

Growth and yield promoting effect of artificial mycorrhization combined with different fertiliser rates on field-grown tomato

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Abstract

Combination of plant inoculation with a commercial mycorrhizal formulation with half or full fertiliser application rates was evaluated for the effects on plant growth and yield and mycorrhization occurrence throughout two consecutive field tomato crops in southern Italy. Mycorrhizal formulation was inoculated on tomato seedling roots both in the nursery and after transplant. Inoculated tomato seedlings were significantly larger than non-inoculated seedlings less than 30 days after the first inoculation in the nursery. Above ground dry biomass and stem number of inoculated plants were found to be higher also at the end of each crop. Positive effects of mycorrhizal inoculation were extended also to marketable yield of both crops, mainly due to an increased number and weight of clusters and fruits. Mycorrhizal treatment also improved crop earliness, seen in terms of anticipating plant

flowering, increasing first harvest yield, and reducing average harvesting time compared to non-inoculated plants. Both rates of mineral fertilisers positively affected tomato growth and marketable yield, but did not influence fruit quality parameters. No significant interaction was found between mineral fertilisation and plant mycorrhization. Crop inoculation with mycorrhizal formulations could reduce the amounts of fertilisers and pesticides being used, and could represent a sustainable technique to improve crop yield and profitability.

Introduction

Microbial activity in the rhizosphere is a primary determinant of plant health and soil fertility (Jeffries *et al.*, 2003). Among mycorrhizal fungi symbiotically associated to plant roots, arbuscular mycorrhizal fungi (AMF) are the most important for agriculture, and are extremely beneficial to host plants (Gosling *et al.*, 2006). Benefits of AMF are mainly related to an improved uptake of relatively immobile phosphate ions, although an increased uptake of macronutrients other than phosphorus (P), including nitrogen, potassium and magnesium, has also been observed (Smith and Read, 2008; Hodge *et al.*, 2001). Increased plant resistance to insects and soil pathogens, and tolerance of salinity, heavy metals and drought, were also reported as further AMF benefits to the host plant (Allen, 2009; Avis *et al.*, 2008; Brundrett, 2009; van der Heijden *et al.*, 1998).

Plant-growth-promoting rhizobacteria (PGPR) and plant-growth-promoting fungi are further components of rhizosphere microflora that can also play a relevant role in plant growth and phytopathogen suppression, mainly due to their synergistic interaction with mycorrhizae (Compant *et al.*, 2005; Jeffries *et al.*, 2003).

Synergistic positive interactions between AMF and PGPR, such as nitrogen fixers, fluorescent *Pseudomonads* and sporulating *bacilli*, have been documented by many researchers (Galleguillos *et al.*, 2000; Hameeda *et al.*, 2007), although some neutral effects of AMF-PGPR interaction have also been reported (Andrade *et al.*, 1997; Walley and Germida, 1997).

Massive application of pesticides and fertilisers, as well as close rotations and deep tillage, resulted in a gradual depletion of soil fertility and microbial diversity in intensive agricultural systems (Daniell *et al.*, 2001; Tilman *et al.*, 2002). Conventionally managed soils were found to exhibit a poorer microflora and a lower biological activity than less intensively or organically managed soils (Mäder *et al.*, 2002). Breeding programmes have also aimed to select high chemical inputs, almost completely ignoring the interactions between plant roots and rhizosphere microorganisms (Wissuwa *et al.*, 2009).

Artificial plant inoculation with appropriate formulations of AMF

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Key words: arbuscular mycorrhizal fungi, fertilisers, yield, mycorrhizal, plant-growth-promoting rhizobacteria, tomato.

Contributions: the authors contributed equally.

Funding: this work was supported by the Italian Ministry of Agricultural and Forestry Policies (MIPAF), research project PROM (*Progetto di Ricerca per potenziare la competitività di Orticole in aree Meridionali* - funds C.I.P.E., Resolution 17/2003, Scientific Co-ordinator: Dr. Agostino Falavigna).

Acknowledgements: the authors thank Paolo Putignano and Cosimo Danzi for their valuable help in conducting the experimental trials.

Received for publication: 11 May 2013.

Revision received: 4 July 2013.

Accepted for publication: 8 July 2013.

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Licensee PAGEPress, Italy
Italian Journal of Agronomy 2013; 8:e22
doi:10.4081/ija.2013.e22

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and PGPR can improve plant adsorption of water and nutrients with a reduction in the use of chemical fertilisers and pesticides resulting in higher crop sustainability. Beneficial effects of AMF inoculation on plant growth and yield were widely documented for vegetable crops (Douds *et al.*, 2007; Hamel and Plenchette, 2007; Larsen *et al.*, 2007). Technical issues raised by the large amount of inoculum needed for AMF application in the field can be effectively avoided by mycorrhizal inoculation of seedlings or cutting beds over a much smaller surface (Jeffries *et al.*, 2003). An improvement in crop productivity following artificial inoculation with AMF formulations has been documented also for tomato, as a consequence of plant phenological, molecular and metabolic variations and systemic effects on fruits (Conversa *et al.*, 2012; Salvioli *et al.*, 2012).

In intensive vegetable cropping systems, potential benefits of mycorrhizal inoculation may be compromised by the massive input of mineral fertilisers. A reduced AMF colonisation of roots and spore density in soil was generally reported, mainly in the presence of an intensive use of P fertilisers (Kahiluoto *et al.*, 2001; Kogelmann *et al.*, 2004), although a negative impact on AMF colonisation and/or diversity has often been documented also for other readily soluble fertilisers, particularly nitrogen (N) fertilisers (Burrows and Pflieger, 2002; Treseder and Allen, 2002).

This paper reports the results of a field experiment in southern Italy aimed at investigating the agronomical effects of tomato inoculation with a commercial AMF and PGPR formulation, and its interactions with different levels of mineral fertilisers throughout two consecutive crops.

Materials and methods

The experiment was carried out during 2006-2007 in a field on the experimental farm Pantanello at Metaponto, in the Province of Matera, southern Italy (40° 24'N; 16° 48'E; 10 m asl).

Field soil was sandy-loamy (51.3% loam, 29.0% sand, 19.7% clay), with a 7.7 pH, 0.8 g/kg total nitrogen, and 21.2 and 215 mg/kg exchangeable phosphorous and potassium, respectively.

Soil was 30-cm ploughed, rotavated and levelled, and the field was subdivided into nine 27 m² plots. At ploughing, 80, 100 and 200 kg ha⁻¹ of N, P₂O₅ and K₂O fertilisers, respectively, were applied to three plots; another three plots received half the quantity of the same fertilisers, and the remaining three plots did not receive any fertiliser at all.

For both years, cherry tomato HF1 PX 02325715 (Seminis srl, Parma, Italy) was sown broadcast in a turf-based substrate (60% organic matter, 6.0-6.5 pH) in a 62×35 cm box. At full extension of cotyledon leaves, bare root seedlings were transferred singly to 60-cell polystyrene sowing trays, 63 mL of the same substrate in each cell. Half seedlings from each tray were inoculated with 1.15 mL/cell of a commercial microbial formulation (Micosat F, CCS AOSTA Srl, Aosta, Italy), containing ground mycorrhizal roots, spores and mycelia of AMF *Glomus mosseae* GP11, *G. viscosum* GC41, *G. intraradices* GB67, as well as PGPR as *Pseudomonas* sp. PN 01, *P. fluorescens* PA28, *Bacillus subtilis* BA41, *Streptomyces* sp. SB14, and the antagonistic saprophytic fungus *Trichoderma viride* TH03.

Forty-day old seedlings were transplanted in the field on 15th May

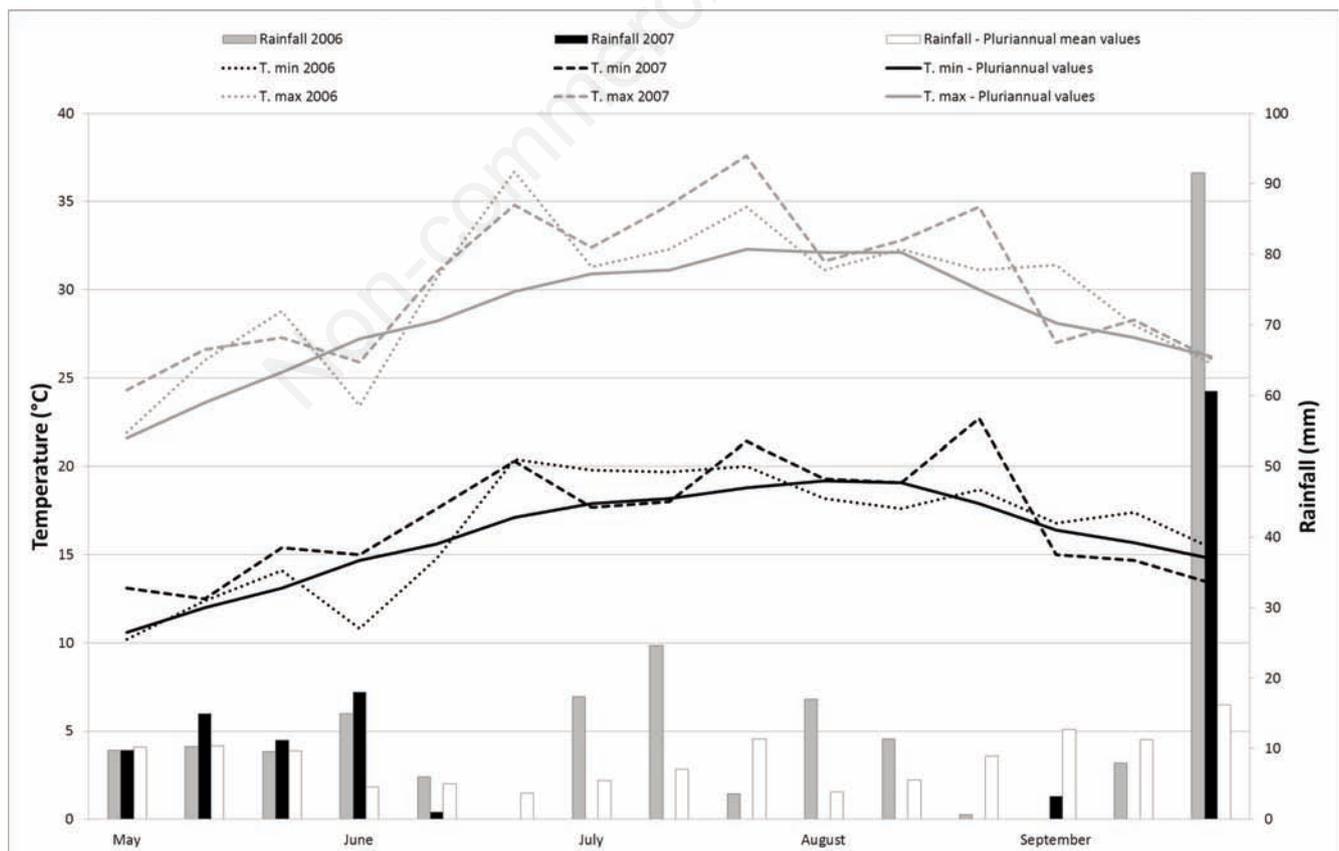


Figure 1. Ten-day minimum and maximum temperature and rainfall trends recorded during the two tomato crop cycles and compared to the pluriannual values.

2006 and 25th May 2007. Each plot was subdivided into two 13.5 m² sub-plots, and each transplanted with inoculated or non-inoculated seedlings. Plants were spaced 30 cm apart within double rows, 1.5 m between each row. A further 1.15 mL of the microbial formulate were poured in the soil beside each previously inoculated seedling so as to reach a total 100 L ha⁻¹ application rate. Therefore, the experiment was arranged in a split plot randomised design, with three replicates of five different treatments and non-treated soil as control.

Top-dress 60 and 30 kg ha⁻¹ N, split into three applications from plant rooting to flowering, were provided to the plots with full and half basal fertilisation, respectively. Plant irrigation was provided throughout both crops by a drip irrigation system: dripper lines 0.5 m apart and emitters (2.5 L h⁻¹ water flow rate) spaced 0.20 m from each other. Non-systemic chemical treatments were applied, as needed, for weed and insect pest control.

Daily maximum and minimum air temperatures and rainfall were recorded by a CR-10X data-logger (Campbell Scientific, Inc., Logan, UT, USA).

Dates of initiation of different plant phenological phases were recorded throughout each crop.

At transplant of each crop, stem height and diameter, above ground and root dry weight, and leaf area (Area Meter LI-Cor, Inc., Lincoln, NE, USA; Model 3100) were recorded on 10 inoculated and non-inoculated seedlings. At half crop cycle and before fruit setting, plant height and number of stems, above ground dry biomass and leaf area index (LAI) were recorded on three plants from each plot.

Tomato fruits were harvested on 28th July, 10th August and 4th September 2006, and on 10th and 31st August 2007 in the first and second cycle, respectively. At each harvest, tomato yield of each plot was estimated by recording number and weight of ripe clusters from 20 plants. Number and weight of marketable and waste fruits were recorded on 10 clusters from each plot. Average fruit weight, soluble solid (°Brix) and dry matter content were recorded on 10 marketable fruits from each plot.

Percentage incidence of first harvest yield on total cumulative yield and mean harvesting time (MHT) were calculated as indicators of crop earliness. MHT was calculated according to the following formula:

$$TMR (dd) = \sum(y_1 \cdot d_1) \dots \dots (y_n \cdot d_n) / Y$$

where:

d is days between crop transplant and harvest beginning;

y is yield at each harvest;

Y is total cumulative marketable yield.

At end of harvest, we counted number of stems per plant and above

ground dry biomass weight was recorded as the cumulative dry weight of oven-dried stem, leaves and fruits of plants from each sampling area. Harvest index was calculated as a ratio between fruits and total dry matter weight. Mycorrhizal root colonisation was estimated on 30 2-cm fragments randomly taken from each root sample of inoculated plants after clearing and staining (Brundrett *et al.*, 1984). Frequency of colonisation (F), intensity of colonisation (m%), and presence of arbuscules and vesicles (a%) were determined according to Trouvelot *et al.* (1986) using MycoCalc software (INRA, Dijon, France).

All data were statistically analysed by ANOVA and means separated by the Student-Newman-Keuls (SNK) test.

Results

Climatic trend

Maximum air temperatures averaged 36.7°C during the last ten days of June 2006, and 34.8°C and 34.3°C in the second ten days of July and the third ten days of August 2007, respectively (Figure 1). In both years, mean maximum temperatures remained over 30°C from the second ten days of June to the last ten days of August, exceeding 40°C on June 20th and August 20th 2006, and on June 2nd and 26th and July 24th 2007. Average minimum temperatures peaked at 20.4°C and 22.2°C in the last ten days of June 2006 and the last ten days of August 2007.

From May to August 2006, rainfall was concentrated in the first ten days of June (15 mm), in the first (17.4 mm) and second (24.6 mm) ten days of July, and in the first (14.6 mm) and second (13.8 mm) ten days of August. Rainfall was lower and less frequent in 2007; rain fell mainly in May (9.8 mm, 15 mm and 11.2 mm in the first, second and third ten days, respectively) and June (18 mm in the first ten days). There was no rain at all during May 2006 or from 13th June to 24th September 2007.

Plant growth parameters

At transplant, almost all tomato seedling growth parameters were affected by the previous mycorrhizal inoculation in the nursery (Table 1). Height, above ground and root dry weight and dry matter content of inoculated seedlings were higher than those of non-inoculated plants. Inoculated seedlings also showed a wider leaf area and a higher number of leaves per plant.

At half crop cycle, plant height, top dry weight and leaf area index were significantly higher in the second than in the first crop (Table 2). In contrast, a larger number of plant branches and a delayed flowering,

Table 1. Effects of mycorrhization on morphological traits of tomato seedlings at transplant.

Year	Mycorrhizal treatment	Stems		Leaves/plant		Epigeal part		Roots	
		Height (cm)	Thickness (mm)	Number	Area (cm ²)	Dry weight/plant (mg)	Dry matter (%)	Dry weight/plant (mg)	Root/shoot dry weight ratio
2006	<i>Micosat F</i>	12.2 ^a	3.3 ^a	5.7 ^a	31.0 ^a	249 ^a	9.8 ^a	103 ^a	2.6 ^a
	Control	9.5 ^b	3.0 ^b	4.7 ^b	12.4 ^b	90 ^b	8.8 ^b	42 ^b	2.2 ^b
	Significance	**	*	**	**	**	**	**	*
2007	<i>Micosat F</i>	21.0 ^a	2.9 ^a	6.0 ^a	76.4 ^a	365 ^a	9.6 ^a	115 ^a	3.2 ^a
	Control	17.4 ^b	2.9 ^a	5.3 ^b	66.9 ^b	318 ^b	9.8 ^a	102 ^b	3.0 ^b
	Significance	**	ns	**	**	**	ns	**	*

^{a,b}Means followed by the same letters in the same column and within each experiment are not significantly ($\alpha \leq 0.05$) different according to the Student-Newman-Keuls Test. * $P \leq 0.05$ was considered significant; ** $P \leq 0.01$. ns, non-significant.

fruit setting and veraison occurred in the first tomato crop.

Mineral fertilisation positively affected plant growth; height, above ground dry biomass, leaf area index and stem number of plants from both full and half fertilised plots were significantly higher than in non-fertilised soil, although the increase in growth was much more evident in plots receiving the full quantity of fertiliser. Compared to non-fertilised plots, mineral fertilisation also resulted in a significantly earlier flowering and fruit veraison, whereas no significant differences were found between the two quantities of fertiliser. Plants inoculated with the mycorrhizal formulation were significantly taller, and had higher above ground dry biomass and LAI, and a significantly earlier flowering and veraison than non-inoculated plants.

Final above ground dry biomass was significantly higher in the second than in the first crop (Table 3). Compared to non-fertilised control, half and full rate mineral fertilisation increased final above ground dry biomass by 2.7 and 4.2 t ha⁻¹, respectively, and number of stems per plant by 2 units. Plant inoculation with the mycorrhizal formulation also resulted in a significant increase in above ground dry biomass and number of stems per plant: +0.6 t ha⁻¹ and +1.1 units, respectively.

Yield parameters

Tomato yield was significantly higher in the second than in the first crop due to a lower number of clusters and fruits per plant, and to a lower fruit dry matter and soluble solid contents (Table 3). A longer length cycle and a 2-week delay in fruit ripening and harvest were also observed in the first crop.

Marketable yield was always significantly higher in soil receiving both half and full fertiliser rates than in non-fertilised control. Half and full fertiliser rates increased tomato yield by 37% and 61% in the first crop and by 21% and 32% in the second crop, respectively, mainly due

to a significantly higher number of clusters per plant. There was no significant difference in average fruit weight when compared to control.

Plants inoculated with the mycorrhizal formulation provided a significantly higher yield compared to the non-inoculated plants, mainly due to a higher number of clusters and fruit.

All yield parameters showed a statistically significant interaction only between crop cycle and mineral fertilisation.

The second tomato crop was earlier than the first, providing a 34.9% larger yield (+) in a 14-day shorter MHT (Table 4).

Both mineral fertilisation and mycorrhizal inoculation also resulted in a significantly earlier crop harvest than the non-treated control, increasing first harvest yield by 4.6% and 4.1% in a 3.5- and 2-day shorter MHT, respectively.

Tomato fruit dry matter and soluble solids content were significantly higher in the second than in the first crop, without any significant influence of either mineral fertilisation or mycorrhizal inoculation.

Mycorrhization parameters

At transplant, presence of fungal mycelium was clearly evident on tomato seedling roots previously inoculated with the mycorrhizal formulation, whereas almost no endomycorrhizal structures were observed in non-inoculated plants (Table 4).

There was only a slight variation in frequency of mycorrhization (F%) and arbuscule presence (a%) between the two crops (Table 5). Mycorrhization frequency and arbuscule presence at half cycle were significantly higher in the second than in the first crop. In contrast, mycorrhization indices at the end of the crop were significantly higher in the first than in the second year.

No significant interaction was found between mineral fertilisation and mycorrhization, although at the end of the crop almost all mycor-

Table 2. Effects of fertilization and mycorrhization on plant growth parameters and occurrence of phenological phases at mid crop cycle.

Treatments	Plant growth parameters				Occurrence of phenological phases (days from transplant)		
	Plant height (cm)	Stems/plant (no.)	Above ground dry biomass (t ha ⁻¹)	Leaf area index (LAI)	Flowering	Fruit setting	Ripening
Years (Y)							
2006	54.7 ^b	7.2 ^a	1.5 ^b	0.9 ^b	32.4 ^a	45.1 ^a	64.8 ^a
2007	74.9 ^a	6.5 ^b	3.8 ^a	3.0 ^a	23.6 ^b	36.8 ^b	58.5 ^b
Significance	**	**	**	**	**	**	**
Fertilization (F)							
0	57.1 ^c	6.4 ^c	1.8 ^c	1.2 ^c	29.2 ^a	41.9 ^a	62.3 ^a
50	65.1 ^b	6.9 ^b	2.8 ^b	2.0 ^b	27.6 ^b	40.6 ^b	61.3 ^a
100	72.2 ^a	7.2 ^a	3.3 ^a	2.7 ^a	27.3 ^b	40.3 ^b	61.3 ^a
Significance	**	**	**	**	**	**	ns
Mycorrhization (M)							
<i>Micosat F</i>	66.2 ^a	7.2 ^a	2.9 ^a	2.2 ^a	27.1 ^b	40.4 ^b	61.4 ^a
Control	63.4 ^b	6.5 ^b	2.3 ^b	1.8 ^b	29.0 ^a	41.5 ^a	61.9 ^a
Significance	*	**	**	**	**	**	ns
Interactions							
Y x F	ns	ns	ns	**	*	**	ns
Y x M	ns	ns	ns	ns	ns	ns	ns
F x M	ns	ns	ns	ns	ns	ns	ns
Y x F x M	ns	ns	ns	ns	ns	ns	ns

^{a,b,c} Means followed by the same letters in the same column and within each experiment are not significantly ($\alpha \leq 0.05$) different according to the Student-Newman-Keuls Test. * $P \leq 0.05$ was considered significant; ** $P \leq 0.01$, ns, non-significant.

Table 3. Effects of fertilisation and mycorrhization on tomato yield, fruit quality, crop earliness and plant growth at harvest.

Treatments	Yield		Clusters per plant (N)	Fruit quality				Crop earliness		Plant growth		
	Total (t ha ⁻¹)	Marketable (t ha ⁻¹)		N plant	Mean weight (g)	Soluble solids (°Brix)	Dry matter (%)	Yield at 1 st harvest (%)	Harvest mean time (d)	Above ground dry biomass (t ha ⁻¹)	Harvest index	Stems (no.)
Years (Y)												
2006	54.1 ^b	51.2 ^b	30.6 ^b	176 ^b	6.3 ^a	7.7 ^b	9.2 ^b	4.4 ^b	104 ^a	9.0 ^b	0.53 ^a	11.9 ^a
2007	61.3 ^a	56.1 ^a	38.7 ^a	215 ^a	6.7 ^a	8.6 ^a	10.1 ^a	39.3 ^a	90 ^b	12.6 ^a	0.46 ^b	9.4 ^b
Significance	**	**	**	**	ns	*	**	**	**	**	**	**
Fertilization (F)												
0	43.4 ^c	40.3 ^c	26.5 ^c	149 ^b	6.4 ^a	8.0 ^a	9.7 ^a	18.8 ^b	99 ^a	8.5 ^c	0.48 ^a	9.2 ^b
50	59.6 ^b	56.2 ^b	35.6 ^b	204 ^a	6.4 ^a	8.2 ^a	9.6 ^a	23.8 ^a	96 ^b	11.2 ^b	0.49 ^a	11.2 ^a
100	70.0 ^a	64.4 ^a	41.8 ^a	233 ^a	6.6 ^a	8.1 ^a	9.7 ^a	23.0 ^a	95 ^b	12.7 ^a	0.51 ^a	11.6 ^a
Significance	**	**	**	**	ns	ns	ns	**	**	**	ns	**
Mycorrhization (M)												
<i>Micosat F</i>	60.6 ^a	56.0 ^a	36.3 ^a	208 ^a	6.4 ^a	8.1	9.7	23.9	96 ^b	11.3 ^a	0.51 ^a	11.2 ^a
Control	54.8 ^b	51.2 ^b	32.9 ^b	183 ^b	6.5 ^b	8.1	9.6	19.8	98 ^a	10.2 ^b	0.50 ^a	10.1 ^b
Significance	**	**	**	**	ns	ns	ns	**	**	**	ns	**
Interactions												
<i>Y x F</i>	**	**	**	*	ns	ns	ns	*	**	*	*	ns
<i>Y x M</i>	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
<i>F x M</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
<i>Y x F x M</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

^{a,b,c}Means followed by the same letters in the same column and within each experiment are not significantly ($\alpha \leq 0.05$) different according to the Student-Newman-Keuls Test. * $P \leq 0.05$ was considered significant; ** $P \leq 0.01$, non-significant.

Table 4. Mycorrhization parameters of tomato seedling roots at transplant.

Mycorrhizal treatments	Mycorrhizal indices									
	F%		M%		m%		a%		A%	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	5.6 ^b	6.7 ^b	0.1 ^b	0.1 ^b	1.0 ^b	1.0 ^b	0.0 ^b	5.0 ^b	0.0 ^b	0.0 ^b
<i>Micosat F</i>	100.0 ^a	100.0 ^a	5.1 ^a	5.5 ^a	5.1 ^a	5.5 ^a	24.3 ^a	9.4 ^a	1.3 ^a	0.6 ^a
Significance	**	**	**	**	**	**	**	**	**	**

^{a,b}Means followed by the same letters in the same column and within each experiment are not significantly ($\alpha \leq 0.05$) different according to the Student-Newman-Keuls Test; ** $P \leq 0.01$ was considered significant. ns, non-significant.

Table 5. Mycorrhization parameters on tomato roots at mid (flowering-fruit setting stage) and end crop cycle.

Treatments	Mycorrhizal indices									
	F%		M%		m%		a%		A%	
	Mid	End	Mid	End	Mid	End	Mid	End	Mid	End
Years (Y)										
2006	67.4 ^b	77.0 ^a	17.1 ^a	26.2 ^a	17.7 ^a	27.2 ^a	40.3 ^b	35.5 ^a	12.2 ^a	14.8 ^a
2007	69.6 ^a	61.9 ^b	19.3 ^a	25.2 ^a	20.4 ^a	25.8 ^a	50.5 ^a	27.8 ^b	14.1 ^a	13.7 ^a
Significance	*	**	ns	ns	ns	ns	**	**	ns	ns
Fertilization (F)										
0	66.4 ^b	67.8	9.9 ^b	23.3 ^b	10.5 ^b	23.3 ^b	47.2 ^a	27.4 ^b	8.1 ^b	11.8 ^b
50	67.8 ^{ab}	70.0	24.0 ^a	23.3 ^b	24.5 ^a	23.9 ^b	37.1 ^b	27.0 ^b	15.8 ^a	9.9 ^b
100	71.4 ^a	70.5	20.7 ^a	30.5 ^a	22.1 ^a	32.2 ^a	51.8 ^a	40.6 ^a	15.5 ^a	20.9 ^a
Significance	**	ns	**	**	**	**	**	**	**	**
Mycorrhization (M)										
<i>Micosat F</i>	100.0	99.4	35.2	49.7	35.2	50.0	76.1	55.1	25.8	28.2
Control	37.0	39.4	1.2	1.6	2.9	3.0	14.7	8.3	0.5	0.2
Significance	**	**	**	**	**	**	**	**	**	**
Interactions										
<i>A x C</i>	*	ns	ns	ns	ns	*	ns	*	ns	**
<i>A x M</i>	ns	**	ns	ns	ns	ns	*	ns	ns	ns
<i>C x M</i>	**	ns	**	**	**	*	**	**	**	**
<i>A x C x M</i>	*	ns	ns	**	ns	**	*	**	ns	**

^{a,b}Means followed by the same letters in the same column and within each experiment are not significantly ($\alpha \leq 0.05$) different according to the Student-Newman-Keuls Test. ** $P \leq 0.01$ was considered significant. ns, non-significant.

rhization indices were significantly higher in the fully fertilised plots than in non-fertilised soil.

Discussion and conclusions

Tomato yield performance was affected in different ways by climatic conditions in the two years studied. High temperatures were recorded in the last ten days of June 2006, and these induced anthoposis and almost completely prevented fruit setting in the first crop.

Low mycorrhization indices of non-inoculated plants demonstrated that natural symbiosis with soil-native AMF populations is of little benefit to conventional tomato crops, particularly in the climatic conditions recorded during this experiment.

Artificial AMF inoculation was shown to be highly and rapidly effective on plant growth. Inoculated tomato seedlings were significantly larger than non-inoculated seedlings after less than 30 days after the first inoculation in the nursery, and dry top biomass and stem number of inoculated plants were found to be higher also at the end of each crop. The positive effects of mycorrhizal inoculation were extended also to marketable yield of both crops, mainly as a result of an increased number and weight of clusters and fruits. The improvement in tomato plant growth and yield through AMF inoculation has already been reported for tomato (Salvioli *et al.*, 2012) and for other different vegetable species (Douds *et al.*, 2007; Regvar *et al.*, 2003). In field conditions, tomato plants inoculated with a commercial formulation of *Glomus intraradices* were recently found to produce larger inflorescences, and a higher number of flowers and total and marketable fruits (Conversa *et al.*, 2012). In contrast, the same commercial mycorrhizal formulate tested in our experiment did not result in any significant increase in either total or marketable yield of an organic tomato crop, probably due to a natural organic soil richness (Bosco *et al.*, 2007).

The higher efficiency of mycorrhizal plants in taking up soil phosphate, and thus improving plant nutritional status, was suggested to be one reason for the positive impact of AMF mycorrhization on tomato plant productivity (Subramanian *et al.*, 2006). It was also hypothesised that enhanced fruit setup and yield could also be related to an increase in pollen quantity and quality in mycorrhizal plants (Poulton *et al.*, 2001; Subramanian *et al.*, 2006).

Mycorrhizal treatment also improved crop earliness, seen as accelerating plant flowering, an increase in first harvest yield and a reduction in the average harvesting time compared to non-inoculated plants.

Accelerated flowering and a faster fruit production following tomato plant inoculation with the AM fungi *G. mossae* or *G. intraradices* have previously been reported by Salvioli *et al.* (2012) and Hildebrandt *et al.* (2002), respectively. Salvioli *et al.* (2012) also hypothesised that accelerated flowering could also be due to a forced meristem transition from the vegetative to floral status or to the effects of mycorrhizal fungus on the expression of flowering-related genes.

In our experiment, quality parameters of tomato fruit were not significantly affected by plant AMF inoculation. This is in contrast to many other studies documenting a positive impact of root inoculation with beneficial rhizosphere microorganisms on fruit quality parameters (Charron *et al.*, 2001; Kaya *et al.*, 2003; Mena-Violante *et al.*, 2006). An increase in antioxidant activity and a higher lycopene and shoot and fruit potassium content were also reported for tomato fruits from AMF-inoculated plants (Ordookhani *et al.*, 2010).

Plant-growth-promoting fungi inoculation was generally found to increase plant nutrient uptake, due to a better water and nutrient absorption by the improved root system (Höfllich and Kühn, 1996; Kloepper *et al.*, 1991; Zimmer *et al.*, 1995). Combination of AMF with PGPR was generally found to result in a synergistic positive interaction

(Galleguillos *et al.*, 2000; Hameeda *et al.*, 2007), although neutral effects were also observed in other studies (Andrade *et al.*, 1997; Walley and Germida, 1997).

In our study, mineral fertilisation had a positive effect on plant growth and marketable yield and accelerated crop flowering, fruit veraison and harvesting time, but did not influence fruit quality parameters. No significant interaction was found between mineral fertilisation and plant mycorrhization, although the few variations in mycorrhization frequency and arbuscule presence in the two tomato crops were more evident when the full quantity of full fertiliser was given. Mineral fertilisation was usually reported to decrease AMF colonisation in agricultural crops, as a lower AMF activity was observed in conventional agricultural systems with high inputs of inorganic fertilisers than in organic crop systems (Douds *et al.*, 1993). However, use of fertiliser in extremely nutrient deficient soils was found to increase AMF colonisation (Hayman, 1975), to mycorrhizal function. (Gryndler *et al.*, 1990). Based on these contrasting responses, the mediation of plant nutritional status in the mycorrhizal response to fertilisers was suggested by Douds and Johnson (2003). In particular, P:N ratio seems to be an important factor governing AMF response to nutrient enrichment, as AMF colonisation was generally found to be reduced by P fertilisation in the presence of adequate N levels, but not necessarily in N-limited plants (Sylvia and Neal, 1990).

Inoculation of tomato plants with AMF, both alone or in combination with PGPR, can provide considerable benefits in terms of growth, nutrient uptake and also yield, although results are difficult to predict and are not always guaranteed. More generally, application of mycorrhizal formulations in intensive tomato systems may improve crop sustainability, due to a reduced impact of fertilisers and pesticides. However, the large variability of plant response to mycorrhization between crops and within crop varieties suggests that it would be useful to extend research to a wider range of tomato genotypes studied over a longer period of time.

References

- Allen MF, 2009. Water relations in the mycorrhizosphere. *Progr. Bot.* 257-76.
- Andrade G, Mihara KL, Linderman RG, Bethlenfalvai GJ, 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant Soil* 192:71-9.
- Avis TJ, Gravel V, Antoun, H, Tweddell RJ, 2008. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. *Soil Biol. Biochem.* 40:1733-40.
- Bosco M, Giovannetti G, Picard C, Baruffa E, Brondolo A, Sabbioni F, 2007. Commercial plant-probiotic microorganisms for sustainable organic tomato production systems. pp 468-471 in *Proc. 3rd QLIF Congress*, Hohenheim, Germany.
- Brundrett MC, 2009. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320:37-77.
- Burrows RL, Pflieger FL, 2002. Host responses to AMF from plots differing in plant diversity. *Plant Soil* 240:169-80.
- Charron G, Furlan V, Bernier-Cardou M, Doyon G, 2001. Response of onion plants to arbuscular mycorrhizae. *Mycorrhiza* 11:187-97.
- Compant S, Duffy B, Nowak J, Clément C, Barka EA, 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* 71:4951-9.
- Conversa G, Lazzizzera, C, Bonasia A, Elia A, 2012. Yield and phospho-

- rus uptake of a processing tomato crop grown at different phosphorus levels in a calcareous soil as affected by mycorrhizal inoculation under field conditions. *Biol. Fertil. Soil* 49:1-13.
- Daniell TJ, Husband R, Fitter A H, Young JPW, 2001. Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *FEMS Microbiol. Ecol.* 36:203-9.
- Douds DD Jr, Johnson NC, 2003. Contributions of arbuscular mycorrhizas to soil biological fertility. In: L.K. Abbott and D.V. Murphy (eds), *Soil biological fertility – a key to sustainable land use in agriculture*. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp 129-62.
- Douds DD Jr, Janke RR, Peters SE, 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. *Agric. Ecosyst. Environ.* 43:325-35.
- Douds DD Jr, Nagahashi G, Reider C, Hepperly P, 2007. Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. *Biol. Agric. Hortic.* 25:67-78.
- Galleguillos C, Aguirre C, Miguel Barea J, Azcón R, 2000. Growth promoting effect of two *Sinorhizobium melilotis* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. *Plant Sci.* 159:57-63.
- Gosling P, Hodge A, Goodlass G, Bending GD, 2006. Arbuscular mycorrhizal fungi and organic farming. *Agric. Ecosyst. Environ.* 113:17-35.
- Gryndler M, Leština J, Moravec V, Píkrýl Z, Lipavský J, 1990. Colonization of maize roots by VAM-fungi under conditions of long-term fertilization of varying intensity. *Agric. Ecosyst. Environ.* 29:183-6.
- Hameeda B, Srijana M, Rupela OP, Reddy G, 2007. Effect of bacteria isolated from composts and macrofauna on sorghum growth and mycorrhizal colonization. *World J. Microbiol. Biotechnol.* 23:883-7.
- Hamel C, Plenchette C, 2007. *Mycorrhizae in crop production*. Haworth Food & Agricultural Products Press, Binghamton, NY, USA.
- Hayman DS, 1975. The occurrence of mycorrhiza in crops as affected by soil fertility. *Endomycorrhizas*. Academic Press, London, UK, pp 495-509.
- Hildebrandt U, Schmelzer E, Bothe H, 2002. Expression of nitrate transporter genes in tomato colonized by an arbuscular mycorrhizal fungus. *Physiol. Plantarum* 115:125-36.
- Hodge A, Campbell CD, Fitter AH, 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature* 413: 297-9.
- Höflich G, Kühn G, 1996. Förderung des Wachstums und der Nährstoffaufnahme bei kruziferen Öl- und Zwischenfrüchten durch inokulierte Rhizosphärenmikroorganismen. *Zeitschr. Pflanzenernähr. Bodenkd.* 159:575-81.
- Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM, 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fertil. Soils.* 37:1-16.
- Kahiluoto H, Ketoja E, Vestberg M, Saarela I, 2001. Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant Soil* 231:65-79.
- Kaya C, Higgs D, Kirnak H, Tas I, 2003. Mycorrhizal colonisation improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.) grown under well-watered and water-stressed conditions. *Plant Soil* 253:287-92.
- Klopper JW, Zablotowick RM, Tipping EM, Lifshitz R, 1991. Plant growth promotion mediated by bacterial rhizosphere colonizers. In: D.L. Keister, P.B. Cregan (eds.) *The rhizosphere and plant growth*. Kluwer Academic Publishers Dordrecht, The Netherlands, pp 315-26.
- Kogelmann WJ, Lin HS, Bryant RB, Beegle DB, Wolf AM, Petersen GW, 2004. A statewide assessment of the impacts of phosphorus-index implementation in Pennsylvania. *J. Soil Water Conserv.* 59:9-18.
- Larsen J, Ravnskov S, Sorensen JN, 2007. Capturing the benefits of arbuscular mycorrhizae in horticulture. In: C. Hamel and C. Plenchette (eds), *Mycorrhizae in crop production*. The Haworth Press Inc., New York, USA, pp 123-49.
- Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U, 2002. Soil fertility and biodiversity in organic farming. *Science* 296:1694-7.
- Mena-Violante HG, Ocampo-Jiménez O, Dendooven L, Martínez-Soto G, González-Castañeda J, Davies Jr FT, Olalde-Portugal V, 2006. Arbuscular mycorrhizal fungi enhance fruit growth and quality of chile ancho (*Capsicum annum* L. cv San Luis) plants exposed to drought. *Mycorrhiza* 16:261-7.
- Ordookhani K, Khavazi K, Moezzi A, Rejali F, 2010. Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato. *Afr. J. Agric. Res.* 5:1108-16.
- Poulton JL, Koide RT, Stephenson AG, 2001. Effects of mycorrhizal infection, soil phosphorus availability and fruit production on the male function in two cultivars of *Lycopersicon esculentum*. *Plant Cell Environ.* 24:841-9.
- Regvar M, Vogel-Mikuš K, Ševerkar T, 2003. Effect of AMF inoculum from field isolates on the yield of green pepper, parsley, carrot, and tomato. *Folia Geobot.* 38:223-34.
- Salvioli A, Zouari I, Chalot M, Bonfante P, 2012. The arbuscular mycorrhizal status has an impact on the transcriptome profile and amino acid composition of tomato fruit. *BMC Plant Biol.* 12:44.
- Smith SE, Read DJ, 2008. (eds.) *Mycorrhizal symbiosis*, 3rd ed. Academic Press, Elsevier, Amsterdam, The Netherlands.
- Subramanian KS, Santhanakrishnan P, Balasubramanian P, 2006. Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. *Sci. Hortic.* 107:245-53.
- Sylvia DM, Neal LH, 1990. Nitrogen affects the phosphorus response of VA mycorrhiza. *New Phytol.* 115:303-10.
- Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S, 2002. Agricultural sustainability and intensive production practices. *Nature* 418:671-7.
- Treseder KK, Allen MF, 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytol.* 155:507-15.
- Trouvelot A, Kough JL, Gianinazzi-Pearson V, 1986. Estimation of VA mycorrhizal infection levels. Research for methods having a functional significance. pp 217-221 in *Proc. 1st European symposium, Physiological and genetical aspects of mycorrhizae*, Dijon. Centre National de la Recherche Scientifique, Dijon; Institut National de la Recherche Agronomique, Dijon; Station d'Amélioration des Plantes, Paris, France.
- Walley FL, Germida JJ, 1997. Response of spring wheat (*Triticum aestivum*) to interactions between *Pseudomonas* species and *Glomus clarum* NT4. *Biol. Fertil. Soils* 24:365-71.
- Wissuwa M, Mazzola M, Picard C, 2009. Novel approaches in plant breeding for rhizosphere-related traits. *Plant Soil* 321:409-30.
- van der Heijden MG, Klironomos JN, Ursic M, Moutoglou P, Streitwolf-Engel R, Boller T, Sanders I R, 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69-72.
- Zimmer W, Kloss K, Hundeshagen B, Niederau E, 1995. Auxin biosynthesis and denitrification in plant growth promoting bacteria. *NATO ASI Ser. G Ecol. Sci.* 37:121.