optimally satisfy demand by the plant, it is unlikely that the above circumstances occur with periodic soil saturation conditions provided by furrow or sprinkler irrigation.

In addition to host crops, *V. dahliae* can infect alternative hosts including many dicot weeds and several gramineous plant species such as oats, barley, and wheat, either symptomatically or asymptomatically (72,128,136,152,153,251). Olive orchards in Mediterranean countries are often heavily infested by susceptible dicot weeds from which *V. dahliae* can be isolated (229). Microsclerotia formed on roots of host and nonhost plants can be a means of replenishment of the pathogen population in soil and persistence in the absence of primary susceptible crops (128–130,163). Whether or not alternative hosts may also play a role in selecting for specific strains from a resident population of *V. dahliae* is not known. This can be of particular significance if highly virulent strains of the D pathotype could multiply on cereals or other nonhosts currently used as cover crops to counteract soil erosion and water loss (252).

V. dahliae can be transported long distances in infected planting stocks and/or infested potting soil, and thus it can be introduced in olive-growing areas free from the pathogen or particular pathogen strains. This spread is enhanced by olive nurseries being established in *V. dahliae*–infested areas (169,173,226) and symptomless infection in the plant (121,160,161,202). Nigro et al. (173) reported that 11 to 22.6% potting mix samples were contaminated with *V. dahliae* in 50% of 29 olive nurseries sampled in the Apulia region in southern Italy; the sand, peat, and pumice components of the mix being infested at rates of 25, 3, and 1%, respectively. However, these authors could not demonstrate *V. dahliae* infection in plants sampled from those nurseries. Conversely, Thanassoulopoulos (226) found *V. dahliae* infecting 3-year-old olive plants in four out of nine nurseries sampled in a *V. dahliae*–infested zone in Greece. In southern Spain, in planta molecular-detection assays by the Plant Health Service of Andalusia indicated that 5.5% of over 600 registered nurseries had *V. dahliae*–infected but symptomless olive planting stocks. Production of planting stocks in those registered olive nurseries is in compliance with the official requirements by the European Union (EU) that do not enforce *V. dahliae*–free certification of the stocks (111). Molecular-detection and plating assays have also shown that *V. dahliae* can be spread in infected olive seeds harvested from symptomatic and asymptomatic trees and be transmitted to seedlings (119). However, the significance of this means of pathogen dispersal would be limited to the use of seed-derived seedlings as root stock and/or use of olive-mill waste as organic amendment.

Observations and inoculum assessments indicate that *V. dahliae* can be disseminated long distances and brought into olive-growing areas and orchards by irrigation water, refuse and residues from olive oil extracting industries used as organic amendments, sheep manure spread over orchards, transport of harvest and residues from affected crops (particularly cotton), and wind-blown dust (68,138,193,207,258; R. M. Jiménez-Díaz, *unpublished*). Easton et al. (68) reported that irrigation settling ponds storing waste water for reuse contained up to 15,000 propagules of *V. dahliae* per liter. In southern Spain, Rodríguez Jurado and Bejarano Alcázar (193) monitored the presence of *V. dahliae* in underground (wells) and surface (ponds storing river water) irrigation water sampled in springtime from irrigation drips of 21 olive orchards affected by Verticillium wilt in Jaén and Seville provinces of Andalusia. They reported that water from 18 (85.7%) of the sampled orchards contained *V. dahliae* in an average amount of 1.5 microsclerotia ≥ 20 µm in size and 3,159 propagules ranging from 1.2 to 20 µm per 1,000 liters. Overall, data and observations indicate the potential of several means for *V. dahliae* long-range dispersal on olive. However, their actual significance remains to be critically assessed. Statistical analyses of distribution data from disease surveys suggested that irrigation water from wells and rivers may have played a role in the dispersal of the D pathotype throughout southern Spain (111,145).

Potential means for *V. dahliae* dispersal within and among olive orchards include the dispersal of pathogen propagules in soil by furrow and flood irrigation or cultivation machinery, as well as dispersal of leaves and refuse from neighboring *V. dahliae*–infected olive and cotton crops (3,68,171,207,228,240,258; R. M. Jiménez-Díaz, *unpublished*). Easton et al. (68) reported an average of up to 301,290 propagules per liter of waste water at the distal end of furrows in irrigated potato fields in Washington, USA; and Thanassoulopoulos et al. (228) demonstrated the presence of *V. dahliae* microsclerotia floating in water of furrow-irrigated olive orchards in Greece. The aggregation of wilt-affected olive trees at the border

of an orchard neighboring olive or cotton fields, or at the entrance of orchards, may be a diagnostic clue for the source of incoming *V. dahliae* inoculum (R. M. Jiménez-Díaz, *unpublished*).

Green leaves fallen from trees infected by the D pathotype are an effective means of *V. dahliae* dispersal and an inoculum source for Verticillium wilt in olive. A single 'Arbequina' olive tree was estimated to lose an average number of 5,580 leaves per month during the period of November through May (Fig. 2C). Of those leaves, an average of 67.7% were colonized by the pathogen and proved effective for infection of 'Arbequina' plants grown in sterile, potted soil artificially infested with chopped leaves and incubated under natural conditions in the orchard (112). Short-distance airborne dispersal of those leaves was hypothesized to determine a contagious aggregation of D-infected trees in an orchard, as well as the increase in Verticillium wilt incidence from 0.011 to 6.83% in a 44-month period. Comparatively, trees infected by the ND pathotype showed a non-contagious aggregation, and their incidence increased from 0.005 to 1% only (171) (see "Epidemiology of Verticillium Wilt in Olive" below). Although trees infected by the ND pathotype also shed infected, though necrotic, leaves (see "Syndromes and Symptoms of the Disease" above), fewer leaves drop from these trees than the green leaves from D-infected trees, and they do not contribute significantly to the spread of the ND pathotype within an orchard.

Infection and colonization of the plant, and development of disease. Microsclerotia of *V. dahliae* in soil are under fungistatic dormancy from which they are released by root exudates from host and some nonhost plants or by air-drying the soil for at least 5 weeks (39,130,164,209). The effective rhizosphere influence of roots on microsclerotial stimulation of germination averages about 100 µm (104). Microsclerotia can germinate multiple times from thin-walled, hyaline microsclerotial cells, but the melanized, thick-walled ones do not germinate (205,209). Germination rate is related to microsclerotial size, with microsclerotia in the range of 75 to 106 µm in size germinating faster and more synchronously than those <75 µm in size (99). At room temperature, germination of microsclerotia produced in liquid culture decreased linearly with matric potential, decreasing from 0 to –1.5 MPa. Germination at –0.9 MPa and –1.5 MPa was 33.8 and 16.9% of that in free water (198). Osmotic potential, which is related to saline concentration, influenced microsclerotia germination less than matric potential. The rate of microsclerotia germination increased with decreasing osmotic potential up to –0.6 MPa, but any further decrease in osmotic potential caused germination to decrease (198).

Suscept–pathogen relationships in Verticillium wilt in olive have been studied under non-gnotobiotic conditions using 4- to 9-month-old, own-rooted plants of susceptible 'Arbequina', 'Amfissis', and 'Picual', resistant 'Kalamon', Koroneiki', and 'Oblonga' olives, and resistant wild olive 'Acebuche L' (148,158,189,192, 194) inoculated with the D or ND *V. dahliae* pathotypes. Plants were inoculated by dipping their bare root system in a *V. dahliae* conidial suspension either without intentional wounding (158,189) or slightly trimmed prior to inoculation (192,194), or by transplanting to soil infested with microsclerotia of the pathogen (148).

Use of a fluorescently tagged D isolate and confocal laser scanning microscopy allowed the observation of early and profuse colonization of the root surface of 'Arbequina' plants by the pathogen. This colonization was more intense at the root differentiation and elongation zones, where microsclerotia were formed by 6 days after inoculation, than at the meristematic zone (189). These authors claimed that ingress of the pathogen into the olive root tissues was predominantly through micro or macro injuries, although they also appear to have observed penetration at sites of emergence of lateral rootlets but not through intact epidermal cells. These injuries in the root elongation and nearby root zones are likely to occur when the root system of a plant grows in an abrasive medium of soil. Thereafter, hyphae of D *V. dahliae* were observed to grow inter- and intracellularly within the root cortex reaching the xylem vessels without much hindrance by 9 days after inoculation of 4-month-old plants, but no account was given for the rate of success of this event (189). In other hosts, researchers have shown that *V. dahliae* can successfully penetrate the root epidermis and reach the cortical tissues; although most cortical infections fail to reach the vascular tissues and establish vascular infections (22,85). Rodríguez Jurado (192), using conventional histological staining techniques, could not determine how hyphae of D *V. dahliae* in the root cortex of 'Picual' olives accessed xylem vessel because outer cortical cell layers were often colonized by other fungi. However, scarcely branched, thin hyphae (1.1 to 1.7 µm diameter) and conidia (3.4 to 6.8 × 1.1 to 2.0 µm) were observed in root xylem vessels by 14 days after inoculation of 9-month-old plants (Fig. 5A). At this time, *V. dahliae* could be isolated from stems and shoots, suggesting that conidia were carried upward in the xylem fluid (194). Thereafter, a rapid and systemic colonization of the plant by hyphae and conidia occurred before symptoms developed (Fig. 5B). By the time of first symptom appearance, 24 days after inoculation, thicker, usually branched hyphae (1.7 to 3.5 µm diameter) and phialide-like structures developed in root xylem vessels. Hyphae grew along xylem elements through pit membranes in perforation plates, occasionally adhered to vessel walls, and colonized adjacent vessels by growing through lateral pits (Fig. 5C) (15,192). Also, large numbers of conidia were seen aggregated at single perforation plates. Thereafter, extensive colonization of the root vascular system occurred: hyphae packed to completely occlude the vessel lumen, grew outside the xylem to colonize xylem parenchyma and phloem tissues, and formed microsclerotia (average 48 × 18 µm) in the invaded vessels but mainly in the cortical tissues (Fig. 4C) (192). *V. dahliae* reached aerial tissues by the time it had colonized the root xylem. The fungus was isolated from the lower stem internodes and shoots 14 and 24 days after inoculation, respectively (192), and mycelia were observed within the secondary xylem vessel cells in the main stem and shoots 25 days after inoculation, as well as in leaf petioles 5 days later (189).

Early work using conventional histological staining techniques and microscopy, together with assessment of pathogen biomass in tissue macerates, led to the conclusion that differences in severity of symptoms induced by the D and ND pathotypes in 'Picual' plants were related to faster and more extensive and intensive colonization of the plant by the D pathotype (192,194). Moreover, the biomass of ND *V. dahliae* in root tissues of symptomatic plants was found to decrease over time, but that did not occur in infections by the D pathotype (192). Subsequent use of real-time quantitative PCR (qPCR) technology confirmed to an extent those findings and allowed us to improve our understanding about the dynamics of *V. dahliae* biomass in infected olive tissues and its relationship with differences in virulence between pathotypes or in susceptibility among olive cultivars. Mercado-Blanco et al. (158) monitored colonization of susceptible olive cultivars ('Picual' > 'Arbequina') and resistant wild olive 'Acebuche L' in a 100-day time course following root-dip inoculation with D and ND isolates. Authors concluded that the amount of pathogen DNA detected in root and stem of susceptible cultivars correlated with their susceptibility to disease to a larger extent than with virulence of pathotypes. Maximal pathogen DNA occurred in root and stem tissues before symptoms fully developed, the DNA amount in stems being lower than that in roots. Thereafter, pathogen DNA decreased sharply in the roots of the less susceptible 'Arbequina' but remained at a high level in the more susceptible 'Picual' for several weeks. This decrease in pathogen DNA also occurred in stem tissues in the least severe ('Arbequina'/ND) and most severe ('Picual'/D) disease reactions. Conversely, a subsequent increase in pathogen DNA occurred in stems of moderately affected 'Arbequina' and 'Picual' plants infected with D or ND *V. dahliae*, respectively, by the time symptoms reached their maximum extent, 70 days after inoculation. The decrease in the amounts of detected pathogen DNA may correspond with partial lysis of *V. dahliae* propagules as a consequence of defense mechanisms in the infected plant that are not sufficient to interfere with subsequent events of fungal conidiogenesis and cyclic colonization during host

growth (100). Recently, Markakis et al. (148) carried out a study similar to that of Mercado-Blanco et al. (158) to correlate the amount of D and ND *V. dahliae* DNA with reactions of susceptible ('Amfissis') and resistant ('Kalamon' and Koroneiki') Greek cultivars. These authors simulated natural infections by transplanting 8-month-old rooted plants into soil artificially infested with microsclerotia of D or ND isolates and monitored colonization of infected plants by means of qPCR assays and isolation of the fungus for over 1 year. Contrary to results by Mercado-Blanco et al. (158), Markakis et al. (148) found that the amounts of *V. dahliae* DNA in roots, stem, and shoots of susceptible 'Amfissis' plants clearly correlated with virulence of the D and ND pathotypes, and also that the amount of pathogen DNA in roots was significantly lower than that in stems and shoots. Possibly the nature of the inoculum (microsclerotia instead of conidia) could have affected the differential response of the plants between the two studies. However, both qPCR assays and isolations indicate a steady decline of *V. dahliae* in olive tissues over time.

Infection of resistant olive and wild-olive plants by isolates of the D and ND pathotype were either asymptomatic ('Oblonga'; 194) or resulted in very mild chlorosis ('Acebuche L', 'Kalamon', and Koroneiki'; 148,158). However, the host–pathogen interaction

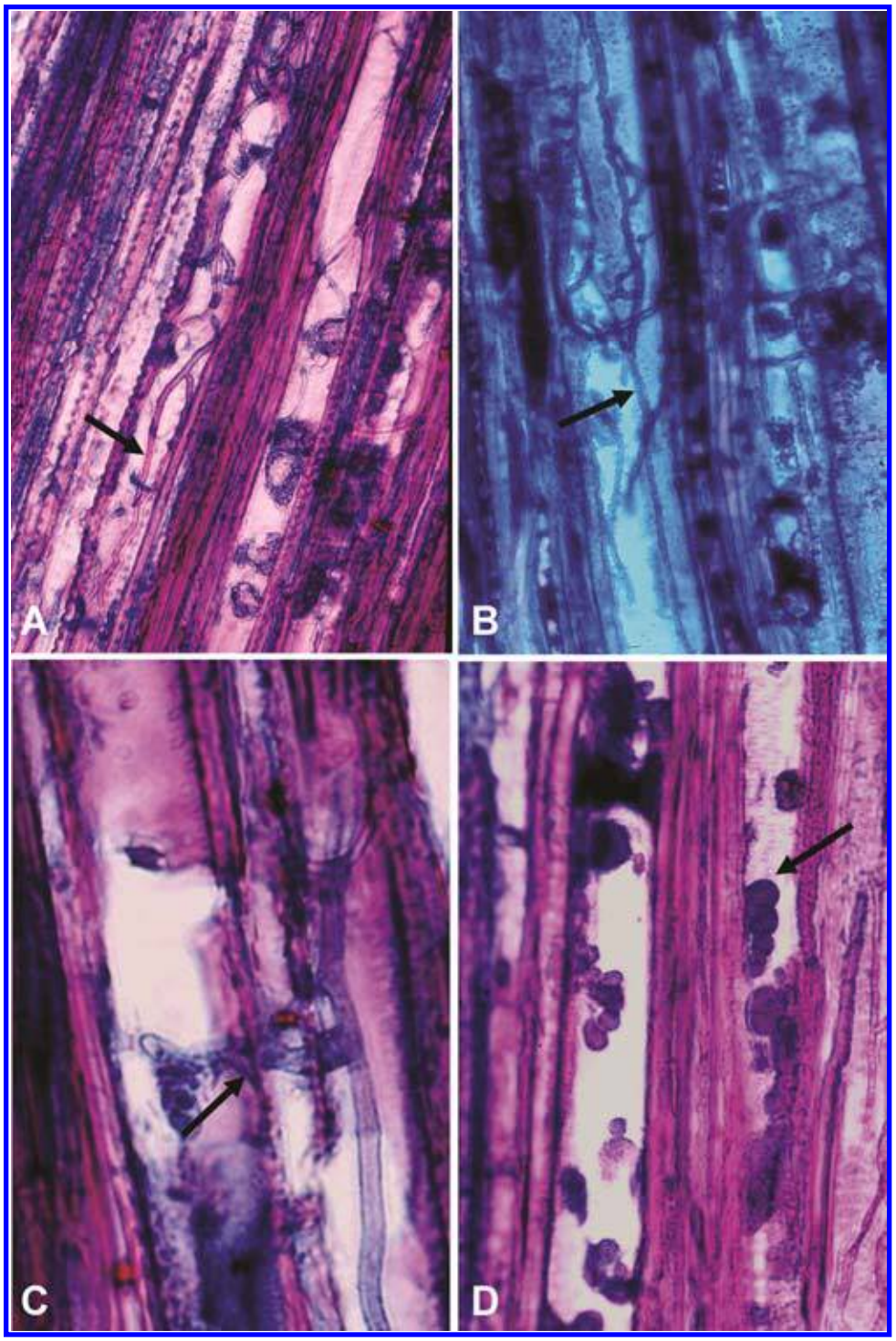

Fig. 5. Systemic colonization of olive xylem by a defoliating isolate of *Verticillium dahliae*: **A,** scarcely branched, thin hyphae growing within root xylem vessels by 14 days after inoculation (×400); **B,** branched hyphae growing within stem xylem vessels by the time of first symptom appearance, 24 days after inoculation (×400); **C,** colonization of adjacent vessels by hyphae growing through lateral pits (arrow) (×1000); and **D,** tyloses (arrow) formed in noncolonized root vessels adjacent to vessels colonized by *V. dahliae* (×400).

in resistant genotypes paralleled that observed in susceptible cultivars, except that growth of the pathogen in infected plants occurred at a much lower rate and to a lesser extent. Thus, Rodríguez-Jurado et al. (194) observed conidia of D and ND *V. dahliae* in the root xylem of 'Oblonga' plants early after inoculation and isolated the pathogen from asymptomatic stems and shoots to a similar extent as from susceptible 'Picual'. This observation is of paramount importance in selecting resistant rootstocks since similar experiments in Greece demonstrated that susceptible 'Amfissis' grafted on 'Oblonga' rootstock was infected and developed severe symptoms, while the root stock alone did not show any symptoms and the pathogen was scarcely isolated (234). Similarly, Markakis et al. (148) found that those two pathotypes colonized the roots, stems, and shoots of resistant 'Kalamon' and 'Koroneiki' olives, though to a much lesser extent than susceptible 'Amfissis'. Moreover, the level of root colonization in 'Kalamon', 'Koroneiki', and 'Acebuche L' indicated by the amount of quantified *V. dahliae* DNA was similar in resistant and susceptible cultivars, suggesting that restriction of systemic spread of the pathogen into the stem might be responsible for the resistant reactions of those cultivars (148). As found for susceptible cultivars, both DNA quantification and pathogen isolation indicated a steady and progressive decline of *V. dahliae* colonization in the infected tissues of resistant cultivars (148,158).

Roots and stems of susceptible 'Picual' olives react to xylem colonization by D and ND pathotypes by producing polysaccharide-type materials, optically dense aggregates, and tyloses extruded from the paravascular parenchyma in the vessels' elements (15,192). Aggregates formed close to vessel walls and might result from degradation of secondary walls of vessel cells by hyphae growing attached to the walls. Tyloses varying in size formed in colonized as well as in noncolonized, adjacent vessels, and together with aggregates were observed in root xylem of asymptomatic, infected 'Picual' plants 14 days after inoculation with D and ND *V. dahliae* (Fig. 5D) (192). Tylose formation did not interfere with fungal growth since hyphae grew along xylem vessels intermingled with tyloses. Tyloses increased in number and size as symptoms developed and partially or completely occluded the vessel lumen (15,192). Occlusion of xylem vessels by fungal structures, tyloses, aggregates, and polysaccharide-type materials can contribute to retarded vascular flow in roots, stems, and shoots of infected olive plants to a larger extent than growth of the pathogen (Fig. 6). As the severity of symptoms increased in plants infected by D *V. dahliae*, xylem vessel were distorted and crushed, and callose deposits formed in the paravascular parenchyma. At this time, nonlignified cells of cambial origin formed adjacent to infected vessels, and small cavities developed in xylem tissues where the pathogen grew extensively. This fungal growth was associated with disappearance of starch in xylem parenchyma, which did not occur in plants infected by the ND pathotype (192). Callose deposits on parenchyma cells and formation of cambial derivative cells between adjacent vascular bundles can contribute to lessening lateral spread of *V. dahliae* in root xylem.

Epidemiology of Verticillium Wilt in Olive

Verticillium wilt of olive is a monocyclic disease at the scale of a single growing season, for which the primary inoculum can be either soilborne microsclerotia or *V. dahliae* structures in infected planting material; i.e., it has only one cycle of pathogenesis per cropping season and the resulting inoculum does not give rise to new infections and disease within the same season. Disease development at that time scale is thus driven by the density and efficacy of inoculum in soil, as well as by the frequency of encounters of inoculum with the host root system (86). Epidemics of Verticillium wilt in olive based on seemingly simple annual infection cycle were described by sigmoid-shaped curves of increasing disease incidence (DI) during an annual cropping season. Over the course of four cropping seasons, polyetic epidemics were described by a generalized logistic model with a multiple sigmoid pattern (171). However, usual symptom development during spring, early sum-

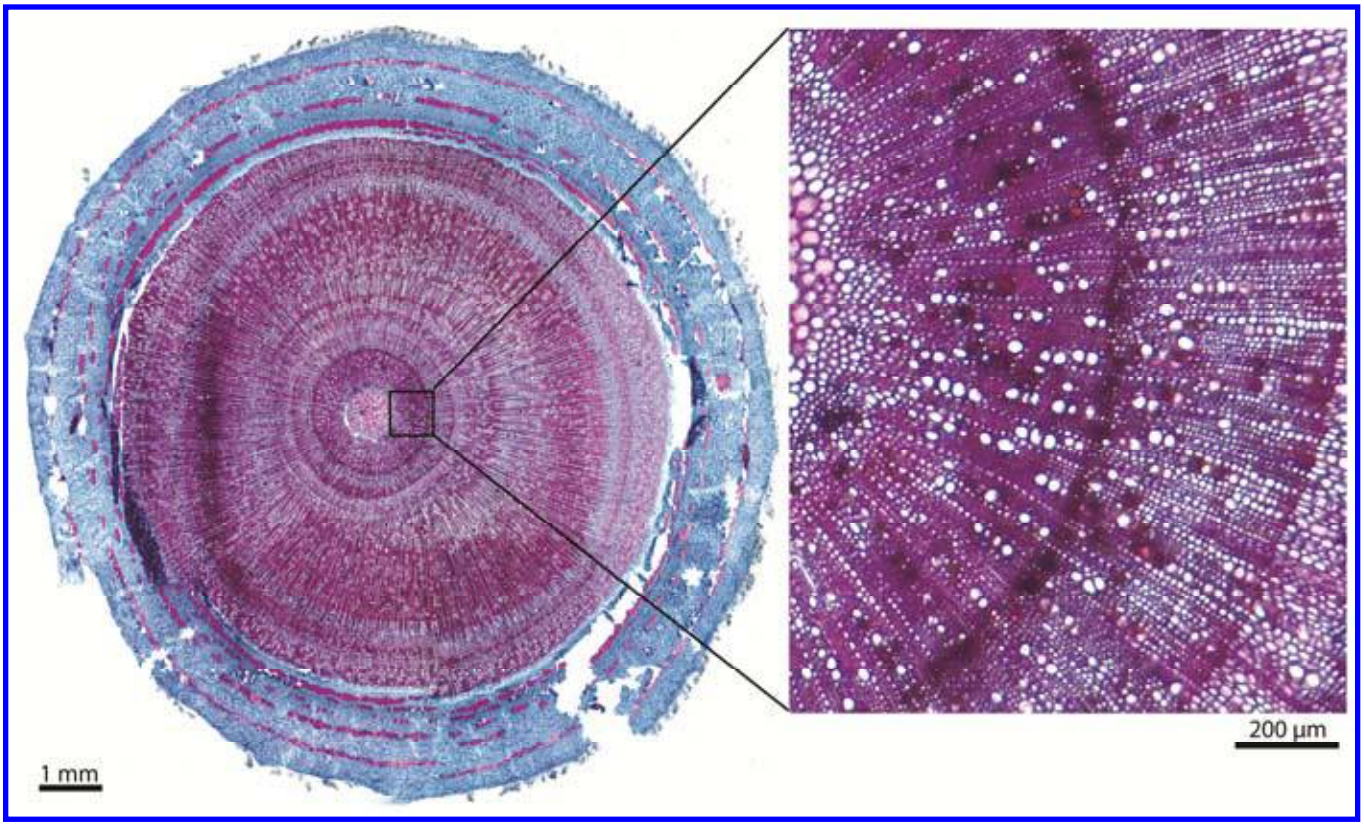

Fig. 6. Transverse, 25-µm-thick trunk section of resistant 'Frantoio' 90 days after artificial inoculation with a defoliating isolate of *Verticillium dahliae*. Fresh sections were stained with safranin and aniline blue. The close-up on the first annual xylem ring shows plugged xylem vessels (dark circular spots) and normal xylem vessels (clear spots). Plugging of xylem vessels is a plant reaction to infection by *V. dahliae*.

mer, and late autumn indicates that Verticillium wilt could be partially polycyclic if spring defoliation provides infected leaves as a potential source of microsclerotia able to invade the plants during the same season. The time to disease onset, disease progress, and distribution of affected plants in an orchard are influenced by the nature of the pathotype infecting the trees.

In Israel, first symptoms of Verticillium wilt in irrigated 'Barnea' and 'Picual' olives, presumably infected by ND *V. dahliae,* developed by 18 and 21 months after planting, respectively, the DI reaching 18.9 and 22% by 3 and 9 months later (133,134). The amount of *V. dahliae* inoculum in those orchards must have been rather high as suggested by the early development and high level of disease, as well as by the cropping history of the orchard soil that had been previously grown to vegetables susceptible to *V. dahliae* (including several cycles of potatoes and tomatoes) for 30 years. Comparatively, 9-month-old 'Picual' plants transplanted into irrigated microplots infested with microsclerotia of the D pathotype produced on agar medium developed the D syndrome 8 months after planting in soil infested with 3.3 or 10.0 ms g^{-1} soil, and 16 months after planting in soil infested with 1.1 ms g^{-1} soil (143). Apparently, 1.1 ms g^{-1} soil (determined by the soil dilution plate assay on NP-10 medium, 112; see below) was an ID threshold for the D pathotype on 'Picual' since there was no further increase in disease incidence over time. Conversely, an initial ID of 3.3 ms g^{-1} soil caused 15.0% incidence of Verticillium wilt 1 year after planting that increased to 47.5% a year later and leveled off thereafter (Fig. 7). Comparatively, a threefold increase in this latter initial ID gave rise to a DI increase from 25.0% affected plants the first year after planting to 55.5% the second year and 63.8% by the third year (143). Interestingly, no Verticillium wilt had developed by this time in similar 'Picual' plants planted in soil infested with 10 ms g^{-1} soil or less of the ND pathotype (31). Similar low ID ranges for severe disease have been reported for herbaceous crops such as artichoke (10), cauliflower (259), cotton (20), and strawberry (96). However, the ID-DI relationships in olive may be as influenced by cultivar susceptibility as they were by virulence of pathotypes.

The significant role of the nature of *V. dahliae* pathotypes on Verticillium wilt progress in olive orchards was highlighted by the monitoring of disease epidemics carried out by Navas-Cortés et al. (171) in a nontilled, drip-irrigated orchard of 'Arbequina' olives. Over four cropping seasons, the number of affected trees increased from three to 141, of which 123 trees were infected by the D pathotype and 18 by the ND pathotype. While most ND-infected trees were affected by the end of the first cropping season, the number of symptomatic trees infected by the D pathotype increased throughout the period of the study. Moreover, D-infected trees showed a pattern of aggregation around initial infections, but mainly within-row aggregation, compared with a random distribution of ND-infected trees. The authors attributed these patterns of distinct disease increase and aggregation to successful infections of new olive trees by *V. dahliae* inocula provided through wind dispersal of infected green leaves fallen from D-infected trees. Defoliation from these latter olive trees occurred extensively during the late winter to early spring period, while almost none fell from trees infected by the ND pathotype. Those new infections were probably caused by conidia washed into the soil, which form in large quantities on infected leaves and/or from microsclerotia in the wet zones developed from drip irrigation (141,203; D. Rodríguez-Jurado and R. M. Jiménez-Díaz, *unpublished*).

Verticillium wilt of olive shows seasonal development within a cropping season, the rate of disease increase both in incidence and severity being highest in the late winter–early spring period then decreasing with time to minimum values in the summer–fall period and increased again thereafter (133,171). Successful isolation of the pathogen from affected tissues also varies seasonally. In a three-season study in Israel, Levin et al. (133) found that the frequency of *V. dahliae* isolation from affected olive tissues was highest from December through May and lowest from June through September. Other authors found similar results except for failure to isolate the pathogen in winter that was associated with average minimum temperature below 0°C (234). Whether this differential isolation reflects seasonal differences in the presence of the pathogen, or some barrier when the pathogen is present, was not determined. Altogether, seasonal disease development and results from isolations suggest that infections of olive trees by *V. dahliae* start mainly in early autumn followed by progressive xylem colonization of stem and shoots during winter and spring.

Additional factors influencing disease development. Besides pathogen virulence and inoculum density, and susceptibility of olive cultivars, the epidemiology of Verticillium wilt in olive can be influenced by the combined effects of environmental, agronomic, and biotic factors. However, information on those effects derives mainly from observational or correlation studies. For instance, as for other Verticillium wilt diseases, development of Verticillium wilt in olive is favored by air and soil temperatures close to the optimum growth range of *V. dahliae.* In Mediterranean-type climates, severity of disease attacks is favored by 20 to 25°C air temperature during spring, but summer temperatures higher

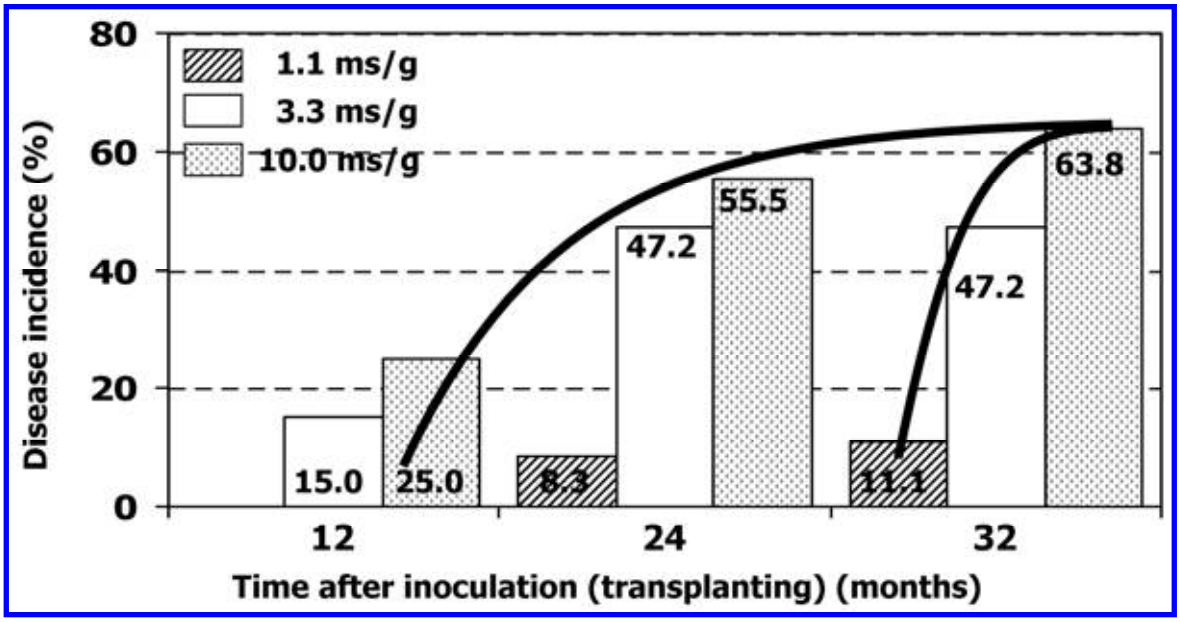

Fig. 7. Increase in Verticillium wilt incidence over time in 'Picual' olive plants transplanted into microplot soil artificially infested with given densities of microsclerotia (ms g^{-1} soil) of defoliating *Verticillium dahliae.* Curves describe estimated incidence increase. (Adapted from López-Escudero and Blanco-López [142]).

than 30°C suppress further development of the disease (133,171, 243,258). Nevertheless, a major lack of knowledge exists of how the physical environment (temperature, soil moisture, plant water use, solar radiation, etc.) influences Verticillium wilt in olive, which limits our understanding of the disease as much as any other area of research.

Several studies indicated that incidence of Verticillium wilt and/or prevalence of *V. dahliae* pathotypes may be correlated with agricultural factors that characterize olive husbandry and management. Rodríguez et al. (191) surveyed 123 olive orchards in Granada Province in southern Spain with trees infected mainly by the ND pathotype and found that disease incidence was significantly higher in irrigated than in nonirrigated orchards, as well as in orchards with trees less than 12 years old at planting densities of 300 trees ha^{-1} or higher. In a similar survey of 90 orchards in Córdoba, Jaén, and Seville provinces of Andalusia, southern Spain, where the D *V. dahliae* pathotype prevails, López-Escudero et al. (145) also found a significant positive correlation between disease incidence and irrigation, as well as nontilled management of orchard less than 25 years old. However, the incidence of disease was significantly lower in orchards with >200 trees ha^{-1}. Log-linear analysis of data from a study over a larger geographic range in the same region revealed that the D pathotype was significantly more prevalent in irrigated than in nonirrigated orchards, occurred with high or moderate frequency in orchards >30 years old and <20 years old, respectively, and it was less prevalent in orchards of high tree density (>350 trees ha^{-1}) than in those of low density (<125 trees ha^{-1}) (111). This consistent association of irrigation with high incidence of Verticillium wilt, which was also observed by other authors (3,30,211), makes irrigation in olive orchards one of most significant factors for severe Verticillium wilt.

In spite of apparent effects of irrigation on Verticillium wilt in olive, the relevant mechanisms contributing to them under field conditions have not yet been elucidated. Irrigation water can recurrently provide exogenous inoculum to the tree root system, as well as creating an environment conducive to the increase of existing *V. dahliae* inoculum and germinating microsclerotia already in the vicinity of olive rootlets (141,198). Additionally, irrigation can exacerbate Verticillium wilt in olive by enhancing both development of a high density of fine roots suitable for infection in a wet soil profile, as well as translocation of *V. dahliae* conidia within xylem vessels of infected trees as a result of increased leaf transpiration (77). Moreover, the effects of irrigation on disease development could be mediated by its influence on tree physiology. Olive is well adapted to the Mediterranean type of climate (long dry season with rainfall not meeting evaporative demand), whereby stomatal conductance is higher in morning hours, coincidental with lowest transpiration and vapor pressure deficit (VPD), and becomes reduced by midday when solar radiation, VPD, and transpiration are higher (175). These diurnal variations occur superimposed to seasonal variation in evapotranspiration, which is highest during spring and summer and lowest in fall and winter (176). However, information is lacking on the cycle of vascular activity and environmental effects on the diameter of xylem vessels. All of those factors above may have an influence on translocation of *V. dahliae* conidia within xylem vessels. Understanding the role(s) of irrigation in Verticillium wilt of olive through research would help in the control of the disease by harmonizing the supply of water for production with irrigation management to counteract the effects promoting disease.

Other factors that may influence the development of Verticillium wilt in olive, as suggested by observations in olive orchards or their effects on other Verticillium wilt diseases, include salinity in soil and/or water, soil texture and fertility, and infection by plant-parasitic nematodes (179,196). For example, severe attacks of Verticillium wilt in 'Picual' olive in Israel (133) occurred associated with use of saline sandy-loam soil and irrigation water. Use of saline water for irrigation enhanced severity of Verticillium wilt in pistachio (199). Similarly, the incidence of Verticillium wilt in olive was found significantly lower in Alfisol than in Entisol, Inceptisol, or Vertisol soils (USDA Soil Taxonomy) in southern Spain (145), and prevalence of the D pathotype in this same area was lowest in clay soil compared with that in sandy, loam, clay-loam, or sandy-loam soils (111). Soil texture influences matric water potential at a given water content in soil (35), but whether or not that influence has an effect on Verticillium wilt in olive has not been determined. Conidia and microsclerotia germination, mycelial growth, and sporulation in *V. dahliae* decrease with decreasing water matric potential, but still can take place at –10 MPa, a matric potential much lower than levels host plants can tolerate without abiotic wilting (usually higher than –1.5 MPa) (179,198). Understanding the factors and mechanisms associated with soil texture and fertility that might underlie putative reduction in the development of the disease would help in the management of Verticillium wilt of olive.

The natural recovery of diseased plants. Development of Verticillium wilt epidemics in olive orchards can eventually result in a progressive decrease of disease incidence and/or severity over time, which is associated with the recovery of affected trees from disease following the initial attack. This is one of the most intriguing phenomena occurring in Verticillium wilt diseases of woody plants, the nature of which still remains to be fully understood (48,102,113). Natural recovery of olive trees from symptoms of Verticillium wilt has been observed to develop in untreated, affected orchards (33,133,134,227,258), as well as following solarization of soil under the canopy of affected young (139) and adult trees (235). Blanco López et al. (33) monitored epidemics of Verticillium wilt in two 4-year-old, irrigated or unirrigated orchards of 'Picual' olive trees infected by ND *V. dahliae* over five consecutive years. They found that the percentage of trees affected over consecutive years decreased in the two orchards from 37 and 40% in the first year of monitoring to 0.0 and 4.2% in the fifth year, respectively, with changes in disease incidence over time being described by negative exponential functions ($DI = 158.1 \times e^{-0,72t} + 2.66$, $R^2 = 0.99$; $DI = 4 \times 10^4 \times e^{-1,41t} + 0.42$, $R^2 = 0.99$, J. A. Navas-Cortés and R. M. Jiménez-Díaz, *unpublished*) (Fig. 8). In addition, the monitoring indicated that the incidence of new infections also decreased as trees aged.

A progressive reduction of disease symptoms has also been observed in olive plants artificially inoculated with *V. dahliae* and incubated for over 1 year under conditions favorable for Verticillium wilt development (140,148,161,192,194). Both the field observations and artificial-inoculation studies have shown that the reduction in symptoms was associated with a steady decrease, and eventually complete failure, in isolation of the pathogen from, or detection of *V. dahliae* DNA in newly developed, asymptomatic shoots (133,148,161,192). Also, studies have indicated that the recovery phenomenon is strongly influenced by factors that determine disease development, such as pathogen virulence (i.e., reduced expression in infections by the D pathotype), cultivar susceptibility (i.e., lack of expression in highly susceptible cultivars) (133,140), and possibly high inoculum load, but the influence of this last factor has not been studied yet.

The actual mechanisms underlying recovery of affected olive trees from Verticillium wilt are not yet understood. Presumably, disease recovery might result from the reduced ability of *V. dahliae* of radial growth between adjacent vascular bundles. This growth is limited by the formation of cambial derivatives and callose deposits on parenchyma cells, and/or sealing of the infected xylem bundles by annual development of new vascular rings from vascular cambium (192,223,231). Furthermore, that effect might be strongly enhanced by *V. dahliae* being killed by high air temperatures during the summer as suggested by Wilhelm and Taylor (258), although the fungus can also be isolated from symptomless shoots (133). In any case, the inactivation or compartmentalization of *V. dahliae* within the xylem infected in one season makes it necessary that new root infections take place for disease to develop in new tissues the following season (258). Another potential mechanism that could be contributing to recovery of affected trees is a potential reduction of the likelihood of infection as roots of older trees penetrate deeper layers of soil presumably harboring lower levels

of inoculum. However, in irrigated olive trees the density of feeder roots is highest in the shallow layer of wetted soil (77). Even if the natural recovery phenomenon is not yet well understood, it offers interesting opportunities for the control of the disease within an integrated management strategy (see below).

Limited information is available about the biochemistry of resistance in most of the olive cultivars against Verticillium wilt. Most recently, Markakis et al. (149) studied the role of the phenolic responses of the *V. dahliae*–susceptible olive 'Amfissis' and the resistant 'Koroneiki' after D and ND *V. dahliae* infection. Phenolic responses were monitored in relation to the fungal DNA levels detected in the vascular tissues with the purpose of exploring the possible biochemical defense mechanisms of olive trees against *V. dahliae*. Quantitative PCR revealed that the decrease in symptom severity shown in resistant 'Koroneiki' trees was associated with significant reduction in the growth of both *V. dahliae* pathotypes in the vascular tissues compared with 'Amfissis'. In 'Koroneiki' trees, the levels of *o*-diphenols and verbascoside were positively associated with the levels of DNA detected of the D and ND pathotypes. In addition, a positive correlation was observed between the levels of verbascoside and the fungal DNA level detected in 'Amfissis' trees, whereas a negative correlation was revealed between the fungal DNA detection and the total phenols and oleuropein content in both cultivars. The levels of verbascoside were clearly higher in 'Koroneiki' trees compared with 'Amfissis' trees, indicating for the first time the potential involvement of verbascoside in the defense mechanism of olive trees against *V. dahliae*.

Disease Diagnosis

Diagnosis of Verticillium wilt in olive trees is based primarily on observations of foliar symptoms and vascular browning in shoots and stems. Early symptoms of foliar chlorosis and wilting may be of help for diagnosis. However, advanced stages of symptom development including shoot wilting and dieback, and foliar necrosis, might be confused with desiccation resulting from Phytophthora root rot (*Phytophthora megasperma* and *P. inundata*; 200), and Phoma dieback (*Phoma incompta*; 147,242). Nevertheless, these latter diseases are characterized by other more distinctive symptoms on roots and stem bark that do not overlap with those of Verticillium wilt. Presence of vascular browning on cross-sections of stem, shoots, and twigs is a more conclusive diagnostic symptom, although it may not always develop.

A further step for conclusive disease diagnosis is direct isolation of *V. dahliae* from olive tissues on agar media. Efficiency of isolation is influenced by the nature and age of sampled plant organs and tissues, as well as by the season of sampling (38,133). The best results for positive isolations are obtained by sampling slightly lignified, living tissues associated with symptomatic parts, almost any time of the year but preferably from September through June. For isolations, woody chips or rings are thoroughly washed under tap water with or without detergent, the bark removed, surface-disinfested in 2.5% NaClO for 60 s, rinsed in sterile distilled water, dried on sterile filter paper, subdivided into small pieces, plated on agar medium in petri dishes, and incubated at 20 to 24°C in the dark for 7 to 10 days. General agar media such as potato dextrose agar (PDA), malt agar, and water agar (WA) can be successfully used for isolations. Although bacteria are not frequently isolated from bark-deprived woody chips, agar media can be amended with antibiotics such as oxytetracyclin and aureomycin to suppress bacterial contaminants (e.g., 1 liter of distilled water, 20 g of agar, 30 mg of aureomycin) (streptomycin can be used, but it is generally not recommended). Ensuing colonies of *V. dahliae* can be identified by their white mycelium, verticillate conidiophores, and microsclerotia. These colonies should not be confused with those of *V. tricorpus*, which can sometimes be isolated, especially from roots, and forms verticillate conidiophores, microsclerotia, resting mycelium, and chlamydospores. *V. tricorpus* develops large and irregularly shaped microsclerotia on PDA, which can be morphologically differentiated from those formed by *V. dahliae* (i.e., smaller and oval to elongated microsclerotia) and often produces a diffusible yellow pigment that helps in differentiating it from *V. dahliae* (19,107). Also, *V. dahliae* forms large microsclerotia and abundant dark hyphae on ethanol agar, whereas *V. tricorpus* does not form microsclerotia, but always forms dark mycelium on this medium (89).

Colonies of *V. dahliae* can be also identified using specific PCR-based protocols and primers derived from the mitochondrial small subunit rDNA gene region (135) or a genomic library (43). The use of primers DB19/DB22 produces *V. dahliae*–specific polymorphic DNA bands of 539 or 523 bp. The 539-bp marker is present in VCG1A/D isolates and ND isolates of VCG1B, whereas the 523-bp marker is associated with the ND pathotype irrespective of VCG (56,160). Sequencing of the 539-bp DNA band allowed designing primer espdef01. The joint use of primers DB19, DB22, and espdef01 in a single PCR assay yields one of the 539- or 523-bp markers, together with a 334-bp amplicon that is present in D isolates and also in some ND isolates of VCG1B and VCG2B (56,110,160). In addition, the joint use of primer pairs INTD2f/2r

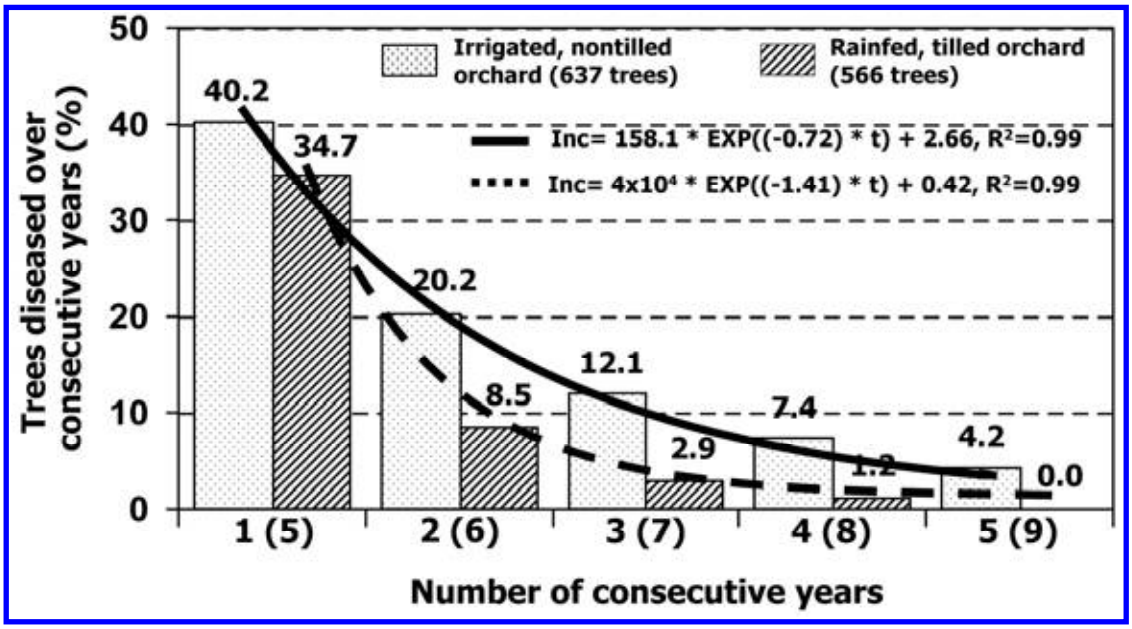

Fig. 8. Decrease of Verticillium wilt incidence over five consecutive years in two 5-year-old 'Picual' olive orchards infested with nondefoliating *Verticillium dahliae* and managed with different practices. Curves describe estimated incidence decreasing over time. Numbers in parentheses indicate age of trees.

and INTND2f/2r produces an amplicon of either 462 or 824 bp that previously were associated with the D and ND *V. dahliae* pathotypes, respectively (161,162). These latter primers were designed from DNA sequences amplified using primers derived from sequence characterized amplified regions (180). The defoliating or nondefoliating nature of *V. dahliae* isolates can also be inferred from phenotypes of cultures in agar media. Isolates of the D pathotype form elongated and round microsclerotia on WA, can grow on sanguinarine-amended PDA, and fluoresce under UV light, whereas ND isolates neither grow on sanguinarine-amended PDA nor fluoresce under UV light and form only rounded microsclerotia (19).

Diagnosis of Verticillium wilt in olives can also be achieved without need of culturing the pathogen by use of a duplex, nested-PCR protocol for in planta detection of *V. dahliae* in symptomatic or asymptomatic plant tissues (160). This protocol is based on the simultaneous amplification of both the 824-bp ND marker (161) and the D-associated 334-bp amplicon. Use of this protocol proved more consistent and sensitive than conventional microbiological isolations for diagnosis of the disease and allowed simultaneous detection of the two infecting *V. dahliae* pathotypes in both artificially inoculated young plants and naturally infected adult trees (160).

Management Strategies

Currently, no control measure applied singly is fully effective for the management of Verticillium wilt of olive. Rather, the disease is best managed by an integrated disease management (IDM) strategy that combines the use of preventive, preplanting control measures and postplanting measures that mitigate risks of pathogen spread and successive infections of other trees (37,234,237). Integrated disease management strategies and minimum use of chemicals will be enforced in EU member countries by year 2014, according to Directive 2009/128/CE of the European Parliament and the Council of 11/24/2009 (http://europa.eu/legislation_summaries/internal_market/single_market_for_goods/chemical_products/l28178_en.htm). Preventive disease control measures for the management of Verticillium wilt in olive include: (i) site selection to avoid planting into high risk soils; (ii) use of *V. dahliae*–free planting material; (iii) reduction or elimination of *V. dahliae* inoculum in soil; (iv) protection of healthy planting material from infection by residual inoculum in soil; and (v) use of resistant cultivars and rootstocks. Postplanting control measures are: (vi) cultural practices; (vii) soil solarization; and (viii) organic or biological amendments. The efficiency of those control measures may be compromised in olive-growing areas where the VCG1A/D pathotype prevails (such as in Andalusia at southern Spain and the Aegean coastal region in Turkey) because of the distinctive high virulence and low inoculum threshold of this pathotype, as well as the increased susceptibility of olive cultivars to infections by D *V. dahliae* and the lessened ability to recover from them (60,61,111,140,144).

Preplanting Measures

Choice of site for establishment of new orchards. Selection of a proper field site to establish an olive orchard should be done with care. Accurate information on the disease history of susceptible crops of the soil to be planted is helpful; particularly with regard to those such as cotton for which cross virulence of D *V. dahliae* is known to occur (see "Population biology", above). Also, planting sites near neighboring fields with a known history of susceptible crops should be avoided because of the likelihood that sources of inoculum of varying nature could be transported to the site by different means of dispersal (see "Pathogen survival and dispersal", above). Fields cultivated with nonhost crops such as gramineous plant species should be preferred. However, this does not ensure absence of *V. dahliae* in the soil because gramineous plants are not immune to infection by the pathogen, which can also persist through infection of symptomless carrier weeds. On the other hand, susceptible crops near or in the candidate field for olive planting may already serve as indicator plants if they show wilting symptoms.

Site selection should ultimately be made based on assessment of inoculum density and distribution in soil, and identification of the prevailing pathotype. In particular, information on VCG or pathotyping of *V. dahliae* isolates prevailing in potential planting sites would allow the avoidance of soils infested with highly virulent isolates of VCG1A/D. In this latter case, disease risk assessment based on the relationship between inoculum density and disease incidence can be done by estimating the inoculum density in soil at planting sites, thus avoiding sites with high risk for severe disease (142; see "Epidemiology and Diagnosis", above). Quantifying *V. dahliae* microsclerotia in soil faces two main difficulties: (i) random versus aggregate distribution of the pathogen propagules in soil, which needs to be considered while designing a strategy for sampling a field; and (ii) protocols for assaying the pathogen in the soil that is collected. Existing methods used to quantify *V. dahliae* microsclerotia in soil were reviewed by Gould and Termorshuizen (88). Methods often used in risk analysis studies are based mainly on dilution plating, dry sieving, or wet sieving, and incubating on semiselective media such as modified NP-10 medium at optimum growth temperature for 2 to 6 weeks (118). All of those methods require a 4- to 6-week time period for drying the soil before analysis to kill conidia and mycelium of *V. dahliae* and to annul fungistatic dormancy of microsclerotia imposed by bacteria, and subsequent grinding and mixing of the soil sample (39). Also, results can be influenced by a number of factors, including soil characteristics (e.g., texture), amount of soil plated, culturable soil microbiota, and the skill of operators to differentiate the typical star-shaped microsclerotial colonies formed from plated *V. dahliae* soil microsclerotia under the stereomicroscope (88,225). Soil dilution plating consists of suspending an amount of air-dried soil in sterile water (e.g., 10 g soil in 100 ml water) in flasks, mixing by continuous stirring, and plating an aliquot of the suspension (172). The dry sieving method consists of distributing soil particles as evenly as possible onto agar plates placed in stages of an Andersen air sampler; this allows soil particles impacting onto agar to be selected according to the size of holes in the botton of a stage (39). The wet sieving method includes sieving the suspension of dried, ground soil to recover the 35 to 150 μm soil fraction in water (105). An interlaboratory comparison of dry and wet sieving methods concluded that they do not differ in detection limits, but the former is less variable at higher inoculum densities and more variable at lower ones than wet sieving (88,225). Nicot and Rouse (172) found that inoculum levels estimated by dry sieving and dilution plating were 2 to 3 times higher than those estimated by wet sieving. Dry sieving was found to be unbiased (i.e., nearness to true value) and efficient but also the least precise (i.e., higher standard error) of the three methods compared, whereas dilution plating was the least time-consuming, had the highest recovery rate, and was moderately variable.

Assessment of *V. dahliae* inoculum density in soil has also been approached by use of bait plants properly cultivated in unknown soils under standardized conditions and subsequent isolation of the pathogen from them. Reported detection limits of those bioassays were 1 to 3 ms g^{-1} soil (73,168,217). However, the baiting assays have not reached large-scale application. Both the plating and baiting methods suffer from inconsistency and soil-type dependence, and are time-consuming and not informative of *V. dahliae* pathotypes infesting the sampled soil.

Research to overcome those limitations has been explored by the use of serological and molecular detection methods. Enzyme-linked immunosorbent assays using the double-antibody sandwich technique detected as little as 2.4 μg ms g^{-1} soil, corresponding to 1 to 2 ms g^{-1} soil (101); however, application of this assay in practical conditions remains challenging. Several protocols have been reported for the quantification of *V. dahliae* DNA extracted from soil. Use of the same INTD2f/2r and INTND2f/2r primer pairs and a nested-PCR protocol for in planta detection together with an efficient method for grinding soil and extracting total DNA from it allowed differential detection of D and ND *V. dahliae* from artificially and naturally infested soil (181). However, no PCR-based

detection protocol has yet been published that can quantify the density of microsclerotia in soil based on estimates of pathogen DNA.

Use of *V. dahliae*–free planting material. The natural recovery from symptoms of Verticillium wilt allows for asymptomatic infections in olive trees (133,161). Thus, selection of planting material based solely on absence of disease symptoms can lead to the spread of *V. dahliae* in planting material originating from apparently healthy plants. The role of nurseries in spreading *V. dahliae* in olive has been documented (173,226). Therefore, the practice of producing olive planting stocks based on visual diagnosis requested under the *Conformitas Agraria Communitatis* mandatory in the EU member countries should be replaced by implementation of true certification systems that guarantee the absence of *V. dahliae* from plant tissue and soil of root-balls (111,173). Use of planting stocks truly certified to be free of *V. dahliae* will optimize the efficiency of selecting planting sites for establishing new orchards.

Soil disinfestation. Soil fumigation, soil solarization, and organic amendments, either individually or in combination, are useful for reducing *V. dahliae* inoculum in soil and have been successfully used for the control of Verticillium wilt diseases (179). However, these control measures are not yet common preplanting practices in olive production as they are in intensive horticulture.

Broad-spectrum soil fumigants used for the control of Verticillium wilts include methyl bromide, chloropicrin, methyl isothiocyanate–generating compounds (e.g., dazomet, metam-sodium, metam-potassium), and 1,3-dicholopropene, either alone or in mixtures. The efficiency of soil fumigation can be curtailed by either survival of *V. dahliae* microsclerotia in soil layers below the depth of effective fumigation, or reintroduction of the pathogen inoculum through infected planting material, infected olive leaves, or infested irrigation water and crop debris. The combination of methyl bromide + chloropicrin is considered to be synergistic and more effective than either single fumigant alone (67,257). Methyl bromide is scheduled for worldwide withdrawal from routine use as a fumigant in 2015 under the directive of the Montreal Protocol on ozone-depleting substances. Chloropicrin seems to be the best replacement for methyl bromide (93). Methyl isothiocyanate is prone to enhanced biodegradation in soil by adaptation of microbial populations to metabolize the compound as an energy source. This adaptation may be induced by repeated or even single applications of methyl isothiocyanate–generating formulations to a field, thus seriously compromising its efficacy (155). None of the fumigants tested against Verticillium wilt diseases have satisfied the harmonizing criteria established by the EU within the framework of EEC Directive 91/414 and will not be allowed for use in the future. A recent revision of nearly 1,000 phytosanitary active ingredients marketed since 1993 concluded that only 71 fungicides and 16 microbial biocontrol agents, but no fumigants are now approved for use in the EU. Many of fumigants are also subjected to increased regulations in other countries besides the EU because of environmental and health concerns and may not be available in the near future; therefore, they do not seem to be viable long-term options for Verticillium wilt management over the long term (221).

Soil solarization is a hydrothermal process that occurs when thoroughly tilled, moist soil is covered with thin (25 to 50 μm), transparent polyethylene or polyvinyl plastic sheets tightly anchored to soil during a period of high temperature and intense solar radiation (Fig. 8). Soil solarization has become a widely and extensively used technology for the management of soilborne plant pathogens after the pioneering work of Katan et al. (122). In this landmark publication, those authors showed that soil solarization for 2 weeks during summer in Israel reduced the populations of buried inoculum of *V. dahliae* by 94 to 100% at 5 cm, 67 to 100% at 15 cm, and 54 to 74% at 25 cm. This effect is based mostly on high thermal sensitivity of *V. dahliae* to temperatures above 40°C. Average maximum temperatures of the solarized soil were 50.7°C at 5 cm and 40.8°C at 15 cm, while the average maximum temperatures of the nonsolarized soil were 37.6 and 32.4°C, respectively. The thermal decline of the pathogen during solarization depends on both soil temperature and exposure time, which are inversely related. Since the upper layer of soil is heated more intensively than the lower ones, the solarization period should last at least 4 to 5 weeks to achieve control at all desired depths; therefore, the longer the solarization, the greater the depth of effective activity, and the higher the pathogen-killing rates. In the Mediterranean Basin, solarization treatment should be practiced during July and August. Sublethal heating may have a weakening effect in surviving *V. dahliae* microsclerotia and reduce their inoculum potential as demonstrated for chlamydospores of *Fusarium oxysporum* ff. spp. (11,84). That effect may facilitate a synergistic interaction between weakened *V. dahliae* microsclerotia and microbial antagonists or low dosages of fumigants leading to an increased effectiveness of combining both soil treatments for disease management (179,238).

Soil solarization has not yet been tested at a large scale as preplanting practice in olive production. However, soil solarization has proved to drastically reduce the number of *V. dahliae* microsclerotia in soil resulting in a variable degree of Verticillium wilt control in numerous annual crops, including artichoke, cotton, strawberry, tomato, etc. For instance, preplanting soil solarization successfully controlled Verticillium wilt of artichoke in Greece, showing even a long lasting effect over multiple seasons (237). Similarly, Melero-Vara et al. (156) found that solarization of clay soil in southern Spain reduced the population of D *V. dahliae* in the 0- to 40-cm soil layer to very low or even undetectable levels and efficiently controlled Verticillium wilt in cotton. For olive production, preplanting soil solarization can be practiced over the entire field or limited to bands over a row to be planted or a 9 to 16 m^2 area comprising a planting hole (Fig. 9A and B). These limited treatments of soil solarization may be adequate to protect young planting stocks from infection during the highly susceptible juvenile stage and can be useful for replanting after removal of affected trees (R. M. Jiménez-Díaz, *unpublished*).

Limited disinfestation of olive planting sites could also be achieved with organic amendments. Organic amendments cover a range of inputs, including animal (cattle, poultry, swine) and green manures, composts, high N-containing products (blood, bone and meat meal, fish meal, soy meal, etc.). Efficient use of organic materials in the management of soilborne plant pathogens is undermined by a still-limited understanding of the mechanisms involved and factors influencing their efficacy (16). Elegant research by Lazarovits and co-workers has convincingly shown that production of ammonia (NH_3) and nitrous acid (NO_2H) upon microbial degradation of N-containing products eradicates soilborne *V. dahliae* microsclerotia, but the efficacy of these materials is related to soil properties, e.g., amendment of an acidic sandy soil with high-N products at 2% soil mass killed most *V. dahliae* microsclerotia in within a week, but the same rate had no effect on microsclerotia viability in an alkaline loam soil (131). Accumulation of nontoxic ammonium (NH_4^+) from that microbial degradation increases soil pH and favors some NH_4^+ being converted to toxic ammonia, with an equilibrium between NH_4^+ and NH_3 being reached at high pH (8.5 to 9.5). However, this accumulation and production of NH_3 are impaired by nitrification (NH_4^+ to NO_2^- and NO_3^-) in soils with high organic C. Enhancement and maintenance of NH_3 formation can be achieved by inhibiting nitrification in organic soils or diluting the organic matter content to below 2% by addition of sand. Conversion of NH_4^+ to NO_2^- results in lowering the soil pH, and when it falls below 5.5, some NO_2^- is converted into NO_2H, which is 300 to 500 times more toxic than NH_3 to *V. dahliae* microsclerotia. This higher toxicity allows for eradication of the pathogen by 2 to 3 weeks after application of rates (0.25 to 0.50%) of high-N products lower than that needed for eradication through formation of NH_3. Amendment of soil with liquid swine manure (55 hl ha^{-1}) is also effective in eradicating *V. dahliae*, but again soil pH is critical to the activity of the amendment. At pH lower than 5, the eradicating activity against *V. dahliae* microsclerotia is related to formation of NO_2H and presence of the nonionized, acidic form of volatile fatty acids, with acetic acid representing 60% of the active

ingredients, and butyric, caproic, isobutyric, isovaleric, propionic, and valeric acids the remainder. However, at pH > 8.5, the killing of those propagules is due to formation of NH_3 (57,131).

Biofumigation (i.e., suppression of pathogens by release of biocidal products after microbial degradation of fresh plant material incorporated into soil) can also be efficient in the eradication of *V. dahliae*. For instance, Blok et al. (34) showed that *V. dahliae* was reduced if soil amended with 3.4 to 4.0 kg fresh weight m^{-2} of fresh broccoli or grass were covered with an airtight plastic sheet, but the pathogens were not or only slightly inactivated in amended, noncovered soil or nonamended, covered soil. This suggests that the eradicating activity may be due to anaerobic and strongly reducing soil conditions that develop in the covered soil. Nevertheless, additional mechanisms might be involved in the eradication of *V. dahliae*, including release of biologically active products such as isothiocyanates, volatile fatty acids, and ammonia (131), and/or the increase of total microbial populations in soil, many of which can be antagonists to the pathogen. Organic amendments and biofumigation have not yet been tested in extensive field experiments as a preplanting practice in olive production.

Protection of healthy planting material from infection by residual or incoming inoculum after planting. The efficacy of combining soil disinfestation with use of pathogen-free planting material in the management of Verticillium wilt of olive would be enhanced if the plant root system were further protected from infection by *V. dahliae* inoculum residual in soil, or incoming inoculum in infected olive leaves, infested debris or irrigation water, by using biocontrol agents (58). Furthermore, such a protection can reduce the potential for severe Verticillium wilt in young trees and facilitate expression of the natural recovery from symptoms.

Several studies have identified bacterial and fungal strains with potential for effective biocontrol of Verticillium wilt in olive, but none has yet been tested for its efficacy at the nursery or orchard level. For example, Mercado-Blanco et al. (159) found that root treatment with two *Pseudomonas fluorescens* strains isolated from nursery-propagated 'Picual' plants significantly reduced Verticillium wilt in 3- to 4-month-old 'Picual' plants in a monocycle of infection with the D pathotype under growth chamber or greenhouse conditions. Interestingly, the most efficient strain, PICF7, neither inhibited pathogen growth in vitro nor colonized the olive rhizosphere at high densities. Further work using fluorescently tagged isolates and confocal laser scanning microscopy indicated that the PICF7 strain is endophytic in olive roots and led to the speculation that direct antagonism against *V. dahliae* may be important in biocontrol (189). Unfortunately, that work was not extended to determine whether or not bacterization provided a long-lasting effect against reinfections by the pathogen under nursery production conditions or after planting in the field. Similarly, *V. dahliae*–antagonistic strain HRO-C48 of *Serratia plymuthica* from oilseed rape rhizosphere reduced Verticillium wilt severity to some extent in olive plants in greenhouse bioassays using the D pathotype, but its efficacy under nursery production conditions or after planting in the field was not determined (167). Recently, Jiménez-Díaz et al. (114) showed that a commercial formulation of *Trichoderma asperellum* strain ICC012 + *T. gamsii* strain ICC080 consistently reduced severity of infections by D *V. dahliae* by over 30% in micropropagated or self-rooted planting stocks of 'Picual' olives under conditions conducive for severe disease in growth chambers. Furthermore, transplanting *Trichoderma* spp.–treated 'Picual' plants to D *V. dahliae*–infested soil in microplots, and additional treatment at planting, also suppressed Verticillium wilt severity by 60% and prevented tree mortality from infection 2 years after planting; it did not, however, reduced disease incidence. During that time, recurrent infections from inoculum provided by infected leaves fallen from affected trees in untreated control microplots led to 100% incidence and 30% mortality.

Studies in other *Verticillium* pathosystems suggest that other microbial antagonists of *V. dahliae* might be effective against Verticil-

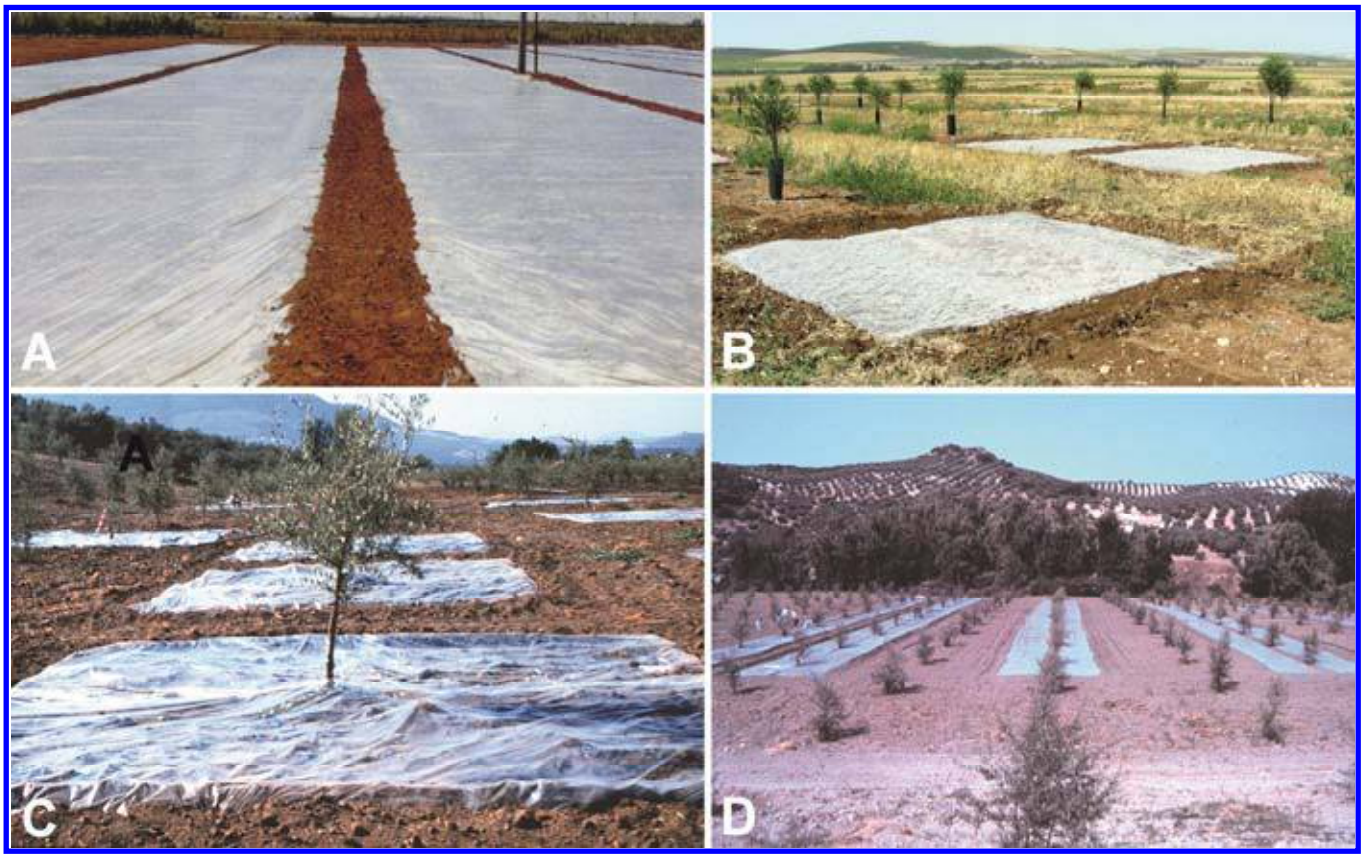

Fig. 9. Soil solarization for management of Verticillium wilt in olive practiced: **A,** over the entire field prior to planting; **B,** limited to an area comprising a planting hole prior to replanting diseased trees; and **C and D,** in established orchards by covering the soil under affected trees either individually **(C)** or on a line **(D)**. Figures C and D are courtesy of F. J. López-Escudero.

lium wilt in olive. For example, Antonopoulos et al. (9) reported that *Paenibacillus alvei* strain K165 reduced germination of *V. dahliae* microsclerotia by 50% in the tips and zone of elongation of eggplant roots, and by 26 and 40% at 10 and 12 cm away from root tips and in soil without plants, respectively. This bacterial strain triggered induced systemic resistance in eggplants against *V. dahliae* and reduced disease severity and microsclerotia germination by 27 and 20%, respectively. Also, *P. alvei* K165 successfully controlled Verticillium wilt of eggplants and potato in greenhouse experiments (236,239). Similarly, *Talaromyces flavus* appears to be a suitable candidate for the biocontrol of Verticillium wilt in olive according to its ability to kill *V. dahliae* microsclerotia and effectively suppress Verticillium wilt in several vegetable crops, both in growth chamber and field experiments (82,83,150,168,238). In contrast, use of arbuscular mycorrhizal fungi such as *Glomus claroideum*, *G. intraradices*, and *G. mosseae* in the control of Verticillium wilt in olive has yielded contradictory results, although those mycorrhizae effectively promote olive growth (115,120,184).

Use of resistant cultivars and rootstocks. Use of resistant cultivars or rootstocks is the best long-term and economically efficient control measure for Verticillium wilt in olive, and should be the core of integrated disease management strategies. However, resistance has not been widely used to manage Verticillium wilt in olive. Rather, choice of olive cultivar for establishing new orchards has been guided mainly by pedoclimatic conditions and market requirements, and most cultivars satisfying them are susceptible to Verticillium wilt. For example, modernization of olive cultivation in southern Spain during the last decade has led to an increase in crop area to 1.5×10^6 ha, but such an expansion has been largely based on the use of two main cultivars, namely 'Arbequina' and 'Picual', which are most demanded by the market. These two cultivars are highly susceptible to the D *V. dahliae* pathotype.

During the last 50 years, evaluations for Verticillium wilt resistance of near 200 commercial olive cultivars and wild olive germplasm accessions have been carried out in several olive-growing countries, including Greece, Italy, Spain, Turkey, and the United States (California) (8,37,49,52,71,143,144,151,212). Evaluation of resistance was done earlier in naturally infested orchards and more recently under controlled conditions using standardized protocols and *V. dahliae* isolates of the D or ND pathotypes. In this latter case, own-rooted plants, usually 6 to 36 months old, are inoculated by dipping their bare root system in a 10^7 conidia ml^{-1} suspension for 30 min (144,194,212), injecting a heavy conidia suspension (0.1 to 4×10^8 conidia ml^{-1}, 100 to 500 µl) into a hole drilled in the stem (8,71,143), or transplanting the plants into soil infested with 3 to 10 ms g^{-1} soil (8). Resistant phenotypes are identified based on the severity of symptoms in inoculated plants (8) or the area under disease progress curve (AUDPC) and percentage of dead plants, with cultivars having AUDPC $\leq$ 10% or AUDPC = 11 to 30% and low percentage or no dead plants being rated highly resistant and resistant, respectively (71,143,144,212). Root dipping and stem injection are equally effective in resistance evaluation of olive to Verticillium wilt and more reliable than transplanting into infested soil (8,49,143).

Comparisons of results from resistance evaluations can be masked by uneven distribution of the pathogen in soil, the efficacy of inoculation methods and/or incubation conditions, and/or erroneous cultivar nomenclature (or synonyms) and intravarietal variability. For example, cultivars designated with the same name may actually be different (erroneous names), and, by contrast, clones with different names may be the same cultivar (e.g., 'Oblonga' seems to be a clone of 'Frantoio') (18). In particular, lack of information on the nature of the testing pathotype may mislead comparisons. López-Escudero et al. (144) showed that cultivars highly resistant to the ND pathotype (e.g., 'Coratina', 'Fragivento', 'Manzanilla de Sevilla', 'Verdial de Alcaudete') are highly susceptible to the D pathotype. 'Changlot Real', 'Empeltre', 'Frantoio', and 'Oblonga' have been repeatedly shown to possess a degree of resistance against D *V. dahliae* under artificial and natural infections (37,50,143,144,151). 'Kalamon' and Koroneiki' were rated moderately resistant to the D pathotype in Greece and Spain (8,144,148,149). 'Changlot Real', 'Empeltre', 'Frantoio', 'Oblonga', and 'Koroneiki' are consistently resistant to the ND pathotype across Mediterranean locations (8,144,148,149). No differential interactions have yet been identified between olive cultivars and *V. dahliae* pathotypes. Recently, 'Sinop No. 1', 'Eğriburun Nizip', 'Erkence', 'Eğriburun Tatayn', 'Girit Zeytini', and 'Marantelli' olives were found to be highly resistant to the D pathotype in Turkey, in assays where 'Arbequina' and 'Frantoio' were rated as resistant (71). In all cases, the resistant reaction of olive cultivars does not exclude the pathogen from reaching the upper plant parts and being isolated from symptomatic or symptomless leaves. This has led some authors to use the term 'tolerance' when referring to the disease reaction to inoculation. However, both histopathological observations and quantification of the pathogen DNA in root and stem tissues convincingly indicated that colonization by the pathogen is hindered in resistant plants and thus should be considered true resistance (8,144,148).

Resistant rootstocks would be of much interest for production of Verticillium-susceptible olive cultivars in soils infested with D *V. dahliae.* In the last few decades, efforts have been made to identify olive clones and wild olives that would be of use for rootstocks, including 'Oblonga' (98), clone Berkeley 117 (256), seven 'Yarmouk' lines (5), and more recently one resistant rootstock in Greece (8) and four wild olives named OffVert, OutVert, and StopVert (52; M. Cirulli, *unpublished*) and D36 (71). Grafting of susceptible cultivars may be carried out either on resistant rootstocks or resistant and tolerant cultivars. Its effectiveness in the control of Verticillium wilt of olive has been demonstrated by several authors (185,234). Recently, Bubici and Cirulli (36) found that grafting susceptible 'Coratina' and 'Lecino' onto resistant 'Frantoio' as a rootstock provided excellent control against infection by D *V. dahliae*. Grafting of 'Frantoio' onto the susceptible cultivars did not provide protection from disease, suggesting that the resistance of 'Frantoio', is determined in the plant roots.

Postplanting Measures

Cultural practices. Proper management of an olive orchard can mitigate risk of severe Verticillium wilt by reducing inoculum spread, its increase in soil, and/or its efficacy in causing new infections. To those aims, it is recommended to: (i) avoid intercropping olive with susceptible crops (3,30,47); (ii) minimize the frequency of plowing since it can increase disease incidence through inoculum spread and mechanical wounding of the root system (3,4,234); (iii) avoid use of tilling machinery and vehicles previously employed in Verticillium-affected orchards, and eventually disinfest them, to prevent transportation of *V. dahliae* inoculum from one field to another (258); (iv) control weed infestations periodically, particularly dicots, and preferably by means of herbicides (not tilling) (229); (v) use drip irrigation in preference to flood and furrow methods to avoid spread of soil inoculum within orchards (228); (vi) prune affected trees in the summer to enhance recovery from disease (133) and before they lose their leaves in late winter to prevent inoculum increase by microsclerotia formed in them; also, fallen green leaves from trees infected by D *V. dahliae* should be eliminated when possible (112,171,234,240); (vii) remove and burn pruning debris from affected trees; and (viii) avoid use of refuse and residues from olive-oil extracting industry as organic amendment.

Soil solarization. Soil solarization can be applied in orchards already established just as it is practiced on bare soil, by covering the soil under affected trees with a transparent polyethylene sheet slightly larger than the tree canopy diameter (see "Soil disinfestation", above). This solarization can be practiced on individual trees or tree lines (139) (Fig. 9C and D). Prior to solarization, weeds under a tree should be eliminated and trees should be pruned to remove symptomatic branches and allow for optimum solar irradiation of soil. After solarization, soil rotovations should be avoided, and weeds should be controlled chemically (234). Tjamos et al. (235) found that effectiveness of solarization of established olive

orchards in Greece includes induced recovery of solarized trees from disease that lasted at least 3 years. The induced recovery was attributed to lack of root reinfections and, in some cases, significantly exceeded the natural recovery: 30 to 87% of recovery in treated plots compared with 37.5 to 50% in the unsolarized controls. Solarization nearly eradicated *V. dahliae* microsclerotia from soil and increased the population of the antagonist *T. flavus*. However, *V. dahliae* inoculum increased and *T. flavus* population decreased in the 2 years after solarization. Solarization reduced mycorrhizae in olive roots, but there were no observable deleterious effects on trees (235). Also, solarization of 3- to 7-year-old trees for 5 to 8 weeks in southern Spain significantly reduced *V. dahliae* microsclerotia in the top 20 cm of soil for at least 3 years, even though that did not correspond to a similar reduction in Verticillium wilt severity (139). Solarization for two consecutive years did not improve the effects of single solarization on control of the disease. López-Escudero and Blanco-López (139) also observed increased natural recovery from Verticillium wilt in some experiments, but solarized trees did not show significant growth increase as determined by measuring the trunk perimeter. Similarly, in Crete, soil solarization of 15- to 20-year-old trees for 12 weeks reduced the number of *V. dahliae* microsclerotia by 97.3 to 85.8% at depths of 0 to 10 cm and 41 to 50 cm, respectively (213). Inconsistency in the effectiveness of soil solarization may be attributed to high inoculum and incorrect application of the method and/or differences in the biotic or abiotic characteristics of the soil.

Al-Ahmad (2) used a particular solarization device to solarize entire olive trees in Syria. Besides mulching the soil, a solar-chamber consisting of a metal framework covered with a plastic sheet was placed over the trees. Using this technique, temperatures reached 55°C in the solar-chamber, and 55 and 45°C at a depth of 5 and 15 cm in soil, respectively. *V. dahliae* could not be isolated from infected trees after 15 to 20 days of treatment, and growth of solarized trees improved compared to nonsolarized controls. No data were reported on disease incidence and severity (2). The solar-chamber was found effective in the control of Verticillium wilt in Jordan either alone or in combination with cryptonol (8-hydroxyquinoline sulfate) and fertilizer treatments (1).

Chemical control. Control of Verticillium wilt in olive by chemical treatment, mainly systemic fungicides, has been approached in several studies since the 1970s, including soil drenching, foliar spray and trunk injection with benomyl, carbendazim, phosetyl-Al, their mixtures with quinosol or prochloraz, etc. (80,166,182,224,244). Overall, none of the tested fungicides successfully controlled the disease under field conditions, and currently available fungicides are not effective to control Verticillium wilt in olive (234,244).

Organic or biological amendments. Early hypotheses about mechanisms underlying the natural recovery of affected trees from disease led Wilhelm and Taylor (258) to recommend amending the rhizosphere soil of affected trees with organic matter (manure, wood sawdust, etc.) to increase activity of microbial antagonists that would facilitate protection of roots from new infections by *V. dahliae*. Use of animal manure should take into account that it may disseminate *V. dahliae* inoculum (4,138). Also, use of organic amendments should be considered with caution because of the possibility of the amendments releasing phytotoxic substances, including volatiles, depending upon their nature and dosage. Overall, however, little research has been done on this subject, including the mode of action of amendments. Microbial agents of use for the protection of healthy planting material (see "Protection of healthy planting material from infection by residual or incoming inoculum after planting", above) could also be employed for postplanting applications. Soil amendment with a selected cured compost obtained from olive oil by products, alone or together with *T. viride*, reduced the number of microsclerotia of *V. dahliae* (137).

Future Prospects for Effective Disease Management

Verticillium wilt is endemic in olive-growing areas worldwide, but it has become an increasing threat to olive production and industry during the last two decades. This increase has occurred concomitant both with changes in cropping practices for increasing olive yields (e.g., irrigation, increased plant density, use of cover crops, mechanization, etc.) and the expansion of olive crops to areas in different regions previously cultivated to cotton, as exemplified in Greece, Spain, and Turkey, together with the spread of the highly virulent D pathotype of *V. dahliae*, as shown in Spain and Turkey. Those factors are likely to recurrently impact olive crops as steps are taken toward the modernization of olive production. Therefore, there is a need of further improving our understanding of how those factors favor development of the disease, as well as the efficiency of strategies for its management.

Effective management of Verticillium wilt in olive is best achieved by means of IDM strategies. These strategies should be based mainly on the use of preplanting control measures that reduce risks of early infections of young trees and enhance natural recovery from symptoms, together with postplanting measures that mitigate risks of pathogen spread and recurrent infections of trees. Use of resistant cultivars would enhance the efficacy of other disease control measures incorporated into an IDM strategy. However, most currently available olive cultivars of commercial interest are susceptible to the D pathotype, which should be the main target for management of the disease. This factor, together with regulations on availability and use of soil fumigants as a result of environmental concerns, are important constraints for the efficient management of this disease.

Development of olive cultivars of commercial interest with improved resistance against D *V. dahliae* is of utmost importance for management of Verticillium wilt. Use of available qPCR protocols should help in indentifying valuable genotypes with true resistance to the pathogen. In addition, use of available resistant cultivars and rootstocks would allow the establishment of Verticillium-susceptible olive cultivars in soils infested with D *V. dahliae.* Conventional olive breeding can face difficulties when combining resistance to *V. dahliae* with agronomic yield or quality traits. Recent research has shown that olive is amenable to genetic transformation through use of selected strains of *Agrobacterium tumefaciens* and subsequent regeneration via somatic embryogenesis, thus offering new possibilities for the biotechnological improvement of olive (44,241). Whether increased resistance to *V. dahliae* in olive is developed by conventional breeding or biotechnological approaches, the possibility of resistance-breaking strains to evolve from resident *V. dahliae* populations as shown for lettuce, sunflower, and tomato (7,94,250) should not be disregarded. The hypothesis that D strains of *V. dahliae* VCG1A might have emerged independently multiple times in different places, as suggested by genetic differences between populations of the pathogen in Spain and Turkey (55,60), deserves to be addressed since multiple origins may be associated with hidden differences in virulence.

Olive planting stocks should be produced under true *V. dahliae*–free certification schemes to replace current EU requirement under *Conformitas Agraria Communitatis* to guarantee use of healthy planting material and rooting substrates by olive producers. The availability of PCR-based protocols for in planta detection of the pathogen would be of use for that aim. The effectiveness of partially resistant cultivars and/or resistant rootstocks and use of *V. dahliae*–free planting material in Verticillium wilt management would be optimized by proper selection of planting site through preplanting assessment of *V. dahliae* population density in soil. Methods presently available for quantifying *V. dahliae* in soil, based on plating soil on semiselective media, are not sensitive or reliable enough to assure efficient implementation of that approach. These limitations can be overcome if new DNA-based methods are developed that would allow sensitive and reliable quantification of specific strains of the pathogen in soil. Development of these methods should be a priority in studies aimed at management of Verticillium wilt of olive.

Soil solarization is useful for suppressing soilborne inoculum of *V. dahliae* and management of Verticillium wilt in olive in the absence of effective soil fumigants. The use of this technique and its

effects on disease suppression can be further enhanced if combined with organic soil amendments and treatment of planting stock with biocontrol agents. Organic soil amendments and formulations of biocontrol agents are becoming commercially available. Their use for soil disinfestations targeting individual olive planting sites should not be disregarded. Nevertheless, better understanding is needed of the mechanisms whereby organic amendments interact with soil microbiota and how that contributes to increased disease suppression, as well as on factors that determine sustained efficiency of biocontrol treatments in the control of the disease. Demonstrating that an organic amendment has the potential to consistently induce resistance to *V. dahliae* in the tree would make it particularly valuable for postplanting treatments of Verticillium wilt–affected olives.

Irrigation is one of the most significant factors affecting the severity of Verticillium wilt in olive, but the relevant mechanisms contributing to this effect under field conditions have not been investigated. The role of irrigation on host versus pathogen versus host–pathogen interaction need to be investigated. Such research addressed to understand the role(s) of irrigation in Verticillium wilt of olive would provide elements for harmonizing irrigation management as agronomic practice and postplanting control measures to mitigate disease development. Management of soil moisture through irrigation practices was shown to reduce root infection by *V. dahliae* in irrigated potato crops (188).

The significant role of infected, fallen leaves as a potential source of *V. dahliae* microsclerotia for development of secondary cycles of pathogenesis, particularly of the D pathotype, points out an urgent need for research to develop control measures targeting this source of inoculum. Development of treatments with microbial agents or new fungicide active ingredients aimed to that task for the management of Verticillium wilt of olive merits research efforts. Similarly, solarization of affected trees in established olive orchards and management of cover crops can be of use as postplanting control measures and should be further investigated for increased efficiency in the integrated management of the disease. Solarization of individual trees can be combined with the application of biocontrol agents and/or organic amendments to extend the efficacy of control against incoming pathogen inoculum. Intercropping olive orchards with cover crops to reduce risks of soil erosion is becoming an extensive practice in modern olive production. Cover crops mostly used in Spain include barley, oat, vetch, and several Brassicaceae species (e.g., *Eruca vesicaria*, *Moricandia moricandioides*, *Sinapis alba*, etc.). However, these plants need to be planted with caution until more research reveals the nature of the interactions between *V. dahliae* and plants traditionally regarded as nonhosts, since some can potentially serve as a reservoir of the pathogen. Long-term, replicated field studies in different production areas should be conducted to determine the effects of that practice on *V. dahliae* strains and microbial populations in the orchard soil and development of Verticillium wilt in olive.

Finally, the integrated management of Verticillium wilt in olive, as is the case for other plant diseases, requires skillful solutions for crop health problems faced by current agricultural production. The practice of IDM strategies requires involvement of well-trained professional plant pathologists able to implement the tenets of that concept at the local level, as well as to incorporate into decision-making frameworks new knowledge and technologies that may be developed from scientific research. As the demand has increased for knowledgeable practitioners capable of integrating multifaceted controls in rigorous IDM programs, institutional support has been reduced through declining or even despairing university education in plant pathology and the loss of extension-related activities in commercial agriculture. Erosion at the top of the trickle-down structure responsible for knowledge transfer to the field is one of the most serious threats to IDM. Lack of appropriate and specific training in plant pathology has consequences: (i) seriously limits proper communication among those that at different levels may be involved in strategic actions concerning IDM programs; (ii) makes more difficult the transferring of new knowledge and technologies derived from research; and most importantly (iii) limits an adequate social perception of the true nature and magnitude of plant diseases as a threat to food production.

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