

Effect of irrigation regimes and artificial mycorrhization on insect pest infestations and yield in tomato crop

**Tonia Colella, Vincenzo Candido,
Gabriele Campanelli, Ippolito Camele &
Donatella Battaglia**

Phytoparasitica

ISSN 0334-2123

Phytoparasitica

DOI 10.1007/s12600-013-0356-3



Your article is protected by copyright and all rights are held exclusively by Springer Science +Business Media Dordrecht. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Effect of irrigation regimes and artificial mycorrhization on insect pest infestations and yield in tomato crop

Tonia Colella · Vincenzo Candido ·
Gabriele Campanelli · Ippolito Camele ·
Donatella Battaglia

Received: 27 February 2013 / Accepted: 25 September 2013
© Springer Science+Business Media Dordrecht 2013

Abstract A 2-year field experiment was carried out to test the effect of root symbionts treatments in combination with different watering levels on tomato yield and pest infestation rates. A split-plot experimental design was followed, where the main treatments were three irrigation regimes, and the subplot factor was the mycorrhizal treatment (two mycorrhizal treatments [M1 and M2] and the control). The M1 treatment consisted in the use of a commercial preparation (Micosat F; CCS Aosta, Italy) containing a mixture of vesicular-arbuscular mycorrhizae (VAM), bacteria of the rhizosphere and saprophytic fungi, while M2 treatment used only arbuscular mycorrhizal fungi. Insect pests sampled in the field during the 2 years of experiment were all sap-feeders (*Trialeurodes vaporariorum*, *Macrosiphum euphorbiae*, *Frankliniella occidentalis* and an unidentified species of leafhopper). Results did not show any impact of root symbionts on pests, whereas water deficit significantly reduced plant infestation rates. Both mycorrhization

treatments and water supply resulted in a significantly positive effect on crop yield.

Keywords *Glomus* · *Lycopersicon esculentum* · Tomato pests · *Trichoderma harzianum* · Water deficit

Introduction

By altering plant physiology, agronomic techniques have a major impact on crop yield but also strongly affect plants' suitability as food for herbivorous arthropods. We address here the effects of using deficit irrigation and root symbionts on tomato yield and on plants' infestation rate by phytophagous insects in the field.

Tomato (*Lycopersicon esculentum* Mill.) is a crop widely distributed in the Mediterranean area where the climate is warm to hot, with dry summers. Water resource scarcity requires an increase in use efficiency of water allocated to agriculture and a possible option is deficit irrigation, an irrigation practice whereby plants are exposed to certain levels of water stress without significant reduction in yields. Tomato is classified as sensitive or moderately tolerant to water stress depending on cultivar, the phenological stage at which the deficit occurs (Patanè *et al.* 2011), and the severity of the stress (Candido *et al.* 2000; Perniola *et al.* 1994). Moreover, water deficit during certain stages of the growing season improves fruit quality (Favati *et al.* 2009; Patanè & Cosentino 2010; Patanè *et al.* 2011).

T. Colella · D. Battaglia (✉)
Dipartimento di Scienze, Università degli Studi della Basilicata, 85100 Potenza, Italy
e-mail: donatella.battaglia@unibas.it

V. Candido · I. Camele
Università degli Studi della Basilicata, Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali,
85100 Potenza, Italy

G. Campanelli
Consiglio per la Ricerca e la Sperimentazione in Agricoltura,
Istituto Sperimentale per l'Orticoltura, Sezione di
Monsampolo del Tronto, 43030 Monsampolo, AP, Italy

Effects of plant water stress on phytophagous insects may be variable and largely dependent upon feeding guilds and stress level (Huberty & Denno 2004). We can expect an inconsistent effect of water stress on chewing insects, which are influenced by the variation of several factors (nitrogen, tissue turgor, accumulation/reduction of allelochemicals in tissues). In contrast, bouts of stress and the recovery of turgor allow sap-feeders, limited mainly by the availability of free amino acids, to benefit from stress-induced increases in plant nitrogen. Plant water stress may also influence host selection by pest insects. In fact, insect orientation and host selection are affected by volatile compounds (Dicke 2000; Webster *et al.* 2008). Climatic conditions and nutrient availability can be important factors in determining the intensity and variability in the release of plant volatiles (Gouinguéné & Turlings 2002). Moreover, herbivores' feeding activity and abiotic conditions can lead to an increase in the same secondary plant chemicals (Dicke 2000). Jasmonates trigger defense response pathways in both biotic and abiotic stresses and stimulate production of volatile isoprenoids (Vickers *et al.* 2009), which play an important role because of their antioxidant activity. This means that stressed plants might be perceived by herbivores as already infested hosts, then colonized by competitors and less suitable because of defense mechanisms already active. Inbar *et al.* (2001) found that *Bemisia argentifolii* and *Liriomyza trifolii* adults, in a choice experiment, prefer ovipositing on vigorous tomato plants rather than on water- and/or fertilizer-stressed ones.

Root symbionts, such as N-fixing bacteria or vesicular-arbuscular mycorrhizae (VAM), supply plants with additional nutrients (mainly nitrogen and phosphorus) (Conversa *et al.* 2007; Harrison & van Buuren 1995; Hodge *et al.* 2001), increasing plant growth and altering foliar chemistry (Goverde *et al.* 2000). Moreover, VAM constitute endotrophic symbiotic associations with plant roots, directly interacting with the host plant. As a consequence of this interaction, VAM can induce a better resistance to drought (Augè 2001; El-Mesbahi *et al.* 2012; Lee *et al.* 2012) and a significantly higher degree of resistance against pests and diseases (Fritz *et al.* 2006; Guerrieri *et al.* 2004; Liu *et al.* 2007; Vos *et al.* 2012). Nevertheless, the tritrophic interaction "host plant – VAM – phytophages" is complex (Cosme *et al.* 2011; Gange 2001; Goverde *et al.* 2000). Insect feeding on the upper plant parts may be either enhanced or reduced by the presence of VAM, or have no observable effect

(Cosme *et al.* 2011; Guerrieri *et al.* 2004; van Dam *et al.* 2003). Root symbionts may also alter volatiles' production of uninfested plants, in the same way as herbivore feeding activity and abiotic stress do. In fact, a significant increase in plant attractiveness toward *Aphydius ervi* (Hymenoptera: Braconidae), an aphid parasitoid, that uses plant volatiles as foraging cues (Du *et al.* 1998; Sasso *et al.* 2007), is observed either after aphid infestation or root colonization by the mycorrhizal fungus *Glomus mosseae* (Guerrieri *et al.* 2004).

The association of water deficit irrigation with plant mycorrhization is an interesting technique, since benefits induced by VAM symbiosis include a better resistance to drought (Augè 2001). VAM can improve drought resistance through several possible mechanisms, including increased root hydraulic conductivity, enhanced water uptake due to extraradical hyphae, osmotic adjustment that promotes turgor maintenance (Davies *et al.* 1993), and accumulation of antioxidant compounds (Baslam & Goicoechea 2011).

Recently, commercial preparations of VAM have appeared on the market; supplying them to seedlings seems at the moment the most promising method for implementation of mycorrhization in horticultural crops (Karagiannidis *et al.* 2002). The commercial inoculum most frequently consists of *Glomus intraradices* Schenck and Smith, either pure or in a mixture with growth-promoting bacteria and with ectomycorrhizal fungi (Dalpé & Monreal 2004). A previous study on plants inoculated with a mixture of bacteria, ectomycorrhizal fungi and VAM seems to support the idea that fungal antagonists do not interfere with arbuscular mycorrhizae; however, modifications of the microbial community structure and ecology take place (Vázquez *et al.* 2000). Therefore, a commercial inoculum containing a mixture of VAM and other microorganisms may have different effects on plant growth and health as compared with a commercial inoculum containing only VAM. To take into account these possible effects, in this work we tested a commercial inoculum containing a mixture of VAM (*Glomus mosseae*, *G. intraradices*, *G. viscosum*), bacteria of the rhizosphere and saprophytic fungi (Micosat), as well as the same mixture of VAM without the other microorganisms (bacteria and saprophytic fungi). The two inoculum types (with or without bacteria and saprophytic fungi) were tested in combination with different levels of water supply. In addition, we monitored insect infestation rates to have a measure of

insects' preference for host plants under different treatments in field conditions.

Materials and methods

Crop cultivation The experiment was carried out at the experiment farm "Pantanello" (40°24'N; 16°48'E; 10 m a.s.l.), situated in the Metapontum plane (Basilicata Region, southern Italy) in the years 2008 and 2009, on a silty-loam soil, with pH 7.68, low total nitrogen content (0.8 g kg⁻¹) and a good level of exchangeable phosphorus (21.2 mg kg⁻¹) and potassium (215 mg kg⁻¹). Soil was plowed to a depth of 30 cm and then rotavated and leveled at the time of basic dressing, before crop planting. Plants of the hybrid (F₁) cultivar 'Faino' (Syngenta Seeds Company; Wilmington, DE, USA) were sown at the end of April into alveolate containers and reared in a plastic greenhouse (PE 200 µm), provided with lateral openings and anti-insect net. Seedlings at the 4–5 true leaf development stage were transplanted into the field at the end of May, with 30 cm spacing between plants and 150 cm between twin-rows (4.94 plants per m²). Two insecticide spray applications were performed in 2008 for control of whiteflies: a spray with imidacloprid (Confidor 200 SL – Bayer; 50 ml hl⁻¹) on June 27 and a spray with methomyl (Lannate® 25 - Du Pont; 150 g hl⁻¹) on July 18. At harvest time, weight of marketable fruits, percentage of waste fruits, fruit mean weight and soluble solids content (°Brix) were assessed on samples taken from 8 m² for each plot.

Experimental design A split-plot experimental design was performed with three replicates. The main treatments were three irrigation regimes, while the subplot factor was the mycorrhizal treatment (two different mycorrhizal treatments and the control). Treatments were as follows:

1. Treatment with the commercial inoculum Micosat F. (produced by CCS Aosta S.r.l.; Quart, Italy) containing VAM (*Glomus mosseae* GP 11, *G. intraradices* GB 67, and *G. viscosum* GC 41), bacteria of the rhizosphere (*Agrobacterium radiobacter* AR 39, *Bacillus subtilis* BA 41, and *Streptomyces* spp. SB 14) and saprophytic fungi (*Beauveria* spp., *Trichoderma harzianum* TH 01, *Pichia pastoris* PP 59) (M1). In particular, 100 g of this product contains 25 g of ground mycorrhizal roots together with spores and

hyphae of *Glomus* (crude inoculum). The percentage of biologically active ingredients was 6.2%.

2. Treatment with only the mycorrhizal fungus *Glomus* spp. (produced by CCS Aosta S.r.l.), as described above, without bacteria and saprophytic fungi (M2);
3. Control, without any treatment (C).

Mycorrhizal treatments were carried out 20 days before transplanting of seedlings, providing 1.15 ml of product into each alveolus. The schedule of mineral fertilization after transplanting was the same in all plots, and two applications of ammonium nitrate were made, with total input of 80 kg ha⁻¹ of nitrogen.

A drip irrigation system (2.5 l ha⁻¹) was used. Hoses, pierced with holes every 30 cm, were placed in the middle of the twin-rows. Watering intervals were 8.8 days in 2008 and 10 days in 2009. In detail, three irrigation regimes were compared: full restoration of crop evapotranspiration (ETc) (V100); 50% restoration of crop evapotranspiration (ETc) (V50); and control, irrigated only at transplanting time (V0).

ETc was calculated according to the evapotranspiration approach of Doorenbos & Pruitt (1977) following the simplified soil water balance ($ET_c = ET_0 * K_c$), where ET_0 is the reference evapotranspiration, calculated according to Hargreaves & Samani (1985), and the crop coefficient (K_c) as reported by Allen *et al.* (1998) for tomato crops. Water was applied on a weekly basis.

Mycorrhizal colonization At transplanting time (20 days after inoculation) and at the middle of the crop cycle (flowering–fruit setting stage), 20 rootlets per treatment were sampled to check on mycorrhizal colonization. Arbuscular and vesicular structures were detected using the technique described by Brundrett *et al.* (1983) and then the rate of roots' mycorrhization (Trouvelot *et al.* 1986) was estimated using the MycoCalc software.

Insect sampling Forty plants, selected at random from the central part of each subplot, were sampled every 7–10 days to check for the presence of pests. Selected plants were examined in the field by using a magnifying lens and without removing plant parts. Plants were gently beaten over a blank sheet of paper to collect thrips. Samples of insects were collected and taken to the laboratory for species identification. Percent of

infested plants was calculated for each sampling date and insect pest.

Statistical analysis Percent data were arcsine transformed before analysis as $\text{Arcsen}\sqrt{(\text{original value}/100)}$ (Zar 1984), and analyzed by applying a full factorial model analysis of variance (ANOVA), including mycorrhizal treatments, water volume and date of sampling as main fixed effects. The following model was used: $X_{ijklr} = \mu + V_i + M_j + D_k + VM_{ij} + VD_{ik} + MD_{jk} + VMD_{ijk} + Er(ijk)$. In this model the measure of a generic data X , belonging to the water regime V_i , to the mycorrhizal treatment M_j , to the sampling date D_k and to the replicate Er , is equal to the sum of the general mean μ , the effects of the water regime i , the mycorrhizal treatment j , the sampling date k , all the interactions and the residuals r .

All agronomic data were statistically processed by analysis of variance (ANOVA); Student–Newman–Keuls (S–N–K) test was performed for the comparison of means at $P \leq 0.05$.

Results

Weather data Climatic conditions in the 2 years of the experiment were quite different (Fig. 1). During June–July, the weather was warm and dry in the year 2008, unsteady and wet (particularly in June) in the year 2009. Temperature gradually increased during spring 2008. Daily average temperatures varied between 20°C and 25°C during the last 10 days of May and the first 20 days of June. In the last 10 days of June and in the whole month of July, the daily average temperatures usually were above 25°C and maximum temperatures often came close to 35°C. A series of small rainy events was recorded in June and only two rainy events in July. Total rainfall was 7.6 mm and 24.8 mm in June and July, respectively. In 2009, after very warm weather in May, there was a rapid drop in temperature in the first 10 days of June, followed by an increase to normal levels in the second 10 days of the month and another rapid decrease during the last 10 days. Temperature finally increased gradually during the month of July. Total precipitation was 63.8 mm in June and 25.2 mm in July.

Roots mycorrhization At transplanting time (20 days after inoculation) the treated seedlings already exhibited

evident development of the fungal symbiont. However, in the seedlings used as control, endomycorrhizal structures were almost or completely absent (Table 1). At the destructive sampling carried out in the middle of the crop cycle (flowering–fruit setting stage), the frequency of root mycorrhization varied significantly between years, with higher values in 2009. The different irrigation regimes did not affect the efficiency of mycorrhization. The artificial mycorrhization positively influenced the frequency of root mycorrhization, thus confirming the success of inoculation.

Insect infestation rate Insect species observed and levels of infestation differed markedly in the 2 years of the experiment (Figs. 2, 3). *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) was the main pest during 2008, with an infestation rate of 100% (Fig. 2a), whereas aleurodids infestation was much lower during 2009 (Fig. 3b). During both years we sampled the potato aphid *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) (Figs. 2b, 3a). During 2008 we also collected the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Fig. 2d) and leafhoppers (unidentified species) (Fig. 2c). The percent of infested plants never differed significantly as a function of mycorrhizal treatments, in both years and for all species sampled, whereas the effect of water management and its interaction with date were significant for the whiteflies, thrips and leafhoppers in 2008 and only the interaction was significant for aphids in 2009 (Tables 2, 3). Regardless of insect species, the infestation rate, as a tendency, was the highest in plots where plants received the full restoration of crop evapotranspiration (V100) and the lowest in plots where plants did not receive water (V0).

In 2008 two insecticide spray applications were performed for control of whiteflies. The effect of insecticide spraying on the infestation rate of *T. vaporariorum* differed depending on the water regime (Fig. 2a). In the V100 plots, the whitefly infestation rate remained high and never dropped below 70%. In the V50 and V0 plots, the infestation rate decreased significantly after insecticide spraying, which took place on June 27, and rose afterwards. On July 22, a 100% infestation rate was recorded in all plots. After the second insecticide spray (on July 18) infestation rates tended to decrease, with trends similar to those observed after the first insecticide spraying. The percentage of plants infested with *F.*

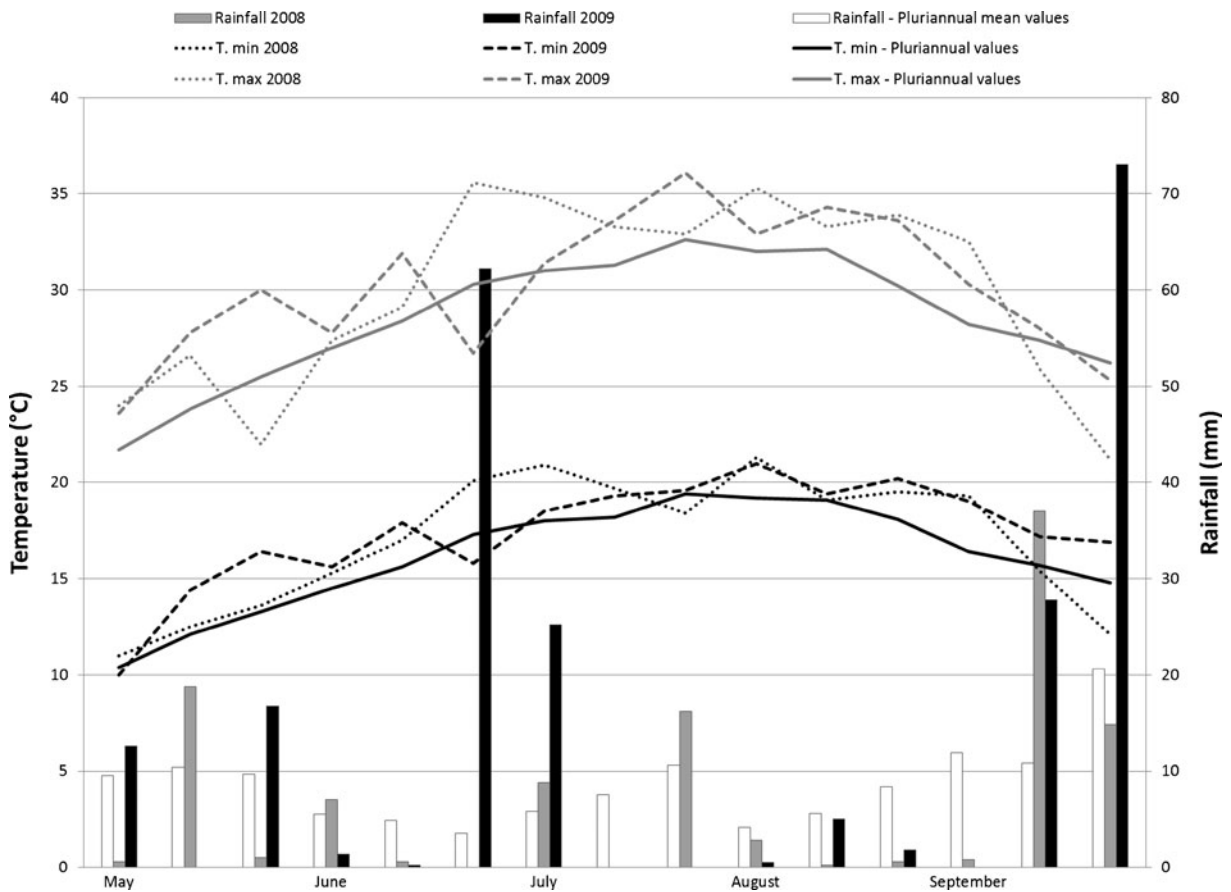


Fig. 1 Ten-day minimum and maximum temperature and rainfall trends recorded during the two tomato crop cycles and compared with the pluriannual values recorded for the 1981–2007 period

occidentalis remained below 10% in V0 plots, whereas it reached 30% in V100 plots, decreasing after each insecticide spraying. The infestation rate in V50 plots had intermediate values (Fig. 2b).

Macrosiphum euphorbiae was the most important pest in 2009 but the infestation was short-lived. Aphids appeared after June 2 and completely disappeared before July 7 (Fig. 3a). The infestation peak on June 16 was significantly lower in V0 plots than in the others ($F_{2,18}=5.25$, $P=0.016$).

A few individuals of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae), all concentrated in M1 plots, were caught at the end of June 2009 (Fig. 4). No mirids were captured in V0 plots. Distribution of *M. pygmaeus* in the experiment field differed significantly as a function of both mycorrhizal treatments and water volumes supplied (Table 4).

Agronomic data All the agronomic traits changed significantly in the 2 years of research (Table 1). In particular, yield decreased from 71.2 t ha⁻¹ in 2008 to 69.7 t ha⁻¹ in 2009; also the fruit mean weight and the waste fruit percentage values were higher in the first year. However, fruit number/plant and fruit soluble solids content (°Brix) were significantly higher in the second year. Irrigation positively influenced tomato productivity. Compared with the unirrigated control, the yield increased by 31.1 and 52.8 t ha⁻¹ with the restoration of 50% and 100% of ETc, respectively. The higher productivity levels of the watered treatments were also accompanied by a significant increase in mean weight and in the number of berries per plant. Besides, the increase in water supply caused an increase in waste berries, from 12.6% of the control (V0) to 15.1% and 16.7%, respectively, in V50 and V100.

The yields of the treatments mycorrhized with Micosat F and VAM did not show significant differences

Table 1 Frequency (%) of mycorrhization on agronomic traits and roots of tomato as affected by irrigation and mycorrhizal treatments during 2 years

FACTORS	Mycorrhization frequency (%)		A g r o n o m i c t r a i t s				
			Yield		F r u i t s		
	Transplant time	Middle of crop cycle	t ha ⁻¹	per plant (no.)	waste (% number)	mean weight (g)	soluble solids content (°Brix)
Years (Y) ^z							
2008	64.7 b	60.6 b	71.2 a	73.1 b	17.4 a	22.2 a	5.1 b
2009	76.3 a	85.5 a	69.7 b	82.7 a	12.2 b	19.1 b	6.6 a
<i>F</i> ^y	10.1*	11.2**	5.6 *	54.7***	163.5***	1674.6***	16.4**
Irrigation regimes (I) ^z							
V0	-	55.5 b	42.5 c	60.8 b	12.6 c	15.2 c	6.6 a
V50	-	82.0 a	73.6 b	82.4 a	15.1 b	20.8 b	5.8 b
V100	-	81.7 a	95.3 a	90.6 a	16.7 a	25.9 a	5.1 c
<i>F</i> ^y	-	7.6**	574.1***	186.1***	34.7***	6411.2***	294.8***
Mycorrhizal treatments (M) ^z							
C	25.0 c	51.0 b	65.7 b	73.1 b	15.6	20.6	5.9
M1	89.5 b	79.4 a	73.2 a	78.8 a	14.3	21.1	5.8
M2	97.0 a	88.8 a	72.5 a	81.9 a	14.5	20.2	5.9
<i>F</i> ^y	10.7**	6.6**	36.7***	11.3***	2.4 ^{ns}	2.8 ^{ns}	0.8 ^{ns}
Interactions (<i>F</i> values) ^y							
Y x I	-	1.9 ^{ns}	40.3***	131.1***	102.9***	223.5***	66.8***
Y x M	-	12.1**	1.6 ^{ns}	2.4 ^{ns}	2.6 ^{ns}	10.9**	1.3 ^{ns}
I x M	5.7*	1.1 ^{ns}	4.0*	0.8 ^{ns}	0.1 ^{ns}	0.9 ^{ns}	2.9*
Y x I x M	-	1.9 ^{ns}	0.7 ^{ns}	0.6 ^{ns}	0.6 ^{ns}	2.2 ^{ns}	3.0*

^z Within the same column, means followed by the same letter within each treatment do not differ significantly ($P \leq 0.05$) according to the SNK test

^y $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; *ns* = not significant

but they exceeded the unmycorrhized control by 7.2 t ha⁻¹, on average. The same trend between mycorrhized treatments and the control was observed for the fruit number/plant, whereas none of the other tomato traits were influenced by mycorrhization treatments.

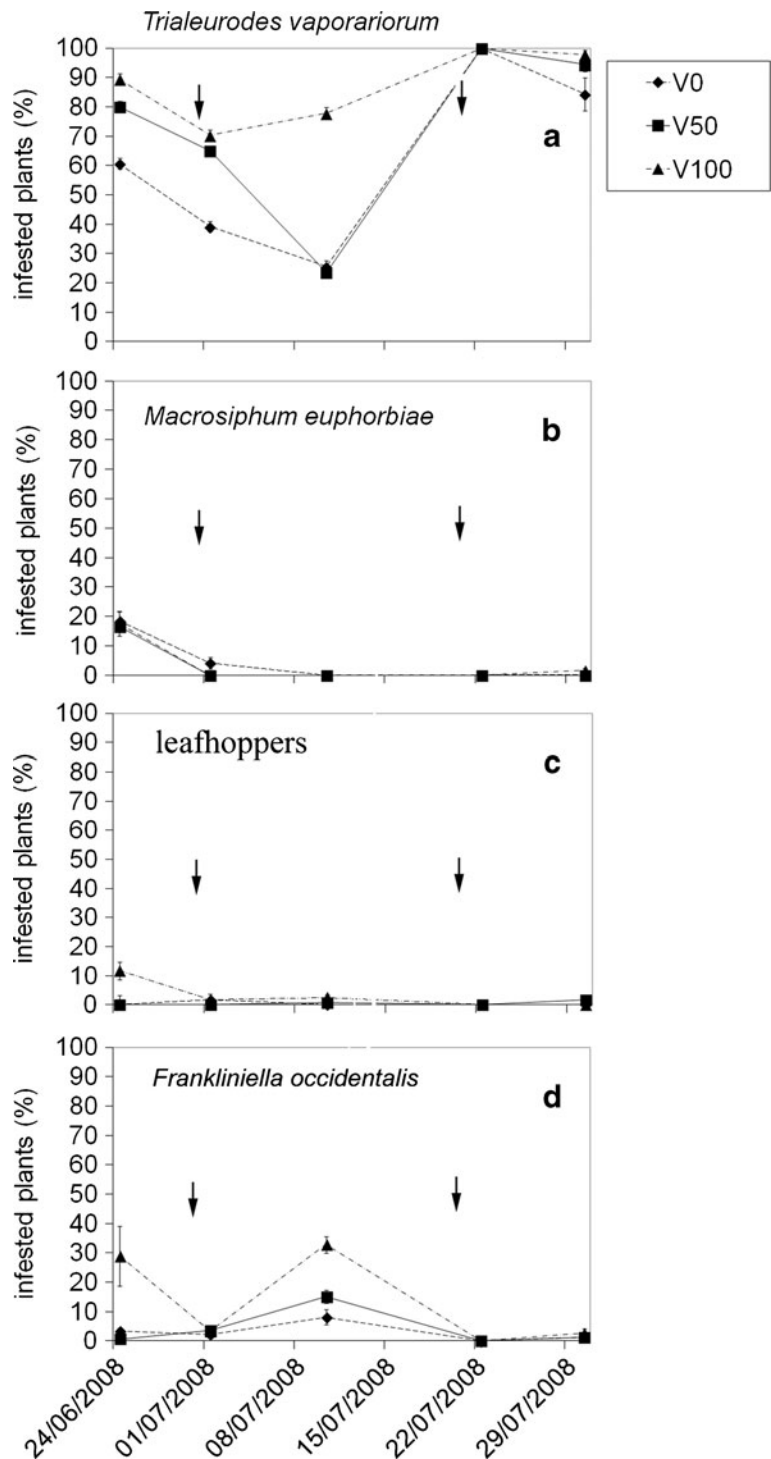
Discussion

Insect pests sampled in the field were all polyphagous sap-feeders. We did not observe the presence of *Tuta absoluta* (Meyrick), detected for the first time in Basilicata in 2009 (personal communication). The rate of infestation of the different species differed considerably in the years 2008 and 2009. This may be partially explained by the different weather trends in the 2 years.

However, many unknown factors, including populations persistence in greenhouses and other refuges in the surroundings, their migration propensity and pathways, and host availability within the study area, might have affected infestations by those polyphagous pests (Lamp & Zhao 1993; Walter 2005). Regardless of insect species and year, there was the same response pattern to the imposed experimental conditions. Infestation rates were affected by water regimes but not by mycorrhizal treatments. The lowest infestation rate was recorded in V0 plots, and the highest in V100 plots.

Water-stressed plants were colonized less than well-watered, so they were less attractive or less suitable. This result may be important for the transmission of viral diseases. In the case of less attractive or less suitable water-stressed plants, we can expect a reduction in the transmission of persistent virus, which requires long

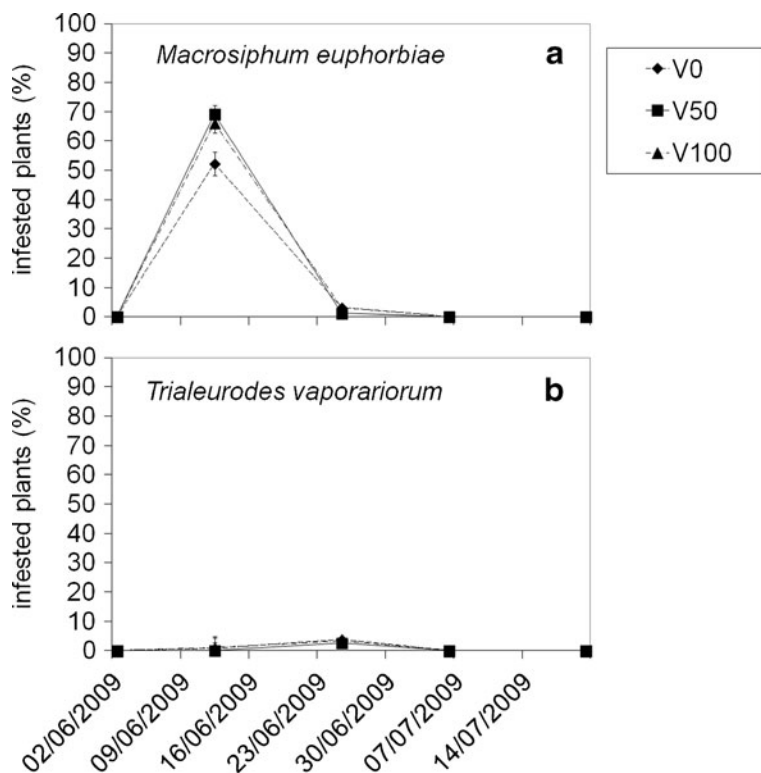
Fig. 2 Infestation rates (means \pm standard error) in the year 2008 as a function of water regimes: a) *Trialeurodes vaporariorum*; b) *Macrosiphum euphorbiae*; c) leaf-hoppers; d) *Frankliniella occidentalis*; V0 = control, irrigated only at transplant time; V50= 50% restoration of crop evapotranspiration (ETc); V100= full restoration of crop evapotranspiration (ETc). The arrows indicate dates of insecticide treatments: imidacloprid on June 27 and methomyl on July 18



acquisition times (hours to days) and long latent periods (one day to several weeks) (Andret-Link & Fuchs 2005). Consequences for non-persistent virus transmission are

more difficult to predict without knowing the influence of water stress on insect behavior. For example, 'non-preference' factors that increase movement and

Fig. 3 Infestation rates (means \pm standard error) in the year 2009 as a function of water regimes: a) *Macrosiphum euphorbiae*; b) *Trialeurodes vaporariorum*; V0 = control, irrigated only at transplant time; V50= 50% restoration of crop evapotranspiration (ETc); V100= full restoration of crop evapotranspiration (ETc)



probing between plants can contribute to disease spread and allow the disease to persist even at very low vector densities (Thomas & Waage 1996). Obviously, the effect of water deficit on virus transmission must be demonstrated with specific experiments under controlled conditions.

Response of pests to non-preferred or less suitable host plants is variable. In particular, we can expect that the response depends largely on population density and on the availability of more suitable hosts in the same area or in the same field (Thomas & Waage 1996). When population density is high, competition

Table 2 Full factorial ANOVA of infestation rates (transformed data) measured in the year 2008

	df	<i>Macrosiphum euphorbiae</i>		<i>Trialeurodes vaporariorum</i>		<i>Frankliniella occidentalis</i>		Leafhoppers	
		MS	F	MS	F	MS	F	MS	F
Date	4	1868.6	96.2***	8461.4	224.7***	1454.4	23.6***	43.1	3.2*
V	2	18.6	1.0	2782.4	73.8***	1073.5	17.4***	225.1	16.9***
M	2	18.4	1.0	6.0	0.2	4.8	0.1	0.6	0.04
Date x V	8	26.9	1.4	538.4	14.3***	337.1	5.5***	146.0	11.0***
Date x M	8	6.7	0.3	8.4	0.2	1.7	0.03	2.8	0.2
V x M	4	13.9	0.7	8.2	0.2	1.2	0.02	2.6	0.2
Date x V x M	16	9.1	0.5	3.6	0.1	4.7	0.08	1.4	0.1
Residuals	90	19.4		37.7		61.6		13.3	

M= mycorrhizal treatments; V= water volume

* $P < 0.05$; *** $P < 0.001$

df, degrees of freedom; MS, mean square; F, variance ratio

Table 3 Full factorial ANOVA of infestation rates (transformed data) measured in the year 2009

	df	<i>Macrosiphum euphorbiae</i>		<i>Trialeurodes vaporariorum</i>	
		MS	F	MS	F
Date	1	23047.5	795.47***	380.2	17.80***
V	2	74.0	2.55	45.7	2.14
M	2	2.1	0.07	4.5	0.21
Date x V	2	221.4	7.64**	2.2	0.10
Date x M	2	10.6	0.37	4.6	0.21
V x M	4	23.2	0.80	10.6	0.50
Date x V x M	4	11.8	0.41	10.8	0.51
Residuals	36	29.0		21.3	

M= mycorrhizal treatments; V= water volume;

** $P < 0.01$; *** $P < 0.001$

df, degrees of freedom; MS, mean square; F, variance ratio

is also high and, accordingly, the acceptance of non-preferred plants increases. This can explain the dynamics of whitefly infestations during 2008, when differences among watering treatments were highly significant after insecticide spraying with imidacloprid and were cancelled afterwards. This trend can be observed also after the second treatment with methomyl but it is less obvious. In fact, we recorded 100% infestation in all plots 4 days after the treatment with methomyl and the decrease after July 22 was much slighter than after the first treatment. Obviously, the insecticide application may have reduced the insect population without eliminating all individuals from the infested plants and then resulting in no or little change of infestation rate. Nevertheless, the results in

Fig. 4 Number (means \pm standard error) of *Macrolophus pygmaeus* per plot in 2009: V0 = control, irrigated only at transplant time; V50= 50% restoration of crop evapotranspiration (ETc); V100= full restoration of crop evapotranspiration (ETc) (V50); M1= Micosat F (AMF + bacteria of the rhizosphere + saprophytic fungi); M2= AMF; C= control

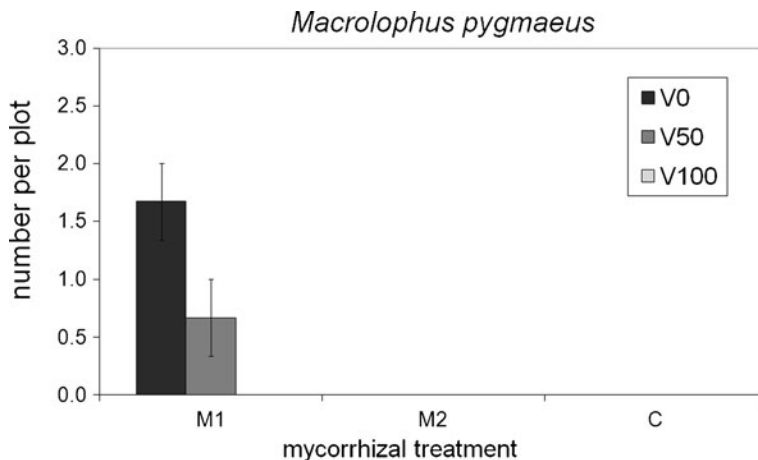


Table 4 Full factorial ANOVA of *Macrolophus pygmaeus* numbers

	df	<i>M. pygmaeus</i>	
		MS	F
V	2	0.7	9.5**
M	2	1.8	24.5***
V x M	4	0.7	9.5***
Residuals	18	0.07	

M= mycorrhizal treatments; V= water volume

** $P < 0.01$; *** $P < 0.001$

df, degrees of freedom; MS, mean square; F, variance ratio

this work can be an indication of poor activity of methomyl against *T. vaporariorum* in the Metapontum area.

There are no accurate data on population density, even though there can be good correlations between infestation rates and yield (Wilson *et al.* 1991); particularly for aphids, the economic threshold can be expressed as infestation rate level (Viggiani 1997). Previous studies under controlled conditions showed that performance of phytophagous insects in tomato may be reduced by water stress, depending on cultivars and stress level (Inbar *et al.* 2001; Rivelli *et al.* 2013). Our results show that water deficit also reduces infestation rate under field conditions. The reduction of infestation, however, was not sufficient to offset the negative effects of water shortage on crop yield. This finding confirms previous observations on concurrent effects of aphids and water stress on tomato plant growth under controlled conditions (Rivelli *et al.* 2012). It is also in agreement

with the results obtained in the case of chickpea and soybean under different irrigation regimes (Çıkman & Civelek 2006; Çıkman *et al.* 2011).

Both mycorrhization and water supply treatments resulted in a significantly positive effect on crop yield but, contrary to expectations, the interaction between mycorrhization and water regimes was not significant. This means that mycorrhization did not confer greater resistance to drought. Moreover, the presence of bacteria of the rhizosphere and saprophytic fungi in the inoculum did not produce significant benefits in terms of agronomic results, but it might have affected the presence of predators. In fact, *M. pygmaeus*, a generalist predator, was collected only in plots treated with Micosat F, containing VAM, bacteria of the rhizosphere and saprophytic fungi all together. In particular, Micosat F contains *T. harzianum*. It has recently been demonstrated that root colonization by another *Trichoderma* species, *T. longibrachiatum* MK1, significantly alters the behavioral response of *M. pygmaeus* to tomato plants (Battaglia *et al.* 2013). In choice experiments and in the complete absence of insect infestation, plants colonized by *T. longibrachiatum* MK1 are significantly more attractive for *M. pygmaeus* females than uncolonized controls. However, plants in the experiment field were infested and the predator was also attracted to pests on the plants. Moreover, *M. pygmaeus* was collected only once, during 2009, and the sampling method was possibly not the most appropriate to sample this species, which is a good flyer. For these reasons it is necessary to be very cautious in interpreting the presence of *M. pygmaeus* in M1 plots, and the possible attraction by Micosat-treated plants needs to be confirmed by further observations.

Acknowledgments This work was supported by the Italian Ministry of Agriculture and Forestry Policies (MIPAF), research project PROM (*Progetto di Ricerca per potenziare la competitività di Orticole in aree Meridionali* – Research Project to strengthen competitiveness of vegetables in southern areas – funds C.I.P.E., Resolution 17/2003, Scientific coordinator: Dr. Agostino Falavigna); and by the Italian Ministry of Education, University and Scientific Research (MIUR), research project PRIN (*Pratiche agronomiche e qualità della pianta: influenza sul secondo e terzo livello trofico* – [Agronomic practices and plant quality: influence on the second and third trophic level] Scientific coordinator: Prof. Donatella Battaglia).

We express our appreciation to Paolo Putignano and Cosimo Danzi for their valuable help in conducting experimental trials and collecting agronomic data. We thank Prof. Luciana Tavella for taxonomic determination of *Macrolophus pygmaeus*.

References

- Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). *Crop evapotranspiration. Guidelines for computing crop water requirements*. Irrigation Drainage, Paper 56. Food and Agriculture Organization of United Nations (FAO), Rome, Italy.
- Andret-Link, P., & Fuchs, M. (2005). Transmission specificity of plant viruses by vectors. *Journal of Plant Pathology*, *87*, 153–165.
- Augè, R. M. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, *11*, 3–42.
- Baslam, M., & Goicoechea, N. (2011). Water deficit improved the capacity of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of antioxidant compounds in lettuce leaves. *Mycorrhiza*, *22*, 347–359.
- Battaglia, D., Bossi, S., Cascone, P., Digilio, M. C., Duran Prieto, J., Fanti, P., *et al.* (2013). Tomato belowground–aboveground interactions: *Trichoderma longibrachiatum* affects the performance of *Macrosiphum euphorbiae* and its natural antagonists. *Molecular Plant-Microbe Interactions*. doi:10.1094/MPMI-02-13-0059-R.
- Brundrett, M. C., Piché, Y., & Peterson, R. L. (1983). A new method for observing the morphology of vesicular arbuscular mycorrhizas. *Canadian Journal of Botany*, *62*, 2128–2134.
- Candido, V., Miccolis, V., & Perniola, M. (2000). Effects of irrigation regime on yield and quality of processing tomato (*Lycopersicon esculentum* Mill.) cultivars. *Acta Horticulturae*, *537*, 779–788.
- Çıkman, E., & Civelek, H. S. (2006). Population densities of *Liriomyza cicerina* (Rondani, 1875) on *Cicer arietinum* L. in different irrigated conditions. *Türkiye Entomoloji Dergisi*, *30*, 3–10.
- Çıkman, E., Çömlekçiöğlü, N., & Şimşek, M. (2011). Effects of different irrigation levels on population densities of *Liriomyza trifolii* (Burgess, 1880) on two vegetable soybean (*Glycine max* (L.) Merr.) cultivars. *Harran Üniversitesi Ziraat Fakültesi Dergisi*, *15*, 29–35.
- Conversa, G., Elia, A., & La Rotonda, P. (2007). Mycorrhizal inoculation and phosphorus fertilization effect on growth and yield of processing tomato. *Acta Horticulturae*, *758*, 333–338.
- Cosme, M., Stout, M. J., & Wurst, S. (2011). Effect of arbuscular mycorrhizal fungi (*Glomus intraradices*) on the oviposition of rice water weevil (*Lissorhoptrus oryzophilus*). *Mycorrhiza*, *21*, 651–658.
- Dalpé, Y., & Monreal, M. (2004). Arbuscular mycorrhiza inoculum to support sustainable cropping systems. *Online Crop Management* doi:10.1094/CM-2004-0301-09-RV, <http://www.plantmanagementnetwork.org/pub/cm/review/2004/amfungi/>. Accessed 5 February 2013.
- Davies, F. T., Jr., Potter, J. R., & Linderman, R. G. (1993). Drought resistance of mycorrhizal pepper plants independent of leaf P concentration - response in gas exchange and water relations. *Physiologia Plantarum*, *87*, 45–53.
- Dicke, M. (2000). Chemical ecology of host-plant selection by herbivorous arthropods: a multitrophic perspective. *Biochemical Systematics and Ecology*, *28*, 601–617.
- Doorenbos, J., & Pruitt, W. O. (1977). *Guidelines for predicting crop requirements*. FAO irrigation and drainage paper no. 24. Food and Agriculture Organization of United Nations, Rome, Italy.

- Du, Y., Poppy, G. M., Powell, W., Pickett, J. A., Wadhams, L. J., & Woodcock, C. M. (1998). Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *Journal of Chemical Ecology*, *24*, 1355–1368.
- El-Mesbahi, M. N., Azcón, R., Ruiz-Lozano, J. M., & Aroca, R. (2012). Plant potassium content modifies the effects of arbuscular mycorrhizal symbiosis on root hydraulic properties in maize plants. *Mycorrhiza*, *22*, 555–564.
- Favati, F., Lovelli, S., Galgano, F., Miccolis, V., Di Tommaso, T., & Candido, V. (2009). Processing tomato quality as affected by irrigation scheduling. *Scientia Horticulturae*, *122*, 562–571.
- Fritz, M., Jakobsen, I., Lyngkjær, M. F., Thordal-Christensen, H., & Pons-Kühnemann, J. (2006). Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. *Mycorrhiza*, *16*, 413–419.
- Gange, A. C. (2001). Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist*, *150*, 611–618.
- Gouinguéné, S. P., & Turlings, T. C. J. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, *129*, 1296–1307.
- Goverde, M., Van der Heijden, M. G. A., Wiemken, A., Sanders, I. R., & Erhardt, A. (2000). Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore. *Oecologia*, *125*, 362–369.
- Guerrieri, E., Lingua, G., Digilio, M. C., Massa, N., & Berta, G. (2004). Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? *Ecological Entomology*, *29*, 753–756.
- Hargreaves, G. H., & Samani, Z. A. (1985). Reference crop evapotranspiration from temperature. *Applied Engineering in Agriculture*, *1*, 96–99.
- Harrison, M. J., & van Buuren, M. L. (1995). A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature*, *378*, 626–629.
- Hodge, A., Campbell, C. D., & Fitter, A. H. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature*, *413*, 297–299.
- Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, *85*, 1383–1398.
- Inbar, M., Doostdar, H., & Mayer, R. T. (2001). Suitability of stressed and vigorous plants to various insect herbivores. *Oikos*, *94*, 228–235.
- Karagiannidis, N., Bletsos, F., & Stavropoulos, N. (2002). Effect of *Verticillium* wilt (*Verticillium dahliae* Kleb.) and mycorrhiza (*Glomus mosseae*) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings. *Scientia Horticulturae*, *94*, 145–156.
- Lamp, W. O., & Zhao, L. (1993). Prediction and manipulation of movement by polyphagous, highly mobile pests. *Journal of Agricultural Entomology*, *10*, 267–281.
- Lee, B. R., Muneer, S., Avicé, J. C., Jung, W. J., & Kim, T. H. (2012). Mycorrhizal colonisation and P-supplement effects on N uptake and N assimilation in perennial ryegrass under well-watered and drought-stressed conditions. *Mycorrhiza*, *22*, 525–534.
- Liu, J. Y., Maldonado-Mendoza, I., Lopez-Meyer, M., Cheung, F., Town, C. D., & Harrison, M. J. (2007). Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *The Plant Journal*, *50*, 529–544.
- Patanè, C., & Cosentino, S. L. (2010). Effects of soil water deficit on yield and quality of processing tomato under a Mediterranean climate. *Agricultural Water Management*, *97*, 131–138.
- Patanè, C., Tringali, S., & Sortino, O. (2011). Effects of deficit irrigation on biomass, yield, water productivity and fruit quality of processing tomato under semi-arid Mediterranean climate conditions. *Scientia Horticulturae*, *129*, 590–596.
- Perniola, M., Rivelli, A. R., & Candido, V. (1994). Yield response to water and stress indexes on tomato. *Acta Horticulturae*, *376*, 215–226.
- Rivelli, A. R., Toma, I., Trotta, V., Fanti, P., De Maria, S., & Battaglia, D. (2012). Combined effect of water stress and *Macrosiphum euphorbiae* infestation on plant growth in tomato. In: F. Stoddard, & P. Mäkelä, *12th Congress of the European Society for Agronomy* (Helsinki, Finland; vol. 1, pp. 334–335).
- Rivelli, A. R., Trotta, V., Toma, I., Fanti, P., & Battaglia, D. (2013). Relation between plant water status and *Macrosiphum euphorbiae* (Hemiptera: Aphididae) population dynamics on three cultivars of tomato. *European Journal of Entomology*, *110*, 617–625.
- Sasso, R., Iodice, L., Digilio, M. C., Carretta, A., Ariati, L., & Guerrieri, E. (2007). Host-locating response by the aphid parasitoid *Aphidius ervi* to tomato plant volatiles. *Journal of Plant Interactions*, *2*, 175–183.
- Thomas, M. B., & Waage, J. K. (1996). *Integration of biological control and host plant resistance breeding: a scientific and literature review*. Wageningen, the Netherlands: Technical Center for Agriculture and Rural Cooperation of the European Union.
- Trouvelot, A., Kough, J. L., & Gianinazzi-Pearson, V. (1986). Mesure du taux de mycorrhization VA d'un système racinaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle, (pp. 217–221) In: V. Gianninazzi-Pearson & S. Gianninazzi (Eds.) *Mycorrhiza: physiology and genetics*. Paris, France: INRA.
- van Dam, N. M., Harvey, J. A., Wäckers, F. L., Bezemer, T. M., Van der Putten, W. H., & Vet, L. E. M. (2003). Interactions between aboveground and belowground induced responses against phytophages. *Basic and Applied Ecology*, *4*, 63–77.
- Vázquez, M. M., César, S., Azcón, R., & Barea, J. M. (2000). Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Applied Soil Ecology*, *15*, 261–272.
- Vickers, C. E., Gershenzon, J., Lerdau, M. T., & Loreto, F. (2009). A unified mechanism of action for volatile isoprenoids in plant abiotic stress. *Nature Chemical Biology*, *5*, 283–291.
- Viggiani, G. (1997). *Lotta biologica e integrata nella difesa fitosanitaria*. Napoli, Italy: Liguori Editore.
- Vos, C., Geerinckx, C. K., Mkandawire, R., Panis, B., De Waele, D., & Elsen, A. (2012). Arbuscular mycorrhizal fungi affect both penetration and further life stage development of root-knot nematodes in tomato. *Mycorrhiza*, *22*, 157–163.

- Walter, G. H. (2005). *Insect pest management and ecological research*. New York, NY: Cambridge University Press.
- Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J., *et al.* (2008). Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology*, *34*, 1153–1161.
- Wilson, L. T., Trichilo, P. J., & Gonzalez, D. (1991). Spider mite (Acari: Tetranychidae) infestation rate and initiation: effect on cotton yield. *Journal of Economic Entomology*, *84*, 593–600.
- Zar, J. H. (1984). *Biostatistical analysis*. Englewood Cliffs, NJ, USA: Prentice-Hall.