esa

ECOSPHERE

Testing the mutualism disruption hypothesis: physiological mechanisms for invasion of intact perennial plant communities

ALISON N. HALE,† STEPHEN J. TONSOR, AND SUSAN KALISZ

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 USA

Citation: Hale, A. N., S. J. Tonsor, and S. Kalisz. 2011. Testing the mutualism disruption hypothesis: physiological mechanisms for invasion of intact perennial plant communities. Ecosphere 2(9):110. doi:10.1890/ES11-00136.1

Abstract. Soil resources derived from mutualistic arbuscular mycorrhizal fungi (AMF) play a critical role in the physiological function of many native plant species. Allelopathic plant invasion studies have revealed declines in AMF inoculation potential of invaded soils, and lost opportunities for plants to form new AMF associations. Yet, if allelochemicals also kill AMF external hyphae already associated with plant roots, this mutualism disruption should result in physiological stress for native plants. We previously demonstrated that forest soils infested with garlic mustard (*Alliaria petiolata*), an allelopathic invader, exhibit reduced fungal hyphal abundance. Here, we demonstrate for the first time that treatment with garlic mustard tissue reduces soil respiration rates and diminishes physiological function of false Solomon's seal (*Maianthemum racemosum*), an AMF-dependant forest understory native. Treated plants exhibited reduced stomatal conductance and photosynthesis relative to controls, consistent with the proposed loss of AMF function. Such physiological declines, if sustained over several growing seasons, could decrease native understory perennials' growth rates and increase their susceptibility to environmental stresses. These data provide an explicit mechanism that can help explain the loss of established native perennials from invaded mature forests. We propose that the physiological costs of mutualism disruption may be a widespread but previously untested mechanism enhancing the invasion of undisturbed ecosystems by allelopathic species.

Key words: allelopathy; *Alliaria petiolata*; arbuscular mycorrhizal fungi (AMF); garlic mustard; invasion; *Maianthemum racemosum*; mutualism; novel weapons; plant physiology.

Received 16 May 2011; revised 19 August 2011; accepted 6 September 2011; **published** 18 October 2011. Corresponding Editor: R. Irwin.

† **E-mail:** alison.n.hale@gmail.com

INTRODUCTION

Plants rely on mutualistic interactions for a number of services that are vital for reproduction, defense, dispersal, and nutrient acquisition. The most widespread plant mutualism is likely the interaction with arbuscular mycorrhizal fungi (AMF): it is estimated that up to 90% of all land plants participate in AMF mutualisms (Smith and Read 2008). The basis for this mutualism is a two-way exchange of resources. Up to 20% of a plant's carbon is shunted to the obligately symbiotic AMF, while AMF improve the supply of water, phosphorus and nitrogen to the plant (Smith and Read 2008). As a result of the numerous benefits that plants derive from AMF, these belowground mutualists can strongly influence the physiology (Fig. 1), overall carbon gain, and likely the competitive ability of their host plants. Disruption of this key plant mutualism is hypothesized to facilitate invasion (Mitchell et al. 2006, Reinhart and Callaway 2006).

The "mutualism disruption" hypothesis sug-

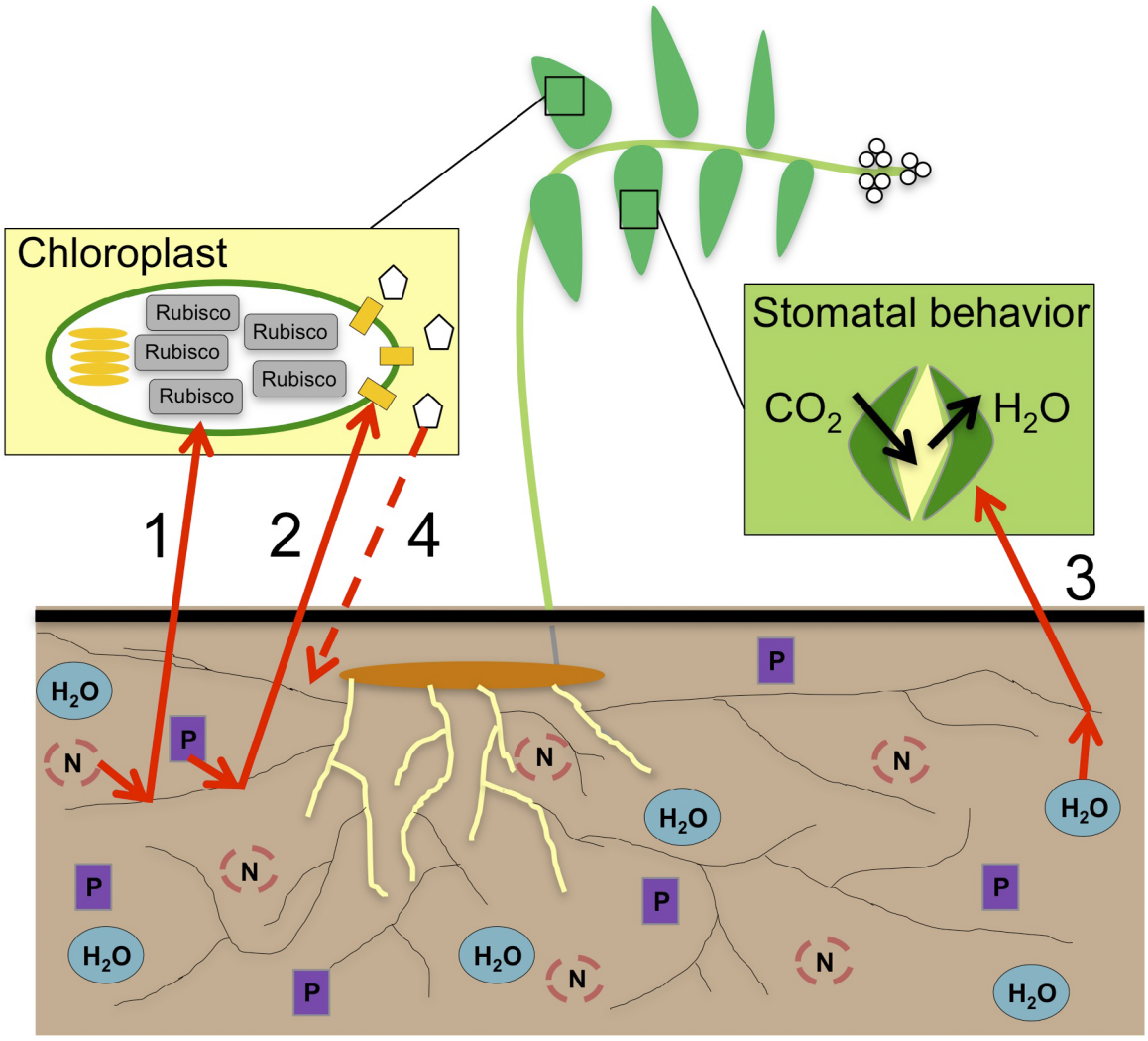

Fig. 1. Plant physiology is dependent on both nutritional benefits (solid arrows) received from AMF and carbon costs (dashed arrows) delivered to AMF by the plant. In the soil, external hyphae of AMF (fine black lines) uptake soil nitrogen (N) and phosphorus (P) that is transported to the host plant. Arrow 1: Nitrogen is essential for the formation of chlorophyll and ribulose bisphosphate-carboxylase/oxygenase (RuBisCO), and as a result, photosynthetic rate (A_n) is highly correlated with total leaf nitrogen content (Evans 1989). Arrow 2: Phosphorus (P) is required to build ATP and other cofactors that play important roles in the Calvin cycle. P is also crucial for the transport of carbon assimilates (represented as pentagons) out of the chloroplast and P deficiencies can lead to a build-up of assimilates and down-regulation of A_n (Sivak and Walker 1986). Arrow 3: AMF enhance the plant's ability to capture water (H_2O) and increase water availability, which results in greater stomatal conductance (g_s) and transpiration (E; Augé 2001). Arrow 4: Maintenance costs and rapid turnover of AMF (Staddon et al. 2003) create an additional carbon sink and plants up-regulate photosynthetic rates in response. Studies in which non-mycorrhizal and mycorrhizal plants are matched for foliar [N] and [P] demonstrate that mycorrhizal plants have higher A_n than non-mycorrhizal plants (Wright et al. 1998, Miller et al. 2002).

gests that inhibition of native mutualists can provide invaders with a competitive advantage over mutualism-dependent native species. While this hypothesis has been tested extensively using reproductive mutualisms (reviewed in Traveset and Richardson 2006), it is increasingly recog-

nized that AMF mutualism disruption may also play an important role in invasions. For example, plant invaders can reduce AMF density (Roberts and Anderson 2001, Vogelsang and Bever 2009) and the diversity and abundance of AMF external hyphae (Mummey and Rillig 2006) in the soil. The adverse effects of invaders on AMF mutualists can result from either negative feedback (Vogelsang and Bever 2009) or novel chemical weapons (Callaway and Ridenour 2004). Many invaders are suspected of employing the latter, including *Alliaria petiolata* (garlic mustard, Brassicaceae), a rampant forest understory invader. If allelochemicals disrupt the function of AMF mutualists, physiological impairment of the plant host could provide a competitive edge for invaders.

Garlic mustard has become a model study system, and as a result, its allelopathic chemicals are increasingly understood. Garlic mustard produces numerous secondary compounds, including glucosinolates (Vaughn and Berhow 1999), which are unique to the Brassicaceae. Upon hydrolysis in the soil, glucosinolates are mainly converted to isothiocyanates, a class of compounds toxic to AMF and other soil organisms (Brown and Morra 1997). Greenhouse experiments demonstrate that soils from garlic mustard-invaded sites or soils pre-conditioned with garlic mustard reduce AMF colonization and biomass in native tree seedlings (Stinson et al. 2006) and increase mortality of herbaceous seedlings (Callaway et al. 2008). Recently, we showed that allyl isothiocyanate (AITC)—an abundant compound exclusive to garlic mustard (Vaughn and Berhow 1999, Barto et al. 2010)—is present in garlic mustard-invaded soils (Cantor et al. 2011). Our companion bioassay revealed that even low AITC concentrations can reduce AMF spore germination by ~60%. Furthermore, garlic mustard-invaded areas at our study site showed reduced colonization by fungal hyphae compared to control areas (Cantor et al. 2011). These new results clearly demonstrate that garlic mustard's allelochemicals are present in field soil, and that even low AITC concentrations are capable of reducing AMF growth and abundance. Thus, the forest invader, garlic mustard, is an ideal species for testing a "mutualism disruption" hypothesis.

The native understory perennial herbs of deciduous temperate forests are a phylogenetically diverse group of plants (Gilliam 2007) that are highly dependent on AMF (Brundrett and Kendrick 1988). The AMF-forest herb mutualism is unique: unlike AMF crop species whose arbuscules are short-lived (4–5 days), arbuscules within forest herbs' roots function for several months (Brundrett and Kendrick 1990). Furthermore, the coarse roots of many understory herbs lack fine root hairs, and AMF external hyphae may function as root hairs for these species (Brundrett 1991). Together these factors indicate high AMF dependence in forest herbs.

In this study we integrate the fields of plant ecophysiology and invasion biology to provide a first step in testing for physiological consequences of mutualism disruption. We propose that if an allelopathic invader depresses the function of AMF external hyphae, the influx of critical resources from the AMF to its host will be reduced, resulting in physiological stress of the host. We hypothesize that this stress could be manifested through several interacting physiological pathways (Fig. 1). Decreases in nutrient supply rates can reduce photosynthetic capacity and demand for CO_2 through reductions in RuBisCO, indirectly causing partial stomatal closure (Fig. 1, arrows 1 and 2). Limited water availability can directly cause partial stomatal closure (arrow 3). The interplay of the conflicting influences of water stress and CO_2 demand for photosynthesis largely determine stomatal conductance. A common physiological measurement—leaf internal CO_2 concentration—can be used to reveal the extent to which water and/or nutrient limitation is driving stomatal closure (Wong et al. 1979, Farquhar and Sharkey 1982). Under nutrient limitation, internal CO_2 concentrations remain unchanged as the partially closed stomata provide sufficient CO_2 to the diminished photosynthetic machinery. In contrast, reduced internal CO_2 concentration occurs under water limitation because photosynthetic CO_2 demand outstrips its diffusion into the leaf. Finally, we expect that loss of sink strength via allelochemical-induced reductions in AMF hyphal function can further lower photosynthetic capacity (arrow 4). Here we use ecophysiological measurements in field and common garden experiments to determine which of these conflicting influences, water stress or nutrient driven reductions in CO_2

demand, impact physiological function. We show that a native perennial and its AMF exhibit reduced soil respiration rates with garlic mustard treatment and implicate water limitation in plant physiological declines.

Methods

Focal species

Garlic mustard is a Eurasian biennial plant introduced to North America circa 1868 that has since spread throughout forest understory habitats (Rodgers et al. 2008). Garlic mustard releases powerful allelochemicals into the soil (Cantor et al. 2011) and can drive declines in native plant abundance and diversity in forests (reviewed in Rodgers et al. 2008).

We chose *Maianthemum racemosum* (false Solomon's seal; Liliaceae) as a focal understory species because it is common in both deciduous and coniferous forests throughout North America, often occurs in sites invaded by garlic mustard (Burke 2008; A. Hale, *personal observation*; Fig. 2A), and is highly dependent on AMF. Like other understory perennial herbs, false Solomon's seal roots are highly colonized by AMF (76–94%; Brundrett and Kendrick 1988, Burke 2008), and lack fine root hairs (Brundrett and Kendrick 1988; Fig. 2B). These attributes led Brundrett and Kendrick (1988) to classify false Solomon's seal as obligately dependent on AMF for growth and survival. AMF external hyphae associated with understory herbs typically exhibit peak growth in mid to late summer (Brundrett and Kendrick 1990). This peak coincides with garlic mustard adults' senescence (Anderson et al. 1996) and the release of allelochemicals (Cantor et al. 2011), making the AMF mutualism in false Solomon's seal particularly susceptible to disruption by garlic mustard. Finally, false Solomon's seal's roots grow at shallow depths (2–4 cm below the soil surface; A. Hale, *personal observation*), which increases their probability of encountering allelochemicals leaching from garlic mustard leaf litter or root exudates.

Common garden experiment: garlic mustard allelochemicals' effect on belowground respiration

We established a 3 × 4 m plot at the University of Pittsburgh's Pymatuning Laboratory of Ecology (PLE) and created a grid of 30 cm deep holes using a post-hole digger. We collected false Solomon's seal plants (N = 34) on 4 June 2009 from Tryon-Weber Woods, a 34-hectare beech-maple forest in northwestern Pennsylvania, USA that is not invaded by garlic mustard. To allow measurement of belowground respiration (described below), we employed a unique pot design. We built pots of 30 cm tall × 10 cm diameter PVC pipe with a mesh bottom (pore size: 1 mm) that allowed air and water to flow through the pot. We potted plants in a 50:50 mixture of Fafard:Turface (Conrad Fafard, Agawam, MA, USA; Profile Products, Buffalo Grove, IL, USA) with inoculum of local field soil collected around false Solomon's seal roots and 3.5 g Nutricote fertilizer (100 day release formula, Florikan E.S.A. Corporation, Sarasota, FL, USA). We randomly assigned each plant to a grid location, making the top of each pot flush with the soil surface. Since AMF colonization begins in late spring for false Solomon's seal (Brundrett and Kendrick 1990), the AMF community was already established in the root systems at the time of collection. However, since we disturbed the external hyphae during transplanting, plants and AMF were allowed to re-establish for 6 weeks prior to treatment (Jakobsen et al. 1992) to allow hyphal regrowth. We enclosed the entire plot with a wire cage to exclude mammalian herbivores and attached a 60% shade cloth to the top of the cage to simulate forest understory light levels.

Treatments.—We randomly applied one of three treatments to each pot: fresh garlic mustard tissue (N = 11), fresh dame's rocket (*Hesperis matronalis*, Brassicaceae) tissue (another exotic mustard (N = 11)), or no plant tissue (N = 12). We collected green adult garlic mustard leaves, stems and roots from Wallace Woods, a mature, second-growth forest owned by PLE that was invaded by garlic mustard within the last decade (T.-L. Ashman, *personal communication*), and also collected green dame's rocket tissue on site at PLE. We placed 100 g of fresh garlic mustard or dame's rocket tissue into 20 × 20 × 1.5 cm fiberglass screen bags (pore size: 1 mm) and transported the bags to the common garden for application to the false Solomon's seal pots. We predicted that allelochemicals leaching from the garlic mustard treatment would kill AMF exter-

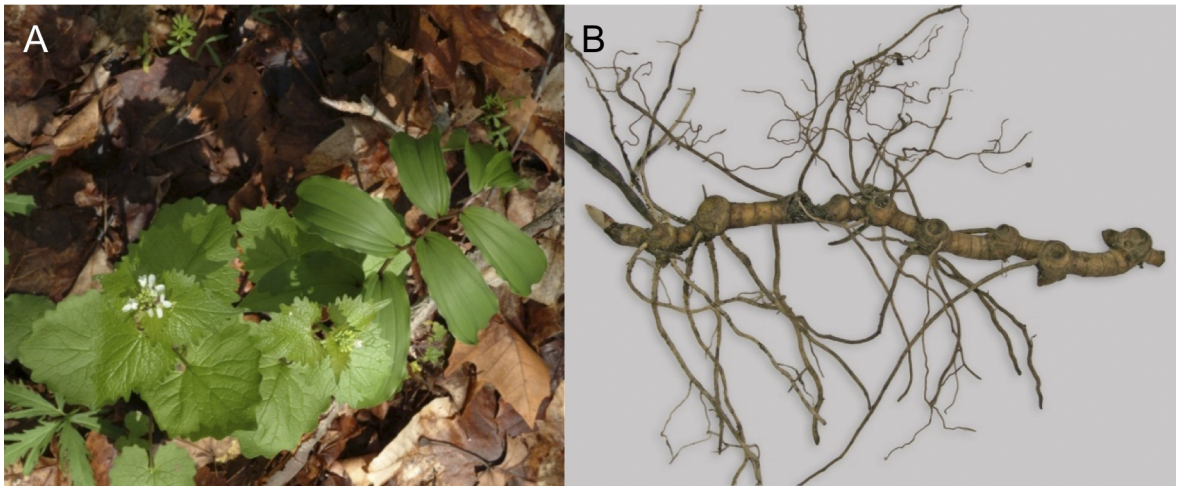

Fig. 2. False Solomon's seal is an ideal native species for studying the impacts of mutualism disruption by garlic mustard because (A) it is commonly found in forests invaded by garlic mustard and (B) it also has very coarse roots that lack fine root hairs, suggesting a high degree of mycorrhizal dependency.

nal hyphae. However, we predicted that the dame's rocket treatment would have a negligible effect on AMF as this species can sustain AMF colonization in its roots (Demars and Boerner 1995) despite its glucosinolate production (Larsen et al. 1992). For the no plant tissue treatment, we left the screen bags empty. Thus, the dame's rocket treatment allows us to separate the effects of garlic mustard glucosinolates from leaf tissue effects, while the empty screen bag treatment allowed us to assess background levels of soil respiration. We placed the screen bags at the base of the plants and fastened them to the soil with stainless steel pins, ensuring that the bags were in direct contact with the soil surface. Treatments were imposed on 20 and 21 July 2010.

The application of garlic mustard tissue to the pots allowed us to closely simulate natural levels of allelochemicals, as decomposition is a major route of allelochemical release into the soil (Rice 1974), and removed the confounding factor of competition when garlic mustard and false Solomon's seal are grown together in pots. Because of the rapid decomposition of garlic mustard tissue (Rodgers et al. 2008) and sorption of isothiocyanates in soil (Matthiessen and Shackleton 2005), coupled with the quick turnover rate of AMF external hyphae (i.e., 5–6 days; Staddon et al. 2003), we determined that a one-week treatment would be sufficient for suppression of AMF external hyphal function.

Belowground respiration.—We measured belowground respiration in each pot using a LI-COR 6400 infrared gas analyzer (IRGA; LI-COR Biosciences, Lincoln, NE, USA). We fabricated a sealed airflow path to pass CO_2-free air through the mesh bottom of each PVC pot, forcing the CO_2 in the soil matrix to flow out of the top of the pot into the IRGA (Appendix A). We recorded the ambient air temperature and the CO_2 concentration (our estimate of belowground respiration) of this air stream at time zero and every two minutes, for a total of 10 minutes. We chose this sampling interval because 1-hour trial runs revealed that $>75\%$ of available CO_2 in the soil was captured in the first 10 minutes. To separate the effect of garlic mustard on microbial vs. root respiration, at the end of the experiment we harvested each plant and recorded its fresh root mass. To assess the potential for direct effects of garlic mustard's allelochemicals on false Solomon's seal, we compared the fresh root mass across treatments.

Statistical analysis.—We fit a curve ($[CO_2] =$ time) to the data for each pot for a total of 34 regressions. The area under each curve, calculated using Mathematica 7.0 (Wolfram Research, Champaign, Illinois, USA), estimates the total CO_2 captured across the sampling duration. We compared belowground respiration in the garlic

mustard vs. dame's rocket treatments using an analysis of covariance (ANCOVA) with root mass and ambient temperature as covariates. We corrected the final means from this analysis by subtracting the mean respiration value in the empty screen treatment, which represented background levels of soil respiration (all uncorrected values are shown in Appendix A). Since our a priori prediction was for lower respiration in the garlic mustard treatment relative to the dame's rocket treatment, we report one-tailed *P*-values. We compared root mass using a Kruskal-Wallis non-parametric test with the model: root mass = treatment.

Field experiment: garlic mustard allelochemicals' effect on native plant physiology

Our experimental site was the Trillium Trail Reserve, a 16-hectare mixed mesophytic forest that is owned and managed by the Fox Chapel Borough, PA, USA. We estimate that 73% of the 79 herbaceous species at Trillium Trail associate with AMF (Appendix B). Because garlic mustard invaded Trillium Trail in 1992 (L. Smith, *personal communication*), the garlic mustard plants in this young population are likely to have high glucosinolate concentrations (sensu Lankau et al. 2009). We have demonstrated that the detected AITC levels in invaded soil at this site can significantly reduce both AMF spore germination and fungal hyphal abundance (Cantor et al. 2011). Thus, the potential for AMF disruption at this site is high.

Experimental design.—We paired false Solomon's seal plants (N = 18, 9 pairs) based on two factors that can influence physiology: individual plant size and microhabitat (Lambers et al. 2008). We used height as our proxy for plant size because it is highly correlated with total leaf area in false Solomon's seal at Trillium Trail ($R^2 = 0.97$, N = 23; S. Kalisz, *unpublished data*). In matching plants for microhabitat, we ensured that paired plants were no more than 1 m apart and experienced similar tree canopy cover and moisture regimes. We again prepared screen bags filled with garlic mustard tissue collected on site and an empty screen bag served as the control. We cleared the natural leaf litter from the immediate area surrounding each focal false Solomon's seal, and then randomly assigned one plant within each pair to the control, while the other received the garlic mustard treatment. We applied these treatments between 23 and 27 June 2008 and left them in place for two weeks.

Leaf gas exchange measurements.—We determined that 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is a saturating irradiance level for false Solomon's seal (Appendix C) and used this light level for all subsequent physiological measurements because this maximizes our ability to detect water and/or nutrients, rather than light, as limiting resources. Between 15 and 21 June 2008, prior to imposing the treatments, we took pre-treatment physiological measures during which mean daily temperatures ranged from 15–22°C. For each plant we recorded net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration rate (E), and leaf internal CO_2 concentration (C_i) every 15 seconds for 1 minute, yielding 5 measures for each physiological trait. Measurements were averaged to provide pre-treatment estimates for each plant. On 8 and 10 July 2008 mean daily temperatures ranged from 21–24°C and we repeated all physiological measurements to obtain post-treatment estimates.

Statistical analysis.—Because all of the physiological response variables are highly correlated, we first used a multivariate analysis of covariance (MANCOVA; physiology (conductance, transpiration, photosynthesis) = temperature + humidity + size + treatment) to determine the overall effect of garlic mustard on false Solomon's seal physiology. Temperature, relative humidity and plant size were covariates in the analyses, as all of these variables are known to affect one or more of the measured physiological traits (Lambers et al. 2008). All of the F-statistics reported for the MANCOVA were identical, therefore, here we report only the F-statistic for Roy's greatest root, as it leads the most naturally to post hoc tests (Scheiner 2001). Upon obtaining a significant F-statistic, we conducted separate analysis of covariance (ANCOVA) tests for each physiological variable (conductance, transpiration, or photosynthesis = temperature + humidity + size + treatment). Because these were planned comparisons, Type I error correction is not necessary.

After obtaining the physiological response results from the ANCOVAs, we then performed a post hoc analysis on the intercellular CO_2 concentration (C_i) data from both treatments with a Bonferroni correction to account for

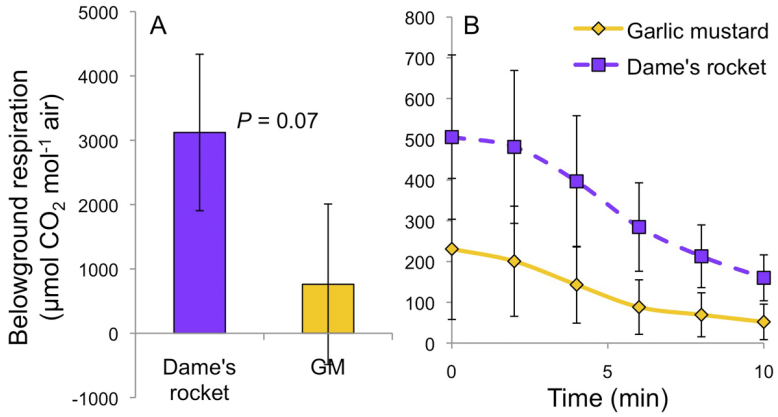

Fig. 3. Belowground respiration from pots treated with garlic mustard (GM) is lower than in pots treated with dame's rocket tissue in our common garden experiment. (A) Total CO_2 captured in a ten-minute sampling period averaged across all plants within a treatment (least squares (LS) means ± 1SE). (B) The mean CO_2 captured per 2-minute sampling interval averaged across all plants within a treatment (LS means ± 1SE).

multiple comparisons ($P = 0.05/4 = 0.0125$; Scheiner 2001). Statistical analyses for the field and common garden experiments were run using SAS version 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

Common garden experiment: belowground respiration

We found that belowground respiration was dramatically lower for garlic mustard-treated relative to dame's rocket-treated (control) pots (Fig. 3A; $F_{3,18} = 2.42$, $P = 0.07$). Over the sampling period, CO_2 levels declined as the residual CO_2 in the soil matrix and current production of CO_2 was pushed through the pot (Fig. 3B). At each time point, CO_2 levels from the garlic mustard-treated pots were significantly lower than those in dame's rocket-treated pots (Fig. 3B). Ambient air temperature and root mass were not significant covariates in this analysis (temperature range = 21–35°C; root mass range = 2.1–8.2 g). Root mass did not differ across treatments (Kruskal-Wallis: df = 2; $P = 0.51$).

Field experiment: plant physiological responses

Prior to treatment, there was no significant difference in physiological rates among false Solomon's seal plants destined for control or garlic mustard treatments (MANOVA; Roy's greatest root, $F = 0.46$, $P = 0.72$). However, after two weeks of treatment, garlic mustard-treated plants displayed significantly lower physiological rates relative to control plants (MANOVA; Roy's greatest root, $F = 3.70$, $P = 0.05$; Fig. 4A–C). Specifically, g_s had the strongest response to garlic mustard treatment: g_s in garlic mustard-treated plants was 36% lower than control plants (Fig. 4B; $F_{4,13} = 13.11$, $P = 0.009$). Similarly, E and A_n were significantly reduced in garlic mustard-treated plants ($F_{4,13} = 8.73$, $P = 0.03$ and $F_{4,13} = 4.58$, $P = 0.05$, respectively). All covariates in the g_s, E and A_n individual analyses were significant ($P < 0.05$), except for the ANCOVA for A_n, where plant size was not significant ($P = 0.47$).

C_i was significantly reduced in garlic mustard-treated plants compared to controls (Fig. 4D; post hoc ANCOVA $F_{4,13} = 10.21$, $P = 0.007$, well below the Bonferroni-corrected $P = 0.0125$). Here, only plant size was a significant covariate ($P = 0.003$).

Discussion

Our field experimental data clearly demonstrate that short-term exposure to garlic mustard tissue, an allelopathic invasive species, can significantly reduce the physiological function of a native understory herb, false Solomon's seal. Stomatal conductance (g_s) in garlic mustard-treated false Solomon's seal adults was reduced by 36%, with concomitant reductions in transpiration (E; 25%) and photosynthesis (A_n; 17%) compared to controls (Fig. 4A–C). This study is

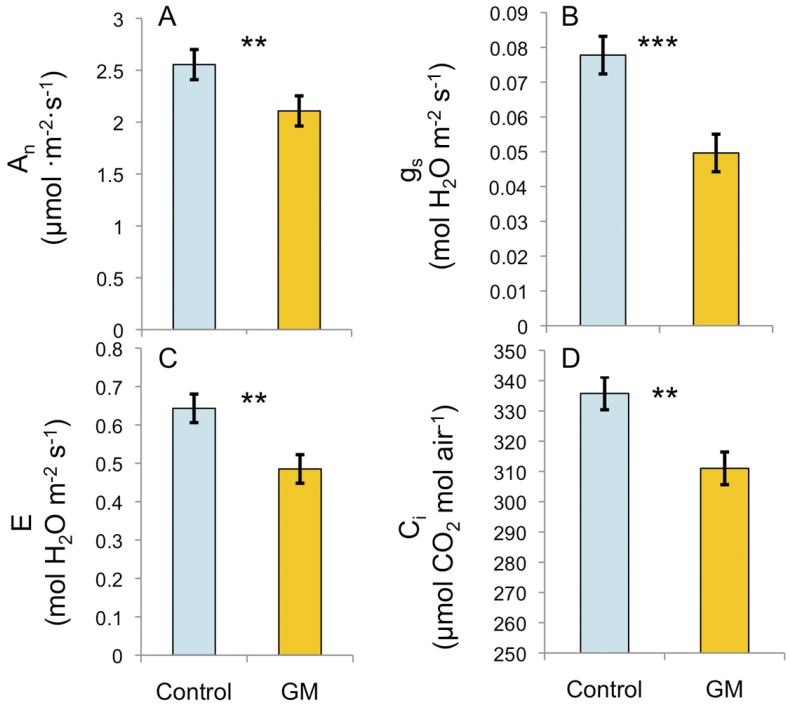

Fig. 4. Garlic mustard tissue negatively affects false Solomon's seal's physiological function. All physiological parameters (A–C), photosynthetic rate (A_n), stomatal conductance (g_s), and transpiration (E) were significantly reduced (*** $P < 0.01$; ** $P \leq 0.05$) after two weeks of garlic mustard (GM) treatment relative to the controls. In a post-hoc analysis (D), intercellular CO_2 concentration (C_i) was also significantly lower (** $P = 0.007$) for garlic mustard-treated plants compared to controls suggesting a potential role of stomatal limitation in the observed A_n declines (least squares (LS) means ± 1SE).

the first to reveal an explicit physiological mechanism underlying an allelopathic species' invasion of an established native plant community.

We attribute the aboveground physiological suppression (Fig. 4A–C) by garlic mustard to allelopathic disruption of AMF external hyphal function. In our common garden experiment, total belowground respiration declined in garlic mustard-treated pots compared to dame's rocket control pots (Fig. 3). Indeed, respiration in the garlic mustard-treated pots did not differ significantly from that measured in the no plant tissue treatment (Appendix A). Thus, while the flush of nutrients from the decomposing dame's rocket tissue stimulated microbial activity above background levels, the garlic mustard tissue did not. These findings are consistent with the prediction that any nutritional benefits of litter decomposition on microbial metabolism are offset in the garlic mustard treatment by loss of AMF external hyphae. AMF are more sensitive to garlic mustard than other soil microbial groups (Lankau 2011) and AMF hyphae can have a disproportionately large impact on overall soil respiration (Johnson et al. 2002). Additionally, our previous work has shown that even low levels of allelochemicals from garlic mustard disrupt AMF spore germination and depress fungal hyphal abundance in field soils (Cantor et al. 2011). Furthermore, we found no direct effects of garlic mustard on false Solomon's seal root mass. Together, our observed reductions in belowground respiration are consistent with a reduction in the function of AMF external hyphae in the garlic mustard treatment.

Our leaf internal CO_2 concentration (C_i) data provide important insight into which soil resource limits false Solomon's seal physiology. We observed significant reductions in both g_s and C_i

in garlic mustard-treated plants (Fig. 4B and D, respectively). This result implicates water limitation as the primary cause of the physiological suppression of false Solomon's seal. Together our data suggest a potential causal chain: garlic mustard inhibits external AMF hyphal function, which limits water availability to false Solomon's seal, which in turn reduces stomatal conductance and lowers photosynthetic rate.

Under natural conditions, native plants in the understory likely experience prolonged periods of exposure to garlic mustard's allelochemicals. In garlic mustard-invaded sites, the continuous presence of garlic mustard rosettes and/or adults, its high seedling and rosette mortality throughout the year (Davis et al. 2006), and a 2-month period of intense leaf litter input as adults senesce (Anderson et al. 1996, Cantor et al. 2011) likely contribute to the year-round release of allelochemicals into the soil. Given the significant physiological suppression shown by our short-term pulse experiments, we anticipate that longer-term exposure of AMF-dependent plants to garlic mustard allelochemicals could affect carbon storage and resource allocation. Indeed, if carbohydrate storage of AMF-dependant species is impacted by season-long declines in physiology, then garlic mustard invasions could have long-term fitness effects on native forest perennial plants. Experiments currently under way in our lab are testing for long-term impacts of mutualism disruption.

Our results have broad implications for understanding how allelopathic invaders can lead to the collapse of established native plant communities in forest understories. Stressful environments diminish mutualism effectiveness (Bronstein 1994, Kiers et al. 2010). Through secretion of allelochemicals in the soil, garlic mustard effectively creates a physiological stress that removes the native plants' mutualistic interactions with AMF. While physical disturbance often facilitates forest invasion (Luken 2003), our data provide a link between novel weapons and native plant declines, implicating physiological disturbance as the intermediate step underpinning invasion. Interference with nutritional mutualisms and subsequent physiological declines in natives may provide both the opportunity for invaders to establish and spread throughout previously stable ecosystems and increase the vulnerability of native species to other stressors that accompany global change.

Acknowledgments

This research was supported by NSF award DEB 0958676 to SK, NSF award IOS 0809171 to SJT, and by a Botany-in-Action Fellowship from Phipps Conservatory, an Andrew Mellon Pre-doctoral Fellowship, Darbaker/Pape grants from the Pymatuning Laboratoy of Ecology (PLE), and a Sigma-Xi Grants-in-Aid of Research to AH. We thank D. Burke for advice about forest mycorrhizae, P. Heckel for photography, T. Harper for image assistance, A. Cantor, C. Kohn, R. Maye, M. Maye, J. Maye, J. Phillips, and M. Ponzo for field and laboratory assistance, and N. Brouwer, R. Spigler and the PEER group at U. Pittsburgh for comments on experimental designs and analyses and H. Maherali for comments. This is PLE contribution 284.

Literature Cited

Anderson, R. C., S. S. Dhillion, and T. M. Kelley. 1996. Aspects of the ecology of an invasive plant, garlic mustard (*Alliaria petiolata*), in central Illinois. Restoration Ecology 4:181–191.

Augé, R. M. 2001. Water relations, drought, and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42.

Barto, E. K., J. R. Powell, and D. Cipollini. 2010. How novel are the chemical weapons of garlic mustard in North American forest understories? Biological Invasions 12:3465–3471.

Bronstein, J. L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology and Evolution 9:214–217.

Brown, P. D. and M. J. Morra. 1997. Control of soilborne plant pests using glucosinolate-containing plants. Advances in Agronomy 61:167–231.

Brundrett, M. C. and B. Kendrick. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Canadian Journal of Botany 66:1153–1173.

Brundrett, M. C. and B. Kendrick. 1990. The roots and mycorrhizas of herbaceous woodland plants. I. Quantitative aspects of morphology. New Phytologist 114:457–468.

Brundrett, M. C. 1991. Mycorrhizas in natural ecosystems. Pages 171–313 *in* A. Macfayden, M. Begon, and A. H. Fitter, editors. Advances in ecological research. Volume 21. Academic Press, London, UK.

Burke, D. J. 2008. Effects of *Alliaria petiolata* (garlic mustard; Brassicaceae) on mycorrhizal colonization and community structure in three herbaceous plants in a mixed deciduous forest. American

Journal of Botany 95:1416–1425.

Callaway, R. M. and W. Ridenour. 2004. Novel weapons: Invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment 2:436–443.

Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. A. Stinson, and J. Klironomos. 2008. Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89:1043–1055.

Cantor, A., A. Hale, J. Aaron, M. B. Traw, and S. Kalisz. 2011. Low allelochemical concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. Biological Invasions. [doi: 10.1007/s10530-011-9986-x]

Davis, A. S., D. A. Landis, V. Nuzzo, B. Blossey, E. Gerber, and H. L. Hinz. 2006. Demographic models inform selection of biocontrol agents for garlic mustard (*Alliaria petiolata*). Ecological Applications 16:2399–2410.

DeMars, B. G. 1996. Vesicular-arbuscular mycorrhizal status of spring ephemerals in two Ohio forests. Ohio Journal of Science 96:97–99.

Demars, B. G. and R. E. J. Boerner. 1995. Arbuscular mycorrhizal development in three crucifers. Mycorrhiza 5:405–408.

Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9–19.

Farquhar, G. D. and T. D. Sharkey. 1982. Stomatal conductance and photosynthesis. Annual Review of Plant Physiology 33:317–345.

Gilliam, F. S. 2007. The ecological significance of the herbaceous layer in temperate forest ecosystems. BioScience 57:845–858.

Hull, J. C. 2002. Photosynthetic induction dynamics to sunflecks of four deciduous forest understory herbs with different phenologies. International Journal of Plant Sciences 163:913–924.

Jakobsen, I., L. K. Abbott, and A. D. Robson. 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytologist 120:371–380.

Johnson, D., J. R. Leake, N. Ostle, P. Ineson, and D. J. Read. 2002. In situ $^{13}CO_2$ pulse-labeling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytologist 153:327–334.

Kiers, E. T., T. M. Palmer, A. R. Ives, J. F. Bruno, and J. L. Bronstein. 2010. Mutualisms in a changing world: an evolutionary perspective. Ecology Letters 13:1459–1474.

Lambers, H., F. S. Chapin III and T. L. Pons. 2008. Plant physiological ecology. Second edition. Springer, New York, New York, USA.

Lankau, R. A., V. Nuzzo, G. Spyreas, and A. S. Davis. 2009. Evolutionary limits ameliorate the negative impact of an invasive plant. Proceedings of the National Academy of Sciences (USA) 106:15362–15367.

Lankau, R. 2011. Resistance and recovery of soil microbial communities in the face of *Alliaria petiolata* invasions. New Phytologist 189:536–548.

Larsen, L. M., J. K. Nielson, and H. Sørenson. 1992. Host plant recognition in monophagous weevils: specialization of *Ceutorhynchus inaffectatus* to glucosinolates from its host plant *Hesperis matronalis*. Entomologia Experimentalis et Applicata 64:49–55.

Luken, J. O. 2003. Invasions of forests in the eastern United States. Pages 283–301 *in* F. S. Gilliam and M. R. Roberts, editors. The herbaceous layer in forests of eastern North America. Oxford University Press, New York, New York, USA.

Matthiessen, J. N. and M. A. Shackleton. 2005. Biofumigation: environmental impacts on the biological activity of diverse pure and plant-derived isothiocyanates. Pest Management Science 61:1043–1051.

Miller, R. M., S. P. Miller, J. D. Jastrow, and C. B. Rivetta. 2002. Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. New Phytologist 155:149–162.

Mitchell, C. E., et al. 2006. Biotic interactions and plant invasions. Ecology Letters 9:726–740.

Mummey, D. L. and M. C. Rillig. 2006. The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288:81–90.

Ögren, E. 1993. Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. Plant Physiology 101:1013–1019.

Reinhart, K. O. and R. M. Callaway. 2006. Soil biota and invasive plants. New Phytologist 170:445–457.

Rice, E. L. 1974. Allelopathy. Academic Press, New York, New York, USA.

Roberts, K. J. and R. C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara and Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. American Midland Naturalist 146:146–152.

Rodgers, V. L., K. A. Stinson, and A. Finzi. 2008. Ready or not, garlic mustard is moving in: *Alliaria petiolata* as a member of eastern North American forests. BioScience 58:426–436.

Scheiner, S. M. 2001. MANOVA: multiple response variables and multispecies interactions. Pages 99–115 *in* S. M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Oxford University Press, New York, New York, USA.

Sivak, M. N. and D. A. Walker. 1986. Photosynthesis *in vivo* can be limited by phosphate supply. New Phytologist 102:499–512.

Smith, S. E. and D. J. Read. 2008. Mycorrhizal symbiosis. Third edition. Academic Press, London, UK.

Staddon, P. L., C. B. Ramsey, N. Ostle, P. Ineson, and A. H. Fitter. 2003. Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of ^{14}C. Science 300:1138–1140.

Stinson, K. A., et al. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. PLoS Biology 4:727–731.

Traveset, A. and D. M. Richardson. 2006. Biological invasions as disruptors of plant reproductive mutualisms. Trends in Ecology and Evolution 21:208–216.

Vaughn, S. F. and M. A. Berhow. 1999. Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). Journal of Chemical Ecology 25:2495–2504.

Vogelsang, K. M. and J. D. Bever. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to invasion. Ecology 90:399–407.

Wang, B. and Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

Wong, S.-C., I. R. Cowan, and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282:424–426.

Wright, D. P., D. J. Read, and J. D. Scholes. 1998. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. Plant, Cell, and Environment 21:881–891.

Appendix A

Table A1. Total belowground respiration (TBR) data (μmol CO_2 mol^{-1} air over 10 min; least square means and SE) for three treatments in common garden experiment at the Pymatuning Laboratory of Ecology.

Treatment	TBR	SE	Sample size
No tissue	10354.57	870.20	12
Dame's rocket	13553.77	869.25	11
Garlic mustard	11162.48	914.78	11

Table A2. Mean belowground respiration (μmol CO_2 mol^{-1} air) at each 2-minute sampling interval for three treatments in common garden experiment at the Pymatuning Laboratory of Ecology.

	0 min		2 min		4 min		6 min		8 min		10 min	
Treatment	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
No tissue	1745.28	120.75	1440.50	93.58	1100.71	59.80	815.28	41.18	609.10	29.07	467.21	21.90
Dame's rocket	2250.68	161.51	1921.82	162.59	1497.50	149.53	1100.00	100.41	822.04	71.21	627.25	51.87
Garlic mustard	1976.18	124.01	1641.23	97.54	1243.78	72.87	903.57	52.71	678.46	45.54	519.12	37.65

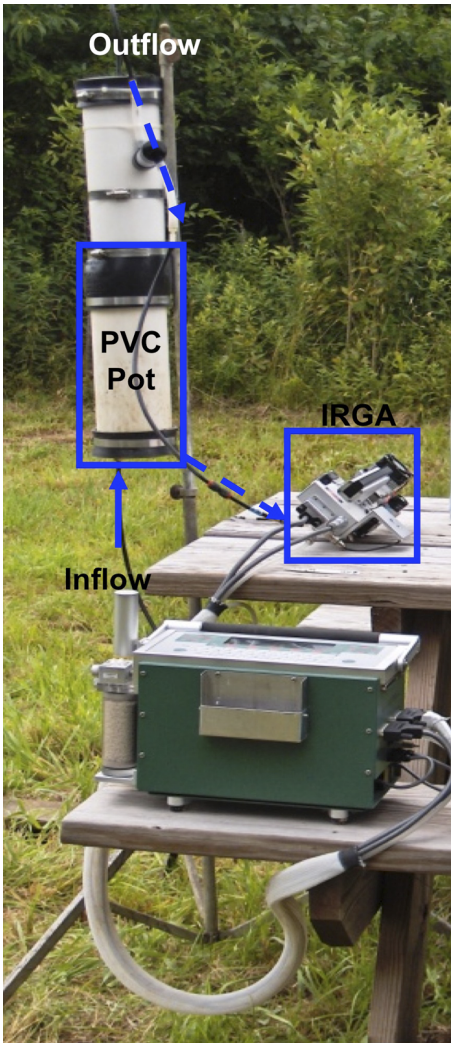

Fig. A1. The sealed air-flow path through the PVC pot and into the IRGA of the LI-COR 6400. CO_2-free air was passed through the bottom of the PVC pot (inflow, solid arrow), forcing the CO_2 in the soil matrix to flow out of the top of the pot into the IRGA (outflow, dashed arrows).

APPENDIX B

Table B1. Mycorrhizal status of 79 herbaceous species at Trillium Trail in Fox Chapel Borough, PA, USA.

Scientific name	Family	AMF status	Native status	Source
Actea pachipoda	Ranunculaceae	AM	Native	1
Alliaria petiolata	Brassicaeae	NM	Invasive	4
Allium tricoccum	Liliaceae	AM	Native	1
Anemone quinquefolia	Ranunculaceae	Unknown	Native	
Aquilegia canadensis	Ranunculaceae	AM	Native	1
Aralia nudicaulis	Araliaceae	AM	Native	1
Arasum canadense	Aristolochiaceae	AM	Native	1
Arisaema triphyllum	Araceae	AM	Native	1
Aster divaricatus	Asteraceae	AM, NM	Native	3
Aster pilosus	Asteraceae	AM, NM	Native	3
Campanula americana	Campanulaceae	AM	Native	3
Cardamine concatenata	Brassicaeae	NM	Native	1
Cardamine diphylla	Brassicaceae	NM	Native	3
Cimicifuga racemosa	Ranunculaceae	AM, NM	Native	3
Circaea quadrisulcata	Onagraceae	AM	Native	1
Claytonia virginica	Portulaceae	NM	Native	1
Clintonia umbellata	Liliaceae	Unknown	Native	
Corylis sempervirens	Papaveraceae	Unknown	Native	
Dicentra canadensis	Papaveraceae	NM	Native	1
Dicentra cucullaria	Papaveraceae	NM	Native	1
Epifagus virginiana	Orobanchaceae	NM	Native	1
Erigeron annuus	Asteraceae	AM	Native	4
Erythronium americanum	Liliaceae	AM	Native	1
Eupatorium purpureum	Asteraceae	AM, NM	Native	3
Eupatorium rugosum	Asteraceae	AM, NM	Native	3
Floerkea proserpinacoides	Limnanthaceaea	NM	Native	2
Galium odoratum	Rubiaceae	AM, NM	Non-native	4
Galium spp.	Rubiaceae	AM	Native	3
Gaultheria procumbens	Ericaeae	Ericoid	Native	4
Geranium maculatum	Geranaceae	AM	Native	3
Glechoma hederacea	Lamiaceae	AM, NM	Invasive	4
Helianthus divaricatus	Asteraceae	Unknown	Native	
Hepatica nobilis	Ranunculaceae	AM	Native	3
Houstonia caerulea	Rubiaceae	Unknown	Native	
Hydrophyllum virginianum	Hydrophyllaceae	NM	Native	1
Impatiens capensis	Balsaminaceae	AM	Native	1
Impatiens pallida	Balsaminaceae	AM, NM	Native	3
Laportea canadensis	Urticaceae	AM	Native	1
Maianthemum canadensis	Liliaceae	AM	Native	1
Maianthemum racemosa	Liliaceae	AM	Native	1
Medeola virginiana	Liliaceae	AM	Native	3
Mertensia virginica	Boraginaceae	NM	Native	3
Microstegium vimenium	Poaceae	AM, NM	Invasive	3
Mitella diphylla	Saxifragaceae	NM	Native	3
Monotropa uniflora	Monotropaceae	Monotropoid	Native	4
Osmorhiza claytonii	Apiaceae	NM	Native	3
Osmorhiza longistylus	Apiaceae	AM	Native	3
Panax trifolius	Araliaceae	Unknown	Native	
Phlox divaricata	Polemoniaceae	AM	Native	1
Phlox stolonifera	Polemoniaceae	Unknown	Native	
Phytolacca americana	Phytolaccaeae	NM	Native	3
Pilea pumila	Urticaceae	AM	Native	1
Podophyllum peltatum	Berberidaceae	AM	Native	1
Polygala paucifolia	Polygalaceae	Unknown	Native	
Polygonatum biflorum	Liliaceae	AM	Native	1
Polygonum cuspidatum	Polygonaceae	AM, NM	Invasive	4
Polygonum persicarium	Polygonaceae	AM, NM	Invasive	4
Ranunculus arbortivus	Ranunculaceae	AM	Native	1
Ranunculus ficaria	Ranunculaceae	AM, NM	Invasive	4
Sanguinaria canadensis	Papaveraceae	AM	Native	1
Sanicula marilandica	Apiaceae	Unknown	Native	
Saxifraga virginiensis	Saxifragaceae	Unknown	Native	
Sedum ternatum	Crassulaceae	NM	Native	3

Table B1. Continued.

Scientific name	Family	AMF status	Native status	Source
Silene virginica	Caryophyllaceae	NM	Native	3
Silene vulgaris	Caryophyllaceae	NM	Invasive	4
Stellaria media	Caryophyllaceae	AM, NM	Invasive	4
Symplocarpus foetidus	Araceae	Unknown	Native	
Thalictrum dioicum	Ranunculaceae	AM	Native	1
Thalictrum thalictroides	Ranunculaceae	AM	Native	2
Tiarella cordifolia	Saxifragaceae	AM	Native	3
Trillium erectum	Liliaceae	AM	Native	1
Trillium grandiflorum	Liliaceae	AM	Native	1
Trillium sessile	Liliaceae	AM	Native	2
Tussilago farfara	Asteraceae	AM, NM	Invasive	4
Urtica dioica	Urticaceae	AM, NM	Native	4
Uvularia perfoliata	Liliaceae	AM, NM	Native	3
Viola blanda	Violaceae	Unknown	Native	
Viola canadensis	Violaceae	AM	Native	1
Viola eriocarpa	Violaceae	AM	Native	1

Notes: Mycorrhizal status was determined through a literature search. Blank cells in the Source column indicate that no articles were found describing the mycorrhizal status of that particular species. Sources are: 1, Brundrett and Kendrick (1988); 2, DeMars (1996); 3, J. M. Trappe, *unpublished data*; 4; Wang and Qiu (2006).

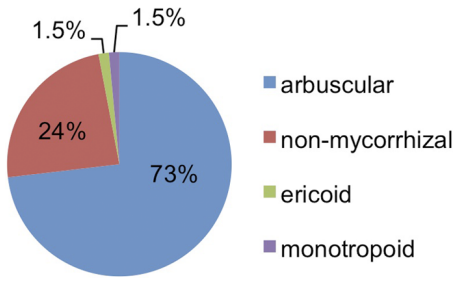

Fig. B1. Pie chart shows the percentage of forest understory herbs at Trillium Trail Reserve that associate with each type of mycorrhizae.

Appendix C

Description of methods used to measure light saturation curves

To determine the appropriate quantum flux density for leaf gas exchange measurements on false Solomon's seal, we calculated light saturation curves in the field on 15 June 2008 at Trillium Trail and 24 June 2009 and 14 July 2009 at PLE between 1000 and 1200 hours using an IRGA. Measurements were made at ambient temperature and humidity, while CO_2 levels were held constant at 400 μmol CO_2 mol^{-1} air using an injector system. We measured photosynthetic rate, A_n at 1500, 1000, 700, 600, 500, 300, 100, 50, 25, 10, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at PLE, and 10 through 700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at Trillium Trail. On each plant, the second distal leaf was placed in the leaf cuvette and allowed to acclimate for 5 minutes before A_n was recorded. This was repeated for each irradiance level. We combined our data with published data for false Solomon's seal (Hull 2002). A non-rectangular hyperbola, which accurately models the shape of light saturation curves (Ögren 1993), was fit to all data points (PROC NLIN, SAS v. 9.2): 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ is a saturating irradiance level for false Solomon's seal and was used for all subsequent physiological measurements.

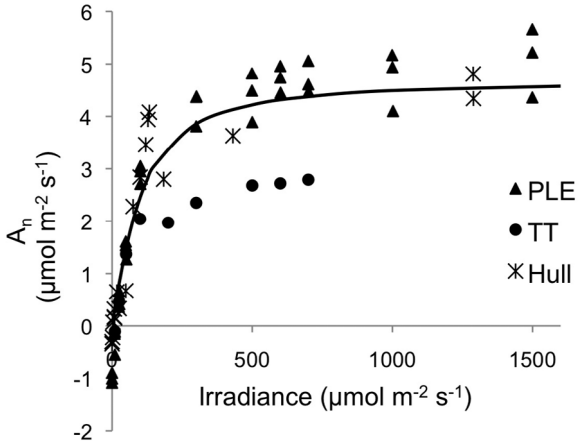

Fig. C1. Light saturation curves for false Solomon's seal. Low observed maximum photosynthetic rates (A_n) and light saturation at low irradiance levels for false Solomon's seal are typical of forest understory herbs. Data from Trillium Trail (TT; 1 plant), Pymatuning Laboratory of Ecology (PLE; 3 plants), and from Hull (2002; 2 plants).