

Colonisation of field pea roots by arbuscular mycorrhizal fungi reduces *Orobanch*e and *Phelipanche* species seed germination

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Summary

Pea (*Pisum sativum*) is an important grain legume crop whose cultivation in the Mediterranean basin and West Asia is severely constrained by infection by the weedy root parasite *Orobanch*e *crenata* (crenate broomrape). Pea is a false host of other species such as *O. minor*, *O. foetida* and *Phelipanche aegyptiaca*. In the present experiments, we demonstrate that seed germination of the *Orobanch*e and *Phelipanche* species examined is reduced in the presence of root exudates from pea plants colonised by arbuscular mycorrhizal (AM)

fungi, *Glomus mosseae* and *G. intraradices*. Reduced germination was due to the effect of the AM fungi and not to the microbial populations potentially present in the soil inoculum. This protective effect of mycorrhizae was noticeable after 35 days of AM colonisation for *O. crenata*, *O. foetida* and *P. aegyptiaca*, but at least 45 days of colonisation were needed to get some reduction of *O. minor* germination.

Keywords: bioprotection, *Pisum sativum*, AM fungi, parasitic plants, broomrape, establishment.

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Introduction

Field pea (*Pisum sativum* L.) is the most commonly produced grain legume in Europe and the second most common in the world (FAOSTAT data, 2008; <http://faostat.fao.org/>). The main values of grain legumes in cropping systems are their ability to fix atmospheric N in association with soil rhizobacteria, commonly referred to as rhizobia. The increasing demand for sustainable food production reinforces the need to reintroduce legumes, not only in subsistence agricultural systems, but also in highly profitable intensive agricultural production systems.

Among the most important constraints for pea cultivation in the Mediterranean basin and West Asia, is the soil infestation by *Orobanch*e *crenata* Forsk. (crenate broomrape) (Rubiales *et al.*, 2003, 2009a,b). *Orobanch*e and *Phelipanche* spp. (the broomrapes) are holoparasitic angiosperms which depend completely on their hosts for nutrients and water. The majority of *Orobanch*e and *Phelipanche* species parasitise only wild hosts in natural ecosystems. However, a few species have become weedy, infecting important crops and causing significant yield losses (Parker, 2009). Their seeds recognise signal compounds released by host roots and germinate as a consequence (Bouwmeester *et al.*, 2003).

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Pea root exudate also stimulates germination of other broomrape species, such as *O. foetida* Poir., *O. minor* Sm. and *Phelipanche aegyptiaca* (Pers.) Pomel (syn. *O. aegyptiaca* Pers.) (Fernández-Aparicio *et al.*, 2009), but infection fails in subsequent parasitic stages. Pea is therefore a false crop for these species, inducing suicidal seed germination (Rubiales *et al.*, 2009c).

The better-known molecules that trigger parasitic plant germination are the strigolactones, which are also recognised by the arbuscular mycorrhizal (AM) fungi in their pre-symbiotic stage (Akiyama *et al.*, 2005; Yoneyama *et al.*, 2008). A role for strigolactones, as hormones involved in the aerial branching of pea and rice plants, has also been recently described (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). Once the parasitic seed germination is induced, an infective radicle arises from the seed coat and grows, following a positive gradient of germination stimulant in search of a host root, to which it can adhere and penetrate. When the parasitic tissue reaches vascular host tissue, a haustorial organ is differentiated, through which the nutritive flux is directed to the parasite.

Arbuscular mycorrhizal fungi belonging to the Glomeromycota phylum (Schüßler *et al.*, 2001), establish the most ancient and widespread symbiosis (Allen, 1996). This symbiosis is based on the improvement of the nutritional status of both partners. The plant provides organic carbon compounds to the fungus, whereas the fungus acquires inorganic mineral nutrients, mainly phosphate. The inorganic compounds acquired by the fungus are translocated to the arbuscules and released to the plant. Vierheilig (2004) suggested that once a plant is colonised by AM fungi, it suppresses subsequent AM colonisation. The root exudates would be responsible for repressing further AM recognition. Collaterally, this effect could repress soil pathogens attracted by the same signal molecules that are active against AM fungi. AM fungi could also be a promising tool for controlling parasitic plants. It has been reported that root exudates from mycorrhizal plants reduced *Striga hermonthica* (Delile) Benth. seed germination (Lendzemo *et al.*, 2007, 2009). The aim of this study was to study the potential effect of colonisation of pea roots by two AM fungi species on *Orobanchae* and *Phelipanche* seed germination.

Materials and methods

Plant material and growth conditions

Seeds of pea cv. 'Frisson' were surface sterilised for 5 min with a solution of commercial bleach (50% v/v) containing 0.02% (v/v) Tween 20 and rinsed thoroughly with sterile distilled water. Pea seeds were then germi-

nated under sterile conditions on wet filter paper for 4 days at 20°C and then transferred individually to 200 ml pots containing a sterile mixture of soil: sand: vermiculite (1:1:1), where they were grown in aseptic conditions (22–18°C, 16/8 h day/night rhythm, 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Plants were watered with Hoagland's nutrient solution (Hoagland & Arnon, 1950), modified to one-quarter phosphorus strength, twice per week by adding 50 ml of nutrient solution per pot.

Experiment 1: Effect of mycorrhizal inoculation on pea root exudates

Effect of pea root colonisation by *Glomus mosseae* and *Glomus intraradices* on *Orobanchae* and *Phelipanche* seed germination was studied in the first experiment carried out in pots. The experimental design consisted of control plants and plants inoculated with *G. mosseae* and *G. intraradices*. Each treatment was replicated four times. Inoculation was performed by adding and mixing 10 ml of a soil-sand based inoculum containing fungal propagules and mycorrhizal roots of either *G. mosseae* or *G. intraradices* to the filling substrate of each pot. Then a 4 days old pea seedling was transplanted into each pot. In order to avoid the differences in bacterial content between treatments and control, a microbial wash for each fungal species was used in the control plants (Pozo *et al.*, 2002). At 35 days after mycorrhizal inoculation (dai), four plants per treatment were harvested, root exudates collected and the degree of root colonisation determined as described below. The pea root, shoot and pod weights were assessed per plant.

Experiment 2: Monoxenic cultures to study the effect of mycorrhizal root colonisation dynamics on germination

Monoxenic cultures of *G. intraradices* growing with transformed carrot roots were established, in order to rule out the possible effects on *Orobanchae* and *Phelipanche* seed germination of bacteria that might live in close association with or attached to AM fungi structures. This experiment was performed using only *G. intraradices*, because the dual fungus-carrot root system does not support the growth of *G. mosseae* up to spore formation (Douds, 1997).

Pea plants were inoculated with a monoxenic culture of *G. intraradices* growing in transformed carrot (*Daucus carota* L.) roots, according to Chabot *et al.* (1992). The mycorrhizal inoculation was carried out at the transplanting time, on 4 days old pea seedlings, by adding to each 200 ml pot, a piece of monoxenic culture in Gel-Gro medium (ICN Biochemicals, Aurora, OH, USA) containing approximately 50 spores of

G. intraradices and infected carrot roots. The monoxenic culture (*G. intraradices* and carrot roots) was produced according to Chabot *et al.* (1992). In the uninoculated treatment, the plants were inoculated with a piece of Gel-Gro medium containing only uninfected carrot roots (Herrera-Medina *et al.*, 2007). Plants were grown in a growth chamber in the conditions described above. At 15, 25, 35 and 45 days after mycorrhizal inoculation, three mycorrhizal and three non-mycorrhizal plants were harvested, root exudates collected and the degree of root colonisation determined as described below.

Collection of root exudates

Mycorrhizal and non-mycorrhizal pea plants were removed from the substrate at each harvest time and the roots carefully washed. Plants were individually placed in 50 ml flasks and the roots immersed for 24 h in sterile distilled water. This allowed the plants to release the root exudates. The root solution containing the root exudates was collected, filtered with sterile gauze and the total root contained in each flask was weighed. As exudation might vary accordingly with the amount of roots in each flask, root solutions were adjusted with sterile distilled water to achieve a final root density of 0.03 g of pea root fresh weight per millilitre of root solution.

Conditioning of *Orobanche* and *Phelipanche* seeds

Seeds of four *Orobanche* and *Phelipanche* species were collected and used for the germination experiments. The species assayed were *O. crenata*, collected on pea in Spain, *O. foetida*, collected on faba bean (*Vicia faba* L.) in Tunisia, *O. minor* collected on red clover (*Trifolium pratense* L.) in Chile, and *P. aegyptiaca* collected on chickpea (*Cicer arietinum* L.) in Israel.

Orobanche and *Phelipanche* seeds were surface sterilised with formaldehyde, rinsed thoroughly with sterile distilled water and dried for 60 min in a laminar air flow cabinet. Approximately 150 seeds of each broomrape species were placed separately on discs of 2 cm diameter glass fibre filter paper (GFFP) moistened with 250 µl of sterile distilled water. Discs were placed in the dark at 20°C for 11 days, to promote the necessary conditioning for germination (Fernández-Aparicio *et al.*, 2008a).

Germination bioassay

After the conditioning period of *Orobanche* and *Phelipanche* seeds, the GFFP discs were dried in a laminar airflow cabinet to remove moisture. The ability of root exudates to induce seed germination was tested for each plant from the two experiments performed. For that purpose,

three aliquots of 125 µl each from the root exudate released from each plant were applied to each of three replicate discs. The strigolactone analogue GR24, previously shown to stimulate germination of most *Orobanche* and *Phelipanche* species (Johnson *et al.*, 1976; Fernández-Aparicio *et al.*, 2009), was used as a positive control at 3.4×10^{-5} M. Sterile distilled water was used as a negative control. Both controls were applied on three additional discs of each *Orobanche* and *Phelipanche* species. Seeds were stored in the dark at 20°C for 7 days to allow germination. The germination was scored for each disc by determining the number of germinated seeds from 100 seeds per disc using a stereoscopic microscope. Seeds were considered germinated when radicle was visible through the seed coat.

Estimation of root colonisation

At each harvest time, after the collection of root exudates, 500 mg of pea roots per plant, representing the total central root system, was stained with trypan blue according to the method of Phillips and Hayman (1970). Mycorrhiza development, determined by percentage of root length colonisation, was evaluated by the grid-line method described by Givannetti and Mosse (1980) under a stereoscopic microscope. Data corresponding to percentage of mycorrhizal colonisation were calculated from 300 intersected root fragments with a 1×1 cm grid.

The proportion of phosphatase-active mycorrhizal structures was determined in Expt 1 using the vital stain for alkaline phosphatase activity (ALP), allowing detection of active AM fungi as described by Tisserant *et al.* (1993). This activity was measured as the percentage of root intersections showing any active structure using the grid-intersect method under a stereoscopic microscope. The mean value of active structures in the root was then standardised relative to the mean value of total root colonisation (total structures with and without activity measured before), giving a final value of activity in each *Glomus* species. Thus the percentage of arbuscules being alkaline phosphatase active was assessed from 300 intersected root fragments and related as percentage from the total mycorrhizal colonisation.

Statistical analysis

Assays were developed using a completely randomised design. Data recorded as percentages were transformed to arcsine square roots (transformed value = $180/\pi \times \arcsin [\sqrt{(\%/100)}]$) to normalise data and stabilise variances throughout the data range. Data were subjected to analysis of variance using SPSS 15.0 (SPSS Inc., Chicago, IL, USA), after which residual

plots were inspected to confirm the data conformed to normality. For comparison between treatments, Tukey tests were performed on transformed germination data.

Results

Experiment 1

Microscopic observation of stained control roots confirmed the absence of mycorrhizal colonisation in non-mycorrhizal control plants. Colonisation of pea roots at the time of root exudate collection (35 dai) was similarly high for both *G. intraradices* (36.7%) and *G. mosseae* (42.2%). *Glomus intraradices* showed a lower proportion (32.8%) of alkaline phosphatase-active arbuscules than *G. mosseae* (55.5%) ($P = 0.010$) (Table 1).

Pea root colonisation by *G. mosseae* did not affect pea aerial or root biomass by the time of sampling, but significantly increased pod weight ($P = 0.035$) (Table 1). Colonisation by *G. intraradices* did not affect aerial biomass, but increased root biomass ($P = 0.032$) by 35 dai and decreased pod weight ($P = 0.024$) (Tables 1 and 2).

Induction of *Orobanche* and *Phelipanche* seed germination was significantly reduced in the presence of

mycorrhizal pea root exudate (Fig. 1). This reduction effect was observed in pea roots colonised by both mycorrhizal species, although the reduction was higher in pea roots colonised by *G. mosseae*. The reduction was higher for *O. crenata*, *O. foetida* and *P. aegyptiaca*, than for *O. minor* seeds. *Glomus intraradices* had no effect on *O. minor* seeds (Fig. 1). *Orobanche* and *Phelipanche* seed germination was negatively correlated with the level of alkaline phosphatase-activity shown by the arbuscules formed by *G. mosseae* ($r = -0.898$, $P = 0.002$ for *P. aegyptiaca*; $r = -0.914$, $P = 0.002$ for *O. crenata*; $r = -0.903$, $P = 0.002$ for *O. foetida*; $r = -0.951$, $P < 0.001$ for *O. minor*).

Experiment 2

The experiment with monoxenic culture of *G. intraradices* (Table 2) showed that pea roots were well colonised only from 35 dai (34.5%). No colonisation was observed by 15 dai and was still low by 25 dai (4.7%). Accordingly, no significant or little reduction of *Orobanche* and *Phelipanche* seed germination was observed at 15 or 25 dai (Fig. 2). A marked reduction in *O. crenata*, *O. foetida* and *P. aegyptiaca* seed germination was evident by 35 dai. The effect on

Table 1 Effect of mycorrhizal colonisation by *Glomus intraradices* (*G.i.*) and *G. mosseae* (*G.m.*) in comparison with non-mycorrhizal plants (Nm) on pea root, shoot and pod growth (g per plant) 35 days after mycorrhizal inoculation

	Mycorrhizal frequency and metabolic activity		Host weight		
	Mycorrhizal colonisation (%)	Arbuscules being alkaline phosphatase active (%)	Fresh root biomass (g per plant)	Fresh aerial biomass (g per plant)	Pods (g per plant)
Nm	0.0 ± 0.0b	–	6.2 ± 0.4ab	6.1 ± 0.6a	2.0 ± 0.01c
<i>G.i.</i>	36.7 ± 4.16a	32.8 ± 5.3b	7.5 ± 0.2a	6.0 ± 0.4a	1.6 ± 0.1c
Nm	0.0 ± 0.0b	–	5.4 ± 0.7b	5.7 ± 0.6a	2.1 ± 0.05b
<i>G.m.</i>	42.4 ± 3.48a	55.5 ± 1.6a	5.2 ± 0.2b	6.2 ± 0.3a	2.4 ± 0.1a

Values represent the mean ± SE. $n = 4$. The same letter per column indicates that differences are not statistically significant (Tukey test, $P < 0.05$).

Table 2 Effect of *G. intraradices* (*G.i.*) colonisation on pea root, shoot and pod growth at 15, 25, 35 and 45 days after mycorrhizal inoculation (Dai) with a monoxenic culture

Dai	Treatment	Mycorrhizal colonisation (%)	Root (g per plant)	Shoot (g per plant)	Pod (g per plant)
15	Nm	0.0 ± 0.0b	1.4 ± 0.3c	2.1 ± 0.2d	0.0 ± 0.0c
	<i>G.i.</i>	0.0 ± 0.0b	2.2 ± 0.2c	2.4 ± 0.4d	0.0 ± 0.0c
25	Nm	0.0 ± 0.0b	3.0 ± 0.4c	4.0 ± 0.4cd	0.0 ± 0.0c
	<i>G.i.</i>	4.7 ± 1.7b	3.7 ± 0.9c	4.5 ± 0.6cd	0.0 ± 0.0c
35	Nm	0.0 ± 0.0b	5.9 ± 0.6cb	7.3 ± 0.7ab	0.9 ± 0.1bc
	<i>G.i.</i>	34.5 ± 6.9a	11.5 ± 2.1a	6.9 ± 0.7abc	0.7 ± 0.3c
45	Nm	0.0 ± 0.0b	9.9 ± 1.1ab	8.9 ± 0.8a	2.5 ± 0.4a
	<i>G.i.</i>	42.4 ± 2.1a	10.4 ± 0.8ab	8.4 ± 0.6a	1.8 ± 0.2b

Values represent the mean ± SE. $n = 3$.

The same letter per column indicates that differences are not statistically significant (Tukey test, $P < 0.05$).

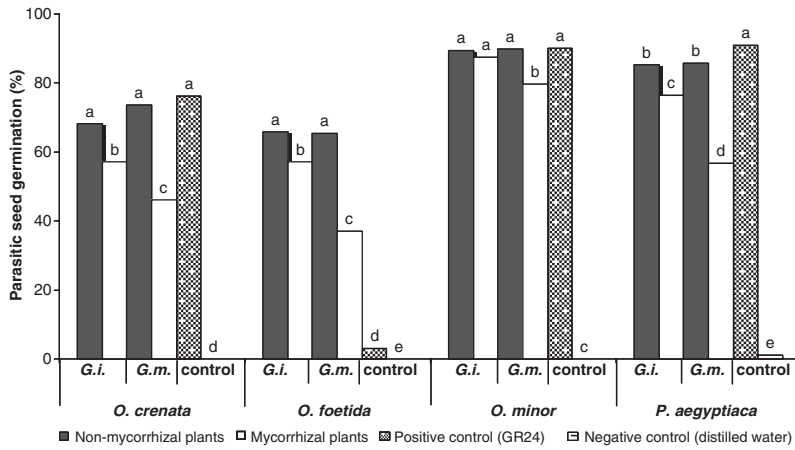


Fig. 1 Percentage germination of seeds of *Orobancha crenata*, *O. foetida*, *O. minor* and *Phelipanche aegyptiaca* exposed to root exudates of pea plants colonised (35 days after inoculation) by *Glomus intraradices* (G.i.) or *G. mosseae* (G.m.) and of non-mycorrhizal control plants.

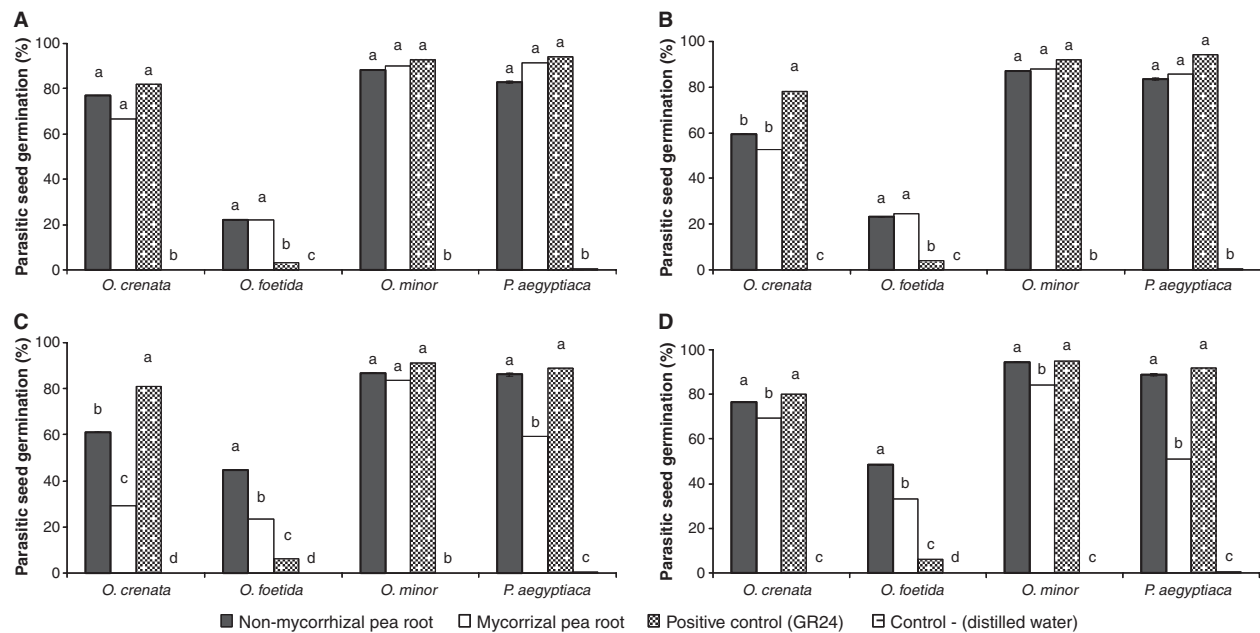


Fig. 2 Percentage seed germination of *O. crenata*, *O. foetida*, *O. minor* and *P. aegyptiaca* exposed to the germination stimulant GR24 and to root exudates of control non-mycorrhizal plants pea and of mycorrhizal pea plants colonised by *G. intraradices* (A) 15 days after inoculation (dai) (B) 25 dai (C) 35 dai and (D) 45 dai.

O. minor was however smaller, being evident only by 45 dai. Taking into account the four sampling times, a negative correlation was observed between percentage of pea root colonisation by *G. intraradices* and percentage germination of *P. aegyptiaca* ($r = -0.658$, $P < 0.001$), *O. crenata* ($r = -0.593$, $P < 0.001$) and *O. foetida* ($r = -0.493$, $P < 0.001$), but not of *O. minor*.

Discussion

The results presented here confirm a reduction in *Orobancha* and *Phelipanche* seed germination following pea root colonisation by *G. mosseae* and *G. intraradices*. Both AM fungi and parasitic weeds are attracted to the host roots by means of strigolactones (Yoneyama *et al.*,

2009). The observed reduction in germination in mycorrhizal pea plants might be due to a decrease in the amount of germination stimulants present in the root exudates. Better nutritive status in mycorrhizal plants could down-regulate the strigolactone exudation and, as a consequence, the autoregulation of further mycorrhizal colonisation. Coincidentally, fewer parasitic seeds would be able to recognise the mycorrhizal roots. Vierheilig (2004) suggested that mycorrhization could modulate the complexity of signal metabolites present in root exudates, thus repressing further mycorrhizal colonisation and also parasitic plant germination.

The reduction of germination was higher in pea roots colonised by *G. mosseae* than by *G. intraradices*. Although the percentage of colonised host root length

was similar between *G. mosseae* and *G. intraradices* inoculated plants, we found that the fungal structures of *G. mosseae* were more active than *G. intraradices*, in terms of ALP. There is no direct evidence that alkaline phosphatase is involved in P turnover. However, it has been reported as a marker for the efficiency of the mycorrhizal symbiosis and its involvement in P transport (Tisserant *et al.*, 1993; Kojima & Saito, 2004) and arbuscular functionality has been suggested (Herrera-Medina *et al.*, 2007).

In agreement with previous reports (Fernández-Aparicio *et al.*, 2008a,b, 2009), *O. crenata*, *O. minor* and *P. aegyptiaca* seed germination was particularly induced by the synthetic strigolactone GR24, whereas *O. foetida* germination was negligible. However, all these species germinated in the presence of pea root exudates. Different germination stimulants, such as fabacyl acetate (Xie *et al.*, 2009), peagol and peagoldione (Evidente *et al.*, 2009), have been identified in pea root exudates. The specialisation in host recognition by the different *Orobanchae* and *Phelipanche* species could be mediated by unique combinations between the type and amount of stimulant exuded by each host plant (Bouwmeester *et al.*, 2003). There are some indications of different levels of activity of each stimulant on each parasite species (Yoneyama *et al.*, 2009). For instance, peagol differentially stimulates *O. foetida* and *P. aegyptiaca*, but not *O. crenata* and *O. minor* (Evidente *et al.*, 2009). In addition, specific mixtures of different strigolactones at specific combinations could stimulate the seeds of the more specialised broomrapes in the soil. Not only strigolactones, but other signalling chemicals that are synergistic or antagonistic to strigolactone action, might be involved in broomrape seed germination.

Our results are in agreement with those of Lenzemo *et al.* (2007), who showed a decrease of *S. hermonthica* seed germination by AM colonisation of sorghum (*Sorghum vulgare* Pers.) roots. However, Lenzemo *et al.* (2007) could not discard inhibitory effects due to other microorganisms present in the mycorrhizosphere. Some bacteria lived in close association or attached to AM fungi structures. We have eliminated this effect by using monoxenic cultures of *G. intraradices* growing in transformed carrot roots. Unfortunately, it should be noted that the dual fungus–carrot root system does not seem to be universally applicable, since it does not support the growth of *G. mosseae* up to spore formation (Douds, 1997).

Reduction of germination inhibition does not take effect at initial stages of symbiotic recognition, but when the pea roots were well colonised (only after 35 dai for *O. crenata*, *O. foetida* and *P. aegyptiaca*; 45 dai for *O. minor*). This reduction affects not only *O. crenata*, but also *O. foetida*, *O. aegyptiaca*, and *O. minor* to which pea

is a false host inducing suicidal germination, thereby diminishing their effectiveness. This is in agreement with Lenzemo *et al.* (2009), who also found inhibition of *S. hermonthica* seed germination by mycorrhizal colonisation of both host and non-host plants.

In addition to this direct effect on *Orobanchae* and *Phelipanche* seed germination, mycorrhizal colonisation might induce defence responses to infection, which deserves further investigation. Indeed, mycorrhizal colonisation is known to increase levels of cell wall-bound phenolics (Grandmaison *et al.*, 1993) and to induce expression of defence-related genes (Taylor & Harrier, 2003), providing direct protection against root pathogens (Pozo & Azcón-Aguilar, 2007). This might also protect against broomrape infection. Similarly, some *Rhizobium leguminosarum* strains have been reported to induce defence against *O. crenata* in pea through activation of the oxidative process, LOX pathway and production of possible toxic compounds, including phenolics and pisatin (Mabrouk *et al.*, 2007). Improved nutrient status as a consequence of mycorrhizal colonisation can also result in enhanced protection against pathogens (Borowicz, 2001).

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References

- AKIYAMA K, MATSUZAKI K & HAYASHI H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**, 824–827.
- ALLEN MF (1996) The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. *Mycological Research* **100**, 769–782.
- BOROWICZ VA (2001) Do arbuscular mycorrhizal fungi alter plant - pathogen relations? *Ecology* **82**, 3057–3068.
- BOUWMEESTER HJ, MATUSOVA R, ZHONGKUI S & BEALE MH (2003) Secondary metabolite signalling in host-parasitic plant interactions. *Current Opinion in Plant Biology* **6**, 358–364.
- CHABOT S, BÉCARD G & PICHÉ Y (1992) Life cycle of *Glomus intraradix* in root-organ culture. *Mycologia* **84**, 315–321.
- DOUDS DD (1997) A procedure for the establishment of *Glomus mosseae* in dual culture with Ri T-DNA-transformed carrot roots. *Mycorrhiza* **7**, 57–61.
- EVIDENTE A, FERNÁNDEZ-APARICIO M, CIMMINO A, RUBIALES D, ANDOLFI A & MOTTA A (2009) Peagol and peagoldione, two new strigolactone like metabolites isolated from pea root exudates. *Tetrahedron Letters* **50**, 6955–6958.
- FERNÁNDEZ-APARICIO M, ANDOLFI A, CIMMINO A, RUBIALES D & EVIDENTE A (2008a) Stimulation of seed germination of *Orobanchae* species by ophiobolin A and fusicoccins derivatives. *Journal of Agricultural and Food Chemistry* **56**, 8343–8347.

- FERNÁNDEZ-APARICIO M, ANDOLFI A, EVIDENTE A, PÉREZ-DE-LUQUE A & RUBIALES D (2008b) Fenugreek root exudates with *Orobanche* species specific seed germination stimulatory activity. *Weed Research* **48**, 163–168.
- FERNÁNDEZ-APARICIO M, FLORES F & RUBIALES D (2009) Recognition of root exudates by seeds of broomrape (*Orobanche* and *Phelipanche*) species. *Annals of Botany* **103**, 423–431.
- GIVANNETTI M & MOSSE B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489–500.
- GOMEZ-ROLDAN V, FERMAS S, BREWER PB *et al.* (2008) Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194.
- GRANDMAISON J, OLAH GM, VANCALSTEREN MR & FURLAN V (1993) Characterization and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots. *Mycorrhiza* **3**, 155–164.
- HERRERA-MEDINA MJ, STEINKELLNER S, VIERHEILIG H, OCAMPO JA & GARCÍA GARRIDO JM (2007) Abscisic acid determinates arbuscule development and functionality in the tomato arbuscular mycorrhiza. *New Phytologist* **175**, 554–564.
- HOAGLAND DR & ARNON DI (1950) *The Water-Culture Method for Growing Plants Without Soil*. University California Experimental Station Circular 347.
- JOHNSON AW, ROSEBERY G & PARKER C (1976) A novel approach to *Striga* and *Orobanche* control using synthetic germination stimulants. *Weed Research* **16**, 223–227.
- KOJIMA T & SAITO M (2004) Possible involvement of hyphal phosphatase in phosphate efflux from intraradical hyphae isolated from mycorrhizal roots colonized by *Gigaspora margarita*. *Mycological Research* **108**, 610–615.
- LENDZEMO VW, KUYPER TW, MATUSOVA R, BOUWMEESTER HJ & VAN AST A (2007) Colonization by arbuscular mycorrhizal fungi of sorghum leads to reduced germination and subsequent attachment and emergence of *Striga hermonthica*. *Plant Signalling Behaviour* **2**, 1–5.
- LENDZEMO V, KUYPER TW & VIERHEILIG H (2009) *Striga* seed-germination activity of root exudates and compounds present in stems of *Striga* host and nonhost (trap crop) plants are reduced due to root colonization by arbuscular mycorrhizal fungi. *Mycorrhiza* **19**, 287–294.
- MABROUK Y, ZOURGUI L, SIFI B, DELAVAUULT P, SIMIER P & BELHADJ O (2007) Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitized by *Orobanche crenata*. *Weed Research* **47**, 44–53.
- PARKER C (2009) Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Management Science* **65**, 453–459.
- PHILLIPS JM & HAYMAN DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.
- POZO MJ & AZCÓN-AGUILAR C (2007) Unraveling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* **10**, 393–398.
- POZO MJ, CHRISTELLE C, DUMAS-GAUDOT E, GIANINAZZI S, BAREA JM & AZCÓN-AGUILAR C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany* **53**, 525–534.
- RUBIALES D, PÉREZ-DE-LUQUE A, CUBERO JI & SILLERO JC (2003) Crenate broomrape (*Orobanche crenata*) infection in field pea cultivars. *Crop Protection* **22**, 865–872.
- RUBIALES D, FERNÁNDEZ-APARICIO M, PÉREZ-DE-LUQUE A *et al.* (2009a) Breeding approaches for crenate broomrape (*Orobanche crenata* Forsk.) management in pea (*Pisum sativum* L.). *Pest Management Science* **65**, 553–559.
- RUBIALES D, FERNÁNDEZ-APARICIO M, MORAL A, BARILLI E, SILLERO JC & FONDEVILLA S (2009b) Disease resistance in pea (*Pisum sativum* L.) types for autumn sowings in Mediterranean environments. *Czech Journal of Genetics and Plant Breeding* **45**, 135–142.
- RUBIALES D, FERNÁNDEZ-APARICIO M, WEGMANN K & JOEL D (2009c) Revisiting strategies for reducing the seedbank of *Orobanche* and *Phelipanche* spp. *Weed Research* **49**, 23–33.
- SCHÜBER A, SCHWARZOTT D & WALKER C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**, 1414–1421.
- TAYLOR J & HARRIER LA (2003) Expression studies of plant genes differentially expressed in leaf and root tissues of tomato colonised by the arbuscular mycorrhizal fungus *Glomus mosseae*. *Plant Molecular Biology* **51**, 619–629.
- TISSERANT B, GIANINAZZI-PEARSON V, GIANINAZZI S & GOLLOTTE A (1993) In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections. *Mycological Research* **97**, 245–250.
- UMEHARA M, HANADA A, YOSHIDA S *et al.* (2008) Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195–200.
- VIERHEILIG H (2004) Regulatory mechanisms during the plant-arbuscular mycorrhizal fungus interaction. *Canadian Journal of Botany* **82**, 1166–1176.
- XIE X, YONEYAMA K, HARADA Y *et al.* (2009) Fabacyl acetate, a germination stimulant for root parasitic plants from *Pisum sativum*. *Phytochemistry* **70**, 211–215.
- YONEYAMA K, XIE X, SEKIMOTO H *et al.* (2008) Strigolactones, host recognition signals for root parasitic plants and arbuscular mycorrhizal fungi, from Fabaceae plants. *New Phytologist* **179**, 484–494.
- YONEYAMA K, XIE X, YONEYAMA K & TAKEUCHI Y (2009) Strigolactones; structures and biological activities. *Pest Management Science* **65**, 467–470.