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The effect of pretransplant inoculation with VA mycorrhizal fungi on the subsequent growth of leeks in the field

M. SASA, G. ZAHKA
Institute of Thallophytes, University of Copenhagen DK-1353 Copenhagen, Denmark

and I. JAKOBSEN
Agricultural Research Department, Risø National Laboratory DK-4000 Roskilde, Denmark

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Summary Leek plants were preinoculated with a mixed inoculum of *Glomus caledonium, Glomus fasciculatum* and *Glomus* sp., and transplanted to Dazomet disinfected and untreated field plots of moderate P deficiency. Successive harvests were made until 99 days after transplanting. Preinoculated leeks attained marketable weights 25 days earlier than uninoculated leeks from untreated soil and their final dry matter yields were 5.7 and 1.5 times as high as those of uninoculated leeks from disinfected and untreated soil, respectively. Phosphorus concentrations in preinoculated leeks remained highest for at least 22 and 75 days after transplanting in untreated and disinfected soil, respectively. Preinoculation had a similar, although smaller, influence on Cu and Zn concentrations. Infection levels produced by introduced and indigenous VA endophytes in leeks reached plateaus of 90% and 40%, respectively, 47 days after transplanting. It is concluded that VAM is essential to leeks grown in moderately P deficient soils, and the potential for inoculating seedlings in commercial leek production is discussed.

Introduction

The benefits of inoculation with vesicular-arbuscular mycorrhiza (VAM) fungi on nutrient uptake and plant growth have been well documented both in pot and field experiments[13]. Most experiments, however, have been performed in soil with no or very few natural endophytes. At present, it is appropriate to define the areas where VAM inoculation may have practical significance.

VAM inoculation may offer benefits to plants which are traditionally transplanted into the field from the greenhouse or the nursery, as a well established VAM infection at time of transplanting may diminish nutrient and water stress. Pretransplant inoculation has a practical advantage as it implies reduction of inoculum needed for infection establishment and the possibility of preselecting effective endophytes.

The effect of preinoculating plants which are traditionally transplanted into the field has been studied for apple[12], chilli[3], finger millet[14], onions[15] and guayule[4]. In most cases, pretransplant inoculation improved subsequent growth in unsterilized field soil. Leeks have been categorized as mycorrhizal dependent[11], and the objective of the present study was to determine the effect of greenhouse preinoculation on subsequent growth of leek transplants in untreated and disinfected field plots.

Materials and methods

Leek seedlings were transplanted into the field according to a split-plot design with disinfection as the main factor and preinoculation with VAM fungi as the subfactor. The field site was located

on a moraine clay loam west of Copenhagen and had not been fertilized with phosphorus nor potassium for nearly 20 years. Prior to this experiment, the field site had been cropped with cereals for several years.

Seedling preparation

Seeds of *Allium porrum* L. cv. Titan were germinated in moist autoclaved sand and after three weeks seedlings were planted singly in 8 cm PVC pots. Each pot contained 200 g of a 12:1 (w/w) mixture of sand and a commercial peat-clay potting medium (ABW Plantin and Co., Oxie, Sweden). The mixture containing 13 mg P kg^{-1} extractable with 0.5 *M* $NaHCO_3$[10] was irradiated (800 krad, 10 MeV electron beam) to ensure the absence of viable mycorrhizal propagules. Half of the pots received a teaspoon each of crude inoculum (approx. 9 g) consisting of infected roots, approx. 200 spores and soil. The inoculum was mixed from separate maize pot cultures with *Glomus caledonium*, *G. fasciculatum* and *G.* sp. Inoculum leachings ($< 38\ \mu m$) were added to the uninoculated controls.

After planting, groups of inoculated and uninoculated control plants were randomly placed on the greenhouse bench. Air temperature was 18–25/14–18°C (day/night), and pots were watered daily. All pots received biweekly 20 ml of a low P (0.1 mg P/pot) nutrient solution modified from that of Hewitt[6]. In order to obtain similar sized plants, 2 mg P as NaH_2PO_4 solution was added to the uninoculated pots with the nutrient solution 4, 5 and 6 weeks after planting. Leeks were transplanted to the field site ten weeks after planting in pots.

Field design

The field site measuring 24 × 5 m was divided into four blocks of 6 × 5 m. Dazomet (active ingredient methylisothiocyanate) was applied to half of each block (3 × 5 m) at the rate of 75 g m^{-2} in September 1983, and immediately rotavated to a depth of 15 cm. Dazomet was previously shown to be efficient against VAM fungi[9]. The treated areas remained covered with polyethylene sheeting for one month. In April 1984, the soil was limed to pH 7.4 (10^{-2} *M* $CaCl_2$) and amended with 150 kg KCl-K ha^{-1} and 50 kg NH_4NO_3-N ha^{-1}, the latter applied again on 3 July. The Dazomet treatment increased the content of 0.5 *M* $NaHCO_3$ — extractable P from 15.5 to 19.3 mg kg^{-1}.

Each halfblock was divided into two plots of 1.75 × 2 m, each being surrounded by a 0.5 m wide fallow strip. One of the plots was randomly selected for preinoculated plants while uninoculated plants were grown in the other. On 30 May the half of each plot (1.75 × 1 m) was planted with leeks at 15 cm intervals in five rows. Preinoculated or uninoculated lettuce plants were grown in the other half of the plots. The lettuce data are not presented in the present report.

Sampling and analyses

Fifteen inoculated and 15 uninoculated leeks were harvested at transplanting. Harvesting from the field plots occurred at days 22, 47, 75 and 99 after transplanting. Samples were taken from the midrows of each plot and consisted of groups of six leeks. Care was taken to collect as much of the root system as possible down to 15–20 cm.

Leek shoots were washed and dried, and fresh weights were recorded. Dry weights were recorded after drying of shoots to constant weight at 80°C. Samples of finely ground shoots were subjected to mixed acid digestion[2]. Phosphorus content was determined colorimetrically with vanadium-molybdate[7], while copper and zinc content were determined by atomic absorption spectrophotometry. Roots were washed and cut into one cm segments and representative samples were cleared in KOH and stained with trypan blue in lactic acid[8]. The proportion of root length with VAM infection was determined by noting the presence or absence of infection at 100 intersections of root segments and grid lines in a petri dish. The data was tested by a two-way analysis of variance. Means were compared by LSD ($P = 0.05$) when the F test showed significant treatment effects.

Results

The shoot dry weight of inoculated leeks was 1.6 times that of the controls at time of transplanting (Table 1a, day 0). This difference increased dramatically in the disinfected field plots, where the maximum relative effect of pretransplant inoculation was observed 47 days after transplanting.

Table 1. Shoot dry weight, P concentration and P content of uninoculated (C) and preinoculated (I) leeks transplanted into untreated or disinfected field soil

Treatment		Days after transplanting				
		0	22	47	75	99
a. *Shoot dry weight (g/plant)*						
Untreated	C	0.17	0.51	2.04	9.84	16.92
	I	0.27	0.76	4.06	16.74	25.57
Disinfected	C	0.17	0.47	0.67	2.55	4.90
	I	0.27	0.84	5.67	16.25	28.07
LSD (0.05)		nd	0.15	0.55	3.13	6.31
b. *P concentration (mg/g dry weight)*						
Untreated	C	0.60	0.55	1.88	2.10	1.76
	I	1.30	0.99	1.83	2.21	1.68
Disinfected	C	0.60	0.50	0.69	1.64	1.41
	I	1.30	1.32	1.71	2.11	1.54
LSD (0.05)		nd	0.11	0.26	0.40	ns
c. *P content (mg/plant)*						
Untreated	C	0.10	0.28	3.83	20.67	30.03
	I	0.35	0.75	7.43	36.42	42.77
Disinfected	C	0.10	0.23	0.46	4.36	7.02
	I	0.35	1.11	9.69	34.01	43.51
LSD (0.05)		nd	0.13	0.95	7.25	12.77

nd, not determined; ns, nonsignificant

In untreated soil, the initial relative response to inoculation was maintained throughout the growth period except for a slight increase after 47 days. Dry matter production differed only little between the preinoculated plants grown at the two soil treatments. Preinoculated and uninoculated leeks grown in untreated soil attained marketable fresh weights 75 days (159 $\pm$ 14 g) and 99 days (168 $\pm$ 14 g) after transplanting, respectively.

At transplanting, the P concentration of inoculated plants was twice as high as that of control plants, and this difference was maintained in plants harvested from disinfected plots at 22 and 47 days (Table 1b). Accordingly, preinoculation increased the P content in shoots relatively more than the shoot dry weights (Table 1c). In untreated soil, the phosphorus concentrations were significantly higher in preinoculated than in uninoculated leeks only in 22-days-old plants, after which P concentrations remained similar. Hence, preinoculation increased the P content of leeks grown in untreated soil in accordance with the effects on dry matter production. Copper and zinc concentrations in the shoots were also increased by preinoculation (Table 2). The various treatments influenced Cu concentrations in a similar way as the P concentrations, while Zn concentrations were significantly increased by preinoculation only in leeks harvested from disinfected soil 22 and 47 days after transplanting. The concentrations of Cu and especially of Zn tended to be highest in preinoculated leeks from disinfected soil.

VAM infection levels were high (80%) in preinoculated leeks at transplanting, whereas no infection was observed in the uninoculated controls (Fig. 1). The infection in preinoculated plants had reached 90%, 47 days after transplanting. This remained constant at later harvests in disinfected soil but decreased to slightly lower levels in untreated soil. VAM infection was also established in uninoculated leeks grown in untreated soil, the infection spreading rapidly between 22 and 47 days after transplanting to a final plateau of 40%. In disinfected soil, uninoculated plants remained nonmycorrhizal until 22 days after transplanting after which infection gradually increased to a final level of 22%.

Table 2. Cu and Zn concentrations ($\mu g\,g^{-1}$ dry weight) in shoots of uninoculated (C) and preinoculated (I) leeks transplanted into untreated or disinfected field soil

Treatment		Days after transplanting							
		22		47		75		99	
		Cu	Zn	Cu	Zn	Cu	Zn	Cu	Zn
Untreated	C	3.01	20.13	6.79	27.06	6.89	25.75	6.50	27.00
	I	5.40	25.06	7.44	27.90	8.16	27.50	6.18	26.25
Disinfected	C	3.17	17.63	4.61	19.31	5.53	29.13	6.67	29.31
	I	6.23	32.88	7.96	31.81	7.98	32.50	7.58	30.19
LSD (0.05)		1.25	5.64	ns	3.30	2.07	ns	1.06	3.47

ns, nonsignificant

Discussion

The present study shows that VAM is essential for growth of leeks in a field with moderate P deficiency. Levels of bicarbonate extractable P in the field soil (15–19 $mg\,kg^{-1}$) were very low compared to optimum P levels of 50 and 100–150 $mg\,kg^{-1}$ for inoculated and nonmycorrhizal leeks grown in pots[16]. Accordingly, there is little doubt that phosphorus was the primary growth limiting factor and that the effect of VAM on growth was due to an increased P uptake. This is also suggested by the low P concentrations in the stunted uninoculated leeks from disinfected soil. Copper and zinc were probably not growth limiting, as the concentrations in the uninoculated control plants were not less than those of commercially grown leeks (unpublished results, National Reseach Center for Horticulture, Institute of Vegetables, Årslev, Denmark).

The different size and P concentration of inoculated and uninoculated plants at time of trans-

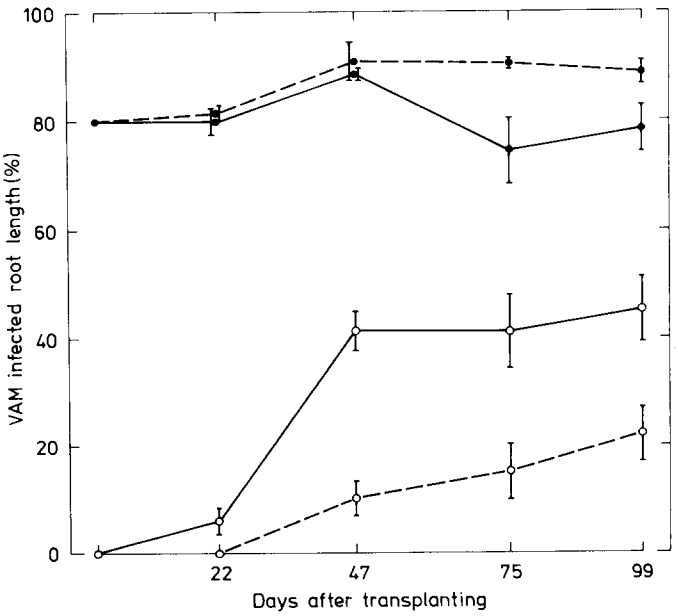

Fig. 1 VAM infection in leeks transplanted into untreated (——) or disinfected (– – – –) field soil. ○, uninoculated control; ●, preinoculated. Bars indicate standard errors of the mean.

planting indicate that more P should have been added to the uninoculated pots to counterbalance the VAM effect. Due to these initial differences, caution must be taken in drawing final conclusions concerning pre-inoculation effects on leeks transplanted into the untreated soil.

The effectiveness of the indigenous VAM population in the field soil used in the present work is not known, but the infection potential seems to be high. Hence 40% infection was obtained in uninoculated leeks 47 days after transplanting into untreated soil. In spite of this, infection levels remained markedly lower than those in preinoculated leeks. This difference may be attributed to the presence of high infection levels in preinoculated leeks at time of transplanting or to a difference in performance of introduced and indigenous endophytes. Generally, *Glomus fasciculatum* produces very high infection levels, perhaps due to a high rate of internal spread in the roots[18]. The lower infection levels observed at the last two harvests in preinoculated leeks in untreated soil as compared with those in disinfected soil might be due to competition from the indigenous endophytes[1], or to grazing soil arthropods[17] which were probably eliminated by disinfection. The minimal infection observed at late harvests in uninoculated leeks grown in disinfected soil was probably due to spread of propagules from adjacent plots or to an inefficacy of the soil disinfection treatment. It remains to be determined whether VAM infection is depressed at the higher P levels in soils used for commercial leek production. However, infection levels in pot-grown leeks were only slightly decreased by P additions as high as 480 mg P kg^{-1} [5].

The influence of preinoculation on leek growth at higher and more common soil P levels cannot be determined from the present work. However, growth responses to VAM infection have been observed in leeks grown in irradiated and untreated soils containing up to 100 mg kg^{-1} of $NaHCO_3$ — extractable P[16]. Many field soils used for commercial leek production in Denmark contain less P than these high levels.

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References

1 Abbott L K and Robson A D 1981 Aust. J. Agric. Res. 32, 621–630.
2 Allen S E 1974 Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, London, 565 p.
3 Bagyaraj D J and Sreermulu K R 1982 Plant and Soil 69, 375–381.
4 Bloss H E and Pfeiffer C M 1984 Ann. Appl. Biol. 104, 175–183.
5 Buwalda J G *et al.* 1982 New Phytol. 92, 391–399.
6 Hewitt E J 1952 Commonwealth Bureau of Horticulture and Plantation Crops. Technical Communication 22.
7 Jackson M L 1958 Soil Chemical Analysis. Prentice-Hall Int., N.J. 498 p.
8 Kormanik P P and McGraw A C 1982 *In* Methods and Principles of Mycorrhizal Research. Ed. N C Schenck, pp 37–46 The American Phytopathological Society, St. Paul, Minnesota.
9 McEwen J *et al.* 1973 J. Agric. Sci., 80, 105–110.
10 Olsen S R *et al.* 1954 US. Dept. Agric. Cir. No. 939, 19.
11 Plenchette C *et al.* 1983 Plant and Soil 70, 199–209.
12 Plenchette C *et al.* 1981 Can. J. Bot. 59, 2003–2008.
13 Powell C L 1984 *In* VA Mycorrhiza. Eds. C L Powell and D J Bagyaraj. pp 206–223. CRC Press Inc., Boca Katon, Florida.
14 Rao G Y S *et al.* 1983 Zbl. Mikrobiol. 138, 415–419.
15 Stribley D P and Snellgrove R C 1984 Rothamsted Report for 1983, Part I.
16 Stribley D P *et al.* 1980 J. Soil Sci. 31, 655–672.
17 Warnock A J *et al.* 1981 New Phytol. 90, 285–292.
18 Wilson J M. 1984 New Phytol. 97, 413–426.