

Verticillium wilt of olive and its control: The heat is on

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Plant and Soil recently published a comprehensive and critical Marschner review about Verticillium wilt (López-Escudero and Mercado-Blanco 2011), one of the most threatening biotic constraints for olive (*Olea europaea* L.) production. The authors reviewed research efforts aimed to understand the diversity of the causal agent (the soil-borne fungus *Verticillium dahliae*), the mechanisms underlying the interaction between the host plant and the pathogen, and the range of factors contributing to this disease becoming a major phytopathological problem in many traditional olive-growing areas.

Since currently available measures to control Verticillium wilt of olive have proven ineffective when applied individually, an integrated management strategy is required to prevent significant economic losses, and more importantly, to avoid pathogen spread to new geographical areas where olive is increasingly being introduced (Tjamos 1993; Tjamos and Jiménez-Díaz 1998; López-Escudero and Mercado-Blanco 2011). Such a holistic strategy, encompassing biological, chemical, cultural and physical control measures, was presented and discussed as the only conceivable framework for an effective management of the disease.

Despite the indisputable historical and socio-economic importance of olives in specific geographical areas (i.e., the Mediterranean basin), and Verticillium wilt being a major threat to nurseries and to olive oil/table olive industries, this could be considered as a topic of regional interest, at least a priori. However, attention garnered by the review by López-Escudero and Mercado-Blanco (2011) has exceeded the authors' expectations. During the first 3 months after publication (July–September 2011), the electronic version of the article was downloaded more than 1000 times from the *Plant and Soil* web site. This confirms that the compilation effort was a worthy task, probably catching the attention of researchers in diverse fields such as soil microbiology, plant pathology, agronomy, epidemiology, molecular biology, etc., and by extension plant pathologists, olive farmers and researchers not directly involved in this pathosystem.

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In addition, the authors' received comments from colleagues elsewhere require us to respond here. First, despite our extensive literature search, some references were inadvertently overlooked. For instance, Verticillium wilt of olive was reported in Argentina by Docampo et al. (1981), an early report that was not detected in our survey. This study is important because it documented the presence of the disease in olive-growing areas of South America, and that it could probably be traced back as early as the 1950s, when a syndrome resembling Verticillium wilt was observed in olive trees (Nicolini and Traversi 1950). Second, we failed to encounter the report on the detection of *V. dahliae*-infected olive trees in Montenegro (Latinovic and Vucinic 2010), a country that must be added to the list of places where the disease has been described suggesting that it is perhaps present in all areas where olive is cultivated. Finally, it is worth mentioning the important body of knowledge that Iranian researchers have gathered over the years on Verticillium wilt of olive. Some of their studies were opportunely mentioned in our paper, but others escaped our literature search. As extensive as the research on Verticillium wilt of olives in Iran, publications from these efforts are unavailable to wider research community because they are published in Farsi (Persian language) journals or books (for instance, Sanei et al. 2010). We thank colleagues who alerted us to this wider body of literature. Our literature survey focussed on broadly-accessible databases such as ISI web of Knowledge, SciVerse Scopus, with the exception of some references from national journals, meeting proceedings, or even PhD Theses, when they were thought to be essential for illustrating a specific comment. We obviously could not source research published in technical reports, meeting abstracts and research articles in local languages, etc. Nonetheless, we trust that most of the relevant, peer-reviewed, and easily-accessible reports and research articles were compiled in our appraisal.

Because of the alarming expansion of the disease, the heavy losses caused to this strategic commodity in the Mediterranean region, and the difficulties associated with controlling the pathogen have likely fuelled the number of studies and associated publications (for instance, Tsror 2011). Between the submission and publication of our review, a number of interesting papers were published that could not be cited. It is pertinent to briefly review some of them here since they enhance our understanding of the relevant aspects of the disease.

Lo Giudice et al. (2010) informed us that decline of young olive trees in Sicily is caused by simultaneous infections of *V. dahliae* and *Phytophthora palmivora*. The possible implications of synergistic interactions between diverse species of this Oomycete genus and *V. dahliae* were already highlighted (López-Escudero and Mercado-Blanco 2011). On the other hand, new epidemiological studies confirm that the most damaging *V. dahliae* pathotype (defoliating, highly virulent, belonging to vegetative compatibility group 1A [VCG1A]) is expanding alarmingly (Triki et al. 2011), and is clearly prevalent over other genetic and/or molecular groups of the pathogen in olive-growing areas in southern Spain (López-Escudero et al. 2010; Jiménez-Díaz et al. 2011). These surveys, and also that reported by Rodríguez et al. (2011), also provide interesting information on the correlation between several agronomical factors and the prevalence of *V. dahliae* (or some of its pathogenic groups) in defined areas. They also demonstrate that PCR-based tools are routinely and consistently used for the effective assignment of *V. dahliae* isolates to a given genetic (VCG) or pathogenic (pathotype) group. Available molecular markers therefore provide useful epidemiological information along with their diagnostic effectiveness. Maruthachalam et al. (2010) have also employed molecular markers and PCR-based approaches to study molecular variation among *V. dahliae* isolates, including some representatives from olive. Likewise, new molecular markers (microsatellites and polymorphic sequences) have also been developed by Berbegal et al. (2011). Despite these markers have been tested to analyze the genetic diversity of potato and artichoke populations of *V. dahliae*, their application to other hosts, including olive, is obviously feasible. Finally, from an epidemiological perspective, Trapero et al. (2011) demonstrated that olive inflorescences can be colonized by *V. dahliae*. Indeed, microsclerotia (survival structures which represent the major infecting propagule of the pathogen in soils) were observed inside the peduncles. Infected inflorescences which are easily decomposed in the soil after falling down can act as an additional source of inoculum in Verticillium wilt epidemics.

Searching and exploiting sources of genetic resistance is one of the most effective measures in an integrated disease management strategy (López-Escudero and Mercado-Blanco 2011). Two Turkish groups have recently evaluated diverse olive cultivars in relation to

their resistance to *V. dahliae*. Sesli et al. (2010) found that all cultivars examined in their study were susceptible to the pathogen. But a broader survey involving more than 70 cultivars have yielded more promising results (Erten and Yildiz 2011). Despite the fact that most of the genotypes analyzed in their study were extremely susceptible to the disease, 17 Turkish cultivars were found to be resistant or highly resistant to a defoliating isolate of *V. dahliae*. Even though confirmation of these results may need longer periods of evaluation, particularly under field conditions, uncovering new sources of resistance offers a promising perspective for Verticillium wilt resistance breeding programs.

The genetic and molecular basis of *V. dahliae* pathogenicity and virulence remains largely unknown. However, a new phytotoxin (VdT) produced by an olive-infecting isolate has been purified from cultures (Laouane et al. 2011). This phytotoxin has been associated with disease symptoms in susceptible hosts. The authors claim that VdT can be used to differentiate between *Verticillium*-tolerant and susceptible olive cultivars. A significant breakthrough will definitively help in shedding light to uncover pathogenicity traits of this vascular pathogen: the availability of the *V. dahliae* genome and that of its close relative *V. albo-atrum* (Klosterman et al. 2011). An important conclusion from this comparative genomics study, among others, is that both genomes encode more pectin-degrading enzymes and carbohydrate-active enzymes than other fungi, suggesting an unusual ability to degrade pectin barriers produced by the plant. There is no doubt that with this powerful tool in hand, new control strategies based on an in-depth knowledge of the pathogen genetics will be developed in the future.

Studies focussing on control strategies are few and far between and will need undoubtedly a long and arduous process to assess their effectiveness. This is particularly true for a pathosystem with very specific characteristics described in our review paper (López-Escudero and Mercado-Blanco 2011). However, a few studies to develop control measures have been published in the last few months. For instance, the potential use of composted olive mill wastes as fungicides against different pathogens, including *V. dahliae*, has been evaluated (Alfano et al. 2011; Yanguí et al. 2010). Similarly, Arslan and Dervis (2010) have examined the antifungal activity of essential oils from different plant species against diverse *V. dahliae* vegetative compatibility

groups. Interestingly, essential oils from some species proved to be natural fungicides effective against *V. dahliae* at relatively low concentration. However, these results will need further and prolonged evaluations under field conditions to assess their effectiveness on a commercial scale. Combined use of soil solarization and diverse amendments has been examined under field conditions by Yildiz and Benlioglu (2010). Results indicate that olive trees undergoing solarization alone or solarization plus amendment with olive processing waste showed disease recovery and symptom remission. Saremi et al. (2010) have also provided new information about the efficacy of soil solarization to reduce the inoculum density of the pathogen in olive and pistachio orchards. Reports on biological control approaches of Verticillium wilt of olive have deserved attention as well (for instance, Sanei and Razavi 2011), although the most recent published information is either preliminary or with very limited success. Thus, Aranda et al. (2011) have explored the potential of bacterial communities residing in the rhizosphere and endosphere of wild olive trees (*O. europaea* L. subsp. *europaea* var. *sylvestris*). Their results indicate that both niches are important reservoirs of bacteria (mainly *Bacillus* spp., *Pseudomonas* spp., and *Paenibacillus* spp.) showing in vitro antagonism against the defoliating pathotype of *V. dahliae*. But their effectiveness *in planta* still needs to be verified. The effect of arbuscular mycorrhizal fungi (*Glomus intraradices* alone or in combination with *G. mosseae*) on growth and development of different olive cultivars was examined in saline water-irrigated orchards under arid conditions and in the presence of *V. dahliae* (Kapulnik et al. 2010). Setting aside the positive results obtained on transplant growth and adaptation to arid areas after mycorrhization at the nursery, mycorrhizal fungi colonization did not appear to improve tolerance of cultivars Picual (highly susceptible) or Barnea (relatively tolerant) to *V. dahliae*. Finally, Bubici and Cirulli (2011) have elegantly demonstrated that grafting of susceptible olive cultivars onto resistant rootstocks of cultivar Frantoio provides very good control of Verticillium wilt caused by the defoliating pathotype under specific conditions (root-dipping inoculation of the pathogen and greenhouse incubation). The authors suggest that resistance in ‘Frantoio’ plants takes place mainly in roots, involving biochemical mechanisms rather than structural barriers.

We trust that addition of above-mentioned, totally-unintentional oversights and hot-off-the-press studies,

whose number has increased rapidly during the last few months, will serve to complete and improve our contribution. The more research efforts performed worldwide the better to properly understand and effectively face this threatening disease.

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