Functional identity has a stronger effect than diversity on mycorrhizal symbiosis and productivity of field grown organic tomato

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Highlights

- Tomato yield was enhanced by the use of Vicia villosa – in the 2nd year of the experiment- and Mix 7 (a mixture of seven species) – during the 3rd year – as cover crops.
- Tomato genotype was the most important factor affecting fruit quality parameters.
- Tomato plants inoculated with beneficial AMF symbionts before transplant show higher rates of root colonization in-field.

Abstract

Beneficial soil biota, and in particular, arbuscular mycorrhizal fungi (AMF) are increasingly being recognized as key elements of organic and low-input agriculture where agrobiodiversity is central to enhanced crop production. However, the role of AMF in diversified organic systems, especially in field crops, is still poorly understood. A 3-year field experiment was carried out in Central Italy to investigate whether organic cropping systems that promote species and genetic diversity are more prone to mycorrhizal symbiosis increasing tomato growth, production and yield quality. Three tomato cultivars with varying genetic diversity were grown following four cover treatments: Indian mustard (Brassica juncea L. Czern.), hairy vetch (Vicia villosa Roth), a commercial mixture of seven cover crop species (Mix 7) and no-till fallow. Plants were either inoculated or not in nursery, with the two AMF isolates Funneliformis mosseae (IMA1) and Rhizoglomus intraradices (IMA6) used alone or mixed in a 1:1 volume ratio. On average, Mix 7 produced higher shoot dry matter (5.0 t ha−1) than V. villosa (3.5 t ha−1) or B. juncea (2.5 t ha−1). Pre-transplant inoculation increased tomato root colonization at flowering and harvest compared to the non inoculated plants (31.8 vs 23.6%) and cv. Rio Grande was on average the best colonized. The mean fresh weight of marketable fruits was 18.4, 28.0 and 28.6 t ha−1 for cvs. Rio Grande, Roma and Perfect Peel, respectively. Cover crops inconsistently affected tomato marketable fruit production in year 1, while in years 2 and 3, Vicia villosa and Mix 7 showed the best effect respectively. In year 3, among the pre-inoculated plants those treated with isolate IMA6 showed a higher production of marketable fruit number m−2 (56.7) than those inoculated either with IMA1 (51.5) or the mixed inocula (52.1). Most fruit quality parameters were affected by tomato genotype. This study shows that while increased agrobiodiversity is important to increase agroecosystem resilience, AMF, crop

and cover crop functional identity may be more important than diversity per se to promote mycorrhizal symbiosis and productivity of field grown organic tomato.

Keywords

Agrobiodiversity, Agroecosystem services, Arbuscular mycorrhizal fungi, Cover crop, Functional biodiversity, Genetic diversity

1. Introduction

Organic agriculture has risen considerably in the last two decades motivated by burgeoning consumer demand for healthier food and the need to conserve the environment and maintain biodiversity (Willer and Kilcher, 2012). Soil fertility and crop production in organic agriculture are maintained through diverse crop rotations and enhanced nutrient cycling where soil biota play a critical role. Arbuscular mycorrhizal fungi (AMF) are an important group of soil microorganisms living in symbiosis with the majority of cultivated crops. AMF enhance crop nutrition and protection through a large network of extraradical hyphae spreading from colonized plant host roots to the surrounding environment (Avio et al., 2006, Oruru and Njeru, 2016). The composition and function of AMF communities is expected to vary upon crop genotype, agronomic management practices and soil conditions (Njeru et al., 2014, Turrini et al., 2016). Among the former, the importance of crop genotype is gaining pace. Recent evidence shows that modern hybrids, bred to exploit high input conditions, may be less prone to mycorrhizal symbiosis compared to older crop varieties and landraces (Lehmann et al., 2012, Singh et al., 2012).

Cover crops are important in organic cropping systems where they are used as green manure, dead or living mulch. The role of cover crops in supplying essential plant nutrients, suppression of plant diseases, weeds and parasitic nematodes is well known (Clark, 2007, Moonen and Bàrberi, 2006). Indeed, cover crops are crucial in maintaining and restoring soil biodiversity, especially of AMF which are obligate mutualists (Kabir and Koide, 2000, Kabir et al., 2008). However, some cover crops, in particular members of the Brassicaeae family, are non-AMF hosts and may be detrimental to AMF although they provide other agroecosystem services (AES) e.g. reduction of nitrate leaching, and suppression of weeds and soil borne pathogens (Weil and Kremen, 2007, White and Weil, 2010). While there is growing interest in the use of cover crops, farmers often choose to grow single cover crop species depending on their preferences and on prevailing environmental conditions (Zibilske and Makus, 2009). Since different cover crops generally provide varying AES and perform differently based on agro-climatic conditions, we can hypothesize that increased cover crop diversity (i.e. mixtures) may foster production as well as resilience and stability in organic farming systems.

Tomato (Solanum lycopersicum L.) is a major vegetable crop that readily benefits from mycorrhizal symbiosis through increased nutritional uptake, general plant health (Cavagnaro et al., 2006), production and yield quality (Giovannetti et al., 2011, Ortas, 2012). Moreover, AMF colonization in tomato induces resistance to diseases (Fritz et al., 2006, Song et al., 2015) and nematodes (Vos et al., 2012) as well as tolerance to abiotic stresses, such as drought (Ruiz-Lozano et al., 2016) and salinity (Al-Karaki, 2006).

To increase AMF symbiosis in tomato and other horticultural crops, plants can be pre-inoculated with exotic AMF isolates at nursery, where the mycorrhizal inoculum is mixed with a sterile substrate used for seedling preparation. In this case, AMF colonization is achieved at a juvenile

stage and in absence of competition from indigenous fungal endophytes (Jeffries et al., 2003). After transplanting, the AMF inoculated plants have higher root colonization, which may increase AMF symbiosis in the field, enhancing growth and production. Although the potential of AMF to improve tomato production and quality has been previously demonstrated in greenhouse experiments (Subramanian et al., 2006) their application under field conditions remains relatively limited. Field experiments on AMF are challenging, e.g. in establishing non mycorrhizal control treatments and manipulating AMF on a large scale (Martinez and Johnson, 2010).

Empirical evidence from previous studies suggested that AMF colonization and responsiveness vary among crop cultivars, depending on age, origin and type of cultivar (An et al., 2010, Singh et al., 2012, Turrini et al., 2015). Choice of crop genotype, preceding cover crop and inoculated fungal isolate are expected to influence AMF colonization. The diversity level of these factors may affect mycorrhizal symbiosis and expression of related AES, upon effects driven either by functional identity (i.e. choosing the right species), functional composition (i.e. heterogeneity between species) or functional diversity (i.e. heterogeneity within species) (Costanzo and Bàrberi, 2014).

In this study, we hypothesized that cropping systems based on higher diversity of biological components (cover crops, cash crop and fungal symbionts) at the genetic or species level would give better growth and production of field grown processing tomato. Therefore, an organically managed field study based on increasing levels of genetic and species agrobiodiversity was carried out to investigate whether the ability of processing tomato to associate with AMF, form effective symbiosis and increase yield and produce quality is better with use of (a) open pollinated varieties vs a modern hybrid; (b) a cover crop species mixture vs single cover crop species; (c) pre-transplant AMF inoculation with two AMF strains vs one.

2. Materials and methods

2.1. Experimental site and soil characteristics

Field experiments were carried out in three nearby fields (one per year) at CIRAA (Interdepartmental Centre for Agri-environmental Research “Enrico Avanzi”, University of Pisa, S. Piero a Grado, latitude 43°40′ N, longitude 10° 20′ E), in the coastal plain of Tuscany, central Italy. The soil in the 0–30 cm layer is a sandy-loam with physical and chemical properties ranging from; clay 11.1–21.1%, silt 16.9–21.5%, sand 57.4–72.0%, pH (H2O) 6.5–7.9, Organic C 2.2–3.5%, total N (Kjedahl) 1.1–2.8 g kg−1, P (ppm, Olsen) 3.1–4.6. The climatic conditions are typical of Mediterranean areas, with rainfall mostly concentrated in autumn (October to December) and spring (March to April) (see graphs in Fig. A.1 and Fig. A.2 in Supplementary Material).

2.2. Experimental design

Field experiments involving a cover crop-tomato sequence and maintained under certified EU organic standards were carried out from September 2010 to September 2013. The experiments were laid as split-split-plot design with three blocks (replicates). Main plots consisted of four cover crop treatments, namely Brassica juncea (L.) Czern. cv. ISCI 20 (Indian mustard), Vicia villosa Roth cv. Latigo (hairy vetch), a mix of seven species (hereafter ‘Mix 7’) and a no-till fallow with natural vegetation (hereafter ‘Control’). The Mix 7 treatment, supplied as a commercial seed mixture by Arcoiris s.r.l (Modena, Italy), included Fagopyrum esculentum Moench (buckwheat), Lupinus albus L. (white lupin), Phacelia tanacetifolia Benth. (lacy phacelia), Pisum sativum L. (common pea), Trifolium alexandrinum L. (berseem clover), Trifolium incarnatum L. (crimson clover) and V.

villosa. Subplots included a commercial hybrid (cv. Perfect Peel) and two open pollinated varieties (cv. Roma and cv. Rio Grande), all from Arcoiris s.r.l. (Modena, Italy). Cvs. Roma and Rio Grande are old varieties, while Perfect Peel is a modern hybrid commonly grown for industrial use in Italy (www.sementi.it accessed on 4 April 2016). Sub-sub-plots hosted four mycorrhizal treatments, including Funneliformis mosseae (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler comb. nov., isolate IMA1, Rhizoglomus intraradices Schenck & Smith, isolate IMA6, IMA1 + IMA6 (1:1 v/v%) and a mock treatment used as non inoculated control (hereafter Mock). The experimental trial comprised 144 sub–sub plots each measuring 3 × 5 m in the first year 3 × 4 m in the second year and 3 × 3.5 m in the third year.

2.3. Cover crop management

The cover crops were sown on 18 October 2010 at a seeding rate of 9 kg ha−1 (B. juncea), 100 kg ha−1 (V. villosa), 50 kg ha−1 (Mix 7). In the second and third year, cover crops were sown on 19 October 2011 and 15 October 2012 at an increased seed rate of 12 kg ha−1 (B. juncea), 120 kg ha−1 (V. villosa), 65 kg ha−1 (Mix 7) to enhance establishment. The Control plots were left fallow and weeds were not controlled, like in all the other cover crop treatments. The cover crops and weeds were then mown and immediately incorporated into the soil by disc harrowing. Seeding beds were then raised and black plastic mulch film and drip irrigation tapes were laid onto the soil.

2.4. AMF material

The AMF isolates were obtained from pot cultures maintained in the collection of the Soil Microbiology Laboratory (International Microbial Archives, IMA) of the Department of Food, Agriculture and Environment, University of Pisa, Italy. Six 8 l pots containing a mixture (1:1 v/v) of soil and Terragreen (calcinated clay, ORLDRI Chicago, IL, USA) were prepared. The soil was a sandy loam collected near S. Piero a Grado. Chemical and physical characteristics of the soil used were as follows: pH (H2O), 8.0; clay, 15.3%; silt, 30.2%; sand 54.5%; organic matter, 2.2% (Walkley-Black); extractable P, 17.6 mg kg−1 (Olsen); total N (Kjedahl) 1.1 g kg−1. The mixture was then autoclaved (121 °C for 30 min, on two consecutive days) to kill naturally occurring AMF. The pots were inoculated with a crude inoculum (500 ml) containing mycorrhizal roots, spores and extraradical mycelium. Mixed seeds of forage legumes (T. alexandrinum cv. Tigri and Medicago sativa L. cv. Messe) were surface sterilized, planted in the pots and maintained in greenhouse in a completely randomized design from October to April of the subsequent year. At harvest, the shoots were excised and discarded while the roots were cut into small fragments (<1 cm) and homogeneously mixed with the substrate to form the crude inoculum used in tomatoes inoculation. In the following years, the inoculum was prepared with the same procedure utilizing the fungal material produced previously and growing Plantago lanceolata L. in addition to the other two mycorrhizal host plant species.

2.5. Tomato crop establishment

Tomato seedlings were prepared in peat substrate Hochmoor Hortus from TERFLOR® s.n.c. (Capriolo, Brescia, Italy) which was hand mixed with the crude inoculum at a ratio of 4:1 (v/v). Mixtures were prepared with single or mixed inocula to obtain the four treatments: IMA1 (containing only F. mosseae), IMA6 (containing only R. intraradices), IMA1 + IMA6 (containing both AMF 1:1 v/v) and a non mycorrhizal Mock (containing inoculum made of IMA1 + IMA6 mixture, autoclaved for two consecutive days). All the substrate-inocula mixtures were supplied with a filtrate obtained by sieving an aliquot of living mixed inocula through a 40 μm sieve to provide the substrate with an equivalent soil microbiota.

The Mycorrhizal Inoculum Potential (MIP) bioassay was performed to test the AMF colonization potential of the substrate–inoculum mixtures and the occurrence of natural endophytes in peat by using Cichorium intybus L. cv. Zuccherina di Trieste as test plant. The plants were maintained in a growth chamber (24 °C constant temperature in 16/8 h light/dark daily cycle) and harvested after 30 days. Roots were cleaned with tap water, cleared with 10% KOH in a 80 °C water bath for 10 min, neutralized with 2% HCl and stained with 0.05% trypan blue in lactic acid (Phillips and Hayman, 1970). The percentage root length colonized was assessed under a dissecting microscope (Wild, Leica, Milano, Italy) at 25 × or 40 × magnification by gridline intersect counts (Giovannetti and Mosse, 1980).

Tomato seeds were mechanically sown in 160 hole polystyrene trays (1 seed hole−1), filled with the substrate-inocula mixtures (20 ml hole−1), and topped with sterile vermiculite. The seedlings were maintained in the greenhouse for 40–50 days and fertilized twice with (9:15:30 NPK + 30:10:10 NPK including B, Cu, Fe, Mn and Zn) at the rate of 112.5 g L−1. Tomato plantlets were transplanted on 24 May 2011 and 30 May 2012 and 5 June 2013, when they had four to five true leaves. Transplanting was done manually at spacing of 1.5 × 0.5 m.

The tomato crop was drip irrigated throughout the season, as needed. The crop was periodically sprayed with either copper (II) sulphate (Cuproxat, 429 g ha−1) or copper oxychloride (Cu, 750 g ha−1) to control fungal pathogens and Bacillus thuringiensis var. kurstaki strain EG 2348 (Rapax, 7.5%) to control tomato fruit worms. Weeds emerging from the plastic mulch were regularly controlled by manual uprooting.

2.6. Assessments

2.6.1. Cover crop biomass

On 5 May 2011, 26 April 2012 and 13 May 2013 the cover crops + weeds biomass was sampled from three randomly selected 0.25 m2 quadrates plot−1. The sampled aboveground biomass was separated into either cover crop or weeds and oven dried at 80 °C to a constant weight.

2.6.2. Mycorrhizal root colonization of tomato

Before transplanting, three tomato seedlings from each treatment were assessed for mycorrhizal colonization by staining the roots with 0.05% trypan blue in lactic acid as previously described. To determine AMF colonization at flowering (48 days After Transplanting, DAT) and harvest (90 DAT), a sample of roots was obtained from four randomly selected plants per sub-sub-plot, stained as described above. The percentage of colonized roots was determined by the gridline intersect method (see Section 2.5). AMF root colonization at flowering was only determined in the second year.

2.6.3. Tomato leaf chlorophyll content

On 17 July 2012 and 24 July 2013 hand held (Soil Plant Analysis Development) SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan) was used for non-destructive estimation of tomato leaf chlorophyll content in the field. Measurements were performed in triplicates on two of the young fully expanded leaves on four different plants per plot and results expressed as SPAD values.

2.6.4. Days to anthesis and maturity

Anthesis was considered to have occurred when 50% of the tomato plants in each sub-sub-plot had at least one completely open flower with clearly visible stamens. Days to anthesis were determined by recording the number of plants with at least one completely open flower at three-day intervals

between 20 and 30 DAT. This measurement was only performed comprehensively in year 2 and 3, since only preliminary observation was done in year 1. The number of days to maturity (IPGRI, 2002) was considered when at least 50% of the plants achieved at least one completely ripe fruit. The number of plants in each sub-sub-plot with at least one completely ripe fruit was recorded every fourth day from 66 to 82 DAT.

2.6.5. Fruit setting

Fruit setting was assessed by counting the total number of green fruits produced by four plants per sub–sub plot, 60 DAT every year. Sampling was done at this period to evaluate earliness of fruit set and to capture all fruits including the ones damaged by blossom end rot and pests (about 5%) and could not reach maturity to be accounted for in the final harvest.

2.6.6. Tomato fruit number and fresh weight at harvest

Tomato harvest was done within the first two weeks of September in every year. Two plants in the middle of each sub-sub-plot were harvested manually in the first year, while four plants were sampled in the second and third year. All the fruits from each sampled plant were handpicked, separated into marketable (fully ripe and healthy fruits) and non marketable (green, decomposed or infested fruits) and counted. The total fresh weight of the marketable fruits and of unmarketable fruits (green fruits only) was then recorded per plant. The dry weight of marketable fruits was recorded after oven-drying (80 °C for 5 days) a subsample of four fruits selected after pooling all the fruits of each sub-subplot. The Harvest index (HI) was determined as a ratio between the fresh weight of marketable and unmarketable fruits, and the sum of shoot, marketable and unmarketable fruits fresh weight.

2.6.7. Fruit quality

In each sampled plant, two marketable fruits were randomly selected and measured for fruit firmness, pH and total soluble solid content (TSS). Fruit firmness was determined using a penetrometer while pH was determined using a glass electrode pH meter. TSS expressed in Brix (%) was determined using a refractometer after squeezing and sieving the tomato fruit juice to remove seeds and other insoluble substances. An additional quality parameter (lycopene content) was assessed in the first two experimental years, selecting randomly four marketable fruits from IMA1 + IMA6 and Mock plots. Lycopene was extracted and determined by high performance liquid chromatography (HPLC) analyses (Heredia et al., 2010, Leonardi et al., 2000).

2.7. Statistical analyses

All data were tested for homogeneity of error variances (Bartlett test) before analyses. Percent data were arcsine ($\sqrt{x}$) transformed, while other data were $\log(x + 1)$ transformed wherever necessary, to fulfill the assumptions of ANOVA. The data reported in tables were back transformed. Data on cover crops and weed biomass taken before cover crop destruction were analyzed by one-way ANOVA according to a completely randomized block design. Pearson correlation coefficient was determined for cover crop dry matter vs weed dry matter relationship. Data on tomato AMF root colonization at transplant were analyzed by two-way ANOVA utilizing a fixed effects model with interactions (genotype × AMF). All the other data were analyzed as a split-split-plot ANOVA separately for each year with cover crop as the main factor, tomato genotype as subplot factor and AMF inoculation as sub-sub-plot factor. Wherever feasible, post hoc test was performed using Tukey's HSD test ($P < 0.05$). All the statistical analyses were performed with the SPSS 20.0

software (SPSS Inc., Chicago, IL, USA). Graphs were created using ggplot2 package (Wickham, 2009) for R (R Development Core Team, 2016).

3. Results

3.1. Cover crop and weeds dry matter

During the three years of this study, cover crops emerged well except for L. albus and F. esculentum in the Mix 7 treatment. The total aboveground biomass (cover + weeds) was on average lower in the last year (2.9 t ha−1) compared to the second (8.3 t ha−1) and the first year (6.8 t ha−1). Indeed, Mix 7 cover crop produced the highest shoot dry matter on average (5.0 t ha−1) compared with V. villosa (3.5 t ha−1) and B. juncea (2.5 t ha−1). Cover crop relative performance was inconsistent among years. In year 1, V. villosa had a higher aboveground biomass than B. juncea; in year 2, Mix 7 outyielded both other cover crops whereas in year 3 it showed a significantly higher biomass than B. juncea only (Table 1). In Mix 7, there was a shift in species composition between year 2 and 3. In year 2, about 80% of the biomass was composed of P. tanacetifolia while in year 3, biomass was mainly composed of clovers, i.e. T. alexandrinum and T. incarnatum.

Table 1. Cover crop and weed aboveground dry matter biomass at the time of incorporation into the soil.

	Cover crops (t ha−1)			Weeds (t ha−1)		
Cover crop	2011	2012	2013	2011	2012	2013
V. villosa	2.97 (0.48) a	4.92 (0.55) b	2.54 (0.26) ab	3.53 (0.50) b	0.66 (3.56) c	0.40 (0.13) b
Mix 7	1.96 (0.61) ab	10.00 (1.34) a	3.25 (0.23) a	5.48 (0.75) ab	2.10 (0.39) b	0.48 (0.14) b
B. juncea	0.74 (0.28) b	5.02 (0.85) b	1.77 (0.19) b	5.87 (0.65) ab	2.36 (3.56) b	1.02 (0.16) b
Control	–	–	–	6.46 (0.57) a	8.00 (1.11) a	2.17 (0.26) a
P value	<0.001	0.002	<0.001	0.019	<0.001	<0.001

Values followed by the same letter in a column are not significantly different at $P < 0.05$ (Tukey's HSD test). Standard errors of the means are shown in parentheses.

The highest absolute weed biomass was always observed in the Control plots, although in year 1 it was significantly different only from that of V. villosa. In year 2, weed biomass in V. villosa plots was only 30% of that found on average in the other two cover crops (Table 1). Compared to the Control, weed suppression was most effective in year 2, when a significant negative correlation between cover crop and weed biomass was observed in V. villosa ($P = 0.048$, $r2 = 0.45$) and Mix 7 ($P = 0.015$, $r2 = 0.59$). Most of the weeds occurring in the cover crop treatments were AMF hosts (data not shown).

3.2. Tomato AMF root colonization

The MIP bioassay performed on the tomato seedling substrate showed that the fungal inocula were active and that the Mock treatment yielded no colonization in all the years. MIP values, determined

on the inoculated substrates using C. intybus as test plant, were on average 26.1% for IMA1, 45.0% for IMA6, and 40.9% for IMA1 + IMA6.

Accordingly, root colonization of tomato plants at transplant occurred only in inoculated treatments and thus only the three fungal treatments were statistically analyzed. Mycorrhizal treatments and tomato genotypes significantly influenced the level of colonization in the first ($F = 18.6$, $P < 0.001$ and $F = 3.5$, $P = 0.038$, respectively) and third year ($F = 18.1$, $P < 0.001$, for mycorrhizal treatment; $F = 4.1$, $P = 0.034$, for genotype), while in the second year only the mycorrhizal treatment significantly ($F = 13.3$, $P < 0.001$) affected root colonization at transplant. On average, IMA6 inoculation resulted in the highest AMF colonization (21.7%) compared to IMA1 + IMA6 (16.1%) and IMA1 (9.7%), while cvs. Rio Grande (16.8%) and Perfect Peel (15.8%) performed better than cv. Roma (12.5%). There was no significant interaction between tomato genotypes and mycorrhizal treatments in any year.

In the field, tomato root colonization at flowering in 2012 was significantly influenced by both mycorrhizal treatment and tomato genotype in all years. Similar results were obtained at tomato harvest in all years (Table 2). AMF inoculated tomato had a significantly higher level of root colonization at all sampled times. Additionally, cv. Rio Grande was always the best colonized although cv. Perfect Peel had similar level of colonization in year 2.

Table 2. Mycorrhizal colonization (%) of tomato at flowering and harvest as influenced by cover crop, genotype and AMF treatments in the three experimental years.

	2011	2012		2013
	Harvest	Flowering	Harvest	Harvest
Cover crop				
V. villosa	24.3 (1.97) a	38.4 (1.98) a	34.3 (1.26) a	42.1 (1.35) a
Mix 7	22.7 (1.68) a	36.2 (1.24) a	32.5 (1.47) a	40.6 (1.76) a
B. juncea	21.1 (2.31) a	29.7 (1.31) a	30.2 (1.32) a	40.4 (0.88) a
Control	21.7 (1.91) a	33.0 (1.94) a	32.8 (1.22) a	41.0 (1.44) a
Tomato genotype				
Rio Grande	29.3 (1.87) a	38.3 (1.43) a	35.4 (1.07) a	46.3 (1.11) a
Roma	19.5 (1.45) b	28.8 (1.14) b	29.6 (0.93) b	37.2 (0.97) b
Perfect Peel	18.5 (1.32) b	36.0 (1.56) a	32.3 (1.28) ab	39.6 (1.12) b
AMF				
IMA1	25.2 (2.08) a	35.6 (1.55) a	32.7 (1.24) a	42.6 (1.20) a
IMA6	23.8 (1.91) a	37.4 (1.61) a	35.4 (1.33) a	41.7 (1.45) a
IMA1 + IMA6	23.4 (2.09) ab	38.2 (1.60) a	34.3 (1.19) a	42.4 (1.50) a
Mock	17.3 (1.57) b	26.1 (1.39) b	27.5 (1.20) b	37.4 (1.22) b

	2011 Harvest	2012 Flowering	2012 Harvest	2013 Harvest
P values on the main factors and their interactions				
Cover crop	0.527	0.099	0.484	0.870
Tomato genotype	0.005	<0.001	0.012	<0.001
AMF	0.004	<0.001	<0.001	<0.001
Genotype × Cover	0.899	0.065	0.249	0.034
AMF × Cover	0.699	0.584	0.852	0.059
AMF × Genotype	0.609	0.849	0.712	0.085
AMF × Genotype × Cover	0.548	0.368	0.783	0.078

Values followed by the same letter in a column within each treatment are not significantly different at $P < 0.05$ (Tukey's HSD test). Standard errors of the means are shown in parentheses. Statistically significant P values are shown in bold.

In contrast, previous cover crop did not affect the level of colonization in tomato roots. Nonetheless, a significant cover crop × genotype interaction was observed in year 3 (Table 2). During this year, cv. Rio Grande showed a higher level of root colonization when grown after Mix 7 (51.9 ± 2.7%) and V. villosa (48.3 ± 1.77%) cover crops than when grown after B. juncea (43.2 ± 1.53%) and Control (41.7 ± 1.58%) treatments.

3.3. Tomato growth

3.3.1. Leaf chlorophyll content

Leaf chlorophyll content (SPAD values) was significantly affected ($P < 0.001$) by tomato genotype in both years (2012 and 2013), where cv. Rio Grande showed higher values. In 2012 chlorophyll content was also affected ($F = 2.97$, $P = 0.037$) by AMF treatment, with the highest SPAD values (61.7 ± 0.48) observed in IMA6 and the lowest (60.5 ± 0.44) in IMA1. In 2013, leaf chlorophyll content was also affected ($F = 9.50$, $P = 0.011$) by cover crop: tomato grown after V. villosa had higher SPAD values (56.0 ± 0.61) than after Mix 7 (54.7 ± 0.51), which in turn was higher than the Control (52.9 ± 0.42) and B. juncea (52.2 ± 0.36).

3.3.2. Days to anthesis and maturity

Cv. Perfect Peel reached anthesis earlier than the other cultivars in 2012, and earlier than cv. Rio Grande in 2013 (Table 3). Similarly, cv. Perfect Peel showed earlier maturity compared to the other cvs. in year 1, and compared to cv. Rio Grande in year 2 and 3. During year 2, tomato maturity was additionally affected ($F = 4.2$, $P = 0.009$) by mycorrhizal treatments where plants pre-inoculated with IMA6 showed a general earlier maturity compared to those pre-inoculated with IMA1 (Table 3). Moreover, in the same year, there was a significant ($F = 4.6$, $P < 0.001$) AMF × genotype interaction, e.g. among Roma plants, those pre-inoculated with IMA1 were usually the latest to

reach maturity, while for Rio Grande the same happened for those plants which were pre-inoculated with both IMA6 and IMA1 (Fig. 1).

Table 3. Number of days between transplanting and anthesis or fruit maturity mean as influenced by cover crop, genotype and AMF treatments in the three experimental years.

	Days to anthesis		Days to maturity		
	2012	2013	2011	2012	2013
Cover crop					
V. villosa	22.6 (0.33) a	24.0 (0.31) a	70.4 (0.71) a	69.4 (0.45) a	69.1 (0.68) a
Mix 7	22.6 (0.39) a	24.3 (0.33) a	72.2 (0.52) a	69.8 (0.47) a	68.6 (0.66) a
B. juncea	22.2 (0.26) a	24.3 (0.31) a	71.8 (0.71) a	69.0 (0.35) a	69.0 (0.68) a
Control	22.9 (0.32) a	24.2 (0.38) a	73.6 (0.72) a	69.6 (0.50) a	68.9 (0.66) a
Tomato genotype					
Rio Grande	23.2 (0.31) a	25.1 (0.30) a	75.2 (0.43) a	71.7 (0.42) a	73.4 (0.53) a
Roma	22.7 (0.28) a	23.9 (0.28) b	72.4 (0.46) b	68.6 (0.31) b	66.5 (0.16) b
Perfect Peel	21.8 (0.21) b	23.6 (0.25) b	68.5 (0.36) c	68.0 (0.15) b	66.6 (0.17) b
AMF					
IMA1	22.8 (0.31) a	24.3 (0.32) a	71.8 (0.66) a	70.0 (0.48) a	68.7 (0.64) a
IMA6	22.1 (0.22) a	24.3 (0.32) a	72.3 (0.72) a	68.6 (0.26) b	68.9 (0.65) a
IMA1 + IMA6	23.1 (0.40) a	24.4 (0.38) a	72.0 (0.64) a	69.6 (0.44) ab	69.1 (0.69) a
Mock	22.3 (0.34) a	23.8 (0.31) a	72.0 (0.67) a	69.4 (0.53) ab	68.7 (0.69) a
P values on the main factors and their interactions					
Cover crop	0.656	0.978	0.117	0.689	0.930
Tomato genotype	<0.001	0.004	<0.001	<0.001	<0.001
AMF	0.053	0.362	0.730	0.009	0.807
Genotype × Cover	0.207	0.082	0.021	0.821	0.931
AMF × Cover	0.118	0.141	0.632	0.333	0.631
AMF × Genotype	<0.001	0.251	0.015	0.001	0.505
AMF × Genotype × Cover	0.607	0.822	0.214	0.693	0.811

Values followed by the same letter in a column within each treatment are not significantly different at $P < 0.05$ (Tukey's HSD test). Standard errors of the means are shown in parentheses. Statistically significant P values are shown in bold.

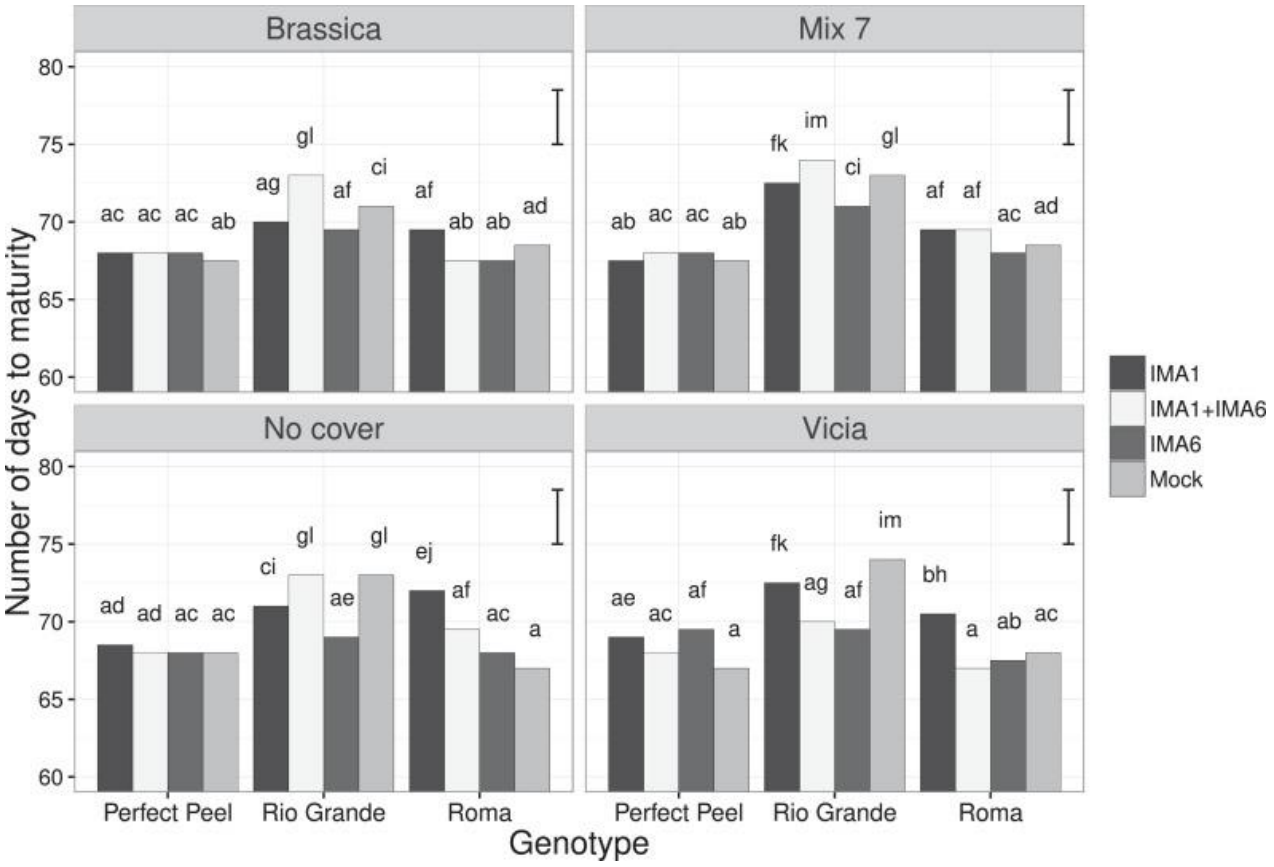

Fig. 1. Number of days to maturity (i.e. when at least 50% of the plants achieved at least one completely ripe fruit) as affected by the experimental factors in year 2 of the experiment. Different letters indicate least significant differences between treatments' means; the bar on the top right corner of each graph represents LSD value at P = <0.05 (3.5).

3.4. Tomato production

3.4.1. Fruit setting

In all years, fruit setting was affected by tomato genotype ($P < 0.001$, $F = 22.3, 21.6, 42.1$ for 2011, 2012 and 2013 respectively). In year 3, fruit setting was also marginally affected ($F = 2.63$, $P = 0.056$) by AMF treatment, with IMA6 showing the highest number of fruits set (42.0 ± 2.57) and IMA1 the lowest (38.3 ± 2.24). Over the three years, cv. Perfect Peel had a higher number of fruits plant−1 (49.7 ± 1.01) compared to Roma (44.5 ± 1.00) which was higher than Rio Grande (24.3 ± 0.68). In year 2, fruit number was additionally affected by the AMF × genotype interaction ($F = 2.6$, $P = 0.025$). Cv. Roma pre-inoculated with IMA6 had a higher number of fruits plant−1 (69.2 ± 2.37) compared with the same cv. pre-inoculated with IMA1 (56.4 ± 4.70).

3.4.2. Fruit number, yield and harvest index

Results of ANOVAs for marketable tomato fruit number and fresh weight are reported in Table 4. In all years, the number and fresh weight of marketable fruits was significantly influenced by tomato genotype. Cvs. Roma and Perfect Peel performed in similar way while cv. Rio Grande usually showed lower number of fruits and fresh weight. Marketable fruits fresh weight and number were also affected by cover crop in year 2 and 3. In year 2, tomato grown after V. villosa had 46 and 23% higher values than Mix 7 and the Control respectively, while in year 3 tomato grown after

Mix 7 had 44 and 38% higher values than B. juncea and the Control. In year 1 a significant AMF × genotype interaction was observed: the non inoculated Perfect Peel hybrid had the lowest marketable number of fruits m−2 (35.7 ± 4.05), while the non inoculated cv. Roma had 45% higher values (51.7 ± 6.06). In year 3, number and fresh weight of marketable fruits were also affected by the AMF × genotype × cover crop interaction (Fig. 2). Cv. Perfect Peel inoculated with IMA6 and grown after Mix 7 produced the highest fruit fresh weight (50.6 ± 2.4 t ha−1) while yield of the same cv. non inoculated and grown after B. juncea was 49% lower. Similarly, yield of cv. Rio Grande was highest when inoculated with IMA6 and grown after V. villosa (39.8 ± 3.20 t ha−1), 2.3 times higher than yield of the same cv. non inoculated and grown in a no cover crop system (17.5 ± 1.82 t ha−1).

Table 4. Number m−2 and fresh weight (t ha−1) of tomato marketable fruits as influenced by cover crop, genotype and AMF treatments in the three experimental years.

	2011		2012		2013	
	Fruit no.	Fruit weight	Fruit no.	Fruit weight	Fruit no.	Fruit weight
Cover crop						
V. villosa	30.2 (2.51) a	17.7 (1.46) a	59.2 (4.03) a	36.8 (2.54) a	57.3 (2.67) a	34.6 (1.10) ab
Mix 7	32.8 (3.41) a	17.9 (1.91) a	46.9 (4.13) b	25.2 (1.99) c	65.1 (4.00) a	38.0 (1.56) a
B. juncea	31.7 (2.59) a	18.2 (1.64) a	55.0 (3.92) ab	32.0 (2.37) ab	44.8 (2.21) b	26.3 (0.84) b
Control	37.4 (3.35) a	22.3 (2.04) a	49.9 (4.33) b	30.0 (2.71) bc	47.7 (2.87) b	27.5 (1.11) b
Tomato genotype						
Rio Grande	18.6 (1.53) b	11.5 (1.08) b	31.3 (2.18) b	25.3 (2.00) b	32.8 (1.21) b	26.9 (1.06) b
Roma	38.8 (2.26) a	21.9 (1.31) a	64.9 (3.39) a	34.1 (2.19) a	63.2 (1.72) a	33.4 (1.02) a
Perfect Peel	41.6 (2.51) a	23.7 (1.61) a	62.0 (2.85) a	33.6 (2.08) a	65.1 (2.44) a	34.5 (1.32) a
AMF						
IMA1	32.8 (2.68) a	19.2 (1.68) a	51.0 (4.04) a	29.9 (2.48) a	51.5 (3.15) b	30.9 (1.28) a
IMA6	32.4 (2.83) a	19.7 (1.76) a	52.2 (4.24) a	30.9 (2.37) a	56.7 (3.63) a	33.0 (1.69) a

	2011		2012		2013	
	Fruit no.	Fruit weight	Fruit no.	Fruit weight	Fruit no.	Fruit weight
IMA1 + IMA6	30.8 (3.16) a	17.2 (1.93) a	53.6 (3.98) a	31.2 (2.53) a	52.1 (2.83) b	31.0 (1.15) a
Mock	35.9 (3.34) a	19.9 (1.80) a	54.1 (4.44) a	32.0 (2.67) a	54.5 (3.46) ab	31.6 (1.54) a
P values on the main factors and their interactions						
Cover crop	0.596	0.443	0.032	0.04	<0.001	0.008
Genotype	<0.001	<0.001	<0.001	0.022	<0.001	0.001
AMF	0.356	0.508	0.799	0.825	0.014	0.128
Genotype × Cover	0.316	0.082	0.933	0.790	0.117	0.276
AMF × Cover	0.310	0.214	0.461	0.591	0.068	0.007
AMF × Genotype	0.002	0.011	0.260	0.151	0.167	0.042
AMF × Genotype × Cover	0.274	0.397	0.795	0.638	0.009	<0.001

Values followed by the same letter in a column within each treatment are not significantly different at $P < 0.05$ (Tukey's HSD test). Standard errors of the means are shown in parentheses. Statistically significant P values are shown in bold.

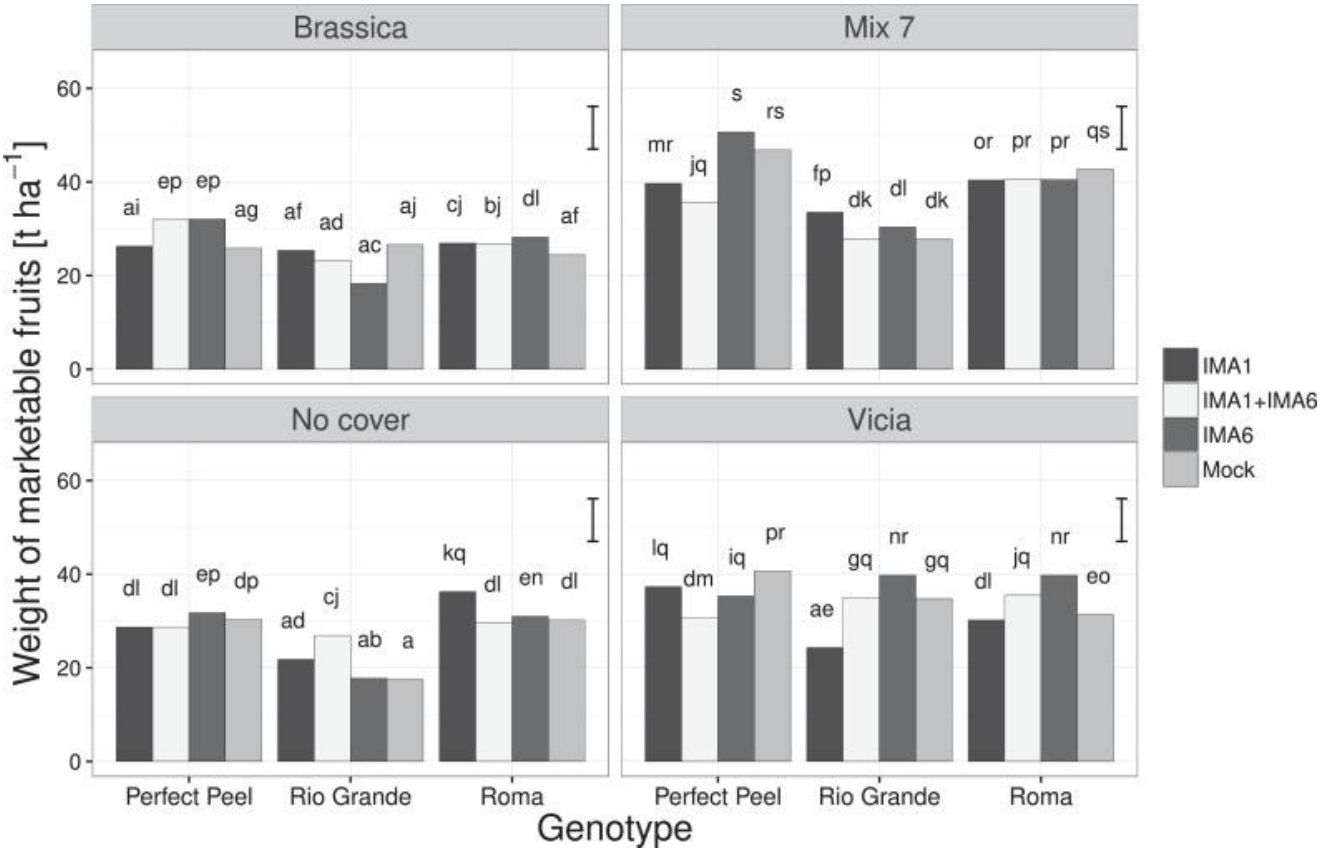

Fig. 2. Marketable tomato fruits fresh weight (t ha−1) in year 3 as affected by the cover crop type, tomato genotype and AMF treatments. Different letters indicate least significant differences between treatments' means; the bar on the top right corner of each graph represents LSD value at P = <0.05 (9.13).

Dry matter (DM) of marketable fruits was significantly influenced by tomato genotype only in year 1 and 2, when cv. Perfect Peel showed the highest values. Tomato fruit DM biomass was also affected by the AMF × genotype interaction (F = 2.4, P = 0.036) in year 1 and by cover crop (F = 14.14, P = 0.004) and the AMF × cover crop interaction (F = 2.41, P = 0.019) in year 3. In year 1, the non inoculated cv. Roma showed the highest fruit DM biomass (146.8 ± 15.8 g m−2), 33% higher than non inoculated cv. Perfect Peel. In year 3, tomato inoculated with IMA6 and grown after V. villosa produced 177.8 ± 29.0 g m−2 of fruit DM biomass, 53% higher than tomato grown after the same cover crop but inoculated with IMA1.

Tomato harvest index was affected by genotype (F = 57.8, P < 0.001), genotype × cover crop interaction (F = 3.3, P = 0.027) and AMF × genotype × cover crop interaction (F = 2.4, P = 0.006) in year 1. Cv. Perfect Peel recorded the highest HI (0.79 ± 0.01), compared to cvs. Roma (0.74 ± 0.01) and Rio Grande (0.64 ± 0.02). In year 2, HI was affected by genotype (F = 7.7, P = 0.005) and AMF × genotype interaction (F = 2.4, P = 0.039) when cv. Roma had the lowest HI (0.66 ± 0.02) compared to cvs. Rio Grande (0.73 ± 0.01) and Perfect Peel (0.75 ± 0.01). Following inoculation with mixed AMF inocula, cv. Rio Grande showed the highest HI (0.78 ± 0.01), while the same non inoculated genotype showed lower HI (0.71 ± 0.03). In year 3, HI was significantly lower (F = 9.7, P = 0.01) after B. juncea than after V. villosa and Mix 7 cover crops. Moreover, there was a significant AMF × cover crop interaction (F = 2.3, P = 0.024): tomato crop grown after V. villosa and inoculated with IMA6 had the highest HI (0.83 ± 0.03), while the crop grown after B. juncea but inoculated with mixed inocula had lower HI values (0.375 ± 0.02).

3.5. Tomato fruit quality

Fruit firmness was generally influenced by tomato genotype in all years (cv. Perfect Peel > Rio Grande > Roma; Table 5). In year 1, we also detected an effect of AMF treatment (F = 2.7, P = 0.052) and a significant AMF × cover crop interaction (F = 2.4, P = 0.018; Fig. 3): e.g. within Rio Grande variety, plants inoculated with IMA1 showed higher firmness values compared to those inoculated with IMA6 when the crop followed V. villosa, while this trend disappeared when the cover crop used was either Mix 7 or B. juncea − or was reversed when no cover crop was used. There was a general reduction of fruit firmness in years 2 and 3.

Table 5. Tomato fruit firmness (kg cm−2), pH and total soluble solids (TSS, % Brix) as influenced by cover crop, genotype and AMF treatments in the three experimental years.

	2011			2012			2013		
	Firmness	pH	TSS	Firmness	pH	TSS	Firmness	pH	TSS
Cover crop									
V. villosa	2.02 (0.14) a	4.37 (0.02) a	5.80 (0.08) a	1.30 (0.07) a	4.24 (0.02) a	5.18 (0.13) a	1.44 (0.09) a	4.44 (0.03) a	4.67 (0.07) a

	2011			2012			2013		
	Firmness	pH	TSS	Firmness	pH	TSS	Firmness	pH	TSS
Mix 7	2.16 (0.12) a	4.46 (0.03) a	6.09 (0.08) a	1.31 (0.06) a	4.26 (0.02) a	5.35 (0.15) a	1.44 (0.09) a	4.42 (0.02) a	4.60 (0.05) a
B. juncea	2.08 (0.13) a	4.40 (0.03) a	6.12 (0.08) a	1.33 (0.08) a	4.23 (0.02) a	4.97 (0.09) a	1.64 (0.10) a	4.43 (0.03) a	4.79 (0.07) a
Control	2.10 (0.16) a	4.37 (0.02) a	5.81 (0.08) a	1.32 (0.08) a	4.27 (0.01) a	5.27 (0.15) a	1.59 (0.10) a	4.48 (0.03) a	4.68 (0.06) a
Tomato genotype									
Rio Grande	2.24 (0.10) b	4.39 (0.02) b	5.76 (0.07) b	1.32 (0.04) b	4.29 (0.01) a	4.82 (0.08) b	1.52 (0.07) a	4.56 (0.02) a	4.58 (0.05) b
Roma	1.27 (0.05) c	4.48 (0.02) a	6.28 (0.05) a	0.91 (0.03) c	4.31 (0.01) a	5.81 (0.12) a	1.40 (0.08) a	4.47 (0.02) a	4.89 (0.06) a
Perfect Peel	2.75 (0.10) a	4.33 (0.02) b	5.82 (0.07) b	1.72 (0.04) a	4.14 (0.01) b	4.94 (0.08) b	1.65 (0.09) a	4.30 (0.01) b	4.61 (0.06) ab
AMF									
IMA1	2.16 (0.15) a	4.37 (0.02) a	5.96 (0.08) a	1.29 (0.06) a	4.25 (0.02) a	5.24 (0.14) a	1.49 (0.09) a	4.47 (0.02) a	4.70 (0.07) a
IMA6	2.16 (0.15) a	4.45 (0.02) a	5.91 (0.08) a	1.27 (0.07) a	4.25 (0.02) a	5.20 (0.14) a	1.58 (0.10) a	4.44 (0.03) ab	4.70 (0.06) a
IMA1 + IMA6	2.11 (0.13) a	4.41 (0.02) a	6.03 (0.08) a	1.36 (0.07) a	4.24 (0.02) a	5.16 (0.14) a	1.52 (0.10) a	4.41 (0.02) b	4.65 (0.06) a
Mock	1.93 (0.12) a	4.37 (0.03) a	5.93 (0.09) a	1.35 (0.08) a	4.25 (0.02) a	5.17 (0.12) a	1.50 (0.10) a	4.46 (0.03) ab	4.73 (0.07) a
P values of the main factors and their interactions									
Cover crop	0.422	0.3	0.146	0.983	0.163	0.059	0.107	0.627	0.858
Tomato genotype	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.098	<0.001	0.035

	2011			2012			2013		
	Firmness	pH	TSS	Firmness	pH	TSS	Firmness	pH	TSS
AMF	0.155	0.066	0.545	0.208	0.933	0.718	0.457	0.009	0.443
Genotype × Cover	0.218	0.318	0.154	0.125	0.744	0.046	0.712	0.505	0.33
AMF × Cover	0.020	0.863	0.252	0.958	0.509	0.791	0.584	0.061	0.615
AMF × Genotype	0.216	0.943	0.217	0.121	0.665	0.021	0.448	0.624	0.952
AMF × Genotype × Cover	0.265	0.206	0.644	0.198	0.593	0.654	0.275	0.116	0.05

Values followed by the same letter in a column within each treatment are not significantly different at P < 0.05 (Tukey's HSD test). Standard errors of the means are shown in parentheses. Statistically significant P values are shown in bold.

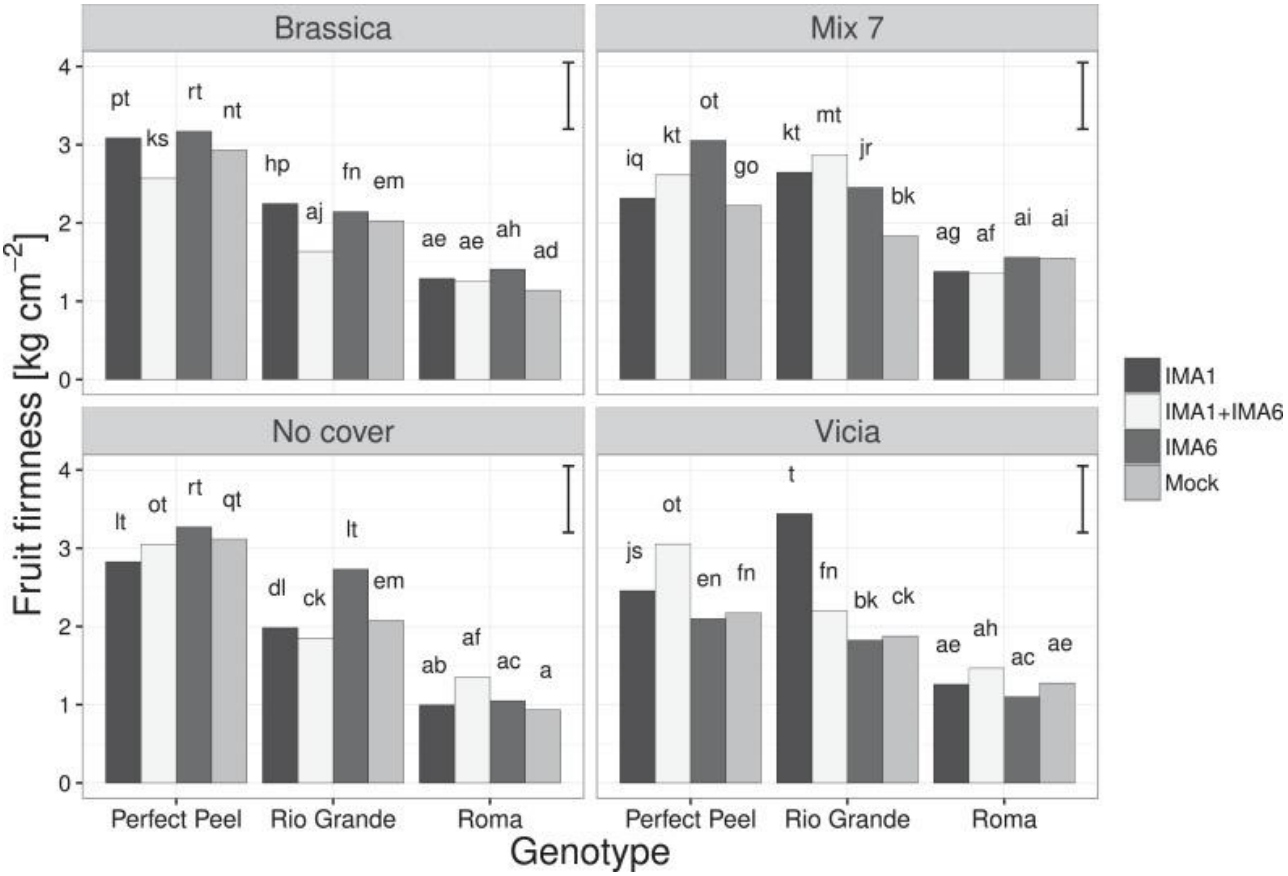

Fig. 3. Tomato fruit firmness (kg cm−2) as affected by AMF, tomato genotype and cover crop type during the first year. Different letters indicate least significant differences between treatments' means; the bar on the top right corner of each graph represents LSD value at P = <0.05 (0.85).

Tomato fruit pH was significantly affected by genotype in all years. Cv. Perfect Peel fruits had the lowest pH except in year 1, when values were comparable to those of cv. Rio Grande. In addition, in year 3 fruit pH was lower after inoculation with IMA1 + IMA6 than IMA1 treatment (Table 5).

Total soluble solid (TSS) content was significantly affected by tomato genotype in all years. Generally, cv. Roma had the highest TSS content although it was not statistically different from that of cv. Perfect Peel in year 3 (Table 5). In year 2, TSS content was additionally influenced by the genotype × cover crop ($F = 2.8$, $P = 0.046$), and the AMF × genotype ($F = 2.7$, $P = 0.021$) interactions. In year 2, cv. Roma had the highest TSS content (6.28 ± 0.28%) when grown after Mix 7 and 27% lower values when grown after B. juncea. In year 3, we also detected a significant AMF × genotype × cover interaction ($F = 1.75$, $P = 0.050$), showing that IMA1 inoculated cv. Roma grown after B. juncea had 12% higher TSS content than the same cv. non inoculated and grown with no cover crop. No treatment affected the lycopene content in the first two years (when this parameter was assessed) although values were more than double in year 2 than in year 1 (24.2 vs 11.2 mg 100 g−1 fresh weight).

4. Discussion

4.1. Cover crops and weeds

Cover crops produced considerable amounts of biomass except in year 1, probably due to lower seed rate and higher weed abundance. On average, Mix 7 and V. villosa cover crops produced a higher biomass compared to B. juncea and suppressed weeds more effectively, likely due to higher competition for resources and their ability to establish a more homogenous ground cover. B. juncea was also expected to be effective in weed suppression due to allelopathy (Haramoto and Gallandt, 2004), but this effect was not observed.

Interestingly, a shift in species composition was observed in Mix 7, in which lacy phacelia was dominant in 2012 and clovers in 2013, despite the same seed rate used in both years. This species shift may be attributed to the different agro-climatic conditions between the two seasons: 2012 had a colder and drier winter, while 2013 was characterized by a warmer and exceptionally rainy winter, i.e. more favourable conditions for clovers growth. This shift highlights a functional insurance effect (Yachi and Loreau, 1999) from the more diverse cover crop: the co-presence of seven species with different environmental optima increases mixture resilience against climatic fluctuations. On a three-year basis, Mix 7 produced the highest biomass of all cover crop treatments, 46 and 105% higher than hairy vetch and Indian mustard respectively.

4.2. AMF colonization

At transplant, the percent mycorrhizal colonization was on average higher in tomato pre-inoculated with IMA6 compared with IMA1. The mixed inoculum, representing a higher level of AMF diversity, had an average behaviour indicating a prevailing effect of AMF symbiont identity over diversity on root colonization. Differences among symbionts observed at transplant disappeared at tomato flowering and harvest.

These results show that at transplant root colonization never reached the plateau, which level is known to depend on the interactions among field natural endophytes and introduced AMF inocula, host genotypes and environmental conditions (Smith and Read, 2008). On average, cv. Rio Grande showed better root colonization than cvs Roma and Perfect Peel. Cv. Roma, an old tomato landrace (www.sementi.it), did not show higher levels of root colonization compared to the modern Perfect Peel hybrid. Our results confirm recent findings (Steinkellner et al., 2012) which showed that although tomato cultivars may differ in AMF susceptibility, such differences are not dependent on genotype age or genetic diversity level. Rather than just focusing on the conservation of heritage cultivars, future work aimed to improve organic systems should identify traits that selectively

favour AMF symbiosis in presently available tomato cultivars or introducing them through targeted breeding programmes.

Cover crop treatments did not generally affect field AMF colonization although a higher level of AMF colonization was observed after V. villosa. We can hypothesize that the resident weed community, mainly comprising AMF hosts, may have provided alternative host plants to AMF even in the B. juncea and Control treatments, buffering any discernible effect of cover crop. Moreover, since most of our root sampling was done at tomato harvest, AMF are likely to have recovered from any temporal mycotoxic effects of allelochemicals released after disruption and incorporation of B. juncea tissues in soil, as previously demonstrated in an organic maize agroecosystem (Njeru et al., 2013).

4.3. Tomato growth and production

Leaf chlorophyll content, as suggested by SPAD values, was higher in cv. Rio Grande than in the other two cultivars. Inoculation of cv. Rio Grande with IMA6 resulted in higher SPAD values in year 2 although not in year 3. SPAD values are directly correlated to N content (Güler and Büyük, 2007) which is expected to be increased by AMF (Hodge and Fitter, 2010). This could explain the higher SPAD values of cv. Rio Grande, which was the most colonized after AMF inoculation.

Days to anthesis and maturity were mainly influenced by the tomato genotype and, as expected, were shorter in the hybrid (cv. Perfect Peel).

Pre-transplant inoculation with IMA6 generally promoted earlier fruit set in year 2 (for cv. Roma) and 3 (for all cvs.). Moreover, isolate IMA6 accelerated tomato maturity in year 2 and promoted higher fruit production in year 3. In contrast, we did not observe any significant effect from IMA1, while the mixed inoculum had only marginal effects. Therefore, AMF functional identity in this case appeared more important than functional composition (Costanzo and Bàrberi, 2014) in delivering production-related agroecosystem services. Functional differences between AMF isolates have been confirmed in carrot, and green onion and lucerne (Wang et al., 2011).

Cvs Perfect Peel and Roma consistently produced higher fruit yield than cv. Rio Grande despite the higher mycorrhizal colonization in the latter. We did not find any significant correlation between mycorrhizal colonization, at either flowering or harvest, and tomato production. It follows that, even if a higher genetic diversity in cv. Rio Grande could have promoted AMF root colonization, this did not turn into a productive advantage for this genotype. This is in line with findings obtained with sorghum and other cereals (Leiser et al., 2016, Ryan and Kirkegaard, 2012).

villosa and Mix 7 cover crops determined the highest tomato marketable fruit yield in year 2 and 3 respectively. B. juncea performed better than Mix 7 only in year 2. As a leguminous crop with easily mineralisable nitrogen in its biomass, V. villosa is likely to have provided sufficient nitrogen promoting tomato growth and production (Campiglia et al., 2010). Similar work in our study area testing the effect of five different cover crops on tomato production and quality only found marginal yield differences, mainly ascribed to Vicia faba (Lenzi et al., 2009). Interestingly, Mix 7 cover crop increased tomato production in year 3, when its biomass was mainly composed of clovers (i.e. leguminous species) while, in contrast, it decreased tomato production in year 2 when it was mainly composed of lacy phacelia (a non leguminous species). Such findings support the key role of functional identity in cover crop mixtures, highlighting the importance of the choice of species to be included in mixtures upon selected functional traits based on the expected agroecosystem services.

In this study, we observed higher fruit firmness in pre-inoculated tomato in year 1, especially with IMA1 or IMA1 + IMA6 after a V. villosa cover crop. However, these results were not confirmed in the following years. In contrast, cv. Roma consistently showed lower fruit firmness, usually considered a disadvantage for processing tomato production. Lower fruit firmness sometimes observed after V. villosa may be due to higher N supply since high N has been reported to affect fruit firmness in tomato (Wang et al., 2007). All tomato cultivars had a fruit pH of ca. 4.0-4.5 which is suggested as the most desirable to ensure safety, which is an essential attribute in tomato processing (Anthon et al., 2011).

In this work, total soluble solid content was higher in cv. Roma than in cvs Rio Grande and Perfect Peel. High soluble solids was an important target for breeding of processing tomatoes (Garcia et al., 2006), which may explain the higher values in the old cv. Roma.

5. Conclusions

Our study shows that both mycorrhizal isolates (as single or mixed inocula) enhanced tomato root colonization in the field. Isolate IMA6 was generally more effective in promoting pre-transplant root colonization, tomato growth and production compared with isolate IMA1 or the mixed inoculum. The two open pollinated tomato varieties, representing higher genetic diversity in tomato genotypes, did not outyield the Perfect Peel hybrid notwithstanding the higher AMF colonization in cv. Rio Grande. Mix 7 cover crop performed better than B. juncea but did not outyield V. villosa in weed suppression, AMF root colonization and tomato productivity. However, between years 2 and 3 we observed interesting changes in tomato production within Mix 7. From these results, we can conclude that functional identity at different levels (i.e. choosing the right cover crop, tomato genotype, and AMF strain) seems to play a more important role in promoting mycorrhizal symbiosis and organic tomato production than functional composition (i.e. mixing two or more species) or functional diversity (using more genetically heterogeneous tomato cultivars). However, the seven-species mixture was more resilient in terms of biomass production under different weather conditions. To develop more efficient mixtures enhancing resilience, productivity and stability in organic systems it will be important to focus on combination of traits likely to increase the provision of agroecosystem services, including those mediated by AMF.

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