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Identity and combinations of arbuscular mycorrhizal fungal isolates influence plant resistance and insect preference

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Abstract. 1. Accumulating evidence indicates that plant resistance against above-ground herbivores can be affected by the presence of arbuscular mycorrhizal fungi (AMF) in association with the host plant. Little is known, however, about how AMF composition can influence herbivore choice to feed on a particular plant.

2. Unravelling the preference–performance hypothesis in a multitrophic context is needed to expand our knowledge of complex multitrophic interactions in natural systems. If given mycorrhizal fungal genotypes increase attractiveness for a herbivore (reduced plant resistance), then the benefits of increased unpalatability provided by the mycorrhizal fungi (increased plant resistance) might be outweighed by the increased herbivore recruitment.

3. This was addressed by designing three experiments to test the effects of different AMF genotypes, inoculated either alone or in combination, to measure intraspecific AMF effects on plant resistance and insect herbivore preference. Using strawberry (*Fragaria vesca* L.) plants that were colonised by eight different combinations of *Rhizophagus irregularis* isolates, we measured effects on plant growth, insect growth and survival, as well as feeding preferences of a generalist herbivore caterpillar (*Spodoptera littoralis* Boisduval).

4. Overall, it was found that: (i) AMF influenced plant resistance in an AMF genotype-specific manner; (ii) some AMF inoculations decreased insect performance; (iii) insects preferentially chose to feed more on leaves originating from non-mycorrhizal plants; but also that (iv) in a whole plant bioassay, insects preferentially chose the biggest plant, regardless of their mycorrhizal status.

5. Therefore, AMF-mediated trade-offs between growth and resistance against herbivores have been shown. Such trade-offs, particularly driven by plant attractiveness to herbivores, buffer the positive effects of the mycorrhizal symbiosis on enhanced plant growth.

Key words. Above-ground–below-ground interactions, *Glomus intraradices*, *Glomus irregularis*, intraspecific genetic variation, plant vigour hypothesis, plant–herbivore interaction, *Rhizophagus irregularis*, *Spodoptera littoralis*, strawberry (*Fragaria vesca*).

Introduction

Arbuscular mycorrhizal fungi (AMF) can form symbioses with more than 80% of vascular plants (Smith & Read, 2008). Plants receive nutrients such as phosphate and nitrogen, and in exchange they provide the fungal partner with carbohydrates (Smith & Read, 2008). Interactions between plants and AMF play a considerable role in ecosystem functioning, as arbuscular mycorrhizal fungi can alter plant growth, community structure, and nutrient cycling (Grime *et al*., 1987; van der Heijden *et al*., 1998a; Smith & Read, 2008). They also affect plants in different ways, ranging from being highly mutualistic to being parasitic (Johnson *et al*., 1997). Species identity of both AMF and plants explain a large part of the

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variation in nutrient uptake (Hart & Reader, 2002; Klironomos, 2003). However, large variation in plant growth responses can also be explained by intraspecific genetic variation in the fungus (Munkvold *et al*., 2004; Koch *et al*., 2006). It has been shown that plants can benefit by being colonised by several genetically different AMF lines or species simultaneously (Gustafson & Casper, 2006; Jansa *et al*., 2008).

Arbuscular mycorrhizal fungal colonisation has also been shown to influence plant protection against insect herbivores (Vannette & Hunter, 2011), and therefore to influence insect population fitness (Wooley & Paine, 2007). Such effects on insect fitness vary depending on the species identity of the fungus, the plant or the herbivores (Gange & West, 1994; Gange *et al*., 2002, 2003; Bennett *et al*., 2006; Gange, 2007; Borowicz, 2009; Gehring & Bennett, 2009; Koricheva *et al*., 2009; Vannette & Hunter, 2011). Despite large observed variations in the effects of AMF on the outcome of plant–herbivore interactions, Hartley and Gange (2009) suggested a pattern in which generalist insect herbivores are negatively affected by the presence of AMF, most of the time. The reason for this was thought to be improved nutrition, allowing the plant to develop stronger defences against herbivores. In particular, this was suggested to be the case for generalist leaf-chewing insects, whereas specialist herbivores often benefit from the presence of AMF. Indeed, AMF can indirectly influence insect population growth rates or survival by increasing plant quality through a better uptake of nutrients, but they can also cause increases in foliar concentrations of carbon-based plant defence compounds (Gange & West, 1994). These changes in plant defence chemistry triggered by AMF have been shown to be species-specific (Bennett *et al*., 2009; Nishida *et al*., 2010). However, Wooley and Paine (2007) have shown that even intraspecific genetic variation in the fungus has the potential to impact the structure and growth rate of insect populations.

One classic hypothesis in plant–insect interactions is that the allocation of resources by plants to chemical and structural defences decreases growth by diverting resources from the production of leaf area and other vegetative structures (Herms & Mattson, 1992). However, it has been suggested that the additional resources provided by AMF should relax the trade-off between growth and defence of the plant (Bennett *et al*., 2006). In light of this, we can predict that in natural ecosystems, mycorrhizal plants should be bigger and more attractive to some herbivores, as well as being more unpalatable (Price, 1991; Kula *et al*., 2005; Bennett & Bever, 2007). Thus, it appears that opposing effects of AMF on plant resistance may occur. Mycorrhizal fungi can increase the growth of plants, which should make them more attractive to herbivores. However, at the same time, more vigorous plants should be better able to defend themselves by increasing the production of toxic and anti-nutritive secondary metabolites, replacing herbivore-damaged tissues as well as being more prone to attract enemies of the herbivore (Guerrieri *et al*., 2004).

Here we aimed at addressing the paradox of plant vigour, attractiveness and resistance by investigating the effect of different AMF genotype colonisation, and combinations thereof, on plant growth, insect performance, and insect preferences. We designed a set of experiments and specifically asked: (i) whether different genotypes of the AMF *Rhizophagus irregularis* differentially affect the growth and the resistance of strawberry plants (*Fragaria vesca* L.); (ii) whether the growth and survival rate of caterpillars of the generalist leaf-chewing insect (*Spodoptera littoralis* Boisduval) are influenced by mycorrhizal treatments; and (iii) whether insects exhibit a preference for plants depending on their mycorrhizal status.

Materials and methods

Study system

Plant material. Seeds of strawberry (*F. vesca*) were obtained from Wyss Samen und Pflanzen AG, Zuchwil-Solothurn, Switzerland. Before planting, seeds were surface-sterilised with 2% bleach, after which they were left to germinate for 3 weeks in Petri dishes filled with sterilised filter paper and 20 ml of tap water. Seedlings were then transplanted into vermiculite for an additional 2 weeks, before being transplanted into plastic pots (diameter 12 cm, height 7 cm) filled with autoclaved mixed loam with a low P concentration (horticol number 1, Orflor, Inc., Geneva, Switzerland) and sand (ratio 1 : 1, v/v). At the same time as transplanting, *R. irregularis* isolates were directly added to the roots (see the section on experimental design). All three experiments (described later) were conducted in a greenhouse under controlled conditions (temperature 22–25 °C, RH 40–60% and 14 h daylight).

Arbuscular mycorrhizal fungi. Four isolates of *R. irregularis* were used to inoculate strawberry plants either as single isolates or in pairs. These isolates were previously assigned to the species *Glomus intraradices* or *Glomus irregulare* (Stockinger *et al*., 2009). Strawberry growth has already been shown to be dependent on *R. irregularis* colonisation (Hernandez-Sebastia *et al*., 2000), and this plant species has already been studied in tripartite plant–fungal–insect systems (Gange, 2001). All fungal isolates originated from the same field located at Hausweid, Tänikon, Switzerland (Koch *et al*., 2004). These different fungal isolates of *R. irregularis* differentially affect plant growth (Koch *et al*., 2006), and intraspecific functional diversity has been shown in other AMF species (Munkvold *et al*., 2004). Moreover, when several *R. irregularis* isolates coexist within a single plant, the relatedness of the coexisting isolates can influence their interaction and, therefore, the growth of the host plant (Roger, 2012). The relatedness of these isolates was already known (Croll *et al*., 2008), which allowed us to select pairs of mycorrhizal isolates representing a gradient of genetic relatedness. *R. irregularis* isolates B2, B12, C2 and C5 (Koch *et al*., 2004) were cultivated *in vitro* on plates containing non-mycorrhizal (NM) Ri T-DNA-transformed carrot roots (Becard & Piche, 1992; StArnaud *et al*., 1996). Each isolate was then cultivated on split plates with two compartments (StArnaud *et al*., 1996). The first compartment was filled with M medium to allow carrot root

development (Becard & Piche, 1992). The second compartment was sucrose-free, in order to promote fungal growth. After 15 weeks of growth, spores were extracted from the medium by dissolving it (Angelard *et al*., 2010). Spores were then stored in tap water, and spore concentration was measured by counting three times independently under a dissecting microscope. Spore concentration was then standardised among the AMF treatments. To inoculate freshly transplanted strawberry plants, 300 spores in 500 µl tap water per plant were applied directly to the roots of the seedlings. For double-inoculation treatments (see later), 150 spores of each isolate were mixed and applied to the roots. Control (NM) plants were inoculated with 500 µl of tap water.

Insects. Egyptian cotton leafworms *S. littoralis* (Lepidoptera: Noctuidae) were obtained from Syngenta Inc (Stein, Switzerland). Eggs were kept at 4 °C for a maximum of 3 days before allowing the larvae to hatch at 20 °C. This polyphagous insect species is known to feed on plants of at least 40 different families, including the Rosaceae (Brown & Dewhurst, 1975). Moreover, chewing herbivores such as *S. littoralis* have been exploited in a variety of studies testing plant resistance (Hartley & Gange, 2009; Kempel *et al*., 2010).

Experimental design

To test the effect of AMF on plant traits and herbivore preference and performance, plants were inoculated with eight different mycorrhizal treatments: one control treatment with no fungus (NM), four single AMF isolate inoculations (B2, B12, C2 and C5) and three double AMF isolate inoculations: C2 + C5 (pair of closely related isolates), B2 + B12 (moderate genetic distance between the pair of isolates) and B2 + C2 (very distant isolates) [see Croll *et al*. (2008) for more details about genetic relatedness among isolates]. Each plant was randomly placed in the greenhouse and their position followed a weekly rotation. Throughout the experiment, plants were watered three times a week with 50 ml of water; no fertiliser was added. Each treatment was replicated 24 times, giving a total of 192 experimental units. Half of these plants ($N = 96$, 12 replicates per treatment) were used to investigated the effect of AMF on plant performance in the absence of herbivores, whereas the second half were used to measure plant resistance against herbivores.

Effect of AMF isolates or combinations of isolates on plant performance. Eight weeks after fungal inoculation, we estimated the effect of mycorrhizal treatments on plant performance. We measured plant height, leaf thickness, and chlorophyll level (Pons & Anten, 2004). In a preliminary experiment, we found that the dry mass of strawberry plants was strongly correlated with plant height (Spearman correlation test, $n = 96$, $r = 0.937$, $P < 0.001$). Thus, we used plant height as an estimator of biomass production. Leaf thickness was measured on randomly chosen, fully expanded leaves with a precision calibre, and chlorophyll levels were measured using a Konica Minolta SPAD-50 chlorophyllometer. Leaf thickness and chlorophyll levels were scored as the average value of three different measures per leaf and taken on three different leaves for each plant.

We did not measure the colonisation of the roots by AMF in the different AMF treatments. While this means that we could not be completely sure that all treatments were colonised by AMF, the inability of the isolates to colonise the roots is unlikely. Many experiments have been conducted by our group using these AMF isolates and they only rarely fail to colonise the plant roots, in a very small number of plants (e.g. Koch *et al*., 2006; Angelard *et al*. 2010; Roger, 2012). Furthermore, extreme care has always been taken to ensure that there is no cross-contamination and that the uninoculated plants do not become accidentally colonised. Following this approach, we have almost never had a case where uninoculated plants become colonised (Streitwolf-Engel *et al*., 1997, 2001; van der Heijden *et al*., 1998a,b, 2006; van der Heijden *et al*., 2003; Koch *et al*., 2006; Angelard *et al*., 2010; Roger, 2012).

Effect of AMF isolates or combinations of AMF isolates on plant resistance to herbivores. With the second half of plants, 4 weeks after fungal inoculation, five neonate *S. littoralis* caterpillars were placed on each plant (one caterpillar per leaf) and were allowed to feed on the plants for 7 days. All insect-infested plants were enclosed in a plastic vessel to prevent the caterpillars escaping. After caterpillar removal, strawberry plants were grown for an additional 3 weeks (8 weeks in total). We then measured plant height (as well as shoot dry mass) in order to compare it with plants that did not suffer from herbivory.

The survival rate of caterpillars, the fresh weight of caterpillars and the amount of plant material eaten by caterpillars were used as measures of plant effect on the herbivores (*sensu* Karban & Baldwin, 1997). The number of surviving individuals was used as a measure of the survival rate. The fresh weight of each individual caterpillar was measured after 7 days' feeding on the plant. The average weight of the caterpillars per plant was used for the analysis. We next scanned all damaged leaves to estimate the total leaf area eaten. The area of all the holes in each leaf that were caused by herbivory was measured using the image analysis software IMAGEJ (http://rsbweb.nih.gov/ij/). The area missing due to herbivory was added to the digitalised leaf area by substituting the consumed leaf area. Total leaf area in mm^2 was determined using the pixel number of the reference area. Area values were then multiplied by leaf thickness values of the respective plants, in order to estimate the volume of leaf eaten.

Effect of AMF isolate combinations on herbivore preference. Using the plants that had not been subjected to herbivory (see earlier), we tested caterpillar preference in two separate dual choice tests: (i) a bioassay using detached leaves (DLs); and (ii) a whole-plant bioassay (WP). The first experiment was designed to test the effect of mycorrhizal status of the plants on insect preference, whereas the second was designed to include

the height (i.e. the vigour) of the plant, which could potentially drive insect choice.

The DL bioassay involved placing two equal-sized leaves from plants of two different treatments in a 10-cm-diameter Petri dish. Equal-sized leaves were selected based on leaf area. This was repeated 20 times for each pairwise comparison. Unfortunately, it was not possible to standardise or determine in advance how many plant individuals would yield a sufficient number of leaves for the DL tests. Thus, the 20 leaves came from seven different plants for four treatments (B2, B12, C5 and B2 + C2: $n = 7$). The 20 leaves came from two different plants for the remaining four treatments (NM, C2, B2 + B12, C2 + C5: $n = 2$). Seven-day-old caterpillars (having previously fed on artificial media) were then allowed to feed for 12 h overnight. Following herbivory, leaves were photographed and scored to measure the percentage of leaf area eaten using imaging software as described earlier, and the values were then converted into volume consumed.

The WP bioassay involved placing two plants next to each other (one plant per treatment) in a single plastic container (diameter 32 cm, height 10 cm). Empty space between the two plants was filled with sterile soil to allow caterpillars to move easily from one plant to the other. Ten third-instar *S. littoralis* caterpillars were then placed halfway between the two plants and allowed to choose between and feed on either plant for 12 h overnight. Percentage leaf volume eaten was scored as described earlier.

For both the DL and WP bioassays, based on results from plant trait measurements (first experiment described earlier), we designed six different pairwise comparisons to investigate caterpillar preference. These six pairwise comparisons were replicated 20 times each in the DL bioassay and five times each in the WP bioassay. Owing to limited availability of AMF spores for inoculum and, thus, inoculated plants, not all treatment combinations could be tested. We therefore chose comparisons based on plant size differences between inoculum treatments, in terms of variation in plant height. The six different pairwise comparisons were as follows: (1) uninoculated (NM) plants against plants of the treatment that yielded the tallest plants (NM with B2 + B12); (2) NM treatment plants against plants of the treatment yielding the smallest plants (NM with C2); (3) and (4) double-inoculation versus single-inoculation treatments, in which plants of the double-inoculation treatments were significantly taller than plants in single-inoculation treatments comprising the same genotype (B2 with B2 + B12, and C2 with C2 + C5, respectively); (5) single-inoculation treatments that yielded plants of equal size (C5 with B12) and; (6) double-inoculation treatments that yielded plants of equal size (B2 + C2 with C2 + C5). The comparisons 5 and 6 were considered as positive controls, in which we did not expect any preference in insect preference driven by plant height.

Statistical analyses. The effect of different mycorrhizal treatments on plant and insect traits was tested using one-way ANOVAs. When a significant effect was detected, we ran Tukey–Kramer honestly significant difference (HSD) *post hoc* comparisons among all treatments. We also investigated the effect of herbivory and AMF treatments on plant size using a two-way ANOVA. Then, we compared the height of plants that remained undamaged with the height of those that were eaten by caterpillars within each AMF treatment, using eight independent Wilcoxon tests. In both preference bioassays (DL and WP), values of leaf volume eaten were turned into percentage values in each particular comparison. We arcsine-square-root-transformed the percentage data in order to fit a normal distribution of the residuals. We tested whether the caterpillars preferentially fed on one of the two plants using a pairwise Student's *t*-test on transformed data. All the statistical tests were done using the statistical software R 2.7.2 (R Development Core Team, 2008).

Results

Effect of R. irregularis *isolates on strawberry plant performance*

When we investigated the sizes of all the plants, we found a significant effect of both AMF treatments ($F_{7,184} = 9.70$, $P < 0.001$), herbivory ($F_{1,184} = 60.56$, $P < 0.001$) and the interaction between AMF and herbivory ($F_{7,184} = 8.61$, $P < 0.001$).

Within the set of plants that was not subjected to herbivory, mycorrhizal treatments affected plant height (Fig. 1a; $F_{7,96} = 25.314$, $P < 0.001$), chlorophyll level (Fig. 1b, $F_{7,96} = 21.275$, $P < 0.001$) and leaf thickness (Fig. 1c, $F_{7,96} = 9.685$, $P < 0.001$). Two single-inoculation treatments (B2 and C2) decreased plant height and chlorophyll level compared with NM plants, whereas, one double-inoculation treatment (B2 + B12) significantly increased plant size, compared with NM plants. Indeed, the height of plants inoculated with B2 + B12 isolates were 20% taller than plants in the NM treatment, whereas leaves of plants inoculated with the B12 isolate were 19% thicker than leaves of NM plants. On the other hand, the height of plants inoculated with B2 and C2 isolates were reduced by 35% and 39%, respectively, compared with NM plants (Fig. 1a). Chlorophyll level was reduced by 23% and 22% when plants were inoculated with isolates B2 and C2, respectively (Fig. 1b). Leaf thickness also decreased by 22% when plants were inoculated with the C2 isolate (Fig. 1c).

Within the set of plants subjected to herbivory, we found no difference in plant height among the different AMF treatments, leading to equal plant size (Fig 1a; $F_{7,88} = 1.12$, $P = 0.358$) and dry mass (data not shown).

The significant effect of the interaction between AMF treatments and herbivory on plant height, found when investigating the whole plant set, needed more detailed investigations. Hence, within-AMF treatment comparisons revealed that, in six out of eight cases, herbivory significantly reduced plant size compared with plants not subjected to herbivory [Wilcoxon rank test for all treatments, all $P < 0.05$, except for treatments B2 and C2 ($P > 0.05$), Fig. 1a].

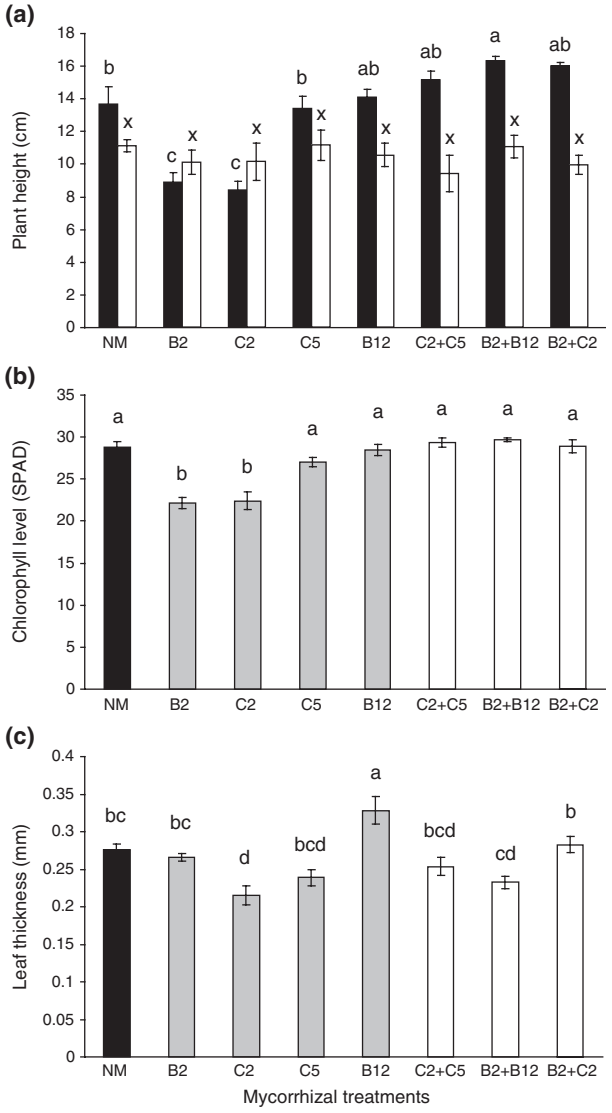

Fig. 1. Strawberry plant growth measured as mean plant height (a), mean chlorophyll level (b), and mean leaf thickness (c). Error bars indicate ± 1 SE and the number of replicates corresponds to the number of individual plants, which is 12 for each treatment. (a) Height of strawberry plants with the eight mycorrhizal treatments, 8 weeks after fungal inoculation. Black bars correspond to plants that were not subjected to herbivory; white bars correspond to plants that were subjected to herbivory during the fifth week of growth. Different letters above columns indicate a significant difference ($P < 0.05$) according to Tukey–Kramer honestly significant difference (HSD) test within each set of plants [letters a, b or c for the black bar set, and letter x (no difference) for the white bar set]. (b, c) Black bar corresponds to the non-mycorrhizal treatment (NM), grey bars to single-inoculation treatments and white bars to double-inoculation treatments. Different letters above columns indicate a significant difference ($P < 0.05$) according to Student's *t*-test. SPAD, Konica Minolta SPAD-50 chlorophyllometer.

Effect of R. irregularis *isolates on plant resistance to herbivores*

The leaf volume eaten and *S. littoralis* fresh weight were both significantly affected by AMF treatments [$F_{7,84} = 5.956$, $P < 0.001$ (Fig. 2a) and $F_{7,73} = 2.518$, $P = 0.021$ (Fig. 2b), respectively]. While insect fresh weight was reduced by AMF inoculations most of the time, leaf volume eaten can be either significantly increased (treatment B12) or decreased (treatment B2) depending on AMF treatments (Fig. 2a,b). Also, AMF treatments led to a marginally significant difference in insect survival (Fig. 2c, $F_{7,73} = 2.104$, $P = 0.0513$), with an average survival rate of 40% across all treatments, and ranging from 27 to 50% depending on the treatments. We also found a highly significant correlation between insect fresh weight and leaf volume eaten ($N = 92$, $r = 0.83$, $P < 0.001$).

Effect of R. irregularis *isolates on herbivore preference*

When insects had to choose between leaves of the same size (DL bioassay), in two out of six comparisons, insects showed a significant choice ($t = -6.224$, $P < 0.001$, comparison 1, NM against B2 + B12 isolates; $t = -5.307$, $P < 0.001$, comparison 2, NM against C2 isolate). In these cases, caterpillars preferentially fed on the leaf from the NM strawberry plants, independently of the size of the plant from which the leaf originated (Fig. 3a). Indeed, 77% and 82% of the plant material eaten by caterpillars occurred on leaves coming from NM plants, when they had to choose between NM plants and plants inoculated with B2 + B12 (comparison 1) or C2 isolates (comparison 2), respectively.

When insects had to choose between whole plants (WP bioassay), in two out of the six comparisons, insects showed a significant choice ($t = -9.422$, $P < 0.001$ for comparison 3, B2 + B12 isolates against B2 isolate; and $t = -5.481$, $P < 0.01$ for comparison 4, C2 + C5 isolates against C5 isolate). This time, *S. littoralis* caterpillars preferentially fed on taller plants inoculated with two *R. irregularis* isolates than on smaller plants inoculated with only one fungal isolate (Fig. 3b). In those particular cases, caterpillars fed preferentially on leaves originating from plants inoculated with B2 + B12 isolates (91% of the plant material eaten) than on leaves coming from plants inoculated with only the B2 isolate (comparison 3). We observed the same pattern in comparison 4. Caterpillars consumed 85% (leaf volume) on leaves originating from plants inoculated with C2 + C5 isolates and only 15% (leaf volume) on leaves originating from plants inoculated with only the C2 isolate.

Discussion

Different combinations of AMF genotypes led to differences in plant performance, resistance and insect preference. Even if insects preferred tissue coming from NM plants, when presented with whole plants, they mostly attacked bigger, healthier plants, regardless of the identity of mycorrhizal

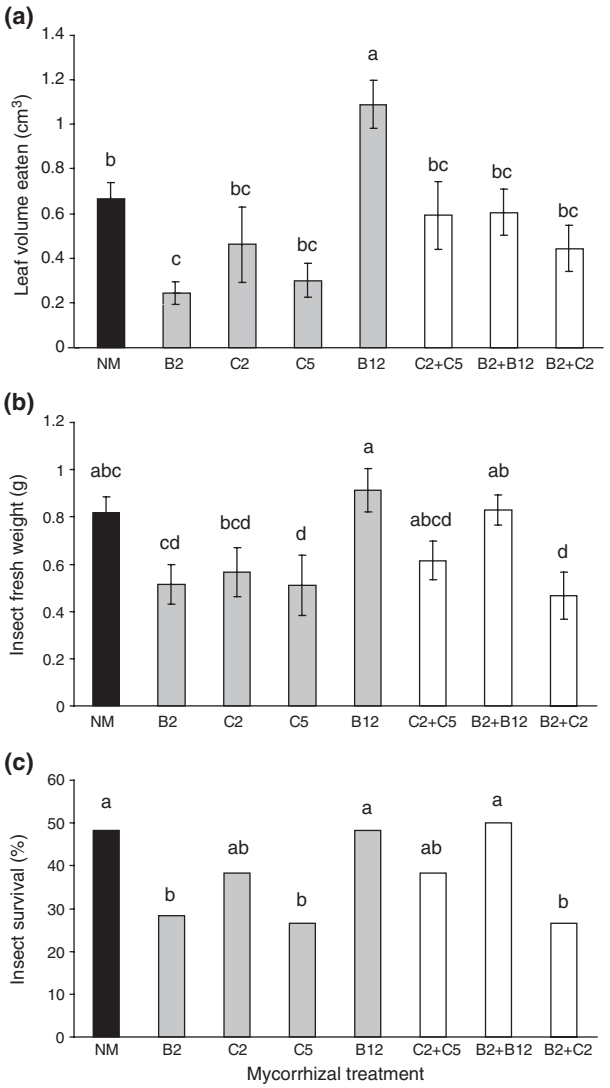

Fig. 2. *Spodoptera littoralis* caterpillar performance and plant resistance measured as mean leaf volume eaten ($n = 12$ in each treatment) (a), mean insect fresh weight (b) and insect survival (c) in the eight mycorrhizal treatments. Error bars indicate ± 1 SE. Black bar corresponds to the non-mycorrhizal treatment (NM), grey bars to single *Rhizophagus irregularis* inoculation treatments and white bars to double-inoculation treatments. Different letters above columns indicate a significant difference ($P < 0.05$) according to Tukey–Kramer honestly significant difference (HSD) test.

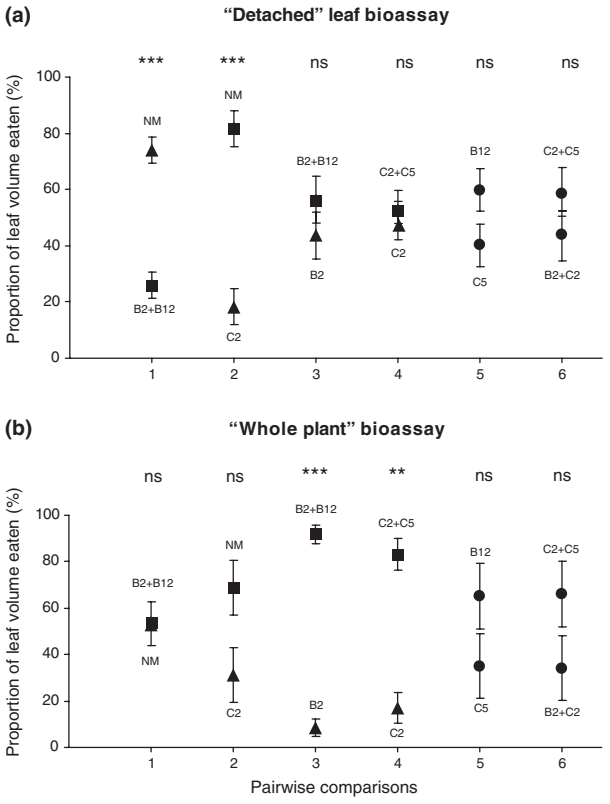

Fig. 3. Mean proportion of leaf volume eaten depending on the mycorrhizal status of the plant in the detached leaf bioassay (DL, $n = 20$ different leaves of variable interdependency) (a) and in the whole plant bioassay (WP, $n = 5$ independent plants) (b). When a significant difference in plant size was found between treatments of the same comparison (comparisons 1–4), treatments with the tallest strawberry plants were represented by a square and treatments with the smallest strawberry plants by a triangle. When no difference in plant size was observed (comparisons 5 and 6), both treatments were represented by a circle. Significance of the pairwise Student's t-test is presented as follows: NS, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$. Error bars indicate ± 1 SE.

colonist. The consequences of these results are discussed below.

Effect of R. irregularis *isolates on growth of strawberry plants*

Different mycorrhizal treatments led to differential growth of strawberry plants. We observed that intraspecific genetic variation in the fungal species led to very different effects on plant performance, which is in agreement with previous studies (Munkvold *et al*., 2004; Koch *et al*., 2006). Double-inoculation treatments resulted in larger plants in two out of four single-inoculation treatments (Fig. 1a,b). Although generalisations cannot be drawn overall regarding dual versus single AMF inoculation treatments (from a total of three double inoculations), our results are in line with other studies that showed that plants benefited from being colonised by several AMF species or isolates simultaneously (Koide, 2000; Gustafson & Casper, 2006; Jansa *et al*., 2008). Among the double-inoculation treatments, almost no difference in growth was observed, despite the different relatedness of the coexisting AMF isolates in those treatments, even if leaves from plants inoculated with treatment B2 + C2 (distantly related) were thicker than the ones from plants inoculated with treatment B2 + B12 (closely related) (Fig. 1c).

Overall, we observed that herbivory reduced the size of plants within each AMF treatment, except for treatments B2 and C2, where plant growth was already reduced without herbivory as compared with NM plants. This plant growth

reduction due to herbivory led to equal plant size among AMF treatments, even several weeks after the herbivores were removed. This supports the hypothesis that herbivore attack triggered a shift in resource allocation from growth to defence (Herms & Mattson, 1992), as previously shown by Kempel *et al*. (2010) with different grass and dicot species.

Colonisation of the roots by the AMF isolates was not measured in this study. However, because of a standardised protocol, routinely used in our group as well as in other mycorrhizal research groups, and because of the significant differences observed among AMF treatments, it is unlikely that any of the AMF isolates failed to colonise the plants or that the uninoculated plants accidentally became mycorrhizal by contamination (see the Materials and methods section). Consequently, we are confident that our results indicate that different AMF isolates altered growth and this is also consistent with previous studies using some of these AMF isolates and where colonisation was measured (Koch *et al*., 2006; Angelard *et al*., 2010).

It could also be argued the percentage of root length colonised by each AMF affects plant growth and nutritional status and, therefore, alters attractiveness or palatability for insect herbivores. Indeed, these isolates are genetically and phenotypically different and, therefore, probably differ in how much they are able to colonise the plant. Thus, we cannot rule out the possibility that the percentage of root length colonised altered attractiveness or palatability. However, we consider this unlikely, because in another study using the same isolates and combinations of isolates, we did not detect significant correlations between the percentage that each isolate occupied the roots and plant growth variables (Roger, 2012). This is consistent with a meta-study of the relationship between AMF colonisation and plant growth, comprising data from 78 published studies, where no obvious relationships were found between these two variables (McGonigle, 1988). Furthermore, since the percentage of colonisation by AMF cannot be experimentally manipulated, any such relationships would be purely correlations revealing no cause or effect.

Effect of R. irregularis *isolates on insect performance*

Along with changes in plant performance, AMF treatments were also responsible for significant changes in *S. littoralis* fitness. Indeed, we observed that, among the seven treatments, both plant and insect growth followed a parallel variation, except for the AMF treatments C2 and B2 + C2. For example, treatment B12 increased both leaf thickness and insect performance. We estimated insect performance directly by measuring growth and survival, and indirectly by measuring leaf volume eaten. These traits, among others such as insect population growth rate, fecundity, development time, or oviposition preference have previously been shown to be influenced by AMF inoculation (Koricheva *et al*., 2009). Enhanced plant growth by AMF must increase the total amount of food available to herbivores and probably the quality. However, the amount of plant material was never a limiting factor for caterpillar development in any treatment in this experiment. Hence, we can be sure that differences in larval weight, their survival rate and the amount of leaf volume eaten were not due to a lack of plant material, but most probably to changes in plant nutritional quality and increased plant defence. Indeed, chewing insects are known to benefit from increased carbon, nitrogen and phosphate content in plant material (reviewed by Pineda *et al*., 2010), which is a likely effect of AMF on plants (Gange & West, 1994). Combinations of different strains or species of beneficial symbiotic microbes are known to improve plant resistance and tolerance. Indeed, the combination of different AMF species was shown to improve plant tolerance to a root-feeding insect (Currie *et al*., 2011), and a combination of different *Glomus* species modulated plant acceptance by different insects (Gange *et al*., 2005). The results of the present experiments suggest that insect performance is linked with plant performance. This is also in agreement with another study involving a sister caterpillar species to the one we used, *Spodoptera exigua* (Kempel *et al*., 2009). However, generalisations of a positive link between plant performance and herbivore performance/preference are difficult to make (Mody *et al*., 2009).

Effects of R. irregularis *isolates on* S. littoralis *caterpillar preference*

The two first comparisons showed that when the amount of plant material was similar in the choice experiment (in the DL bioassay), caterpillars preferred leaves coming from NM plants (Fig. 3a, comparisons 1 and 2). Indeed, leaves from NM plants were eaten more than the ones from the genotype-specific mycorrhizal treatments, regardless of the differential plant growth provided by AMF. In the DL bioassay, leaves were cut from healthy, undamaged plants. Hence, it is likely that observed preference is due to changes in constitutive chemical plant traits that were manipulated by the fungi. This result is supported by other studies demonstrating that fungal presence can enhance production of defensive metabolites (Gange & West, 1994; Bennett *et al*., 2009). Some caution has to be taken with the interpretation of the DL assay results, because due to the difficulty of obtaining enough pairs of leaves of similar size in some treatments, pseudoreplication was unavoidable (see the Materials and methods section). Thus, the choice of plants may be biased to those exhibiting a certain leaf size, which might not necessarily have been representative of the leaf size of the treatment, and this is difficult to avoid in such a design. On the other hand, when whole plants were available to caterpillars, we could not detect differences in leaf volume eaten by caterpillars between NM and mycorrhizal plants. Instead, when two mycorrhizal plants of different sizes were available, herbivores preferentially fed on the tallest (Fig. 3b, comparisons 3 and 4).

Plant height could be assumed to be a good estimator of plant health. Here, we demonstrated that this parameter may affect attractiveness to insects. Wurst and Forstreuter (2010) did not find any effect of mycorrhizal status on aphid preference, even though mycorrhizal plants produced significantly more biomass than NM plants. However, in general, plants with increased performance were more attractive to a wide variety of herbivores, including Lepidoptera, Diptera, Hymenoptera or Homoptera (see Cornelissen *et al*., 2008 for a meta-analysis).

Feeding choice of insects can be altered by the presence of different AMF species or genotypes. Different combinations of AMF, which enhance plant growth and vigour, might indirectly increase plant attractiveness, and therefore the plant's susceptibility to insect herbivores. We therefore suggest that AMF should be incorporated as potential mechanisms underlying classic plant–herbivore interaction theories, such as the plant vigour hypothesis (Price, 1991).

Conclusions

We found a variable effect of AMF genotypes and their combinations on plant growth in this study, which only included one AMF species (*R. irregularis*). This influence of AMF on plant growth sometimes resulted in modification of insect performance triggered by AMF inoculations. In the two pairwise interactions, we also saw a particular preference of insects for NM plants when only leaves were available. However, we found no significant difference in insect feeding choice when comparisons occurred between whole mycorrhizal plants of equal sizes. Hence, we suggest a dual role for AMF, in which they can enhance plant defences and simultaneously increase plant attractiveness to herbivores that seek taller, healthier plants. This cannot be extrapolated to insect herbivores in general, as some of them are more attracted by specific volatiles. Hence, future work is needed to elucidate cues involved in larval orientation, more likely involving changes in odour profiles of plants.

In natural settings, land plants are colonised by different AMF amounts and genotypes (Allen, 1996; Klironomos *et al*., 2000; Croll *et al*., 2008). However, we think that the direct benefits of different AMF taxa to plants may be in opposition to the indirect negative effects of higher attractiveness for plant antagonists, such as insect herbivores.

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