

Control of *Orobanche crenata* in legumes intercropped with fenugreek (*Trigonella foenum-graecum*)

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Abstract

Grain legume production in the Mediterranean Area is threatened by the holoparasitic plant *Orobanche crenata*, to which little resistance is available in affected crops. Control strategies have centred around agronomic practices and the use of herbicides, although success has been marginal. Several authors have described fenugreek as a suitable crop for intercropping with legumes, reducing the infection level of *O. crenata*; however, there is an important lack of experimental data and of a systematic research of the mechanisms involved in the reduction of parasitic infection. Two years of field experiments and further investigation by mini-rhizotron and pot experiments showed a decrease of *O. crenata* infection due to an allelopathic interference on the parasitic life cycle at the level of germination. Inhibition of *O. crenata* seed germination by allelochemicals released by fenugreek roots is suggested as the mechanism for reduction of *O. crenata* infection.

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1. Introduction

The broomrapes (*Orobanche* spp.) are widespread in Mediterranean Areas in Asia and Southern Europe, attacking dicotyledonous crops and depend entirely on their hosts for all nutritional requirements. Crenate broomrape (*Orobanche crenata* Forsk.) has threatened legume crops since antiquity, being of economic importance in grain and forage legumes (Joel et al., 2007; Rubiales et al., 2006).

Orobanche species exert their greatest damage prior to their emergence; therefore, the majority of field loss may occur before diagnosis of infection. A wide variety of approaches—physical, cultural, chemical and biological—have been explored against root parasites, but most of them are not effective, or not selective to the majority of susceptible crops. The intimate connection between host and parasite also hinders efficient control by herbicides. Unfortunately, in many crops no resistant varieties have

been produced to date. Control approaches applied as a single means often are only partially effective and sometimes inconsistent and affected by environmental conditions. Altogether, the above-mentioned control methods have not proven to be as effective, economical and applicable as desired (Parker, 1991; Joel, 2000; Joel et al., 2007).

Current means for controlling parasitic weeds are focusing on reducing soil seed bank, preventing seed set and inhibiting seed movement from infested to non-infested areas. Rotation into non-host crops continues to be mostly the only valid recommendation to farmers, which is not always possible due to the importance of the host crops for the economy and living of subsistence farmers. There are some reports on potential ‘trap crops’ or ‘false crops’ that offer the advantage of stimulating germination of the root parasites without themselves being parasitised (Parker, 1991). An alternative is mixed cropping or intercropping. Intercropping is regarded as an ecological method to manage pests, diseases and weeds via natural competitive principles that allow for more efficient resource utilisation (Liebman and Dyck, 1993). Many

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African farmers traditionally intercrop maize or sorghum with legumes to increase crop production, achieving better returns on fertiliser, pesticide, energy and manpower resources. These intercrops also reduce infection by *Striga hermonthica* (Carson, 1989; Carsky et al., 1994; Oswald et al., 2002). It has recently been shown that intercrops with oat reduce the infection by *O. crenata* on legumes (Fernández-Aparicio et al., 2007).

Fenugreek (*Trigonella foenum-graecum* L.) is a self-pollinated, small-seeded annual legume that is grown as a spice and a forage crop. It is widely cultivated in warm temperate and tropical regions in the Mediterranean, Europe and Asia. Fenugreek is suitable for intercropping with legumes (Petropoulos, 2002). The objective of the present studies was to access the feasibility of *O. crenata* control in various grain and forage legumes by intercropping with fenugreek.

2. Materials and methods

2.1. Field experiments

A field experiment was performed during seasons 2005–2006 and 2006–2007 at Kalen, Kafr El-Sheikh, Egypt. Sowing was done in November. The host faba bean (*Vicia faba* L., cv. Giza Blanca) was grown both as a sole crop (10 plants per metre) and mixed intercropped with fenugreek (local landrace) at a ratio of 1:8 (10 plants of faba bean and 80 fenugreek plants per meter) (Table 1). Experimental units contained six ridges. Ridge width was 60 cm and length 1 m. One ridge of faba bean monocrop was sown in each of the four ridges of the same treatment. Five replications of each treatment were laid out in a complete randomised block design in a clay loam soil, with an alluvial substratum. No inoculation was done as the plots were known to be heavily and uniformly infested with *O. crenata* seeds from previous trials. Numbers of emerged *O. crenata* shoots and host plants were counted at plant maturity in each row and referred as the number of

Table 1
Infection by *O. crenata* on faba bean (*Vicia faba*) intercropped with fenugreek (*Trigonella foenum-graecum*) in field experiments performed during 2005–2006 and 2006–2007 at Kafr El-Sheikh, Egypt

Crop system	Relative number ^a of emerged <i>O. crenata</i> plants	
	2005–2006	2006–2007
Faba bean monocrop	100 (7.6) ^b	100 (8.3)
Faba bean + fenugreek intercrop (1:4 ratio)	59.5*	69.8*
Fenugreek monocrop	0.0	0.0

^aNumber of emerged *O. crenata* plants per host plant standardised relative to the mean value of broomrapes emerged per plant in the two ridges of faba bean sowing as a check.

^bActual value of emerged broomrapes per faba bean plant in brackets.

*Intercrop significantly different from monocrop (Tukey test, $p < 0.05$).

emerged *O. crenata* plants/host plant, and was then standardised relative to the mean value of broomrapes emerged per plant in the two ridges of faba bean sowing as a check laterally to each of the four ridges of treatments (Rubiales et al., 2006). Surface irrigation took place four times by the application of $600 \text{ m}^3 \text{ ha}^{-1}$ each time. The first irrigation was done after sowing, the second one in December the third in March and the fourth one in April.

2.2. Growth chamber experiments

2.2.1. Pot experiments

Fenugreek intercropping effects on infection by *O. crenata* was also validated in pot experiments. In a first experiment (Table 2), host legumes studied were faba bean (cv. Brocal) and chickling pea (*Lathyrus cicera* L., accession BG-1043). Host plants were grown individually (one per pot) or mixed with fenugreek (one plant of the host legume with one fenugreek) in pots filled with 11 of black peat:perlite:vermiculite (2:2:1, v:v:v) mixed with 40 mg per pot (about 6000 seeds) of *O. crenata* seeds collected in the previous season from parasitic plants on faba bean (Rubiales et al., 2004, 2006). Each combination was represented by six pots in a completely randomised design. The plants were grown during 3 months, in environment growth chambers (20 °C, 12/12 h day/night regime, $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Then, the plants were removed from the pots, the roots gently washed in water and the number of *Orobanche* attachments counted.

A second experiment was carried out to test the inhibitory effect on *Orobanche* infection under two different temperatures (20 and 28 °C) with perlite as the inert substrate (Table 3). Thirteen litre pots filled with sterile perlite were inoculated with 0.34 g of *O. crenata* seeds per pot. Eight seedlings of faba bean were sown by pot in the monocrop treatment. In the intercrop treatment, four faba bean seedlings were intercropped with 16 seedlings of fenugreek (ratio 1:4). The plants were grown during 2 months, at 20 and 28 °C simultaneously in two different environment growth chambers (12/12 h day/night regime, $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$). After that period the plants were removed from the pots and the number of *Orobanche* attachments counted.

Table 2
O. crenata infection on faba bean and chickling pea (*Lathyrus cicera*) in 11 pots filled with peat:perlite:vermiculite (2:2:1) at 20 °C when monocropped and when intercropped with fenugreek (1:1)

Treatment	Number of <i>O. crenata</i> tubercles/host plant
Faba bean monocrop	78.0
Faba bean + fenugreek intercrop (1:1)	40.0*
Chickling pea monocrop	41.8
Chickling pea + fenugreek intercrop (1:1)	17.8*
Fenugreek monocrop	0.0

*Intercrop significantly different from monocrop (Tukey test, $p < 0.05$).

2.3. Mini-rhizotron experiments

2.3.1. Testing the allelopathic effect of fenugreek roots on *Orobanche* infection

The allelopathic effect of fenugreek root exudate on all phases of *O. crenata* infection process on roots of three species (chickling pea, lentil and pea) was studied by growing intercrops in mini-rhizotrons (Table 4) following the experimental design described by Fernández-Aparicio et al. (2007). Due to the fact that the mini-rhizotron system does not work successfully for faba bean because of the early darkening of roots, probably by oxygen depletion (Cubero et al., 1994; Rubiales et al., 2006), faba bean could not be studied in this system, being replaced by a susceptible lentil (*Lens orientalis*, accession L-317).

O. crenata seeds were sterilised with formaldehyde and spread over a 12 × 12 cm sheet of glass fibre filter paper (GFFP, Whatman GF/A) at a density of 50 seeds cm⁻². The GFFPs were placed over a square Petri dish (12 × 12 cm) filled with sterile perlite that was moistened with sterile deionised water. Petri dishes were placed in the dark at 20 °C for 11 days to promote conditioning of broomrape seeds. Ten seeds of each species per treatment were sterilised with sodium hypochlorite solution (5% w/v) during 20 min, rinsed three times in sterile deionised water and germinated on wet filter paper at 20 °C. Four-day-old

seedlings of each species together with fenugreek were transferred to the GFFP sheets containing the conditioned broomrape seeds, and placed into the Petri dishes that were punctured on the top so that plant foliage can develop outside of the dish. Ten seedlings of each host species were transferred individually in control treatments. Petri dishes were sealed with parafilm, wrapped in aluminium foil and stored vertically in a growth chamber (20 °C, 12/12 h day/night regime, 200 μmol m⁻² s⁻¹ irradiance). Plants received Hoagland's nutrient solution (30 ml per dish) twice per week (Hoagland and Arnon, 1950).

Twenty-five days after placement in the growth chamber, 400 broomrape seeds that were close (<3 mm) to host roots, which were grown on the GFFP near the fenugreek roots, were studied per Petri dish under a stereoscopic microscope at 30 × magnification to determine the percentage of germination. Germination was assessed as seeds with an emerged radicle. After another 20 days of incubation, the total number of broomrape attachments per host plant was recorded. The total length of each root plant was estimated by counting the number of intersections between root and a 1 × 1 cm grid, according to Tennant (1975), with a total length of 12 × 12 cm. The number of broomrape attachments per plant was expressed as the number of attachments per root length unit.

Table 3

O. crenata infection on faba bean in 131 pots filled with perlite at 20 and 28 °C when monocropped and when intercropped with fenugreek (ratio 1:4)

	Number of <i>O. crenata</i> attachments per host plant	
	20 °C	28 °C
Faba bean monocrop	20.3	16.4
Faba bean + fenugreek intercrop (4:16)	16.3*	13.4
Fenugreek monocrop	0.0	0.0

*Intercrop significantly different from faba bean monocrop (Tukey test, $p < 0.05$).

2.3.2. Testing the fenugreek inhibitory effect on *Orobanche* seed germination

The inhibitory effect of fenugreek root exudate was studied on *O. crenata* seeds that were conditioned and stimulated by the exogenous application of the synthetic germination stimulant GR24 (Johnson et al., 1976, Mangnus et al., 1992). This was tested at 20 and 28 °C in order to assess the possible influence of temperature on the allelopathic effect of fenugreek root exudates (Table 5). *O. crenata* seeds were sterilised and conditioned as described above. Ten plants of fenugreek per treatment were grown individually in mini-rhizotrons in different growth chambers at 20 and 28 °C. After 11 days of conditioning, *O. crenata* seeds belonging to the 28 °C

Table 4

Infection by *O. crenata* on the hosts pea (*Pisum sativum*), wild lentil (*Lens orientalis*) and chickling pea (*Lathyrus cicera*) monocropped and intercropped with fenugreek (ratio 1:1) in mini-rhizotrons in a growth chamber at 20 °C

	% <i>O. crenata</i> seed germination	Anchorage success	Number of <i>O. crenata</i> tubercles per plant	Number of <i>O. crenata</i> tubercles cm ⁻¹ host root	Total host root length
Pea monocrop	54.2	29.2	233.6	2.2	112.1
Pea + fenugreek intercrop	40.0*	25.3	168.0*	1.4*	124.1
Lens monocrop	62.2	18.2	78.6	4.3	18.4
Lens + fenugreek intercrop	27.6*	25.7	28.7*	2.5*	11.4*
Chickling pea monocrop	65.5	11.3	120.4	1.7	70.0
Chickling pea + fenugreek intercrop	45.2*	10.6	71.2*	0.8*	87.5*
Fenugreek monocrop	5.6	32.4	15.4	0.2	83.7
Negative control: water	0	na	na	na	na

na: not applicable.

*Intercrop significantly different from monocrop (Tukey test, $p < 0.05$).

Table 5
Germination of *O. crenata* seeds in mini-rhizotrons at 20 and 28 °C in the vicinity of fenugreek roots after being or not being challenged by the synthetic germination stimulant GR24 (100 ppm)

Treatment	% <i>O. crenata</i> seed germination	
	20 °C	28 °C
Negative control: just water, no fenugreek plants	0.0d	0.0c
Positive control: GR24, no fenugreek plants	51.6a	38.7a
Vicinity of fenugreek roots + GR24	43.4b	41.4a
Vicinity of fenugreek roots	4.9c	3.1b

O. crenata seeds treated with GR24 (100 ppm) in the absence of fenugreek roots served as positive control, and those treated only with water served as negative control.

¹Same letter per column indicates that differences are not statistically significant (Tukey test, $p < 0.05$).

treatment were transferred to the growth chamber with 28 °C temperature, and *O. crenata* seeds belonging to the 20 °C remained at the same temperature that was used for conditioning. At this time, one fenugreek plant was placed on each Petri dish containing the conditioned *Orobanche* seeds and simultaneously GR24 (100 ppm) was distributed on the *Orobanche* seeds. Petri dishes with fenugreek without GR24 were used as negative control. Petri dishes with GR24 and without fenugreek were used as positive control. Dishes were sealed with parafilm, wrapped in aluminium foil and placed vertically in a growth chamber at 20 and 28 °C applying 12 h of supplemental light. Plants were irrigated using a Hoagland's nutrient solution twice per week.

Fifteen days after GR24 application, the percentage of germination was determined in all experiments. A total of 400 broomrape seeds that were close (<3 mm) to the fenugreek roots (in negative control and fenugreek treatment) were examined in each Petri dish under a stereoscopic microscope at 30 × magnification to determine the percentage of germination. In positive control (dishes with GR24 without fenugreek), 400 seeds were randomly chosen in the dish. Seeds having an emerged radicle were scored as germinated.

2.4. Statistical analysis

Data collected on field, mini-rhizotron and pot experiments were approximated to normal frequency distribution by means of angular transformation when necessary, and ANOVA and Tukey's test were conducted using SPSS14.0. Null hypotheses were rejected when $p < 0.05$.

3. Results

3.1. Field experiments

O. crenata infection on faba bean was high and uniform in both seasons (7.6 and 8.3 emerged broomrapes per faba

bean plant in 2005–2006 and 2006–2007, respectively) as weather conditions were conducive for infection (Fig. 1). Infection was significantly reduced (Table 1) on faba bean intercropped with fenugreek, with a 40.5% and 30.25% reduction in the number of emerged shoots per faba bean plant in the 2005–2006 and 2006–2007 season, respectively. The effect of intercrop on the infection reduction was significant ($p = 0.001$), with no year × treatment interaction.

3.2. Pot experiments

Reduction of *O. crenata* infection in faba bean intercropped with fenugreek was confirmed in 11 pot experiments at 20 °C (Table 2), with a 49% reduction ($p = 0.022$). Infection was also markedly reduced (57% reduction) in *L. cicera* intercropped with fenugreek ($p = 0.044$).

The second experiment performed in bigger pots (Table 3), to avoid possible effects on root confinement, further confirmed the reduction in *O. crenata* infection on faba bean at 20 °C ($p = 0.036$). However, reduction was not significant at 28 °C ($p = 0.247$), suggesting a temperature effect ($p = 0.03$).

3.3. Mini-rhizotron experiments

O. crenata seed germination was significantly reduced ($p < 0.001$ for all species) (Table 4) in the vicinity of pea, chickling pea and lentil roots when intercropped with fenugreek. We did not observe a germination gradient when moving away from the roots, as would be expected as results of the concentration gradient host root germination stimulants. Once a broomrape seed had successfully germinated, no differences were observed in its success to attach to the host roots; either this was cultivated as a sole crop or was intercropped. Intercrop did not alter the chance of success of germinated seeds to anchor to host roots ($p = 0.272$, 0.199 and 0.793 for pea, lentil and chickling pea, respectively). As a result of reduced germination, the total number of *O. crenata* tubercles per host plant was significantly reduced in pea, chickling pea and lentil when intercropped with fenugreek ($p = 0.010$, 0.001 and 0.008 for pea, lentil and chickling pea, respectively).

Intercropping with fenugreek resulted in a significant increase in the root length of lentil and chickling pea ($p = 0.023$ and 0.020, respectively). The same tendency was observed in pea roots, but the increase was not statistically significant ($p = 0.438$) (Table 4). Even when pea, chickling pea and lentil roots were longer when intercropped with fenugreek, which would have represented and increased the chance of contact with *O. crenata* seedlings, and thus increased infection, this did not increase infection, and in fact, the number of tubercles per cm of host root unit was significantly reduced in all cases ($p = 0.010$, 0.006 and 0.001 for pea, lentil and chickling pea, respectively). In order to confirm the reduction in germination of *O. crenata*

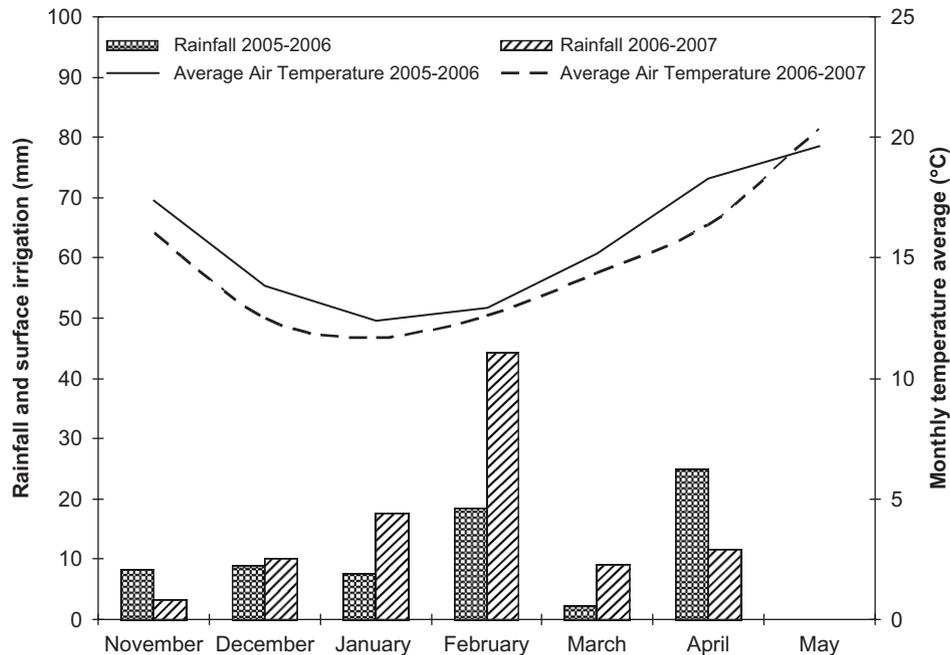


Fig. 1. Average air temperature and rainfall during the seasons 2005–2006 and 2006–2007 at Kalen, Kafr El-Sheikh, Egypt.

seeds induced by fenugreek, the inhibitory effect was tested on *O. crenata* seeds stimulated by GR24. Fenugreek roots significantly reduced germination when the seeds were exposed simultaneously to the influence of GR24 (positive stimulus) and fenugreek roots (inhibitory stimulus) at 20 °C (Table 5). However, this reduction was not significant at 28 °C, confirming the temperature effect suggested in Table 3. The influence of temperature on the germination of *O. crenata* seeds stimulated by GR24 was significantly decreasing ($p = 0.020$) from 51.6% at 20 °C to 38.7% at 28 °C. The difference of temperature was only at the level of germination time due to the fact that all seeds were conditioned at 20 °C in the dark for 11 days until the time to increase the temperature in the 28 °C treatment.

4. Discussion

Intercropping is a method for simultaneous crop production and soil fertility building (Willey, 1985). Intercrops of legumes and grasses in pastures are still widely used, but arable intercropping (cereals, grain legumes, oil seeds) for feed and human consumption have declined in the past decades in industrialised countries, although it is still a common practice in many areas with less intensified agriculture. There is, however, a renewing interest in intercropping linked to the need for reducing nitrogen cost and soil erosion and the potential for increasing land-use intensity (Francis, 1986). There is also interest in intercropping in organic farming to produce animal feed sources of organic origin, with a need to increase organic cereal and grain legume (protein) crop production.

Intercropping is already used in Africa as a low-cost method of controlling *Striga* (Carson, 1989; Carsky et al., 1994; Oswald et al., 2002). Only recently has it been shown that intercropping with oat reduce the infection by *O. crenata* on legumes (Fernández-Aparicio et al., 2007). Previous reports on the beneficial effect of intercropping with fenugreek were inconclusive and conflicting, with some authors suggesting a beneficial effect of fenugreek when intercropped with faba bean for *O. crenata* (Bakheit et al., 2002) or *O. foetida* (Kharrat and Halila, 2005) control in the field, and others denying it (Khalaf, 1994). In the present paper, we show a consistent control of *O. crenata* infection in faba bean, pea, lentil and chickling pea when intercropped with fenugreek over field, pots or mini-zizotrons. From the present experiments, we can speculate on the mechanism for the reduction of *O. crenata* infection in legumes by the intercrop with fenugreek, allelopathy being a major component for the reduction. Our finding that germination challenged with exogenous applications of the synthetic germination stimulant GR24 is partially inhibited in the presence of fenugreek roots (Table 5) suggests that roots might exudate substances that inhibit *O. crenata* seed germination. This has been confirmed in a subsequent work, and trigoxazonane identified from fenugreek root exudates might be responsible for the inhibition of *O. crenata* seed germination (Evidente et al., 2007). Here we show that inhibition is at the level of germination only, with fenugreek roots inhibiting *O. crenata* seed germination in the vicinity of a range of host roots, but not affecting the chance of contact and establishment on host roots of germinated seeds.

Allelopathy has been reported to be the cause for the reduction of *S. hermonthica* infection in intercropping with

Desmodium uncinatum by inhibition of the development of *Striga* haustoria although not of seed germination (Khan et al., 2002). Also, coumarins excreted by sunflower roots have been shown to inhibit *O. cumana* seed germination and seedling growth (Serghini et al., 2001). Considerable genetic variation in allelopathic activity has been found within cereals (Grimmer and Masiunas, 2005), which allows for the selection of more allelopathic cultivars.

Another suggested mechanism for *Striga* control is that the intercropped non-host legumes might be acting as trap crops, stimulating suicidal *Striga* germination (Parker and Riches, 1993). Our *in vitro* trials (Table 5) show that fenugreek induces very little *O. crenata* seed germination, neglecting its potential role as effective trap or catch crops for *O. crenata* inducing suicidal germination.

Nitrogen fixed by the legumes has also been pointed as a reason for *Striga* control. Incidence of *Striga* is known to negatively correlate with soil fertility, particularly nitrogen availability (Cechin and Press, 1993). In contrast to the *Striga*/cereal systems, N is unlikely to play any effect, as in our case both the host and the intercrop improve N by rhizobial fixation, although they are specific in the symbiosis, with *Pisum*, *Vicia*, *Lens* and *Lathyrus* having *Rhizobium leguminosarum* bv. *viciae* as the major symbiotic agent, whereas *Trigonella* have *Rhizobium meliloti* (van Rhijn and Vanderleyden, 1995). Only recently has it been found that arbuscular mycorrhizal hyphal branching is induced by plant sesquiterpenes, which is the same signal required for *Orobanchae* and *Striga* seed germination (Akiyama et al., 2005). Assuming that the signal required for *Orobanchae* germination also has an ecological role in *Rhizobium* symbiosis, the fact that the host species interact with a different *Rhizobium* species than *Trigonella* suggests that the signals produced are specific. In our system, the allelopathic effect of fenugreek roots on *O. crenata* seed germination was also confirmed in a sterile substrate, both in pots and in mini-rhizotrons, confirming that the effect is not due to effects of microbial interactions in the rhizosphere. However, we cannot exclude the possibility that microbial interactions in the rhizosphere might have additional influence. For instance, the nitrogen-fixing bacterium *Azospirillum brasilense* has been reported to inhibit germination and radicle growth of *O. aegyptiaca* (Dadon et al., 2004) and *Striga hermonthica* (Miché et al., 2000). Also, arbuscular mycorrhizal fungi have been reported to increase phosphorous uptake and to reduce weedy root parasite infection (Salonen et al., 2000).

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