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SHORT NOTE

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Field application of vesicular-arbuscular mycorrhizal fungi improved garlic yield in disinfected soil

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Abstract The production of certified garlic propagation material requires measures to be taken against pathogenic nematodes. Methyl bromide (MB) may be used for this purpose, but is known to cause stunting in *Allium* spp. Vesicular-arbuscular mycorrhizal (VAM) fungal inoculum was applied to the planting furrow after MB treatment. VAM-inoculated plants were larger, had more green leaves, an increased photosynthesis rate, especially at low light intensities, and higher fresh and dry weights than plants in uninoculated plots. The mean bulb weights from uninoculated and VAM-treated plots were 27 g and 51 g respectively. The native or an improved VAM population should be reintroduced after soil disinfection to ensure satisfactory garlic yields.

Key words *Allium sativum* · Methyl bromide · Photosynthesis

Introduction

About 500 000 ha of garlic (*Allium sativum* L.) are cultivated worldwide (FAO 1994). A significant portion of this area is for production of propagation material. In many countries, programs now exist to produce high-quality propagation material free of endemic viruses (Walkey 1990) and other soil-borne pests, especially the stem and bulb nematode *Ditylenchus dipsaci*.

Certified propagation material must be grown from cloves tested for the absence of pathogens. Because of the long survival and wide host range of *D. dipsaci*, fields must be treated before planting to eliminate nematodes (Green 1990). Methyl bromide (MB) is one of the most effective agents for this, but causes stunting in garlic (Siti et al. 1982).

One of the recognized side effects of soil disinfection is the destruction of vesicular-arbuscular mycorrhizas (VAM) (Haas et al. 1987; Menge 1982, 1983). *Allium* spp., including garlic, are extremely responsive to VAM symbiosis and stunting of onion growth in the absence of VAM fungi has been well documented (Gerdemann 1968; Boatman et al. 1978). Since the size of the harvested garlic bulb is directly related to the planted clove size (Jones and Mann 1963), stunting in propagation material reduces not only the yield of the crop of propagation material, but also the yield of the subsequent commercial crop.

Application of VAM fungi to soil or propagation material has been shown to be effective in suppressing stunting syndrome in other crops following soil disinfection (Menge 1983; Kapulnik et al. 1994). A field experiment was carried out to test the effect of VAM inoculation on garlic growth following soil fumigation with MB.

Materials and methods

Plant and mycorrhizal material

Cloves of the Israeli cultivar 'Frankon' were prepared from bulbs harvested in May 1993. Inoculum of *Glomus intraradices* (Smith and Schenck) was raised on leek (*A. porrum*) plants using the technique reported by Patterson et al. (1990).

Field preparation and experimental layout

The experiment was carried out on a sandy loam soil in Bet Dagan. Raised planting beds were prepared in early September 1993. The equivalent of 84 kg N, 87 kg P, and 105 kg K per ha was

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applied as ammonium nitrate, superphosphate, and potassium chloride during soil preparation. Final P concentration at 0–30 cm soil depth at the time of planting was 60 ppm as tested by the method of Olsen and Dean (1965). Four weeks before planting, two parallel 10-1-m beds were covered with plastic sheeting. The equivalent of 400 kg MB/ha was applied, the cover was removed after 48 h and the field was irrigated by overhead sprinklers with a total of 2000 m^3 water/ha. Two 10-cm-deep planting furrows per bed were then divided into five 1.5-m-long plots plus 1.25-m border plots. Parallel plots were paired to create five replicated experimental units of a randomized-block experimental design.

VAM inoculum (dry roots and spores) was spread in the furrows of one randomly chosen plot per unit and lightly covered with soil from the furrow; the other furrow was similarly partly filled with soil. Cloves were planted on 11 October 1993 (28 plants/m^2).

Crop growth and sampling procedures

Plants were irrigated as necessary from overhead sprinklers and fertilized four times with ammonium nitrate to a total of 57 kg N/ha. The field was sprayed against pests and foliar pathogens as needed.

Roots of two plants per plot were randomly sampled 44 and 140 days after planting (dap) and assessed for VAM colonization according to the method of Phillips and Hayman (1970). Roots were digested in 10% KOH and stained with 0.1% trypan blue in lactophenol. Thirty root samples per treatment were observed under a light microscope and roots showing signs of infection were counted.

Plots were sampled 74, 119, 141, 155 and 176 dap for plant height (stem base to tip of longest leaf), number of green leaves, and plant fresh and dry weights. Photosynthesis rates of leaves were measured on four intact leaves of four individual plants in each of the VAM fungus and untreated replicate plots in the field by means of a leaf gas exchange meter (LCA-4 Analytical Development Company, Hoddeson, England), which measures CO_2 production, leaf temperature and incident light intensity of a 2 × 3-cm leaf section. Photosynthesis measurements were carried out on two separate occasions.

When bulbs were mature, plants of a 1-m portion of each experimental plot were harvested, dried and cleaned. Bulbs were sorted into four classes per treatment per replication according to diameter, and each group was weighed and counted. Clove number per bulb and bulb dry weight were measured on two bulbs from each diameter class per treatment per replication. Five-meter-rows of bulbs were also harvested from 15 m long rows of cv. 'Frankon' grown in the same field at the same time, but in soil not previously treated with MB or VAM. Bulb weights and diameters were measured as described above.

Tests for significance of difference between VAM-inoculated and uninoculated plots was carried out using a *t*–test for paired sets of data (Snedecor and Cochran 1980). Tests for significance of difference between harvest data of the different groups were carried out using Duncan's Least Significant Difference.

Results

The field showed greater than 50% emergence at 9 dap. The roots of all sampled plants of VAM-inoculated plots showed a high level (>85%) of VAM infection 44 dap. Roots of all plants sampled from control plots showed no mycorrhizal infection when sampled 44 and 140 dap.

Differences in plant growth between treatments were first visible 100 dap, when plots inoculated with VAM appeared darker green and slightly larger than uninoculated plots. By 119 dap VAM-inoculated plants were taller, had more green leaves, and higher dry weights than those of uninoculated plots (Table 1). Plants of both treatments began bulbing about 130 dap. Differences between the treatments in plant height and fresh and dry weight increased greatly during bulb-filling and maturation.

The rate of photosynthesis was measured in the field 167 and 168 dap, during the bulb-filling phase. With light intensities varying from hazy to full noon sun, photosynthesis rates varied from 0–8 mole CO_2 $m^{-2}s^{-1}$, with a mean value of 2.6. Plants colonized by VAM showed higher rates of photosynthesis than uncolonized plants. The difference between treatments was greater at low light intensities (<800 $mEm^{-2}s^{-1}$) than at full light (>800 $mEm^{-2}s^{-1}$) (Table 2).

Plots were harvested 200 dap (29 April 1994). Mean bulb weights and diameters were significantly higher for VAM-inoculated plants than for uninoculated plants (Table 3). Yields from VAM-inoculated plants compared favorably with cv. 'Frankon' plants grown in the same field in soil not treated with MB or VAM (Table 3).

Discussion

The degree of stunting of garlic grown in MB disinfected soil was similar to that reported by Siti et al.

Table 1 Effect of VAM fungal inoculation following soil disinfection with methyl bromide (MB) on plant growth of garlic cv. 'Frankon'

Days after planting	Plant length (cm)		No. green leaves		Fresh weight (g)		Dry weight (g)	
	MB	VAM	MB	VAM	MB	VAM	MB	VAM
74	ns[a]	ns	ns	ns	ns	ns	2±0	2±0
119	50±3	59±1**	7±0	8±0	21±3	35±2	4±1	5±0*
141	67±4	73±2	9±0	9±0	43±3	68±7*	8±1	11±1*
155	70±4	80±2	9±0	10±0*	52±9	90±7*	10±2	15±1*
176	66±2	80±0*	8±0	9±0	61±8	130±2*	14±1	25±0**

[a] Not sampled
* or ** Values significantly greater than the paired MB value at $P<0.95$ or 0.99, respectively

Table 2 Effect of VAM fungal inoculation following soil disinfection with MB on the rate of photosynthesis (mole CO_2 m^{-2} s^{-1}) of garlic leaves at two ranges of light intensities: low = 200–799 mEm^{-2} s^{-1}, high = 800–1400 mEm^{-2} s^{-1}. Numbers in parentheses are the number of plants measured in each treatment

Treatment	Means photosynthesis rates		Means
	Low	High	
MB	1.23 (17)	2.32 (22)	1.84b
MB + VAM	3.13 (18)	3.37 (17)	3.25a
Mean	2.21	2.78	

Table 3 Effect of VAM fungal inoculation following soil disinfection with MB on the diameter class distribution of harvested bulbs and on mean bulb weight of garlic cv 'Frankon'. Numbers within a column followed by different letters differ significantly ($P < 0.05$, DMRT)

Treatment	Percentage of bulbs in class (cm diameter)				Mean bulb weight (g)
	>4	5	6	>7	
MB	30a	57a	13c	0c	27 ± 3b
MB + VAM	0b	19b	59b	22a	51 ± 3a
Untreated	0b	19b	73a	14b	48 ± 3a

(1982). We have shown that this stunting can be completely eliminated by the application of VAM fungi, indicating that the bromide residues are not the main reason for the stunting phenomenon, as was suggested by Siti et al. (1982).

Phosphorus uptake is widely considered to be the most important physiological effect of VAM colonization, and it is possible that the stunting could have been avoided by extra phosphorus fertilizer. However, studies have shown that garlic grown in a wide range of soils with available phosphorus levels of 8 ppm, or even less, did not respond to additional P fertilizers (Tyler et al. 1988). Our plots had 60 ppm P at the root level at the time of planting, but exhibited stunting in the control plots. Yost and Fox (1979) have estimated in a pot experiment that the addition of VAM inoculum to leek was equivalent to adding 250 kg P (or more than 2000 kg superphosphate) per ha.

VAM inoculation of plants greatly increased the rate of photosynthesis compared with noninoculated plants, and we attribute the increased growth of the mycorrhizal plants to this increased photosynthesis. Higher photosynthesis in mycorrhizal plants in pot-grown plants of a variety of species has been found by several workers(Johnson 1983; Allen et al. 1981; Brown and Bethenfalvay 1987). Increased accumulation of photosynthates in the roots of pot-grown onion plants was also attributed to increased photosynthesis, rather than to changes in the partioning of photosynthates (Lee and Cooper 1979). The relatively higher rates of photosynthesis found by us, especially under low-light conditions, is similar to the effect seen by Allen et al. (1981), who reported a significant difference between mycorrhizal and non-mycorrhizal plants in reponse to light/dark transitions. Brown and Bethlenfalvay (1987) concluded that the increased growth of pot-grown mycorrhizal soybean plants was not related to the level of phosphorus available, but rather to the increased photosynthesis, which was shown by them to be unrelated to the concentration of phosphorus in the leaves. Druge and Schonbeck (1992) also concluded that the increased CO_2 assimilation rate of mycorrhizal flax was not related to increased phosphorus availability. They gave evidence that changes in the endogenous levels of plant growth hormones, especially cytokinins, were responsible for changes in stomatal movements and perhaps in the photosynthetic apparatus itself, which resulted in the increased levels of CO_2 assimilation. This change in the relationship between the level of photosynthesis and light conditions was large enough for us to detect in field-grown plants, and is evidently a major part of the total added benefit of mycorrhizal infection on garlic growth.

Thus, increased phosphate fertilization following chemical soil treatments which destroy the local mycorrhizal populations cannot be assumed to replace the beneficial effects of mycorrhizal infection in garlic. The use of VAM inoculum, if this can be made available at a reasonable cost, would be an integral part of a program to produce healthy, virus and pest-free stocks of garlic for commercial use.

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