

## Mycorrhizal fungi influence competition in a wheat–ryegrass association treated with the herbicide diclofop

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### Abstract

Vesicular–arbuscular mycorrhizal (VAM) fungi interconnect the root systems of adjacent plants, and mediate the transfer of nutrients between them. The objective of this study was to determine if the VAM fungus *Glomus deserticola* Trappe, Bloss and Menge enhanced such a transfer in a crop–weed association when one associated plant was selectively weakened by an herbicide. Wheat (*Triticum aestivum* L.) or perennial ryegrass (*Lolium perenne* L.) plants were grown with or without VAM fungi either in monocultures, or together as an intercrop for 7 weeks, when the herbicide diclofop was applied as a soil drench at dose rates of 0%, 10%, 50%, 100% and 1000% of the field recommendation (0.9 kg ha<sup>-1</sup>). Seed yield at harvest (13 weeks) was significantly greater for the +VAM than for the –VAM intercropped wheat plants at all dose rates. When grown in monoculture, wheat yield was greater in the –VAM plants. Shoot growth of intercropped +VAM wheat was enhanced, while that of ryegrass was inhibited. In monoculture, the plant dry masses of both –VAM wheat and ryegrass were greater than those of the +VAM plants, while in intercrop the +VAM wheat plants fared better. The data showed that (1) the herbicide inhibited root colonization by *G. deserticola* in wheat but not in ryegrass, and (2) when grown together, wheat growth and yield were enhanced and ryegrass growth was inhibited in +VAM herbicide-treated associations. We interpret the findings as a change in interplant source–sink relations upon treatment with diclofop: ryegrass roots became sources of nutrients to the tolerant wheat roots (stronger sinks), and the transfer process was enhanced by the VAM mycelium common to both. © 1997 Elsevier Science B.V.

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### 1. Introduction

The need to reduce biocide use as stated in the Farm Bill of 1990 (U.S. Congress, 1990) is focusing weed-control research on finding the lowest effective herbicide dose rates (Kropff et al., 1993). One sequel of these efforts is the development of a concept for

‘factor-adjusted doses’ (Kudsk, 1989). Factors which permit herbicide application to be adjusted below the recommended rate without sacrificing control depend on the status of the plant–soil system to be treated and relate mostly to aboveground aspects, such as weather conditions, time-of-day, growth stage and row spacing.

Among belowground factors that may contribute to a downward adjustment of herbicide dose rates are vesicular–arbuscular mycorrhizal (VAM) fungi (Be-

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thlenfalvai et al., 1996a). Hyphae of VAM fungi interconnect the root systems of adjacent plants (Newman et al., 1992) and facilitate the transfer of nutrients between them (Martins, 1993). Such fluxes mediated by mycorrhizal fungi appear to be sink-driven and predominate in the direction of the larger and more active plant (Bethlenfalvai et al., 1991; Waters and Borowicz, 1994). When the weed plant is selectively weakened by an herbicide in a crop-weed association, the ability of the weed to compete may be further reduced by a transfer and redistribution of organic nutrients from weed to crop plants by the VAM mycelium (Bethlenfalvai et al., 1996b).

We chose the herbicide diclofop (Thomson, 1993) to inhibit the growth of ryegrass (*Lolium perenne* L.) in the facultatively mycorrhizal wheat (*Triticum aestivum* L.)–ryegrass association because its unique capability of controlling annual grasses in graminaceous crops is of interest agriculturally and scientifically. The purpose of this experiment was to test the hypotheses that (1) tolerant plants benefit from being connected to susceptible plants by VAM fungi upon herbicide application, (2) herbicide effectiveness is enhanced in VAM–plant associations by inhibiting the growth of the susceptible associate, and (3) VAM fungi influence herbicide effects even in plant associations that are not obligately VAM-dependent.

## 2. Materials and methods

### 2.1. Experimental design and statistics

The experiment was designed as a  $2 \times 3 \times 5$  factorial, with VAM colonization, plant, and herbicide dose rate as factors. The experimental units (potted plants) were arranged in a completely random manner and rotated weekly. The main effects were due to: (1) presence (+VAM) or absence (–VAM) of a VAM fungus; (2) plant species as wheat only, ryegrass only (monocultures), or a wheat–ryegrass combination (intercrop); and (3) herbicide soil drenches at the rates of (approximately) 0, 0.1, 0.5, 1.0 or 10 times those of the field-recommended (FR) rate (0% FR, 10% FR, 50% FR, 100% FR or 1000% FR). The resulting 30 treatments were replicated five times. The results were evaluated by analysis of variance. We present actual probability values, where appro-

priate, instead of the traditional, arbitrary significance criteria ( $p \leq 0.01$  or  $p \leq 0.05$ ) to permit the reader to interpret significance (Nelson, 1989). We may interpret differences as biologically significant up to  $p = 0.1$ .

### 2.2. Biological materials and soil

*T. aestivum* L., cv. Anza and *L. perenne* L., var. Tove seeds were pregerminated, selected for uniformity prior to planting, transplanted to 0.5-l plastic pots, and thinned to two wheat, two ryegrass, or one wheat plus one ryegrass plant per pot. Pots were filled with a grey loam soil obtained from the garden of the Estación Experimental del Zaidín (Granada, Spain). The soil (pH 8.1; 1:1, soil:water method) contained ( $\text{mg kg}^{-1}$ ): P ( $\text{NaHCO}_3$ -extractable), 6.2; N, 0.3; and K, 132; and consisted of (%): sand, 35.8; silt, 43.6; clay, 20.5; and organic matter, 1.8. It was mixed with sand (v:v, 1:1) and was steam-sterilized at  $100^\circ\text{C}$  three times at 24-h intervals.

The VAM inoculum consisted of rhizosphere soil containing spores and root fragments (*Medicago sativa* L.) of the VAM fungus *Glomus deserticola* Trappe, Bloss and Menge (provided by the Instituto de Investigaciones Agrobiológicas de Galicia, Spain) in amounts (5 g) predetermined to achieve high levels of root colonization. Plants of the –VAM treatment were given a filtrate (Whatman no. 1 paper) of the inoculum containing the common soil microflora, but free of VAM propagules.

### 2.3. Growth conditions

Plants were grown in a greenhouse in Granada, Spain from October 1993 to January 1994. Sunlight was supplemented by Sylvania incandescent and cool-white fluorescent lamps ( $400 \text{ nmol m}^{-2} \text{ s}^{-1}$ , 400 to 700 nm PAR), providing a light/dark cycle of 16/8 h. Temperature was maintained at  $19$  to  $25^\circ\text{C}$  by automatic controls, with relative humidity averaging 50%. Plants were watered from below as needed to maintain the soil moisture to surface level. Watering was supplemented ( $10 \text{ ml wk}^{-1} \text{ pot}^{-1}$ ) with a nutrient solution (Hewitt, 1952) which was adjusted to be P-free for the +VAM plants.

## 2.4. Herbicide application

The mode of action of the herbicide diclofop (methyl, 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate; 0.36 kg l<sup>-1</sup> emulsifiable concentrate; FR, 0.9 kg ha<sup>-1</sup>; Hoechst-Iberia, Barcelona, Spain) is the inhibition of lipid biosynthesis. The major plant function disrupted is structural organization, and herbicides in this chemical group have the unique capability of selectively removing annual grasses from gramineous crops, such as wheat (Zimdahl, 1993). A suspension of diclofop was prepared (360 g diclofop l<sup>-1</sup> water). Different quantities of the suspension (0, 1.1, 6.0, 11.5 and 115 µl, equivalent to 0.39, 2.16, 4.14 (FR) and 41.4 mg, respectively) were mixed with 100 ml of water and applied to the pots 7 weeks after planting.

## 2.5. Assays

Plants were harvested 13 weeks after planting, when the wheat seeds were mature. Root and shoot weights for all plants, and the head weights of wheat were taken after drying (70°C, 2 days). The roots of the two plants in each pot were intermingled and could not be separated. Whole plant results were therefore based on the experimental unit (two wheat plants, two ryegrass plants, and the sum of the wheat and ryegrass plants grown together). Wheat-head and grain-number data and derived treatment compar-

isons (percent changes due to VAM [(VAM – non-VAM)/non-VAM] · 100 or to the herbicide [(x% FR – 0% FR)/0% FR] · 100) and the wheat/ryegrass shoot dry-weight ratio were based on the average value of the two plants in each pot (monoculture), or on the individual plants of the wheat–ryegrass intercrop. Root colonization by the VAM fungus was determined after staining (Phillips and Hayman, 1970) by the gridline intersect method (Giovannetti and Mosse, 1980). Roots of the –VAM plants were found to be free of VAM colonization.

## 3. Results

### 3.1. Whole plant data

The dry weights of +VAM wheat plants declined with increasing herbicide doses (Fig. 1). The decline was correlated ( $r = 0.854$ ,  $p = 0.063$ ) with an inhibition of root colonization (Table 1). Since there was no correspondingly significant decline in the –VAM plants, the herbicide effect on the +VAM plants may have been mediated by the direct inhibition of mycorrhiza formation. Such effects have been described by others (Johnson and Pflieger, 1992; Ocampo, 1993). In ryegrass, there was no consistent herbicide effect on root colonization (Table 1) and the biomass of both +VAM and –VAM plants declined with increasing dose rates (Fig. 1b). The

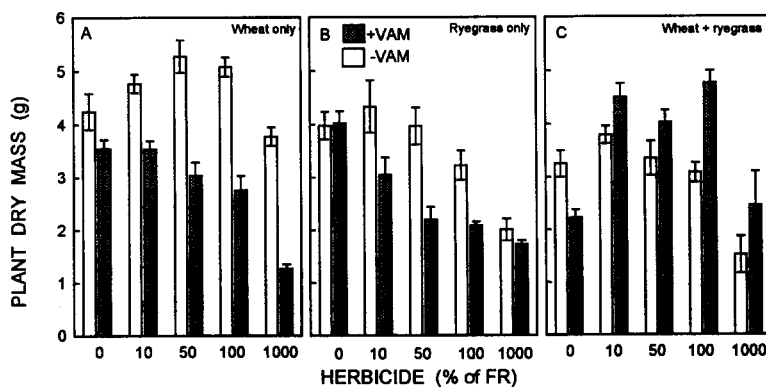


Fig. 1. Plant dry weights of wheat or ryegrass plants in monoculture, or of a wheat–ryegrass intercrop (numbers represent the sum of two plants per pot: two wheat or two ryegrass plants, or one wheat plus one ryegrass plant grown together). Plant roots were colonized by the VAM fungus *G. deserticola* (+VAM) or not so colonized (–VAM), and drenched with the herbicide diclofop at different percentages of the field-recommended (FR) rate. Values are the means of five replicates  $\pm$  SE.

– VAM plants grew better in both monocultures, in contrast to better growth of the + VAM plants in the herbicide-treated intercrop (Fig. 1c).

### 3.2. Mycorrhiza effects

Changes in shoot dry weight due to root colonization differed for the wheat and ryegrass monocultures and the intercrops (Fig. 2) and helped to explain the monocrop vs. intercrop reversal noted in Fig. 1. In both wheat (Fig. 2a) and ryegrass (Fig. 2b), the VAM effect was negative. In the intercrop (Fig. 2c), however, the VAM effect was negative only on ryegrass, while wheat growth was enhanced. It was apparently this increase in + VAM wheat (shoot) biomass that produced the + VAM reversal in the intercrop.

A similar reversal between monoculture and intercrops was noted in wheat seed development (Fig. 3). The + VAM values in the ‘wheat only’ treatment were lower for both head dry weight and grain number than the corresponding – VAM values (Fig. 3a and c), while those of the ‘wheat with ryegrass’ treatment were higher (Fig. 3b and d). The presence of a susceptible plant enhanced shoot and seed development of the herbicide-tolerant associate.

The dry-weight ratios of wheat and ryegrass shoots illustrate the interaction between herbicide treatment and the VAM condition, and the influence this interaction had on the relationship between these plants when each was grown in monoculture (Fig. 4a) or as

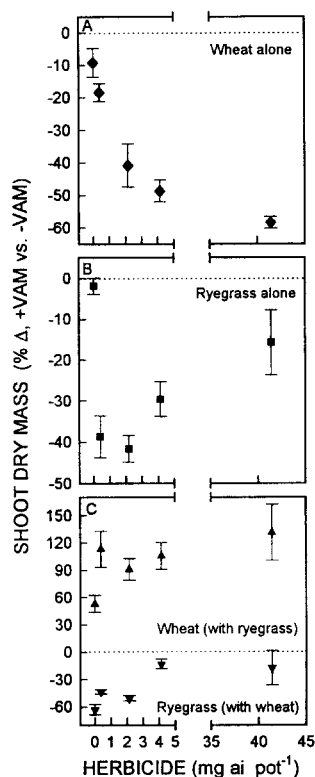


Fig. 2. The effect of root colonization by the VAM fungus *G. deserticola* on shoot growth. Wheat or ryegrass plants were grown in monoculture or as an intercrop. Numbers are the average of two plants. Percent change ( $\Delta\%$ ) due to VAM colonization was calculated as  $((\text{VAM} - \text{nonVAM}) / \text{nonVAM}) \cdot 100$ . The herbicide diclofop was applied at the rates of 0, 0.39, 2.16, 4.14 (field recommendation), or 41.4 mg pot<sup>-1</sup>. Values are the means of five replicates  $\pm$  SE.

Table 1

Root colonization of wheat, ryegrass and wheat plus ryegrass (W+R) plants by the VAM fungus *G. deserticola* in soils drenched with different doses of the herbicide diclofop

Plant	Herbicide (percent of FR) <sup>a</sup>				
	0	10	50	100	1000
	Percent colonization <sup>b</sup>				
Wheat	65.8a	65.4a	56.6b	55.2b	52.2b
Ryegrass	53.4b	57.4a	61.6a	54.4ab	58.6a
W + R	49.8b	64.2a	68.4a	57.6a	61.4a

<sup>a</sup>The dose at field recommendation (FR) was 4.14 mg pot<sup>-1</sup>.

<sup>b</sup>Main effects due to plant ( $p = 0.222$ ) were not significant, but herbicide effects ( $p = 0.042$ ) and the herbicide  $\times$  plant interaction were significant ( $p = 0.010$ ) by analysis of variance; values followed by the same letter (horizontally) are not significant ( $p < 0.05$ ).

an intercrop (Fig. 4b). In the intercrop, the growth of + VAM wheat was superior to that of – VAM wheat relative to ryegrass at all levels of herbicide dosage.

### 3.3. Herbicide effects

Changes in shoot dry weight of + VAM plants due to herbicide treatment were negative at all herbicide dose rates for both wheat and ryegrass grown in monoculture (Fig. 5a, c). This relationship was reversed in the intercropped plants where the herbicide affected the – VAM plants more negatively (Fig. 5b, d). In the intercrops, an enhancement of wheat shoot development was related to a decline in the dry

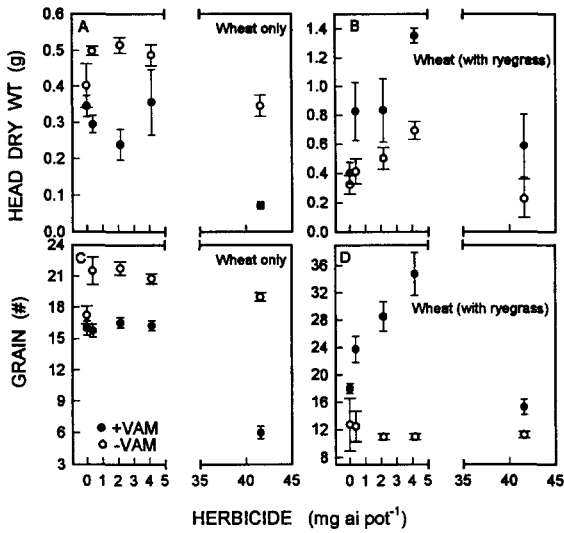


Fig. 3. Head dry weights and grain numbers of wheat grown in monoculture or as an intercrop with ryegrass. Plants were colonized (+VAM) or not colonized (-VAM) by the VAM fungus *G. deserticola* and treated with the herbicide diclofop at different rates. For explanations, see Fig. 2.

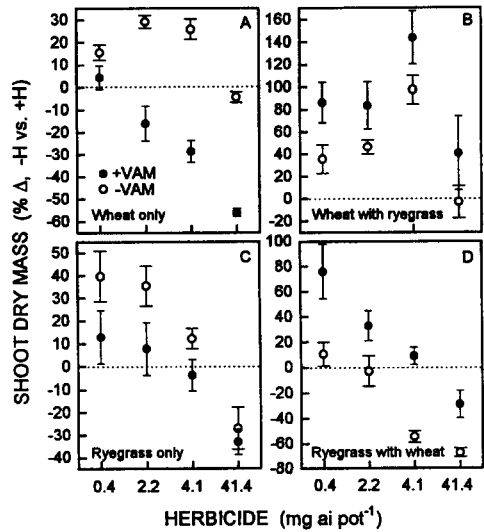


Fig. 5. Effect of the herbicide diclofop on shoot growth of wheat or ryegrass plants grown in monoculture or as an intercrop. Percent change ( $\Delta\%$ ) due to the herbicide at different dose rates was calculated as  $[(x\% \text{ FR} - 0\% \text{ FR}) / 0\% \text{ FR}] \cdot 100$ , with  $x\% \text{ FR}$  as 0.39, 2.16, 4.14 (field recommendation), or 41.4  $\text{mg pot}^{-1}$ , and 0% FR as the herbicide-free treatment.

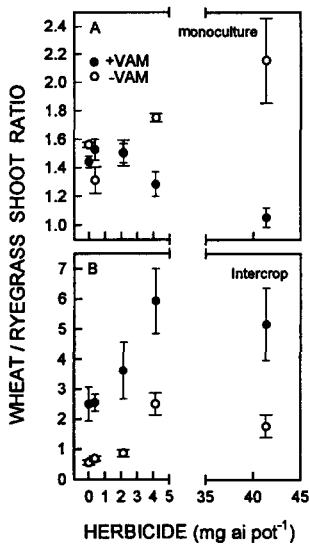


Fig. 4. Shoot dry-weight ratios of wheat and ryegrass plants grown in monoculture or as an intercrop. For explanations, see Fig. 2.

weight of ryegrass shoots relative to the plants not treated with the herbicide (0% FR).

### 3.4. The herbicide–mycorrhiza interaction

The responses of wheat and ryegrass to herbicide application differed with colonization by VAM fungi and with their status as a monoculture or as an intercrop (crop–weed association). The data suggest that in an association of tolerant and intolerant plants, VAM colonization increases growth inhibition of the intolerant plant and enhances growth of the tolerant one (Fig. 2c, Fig. 4d). The enhancement of +VAM wheat when grown together with ryegrass, but not in monoculture (Fig. 2c, Fig. 3b, Fig. 3d, Fig. 4b), and the lack of such an enhancement in -VAM wheat (Fig. 2a, Fig. 3a, Fig. 3c, Fig. 4a, Fig. 5b) suggests that the VAM fungus is involved in altering the competitive relationships between the herbicide-susceptible and the herbicide-tolerant plant associates when the susceptible plant is weakened by the herbicide.

#### 4. Discussion

The nature of competition between plants is known more by its manifestations than by its mechanisms, although mechanisms can sometimes be inferred from physiological performance of individual plants tested in isolation (Caldwell et al., 1985). In weed–crop relations in particular, the direction of nutrient movement is an important determinant of physiological performance: it will be in the direction of the stronger sink (sink strength = sink size  $\times$  sink activity, Zeevaart, 1979). When VAM fungi connect the roots of adjacent plants, nutrients move not only from soil to plant but also from plant to plant via the interconnecting VAM mycelium (Newman, 1988). Whether this mycorrhiza-mediated plant-to-plant transfer of nutrients can significantly affect competitive relationships is related to the quantity of nutrients that can be transported through the hyphae (Allen and Allen, 1990), to nutrient gradients between sources and sinks (Read et al., 1985), and to the relative sink strengths of the associated plants (Bethlenfalvay et al., 1996b). Sink relationships, however, are dramatically altered in favor of the tolerant plant, when an association of susceptible and tolerant plants is treated with a selective herbicide. Since the direction of interplant nutrient fluxes appears to be sink-driven (Bethlenfalvay et al., 1991; Newman et al., 1992; Waters and Borowicz, 1994), the nonstressed plant may draw on the resources of the stressed one. Thus, in the herbicide-treated weed–crop system, VAM fungi may potentially enhance crop growth and aggravate weed injury.

The responses of our tolerant and susceptible plants to the joint herbicide–VAM influence may be interpreted in terms of source–sink relationships between the associated plants. As the balance in sink strength shifted in favor of the tolerant plant as a result of herbicide application, the necrotic roots of the susceptible plant became a source of nutrients, and the cycling of these nutrients to the tolerant plant roots (strong, surviving sink) was accelerated by the VAM mycelium that connects both (Martins, 1993; Newman et al., 1992). Since soil permeation by VAM mycelia is influenced by cultural practices (Johnson and Pflieger, 1992) and environmental stresses (Sylvia and Williams, 1992), knowledge of the VAM status of a soil, and of VAM effects on

crop–weed relations may lead to reduced effective herbicide dose rates.

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