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Influence of the Herbicides Chlorsulfuron and Glyphosate on Mycorrhizal Soybean Intercropped with the Weeds *Brassica campestris* or *Sorghum halepensis*

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Abstract

The effect of the herbicides chlorsulfuron and glyphosate on arbuscular mycorrhizal (AM) colonization and plant dry matter was examined in soybean cultivated either alone or as an intercrop with the weeds *Brassica campestris* (chlorsulfuron) or *Sorghum halepensis* (glyphosate). There were 48 treatments, altogether, 24 with chlorsulfuron and 24 with glyphosate. Each set of 24 was designed as 2 × 3 × 4 factorial with 1) plus or minus *Glomus mosseae,* 2) soybean alone, weed alone or soybean plus weed combination, 3) herbicide applied at the rates 0, 0.1, 0.5 and 1 × the field recommendation dose. The shoot dry mass of AM soybean treated with low doses of herbicides, when grown together with *B. campestris* or *S. halepensis,* but not when grown alone, was increased. This fact together with the absence of an increase in plant dry mass in intercropped non-AM soybean plants, suggest that the AM fungus mediates nutrient transfer from weeds to soybean. Neither herbicide affected AM colonization of plants except when glyphosate was applied at field recommendation dose to the weed *S. halepensis* grown as an intercrop. The most beneficial effect of *G. mosseae* on soybean was found when chlorsulfuron and glyphosate were applied at low doses, but this beneficial effect disappeared when the herbicides were applied at high doses.

Keywords: *Brassica campestris,* chlorsulfuron, *Glomus mosseae, Glycine max,* glyphosate, *Sorghum halepensis*

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1. Introduction

Arbuscular mycorrhizal (AM) symbioses are widespread throughout the plant kingdom. Among other things, they benefit their hosts by increasing the capability of the root system to absorb and translocate phosphorus through an extensive network of external hyphae (Hayman, 1983). Because the fungi are partly inside and partly outside the host, external factors such as the application of pesticides will affect the development of the symbiosis. Herbicides are expected to affect AM symbiosis because of their toxicity to a wide range of plants, many of which are hosts to AM fungi (Ocampo, 1993). When crop and weed plants live together, hyphae of AM fungi interconnect the root systems of adjacent plants (Bethlenfalvay et al., 1996b; Rejón et al., 1997). Although most weed-crop combinations are mycorrhizal (Hayman, 1983), many weeds are non-AM (Franz, 1985). However, host competition with non-host plants for nutrients throughout AM mycelia have been found (Ocampo, 1986).

Chlorsulfuron (2-chloro-N-(((4-methoxy-6-methyl-1,3,5 triazin-2-yl) amino) carbonyl)-benzene-sulfonamide) inhibits aminoacid biosynthesis and cell division, and causes extreme stunting of shoots and roots of plants. This broad-spectrum sulfonylurea herbicide has prolonged persistence in soil, with important residual effects on plants (Beyer et al., 1988), especially on legumes (Ferris and Haigh, 1993). Its application to soybean has been limited by the sensitivity of this legume to sulfonylurea herbicides, but herbicide-resistant cultivars have been developed with mutational techniques (Sebastian et al., 1989).

Glyphosate ((N-(phosphonomethyl)glycine) is a potent, broad-spectrum, non-selective, postemergence herbicide capable of effectively controlling 97% of the world's worst weeds (Franz, 1985). This herbicide inhibits aminoacid synthesis and acts systematically, being rapidly translocated in the phloem of both annual and perennial plants from foliar tissue to meristematic areas such as shoot and root apices. Many studies on the effects of glyphosate on the growth of saprophytic, pathogenic and ectomycorrizal fungi alone have been reported, and it is clear that glyphosate exerts some inhibitory effect on the growth of many ectomycorrhizal fungi (Quinn, 1993). Legumes are sensitive to the application of glyphosate, but resistant cultivars have been obtained by molecular techniques (Quinn, 1993).

The weeds *Brassica campestris* (AM non-host) and *Sorghum halepensis* (host) often grow in soybean crops, and are treated with chlorsulfuron and glyphosate respectively. To date the effect of these herbicides on AM fungi is not well known. Knowledge of the effect of AM colonization on crop-weed relations can help to determine lower effective herbicide dose rates that are still able to effectively control weeds.

2. Materials and Methods

Effect of herbicides on plants

The experimental plants were grown in 500-ml pots with soil collected from the province of Buenos Aires, Argentina. The soil (Silty-clay-loam of argiudol type, pH 5.4), contained 2,28% C, and (mg/kg) 331 N, 9.5 P ($NaHCO_3$-extractable) and 3.2 Ca. It was steam-sterilized at 100°C three times at 24 h intervals and mixed 1:1 (v/v) with sterilized quartz sand. Soybean (*Glycine max* cv. Nidera), resistant to sulfonylurea and glyphosate herbicides, and the weeds *Brassica campestris* and *Sorghum halepensis*, were selected as test plants. Roots of *S. halepensis* and seeds of the other plants were surface-sterilized with 50% NaOCl for 40 and 10 min, respectively, and thoroughly rinsed with sterilized water. After germination, seedlings were selected for uniformity before planting. There were 2 soybean, 2 *B. campestris* or 2 *S. halepensis* plants per pot (as separate treatment) or 1 soybean plus 1 *B. campestris* or 1 soybean plus 1 *S. halepensis* per pot (intercrop treatment). Plants were watered from below in a greenhouse with a day/night cycle of 25/19°C and 50% relative humidity.

The AM inoculum consisted of 5 g of rhizosphere soil from alfalfa (*Medicago sativa*) pot cultures of an isolate of *G. mosseae* (Nicol. & Gerd.) Gerd. and Trappe. The sporocarps and spores were isolated from the soil of the University of Buenos Aires Farm and identified as *G. mosseae* according to Gerdemann and Trappe (1974). Uninoculated plants were given filtered leachings from the inoculum soil. Soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of AM plants was added to the non-AM treatment. The filtrate contained common soil microorganisms but no propagules of *G. mosseae*.

Chlorsulfuron (Monsanto, 70% active ingredient) and glyphosate (Glicep from Fitoquim, 48% active ingredient) were applied to 30-day-old soybean and either *B. campestris* or *S. halepensis*, respectively. Two ml per pot was sprayed onto the foliage at concentrations of 0, 1, 5 and 10 g ha^{-1} (field recommendation dose) of chlorsulfuron and of 0, 0.4, 2 and 4 l ha^{-1} (field recommendation dose) of glyphosate.

Plants from the five replicate pots per treatment were harvested 30 days after the herbicides were applied (60 days after planting), and dry mass was determined. The crop-alone, the weed-alone and the mixed-set numbers are based on one plant. Part of the root system was cleared and stained (Phillips and Hayman 1970), and the percentage of root colonization was determined (Giovannetti and Mosse, 1980).

Experimental design and statistics

There were 48 treatments, altogether, 24 with chlorsulfuron and 24 with glyphosate. Each set of 24 was designed as 2 × 3 × 4 factorial. Pots were arranged in a completely random manner. The factors were 1) inoculation with *G. mosseae* (+M) or no inoculation (–M); 2) plant species as soybean only, weed only (alone), or a soybean-weed combination (intercrop); and 3) herbicide spraying at the rates of 0, 0.1, 0.5 and 1 × the field recommendation dose.

The percentage values were arcsine transformed for statistical analysis. The data were subjected to ANOVA. Mean values were compared by the new Duncan's multiple range test ($p = 0.05$).

3. Results

The results from the ANOVA showed a significant effect of inoculation with *G. mosseae* ($p = 0.001$) and plants grown alone or as intercrop ($p = 0.0001$) on shoot dry mass of soybean plants. There was a significant interaction between application of chlorsulfuron on plant growth alone or as intercrop ($p = 0.03$) and between application of chlorsulfuron and inoculation with *G. mosseae* ($p = 0.04$) on shoot dry mass. Table 1 shows that AM fungi increased shoot dry mass of plants treated with chlorsulfuron except when 5 and 10 g ha^{-1} of the herbicide were applied to soybean alone. When 1 g ha^{-1} of herbicide was applied to soybean grown together with *B. campestris*, shoot dry mass of soybean was higher than the non-herbicide treated control. Shoot dry mass of non-AM soybean alone were lower than those of plants grown as an intercrop. Root dry mass were similar in AM and non-AM soybean plants treated with herbicide regardless of whether they were grown alone or as an intercrop. However, the root dry mass of non-AM relative to AM plants was lower when the herbicide was applied to soybean grown as an intercrop ($p = 0.002$). Chlorsulfuron did not affect the percentage of root length colonization of soybean plants. However, colonization was significantly greater in soybean plants grown with *B. campestris* (Table 1).

Shoot dry mass of *B. campestris* was significantly affected by the application of chlorsulfuron ($p = 0.001$), the alone or intercrop treatments ($p = 0.002$) and the interaction between the herbicide chlorsulfuron and the alone or intercrop treatments ($p = 0.006$). Shoot dry mass of *B. campestris* grown alone was lower after treatment with 5 and 10 g ha^{-1} chlorsulfuron than at the lower dose. When grown as an intercrop, this value was even lower at all herbicide doses (Table 2). Shoot dry mass in *B. campestris* was higher when grown alone than as an intercrop. Root dry mass was affected by the herbicide ($p = 0.002$) and by the interaction between chlorsulfuron and the alone or intercrop treatments ($p = 0.01$). The root dry mass of plants grown alone and treated with 1 g ha^{-1} of the herbicide were higher than the other treatments. However, root mass decreased when plants were grown as an intercrop with herbicide doses of

5 and 10 g ha^{-1} (Table 2). At all doses of chlorsulfuron the apical meristem of *B. campestris* became necrotic, and at 10 g ha^{-1}, excessive numbers of lateral branches appeared together with yellow flowers (not shown). Although aborted entry points and external hyphae around *B. campestris* roots were seen when this weed was grown together with soybean, no AM colonization of *B. campestris* ocurred in any of the treatments (Table 2).

A significant effect of glyphosate ($p = 0.001$), AM fungi ($p = 0.006$), alone or as intercrop treatments ($p = 0.0001$) and the interactions between alone or intercrop treatments and application of glyphosate ($p = 0.007$) and between alone or intercrop plants culture and AM fungi ($p = 0.008$) was observed. In the glyphosate-treated intercropped plants, the decrease in shoot mass caused by the herbicide was greater in non-AM than in AM plants (Table 3). AM colonization increased soybean shoot dry mass in both alone and as intercrop, except when the field recommendation dose of glyphosate (4.0 l ha^{-1}) was applied to plants grown alone (Table 3). The differences in root dry mass between AM and non-AM soybean plants grown alone were not significant at all doses of glyphosate (Table 3). However, intercrop root dry mass was significantly lower in AM than in non-AM soybean plants resulting from all doses of herbicide except the field recommendation dose ($p = 0.04$). Root dry mass in non-AM soybean plants grown as an intercrop was lower (except with 0.4 l ha^{-1}) in plants treated with glyphosate than in untreated plants ($p = 0.03$). In AM soybean, root dry mass was lower in intercropped than in plants grown alone ($p = 0.001$). The herbicide did not affect percentage root length colonization in soybean plants (Table 3).

Table 4 shows that when *S. halepensis* was grown alone and treated with glyphosate, AM colonization had no effect on shoot dry mass. However, this herbicide led to lower shoot dry mass in intercrops of this weed when inoculated with *G. mosseae* ($p = 0.02$). Shoot dry mass in *S. halepensis* was lower when this weed was grown as an intercrop than alone ($p = 0.001$). In plants grown alone, glyphosate at a dose of 0.4 l ha^{-1} significantly increased root dry mass in comparison with the other treatments ($p = 0.002$). However, when used at field recommendation dose with AM *S. halepensis* plants grown as an intercrop, the herbicide decreased root dry mass in comparison with untreated plants ($p = 0.007$). The field recommendation dose of glyphosate decreased AM colonization of *S. halepensis* grown as an intercrop (Table 4).

4. Discussion

The shoot dry mass of AM soybean treated with low doses of herbicide were higher than AM controls when grown together with *B. campestris* or *S. halepensis*, but not when the crop was grown alone. This finding together with

Table 1. Effect of chlorsulfuron on the percentage of root colonization (%) and on the shoot and root dry mass (dm) of soybean grown alone or as an intercrop with *B. campestris* inoculated with *G. mosseae* or non-inoculated.

Plant combination Herbicide dose (g ha^{-1})	Shoot dm (mg)		Root dm (mg)		AM root colonization (%)
	−M	+M	−M	+M	
G. max					
0	1790 c1	1990 d2	940 bc1	931 a1	16 a
1	1747 c1	1890 cd1	977 bc1	943 a1	15 a
5	1707 c1	1885 cd1	974 bc1	1016 a1	18 a
10	1746 c1	1771 c1	1019 c1	1055 a1	12 a
G. max with B. campestris					
0	1449 b1	1700 b2	982 bc1	953 a1	45 b
1	1356 b1	2508 c2	814 ab1	943 a1	40 b
5	998 a1	1496 a2	815 ab1	909 a1	50 b
10	945 a1	1407 a2	782 a1	895 a1	41 b

Each value is the mean of five replicates. Within percent of root colonization, shoot and root dry mass of soybean, column values followed by the same letter or rows values followed by the same number are not significantly different according to Duncan's multiple range test (p = 0.05).

Table 2. Effect of chlorsulfuron on the percentage of root colonization (%) and on the shoot and root dry mass (dm) of *B. campestris* grown alone or as an intercrop with soybean and inoculated with *G. mosseae* or non-inoculated.

Plant combination Herbicide dose (g ha^{-1})	Shoot dm (mg)		Root dm (mg)		AM root colonization (%)
	−M	+M	−M	+M	
B. campestris					
0	1871 e1	1694 e1	375 c1	393 d1	0
1	1646 e1	1791 e1	641 d1	578 e1	0
5	871 cd1	836 cd1	423 c1	402 d1	0
10	628 bc1	645 bc1	345 c1	334 d1	0
B. campestris* with *G. max					
0	805 cd1	1069 d1	102 b1	196 c2	0
1	418 b1	462 b1	274 c1	440 d2	0
5	172 a1	183 a1	23 a1	64 b2	0
10	159 a1	141 a1	14 a1	17 a1	0

Each value is the mean of five replicates. Within shoot and root dry mass of *B. campestris*, column values followed by the same letter or rows values followed by the same number are not significantly different according to Duncan's multiple range test (p = 0.05).

Table 3. Effect of glyphosate on the percentage of root colonization (%) and on the shoot and root dry mass (dm) of soybean grown alone or as an intercrop with *S. halepensis* and inoculated with *G. mosseae* or non-inoculated.

Plant combination Herbicide dose ($g\ ha^{-1}$)	Shoot dm (mg) −M	Shoot dm (mg) +M	Root dm (mg) −M	Root dm (mg) +M	AM root colonization (%)
G. max					
0	1564 c1	1999 d2	487 b1	512 b1	36 b
0.4	1571 c1	1891 cd2	508 b1	481 b1	38 b
2.0	1670 c1	1921 d2	499 b1	464 b1	36 b
4.0	1515 c1	1560 c1	517 b1	508 b1	26 b
G. max* with *S. halepensis					
0	1421 bc1	1707 bc1	496 b1	313 a2	36 b
0.4	1265 b1	1944 d2	464 b1	353 a2	30 b
2.0	723 a1	1379 a2	572 b1	331 a2	36 b
4.0	719 a1	1233 a2	357 a1	346 a1	41 b

Each value is the mean of five replicates. Within percent of root colonization, shoot and root dry mass of soybean, column values followed by the same letter or rows values followed by the same number are not significantly different according to Duncan's multiple range test ($p = 0.05$).

Table 4. Effect of glyphosate on the percentage of root colonization (%) and on shoot and root dry mass (dm) of *S. halepensis* grown alone or as an intercrop with soybean and inoculated with *G. mosseae* or non-inoculated.

Plant combination Herbicide dose ($g\ ha^{-1}$)	Shoot dm (mg) −M	Shoot dm (mg) +M	Root dm (mg) −M	Root dm (mg) +M	AM root colonization (%)
S. halepensis					
0	1997 b1	1655 d1	729 bc1	559 bc1	32 b
0.4	1981 b1	1761 d1	1257 d1	1123 d1	28 b
2.0	1868 b1	1844 d1	743 bc1	820 c1	40 b
4.0	1977 b1	1612 d1	650 ab1	578 bc1	28 b
S. halepensis* with *G. max					
0	971 a1	1111 c1	816 c1	831 c1	36 b
0.4	1049 a1	567 ab2	561 a1	625 bc1	26 b
2.0	1096 a1	472 ab2	683 ab1	661 bc1	18 ab
4.0	1088 a1	206 a2	784 bc1	286 a2	8 a

Each value is the mean of five replicates. Within percent root colonization, shoot and root dry mass of *S. halepensis*, column values followed by the same letter or rows values followed by the same number are not significantly different according to Duncan's multiple range test ($p = 0.05$).

the absence of an intercrop effect in non-AM plants suggests that after herbicide application the AM fungus mediates nutrient transfer from weeds to soybean plants. Herbicides can change the weed plant root from a strong sink to a source of nutrients (Bethlenfalvay et al., 1996a; Rejón et al., 1997). The AM fungi that colonize these roots are the immediate sink, and through them the roots of associated plants (crops) benefit from sharing these fungi. Direct hyphal transfer from root to root may account for part of the nutrient flux between plants (Bethlenfalvay et al., 1996b). This transfer between soybean and weed plants may also be responsible for the enhanced growth of intercropped AM soybean under our experimental conditions, for the inhibition of growth in *S. halepensis* and for the absence of growth inhibition in the non-host *B. campestris*. However, some nutrient transfer may be due to hyphal uptake of nutrients released by the donor to the soil, with subsequent transfer to the recipient root. Alternatively, the neighbouring root may directly absorb nutrients in a manner that is not mediated by the AM mycelium (Ocampo, 1986). This may explain the enhanced growth in soybean grown as an intercrop with *B. campestris*. The increased mycorrhization of soybean when grown together with the non-host *B. campestris* may also have contributed to enhanced growth in soybean. The beneficial effect of non-host plants on AM colonization of host plants has been reported in other situations (Ocampo et al., 1980).

Under field conditions crop and weed plants live together; thus their responses to AM and herbicide treatments may be interdependent. However, as found for other herbicides, the beneficial effect of AM fungi disappeared when herbicides were applied at high doses (Ocampo, 1993). At high doses the herbicide inhibited both weed and crop growth. Our results show that root growth in *S. halepensis* decreased after treatment with high doses of herbicide, and percent root colonization also declined, leading to a decrease in hyphal contact with the soil and potentially with the roots of associated plants. However, at lower doses of either herbicide, root dry mass increased in both species of weed, and this combination of conditions led to the most favourable AM effect on soybean.

The influence of mycorrhizas in the crop-weed interactions, open possibilities for the utilization of AM fungi in herbicide reduction (Bethlenfalvay et al., 1996a; Rejón et al., 1997). Our finding indicates that the AM fungi can be a factor for a reduced biocide environment of sustainable agriculture.

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