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Influence of simazine on formation of vesicular-arbuscular mycorrhizae in *Chenopodium quinona* Willd

S. M. SCHWAB,
Department of Botany and Plant Sciences, University of California, Riverside, California 92521

E. L. V. JOHNSON and J. A. MENGE
*Department of Plant Pathology**, University of California, Riverside, California 92521*

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Summary Low levels of vesicular-arbuscular mycorrhiza formation were found in a normally nonmycorrhizal species, *Chenopodium quinona,* when sprayed with sublethal doses of simazine. Application of simazine also increased exudation of sugars and amino acids from roots. It is suggested that this increase in root exudation is responsible for the unusual formation of mycorrhizae in this species.

Introduction

Symbiotic vesicular-arbuscular mycorrhizal associations between certain fungi and the roots of plants are common throughout the plant kingdom. Members of the Chenopodiaceae (Chenopodium, Atriplex, Beta, Spinacia, *e.g.*) and several closely related families are unusual in that they typically do not form vesicular-arbuscular mycorrhizae (VAM). The occurrence of low levels of VAM formation in some members of the Chenopodiaceae when grown in the presence of a mycorrhizal 'nurse plant'[4,8] suggests that VAM are not eliminated from this group of species because of the presence of an inhibitor in the roots. Instead, it seems that the barrier to VAM formation can be partially overcome by the addition of factors associated with the living roots of a host plant. Based on experiments with plants grown at high and low tissue phosphorus concentrations, it has been suggested that root exudation may be an important factor in regulating the extent of VAM formation in normally mycorrhizal species[2,11].

The herbicide simazine (2-chloro-4, 6-bis(ethylamino)-*s*-triazine, 80% W.P.) has been reported to alter the amino acid and sugar content of extracts of shoots[10,14] and roots[3] of several plant species. If this change in composition of extracts also is reflected in changes in root exudates, application of simazine could stimulate VAM formation. Ectomycorrhiza formation has been reported to increase with simazine application in two species of pine[12]. This paper reports the results of experiments on the effect of simazine on root exudation and VAM formation in a nonmycorrhizal plant species, *Chenopodium quinona* Willd.

Materials and methods

C. quinona seeds were sown in 10 cm clay pots filled with autoclaved sand mixed with 15 g per pot of mycorrhizal inoculum consisting of soil, roots, hyphae, and chlamydospores from pot cultures of barley (*Hordeum vulgare* L.) infected with *Glomus deserticola* Trappe, Bloss, Menge. Pots were divided into six treatments of five plants each. Each plant was sprayed once weekly with one of the following concentrations of simazine: 2 ppm, 1 ppm, 0.5 ppm, 0.05 ppm, 0.005 pm, or a distilled water control. To enhance foliar penetration, 0.1% Triton-X was added to each spray solution. Plastic sheets were placed over soil while spraying to prevent soil contamination. Plants were harvested eight weeks after sowing. Roots were washed to remove any adhering soil or organic

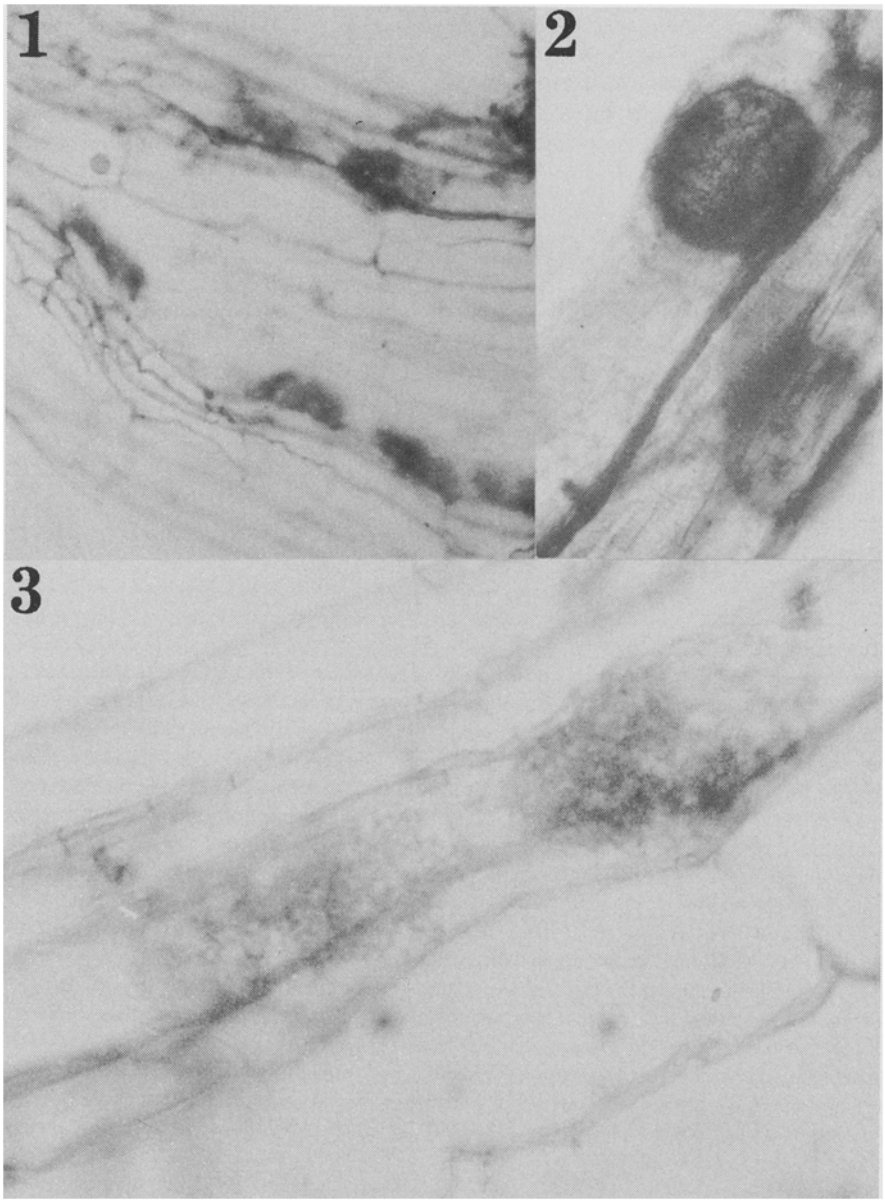

Plate 1.

Fig. 1. *C. quinona* root with internal hyphae and arbuscules of *G. deserticola.*
Fig. 2. Chlamydospores of *G. deserticola* in *C. quinona* root.
Fig. 3. Arbuscules and hyphae in cortex of *C. quinona.*

debris, then cleared in KOH and stained in trypan blue[9]. Approximately 1/3 of each root system was examined for the presence of internal hyphae, vesicles, and arbuscules. An estimate of the level of VAM infection was made by recording the percent of 1 mm root segments containing vesicles or arbuscules in a random subsample of each root system.

A similar experiment was done five months later, this time using sprays of 10 ppm and 2 ppm simazine and a distilled water control. Growing conditions and assessment of mycorrhiza formation were as in the first experiment. At the same time 18 plants were grown in noninoculated soil and divided into the same three spray treatments as above. Five weeks after sowing four plants in each treatment were selected for uniformity and carefully washed free of soil for root exudate collection. Immediately after washing away soil, roots of intact plants were immersed in an aerated solution of 0.5 m*M* $CaCl_2$ containing 0.05 g/l rifampicin and 0.025 g/l tetracycline to reduce bacterial contamination of root exudates. After 2 h in the antibiotic solution, roots were rinsed in sterile water, then reimmersed in aerated sterilized water containing 0.05 m*M* $CaCl_2$ for 6 h, followed by a transfer to a second flask of sterilized water for another 6 h. Contents of the two flasks were pooled for each plant and condensed to 10 ml on a rotary evaporator at 45°C. Duplicate 1 ml aliquots of each exudate sample were analyzed for amino acids using ninhydrin[13], reducing sugars using Nelson's reagent[7], and total soluble carbohydrates using anthrone[6].

Plant heights and numbers of leaves were measured once per week throughout the experiment. Root and shoot dry weights were recorded at the end of the experiment. Dried roots and shoots were pooled for each treatment and their P content determined by the Vanadate-Molybdate-Yellow method[1]. Differences in growth parameters and root exudation among treatments were analyzed for significant differences using one way AOV and Duncan's test.

Results and discussion

Over half the *C. quinona* plants sprayed with 1 ppm or more simazine showed some VAM formation (Table 1). In all plants less than 5% of the total root length was infected, and in all but two cases 1% or less was infected. However, the infections present were very similar to infections in species that readily form VAM (Plate 1). Both arbuscules and thin-walled vesicles, as well as thick-walled chlamydospores, were found in the cortex of apparently healthy roots with no hyphal penetration into the stele. Where infections occurred, they were extensive (up to 1 cm in length) and more than one penetration at a site were commonly observed. External hyphae and appressoria without further penetration were also common around noninfected roots in all treatments.

Table 1. Percent of eight-week-old *Chenopodium quinona* plants showing VAM infection when sprayed with sublethal doses of simazine. Percentages are based on five plants per treatment

Simazine concentration (ppm)	Plants infected (%)
1st experiment	
0	0
0.005	0
0.05	0
0.50	0
1.00	20
2.00	60
2nd experiment	
0	0
2.00	60
10.00	60

Table 2. Shoot growth, shoot P concentration, and carbohydrate and amino acid concentrations in root exudates of *Chenopodium quinona* sprayed with sublethal doses of simazine. Mean and standard deviation of four plants per treatment

	Growth parameters				Root exudates (mg/g dry root)		
Spray	Height (cm)	Shoot dry wt. (gram)	Leaves (no. per plant)	Shoot P concentration (% of dry wt.)	Total carbohydrate	Reducing sugars	Amino acids
Distilled water	$12.3 \pm 1.3_a$*	$.30 \pm 0.09_a$	$18.0 \pm 4.5_a$	.21	$.64 \pm 0.09_a$	$0.21 \pm 0.03_a$	$0.10 \pm 0.01_a$
2 ppm simazine	$7.9 \pm 1.0_b$	$.04 \pm 0.02_b$	$12.0 \pm 1.2_b$	.19	$3.48 \pm 1.54_a$	$1.51 \pm 0.20_b$	$1.98 \pm 0.89_b$
10 ppm simazine	$7.1 \pm 1.0_b$	$.02 \pm 0.01_b$	$8.5 \pm 1.3_b$	.20	$9.52 \pm 3.23_b$	$2.29 \pm 1.01_b$	$2.42 \pm 0.51_b$

* Values with different subscripts are statistically different, $P = 0.01$.

Foliar application of simazine reduced growth of *C. quinona* but had no effect on tissue P concentrations (Table 2). Application of 2 ppm or more simazine significantly increased the concentration of reducing sugars and amino acids in root exudates, compared to unsprayed controls, and 10 ppm simazine significantly increased the concentration of total carbohydrates in root exudates. Although there was a trend toward increasing amino acid and reducing sugar exudation at 10 ppm compared to 2 ppm simazine, differences between these treatments were not statistically significant. There was also no increase in VAM formation with increasing simazine dose above 2 ppm.

Although the limited amount of infection observed seems unlikely to noticeably affect growth of *C. quinona*, the stimulation of even small amounts of VAM infection in a 'nonmycorrhizal' species is significant from the standpoint of understanding how the physiology of the host can affect VAM formation. In species that typically form VAM, the extent of infection has been correlated with greater exudation of sugars and/or amino acids under various levels of P nutrition[2,11] and photoperiod[5]. This has led to the suggestion that some factor(s) in root exudates is (are) important in regulating VAM formation.

C. quinona shows significantly lower levels of exudation of both sugars and amino acids than do three unrelated mycorrhizal species (unpubl. data). Increasing the supply of root exudates available to the fungal symbiont, either with a mycorrhizal 'nurse plant'[4,8] or by application of simazine has been assoicated with at least limited VAM formation in what would otherwise be a nonmycorrhizal plant species.

Although simazine has been reported to increase amino acid content in shoots[10,14] and roots[3], in these experiments the concentrations of sugars as well as amino acids were increased in the root exudates. Thus it is not possible to determine from this study whether some components of exudates are more important than others in stimulating VAM formation. It is also quite possible that other major components of root exudates which have not been measured, such as organic acids, are also correlated with increases in infection. These experiments do suggest, however, that VAM can be formed even in poor host plants when the overall level of root exudation is sufficiently high.

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References

1 Chapman H D and Pratt P F 1961 Methods of Analysis for Soils, Plant, and Waters. University of California, Agric. Sci., Berkeley.
2 Graham J H *et al.* 1981 Pl. Physiol. 68, 548–552.
3 Graser H 1967 Flora A. 158, 493–504.
4 Hirrel M C *et al.* 1978 Can. J. Bot. 56, 2813–2817.
5 Johnson C R *et al.* 1981 New Phytol. *In press.*
6 Morris D L 1948 Science 107, 254.
7 Nelson N 1944 J. Biol. Chem. 153, 375–380.
8 Ocampo J A *et al.* 1980 New Phytol. 84, 27–35.
9 Phillips J M and Hayman D S 1970 Trans. Br. Mycol. Soc. 55, 158–161.
10 Ploszynski M 1972 Pamiet. Pulawski 51, 51.
11 Ratnayake M *et al.* 1978 New Phytol 81, 543–552.
12 Smith J R and Ferry B W 1979 Ann. Bot. 43, 93–99.
13 Spies J R 1957 Methods in Enzymology III. Academic Press, New York.
14 Swietochowski B *et al.* 1966 Pamiet. Pulawski 21, 211–226.