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Responsiveness of certain agronomic weed species to arbuscular mycorrhizal fungi

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Abstract

Arbuscular mycorrhizal fungi (AMF) are plant root symbionts that provide many benefits to crop production and agro-ecosystem function; therefore, management of AMF is increasingly seen as important to ecological farming. Agronomic weeds that form a symbiotic relationship with AMF can increase diversity and abundance of agronomically beneficial AMF taxa. Also, AMF can strongly affect plant community composition, and may thus provide some degree of biological control for weeds. Therefore, relationships between weeds and AMF have a dual significance in ecological farming, but are relatively unexamined. In glasshouse experiments, seedlings of 14 agronomic weed species were grown in the presence or absence of AMF inocula sampled from each of three types of cropping systems: organic, transitional-organic or high-input/conventional. For each weed species, AMF root colonization rates and growth responses to AMF were assessed. On the basis of observed colonization levels, the species were classified as strong hosts (five species), weak hosts (three) and non-host species (six). Among species, biomass responses to AMF were highly variable. Strong hosts showed more positive responses to AMF than weak hosts, although the range of responses was great. Non-hosts did not suffer consistent negative biomass responses to AMF, although strong biomass reductions were noted for certain species–inoculum combinations. Biomass responses to inocula from different cropping systems varied significantly among weed species in one of two experiments. Results suggest that weed–AMF interactions can affect weed community dynamics. We recommend investigation of these interactions in agro-ecosystems that use management methods likely to intensify weed–AMF interactions, such as conservation tillage and cover cropping.

Key words: weed ecology, agro-ecological restoration, mycorrhizae, mycorrhizal responsiveness, weed biocontrol

Introduction

The limited biological diversity characteristic of current high-input 'industrialized' farms is an important cause of many problematic aspects of these agro-ecosystems[1], such as high losses of nutrients and soil, and dependence on pesticide and fertility inputs[2]. Consequently, restoration of biodiversity is an important strategy for mitigating these problems[3]. The plant component of agro-ecosystem biodiversity includes crops and weeds. Current evidence[4] suggests that weed species can provide certain agro-ecological benefits and therefore could be a useful biodiversity component, if beneficial weed species can be identified and managed at tolerable levels of abundance. Most ecological benefits from weeds result from interactions between weeds and organisms at other trophic levels[4]. Relations between weeds and arbuscular mycorrhizal fungi (AMF) are an important class of such interactions[4], because of the agro-ecological importance of AMF.

AMF symbiosis has a variety of effects on crop plant biology and functional ecology, including increased uptake of soil nutrients, protection from drought and other environmental stressors, and protection from soil pathogens[5]. Moreover, AMF evidently can positively affect certain important agro-ecosystem functions. For example, diversity and abundance of AMF is increasingly understood to be an important influence on soil quality and tilth[6].

Interactions between AMF and weeds, in particular, could be agro-ecologically significant for the following reasons. First, weeds may serve to maintain diversity and abundance of agronomically beneficial AMF taxa. Several studies have demonstrated that removal of host weeds from agro-ecosystems causes changes in diversity, abundance and functioning of AMF, reducing beneficial AMF effects

on crop growth[7,8]. Secondly, population dynamics of weed species that host AMF may be affected by interactions with AMF, since these fungi have been shown to affect plant community composition and dynamics[9–13]. In particular, interactions with AMF might serve to increase or maintain populations of weeds that provide some agro-ecological benefit[4], and/or to decrease populations of weeds that are problematic.

In limited studies of relations between AMF and host weed species, AMF symbiosis has been shown to increase growth, seed production and seed quality[14–19]. However, these findings are based on detailed studies of only six agronomic weed species; thus, relations with AMF have scarcely been assessed among agronomic weeds. In particular, little is known of interspecific variation in AMF effects among co-occurring weeds. The magnitude of this variation is important because theory indicates that it strongly influences the potential for AMF to affect weed community composition[20]. In the only comparative study that we are aware of, substantial differences in biomass response to AMF infection were noted in a comparison of two host species, velvetleaf (*Abutilon theophrasti* L.) and yellow foxtail (*Setaria lutescens* L.)[21,22]. In studies of other plant communities[23–26], a large range of plant responses to AMF infection has been observed consistently, suggesting that a similar range may occur among agronomic weeds.

In addition to AMF effects on mutually beneficial interactions with desirable weed species, there is another weed–AMF interaction of potential agronomic significance. AMF can exert strong antagonistic effects on certain non-host plant species, some of which are important agronomic weeds[10,27–32]. Note that these effects are direct, rather than occurring through advantages conferred by AMF to host species growing in mixture with non-hosts. For example, relative growth rate and survivorship of lambsquarters (*Chenopodium album*) was reduced by 42% and 33%, respectively, when grown with AMF[10]. Similarly, in a pilot experiment with six non-host species, we found a consistent pattern of growth inhibition when seedlings of single weed species were exposed to a diverse AMF assemblage. Some of these inhibitory effects were very strong, e.g., a 90% reduction in biomass production by pigweed (*Amaranthus retroflexus* L.), and an 80% reduction in lambsquarters[33]. The mechanistic basis of these antagonistic effects on non-hosts is not clear, although it may result from inhibitory effects of AMF on root development[10]. Many problematic agricultural weeds belong to families that are typically non-hosts[10,34]. Therefore, these observations of antagonistic effects of AMF on non-host species raise the possibility that AMF could provide a broad-spectrum biocontrol measure against non-host weed species.

In the present study, we determined colonization rates and biomass responses to AMF among 14 weed species of agronomic importance, to assess interspecific variation in colonization and growth responses to AMF infection, and AMF antagonism to non-host species. The weed species used in this study were chosen after farmers belonging to a Minnesota (USA) sustainable-agriculture organization were surveyed to identify weeds they found highly problematic. Based on observed patterns of hosting among plant families, we anticipated that eight of these species were potential AMF hosts. To increase the range of inferences regarding weed responses to AMF, we examined weed responses to AMF collected from three different farm management systems: organic, transitioning to organic, and high-input/industrial (hereafter, 'conventional') farms, and repeated our experiment over time.

Materials and Methods

Experimental design

Two similar glasshouse experiments were conducted. The first (Experiment 1) was conducted from May to July 2001; the second (Experiment 2) occurred from November 2001 to January 2002 and used different sources for one inoculum treatment (Table 1). These experiments took place in a single glasshouse, with growth conditions of 27 : 23°C day : night (Experiment 1) and 20 : 19°C (Experiment 2). In Experiment 2, natural sunlight was supplemented with artificial light (400 watt high-pressure sodium lamps, 14–16 h day^{-1}). The light intensity at the bench surface was $\sim$1050 µmol photon $m^{-2}\,s^{-1}$ in Experiment 1, and 600 µmol photon $m^{-2}\,s^{-1}$ in Experiment 2. The experimental design was a randomized complete block with three factors and 13 replicates. Factors were: (1) three sources of soil inocula (organic, transitional, conventional); (2) presence or absence of AMF (+AMF, −AMF); and (3) 14 weed species (Table 2), seeds from Valley Seed Service (Fresno, California, USA).

Soil media and inocula

A Waukegan silt loam was collected from the Rosemount Experiment Station (Minnesota), sieved to remove large roots and stones, mixed 1 : 1 with sand, and pasteurized (2 h at 77°C, repeated after 48 h) for use as a 'base soil' mixture. Soil tests were conducted prior to planting and at weed harvest on samples taken from each of the base soil/treatment mixtures. No deficiencies or toxic nutrient levels due to pasteurization were observed (Table 3). Inoculum soils were collected from central Minnesota farms in each of three categories (organic, transitioning-to-organic, or conventional field management) in August 2000 for Experiment 1, and in July 2001 for Experiment 2. Inocula were put in cold storage to preserve AMF diversity and abundance. Infection levels in these experiments were similar to levels observed in earlier studies using freshly collected inocula, suggesting that inocula retained viability during storage. Soils were dried, sieved and mixed within each category to create inocula. We sampled from four organic (Org) farms; each used long rotations of 5–6 crops and cover crops at various points in the rotations, and tillage for weed management (Table 1). Three transitional farms (Trans) were sampled; each used three-crop rotations

Table 1. Cropping system history and weed presence for inocula source fields.

Experiment	Category	History	Weeds present at sampling time
1,2	Organic	Soy, grain, pasture, corn, small grain, alfalfa, corn	Lambsquarters (*Chenopodium album*), pigweed (*Amaranthus retroflexus*)
1,2	Organic	Alfalfa, alfalfa, corn, grain, soy or forage, grain/fall alfalfa, alfalfa, corn	Velvetleaf (*Abutilon theophrasti*), pigweed (*A. retroflexus*), giant ragweed (*Ambrosia trifida*), lambsquarters (*C. album*), foxtail (*Setaria* spp.)
1,2	Organic	Corn, barley, clover, soybean, corn	Dandelion (*Taraxacum officinale*), orchard grass (*Dactylis glomerata*), sweet clover (*Melilotus officinalis*)
1,2	Organic	Soy, small grain (oats)/underseed legume (alfalfa), alfalfa, corn	Foxtail (*Setaria* spp.)
1	Transitional	Wheat/peas, corn, soy-cultivated, corn	–
1	Transitional	Soy, alfalfa transition, corn	–
1	Transitional	Soy, corn, soy, corn	–
1	Transitional	Soy, wheat, soy, corn	Foxtail (*Setaria* spp.)
2	Transitional	Corn, fallow, vetch/rye mix, corn	Milkweed (*Asclepias* spp.)
2	Transitional	Soy, corn, fall rye, pumpkins	Pigweed (*A. retroflexus*), rye volunteer (*Secale cereale* L.)
2	Transitional	Corn, soy, rye, hay, corn	Green foxtail (*Setaria viridis*), lambsquarters (*C. album*), ragweed (*A. artemisifolia*), pigweed (*A. retroflexus*), sowthistle (*Sonchus arvensis*), orchard grass (*Dactylis glomerata*), wild buckwheat (*Polygonum convolvulus*), hairy vetch (*Vicia villosa*), Canada thistle (*C. arvense*), mustard (*B. kaber*), Shepherd's purse (*Capsella bursa-pastoris*)
1,2	Conventional	Corn, corn, soy, corn	None
1,2	Conventional	Corn, soy, corn	None
1,2	Conventional	Corn, soy, corn	Shepherd's purse (*Capsella bursa-pastoris*), lambsquarters (*C. album*), pigweed (*A. retroflexus*), purslane *(P. oleraceae*), milkweed (*Asclepias* spp.), Canada thistle (*C. arvense*)

and tillage for weed management. Four conventional farms (Conv) were sampled; each used corn–soybean rotations and herbicidal weed management; with one exception (Table 1), all inocula were collected from corn fields.

To create sterile inocula lacking AMF and other soil biota, half of each inoculum was pasteurized (30 min at 77°C, repeated once after 24 h) for control treatments (denoted Org−, Trans−, Conv−; live inocula denoted

Table 2. Mean percent root colonization of agricultural weed species to three inoculum types (organic, Org+; transitional, Trans+; conventional, Conv+) and standard error of the mean.

				Root colonization[1]		
Category	**Species**	**Common name**	**Family**	**Org+**	**Trans+**	**Conv+**
Strong host	*Abutilon theophrasti*	Velvetleaf	*Malvaceae*	15 (4.2)	24 (5.8)	32 (5.5)
	Ambrosia artemisifolia	Ragweed	*Asteraceae*	51 (5.0)	52 (3.5)	51 (5.4)
	Cirsium arvense	Canada thistle	*Asteraceae*	32* (8.8)	55 (8.1)	60* (7.5)
	Solanum nigrum	Nightshade	*Solanaceae*	37 (4.4)	36 (9.7)	26 (7.2)
	Xanthium strumarium	Cocklebur	*Asteraceae*	33 (4.3)	37 (3.4)	43 (4.6)
Weak host	*Agropyron repens*	Quackgrass	*Poaceae*	16 (6.2)	8 (3.5)	4 (1.2)
	Setaria faberi	Giant foxtail	*Poaceae*	3 (1.7)	2 (1.6)	0.3 (0.3)
	Setaria lutescens	Yellow foxtail	*Poaceae*	15 (5.3)	14 (4.9)	4 (1.5)
Non-host	*Amaranthus retroflexus*	Pigweed	*Amaranthaceae*	nd	nd	nd
	Brassica kaber	Mustard	*Brassicaceae*	1.3 (0.5)	0.9 (0.3)	0.7 (0.2)
	Chenopodium album	Lambsquarters	*Chenopodiaceae*	0.8 (0.3)	0* (0)	1.4* (0.4)
	Polygonum lapathifolium	Smartweed	*Polygonaceae*	0 (0)	0 (0)	0 (0)
	Portulaca oleracea	Purslane	*Portulacaceae*	nd	nd	nd
	Rumex crispus	Curly dock	*Polygonaceae*	0.9 (0.0)	0.2 (0.2)	2.3 (1.3)

[1] Mean root colonization (%) and standard error (in parentheses) = root length colonized by AMF in Experiments 1 and 2 combined (data were not significantly different between the two experiments).

* Root colonization levels significantly different between inoculum types as determined by ANOVA ($P < 0.05$).

Table 3. Soil chemistry data of greenhouse soils (base soil/inocula mixtures) sampled at time of weed seed planting (a) and weed harvest (b)[1].

Soil		Bray-P[a] ppm	Bray-P[b] ppm	pH[a]	pH[b]	EC 1:1[a] Mhos cm^{-1} × 10^{-5}	EC 1:1[b] Mhos cm^{-1} × 10^{-5}	OM%[a] Loss on ignition	OM%[b] Loss on ignition	NO_3[a] ppm in solution	NO_3[b] ppm in solution	NH_4[a] ppm in solution	NH_4[b] ppm in solution	P[a] Total P units	P[b] Total P units	K[a] Total K units	K[b] Total K units
Experiment 1	Org +	24	27	7.7	7.8	27	22	1.5	1.4	5.0	< 5	1.3	0.9	0.516	0.521	< 0.707	2.613
	Org −	20	22	7.8	7.7	24	23	1.2	1.1	< 5	< 5	1.3	1.4	0.551	0.291	< 0.707	1.402
	Trans +	21	27	7.9	7.7	52	54	1.4	1.4	< 5	5.0	1.4	1.0	0.792	0.530	< 0.707	2.369
	Trans −	23	19	8.0	8.0	40	28	0.9	1.2	5.0	< 5	1.6	1.0	0.683	0.619	< 0.707	2.116
	Conv +	32	28	7.9	8.1	28	24	1.3	1.1	7.0	< 5	1.3	1.0	0.948	0.784	< 0.707	2.267
	Conv −	31	28	7.8	8.0	38	28	1.7	1.3	7.0	< 5	1.6	1.0	0.748	0.733	< 0.707	2.033
Experiment 2	Org +	12	11	7.6	7.8	18	21	1.06	1.1	5.0	< 5	0.9	< 0.5	0.204	0.313	1.798	1.949
	Org −	15	16	7.6	7.6	20	21	1.41	1.6	< 5	< 5	1.2	< 0.5	0.260	0.448	2.729	2.835
	Trans +	38	27	6.8	7.4	8	15	0.81	1.2	< 5	< 5	1.2	< 0.5	0.416	0.379	2.788	2.034
	Trans −	37	25	6.9	7.4	8	19	0.67	1.2	< 5	< 5	1.4	< 0.5	0.378	0.523	2.391	2.781
	Conv +	28	19	6.6	7.4	24	15	1.02	1.2	14.9	< 5	1.4	< 0.5	0.474	0.237	3.812	1.622
	Conv −	25	17	6.7	7.4	13	17	0.95	1.2	5.9	< 5	1.6	< 0.5	0.273	0.271	3.108	1.573

[1] Specific soil test information can be found at http://soiltest.coafes.umn.edu/ (accessed 5 February 2005).

Org+, Trans+, Conv+). Inoculum soils for the second experiment were collected from the same farm sites, except for transitional farms as these had become fully organic at the time of the second soil collection. Inoculum soils were generally similar across farm types in a variety of parameters (Table 3). The AMF taxa present in experimental inocula were not identified. A 'microbial wash'[35] was prepared from each inoculum type through an 11 µm sieve and applied to all pots (10 ml/pot), specific to inoculum type, to equalize soil–microbial components between pots containing live-soil and control inocula, such that the main difference between the pots of each soil was AMF. We note, however, that the microbial-wash treatment does not fully equalize non-AMF soil biota between live- and killed-soil inocula; in particular pathogenic fungi whose spores are too large to be present in the wash treatment may be present in live inocula but not in controls. Therefore, weed responses to live inocula should be interpreted with some caution, as they may reflect effects of pathogens in addition to AMF. However, roots of all weed plants were examined for visually apparent disease symptoms after harvest. No symptoms were evident, suggesting that substantial pathogen effects did not occur in these experiments.

Plant growth conditions

Pots (7 cm × 30 cm) were filled with a mixture of base and inoculum soils (550 ml : 80 ml; 13% inoculum), and topped with base soil (80 g) to reduce contamination by water splashing and by air movement in the greenhouse. In agroecosystems, particularly where frequently-recommended measures such as conservation tillage and cover crops are used, weed seedlings are likely to interact with AMF mycelia associated with existing plant roots. To model this situation, we developed an AMF mycelium in the soil prior to weed seeding. After pots were filled with experimental soil mixes, an AMF host species, red clover (*Trifolium pratense*, inoculated with a commercial rhizobium strain, 'Nitragin', LiphaTech, Inc. Milwaukee, Wisconsin), was planted and thinned to 5 plants per pot 7–10 days after planting (DAP) and allowed to grow for 42 days to develop an AMF mycelium in pots containing live inoculum. Clover shoots were harvested after 6 weeks by cutting plants at the soil surface or just under the surface; roots were undisturbed. Weed seeds were then planted and thinned at 7–14 DAP to one seedling per pot. After 42 DAP all plants were harvested. Roots were washed to remove soil and clover roots. Clover roots were easily distinguishable and separated from weed roots. All harvested material (plant shoots and roots) was dried at 70°C for 3–5 days, and total dry biomass was determined. Effects of AMF on biomass production were estimated by calculating mycorrhizal response[25] values, defined as the difference between inoculated and uninoculated biomass means, expressed as a percentage of the inoculated biomass mean.

Table 4. Mycorrhizal responsiveness of agricultural weed species to three inoculum types (organic, Org; transitional, Trans; conventional, Conv) in Experiments 1 and 2.

			Mycorrhizal responsiveness					
			Experiment 1[1]			Experiment 2[1]		
Category	Species	Common name	Org	Trans	Conv	Org	Trans	Conv
Strong host	*Abutilon theophrasti*	Velvetleaf	9	4	20	−179*	29	55
	Ambrosia artemisifolia	Ragweed	45**	54**	49**	14	26	25*
	Cirsium arvense	Canada thistle	51	68*	67	20	42	27
	Solanum nigrum	Nightshade	4	12	−1	−14	−28	−15
	Xanthium strumarium	Cocklebur	nd	nd	nd	38	−74	18
Weak host	*Agropyron repens*	Quackgrass	−51	−24	−106	−2	−6	−21
	Setaria faberi	Giant foxtail	24	22	16	−23	−32	−45
	Setaria lutescens	Yellow foxtail	−15	31	−10	59*	−54*	−87
Non-host	*Amaranthus retroflexus*	Pigweed	17	42	−24	nd	nd	nd
	Brassica kaber	Mustard	6	23	16	−1	12	−76**
	Chenopodium album	Lambsquarters	13	26	32	68	63	−116
	Polygonum lapathifolium	Smartweed	31	−57	9	28	42	44
	Portulaca toleracea	Purslane	21	−11	−3	−83	58	−51
	Rumex crispus	Curly dock	−85*	12	−24	−44	−19	−42

[1] Mycorrhizal response (%) = [(biomass in AMF presence (g) – biomass in AMF absence)/biomass in AMF presence] × 100.
Total biomass of inoculated plant significantly different from non-inoculated control as determined by ANOVA (* $P < 0.05$; ** $P < 0.005$).
nd signifies no data available.

AMF colonization assays

To verify effectiveness of live inocula and absence of contamination in killed-inoculum treatments, colonization rates were assessed in velvetleaf (*A. theophrasti*), a weed species that typically forms abundant mycorrhizae[22] (Table 2). A sample of roots from velvetleaf plants grown in all soil treatments were stained using aniline blue[36] and colonization rates were determined by counting the number of arbuscules, vesicles and hyphae present in each root segment under 200 × magnification using the magnified intersection method[37]. To confirm host/non-host status and estimate AMF colonization rates for each host species, similar counts were taken on roots sampled from five plants of each weed species, grown in the inoculated treatments of both experiments (Table 2).

Statistical analyses

ANOVA was used to test treatment main effects and interactions. Separate analyses were carried out for host and non-host species, and for Experiments 1 and 2. An indication of the significance of individual weed species responsiveness to AMF inoculation in each experiment (Table 4) was obtained by doing a *t*-test of biomass differences between AMF+ and AMF- treatments for each weed species in each inoculum[27]. All analyses were done using SAS[38].

Results

AMF colonization

No AMF contamination of control pots was observed in either experiment. Root colonization rates (Table 2) were not significantly different between experiments for any species, though they did vary sharply among species. Based on observed colonization rates, host weed species were found to comprise two groups. Five species were relatively strong hosts, each with mean colonization rates ≥29%. These species were velvetleaf (*A. theophrasti*), ragweed (*Ambrosia artemisifolia*), Canada thistle (*Cirsium arvense*), nightshade (*Solanum nigrum*), and cocklebur (*Xanthium strumarium*). The three other host species, giant foxtail (*Setaria faberi*), yellow foxtail (*S. lutescens*) and quackgrass (*Agropyron repens*) showed colonization rates <16%, and were therefore categorized as 'weak' hosts. Other weed species were regarded as non-hosts. These were pigweed (*A. retroflexus*), mustard (*Brassica kaber*), lambsquarters (*C. album*), smartweed (*Polygonum lapathifolium*), purslane (*Portulaca oleracea*), and curly dock (*Rumex crispus*). Though mustard, lambsquarters and curly dock all were found to have low levels of root colonization of typical morphology (between 0.2 and 2.3%), these species were classified as non-hosts in analyses of growth responses because they have previously been reported only as non-mycotrophic[7,10,25,39]. Likewise, pigweed and purslane were classified as non-hosts, though there was

insufficient root biomass to calculate colonization rates for these species. Generally, colonization levels were fairly consistent across inocula from different cropping systems (Table 1), with the exceptions of Canada thistle (*C. arvense*) and lambsquarters (*C. album*).

Host weed growth responses to AMF inoculation

In Experiment 1, mycorrhizal response values of host species, [(biomass in AMF presence (g) − biomass in AMF absence)/biomass in AMF presence] × 100 (Table 4), indicated large and strongly significant (Table 5) differences in seedling growth responses to mycorrhizal colonization. In this experiment, strong-host species generally benefited more strongly from AMF colonization (mycorrhizal response values ranged from −1 to 68%; Table 4) than did weak-host species (mycorrhizal responses ranged from −106% to 31%), although a large range of values occurred within each host type (Table 4). Only the strong-host species ragweed *(A. artemisifolia*) and Canada thistle (*C. arvense*) showed unequivocally positive responses to AMF colonization, indicating that colonization was beneficial to seedling growth. Mycorrhizal responses of all other host species were not significantly different from zero, and negative estimates were common, especially among weak-host species. Therefore, most host species showed little sign of mycorrhizal dependency (i.e., dependence on AMF symbiosis for growth) under these experimental conditions, despite abundant colonization in certain cases (Table 2).

Weed seedling growth was somewhat reduced in Experiment 2, in which temperatures and light levels were lower (see methods) than in Experiment 1; mean seedling biomass was 0.28 and 0.18 g in Experiments 1 and 2, respectively. In the conditions of Experiment 2, there were no statistically significant differences in mycorrhizal responsiveness among host weed species (Table 5). However, in contrast to Experiment 1, there was significant heterogeneity among weed species in their responses to inoculation from different sources (Table 5). This interaction arose because certain host species, including velvetleaf (*A. theophrasti*), yellow foxtail (*S. lutescens*), and cocklebur (*X. strumarium*), varied sharply in responsiveness across inoculum sources. For example, responsiveness of velvetleaf ranged from −179% in organic inoculum to 55% in conventional soil (Table 4).

Table 5. ANOVA of biomass response to weed species, inoculation (AMF factor) and inoculum source (soil factor), for weed species grouped into host and non-host categories; non-significant effects denoted ns.

		Experiment 1			Experiment 2		
Category	**Source of variation**	**df**	***F***	***P***	**df**	***F***	***P***
Host	AMF	1	16.2	0.0001	1	0.1	ns
	Species	6	43.7	0.0001	7	52.0	0.0001
	Soil	2	18.7	ns	2	1.2	ns
	AMF × Species	6	3.4	0.0027	7	1.0	ns
	AMF × Soil	2	0.5	ns	2	2.4	0.10
	Species × Soil	12	2.1	0.02	14	0.9	ns
	Species × Soil × AMF	12	0.4	ns	14	2.7	0.001
Non-host	AMF	1	2.71	0.103	1	0.4	ns
	Species	5	24.8	0.0001	4	38.4	0.0001
	Soil	2	4.8	0.012	2	1.3	ns
	AMF × Species	5	1.2	ns	4	1.5	ns
	AMF × Soil	2	0.5	ns	2	2.3	0.11
	Species × Soil	10	0.6	ns	8	0.6	ns
	Species × Soil × AMF	10	1.3	ns	8	0.8	ns

Non-host responses to AMF presence

Overall, non-host biomass production was not significantly affected by AMF (Table 5). In particular, there was no indication of strong, consistent negative AMF responses by the non-host weed species in either experiment (Table 4), although in a few instances strong and significant negative mycorrhizal responses were observed, e.g., an 85% reduction of curly dock (*R. crispus*) biomass in the organic inoculum of Experiment 1, and 76% reduction of mustard (*B. kaber*) in conventional inoculum of Experiment 2. These negative responses fall below the range of non-significant negative responses that was observed in a number of host species (Table 4).

Discussion

We found substantial variation in mycorrhizal responsiveness and hosting behavior among 14 weeds of temperate field-crop agro-ecosystems. This finding parallels results of previous studies in other ecosystems. Among a group of hosts from a prairie plant community, mycorrhizal responsiveness ranged from 24.5% to 99.4% among warm-season grasses, and ranged from −4.9% to −33.3% among cool-season grasses[25]. Similarly, host species from early-successional temperate grasslands[24,40–42] varied widely in responsiveness. Given the wide range of biomass responses observed among these weed species, it is possible that AMF could have a substantial effect on the dynamics of weed communities containing these species, particularly in agro-ecosystems that minimize soil disturbance and mechanical weed control for soil and water conservation purposes. In such agro-ecosystems, AMF are likely to be more diverse and abundant, and other effects on weed community dynamics (e.g., those of selective tillage) are likely to be less.

We also found that mycorrhizal responsiveness was generally less positive under the reduced light and temperature levels of Experiment 2, a result consistent with the hypothesis that AMF may typically provide lower net benefits to hosts when photosynthesis is restricted[29]. Therefore, our results suggest that weeds under a crop canopy might respond differently to AMF than weeds that

are above that canopy. AMF interactions with sub-canopy weed plants may be a very important facet of weed–AMF interactions, since small, sub-canopy weed plants can produce considerable numbers of seeds[43] and are probably important to the persistence of populations of many weed species. An adequate assessment of AMF effects on weeds requires characterization of variation in AMF–weed relations across relevant ranges of environmental factors. Differences among cropping systems in management or other factors have been shown to cause functional differences in relations between AMF and crop plants[44]. However, we observed little evidence that AMF from different cropping systems had differential effects on weed growth or colonization, although large differences were observed in biomass responses to different inocula in a few cases.

Our finding of considerable variation among common agronomic weed species in colonization and biomass response to AMF raises questions about the effect of weed communities on AMF diversity and abundance. Although critical data on this point are lacking, the higher levels of AMF root colonization observed in strong host species may be associated with higher levels of AMF biomass and spore production by some or all colonizing AMF species in strong hosts, relative to biomass and spore production of these species when colonizing weaker host species. If so, then a weed community composed predominantly of strong hosts would be expected to cause different AMF community dynamics than a weed community of weak hosts. Given that several studies have shown that experimental alteration of weed communities can reduce beneficial AMF effects on crop growth[7,45], attention should focus on identifying weed species that can play an important role in maintaining the beneficial effects of AMF on crop growth and other desirable agro-ecosystem attributes, such as good soil tilth.

We did not observe consistent antagonistic effects of AMF on non-host weed species. Some strong antagonistic effects were observed, but there was no indication of any broad-spectrum biocontrol effect. These findings contrast distinctly with results from our preliminary experiment[33], in which we observed moderate to very large biomass reductions in response to AMF among the same group of non-host species examined in the present experiment. Similarly, a group of eight non-host species (mostly ruderal weeds) showed consistent negative biomass and survivorship responses to AMF[10]. Given the potential value for weed biocontrol of AMF non-host antagonism, it is important to consider reasons for the discrepancy between our results and previous observations of strong antagonistic effects. If non-host antagonism occurs via damaging effects of AMF on seedling roots, as proposed by Francis and Read[10], then it is plausible that non-host weed seedlings may be vulnerable to AMF antagonism only during a certain early phase of development. Our preliminary experiment was carried out in greenhouse conditions similar to those of Experiment 2, and seedling growth rates were clearly slower than in Experiment 1 or 2. Relative to our experiment, the protocol used by Francis and Read[10] may have achieved a higher density or physiological activity of AMF mycelia, by using a mesh screen to create a root-free soil zone containing mycelia supported by living plants. Similarly, seedling growth rates in our current experiment were greater than those in our preliminary experiment. Thus, in previous experiments showing strong antagonism, weed seedlings could have been exposed to stronger AMF effects, or exposed for a longer period, than was the case in our present experiments, and these differences may explain the lack of consistent antagonistic effects in these latter experiments.

We particularly encourage further investigations of weed–AMF communities in agro-ecosystems that make use of conservation tillage and cover cropping techniques. Weed–soil microbiota interactions are likely to be generally stronger in such situations; e.g., weed-suppressive bacterial activity was increased by reductions in tillage intensity[46]. Such management approaches are likely to intensify weed–AMF interactions, including possible weed biocontrol effects, and increases in abundance of beneficial weeds and AMF taxa.

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