Plant and Soil **145**: 298–300, 1992.
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PLSO 9508

Absence of VA colonization in *Oxalis pes-caprae* inoculated with *Glomus mosseae*

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Received 14 February 1992. Revised June 1992

Key words: *Glomus mosseae*, *Medicago sativa*, non-host plants, *Oxalis pes-caprae*, VA mycorrhizas

Abstract

Although members of the Oxalidaceae family have been described as host plants of vesicular-arbuscular mycorrhizal fungi, *Oxalis pes-caprae* did not become colonized by *Glomus mosseae*. Extracts of *Ox. pes-caprae* root inhibited the germination of *G. mosseae* spores. However, the presence of *G. mosseae* in the rhizosphere of *Ox. pes-caprae* produced browning of the roots, which was interpreted as a hypersensitivity response of the plant to the presence of VA fungus.

Introduction

The absence of vesicular-arbuscular mycorrhizal (VAM) symbiosis has been described not only in members of Cruciferae and Chenopodiaceae families, but also in members of families that usually form VAM (Harley and Harley, 1987). The lack of VAM colonization in non-susceptible plants has been attributed to toxic compounds in the root (Allen et al., 1989; Vierheilig and Ocampo, 1990a), or to the lack of factors that promote VAM colonization (Becard and Piche, 1989). However, most of these studies have been done in the Cruciferae and Chenopodiaceae families. There is very little information on the barriers to colonization of other non-host plants which can be important for understanding the development of VAM colonization in general.

Members of the Oxalidaceae family have been described as VA host plants (Harley and Harley, 1987). Nevertheless, *Oxalis pes-caprae* does not form VAM, hence the aim of this work was to study the possible nature of the factors involved in the absence of VA colonization of *Ox. pes-caprae*.

Methods

Experiment 1

Plants were grown in 300 mL capacity open pots of soil collected from the Province of Granada, Spain. The soil, of calcexerollic xerochrept type, 41 mg kg^{-1} of P and pH 7.6 (for full details see Garcia-Romera and Ocampo, 1988), was steam-sterilized and mixed with sterilized quartz sand at a proportion of 1:1 (V:V). Alfalfa (*Medicago sativa* cv. Aragon) and *Oxalis pes-caprae* were used as test plants. Seeds were sown in moistened sand, and after 2 weeks, seedlings were transplanted to the pots and grown under greenhouse conditions. Natural light was supplemented by Silvania incandescent and cool-white lamps, 400 nmol m^{-2} s^{-1}, 400–700 nm length wave spectrum; with a 16–8 h light-dark cycle at 25–19°C and 50% relative humidity. Plants were watered from below using a capillary system, and fed with a nutrient solution (Hewitt, 1952) lacking phosphate for VA-inoculated plants.

The VA inoculum consisted of 5 g of rhizosphere soil from a maize plant pot culture of a

G. mosseae isolate that contained spores, mycelia and colonized root fragments. Uninoculated plants were given filtered leachings from the inoculum soil. The VA uninoculated (control) treatment consisted of soil filtrate (Whatman No. 1 filter paper) from the rhizosphere of mycorrhizal plants containing common soil microorganisms, but no propagules of *G. mosseae*.

Plants were harvested after 8 weeks and roots samples were cleared and stained (Phillips and Hayman, 1970), cut into 1-cm segments that were mixed and repeatedly subdivided to yield random samples of 40 root segments per replicate, which were mounted on slides and examined under a compound microscope at ×160 magnification. The percentage of total root length which was colonized with VA mycorrhizas was measured as described by Ocampo et al. (1980).

Experiment 2

Alfalfa and *Ox. pes-caprae* were grown in pots containing a sterilized soil:sand mixture inoculated with *G. mosseae* as in Experiment 1. When plants were 8 weeks old, roots samples were extracted by grinding 2 g root material with 1 mL 0.1 *M* Tris-HCl buffer, pH 7. After centrifugation (20 min 8000 × g) the extract was passed through a Millipore filter (0.45 μm). The sterilized extracts were used fresh or autoclaved for 20 min at 120°C. Twenty-five surface-sterilized (Mosse, 1962) spores of *G. mosseae* were flooded with the extract or with 1 ml of buffer as control for 3 h and then placed in Petri dishes with agar (1%, pH 7). Dishes were sealed with nescofilm and stored at 25°C in the dark. Spore germination was examined after 1 week in 10 replications per treatment.

Results

No VA colonization was observed in *Ox. pes-caprae* plants grown singly or together with alfalfa (Table 1). VA colonization of alfalfa plants was not decreased by the presence of the 'non-host' *Ox. pes-caprae*. Root turned brown only when *Ox. pes-caprae* was grown in the presence of *G. mosseae*.

Fresh *Ox. pes-caprae* extracts completely inhibited spore germination, but inhibition decreased when the extracts were autoclaved for 20 min at 120°C. When the spores were exposed to alfalfa root extract, percentage germination did not differ significantly from controls (Table 2).

Table 1. VA colonization of alfalfa grown alone or together with *Oxalis pes-caprae* and inoculated with *Glomus mosseae*

Plant	Root length colonization (%)
Alfalfa alone	60 ± 9
Alfalfa together with *Ox. pes-caprae*	56 ± 7
Ox. pes-caprae alone	0
Ox. pes-caprae together with Alfalfa	0

Each figure is the mean for 10 pots. Standard errors of the mean are given.

Table 2. Percentage germination of *G. mosseae* spores in the presence of different root extracts

Root extracts	Spore germination (%)
Control	30 ± 3
Alfalfa	40 ± 8
Ox. pes-caprae	0
Alfalfa autoclaved for 20 min. at 120°C	38 ± 6
Ox. pes-caprae autoclaved for 20 min. at 120°C	16 ± 5

Each figure is the mean for 10 replicates. Standard errors of the mean are given.

Discussion

Several observations suggest different possible explanations for the lack of VAM colonization in non-host plants. The lack of promoting-factors can influence the non-susceptibility of non-host plants (Becard and Piche, 1989). However, toxic compounds present in the root extracts of Cruciferae and Chenopodiaceae also seem to be involved in the non-colonization of these plants (Vierheilig and Ocampo, 1990a). The absence of VAM colonization in *Ox. pes-caprae* root in the presence of alfalfa indicates that the non-suscep-

tibility of this plant cannot be attributed solely to the lack of promoting substances.

The root extracts of Ox. pes-caprae, like those of some Cruciferae and Chenopodiaceae plants, inhibit spore germination of *G. mosseae* (Vierheilig and Ocampo, 1990a). The lack of VAM colonization may therefore be attributed to the presence of toxic compounds released in the root extracts, rather than to compounds released in its exudates (Ocampo et al., 1980).

Our observations suggest that *Ox. pes-caprae* reacts like some Chenopodiaceae plants (Vierheilig and Ocampo, 1990b) in producing a classic incompatibility response. Allen et al. (1989) concluded that this reaction might reflect active rejection of the plant towards the fungus. Anderson (1988) suggested that the accumulation of phenol by the plant could cause the roots to turn brown. Fresh *Ox. pes-caprae* extracts inhibited spore germination, but inhibition decreased when the root extracts were autoclaved for 20 min at 120°C. This suggests that the formation of inhibitory substances produced by root extracts were sensitive to high temperatures but its inhibitory effect did not dissapear completely. Although these results indicate that the nature of the toxic compounds of *Ox. pes-caprae* seem to be non proteic, further studies are needed to determine the nature of these compounds and to understand the interaction between *Ox. pes-caprae* and VAM.

Acknowledgements

The authors thank Karen Shashok for grammatical correction of the text. Financial support for this study was provided by the Comision Interministerial de Ciencia y Tecnologia, Spain.

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