Interactions between vesicular-arbuscular mycorrhizae (VAM) and fungal pathogens in wheat.

A thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Curtis Brian Rempel

In partial fulfillment of the

Requirements for the degree

of

Master of Science

Department of Plant Science

August 1989

ISBN 0-315-57143-8

INTERACTIONS BETWEEN VESICULAR-ARBUSCULAR MYCORRHIZAE (VAM) AND FUNGAL PATHOGENS IN WHEAT

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FOREWARD

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GENERAL ABSTRACT

Rempel, C. B. 1989. Interactions between vesicular-arbuscular mycorrhizal (VAM) and fungal pathogens in wheat. Major Professor: C.C. Bernier.

A study was conducted under controlled environmental conditions to evaluate the impact of *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita* and the wheat genome on root colonization by the vesicular-arbuscular mycorrhizal (VAM) fungus, *Glomus intraradices*. Four wheat cultivars, possessing various resistance genes to the pathogens, were inoculated with appropriate virulent and avirulent races of wheat stem and leaf rust. Simultaneous inoculation with a virulent race of stem and leaf rust significantly reduced *G. intraradices* colonization in all four cultivars. Simultaneous inoculation with an avirulent stem and leaf rust race also reduced VAM formation. When a race of stem or leaf rust was inoculated onto the cultivars individually, reduction in *G. intraradices* also occurred. No differences in *G. intraradices* root colonization were found when the cultivars were not challenged with the pathogens.

The effect of *G. intraradices* colonization on disease severity of wheat stem rust was also studied. *G. intraradices* colonization significantly increased stem rust disease severity on adult wheat plants. *G. intraradices* colonization also increased wheat biomass production and yield. Inoculation with stem rust alone resulted in significantly reduced plant biomass production and yield. Wheat plants inoculated with both organisms were the lowest yielding, with grain yields less than nonmycorrhizal rust-inoculated plants. The stimulatory effect of *G. intraradices* colonization was lost due to stem rust infection.

Growth responses in wheat colonized by *Glomus intraradices* and the root pathogen *Cochliobolus sativus* were studied under two moisture regimes. VAM inoculation decreased root rot disease intensity on wheat plants under both moisture regimes while *C. sativus* decreased VAM colonization under adequately watered and water-stressed conditions. Mycorrhizal wheat plants infected with *C. sativus* had higher root weights than did nonmycorrhizal plants infected with root rot. Water stress significantly decreased shoot dry weights of wheat plants. VAM plants had the highest yields and 1000 kernel weights

regardless of moisture regime. Plants inoculated with *C. sativus* alone had the lowest yields. *G. intraradices* offset the decreased yields caused by *C. sativus* when both organisms were inoculated simultaneously. These results were coraborated by results obtained from a field study.

A study was also conducted to determine the natural infection levels of vesicular-arbuscular mycorrhizae in spring- and winter-sown wheat on the Manitoba prairie. Cultivar and soil type had no effect on VAM infection levels in spring wheat. VAM infection levels increased as the growing season and plant growth progressed. However, infection levels remained relatively low. There were no differences in VAM colonization levels between winter wheat plants grown at two locations. VAM colonization levels remained low on winter wheat plants harvested in August.

GENERAL INTRODUCTION

In 1885, Frank observed a symbiotic association between plant roots and fungi while working in Prussia. He appropriately called this symbiotic association "mycorrhizae" which in Greek literally means "fungus root". There are various types of mycorrhizae but the most common mycorrhizal association is the vesicular-arbuscular type. Vesicular-arbuscular mycorrhizae (VAM) occur in most plant families and are geographically and ecologically ubiquitous in their distribution.

VAM fungi are obligate symbionts and have not been cultured *in vitro*. The VAM endophytes are also non-host specific, although there is considerable evidence that preferential symbiotic associations do form between certain hosts and endophytic species.

VAM are considered mutualistic symbionts based principally on the interchange of carbon and phosphate between plant and endophyte. The passage of phosphate from fungus to plant and of carbon from plant to fungus has been unequivocally demonstrated (Cox et al., 1975). Studies conducted on a wide range of infected host plant species grown in sterile P-deficient soil in pot culture demonstrated that the mycorrhizal plants typically outyielded non-mycorrhizal plants several-fold (Fitter, 1985). The association is facultative, as plants of high phosphate status are rarely infected (Sanders, 1975; Graham et al., 1981).

Several factors have been found to affect the intensity of VAM infection. These include soil fertility and plant nutritional status (Baylis, 1970; Mosse, 1973a; Hayman, 1975; Menge et al., 1978; Sparling and Tinker, 1987a and b; Stribley et al., 1980; Singh et al., 1986), light intensity (Hayman, 1974; Daft and El-Giahini, 1978) air and soil temperature (Furlan and Fortin, 1973; Volkmar, 1981; Volkmar and Woodbury, 1989), soil moisture (Rabatin, 1979), pH (Mosse, 1973b), inoculum density and plant susceptibility.

The importance of the mycorrhizal association has been widely recognized (see Mosse, 1973b; Hayman, 1976). The attributes of symbiosis are as follows. Mycorrhizae have been shown to help plants acquire mineral nutrients from the soil, especially "immobile" elements such as P, Zn, and Cu but also more mobile ions such as S, Ca, K, Fe, Mg, Mn, Cl, Br and N (Tinker, 1984). Increased uptake of mineral elements from soil will obviously alter the nutrient balance of the plant tissues. Mycorrhizae have also been shown to enhance water uptake or transport in plants (Allen, 1982; Nelson and Safir, 1982), to increase drought tolerance of plants (Sieverding, 1981; Allen and Boosalis, 1983; Levy et al., 1983), and decrease transplant injury (Menge et al., 1978).

VAM have also been shown to increase and, in some instances, decrease the general resistance of plants to pathogens (Schenck, 1981; Dehne, 1982). The general hypothesis presented is that root infection by VAM fungi or the associated increase in mineral absorption by VAM reduce the severity of diseases caused by root-invading fungi or nematodes while diseases on the foliar plant parts caused by fungi and viruses are usually more severe in mycorrhizal plants. These reductions (or increases) are associated with morphological or physiological changes in the plant (Dehne, 1982).

The carbon balance in plants will also change, due to increased photosynthetic rate and altered carbon partitioning in mycorrhizal plants (Paul et al., 1985). Mycorrhizae can also play a key role by influencing plant regulatory systems (Slankis, 1973; Allen et al., 1980; Graham et al., 1981; Barea and Azcon-Aquilar, 1982). Mycorrhizal fungi induce changes, usually increases, in phytohormone production (i.e. cytokinins, gibberellins, and ethylene) (Cooper, 1984).

One of the most dramatic changes that occurs in mycorrhizal plants is a decrease in root membrane permeability (Graham et al., 1981). This alteration is primarily due to an increase of phosphorus in plant root tissue (Ratnayake et al., 1978). A decrease in root membrane permeability results in a corresponding change in the quality and quantity of root

exudates (Schwab et al., 1983) which, in turn, has the potential to alter rhizosphere microflora and microfauna.

It is well established that VAM improve phosphorus uptake and growth in a wide range of plants (Hayman, 1983), and that VAM occur in many field crops under a wide range of environmental conditions (Mosse et al., 1981) especially when the availability of phosphorus in the soil is limited. However, Jakobsen and Nielsen (1983) demonstrated that phosphorus uptake in annual crops may be significantly affected by VAM only if infection is well established shortly after seedling emergence. Cereals, such as barley (*Hordeum vulgare* L.), have a fine, extensive root system and have been shown to respond less to inoculation with VAM fungi than onion (*Allium cepa* L.) or alfalfa (*Medicago sativa* L.) in the field (Owusu-Bennoah and Mosse, 1979). Bolan et al. (1983) suggested that plants with thick roots (ie. subterranean clover) are inefficient without VAM whereas fine rooted plants such as rye grass (*Lolium multiflorum* Lam.) do not benefit greatly from VAM colonization.

Rapid development of VAM infection can be a major determinant of response in annual crops. Even low percentage root infections by VAM (<10%) can be beneficial to the host, especially when the plant is young and a well developed external mycelium is present (Sanders et al., 1977). Most previous studies in temperate climates have suggested that infection of cereals by VAM does not reach appreciable levels (>30% root length) until late spring or early summer (Hayman, 1970; Jakobsen and Nielsen, 1983; Hetrick and Bloom, 1983).

The major focus of this research will be on "wheat genome - VAM-pathogen interactions." VAM infection of host plants has been shown to increase or decrease resistance to disease. Conversely, plants with genes for resistance to various fungal pathogens may have increased resistance to mycorrhizal infection. In selecting cultivars for resistance to pathogens, plant breeders may not consider mycorrhizae in their experiments. Under natural field conditions, the plant grows in association with the VA endophyte.

Infection by a foliar pathogen such as stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) or leaf rust (*Puccinia recondita* Rob. ex Desm.) may set up a generalized resistance phenomenon in a cultivar that has been selected for genetic resistance to the pathogen(s). Such a resistance "phenomenon" may limit the VAM association or exclude it entirely.

It is important to look at challenged and unchallenged plants using susceptible and resistant cultivars. If a plant with genetic resistance to a pathogen is not challenged by the pathogen, will VAM fungal infection still occur, or does the plant need to set up resistance mechanisms triggered by the pathogen before VAM infection is affected?

Furthermore, for a number of host-pathogen systems where the gene-for-gene hypothesis applies, the specific interaction of genes or gene products is associated with incompatibility or compatibility. The consensus is that incompatibility is the active process requiring a gene product for resistance produced by the host and a gene product for avirulence produced by the pathogen (Ellingboe, 1976; 1981). Knowing this, we can further assess the impact that resistance genes have on mycorrhizal infection. By using both virulent and avirulent races of the pathogen, it may be possible to determine whether products of resistance genes in the host (or fungal avirulence gene products) are responsible for reduced colonization by VAM (i.e. inoculation with an avirulent race) or whether pathogen virulence results in reduced infection by VAM fungi. If the latter is the case, it may be concluded that the pathogen itself competes (ie. by reducing photosynthesis or plant growth) against the mycorrhizal fungus for host resources and in doing so reduces VAM fungal infection and the host genome has no effect on infection.

Yield Loss Surveys in the Canadian prairie provinces have shown that common root rot is a widespread and economically important disease in cereals, with annual yield losses of about 5.7% in spring wheat (Ledingham et al., 1973). Conidia of the causal agent, *Cochliobolus sativus* (Ito & Kuribay) Dreschl. ex Dastur. (conicial state:*Bipolaris sorokinia* (Sacc.) Shoem. syn *Helminthosporium sativum* Pamm., King & Bakke), persist

in the soil for long periods of time. The fungus is an aggressive pathogen especially when plants are under stress. Drought and high temperatures are the most important predisposing factors. Plants under nutritional stress are also subject to attack (Wiese, 1977). At present there are no biological control agents which have been found effective against *Cochliobolus sativus*.

The first section of the thesis involves an assessment of effects of the host genome on VAM infection. A model system to assess the impact of resistance genes on VAM infection was employed using a series of wheat (*Triticum aestivum* L.) cultivars with differing numbers of genes conferring resistance to both stem and leaf rust. The wheat cultivars were "challenged" with both virulent and avirulent races of the pathogens. VAM infection of these plants was then determined along with that of non-rust-inoculated control plants.

The effect of VAM colonization on the disease severity of wheat stem rust, inoculated at the adult plant stage, and the resulting effects on wheat growth and yield were also determined. These results are presented in the second section of the thesis.

The third section deals with an evaluation of the effects of VAM on incidence of common root rot disease in wheat. As well, the effects of VAM and pathogens on water uptake by wheat were assessed.

The fourth section of the thesis deals with the infection kinetics of VAM in field-grown spring and winter wheat in Manitoba.

REVIEW OF LITERATURE

1. INFECTION LEVELS AND RESPONSES TO VAM COLONIZATION IN CEREALS

In the United Kingdom, Hayman (1970) reported moderate levels of root colonization, sampling from May through September. He found that mycorrhizal colonization in winter wheat was sparse during the spring months, but increased gradually during the summer months to a peak at harvest time in September. The abundance of *Endogone* spores in wheat field soil was strongly influenced by season, with spore numbers increasing during the summer after the period of maximum root growth. The wheat roots had become appreciably colonized only after heading when maximum root growth had occurred. These observations were consistent with those of Mason (1964) and Sutton and Barron (1972).

In Denmark, very low levels (<10%) of colonization by VAM in winter cereals were found until mid-April after which there was a gradual increase in infection to levels that approached 50% at harvest three months later (Jakobsen and Nielsen, 1983).

Similarly, Hetrick and Bloom (1983) observed no VAM infection in winter wheat, regardless of soil fertility, until after anthesis in May, although moderate to high levels of infection were maintained in perennial native grasses throughout the year. They identified the occurrence of a wider diversity of VAM fungal species in the prairie than in the cultivated wheat soils and significantly more fungal spores were recovered from undisturbed prairie soils than four winter wheat field soils. Though variable, VAM root colonization was evident in all prairie grass roots sampled throughout the year. In contrast, no identifiable VAM root colonization was evident in wheat until May after flowering when 27% root colonization was evident.

In a follow-up study, Hetrick et al. (1984) found little VAM colonization of hard red winter wheat until flowering in May after the soil had warmed. However, only a small amount of infection developed (<1% to 10%) "just prior to harvest". No yield benefits

occurred from late-season colonizations. Also, in-furrow field inoculation with *Glomus mosseae* (Nicol. & Gerd.) Gerd & Trappe or *Glomus epigaeum* Daniels and Trappe failed to provide earlier colonization or increased yield. The authors suggested that resistance of winter wheat cultivars to VAM colonization might explain the absence of typical levels of VAM infection. However, in their greenhouse experiments, all of the eight wheat cultivars tested became mycorrhizal when the plants were grown in field soil containing indigenous or amended VAM fungal endophytes. Therefore, the observed failure of colonization could not be explained by cultivar resistance. Significant differences in the intensity of VAM colonization were observed between cultivars. However, the cultivars showing the greatest VAM colonization varied from soil to soil. The researchers grouped the cultivars as highly or moderately colonized based on their response in the three soils considered together.

As a second explanation for these low infection levels, Hetrick et al. (1984) considered that low VAM populations might retard development of mycorrhizae in the field. They previously observed extremely low VAM fungal spore numbers in wheat field soils, suggesting that inoculum concentrations in the soil were insufficient to support extensive infection. However, in greenhouse experiments, wheat became colonized in field soils previously cropped to wheat or corn (*Zea mays* L.) and the researchers concluded that nonsterile field soils do contain enough indigenous inoculum to initiate colonization. This is further supported by the fact that late emerging volunteer wheat in several fields became colonized within a few months after germination.

The most plausible explanation for low infection levels is that soil temperature limits colonization until May when winter wheat plants are already nearing maturity. The first observed colonization of wheat in April and May probably occurs when soil temperatures are first conducive to germination and colonization of VAM fungi. There is strong evidence that germination of some VAM fungal species including *G. epigaeum* occurs very slowly or is entirely inhibited at soil temperatures below 18°C (Furlan and Fortin, 1973; Daniels and Trappe, 1980; Koske, 1981; Volkmar, 1981). The fact that wheat is not

colonized because of low soil temperature is supported by the results of temperature experiments conducted in the greenhouse. Hetrick and Bloom (1984) noted that wheat failed to become colonized at 10°C but was 7.8% colonized at 25°C. The fact that red clover (*Trifolium pratense* L.) plants were similarily free of colonization when grown at low temperature suggests that this phenomenon may be associated with other fall sown crops as well. Therefore, the researchers concluded that the observed failure of winter wheat in the field to become colonized until late in the growing season could not be explained by cultivar resistance or insufficient inoculum levels in field soils but may be attributed to low soil temperatures which may inhibit spore germination or root colonization.

A survey of four fields growing various commercial and breeder wheat and triticale (*XTriticosecale* Wittmach) cultivars revealed the presence of several VAM fungi in soils with elevated levels of available P (Young et al., 1985). Extractable P (Bray) levels of sampled fields ranged from 73 to 119 mg/kg soil. Cortex colonizations (sampled just prior to harvest) were variable and relatively low (range <2 to >18%) partly due to declining root tissue.

Detection of few VAM structures in roots of some breeder-plot cultivars contrasted with appreciable (for winter wheat) internal arbuscles/vesicles and external mycelium found in several other cultivars. This suggests differences in susceptibility of certain cultivars to colonization by particular VAM fungi as Azcon and Ocampo (1981) reported for 13 Spanish wheat cultivars tested against a *Glomus mosseae* isolate in a 10 week experiment.

Bertheau et al. (1980) also suggested that VAM development in a host plant is partially controlled by the plant genome. They found that among 20 wheat cultivars colonized with *Glomus mosseae* responses varied from yield increases to yield depressions.

Infection levels and response to VAM colonization in spring cereals are somewhat different than in winter cereals. Khan (1972; 1975) and Saif and Khan (1977) reported

field responses to inoculation with mycorrhizal fungi in corn, wheat and barley in soil containing 15 ppm Olsen P. In experiments conducted with wheat, the addition of P and fungus or fungus alone increased total yield, number of spikes per plant, and the number of fertile spikelets per plant. Yield was increased three-fold by the fungus. However, the 1000 kernel weight was slightly lower than in nonmycorrhizal plants. Khan (1975) reasoned that the greater number of seeds per spike in the mycorrhizal treatments was probably determined quite rapidly at heading (or during the vegetative to reproductive transition) and thus may account for slightly smaller 1000 kernel weights. The differences between mycorrhizal and nonmycorrhizal wheat plants were eliminated by the application of P fertilizer, indicating that the fungus does not enhance cereal growth in soils containing enough available P. They also found that there were direct relationships between spore numbers in the soil and mycorrhizal development and between extent of root infection and increased growth. Root infection was greatest in non-P supplemented plots and decreased in inoculated plants when they were supplemented with superphosphate. However, the results of these experiments may not be representative due to seedlings being transplanted into the field and not sown *in situ*, and more seriously, the transplanted mycorrhizal and nonmycorrhizal seedlings, although of equal size, may have been unequal in P content when planted out.

The effects of inoculation *in situ* were examined in a field experiment using onion, alfalfa, and barley (Owusu-Bennoah & Mosse, 1979). The plants were inoculated with one of two endophytes placed below the seed. *Glomus mosseae* increased growth by 77% (onion), 79% (alfalfa), and 33% (barley), while *G. caledonicum* (Nicol. & Gerd.) Trappe & Gerd. increased growth by 40%, 60% and 30% respectively.

The onions and alfalfa benefitted most from inoculation in plots with high available P (13 mg P/kg) while barley responses to inoculation were confined to those plots with less available P (9 mg P/kg). An infection level of approximately 45% occurred in plants

infected with indigenous endophytes while inoculated plants reached infection levels of approximately 70%. Inoculation responses were not related to infection level.

This experiment is important as it showed that VAM fungi introduced at seeding time must become established quickly to affect the growth of an annual crop in the presence of an appreciable populations of indigenous endophytes. One of the desired effects of inoculation is increased uptake of P in the early developmental stages of plant growth because this is generally reflected in greater final yield. This experiment also shows that the effects of inoculation may be due to inherent differences in the effectiveness of a particular endophyte rather than to differences in the rate and extent of infection caused by mycorrhizae. While the extent of infection by *Glomus mosseae* and *Glomus caledonicum* was very similar, their effects on the growth of onions and alfalfa were quite different.

Black and Tinker (1979) studied the effects of crop rotations on the development of mycorrhizal infections in barley and on the spore population in the soil. They showed that with different crop rotations of barley, kale (*Brassica oleracea* L.-a nonhost) and fallow, spore numbers and subsequent infections of barley crops were largest following barley. Both kale and fallow breaks reduced spore populations and infections similarly. They also surveyed levels of mycorrhizal infection on barley grown commercially. Infection levels ranged from 4 to 40% root length infected, with averages of 14 and 23% in two years. They concluded that mycorrhizal infection in barley developed very slowly and was unlikely to improve crop nutrition. They also concluded, with some reservations, that final yield was negatively related to mycorrhizal infection.

Clarke and Mosse (1981) compared the growth and yield of barley inoculated with three different endophytes with those of non-inoculated plants infected only with indigenous endophytes in the field, in plots with and without added superphosphate. In the non-P amended plots, inoculation by each of the three different endophytes approximately doubled all measured growth parameters over the non-inoculated control. There was little difference between the three inoculants. This confirms the previous trial of Owusu-

Bennoah and Mosse (1979). In the P amended plots, *Glomus caledonicum* appeared to be a better inoculant for alfalfa and onions (Owusu-Bennoah & Mosse, 1979). *Glomus caledonicum* increased the dry mass of barley heads by 35% over P-amended non-inoculated control plants. At the high P levels, *Glomus mosseae* and *Glomus fasciculatum* (Thawter sensu Gerd.) Gerd. & Trappe were, if anything, growth depressing compared to uninoculated plants. Clark and Mosse (1981) obtained high infection levels in inoculated barley plots only 21 days after seedling emergence. Inoculation greatly increased mycorrhizal infection in the three-week-old seedlings and added P decreased it. The relatively high level of infection which was established shortly after seedling emergence shows one important benefit of inoculation. Infection of indigenous endophytes was depressed to a much larger extent by added P than that of introduced endophytes. It is also interesting to note that although *Glomus caledonicum* was the most beneficial of the three inoculants, particularly in P-amended plots, it tended to produce less infection than the other two species at most sampling dates.

Jensen (1982) conducted an experiment to determine the influence of four VAM fungi on nutrient uptake and growth in barley. The barley plants were inoculated with *G. constrictum* Trappe, *G. fasciculatum* isolate number 185, *G. fasciculatum* isolate number 0-1, and *Gigaspora margarita* Becker & Hall. Plants inoculated with *G. constrictum* and with *G. fasciculatum* isolates showed an increased yield of grain and straw. These two fungal isolates also increased the total plant uptake of P, Cu, and Zn. Barley plants inoculated with *G. margarita* did not differ from uninoculated control plants in growth or P, Cu, and Zn uptake. Also, growth responses brought about by inoculation with these endophytes were not correlated with intensity of infection. This is in accordance with earlier results obtained with *Glomus fasciculatum* (Daniels and Menge, 1981) and with other VAM fungi (Daniels and Menge, 1981; Owusu-Bennoah and Mosse, 1979).

Singh et al. (1986) conducted a study on the effect of P on zinc uptake in wheat. They found that a one-time heavy P application resulted in high residual P levels such that

Zn uptake was still depressed 5 years after the P application. High P levels significantly reduced VAM colonization levels in wheat roots (ie. from 40% at 0 P to 15-20% at 80 and 100 kg P/ha). They also found a linear relationship between Zn concentrations in the plant and % VAM colonization in the roots and postulated that a P x Zn interaction may be due, in part, to a reduction in VAM infection by high soil P levels.

Jensen and Jakobsen (1980) reported on the occurrence of VAM fungi in fields of wheat and barley grown in different types of soils with different fertilizer treatments. VAM infection was found at all locations. An inverse relationship was found between soil P levels and intensity of infection. Infection was also decreased by increasing levels of nitrogen fertilizer. Infection intensity ranged from 2% of the root cortex infected (100N/30P) to 44% (50N/0P).

Jakobsen (1983) studied the effect of inoculation with VAM fungi on the growth of barley in the field at two levels of soil P. He found that production of dry matter was significantly increased by soil P at all sampling times, whereas it was significantly increased by inoculation only at crop maturity. Both inoculation with *G. caledonicum* and added P increased the number of heads. Inoculation also increased the uptake of Zn and Cl significantly. Mycorrhizal infection was first noted 25 days after seedling emergence when barley was inoculated with *G. caledonicum* and reached infection plateaus of approximately 50%. In the uninoculated plots, infection was first observed later and the final infection levels were approximately 12%.

In another study, infection in spring-sown cereals progressed very rapidly and an infection plateau of about 50% was reached only 15 days after seedling emergence (Jakobsen and Neilsen 1983). The lag phase and phase of rapid spread of infection was unusually short compared with other reports (Sutton, 1973; Saif, 1977) but similar to results of Clark and Mosse (1981). An infection plateau was reached somewhat later in peas (*Pisum sativum* L.) than in spring cereals, but the peak occurred at a higher level (70-75%). Such high infection levels in peas have been found by others (Sutton, 1973;

Strzemska, 1975; Saif, 1977). The results presented here contrast with the slow infection rate and rather low final infection levels reported by Black and Tinker (1979). Jakobsen and Neilsen (1983) discussed several factors of potential significance for infection spread. They believed that of the factors considered, an adequate soil moisture, higher soil temperatures, and possibly also a faster decrease of P concentrations in plant tissues due to more rapid growth were the main factors responsible for a more rapid infection development in their work compared with that of Black and Tinker (1979).

Buwalda et al. (1985) compared responses of cereals to inoculation on fumigated and non-fumigated plots at a range of soil P levels. They tested the effects of fumigation, inoculation and added P first on spring wheat in the treatment year followed by winter barley. Testing of residual effects on winter barley allowed the spread of introduced or natural endophytes to be monitored.

In this experiment, the levels of mycorrhizal infection in both fumigated, inoculated plots and non-fumigated non-inoculated plots were similar, reaching infection plateaus of approximately 30% in wheat. The nonfumigated inoculated wheat plots had infection levels of 40%. Generally in wheat, the levels of infection were only slightly reduced by applied P. Within all treatments, levels of infection reached plateaus well before harvest. It is quite surprising that dissimilar endophytes, native and introduced, should follow such similar time courses in the development of infection.

The levels of infection on fumigated and non-inoculated plots consequently increased with length of cropping. In contrast, the maximum levels of infection attained on non-fumigated and inoculated plots were remarkably similar in the first, second and third crops sown after the treatments were applied. These levels were also similar to those reported by Black and Tinker (1979), Clark and Mosse (1981), Powell (1981) and for barley grown in the field, and may reflect a maximum host controlled level of infection for field grown cereals. Levels of infection on all plots tend towards this maximum as soil inoculum builds up and the effects of the fumigation and inoculation treatments

consequently disappear. The effects of applied P on levels of mycorrhizal infection were still evident at 27 months after the treatment was applied.

In wheat, artificial inoculation on fumigated plots increased uptake of P by 50% where 60kg/ha gave an increase of 89%. The corresponding figures for increase in grain yield are 36 and 69% respectively. This suggests that mycorrhizal infection is less effective in cereals than P fertilizer.

The increases in yields of barley on inoculated plots compared to noninoculated plots were significant only where levels of infection in the noninoculated plots were very low. In some cases, inoculation failed to increase yields, although levels of infection were increased. It therefore appears that the number of infective propagules of mycorrhizal fungi required to produce maximum growth response may be less than the number required for maximum levels of mycorrhizal infection. All of the barley experiments showed similar responses to applied P, confirming that the diminishing effect of inoculation in successive crops was a result of increases in infection on noninoculated plots, rather than of any change in crop P status.

2. VAM AND DISEASE INTERACTIONS

The first good evidence that mycorrhiza can decrease disease severity in natural soils and against several pathogens on the same host is given by Zambolin and Schenck (1983). They noted that *Glomus mosseae* reduced the effects of *Macrophomina phaseoli* (Tassi) Goid., *Rhizoctonia solani* Kuehn, and *Fusarium solani* (Mart.) App. & Wr. emend. Synd. & Hans. individually on soybean (*Glycine max* (L.) Merr.) in autoclaved soil. The pathogens were added to the soil in amounts and forms that were encountered in the field. In each instance the mycorrhizal plant inoculated with the pathogen was significantly superior to the nonmycorrhizal plant in growth after 45 days. Thus the mycorrhizal plants were able to counteract each pathogen's reductions in plant growth. The same effect occurred in natural field soils and resulted in higher yield compared to control plants. The

soybean plants were well colonized (49-65%) by *Glomus etunicatum* Becker & Gerd. and *Gigaspora margarita* despite the fact that the soil had a high level of available P (92 ppm, double acid extraction).

Chakravarty and Mishra (1986) found that the wilting of cassava (*Cassea tora* L.) caused by *Fusarium oxysporum* Schlecht was reduced after inoculation with VAM. When *G. fasciculatum* and *G. tenue* (Green) Hall were added to soil infected with *F. oxysporum*, plant growth was increased. The population of *F. oxysporum* in the rhizosphere was significantly lower on mycorrhizal than nonmycorrhizal root systems. Schonbeck and Dehne (1977) tested the ability of mycorrhizal cotton (*Gossypium hirsutum* L.) plants to resist *Thielaviopsis basicola* (Berk & Br.) Fr. The extent of root damage and root dry mass was similar in both nonmycorrhizal and mycorrhizal plants five weeks after simultaneous inoculation with the pathogen and *Glomus mosseae*. However, shoot dry mass was significantly greater in mycorrhizal plants. Baltruschat and Schonbeck (1972) reported increased resistance of mycorrhizal tobacco (*Nicotiana tabacum* L.) and alfalfa to *T. basicola*. The number of chlamydospores was 10-fold lower in mycorrhizal tobacco plants at the low inoculum concentration (10,000 endoconidia/ml). Interactions between *G. fasciculatum* and the root pathogen *Sclerotium rolfsii* Curzi resulted in a reduced severity of disease and number of sclerotia produced by the pathogen in peanut (*Arachis hypogaea* L.) plants (Krishna and Bagyaraj, 1983).

The sequence of inoculation of VAM and pathogen as well as the pathogen inoculation dosage will also have some bearing on the outcome of these interactions. For instance, when the mycorrhizal fungus was inoculated simultaneously with the pathogens *Pythium ultimum* or *Rhizoctonia solani*, it had no effect on disease resistance in poinsettia (*Euphorbia pulcherima* Wiild.) (Stewart and Pfleger, 1977). However, inoculation with mycorrhiza conferred resistance to infection by the pathogens added 20 days later.

Prior colonization by the mycorrhizal fungus *G. mosseae* reduced the damage to tomato (*Lycopersicon esculentum* Mill.) by *Fusarium oxysporum* Schlecht f. sp. *radicis-*

lycopersici Jarvis & Shoemaker (Dehne and Schonbeck, 1979). The mycorrhizal plants had reduced wilt symptoms, vascular invasion, and sporulation of the pathogen. Mycorrhizal plants had 125 pathogen spores/ plant compared to 280 for nonmycorrhizal plants and the stem height infected by the pathogen was 12.4 cm in mycorrhizal plants as opposed to 23.6 cm for nonmycorrhizal plants. Increasing inoculum of the pathogen altered this effect.

In a follow-up study the pathogen and mycorrhizal fungus were added simultaneously in a propagule form (chlamydospores), and at a rate comparable to that found in natural soils (McGraw, 1983). Under these conditions, wilt symptoms occurred on mycorrhizal plants 8 - 10 days before symptoms appeared on nonmycorrhizal plants. However, after two months, disease severity was significantly less on mycorrhizal plants than on nonmycorrhizal plants. Tomato plants inoculated with *G. mosseae* showed greater disease symptoms than plants inoculated with *G. etunicatum* but plants colonized with either VAM fungal species had less disease than nonmycorrhizal plants.

Davis et al. (1978) evaluated the effect of the mycorrhizal fungus *Glomus fasciculatum* on *Phytophthora parasitica* Dast. on citrus. The sweet orange (*Citrus sinsensis* (L.) Osbeck) roots were precolonized with *G. fasciculatum*. After 8 weeks, the precolonized roots were submerged in a *P. parasitica* spore suspension containing 1000 zoospores/ml. Plants inoculated with *G. fasciculatum* showed a 68% increase in dry plant mass when compared to nonmycorrhizal controls. A visual examination of roots showed that decay and discoloration, indicative of the effects of the pathogen, were much less pronounced in mycorrhizal roots. Mycorrhizal citrus plants inoculated with *P. parasitica* had a healthier root system than did nonmycorrhizal roots inoculated with *P. parasitica*.

Davis and Menge (1981) used chlamydospores of *P. parasitica* at three different levels (10, 20, and 50 chlamydospores/g soil) as an inoculum source and in a second experiment (using 500 zoospores/ ml inoculum), evaluated several species and isolates of mycorrhizal fungi for their effect on *P. parasitica*. The pathogen significantly reduced the

total and root dry mass of mycorrhizal and nonmycorrhizal sweet orange, when chlamydospores were used as inoculum. However, the mycorrhizal plants had 2 - 2.5 times the total dry mass as the pathogen-inoculated nonmycorrhizal plants and were superior always to nonmycorrhizal, noninoculated plants in dry root mass. *G. fasciculatum* produced the greatest plant growth response but was not the best in reducing the effects of the pathogen on citrus. Plants infected by *G. mosseae* and *Gigaspora margarita* had the least reduction in root and shoot mass by *P. parasitica.* Mycorrhizal fungi are specific in their effects on plants and the selection of a species or isolate may influence the results obtained in mycorrhizal-disease interaction studies.

Interactions between *G. fasciculatum* and *Aphanomyces euteiches* (Dreschsl.) root rot of peas were studied in pot experiments using irradiated soil (Rosendahl, 1985). The influence of the time interval between inoculation with the mycorrhizal fungus and the pathogen on the interaction between the two fungi was assessed. VAM suppressed infection of the pathogen when the challenge inoculation was made two and four weeks after planting. However, no reduction of the pathogen was detected when the plants were inoculated with both fungi at the same time.

Six different zoospore concentrations were used to determine the influence of increasing inoculum on infection by *A. euteiches* . Infection increased with increasing inoculum level in the non-VAM pea plants, whereas the infection in the VAM plants was independent of the zoospore concentration. Oospore production was reduced in the VAM plants, compared to the non-VAM plants.

Caron et al. (1985; 1986a and b), demonstrated that the presence of the VAM fungus *Glomus intraradices* Schenck & Smith decreased both root rot of tomatoes and the development of the pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici.* Caron et al. (1986c) then conducted a study to determine if such effects are influenced by the sequence of inoculation of the two fungi. They inoculated tomato plants with *G. intraradices* four weeks before, simultaneously, and four weeks after inoculation with *F. oxysporum.* This

is the first study in which a VAM fungus was inoculated following inoculation by a pathogen.

The presence of *G. intraradices* resulted in a decrease in root rot (%root necrosis) and in the population of *F. oxysporum* in all treatments The effects of the VAM fungus were not influenced by the sequence of inoculation with the 2 microorganisms. Plant dry mass was not increased by the presence of *G. intraradices* alone, but compensation for dry mass loss due to *F. oxysporum* occurred when the VAM fungus was inoculated after the pathogen. Root colonization by *G. intraradices* was significantly increased when *F. oxysporum* was inoculated simultaneously with or 4 weeks prior to inoculation with the VAM fungus.

Kaye et al. (1984) reported a greater colonization of roots of poinsettia by *G. fasciculatum* when *Pythium ultimum* was inoculated after the VAM fungus than in the absence of *P. ultimum*. In contrast, Baath and Hayman (1983) observed a lower root colonization of tomatoes by *Glomus caledonicum* challenged by *Verticillium albo-atrum* Reinke & Berthold than in the absence of the pathogen. They suggested that VAM infection was decreased because the pathogen reduced the photosynthetic efficiency of the leaves and therefore the rate of root exudation.

G. intraradices inoculated 4 weeks after *F. oxysporum* resulted in a substantial increase in VAM colonization which demonstrates that *G. intraradices* can colonize roots extensively despite the presence of a pathogen. This was the first report of an increase in root colonization by a VAM fungus when inoculated after a pathogen. Clearly, the effect a fungal root pathogen has on root colonization by a VAM fungus may be consistent within a system but can vary in different VAM/pathogen/ host combinations.

Studies of interactions between VAM fungi and root fungal pathogens have shown that the effect of VAM fungi on pathogen and disease development can be related to enhanced P nutrition, which was wholly or partially responsible for a decrease in disease

severity in numerous studies (Davis and Menge, 1980; Graham and Menge, 1982; Krishna and Bagyaraj, 1983; Zambolin and Schenck, 1983; Kaye et al., 1984).

An increase in the level of host resistance to take-all disease in mycorrhizal wheat was attributed to improved P nutrition (Graham and Menge, 1982). This effect is consistent with the recognition that take-all disease is favored by inadequate plant nutrition, especially P deficiency. Several reports indicate that application of P reduces take-all disease in the field (Slope et al., 1978; Reis et al., 1981). High levels of VAM root colonization in plants grown in a P deficient soil (0.5 mg P/kg soil) or the addition of 50 mg P/kg soil equally suppressed disease severity (Graham and Menge, 1982). For plants grown in P-deficient soil, the formation of high levels (>70% root colonization) of VAM increased root P content nearly to the level of P-amended plants. Fifty mg P/kg soil severely inhibited VAM colonization (<10%). The inhibition of VAM infection in P-sufficient plants is widely recognized (Sanders, 1975; Menge et al, 1978). Thus, in P deficient plants, mycorrhizal formation was dramatically higher and as a result of infection the P content of roots increased to a level more comparable with P-amended plants.

Wheat plants of each P-VAM treatment combination were inoculated with *Gaeumannomyces graminis* (Sacc.) von Arx & Olivier var *tritici* Walker (Ggt) at two inoculum levels (Graham and Menge, 1982). The P-deficient nonmycorrhizal plants developed more take-all at the high inoculum level than at the low inoculum level as determined by either visual or quantitative disease assessment. In contrast, disease severity was uniformly low for plants grown at 50 mg P/kg soil, regardless of concentration of pathogen inoculum or the presence of low levels of mycorrhizal infection. In the P-amended wheat plants, mycorrhizal infection of less than 15% had no significant effect on disease development compared to nonmycorrhizal treatments. High levels of VAM (>90% root colonization) in plants grown in P-deficient soil were correlated with significant decreases in disease severity at the low inoculum level but not at the higher level (Graham and Menge, 1982).

Reis et. al. (1981) proposed that certain nutrients control take-all disease severity through either an increase in resistance of the host tissues to the pathogen or a greater plant tolerance of the pathogen as a result of more root formation. Graham and Menge (1982) found that the decrease in take-all severity in P and VAM treatments was not a result of significant increases in root growth, but rather was related to improved root P status and decreased root exudation prior to pathogen inoculation. Root exudation of amino acids and reducing sugars, which was lower in heavily mycorrhizal and P-treated plants than the untreated controls, was inversely correlated with root P content (Graham and Menge, 1982). The relationship between P deficiency and increased root exudation has been observed in a wide variety of plants including Monterey pine (*Pinus radiata* D. Don) (Bowen, 1969), citrus and Sudangrass (*Sorghum sudanense* (Piper) Stapf) (Ratnayake et al., 1978).

Changes in root exudation have been correlated with P-induced decreases or increases in phospholipid content of cells with resultant changes in root membrane permeability. Membrane-mediated effects on root exudation have been shown to be responsible for the observed effect of P on VAM formation (Sanders, 1975; Menge et al., 1978; Graham et al., 1981). That is, increased levels of exudation (due to P deficiency) promoted high VAM infection levels which resulted in improved P nutrition and eventually a decrease in membrane permeability and root exudation compared to nonmycorrhizal plants. Graham and Menge (1982) confirmed the relationship between VAM improvement of P and lower rates of root exudation in wheat.

Root exudates play an important role in the take-all infection process. Pope and Jackson (1973) found that Ggt hyphae emerging from pot culture inocula had a positive growth response to wheat roots or wheat root exudates. Changes in quality or quantity of exudates reaching inocula of Ggt may alter hyphal response and therefore the infection process. Graham and Menge (1982) showed that root exudation influences pathogen activity since P-induced decreases in root exudation were correlated with decreased disease

severity. The influences of soil P and VAM on take-all appear to be the same. Both factors increase the P status of the host, leading to a decrease in net leakage of root exudates and therefore reduced pathogen activity. Thus, reduction of take-all disease by VAM is indirect and results from improved P nutrition.

Ggt has been shown to be a poor saprophytic competitor in the soil. VAM fungi may successfully compete for the root exudates which are required by the Ggt to initiate or sustain infection thereby producing or preventing disease. This competative advantage perhaps is lost as inoculum concentration of the pathogen increases, which would explain why the VAM did not decrease disease at the high inoculum potential (Graham and Menge, 1982).

Davis and Menge (1980) also reported on the effect of P and *G. deserticola* Trappe, Bloss & Menge on *P. parasitica* disease severity in citrus. Three different levels of P (6, 56, and 60 mg/kg soil) were tested. The addition of 56 and 60 mg P/kg of soil significantly reduced the disease produced by the pathogen on citrus. This reduction in disease was equal to the reduction of disease produced by *G. deserticola* at 6 mg/kg soil P indicating that the benefit of VAM fungi was a result of increased P uptake by the mycorrhizal plant.

Caron et al. (1986b) conducted a study to determine the effect of colonization of tomato roots by *Glomus intraradices* on the development of tomato crown and root rot and on the population of *Fusarium oxsporum* f. sp. *radicis-lycopersici* in relation to increasing phophorus concentrations in the substrate. They found that increased P levels in the roots and leaves following an increase in the available P in the substrate had no effect on the pathogen population nor the development of the disease but it did result in a decrease in root colonization by *G. intraradices*. VAM colonization varied from 8% at the high P level to 65% at the lowest P level. Only plants inoculated with *G. intraradices* showed significantly decreased root necrosis and *Fusarium* populations in the substrate at all P levels (ranging from 0 - 1312 mg P/3.2 L substrate applied weekly). There were no significant differences in the percentage of root necrosis and in the number of propagules of

Fusarium oxysporum f. sp. *radicis-lycopersici* among concentrations of applied P and no significant interactions between any inoculation treatment and any concentration of applied P. The presence of *G. intraradices*, even in the presence of high P levels, decreased root necrosis and limited the population of *Fusarium oxysporum* f. sp. *radicis-lycopersici* . Caron et al. (1986b) demonstrated that a higher concentration of P in the plant is not responsible for lower disease development and a lowered *Fusarium* population and that root necrosis and pathogen population could be reduced even by low levels of endomycorrhizal colonization. Caron et al. (1986b) also showed that the observed reduction of the disease and pathogen development could not be explained by the improved P nutrition attributed to the presence of VAM fungi.

Not all reports indicate that mycorrhizae decrease disease. In some studies VAM either had no effect on the pathogen or disease severity was actually increased by the presence of VAM. Ross (1972) inoculated Lee soybeans with an Endogone species and with *Phytopthora megasperma* Dreschsl. Plants inoculated with both organisms weighed 204g/plant, the *Phytopthora* infected plants weighed 153g and the controls weighed 181g. A numbered soybean line weighed 126g when inoculated with *Phytopthora* alone, the survivors of plants inoculated with both fungi weighed 126g each, and healthy plants weighed 284g.

Internal stem discoloration developed on 88% of *Phytopthora* rot susceptible soybeans which were also inoculated with a VAM fungus and 33% of the plants died. In microplots infected with *P. megasperma* alone, 17% of the plants of the numbered line developed stem discoloration, but none died. There were no differences in the amount of root and stem discoloration between mycorrhizal and nonmycorrhizal Lee plants growing on *Phytopthora* infested soils.

The VAM fungus made the numbered line more susceptible to *P. megasperma* by either predisposing the host to infection or enhancing the disease in doubly infected plants. The development of stem rot symptoms on plants in mycorrhizal inoculated plots under

conditions of high soil P indicates that the effect exerted by *Glomus macrocarpum* Tul. & Tul. var. *geosporum* on susceptibility is related to VAM infection per se rather than to alterations in host nutrition. Since stem rot developed much more severely in the VAM colonized plants, the appearance of these symptoms under field conditions may be associated with the presence of VAM. Very large vesicles and chlamydospores produced by the VAM fungus in the root cortex may have damaged host root tissue and facilitated development of the pathogen.

Davis et al. (1979) initiated a study to determine the influence of VAM on Verticillium wilt of cotton caused by *Verticillium dahlia* Kleb. Two soil P regimes were used in the study. In plants fertilized with 20 mg P/ kg soil, Verticillium wilt was more severe in plants infected with the VAM fungus *G. fasciculatum* than in nonmycorrhizal plants. Also, there were more propagules of *V. dahlia* in petioles of mycorrhizal plants than in petioles of nonmycorrhizal plants. However, in plants fertilized with 300 mg P/ kg soil, Verticillium wilt symptoms were equally severe in mycorrhizal and nonmycorrhizal plants and the numbers of propagules were not significantly different in mycorrhizal and nonmycorrhizal plants. Adequate P nutrition of cotton, whether due to P fertilizer in the soil or increasd P uptake by mycorrhizal fungi, resulted in more severe wilt of plants than inadequate P nutrition.

The authors postulated several reasons for the increase in disease severity in mycorrhizal plants. These included dilution of the concentration of potassium in mycorrhizal plants, a larger population of *V. dahlia* in the mycorrhizal plants due to their improved nutrient status, and more movement of microconidia in the mycorrhizal plants where larger leaf area resulted in greater transpiration.

There has been only one report on the effect of VAM on the incidence of disease caused by fungi on the aerial parts of the plant. Disease severity of *Cochliobolus sativus* (Ito & Kurib.) Dreschsl. ex Dastur (conidial state: *Bipolaris sorokinia* (Sacc. in Sorokin) Shoemaker (syn. *Helminthosporium sativum* P.K. & B.)) and *Erysiphe graminis* DC. ex

Merat of barley, of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav. and *Uromyces phaseoli* (Rebent.) Wint.on kidney beans (*Phaseolus vulgaris* L.), of *Erysiphe cichoracearum* DC. ex Merat on cucumber (*Cucumis sativus* L.), and of *Botrytis cinerea* Pers. ex Fr. on lettuce (*Lactuca sativa* L.) was increased in mycorrhizal plants when compared with nonmycorrhizal plants (Dehne, 1982). Both obligate and facultative foliar pathogens are included in the above list. However, no attempt was made to quantify the disease nor was there any mention of the interaction on plant growth.

The specific effects of VAM fungi on disease is not well known and is far from being completely understood. Several factors have been implicated with ectomycorrhizae (Marx, 1973). Ectomycorrhizae (EM) can produce antibiotic compounds which affect both bacteria and fungi. They also produce a hyphal shell, called the mantle, around plant roots and this forms a physical barrier to pathogens. The antibiotics presumably "protect" noncolonized root areas from infection by pathogens. EM have also been shown to stimulate the production of volatile and nonvolatile compounds, and possibly phytoalexins, that are fungistatic and probably inhibit mycorrhizal development in the host and pathogen establishment in the rhizosphere (Duschene et al., 1987; Malajczuk et al., 1982). EM can affect rhizosphere microorganisms selectively and can stimulate a mycoflora antagonistic to plant-pathogenic organisms.

VAM fungi, however, have never been shown to interact directly with pathogens through antagonism and antibiosis, nor do they have a predatory nature. They do not produce a root mantle or antibiotics and therefore have a different effect on pathogens and disease than EM (Schenck and Kellam, 1978). VAM fungi have been shown to affect root growth, root exudation, nutrient absorption and host physiological reponses to environmental stress. Many of these factors are related to P physiology and nutrition (Safir and Nelson, 1981; Wallace, 1983).

Dehne (1982) concluded that interactions between VAM and root-pathogens are based on modifications of the infection process since very virulent isolates or high

inoculum levels of the pathogen may reduce or even eliminate the benificial effects of VAM.

Successful suppression of a pathogen may not only depend on the mode of parasitism and the virulence of the pathogen, but also on the particular potential of the VAM fungus to induce resistance. Differences in effectiveness have been observed in different VAM fungal species. Bartschi et al. (1981) found that an unidentified, mixed population was considerably more effective in reducing pathogen development than a pure culture of *Glomus mosseae*. The single endophyte species caused only a delay of disease development, whereas the mixed population was able to reduce the damage to *Chamaecyparis lawsoniana* A. Murr. almost completely.

Hypotheses proposed to explain VAM fungal effects on soilborne plant pathogens generally invoke either a physical or physiological basis. With few exceptions, the primary effects of mycorrhizal symbiosis on the host-pathogen relationship have been intimately related to improved P nutrition because substitution of VAM fungi and/or P fertilization produce parallel changes in the mycorrhizal host response. However, several recent interaction studies on sedentary endoparasitic nematodes have provided the most convincing evidence that VAM fungi may affect the host-pathogen relationship by other mechanisms. The clarification on nonnutritional effects of VAM fungi on this group of plant-parasitic nematodes may yield more definitive results because of the obligately biotrophic requirements shared between these two groups of organisms (Smith, 1988).

The interactions between VAM fungi and plant pathogens can be described in two general statements about mechanisms of resistance: 1) Mycorrhizal fungi are able to retard pathogen development in the root system. This influence is restricted to the site of mycorrhizal establishment. 2) Mycorrhizal fungi are able to increase disease incidence systematically, especially in nonmycorrhizal plant parts. Presently, the systemic influence seems to be related to better nutrition, enhanced plant growth, and physiological stimulations in mycorrhizal plants. With increased concentration of assimilates, these

plants can serve as better nutrient sources for plant parasitic organisms thereby leading to an increase in disease incidence according to the rule: what is good for the plant is also good for the pathogen (Dehne, 1982).

3. VAM AND PLANT WATER RELATIONS

There are now several reports that VAM play a role in the water economy of plants and of VAM plants being less sensitive to temporary periods of water stress than nonmycorrhizal plants. This is important agriculturally because in many arid and semi-arid regions, the factor limiting plant production and nutrient uptake is water availability.

Mycorrhizal plants can have a lower resistance to water flow (ie. higher hydraulic conductivity) than nonmycorrhizal plants. Safir et al. (1971) showed an increase of about 60% in hydraulic conductivity of liquid water flow in soybeans when they were infected with the mycorrhizal fungus *Glomus mosseae*. Later, these same authors demonstrated that the differences in hydraulic conductivity between mycorrhizal and nonmycorrhizal soybeans were eliminated after addition of a complete nutrient solution to the soil (Safir et al., 1972). In this experiment, resistance to water transport was about 40% lower than in nonmycorrhizal controls. However, stem plus leaf resistances were similar in mycorrhizal and nonmycorrhizal plants. Thus, differences in resistance of whole plants must be associated with the root system. Root resistance accounts for a major part of the whole plant resistance.

In mycorrhizal plants under low P conditions, higher hydraulic conductivity may be associated with changes in other parameters such as higher leaf water potentials, higher transpiration rates, and lower stomatal resistances, than those measured in nonmycorrhizal plants. However, at high soil P levels, these parameters were similar in infected and uninfected plants (Safir et al., 1972). Furthermore, the addition of the fungitoxicant p-chloronitrobenzene (PCNB), which inhibits mycorrhizal development, 48 hr prior to measurement of resistances did not affect hydraulic conductivity (Safir et al., 1972).

The anatomical characteristics of the mycorrhizal roots suggest several means by which the fungus could lower resistance of root tissues to water transport. The fungus might act as a low resistance pathway through the root cortex, or it could increase the surface area available for water absorption through hyphal growth. Alternatively, the fungus may increase surface area indirectly by stimulating root growth. Root growth, however, does not appear to be involved, since in the low nutrient experiments, the dry mass and volume of mycorrhizal and nonmycorrhizal roots were similar when resistances to water transport were different. The absence of an effect of the PCNB suggests that the additional surface area as well as the pathway to the endodermis provided by the fungus are not involved in lowering the resistance to water transport within the root.

To carry this work further, the water relations of mycorrhizal onions were compared with those of nonmycorrhizal controls grown under low and high soil phosphorus conditions (Nelson and Safir, 1982). Leaf water potentials, transpiration rates and hydraulic conductivity are always higher for the mycorrhizal plants when compared to the nonmycorrhizal non-P plants, while leaf resistances are always lower. Treatment of nonmycorrhizal plants with phosphorus (NM+P) always eliminated the differences between mycorrhizal and nonmycorrhizal plants.

The differences in leaf water potential, transpiration, and leaf resistance are probably caused by the differences in hydraulic conductivity. For a given evaporative demand, a decrease in hydraulic conductivity (as in the NM-P plants) would lead to a lower leaf water potential. A plant can counter this decrease by partial or cyclic closing of the stomates, thus allowing partial (or total) recovery of the leaf water status. This increase in leaf resistance to vapor transfer would then reduce the transpiration rate.

The fact that added P and VAM both affect water relations in the same way suggests that the primary cause of the change is nutritional. Changes in root membrane permeability can be altered by the presence of P and this can have large effects on root resistance to water flow. This may explain the effects of VAM on hydraulic conductivity. These results

also suggest that under conditions of high water and P availability, mycorrhizal infection may not have major effects on plant-water relations.

Hardie and Leyton (1981), in a direct argument against the role of P nutrition in VAM-water relations, indentified the ability of VAM to improve water relations of red clover (*Trifolium pratense* L.) regardless of P status; infected plants were found to extract soil water to lower water potentials compared with noninfected plants over a range of P fertilization. The nonmycorrhizal plants wilted more easily at a higher soil water potential (-0.8 to -1.2 MPa) than mycorrhizal plants (-1.8 to -2.4). Two explanations were proposed: first, larger root systems of mycorrhizal plants explored greater volumes of soil; second, VAM infection resulted in lower leaf water potentials, allowing plants to remain turgid at lower soil water potentials. Increased hormone production of VAM plants, with resultant changes in stomatal physiology, could explain their tolerance to decreased leaf water potentials.

Of particular interest are reports that VAM can improve drought tolerance of plants. Allen et al (1981) showed that resistance to water transport in *Bouteloua gracilis* (H.B.K.) Lag. ex Steud. was decreased as much as 90% in mycorrhizal plants under conditions of water stress. As moisture stress increased, leaf water potentials of mycorrhizal plants dropped more rapidly than those of nonmycorrhizal controls, and throughout, stomatal resistance remained lower (by 50 to 70%) in inoculated plants. The authors postulated that reduced resistance in mycorrhizal plants was due to improved water uptake, increased photosynthesis, or elevated cytokinin levels which stimulated stomatal opening.

Water relations were studied for wheat which was infected with *G. fasciculatum* or *G. mosseae* in wet and dry soils to determine the plant's tolerance of continued drought (Allen and Boosalis, 1983). Conductance was higher in mycorrhizal than in nonmycorrhizal plants in both wet and dry treatments. When nonmycorrhizal wheat plants and plants infected with *G. fasciculatum* were watered to soil saturation and then allowed to continue transpiring while the soil dried, stomata in nonmycorrhizal plants began to close at

leaf water potentials of -1.8 MPa and were closed after 4 days but in mycorrhizal plants they did not begin to close until water potentials of -2.2 to -2.7 MPa were reached and were still transpiring after 6 to 7 days. Some leaves of *G. mosseae* infected wheat plants showed no stomatal response to drought and continued to transpire at leaf water potentials as low as -4.1 MPa. Leaf osmotic adjustment was greatest for *G. fasciculatus* infected wheat plants. Infection by *G. fasciculatum* appeared to increase wheat drought tolerance while infection by *G. mosseae* did not.

In a further study, drought resistance of wheat inoculated with two VAM species was evaluated in plants growing in soil columns (Ellis, et al., 1985). With initial soil water at 0.5 MPa, plants were subjected to low-level water stress throughout the experiments and severe water stress for 24 hours for 1-3 periods before harvesting. After each stress period, one set of plants was watered and grown to maturity without subsequent water stress and a second set of plants was harvested 1 week after stress. Both *G. deserticola* and *G. fasciculatum* plants harvested 7 days after stress at 55 days had greater leaf area and leaf, total plant, and root mass than non-VAM plants. After stress at 55 and 63 days the same was true but after 3 periods of stress (55, 63, and 70 days), differences in above ground biomass between VAM and non-VAM plants were not significant. However, *G. fasciculatum* inoculated plants produced more tillers after stress at 55 days. When grown to maturity, VAM plants which had undergone three periods of stress had twice the biomass and grain yield as non-VAM plants subjected to the same stress. Also, wheat plants inoculated with *G. fasciculatum* consistently had increased root mass and rooting depth.

Busse and Ellis (1985) evaluated the effect of *G. fasciculatum* on soybean drought tolerance in a high P soil. They found that plants harvested immediately after stress (51 days after emergence) had a root-to-shoot ratio reduction of 24% when inoculated with VAM as compared to noninoculated plants. Total seed mass of VAM colonized plants increased 10% as a result of reduced pod abortion. Significant *G. fasciculatum* x drought

stress interactions were found for total seed mass, pod number, seed number, and root-to-shoot ratio, showing that *G. fasciculatum* had a more positive effect on drought-stressed compared with control plants. Enhanced yields may have been the result of improved P nutrition or more extensive soil water extraction.

If there is the possibility of VAM fungi lowering resistance to water flow in roots under low P conditions, then the site of such an effect must be considered. VAM hyphae ramifying into the soil are likely to increase the water absorbing area and may also be able to bypass the dry soil zones surrounding slow growing roots during drought periods. Hyphae may also bypass cortical resistance in colonized roots (Cooper, 1984).

Allen (1982) suggested that the effect of infection on plant water relations was due to increased water uptake by hyphae. While some bulk water movement obviously does occur in hyphae Sanders and Tinker (1973) and Cooper and Tinker (1978) showed evidence that hyphal water movement alone could not account for increased water uptake by mycorrhizal plants.

Reid (1984) showed that an indirect mechanism enabling water uptake by VAM plant roots was to prevent the development of significant gaps between the root and soil, thereby maintaining liquid continuity across the soil-root interface. VAM hyphae have also been shown to bind soil particles into water-stable aggregates (Tisdall and Oades, 1979). Soil aggregation at the root surface may be increased thereby preventing the separation of root from soil during periods of low soil water potential.

VAM fungi have a role in improving the water relations of plants. However, it is not clear whether this effect is a direct result of fungal colonization and subsequently increased water through hyphae, or a secondary response due to improved nutrition on physiological alterations of the host. Since water flow through growing roots involves crossing of membrane barriers, water absorption can be affected by membrane permeability. VAM may alter membrane permeability through either improved P nutrition

or increased production of phytosterols, thereby influencing water transport (Cooper, 1984).

RESULTS OF RESEARCH

1. Interaction between *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita*, *Glomus intraradices* and genome in wheat

ABSTRACT A model system using four wheat (*Triticum aestivum*) cultivars possessing differing numbers of resistance genes was employed in order to determine the impact of *Puccinia graminis tritici* and *Puccinia recondita* and the wheat genome on root colonization by the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. Simultaneous inoculation with a virulent stem and leaf rust race reduced VAM colonization in each of the four wheat cultivars as did simultaneous inoculation with an avirulent stem and leaf rust race. Wheat cultivars challenged with virulent races of each rust pathogen had significantly lower VAM colonization levels when compared to wheat plants inoculated simultaneously with avirulent rust races. When the rust races were inoculated onto the cultivars individually, reduction in VAM colonization also occurred. The plant genome itself did not have an impact on VAM colonization as significant differences were not detected when the pathogens were absent on each of the four cultivars.

Introduction

Vesicular-arbuscular mycorrhizae (VAM) have been shown to increase (Schonbeck and Dehne, 1979; Davis and Menge, 1981; Graham and Menge, 1982; Krishna and Bagyaraj, 1983) and in some instances decrease (Ross, 1972; Davis et al., 1979) the resistance of plants to pathogens. Conversely, pathogen infection may lead to reduced VAM colonization levels. Incorporation of genes which impart resistance to plant

pathogens is essential for sustained crop productivity. However, when selecting plants for disease resistance, plant breeders may not consider the impact of (VAM) in their breeding programs. Under natural field conditions plants may grow in symbiotic association with VA endophytes. However, plants selected for genetic resistance to pathogens may also have an increased resistance to colonization by mycorrhizal fungi. For instance, infection of a plant by a foliar pathogen such as wheat stem and/or leaf rust may induce a generalized resistance response in a wheat cultivar that has been selected for genetic resistance to the pathogens (Heath, 1981). Such a resistance response may restrict the VAM association or exclude it entirely.

A model system to assess the impact of resistance genes on VAM infection was employed using a differential series of wheat (*Triticum aestivum* L.) cultivars, each cultivar carrying specific genes conferring resistance to races of stem (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn.) and leaf rust (*Puccinia recondita* Rob. ex Desm.). These wheat cultivars were "challenged" with both virulent and avirulent races of the two pathogens. VAM colonizaton of these plants was then determined along with that of non-rust-inoculated control plants.

The hypothesis tested were that root colonization by VAM will be inhibited or reduced when cultivars with genetic resistance to fungal pathogens are challenged with avirulent or virulent races of the pathogen and that cultivars possessing increasing numbers of resistance genes to fungal pathogens will have diminishing levels of VAM root colonization even when plants are not challenged with pathogens.

Materials and Methods

The influence of fungal resistance genes on VAM colonization was assessed using wheat cultivars challenged with an appropriate virulent or avirulent race of stem (*Puccinia graminis* Per. f. sp. *tritici* Eriks. and E. Henn) and/or leaf rust (*Puccinia recondita* Rob. ex Desm.). These races were inoculated on the wheat cultivars Little Club, Katepwa,

Neepawa, and Selkirk which possess 0, 5, 7, and 9 stem and leaf rust resistance genes respectively. The virulent and avirulent races of stem and leaf rust used on each cultivar are given in Table 1. The cultivar Little Club has no resistance genes for stem and leaf rust. Therefore all races of the pathogens used in the experiments are virulent on Little Club.

Three separate experiments were established to determine the influence of fungal resistance genes on VAM colonization. In experiments one and two, the wheat cultivars Neepawa and Katepwa respectively, and the universal suscept Little Club, were inoculated with one of five treatments: (a) no rust - NR, (b) an avirulent leaf rust race - ALR, (c) a virulent leaf rust race -VLR, (d) an avirulent stem rust race - ASR, and (e) a virulent stem rust race - VSR. All of the plants were inoculated with *Glomus intraradices*. Both experiments were organized as 2 x 5 factorials (2 cultivars, 5 rust treatments) in a randomized complete block design. Each experiment was repeated twice. Each treatment was replicated five times per experiment. In experiment three, all four wheat cultivars.were inoculated with one of three treatments: (a) no rust - NR, (b) a virulent leaf rust race and a virulent stem rust race - VR, and (c) an avirulent leaf rust race and an avirulent stem rust race - AR. All of the plants were inoculated with *G. intraradices*. Experiment three was organized as a 4 x 3 factorial (4 cultivars, 3 rust treatments) in a randomized complete block design. The experiment was repeated three times. Each treatment was replicated five times per experiment.

All experiments were conducted using an Almissippi very fine sandy loam soil. The soil had a pH of 7.8, and available nutrients were as follows: 2.8 $\mu g\ g^{-1}$ NO_3 - N (Sodium Bicarbonate Extractable), 5.2 $\mu g\ g^{-1}$ available P (Sodium Bicarbonate Extractable), 122 $\mu g\ g^{-1}$ K (Ammonium Acetate Exchangeable), and 1.2 $\mu g\ g^{-1}$ SO_4 (Water Soluble). Prior to use, the soil was sieved through a 1 cm mesh screen and stored in polyethylene bags. The soil was exposed to 0.8 Mrad of gamma irradiation from a Cobalt-60 source for 20 minutes in order to eradicate mycorrhizal propagules. The soil was amended with a fertilizer treatment consisting of K_2SO_4, NH_4NO_3, and $NH_4H_2PO_4$ adjusted to 20 $\mu g\ g^{-1}$

P (see Appendix C). Each pot received 140 ml of the nutrient solution just prior to planting. Nutrients were amended with the same nutrient solution, minus P, after four weeks.

Wheat seeds were surface sterilized in a 0.5 % sodium hypochlorite solution for 10 minutes and pregerminated on moist filter paper for 48 hr. Four wheat seeds were sown in 1 L milk cartons containing 1050 g of soil. Seedlings were thinned to one plant per pot 12 days after planting. At planting, soil and corn roots were added to each pot as layers as follows; 400 gm of soil and 2.5 g of roots; 250 g soil and another 2.5 g of roots; 200 g of soil were then added to each pot. The seeds were then placed on the soil and covered with the final 200 g of soil.

The original source of mycorrhizal inoculum consisted of *Glomus intraradices* infected leek roots obtained from Dr. V. Furlan (Department de Phytologie, Universite Laval, Ste. Foy, Quebec). The *G. intraradices* inoculum used in all experiments was produced on corn grown in steam sterilized, calcined Montmorillonite clay (Turface, IMC Imcore, Mandelein, Illinois, 60060) for approximately three months (see Appendix A). To prevent establishment of microorganisms on corn that may be debilitating to wheat, the corn inoculum was rotated to peas so that the inoculum producing rotation was corn-peas-corn-wheat. All *Glomus* treatments were inoculated with 5 g (wet weight) chopped root inoculum (50-70 spores/gm) per pot at planting. All soils not receiving *G. intraradices* received a similar amount of uninfected corn roots. Microflora associated with VAM roots in pot culture may influence plant growth or nutrient availability in *G. intraradices* inoculated treatments (Williams, 1981). Therefore, to establish the microflora associated with mycorrhizal roots, a water extract from pot cultured mycorrhizal corn root inoculum was prepared by leaching the inoculum (in amounts equivalent to that received by mycorrhizal treatments) with sterile distilled water on a 45 µm screen to exclude mycorrhizal propagules, and 5 mL of the extract were added to each nonmycorrhizal pot.

When the host seedlings were 7-9 days old (2-leaf stage) they were sprayed with

0.025% Polyoxyethylene sorbitan monolaurate (Tween 20), a surface tension depressant. The plants were inoculated by rubbing urediospores of stem and/or leaf rust on the leaves. Following inoculation, the plants were placed into a humidity chamber for 12-24 hr and then moved to growth chambers under high-intensity fluorescent light (16 hr day, 380 $\mu E/(m^2 \cdot sec)$, 20-15°C day/night temperature regime) or into the greenhouse (max. light intensity 1000 $\mu E/(m^2 \cdot sec)$ and approx. 20-25°C day and 15-18°C night temperature regime). Rust infection types were rated 12-14 days after incubation.

After 8 weeks the plants were harvested, dried at 72°C for 3 days, and shoot dry mass was recorded. Roots were washed, heat dried, and weighed. The roots were sectioned into four segments each about 9 cm long. The bottom segment from each root was discarded because considerable root disfiguration occurred as a consequence of the root system coming into contact with the bottom of the pot. The three remaining root segments were then cut into 1-cm pieces. A random sample of these segments was stained with Phillips and Hayman's technique (1970) using Trypan blue instead of Cotton blue.

To determine mycorrhizal colonization, a randomly selected aliquot of stained root segments suspended in lactoglycerin was spread in a Petri dish and viewed under a stereomicroscope at 12 to 15 X magnification. The extent of VAM fungal colonization (either hyphae, vesicles, spores, or arbuscules) was estimated to the nearest 10% and was recorded as a frequency distribution with classes arranged in 10% intervals from 0 to 100%. The total % of the root length colonized was then based on an average of the frequency distribution of 5 samples of 50 root segments each (Bierman and Lindermann, 1980).

Data was analyzed using a General Linear Model procedure (SAS-GLM, SAS Institute, Box 8000, Cary, NC 27511-8000). Significant differences were resolved using Tukey's Studentized Range test at the 5% level.

Results

In experiment one, inoculation with a stem or leaf rust race, either virulent or avirulent, reduced root colonization of Neepawa and Little Club plants by *Glomus intraradices* (Table 2). Significant differences (p=0.05) in root colonization occurred between non-rust-inoculated (NR) Neepawa and Little Club plants and (i) Neepawa and Little Club plants inoculated with a virulent leaf rust race; (ii) Neepawa and Little Club plants inoculated with a virulent stem rust race; and (iii) Neepawa and Little Club plants inoculated with an avirulent stem rust race. Non-rust-inoculated (NR) Little Club plants had higher levels of *G. intraradices* colonization than did roots of non-rust-inoculated (NR) Neepawa plants, but the difference was not significant. There were no significant differences in *G. intraradices* colonization between non-rust-inoculated Neepawa and Little Club plants and plants inoculated with an avirulent leaf rust race nor were there significant differences in *G. intraradices* colonization in plants inoculated with either virulent or avirulent leaf or stem rust races (Table 2).

Rust inoculation also reduced VAM colonization of wheat plants in experiment two (Table 3). Significant differences (p=0.05) in root colonization occurred between non-rust-inoculated Little Club and Katepwa wheat plants and (i) Little Club and Katepwa plants inoculated with an avirulent race of stem rust ; (ii) Little Club and Katepwa plants inoculated with a virulent race of stem rust; and (iii) Little Club and Katepwa plants inoculated with a virulent race of leaf rust. *Glomus intraradices* colonization was also significantly greater in Little Club and Katepwa plants inoculated with an avirulent leaf rust race than Little Club plants inoculated with a virulent stem rust race. Again, non-rust-inoculated (NR) Little Club plants had higher levels of *G. intraradices* colonization than did roots of non-rust-inoculated (NR) Katepwa plants, but the difference was not significant.

In experiment three, the avirulent and virulent races of stem and leaf rust were inoculated simultaneously onto four different wheat cultivars. Non-rust-inoculated Little Club, Katepwa, Neepawa, and Selkirk plants all had significantly greater (p=0.05) levels

of VAM colonization than all plants inoculated with rust (Table 4). Katepwa, Neepawa, and Selkirk plants inoculated simultaneously with an avirulent leaf and stem rust race had significantly higher (p=0.05) percentage VAM colonization levels than plants inoculated simultaneously with a virulent stem and leaf rust race. There were no significant differences in *G. intraradices* colonizationbetween any of the four wheat cultivars tested when the plants were not "challenged" with rust. However, Little Club plants had higher levels of *G. intraradices* colonization than the other cultivars tested. Also, there were no significant differences in *G. intraradices* colonization between Little Club plants inoculated with virulent and avirulent leaf and stem rust races. The cultivar possesses no rust resistance genes and leaf and stem rust races that would otherwise be avirulent on other wheat cultivars are virulent on Little Club. This explains why Little Club plants inoculated with an "avirulent" race of leaf and stem rust have significantly lower *G. intraradices* colonization levels than Katepwa, Neepawa, and Selkirk plants inoculated with an avirulent race of leaf and stem rust.

Discussion

The data reported in Table 2 show that there was no significant difference between VAM colonization of NR Neepawa and Little Club wheat. Also, inoculation with the ALR race did not significantly reduce VAM colonization. However, inoculation with the VLR, VSR, and ASR races significantly reduced VAM root colonization. Similar results were found when Katepwa and Little Club were tested (Table 3). Also, on Katepwa the VSR race reduced VAM colonization significantly when compared to plants inoculated with the ALR race.

The data reported in Table 4 show that there is no significant difference in VAM colonization betweeen any of the NR plants of the four wheat cultivars tested. Inoculation of wheat plants with AR races significantly reduced VAM colonizationwhen compared to NR plants. Inoculation with VR races resulted in the lowest VAM colonization levels, with

VAM colonization levels being significantly lower than plants inoculated with AR races or NR plants.

Selecting cultivars with increasing numbers of resistance genes to stem and leaf rust apparently has no adverse effect on *G. intraradices* colonization if cultivars are not "challenged" with the appropriate virulent or avirulent races of the pathogens. In all three experiments there were no significant differences between VAM colonization levels in non-inoculated (NR) plants of all cultivars tested. However, there does seem to be a trend toward reduced *G. intraradices* colonization as the number of resistance genes increases in the non-inoculated (NR) plants. The cultivar containing the highest number of resistance genes (Selkirk) had the lowest levels of VAM colonization when plants were not inoculated with rust (Table 4). Also, non-inoculated Neepawa plants, which possess 7 rust resistance genes, had 36.8% *G. intraradices* colonization (Table 2) whereas non-inoculated Katepwa plants with 5 rust resistance genes had43.3% *G. intraradices* colonization (Table 3)

The experiments indicate that infection with a virulent pathogen results in diminished VAM root colonization. Cultivars inoculated with virulent races of rust had the lowest VAM colonization levels. Perhaps the stem and leaf rust compete with *G. intraradices* for host photosynthates, thereby reducing VAM colonization. Both rust pathogens and *G. intraradices* are obligate organisms and require a living host to provide carbon compounds essential for growth and reproduction. It has been unequivocally demonstrated that carbon requirements of VAM fungi are supplied by the plant host (Bevege et al., 1975; Ho and Trappe, 1973). Rust fungi have been shown to be strong sinks (Shaw and Samborski, 1957; Doodson et al., 1965; Zaki and Durbin, 1967) for host photosynthates and depletion of photosynthates by the pathogen at an early stage of plant development may be detrimental for the establishment and growth of *G. intraradices.*

There have been numerous reports which indicate that plants can be systemically immunized against fungal infection by restricted prior infection with the fungus (Caruso and Kuc´, 1977; Kuc´ and Richmond, 1977; Gessler and Kuc´, 1982). Immunization was

shown to be effective against both obligate and facultative fungi (Kuz´, 1983). Perhaps such an "immunization" effect occurs when avirulent rust races are inoculated onto wheat leaves for the resistance response to stem and leaf rust on wheat leaves also induces resistance to *G. intraradices* colonization in wheat root systems. In host-pathogen systems where a gene-for-gene relation exists, incompatibility or avirulence is the active process requiring a gene product for resistance produced by the host and/or a gene product for avirulence produced by the pathogen (Ellingboe, 1976; 1981). An incompatible reaction results in early molecular recognition followed by rapid expression of defense responses (Lamb et al., 1989). These defense responses may reduce VAM colonization. Defense responses in plants may be induced in uninfected tissue at a distance from the initial site of microbial attack, associated with establishment and/or subsequent expression of induced systemic resistance (Sequeira, 1983).

Chitinase and ß-1,3-glucanase are enzymes which are present in many higher plants and are both involved in plant defense reactions to potential pathogens (Abeles et al., 1971; Boller et al., 1983; Dixon, 1986). These hydrolases accumulate rapidly following pathogen attack or elicitor treatment. Their substrates, chitin and ß-1,3-glucan, are major constituents of many fungal cell walls (Wessels and Sietsma, 1981) and consequently these enzymes appear to be part of an inducible defense response of higher plants. The ß-1,3-glucanase and chitinase become activited, through the action of specific elicitors, when host cells are lysed during pathogenesis i.e., the pathogen triggers a hypersensitive response of the surrounding host tissue. (Mauch and Staehlin, 1989). Challenge inoculation with avirulent stem and leaf rust races results in a hypersensitive response in a cultivar that has been selected for genetic resistance to specific races of the pathogens. The release of hydrolases such as chitinase and ß-1,3-glucanase during the hypersensitive response may limit *G. intraradices* colonization of wheat roots if the enzymes are transported systemically to the roots. Physiological concentrations of chitinase and ß-1,3-glucanase strongly inhibit growth of pathogenic fungi *in vitro* and these hydrolases have been shown to kill fungi by

lysing their hyphal tips (Mauch et al., 1988). Ultrastructural and cytochemical studies have shown that hyphal walls of *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe are formed by proteins and polysaccharides and probably chitin (Bonfante-Fasolo and Grippiolo, 1982). Conversely, high chitinase activity of mycorrhizal tissue has been implicated in delimiting growth of soil-borne pathogens in the host and the enzyme would then have no affect on VAM formation (Bagyaraj 1984).

Alternatively, perhaps the plant expends some of the energy it would normally use for growth in order to set up a resistance mechanism to the pathogens. If VAM formation is dependent upon host carbon, a decrease in available carbon may explain the reduction of VAM colonization

In conclusion, VAM colonization is not reduced significantly in cultivars possessing resistance genes to leaf and stem rust when the plants were not challenged with the pathogens. Virulent and avirulent combinations of leaf and stem rust races affect VAM colonization. The establishment of a completely parasitic relationship between the pathogens and the host significantly reduced VAM root colonization. The pathogen resistance genes present in the cultivars Selkirk, Neepawa, and Katepwa must have some impact on *G. intraradices* colonization when activated by avirulent races of leaf and stem rust. Resistance gene products (or fungal avirulence gene products) may be responsible for reduced VAM colonization. Further research using VAM fungi and avirulent pathogen races is needed to explain the mechanisms involved. However, lowered *G. intraradices* root colonization levels due to host plant resistance mechanisms or pathogen virulence may not be important agronomically since VAM root colonization is not restricted to very low levels or excluded entirely.

TABLE 1. Virulent and avirulent leaf and stem rust races inoculated onto four wheat cultivars possessing increasing numbers of resistance genes.

CULTIVAR	RESISTANCE GENES	LEAF RUST		STEM RUST	
		Avirulent Race	Virulent Race	Avirulent Race	Virulent Race
Little Club	0	C53*	C63	1*	15
Katepwa	5	C17	C63	98x96	123
Neepawa	7	C53	C63	98x96	30
Selkirk	9	C53	C63	1	15

* Avirulent races of leaf and stem rust are also virulent on Little Club as the cultivar possesses no resistance genes for the pathogens.

TABLE 2. Percentage VAM root colonization of Neepawa and Little Club wheat inoculated with virulent and avirulent stem or leaf rust races.

	CULTIVAR	
RUST RACE	LITTLE CLUB	NEEPAWA
NR	37.4b	36.8b
ALR	32.0ab	31.3ab
ASR	29.6a	29.1a
VLR	30.3a	30.3a
VSR	26.8a	25.4a
GENES	0	7

Values in rows and columns followed by the same letter are not significantly different (P=0.05).

NR = no rust, ASR = avirulent stem rust race, ALR = avirulent leaf rust race,

VSR = virulent stem rust race, VLR = virulent leaf rust race.

Values are means of two experiments. Each treatment was replicated five times per experiment.

TABLE 3. Percentage VAM root colonization of Katepwa and Little Club wheat inoculated with virulent and avirulent stem or leaf rust races.

	CULTIVAR	
RUST RACE	LITTLE CLUB	KATEPWA
NR	43.1c	43.3c
ALR	38.7bc	39.0bc
ASR	38.2ab	38.3ab
VLR	37.0ab	37.9ab
VSR	35.6a	35.3a
GENES	0	5

Values in rows and columns followed by the same letter are not significantly different (P=0.05).

NR = no rust, ASR = avirulent stem rust race, ALR = avirulent leaf rust race,

VSR = virulent stem rust race, VLR = virulent leaf rust race.

Values are means of two experiments. Each treatment was replicated five times per experiment.

TABLE 4. Percentage VAM root colonization of four wheat cultivars inoculated with virulent and avirulent stem and leaf rust races.

	CULTIVAR			
RUST RACE	LITTLE CLUB	KATEPWA	NEEPAWA	SELKIRK
NR	56.0a	53.8a	51.7a	50.7a
AR	29.9c	38.7b	38.6b	37.7b
VR	27.7c	30.8c	29.5c	30.9c
GENES	0	5	7	9

Values in rows and columns followed by the same letter are not significantly different (P=0.05).

NR = no rust, AR = avirulent race of leaf and stem rust , VR = virulent race of leaf and stem rust.

Values are means of three experiments. Each treatment was replicated five times per experiment.

2. The Effect of *Glomus intraradices* colonization on *Puccinia graminis* f. sp. *tritici* disease intensity in a hard red spring wheat.

ABSTRACT The effect of vesicular-arbuscular mycorrhizal (VAM) colonization on disease severity of wheat stem rust was studied. *Glomus intraradices* significantly increased the disease severity of *Puccinia graminis tritici* in wheat (*Triticum aestivum*) plants. The influence of *G. intraradices* and *P. graminis* f. sp. *tritici* on Columbus wheat growth and yield was determined. Plants inoculated with the VA endophyte alone produced the greatest amount of plant biomass and were the highest yielding. Inoculation with stem rust alone resulted in reduced yields and plant dry matter production. Plants inoculated with both organisms showed the lowest yields.

Introduction

Vesicular-arbuscular mycorrhizae (VAM) have been reported to increase the general resistance of plants to pathogens (Schenck, 1981) This is especially true for root pathogens (Davis et al., 1978; Rosendahl, 1985; Caron et al., 1985). Little is known about the consequence of VAM colonization on disease incidence caused by foliar pathogens but, VAM colonization has been reported to increase disease severity of foliar pathogens (Schonbeck and Dehne, 1979). VAM colonization resulted in increased disease severity of *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur [anamorph: *Bipolaris sorokinia* (Sacc) Shoem., syn. *Helminthosporium sativum* P.K. & B.] and *Erysiphe graminis* DC. ex Merat in barley (*Hordeum vulgare* L.); of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. & Cav. and *Uromyces phaseoli* (Rebent.) Wint. in kidney beans (*Phaseolus vulgaris* L.); of *Erysiphe cichoracearum* DC. ex Merat in cucumber (*Cucumis sativus* L.); and of *Botrytis cinerea* Pers. ex Fr. in lettuce (*Lactuca sativa* L.), when compared with

nonmycorrhizal plants. Dehne (1982) concluded that the protective effects brought about by VAM formation are localized in the roots of the plant while the shoots of VAM plants were no more resistant to aerial pathogens than those of nonmycorrhizal plants. No attempt was made to quantify the disease nor was there any assessment of the interaction between VAM endophyte and foliar pathogen and its impact on plant growth.

In this study, we have determined the effect of VAM colonization on disease severity of wheat stem rust as well as the influence of the VAM-pathogen interaction on wheat (*Triticum aestivum* L.) growth and yield.

Materials and Methods

The effect of VAM colonization on rust infection intensity was determined on adult Columbus wheat plants inoculated at the flag leaf stage. Plants were grown and inoculated with *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. and *Glomus intraradices* Schenck & Smith as described previously (Section 1). The experiment was a 2 x 2 factorial combination with treatments as follows: (a) *G. intraradices* plus stem rust (+G+R) (b) *G. intraradices* alone (+G-R) (c) stem rust alone (-G+R) and (d) control (-G-R). Each treatment was replicated 8 times in a randomized complete block. The experiment was repeated three times.

Twenty days after inoculation, per cent leaf infection by rust pustules was determined by computer image analysis (Lamari and Bernier, 1987) using two leaves from each plant. At maturity, the plants were harvested and dry weights were recorded. In addition, grain yield and yield components were determined.

Arcsin transformation for percentage rust infection and analysis of variance were conducted on each experiment . Data were analyzed using a General Linear Model procedure (SAS-GLM, SAS Institute, Box 8000, Cary, NC 27511-8000). Significant differences were resolved using Tukey's Studentized Range test at the 5% level. Results of the three experiments were combined for statistical analysis.

Results and Discussion

Wheat plants inoculated with *G. intraradices* had 39.5% of the leaf area infected with stem rust pustules while nonmycorrhizal wheat plants had 16.7% leaf area infection (Table 5). The analysis of variance for rust infection (Table 6) indicates a significant effect due to the presence of *G. intraradices* as well as stem rust. The presence of a significant interaction between the two organisms reflects the significant increase in stem rust disease severity due to prior colonization with *G. intraradices*. VAM colonization results in increased rust infection thereby indicating that the symbiotic mycorrhizal relationship affects the entire plant. The increased rust infection in VAM plants may be due to increased plant nutrition or hormonal alterations in the plant. These results are in agreement with those of Schonbeck and Dehne (1979) who found increased disease severity of several obligate and facultative foliar pathogens on mycorrhizal plants.

Mycorrhizae can absorb several times more phosphate than uncolonized roots from soils resulting in dramatic increases in foliar phosphate concentrations and host plant growth (Gerdemann, 1968; Mosse, 1973a). Johnson et al. (1966) reported that ^{32}P accumulates at rust infection sites. MacDonald and Strobel (1970) reported that the changes in starch synthesis in infected wheat leaves could be explained by changes in the concentration of orthophosphate in the host/parasite complex. Starch synthesis is very rapid immediately prior to sporulation of the fungus and is dependent upon phosphate concentration. Increased foliar P supply provided by *G. intraradices* root colonization may account for the significant increase in *P. graminis tritici* infection on Columbus wheat leaves. In any case, the increase in rust infection due to VAM formation seems to follow the rule for obligate parasites - "what is good for the plant is also good for the pathogen."

Inoculation with both organisms also influenced wheat biomass production (Table 5). Root weight was significantly affected by *G. intraradices* colonization ($p=0.01$) and stem rust infection ($p=0.05$) (Table 6). The root weight of all VAM plants, whether non-inoculated (+G-R) or inoculated with stem rust (+G+R) were significantly greater than the

root weights of all nonmycorrhizal plants (Table 5). There were no significant differences in root growth between plants inoculated with *G. intraradices* alone (+G-R) and dual-inoculated (+G+R) plants. *Glomus intraradices* colonization also overcame any affects that rust inoculation may have on root growth.

There were no significant differences between any of the treatments in terms of their shoot weights. There was, however, a general trend for VAM plants to have greater shoot weights than nonmycorrhizal plants. The analysis of variance shows that the root/shoot ratio was significantly affected by *G. intraradices* colonization (Table 6). Plants inoculated with *G. intraradices* (+G-R) alone had the highest root/shoot ratios while plants inoculated with stem rust (-G+R) alone had the lowest root/shoot ratios (Table 5). Plants inoculated with both organisms (+G+R) had higher root/shoot ratios than non-inoculated (-G-R) control plants. Significant differences in root/shoot ratio occurred between (+G-R) plants and (-G-R) plants as well as between (+G-R) plants and (-G+R) plants.

Significant differences for the number of heads/plant occurred between plants inoculated with *G. intraradices* (+G-R) alone and control (-G-R) plants and between (+G-R) plants and plants inoculated with stem rust (-G+R) alone (Table 5). The number of heads/plant were significantly affected by *G. intraradices* colonization ($p=0.01$) and rust infection ($p=0.05$). VAM plants showed an increase in head number/plant over nonmycorrhizal plants.

Root weight, root/shoot ratio, and the number of heads/plant all have a similar response to treatments. Wheat plants inoculated with *G. intraradices* alone (+G-R) produced the greatest biomass. Plants inoculated with only the pathogen made poor growth. Plants inoculated with both *G. intraradices* and stem rust (+G+R) produced more plant dry matter and had more heads/plant than control plants (-G-R). The parameters indicating biomass production of wheat are all parameters of wheat growth determined prior to rust inoculation at the flag leaf stage and are influenced primarily by *G. intraradices* colonization.

The increased severity of stem rust in wheat plants inoculated with *G. intraradices* is expressed in wheat yield components. Plants inoculated with *G. intraradices* alone (+G-R) and non-inoculated control (-G-R) plants produced the highest number of seeds per plant (Table 7). Stem rust inoculation led to a significant (p=0.01) reduction in the number of seeds per plant (Table 8). Plants inoculated with both organisms produced the lowest number of seeds (Table 7). Significant (p=0.05) differences in seed number occurred between plants inoculated with*G. intraradices* (+G-R) alone and plants inoculated with stem rust (-G+R) alone as well as between (+G-R) plants and dual-inoculated (+G+R) plants (Table 7). There were also significant differences between control (-G-R) plants and plants inoculated with stem rust alone (-G+R) in terms of seed weight.

Thousand kernel weights were also significantly (p=0.01) decreased by stem rust infection (Table 8). Significant differences in thousand kernel weight occurred between (+G-R) and (+G+R) plants and between (+G-R) and (-G+R) plants.

The increased severity of stem rust due to *G. intraradices* colonization is also expressed in wheat yield. Wheat yields are expressed as seed weight (Table 7). Again, (+G-R) plants produced the highest seed weight/plant followed by control (-G-R) plants (Table 7). When VAM or nonmycorrhizal wheat plants are inoculated with stem rust the seed weight is significantly (p=0.01) reduced. Plants inoculated with *G. intraradices* and stem rust had lower seed yields than plants inoculated with stem rust alone but the effect was not significant. Significant (p=0.05) differences occurred (a) between plants inoculated with *G. intraradices* (+G-R) alone and dual-inoculated (+G+R) plants; (b) between (+G-R) plants and plants inoculated with stem rust (-G+R) alone; (c) between control (-G-R) plants and dual-inoculated (+G+R) plants, and (d) between (-G-R) plants and plants inoculated with stem rust (-G+R) alone (Table 7).

There were no significant differences between plants inoculated with *G. intraradices* (+G-R) alone and control (-G-R) plants in terms of grain yield and yield components nor were there any differences between dual-inoculated (+G+R) plants and plants inoculated

with stem rust (-G+R) alone. Wheat plants inoculated with *G. intraradices* alone produced the greatest amount of plant dry matter and had the highest yields and yield component values. Perhaps the significantly greater differences in root weight which occurred between plants inoculated with *G. intraradice* and control plants could not be reflected entirely in grain yield and yield components due to the size of the pots. The roots of all treatments were pot-bound in the one litre milk cartons by harvest time.

It is important to note that for dry weight parameters determined before rust inoculation, dual-inoculated (+G+R) plants produced more dry matter than non-inoculated control (-G-R) plants (Table 7). At this stage of the experiment, the plants have only been inoculated with *G. intraradices.* It is also important to note that VAM formation or the resulting symbiosis increases stem rust disease incidence on aerial parts of the plant. Following stem rust inoculation, the dual-inoculated (+G+R) become the lowest yielding and have the lowest thousand kernel weights and seed numbers/plant.

Results obtained from experiments in which mycorrhizal plants are allowed to photosynthesize in the presence of $^{14}CO_2$ provide clear evidence that the carbon requirements of VA mycorrhizal fungi are supplied by the host plant (Bevege et al., 1975; Ho and Trappe, 1973). Competition for host carbon by VAM fungi has been suggested as a cause for growth depression in VAM plants (Buwalda and Goh, 1982; Stribley et al., 1980). Mycorrhizae incorporate greater amounts of carbon derived from photosynthate than nonmycorrhizal roots (Losel and Cooper, 1979). The amount of carbon retained by shoots of mycorrhizal legume plants is only 88-90% of that in shoots of nonmycorrhizal plants (Pang and Paul, 1980; Kucey and Paul, 1981). Under normal growing conditions, mycorrhizal plants may however, compensate for this carbohydrate drain. Increased photosynthetic and CO_2 fixation rates increased CO_2 in mycorrhizal plants by as much as 8-17% (Allen et al., 1981; Kucey and Paul, 1982). VAM also increased chlorophyll concentrations in *Bouteloua gracilis* (H.B.K.) Lag. ex Steud. (Allen et al., 1981) and

bundle sheath chloroplasts were larger and more numerous than those of nonmycorrhizal millet (*Eleusine coracanna* Gaertn.) plants (Krishna et al., 1981).

The rate of photosynthesis in heavily rusted leaves is usually reduced by 1/3 to 2/3 of corresponding uninfected leaves 8-12 days after inoculation (Livne, 1964; Doodson et al., 1965; Mitchell, 1979; Owera et al., 1981). The rate of photosynthesis per unit of chlorophyll declined with wheat stem rust to 70-85% of rates in healthy leaves (Mitchell, 1979). The amount of chlorophyll also declined, suggesting that both factors contribute to loss of photosynthetic capacity. Repiratory rates of rusted wheat leaves infected with wheat stem rust are usually two to three times greater than rates of uninfected leaves (Antonelli and Daly, 1966; Mitchell, 1979; Shaw and Samborski, 1957) and tissues excised from pustules can have rates 10-15 times higher than those of uninfected tissues (Bushnell, 1970; Samborski and Shaw, 1956). Rust infections also have a significant effect on translocation patterns of the host (Shaw and Samborski, 1957). Photosynthate movement to the stem apex from infected primary bean leaves is reduced fivefold, and to the root eightfold (Zaki and Durbin, 1967). Wheat plants infected with *Puccinia striiformis* West. had ^{14}C translocation reduced as much as 99% when compared to healthy leaves (Doodson et al., 1965). Thus the fungus creates an extremely active sink. The net result is that the vegetative and /or reproductive meristems are starved (Doodson et al., 1965; Siddiqui and Manners, 1971).

The experimental results of the present study may be explained by the literature. Perhaps there are two carbon sinks operating such that yield components of plants inoculated with rust will be reduced but the added sink of the mycorrhiza further reduces yields. In the symbiotic relationship the carbon requirements of VAM fungi are supplied by the plant. VAM can compensate for their carbon uptake by enhancing photosynthesis. A higher incidence of stem rust on wheat colonized by *G. intraradices* may be due to increased photosynthesis as a result of VAM formation. However, when the mycorrhizal plants are inoculated with an obligate foliar pathogen such as *P. graminis* f. sp. *tritici* the

extra photosythates produced by the plant are utilized by the pathogen and the plant is then in fact succumbing to two parasites. This could explain the yield reduction which occurred when plants were inoculated with both organisms. When *P. graminis* f. sp. *tritici* is not present, this loss of yield would not occur either because the plant would be gaining benefit from the additional P being transported by the fungus, or because the plant was able to compensate in some way for the loss of carbon. If no compensation occurred, then a carbon loss may represent a serious loss in wheat productivity.

TABLE 5. Effects of VAM and wheat stem rust on percentage stem rust infection and plant dry matter production on Columbus wheat leaves.

TREATMENT	RUST INFECTION (%)	ROOT WEIGHT (g)	SHOOT WEIGHT (g)	ROOT/SHOOT RATIO	HEADS/ PLANT
+G-R	-	2.9	5.4	0.58	6.7
+G+R	39.5	2.5	5.5	0.48	6.0
-G-R	-	2.0	5.1	0.41	5.7
-G+R	16.7	1.8	4.9	0.38	5.3
SE	3.3	0.02	0.06	0.002	0.06
	+G+R -G+R	+G-R -G-R +G-R -G+R +G+R -G-R +G+R -G+R	n.s.d.	+G-R -G-R +G-R -G+R	+G-R -G-R +G-R -G+R

All possible pairwise comparisons were made for each variable using Tukey's Studentized Range (HSD) test. The contrasts significant at the 0.05 level are indicated below each variable.
G = *G. intraradices*, R = *P. graminis* f. sp. *tritici*, + = inoculated with organisms, - = minus organisms.
Values are means of three experiments. Each treatment was replicated eight times per experiment.

TABLE 6 Analysis of variance for % leaf infection and dry matter production of adult myorrhizal wheat plants inoculated with *P. graminis* f. sp. *tritici*.

		MS				
SOURCE	df	Rust Infection	Root Weight	Shoot Weight	Root/Shoot Ratio	Heads/ Plant
Expt. (E)	2	16.3	2.0	0.80	0.15	0.05
VAM (V)	1	2984.2**	11.65**	4.87	0.26	16.96**
Rust (R)	1	18913.7**	2.36*	0.05	0.07	6.81*
V x R	1	3093.3**	0.13	0.39	0.02	0.72
E x V	2	16.7	1.09*	1.10	0.08	0.22
E x R	2	6.8	0.06	0.07	0.00	0.02
E x V x R	2	16.4	0.00	0.09	0.00	3.19
Error	83	82.9	0.32	1.38	0.02	1.37

*,**Significant at P=0.05 and 0.01, respectively.

TABLE 7. Effects of VAM and wheat stem rust on the components of grain yield in Columbus wheat.

TREATMENT	SEED WEIGHT (g)	SEED NUMBER (g)	THOUSAND KERNEL WEIGHT (g)
+G-R	3.8	137.1	27.3
+G+R	2.4	109.2	21.8
-G-R	3.3	132.1	24.7
-G+R	2.5	113.0	22.7
SE	0.04	34.9	0.83
	+G-R +G+R	+G-R +G+R	+G-R +G+R
	+G-R -G+R	+G-R -G+R	+G-R -G+R
	-G-R +G+R	-G-R +G+R	
	-G-R -G+R		

All possible pairwise comparisons were made for each variable using Tukey's Studentized Range (HSD) test. The contrasts significant at the 0.05 level are indicated below each variable.
G = *G. intraradices*, R = *P. graminis* f. sp. *tritici*, + = inoculated with organisms, - = minus organisms.
Values are means of three experiments. Each treatment was replicated eight times per experiment.

TABLE 8. Analysis of variance for components of grain yield of adult myorrhizal wheat plants inoculated with *P. graminis*f. sp. *tritici*

		MS		
SOURCE	df	seed weight	seed number	1000 kernel weight
Expt.(E)	2	0.42	48.14	1.02
VAM (V)	1	0.90	15.78	17.34
Rust (R)	1	28.11**	13136.51**	343.40**
V x R	1	2.17	468.28	74.74
E x V	2	0.06	1.34	0.00
E x R	2	0.36	490.50	5.06
E x V x R	2	0.10	576.32	0.48
Error	83	0.94	852.20	20.79

*,**Significant at P=0.05 and 0.01, respectively.

3. *Glomus intraradices* and *Cochliobolus sativus* interactions in wheat grown under two moisture regimes.

ABSTRACT. Interactions and growth responses in wheat (*Triticum aestivum*) colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus intraradices* and the root pathogen *Cochliobolus sativus* were studied under two moisture regimes. VAM inoculation decreased root rot disease intensity on wheat plants under both moisture regimes. Common root rot decreased VAM colonization under adequately watered and water-stressed conditions. Mycorrhizal wheat plants infected with *C. sativus* had higher root weights than did nonmycorrhizal plants infected with root rot. Water stress significantly decreased shoot dry weights of wheat plants. VAM plants had the highest yields and 1000 kernel weights irregardless of moisture regime. Plants inoculated *C.sativus* alone had the lowest yields. *Glomus intraradices* offset the decreased yields caused by *C. sativus* when both organisms were inoculated simultaneously. These results were coraborated by results obtained from a field study.

Introduction

Vesicular-arbuscular mycorrhizal (VAM) fungi form a symbiotic relationship with their host by colonizing the inter- and intracellular regions of the root cortex. The symbiosis aids plants in acquiring mineral nutrients from soils, especially phosphorus (Tinker, 1984). Mycorrhizae have also been shown to enhance water uptake and transport in plants (Allen, 1982; Nelson and Safir, 1982), and to increase drought tolerance of plants (Sieverding, 1981; Allen and Boosalis, 1983; Levy et al., 1983).

Studies have shown that VAM fungi influence plant disease (Schonbeck and Dehne, 1977; Schenck, 1981). Many of the studies conclude that root colonization by the

fungal endophyte or the associated increase in mineral uptake reduce the severity of root pathogens (Davis and Menge, 1980; Graham and Menge, 1982; Rosendahl, 1985; Caron et al., 1986b). The VAM association has also been shown to predispose plants to or have no effect upon root disease (Ross, 1972; Davis et al., 1979).

Common root rot (CRR) is considered to be the most important root disease of hard red spring wheat on the Prairie provinces (Burrage and Tinline, 1960; Harding, 1972; Huang and Tinline, 1976). In Western Canada the disease is responsible for an average grain yield loss of 5.7% in hard red spring wheat (Ledingham et al., 1973). The disease occurs wherever wheat or barley are grown. CRR is characterized by blighting, stunting, and death of seedlings, stunting of mature plants, formation of necrotic lesions on seminal roots, crown roots, subcrown internodes, and crown and basal stem tissues (Ledingham et al., 1973; Piening et al, 1976). Conidia of the causal agent, *Cochliobolus sativus* (Ito & Kurib.) Dreschsl. ex Dastur, anamorph *Bipolaris sorokinia* (Sacc. in Sorok.) Shoem., syn. *Helminthosporium sativum* (Pamm., King & Bakke), persist in the soil for long periods of time. The fungus is an aggressive pathogen especially when plants are under stress. Water stress, high temperatures, and nutritional stress all predispose plants to the disease (Smith et al.,1962; Sallans, 1965; Piening et al., 1967; Piening et al., 1969; Wiese, 1977).

Infection can arise either from seed-borne or from soil-borne inoculum, but on the Canadian prairies the latter appears to play a major role in both initiation and progression of disease (Verma et al., 1974). Because infection from soil-borne inoculum can attack cereal plants at any time during plant development, attempts to control root rot in the post-seedling to mature stages with protective fungicides, used as seed dressings, have generally not been effective (Hampton, 1978; Wallace and Mills, 1986). Several systemic fungicides, including imazalil [1-(ß-allyloxy-2,4-dichlorophenethyl) imidazole], have been shown to be effective in reducing disease intensity, but yields have not usually been increased (Piening et al., 1983; Verma, 1983). Imazalil as a seed treatment is potentially phytotoxic and may reduce germination, slow emergence, and alter the normal tillering patterns of plants

(Chinn, 1978; Chinn et al., 1980; Verma, 1983; Verma et al., 1981; Verma et al., 1986). Chloride fertilization has also been established as a means of reducing CRR (Goos et al., 1987; Sheffelbine et al., 1986; Timm et al., 1986). Significant disease control effected by chloride has not been shown to increase grain yield. Goos et al. (1989) found that potassium chloride fertilization significantly increased yield in one experimental site, but this yield increase could not be attributed to control of CRR. Crop rotation (Ledingham, 1961), residue management (Ledingham, 1970; Piening et al., 1967; Pittman and Horricks, 1972), seeding depth and date, and seed size (Duzcek and Piening, 1972) all affect CRR severity, but do not provide adequate control of the disease. The development of resistant cultivars is the most practical means of controlling the disease (McKenzie and Atkinson, 1968; Harding, 1972), but most wheat cultivars presently grown are only moderately resistant to CRR (Conner et al., 1989). At present, there are no biological control agents which have been found effective against *C. sativus*.

The objectives of this study were to determine the relationship between the VAM fungus *Glomus intraradices* Schenck & Smith and disease caused by *Cochliobolus sativus* and to determine the effects of VAM and the pathogen on water uptake and yield in wheat (*Triticum aestivum* L.).

Materials and Methods

Eight treatment combinations from three factors (2 x 2 x 2) were planted in a randomized complete block design. The experiment consisted of four biotic treatments and two abiotic treatments, high vs low water. There were eight replications for each treatment viz. (i) control (no inoculation, -G-C) (ii) *Glomus intraradices* alone (+G-C) (iii) *Cochliobolus sativus* alone (-G+C) (iv) *G. intraradices* and *C. sativus* inoculated together (+G+C). The experiment was repeated three times. Data from all three experiments were combined for statistical analysis.

All experiments were conducted using gamma-irradiated Almissippi soil amended with a nutrient solution adjusted to supply 20 $\mu g\ g^{-1}$ phosphorus. Each pot was fertilized, inoculated with *G. intraradices*, and planted as described previously (Section 1). Wheat seeds (cv. Glenlea) were surface sterilized with 0.5% sodium hypochlorite, triple rinsed with sterile distilled water, and pregerminated on moistened filter paper in petri plates for 24-48 hr. Three to four seeds were placed into each 1-L pot and thinned to 1 seedling after 12 days.

Inoculum of *G. intraradices* was obtained from cultures on corn plants grown in Turface (IMC Imcore, Mundelein, Illinois 60060). Five g of chopped mycorrhizal corn roots were added to each pot. Nonmycorrhizal treatments received 5 g of uncolonized corn roots and 5 mL of mycorrhizal root washings passed through a 45 μm sieve.

To produce *C.sativus* conidia, isolates were collected from leaf pieces containing disease lesions and were placed in a humidity chamber for 24-48 hr. Spores were isolated and placed on petri plates containing V8-PDA media (Difco). Petri dishes were incubated for 10-15 d at 20°C, allowing for spore germination and colony growth. Each culture was a single-lesion isolate. One culture with growth typical of *C. sativus* was selected. Hyphal disks were cut out of the media and placed in petri plates containing V8-PDA and incubated for 7-10 days and then placed 30 cm below warm white fluorescent tubes with a 24 hr photoperiod to facilitate sporulation.. Conidia were scraped off the petri dishes, blended at full speed, and filtered through a J-cloth. Spores were sprayed onto the soil using an atomizer, for a final concentration of 1000 conidia/g of soil. The soil was turned periodically to facilitate mixing of the conidia. Pots not receiving conidia were sprayed with an equal volume of distilled water. The soil was allowed to dry before planting.

Plants were watered regularly for 4 weeks to permit plant and VAM establishment. High and low water treatments were then created by daily watering half of the pots to field capacity (high water) while the other half of the pots were watered to 50% field capacity

(low water). The field capacity and permanent wilting point of the Almissippi soil were 20% and 12% by weight, respectively.

Plants were grown in a growth chamber at a day/night ambient air temperature of 20/15°C. In two of the experiments the plants were exposed to 16 hrs daylight and a constant relative humidity of 65%. The photon flux density (400-700 nm) was 380 $\mu E/(m^2 \cdot sec)$. The third experiment was conducted in a growth chamber where the relative humidity was not held constant and the photon flux density was 295 $\mu E/(m^2 \cdot sec)$.

At harvest, plant shoots were separated from their root systems and shoot and head dry weights were obtained following oven drying for 48 hr at 80°C. Numbers of grains per spike, spikes per plant, fertile spikelets per spike, and weight per 1000 kernels were also recorded. After removal of the top growth, the pots containing roots were filled with water and allowed to soak overnight. The roots were then carefully removed from the pots, gently washed under a stream of water to remove soil, dried at 40°C for 72 hr and dry weights were determined. Following this the roots were sectioned and stained as described previously (Section 1). Mycorrhizal colonization was estimated using the method of Biermann and Lindermann (1980).

Plants infected with *C. sativus* were individually scored for disease on the basis of subcrown internode lesioning (Ledingham et al., 1973) and on overall root discoloration. Plants were scored as clean, slight, moderate, and severe using numerical values of 0, 1, 2, and 4, respectively.

In addition to the growth chamber experiments, a field trial was conducted during the summer of 1987, at the University of Manitoba experimental plots at the Winnipeg campus. The trial consisted of 4 treatments: (1) *C. sativus* (2) *G. intraradices* (3) *C. sativus* and *G. intraradices* and (4) control (no VAM or pathogen added). The experimental design was a randomized complete block and each treatment was replicated five times. Each treatment was planted in a 4 m row with a guard row situated 16 cm on either side of

the treatment row. Each guard row of was separated by a distance of 3 m from the next treatment to prevent root contact between treatments.

Treatments inoculated with *C. sativus* received 40 g of *Cochliobolus* infested millet per 4 m row. The pathogen was grown on millet seed which was gamma-irradiated, allowed to imbibe water overnight, and then autoclaved three times in jars. The millet was then inoculated using agar discs of mycelium from fungal colonies growing on PDA (Difco) agar. Plants inoculated with *G. intraradices* received 240 g of corn root inoculum (60-70 spores/g) per 4 m row. The corn root inoculum was produced on plants grown on Turface as described previously (Appendix B). Non-VAM treatments received equivalent amounts of noninfected corn roots. The *G. intraradices* and *C. sativus* inocula were placed into furrows, and lightly covered with soil. Wheat seeds (cv. Glenlea) were placed on top. The furrows were then completely covered. Each 4 m row was sown with 200 Glenlea seeds.

Plots were harvested when the control and VAM inoculated wheat plants reached the firm dough stage (11.3 Feeke's scale) of development. Plants in a two meter length of the center treatment row were harvested. Shoot dry weight, head weight, seed weight, seed number, and 1000 kernel weight were determined. The roots of 4 plants per treatment were removed and rated for common root rot disease intensity as well as extent of VAM colonization as described previously (Section 3).

Prior to statistical analysis, Arcsine transformation was applied to all percentage measurements to ensure normal distribution of these variates. Data were analyzed using a General Linear Model procedure (SAS-GLM, SAS Institute, Box 8000, Cary, NC 27511-8000). Significant differences were resolved using Tukey's Studentized Range test at the 5% level.

Results and Discussion

Control plants not inoculated with *G. intraradices* or with *C. sativus* were free of mycorrhiza and root rot, respectively. Thus data from treatments for controls for *G. intraradices* (i.e. *C. sativus* inoculated alone and absence of both microorganisms) were not included in the analysis of variance of % VAM colonization. Likewise, data from treatments for controls for *C. sativus* (i.e. *G. intraradices* inoculated alone and absence of both microorganisms) were not included in the analysis of variance of root rot disease rating.

Glomus intraradices colonization was significantly (p=0.01) affected by water stress and inoculation with *Cochliobolus sativus* (Table 9). Inoculation with *C. sativus* reduced VAM colonization in plant roots by about the same magnitude in both water regimes (Figure 1). Water stressed plants inoculated with both organisms (L+G+C) had the lowest colonization of roots by VAM. However, VAM plants growing under water stress (L+G-C) had higher colonization levels than adequately watered plants inoculated with both organisms (H+G+C). Lower VAM root colonization in the presence of a root pathogen may be due to early colonization of readily infectable sites by the pathogen (Zambolin and Schenck, 1983). Reductions of mycorrhizal colonization by plant pathogens have been reported in other studies (Menge et al., 1978; Davis et al., 1979). There was also a significant effect due to experiment for VAM colonization and all other parameters measured (see Table 9). This most likely due to the fact that one of the three experiments was conducted in a different growth chamber than the chamber used for the other two experiments.

G. intraradices inoculation significantly (p=0.01) reduced common root rot (CRR) disease intensity (Table 9). Plants inoculated with both organisms had lower disease ratings under both water regimes than plants inoculated with *C. sativus* alone (Figure 2). Water regime significantly (P=0.05) affected CRR disease intensity (Table 9). L-G+C plants had a higher disease rating than H-G+C plants. Water stress has been shown to

predispose plants to attack by *C. sativus*. In these experiments, dual inoculated plants growing in a water-stressed regime had a lower disease intensity rating than plants inoculated with *C. sativus* alone growing under a high water regime. Therefore, *G. intraradices* colonization does offer some protection against CRR disease even under water-stressed conditions.

Caron et al. (1986c) observed that the presence of *G. intraradices* resulted in a decrease in *Fusarium* root rot and in the population of *Fusarium oxysporum* Schlecht. f. sp. *radicis-lycopersici* Jarvis & Shoemaker when *G. intraradices* was inoculated onto tomato (*Lycopersicon esculentum* Mill.) plants 4 weeks before, simultaneously, and 4 weeks after inoculation with *F. oxysporum*. Compensation for dry mass lost due to *F. oxysporum* occurred when the VAM fungus was included in the treatment. Thompson and Wildermuth (1989) found an inverse relation between infection of root segments with *C. sativus* and percent VAM root colonization. In this experiment, plants with reduced VAM colonization also had higher disease intensity ratings. The cause and effect of this relationship may be difficult to determine. The interaction between *C. sativus* and VAM colonization appears to be a direct one within the roots rather than mediated via indirect effects of VAM colonization on host nutrition or physiology (Thompson and Wildermuth, 1989). Dehne (1982) indicated that, under the influence of VAM fungi, root tissues may become less suitable for or more resistant to the growth of some pathogens.

Water stress significantly ($p=0.01$) reduced shoot dry weights of wheat plants (Table 9). Under a high water regime, the presence of *G. intraradices* increased shoot weights when compared to nonmycorrhizal plants but the effect was not significant. Mean shoot weights of both water-stressed and unstressed mycorrhizal (+G-C) plants were slightly higher than those of control (-G-C) plants (Figure 3). Dual-inoculated plants produced the same shoot dry weights as control plants growing under the same water regime. When plants were water-stressed, L-G+C plants had shoot weights as high as those of L+G-C plants. This is probably due to the fact that L-G+C plants were so much

later in development when compared to plants from any other treatment. Many of these plants were still producing green shoot growth and were just beginning to produce heads at harvest.

These data are in agreement with those of Nelson and Safir (1982) and Busse and Ellis (1985) who found that shoot weight of onion (*Allium cepa* L.) and soybean (*Glycine max* L.) increased with VAM formation. Yocom et al. (1987) found that plants inoculated with *Glomus deserticola* Trappe, Bloss & Menge produced higher shoot dry weights when water stressed than nonmycorrhizal plants grown under high moisture conditions. However, this was not the case in our experiments.

Root dry weight was significantly ($p=0.01$) affected by all three factors - water regime, VAM, and CRR (Table 9). Non-water-stressed plants for each combination of *G. intraradices* and *C. sativus* treatments had higher root weights than did their respective water-stressed counterparts (Figure 3). Inoculation with *G. intraradices* increased root weight over non-inoculated control plants while inoculation with *C. sativus* decreased root weight. Plants inoculated with both organisms had higher root weights than did plants inoculated with *C. sativus* alone. Plants inoculated with *G. intraradices* alone had the highest root weights. It is interesting to note that the L+G-C plants had higher root weights than all plants growing under non-stressed conditions with the exception of H+G-C plants. Plants inoculated with *C. sativus* alone had the lowest root weights. There was a significant ($p=0.05$) interaction between water and *Cochliobolus* indicating that the pathogen reduced root weight more severely under conditions of water stress.

The root/shoot ratio was also affected by all three factors in a similar manner (Table 9). With the exception of mycorrhizal (+G-C) plants, the drought stressed plants had higher root/shoot ratios (Figure 4). Mycorrhizal wheat plants also had the highest root/shoot ratios. This is a reflection of the increase in root weight and decrease in shoot weight of mycorrhizal plants as compared to nonmycorrhizal plants. The presence of

Cochliobolus also reduced the root/shoot ratio regardless of moisture conditions or VAM colonization.

Plant water use was significantly (p=0.01) affected by all three factors (Table 9). Under both water regimes, inoculation with *C. sativus* alone resulted in greater water use by wheat plants than plants which were inoculated with both *C. sativus* and *G. intraradices*. Control plants used the most water for growth, -G-C plants used more water for growth than +G+C plants (Figure 5). Plants inoculated with *G. intraradices* alone (+G-C) also used more water under each respective water regime than dual-inoculated plants (+G+C) or plants inoculated with *C. sativus* alone. They also produced higher yields, 1000 kernel weights, and plant dry matter and this reflects on their greater water use. In some instances root rot damage was so severe in plants inoculated with *C. sativus* alone that very few plants produced heads. Under high moisture conditions the effect of *Cochliobolus* was not as severe.

Cochliobolus infection significantly (p=0.01) reduced 1000 kernel weights (Table 9). Non-inoculated control plants and plants inoculated with *G. intraradices* alone, growing under both water regimes, had 1000 kernel weights of approximately 40 g while dual-inoculated plants and plants inoculated with *C. sativus* alone had 1000 kernel weights of approximately 31 g (Figure 6). Dual inoculated plants showed greater dry matter production than plants inoculated with *C. sativus* alone but this effect was not evident in 1000 kernel weight.

Plant grain yield corresponds to the seed weight/plant. Grain yield was significantly (p=0.01) affected by water regime and *C. sativus* infection (Table 9). *Cochliobolus sativus* infection resulted in significantly lower yields at both water regimes but yields were reduced to a much greater by *C. sativus* when plants were water-stressed (Figure 7). Plants inoculated with *C. sativus* alone had the lowest yields. Under nonstressed conditions, *G. intraradices* inoculation offset the decreased yields caused by *C. sativus* when both organisms were inoculated at the same time but this effect disappeared

when plants were subjected to water stress. Plants inoculated with *G. intraradices* alone had the highest yields and 1000 kernel weights regardless of water regime .

Ellis et al. (1985) found that water stressed wheat plants infected with two VAM species had greater leaf area and leaf, root, and total plant weight than non-VAM plants. When grown to maturity, water-stressed VAM plants had twice the biomass and grain yield as non-VAM plants subjected to the same stress but such large differences were not evident in our experiments.

In these pot experiments, plants inoculate with *G. intraradices* alone had the highest yields, 1000 kernel weights, root weights, and shoot weights. When plants were inoculated with *C. sativus* alone 1000 kernel weight, yield, and plant dry matter production decreased when compared to other treatments. Inoculation with *G. intraradices* also reduced *C. sativus* intensity. Plants inoculated with both organisms had reduced CRR disease intensity and used less water for growth than plants inoculated with *C. sativus* alone. The presence of *C. sativus* reduced root colonization by *G. intraradices*. Water stress significantly increased CRR disease intensity, reduced *G. intraradices* colonization, and decreased root weight, shoot weight, and grain yield.

In the field trials, *G. intraradices* significantly ($p=0.01$) reduced CRR disease intensity (Table 11). Subcrown internodes of plants inoculated with both organisms were either clean or had moderate lesions present while subcrown internodes of plants inoculated with *C. sativus* alone showed severe lesion development. These results are in agreement with those obtained from our pot trials. Dehne (1987) also found a positive influence of VAM on the resistance and/or tolerance of barley (*Hordeum vulgare* L.) plants under field conditions. Inoculation with *G. etunicatum* Beck. & Gerd. markedly reduced root necrosis and grain yield loss in barley caused by *C. sativus* in both field and pot trials. Unlike our pot trials however, CRR had no affect on VAM colonization on wheat plants grown in the field (Table 11). Colonization levels between plants inoculated with both organisms

(+G+C) and those inoculated with only *G. intraradices* (+G-C) were not significantly different (Table 10).

Plant dry weight was significantly influenced by both *G. intraradices* and *C. sativus* inoculation (Table 11). *Cochliobolus sativus* led to a significant reduction of plant dry matter from 60 g/plot to 20 g/plot when *G. intraradices* was not present (Table 10). Non-inoculated control plants and plants inoculated with *G. intraradices* alone produced the same amount of dry matter.

The significant reduction of CRR disease intensity by *G. intraradices* is reflected in grain yield and yield components. Head weight, seed number, 1000-kernel weight and grain yield were all significantly affected (p=0.01) by *G. intraradices* and *C. sativus* (Table 11). There were no significant differences in the number of seeds per plot between control plants (-G-C) and those plants inoculated with *G. intraradices* (+G-C) alone (Table 10). Plants inoculated with *G. intraradices* (+G-C) and control plants had significantly (p=0.01) greater seed numbers than plants inoculated with both organisms or *C. sativus* alone. Plants inoculated with *G. intraradices* alone had significantly (p=0.01) greater 1000-kernel weights than all other treatments. Plants inoculated with both organisms and noninoculated controls were not significantly different in 1000-kernel weights (Table 10). Significant differences (p=0.01) in head weight per plot and grain yield per plot occurred between every treatment (Table 10). Plants inoculated with *G. intraradices* were the highest yielding while plants inoculated with *C. sativus* alone were the lowest yielding. There was a significant (p=0.01) VAM x CRR interaction present (Table 11) for all parameters measured. This indicates that CRR disease expression was decreased when plants were also inoculated with *G. intraradices*. Several researchers have reported increases of yields by cereals that have been field inoculated with VAM species. Khan (1975) found that addition of a VAM fungus increased total yield, number of spikes per plant, and the number of fertile spikelets per spike per plant in wheat. Owusu-Bennoah and Mosse (1979) and Clarke and Mosse (1981) reported similar results with barley.

The effect of CRR on yield components of spring wheat growing under Canadian dryland prairie conditions in the presence of soil-borne *C. sativus* has been determined. Values for the mean number of heads/plant, mean weight/head, mean weight and number of grains/head, and mean 1000-kernel weights decreased with increasing disease intensity (Ledingham et al., 1973; Verma et al., 1976). Our experimental results show similar trends for plants inoculated with *C. sativus* alone.

Inoculation of plants with both *G. intraradices* and *C. sativus* decreased the disease intensity of CRR in both field and pot trials. Our data showed consistent trends with increasing weight per head, number grains per plant, weight of grains per plant, and 1000-kernel weight with decreasing CRR disease intensity due to *G. intraradices* inoculation. Yield appears to be one of the most important factors in assessing efficacy of potentially useful organisms for biological control of plant diseases. These experimental results show that *G. intraradices* can increase wheat yields in the prescence or abscence of *C. sativus*.

To successfully control a soil-borne disease like common root rot of cereals, one must provide continued protection against ever-present soil-borne inoculum throughout the entire life of the plant. Our experimental results show a significant reduction in common root rot intensity in VAM plants at the mature stage of plant development and this indicates that *G. intraradices* may have long term effectiveness in reducing CRR disease intensity.

FIGURE 1. The effect of common root rot and water stress on Glomus intraradices root colonization.

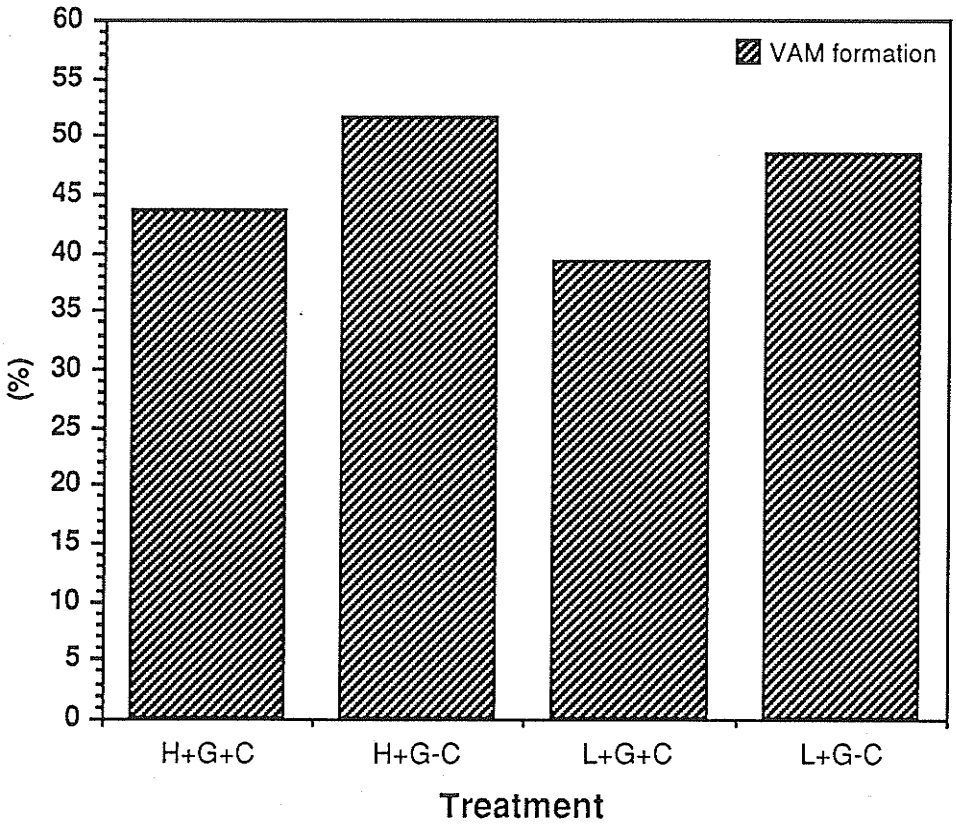

FIGURE 2. The effect of VAM and water stress on common root rot disease intensity.

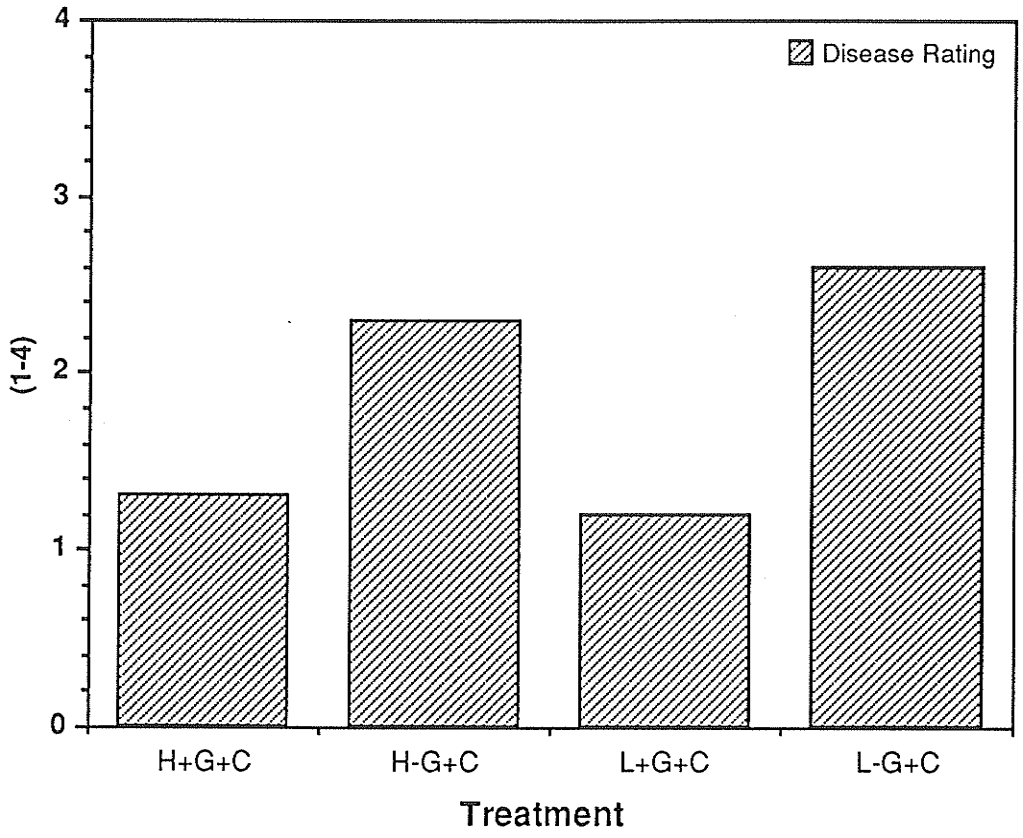

FIGURE 3. The effect of VAM, root rot, and water stress on wheat shoot and root weight.

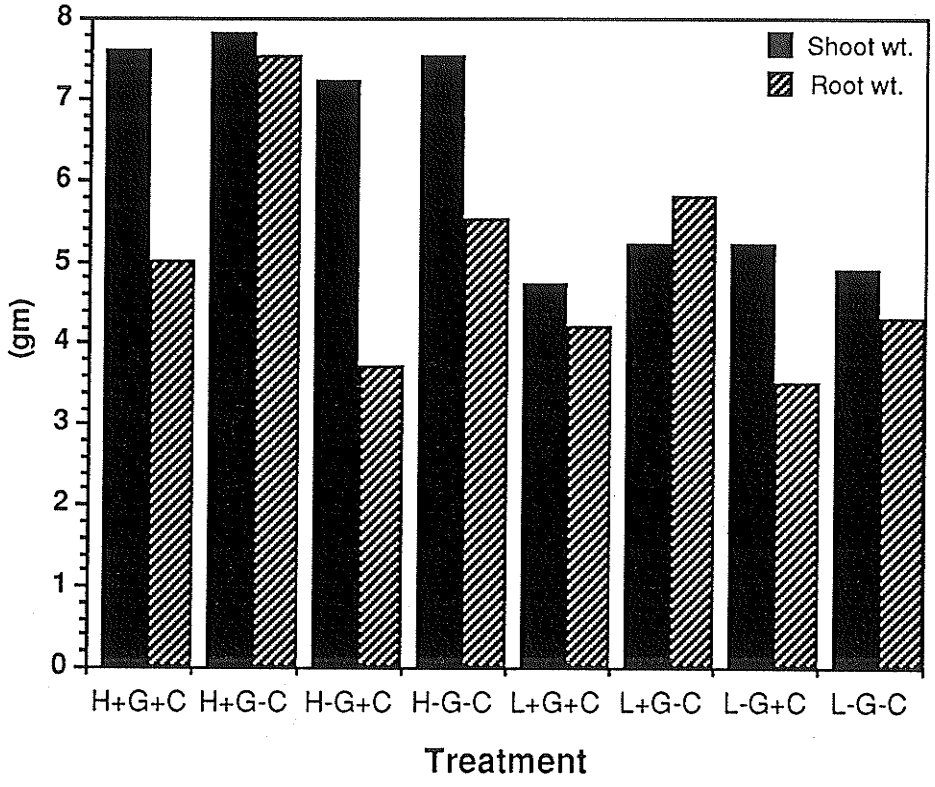

FIGURE 4. The effect of VAM, root rot, and water stress on root/shoot ratio of Glenlea wheat.

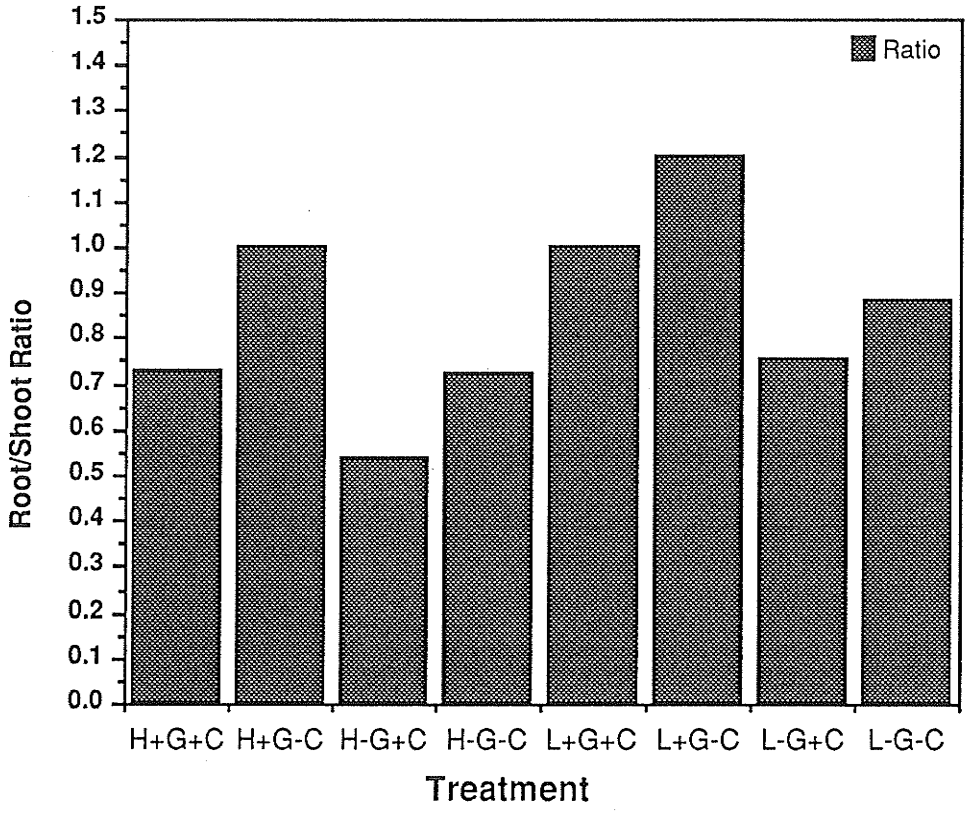

FIGURE 5. The effect of VAM and root rot on water use of Glenlea wheat plants.

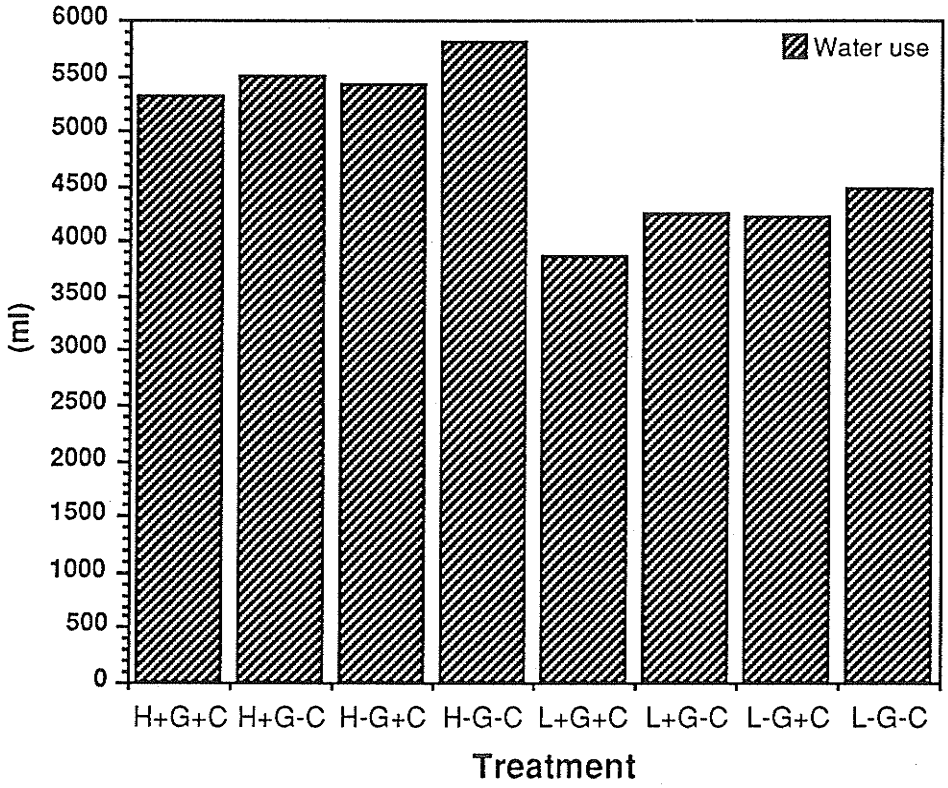

FIGURE 6. The effect of VAM, root rot, and water stress on thousand kernel weight of Glenlea wheat.

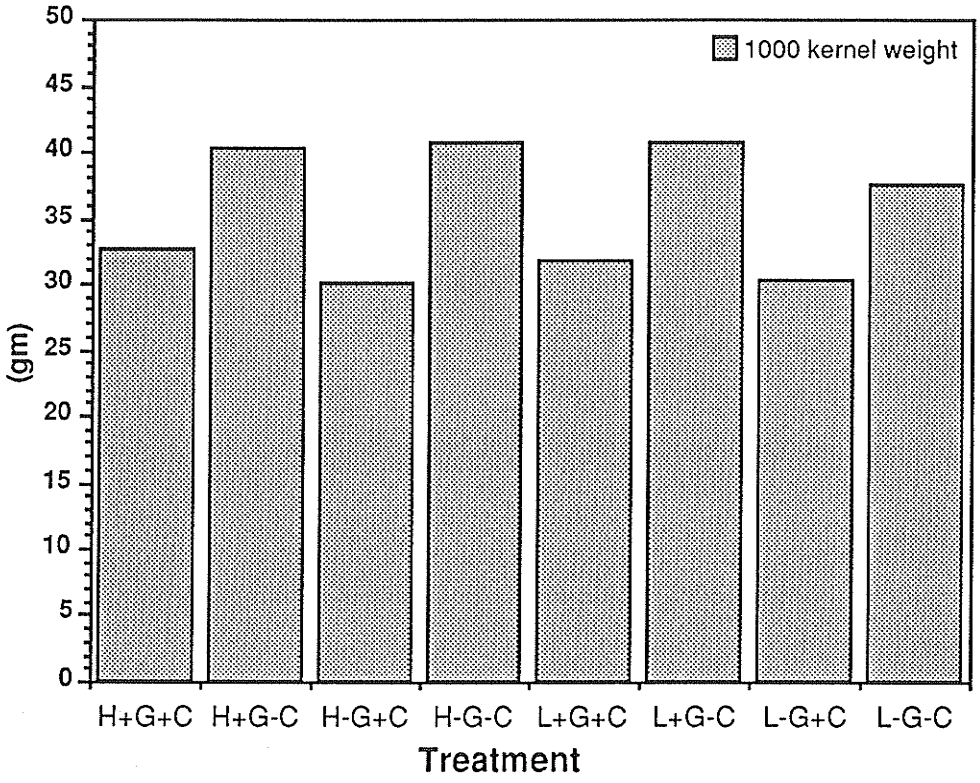

FIGURE 7. The effect of VAM, root rot, and water stress on grain yield of Glenlea wheat.

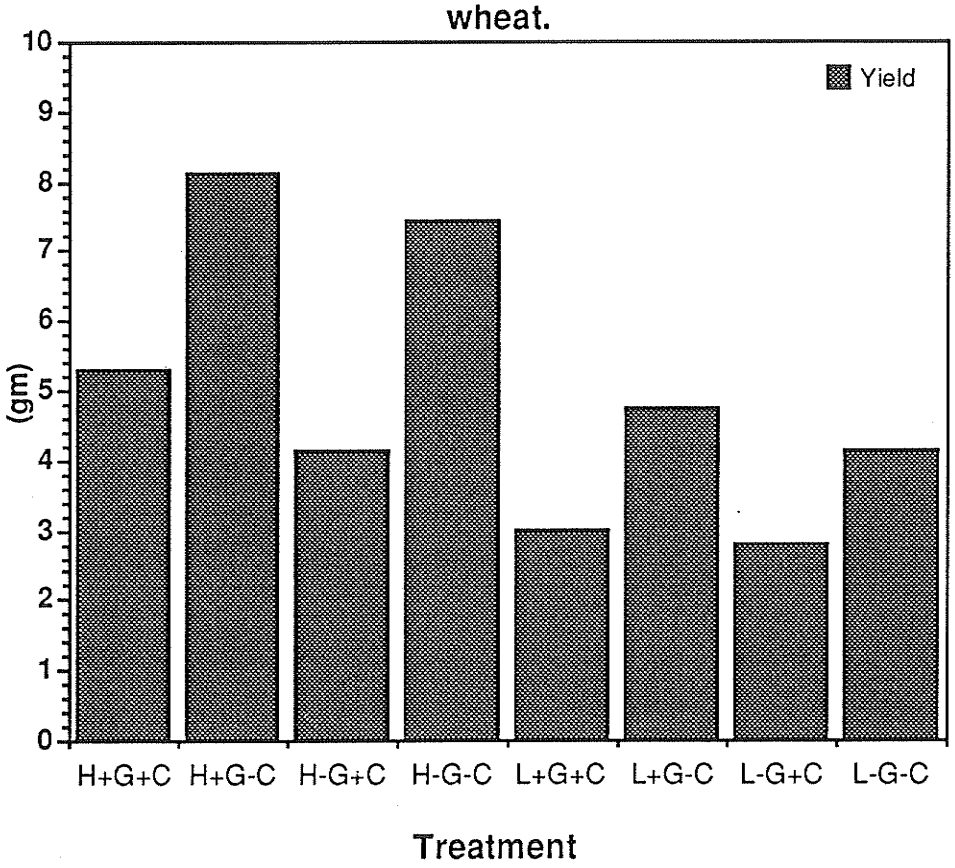

TABLE 9. Analysis of variance for root rot disease intensity, percentage VAM colonization, dry weight, and yield of Glenlea wheat plants grown under two moisture regimes.

		MS								
Source	df	Disease Rating	VAM Colonization	df	Shoot Weight	Root Weight	Root/Shoot Ratio	Plant Water Use	1000 Kernel Weight	Grain Yield
Expt.	2	53.3**	312.5**	2	1025.0**	500.3**	1.88**	2202361231**	11661.0**	2.86
Water (W)	1	6.5*	348.1**	1	310.4**	46.4**	1.89**	81691052**	34.5	288.61**
VAM (G)	1	15.8**	-	1	0.62	90.8**	47.80**	2871188**	138.6	16.57
Root rot (C)	1	-	1773.3**	1	2.0	134.7**	1.74**	4422389**	3572.8**	264.14**
W x G	1	0.51	-	1	1.9	3.6	0.01	84894	15.2	5.33
W x C	1	-	5.8	1	0.4	10.6*	0.09	31199	10.5	23.80*
G xC	0	-	-	1	1.7	6.9	0.06	6105	5.8	0.32
W x G x C	0	-	-	1	1.7	0.0	0.02	363616	65.9	1.24
Error	90	142.7	2504.8	182	320.2	433.7	12.1	64469504	13129.3	789.40

*,** Significant at p=0.05 and 0.01, respectively.

TABLE 10. Effect of *G. intraradices* and *C. sativus* on root rot disease intensity, VAM colonization, growth, and yield of field grown wheat.

TREATMENT	DISEASE INTENSITY (1-4)	VAM COLONIZATION (%)	HEAD WEIGHT (g/plot)	GRAIN YIELD (g/plot)	PLANT WEIGHT (g/plot)	SEED NUMBER (/plot)	1000 KERNEL WEIGHT (g/plot)
+G-C	-	41.8	73.2	54.9	96.0	1467.0	37.4
-G-C	-	-	61.3	44.7	96.4	1443.2	30.8
+G+C	1.0	34.2	51.9	38.2	60.5	1171.6	32.6
-G+C	4.0	-	16.1	11.0	20.3	412.5	26.5
SE	0.04	3.0	0.77	0.57	0.89	23.3	0.44
		+G-C +G+C			+G-C -G-C	+G-C -G-C	+G+C -G-C

All possible pairwise comparisons for treatments were made using Tukey's Studentized Range (HSD) test.
The contrasts listed below each variable are <u>not</u> significantly different. All other pairwise comparisons are significant at the 0.05 level.
G = *G. intraradices*
C = *C. sativus*
Values given for biomass and yield are means of all plants harvested in a two meter row per plot, replicated five times.
Values given for disease intensity and VAM colonization are means of four plants per plot, replicated five times.

TABLE 11. Analysis of variance for root rot intensity, VAM colonization, plant weight and yield components of field grown wheat inoculated with *G. intraradices* and *C. sativus*.

		MS						
SOURCE	df	Disease Intensity	VAM Colonization	df	Grain Yield	Seed Weight	Seed Number	Plant Weight	1000 Kernel Weight
Rep	4	0.02	57	5	246.5**	140.8**	96552.7**	526.5**	7.7
VAM (G)	1	19.5**	-	1	1886.3**	1184.8**	415872.8**	752.8**	176.4**
Root rot (C)	1	-	144.4	1	5346.0**	3063.0**	2105629.9**	15051.1**	100.37**
G x C	1	-	-	1	749.8**	354.7**	608388.0**	1790.0**	0.0
Error	3	0.03	226.4	11	14.5	8.1	7886.3	19.8	4.6

*,** Significant at P=0.05 and 0.01, respectively.

4. The development of vesicular-arbuscular mycorrhizae in spring- and winter-sown wheat.

ABSTRACT A study was conducted to determine the natural infection levels of vesicular-arbuscular mycorrhizae (VAM) in spring- and winter-sown wheat (*Triticum aestivum*) on the Manitoba prairie. Additionally, host cultivar and soil type were studied to determine their effect on VAM colonization. Cultivar and soil type had no effect on VAM infection levels in spring wheat. VAM infection levels increased as the growing season and plant growth progressed. However, infection levels remained relatively low. There were no differences in VAM colonization levels between winter wheat plants grown at two locations. VAM colonization levels remained low on winter wheat plants harvested in August.

Introduction

The ecophysiology and infection kinetics of vesicular-arbuscular mycorrhizal (VAM) associations has been extensively studied in recent years. Jensen and Jakobsen (1980) studied the occurrence of VAM formation in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) grown in different Danish soils under different fertilizer regimes. VAM colonization was found at all locations. An inverse relationship occurred between soil P levels and intensity of infection such that VAM infection was greatest at the three locations with low soil P levels and lowest at the two locations with elevated soil P levels. Infection levels also decreased with increasing levels of nitrogen fertilizer. When barley plants were inoculated with *G. caledonicum* (Nicol. & Gerd.) Trappe & Gerd. in the field, mycorrhizal infection was first noted in barley plants 25 days after seedling and reached infection plateaus of approximately 50%. In uninoculated plots, infection was first observed later and final levels were approximately 12% (Jensen and Jakobsen, 1980).

In another study infection development in spring-sown cereals was very rapid and an infection plateau of about 50% was reached only 15 days after seedling emergence (Jakobsen and Neilsen, 1983). They noted that VAM infection progressed more rapidly and to higher levels of colonization than the slow infection rate and rather low final infection levels reported by Black and Tinker (1979). Buwalda et al. (1985) found that levels of VAM in cereals reached plateaus well before harvest. The infection levels during mycorrhizal development in both fumigated, inoculated plots and non-fumigated non-inoculated plots followed similar time courses. Infection levels in both fumigated, inoculated and non-inoculated plots increased with length of cropping.

Infection levels in winter cereals are somewhat different than in spring cereals. Hayman (1970) reported moderate levels of colonization, sampling from May through September. He found that mycorrhizal colonization in winter wheat was sparse during the summer months and rose to a peak at harvest time in September. *Endogone* spore numbers also increased during the summer after the period of maximum root growth. The wheat roots had become appreciably colonized only after heading when maximum root growth had occurred.

In Denmark, very low levels (<10%) of colonization by VAM in winter cereals were found until mid-April after which there was a gradual increase in infection to levels that approached 50% at harvest three months later (Jakobsen and Nielsen, 1983).

Similarly, Hetrick and Bloom (1983) observed no VAM infection in winter wheat, regardless of soil fertility, until after anthesis in May, although moderate to high levels of infection were maintained in perennial native grasses throughout the year. They identified the occurrence of a wider diversity of VAM fungal species in the prairie than in the cultivated wheat soils and significantly more fungal spores were recovered from undisturbed prairie soils than from four winter wheat field soils. Though variable, 11% - 50% VAM root colonization was evident in all prairie grass roots sampled throughout the

year. In contrast, no identifiable VAM root colonization was evident in wheat until May after flowering when 27% root colonization was evident.

In a follow-up study, Hetrick et al. (1984) found little VAM colonization of hard red winter wheat until flowering in May after the soil had warmed. However, only a small amount of infection developed (<1% to <10%) "just prior to harvest".

Bertheau et al. (1980) suggested that VAM development in a host plant is partially controlled by the plant genome. They found that among 20 wheat cultivars colonized with *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, responses varied from yield increases to yield depressions. Young et al. (1985) detected large differences in external fungal mycelia and internal arbuscles/vesicles in roots of several breeder-plot cultivars. This suggests differences in susceptibility of certain cultivars to colonization by particular VAM fungi as Azcon and Ocampo (1981) reported for 13 Spanish wheat cultivars tested against a *Glomus mosseae* isolate in a 10 week experiment.

VAM have been shown to improve phosphorus uptake and growth in a wide range of plants (Hayman, 1983) and VAM have been found to occur in many field crops under a range of environmental conditions (Mosse et al., 1981), especially when the availibility of soil phosphorus is limited. Jakobsen and Neilsen (1983) found that P uptake in annual crops may be significantly affected only if VAM fungal colonization is well established shortly after seedling emergence. Rapid development of a VAM association may be a major determinant of response in annual crops.

The research reported here was conducted to determine the natural infection levels of spring- and winter-sown wheat crops in Manitoba. The influence of host cultivars and soil types on VAM colonization of spring-sown wheat was also determined.

Materials and Methods

During the first summer, plots at Portage la Prairie and Minto were sampled to determine mycorrhizal infection under field conditions. Six different wheat cultivars were

sampled: Glenlea, Katepwa, Marshall, HY320, Oslo, and Wheaton. The later four are semi-dwarf varieties. All of the plants were seeded at a rate of 400 seeds/m^2 with 150 kg N/ha.

The Minto field trial site soil classification was Ry7 Cs2 Tn1/ c, where Ry = Ryerson series, Cs = Coatstone series, Tn = Tilston series and c indicates an undulating topography (Eilers et al., 1987). Ryerson soils are loam to clay loams and well drained. The Coatstone and Tilston components of this soil drain imperfectly, have problems maintaining tilth and aeration, and hence take longer to warm up and dry out. The Minto site was previously cropped to flax (*Linum usitatissimum* L.) (1985) and winter wheat (1984). The soil received 60 kg/ha P_2O_5 and 80 kg/ha K_2O banded prior to seeding. Prior to fertilization, available soil P was estimated to be 16 $\mu g\ g^{-1}$. The soil composition and nutrient status of this site are presented in Table 12. The wheat cultivars were seeded May 23.

The Portage field trial site soil was classified as a Neuhorst series. Neuhorst soils are imperfectly drained clay loams. Runoff is slow and permeability is moderately slow to slow. The terrain is level to very gently sloping (Michalyna and Smith, 1972). The ratio of sand, silt, and clay was 25:44:31. The soil composition and nutrient status of the Portage site are presented in Table 12. The Portage la Prairie site was previously cropped to mustard (*Brassica hirta* Moench) (1985) and winter wheat (1984). The soil was prepared with a fertilizer base of 50-50-30 NPK. Prior to fertilization, soil P content was determined to be 13 ppm . The wheat cultivars where seeded on May 24.

Samples were taken on June 17, July 9, and August 5. Five replicates were sampled at each site. Five plants were sampled in each replicate for a total of 25 plants of each cultivar per site. The roots and surrounding soil of each plant were collected from the top 20 cm of soil at each sample site. All of the roots and soil from each of the 6 cultivars sampled at each location were bulked and the roots of 12 plants of each cultivar from each location were chosen at random. The roots were then washed, stained using Phillips and

Hayman's method (1970), and examined for % infection using the grid-line intersect method (see Appendix A).

During the second summer, root and soil samples were collected from fields cropped with winter wheat at the University of Manitoba experimental farm sites on the Winnipeg campus and at Portage la Prairie. Sites were selected on the basis of monocultered wheat (>3 years) and liberal fertilization.

Both sites were sampled in the beginning of August immediately prior to the winter wheat harvest. Twenty plant samples were taken from the top 10 cm of soil at approximately 10 m intervals along a transect across the field. The samples were bulked and 10 subsamples consisting of plants with seminal and nodal roots attached were obtained by washing them free of soil. The roots were then stained as above and examined for mycorrhizal infection using the method described by Biermann and Lindermann (1981). Data for each year were analyzed using a General Linear Model procedure (SAS-GLM, SAS Institute, Box 8000, Cary, NC 27511-8000).

Results and Discussion

The spring wheat cultivars were sampled at two locations at three different times (Table 13). Significant differences occurred in root colonization levels for each sampling date (Table 13). The most important factor influencing VAM colonization was harvest date (Table 14). As the growing season and plant growth progressed, colonization levels increased. These results are in agreement with those of Buwalda et al. (1985). VAM infection levels in wheat roots remained relatively low (<20%) throughout most of the growing season. Young et al. (1985) sampled breeder and commercial wheat cultivars just prior to harvest and found that root cortex colonizationswere variable and relatively low (range <2 to >18%). Black and Tinker (1979) concluded that such low and slow infection by indigenous endophytes in the field was of no benefit for cereal growth.

In our experiments differences in colonization levels between cultivars occurred but only at the last sampling date. Azcon and Ocampo (1981) reported differences in susceptibility of certain wheat cultivars to colonization by VAM fungi and Bertheau et. al. (1980) also suggested that VAM development in host plants is partially controlled by the plant genome. However, in our field samples, wheat genome or cultivar did not influence VAM colonization significantly (Table 14). The four semi-dwarf varieties showed higher colonization levels when grown at Minto than did the two hard red spring cultivars at the last harvest.

Bethlenfalvay et al. (1985) found that VAM-fungal biomass in sorghum (*Sorghum vulgare* Pers.) depended on soil type. In the present experiment, soil type also had a significant ($p=0.05$) on VAM colonization levels in wheat (Table 14). Significant differences in VAM colonization only occurred between plants grown on the two different soil types (locations) on the last sampling date. Significant two and three way interactions occurred between all of the factors (Table 14).

The winter wheat was sampled at two locations (Table 15). There were no differences in VAM colonization levels between plants grown at Winnipeg or Portage (Table 16). VAM colonization levels remained below 24%. Low colonization levels of plants sampled in August may be due in part to declining root cortical tissues of mature wheat plants. These field colonization levels for both the spring and winter wheat cultivars were similar to the "slow and low" colonization levels found by Hetrich and Bloom (1983), Hetrick et al. (1984) and Black and Tinker (1979). Hetrick et al. (1984) found little VAM colonization of winter wheat until May and only a small amount of infection developed (<1% to 40%) before harvesting. The fact that winter wheat had higher VAM infection levels than the spring wheat cultivars may be due to higher inoculum densities associated with long term winter wheat monoculture at both locations. In Manitoba, low soil temperatures may not be conducive to early VAM colonization in spring. The levels of VAM colonization which occur as a result of indigenous infection are probably not

sufficient so as to have a beneficial effect on wheat in terms of plant dry matter production and yield. This is especially important when one considers that cereals such as wheat are reported to have a relatively low "field mycorrhizal dependency" (Saif and Khan, 1977).

Hetrick et al. (1984) suggested that resistance of winter wheat cultivars to VAM colonization might explain the absence of typical levels of VAM infection. However, in their greenhouse experiments, all of the eight wheat cultivars tested became mycorrhizal when the plants were grown in field soil containing indigenous or amended VAM fungal endophytes. Therefore, the observed failure of colonization could not be explained by cultivar resistance. Significant differences in the intensity of VAM colonization were observed between cultivars. However, the cultivars showing the greatest VAM colonization varied from soil to soil. The researchers grouped the cultivars as highly or moderately colonized based on their response in the three soils considered together.

As a second explanation for these low infection levels, Hetrick et al. (1984) considered that low VAM populations might retard development of mycorrhizae in the field. They previously observed extremely low VAM fungal spore numbers in wheat field soils, suggesting that inoculum concentrations in the soil were insufficient to support extensive infection. However, in greenhouse experiments, wheat became colonized in field soils previously cropped to wheat or corn (*Zea mays* L.) and the researchers concluded that nonsterile field soils do contain enough indigenous inoculum to initiate colonization. This is further supported by the fact that late emerging volunteer wheat in several fields became colonized within a few months after germination.

The most plausible explanation for low infection levels is that soil temperature limits colonization until May when winter wheat plants are already nearing maturity. The first observed colonization of wheat in April and May probably occurs when soil temperatures are first conducive to germination and colonization of VAM fungi. There is strong evidence that germination of some VAM fungal species including *G. epigaeum* Daniels & Trappe occurs very slowly or is entirely inhibited at soil temperatures below 18°C (Daniels

and Trappe, 1980; Furlan and Fortin, 1973; Koske, 1981; Volkmar, 1981). The results of experiments conducted in the greenhouse support the observation that wheat is not colonized in the field due to low soil temperature. Hetrick and Bloom (1984) noted that wheat failed to become colonized at 10°C but was 7.8% colonized at 25°C. Red clover (*Trifolium pratense* L.) plants were similarily free of colonization when grown at low temperature suggesting that this phenomenon may be associated with other fall sown crops as well. Therefore, the researchers concluded that the observed failure of winter wheat in the field to become colonized until late in the growing season could not be explained by cultivar resistance or insufficient inoculum levels in field soils but may be attributed to low soil temperatures which may inhibit spore germination or root colonization. Low soil temperatures may also affect VAM establishment in spring-sown wheat as well. In order to have any beneficial effect in cereals, it is most likely that suitable VAM species will have be inoculated into the field at or just prior to planting in order to establish early and rapid colonization of roots.

The grid-line intersect method used to sample VAM colonization levels in the field is based on determination of the percentage of variously sized root segments which contain VA mycorrhizal fungus structures. However, this method may overestimate the extent of colonization for a segment which is counted as positive may not be mycorrhizal for its entire length, whereas those which are counted as nonmycorrhizal are completely nonmycorrhizal. As the size of root segments increases, this inaccuracy is magnified due to the fact that there will be a decrease in the proportion of the length of the segments which is colonized.

The method of sampling outlined by Bierman and Lindermann (1981) does not have the inherent inaccuracy discussed above. This is probably a more sensitive measure of infection at high or low levels of VAM colonization than the percentage of root segments with mycorrhizae because almost all or almost none of the root segments are colonized.

However, this method does not take into account the three-dimensional structure of the root. A root segment which has extensive hyphal coiling and spores occupying 20% of the length of the segment is estimated at 20% colonization while another segment with a thin mycelial thread extending the length of the segment will be counted as having 100% colonization. In either case, we do not know which morphological VAM feature has the most beneficial effect on nutrient uptake and plant growth.

Sampling plants in the field is also very difficult in that only a small portion of the plant host system is obtained and it may be contaminated with old roots and roots of other plant species. Removing soil and debris from roots also results in significant root damage and loss. A small nonrepresentative root sample coupled with root loss and damage during handling leads to a widely inaccurate percentage VAM root colonization level.

Notwithstanding the limitations imposed by the experimental techniques employed, the results of this study show that in Manitoba, VAM colonization of field grown spring and winter wheat occurs slowly and that colonization levels remain low when compared with VAM colonization levels of pot-grown wheat (Section 1 and 2).

TABLE 12 Composition of soil at the field trial site at Minto and Portage, Manitoba.

CHARACTER[a]	Minto	Portage
Organic Matter (%)	3.5	8.5
pH	7.5	8.0
Carbonate	Very low	High
Salinity (ms/cm)	0.52	0.93
Nitrate ($\mu g\ g^{-1}$)*	45.4	71.9
Phosphate ($\mu g\ g^{-1}$)*	16.3	13.4
Potassium ($\mu g\ g^{-1}$)**	321.7	198.0
Sulphur ($\mu g\ g^{-1}$)***	12.9	20.0+

a Soil analysis performed by the Manitoba Provincial Soils Test Laboratory.

* Sodium bicarbonate extractable

** Ammonium acetate extractable

*** Water soluble

TABLE 13. Percentage VAM root colonization of six spring wheat cultivars harvested at monthly intervals at two locations.

		HARVEST DATE		
LOCATION	CULTIVAR	JUNE	JULY	AUGUST
		% Root Colonization		
Portage	Glenlea	4.2g	18.8de	27.6bcd
Portage	Katepwa	4.3g	18.2ef	27.8bcd
Portage	HY320	3.9g	15.7f	24.3de
Portage	Wheaton	4.0g	16.4f	26.3cd
Portage	Oslo	3.7g	15.8f	26.6cd
Portage	Marshall	3.6g	16.5f	26.0cd
Minto	Glenlea	3.9g	13.6f	25.2cd
Minto	Katepwa	3.7g	16.3f	26.1cd
Minto	HY320	4.6g	17.8f	35.7ab
Minto	Wheaton	4.6g	16.2f	31.4abc
Minto	Oslo	3.9g	16.2f	33.7ab
Minto	Marshall	4.1g	17.2f	36.5a

Values followed by the same letter are not significantly different at the P=0.05 level

TABLE 14. Analysis of variance for percentage VAM root colonization of six spring wheat cultivars harvested at monthly intervals at two locations.

SOURCE	df	MS
Replicate	11	178.0
Location (L)	1	243.2**
Cultivar (C)	5	148.9
Harvest Date (H)	2	44646.1**
L x C	5	768.4**
L x H	2	669.7**
C x H	10	323.1**
L x C x H	10	483.2**
Error	358	6142.8

*,**Significant at P=0.05 and 0.01, respectively.

TABLE 15. Percentage VAM root colonization of winter wheat grown at at two locations.

Location	VAM colonization (%)
Winnipeg	23.0
Portage	22.3

Values followed by the same letter are not significantly different at the P=0.05 level

TABLE 16. Analysis of variance for percentage VAM root colonization of winter wheat cultivars grown at two locations.

SOURCE	df	MS
Location	1	2.45
Error	18	92.78

**Significant at P=0.01 level

GENERAL DISCUSSION

These studies conducted with *Puccinia graminis* f. sp. *tritici*, *Puccinia graminis* f. sp. *recondita*, and *Cochliobolus sativus* show that *G. intraradices* increases foliar disease incidence systemically in the plant and retards pathogen development in the root system.

The influence of *G. intraradices* on a soilborne disease such as common root rot may prove to be the most important effect on wheat plant disease resistance. The symbiotic association also reduced the susceptibility of wheat plants to an abiotic stress such as water shortage. The efficiency of *G. intraradices* to increase disease resistance varied with environmental conditions such as water stress. *Glomus intraradices* may be able to keep root rot damage below an economic threshold and in so doing may offer a potential biological control agent against *Cochliobolus sativum.*

The various experiments have also shown that inoculation with *G. intraradices* has some benefit in terms of plant dry matter production and yield. It would be beneficial to screen various VA endophytes on several cultivars of wheat to determine if other species enhance yields of wheat via improved root growth and P uptake. Also, VAM fungal species must be screened for cold tolerance in hopes of finding species which are adapted to the cool spring soil temperatures prevelant in the prairie region.

Finally, in conducting research such as this, one must keep in mind that plants respond to VAM in a number of ways. These responses may not be due to mycorrhizae alone but to other beneficial rhizosphere microbes or to their combination. Perhaps reduced root infection by pathogens such as *C. sativus* are due to the effects of *G. intraradices* along with bacterial associates acting in concert rather than *G. intraradices* alone. Williams (1981) observed that certain "companion fungi" occurred in high frequency in VAM pot cultures. Perhaps these companion fungi stimulate yield and plant growth or reduce disease intensity. Knowledge of the "mycorrhizosphere" is not complete and a better understanding is needed before absolute conclusions can be drawn about VAM and their effect on plant growth and development.

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APPENDIX A: Means and ANOVA's for experimental data.

TABLE 17. Analysis of variance for percentage VAM root colonization of wheat cultivars inoculated with virulent and avirulent stem and leaf rust races.

		MS	
Source	df	Expt.1	Expt.2
Replication	4	83.05	43.53
Cultivar (C)	1	12.03	7.50
Rust (R)	4	1895.46**	1196.04**
Expt. (E)	1	3520.83**	136.53*
E x C	1	3.33	4.80
E x R	4	186.07	368.54**
C x R	4	21.77	12.58
E x C x R	4	85.77	35.68
Error	119	8235.47	1473.17

*, ** Significant at P=0.05 and 0.01 respectively.

TABLE 18. Analysis of variance for percentage VAM root colonization of wheat cultivars inoculated with virulent and avirulent stem and leaf rust races.

		MS
Source	df	Expt.3
Replication	4	75.02
Cultivar (C)	3	242.73*
Rust (R)	2	17401.10**
Expt. (E)	2	137.64*
E x C	6	36.36
E x R	4	341.82**
C x R	6	919.42**
E x C x R	12	78.44
Error	140	2991.78

*,**Significant at P=0.05 and 0.01, respectively.

TABLE 19. The effect of *Glomus intraradices*, *Cochliobolus sativus*, and water stress on disease intensity, VAM formation, and plant dry matter production of Glenlea wheat plants.

Treatment	Disease Rating (1-4)	VAM Colonization (%)	Shoot Weight (gm)	Root Weight (gm)	Root/Shoot Ratio
H+G+C	1.3	43.5	7.6	5.0	0.73
H+G-C	-	51.6	7.8	7.5	1.02
H-G+C	2.3	-	7.2	3.7	0.53
H-G-C	-	-	7.5	5.5	0.72
L+G+C	1.2	39.2	4.7	4.2	1.00
L+G-C	-	48.3	5.2	5.8	1.16
L-G+C	2.6	-	5.2	3.5	0.75
L-G-C	-	-	4.9	4.3	0.88

H = high moisture regime
L = low moisture regime
G = *G. intraradices*
C = *C. sativus*
Values are means of three experiments. Each treatment was replicated eight times per experiment.

TABLE 20. The effect of *Glomus intraradices*, *Cochliobolus sativus*, and water stress on water use and yield of Glenlea wheat plants.

Treatment	Plant Water Use (ml/pot)	1000 Kernel Weight (gm)	Grain Yield (gm)
H+G+C	5304.6	32.6	5.3
H+G-C	5484.3	40.2	8.1
H-G+C	5408.8	30.0	4.1
H-G-C	5785.2	40.6	7.4
L+G+C	3845.4	31.6	3.0
L+G-C	4250.2	40.6	4.7
L-G+C	4207.8	30.2	2.8
L-G-C	4461.1	37.5	4.1

H = high moisture regime
L = low moisture regime
G = *G. intraradices*
C = *C. sativus*
Values are means of three experiments. Each treatment was replicated eight times per experiment.

APPENDIX B: *Glomus intraradices* inoculum production

An experiment was conducted to determine the effect of phosphorus on *Glomus intraradices* root colonization. The experiment was a factorial combination of: (a) two levels of phosphate supply (10 and 50 $\mu g\ g^{-1}$ phosphorus) (b) two inoculation treatments, inoculated or not with a VA mycorrhizal fungus - *Glomus intraradices* Schenck & Smith and (c) two harvests (30 and 90 d after sowing). There were 20 replicates of each treatment and all treatments were randomized within blocks.

The Golden Beauty variety of corn (*Zea mays* L.) was grown in 16 cm diameter pots filled with a steam sterilized (70°C for 8 h) calcined montmorillonite clay (Turface, ICM Imcore). Corn seeds were surface sterilized in a 0.5% sodium hypochloride solution for 10 min prior to planting. Four seeds were placed in each pot and later thinned to one seedling per pot.

VAM treatments were inoculated with 5 g of corn roots (40-50 spores g^{-1}) placed at two separate layers in each pot. One layer was placed approximately 2.5 cm below the seed and the other approximately 10 cm below the seed. Control pots recieved 5 g of noninfected corn roots inoculated in the same fashion as well as 5 mL of mycorrhizal root washings sieved through a 45 micron mesh screen to exclude mycorrhizal propagules.

The plants were grown in a growth cabinet under high-intensity lamps with a photon flux density of 380 $\mu E/(m^2 \cdot sec^{-1})$. A light-dark period of 16/8 hours was used with corresponding temperatures of 25/20°C. Relative humidity was approximately 50%.

The plants were watered daily. No fertilizer was added for the first three weeks. After that, the plants were watered twice a week with a modified Long Ashton nutrient solution adjusted to 10 and 50 $\mu g\ g^{-1}$ phosphorus respectively. Half of the pots recieved 10 $\mu g\ g^{-1}$ P (low P) while the other half recieved 50 $\mu g\ g^{-1}$ (high P). The pots were fertilized until runoff occurred.

The modified Long Ashton solution contained the following:

KNO_3	202 g	
NH_4NO_2	160 g	
$MgSO_4 \times 7H_2O$	184 g	
CaCl	111 g	
$NaH_2PO_4 \times H_2O$	174 g	(high P)
	43 g	(low P)

added to 5 L of water to make the stock solution. 100 mL of the stock solution was added to 20 L of water to make the watering solution.

Half of the plants were harvested at the 4 leaf stage and the other half were harvested following anthesis. Plant height was measured and shoot dry mass (dried at 72°C for 48 hr) determined. The roots were washed, dried (40°C for 72 hr), and weighed. The roots were then chopped into approx. 1 cm pieces, cleared in 10% KOH and 0.1 M HCl at 22°C, stained in 0.05% Trypan blue, and suspended in lactoglycerol.

A randon subsample of each stained mycorrhizal root was then used to determine the extent of mycorrhizal infection. The subsample, containing approximately 50 1 cm root pieces, was placed in a Petri dish containing 50% glycerol. The Petri plate was then placed on a specially prepared plastic platform on which a grid-line pattern had been etched to form 1 cm squares. The plate was scanned along the gridlines under a Zeiss dissecting microscope at 40X magnification with high intensity direct illumination. Roots that intersected the gridlines were counted. Percentage mycorrhizal infection was obtained by dividing the number of mycorrhizal root pieces intersecting the gridlines by the total number of root pieces counted and multiplying by 100. This was repeated another 4 times per root using approx. 50 root segments per sample. The average of the 5 samples was then used as the % colonization of *G. intraradices* in that particular plant. *Glomus intraradices* colonization was detected on the basis of recognition of characteristic, readily indentifiable vesicles, mycelium, and arbuscles.

In addition, three samples consisting of five - 1 cm root pieces were sampled to determine spore numbers per cm of root. The stained segments (15 per plant) were mounted and examined under 100X magnification using phase contrast microscopy. The spores in each segment were recorded using a hand-held counter and an average spore number per plant was calculated based on the 3 samples.

TABLE 21. ***Glomus intraradies*** **inoculum production.**

		Harvest 1		Harvest 2	
Experiment	Phosphate	Spores*	VAM†	Spores	VAM
1	10	19.6a††	92.5a	21.0a	95.0a
1	40	13.5b	74.1b	14.8b	80.5b
2	10	5.5a	64.4a	11.4a	75.6a
2	40	5.2a	41.3b	6.7b	67.7b
3	10	6.1a	64.3a	10.7a	85.4a
3	40	4.8b	47.7b	7.5b	68.5b

†† Values in the same vertical column followed by the same letter are not significantly different at the P=0.05 level.
* Spores = the number of spores/cm of root.
† VAM = prcentage root colonization by *G. intraradices*.

APPENDIX C: Nutrient Solution for Wheat

K_2SO_4	35.5g
NH_4NO_3	35.5g
$NH_4H_2PO_4$	15.0g

are added to 1 L of water to make the stock solution. 300 mL of the stock solution are then added to 6.2 L of water and 140 mL of the watering solution is added to each 1 L pot.