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Mycorrhizae, biocides, and biocontrol. 2. Mycorrhizal fungi enhance weed control and crop growth in a soybean–cocklebur association treated with the herbicide bentazon [1]

G.J. Bethlenfalvay *, R.P. Schreiner, K.L. Mihara, H. McDaniel

USDA–ARS HCRL, 3420 NW Orchard Ave.Corvallis, OR 97330 USA

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Abstract

The mediation of herbicide effects on weed–crop combinations by vesicular–arbuscular mycorrhizal (VAM) fungi is essentially unknown. We conducted a greenhouse study to determine if VAM fungi, whose hyphae interconnect the roots of adjacent plants, modify herbicide effects by enhancing nutrient fluxes between associated plants. Soybean (*Glycine max* (L.) Merr.) and common cocklebur (*Xanthium strumarium* L.) plants were grown together in pots (1.5 L) in a high-P (28 mg kg^{-1}) soil. They were sprayed with the herbicide, bentazon (BEN, 3–isopropyl–1H–2,1,3–benzothidiazine–(4)3H–one 2,2–dioxide), at dose rates of 0, 1/3, 2/3, 3/3, and 4/3 of field recommendation (FR, 1.12 kg a.i. ha^{-1}) while in the 7- to 8-leaf stage. Labelled N (1 mL of 100 mM $^{15}NH_4NO_3$, 98 atom percent ^{15}N) was applied to the cocklebur leaves 4 days before spraying and then assayed in the soybean leaves at harvest. Growth and nutrient contents of +VAM soybean shoots were enhanced only at the intermediate FR levels, while shoot growth in adjacent cocklebur was inhibited beyond the extent measured in −VAM plants. Labelled N was at natural abundance in both +VAM and −VAM soybean leaves at 4/3–FR, but at 1/3–FR to 3/3–FR ^{15}N abundance was significantly higher in +VAM than in −VAM plants. These results suggest that shifts in source–sink relations occurred both within each plant and between plants as a result of the selective stress imposed on cocklebur. Moreover, this shift in competitiveness permitted a VAM-mediated flux of nutrients from weed to crop.

Keywords: *Glycine max*; Inter-plant nutrient transfer; Mycorrhiza; Source–sink relations; Weed control; *Xanthium strumarium*

1. Introduction

Weed control strategies must effectively balance the use of herbicides with environmental protection, necessitating a search for a better understanding of interactions between fertilization, biocides, cultivation, and crop rotation (Senft, 1993). In the past, this search has often led to innovative mechanical weed-control measures (Buhler et al., 1992; Klonsky and Livingston, 1994; Wyse, 1994). However, cultivation disrupts soil structure, along with the network of vesicular–arbuscular mycorrhizal (VAM) hyphae that inter-connects roots of plants within the community

* Corresponding author: Tel. 541 750 8785 or 8760; Fax. 541 750 8764.

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and with the soil system (Miller and Jastrow, 1992). Damage to the VAM soil mycelium by soil disturbance (McGonigle and Miller, 1993) or fallowing (Thompson, 1994) can impair plant growth by interfering with mycorrhiza-mediated nutrient transport between plant and soil (Johansen et al., 1993) and between plants (Martins, 1993).

In weed–crop relationships, the direction of nutrient movement is important. The flux of nutrients from soil to plant in a cocklebur–soybean association will predominate in the direction of the stronger sink, the cocklebur root (Wilkerson et al., 1990; Bethlenfalvay et al., 1995). Nutrient fluxes between these plants are likely to depend both on the physiological balance between the associated plants (Frey and Schüepp, 1992) and the strengths of the respective sinks (Bethlenfalvay et al., 1991). If VAM fungi alter the outcome of competitive interactions among plants (Allen, 1991; Goodwin, 1992), then VAM mycelia shared by such plants may influence stress-mediated changes in competitive ability.

The purpose of this experiment was to test the hypotheses that (1) a strongly competitive but herbicide-susceptible plant (cocklebur) may become a source of nutrients to an associated herbicide-tolerant plant (soybean) upon treatment with an herbicide, (2) nutrient transfer depends on herbicide dose rate between source (weed) and sink (crop) plants and is measurable as increased growth enhancement of the sink and growth inhibition of the source plant, and (3) the transfer can be detected by VAM-enhanced transfer of label (^{15}N) from the source to the sink.

2. Materials and methods

2.1. Experimental design and statistics

The experiment had a 5 × 2 factorial arrangement of treatments with six replications. The experimental units (1 crop and 1 weed plant grown together in a pot) were arranged in a completely random manner and rotated weekly. Factors were herbicide application (five dose rates) and the presence (+VAM) or absence (−VAM) of VAM root colonization. The results were evaluated by analysis of variance. Individual treatment differences were tested for significance by Student's *t*-test. Competitive relationships between crop and weed plants within treatments were subjected to regression analysis. Instead of an arbitrary indication of significance ($p >$ or ≤ 0.05), actual probability values (p) were shown, to permit individual interpretation by the reader (Nelson, 1989). We may interpret differences as significant up to $p = 0.1$.

2.2. Biological materials, soil, and growth conditions

Soybean (*Glycine max* (L.) Merr., cv. Hobbit) and cocklebur (*Xanthium strumarium* L.) seeds were established in 1.5 L plastic pots filled with a silty-clay loam soil high in plant-available P (28 mg kg^{-1}) as described previously (Bethlenfalvay et al., 1995). The high P-content soil was chosen to maximize herbicide–VAM interactions and minimize host nutritional responses to the VAM fungi. A mixture (40 mL of each) of soil inocula of the VAM fungi *Glomus etunicatum*, *Glomus leptotichum*, and *Glomus mosseae* (isolates UT316A–2, FL184–1, and CA110, respectively, of the International Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi, University of West Virginia, Morgantown) was mixed evenly into the soils of the VAM treatments. Washings of the inocula free of VAM propagules (45 μm sieves) were applied to the soils of all treatments. Plants were grown in a greenhouse, with temperatures between 18 and 28°C, and light supplementation (16 h, 400 μmol m^{-2} s^{-1} PAR at soil surface) by 1000-W metal halide lamps. Plants were watered once a week for the first 3 weeks and twice a week thereafter. They were fertilized as needed with a solid (20–0–15, N–P–K) fertilizer.

2.3. Herbicide application

Plants were sprayed with bentazon 26 days after planting (7–8 leaf stage) as described previously (Bethlenfalvay et al., 1995). The field recommendation (FR) for a foliar spray of bentazon to control cocklebur (2–10 leaf stage) was 1.12 kg AI ha^{-1} (2 pints $acre^{-1}$ of liquid formulation, 42% active ingredient (AI)). The rates applied were 0–FR, 1/3–FR, 2/3–FR, 3/3–FR, or 4/3–FR (0, 0.37, 0.75, 1.12, or 1.50 kg AI ha^{-1}). The spray also contained 2.3 mL L^{-1} of a surfactant oil. Control plants (0–FR) were sprayed with the surfactant only.

2.4. Plant responses

Plants were harvested 2 weeks after spraying (40 days after planting). Shoots were excised and weighed immediately. Dry weights were determined after drying for 3 days at 70°C. Soybean leaf nutrient concentrations were determined by A and L Western Agricultural Laboratories, Modesto, CA, according to "Official Methods of Analysis" (AOAC, 1980). Colonization of intermingled soybean and cocklebur roots by VAM fungi was determined by the grid-line intersect method (Ambler and Young, 1977). Percent changes [(VAM − nonVAM)/(nonVAM) × 100] reflect the effects of VAM colonization on dry mass, ^{15}N abundance, or soybean leaf nutrient status. Leaf dry-weight and nutrient-content comparisons were made between associated soybean plants (this experiment) and individual soybean plants grown under identical conditions reported previously (Bethlenfalvay et al., 1995) as a measure of competition between soybean and cocklebur plants.

2.5. Nitrogen transfer

Labelled N (1 mL of 100 mM $^{15}NH_4NO_3$, 98 atom percent ^{15}N, Sigma Chemical Co., St. Louis, MO) was applied to the upper surfaces of all leaves of each cocklebur plant 22 days after planting (6–7 leaf stage). To facilitate foliar uptake of N, the leaves were moistened with distilled water applied with an atomizer twice a day for 4 days following application. Care was taken to avoid dripping of labelled N to the soil or its direct transfer from leaf to leaf. Plants were sprayed with the herbicide 4 days after ^{15}N application.

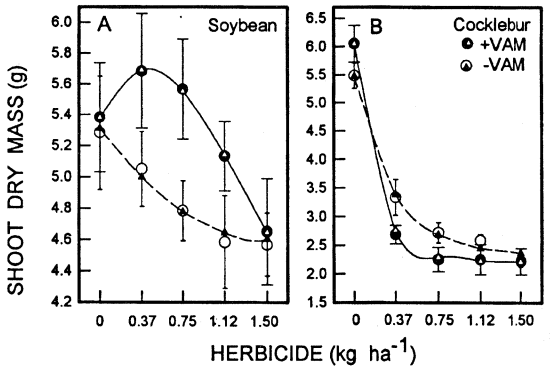

Fig. 1. Shoot dry masses of soybean (A) and cocklebur (B) plants treated with the herbicide bentazon. Plants were colonized by vesicular–arbuscular mycorrhizal fungi (+VAM, ●) or were nonmycorrhizal (−VAM, ○). Leaves were sprayed with the herbicide at dose rates of 0, 1/3, 2/3, 3/3, or 4/3 (0, 0.37, 0.75, 1.12, or 1.50 kg ha^{-1}) of field recommendation. Soybean data points were fitted to third-degree polynomial equations (best fit), and those of cocklebur to the equation $y = A - B(1 - e^{-kx})$. Circles represent actual data (means and SE of six replications), triangles indicate values derived from the equations.

3. Results

3.1. Plant growth and dry matter content

The effects of bentazon on +VAM and −VAM plants diverged both in soybean and cocklebur (Table 1). In soybean, +VAM and −VAM plants had similar shoot biomass at the 0–FR and 4/3–RF dose

Table 1
Analysis of variance of soybean and cocklebur plant parameters. The effects were due to root colonization by vesicular–arbuscular mycorrhizal (VAM) fungi and treatment with the herbicide bentazon (BEN)

Parameter	Soybean			Cocklebur		
	VAM	BEN	VxB	VAM	BEN	VxB
	p					
Shoot						
Dry mass	0.030	0.075	0.625	0.275	< 0.001	0.077
Fresh mass	0.193	0.056	0.617	0.163	< 0.001	0.811
Dry/fresh mass ratio	0.178	0.057	0.550	0.281	0.002	0.167
15N Abundance atom percent	0.022	0.008	0.385			

Interactions are denoted V × B.

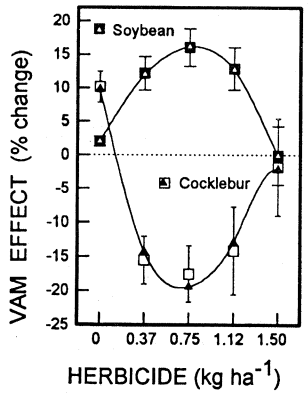

Fig. 2. Modification of herbicide effects on soybean and cocklebur shoot dry masses by VAM fungi. The VAM effect is expressed as percent change in the response variable due to VAM colonization [100 × (VAM − nonVAM)/nonVAM]. Data points were fitted to third-degree polynomial equations (best fit). Squares represent actual data (means and SE of six replications), triangles indicate values derived from the equations.

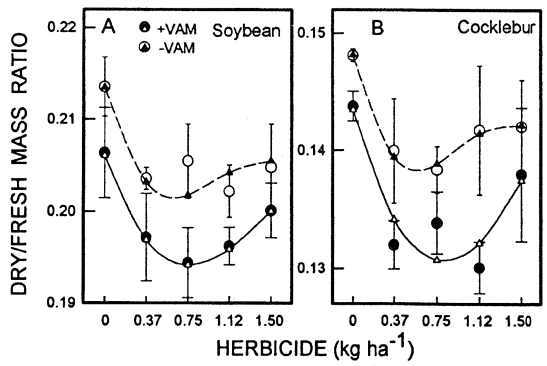

Fig. 3. Shoot dry/fresh mass ratios of soybean (A) and cocklebur (B) plants: The effects of treatment with the herbicide bentazon and root colonization by vesicular–arbuscular mycorrhizal (VAM) fungi. Data points were fitted to third-degree polynomial equations. Circles represent actual data of −VAM (○) and +VAM (●) plants (means and SE of six replications); triangles indicate the best-fit equations.

rates, but at intermediate rates +VAM shoots had more dry mass than −VAM shoots (Fig. 1A). Best fit of the soybean shoot dry mass data was with cubic polynomials (+VAM, $y = 0.03x^3 - 0.32x^2 + 0.60x + 5.38$, $r = 0.449$; −VAM, $y = 0.02x^3 - 0.07x^2 - 0.18x + 5.28$, $r = 0.421$). The +VAM curve showed a maximum within the data range, while the −VAM curve suggested a minimum near the high dose rate. These deviations from linearity indicate that soybean shoot-growth responses to bentazon were dose-dependent: positive for +VAM (enhancement) and negative for −VAM (inhibition) at intermediate doses.

The effects of VAM colonization in cocklebur (Fig. 1B) were the opposite of those in soybean. The −VAM cocklebur plants had greater shoot dry masses at intermediate herbicide doses than the +VAM plants (1/3–FR, $p = 0.048$; 2/3–FR, $p = 0.062$), while at the highest dose rate (4/3–FR), the +VAM vs. −VAM difference was not significant ($p > 0.1$). The response data of cocklebur shoot dry mass fitted the equation: $y = A - B(1 - e^{-kx})$, with A as shoot dry mass at 0–FR ($x = 0$, $e^{-xk} = 1$), k as a decay constant, and 'A–B' as the y-value approached asymptotically by shoot dry mass with increasing dose rates ($x \rightarrow \infty$, $Be^{-xk} \rightarrow 0$) of the herbicide (i.e. the level of maximum control).

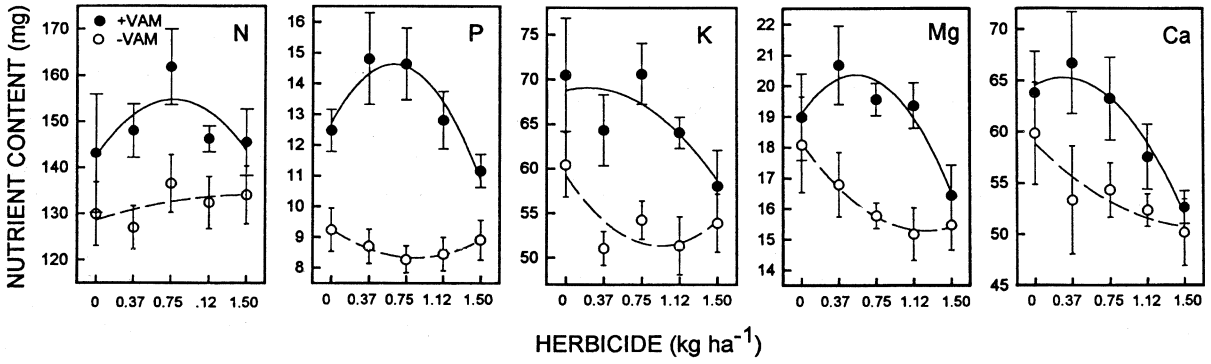

Fig. 4. Nutrient contents of soybean leaves, as influenced by treatment with the herbicide bentazon and colonization by vesicular–arbuscular mycorrhizal (VAM) fungi. Circles represent data of −VAM (○) and +VAM (●) plants as means and SE of six replications.

A representation of soybean and cocklebur shoot dry-mass data as percent change due to VAM effects [(VAM − nonVAM)/nonVAM × 100] showed the strongest BEN effect (growth enhancement) to occur in soybean at 2/3–FR (Fig. 2). This maximum in the soybean response curve coincided with the greatest VAM-mediated inhibition in cocklebur, with a minimum also at 2/3–FR. Best-fit curves were third-degree polynomials (soybean, $y = 0.3x^3 - 2.0x^2 + 12.4x + 2$; cocklebur, $y = -1.2x^3 + 13.1x^2 - 36.0x + 9.8$).

Shoot dry matter content (dry/fresh-mass ratio) was also a function of herbicide dosage, showing minima corresponding to the rate of 2/3–FR in both soybean (Fig. 3A) and cocklebur (Fig. 3B). Best fit for these curves was the cubic polynomial. As observed previously with individual plants (Bethlenfalvay et al., 1995), shoot dry matter content was smaller in +VAM than in −VAM plants, but the difference was greater proportionately in cocklebur than in soybean and greatest at intermediate herbicide application rates.

Since the roots could not be separated, percent VAM colonization was determined on the intermingled roots (means and ± SE: 0–FR, 40 ± 2; 1/3–FR 45 ± 2; 2/3–FR, 46 ± 3; 3/3–FR, 43 ± 2; and 4/3–FR, 46 ± 3). While the differences between VAM colonization of the 0–FR roots and those of the other herbicide treatments were not significant ($0.13 > p > 0.10$), there was a trend towards higher colonization in the roots of all herbicide-treated plants.

3.2. Nutrients

Nutrient contents in soybean leaves showed herbicide-dose responses similar to those of shoot dry mass, with maxima by the +VAM plants and minima (except for N) by the −VAM plants at intermediate application rates (Fig. 4). While VAM effects on leaf nutrient contents were highly significant, BEN effects were significant only for Ca, K, and Mg (Table 2). The lack of significant VAM × BEN interactions (except for P) indicated that the responses of nutrient contents to VAM were similar at each level of BEN (Table 2). For P, however, there was a significant difference in the magnitude of the VAM response at different dosages of BEN.

Table 2
Analysis of variance of soybean leaf nutrient contents and concentrations. The effects were due to root colonization by vesicular–arbuscular mycorrhizal (VAM) fungi and treatment with the herbicide bentazon (BEN)

✠vertical join✠Nutrient	Content			Concentration		
	VAM	BEN	V × B	VAM	BEN	V × B
	p					
N	< 0.001	0.419	0.840	0.601	0.001	0.540
P	< 0.001	0.254	0.059	< 0.001	0.009	0.026
K	< 0.001	0.093	0.348	< 0.001	0.104	0.021
Mg	< 0.001	0.065	0.332	0.058	0.086	0.928
Ca	0.006	0.060	0.619	0.676	0.711	0.662

The evaluation of VAM-mediated changes in leaf dry mass and nutrient concentrations shows that there were no nutrient dilution or concentration effects (sensu Jarrell and Beverly, 1981) as a result of the treatments. The actual condition observed here was described by these authors as "synergism" and was defined as simultaneous increases in dry mass, nutrient content, and nutrient concentration (or no change in the latter). Such synergistic responses occurred in all three response variables at the intermediate herbicide dose rates of 1/3–FR, 2/3–FR, and 3/3–FR (Fig. 4, Table 3). The increases

Table 3
Percent changes (%Δ) in soybean leaf dry mass and nutrient concentrations. The %Δ was calculated as 100 × (VAM − nonVAM)/(nonVAM). Soybean plants were grown in pots associated with cocklebur plants and sprayed with the herbicide bentazon at rates expressed as fractions of field recommendation (FR)

Leaf parameter		Herbicide (fraction of FR)				
		0	1/3	2/3	3/3	4/3
Dry mass	%Δ	1.8	22.1	14.2	15.3	6.2
	p	0.855	0.030	0.028	0.032	0.317
N conc.	%Δ	6.6	−2.5	3.6	−5.2	4.9
	p	0.126	0.361	0.262	0.173	0.196
P conc.	%Δ	29.1	38.7	53.7	29.8	22.0
	p	< 0.001	< 0.001	< 0.001	< 0.001	0.006
K conc.	%Δ	3.9	13.8	20.8	7.7	3.7
	p	0.119	0.011	< 0.001	0.037	0.112
Mg Conc.	%Δ	3.4	2.0	8.0	6.1	1.1
	p	0.193	0.343	0.050	0.210	0.267
Ca conc.	%Δ	4.8	4.1	1.0	−6.5	2.6
	p	0.213	0.311	0.435	0.115	0.297

Probability values (*p*) denote differences between +VAM and −VAM comparisons (*t*-test).

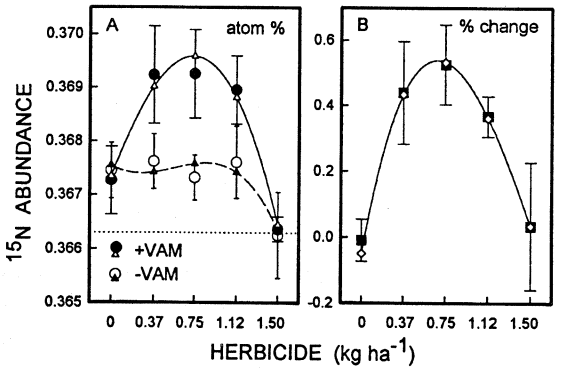

Fig. 5. Abundance of ^{15}N (atom %) in soybean leaves (A) and its percent change [100× (VAM − non VAM)/non VAM] in -VAM- vs. +VAM-plant comparisons (B). Soybean plants were grown in association with cocklebur plants, treated with the herbicide bentazon, and colonized (+VAM) or not colonized (−VAM) by vesicular-arbuscular mycorrhizal (VAM) fungi. Labelled N was applied to the cocklebur leaves. Curves were fitted to third-degree polynomial equations (best fit). Circles (A) represent actual data of −VAM (○) and +VAM (●) plants (means and SE of six replications); triangles indicate values derived from the best-fit equations. Squares (B) represent actual percent changes, diamonds the best fits. Dotted line (A) indicates natural abundance of ^{15}N.

(+VAM vs. −VAM) in nutrient concentrations were greatest (and significant) at 2/3–FR for P, K, and Mg, while for N and Ca the VAM response was not significant ($p > 0.1$) throughout the range of BEN dosages (Table 3). In contrast to nutrient contents, BEN effects on the concentrations of N and P were significant (Table 2). We do not know how to interpret this effect on N in view of the lack of change in N concentration with herbicide dosage, but with P the responses were distinct at all levels of BEN (Table 3).

3.3. Nitrogen transfer

Labelled N applied to cocklebur leaves 4 days before herbicide application did not appear in +VAM and −VAM soybean leaves of the 4/3–FR treatment 14 days after spraying (Fig. 5A). Leaf ^{15}N contents of −VAM soybean of the other herbicide treatments were higher ($0.14 > p > 0.11$) than those of the 4/3–FR plants. The ^{15}N contents of the +VAM 4/3–FR and 0–FR leaves differed slightly

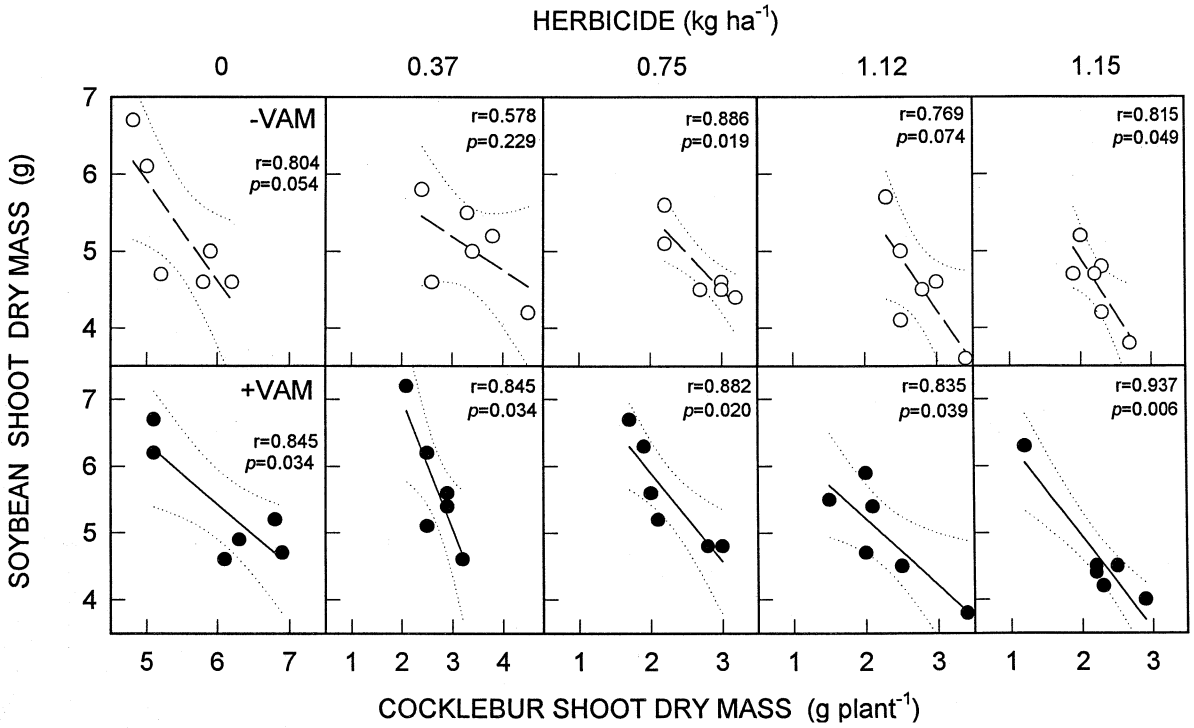

Fig. 6. Competitive relationships between associated soybean and cocklebur plants. Plants were treated with the herbicide bentazon at different rates (1.12 kg ha^{-1} = field recommendation), and colonized (+VAM, ●) or not colonized (-VAM, ○) by vesicular-arbuscular mycorrhizal (VAM) fungi. Statistics represent the coefficients and significance of linear correlations. Dotted lines represent 95% confidence intervals.

($p = 0.104$) but the difference between the 4/3–FR and the other +VAM treatments was significant (1/3–FR, $p = 0.006$; 2/3–FR, $p = 0.006$; 3/3–FR, $p = 0.002$). Expressed as percent change, these data showed no VAM effect in leaf ^{15}N content at 0–FR and 4/3–FR, but significant changes at 1/3, 2/3, and 3/3–FR. The maximum was near 2/3–FR (Fig. 5B), the same as the dry mass and nutrient data. The ^{15}N data fit third-degree polynomial curves.

3.4. Competition

There was a significant inverse relationship between the shoot dry masses of the associated soybean and cocklebur plants in all but one (−VAM, 1/3–FR) of the ten treatments (Fig. 6). This competitive relationship was tighter in the +VAM than in the −VAM plants, as shown by the higher correlations of the former. A comparison of noncompeting soybean plants grown under identical conditions (Bethlenfalvay et al., 1995) with plants from this experiment showed declines in leaf parameters (except for P content of the +VAM plants) due to the presence of the cocklebur for both herbicide-treated (3/3–FR) and nontreated (0–FR) plants (Table 4). Enhancement of P nutrition by VAM fungi was significant, but the fungi did not protect soybean from competition by cocklebur for other nutrients (N and K). However, the decline in P content of −VAM soybean grown in competition with cocklebur suggested that the added P stress contributed to their sensitivity to the herbicide.

4. Discussion

Inhibition of cocklebur growth by bentazon at only 1/3–FR to almost the level attained at full (3/3 −) FR indicates that the recommended rate was excessive (Fig. 1B). However, our plants were grown in a greenhouse and may therefore have been more vulnerable (e.g. thinner cuticles) than field-grown plants (Martin and Juniper, 1970). The appearance of VAM effects at 1/3–FR and their absence at only one-third above FR (4/3–FR, 1.5 kg ha^{-1}) in both soybean and cocklebur (Fig. 2) showed the importance of dose rates in evaluating modifications of herbicide effectiveness by VAM fungi. These modifications were: (1) growth promotion of +VAM soybean counteracting the herbicide effect, and (2) growth inhibition of +VAM cocklebur in excess of that shown by −VAM plants.

The high C requirement of the VAM endophyte appears to make the +VAM root a stronger sink for over-all plant resources than the −VAM root (Harris et al., 1985; Amijee et al., 1993), a process that manifests itself in generally lower shoot dry matter content (Snellgrove et al., 1982) and higher soluble C concentration in the roots (Amijee et al., 1993) of +VAM plants. Inhibition of shoot activity by a

Table 4
Comparisons between soybean leaf parameters from potted soybean plants growing alone (1plt)[a] or in association with a cocklebur plant (2plt)

Leaf parameter		−BEN				+BEN			
		1plt	2plt	%Δ	p	1plt	2plt	%Δ	p
Dry mass (g)	−VAM	4.8	3.4	−29	0.001	3.9	3.0	−28	< 0.001
	+VAM	4.9	3.4	−31	0.001	4.5	3.5	−25	0.003
Nutrient content (mg)									
N	−VAM	170.6	129.9	−24	0.001	147.0	132.8	−10	0.093
	+VAM	186.9	143.2	−23	0.070	180.3	146.1	−19	0.011
P	−VAM	12.3	9.2	−25	0.023	10.0	8.4	−16	0.083
	+VAM	13.9	12.1	−13	0.378	13.8	12.8	−7	0.420
K	−VAM	99.9	60.4	−40	< 0.001	83.0	51.3	−38	< 0.001
	+VAM	98.9	70.5	−29	0.017	101.1	64.0	−37	< 0.001

Plants were either colonized by vesicular–arbuscular mycorrhizal (VAM) fungi (+VAM) or not (−VAM), or sprayed with the herbicide bentazon at field recommendation (+BEN) or were left untreated (−BEN). Percent change (%Δ) reflects a competition effect (1plt vs. 2plt) horizontally.

[a] Data from Bethlenfalvay et al., 1995.

nonsystemic herbicide makes the root the strongest (or only) sink within the plant (sink strength = sink size × sink activity, Zeevaart, 1979), and enhances transfer of mobile (organic and mineral) nutrients to the root. If the shoot is severely or permanently damaged (high herbicide dose rate), the root also becomes necrotic eventually; the stability of its membranes declines, and nutrients are released to the soil. The most likely beneficiary of this release of nutrients is the largest organism (strongest sink) in contact with the root, the VAM fungus (Newman and Eason, 1989), which colonizes most of the root's cortical cells and the rhizosphere.

The VAM fungus, however, is not only an endophyte of the stressed root systems of the susceptible (weed) plants, but also of those of adjacent, herbicide-tolerant (crop) plants. Source–sink relationships between such roots undergo a dramatic shift as a result of herbicide effects: with their sink strength unimpaired, crop-plant roots are in a position to benefit from a decline in sink strength of competing weeds, and VAM fungi, which maintain their own source–sink relationships (Koch and Johnson, 1984) with both potential donor (weed) and receiver (crop) roots, may adjust to these changes in favor of the stronger sink.

An enhancement of nutrient and C transfer between roots by VAM fungi is well-known (Francis and Read, 1984; Newman and Ritz, 1986; McGee, 1990), but the direction of net flux has been controversial (Bergelson and Crawley, 1988). One hypothesis favored source-driven fluxes from nonstressed donors to stressed recipient plants (Francis and Read, 1984; Grime et al., 1987; Newman, 1988). Much of this work focussed on N transfer from (N-sufficient) legumes to (N-deficient) nonlegumes (see introduction, Ikram et al., 1994), concluding that VAM links do not play a significant role in nutrient transport between plants (Ikram et al., 1994). The alternative (and more likely) hypothesis considers sink-driven fluxes between VAM plants, where the nonstressed plant draws on the resources of an adjacent stressed one. This phenomenon has been observed in VAM-mediated P (Bethlenfalvay et al., 1991) and C (Newman et al., 1992, Waters and Borowicz, 1994) transfer. Thus, in the herbicide-treated crop–weed system, VAM fungi could potentially either accelerate weed recovery (source-driven flux from strong to weak plant) or aggravate weed injury (sink-driven flux, crop plant as sink), depending on the direction of inter-plant fluxes that they enhance. Our data support the second hypothesis: sink-driven fluxes and enhanced weed injury.

While the mediation of the processes of interaction between our soybean and cocklebur plants was measurable as enhancement of the herbicide-tolerant and inhibition of the susceptible plants (Figs. 2 and 4, Table 3), the data did not show the extent to which the decline of cocklebur and promotion of soybean growth was due to direct hyphal transfer. Others (Martins, 1993), however, have demonstrated that direct hyphal transfer from root to root may account for almost 50% of nutrient fluxes between plants, while the rest was due to hyphal uptake of nutrients released by the donor root to the soil with subsequent transfer to the recipient root, or due to direct absorption by neighboring roots not mediated by the VAM mycelium. The drastic changes in inter-plant source–sink relationships as a result of herbicide application are likely to enhance the share of direct hyphal transfer from the weaker to the stronger plant.

The passage of organic (C and N) compounds from the fungal component of the recipient mycorrhiza (fungus-root) to the rest of the plant (and thus detectability in leaves) remains problematic, on theoretical and evolutionary grounds (Smith and Smith, 1990): the natural flow of C is from plant to fungus (not from fungus to plant), in keeping with the status of the endophyte as an obligate biotroph. Fungus-to-host C transfer may therefore take place as a result of the digestion and absorption by the host of the short-lived VAM arbuscules (Smith, 1980). In an attempt to show movement of donor-plant organic compounds to the recipient, we applied N as labelled ammonia ($^{15}NH_4NO_3$) to the cocklebur leaves to encourage its rapid assimilation into amino acids (Robinson and Baysdorfer, 1985). Its appearance in detectable amounts only in the leaves of the +VAM (but not the −VAM) soybean plants indicated that the pathways of VAM-hyphal plant-to-plant nutrient transfer (direct or indirect) were significant in comparison to that part of exudate uptake that is not mediated by VAM fungi. It also suggests that a transfer of organic compounds from endophyte to host plant does take place, perhaps in the form of amino acids (see Cliquet and Stewart, 1993).

We explain the lack of differences in ^{15}N abun-

dance of the 4/3–FR +VAM and −VAM soybean leaves by a rapid and pervasive necrosis (see Browde et al., 1994) of cocklebur leaves (high herbicide rate) which prevented mobilization and export of organic compounds. Shoots of the 0–FR cocklebur plants, on the other hand, remained strong sinks for their products (assimilated ^{15}N), while the large cocklebur root system, the dominant competitor in this weed-crop association (Wilkerson et al., 1990), apparently precluded VAM-mediated export of nutrients, or rapidly reabsorbed its (^{15}N-labelled) exudates (Jones and Darrah, 1993). Under the present conditions, 2/3–FR was the dose rate which permitted maximum mobilization of nutrients from shoot to root (Schubert, 1987), and their export to the endophytes and to the associated soybean plant.

Our findings indicate that herbicides affect weed–crop relations in more subtle ways than can be judged by above-ground observations alone: their use also involves below-ground components of the agrosystem (Bethlenfalvay and Schüepp, 1994), such as VAM fungi. Possibilities for the utilization of VAM fungi in herbicide reduction is promising, since the plasticity of the VAM–plant–herbicide system leaves a lot of freedom for manipulation: most weed–crop combinations are mycorrhizal (Baltruschat, 1986), host–endophyte preferences (Dhillon, 1992) vary widely, and VAM–herbicide interactions (Sieverding and Leihner, 1984; Ocampo, 1993) depend on the conditions of use. Cultivation is one of the conditions of particular interest, since soil disturbance is particularly destructive to VAM soil mycelia (McGonigle and Miller, 1993). In efforts to determine the lowest effective rates of herbicide application (Kropff et al., 1993), our findings hold promise to make VAM fungi a factor in the 'factor adjusted dose' (Kudsk, 1989) to be used in the reduced-biocide environment of sustainable agriculture (Bethlenfalvay and Linderman, 1992).

Benefits, however, are likely to be gained only under the right conditions: endophytes compatible with both crop and weed host, cultural practices not inhibitory to a VAM soil mycelium, and herbicide dosage maximizing VAM enhancement of its effectiveness. To determine these conditions is a labor- and knowledge-intensive process, and recommendations based on it will be as variable as the prevailing edaphic conditions. But, used properly, the VAM factor holds promise to advance the goals of the reduced-chemical environment prescribed by the 1990 Farm Bill (U.S. Congress, 1990).

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