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Does mycorrhizal colonisation vary between maize and sunflower under limitations to radiation source or carbohydrate sink?

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Abstract. The aim of this work was to analyse and compare indigenous arbuscular mycorrhizal colonisation (AMC) in relation to growth and total soluble carbohydrates (TSC) in two major, physiologically contrasting crop species: maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.). In order to promote contrasting TSC concentrations, we modified the radiation source by shading and the carbohydrate sink by manipulating reproductive sinks at different phenological stages during the grain-filling period in two field experiments. We assessed plant dry matter, TSC in stems, and root AMC from flowering until final harvest. AMC during the grain-filling period decreased in maize and increased in sunflower. A sink limitation increased AMC in maize, and reduced it in sunflower. A source limitation decreased AMC in both species, especially in sunflower. AMC was positively related to TSC in maize, but negatively in sunflower. The relationship was affected by shading in sunflower, but not in maize. In both species, a different linear model described the relationship between AMC and TSC in plants submitted to the removal of the reproductive organs. The results highlight the role of carbohydrates in mediating mycorrhizal formation, and show for the first time the opposite AMC–TSC relationships in maize and sunflower.

Additional keywords: agronomy, annual crops, endophytes, plant–microbe interactions, soil fungi, soluble carbohydrates, symbiosis.

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Introduction

Mutualistic interactions between plants and soil microorganisms related to biogeochemical cycles are an important component of plant diversity and ecosystem productivity. One of the most important fungal groups of plant-associated microbes is the arbuscular mycorrhizal fungi (AMF), which form symbiosis with plant roots. Root colonisation with AMF occurs in >80% of plant species globally (Smith and Read 2008) and mycorrhizae is the most widespread and ancient symbiosis in the plant kingdom (van der Heijden *et al*. 2015). AMF are obligate symbionts, consuming up to 20% of their host plant's photosynthetically fixed carbon (Bryla and Eissenstat 2005); in return, they forage the soil with their extraradical mycelium and deliver mineral nutrients, mainly of those of low mobility in the soil such as phosphorus (P) and zinc (Zn), to their host plants (Smith and Read 2008). Owing to the exchange of carbohydrates and nutrients with a positive balance towards the host plant, root colonisation with AMF generally has positive effects on plant growth (Chalk *et al*. 2006), and mycorrhizal inoculation have been applied to increase crop plant productivity (Li *et al*. 2005; Ortas 2012; Astiz Imaz *et al*. 2014). Plants can respond with sink stimulation of carbohydrates when colonised with AMF, compensating costs of maintenance of the symbiosis.

Many reports have focused on the effects of mycorrhizal fungi in a broad range of plant families (Barea and Azcon-Aguilar 1983; Liu *et al*. 2003; Scheublin and Ridgway 2004; Astiz Imaz *et al*. 2014), and on environmental factors (e.g. P, iron, Zn, organic matter, salinity, drought, pesticides, host plant) that may affect mycorrhizal colonisation (Tahat and Sijam 2012; Gosling *et al*. 2013; Thougnon Islas *et al*. 2016). However, understanding is often lacking of intrinsic factors of the host plant that can determine the formation of the symbiosis. The benefit of AM colonisation (AMC) is determined via the impact on host nutrition and probably by host identity. Interspecific variation in AMC has rarely been explored among

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crops under identical management conditions. Fernandez *et al*. (2009) assessed interspecific variation of indigenous AMC among maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* (L.) Merr.) crops of the Pampean Region, Argentina, across different levels of available P in the soil. Gosling *et al*. (2013) compared changes in colonisation and molecular diversity between maize and soybean along a P gradient in a field in the United Kingdom.

Cultivated sunflower is one of the five most important oilseed crops in the world (USDA Economic Research Service: https://www.ers.usda.gov/topics/crops/soybeans-oil-crops/sunflowerseed/), and in addition to soybean, one of the most seeded in temperate countries. Maize is the second-most plentiful cereal (after wheat) grown for human consumption (Bolsa de Cereales de Buenos Aires: http://www.bolsadecereales.com). In Argentina, areas of 6.0 and 1.4 Mha are annually cropped with maize and sunflower, respectively, reaching annual production of 33.8 and 3.2 Mt in the 2014–15 growing season (Secretaría de Agroindustria de la República Argentina: http://www.siia.gob.ar/monitorsiogranos/tablerov5.html). Both species are mycorrhizal (Mc Gonigle *et al*. 1999) but differ in how they metabolise environmental carbon. Maize is classed as a C_4 species because the first organic product from its carbon fixation is a 4-carbon sugar, whereas sunflower is placed within the C_3 species, its first organic product from photosynthesis being a 3-carbon sugar. The C_4 species are more efficient at metabolising carbon than C_3 species, through both anatomical and physiological traits (Gowik and Westhoff 2011). In addition, maize and sunflower differ in the position of the reproductive organs on the stem. The principal sink in maize, the ear, is in an axillar, non-dominant position, whereas the head in sunflower is in an apical and dominant position, which is related to leaf-senescence pattern (Ho *et al*. 1987). These differences may lead to a differential flux and availability of carbohydrates in the host, which could be strongly related to mycorrhizal colonisation.

Andrade and Ferreiro (1996) analysed effects of changes in the source of radiation (by shading or thinning) on the growth and concentration of carbohydrates in maize and sunflower. However, to our knowledge, there are no reports showing how changes in host factors (such different species or changes in the source or the sink) can affect plant growth and carbon accumulation, and consequently the relationship with AMC. Even though the exact mechanisms involved are uncertain, it is probable that changes in the carbon provided to maintain the symbiosis affect AMF colonisation.

Reproductive growth in annuals is related to leaf senescence because senescent leaves increase after flowering (Thomas and Stoddart 1980; Gan and Amasino 1997; Yoshida 2003). Thus, the demand of grains converges with a decreasing green coverage during the grain-filling period. In oilseeds species (including sunflower), experiments preventing grain formation showed a delay in leaf senescence (Lindoo and Nooden 1977; Ho *et al*. 1987; Sadras *et al*. 2000). In most cases, an increase in the source : sink ratio in maize produced an advance in leaf senescence (Allison and Weinmann 1970; Rajcan and Tollenaar 1999; Sadras *et al*. 2000); however, in some instances, removal of the ear produced an advance or a delay in leaf senescence in this species (Thomas and Smart 1993).

In any case, carbohydrate metabolism always plays a principal role (Wingler *et al*. 2006), so an effect of changes in the source or the sink on mycorrhizal colonisation is expected. During the grain-filling period, the source and the sink adjust their relative magnitudes in response to environmental (incident radiation, temperature, etc.) and plant (leaf area, grain number, etc.) factors. Therefore, we could also hypothesise that a source or sink effect on mycorrhizal colonisation can change as plant development progresses. We found no reports in the literature about the effect of changes in the source or the sink at different times in the grain-filling period on mycorrhizal colonisation of maize and sunflower. Furthermore, the relationship between host carbon and mycorrhizal colonisation as affected by changes in the source, the sink or host identity is also uncertain. We therefore sought to address the following three questions. Do modifications in the source or the sink have a negative impact on indigenous colonisation by AMF? If so, is the magnitude of the response the same throughout the grain-filling period? Does the effect of carbon availability in plants on AMC depend on host identity? To answer these questions, we compared AMF colonisation, growth and total soluble carbohydrates (TSC) in field-grown sunflower and maize crops under contrasting source and sink conditions during the grain-filling period.

Materials and methods

Cultural details

Two experiments were conducted at the INTA Experimental Station, Balcarce, Argentina (37°45′S, 58°18′W) during two consecutive growing seasons (November–April 2013–14 and 2014–15). The soil in both experiments was a Typic Argiudol (USDA Soil Taxonomy, Soil Survey Staff 2014). Chemical properties of the soil are shown in Table 1.

Maize hybrid Ax 820 (Nidera Semillas, Venado Tuerto, Argentina) and sunflower hybrid VDH 487 (Advanta Semillas, Balcarce, Argentina) were sown, respectively, on 28 October and 4 November 2013 in Expt 1, and on 27 and 13 November 2014 in Expt 2. Using a manual sowing device, three seeds were placed at 5 cm depth in the soil every 18 cm in the row for maize, and every 25 cm for sunflower. Rows were 0.7 m apart. Emergence occurred 9 and 10 days after sowing (DAS) in maize, and 12 and 8 DAS in sunflower, for Expt 1 and Expt 2, respectively. At the V2 phenological stage in each species (Schneiter and Miller 1981; Ritchie *et al*. 1986), plots were thinned to retain only one of the three emerged seedlings, adjusting plant density to 7.9 plants m^{-2} for maize and 5.7 plants m^{-2} for sunflower. According to the chemical characteristics of the soil, both experiments were fertilised with sufficient nitrogen, P and sulfur to insure that nutrient availability did not limit crop yield (Echeverría and García

Table 1. Chemical properties of the experimental soil

	Expt 1		Expt 2	
	Maize	Sunflower	Maize	Sunflower
pH	6.9	6.8	6.8	6.7
Bray phosphorus (mg kg^{-1})	28.5	31.7	31.9	32.5
Organic matter (g kg^{-1})	55.1	54.2	59.3	61.0

2015). Supplementary sprinkler irrigation (central pivot) was applied when necessary (water balance) to maintain soil-water availability at non-limiting levels for crop yield. Weeds, insects and diseases were controlled adequately through cultural, chemical and manual techniques.

Solar radiation, air temperature and rainfall were obtained from the INTA Balcarce weather station, 700 m from the experimental site. Meteorological conditions of the experiments were compared with a 45-year series (1971–2015) (Table 2).

Treatments and experimental design

Experiments were designed as complete blocks arranged as split-plots and three replications, with species assigned as the main plot and variation in the source or the sink as the subplots. Each subplot was 2.8 m wide and 10 m long.

Treatments applied to vary the source or the sink were: (*i*) removal of the reproductive organ, at phenological stage R2, R3, R4 or R5 in maize (Ritchie *et al*. 1986) and at R6, R7 or R8 in sunflower (Schneiter and Miller 1981) (treatments R2–R8); (*ii*) 50% reduction of incident solar radiation from phenological stage R3 in maize and R7 in sunflower to final harvest (shaded treatments); and (*iii*) untreated control.

Subplots were considered to be at a given stage when 95% of the plants had achieved this stage. Flowering of maize and sunflower, respectively, occurred in Expt 1 at 65 and 63 DAS, and in Expt 2 at 59 and 61 DAS. Physiological maturity in control and shaded subplots, respectively, occurred in Expt 1 at 62 and 67 days after flowering (DAF) in maize and at 66 and 56 DAF in sunflower, and in Expt 2 at 63 and 59 DAF in maize and at 44 and 40 DAF in sunflower.

Removal of the reproductive organ was performed to allow a decrease in the sink (alternative sinks like stems must be considered), whereas the reduction of incident radiation was performed to decrease the source. Ears and heads were removed by hand from all plants of the subplot through a sharp cut with a bladed instrument. Incident radiation was reduced by the application of a plastic neutral mesh, which retained ~50% of the total incident radiation.

Aboveground dry matter and total soluble carbohydrates

Aboveground dry matter (DM) was measured periodically from flowering. Three to five plants were cut at the ground level. Each plant was separated into stem, leaves, and ear or head. Samples were oven-dried (with air circulating at 60°C) to constant weight and weighed. TSC from stem was quantified by spectrophotometry at 490 nm (Spectronic 20; Bausch and Lomb, Rochester, NY, USA) by applying the phenol–sulfuric acid method (DuBois *et al*. 1956) after extraction (100°C water bath and centrifugation at 1096*g*, three times) from a 50-mg milled sample. Both TSC and DM are considered indicators of the photosynthetic rate of the plant.

Table 2. Daily mean air temperature and solar radiation during the experiments compared with the 45-year average

	Temperature (°C)			Solar radiation (MJ)		
	Expt 1	Expt 2	1971–2015	Expt 1	Expt 2	1971–2015
Nov.	17.2	17.6	16.1	19.0	20.6	20.1
Dec.	21.6	21.0	19.1	22.6	22.9	22.1
Jan.	22.2	21.0	20.7	21.9	21.4	22.0
Feb.	19.9	21.5	19.9	18.1	19.8	19.6
Mar.	17.7	21.2	18.1	14.9	15.8	15.2
Apr.	14.7	17.0	14.6	9.6	11.3	10.9
Average	18.9	19.9	18.1	17.7	18.6	18.3

Arbuscular mycorrhizal colonisation

Maize and sunflower roots were sampled periodically from flowering until final harvest for determination of AMC (six soil samples per subplot, 5 cm diameter and 20 cm depth, collected in the row). In Expt 2, treatments R2–R8 and shaded subplots were sampled only at final harvest. Roots were separated from soil, washed to remove soil particles, collected on a sieve (2 mm), cut thoroughly, mixed and stained according to the Phillips and Hayman (1970) modified method. Briefly, roots were cleared with KOH (10%, 30 min, 100°C), acidified with HCl (0.1 N, 2 min), placed in 60% H_2O_2 for 10 min, washed, and stained with Trypan Blue (0.05%, 5 min, 100°C) in lactoglycerol (lactic acid, glycerol, distilled water 1 : 1 : 1). The occurrence of AMC was assessed by microscopic examination (40× and 100×) of the stained root system. For determination of frequency of AMC, a segment was considered colonised if it contained arbuscules, coils plus hyphae and/or vesicles. Colonisation was assessed by using the Trouvelot *et al*. (1986) method, which allowed the simultaneous evaluation of the intensity of AMC, and the proportion of arbuscules of roots.

Statistical analyses

Homoscedasticity and normality of the data were checked by Levene and Shapiro–Wilk tests, respectively ($P = 0.95$). Results were evaluated by analysis of variance procedures according to the statistical design of the experiments, using InfoStat Professional v.1.1 (Di Rienzo *et al*. 2016). DM was analysed by species. Control data were used to evaluate the effect of species. Differences among treatment means were evaluated with the Fisher's l.s.d. test ($\alpha = 0.05$). Irrespective of the significance of the interactions, for each parameter, we showed the effect of variations in the source or sink treatments separately for each species and experiment. Regression analyses were also conducted by the least-squares method, using Sigma Plot version 11.0 (Systat Software, Chicago). Relationships between stem DM and TSC during the grain-filling period were fitted by using linear and quadratic functions. Similarly, the relationship between AMC and TSC was explored by using linear functions. In both cases, regression coefficients (R^2), residuals and significance of regression parameters ($P \leq 0.05$) were used to assess the appropriateness of the regressions.

Results

Meteorological conditions

Air temperature during both experiments was higher than the 45-year average (by 0.8°C in Expt 1 and 1.8°C in Expt 2, average of entire season; Table 2). Temperatures in Expt 1 were higher than those in Expt 2 during vegetative stages (December–January), but were lower during reproductive stages (February–March). Solar radiation during both experiments was rather similar to that of the 45-year data series (0.6 MJ lower in Expt 1 and 0.3 MJ higher in Expt 2, average of entire season).

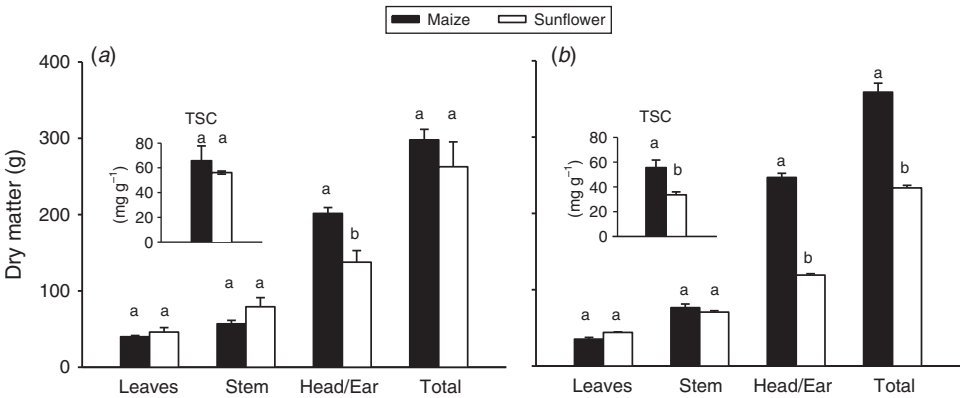

Fig. 1. Dry matter and total soluble carbohydrates (TSC, insets) at physiological maturity in (*a*) Expt 1 and (*b*) Expt 2. Capped lines indicate standard error of the mean. Within experiments, for each plant part, means with the same letter are not significant different between species (l.s.d. Fisher, $\alpha \leq 0.05$).

Solar radiation was higher in Expt 2 than Expt 1 during most of the growing season (by 0.9 MJ, average of entire season), and particularly during reproductive stages (by 1.4 MJ, average of February–April).

Plant growth and carbohydrate concentration at final harvest: comparison between species and effect of a source or a sink limitation

Maize ear DM was higher than sunflower head DM, by 32% in Expt 1 and 52% in Expt 2 ($P \leq 0.05$; Fig. 1*a*, *b*). Total DM and TSC were also higher for maize than for sunflower in Expt 2 (35% and 39%, respectively; $P \leq 0.05$). No differences between species were observed with respect to DM of leaves or stem ($P > 0.05$).

In maize, compared with control plants, shading induced a decrease in ear DM and total DM, by ~22% and 27%, respectively (average of Expts 1 and 2) ($P \leq 0.05$, Table 3). Shaded plants tended to show diminished TSC in both experiments, although differences from control plants did not reach significance (l.s.d. at $P = 0.05$, 25.4). As expected, removal of the ear at any phenological stage also reduced total DM compared with control plants (by 25–61% in Expt 1, and 37–70% in Expt 2, $P \leq 0.05$). Conversely, stem DM mostly increased after ear removal, the increments being smaller as the ear was removed at stages closer to physiological maturity (193% and 105% in treatments R2 and R3, respectively (average of Expts 1 and 2), and 67% in treatment R4 (Expt 1 only); $P \leq 0.05$). TSC in the stem increased by 194–344% in Expt 1 and by 130–291% in Expt 2 after removal of the ear at any phenological stage ($P \leq 0.05$).

Shading of sunflower induced a decrease in head DM and total DM by ~25% and 20%, respectively, in Expt 1 only ($P \leq 0.05$, Table 3). As expected, removal of the head induced a decrease in total DM compared with control plants, but only in treatments R7 and R8 (by 30% and 36%, respectively, average of Expts 1 and 2; $P \leq 0.05$). Stem DM increased by 66% and 46% in treatments R6 and R7, respectively (average of Expts 1 and 2), and by 29% in treatment R8 (Expt 1 only), compared with the control ($P \leq 0.05$). Leaf DM also increased by 77% and 81% in treatment R6 (Expts 1 and 2, respectively) and by 60% in treatment R7 (Expt 1 only), compared with the control ($P \leq 0.05$). Finally, head removal increased TSC in stems by 105–202% in Expt 1 and by 160–239% in Expt 2 ($P \leq 0.05$).

Table 3. Dry matter and total soluble carbohydrates (TSC)
Treatments: removal of the reproductive organ at phenological stages R2, R3, R4 and R5 in maize, and R6, R7 and R8 in sunflower; 50% shading (S); control (C). For each species and experiment, means followed by the same letter are not significantly different (Fisher's l.s.d. at $P = 0.05$)

	Treatment	Dry matter (g)				TSC
		Leaves	Stem	Ear or head	Total	(mg g^{-1})
			Maize			
Expt 1	C	39.8ab	56.8d	201.3a	297.9a	65.8d
	S	40.5ab	55.2d	155.7b	251.4b	45.4d
	R2	36.2ab	188.7a	–	224.9b	201.8bc
	R3	36.0b	132.4b	–	168.4c	292.4a
	R4	34.0b	95.0c	–	129.0d	236.8b
	R5	41.0a	76.5cd	–	117.5d	193.2c
Expt 2	C	35.2a	76.5cd	247.1a	358.8a	55.5d
	S	31.4ab	58.6d	171.4b	261.5b	31.2d
	R2	32.4ab	194.0a	–	226.3c	127.9c
	R3	32.7ab	134.5b	–	167.2d	184.9ab
	R4	30.4ab	96.2c	–	126.6e	217.0a
	R5	29.2b	77.3cd	–	106.5e	153.6bc
			Sunflower			
Expt 1	C	46.0cd	79.2c	137.5a	262.7a	56.2c
	S	38.6d	67.3c	103.1b	209.0b	34.8c
	R6	81.3a	133.0a	–	214.2ab	139.7ab
	R7	73.7ab	117.9ab	–	191.6bc	169.8a
	R8	57.5bc	102.0b	–	159.5c	115.4b
Expt 2	C	43.9b	70.4cd	119.0a	233.3a	42.8b
	S	45.9b	65.9d	108.6a	220.4a	38.3b
	R6	79.6a	114.9a	–	194.5ab	103.1a
	R7	56.1b	99.7ab	–	155.8bc	113.9a
	R8	51.9b	90.9bc	–	142.8c	87.2a

Stem dry matter and total soluble carbohydrates during the grain-filling period

Stem TSC decreased through the grain-filling period in control plants of both species (Fig. 2*c*, *d*; only Expt 1 is presented).

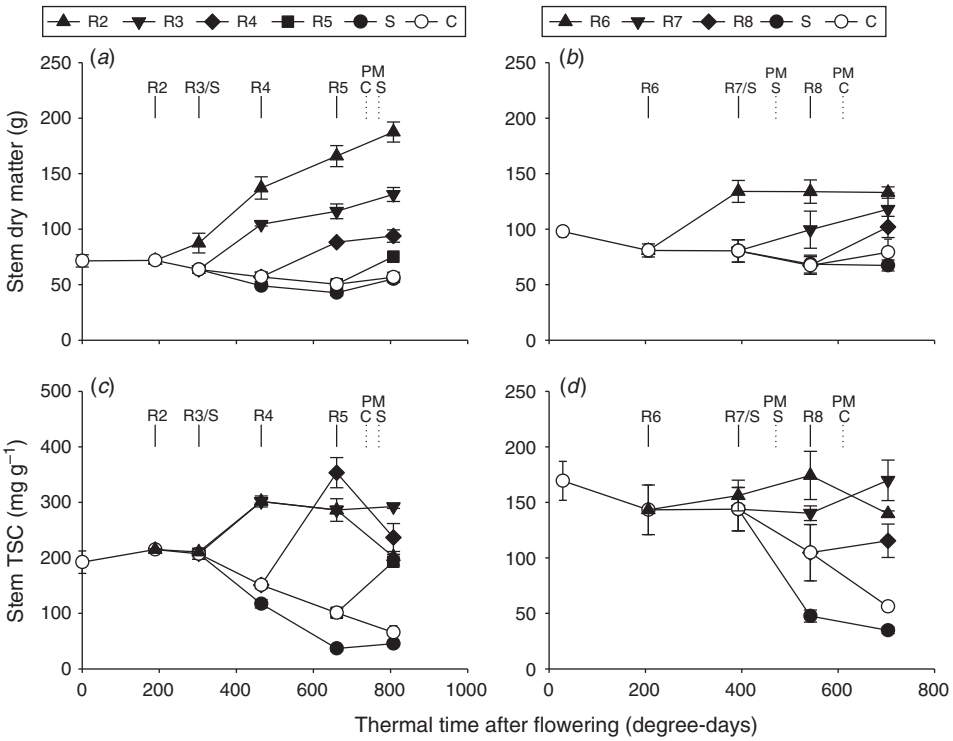

Fig. 2. Stem dry matter and total soluble carbohydrates (TSC) in stem in (*a*, *c*) maize and (*b*, *d*) sunflower after flowering in Expt 1. Treatments: removal of the reproductive organ at phenological stages R2, R3, R4 and R5 in maize, and R6, R7 and R8 in sunflower; 50% shading (S); control (C). Dates of treatments application (solid vertical lines) and physiological maturity (PM, dotted vertical lines) are indicated at the top of each graph. Capped lines indicate standard error of the mean.

Stem DM also decreased, but to a lesser extent (Fig. 2*a*, *b*; only Expt 1 is presented). For maize, TSC range was 70–220 mg g^{-1} in Expt 1 and 60–210 mg g^{-1} in Expt 2, while for sunflower, it was 60–170 mg g^{-1} in Expt 1 and 40–120 mg g^{-1} in Expt 2. A rapid increase in DM and TSC was found after removal of the reproductive organ at any phenological stage in both experiments (Fig. 2). Conversely, a decrease in TSC and, to a lesser extent, in DM of stem was obtained in shaded compared with control plants.

Stem DM and TSC were positively related in control plants of both species (Fig. 3*a*, *b*). This relationship was described by a different linear model in each experiment for maize, and by a single quadratic model including both experiments for sunflower. These models accounted for the effect of the shading in both species, and in maize, for the removal of the ear at R4 and R5 (Fig. 3*c*, *d*). When ear was removed at R2 and R3, the increase was proportionally greater in DM than in TSC (Fig. 3*c*). Stem DM in sunflower increased after removal of the head at R6, R7 or R8, whereas TSC remained approximately within the range of control values (Fig. 3*d*).

Arbuscular mycorrhizal colonisation: comparison between species and effect of a source or a sink limitation

Mycorrhizal fungal structures such as spores, vesicles, arbuscules, mycelium and colonised roots were found in both species at all phenological stages studied, and in all applied treatments. Among control plants, AMC during the grain-filling period mostly decreased in maize and increased in sunflower, regardless of the assessed variable (Fig. 4). On average, AM infection frequency, AM intensity and arbuscules content during the grain-filling period were, respectively, 30%, 43% and 33% higher in sunflower than in maize ($P \leq 0.05$). For maize, AMC was 33% higher in Expt 1 than Expt 2 (average of frequency, intensity and arbuscules content; $P \leq 0.05$), whereas for sunflower, no differences were found between experiments.

In Expt 1, shaded plants showed lower AMC intensity than control plants (Fig. 5*a*, *f*). The effect increased as physiological maturity approached, and was greater in sunflower than in maize. In sunflower, AMC intensity around physiological maturity was 37% and 57% lower in shaded than control plants in Expts 1 and 2, respectively ($P \leq 0.05$, Table 4). Shaded maize plants showed a similar tendency in both experiments; however, differences from control plants did not reach significance ($P > 0.05$, l.s.d. at $P = 0.05$, 9.1; Table 4).

Maize plants with a sink limitation at any of the studied phenological stages showed higher AMC intensity than control plants, whereas the opposite was found for sunflower (Fig. 5*b–e*, *g–i*). For maize, AMC intensity increased by ~49% and 54% after removal of the ear at R2 and R4, respectively, compared with control plants in Expt 1 ($P \leq 0.05$, Table 4). For sunflower, AMC intensity around physiological maturity was 26% and 33% lower when the head was removed at R6 and R7, respectively, than in control plants in Expt 1, and 36% and 37%

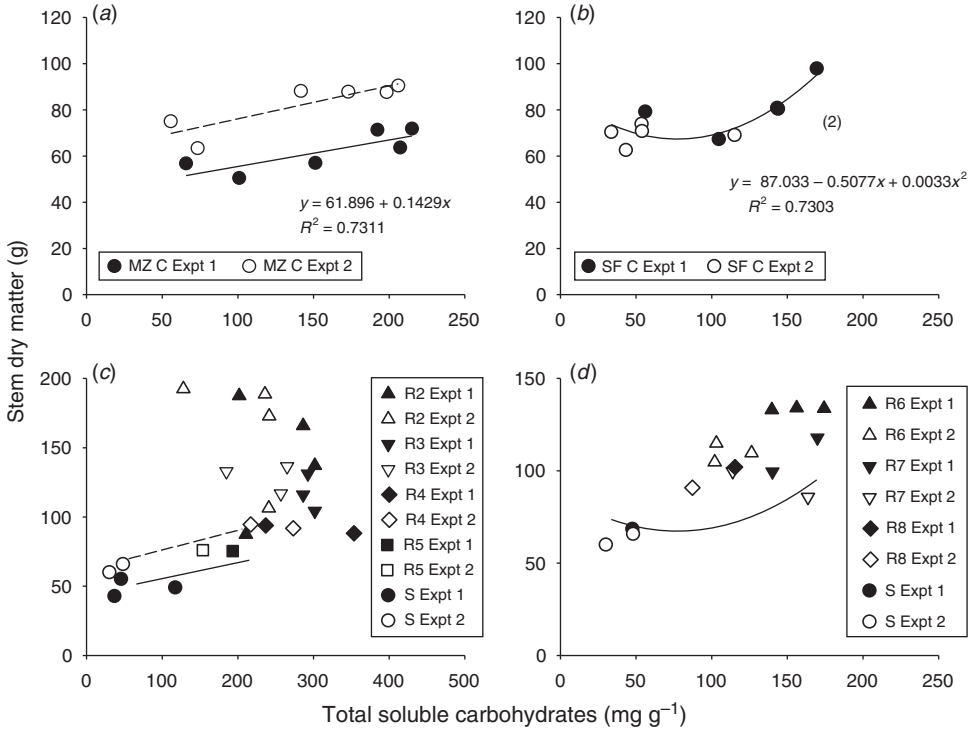

Fig. 3. Stem dry matter as a function of total soluble carbohydrates in stem of (*a*, *c*) maize and (*b*, *d*) sunflower. The relationship between stem dry matter and total soluble carbohydrates in control plants was described by (*a*) a positive linear model in Expt 1 (solid line) and Expt 2 (dashed line) in maize, and (*b*) a single positive quadratic model in sunflower. These models were used as a reference in (*c*) and (*d*) to evaluate the effect of treatments: removal of the reproductive organ at phenological stages R2, R3, R4 and R5 in maize, and R6, R7 and R8 in sunflower; 50% shading (S); control (C). Each value represents the average of the three replications at each sampling date ($n = 9$).

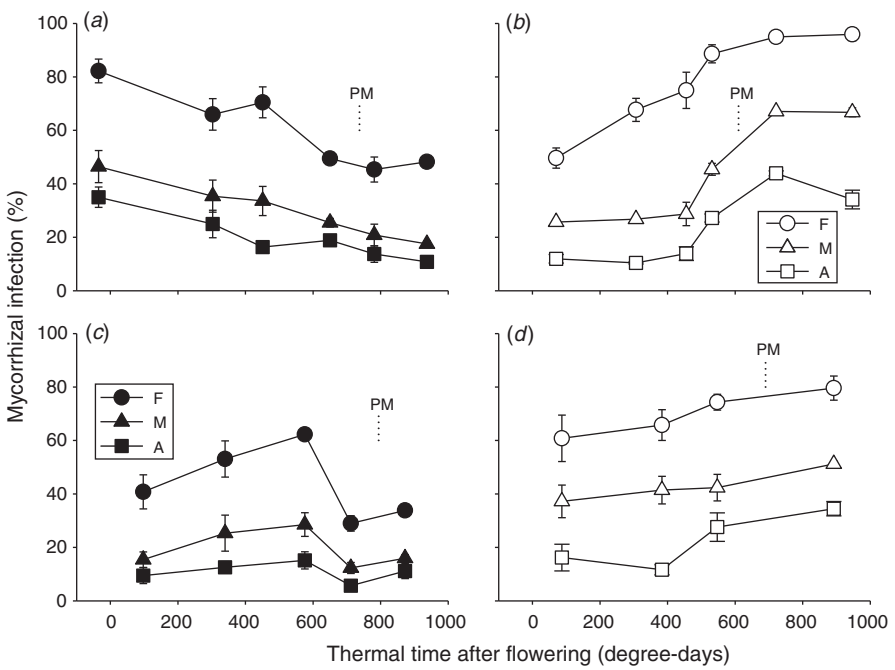

Fig. 4. Arbuscular mycorrhizal infection frequency (F) and intensity (M) and arbuscules content (A) on roots of control (*a*, *c*) maize and (*b*, *d*) sunflower after flowering, in (*a*, *b*) Expt 1 and (*c*, *d*) Expt 2. Dates for physiological maturity (PM) are indicated with dotted vertical lines. Capped lines indicate standard error of the mean ($n = 18$).

Table 4. Intensity of arbuscular mycorrhizal colonisation
Treatments: removal of the reproductive organ at phenological stages R2, R3, R4 and R5 in maize, and R6, R7 and R8 in sunflower; 50% shading (S); control (C). For each species and experiment, means followed by the same letter are not significantly different (Fisher's l.s.d. at $P = 0.05$)

	Treatment	Intensity of colonisation (%)	
		Expt 1	Expt 2
Maize	C	20.9cd	16.0ab
	S	13.5d	9.0b
	R2	31.2ab	14.4ab
	R3	27.6abc	22.4a
	R4	32.2a	21.8a
	R5	23.0bc	16.0ab
Sunflower	C	67.1a	51.2a
	S	42.5b	22.0c
	R6	49.4b	32.7b
	R7	44.8b	32.2b
	R8	71.4a	24.8bc

lower in Expt 2 ($P \leq 0.05$, Table 4). When the head was removed at R8, AMC intensity around physiological maturity was lower than in control plants in Expt 2 only (by 52%, $P \leq 0.05$). After physiological maturity, differences in AMC intensity between each treatment and the control plants tended to diminish or disappear in both species.

Relationships between AMC and TSC: comparison between species and effect of a source or a sink limitation

The relationship between AMC intensity and TSC in stem was described, for both experiments, by a positive linear model in maize, and by a negative linear model in sunflower (Fig. 6). The absolute value of the slope in the model for sunflower was greater than (more than double that) obtained in the maize model, regardless of the different levels of AMC intensity found between experiments.

For maize, the model obtained in control plants accounted for the relationship between the intensity of mycorrhizal colonisation and TSC in shaded plants (Fig. 7*a*). Another positive linear model with a similar slope (0.14 vs 0.12% g mg^{-1}) described this relationship in plants submitted to the removal of the ear at different phenological stages, even if TSC in these plants was considerably higher (180–350 mg g^{-1}).

For sunflower, we obtained a 50% decrease in AMC intensity in shaded plants for the same concentration of TSC, compared with control plants (Fig. 7*b*). The relationship between AMC intensity and TSC in plants submitted to the removal of the head at different phenological stages was described by a negative linear model, but with a slight increase in AMC intensity at high concentrations of TSC in relation to the model obtained for control plants.

Discussion

We set up a field experimental system in which a C_3 species (sunflower) and a C_4 species (maize) were subjected to manipulation of the source of radiation or the sink of carbohydrates through the grain-filling period. Treatments applied caused a limitation in the source or in the sink of assimilates, and differential responses in growth and accumulation of carbohydrates between species, as well as in formation of mycorrhizae.

Crop production was in agreement with Andrade and Ferreiro (1996), who reported values of total DM per plant within the range 250–350 g for maize and 200–220 g for sunflower, in field-grown experiments in the same location. The reduction in plant growth after a shading was not surprising, and experimental evidence of the effect of low radiation is extensive, mainly of decreased biomass, in several plants species (Daft and El-Giahmi 1978; Bethlenfalvay and Pacovsky 1983; Tester *et al*. 1986; Olsson *et al*. 2010) including maize and sunflower (Andrade and Ferreiro 1996).

Soluble sugars are an important source of reserves in the plant, the stem being the main organ where they are accumulated in most crops, including maize and sunflower (Setter and Flannigan 1986; Hall *et al*. 1989). The concentrations of TSC found in sunflower in our experiments ranged between those reported by Andrade and Ferreiro (1996) and Hall *et al*. (1989). Although the former authors reported concentrations of TSC in maize somewhat higher than we found (by 14–28%), this could be explained by the fact that they used an older hybrid. In our work, although the trend in shaded plants was always towards a lower TSC concentration than in the control, the effect was not significant. Andrade and Ferreiro (1996) stated that TSC concentration was more affected by shading in sunflower than in maize; they therefore considered that maize had greater capacity to buffer source reduction than sunflower. However, in our work, the difference in TSC found between species was not so large as to confirm this.

For maize, the relationship between DM and TSC in stem was described by two positive linear functions, one for each experiment. Both the stem and the whole plant in this species weighed more in Expt 2 than in Expt 1, but TSC remained rather similar. The higher incident radiation registered during the reproductive period in Expt 2 was probably the cause of the difference in growth, considering that development was not modified by temperature (similar number of days from flowering to physiological maturity).

High indigenous colonisation of agricultural crops, including maize and sunflower, has been detected in soils of the Pampean Region, Argentina (Fernandez *et al*. 2009; Astiz Imaz *et al*. 2014; Barbieri *et al*. 2014). However, there are evident differences of colonisation related to different habitats, and among different host species in the same habitat (Scheublin and Ridgway 2004; Uibopuu *et al*. 2009; Hazard *et al*. 2013).

Some studies have found that AMC of legumes and of maize was reduced, but not eliminated from roots, under low light intensities (Daft and El-Giahmi 1978; Konvalinková *et al*. 2015). In our experiment, incident radiation was reduced to 50% after flowering and AMC of both crops was decreased, especially in sunflower, but it was not eliminated. This was in agreement with Walder and van der Heijden (2015), who stated that, in shaded host plants, AMC could be maintained as an investment for potentially more favourable future conditions.

Arbuscular mycorrhizal fungi are largely dependent on supply of fixed carbon by the hosts for metabolism and growth (Bago *et al.* 2000). It was suggested that the sugar content of host plants plays a crucial role in AMC and that the amount of sugar would affect the frequency of penetration by

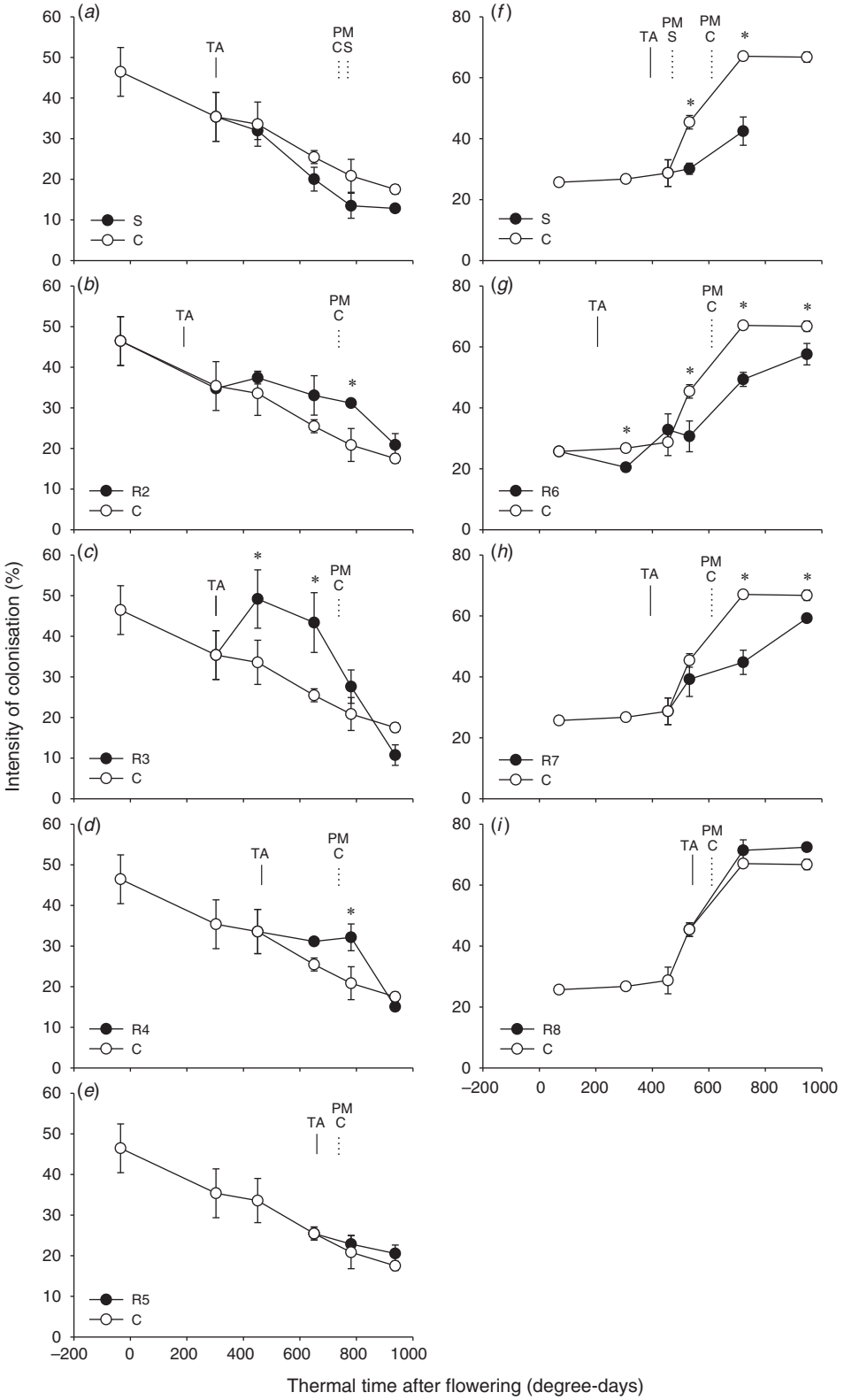

Fig. 5. Intensity of arbuscular mycorrhizal colonisation in roots of (*a–e*) maize and (*f–i*) sunflower after flowering in Expt 1. Treatments: 50% shading (S); removal of the reproductive organ at R2, R3, R4 and R5 in maize, and R6, R7 and R8 in sunflower; control (C). Dates of treatments application (TA, solid vertical lines) and physiological maturity (PM, dotted vertical lines) are indicated at the top of each graph. Capped lines indicate standard error of the mean ($n = 18$). Asterisks indicate significant differences (l.s.d. Fisher, $\alpha \leq 0.05$).

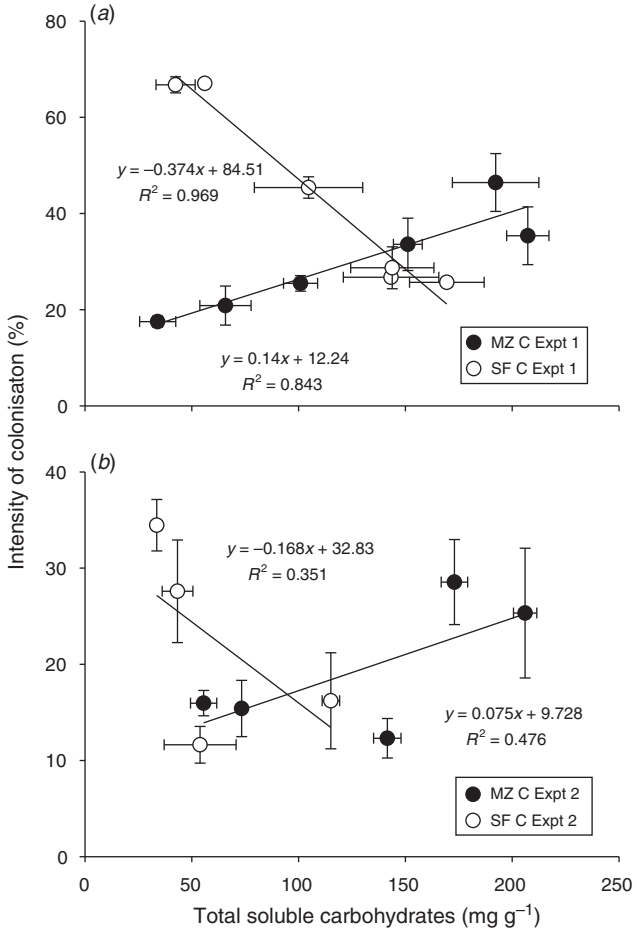

Fig. 6. Intensity of arbuscular mycorrhizal colonisation in roots of control (C) maize (MZ) and sunflower (SF) as a function of total soluble carbohydrates in the stem: (*a*) Expt 1, (*b*) Expt 2. The relationship was described by linear models in both species. Capped lines indicate standard error of the mean of intensity of colonisation ($n = 18$) and total soluble carbohydrates ($n = 9$).

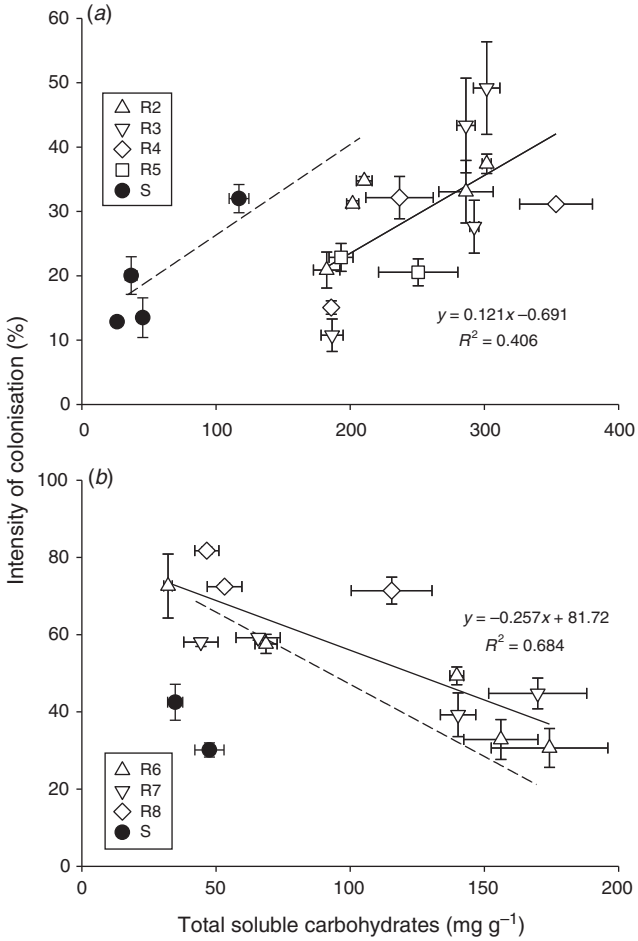

Fig. 7. Intensity of arbuscular mycorrhizal colonisation in roots of (*a*) maize and (*b*) sunflower as a function of total soluble carbohydrates in the stem in Expt 1. The models obtained in control plants (dashed lines) were used as a reference to evaluate the effect of the removal of the reproductive organ at phenological stages R2, R3, R4 and R5 in maize (solid line), and R6, R7 and R8 in sunflower (solid line), and under 50% shading (S). Capped lines indicate standard error of the mean of intensity of colonisation ($n = 18$) and total soluble carbohydrates ($n = 9$).

the mycorrhizal endophyte (Same *et al*. 1983). Thus, from the host plant's perspective, the amount of carbon provided to the AMF represents the symbiotic cost and this could be considered an investment by the host plant. In this sense, the decrease in plant growth in shaded plants, without a significant decrease in TSC found in our experiment, could be considered an investment of shaded plants to maintain the mycorrhizal symbiosis.

The interacting effects of the dynamics of carbon allocation under changing light conditions on mycorrhizal colonisation, plant nutrition and growth have been studied for 40 years and were summarised in Konvalinková and Jansa (2016). A pioneer experiment comparing AMC of maize, sunflower and other host plants showed that plant susceptibility to AMC was independent of the level of sugar content in their roots (Ocampo and Azcon 1985). Other studies stated that by reducing the radiation intensity, the carbon cost of the symbiosis is expected to increase because the mycorrhizal activities will consume a higher proportion of the carbon-flow in shaded plants (Peng *et al*. 1993; Johnson *et al*. 1997). Schmitt *et al*. (2013) showed that *Medicago sativa* is very sensitive to shading, with reductions of carbon allocation to the root system, leading to a higher carbon allocation to the shoot meristem, to compensate for the decrease in photosynthetic activity. Although we found no significant changes in TSC in response to shading, we found well-marked differential relationships between AMC and TSC that were associated with the host plant.

Although Kiers *et al*. (2011) stated that AMF preferentially colonise plants with high carbon supply, we found that maize had higher TSC in stems than sunflower; even so, we quantified higher levels of mycorrhizal formation in sunflower. The negative relationship between TSC and AMC in sunflower, a species less efficient in the metabolism of carbon than maize, could be explained by metabolic regulations favouring TSC transfer to the seeds. This coincides with the large drop in mycorrhizal intensity that we found after shading in sunflower. By contrast, the positive TSC–AMC relationship in maize, which was not affected by shading, would indicate a greater efficiency in the carbon metabolism of this species

to sustain and favour carbon consumption by colonisation. Furthermore, the observation that a sink limitation in maize increases the content of TSC for the same AMC production could suggest that maize did not compromise photosynthates to maintain the symbiosis at levels that can affect plant growth. Conversely, in sunflower, AMC decreased linearly with increasing TSC after removal of the reproductive organ, as well as in control plants, but seemed not related to TSC in shaded plants. It could be hypothesised that in this species, AMC is downregulated in an excess of carbohydrate, but also that sunflower cannot regulate AMC under decreases in carbohydrate content. Future work should focus on the study of the regulatory mechanisms underlying this effect. Furthermore, considering that the removal of reproductive structures and the shading treatment caused the decreases in sink and source, respectively, our results showed maize to be the crop with a larger capacity to buffer indigenous mycorrhizal colonisation in response to source or sink decreases during the grain-filling period. We also found that although a reduction in light intensity in both crops decreased mycorrhizal formation (especially in sunflower), an increase in TSC positively stimulated mycorrhizal formation in maize but not in sunflower.

Conclusions

This work describes for the first time how modifications in maize and sunflower source or sink differentially affected AMC and its relationship with TSC. The results support the idea that AMC are controlled by carbon, which could be related, at least in part, to their obligate dependence on the host. The higher efficiency of maize to metabolise carbon was evidenced from the point of view of mycorrhizal colonisation and by the positive relationship with TSC, which was not affected by shading. Finally, our results contribute to understanding of the distribution of resources in the plant, a key goal in elucidating mycorrhizal symbiosis and its contribution to crop production.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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