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ORIGINAL PAPER

Arbuscular mycorrhizal fungi influence whitefly abundance by modifying habanero pepper tolerance to herbivory

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Abstract

Arbuscular mycorrhizal fungi (AMF) develop strong mutualistic associations with roots of about 70% of all vascular plants. By modifying host plant nutrient uptake, growth and defense, AMF also indirectly influence the aboveground herbivorous insect community. Since plant response to AMF depends on the extent of mycorrhization, outcomes of the bottom-up effects of AMF on herbivores are extremely variable and not well understood. We thus tested whether the generalist phloem feeder *Bemisia tabaci* (tobacco whitefly) was affected by the colonization of habanero pepper seedlings (*Capsicum chinense*) by different densities of the AMF *Rhizophagus irregularis*. After AMF inoculation (with control, low and high doses of liquid inoculum), seedlings were grown in a greenhouse without (control) and with whiteflies (10 adults per seedling). We measured plant traits and growth and biomass allocations at 2, 16 and 30 days after transplantation of emerged seedlings. We estimated whitefly adult, egg and nymph densities 28 days after transplantation. *B. tabaci* abundance significantly increased after *C. chinense* inoculation with low AMF density (120% increase for adults, 97% for eggs) through an augmentation of seedling nutritional status. By enhancing plant tolerance and primary metabolism, the higher density of AMF did not affect *B. tabaci* fitness on seedlings. We highlight here that whitefly abundance on mycorrhizal *C. chinense* varies widely depending on AMF inoculum concentration.

Keywords Arbuscular mycorrhizal fungi · *Rhizophagus irregularis* · Plant–herbivore interactions · *Capsicum chinense* · *Bemisia tabaci* · Plant tolerance

Introduction

Both above- and belowground organisms such as viruses, bacteria, fungi, oomycetes, nematodes and insects can positively and negatively influence plant growth, nutrition, tolerance and plant defense against herbivores and other processes (Bezemer and Van Dam 2005; Pineda et al. 2010). Mycorrhizal fungi, that live in symbiosis with roots of most (80%) vascular plants (Wang and Qyu 2006), for example, increase the root surface area for absorption and therefore the plant's nutrient supply (especially P and N), while plants provide photosynthetically derived carbohydrates to the fungi (Smith and Smith 2011). Arbuscular mycorrhizal fungi (AMF; phylum Glomeromycota) are the most ubiquitous and widely distributed mycorrhiza among these associations. Mycorrhizal fungi can also stimulate or inhibit plant tolerance and resistance against herbivore insects (Bennett et al. 2005; Koricheva et al. 2009; Jung et al. 2012); Colonization of roots by AMF has been shown to have positive (45%), negative (35%), and variable to no (20%) effect on aboveground herbivorous insects (Gehring and Bennett 2009; Pineda et al. 2013). These bottom-up effects cause variable outcomes related to the degree of specialization and to the feeding guild of the insect, but conditions that influence these interactions and their mechanisms are not fully understood. In fact, the effects of AMF on herbivorous insect performance first depend indirectly on the effects of AMF on the nutrient concentration, growth, tolerance and defensive traits of the host plant (Gehring and Whitham 2002).

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Within the objective of testing the effect of AMF colonization on an aggressive pest insect, we selected *Bemisia tabaci* (Hemiptera: Aleyrodidae; tobacco whitefly) in part because only 14% of all AMF–insect studies have focused on species of the order Hemiptera (Hartley and Gange 2009), and in a broader context, effects of AMF on pests and pathogens attacking shoots have generally been studied less than those attacking roots (Jung et al. 2012). In addition, *B. tabaci* is a very destructive, invasive species that feeds on the phloem of more than 600 cultivated plant species (Oliveira et al. 2001) in tropical, subtropical and temperate zones of all continents and of open and protected cropping systems. Beside direct damage caused by plant consumption (weakened vigor and growth, decreased fruit ripening, silver leaf damage and honeydew deposition), *B. tabaci* is also recognized to transmit more than 100 species of plant viruses, in particular begomoviruses (Oliveira et al. 2001). Although the influence of AMF is generally positive on generalist phloem-feeding insects (Gehring and Whitham 2002; Gehring and Bennett 2009; Koricheva et al. 2009), a positive outcome has not always been observed (Pacovsky et al. 1985; Guerrieri et al. 2004; Wooley and Paine 2011). Only a few published studies have focused on the effects of AMF on *Bemisia* sp., and the effects have ranged from positive (Wahba 2015) to null (Wooley and Paine 2011).

Bemisia tabaci is well adapted to the temperatures of the Yucatan Peninsula where habanero pepper seedlings (*Capsicum chinense*) are grown and found in association with spores of the commonly studied AMF *Rhizophagus irregularis* Schüßler and Walker (2010). Although the effects of *R. irregularis* inoculation on *Capsicum annuum* have been reported to be largely positive (Pereira et al. 2016), the response of *C. chinense* to AMF is poorly documented. The only study reporting interactions between AMF and *C. chinense* (Constantino et al. 2008) revealed positive effects of commercial strains of AMF on *C. chinense* growth and nutrition. The response of plants associated with AMF and herbivore insect fitness can also vary depending on the density of the fungal symbiont in the soil (Vannette and Hunter 2011, 2013).

The bottom-up effects of various AMF densities on *C. chinense* and on *B. tabaci*, however, have not been studied. Our main objective here is thus to contribute to the knowledge on the response of generalist phloem feeder insects such as *B. tabaci* to AMF colonization of solanaceous species exposed to different AMF inoculum concentrations. In addition, exploring responses of habanero pepper to AMF colonization may lead to the identification of an interesting mycorrhiza-induced form of resistance against *B. tabaci*. Firdaus et al. (2011), for example, studied eight *C. chinense* varieties and revealed repellent effects of *C. chinense* AC 2212 and No. 1720 against *B. tabaci* feeding and oviposition, whereas other varieties were more susceptible to whitefly attacks. Plant variations in toxic compound content, physical barriers, leaf architecture and palatability also influence these resistance mechanisms and/or susceptibility. We expected a positive effect of colonization by the arbuscular mycorrhizal fungus *R. irregularis* on *C. chinense* nutrition, and thus a possible advantage for *B. tabaci* from greater plant biomass availability and/or nutrients. We hypothesized that this advantage might be enhanced by better foliar growth, but it could also be limited by plant defenses and tolerance, both of which can be stimulated by AMF colonization. Such research in a tropical system should help determine the optimal conditions for mycorrhizal inoculation of plants, especially in the context of global change that promotes generalist herbivore invasions.

Material and methods

Plant, AMF and whitefly material

Hybrid PX-11459057 (Seminis Vegetable Seeds Inc., Mexico City, Mexico) of habanero pepper (*Capsicum chinense* Jacquin, 1777; Solanales: Solanaceae) was chosen as the host plant because it produces vigorous plants that mature precociously (Berke 2017). As the AMF material, we used Micorrizafer powder mix (BIOfabrica Siglo XXI, Mexico City, Mexico) which is composed of 60% (w/w) mycelium, hyphae and spores of *Rhizophagus irregularis* (renamed from *Glomus intraradices*, Schenck and Sm 1982 by Schüßler and Walker 2010; Glomerales: Glomeraceae) and 40% sterile soil. The mix also contains carbosil methyl cellulose, an adherent substance that will allow the powder to coat on the plant seeds during inoculation. The mix contains between 30 and 35 spores g^{-1}. Individuals of B-biotype *Bemisia tabaci* Gennadius 1889 (Hemiptera: Aleyrodidae) were reared on eggplant (*Solanum melongena*) and tomato (*Solanum lycopersicum*) plants in a greenhouse at the Instituto Tecnológico de Conkal (ITC; Yucatan, Mexico). Live *B. tabaci* adults were collected and manipulated using entomological aspirators (also called pooters).

Mycorrhizal inoculation

Habanero seeds were sterilized in a 20% (v/v) bleach solution (containing 1% sodium hypochlorite and 0.015% sodium hydroxide after dilution, v/v) for 15 min, then rinsed three times with distilled water. Mycorrhizal inoculum was prepared for $n = 16$ seeds lots in 50 mL of distilled water for the following treatments: control without Micorrizafer (−M), low dose of 4 g of Micorrizafer (LM) containing 130 spores or 2.6 spores mL^{-1} of inoculum, and high dose of 12 g of Micorrizafer (HM) containing 400 spores or 8 spores mL^{-1} of inoculum. Seeds were coated

with AMF spores by incubating them in the inoculum solution in a stirrer (90 rpm at 29 °C) during 24 h. Agitation provides more opportunity for the spores to contact the seeds. Moreover, seeds of *C. chinense* subjected to a pre-sowing treatment with distilled water had higher germination and emergence rates (Garruña-Hernandez et al. 2014). Seeds were removed from the inoculum solution without rinsing and allowed to air-dry before sowing.

At the end of the experiments to corroborate root mycorrhization, we quantified the mycorrhizal structures on the seedling roots (percentage of mycorrhization, proportion of arbuscules, vesicles and mycelia) in 10 fragments of 1 cm per seedling (3–6 seedlings per mycorrhizal treatment). As detailed by Rodríguez et al. (2015), roots were washed, dried to constant mass, clarified with KOH, washed, then stained with trypan blue, and examined for mycorrhizal structures using a stereoscope (Leica DM500).

Soil characteristics

We used Sunshine Professional Peat Lite Mix #3 (LG3; Sun Gro Horticulture, Agawam, MA, USA) consisting principally of sphagnum peat moss, fine vermiculite, dolomite and gypsum (typical extractable nutrient range: pH: 4.5–6, NO_3-N: 0–55 ppm, NH_4-N: 1.5–17 ppm, P: 1–17 ppm, K: 10–90 ppm, calcium Ca: 50–190 ppm). For increasing colonization potential and avoiding contamination by soil pathogens, soil was autoclaved twice for 40 min at 120 °C before use. Soil was mixed with distilled water at sowing time. All seeds were sown on this soil in 2.5 × 2.5 × 6 cm cells of a polystyrene seed tray.

Experimental set-up

All experiments were performed at the ITC. Seedlings were grown in a greenhouse in natural light and ambient temperatures between 32 and 46 °C and relative humidity between 13 and 52% (THWD-3 Humidity Temperature Relative Meter; Amprobe, Everett, WA, USA). Twenty-four days after sowing in the seed tray, the emerged seedlings were transplanted to 500 mL polystyrene pots. Plants were then placed in a randomized block design in entomological metal cages (30 × 30 × 40 cm, four plants maximum) with anti-aphid mesh (0.26 × 0.82 mm mesh). Each cage was intended for one of the six treatments: Mycorrhiza (−M: control, LM: low mycorrhiza and HM: high mycorrhiza) × *B. tabaci* herbivory (Control and Whitefly). An irrigation system of plastic tubes was used for each plant so that the cages were not opened and insects could not escape.

Whitefly experiment and measurements

Seven days after transplanting, half of the remaining plants were exposed to 10 adult *B. tabaci*. Whiteflies were then counted 28 days after transplanting. To estimate whitefly abundance, two well-developed young leaves of the upper third of each plant were detached ($n = 6$ plants per mycorrhizal treatment), and the adults on the abaxial side of the leaves were counted. In the laboratory, the same detached leaves were individually observed with a stereomicroscope to determine the density of whitefly eggs and nymphs (from first to fourth instar) in a square (2 × 2 cm) randomly placed on leaf lamina. The area of each leaf was then determined using ImageJ 1.51 software (National Institutes of Health, Bethesda, MD, USA) to express results in number of adults, eggs and nymphs per square centimeter.

Plant measurements

Variables for plant morphology, physiology and growth were measured after three destructive harvests: 2, 16 and 30 days after transplantation of the seedlings. Seven abnormal plants were excluded from the initial 96 seedlings, and six died in early development. At each harvest, plant traits were directly measured in the greenhouse: basal diameter, plant height (using a Gripper flexible rule; Truper, Mexico City, Mexico), leaf thickness (Quantumik micrometer; Mitutoyo, Kawasaki, Japan), and leaf toughness (using a punch-and-die penetrometer). In the laboratory, plant roots, stems and leaves were carefully cleaned and separated. Leaf area was calculated with ImageJ. Roots, stems and leaves were dried at 40 °C for 48 h or more (until constant mass) and then weighed. Folin and Ciocalteu (1927) assay was used to measure total foliar phenolic content; results were expressed as a percentage of leaf dry mass based on a standard curve prepared with dilutions of tannic acid.

Calculations

Relative growth rate in biomass ($RGR_{Biomass}$, g g^{-1} day^{-1}) represents the increase in plant total dry biomass per unit of biomass and per unit of time (Poorter and Remkes 1990). Similarly, we calculated relative growth rate in diameter ($RGR_{Diameter}$, mm mm^{-1} day^{-1}), relative growth rate in height (RGR_{Height}, cm cm^{-1} day^{-1}) and relative growth rate in leaf area ($RGR_{Leaf\ area}$, cm^2 cm^{-2} day^{-1}). All relative growth rates were calculated as:

$$RGR_X = \left(\ln_e X_2 - \ln_e X_1\right) / \left(t_2 - t_1\right), \quad (1)$$

where X represents, respectively, total plant dry mass (M_P, g), stem basal diameter (D, mm), plant height (H, cm) or

plant leaf area (A, cm^2); t is time expressed in days, subscripts 1 and 2 correspond respectively to $Harvest_n$ and $Harvest_{n+1}$. $RGR_{Biomass}$ can be decomposed into the product of net assimilation rate (NAR, g cm^{-2} day^{-1}) and leaf area ratio (LAR, cm^2 g^{-1}). NAR is the physiological component of $RGR_{Biomass}$ and can be interpreted as the outcome between photosynthetic carbon gain and carbon losses (through respiration, exudation, volatilization) per unit leaf area per day (Poorter and Remkes 1990). LAR is the ratio between plant leaf area and total plant dry mass. These variables were calculated as following:

$$\mathrm{NAR} = \left(M_{P,2} - M_{P,1}\right) / \left(t_2 - t_1\right) \times \left(\log_e A_2 - \log_e A_1\right) / \left(A_2 - A_1\right) \quad (2)$$

$$\mathrm{LAR} = \left(A_1 / M_{P,1} + A_2 / M_{P,2}\right) / 2, \quad (3)$$

where LAR is the morphological component of $RGR_{Biomass}$ and is the product of specific leaf area (a measure of leaf density or relative thinness; SLA, cm^2 g^{-1}) and leaf mass ratio (LMR, g g^{-1}).

$$\mathrm{SLA} = \left(A_1 / M_{L,1} + A_2 / M_{L,2}\right) / 2 \quad (4)$$

$$\mathrm{LMR} = \left(M_{L,1} / M_{P,1} + M_{L,2} / M_{P,2}\right) / 2, \quad (5)$$

where M_L is the leaf dry mass (g). Like LMR, the stem mass ratio (SMR, g g^{-1}) and root mass ratio (RMR, g g^{-1}) were calculated by replacing M_L in Eq. (5) with M_S (stem dry mass; g) and M_R (root dry mass; g), respectively. These biomass allocation indices indicate the fraction of total plant dry biomass allocated to leaves (LMR), stems (SMR) and roots (RMR).

Statistical analyses

Models and graphs were generated using R 3.4.4 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria) and R packages lme4 (Bates et al. 2015) and emmeans (Searle et al. 1980). For each group defined by levels of fixed effects (time and mycorrhiza), all plant variables were tested for homoscedasticity using Levene's test for homogeneity of variance and for normality using Shapiro–Wilk's normality test. Variables that did not meet parametric assumptions were $\log_{10}$-transformed. Cage reference and/or number of days after emergence of the seedlings were both considered as random effects in linear mixed models (LMMs) with restricted maximum likelihood estimation to determine for each variable whether it was affected by time, mycorrhiza and/or by their interaction. In submodel routines, we parsimoniously removed each fixed effect and/or each random effect from the full models and compared them to the submodels. We principally examined quantile–quantile plots, plots of residuals against fitted values and AICc (corrected Akaike's information criterion for small samples) to select the best-fitting models and to decide whether to use mixed models or not. We assessed significance of fixed effects using two-way ANOVAs for unbalanced data. We used type II sum of squares (SS) in the case of linear models without interaction term, type III SS for linear models with interaction term and type III SS in the case of LMMs with a Satterthwaite's approximation for the degrees of freedom. The significance of random effects in our mixed models was assessed using a likelihood-ratio χ^2 test (LRT). We calculated conditional pseudo R^2 for LMMs using Nakagawa and Schielzeth's equation. We fitted generalized linear mixed models (GLMMs) and generalized linear models (GLMs) to visualize effects of mycorrhiza on *B. tabaci* abundance (number of adults, eggs and nymphs per cm^2). We used gamma error distributions for positive data and log x as link functions. Because we collected abundance data for adults, eggs and nymphs on two leaves per seedling, we considered a random subject effect. After using the same submodel routine as before, we used LRT to assess significance of effects. We calculated pseudo R^2 for GLMMs and GLMs according to the R function r2.corr.mer proposed by Byrnes (2008), calculated as the multiple R^2 of linear regression between fitted and observed values of each model. We computed estimated marginal means (EMMs or least square means) and compared them in multiple pairwise comparisons tests using Tukey's P-value adjustment method as a post hoc analysis. To highlight effects of *C. chinense* traits and growth on abundance of *B. tabaci*, we tested for correlations between whitefly abundances and plant variables measured 30 days after transplanting. We used PCAs to identify trends for the influence of plant variables on *B. tabaci* abundance variables. We modelled these trends using GLMs with gamma error distributions and either log x or $1/x$ as link functions. We used ANOVA after type III SS with LRTs to assess significance of fixed effects (plant variables). In addition, to test differences in mycorrhization among treatments (−M: control, LM: low mycorrhiza and HM: high mycorrhiza) we used a one-way ANOVA. When the response variable did not meet the assumptions of normality and homoscedasticity, they were arcsine–square root-transformed. Tukey's paired tests ($P < 0.05$) were used to identify differences in treatments. This analysis was carried out in software InfoStat (Di Rienzo et al. 2018).

Results

Quantification of mycorrhization

We found significant differences in the percentages of mycorrhization ($F = 10.45$, $df = 2$, $P = 0.003$), proportion of arbuscules ($F = 7.95$, $df = 2$, $P = 0.008$) and mycelia

($F = 6.34$, $df = 2$, $P = 0.01$) between the treatments. For vesicle proportion, we found only marginal differences ($F = 3.18$, $df = 2$, $P = 0.08$). LM and HM yielded similar percentages of mycorrhization from 97.33% (± 1.01) to 98% (± 0.71); in contrast, the control treatment (−M) had the lowest percentage (93% ± 0.88). Similarly, for arbuscules (0.38 ± 0.05 for LM and 0.35 ± 0.07 for HM) and mycelial proportion (0.97 ± 0.01 for LM and 0.97 ± 0.01 for HM), the control treatment (−M) had the lowest proportion of the mycorrhizal structures (arbuscules 0.39 ± 0.08 and mycelia 0.93 ± 0.01). Although the vesicle proportion did not differ significantly among the treatments, we found that roots in the LM (0.38 ± 0.05) and HM (0.35 ± 0.07) treatments had a higher proportion of vesicles than those in the control treatment (0.20 ± 0.06).

Effects of AMF on C. chinense

Morphological and physiological traits

Effects of mycorrhiza on *C. chinense* were highly variable-dependent (Table 1). Total polyphenol concentration tended to be lower in inoculated seedlings (Fig. 1A) compared to the controls; however, results were highly variable, so differences were not significant. Overall, NAR was 25% higher for seedlings treated with the HM dose (7.15 ± 0.61 g m^{-2} day^{-1}; Fig. 1D) than for seedlings treated with the LM (5.74 ± 0.61 g m^{-2} day^{-1}) and 34% higher than for controls (5.35 ± 0.66 g m^{-2} day^{-1}). SLA was lower for both AMF treatments (10% for LM: 413.44 ± 8.19 cm^2 g^{-1}; 11% for HM: 407.81 ± 8.08 cm^2 g^{-1}; Fig. 1G) than for controls (459.42 ± 10.79 cm^2 g^{-1}). LAR was 27% lower under HM (150.15 ± 5.17 cm^2 g^{-1}; Fig. 1H) than under −M (206.57 ± 10.06 cm^2 g^{-1}) 2 days after transplanting. Sixteen days after transplantation, leaves on HM seedlings (0.22 ± 0.01 kg cm^{-2}; Fig. 1B) were 47% harder than on controls (0.15 ± 0.01 kg cm^{-2}) and 83% harder than on LM seedlings (0.12 ± 0.01 kg cm^{-2}). Also after 16 days, leaves were significantly thinner on seedlings treated with AMF (27% for LM: 0.43 ± 0.02 mm and 35% for HM: 0.38 ± 0.02 mm) than for controls (0.59 ± 0.03 mm; Fig. 1C).

Seedling growth

We did not find any effect of mycorrhiza or of a mycorrhizal interaction with time on plant total biomass and $RGR_{Biomass}$ (ANOVA, all $P > 0.05$). Only 2 days after transplantation, RGR_{Height} was 12% lower for LM seedlings than for HM seedlings (LM: 0.15 ± 0.01 cm cm^{-1} day^{-1}; vs HM: 0.17 ± 0.01 cm cm^{-1} day^{-1}; Tukey, $P = 0.003$), but with no differences compared to controls (0.16 ± 0.01 cm cm^{-1} day^{-1}; Tukey, $P > 0.05$). Nevertheless, 16 days after transplantation, $RGR_{Leaf\ area}$ was 21% higher for HM seedlings (0.23 ± 0.01 cm^2 cm^{-2} day^{-1}; Fig. 1F) than for controls (0.19 ± 0.01 cm^2 cm^{-2} day^{-1}). Thirty days after transplantation, $RGR_{Diameter}$ was 35% higher for HM seedlings (0.031 ± 0.002 mm mm^{-1} day^{-1}; Fig. 1E) than for controls (0.023 ± 0.002 mm mm^{-1} day^{-1}), but 36% lower for LM seedlings (0.016 ± 0.001 mm mm^{-1} day^{-1}) than for controls.

Exposing non-inoculated seedlings (−M) to whiteflies reduced plant biomass (0.53 ± 0.04 g for −M seedlings without whiteflies against 0.34 ± 0.04 g with whiteflies; Fig. 2A) and their $RGR_{Biomass}$ (0.22 ± 0.01 g g^{-1} day^{-1} for −M seedlings without whiteflies against 0.20 ± 0.01 g g^{-1} day^{-1} with whiteflies; Fig. 2B). These biomass and $RGR_{Biomass}$ differences between controls and infested plants were lower for LM seedlings and even lower for seedlings inoculated with the HM dose. We observed the same pattern with $RGR_{Leaf\ area}$, but the difference between controls and infested −M seedlings was lower and not significant (0.21 ± 0.01 cm^2 cm^{-2} day^{-1} for −M without whiteflies against 0.18 ± 0.01 cm^2 cm^{-2} day^{-1} with whiteflies; Fig. 2C).

Biomass allocations

Although results of ANOVA revealed a significant effect of mycorrhiza on SMR (Table 1), no significant differences among mycorrhizal treatments were detected in the post hoc analysis (Fig. 3). LMR and RMR showed a stable response pattern to AMF over time (Fig. 3). LMR was 11% higher for LM seedlings than for controls at 16 days (for LM: 0.63 ± 0.01 g g^{-1} against 0.57 ± 0.01 g g^{-1} for −M). Thirty days after transplantation, both AMF treatments had higher LMR (17% increase for LM: 0.56 ± 0.01 g g^{-1}; and 12% increase for HM: 0.54 ± 0.01 g g^{-1}) than for the controls (0.48 ± 0.01 g g^{-1}). However, RMR was 25% lower for LM seedlings than for controls after 16 days (0.21 ± 0.01 g g^{-1} for LM against 0.28 ± 0.01 g g^{-1} for −M), and both AMF treatments had lower RMR after 30 days after transplantation (19% decrease for LM: 0.29 ± 0.01 g g^{-1}; and 14% decrease for HM: 0.31 ± 0.01 g g^{-1}; vs −M: 0.36 ± 0.01 g g^{-1}).

Effects of AMF on Bemisia tabaci

Mycorrhiza significantly affected *B. tabaci* adult and egg densities (Table 2); seedlings treated with LM (0.11 ± 0.02 adults cm^{-2}) had more adults (120%) than on controls (0.05 ± 0.01 adults cm^{-2}; Fig. 4A) and significantly more eggs (97% increase; LM: 12.12 ± 2.51 eggs cm^{-2}; vs −M: 6.15 ± 1.16 eggs cm^{-2}; Fig. 4B). The density of eggs and adults on HM seedlings did not differ significantly from controls. Although we observed a similar tendency for the density of nymphs (−M: 4.13 ± 1.21 nymphs cm^{-2}; LM: 5.75 ± 1.84 nymphs cm^{-2}; HM: 3.73 ± 1.10 nymphs cm^{-2}; Fig. 4C), the selected model did not show any significant effect of mycorrhiza on nymph abundance (Table 2).

Table 1 Effect of time (days after transplantation) and mycorrhiza (*Rhizophagus irregularis*) inoculum concentrations on traits of *Capsicum chinense*

Dependent variable	Leaf toughness (kg cm^{-2})		Leaf thickness^{10} (mm)		Total polyphenols (% of leaf dry mass)		$RGR_{Diameter}$^{10} (mm mm^{-1} day^{-1})		$RGR_{Leaf\ area}$ (cm^2 cm^{-2} day^{-1})		RGR_{Height} (cm cm^{-1} day^{-1})	
Random effects	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
Cage	–	–	–	–	–	–	–	–	–	–	$\chi^2_{1,48}=8.10$	0.004
Days after emergence	–	–	–	–	–	–	–	–	$\chi^2_{1,49}=2.75$	0.097	$\chi^2_{1,48}=11.02$	<0.001
Fixed effects	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*
Time	$F_{2,39}=8.00$	<0.001	$F_{2,40}=13.79$	<0.001	$F_{2,33}=35.06$	<0.001	$F_{2,40}=194.75$	<0.001	$F_{2,38.86}=141.69$	<0.001	$F_{2,14.45}=106.04$	<0.001
Mycorrhiza	$F_{2,39}=3.58$	0.037	–	–	$F_{2,33}=0.32$	0.725	–	–	–	–	–	–
Time × Mycorrhiza	$F_{4,39}=3.84$	0.010	$F_{6,40}=5.55$	<0.001	–	–	$F_{6,40}=15.61$	<0.001	$F_{6,39.01}=3.98$	0.003	$F_{6,10.87}=6.68$	0.004
Adj R^2	0.64		0.68		0.64		0.97		0.98*		0.99*	
Dependent variable	NAR (g m^{-2} day^{-1})		LAR^{10} (cm^2 g^{-1})		SLA^{10} (cm^2 g^{-1})		LMR (g g^{-1})		SMR (g g^{-1})		RMR (g g^{-1})	
Random effects	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*	LRT	*P*
Cage	$\chi^2_{1,49}=3.22$	0.073	–	–	–	–	–	–	$\chi^2_{1,49}=5.53$	0.019	–	–
Days after emergence	–	–	–	–	–	–	$\chi^2_{1,49}=5.65$	0.017	–	–	–	–
Fixed effects	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*	*F*-test	*P*
Time	$F_{2,5.50}=21.49$	0.003	$F_{2,40}=36.50$	<0.001	$F_{2,44}=309.38$	<0.001	$F_{2,13.66}=78.66$	<0.001	$F_{2,8.30}=106.89$	<0.001	$F_{2,40}=42.43$	<0.001
Mycorrhiza	$F_{2,40.50}=7.47$	0.002	$F_{2,40}=15.26$	<0.001	$F_{2,44}=8.55$	<0.001	$F_{2,36.95}=40.61$	<0.001	$F_{2,37.33}=4.20$	0.023	–	–
Time × Mycorrhiza	–	–	$F_{4,40}=5.74$	<0.001	–	–	$F_{4,36.57}=15.92$	<0.001	–	–	$F_{6,40}=13.61$	<0.001
Adj R^2	0.84*		0.92		0.93		0.95*		0.97*		0.80	

Results are from GLMMs with restricted maximum likelihood estimation after submodel routine.

We used ANOVA for unbalanced data ($n=49$) to assess significance of fixed effects, and significance of random effects was assessed with likelihood-ratio χ^2 tests (LRT). $^{10}\log_{10}$-transformed during model selection routine

$RGR_{Diameter}$ diameter relative growth rate, $RGR_{Leaf\ area}$ leaf area relative growth rate, RGR_{Height} height relative growth rate, *NAR* net assimilation rate, *LAR* leaf area ratio, *SLA* specific leaf area, *LMR* leaf mass ratio, *SMR* stem mass ratio, *RMR* root mass ratio

*We calculated adjusted R^2 for GLMs and Nakagawa and Schielzeth's conditional pseudo R^2 (*) for GLMMs

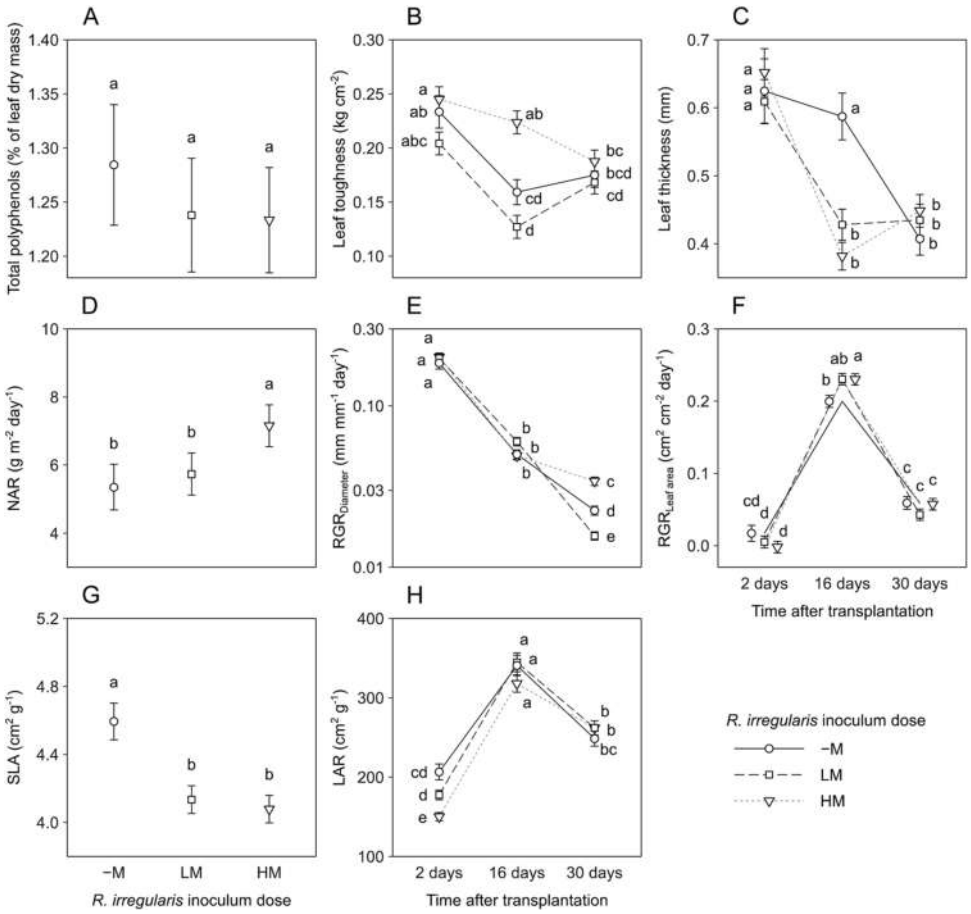

Fig. 1 Effects of time (days after seedlings transplantation) and mycorrhiza (levels of *Rhizophagus irregularis* inoculum concentrations: −M = control without mycorrhiza; *LM* low dose, (2.6 spores mL^{-1}; *HM* high dose, 8 spores mL^{-1}) on variables for *Capsicum chinense* ($n = 49$). Values are marginal estimated means for LMMs (calculated over levels of time × mycorrhiza) ± SE. Intervals were back-transformed from the $\log_{10}$ scale (see Table 1). Different letters indicate a significant difference in Tukey's pairwise comparisons at $\alpha = 0.05$. *NAR* net assimilation rate, $RGR_{Diameter}$ diameter relative growth rate, $RGR_{Leaf\ area}$ leaf area relative growth rate, *SLA* specific leaf area, *LAR* leaf area ratio

Interactions between C. chinense and B. tabaci

In Table 3, we only report the significant relationship between *C. chinense* traits and *B. tabaci* abundance. Both the adult and egg densities were positively related to LAR (Fig. 5A and E), and NAR augmentation decreased egg abundance (Fig. 5F). We found more whiteflies on seedlings that had a high leaf mass ratio. In fact, LMR augmentation significantly increased the abundance of adults (Fig. 5B), and adult density decreased with RMR diminution (Fig. 5C). We observed a negative relationship between nymph density and SMR (Fig. 5H). Increases in basal diameter and plant height relative growth also had a negative impact on *B. tabaci*. Egg abundance decreased significantly as $RGR_{Diameter}$ increased (Fig. 5D), and nymph density significantly decreased depending on RGR_{Height} augmentation (Fig. 5G).

Discussion

Habanero pepper response to AMF and subsequent whitefly fitness depends on AMF density

Responses of *C. chinense* to mycorrhizal inoculation vary through AMF density. Leaf thickness, $RGR_{Leaf\ area}$, NAR, SLA and LAR responded similarly to both mycorrhizal treatments, with a stronger effect at the higher AMF inoculum concentration. Effects of AMF on leaf toughness, $RGR_{Diameter}$, LMR and RMR varied, following a nonlinear curve with increasing inoculum density. All these relationships between plant traits and AMF density might be nonlinear (e.g., quadratic or exponential) as predicted by the resource exchange model of plant defense (REMPD) of Vannette and Hunter (2011). They suggested that the ratio between plant nutrient benefits and carbon costs in a mycorrhizal association is nonlinearly related to mutualist density. In fact, the REMPD revealed an optimal AMF density where net plant benefit is maximal. Beyond that optimum, as AMF density increases, the benefit to cost ratio might decrease, thus leading eventually to fungal parasitism (see also Gange and Ayres 1999). Herbivorous insect performance will be related to these nonlinear variations in plant

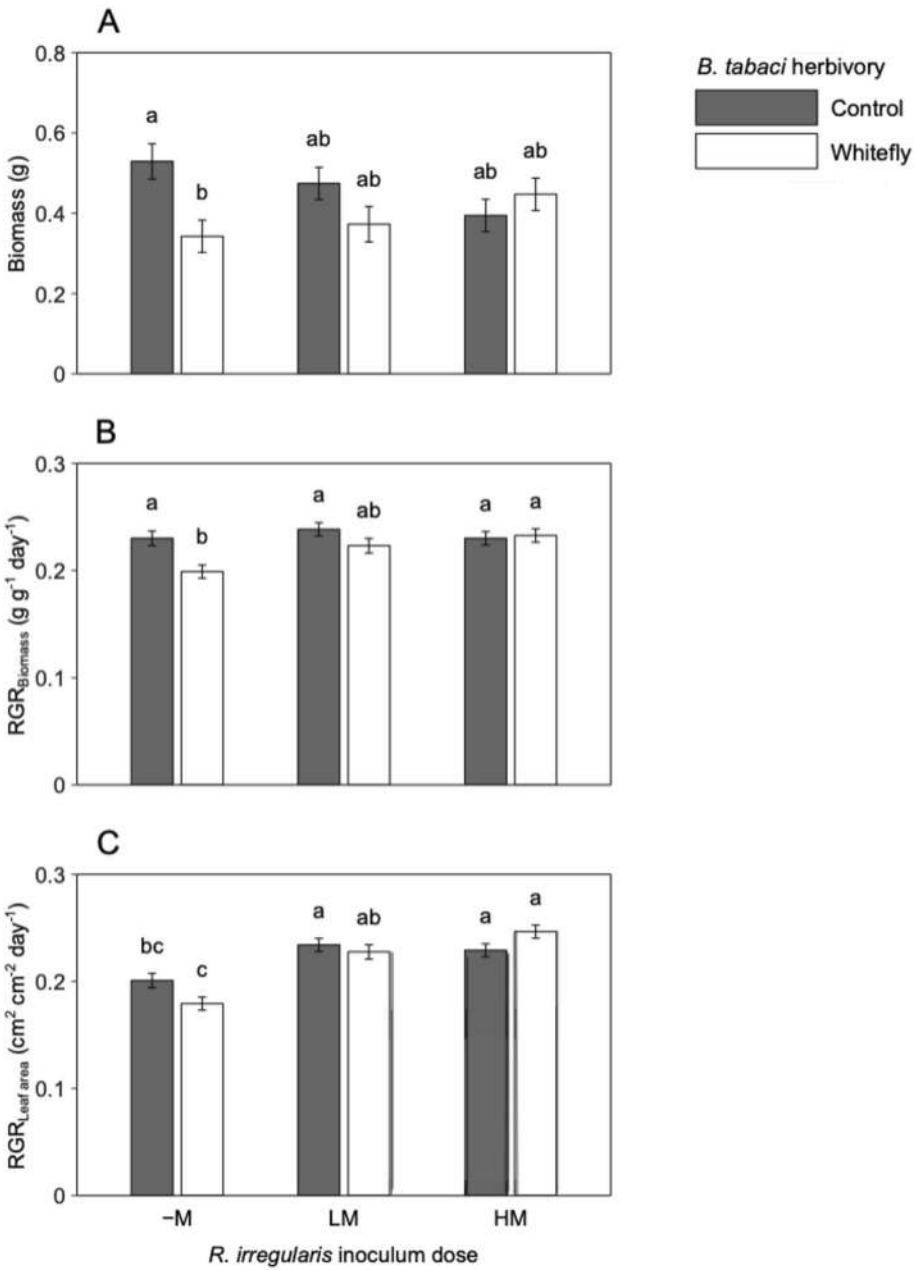

Fig. 2 Effects of mycorrhiza (levels of *Rhizophagus irregularis* inoculum concentrations: −M = control without mycorrhiza, *LM* low dose, 2.6 spores mL^{-1}; *HM* high dose, 8 spores mL^{-1}) on mean biomass allocations in *Capsicum chinense* ($n = 49$). Different letters in the same organ indicate a significant difference in Tukey's pairwise comparisons at $\alpha = 0.05$. *LMR* leaf mass ratio, *SMR* stem mass ratio, *RMR* root mass ratio

growth, defense expression and nutritional quality driven by AMF density. We believe that the response of *B. tabaci* to *R. irregularis* colonization depends, therefore, on the initial inoculum dose. We found more adults and eggs on leaves of seedlings treated with the low mycorrhizal dose, which in this case yielded a clear benefit for *B. tabaci*. This result agrees with that of Wahba (2015) who found a positive effect of mycorrhizal fungi (between 45 and 67.5 g of solid mycorrhizal inoculum were used per plant) on *B. tabaci* (eggs, nymphs and pupae densities) and on other sucking pests, all reared on *Cucumis sativus* (Solanaceae). On the other hand, we did not detect any difference in *B. tabaci* abundance between seedlings treated with the HM dose and the non-inoculated seedlings. Our results revealed an oviposition preference of *B. tabaci* for leaves of seedlings treated with the low AMF dose in contrast to the high dose. Likewise, Wooley and Paine (2011) found no significant effect of AMF (*Glomus* sp.) on silver whitefly populations (*Bemisia* sp.) reared on tobacco (*Nicotiana rustica*; Solanaceae). They assumed that the mixed responses of phloem feeders such as whiteflies to AMF were due to their broad host range (Oliveira et al. 2001). This variability could also be the consequence of a strong AMF species-specificity (e.g., Bennett and Bever 2007) or as in our case, to variations in mutualist density.

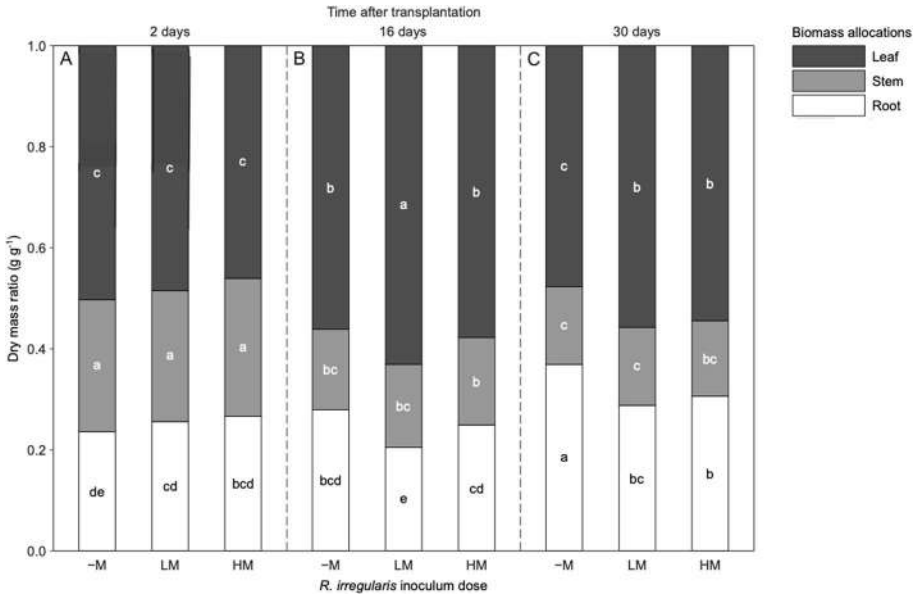

Fig. 3 Effects of *Rhizophagus irregularis* and *Bemisia tabaci* herbivory treatments on *Capsicum chinense* biomass and growth rates (values are means ± SE) at 16 days after transplantation ($n = 34$). Results of mycorrhiza × herbivory interaction term after ANOVAs: **A** $F_{2, 28} = 4.37$, $P = 0.022$; **B** $F_{4, 28} = 4.14$, $P = 0.009$; **C** $F_{2, 28} = 5.02$, $P = 0.014$. Values are means ± SE. Different letters indicate a significant difference in Tukey's pairwise comparisons at $\alpha = 0.05$. −M = control without mycorrhiza, *LM* low AMF dose (2.6 spores mL^{-1}), *HM* high AMF dose (8 spores mL^{-1}), $RGR_{Biomass}$ biomass relative growth rate, $RGR_{Leaf\ area}$ leaf area relative growth rate

Table 2 Gamma GLMs and GLMMs of the effect of mycorrhiza (*Rhizophagus irregularis* density) on *Bemisia tabaci* abundances ($n = 34$) with subject as random effect and log x as link function

Model parameter	No. adults/cm^2		No. eggs/cm^2		No. nymphs/cm^2	
	LRT	P	LRT	P	LRT	P
Subject (random)	–	–	–	–	$\chi^2_{1, 34} = 4.14$	0.042
Mycorrhiza	$\chi^2_{2, 34} = 14.02$	< 0.001	$\chi^2_{2, 34} = 9.49$	0.009	$\chi^2_{2, 34} = 1.06$	0.588
Pseudo R^2	0.31		0.20		0.49	
AICc	− 128.90		200.45		178.46	

Significance of fixed and random effects was assessed with likelihood-ratio χ^2 tests (LRT). Pseudo R^2 was calculated as the multiple R^2 of linear regression between fitted and observed values of each model. We used corrected Akaike's information criterion for small samples (AICc) in our model selection routine

Effects of AMF on aboveground plant growth increased B. tabaci abundance

AMF did not significantly increase whole dry biomass or biomass relative growth rate of seedlings, similar to the results of a nursery experiment on older *C. chinense* plants (plant fresh mass at 30 days after inoculation: 7.6 g for controls vs 10.6 g after AMF liquid inoculation, but values were not significantly distinct) (Constantino et al. 2008). Note that they used much higher concentrations of liquid inoculum (up to 5×10^6 spores mL^{-1}) than we used here (2.6 spores mL^{-1} for LM and 8 spores mL^{-1} for HM seedlings). We observed variable effects on aboveground organ RGRs, depending on AMF density. Seedlings inoculated with the HM dose always had higher $RGR_{Diameter}$ and $RGR_{Leaf\ area}$ compared with non-inoculated plants, whereas LM had a lower $RGR_{Diameter}$, probably due to their earlier investment in leaf biomass, relative to roots and stems. These higher RGRs of HM seedlings support the idea that higher mycorrhizal colonization induces aboveground growth rather than belowground growth (Veresoglou et al. 2012). The negative relationship between egg abundance and $RGR_{Diameter}$ (Fig. 5D) contributed to the higher egg abundance measured on LM seedlings (Fig. 4B), as we reported a lower $RGR_{Diameter}$ on LM seedlings (Fig. 1E). The negative relationship between RGR_{Height} and nymph density (Fig. 5G) does not explain the marginally higher nymph abundance on LM seedlings (Fig. 4C) because the RGR_{Height} did not differ between inoculated and non-inoculated seedlings.

Higher compensatory growth increased plant tolerance to herbivory

Tolerance is considered as the result of a greater capability of a plant to deal with its enemy in optimal growing conditions (Hill 2008). In the context of *B. tabaci* herbivory, mycorrhizal seedlings compensated for biomass losses with stimulated growth (Fig. 3). This effect appears to be stronger for HM seedlings, suggesting that AMF significantly increased plant tolerance to the pest (McNaughton 1983). AMF-induced compensatory growth constitutes a key mechanism in the response of *C. chinense* to herbivory, as for numerous

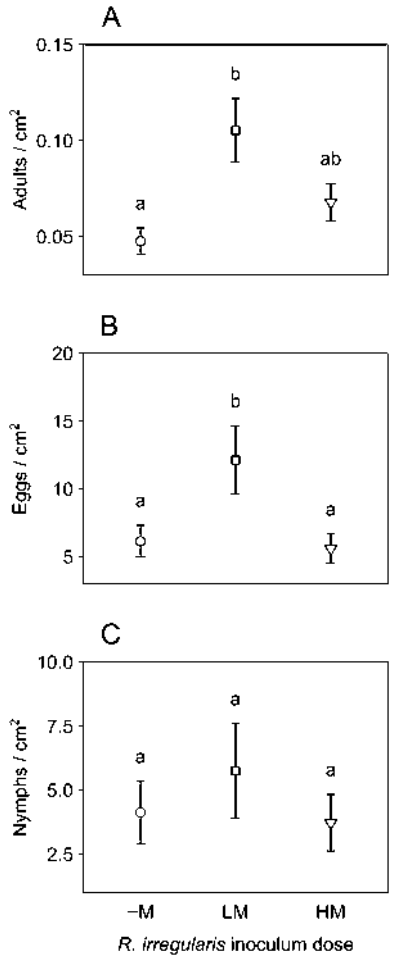

Fig. 4 *Bemisia tabaci* adults (**A**), eggs (**B**) and nymphs (**C**) abundance 28 days after transplantation ($n=34$), after concentrations: −M = control without mycorrhiza, *LM* low dose (2.6 spores mL^{-1}), *HM* high dose (8 spores mL^{-1}). All results were expressed in number of individuals per cm^2. Values are marginal estimated means for GLMs and GLMMs ± SE. Values were back-transformed from the log scale. Different letters indicate a significant difference in Tukey's pairwise comparisons at $\alpha=0.05$

other plant species (e.g., Kula et al. 2005; Hoffmann et al. 2011). Stimulation of compensatory growth in *C. chinense* seedlings by AMF thereby reflects an improvement of plant growing conditions and plant vigor. An improvement in photosynthetic activity and better regulation of reactive oxygen species generation by plants in response to environmental stress are part of the physiological mechanisms contributing to plant tolerance to hemipteran herbivores such as *B. tabaci* (Koch et al. 2016).

Allocations in leaf biomass enhanced whitefly abundance

Mycorrhizal inoculation at both densities enhanced leaf biomass and reduced root biomass, but this allocation pattern appeared earlier for LM seedlings than for HM seedlings (Fig. 2). An increase in LMR associated with a reduction in RMR generally reflects investments in primary metabolism with a probable nutrient gain. In a functional perspective, this pattern agrees with the meta-analysis of Poorter et al. (2012) in that higher nutrient availability (in our case induced by AMF colonization) increases LMR, slightly increases SMR, but lowers RMR. The early increase in LMR for the LM seedlings may therefore reflect a higher net nutrient gain, when HM seedlings had to deal first with the higher carbohydrate demands from the more abundant AMF (Gange and Ayres 1999), thus explaining the lag time for higher investment in foliar biomass. Not surprisingly, insect adult abundance was enhanced by augmentation of foliar biomass ratio (Fig. 5B) and concurrently reduced by diminution of root mass ratio (Fig. 5C). An increase in LMR doubtless induces an advantage for sucking herbivores such as *B. tabaci* that depend on foliar quantity and quality (Bennett et al. 2005). The clear discrimination in LMR and RMR between our two treatments at the second harvest could

Table 3 Gamma GLMs of the effect of *Capsicum chinense* variables on *Bemisia tabaci* abundance ($n=17$)

Dependent variable	Model parameter	Pseudo R^2	AICc	Link function	Intercept	*B*	LRT	*P*
No. adults/cm^2	LAR	0.21	−66.33	Log *x*	−6.19	0.01	$\chi^2_{1,17}=5.24$	0.022
	LMR	0.34	−69.93	1/*x*	88.81	−139.56	$\chi^2_{1,17}=9.17$	0.002
	RMR	0.28	−69.32	1/*x*	−27.41	133.02	$\chi^2_{1,17}=8.69$	0.003
No. eggs/cm^2	$RGR_{Diameter}$	0.47	96.14	1/*x*	−0.02	6.76	$\chi^2_{1,17}=8.71$	0.003
	NAR	0.23	98.19	1/*x*	−0.05	0.06	$\chi^2_{1,17}=5.52$	0.019
	LAR	0.14	100.19	Log *x*	−2.37	0.02	$\chi^2_{1,17}=3.92$	0.048
No. nymphs/cm^2	RGR_{Height}	0.22	81.24	Log *x*	2.50	−36.41	$\chi^2_{1,17}=7.76$	0.005
	SMR	0.20	79.53	Log *x*	6.54	−33.32	$\chi^2_{1,17}=4.00$	0.045

Significance of variables was assessed with likelihood-ratio χ^2 tests (LRT). Pseudo R^2 was calculated as the multiple R^2 of linear regression between fitted and observed values of the models. Corrected Akaike's information criterion for small samples (AICc) was used in our model selection routine

$RGR_{Diameter}$ diameter relative growth rate (mm mm^{-1} day^{-1}), RGR_{Height} height relative growth rate (cm cm^{-1} day^{-1}), *NAR* net assimilation rate (g m^{-2} day^{-1}), *LAR* leaf area ratio (cm^2 g^{-1}), *LMR* leaf mass ratio (g g^{-1}), *SMR* stem mass ratio

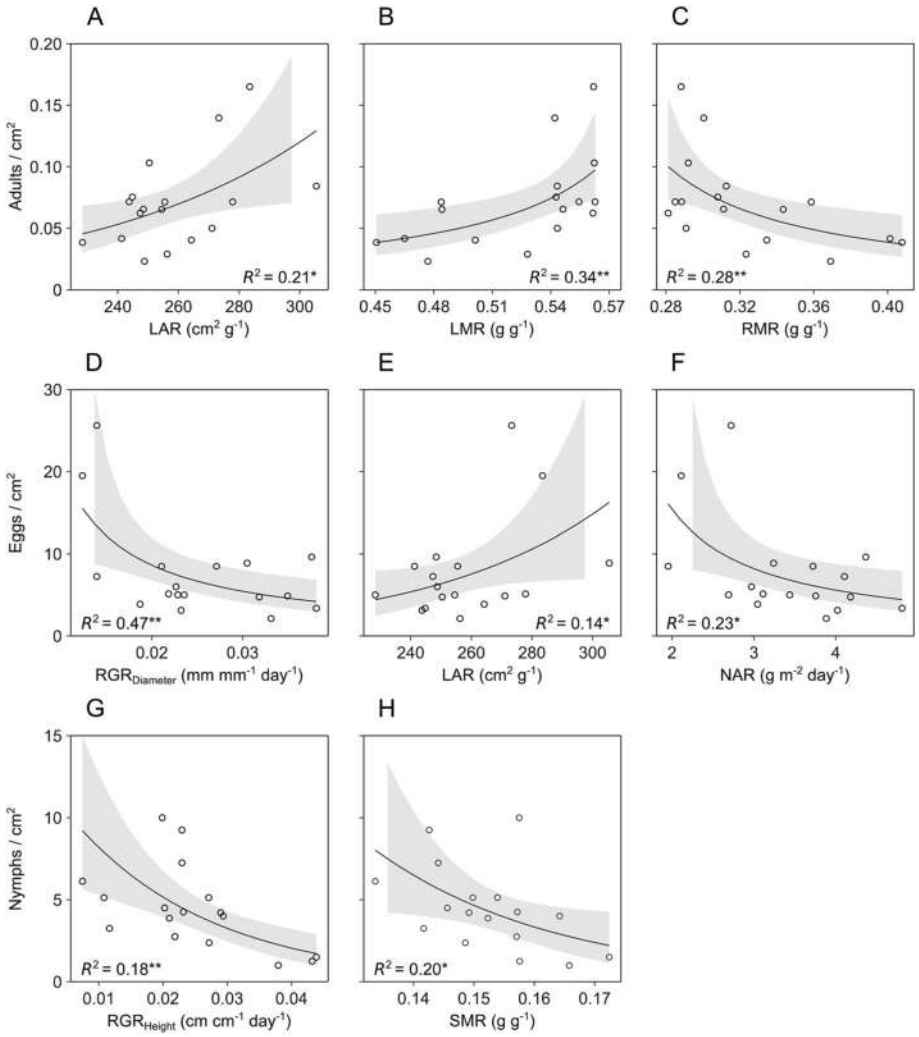

Fig. 5 Fitted regression curves with 95% confidence intervals (in gray) of gamma GLMs (Table 3) of the effect of *Capsicum chinense* variables (30 days after transplantation) on *Bemisia tabaci* abundance ($n = 17$). R^2 symbolizes a pseudo R^2 calculated as the multiple R^2 of the linear regression between fitted and observed values of each model. $^{**}P \leq 0.01$, $^{*}P \leq 0.05$, *LAR* leaf area ratio, *LMR* leaf mass ratio, *RMR* root mass ratio, $RGR_{Diameter}$ diameter relative growth rate, *NAR* net assimilation rate, RGR_{Height} height relative growth rate, *SMR* stem mass ratio

therefore be a major contributor to the higher eggs density found on LM seedlings than on HM seedlings.

Effects on morphological and physiological traits

The increase in NAR for HM seedlings (Fig. 1D) stimulated by greater mycorrhizal colonization might reflect an increase in photosynthetic carbon gain and/or reduced carbon loss through respiration, exudation and volatilization (Poorter and Remkes 1990). Associated with a negative relationship between NAR and eggs abundance (Fig. 5F), the higher NAR also helps explain why more eggs were laid on LM seedlings than on HM seedlings (Fig. 4B). The early diminution of LAR measured for both mycorrhizal treatments may be the result of a diminution of total leaf area or of an increase of total plant mass (Eq. 3). Because we did not measure significant differences between treatments for those two variables, LAR decrease could be explained by the diminution of SLA in both mycorrhizal treatments because LMR was still similar among treatments at the first harvest. Subsequent LMR increases, first in the LM seedlings and then in the HM, probably led to a reduction in the difference in the LAR values between treatments measured at 16 and 30 days after transplantation. LAR diminution appears to negatively affect adult and egg abundance (Fig. 5A, E). However, LAR was lower for both LM and HM seedlings 2 days after transplantation (Fig. 1H), which only slightly affected whitefly abundance because plants were only infested 7 days after transplantation. The lower SLA for both AMF treatments may be due to cellular differentiation induced by mycorrhizal colonization (Lambers and Poorter 1992). In fact, the decrease in SLA found for sun-adapted species such as *C. chinense* (see Jaimez and Rada 2006) can be due to the differentiation of extra layers of palisade parenchyma (Lambers and Poorter 1992). Combined with the higher photosynthetic activity, better regulation of ROS involved in plant tolerance against hemipteran herbivores and increase in NAR, this result corroborates the idea that mycorrhiza promotes photosynthesis in the HM seedlings (Kaschuk et al. 2009; Koch et al. 2016). We hypothesize that

LM seedlings compensated for lower carbon costs through LMR augmentation rather than through foliar tissue differentiation, whereas HM seedlings responded to higher carbon costs with improved photosynthesis at a cellular level.

Effects on plant defense

AMF increased leaf toughness after a triggering threshold that ranged between our two inoculum concentrations (between 2.6 and 8 spores mL^{-1}). Considered as a mechanical defense trait, harder leaves reduce palatability and digestibility of vegetal material (Hanley et al. 2007), thus theoretically limiting herbivore fitness. Similar to other host resistance factors (e.g., hairiness, glandular trichomes presence), harder leaves can limit stylet penetration of *B. tabaci* in *C. chinense* leaf veins (Janssen et al. 1989). Labial chemoreceptors on this obligatory sessile phloem-feeding insect may explain a discriminative sense shortly after contact of the labium with the leaf surface. However, our models did not reveal any effect of plant defensive traits on *B. tabaci* abundance. Moreover, Peeters et al. (2007) and Caldwell et al. (2015) showed that leaf toughness and other leaf mechanical traits were not negatively correlated with sucking-insect density. The tougher cuticle revealed on HM seedlings thus probably does not explain the lower whitefly densities on these plants compared to the LM seedlings (see also Walling 2008). Gange and West (1994) suggested that sucking insects benefit from changes in leaf morphology induced by AMF, specifically from changes in phloem location and size rather than mechanical leaf traits. The thinning between the first and third harvest on all seedlings was accelerated by both levels of AMF inoculations, but this trait did not differ between mycorrhizal treatments, so leaf thickness is probably not related to sucking herbivore fitness (Peeters et al. 2007; Caldwell et al. 2015). No effect of AMF on foliar phenolic concentration was detected, but perhaps a longer experiment would reveal an influence of AMF colonization on alkaloid content in *C. chinense*. Among defensive chemical compounds in *C. chinense*, capsaicin can be exceptionally concentrated in fruits (Sanatombi and Sharma 2008) and induce direct mortality and act as a strong antifeedant and oviposition deterrent against *B. tabaci* (Zhao et al. 2012).

Conclusions

The responses of *C. chinense* seedlings to *R. irregularis* varied depending on the extent of mycorrhizal colonization, thus implying colonization was associated with changes in plant physiology and primary metabolism. A precocious increase in the nutritive value of plants treated with the LM dose benefited *B. tabaci*, whereas a higher mycorrhiza-induced tolerance and an improved photosynthetic activity in HM plants resulted in a null effect on the phloem-sucking pest. Mycorrhizal inoculation induces more than a single positive or negative feedback on herbivore insects, especially when mycorrhizal density is taken into account during AMF–plant–insect interactions.

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Declarations

Conflict of interest No potential conflict of interest was reported by authors.

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