Neutral Indirect Effects of Mycorrhizal Fungi on a Specialist Insect Herbivore

ROBERT A. LAIRD[1] AND JOHN F. ADDICOTT

Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada

Environ. Entomol. 37(4): 1017–1024 (2008)

ABSTRACT Arbuscular mycorrhizal (AM) fungi can indirectly affect insect herbivore performance by altering traits in their host plant. Typically, generalist herbivores are negatively affected by AM fungi, whereas specialists are positively affected. This is thought to be caused by differential abilities of specialists and generalists to tolerate and/or exploit plant secondary compounds, the prevalence of which may be related to mycorrhizal colonization. We performed a feeding experiment in which specialist sunflower beetle larvae (*Zygogramma exclamationis* Fabricius, Chrysomelidae) were fed on mycorrhizal or nonmycorrhizal common annual sunflower plants (*Helianthus annuus* L., Asteraceae). To determine the indirect effects of AM fungi on the sunflower beetle larvae, we measured insect survival and relative growth rate. We also measured leaf area eaten, which allowed relative growth rate to be broken down into two components: relative consumption rate and efficiency of conversion of ingested food. Contrary to several previous studies, we detected no indirect effects of mycorrhizal fungi on larval survival or on relative growth rate or its components. Small effect sizes suggest that this is nonsignificant biologically, as well as statistically, rather than merely an issue of statistical power. Our results support an emerging view that indirect effects of mycorrhizal fungi on insect herbivores may be complex and idiosyncratic. We suggest that future research should emphasize the effects of mycorrhizal fungi on individual plant traits and how these interact to affect insect performance.

KEY WORDS arbuscular mycorrhizal fungi, *Helianthus annuus*, sunflower beetle, trait-mediated indirect effects, *Zygogramma exclamationis*

Below- and aboveground ecological processes are highly dependent on one another, and a rich understanding of either necessitates the study of their reciprocal effects (Porazinska et al. 2003, Wardle et al. 2004). Plants bridge the belowground–aboveground interface, providing a major conduit through which these two subsystems can interact. Therefore, exposing the various mechanisms by which plants propagate belowground–aboveground links is a vital step toward integrating these historically separate aspects of terrestrial ecology (Wardle et al. 2004). To this end, we examined the interplay between two of most common and important interspecific interactions in nature—the mycorrhizal fungus–plant symbiosis and insect herbivory—focusing on the ways that arbuscular mycorrhizal fungi alter the performance of a specialist herbivore.

Insect herbivores and mycorrhizal fungi have the potential to indirectly interact through changes in their host plant (i.e., "trait-mediated indirect effects"; Abrams 1995). Frequently, herbivores negatively affect their host plant's mycorrhizal fungi, resulting in reduced percent-colonization (Gehring and Whitham 1994, 2002, but see Wamberg et al. 2003). This is thought to be caused by carbon limitation: mutualistic mycorrhizal fungi provide their host plants with soil nutrients in exchange for a large proportion of the plant's carbon budget (e.g., 10–20% for arbuscular mycorrhizal fungi; Jakobsen et al. 2002), so when herbivores consume plant tissues, they appropriate carbon that would otherwise be available for mycorrhizal fungi (Gehring and Whitham 1994, Gange and Bower 1997, Gange et al. 2002a and references therein). The indirect effects of mycorrhizal fungi on herbivore feeding patterns, survival, growth, and reproduction are much more variable, and there are examples of positive effects (Gange and West 1994, Borowicz 1997, Manninen et al. 1998, 1999, 2000, Gange et al. 1999, 2002b, 2005, Goverde et al. 2000, Gehring and Whitham 2002), neutral effects (Gehring et al. 1997, Gange et al. 1999, Manninen et al. 1999, 2000, Gange 2001, Koschier et al. 2007), and negative effects (Pacovsky et al. 1985, Rabin and Pacovsky 1985, Tylka et al. 1991, Gange and West 1994, Gange et al. 1994, 2002b, Gange and Nice 1997, Gange 2001, Gehring and Whitham 2002, Vicari et al. 2002, Wamberg et al. 2003, Guerrieri et al. 2004) (reviewed in Gehring and Whitham 2002, Strauss and Irwin 2004).

[1] Corresponding author: Department of Biology, Carleton University, Ottawa, Ontario K1S 5B6, Canada (e-mail: laird.robert@gmail.com).

0046-225X/08/1017–1024$04.00/0

Notwithstanding the view that the direction of the indirect effects of mycorrhizal fungi–herbivore interactions might be idiosyncratic (Strauss and Irwin 2004), several herbivore life history traits have been implicated in determining the position of particular herbivores along this continuum of responses. For example, an insect's feeding mode may be important, with leaf-chewing herbivores tending to be negatively affected by mycorrhizal fungi, whereas the converse is true for phloem-suckers (but see Guerrieri et al. 2004; earlier examples and counterexamples reviewed in Gehring and Whitham 2002). Of these relevant herbivore life history traits, the degree of diet specialization seems to be particularly important. Typically, generalists are negatively affected and specialists are positively affected by their host plant's mycorrhizal symbiosis (Gange et al. 2002b, Gehring and Whitham 2002). This variation is thought to be related to the relative advantage of specialists over generalists in tolerating or exploiting plant secondary compounds (Bowers and Puttick 1988, van der Meijden 1996), the production of which may be promoted by mycorrhizal fungi (Gange and West 1994, but see Mohr et al. 1998).

In this article, we report the results of an experiment on sunflower beetle larvae (*Zygogramma exclamationis* Fabricius) fed on sunflower plants (*Helianthus annuus* L.) in one of two mycorrhizal treatments, "mycorrhizal fungi absent" (M−) or "mycorrhizal fungi present" (M+). Sunflower beetle larvae are *Helianthus* specialists (Rogers 1977), so we predicted that they would perform better on M+ compared with M− plants in terms of increased survival and relative growth rate. To extend the work of previous studies (Goverde et al. 2000, Manninen et al. 2000, Vicari et al. 2002) and to further investigate the mechanism behind the predicted variation in growth, we used leaf area analysis to break down each larva's relative growth rate (RGR) into two of its component parts: relative consumption rate (RCR) and the efficiency of conversion of ingested food (ECI) (see Waldbauer 1968, Scriber and Slansky 1981). Because RGR = RCR × ECI, an increased RGR must necessarily be a result of increases in one or both of RCR and ECI. If mycorrhizal fungi increase RCR, this means that larvae of a given size feed more quickly on mycorrhizal plants than on nonmycorrhizal plants. However, if mycorrhizal fungi increase ECI, this means that leaves from mycorrhizal plants are either more digestible or are more easily converted into larval biomass compared with leaves from nonmycorrhizal plants. Finally, we examined the indirect effects of mycorrhizal fungi on the damage inflicted on host sunflower plants by sunflower beetle larvae to determine whether increased damage by specialists was a cost of mycorrhizal fungi to their host plant. We show that, contrary to our predictions, mycorrhizal fungi had no discernible indirect effects on *Z. exclamationis* larvae and no subsequent effects on plant damage.

Materials and Methods

Study Species. *Helianthus annuus* L. (Asteraceae), the common annual sunflower, is an abundant native plant in disturbed areas of much of western North America (Rogers et al. 1982). The subspecies found in our study region of southeastern Alberta is *H. annuus* L. ssp. *lenticularis* (Lindl.) Cockerell (Moss 1983). *H. annuus* plants can grow up to ≈4 m tall (Rogers et al. 1982), although the plants at our study site rarely exceed 1 m (R.L., unpublished data). Unlike the cultivated sunflower, *H. annuus* L. variety *macrocarpus*, native *H. annuus* plants usually have multiple capitula (inflorescences), with disks ≈2–4 cm in diameter and ray florets ≈2.5 cm long (Rogers et al. 1982, Moss 1983).

Zygogramma exclamationis Fabricius (Chrysomelidae), the sunflower beetle, is a leaf-chewing specialist of several *Helianthus* species, including *H. annuus* (Rogers 1977). Westdal (1975) and Rogers (1977) provide descriptions of the morphology and life history of *Z. exclamationis.* Adult beetles overwinter in the soil, emerge in the spring, and feed, mate, and oviposit on sunflower leaves and bracts (Westdal 1975). The larvae also eat sunflower leaves, feeding at night and hiding in the bracts of the capitula during the day (Westdal 1975, Rogers 1977). The larvae are present at our study sites mainly in late June and July; individual larvae feed for ~15 d, proceeding through four instars before pupating in the soil (Rogers 1977). The beetles that we used started the experiment as a combination of first- and second-instar larvae and ended the experiment as a combination of second- and third-instar larvae. Beetles at comparable latitudes to our study site have only one generation per summer (Gerber et al. 1979). Both adult and larval *Z. exclamationis* are potentially economically important pests for sunflower crops in the Northern Plains and can severely damage entire fields when present at sufficient densities (Westdal 1975).

Sunflower Seed Collection, Germination, and Mycorrhizal Inoculation Treatments. Sunflower achenes ("seeds") were collected at the end of the 2004 growing season from a large population at a field site located on the banks of the Red Deer River, near Gem, Alberta. The seeds were refrigerated over the winter. On 9 May 2005, the seeds were surface-sterilized by washing them in a 2% sodium hypochlorite solution for 20 min and rinsing them five times in sterile (autoclaved) water, each rinse lasting 5 min. We scarified the seeds using sterilized sandpaper and excised the narrow end of each seed using a razor blade. The seeds were placed in petri dishes on filter paper that was moistened with sterile water. The following day, the seed coat of each seed was removed using sterilized forceps. The seeds were left in the dark to germinate.

The seeds germinated within 3 d, and on 12 May 2005, each seedling was planted in a 100-ml pot. The pots contained a soil-free, mycorrhizal fungi-free growth medium composed of a 4:3:3 mixture (by volume) of peat moss, perlite, and crushed clay. During planting, each seedling was randomly assigned to one

of two treatments, "mycorrhizal fungi absent" (M−) and "mycorrhizal fungi present" (M+). There were 34 M− plants and 40 M+ plants. M+ plants were created by dipping the seedlings' emerging radicles in a mycorrhizal inoculant containing the spores of eight common species of arbuscular mycorrhizal fungi (*Glomus aggregatum, G. clarum, G. deserticola, G. intraradices, G. monosporus, G. mosseae, Gigaspora margarita,* and *Paraglomus brailianum;* BioOrganics, Lapine, OR), several of which are known to form symbioses with *H. annuus.* Furthermore, M+ seedlings were planted along with 5 ml of inoculant. M− plants were treated similarly, with the exception that they did not receive any inoculant. Flats of plants were placed in a random spatial arrangement on a greenhouse bench and were watered daily until saturation with distilled water. On 26 May 2005, the plants were transplanted into 7.6-liter pots in the same peat moss-perlite-clay medium and moved on to the roof of the building on which the greenhouse was situated.

Beetle Collection and Feeding Experiment. On 1 July 2005, we collected sunflower beetle larvae from a large patch of sunflowers in Dinosaur Provincial Park, Alberta, Canada. We weighed the larvae as soon as possible on the same day. There was no difference in the initial mass of larvae assigned to feed on M− plants (mean ± SEM: 0.65 ± 0.057 mg) and those assigned to feed on M+ plants (0.55 ± 0.053 mg; Wilcoxon rank sum test: $Z = 0.63$; $n_{M-} = 34$, $n_{M+} = 40$; $P = 0.53$). Immediately after the larvae were weighed, one larva was randomly assigned to each sunflower plant and was placed on the highest fully open leaf, because sunflower beetle larvae prefer to feed on these relatively new leaves near the top of the plant (Rogers 1992). The larvae were allowed to feed for 8 d until 9 July 2005. Sixty-six of the 74 larvae survived until the end of the feeding experiment and were weighed to determine their final mass. We never found more than one larva on the same plant, which supports the assumption of limited between-plant larval mobility in our experimental design. Therefore, we assumed that those that survived stayed on the same plant throughout the feeding experiment.

Leaf Area Analysis. Directly after the conclusion of the feeding experiment on 9 July 2005, the aboveground parts of each plant were harvested. The leaves were removed from each plant and scanned at 200 dpi. Leaf area remaining at harvest was determined for each plant using SigmaScan Pro 2.0 software (Jandel Scientific 1995). Total leaf area was estimated by filling in the eaten portions of the digital image of each plant's leaves and reanalyzing using SigmaScan. Leaf area eaten was calculated as the difference between total and uneaten leaf area. Virtually no herbivore damage was noted at the start of the feeding experiment, so we assumed that all the leaf area eaten on a given plant was eaten by the larva that was assigned to it. We further assumed that leaf thickness was the same between treatments, such that leaf area eaten was an appropriate proxy for larval food consumption. Although this assumption is violated in some species (e.g., in millet; Krishna et al. 1981), Koide (1985) found no difference in leaf thickness in mycorrhizal and nonmycorrhizal *Helianthus annuus* plants.

The digital images of the leaves of 10 randomly chosen plants were analyzed twice in this fashion. These repeat measurements showed that the leaf area analysis was highly repeatable as the correlation coefficient (r) for the first versus second measurements of leaf area eaten was >0.997.

Estimating RGR, RCR, and ECI. A larva's RGR is its rate of biomass accumulation scaled by the current mass of that larva (hence, relative growth rate), with units of milligrams per milligrams per day. Because of the fact that we collected "before and after" data for larval mass, rather than a time series, we assumed that each larva's RGR remained constant over the course of the 8 d of feeding. Therefore,

$$\mathrm{RGR} = \frac{\ln \mathrm{M_A} - \ln \mathrm{M_B}}{\mathrm{t}},$$

where $\mathrm{M_B}$ and $\mathrm{M_A}$ are the masses (mg) of the larva before the feeding experiment and after the feeding experiment, respectively, and t is the duration (d) of the feeding experiment (Kogan and Cope 1974).

The RCR is the rate that a larva consumes leaf tissue, again scaled by the current mass of the larva in question. Variation in RCR largely reflects differences in feeding behavior among the larvae (but see Waldbauer 1968). Because we measured the area of leaf consumed ($\mathrm{mm^2}$), as opposed to the mass, the units of RCR are millimeters squared per milligram per day. As with larval mass, we had "before and after" data for leaf area eaten; initial leaf area eaten was assumed to be zero (see above), and we measured the total area eaten by each larva. For this reason, we assumed that the RCR of each larva was also constant over the 8 d of feeding. Hence,

$$\mathrm{RCR} = \frac{\ln \mathrm{M_A} - \ln \mathrm{M_B}}{\mathrm{t}(\mathrm{M_A} - \mathrm{M_B})}\mathrm{L},$$

where L is the leaf area eaten by the larva ($\mathrm{mm^2}$) over the course of the feeding experiment.

The ECI is the rate of biomass acquisition per unit of food eaten (in this case, measured as leaf area). The units of ECI are milligrams per square millimeter. ECI reflects both the digestibility of ingested food and the larva's ability to convert food into biomass (Waldbauer 1968). As with RGR and RCR, we assumed ECI to be constant over the course of the feeding experiment:

$$\mathrm{ECI} = \frac{\mathrm{M_A} - \mathrm{M_B}}{\mathrm{L}}.$$

Note that RGR is equal to the product of RCR and ECI (Waldbauer 1968, Scriber and Slansky 1981). Therefore, RCR and ECI can profitably be thought of as components of RGR. For instance, if larvae fed on plants in one of the mycorrhizal fungi treatments have a greater RGR than larvae in the other treatment, this must necessarily be reflected by either (1) a greater RCR, (2) a greater ECI, or (3) both.

Root Colonization Analysis. We checked to see whether M+ plants were mycorrhizal and M− plants were nonmycorrhizal, following the root staining procedures outlined in Brundrett (1994) and Brundrett and McGonigle (1994). The day after harvesting the aboveground plant parts, we gently harvested and washed the roots of 10 M− plants and 10 M+ plants (randomly chosen). We stored the roots in 50% ethanol. Later, we rinsed the roots with distilled water and cleared them for 15 min at 121°C in 10% KOH. We then rinsed the roots again and stained them for 15 min at 121°C in 0.03% Chlorazol Black E, a biological stain. We stored the roots in 50% glycerol and allowed them to destain for several days. Next, we used a compound microscope (magnification = ×400) with a micrometer in the eyepiece to examine root-micrometer intersections. We examined at least 100 intersections per root for evidence of arbuscular mycorrhizal colonization (arbuscules, vesicles, mycorrhizal hyphae) to calculate percent colonization.

Statistical Analyses. To test whether the probability of larval survival depended on mycorrhizal treatment, we used a Fisher exact test. To test the effects of the mycorrhizal treatments, means comparisons were done using unpaired *t*-tests or medians comparisons using Wilcoxon rank sum tests for unpaired data. We tested the assumptions of parametric statistics using Levene's test (for homoscedasticity) and Shapiro-Wilks' test (for normality). If the assumptions were met, we used a *t*-test. If the assumptions were not met using nontransformed data, we applied a log transformation. If this did not rectify the situation, we used the nonparametric Wilcoxon rank sum test instead. Other than the Fisher exact test, the statistical analyses were performed using JMP 6.0 software (SAS Institute 2005).

Results and Discussion

The maximum percent colonization rate (percentage of root intersections with arbuscules, vesicles, or hyphae) of arbuscular mycorrhizal fungi was significantly greater for M+ plants than for M− plants (Fig. 1a). There was no overlap between the maximum percent colonization of M+ and M− plants. Seven of the 10 M+ plants tested had a maximum percent colonization >10%, whereas 7 of the 10 M− plants tested had a maximum percent colonization <1% (and 5 had no evidence of mycorrhizal colonization at all). When looking at arbuscules alone (the sites of plant–fungus carbon-nutrient exchange), the results were similar: the percentage of root intersections with arbuscules was significantly greater for M+ plants than for M− plants (Fig. 1b).

Total leaf area was significantly greater in M+ plants compared with M− plants; this was true when all plants were analyzed together (Fig. 2a) and when only the plants whose larvae survived the feeding experiment were analyzed (Fig. 2b). However, there was no difference in the leaf area eaten in the two mycorrhizal treatments, either in absolute terms (Fig. 2c) or relative to the total leaf area (Fig. 2d).

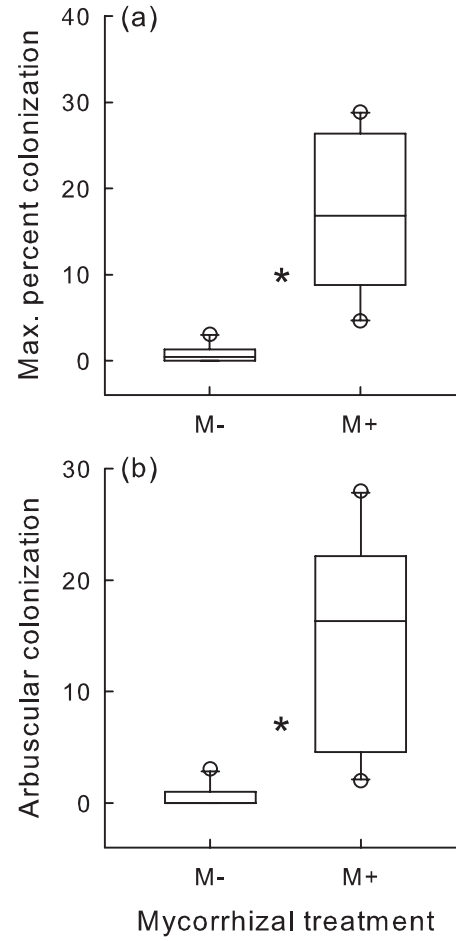

Fig. 1. Box plots for (a) maximum percent colonization by arbuscular mycorrhizal fungi (percentage of intersections with arbuscules, vesicles, or mycorrhizal hyphae; Wilcoxon rank sum test: $Z = -3.77$; $n_{M-} = 10$, $n_{M+} = 10$; $P = 0.0002$), and (b) arbuscular colonization (percentage of intersections with arbuscules; Wilcoxon rank sum test: $Z = -3.72$; $n_{M-} = 10$, $n_{M+} = 10$; $P = 0.0002$), for M− and M+ plants. The whiskers represent the 10th and 90th percentiles, the bottom and top edges of the boxes represent the 25th and 75th percentiles, the lines inside the boxes are the medians, and the points represent data that fell outside the 10th or 90th percentiles. *Significant difference in the means ($P \leq 0.05$). NS, not significant.

Mycorrhizal treatment had no effect on the proportion of larvae that survived the feeding experiment (Fig. 3a). Moreover, of the larvae that did survive, there were no differences with mycorrhizal treatment in the RGR, RCR, or ECI (Fig. 3b–d).

An absence of significance does not necessarily imply an absence of effect. However, in this case, we can be confident that our results are nonsignificant biologically as well as statistically speaking. For example, larvae fed on M+ plants had a greater (nonsignificant) relative growth rate than those fed on M− plants (Fig. 3b). However, to detect this difference as significant with even a modest power of $1\text{-}\beta = 0.5$ (and the usual

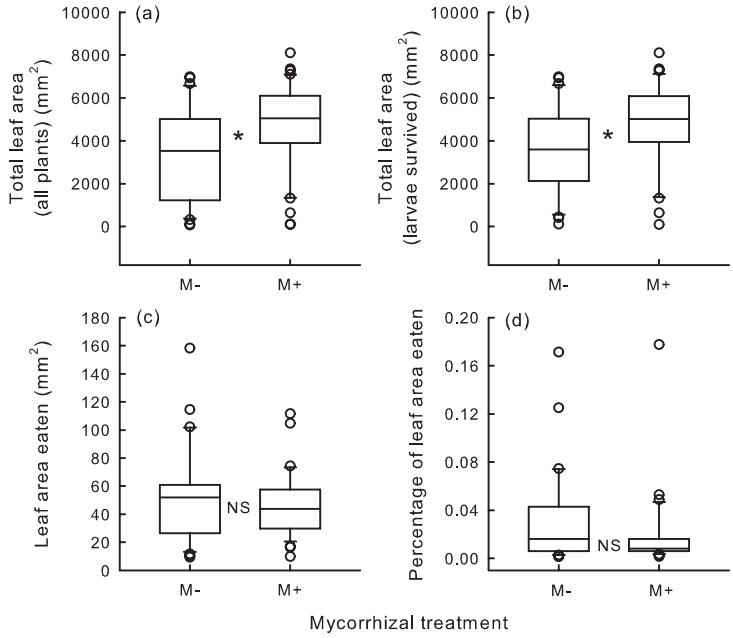

Fig. 2. Box plots for (a) total leaf area (all plants in the experiment; Wilcoxon rank sum test: $Z = -2.72$; $n_{M-} = 34$, $n_{M+} = 40$; $P = 0.0065$), (b) total leaf area (plants whose larvae survived until the end of the feeding experiment; *t*-test: $t = -2.38$; df = 64; $n_{M-} = 32$, $n_{M+} = 34$; $P = 0.021$), (c) leaf area eaten (*t*-test on log-transformed data: $t = 0.15$; df = 64; $n_{M-} = 32$, $n_{M+} = 34$; $P = 0.88$), and (d) leaf area eaten as a percentage of total leaf area (*t*-test on log-transformed data: $t = -0.16$; df = 64, $n_{M-} = 32$, $n_{M+} = 34$; $P = 0.87$) for larvae fed on nonmycorrhizal plants (M−) or mycorrhizal plants (M+). See Fig. 1 for explanation of the symbols in the box plots.

$\alpha = 0.05$) would have required a sample size of almost 1,700 larvae per mycorrhizal treatment, given the size of the effect (the effect size d was well below 0.2, the standard cut-off for small effects; Cohen 1992). It is doubtful that such a small effect has any ecological importance.

One possible explanation for the neutral indirect effects is that there were no mycorrhizal fungi-induced changes to host plants that were relevant to the feeding performance of *Z. exclamationis.* Mycorrhizal fungi may be more selective than previously thought (Helgason et al. 2002), and recent evidence suggests the hypothesis that the mutualistic benefits gained by plants may be positively related to the specificity of their mycorrhizal fungi (Helgason et al. 2007). Commercial inoculants, such as the one used here, typically contain the spores of generalist arbuscular mycorrhizal fungi, because they are relatively easy to culture and form associations with a wide range of plant species. Thus, it is possible that the generalist strains that we used were only weakly mutualistic, which, in turn, led to weak indirect effects on *Z. exclamationis* larvae compared with the indirect effects that might be observed in the presence of more specialized fungi.

However, we consider it unlikely that this explanation fully accounts for our neutral results. Although we did not measure plant physiological traits, we did find that M+ plants had significantly more leaf area than M− plants (Fig. 2a and b), which suggests a positive effect of arbuscular mycorrhizal fungi on *H. annuus* plants and underlying physiological variation between the two treatments. Moreover, many studies have reported mycorrhizal fungi-induced changes in plant nutritional traits (Smith 1980, Bolan 1991) that are potentially important for insect nutrition and performance. Also, mycorrhizal fungi can alter the expression of secondary compounds (but see Mohr et al. 1998). For example, Gange and West (1994) found that plants with mycorrhizal associations produced more of the feeding deterrents aucubin and catalpol, leading to a reduced relative growth rate in the generalist lepidopteran *Arctia caja* when fed on *Plantago lanceolata.* In addition, a number of studies reported mycorrhizal fungi-induced defenses to pathogens (Azcón-Aguilar and Barea 1996, Cordier et al. 1996, Trotta et al. 1996, Borowicz 2001, Lingua et al. 2002), some of which may provide ancillary protection against herbivores (Guerrieri et al. 2004). Thus, variation in colonization by mycorrhizal fungi often leads to variation in host plants' nutritional and defensive qualities, which in turn can lead to positive or negative effects on herbivorous insects (see Awmack and Leather 2002). Therefore, while acknowledging that more trait-based data are necessary to test the hypothesis that mycorrhizal fungi induce no changes in *H. annuus* plants that are relevant to larval *Z. exclamationis* performance, we suggest that the findings of these previous studies, coupled with the observed differences in root colonization and plant growth in this study, make this explanation unlikely.

An alternative explanation is that that the observed neutral indirect effect between mycorrhizal fungi and

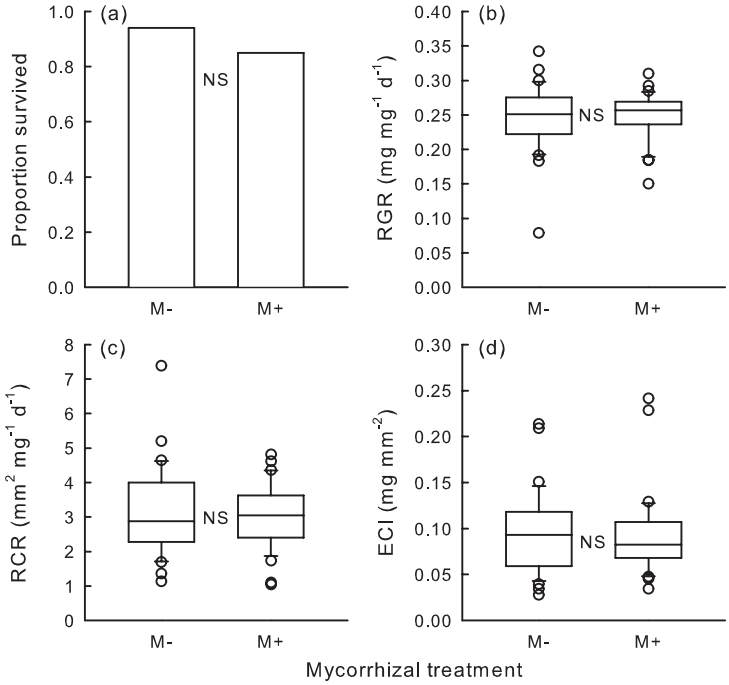

Fig. 3. (a) Proportion of larvae surviving until the end of the feeding experiment (Fisher exact test: $P = 0.28$), and box plots for (b) relative growth rate (RGR; Wilcoxon rank sum test: $Z = -0.29$; $n_{M-} = 32$, $n_{M+} = 34$; $P = 0.77$), (c) relative consumption rate (RCR; Wilcoxon rank sum test: $Z = -0.31$; $n_{M-} = 32$, $n_{M+} = 34$; $P = 0.75$), and (d) efficiency of conversion of ingested food (ECI; t-test on log-transformed data: $t = -0.21$; df = 64; $n_{M-} = 32$, $n_{M+} = 34$; $P = 0.84$) for larvae that survived the feeding experiment and were fed on nonmycorrhizal plants (M−) or mycorrhizal plants (M+). See Fig. 1 for explanation of the symbols in the box plots.

larvae represented the net effect of multiple, opposing positive and negative indirect effects. Net effects will certainly be centrally important when determining how mycorrhizal fungi affect insect fitness. However, to gain a deeper understanding of mycorrhizal fungus–insect interactions themselves, future research should emphasize the effects of mycorrhizal fungi on individual plant traits and how these interact to affect insect performance. In the case of the mycorrhizal fungi-sunflower-sunflower beetle system, this trait-based approach might involve assaying mycorrhizal and nonmycorrhizal plants for nutrient content and putatively defensive secondary compounds and determining how variation in their expression affects the survival, growth, and feeding performance of larvae.

In summary, the results presented here are counter to emerging theory and several previous studies in which arbuscular mycorrhizal fungi had positive indirect effects on specialist insect herbivores (Borowicz 1997, Goverde et al. 2000; but see Gange and Nice 1997, Gehring and Whitham 2002). Moreover, neutral or variably positive and negative effects are frequently observed in studies examining the indirect effects of ectomycorrhizal fungi on insect herbivores (Manninen et al. 1999, 2000). Recently, Strauss and Irwin (2004) proposed that the effects of mycorrhizal fungi on herbivores might be idiosyncratic, because the outcomes of both mycorrhizal–plant and plant–herbivore interactions are diverse and context-dependent. This view emphasizes the need for a trait-based approach to studying mycorrhizal fungi-plant-insect indirect effects.

Acknowledgments

We thank the following people for assistance: A. Camaclang, H. Addy, F. Cusack, K. Foreman, C. Gehring, K. Girard, C. Jacob, G. Laird, C. McCawley, B. Smith, S. Vamosi, and P. Hofer, R. Hugill, and the staff of Dinosaur Provincial Park. This study was funded by the Natural Sciences and Engineering Research Council of Canada, the Alberta Ingenuity Fund, the Alberta Conservation Association and Alberta Cooperative Conservation Unit, and the Killam Trusts.

References Cited

Abrams, P. A. 1995. Implications of dynamically variable traits for indentifying, classifying, and measuring direct and indirect effects in ecological communities. Am. Nat. 146: 112–134.

Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity of herbivorous insects. Annu. Rev. Entomol. 47: 817–844.

Azcón-Aguilar, C., and J. M. Barea. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens: an overview of the mechanisms involved. Mycorrhiza 6: 457–464.

Bolan, N. S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant Soil. 134: 189–207.

Borowicz, V. A. 1997. A fungal root symbiont modifies plant resistance to an insect herbivore. Oecologia (Berl.) 112: 534–542.

Borowicz, V. A. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? Ecology 82: 3057–3068.

Bowers, M. D., and G. M. Puttick. 1988. Response of generalist and specialist insects to qualitative allelochemical variation. J. Chem. Ecol. 14: 319–334.

Brundrett, M. 1994. Clearing and staining mycorrhizal roots, pp. 42–46. *In* M. Brundrett, L. Melville, and L. Peterson (eds.), Practical methods in mycorrhiza research: the ninth North American conference on mycorrhizae. Mycologue Publications, Guelph, Canada.

Brundrett, M., and T. McGonigle. 1994. Estimation of root length and colonization by mycorrhizal fungi, pp. 51–61. *In* M. Brundrett, L. Melville, and L. Peterson (eds.), Practical methods in mycorrhiza research: the ninth North American conference on mycorrhizae. Mycologue Publications, Guelph, Canada.

Cohen, J. 1992. A power primer. Psychol. Bull. 112: 155–159.

Cordier, C., S. Gianinazzi, and V. Gianinazzi-Pearson. 1996. Colonization patterns of root tissues by *Phytophthora nicotianae var. parasitica* related to reduced disease in mycorrhizal tomato. Plant Soil. 185: 223–232.

Gange, A. C. 2001. Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. New Phytol. 150: 611–618.

Gange, A. C., and H. M. West. 1994. Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. New Phytol. 128: 79–87.

Gange, A. C., and E. Bower. 1997. Interactions between insects and mycorrhizal fungi, pp. 115–131. *In* A. C. Gange, and V. K. Brown (eds.), Multitrophic interactions in terrestrial systems. Blackwell, Oxford, United Kingdom.

Gange, A. C., and H. E. Nice. 1997. Performance of the thistle gall fly, Urophora cardui, in relation to host plant nitrogen and mycorrhizal colonization. New Phytol. 137: 335–343.

Gange, A. C., V. K. Brown, and G. S. Sinclair. 1994. Reduction of black vine weevil larval growth by vesicular-arbuscular mycorrhizal infection. Entomol. Exp. Appl. 70: 115–119.

Gange, A. C., E. Bower, and V. K. Brown. 1999. Positive effects of an arbuscular mycorrhizal fungus on aphid life history traits. Oecologia (Berl.) 120: 123–131.

Gange, A. C., E. Bower, and V. K. Brown. 2002a. Differential effects of insect herbivory on arbuscular mycorrhizal colonization. Oecologia (Berl.) 131: 103–112.

Gange, A. C., P. G. Stagg, and L. K. Ward. 2002b. Arbuscular mycorrhizal fungi affect phytophagous insect specialism. Ecol. Lett. 5: 11–15.

Gange, A. C., V. K. Brown, and D. M. Aplin. 2005. Ecological specificity of arbuscular mycorrhizae: evidence from foliar- and seed-feeding insects. Ecology 86: 603–611.

Gehring, C. A., N. S. Cobb, and T. G. Whitham. 1997. Three-way interactions among ecotmycorrhizal mutualists, scale insects, and resistant and susceptible pinyon pines. Am. Nat. 149: 824–841.

Gehring, C. A., and T. G. Whitham. 1994. Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. Trends Ecol. Evol. 9: 251–255.

Gehring, C. A., and T. G. Whitham. 2002. Mycorrhizae-herbivore interactions: population and community consequences, pp. 295–320. *In* M.G.A. van der Heijden and I. Sanders (eds.), Mycorrhizal ecology. Springer, Berlin, Germany.

Gerber, G. H., G. B. Neill, and P. H. Westdal. 1979. The reproductive cycles of the sunflower beetle, Zygogramma exclamationis (Coleoptera: Chrysomelidae), in Manitoba. Can. J. Zool. 57: 1934–1943.

Goverde, M., M.G.A. van der Heijden, A. Wiemken, I. R. Sanders, and A. Erhardt. 2000. Arbuscular mycorrhizal fungi influence life history traits of a lepidopteran herbivore. Oecologia (Berl.) 125: 362–369.

Guerrieri, E., G. Lingua, M. C. Diligo, N. Massa, and G. Berta. 2004. Do interactions between plant roots and the rhizoshpere affect parasitoid behaviour? Ecol. Entomol. 29: 753–756.

Helgason, T., J. W. Merryweather, J. Denison, P. Wilson, J.P.W. Young, and A. H. Fitter. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J. Ecol. 90: 371–384.

Helgason, T., J. W. Merryweather, J.P.W. Young, and A. H. Fitter. 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. J. Ecol. 95: 623–630.

Jakobsen, I., S. E. Smith, and F. A. Smith. 2002. Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition, pp. 75–92. *In* M.G.A. van der Heijden and I. Sanders (eds.), Mycorrhizal ecology. Springer, New York.

Jandel Scientific. 1995. SigmaScan Pro for windows, user's manual. Jandel Scientific, San Rafael, CA.

Kogan, M., and Cope, D. 1974. Feeding and nutrition of insects associated with soybeans. 3. Food intake, utilization, and growth in the soybean looper, Pseudoplusia includens. Ann. Entomol. Soc. Am. 67: 66–72.

Koide, R. 1985. The nature of growth depression in sunflower caused by vesicular-arbuscular mycorrhizal infection. New Phytol. 99: 449–462.

Koschier, E. H., T. Khaosaad, and H. Vierheilig. 2007. Root colonization by the arbuscular mycorrhizal fungus *Glomus mosseae* and enhanced phosphorous levels in cucumber do not affect host acceptance and development of *Frakliniella occidentalis.* J. Plant. Interact. 2: 11–15.

Krishna, K. R., H. M. Suresh, J. Syamsunder, and D. J. Bagyaraj. 1981. Changes in the leaves of finger millet due to VA mycorrhizal infection. New Phytol. 87: 717–722.

Lingua, G., G. D'Agostino, N. Massa, M. Antosiano, and G. Berta. 2002. Mycorrhiza-induced differential response to a yellows disease in tomato. Mycorrhiza 12: 191–198.

Manninen, A.-M., T. Holopainen, and J. K. Holopainen. 1998. Susceptibility of ectomycorrhizal and nonmycorrhizal Scots pine (*Pinus sylvestris*) seedlings to a generalist insect herbivore, *Lygus rugulipennis,* at two nitrogen availability levels. New Phytol. 140: 55–63.

Manninen, A.-M., T. Holopainen, and J. K. Holopainen. 1999. Performance of grey pine aphid, *Schizolachnus pineti,* on ecto-mycorrhizal and non-mycorrhizal Scots pine seedlings at different levels of nitrogen availability. Entomol. Exp. Appl. 9: 117–120.

Manninen, A.-M., T. Holopainen, P. Lyytikäinen-Saarenmaa, and J. K. Holopainen. 2000. The role of low level ozone exposure and mycorrhizas in chemical quality and insect herbivory on Scots pine seedlings. Global Change Biol. 6: 111–121.

Mohr, U., J. Lange, T. Boller, A. Wiemken, and R. Vögeli-Lange. 1998. Plant defence genes are induced in the pathogenic interaction between bean roots and *Fusarium solani,* but not in the symbiotic interaction with the arbuscular mycorrhizal fungus *Glomus mosseae.* New Phytol. 138: 589–598.

Moss, E. H. 1983. Flora of Alberta, 2nd ed. University of Toronto Press, Toronto, Canada.

Pacovsky, R. S., L. B. Rabin, C. B. Montllor, and A. C. Wais, Jr. 1985. Host-plant resistance to insect pests altered by *Glomus fasciculatum* colonization, p. 288. *In* R. Molina [ed.], Proceedings of the 6th North American Conference on Mycorrhizae. Oregon State University Forest Research Laboratory, Corvallis, OR.

Porazinska, D. L., R. D. Bardgett, M. B. Blaauw, H. W. Hunt, A. N. Parsons, T. R. Seastedt, and D. H. Wall. 2003. Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. Ecol. Monogr. 73: 377–395.

Rabin, L. B., and R. S. Pacovsky. 1985. Reduced larva growth of two lepidoptera (Noctuidae) on excised leaves of soybean infected with a mycorrhizal fungus. J. Econ. Entomol. 78: 1358–1363.

Rogers, C. E. 1977. Bionomics of the sunflower beetle. Environ. Entomol. 6: 466–468.

Rogers, C. E. 1992. Insect pests and strategies for their management in cultivated sunflower. Field Crops Res. 30: 301–332.

Rogers, C. E., T. E. Thompson, and G. J. Seiler. 1982. Sunflower species of the United States. National Sunflower Association, Fargo, ND.

SAS Institute. 2005. JMP introductory guide, release 6. SAS Institute, Cary, NC.

Scriber, J. M., and F. Slansky, Jr. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183–211.

Smith, S. E. 1980. Mycorrhizas of autotrophic higher plants. Biol. Rev. Camb. Phil. Soc. 55: 475–510.

Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. Annu. Rev. Ecol. Syst. 35: 435–466.

Trotta, A., G. C. Varese, E. Gnavi, A. Fusconi, S. Sampò, and G. Berta. 1996. Interactions between the soilborne root pathogen *Phytophthora nicotianae var. parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant Soil. 185: 199–209.

Tylka, G. L., R. S. Hussey, and R. W. Roncadori. 1991. Interactions of vesicular-arbuscular mycorrhizal fungi, phosphorus, and *Heterodera glycines* on soybean. J. Nematol. 23: 122–133.

van der Meijden, E. 1996. Plant defence, an evolutionary dilemma: Contrasting effects of (specialist and generalist) herbivores and natural enemies. Entomol. Exp. Appl. 80: 307–310.

Vicari, M., P. E. Hatcher, and P. G. Ayres. 2002. Combined effect of foliar and mycorrhizal endophytes on an insect herbivore. Ecology 83: 2452–2464.

Waldbauer, G. P. 1968. The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229–288.

Wamberg, C., S. Christensen, and I. Jakobsen. 2003. Interaction between foliar-feeding insects, mycorrhizal fungi, and rhizosphere protozoa on pea plants. Pedobiologia 47: 281–287.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setala, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304: 1629–1633.

Westdal, P. H. 1975. Insect pests of sunflower, pp. 475–495. *In* J. T. Harapiak (ed.), Oilseed and pulse crops in Western Canada: a symposium. Western Co-operative Fertilizers Limited, Calgary, Canada.

Received 10 September 2007; accepted 22 April 2008.