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***Verticillium*-induced Wilt in Pepper: Physiological Disorders and Perspectives for Controlling the Disease**

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Abstract: *Verticillium* wilt of pepper is mainly caused by *Verticillium dahliae* Klebahn. This pathogen alters physiology of infected plants, accelerates their senescence and reduces yield. The decrease in photosynthesis and consequently, in the supply of carbohydrates to forming fruits, together with a premature fall of flowers are related to the limited fruit yield observed in diseased plants. The control of *V. dahliae* is difficult because it can survive in field soil for several years. For intensively managed pepper, it has been usual to fumigate with methyl bromide prior to planting. However, as this chemical product causes environmental contamination and reduces the diversity of the microflora in the treated soil, its use was phased out under the 1992 Montreal Protocol. Therefore, finding alternative methods becomes essential for agriculture. At present, an integrated management of the crop would be the best strategy to preserve pepper from *Verticillium*-induced wilt. Such management should include the use of the less susceptible pepper cultivars, the most adequate culture practices and the exploitation of soil symbiotic microorganisms as bioprotectors. More research on the defence signalling pathways and molecular mechanisms employed by plants against pathogens may lead to novel strategies to enhance disease resistance in crop plants, including pepper.

Key words: Biological control, *Capsicum annuum*, cultural practices, integrated management, *Verticillium*, wilt disease

INTRODUCTION

Peppers belong to the family Solanaceae and the genus *Capsicum*. Although this genus includes 25 species, most of the peppers cultivated in temperate and tropical areas belong to the species *C. annuum*, thought to originate in Mexico and Central America (Andrews, 1984). The cultivated peppers are herbaceous, frost-sensitive plants that can be grown as an annual or as a perennial crop in field or greenhouses. This crop is widely consumed as a fresh vegetable or condiment and used for pharmaceutical and cosmetic purposes (Bosland, 2003).

Pepper plants can suffer disorders, diseases and pests that reduce fruit quality and yield. Some of the most common and important physiological disorders affecting this crop are flower bud and flower abscission, sunscald, blossom-end rot, abnormal fruit shape and fruit discoloration (Wien, 1997). Diseases can be caused by a wide range of biological agents, including bacteria, fungi, viruses, parasitic higher plants, insects, nematodes, birds and mammals (Bosland, 2003). The most important diseases caused by fungi and Oomycetes are Anthracnose, Cercospora Leaf Spot, Charcoal Rot, Choanephora Blight, Damping-Off and Root Rot,

Fusarium Stem Rot, Fusarium Wilt, Gray Leaf Spot, Gray Mold, Phytophthora Blight, Powdery Mildew, Southern Blight, White Mold and *Verticillium* Wilt. *Verticillium* wilt of pepper can be caused by both *Verticillium albo-atrum* Reinke and Berthold and *V. dahliae* Klebahn, being the latter the most frequently reported as the causal organism (Golberg, 2003).

Verticillium dahliae Kleb. is a systemic pathogen that causes vascular wilt disease in several plants species (Pegg, 1989). The disease has been reported in pepper in Europe, the Mediterranean, Canada and the United States (Goldberg, 2003). The fungus enters the root through wounds that expose the vascular system or grows between the cells of the apical meristem to gain access to immature xylem elements. Visible symptoms of the syndrome include stunting, epinasty, wilting, foliar chlorosis progressing to necrosis, vascular browning and leaf abscission. The control of this soil-borne pathogen is especially difficult due to its ability to survive in field soil for several years as various types of mycelia, clusters of hyaline cells and microsclerotia (Schnathorst, 1981). Microsclerotia can tolerate a wide range of environmental conditions. Most of them die within two to four years, but even a small residual population is able to cause significant crop loss. Moreover, *V. dahliae* can produce

microsclerotia on nonhost plants, which allows the fungus to persist in soil almost indefinitely (Goldberg, 2003).

EFFECTS OF *Verticillium dahliae* ON PEPPER GROWTH, PLANT PHYSIOLOGY AND FRUIT YIELD

Growth: Symptom severity of *Verticillium* wilt in pepper widely varies with soil and air temperatures and nutrient availability (Goldberg, 2003). When research studies are performed under controlled conditions, the effect of *V. dahliae* on pepper growth is also dependent on plant phenology when the inoculation with the pathogen is carried out. In this sense, no effects on pepper shoot height were observed when the inoculation was performed at the beginning of the flowering period (Goicoechea *et al.*, 2000, 2001; Garmendia *et al.*, 2004a). In contrast, a marked reduction in shoot growth occurred when *V. dahliae* infected pepper plants before flowering (Garmendia *et al.*, 2004a). Moreover, the negative effect of *V. dahliae* on total plant biomass is also dependent on the degree of tolerance exhibited by pepper cultivars. While the decrease in fresh weight was 69% in the tolerant cultivars Padrón and Yolo Wonder, such reduction achieved 81% in the susceptible cv. Luesia (Pomar *et al.*, 2004).

Verticillium dahliae can cause activation of axillary buds in infected pepper (Garmendia *et al.*, 2004a), suggesting that apical dominance of infected plants could be affected by some changes in hormone-like signals involved in the interaction between host plant and pathogen. When the development of axillary branches is close to the fruit set, the growth of secondary shoots may result in enhanced competition for assimilates to the detriment of both fruit production and ripening (Garmendia *et al.*, 2004a).

Plant physiology

Water relations: In studies carried out under controlled conditions, the water potential (ψ) in leaves of *Verticillium*-inoculated pepper declined before any change in the Relative Water Content (RWC) was still observed (Goicoechea *et al.*, 2000). As has been widely demonstrated, most wilt pathogens increase the resistance to water movement as a consequence of reduced diameter of the conductive elements. In pepper, the decrease in leaf ψ could be a mechanism developed by infected plants in order to achieve the required tension to let water move from soil to shoot. In addition, the decline in leaf ψ occurred before the appearance of visible symptoms of the disease, which suggests that *V. dahliae* may release toxic substances that alter the physiology

of the plant host even when the fungus is still localised in the roots. Once the infection had progressed, the leaf RWC sharply declined and this reduction was concomitant with drastic increases in free proline and Total Soluble Sugars (TSS), so that the accumulation of proline and TSS could be sensor of the damage caused by the fungal infection (Goicoechea *et al.*, 2000).

Gas exchange: *Verticillium dahliae* reinforces the reduction in the rate of CO₂ assimilation and leaf conductance experienced by pepper plants as a consequence of the natural senescence process. However, the decline of photosynthesis in infected pepper can be greater than that measured in the leaf conductance even without any significant change in the total chlorophyll content (Goicoechea *et al.*, 2001), suggesting that other factors apart from stomatal closure and chlorophyll degradation can be also involved in the CO₂ exchange rate reduction. In this sense, Pennypacker *et al.* (1990) found that the decrease in net photosynthesis in alfalfa infected with *V. albo-atrum* was due to the reduction in Rubisco activity and Bowden and Rouse (1990) observed that only the initial decrease in photosynthesis caused by *V. dahliae* in potato was associated with stomatal closure. Defoliation is another factor to take into account when talking about gas exchange. When infected with defoliating *V. dahliae* strains, the reduced gas exchange of the entire plant can be mainly due to decreased leaf area rather than to reduced photosynthetic efficiency (Gent *et al.*, 1995).

Recently, Pomar *et al.* (2004) have found that, in the tolerant pepper cultivars, changes in stem lignins (monomer composition and crosslinking) and peroxidase are related with the maintenance of leaf photosynthetic integrity during *Verticillium* wilt. This strategy, however, does not stop fungal hyphae penetration. Conversely, in the susceptible cultivar Luesia, photochemical processes were strongly damaged as a consequence of the disease.

Fruit yield: *Verticillium dahliae* can accelerate the reproductive ability of pepper plants, possibly through some changes in host hormonal balance. However, many of the flowers fall prematurely in inoculated plants (Goicoechea *et al.*, 2001), which produces a significant decline in fruit set and, afterwards, in fruit yield (Garmendia *et al.*, 2004a). Total yield per plant also decreases as a consequence of the small size achieved by individual fruits in *Verticillium*-infected plants (Garmendia *et al.*, 2004a). Fruit ripening may be altered by the infection with *V. dahliae*. In experiments carried out under controlled conditions, it is possible to find contrasting results depending on plant phenology when

the pathogen is inoculated. If the pathogen attack takes place during the vegetative growth of pepper plants, fruit maturation may be delayed. In contrast, when *V. dahliae* is inoculated at flowering, fruit ripening may be accelerated (Garmendia *et al.*, 2004a). Such different behaviours could be due to differences in competition between vegetative and reproductive organs in both cases. As explained above, when the activation of axillary buds in *Verticillium*-infected plants occurs close to the fruit set, the growth of secondary shoots may result in enhanced competition for assimilates to the detriment of both fruit production and ripening.

CONTROL OF *V. dahliae*-INDUCED WILT IN PEPPER

At present, there are not effective measures for controlling *Verticillium* wilt in pepper once the disease has occurred in a field (Goldberg, 2003). In fact, the percentage of infected plants in a soil infested with *V. dahliae* can be up to 25% (Garcia-Mina *et al.*, 1996). For intensively managed pepper, it has been usual to fumigate with methyl bromide prior to planting in order to reduce soil-borne pathogens (Chellemi, 2000). However, as methyl bromide causes soil and water contamination and severely reduces the diversity of the microflora in the treated soil, the use of this chemical product was phased out under the 1992 Montreal Protocol. Therefore, finding alternative methods has become essential for agriculture in the future. Some of these alternatives are the use of tolerant pepper cultivars, the employment of adequate management strategies and the development of biological methods.

Tolerant pepper cultivars: Resistance is considered the most prudent prevention strategy for controlling diseases due to its effectiveness, ease of use and lack of potential negative effects on the environment (Bosland, 2003). In tomato, another plant belonging to Solanaceae, it has been found that genes that confer resistance against *V. dahliae* encode a class of cell-surface glycoproteins with signals for receptor-mediated endocytosis and leucine zipper or Pro-Glu-Ser-Thr (PEST) sequences (Kawchuk *et al.*, 2001). Recently, Bolek *et al.* (2005) have phenotyped a molecular mapping F2 population derived from the interspecific cross of a highly tolerant and a susceptible cotton cultivars for disease incidence and severity induced by *V. dahliae*. Unfortunately, although there are some tolerant pepper cultivars (i.e., Padrón and Yolo Wonder), resistance genes have not been identified for the *C. annuum*-*V. dahliae* interaction (Pomar *et al.*, 2004). In addition, as all cultivated species of *Capsicum* have $2n = 24$ chromosomes, the crossability

among the species is limited and breeders have only been able to make little differences in disease resistance (Greenleaf, 1986). In fact, no commercial cultivars resistant to the disease are currently available (Goldberg, 2003). However, the recent studies of Jung *et al.* (2003, 2005) constitute an important advance in the knowledge on genes involved in the response of pepper plants when subjected to pathogens, abiotic and environmental stresses. Jung *et al.* (2003) reported the isolation and functional analysis of pepper CALTP genes encoding three lipid transfer proteins, CALTPI, CALTPII and CALTPIII. While CALTPI and CALTPIII genes were mainly expressed in various pepper tissues infected by pathogens, infection by *Phytophthora capsici*, a pathogenic fungus that causes wilt disease in pepper (Aguirreola *et al.*, 1995), did not induce the transcription of the CALTPII gene.

Furthermore, manipulation and enhancement of plant defence responses using genetic engineering technology has the potential to produce crop plants with improved resistance to pathogens (McFadden, 2000). In fact, the identification of antifungal proteins and their expression in transgenic plants provides new possibilities of introducing resistance to pathogens in susceptible cultivars. This is the case of potato, a crop severely attacked by the early dying disease in many regions of the United States, being *V. dahliae* the most important causal agent of this disorder (Bowden *et al.*, 1990). Gao *et al.* (2000) demonstrated that the alfalfa antifungal peptide (alfAFP) defensin, a small cysteine-rich peptide isolated from seeds, displayed strong activity against *V. dahliae*. When expressed the alfAFP peptide in transgenic potato, plants exhibited great resistance against such pathogen not only in the greenhouse but also under field conditions. *Capsicum annuum*, however, is one of the most recalcitrant dicotyledoneous species for genetic engineering. In fact, there is not a universal procedure suitable to transform different cultivars within each species via *Agrobacterium tumefaciens*. However, Pozueta-Romero *et al.* (2001) developed a tissue culture regeneration protocol that opens new prospects for *Agrobacterium*-mediated transformation in tomato and bell pepper. Therefore, the use of genetic engineering as an adjunct to traditional breeding methods for generation of new crop lines will become an increasingly important component of modern agriculture (McFadden, 2000).

Adequate management strategies: Soil solarization using clear plastic mulch for preplant soil treatment is a common practice in modern agricultural systems as an alternative to methyl bromide and it has been reported as an effective technique to eradicate microsclerotia of *V. dahliae* in the soil and to control *Verticillium* wilt in several herbaceous

and woody hosts. However, working with olive trees, López-Escudero and Blanco-López, (2000) found that the efficacy of soil solarization can be influenced by particular environmental or soil conditions such as physical soil characteristics and irrigation. Solarization together with soil fumigation (i.e., fumigants containing chloropicrin) may increase the rate of propagule death (Goldberg, 2003). Biofumigation is a non-chemical approach that combines organic matter or green manure with solarization to control diseases, nematodes and weeds. In most cases, biofumigation refers to the agronomic practice used for the suppression of soil-borne pests and pathogens by volatile chemicals containing biocidal compounds released by brassicaceous green manure (Kirkegaard *et al.*, 2000). Lira-Saldivar *et al.* (2004) obtained increased pepper yield as a consequence of solarization alone or in combination with *Larrea tridentata* leaf resin extract as a biofumigant. In addition, the incidence and disease severity of soilborne pathogens clearly decreased in treated soils. However, plant health is not the only objective in the context of the sustainable agriculture. Soil is considered as a fragile, dynamic and essential part of the system and microflora and microfauna must be taken into account. When solarization and biofumigation were applied together to control some pathogens (*Phytophthora capsici* and *Meloidogyne incognita*) that affect pepper and tomato in greenhouses in southern Spain, soil mycoflora drastically decreased (Martínez *et al.*, 2004).

Crop rotation is one of the best ways to minimize pepper diseases caused by soilborne pathogens (Bosland, 2003) including Verticillium wilt (Xiao *et al.*, 1998). In pepper production, the recommendation for farmers is to apply crop rotation for three or four years between crops (Goldberg, 2003). However, the design of crop rotation is complicated by the variation in host range among isolates. A plant species proposed for crop rotation programs concerning pepper production is *Gladiolus* because of its resistance to *V. dahliae* and its commercial application to produce corms and ornamental flowers (Palazón, 1985). Unfortunately, the efficacy of this management strategy may be reduced because some propagules of the fungus can persist by saprofitic colonization of plant debris and reproduction on nonhosts or weed species (Goldberg, 2003).

Another management strategy that can be useful for controlling *V. dahliae* is the application of soil amendments. The efficacy and mode of action of different soil amendments, including nitrogenous and cellulosic amendments, animal and green manures and composts has been exhaustively exposed in the review of Lazarovits *et al.* (2000). According to the latter authors,

nitrogen-containing materials can significantly reduce populations of plant pathogenic fungi without causing a concomitant decrease in other microorganisms, with the subsequent maintenance of ecosystems sustainability. However, the efficacy of an amendment depends on its C:N ratio, the soil to which it is added and the environmental conditions. Two are the mechanisms that appear to be associated with a decrease in populations of plant pathogens after applying a soil amendment. When the release of ammonia from nitrogenous amendments is high enough to increase soil pH above 8.5, a rapid inactivation of pathogens may occur. On the other hand, some amendments can produce a slower destruction of pathogens. This last effect could be considered as a biological control. More recently, Tenuta and Lazarovits (2002) have demonstrated that ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *V. dahliae*.

In the case of organic amendments, the soil-specific response in the ability of these amendments to control disease incidence of plants has contributed to their reduced use over time (Lazarovits *et al.*, 2000). However, Jordana *et al.* (1994) and Iglesias *et al.* (1999) suggested that organic amendments could stimulate natural defence and improve development of seedlings growing under fatigue soil conditions, being an ecological and efficient means for the control of pepper-wilt induced by *V. dahliae* (García-Mina *et al.*, 1996). In agreement with the latter point of view, Goicoechea *et al.* (2004) found that the use of an organic amendment of natural origin (COA H) that contained salicylic acid, soluble ammonium salts and sea weed extract from *Ascophyllum nodosum* alleviated Verticillium wilt symptoms in a susceptible pepper cultivar (Piquillo). Pepper seedlings grown with COA H showed similar leaf chlorophyll and relative water contents, as well as leaf conductance and nutrient levels to their relative healthy controls. In addition, organic amendment allowed *Verticillium*-inoculated seedlings to longer maintain photosynthetic rates and delayed the appearance of disease symptoms. The cytokinin activity showed by the organic product could be involved in the delay of whole plant senescence in *Verticillium*-infected pepper. In addition, the early accumulation of phenolics in peppers grown with COA H could also contribute to their enhanced resistance or tolerance against *V. dahliae*.

Irrigation is another factor to take into account because its possible influence on the spread and severity of the disease caused by *V. dahliae*. In a recent work, López-Escudero and Blanco-López (2005) have proposed rational and moderate irrigation dosage and timing for controlling the disease in affected olive orchards for intensive production. The results of their research clearly

indicated higher inoculum density in wet areas under the influence of drip-irrigation system, which implies relevant epidemiological consequences. Similarly, Pegg and Brady (2002) indicated that the application of semicontinuous irrigation incremented wilt disease incidence and severity in herbaceous host plants. However, the severity of *V. dahliae*-induced wilt in pepper can increase when seedlings are subjected to a moderate water deficit before the pathogen's attack (Garmendia *et al.*, 2005). The latter authors found that drought enhanced the deleterious effect of *V. dahliae* on plant growth, fruit set and yield.

Biological methods: Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. However, they may have potential risks to other organisms present in the ecosystem that should also be considered (Brimmer and Boland, 2003).

As microsclerotia are the most important structures that ensure the survival of *V. dahliae*, several biocontrol strategies have been focused on (a) inhibition of microsclerotia formation on diseased plant tissues after plant death, (b) reduction in survival of microsclerotia, (c) prevention of their germination and/or (d) prevention of root infection by germinating microsclerotia (Tjamos, 2000). The use of antagonistic bacteria is a widely applied strategy for controlling *V. dahliae*, being *Talaromyces flavus* the most studied biological control agent. The release of H₂O₂ in the rhizosphere as a consequence of glucose oxidase activity from *T. flavus* is known to inhibit and kill microsclerotia of *V. dahliae* (Tjamos, 2000). Other genera that have been reported as biocontrol agents against vascular or soil-borne fungal pathogens are *Bacillus*, *Pseudomonas*, *Chryseomonas*, *Sphingomonas*, *Stenotrophomonas* and *Serratia*. In a recent work, Mercado-Blanco *et al.* (2004) have found that root pretreatment of olive plants with some isolates of *Pseudomonas fluorescens* during nursery propagation can help in the biocontrol of *V. dahliae*.

Apart from bacteria, some fungal root endophytes can be used to biologically control plant diseases (Brimmer and Boland, 2003), including wilt induced by *V. dahliae* (Narisawa *et al.*, 2002). A group of rhizospheric fungi with increasing interest are Arbuscular Mycorrhizal Fungi (AMF), because the majority of crop and horticultural plants are associated with them. This symbiotic association develops in two functional phases (Harley and Smith, 1983): the extraradical phase extending from the root into the soil and the intraradical phase with intercellular hyphae and specialised intracellular structures called "arbuscules". The external mycelium

transfers water and minerals from soil to roots. Arbuscules are the structures where exchanges of carbon to the fungus and nutrient and water to the host plant take place. The intraradical phase sometimes includes the "vesicles", structures considered to be mainly for storage. Mycorrhizal fungi play a key role in nutrient cycling in the ecosystem and also protect plants against environmental and cultural stress, including the protection against soil-borne pathogens. Several mechanisms are involved in bioprotection by AMF (Azcón-Aguilar *et al.*, 2002): (a) the improvement of plant nutrient status can help mycorrhizal plants to overcome a pathogen's attack; (b) the increase in root biomass in mycorrhizal plants can compensate tissue damage by the pathogen; (c) mycorrhizal and pathogenic fungi may compete for host photosynthates and root colonization sites; (d) AMF can induce changes in the anatomy and architecture of the root system and in the rhizosphere; (e) AMF can activate plant defence mechanisms. Effective bioprotection is a cumulative result of all these mechanisms working separately and/or together (Harrier and Watson, 2004). Therefore, there is an increasing interest in ascertaining the effectiveness of AMF in particular plant production situations to incorporate them into agricultural practices.

The use of AMF can be effective for alleviating the severity of *Verticillium*-induced wilt in pepper (Garmendia *et al.*, 2004a), although the interaction between mycorrhizal plants and *V. dahliae* is far from behaving as incompatible. Moreover, the effectiveness of AMF depends on several factors, such as fungal species (Garmendia *et al.*, 2004b) and plant phenology at the moment of pathogen attack (Garmendia *et al.*, 2004a). In experiments carried out in greenhouse, Garmendia *et al.* (2004b) found that, in pepper colonized by *Glomus intraradices*, the severity of the disease was even higher than that observed in non-mycorrhizal plants in terms of plant growth and fruit yield. In addition, high effectiveness of AMF in improving plant nutrition and growth is not necessarily related to great effectiveness in protecting pepper against *Verticillium*-induced wilt. In fact, although plants associated with *G. mosseae* had greater biomass and exhibited shorter vegetative period than non-mycorrhizal ones, the earlier beginning of reproductive stage in pepper colonized by *G. mosseae* did not avoid yield reduction when plants were inoculated with *V. dahliae*. On the other hand, plants associated with *G. deserticola* exhibited greater yield than non-mycorrhizal ones when they infected by this pathogen. According to Garmendia *et al.* (2004b), the maintenance of specific P uptake rate could contribute to diminish the deleterious effect of *V. dahliae* on yield in plants

associated with *G. deserticola*. However, the effectiveness of *G. deserticola* depended on plant phenology when *V. dahliae* was inoculated. When pathogen infection occurred during the vegetative growth of pepper plants, *G. deserticola* allowed the leaf RWC to be maintained for longer and delayed both the appearance of disease symptoms and the decrease of photosynthesis in *Verticillium*-inoculated plants (Garmendia *et al.*, 2004a). This bioprotective effect was not so evident when pepper plants were inoculated with *V. dahliae* at flowering. After studying the antioxidant metabolism in asymptomatic leaves of *Verticillium*-infected pepper, Garmendia *et al.* (2004c) concluded that, compared to non-mycorrhizal plants, pepper associated with *G. deserticola* had more balanced Superoxide Dismutase (SOD), catalase (CAT) and guaiacol peroxidase activities in leaves along the first month after pathogen inoculation, which could contribute to delay the development of disease symptoms and maintain control photosynthetic rates for longer in mycorrhizal plants. In addition, the presence of *G. deserticola* induced the appearance of new isoforms of acidic chitinases, SOD and, at early stages, peroxidases in roots of pepper plants (Garmendia *et al.*, 2006). Pomar *et al.* (2004) suggested that through changes in stem lignins (monomer composition and crosslinking) and peroxidase activities in roots, stems and leaves the tolerant cultivars of *C. annuum* can retard the penetration by *V. dahliae* hyphae. Moreover, Garmendia *et al.* (2006) found that the inoculation with *V. dahliae* slightly increased both Phenylalanine Ammonia-lyase (PAL) and peroxidase activities only in mycorrhizal roots. Taken together, these results suggest that the mycorrhizal-specific induction of new isoforms of acidic chitinases and SOD as well as the enhanced peroxidase and PAL activities could also be involved in the biocontrol of *Verticillium*-induced wilt in pepper colonized by *G. deserticola*.

CONCLUSIONS AND PERSPECTIVES

At the present time an effective strategy that preserves pepper plants from the *Verticillium*-induced wilt is still unknown. Further work is required to deep into the molecular aspects implied in the interaction between such pathogen and different varieties of pepper exhibiting different degrees of susceptibility. Understanding the mechanisms employed by plants to defend themselves against pathogens may lead to novel strategies to enhance disease resistance in crop plants. Microarray studies have provided insight into the complexity and arrangement of the defence response in *Arabidopsis thaliana* and other model plants. However, although the

study of the mechanisms developed by model plants has increased the knowledge of defence signalling pathways, there are areas where significant differences between model and other plants are evident. Therefore, it is necessary to investigate into the specific aspects of the defence that cannot be studied using *Arabidopsis* as a model (Anderson *et al.*, 2005).

Taking together all the results obtained by applying different strategies to control the disease caused by *V. dahliae* in pepper, nowadays the best solution would be an integrated management of the crop that would include the use of the less susceptible pepper cultivars, the most adequate culture practices and the exploitation of soil symbiotic microorganisms (especially AMF) as bioprotectors. However, most part of experiments performed to study the potential role of AMF as plant bioprotectors have consisted in the analysis of symbiotic *versus* non-symbiotic plants under laboratory conditions. Researching in more realistic environments, such as microcosms of field plots, is also needed to assess the impact of the selected mycorrhizal strain on the indigenous microbial community and the capability of such AMF for persisting in the soil (Selosse *et al.*, 2004). As suggested by Harrier and Watson (2004), improved understanding of the interactions of agricultural practices with mycorrhizal effectivity is crucial if the potential for biocontrol using AMF is to be realized in practical agriculture.

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