THE AMOUNT AND EFFECT OF V.A. MYCORRHIZAL FUNGI IN SASKATCHEWAN SOILS

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Mycorrhizal Relationships

Mycorrhizal symbioses are part of the great array of relationships between heterotrophic and autotrophic organisms, from corals to beach trees to lichens to legumes. However, the term is more often used to describe a wide variety of associations between fungi and underground structures of vascular plants. The plant appears to be able to control the degree of infection and obtain benefits from the fungal partner in return for the carbon it supplies to the fungi. Therefore, it could be termed a mutualistic symbiosis in that there is very little if any destruction of plant tissues.

The non-sheath forming organisms usually called vesicular-arbuscular (VA) organisms occur on more plant species than any other kind. Most cultivated plants as well as many forest and shade trees, shrubs and wild herbaceous plants have VA mycorrhiza. Plants such as our common poplar appears to carry both VA mycorrhiza and sheath-forming ectomycorrhiza. Cultivated plants of the Cruciferae (rapeseed, cabbage, etc.) and Chenopodiaceae are plant families that have not been found to contain mycorrhiza.

Initially the Phycomyetes causing VA infections were all classified as one genus Endogone. More recently, a number of other genera such as Gigaspora and Glomus in the family Endogonaceae have been classified as shown in Table 1. The classification is dependent on the fruiting bodies or sporocarps, the kind of spore and method spore germination.

The structures formed between the plant and fungal partner are shown in Fig. 1. Many of the hyphae are internal and intracellular. The internal structures in the cortex include hyphae and arbuscules which are finely branched haustoria. The arbuscules, after growth within the cell, are digested by the plants. Nutrient transfer is thought to occur between the finely branched fungal mycelia and from the digestion of the fungal mycelia by the plant.

Expanded fungal tips often containing large concentrations of lipids are known as vesicles. External structures include mycelium that penetrates through the soil, individual spores which can be chlamydospores and fruiting bodies or sporocarps which are large groups of spores.

The purpose of this paper is to show the amount of mycorrhizal fungi in Saskatchewan soils and their effect on the growth of faba-beans.

Table 1. Genera of Endogonaceae: A summary of their presently known characteristics.

Genera	Fruiting	Kind of Spores	Spore Germination	Type of Mycorrhiza
Endogone	Sporocarps	Zygospores[a]	Unknown	Ectomycorrhiza or unknown
Gigaspora	Single Spores	Azygospores?[b]	Through wall	Arbuscular[c]
Acaulospora	Single Spores	Azygospores?	Through wall	Vesicular-arbuscular
Glomas	Sporocarps and single spores	Chlamydospores	Regrowth of attached hyphae	Vesicular-arbuscular
Sclerocystis	Sporocarps[d]	Chlamydospores	Regrowth of attached hyphae?	Vesicular-arbuscular
Glaziella	Sporocarps	Chlamydospores	Unknown	Unknown
Modicella	Sporocarps	Sporangiospores	Through wall	Unknown

a. Sporangial stages uncertain.
b. *Gigaspora* species produce soil-borne vesicles on coiled hyphae that may represent vestigial sporangial stages.
c. Vesicles formed within roots have been reported for *G. calospora* (Furlan & Fortin, 1973).
d. Spores arranged in a single orderly layer.

(Reprinted from Gerdemann and Trappe, 1975).

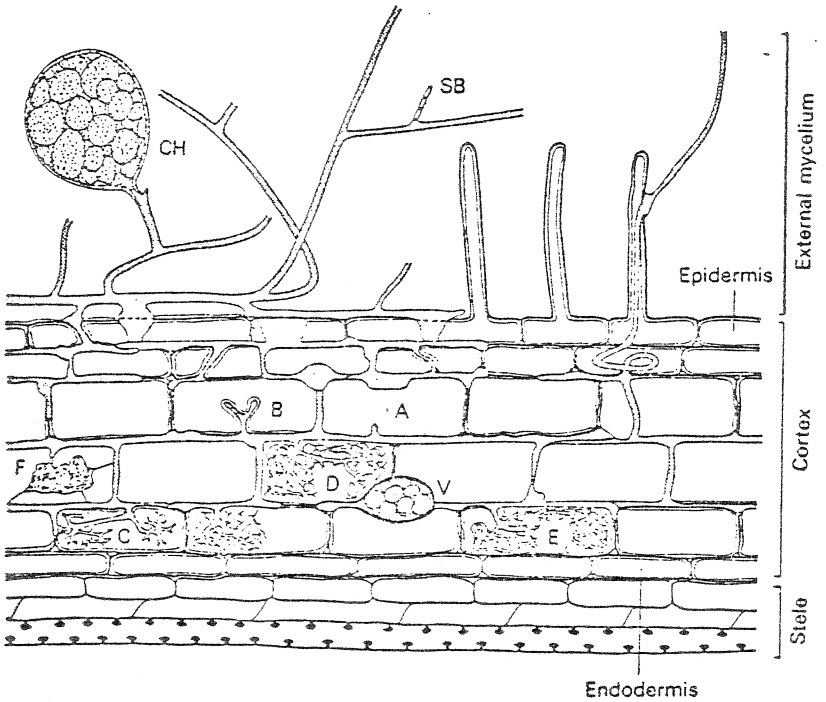

FIG. 1. Schematic diagram of the association of vesicular arbuscular mycorrhizal fungi with plant roots – the external mycelium bears large chlamydospores (CH), and occasional septate side branches (SB) – infection of the plant can occur through root hairs or between epidermal cells; the hyphae then often form broad and flat wedges between cell walls – arbuscles arise after the penetration of hyphae into cells – (A–D) and (E–F) represent respectively progressive stages in their development and senescence – vesicles occur within the root, (V); similar structures also develop on the external mycelium. To avoid confusion cell walls of the root are not indicated when they underlay fungal hyphae (from a drawing kindly provided by Dr F. E. Sanders, University of Leeds).

(Reprinted from Russell, 1977)

Materials and Methods

Field spore survey

Three composite samples were taken at each location tested. Each of these was composed of ten subsamples taken with a 2.5 cm corer to a depth of 15 cm at 10 meter intervals in the field. The composite samples were thoroughly mixed before three subsamples of 100 g wet soil were removed for analyses and one for moisture content and phosphorus measurement. The 100 g samples were sieved through a 60 μm sieve to remove fine particles. The material left on the sieve was floated in 50% glycerol (modified from Furlan and Fortin, 1975). Spores were counted under the dissecting microscope at 25X.

Root infection analyses

Roots were washed from field or pot soil. A random sample of the roots was cleared of cell contents by heating in 5% KOH for 25 min. The roots were rinsed and placed in 0.1% acid fuschin in lactophenol at 80°C for ten minutes. The roots were rinsed and stored in 50% glycerol after staining. Root sections (1-2 cm) were mounted in 50% glycerol on glass slides and squashed under a cover slip. Twenty-four sections of root were mounted per plant. The extent of root infection was determined at 100X. Each microscopic field viewed was assessed as either infected or non-infected. The total number of infected fields viewed was expressed as a percentage of the total number of microscopic fields observed.

Section of mycorrhizal fungal species

Fababeans (Vicea faba) infected with naturally occurring mycorrhizal fungi were harvested from a field near Outlook, Sask. Roots and soil from around these plants were used as inoculum for fababean seedlings in the growth chamber. Seedlings were grown in a 2:1 mixture of sterile Ottawa sand (#100) and Oxbow sandy loam soil. These plants were harvested after 8 weeks. The soil was sieved and floated to remove the mycorrhizal spores. The most common type of spore was hyaline to yellow gold in color, spherical 80-120 μ in diameter with one hyphal attachment. These were later identified as belonging to the genus Glomus. Spores of this fungus were used to inoculate other fababean seedlings growing in mycorrhiza free soil (sterilized in ethylene oxide (15%) for 48 hours). Spores and roots from these plants were used as inoculum for growth chamber experiments and inoculum propagation.

Growth chamber experiments

Fababeans (Vicea faba var Diana) were used in all experiments run in the growth chamber. Seeds were surface sterilized with 10% javex for 10 min before germinating on filter paper. Where used, sand was an Ottawa sand (100 mesh) which had been autoclaved for one hour at 121°C, 15 psi. Soil used was an Oxbow sandy loam which had been air dried and ground to pass through a 2 mm sieve. The soil at the beginning of the experiments contained 16 μg g^{-1} P_2O_5, 21 μg g^{-1} NO_3-N, 320 μg g^{-1} K_2O and 16 mycorrhizal spores 100 g^{-1} dry soil. In cases where mycorrhizal free soil was used, the soil was sterilized in

ethylene oxide (15%) for 48 hours. Mycorrhizal inoculum consisted of approximately 400 spores of the selected Glomus sp. (Gerdemann and Trappe, 1974) or 3 g of dried infected roots and adhering soil. In the latter case, noninfected dried roots and soil were added to controls. The inoculum was placed 8 cm below the seed. Unless otherwise stated, Rhizobium leguminosarum (Legume-aid, Agricultural Laboratories Inc., Columbus, Ohio) was also added. Plants were watered every day to 90% of field capacity with distilled water. Humidity and light were kept at 50% and 19,000 lux, respectively. A light-dark period of 16/8 were used with a temperature of 24/20°C.

The extent of root infection and spore production by this Glomus spp. over time was studied using plants growing in sterilized soil inoculated with roots and soil. Three infected plants were harvested every two weeks for 10 weeks. Roots were stained and analyzed for the extent of mycorrhizal infection. The soil was sieved and floated for spores.

The effect of this Glomus spp. on plant growth was studied in three experiments. Plants in experiment A were grown in 2800 g sand. Nutrients were added as 70 ml of N-free Long Ashton solution (Hewitt, 1966) every 7 days. The effect of N was studied at three levels; 0, 15 and 50 $\mu g\ g^{-1}$ N, added as KNO_3 before planting. At each level of N, plants were grown with and without Rhizobium and with and without Glomus spores. Plants were harvested after 8 weeks and analyzed for root infection, dry weight and N and P content. Nitrogen was measured by semimicro Kjeldahl digestion (Bremner, 1965). Phosphorus was measured by acid digestion and complexing with vanadomolybdate (Hesse, 1971).

Experiments B and C studied the effect of Glomus spore inoculation on plants growing in non-sterile soil, i.e. in the presence of residual mycorrhizal spores. Experiment B used plants growing in 3,200 g of sand:soil (2:1). The effect of added phosphorus was studied at three levels: 0, 6.25 and 12.5 $\mu g\ g^{-1}$ P_2O_5 added as $NH_4H_2PO_4$. ^{32}P as orthophosphate was added at the rate of 0, 10 and 20 μCi per pot, respectively. Non-radioactive phosphorus was mixed with ^{32}P and added in solution 8 cm below the seed. Experiment C differed from B in that 3,200 g Oxbow soil was used and phosphorus levels studied were 0 and 25 $\mu g\ g^{-1}$ P_2O_5 added (0 and 20 μCi ^{32}P). Plants were harvested after 8 weeks. The extent of root infection was determined on root samples taken before drying of the roots. Harvested plants were separated into roots and shoots, oven dried, weighed and analyzed for phosphorus content by vanadomolybdate. ^{32}P uptake was measured in a scintillation counter using PPO, POPOP Triton X-100 scintillant (Rennie and Paul, 1971).

Results

Field spore survey

Numbers of mycorrhizal spores in field sites are shown in Table 2. The natural grassland areas were located adjacent to the cultivated sites. Grasslands consistently showed 1.2 to 3.6 times the number of spores found in adjacent cultivated sites. One Oxbow site near Sheho,

Table 2. Effect of cultivation on mycorrhizal spore populations.

Soil type and location	Spores 100 g^{-1} soil		$NaHCO_3$ extractable phosphorus $\mu g\ g^{-1}$	
	Virgin soil	Wheat field	Virgin soil	Wheat field
Waitville (Nut Mt.)	71±9	41±2	9.7	19.4
Naicam (Annaheim)	204±18	88±4	14.6	19.0
Bradwell (Bradwell)	62±1	51±2	5.6	8.1
Oxbow (Sheho)	2314±1074	68±10	11.4	25.9
Hoey (Hoey)	749±116	211±18	12.9	27.5

± Standar Error

Sask. had 34 times the number of spores in the natural as opposed to the cultivated site. The available phosphorus ($NaHCO_3$ extractable) level in all cases was higher (1.35 - 2.27X) in the cultivated than in the natural sites.

The effect of summerfallowing on field spore populations is seen in Table 3. It appears that for at least the first two years following crop removal, the spore number decreases by one-half to one-third of the population of the previous year. After three years of continuous summerfallowing, there still remained a low level of viable mycorrhizal spores. The data for spores found in rapeseed planted on barley stubble show that the rape roots do not increase spore numbers. This is consistent with other findings that cruciferous plants do not become infected with mycorrhizal fungi (Gerdemann, 1974).

Table 3. Effect of crop and summerfallow on mycorrhizal spore populations.

Soil type and location	State of field	Spores 100 g^{-1} soil
Canora (Canora)	Wheat	302±21
	Barley	326±14
	1-yr. summerfallow	189±23
Oxbow (Watrous)	Barley stubble	332±35
	1-yr. summerfallow*	127±20
	2-yr. summerfallow*	55±9
	Rapeseed*	104±37

*On fields previously planted to barley.

± Standard Error

Figure 2 shows the variations in the number of spores found in a wheat field (Oxbow soil) and in an adjacent grassland area over the growing season. The number of spores found in the wheat field soil increased slowly for the first 10 weeks (36 to 83 spores 100 g^{-1}) than rose rapidly till the end of the growing season (83 to 161 spores 100 g^{-1} dry soil). Spore numbers in the natural grassland showed more variability over the growing season, but lacked the steady rise in spore numbers. After the soil was permanently frozen, a series of soil samples were taken from the cultivated site. The number of viable spores found had dropped drastically to the level of spores found in the previous spring.

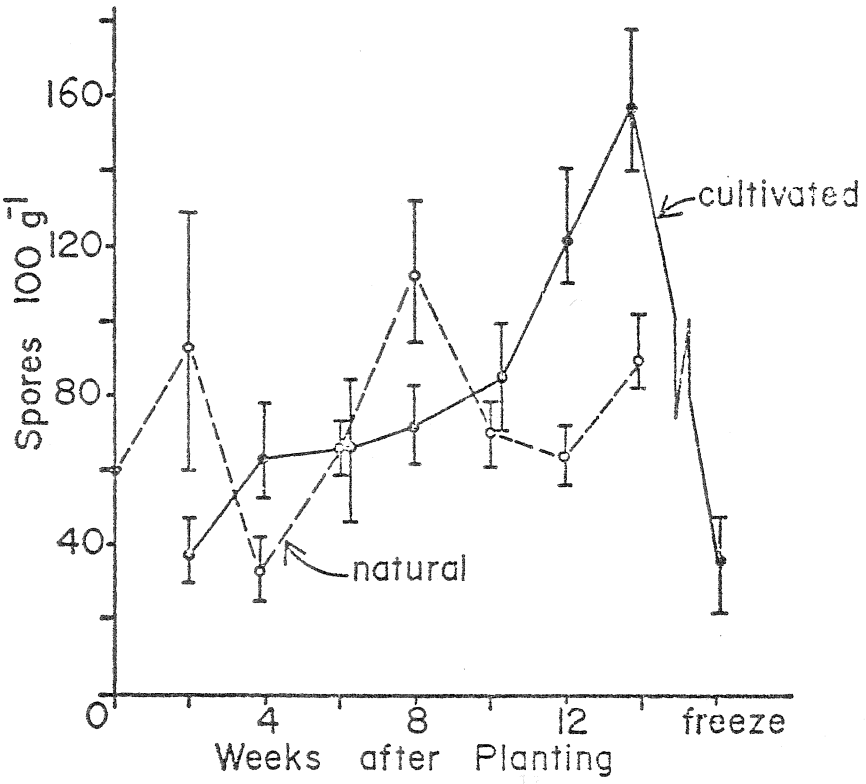

Fig2. Mycorrhizal Spore Populations in a Natural and a Cultivated Area over the growing season.

Figure 3 shows the effect of the phosphorus status of a soil on the extent of roots infected with mycorrhizal fungi in a field at Outlook, and on spore production as determined on samples taken in late summer. The level of infection was not as high in phosphorus rich soils as in phosphorus poor soil (46.7% in non-amended soil to 14.7% in soil which was added 99 kg ha^{-1}P). Spore numbers reflected this decrease, dropping from an average of 67 spores 100 g^{-1} dry soil in non-amended soil to 32 spores 100 g^{-1} in soil with 45 $\mu g\ g^{-1}$ P.

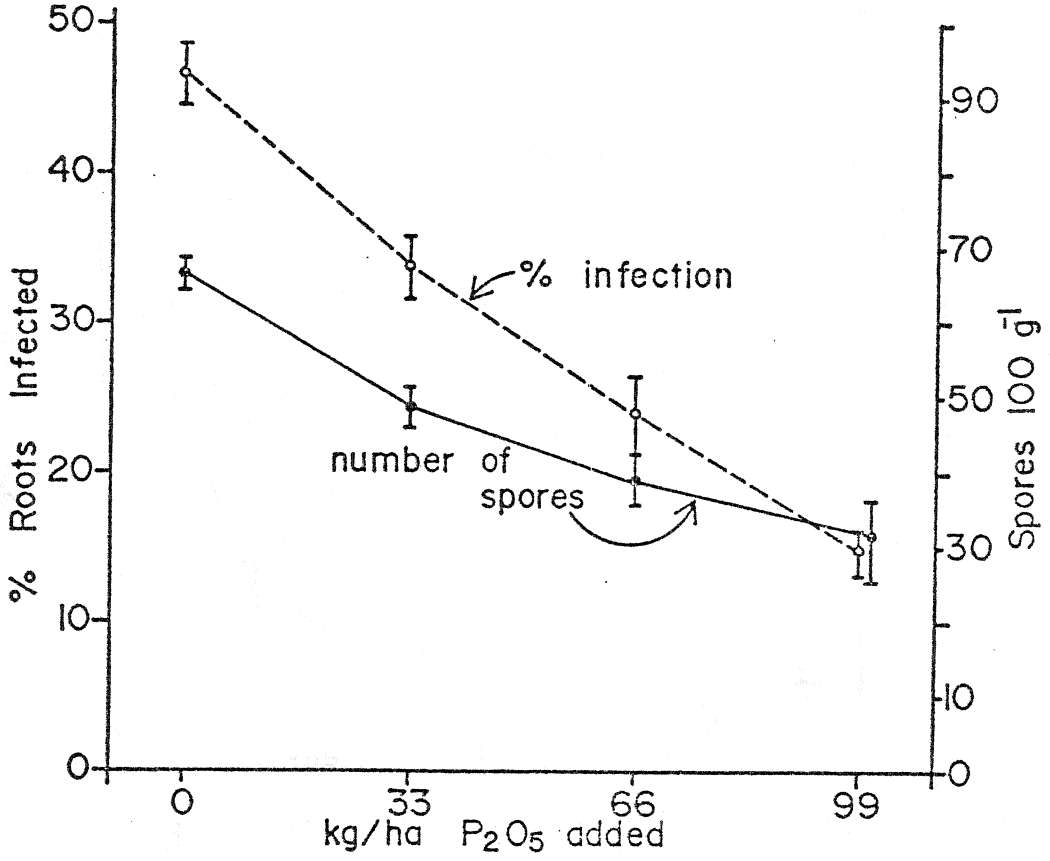

Fig 3. Effect of Added Phosphorus on Mycorrhizal Infection and Spore Production on Fababeans.

These plants were irrigated in the field. Similar plants that were not irrigated were only 24.9% infected in non-amended soil.

Growth chamber experiments

The development of root infection and spore production over time is seen in Fig. 4. For the first two weeks, infection levels were low and consisted mainly of external hyphae and some internal hyphae. Few vesicles or arbuscules were found. Between two and four weeks, the extent of infection began a rise which became exponential. Many intracellular vesicles were seen in the root cortex. The exponential rise continued till six weeks of age, then levelled off at approximately 70% of the root system being infected. Spores were absent or present in low numbers (<19/plant) for the first six weeks. The number of spores rose gradually becoming a sharp rise after eight weeks (658 spores/pot).

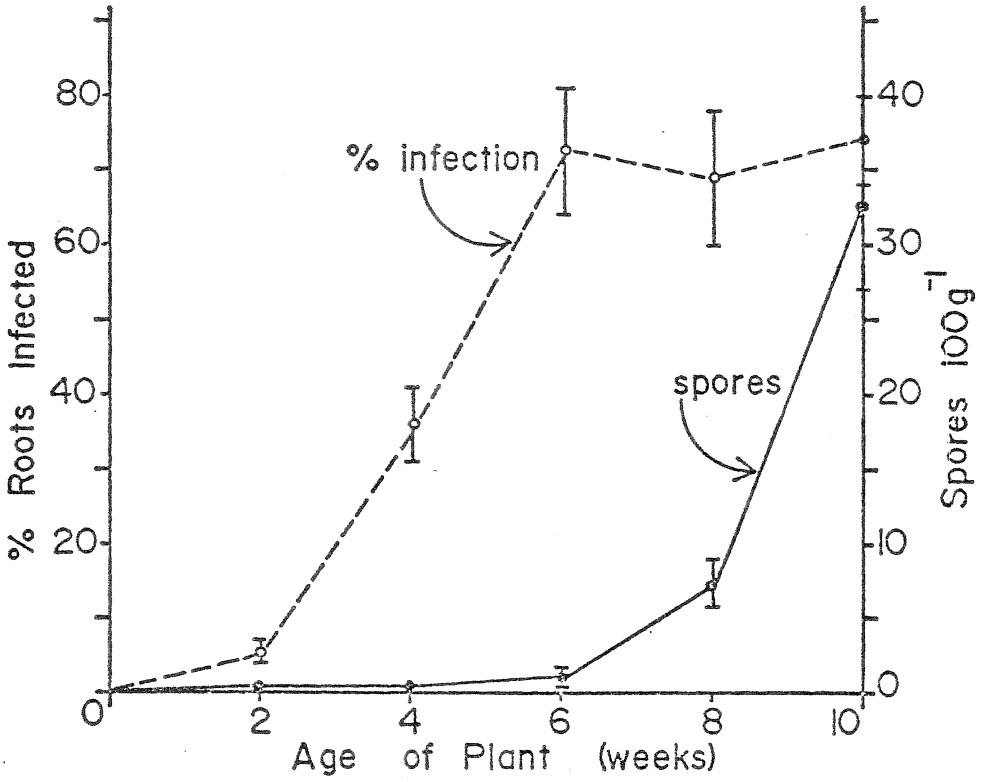

Fig 4. Extent of Root Infection and Spore Production by Glomus sp on Fababeans.

The effect of mycorrhizal and/or Rhizobium infection in solution (sand) culture is seen in Table 4. Rhizobium inoculation, in the absence of mycorrhiza, did not affect shoot weight of plants when compared over the different N levels. Within each level of N, however, Rhizobium inoculation did affect plant growth. Rhizobium inoculated plants are 36.5% heavier than controls at 15 $\mu g\ g^{-1}$ N but 20.1% less heavy than controls at 50 $\mu g\ g^{-1}$ N. In all treatments, mycorrhizal infection increased shoot weight by 54.0% to 221.4% (ave. 138.7%). The combined effect of both mycorrhizal and Rhizobium infections is greater than the additive effect at both 15 and 50 $\mu g\ g^{-1}$ N. At 50 $\mu g\ g^{-1}$ N, the mycorrhiza appear to overcome the negative effect of the Rhizobium inoculation.

Phosphorus contents (mg g^{-1} shoot) of plants grown in solution culture shows that the presence of mycorrhizal fungi not only increase the total amount of phosphorus taken up by 4.5 to 12.4 times, but also increases the phosphorus content per unit weight by 1.7 to 3.8 times. The effect was more drastic in the presence of Rhizobium (2.6 - 3.8X) than for mycorrhiza alone (2.3 - 2.8X). Rhizobium incoulation alone had no effect on phosphorus content except at 50 $\mu g\ g^{-1}$ N where the phosphorus content dropped. The combined

Table 4. Effect of mycorrhizal plus Rhizobium inoculation on fababean growth in solution culture.

		Level of added nitrogen ($\mu g\ g^{-1}$ NO_3-N)				
		0	15		50	
		+ Rhizobium	- Rhizobium	+ Rhizobium	- Rhizobium	+ Rhizobium
Shoot wt. (g)	- mycorrhiza	1.24 ± 0.13	0.96 ± 0.12	1.31 ± 0.35	1.64 ± 0.13	1.31 ± 0.19
	+ mycorrhiza	1.91 ± 0.25	2.65 ± 0.30	3.11 ± 0.32	3.36 ± 0.34	4.21 ± 0.34
N content ($mg\ g^{-1}$)	- mycorrhiza	22.43 ± 1.77	11.71 ± 1.64	26.03 ± 3.45	15.42 ± 0.98	23.53 ± 1.04
	+ mycorrhiza	29.07 ± 3.36	13.03 ± 1.19	19.67 ± 1.09	27.32 ± 1.43	21.75 ± 1.20
N content ($mg\ g^{-1}$ shoot)	- mycorrhiza	27.81 ± 4.38	11.25 ± 2.73	34.10 ± 9.05	25.29 ± 3.23	30.82 ± 2.73
	+ mycorrhiza	55.53 ± 12.85	34.55 ± 4.46	61.17 ± 6.79	91.79 ± 9.62	91.56 ± 10.09
P content ($mg\ g^{-1}$)	- mycorrhiza	1.32 ± 0.13	1.43 ± 0.12	1.21 ± 0.24	1.21 ± 0.10	0.84 ± 0.12
	+ mycorrhiza	3.87 ± 0.45	2.48 ± 0.07	3.38 ± 0.49	2.87 ± 0.14	3.12 ± 0.15
P content ($mg\ g^{-1}$ shoot)	- mycorrhiza	1.64 ± 0.20	1.41 ± 0.24	1.71 ± 0.36	1.99 ± 0.21	1.06 ± 0.08
	+ mycorrhiza	7.76 ± 0.65	6.58 ± 0.94	10.75 ± 2.15	9.70 ± 0.91	13.11 ± 0.58

Plant weight data and nitrogen data courtesy of Dr. M. El Halfawi.

± Standard error.

effect of both infections at both N levels was larger than the additive effects of each.

The mycorrhiza also have a small effect on N content at 15 μg g^{-1} N and a larger effect at 50 μg g^{-1}N. Rhizobium effect on N content was as expected. Rhizobia increased the total N in the shoot and also the N per unit weight by 1.5 to 2 times.

Inoculation of Glomus spores into sand:soil (Experiment B) and soil (Experiment C) cultures increased the level of infection at all phosphorus levels tested (Table 5). The increased level of infection caused corresponding increases in shoot dry weight and total uptake of P. In sand:soil culture, the percentage of root length infected decreased with decreasing fertility status. In soil culture, the extent of infection increased with decreasing fertility. This may be a reflection of plant health affecting the amount of energy available to the fungus. Plants grown in sand:soil were much poorer in appearance than those grown in soil. The amount of fertilizer P taken up was not significantly different for plants at 6.25 μg g^{-1} P_2O_5, but was higher for inoculated plants at 12.5 μg g^{-1} P_2O_5. In soil culture, the amount of fertilizer P taken up was increased by Glomus inoculation. The A values (ratio of soil P taken up to fertilizer P taken up), were not different at 6.25 μg g^{-1} P_2O_5 but were higher for inoculated plants at 12.5 μg g^{-1} P_2O_5 in sand:soil.

Discussion

Field data

Since the mycorrhizal fungi are so closely associated with the host plants, cropping practices have a large effect on the mycorrhiza as well as on the plants. The finding that mycorrhizal spore populations are consistently higher in areas of natural vegetation as opposed to cultivated fields, may be due to several reasons. The first is that areas of natural vegetation have large amounts of active roots all through the growing season. Cultivated fields, on the other hand, have little root material at the beginning of the growing season, and little active root material at the end. Even during the mid-growing season, the soil is not as heavily colorized by roots as the uncultivated area. These active roots serve as host tissue for the growth and propagation of mycorrhizal fungi. Therefore, if roots appear sooner, last longer and are in greater number, greater numbers of mycorrhizal spores are found. Another reason for lower incidence of mycorrhizal fungi in cultivated fields is the practice of fertilization with phosphorus. Figure 2 clearly shows that addition of phosphorus with the seed, reduces the extent of root infection and spore production for that year. Table 1 shows that cultivated fields, in every case studied, had a higher level of $NaHCO_3$ extractable P.

Summerfallowing also appears to reduce the number of mycorrhizal propagules. Mycorrhizal spores are able to germinate in the absence of host plant roots, but are not able to grow extensively. In suitable conditions, the spore will germinate and produce a small amount of hyphae. If no plant root is contacted, the spore will retract much of the cytoplasm in the hyphae and become dormant again. Such a process

Table 5. Effect of Glomus inoculation on fababean growth in the presence of indigenous mycorrhizal spores.

	Glomus inoculum	Sand:Soil (2:1)			Soil	
		0	6.25	12.5	0	25
		$\mu g\ g^{-1}\ P_2O_5$				
Shoot weight (g)	+	1.32 ± 0.03	1.95 ± 0.17	3.05 ± 0.22	9.61 ± 0.10	15.19 ± 0.33
	-	0.95 ± 0.11	1.68 ± 0.17	2.58 ± 0.13	7.91 ± 0.23	12.67 ± 0.39
P content ($mg\ g^{-1}$ shoot)	+	0.75 ± 0.17	0.64 ± 0.04	1.16 ± 0.14	0.97 ± 0.06	1.14 ± 0.05
	-	0.48 ± 0.09	0.66 ± 0.08	0.95 ± 0.07	1.04 ± 0.05	1.09 ± 0.09
P total ($mg\ g^{-1}$ shoot)	+	1.25 ± 0.20	1.25 ± 0.10	3.49 ± 0.23	9.30 ± 0.67	17.37 ± 0.64
	-	0.94 ± 0.24	1.14 ± 0.24	2.44 ± 0.18	8.20 ± 0.55	13.80 ± 0.86
% fertilizer uptake	+	-	9.50 ± 0.50	11.30 ± 0.40	-	36.20 ± 0.61
	-	-	8.60 ± 0.50	8.70 ± 0.20	-	27.70 ± 0.38
A value	+	-	0.51	0.78	-	0.37
	-	-	0.51	0.59	-	0.42
% roots infected	+	7.10 ± 0.70	10.30 ± 1.10	22.00 ± 2.10	32.70 ± 1.80	18.30 ± 1.80
	-	3.60 ± 0.60	5.20 ± 0.80	10.80 ± 1.60	24.00 ± 2.00	13.70 ± 1.20

± Standard Error

can happen only two or three times before the energy reserves of the spore are used up. In a summerfallow field, many of the spores left from the previous crop may become non-viable in this manner. Freezing of the soil may also reduce the viable spore population as seen in Graph 1. The moisture content and soil condition around the spore may affect its survival over winter.

The growth of rape in a field is essentially a summerfallowing process as far as the mycorrhiza are concerned. Rape and other cruciferous plants do not become mycorrhizal for reasons that are yet unknown. Therefore, for the mycorrhizal fungi, there are no host roots present in a rape field, especially if weeds are scarce.

Other factors affect the population of mycorrhizal fungi in a field or natural site. Being highly aerobic, the fungi are absent, or in low numbers in wet or waterlogged soils. Soil type and condition, host species, fungal species and amount of organic matter will all have a direct or indirect effect on the number of mycorrhizal propagules in an area.

The time of sampling is more crucial for a cultivated site than for a natural vegetation site. Spores are produced by a mycorrhizal fungus after it has colonized a root. In the cultivated site (Fig. 2) the spore numbers rise slowly during the period of root colonization. After six to eight weeks, the roots are sufficiently colonized so that large numbers of spores could be produced. Following harvest and snowfall, the number of spores dropped to the level of spores found in the spring.

Spore numbers found in the virgin soil, while fluctuating over the growing season, did not substantially rise over the season. If the variation within a sample is considered, all of the points except for the value at eight weeks, fall on a horizontal line.

This means that since the natural grass roots were already infected from the previous year, spores were produced early in the spring. These spores germinate and infect new roots. The process continues all summer long. The rise at eight weeks may be due to the growth and infection of annual plants causing an increase in spore numbers corresponding to the beginning of the rise occurring in the cultivated field. The fluctuations may also be due to climatic conditions affecting plant health.

Growth chamber data

Since each host-fungus symbioses in a mycorrhizal relationship is unique, the selection of each component of the symbioses is critical. Fababeans were selected as hosts because of their other symbioses with N fixing Rhizobia and also because of their very variable phosphorus response in the field.

The selection of the Glomus sp. used was done in such a way that only an indigenous fungus that highly infects fababeans would be isolated. By isolating the fungus from field grown fabaeans, we were assured that the fungus is able to survive and infect fababeans in the

field where conditions are different than in the growth chamber. Although not initially selected for, this symbioses proved advantageous because both spores produced, and infected roots could be used as inoculum. The use of infected roots is much easier because the sieving and floating of the soil as well as the laborious task of picking the spores can be eliminated.

The data in Fig. 4 was accumulated for two reasons. The first was to obtain a profile of the state of the symbioses at different ages of host for this particular symbioses. The second reason was to determine at what age the host is getting the maximum benefit from the symbioses. Studies by others (Kinden and Brown, 1975; Sutton, 1973) show that a particular section of root undergoes a cycle of infection. First external hypha penetrate the root. Internal structures, such as vesicles and arbuscles, are formed. New external hyphae are produced which produce spores and runner hyphae to infect new areas of root. The internal structures begin to atrophy and are eventually digested. Whether digestion is facilitated by the plant or by the fungus itself is unknown. The length of time needed for the complete cycle varies with the fungus. In the case of Glomus, it appears that the cycle takes six to ten weeks. After six weeks, the degeneration of older infection sites occurs at the same rate as initiation of new infection sites as new root material is produced. Spore production did not occur on a large scale till after six weeks. Thus, it appears that the time needed for initiation of infection sites and external hyphal production takes less than six weeks. Since spore numbers rise while the infection level remains constant, it seems probable that external spores are produced at the same time as internal structures are degenerating.

Since nutrients are released to the host by both functional and degenerating structures (Sutton, 1973), it appears that the plant is obtaining its maximum benefit in the period of six to eight weeks of age. At this point, infection is at its maximum, but spores are not being produced at the maximum rate. Since the fungus derives its energy from the host, production of large numbers of spores would probably decrease the benefit the plant obtains. Thus for maximum benefit, plants used for growth chamber experiments were harvested after 8 weeks growth, i.e. at high infection, but before high spore production.

The health of a plant is reflected in its weight and composition. A plant can only remove from the media what is present. In solution culture, it is assumed that nutrients added to the pot are available to the plant. We have found, however, that mycorrhizal plants are able to take up more of the added phosphorus than non-mycorrhizal plants. Not only is the total phosphorus uptake greater, but also the phosphorus per unit weight. The mycorrhiza may work in three ways. One is that the mycorrhiza act as an increaser of root surface absorbing area. The external hyphae, in effect, act as root hairs that take up phosphorus. The second manner is related to the first. Since more phosphorus is taken up by the plant, phosphorus is no longer limiting plant growth. Thus, the plant grows until some other factor becomes limiting. Since the plant is larger, it has more root area for absorbing phosphorus.

The third manner in which the mycorrhiza may increase plant growth is by producing some sort of growth factor that stimulates growth and increases root area.

The possibility that some other nutrient besides phosphorus is limiting plant growth in the mycorrhizal plants is seen from the P content per gram of shoot (Table 4). The levels of phosphorus are in the luxury consumption range.

Mycorrhiza also affect N content of the plant tissue. Added N also thought to be equally available to all roots was more extensively used by infected than non-infected plants. The effect was greater at 50 ug/g N than at 15 ug/g N, probably due to the fact that more N is present to take up. The increase in added N also has an effect on N content in the absence of either Rhizobium or mycorrhiza.

At both levels of added N, the additive effect of both inocula decreased the N content per gram as compared to the Rhizobium inoculum alone. This, however, may be due to a dilution of the N in a greater plant weight for doubly inoculated plants.

Glomus inoculations in both sand:soil and soil alone, increased plant weight, fertilizer uptake and total phosphorus uptake. The P content per gram did not increase or decrease because while Glomus inoculation increased the extent of root infection, the non-inoculated plants were still infected. Thus, they were able to take up phosphorus from the same sources as the inoculated plants, but to a lesser degree. Again, plant health appeared to affect mycorrhizal infection by altering energy supply. In the sand:soil pots, the plants were small and spindly. Such plants cannot support a large population of symbionts. Consequently, as the fertility decreased, the level of infection decreased from 22 to 7.1%. In the soil pots, where fertility did not play such a large role, the level of infection followed the classical role of decreasing with increasing phosphorus. This Glomus fungus then appears to act the same as the mycorrhizal fungi found in the field, but is able to infect roots to a greater degree.

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