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ORIGINAL PAPER

G. Charron · V. Furlan · M. Bernier-Cardou
G. Doyon

Response of onion plants to arbuscular mycorrhizae

1. Effects of inoculation method and phosphorus fertilization on biomass and bulb firmness

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Abstract Onion (*Allium cepa*) plants were grown in pots in two types of irradiated soil, mineral and organic. Onion development was observed under two or three levels of P fertilization, and three methods of arbuscular mycorrhizal fungus inoculation with two fungus species. In mineral soil, preinoculated onion plants had a higher biomass than non-inoculated control plants or plants inoculated with either colonized root segments or spores. Fungus species had no differential effect on dry biomass or final bulb diameter. Preinoculated onion plants reached marketable size (>25 mm bulb diameter) 2–3 weeks earlier than those inoculated by either of the other two methods. Non-inoculated onion plants remained stunted. Bulbs of onions inoculated with *Glomus versiforme* were firmer than those inoculated with *G. intraradices*. Increasing P fertilizer rates had a significant positive linear effect on the P tissue concentration of plants inoculated with *G. intraradices* or *G. versiforme*, but no effect on bulb firmness. The P tissue concentration of inoculated plants was significantly higher than that of non-inoculated controls, and in inoculated plants, it differed among inoculation methods. The P tissue concentration was higher in onion plants inoculated with *G. versiforme* than in those inoculated with *G. intraradices.* In organic soil, the dry biomass of preinoculated plants was higher than that of plants inoculated by root segments. The highest root colonization levels were obtained under a low soil P level with *G. intraradices*, and with the root segment method of inoculation with *G. versiforme*.

Keywords *Allium cepa* · Texture measurement · Irradiated soil

M. Bernier-Cardou (✉)
Natural Resources Canada, 1055 rue du P.E.P.S., Sainte-Foy, Québec, G1V 4C7 Canada
e-mail: mbernier@cfl.forestry.ca
Tel.: +1-418-6485283, Fax: +1-418-6485849

G. Charron
DREPA, 555 boulevard Roland-Therrien, Longueuil, Québec, J4H 3Y9 Canada

V. Furlan
Agriculture and Agri-Food Canada, ECORC – K.W. Neatby 2057, Central Experimental Farm, Ottawa, Ontario, K1A 0C6 Canada

G. Doyon
Food Research and Development Centre,
3600 boulevard Casavant Ouest, Saint–Hyacinthe, Québec, J2S 8E3 Canada

Introduction

Arbuscular mycorrhizal (AM) fungi are known to stimulate host plant growth mainly by enhancing soil nutrient uptake, particularly that of P (Siquiera et al. 1998; Torrey 1992; Valdés et al. 1993). Although most plant species are colonized by AM fungi (Gerdemann 1971), they are not necessarily infected by the most efficient ones (Rickerl et al. 1994). Also, these fungal endophytes are obligate symbionts (Menge 1983) and their agricultural use remains limited due to the large quantities of inoculum required (Waterer and Coltman 1988).

The use of preinoculated seedlings could be a very promising solution to this problem, especially for crops that are grown in nursery beds for later outplanting (Sasa et al. 1987). Preinoculated seedlings may also be more tolerant to transplant shock than nonmycorrhizal plants or plants inoculated at transplanting (Valdés et al. 1993; Waterer and Coltman 1988). Siquiera et al. (1998) report that if adequate P is applied at planting, preinoculation of outplants with selected AM fungi enhances early crop development and productivity of coffee in low-fertility soils in Brazil. The production of preinoculated seedlings allows for the selection of AM fungus species which are best suited for a given crop (Hayman 1987; Waterer and Coltman 1988) or most able to compete with indigenous AM fungus species (Hayman 1987; Sasa et al. 1987; Valdés et al. 1993).

In organic soils, little research has been conducted on the effect of AM fungus inoculation on plant growth. Bolgiano et al. (1983) and Nelsen et al. (1981) have studied the effect of soil P level and water availability on

the growth of onions colonized by AM fungi, and control plants in organic soil. Neither observed any yield increase due to mycorrhiza formation by onions grown in organic soil with a high P concentration. However, in organic soil with a low P concentration, both obtained a positive response from plants inoculated with AM fungi.

This study was performed to assess the effects of three factors, AM fungus species, inoculation method, and P fertilization, and their interactions on onion plants grown in two soil types: irradiated mineral and organic soils.

Materials and methods

Experiment 1 – mineral soil

Onion transplants (*Allium cepa* L. cv. Improved Autumn Spice) were submitted to five inoculation treatments as follows: preinoculated by *Glomus versiforme* (Karst.) Berch or *G. intraradices* Schenck and Smith, inoculated by root segments containing either AM fungus species, or inoculated with spores of *G. versiforme*. The quantity of inoculum applied was more than optimal to ensure that responses would be comparable in every respect except time. There were three types of control: (1) seedlings produced in contact with non-mycorrhizal leek host plants, (2) seedlings sham inoculated with non-mycorrhizal leek root segments, and (3) seedlings sham inoculated with sterilized spores plus non-mycorrhizal soil microflora. All treatments were repeated with each of three levels of P fertilization (0, 65, and 130 mg P kg^{-1} dry soil) applied as $Ca(H_2PO_4)_2 \cdot H_2O$. All pots received 300 mg N, 76 mg K, and 227 mg Mg per kg of dry soil, applied as $Ca(NO_3)_2 \cdot 4H_2O$, K_2SO_4, and $MgSO_4 \cdot 7H_2O$, respectively. All fertilizer treatments were dry-mixed in the soil in a single application before potting.

To produce preinoculated seedlings, onion seeds (Stokes Seeds) were disinfected for 5 min in 1% sodium hypochlorite plus one drop of Tween 20. These seeds were then soaked for 10 min in a solution of 0.01 N HCl and rinsed with sterile distilled water. Onion seeds were sown in rows that ran parallel to a row of 6-month-old leek plants inoculated with AM fungus and grown in Turface (calcined montmorillonite clay, distributed by Profile Products, Ill.) in 70-l plastic containers; the two rows were 5 cm apart. Containers with leek plants colonized by either *G. versiforme*, *G. intraradices*, or without AM fungi (control) were used. The technique of producing preinoculated seedlings was modified from Brundrett et al. (1985). Seven weeks after sowing, root colonization reached 55%. At this stage, seedlings were transplanted individually into 15-cm pots as the preinoculated treatments. Seven-week-old seedlings produced in trays of the same size as those used to produce preinoculated seedlings and containing sterilized vermiculite were inoculated at transplanting with either colonized root segments or AM fungus spores of *G. versiforme*.

Roots used as root-segment inoculum came from 6-month-old leek plants colonized by the AM fungus species mentioned above and from non-inoculated control leek plants. All root segments, colonized or not, were surface sterilized for 20 min with 2% chloramine T plus streptomycin sulphate, and rinsed with distilled water (Mosse 1959). At transplanting, 1.5 g fresh root inoculum was placed 5 cm below the surface in pots assigned to the root inoculum treatments.

Inoculation with AM fungus spores was performed with *G. versiforme* only. Surface sterilization was as described above. One part of the spores was autoclaved for 15 min at 100°C and the rest was filtered through Whatman no. 4 paper to extract surface microflora. At transplanting, 10 ml of the spore suspension (1,500 spores ml^{-1}), which had been neither autoclaved nor filtered, was poured on the onion seedling roots of the *G. versiforme* spore treatments. For control plants, 10 ml autoclaved spores plus 10 ml of the filtrate containing the surface microflora were poured on the seedling roots.

All onion transplants were grown individually in pots containing a 1:3 (silica:soil) mix of silica no. 10 and mineral soil (sombric brunisol) sterilized by gamma irradiation (^{60}Co, 15 kGy, 22°C). Plants were harvested after 12 weeks. The main soil mixture characteristics before fertilization were as follows: pH (soil:water ratio 1:2) 6.2, 42 g kg^{-1} organic matter (wet oxidation), 4.2 g kg^{-1} total N, 1,268 µg g^{-1} total P, 51 µg g^{-1} available P, 222 µg g^{-1} K, 620 µg g^{-1} Ca, and 94 µg g^{-1} Mg. Plants were kept in a growth chamber under a 16-h photoperiod, 24/18°C (day/night temperature, SD 1°C), 280 µmol m^{-2} s^{-1} light intensity, and 65% relative humidity.

Starting at the end of the second week after transplanting, until harvest, the cross-diameter of all bulbs was measured every 2 weeks. To do so, soil at the base of each plant was gently pushed aside and two bulb diameters were measured at a 90° angle using a Mitutoyo Digimatic calliper. Tools were disinfected in isopropyl alcohol between each bulb measurement.

Twelve hours after harvest, bulb firmness (texture) was measured with an Instron Computerized Compression Testing System (model 4201). This technique is used to measure the texture of different products (fruits, vegetables, etc.) (Bourne 1981, 1982). Onion bulbs were perforated at three equidistant points around their largest circumference. A five-bevelled (45°) branched star-shaped punch with a total contact surface of 60 mm^2 was used. The downward speed of the punch was 150 mm min^{-1} and the bulb rested on a plate with a 15-mm hole centred on the punch axis. Firmness, expressed in N mm^{-1}, is the stress applied on the bulb divided by the compression (mm) of the latter at the point of perforation by the punch. The mean value from three equidistant points was used for statistical analysis.

After firmness measurements, onion plants were dried at 65°C for 5 days and weighed. Tissue from each plant was analysed. A wet digestion with concentrated H_2SO_4 and H_2O_2 was used for the determination of N, P, K, Ca, and Mg (Thomas et al. 1967). Ca, Mg, Fe, Mn, Cu, and Zn were determined by atomic absorption spectrometry. K was determined by flame photometry. Total P was determined by an automated vanado-molybdate method (Colwell 1965). N was determined by an automated indophenol blue method (Ferrari 1960).

The experimental design consisted of seven complete randomized block replicates of 24 treatments: eight fungus species and inoculation method combinations each at three P fertilization levels. Every 2 weeks, blocks and pots within blocks were randomly rotated. The treatment structure was a 3×3×3 factorial arrangement of inoculation methods (preinoculated seedlings, seedlings inoculated with root segments or spores), AM fungus species (*G. versiforme*, *G. intraradices*, and control), and P fertilization (0, 65, and 130 mg P kg^{-1} of dry soil). Inoculation with spores of *G. intraradices* was not possible because of a lack of material, hence 24 combinations resulted instead of 27.

ANOVA was conducted and treatment differences were assessed by means of pre-determined contrasts. It was required that the observed *P*-value of a contrast be smaller than or equal to $\alpha' = 0.05/13 = 0.0038$ to be considered significant at the $\alpha = 0.05$ level. This is known as a Bonferroni procedure. It allows for the fact that the same contrast is tested for 13 variables observed on the same experimental units. The variables were dry biomass, final bulb diameter, bulb firmness, root colonization, and tissue concentrations of nine macro- and micronutrients. The contrast between onions inoculated with spores of *G. versiforme* and those inoculated with the other two methods applied only to this fungus. The contrast between *G. versiforme* and *G. intraradices* was based on the averages for each fungus species over preinoculated plants and those colonized by root segments. Onion dry biomass and final bulb diameter were transformed to their natural logarithms before analysis. The purpose of the transformation was to stabilize the between-pot variance, a key assumption for validity of the *F*-tests in the ANOVA (Steel and Torrie 1980). Means were computed on the logarithmic scale and back-transformed for presentation. Differences among means and their SEs are quoted on the logarithmic

scale. Bulb firmness and root colonization did not require transformation, but control treatments were excluded from the analysis of these variables because their bulbs were too small for firmness measurements, or root colonization was zero on every control plant. Macro- and micronutrient concentrations were also analysed without transformation.

The logarithms of average cross-diameters over time were analysed as a separate set of repeated measures. The analysis accounts for the correlation between successive measures on the same plants (Crowder and Hand 1990).

Experiment 2 – organic soil

For this experiment, muck soil (terric mesisol) from the Saint-Hyacinthe region (Québec) was used. The main soil characteristics were as follows: pH (soil:water ratio 1:2) 5.1, 814 g kg^{-1} organic matter (wet oxidation), 2.1 g kg^{-1} total N, 2,114 µg g^{-1} total P, 826 µg g^{-1} available P, 2,012 µg g^{-1} K, 16,620 µg g^{-1} Ca, and 550 µg g^{-1} Mg. Because of the high available-P concentration of this soil (826 µg P kg^{-1}), it was diluted with silica no. 10 in the following proportions: 1:3 and 3:1 (soil:silica). All pots received 310 mg N and 2.5 mg Cu kg^{-1} dry soil, applied as $Ca(NO_3)_2 \cdot 4H_2O$ and $CuSO_4 \cdot 5H_2O$, respectively, in order to obtain the optimal soil fertility level as recommended by the Conseil des productions végétales du Québec (CPVQ 1989). The soil was sterilized by gamma irradiation (^{60}Co, 13 kGy, 22°C).

Treatments in this experiment were the same as those described for experiment 1, except that only two levels of P were used. The two experiments were conducted simultaneously and placed on the same bench of a growth chamber. Growth conditions, block and pot rotation, and measurements were identical to those of the first experiment except that plants were harvested after 10 rather than 12 weeks.

The experimental design consisted of seven complete randomized block replicates of a 3×3×2 factorial arrangement of inoculation methods (preinoculated seedlings, colonized root segments and spores), AM fungi (*G. versiforme*, *G. intraradices*, and control), and P levels (123 and 271 mg P kg^{-1} of the dry soil mixture). The two soil P levels were achieved through the soil dilution with silica. Inoculation with spores of *G. intraradices* was not possible because of a lack of material. Hence there were 16 treatment combinations rather than 18.

Variables and their statistical analysis were as in experiment 1, except that dry biomass and final bulb diameter did not require transformation. The logarithms of the bulb diameter measured every 2 weeks were also analysed as in experiment 1. Analyses for both experiments were performed with the GLM procedure of SAS (SAS Institute 1989, 1997).

Results

Experiment 1 – mineral soil

Dry biomass and bulb diameter

At harvest, dry biomass and final bulb diameter of onions inoculated with *G. intraradices* or *G. versiforme* were greater than those of the nonmycorrhizal controls ($P \leq 0.0001$ for the contrast between inoculated and control plants; Table 1). The average dry biomass of inoculated plants (mean 2.96 g) was 33 times that of the controls [mean 0.09 g, log-scale difference 3.50±0.09 log(g) (±SE)]. The average final bulb diameter of inoculated plants was 28.13 mm, about 6.7 times larger than that of the controls, which was 4.18 mm [log-scale difference 1.91±0.05 log(mm)]. The dry biomass and final bulb diameter of preinoculated onions were respectively 2.3 and 1.4 times larger than those of seedlings inoculated at transplanting with root segments ($P \leq 0.0001$; Tables 1, 2). Onions inoculated with spores of *G. versiforme* had dry biomasses and final bulb diameters that were intermediate between those of preinoculated plants and those of plants colonized by root segments ($P=0.2668$ and 0.6037, respectively, for the contrast between plants inoculated with *G. versiforme* by root segments or preinoculated with this fungus, and plants inoculated with spores; Tables 1, 2). Arbuscular mycorrhiza species had no apparent effect on dry biomass or final bulb diameter of preinoculated plants or plants inoculated with root segments ($P=0.9115$ and $P=0.9705$, respectively).

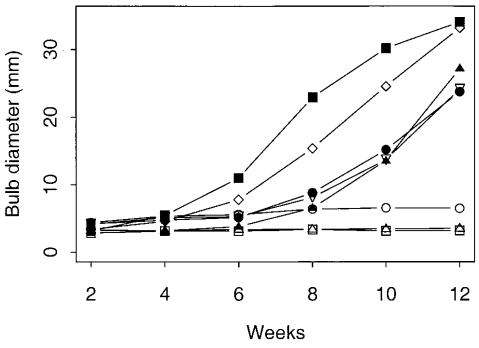

Fig. 1 Average bulb diameter (mm) as a function of time (weeks) for plants preinoculated with *Glomus versiforme* (■), plants inoculated with root segments colonized by *G. versiforme* (●), plants inoculated with spores of *G. versiforme* (▲), plants preinoculated with *G. intraradices* (◇), plants inoculated with root segments colonized by *G. intraradices* (▽), and controls: non-inoculated (○), plants sham inoculated with non-mycorrhizal roots (□) and plants sham inoculated with sterile spores (△)

The linear effect of P on the logarithm of dry biomass of inoculated plants seemed negligible, but the logarithm of the much lower dry biomass of control plants increased in proportion with P, on average ($P=0.0036$ for the interaction between the linear component of the effect of P fertilization and the contrast between inoculated and control plants; Table 1). The slope for inoculated plants was of the same order of magnitude as its SE [0.0010±0.0010 log(g) per mg kg^{-1} of P], whereas that for control plants was about 21 times its SE [0.0272± 0.0013 log(g) per mg kg^{-1} of P]. The effect of this interaction on bulb diameter did not reach statistical significance ($P=0.0524>0.0038$).

Over the 12-week growing period, the diameter of preinoculated onions was generally larger than that of onions inoculated with root segments ($P \leq 0.0001$, Fig. 1). It also increased faster, and the preinoculated onions reached marketable size (25 mm in Québec) 2–3 weeks earlier than onions inoculated with root segments ($P \leq 0.0001$ for the interaction between time and the contrast between preinoculated seedlings and those inoculated with root segments). Bulbs of plants inoculated with spores of *G. versiforme* had smaller diameters than those of plants inoculated with this fungus by other methods ($P \leq 0.0001$ for both the intercept and shape of the curves): they grew

Table 1 ANOVA (*P*-values) of the logarithms of dry biomass (g) and final bulb diameter (mm), of bulb firmness (N mm^{-1}), and of root colonization (%) of onion plants grown in mineral soil. *G. int. Glomus intraradices*, *G. ver. G. versiforme*

Sources of variation and contrasts	*df*	Dry biomass	Final bulb diameter	*df*	Bulb firmness	*df*	Root colonization
Inoculum (I)	7			4		4	
Preinoculated vs colonized roots	(1)	**0.0001**[a]	**0.0001**	(1)	0.0106	(1)	**0.0002**
Preinoculated+colonized roots vs spores[b]	(1)	0.2668	0.6037	(1)	0.0552	(1)	0.1852
Inoculated[c] vs control	(1)	**0.0001**	**0.0001**	–	–[d]	–	–[d]
G. int. vs *G. ver.*	(1)	0.9115	0.9705	(1)	**0.0006**	(1)	**0.0001**
(*G. int.* preinoculated+*G. ver.* colonized roots) vs (*G. int.* colonized roots+*G. ver.* preinoculated)[e]	(1)	0.6834	0.7197	(1)	0.2174	(1)	0.6825
P fertilization (P)	2			2		2	
P linear	(1)	**0.0006**	0.0236	(1)	0.2789	(1)	0.8795
P quadratic	(1)	0.7841	0.4646	(1)	0.8604	(1)	0.7949
I×P	14			8		8	
(Preinoculated vs colonized roots) ×P linear	(1)	0.9314	0.9170	(1)	0.5315	(1)	0.4121
(Preinoculated vs colonized roots) ×P quadratic	(1)	0.7863	0.2004	(1)	0.2167	(1)	0.6297
(Preinoculated+colonized roots vs spores)×P linear[b]	(1)	0.9223	0.7788	(1)	0.5862	(1)	0.0453
(Preinoculated+colonized roots vs spores)×P quadratic[b]	(1)	0.8155	0.4003	(1)	0.9069	(1)	0.8124
(Inoculated vs control)×P linear	(1)	**0.0036**	0.0524	–	–	–	–
(Inoculated vs control)×P quadratic	(1)	0.6805	0.6468	–	–	–	–
(*G. int.* vs *G. ver.*)×P linear	(1)	0.0530	0.2146	(1)	0.5344	(1)	0.8666
(*G. int.* vs *G. ver.*)×P quadratic	(1)	0.5891	0.4716	(1)	0.4809	(1)	0.2095
(*G. int.* preinoculated+*G. ver.* colonized roots) vs (*G. int.* colonized roots+*G. ver.* preinoculated)×P linear	(1)	0.5603	0.7271	(1)	0.3583	(1)	0.6178
(*G. int.* preinoculated+*G. ver.* colonized roots) vs (*G. int.* colonized roots+*G. ver.* preinoculated)×P quadratic	(1)	0.5615	0.6839	(1)	0.0630	(1)	0.7750
Error mean square[f]	137	0.3182	0.0935	79	76.1044	83	0.6366

[a] Significant *P*-values (≤0.0038) appear in *bold*
[b] Contrast valid for *G. versiforme* only
[c] "Inoculated" includes the five combinations of a fungus species and an inoculation method
[d] Root colonization of control plants was zero, and their bulbs were too small to be evaluated for bulb firmness
[e] Interaction between fungus species and the two inoculation methods common to both fungi
[f] Actual error mean square, not a *P*-value

Table 2 Means over all P levels of dry biomass, final bulb diameter, root colonization, and N tissue concentration of onion plants grown in mineral soil, by inoculum species, by inoculation method (n=21). For abbreviations, see Table 1

Inoculation method	Arbuscular mycorrhiza species			
	G. int.	*G. ver.*	*G. int.*	*G. ver.*
	Dry biomass (g)		Final bulb diameter (mm)	
Preinoculated	4.57	4.76	33.12	34.12
Root segments	2.08	1.95	24.29	23.57
Spores	–	2.56	–	27.11
SE (difference)[a]	0.17		0.09	
	Root colonization (%)		N tissue concentration (%)	
Preinoculated	75	61	1.49	1.46
Root segments	62	46	2.17	2.36
Spores	–	59	–	2.20
SE (difference)[b]	4.9		0.10	

[a] SE of the difference between the logarithms of any two of the above means
[b] SE of the difference between any two of the above means

Table 3 ANOVA (*P*-values) of plant tissue macro- and micronutrient concentrations (%) of onions grown in mineral soil. For abbreviations, see Table 1

Sources of variation and contrasts	*df*	N	P	K	Ca	Mg	*df*	Fe	Cu	Mn	Zn
Inoculum (I)	7						7				
Preinoculated vs colonized roots	(1)	**0.0001**[a]	0.3086	**0.0001**	0.0559	**0.0001**	(1)	0.2250	0.8728	0.1182	0.0129
Preinoculated+colonized roots vs spores[b]	(1)	**0.0017**	**0.0031**	0.0443	0.2823	0.0742	(1)	0.5297	0.8775	0.6086	0.6341
noculated[c] vs control	(1)	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	(1)	**0.0001**	0.0062	**0.0001**	**0.0001**
G. int. vs *G. ver*.	(1)	0.2461	**0.0001**	0.5559	0.7937	0.3641	(1)	0.7106	0.0602	0.8770	0.2124
(*G. int*. preinoculated +*G. ver*. colonized roots) vs (*G. int*. colonized roots +*G. ver*. preinoculated)[d]	(1)	0.1368	0.8160	0.6138	0.9202	0.8442	(1)	0.7666	0.2012	0.4745	0.7604
P fertilization (P)	2						2				
P linear	(1)	0.1641	**0.0012**	0.0686	0.1941	0.0091	(1)	0.4013	0.0087	**0.0004**	0.2300
P quadratic	(1)	0.4864	0.8154	0.8677	0.7294	0.0408	(1)	**0.0001**	0.0941	0.0148	0.0171
I×P	14						14				
(Preinoculated vs colonized roots)×P linear	(1)	0.0365	0.4198	0.1700	0.9417	0.1134	(1)	0.7387	0.0814	0.9982	0.9864
(Preinoculated vs colonized roots)×P quadratic	(1)	0.3590	0.5924	0.0746	0.2977	0.1933	(1)	0.6492	0.3845	0.9894	0.7509
(Preinoculated+colonized roots vs spores)×P linear[b]	(1)	0.6618	0.0698	0.9714	0.2240	0.6558	(1)	0.2527	0.1908	0.3807	0.0535
(Preinoculated+colonized roots vs spores)×P quadratic[b]	(1)	0.7816	0.3560	0.2066	0.5708	0.8216	(1)	0.5682	0.9301	0.6401	0.9572
(Inoculated vs control) ×P linear	(1)	0.0638	**0.0016**	0.1493	0.5911	0.0675	(1)	0.5697	**0.0003**	0.0242	0.4476
(Inoculated vs control) ×P quadratic	(1)	0.7002	0.8470	0.6056	0.9695	0.0794	(1)	**0.0001**	0.2993	**0.0022**	0.0144
(*G. int*. vs *G. ver*.)×P linear	(1)	0.4536	0.2526	0.7378	0.2190	0.2355	(1)	0.6491	0.3012	0.5939	0.1092
(*G. int*. vs *G. ver.*)×P quadratic	(1)	0.8286	0.5513	0.4282	0.5280	0.3939	(1)	0.2938	0.4201	0.7269	0.0519
(*G. int*. Preinoculated+ *G. ver*. colonized roots) vs (*G. int*. Colonized roots +*G. ver*. preinoculated) ×P linear	(1)	0.2246	0.4414	0.1854	0.8545	0.3420	(1)	0.6540	0.7716	0.8695	0.5561
(*G. int*. Preinoculated+*G. ver.* colonized roots) vs (*G. int*. Colonized roots+*G. ver*. preinoculated)×P quadratic	(1)	0.9843	0.2716	0.4516	0.4564	0.4409	(1)	0.8836	0.5395	0.8644	0.3752
Error mean square[e]	82	0.1035	0.00251	0.37235	0.13197	5.12 ×10^{-4}	81	3.53 ×10^{-5}	1.95 ×10^{-8}	8.54 ×10^{-6}	4.05 ×10^{-7}

[a] Significant *P*-values (≤0.0038) appear in *bold*
[b] Contrast valid for *G. versiforme* only
[c] "Inoculated" includes the five combinations of a fungus species and an inoculation method
[d] Interaction between fungus species and the two inoculation methods common to both fungi
[e] Actual error mean square, not a *P*-value

more slowly until the eighth week, but by the 12th week, they had reached the same size as those of plants inoculated with this fungus by root segments. Over the two inoculation methods tested with both fungus species, there was no evidence that the average diameter growth curves differed for the two AM fungus species (P=0.2698 for the difference in the overall level of the curves, and P=0.2452 for the difference in their shape). In this mineral soil, bulb diameters of inoculated onions increased with time while those of the controls remained small, and stunted until the 12th week (P≤0.0001 for the interaction between the effect of time and the contrast between inoculated plants and the controls).

Bulb firmness

Bulbs of onions colonized by *G. versiforme* were firmer than those colonized by *G. intraradices* (P=0.0006; Table 1). Onion bulb firmness was 32.1 N mm^{-1} for plants inoculated by *G. versiforme*, and 25.3 N mm^{-1} for those inoculated with *G. intraradices* by the same methods (difference 6.8±1.9 N mm^{-1}). P fertilization had no apparent effect on bulb firmness (P>0.27), nor did the interaction between the inoculation treatments and P fertilization level (P>0.06>0.0038).

Table 4 Means, mean differences (Diff.), and their SEs [SE(diff.)] for plant tissue macro- and micronutrient concentrations (%) of inoculated (Inoc.) and control plants. For abbreviations, see Table 1

Treatments	Sample size	N	P	K	Ca	Mg	Fe	Mn	Zn
Inoc.	105	1.94	0.199	2.18	1.49	0.138	0.0103	0.0079	0.00322
Control	63	2.46	0.058	3.74	2.17	0.195	0.0593	0.0458	0.00479
Diff.		–0.52	0.141	1.56	–0.68	–0.057	–0.0490	–0.0379	–0.00157
SE(diff.)		0.05	0.008	0.10	0.06	0.004	0.0009	0.0005	0.00010

Root colonization

Root colonization was 14% (±3.5%) higher in preinoculated onions than in those colonized by root segments (P=0.0002; Tables 1, 2). Roots of onion plants inoculated with *G. intraradices* were more heavily colonized by 15% (±3.5%) than those of plants inoculated with *G. versiforme* by the same methods (P≤0.0001). For *G. versiforme*, root colonization resulting from preinoculation was similar to that obtained from inoculation with spores. P fertilization had no apparent effect on root colonization (P>0.79), nor did any of the components of the interaction between the inoculation treatments and P level (P>0.04>0.0038).

Plant tissue mineral concentration

The P tissue concentration of onion plants colonized by *G. versiforme* was higher than that of plants colonized by *G. intraradices* (means 0.231% and 0.172%, respectively, difference 0.058±0.016%, P≤0.0001; Table 3). This was the only mineral nutrient affected by fungus species (P>0.06 for all other nutrients).

In plants inoculated with *G. versiforme*, the N tissue concentration was high when plants were inoculated with root segments or spores, and low in preinoculated plants. The difference in N tissue concentration between preinoculated and root-colonized plants also held for plants inoculated with *G. intraradices* because there was no evidence of interaction between fungus species and these two inoculation methods (P≤0.0001 for the average difference between preinoculated plants and root-colonized ones over the two fungus species, P=0.1368 for its interaction with fungus species, and P=0.0017 for the difference between the spore method and the other two in *G. versiforme*; Tables 2, 3). On average over fungus species, K and Mg tissue concentrations were also higher in root-colonized plants (means 2.50% and 0.149%, respectively) than in preinoculated plants (means 1.75% and 0.121%, respectively; differences 0.75±0.13% for K, and 0.028±0.005% for Mg, P≤0.0001 for both K and Mg). In plants inoculated with *G. versiforme*, the P tissue concentration was higher when the fungus was preinoculated or inoculated with root segments than when it was inoculated with spores (means 0.231% for the first two methods and 0.190% for the latter, difference 0.041±0.014%).

Inoculated onion plants had higher P tissue concentrations than the controls, but the opposite effect was observed for N, K, Ca, Mg, Fe, Mn, and Zn tissue concentrations (P≤0.0001 for all these nutrients; Tables 3, 4).

On average over treatments, P, Fe, and Mn tissue concentrations differed among P fertilization levels (P=0.0012 and P=0.0004 for the linear effect of P fertilization on P and Mn tissue concentrations, respectively, and P≤0.0001 for the quadratic effect of P fertilization on Fe concentration; Table 3). The P tissue concentration increased with P fertilization in inoculated plants, but did not respond to P in the controls (P=0.0016 for the interaction between the slope of the regression line on P fertilization level and the "inoculated vs control" contrast; Table 3). The quadratic effect of P on the Fe tissue concentration was mainly because of the controls, P fertilization having little effect on the Fe tissue concentration of inoculated plants (P≤0.0001 for the interaction between the quadratic effect of P and the "inoculated vs control" contrast). P fertilization had no effect on the Cu or Mn plant tissue concentration of inoculated plants, but had a linear, negative effect on the Cu plant tissue concentration of control plants (P=0.0003) and a quadratic effect on their Mn tissue concentration (P=0.0022).

Experiment 2 – organic soil

Dry biomass and bulb diameter

The dry biomass of inoculated plants was 1.06 g (±0.25 g) higher when onions were preinoculated (mean 4.90 g) than when they were inoculated with root segments (mean 3.84 g, P≤0.0001; Tables 5, 6). Inoculation with spores of *G. versiforme* yielded dry biomasses 0.98 g (±0.31 g) larger than preinoculation or inoculation with this fungus by root segments (P=0.0018). On average over the two inoculation methods common to both fungus species, inoculation with *G. intraradices* produced onions with a higher dry biomass (mean 4.79 g) than inoculation with *G. versiforme* (mean 3.95 g, difference 0.84±0.25 g, P=0.0010; Tables 5, 6).

In organic soil, neither inoculation treatment nor P fertilization apparently had any effect on final bulb diameter (P≥0.0040>0.0038; Table 5).

Over time, the bulb diameter of inoculated plants increased somewhat faster than that of the controls (P=0.0016, results not shown). In organic soil, the growth of onion bulbs was not stunted as in mineral soil. Their average diameter followed that of inoculated plants with a small lag.

Table 5 ANOVA (*P*-values) of dry biomass (g), final bulb diameter (mm), bulb firmness (N mm^{-1}), and root colonization (%) of onion plants grown in organic soil. For abbreviations, see Table 1

Sources of variation and contrasts	*df*	Dry biomass	Final bulb diameter	Bulb firmness	*df*	Root colonization
Inoculum (I)	7				4	
Preinoculated vs colonized roots	(1)	**0.0001**[a]	0.0179	0.4014	(1)	**0.0001**
Preinoculated+colonized roots vs spores[b]	(1)	**0.0018**	0.0058	0.1868	(1)	**0.0001**
Inoculated[c] vs control	(1)	0.5037	0.9528	0.5016	–	–[d]
G. int. vs *G. ver.*	(1)	**0.0010**	0.0066	0.8907	(1)	0.8259
(*G. int*. preinoculated+*G. ver*. colonized roots) vs (*G. int*. colonized roots+*G. ver.* preinoculated)[e]	(1)	0.0298	0.0428	0.3499	(1)	**0.0001**
P fertilization (P)	1	0.0257	0.0213	0.6158	1	0.0532
I×P	7				4	
(Preinoculated vs colonized roots)×P	(1)	0.2250	0.3593	0.9191	(1)	0.1277
(Preinoculated+colonized roots vs spores)×P[b]	(1)	0.6636	0.2244	0.6465	(1)	0.7196
(Inoculated vs control)×P	(1)	0.0548	0.0836	0.7618	–	–
(*G. int*. vs *G. ver*.)×P	(1)	0.0419	0.0040	0.2379	(1)	**0.0001**
(*G. int*. preinoculated+*G. ver*. colonized roots) vs (*G. int.* colonized roots+*G. ver*. preinoculated)×P	(1)	0.0360	0.2329	0.5237	(1)	0.5101
Error mean square[f]	90	0.8764	11.1480	0.0304	54	0.3656

[a] Significant *P*-values appear in ***bold***
[b] Contrast valid for *G. versiforme* only
[c] "Inoculated" includes the five combinations of a fungus species and an inoculation method
[d] Control plants were not colonized
[e] Interaction between fungus species and the two inoculation methods common to both fungi
[f] Actual error mean square, not a *P*-value

Table 6 Mean dry biomass (g; *n*=14)) over P levels, and mean root colonization (%; *n*=7) by P level of onion plants grown in organic soil, by inoculum species, and by inoculation method. For abbreviations, see Table 1

Inoculation method	Arbuscular mycorrhiza species					
	G. int.	*G. ver.*	*G. int.*		*G. ver.*	
	Dry biomass		P=1:3	P=3:1	P=1:3	P=3:1
			Root colonization			
Preinoculated	5.60	4.20	61	33	30	33
Root segments	3.98	3.69	53	39	56	64
Spores	–	4.93	–	–	24	27
SE(difference)[a]	0.35	6.5				

[a] SE of the difference between any two of the above means

Bulb firmness

Neither inoculation method, nor P level, nor AM fungus species, nor their interactions had any apparent effect on bulb firmness ($P \geq 0.1868$ for all main effects and interactions; Table 5).

Root colonization

Root colonization in plants inoculated with *G. intraradices* was similar for the two inoculation methods used with this fungus species (average difference 1±4.6%; Tables 5, 6). When plants were inoculated with *G. versiforme*, however, inoculated root segments produced higher root colonization than preinoculation (average difference 28±4.6%, $P \leq 0.0001$ for the interaction between fungus species and the two inoculation methods). On average over P levels, inoculation with spores of *G. versiforme* produced lower root colonization than the other two methods (difference 20±4.0%, $P \leq 0.0001$). A high soil P level decreased the root colonization of plants inoculated with *G. intraradices* (difference 21±4.6%) but had apparently little or no effect on the root colonization of plants inoculated with *G. versiforme* by the same inoculation methods (difference –6±4.6%, $P \leq 0.0001$ for the interaction between fungus species and soil P level).

Table 7 ANOVA (*P*-values) of plant tissue macro- and micronutrient concentrations (%) of onions grown in organic soil. For abbreviations, see Table 1

Sources of variation and contrasts	*df*	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
Inoculum (I)	7									
Preinoculated vs colonized roots	(1)	0.5304	**0.0029**[a]	0.0421	0.4238	0.0165	0.2442	0.1059	0.9425	0.2658
Preinoculated+colonized roots vs spores[b]	(1)	0.6366	0.0965	0.2182	0.6196	0.5898	0.4241	0.2216	0.9531	0.7966
Inoculated[c] vs control	(1)	0.4307	0.2215	0.7080	0.3197	0.3575	0.4640	0.2942	0.1267	0.1632
G. int. vs *G. ver.*	(1)	0.1599	0.8521	0.1217	**0.0009**	0.0656	0.2442	0.3815	0.5357	0.4109
(*G. int*. preinoculated+*G. ver*. colonized roots) vs (*G. int*. colonized roots+*G. ver*. preinoculated)[d]	(1)	0.2944	0.2905	0.0819	0.2205	0.9671	0.8201	0.0627	0.2941	0.5848
P fertilization (P)	1	**0.0001**	**0.0001**	**0.0001**	0.3333	**0.0001**	0.0414	0.0357	**0.0001**	**0.0001**
I×P	7									
(Preinoculated vs colonized roots)×P	(1)	0.9407	0.3353	0.2817	0.5636	0.1931	0.5154	0.1059	0.0222	0.8353
(Preinoculated+colonized roots vs spores)×P[b]	(1)	0.0156	0.1293	0.0898	0.0054	0.0821	0.6773	0.8379	0.1255	0.2268
(Inoculated vs control)×P	(1)	0.6433	0.7847	0.4781	0.7898	0.0962	0.0958	0.0465	0.2258	0.0074
(*G. int*. vs *G. ver*.)×P	(1)	0.4112	0.5737	0.7377	0.1781	0.8580	0.5779	0.7072	0.2941	0.5954
(*G. int*. preinoculated+*G. ver*. colonized roots) vs (*G. int.* colonized roots+*G. ver*. preinoculated)×P	(1)	0.2346	0.0132	0.2936	0.3434	0.6899	0.2142	0.1059	**0.0006**	0.5433
Error mean square[e]	90	0.0340	0.0013	0.0788	0.0441	0.9371 ×10^{-4}	0.2885 ×10^{-5}	1.1315 ×10^{-8}	0.85 ×10^{-6}	0.299 ×10^{-5}

[a] Significant *P*-values (≤0.0038) appear in *bold*
[b] Contrast valid for *G. versiforme* only
[c] "Inoculated" includes the five combinations of a fungus species and an inoculation method
[d] Interaction between fungus species and the two inoculation methods common to both fungi
[e] Actual error mean square, not a *P*-value

Table 8 Means of Mn tissue concentration (%; *n*=7) of onion plants grown in organic soil by inoculum species, by inoculation method, and by soil P level; the SE of the difference between any two means is 0.00049%. For abbreviations, see Table 1

Inoculation method	Arbuscular mycorrhiza species			
	G. int.		*G. ver.*	
	P=1:3	P=3:1	P=1:3	P=3:1
Preinoculated	0.00883	0.00943	0.01039	0.00870
Root segments	0.01053	0.00821	0.00980	0.00873
Spores	–	–	0.00964	0.00920

Table 9 Means over all inoculation treatments and controls by P level, Diff. and SE(diff.) for N, P, K, Mg, Mn and Zn concentrations (%, n=56) in the plant tissue of onions grown in organic soil. For abbreviations, see Table 4

P level	N	P	K	Mg	Mn	Zn
1:3	1.904	0.273	1.782	0.0886	0.00990	0.00490
3:1	2.116	0.393	2.208	0.0796	0.00900	0.00650
Diff.	–0.212	–0.120	–0.426	0.0100	0.00090	–0.00160
SE(diff.)	0.035	0.007	0.053	0.0018	0.00017	0.00033

Plant tissue mineral concentration

P concentration in plant tissue was higher when plants were root-colonized (mean 0.355%) than when they were preinoculated (mean 0.325%, difference 0.030± 0.010%, *P*=0.0029; Table 7).

When plants were inoculated with *G. versiforme*, the Mn tissue concentration decreased by an average of 0.00138% (±0.00035%) as the soil P level increased, irrespective of whether plants were inoculated with root segments or preinoculated (Tables 7, 8). When plants were inoculated with *G. intraradices*, however, a high soil P level reduced the Mn tissue concentration of plants inoculated by root segments (difference 0.00231± 0.00049%), but had no apparent effect on this nutrient when plants were preinoculated with this fungus (differ-

ence –0.00060±0.00049%, P=0.0006 for the three-way interaction between P level, fungus species and the two inoculation methods; Tables 7, 8).

On average over the two inoculation methods used with both fungus species, the Ca tissue concentration of plants inoculated with *G. versiforme* was higher than that of plants inoculated with *G. intraradices* (means 1.61% and 1.41%, respectively, difference 0.20±0.06%, P=0.0009; Table 7).

On average over inoculation treatments, a high soil P level increased the tissue concentrations of N, P, K, and Zn, and decreased those of Mg and Mn ($P \leq 0.0001$; Tables 7, 9).

Discussion

Our results in mineral soil suggest that preinoculation of seedlings enhances plant growth and development compared to the other two inoculation methods. There was no apparent difference between the dry biomass or final bulb diameter of onion plants inoculated with root segments or spores. These results are in agreement with those reported by Biermann and Linderman (1983).

Despite high P fertilization in experiment 1, control plants remained stunted throughout the experiment. This was also observed by Sasa et al. (1987). In this case, the lack of growth of control plants can be attributed to their inability to absorb P from the sterile soil in the absence of arbuscular mycorrhizae.

Onion bulb diameters reveal that preinoculation accelerates the maturation of onion plants compared to the other inoculation methods. Preinoculated onions reached maturity, at 25 mm in diameter in Québec, 2–3 weeks earlier than those inoculated with root segments or spores. If this trend persists under field conditions, the use of preinoculated seedlings appears to be a very promising choice for commercial growers. Sasa et al. (1987) obtained similar results in the field using leek as a test plant.

Bulb firmness was measured to determine which treatment, if any, produces firmer onions for better preservation over long periods of cold storage. There was no indication that preinoculated onions were firmer than those inoculated by other methods, but onions inoculated with *G. versiforme* were firmer than those inoculated with *G. intraradices*. Increased firmness is particularly induced by the formation of calcium pectate, responsible for the cementing action of cells (Van Buren 1991). Smock and Neubert (1950) report that cells become less firmly cemented together as insoluble pectin (or protopectin) is hydrolysed into soluble pectin. The presence of insoluble pectic substances in onions was demonstrated by Conrad (1926). At the end of the 12-week experimental period, there was no evidence that seedlings inoculated with *G. versiforme* benefited from better nutrient and water uptake than those inoculated with *G. intraradices*, but it is possible that this uptake occurred earlier in the growth period when plants were inoculated with *G. versiforme*, resulting in somewhat greater cellular integrity. Calcium plays an important role in maintaining cellular integrity (Mengel and Kirkby 1982). Future work will require physiological analysis such as the chronological turgor pressure of bulb parenchyma cells to verify this hypothesis.

Mosse and Hayman (1971) noted a more uniform distribution of fungal material in preinoculated seedlings compared to those colonized by root segments. When seedlings are inoculated with root segments or spores, the inoculum may not be at its optimum colonization potential and consequently may produce lower root colonization of the host plant than could be achieved. The physiological response of preinoculated seedlings is practically immediate after transplanting, while seedlings inoculated with either colonized root segments or spores take 2–3 weeks to reach the same root colonization level as seedlings inoculated before transplanting. This could explain the difference observed in bulb development time.

Seedlings preinoculated with *G. versiforme* produced onion plants with an average dry biomass of 4.76 g, more than twice as large as 2.24 g, the mean dry biomass of plants inoculated with root segments or spores (Table 2). In inoculated seedlings, this effect of the inoculation method did not depend on the level of P fertilization. The same effect was observed for *G. intraradices* when comparing preinoculation with inoculation through root segments. Bagyaraj and Sreeramulu (1982) obtained similar results with red pepper colonized by *G. fasciculatum* and three indigenous AM fungus species. Both their results and ours show the potential savings in fertilizer when seedlings are preinoculated with arbuscular mycorrhizae. The significant positive linear effect of P fertilization on P uptake by onion plants suggests that AM fungi translocate nutrients efficiently even under high P fertilization. These results corroborate those of Plenchette et al. (1983) and Waterer and Coltman (1988), but they differ from those obtained by Hamel et al. (1996).

The effects of the two fungus species on dry biomass, final bulb diameter, root colonization, and P uptake observed in the mineral soil experiment differed. Daniels and Menge (1981) as well as Schubert and Hayman (1986) reported that different AM fungus species have different effects on the growth of host plants in terms of plant dry mass. Ferguson and Woodhead (1982) explained this phenomenon by differences in colonization levels, development and density of external mycelium, and efficiency in essential nutrient translocation. Extraradical hyphal development was not evaluated in this experiment.

In organic soil, there was some indication that the final bulb diameter of onions colonized by *G. intraradices* remained relatively stable over the two soil P levels compared to those of seedlings colonized by *G. versiforme* which decreased at high P, but this interaction effect did not quite reach significance (P=0.0040; Table 5). Hayman (1987) mentioned that if P fertilization is suffi-

cient to provide what the host plant needs without decreasing the colonization level, then AM fungi may induce C loss from the host plant to the symbiont without the reciprocal transfer of P. This may explain a possible decrease in the final bulb diameter of onion plants colonized by *G. versiforme* at a high P level. *G. intraradices* is likely the more appropriate symbiont to use in soil with a high concentration of available P. Selection tests are thus necessary to determine the most efficient AM fungus species to use in a given soil, and this may depend on available P.

The organic soil with the lowest P level seems to have been sufficiently rich to ensure normal and complete growth of onion plants with or without AM fungus inoculation. The higher P level did not contribute to increased growth. These results agree with those of Bolgiano et al. (1983).

Our results show that in mineral soil, the preinoculation method is superior to inoculation with either colonized root segments or spores; it contributes to earlier maturation of onion plants. It also reduces the quantity of P fertilizer normally required. In mineral soil, there was an indication that *G. versiforme* improves onion bulb firmness compared to *G. intraradices*; this may be important for the preservation of onion bulbs over long periods of cold storage. In organic soil, preinoculation produced onions with a higher dry mass than other inoculation methods. However, onions inoculated with *G. intraradices* produced bulbs with a higher dry biomass than those colonized with *G. versiforme*, in contrast to the effects of these two fungi in mineral soil.

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