Effects of environmental factors on infection of barley by parasitic or symbiotic soil-borne fungi
by William Edward Grey

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University

Abstract:
The reaction of spring barley to common root rot and its effect on yield components and disease severity was studied under varied moisture regimes. Infection of barley from natural soil inoculum, primarily of Cochliobolus sativus. was augmented with inoculum of C. sativus or Fusarium culmorum to insure uniform disease pressure during plant development. To study the effect of varied soil moisture, three locations were utilized that differed in annual precipitation in 1986, and a line-source sprinkler irrigation system was utilized to establish a soil moisture gradient from a high moisture regime that received both irrigation water and rainfall to a dryland regime that received only rainfall in 1986 and 1987. Plant emergence and harvestable tillers were reduced by inoculation with C. sativus as compared to non-inoculated controls. Grain yield loss was associated with C. sativus when the dryland regime received no rainfall during seedling development and in the low rainfall, drought location. No grain yield losses occurred in the irrigated moisture regimes or the moderate and high rainfall locations with inoculation by either pathogen. In the irrigated moisture regimes, the moderate rainfall location, and the high rainfall location, the inoculated plants compensated for reduced plant emergence and harvestable tillers by producing heavier kernels. Disease severity, based on the subcrown internode lesion development during soft dough growth stage, did not differ in the dryland and irrigated moisture regimes. However, disease severity was higher in the drought location as compared with the moderate and high rainfall locations. Selection of a spring barley with low disease severity was independent of location. Prolonged drought and disease pressure during seedling development can result in grain yield reductions, whereas subsequent moisture will affect the crop's ability to compensate for common root rot.

The effects of three soil temperatures on growth of spring barleys and root colonization by vesicular arbuscular mycorrhizal (VAM) fungi from Montana or Syria soils at different inoculum concentrations with pasteurized soil were tested in the glasshouse. Shoot dry weight was reduced by inoculation with high concentration of VAM fungi from Montana or Syria at 16°C. Number of mycorrhizal plants or proportion of mycorrhizal roots colonized increased with higher temperatures. In warm soil at 26°C, an increased mycorrhizal rating was associated with a greater shoot dry weight for Clark and Harmal as compared with Steptoe or Rihane. VAM fungi from Montana, primarily Glomus macrocarpum. were cold tolerant at 11°C while those from Syria, primarily G. hoi, were heat tolerant at 26°C. Inoculum potential of Montana VAM fungi was higher than Syria VAM fungi in cool soils.

EFFECTS OF ENVIRONMENTAL FACTORS ON INFECTION OF BARLEY BY PARASITIC OR SYMBIOTIC SOIL-BORNE FUNGI

by

William Edward Grey

A thesis submitted in partial fulfillment
of the requirements for the degree

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APPROVAL

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William Edward Grey

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 4 May 1990

I dedicate this thesis in memory of my mother, Irma F. Grey, who passed away on February 9, 1989. She and my father, James Robert, encouraged their children, including Robert James, Michael Thomas and Patricia Ann, to live their lives to the fullest and stood with us when we made our decisions.

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ABSTRACT

The reaction of spring barley to common root rot and its effect on yield components and disease severity was studied under varied moisture regimes. Infection of barley from natural soil inoculum, primarily of Cochliobolus sativus, was augmented with inoculum of C. sativus or Fusarium culmorum to insure uniform disease pressure during plant development. To study the effect of varied soil moisture, three locations were utilized that differed in annual precipitation in 1986, and a line-source sprinkler irrigation system was utilized to establish a soil moisture gradient from a high moisture regime that received both irrigation water and rainfall to a dryland regime that received only rainfall in 1986 and 1987. Plant emergence and harvestable tillers were reduced by inoculation with C. sativus as compared to non-inoculated controls. Grain yield loss was associated with C. sativus when the dryland regime received no rainfall during seedling development and in the low rainfall, drought location. No grain yield losses occurred in the irrigated moisture regimes or the moderate and high rainfall locations with inoculation by either pathogen. In the irrigated moisture regimes, the moderate rainfall location, and the high rainfall location, the inoculated plants compensated for reduced plant emergence and harvestable tillers by producing heavier kernels. Disease severity, based on the subcrown internode lesion development during soft dough growth stage, did not differ in the dryland and irrigated moisture regimes. However, disease severity was higher in the drought location as compared with the moderate and high rainfall locations. Selection of a spring barley with low disease severity was independent of location. Prolonged drought and disease pressure during seedling development can result in grain yield reductions, whereas subsequent moisture will affect the crop's ability to compensate for common root rot.

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INTRODUCTION

Roots support the growth of a complex of soil-borne fungi that can have an effect on the growth and survival of the plant. Vesicular-arbuscular mycorrhizal (VAM) fungi are ubiquitous obligate parasites of roots, using carbon but providing phosphorus, that can stimulate growth responses. Common root rot (CRR) fungi are facultative parasites of roots that infect and colonize with a concomitant weakening of the plant. The extent of stimulation or inhibition of plant growth as a result of both root parasites can be strongly influenced by environmental stress. The stress of low plant nutrition can be alleviated by the "benefits" derived from mycorrhizae. The stress of low plant moisture can accentuate the "damage" from common root rot infection.

VAM are fungi that form a characteristic arbuscule and vesicle, whereas CRR is caused by a complex of fungi, primarily Cochliobolus sativus and Fusarium spp. Both VAM and CRR fungi survive as soil-borne spores or as mycelium in infected plant debris. They infect the roots of a wide range of plants. VAM fungi form an arbuscule in a parenchyma cell of the root cortex without killing the cell. Dichotomous branching of the arbuscule and a close contact with the plasmalemma of the parenchyma cell insures a high exchange surface for transport of carbon compounds and phosphorus. Individual parenchyma with arbuscules are connected to one another with intercellular hyphae and with extramatrical hyphae that radiate into the rhizosphere. The

extramatrical hyphae of VAM fungi increase the amount of soil explored by mycorrhizal roots for soil moisture and immobile elements, such as phosphorus. The development of CRR mycelium in cortical tissue can disrupt water flow through the vascular elements and reduce the function of plants roots in supporting shoot growth. Under soil conditions of high fertility and high moisture, presence or absence of these root colonizers may have no effect on plant growth. However, nutrition or drought stress during a stage of crop development coincident with maximum infection could result in growth inhibition by CRR fungi. On the other hand, a mycorrhizal plant may have no change in growth response during fluctuations in fertility or moisture stress. The survival of a mycorrhizal plant may be dependent on a stabile growth response during periods of crop stress.

Research on these two groups has been justified based on responses of stimulation/inhibition in the infected plant. Research on prevention of CRR disease has been aided by the ability to detect visible symptoms of infection and the growth of the causal organisms on artificial media. A great deal is known concerning the epidemiology of CRR disease, the genetics of host and pathogen interactions, and fungal physiology and genetics. In contrast, research on exploitation of the stimulation of plant growth by VAM has been hindered by the effort required to confirm the presence of a mycorrhiza in the roots. Major efforts have been devoted to refinement of techniques to detect mycorrhiza, to prepare inoculum, to quantify mycorrhizal colonization, and to assess the contribution of host and endophyte in the mutual association. More is known concerning the physiology of a mycorrhiza

association than is known about the environmental factors affecting infection and the specificity between isolates and host genotypes. A better understanding of environmental factors that influence a mutualistic or parasitic association will improve our ability to predict, and subsequently select for, a given plant response.

In my studies with CRR disease there has often been a failure to associate consistent grain yield losses with inoculation of plants or to correlate yield response with disease evaluation. We suspected that moisture could influence the infection of the seedling and affect later plant growth. Conditions of optimum moisture may allow the crop to compensate for early infection and experience no yield reductions. However, the timing of moisture stress during development of a diseased crop and the associated responses could explain the inconsistencies as noted above.

Experimentation with VAM fungi required controlled and uniform testing procedures to minimize loss of time and effort. The time period from preparation, implementation, and completion of an experiment with VAM fungi can be 6 to 12 months. In preliminary studies on the influence of temperature on plant mycorrhizal colonization, variability was associated with inoculum preparation, particularly source and type of infectious propagules. Mycorrhizal colonization of plants with inoculum prepared from infected root pieces and associated spores was higher than with inoculum prepared from extracted spores by wet sieving. Also, the slow mycorrhizal colonization in field and greenhouse studies was related to cool soil temperatures and not plant growth stage. It was interesting to

speculate that selection for VAM fungi with rapid plant colonization could be made under conditions of low soil temperature. Once the methodology for inoculation was completed, the question of influence of temperature on plant mycorrhizal colonization by VAM fungi could be examined.

This study was undertaken with the following objectives:

1. To determine the responses of barley to common root rot under varied moisture regimes.
2. To evaluate the influence of temperature on colonization of barley by vesicular arbuscular mycorrhizal fungi.
3. To examine the responses of barley to interaction of common root rot and vesicular arbuscular mycorrhizal fungi.

REACTION OF SPRING BARLEY TO COMMON ROOT ROT UNDER VARIED MOISTURE REGIMES: EFFECT ON YIELD COMPONENTS AND DISEASE SEVERITY

Introduction

Common root rot (CRR) of barley (Hordeum vulgare L.) and other small grains is a ubiquitous disease in the semi-arid climates of the Middle East and North America (Grey and Mathre 1988; Sturz and Bernier 1989). Root, subcrown internode, crown, and lower stem tissue discoloration on seedlings and mature plants, as well as stunting and reduced tillering, are symptomatic of the disease (Ledingham et al. 1973; Piening et al. 1976). In the Northern Great Plains, the predominant pathogens responsible for this disease are Cochliobolus sativus (Ito & Kurib.) Drechsl. ex Dastur. (anamorph: Bipolaris sorokiniana (Sacc. in Sorok.) Shoemaker = Helminthosporium sativum Pammel et al.) and Fusarium culmorum (W.G.Sm.) Sacc. (Sturz and Bernier 1989).

Individual plants with CRR symptoms typically yield less than nonsymptomatic plants, however, over a multitude of locations and site-years, yield losses in wheat and barley from CRR have varied considerably (Ledingham et al. 1973; Piening et al. 1976). This variability may be due to known differences in cultivar tolerance to CRR (Duczek 1984) and/or environmental factors (Sallans 1948), both of which affect disease severity and yield potential of the crop. For instance, the incidence of infection is lower under dryland conditions,

but once moisture is no longer limited, the rate of lesion development is faster with higher temperatures (Bailey et al. 1989). Cool soils combined with low soil moisture favors the seedling blight phase of CRR incited by C. sativus (McKinney 1923), whereas F. culmorum is favored by warm soils with low soil moisture (Colhoun et al. 1968).

In field experiments, low rainfall has been associated with increased CRR disease severity in mature wheat plants and decreased grain yields (Sallans 1948). Conner et al. (1987) have shown in tillage studies that CRR disease severity is greater under low soil moisture conditions. Environmental factors, besides soil moisture, may vary across field sites, which can be a problem when comparing disease severity and grain yield. Therefore, it becomes difficult to isolate the effect of soil moisture on disease severity and grain yield.

The line-source sprinkler irrigation system provides a method for creating a soil moisture gradient under field conditions at a single location (Hanks et al. 1976). Multiple locations with different average annual precipitation also provides a method for creating a moisture variable. In combination with field inoculation techniques for C. sativus and F. culmorum (Grey and Mathre 1984; 1988), the interaction of soil moisture and CRR pathogens on barley growth can be investigated. The objectives of this study were to determine the effect of soil moisture, both timing and quantity, on grain yield, yield components, and disease severity in barley infected with C. sativus and F. culmorum. A preliminary report has been published (Grey et al. 1988)

Materials and Methods

Line-Source Sprinkler Study

Site Description and General Field Methods in 1986 and 1987. Two experiments were conducted at the Southern Agricultural Research Center, near Huntley, MT, in a field that had a previous cropping history of barley-fallow-barley. Soil at this site is a Fort Collins, silty clay loam (Ustollic Haplargids). Soil chemical properties were as follows: pH 7.9, 620 mg kg^{-1} ammonium acetate-extractable K and 12 mg kg^{-1} Na bicarbonate-extractable P. Soil NO_3-N levels in 1986 and 1987 before seeding were equivalent to 23 and 54 kg N ha^{-1}, respectively, in the upper 60-cm soil layer.

A line-source sprinkler irrigation system was utilized to create four (high, medium, low, and dryland) and three (high, medium, and dryland) soil moisture regimes in 1986 and 1987, respectively. The high moisture regime represented minimum moisture stress; the dryland regime represented the area of maximum moisture stress that received only rainfall. Irrigation scheduling was based on neutron probe readings of soil moisture within the high moisture regime. Irrigation was applied when the moisture deficit in the upper 60-cm soil layer was 4-5 cm below field capacity. The irrigation rate returned the soil profile to field capacity within the high moisture regime without water erosion. Due to a dry surface moisture condition in 1987, a uniform application of water (15.5 mm) was applied immediately after planting to insure uniform stand establishment.

Four treatments were included in each moisture regime: inoculation with C. sativus plus an appropriate control, and inoculation with F. culmorum plus an appropriate control. These field sites contained natural soil inoculum of C. sativus and F. culmorum. Treatments were arranged in a randomized complete block design within each moisture regime and replicated four and five times in 1986 and 1987, respectively. Individual row plots were 1.8 m wide and 3.1 m long in 1986, and 1.2 m wide by 6.1 m long in 1987.

A two-rowed cultivar Clark (CI15857) was seeded at a rate of 50 seeds m^{-1} in rows spaced 30.5 cm apart. This cultivar was chosen because of its adaptability under semi-arid conditions (Grey and Mathre 1984; 1988). Nitrogen, as ammonium nitrate, was applied before seeding at a rate of 160 kg ha^{-1} in 1986, and split-applied in 1987, with 90 kg ha^{-1} applied before seeding and 70 kg ha^{-1} applied at stem elongation or Zadoks growth scale (GS) 30-35 (Tottman and Broad 1987). Phosphorus, as triple phosphate, was applied with the seed at a rate of 11 kg ha^{-1} in both years.

Experiments were seeded on 9 April 1986 and 8 April 1987. Plant emergence counts from 1 m of two yield rows were determined at the appearance of the second leaf (GS 12). During the soft dough stage (GS 80-86) plants were uprooted from 1 m of two border rows for counts of harvestable tillers and for disease evaluation. Harvestable tillers were those tillers with spikes in the upper 50% of the plant canopy. The center rows of each plot were trimmed to 2.4 m in 1986 and 4.3 m in 1987, cut, and threshed on 27 July 1986 and 21 July 1987, respectively. Harvested grain was separately recorded for each of the two yield rows.

A 500-kernel sample from each harvested row was used to calculate 1000-kernel weight. Mean values were used for data analysis.

Rainfall was measured with rain gauges placed outside the perimeter of the study area. Irrigation water was measured with catch-cans placed along an axis perpendicular to the line-source sprinkler system. Growth moisture was determined as the accumulative rainfall and irrigation water in each moisture regime from seeding to harvest.

Canopy Temperature. A hand-held infrared thermometer (Model 110, Everest Interscience, Inc., Fullerton, CA) (accuracy ±0.25°C, -30 to +50°C) was used to measure plant canopy temperature and the temperature differential between plant canopy and ambient air as an indicator of plant water status (Chaudhuri et al. 1986). Measurements were made with the instrument held at an oblique angle to the plant canopy to minimize the influence of soil exposure. A reading from the north and south were averaged for each treatment. Measurements were made between 1200-1400 hr on 7 July (GS 75-80) in 1986, and 6 June (GS 40-49), 24 June (GS 75-80), and 2 July (GS 80-85) in 1987.

Statistical Analyses. Multivariate methods described by Johnson et al. (1983) with the SAS MVAR and GLM procedures (1987) were used to compare the effects of moisture regime and treatments. The multivariate method is preferable to a split-plot analysis of variance because the moisture regimes are applied systematically without randomization (Johnson et al, 1983). However, the four treatments of inoculation with C. sativus or F. culmorum and their appropriate controls were randomized within each moisture regime. The least

significant difference (LSD) comparison test was used to contrast inoculated C. sativus or F. culmorum with their respective uninoculated controls.

Location Study

Site Descriptions and General Field Methods in 1986. Experiments were conducted in three Montana locations: Arthur H. Post Field Research Laboratory near Bozeman in Amsterdam silty clay loam (fine-silty, mixed Typic Cryoboroll), a farmer's field near Willow Creek in Amsterdam very fine sandy loam (fine-sandy, mixed Typic Haploborolls), and in the same field as the line-source sprinkler study, near Huntley, in Fort Collins silty clay loam (Ustollic Haplargids). The field sites had a previous cropping history of small grains-fallow. Average ten year annual precipitation was 335 mm, 381 mm, and 432 mm for Willow Creek, Huntley, and Bozeman, respectively. Growth moisture was determined as the accumulative rainfall from seeding to harvest.

The location study included four treatments: inoculation with C. sativus and an appropriate control, and inoculation with F. culmorum plus an appropriate control. Treatments were arranged in a split-plot design as mainplots and ten spring barleys randomly assigned to subplots with four replications. Individual four row plots were 1.2 m wide and 3.1 m long.

Ten spring barleys, chosen for their adaptability under semi-arid conditions, diversity of pedigree, and reaction to CRR, were seeded at a rate of 50 seeds m^{-1} in rows spaced 30.5 cm apart. The two-rowed barleys included: Bowman (PI483237), Gallatin (PI4915340), Lewis

(CI15856), and Piroline (CI9558). The six-rowed barleys included: Glenn (CI15769), Melvin (with susceptibility to C. sativus), Robust (MN36), and Steptoe (CI15229). The composite cross populations included: CC XLV (PI510677), two-rowed, with resistance to net blotch (incited by Pyrenophora teres f.sp. teres), and CC XLIII (Reg.no. GP65), six-rowed, with resistance to scald and net blotch (incited by Rynchosporium secalis and P. teres f.sp. teres). Nitrogen, as ammonium nitrate, was applied prior to seeding at a rate of 160 kg ha^{-1} and phosphorus, as triple superphosphate, was applied with the seed at a rate of 11 kg ha^{-1} at Huntley.

Experiments were seeded on 6 April, 9 April, and 25 April 1986 at Willow Creek, Huntley, and Bozeman, respectively. Plant emergence counts were conducted at the appearance of the second leaf (GS 12). Harvestable tiller counts and disease evaluation were determined during the soft dough stage (GS 80-86). The center two rows of each plot were trimmed to 2.4 m, cut, and threshed on 8 August, 28 July, and 14 August at Willow Creek, Huntley, and Bozeman, respectively. Mean values were used for data analysis.

Statistical Analyses. Analysis of variance with the SAS GLM procedure (1988) was used on plant emergence counts, harvestable tillers counts, mean grain yield, disease rating, and frequency of infected plants for each pathogen and location combination. Kendall's degree of concordance was utilized to measure the relative ranking of spring barleys across locations and treatments with a value of +1.0 as complete agreement (Lund 1989). The ranking and selection method

described by Gibbons et al. (1977) with the statistical procedure described by Lund (1989) was used to identify the set of spring barleys containing the single best entry as determined by low disease rating or high grain yield.

Inoculation Techniques

To augment natural soil inoculum and insure uniform disease pressure, oat kernels colonized by C. sativus were sown concurrently with the barley seed or the barley seed was infested with a macroconidial suspension of F. culmorum. In 1986, a uniform blend of oat kernel inoculum colonized by C. sativus, grown from four isolates tested for pathogenicity on barley, was used as the inoculum source. Isolates of C. sativus recovered from infected barley plants in 1986 were used to infest sterilized oat kernels for the 1987 inoculum source. A portion of the infested oat kernels was heat sterilized (121°C for 45 min) for use in the control plots; colonized or heat-sterilized colonized oats were sown at a rate of 5 g m^{-1} of row (Grey and Mathre 1984).

A single-spore selection of F. culmorum, reisolated from infected barley, was used as the inoculum source in 1986 and 1987. The seed was first coated with a 5% solution of carboxymethylcellulose (10 ml 100 g^{-1} seed) as a sticker, and then a 5 ml macroconidia suspension from liquid shake culture (10^6 macroconidia per milliliter) was atomized on the seed. A portion of the macroconidial suspension was heat sterilized (121°C for 17 min) before treating seed for the control plots (Grey and Mathre 1988).

Disease Evaluation

Each plant was evaluated for the extent of subcrown internode lesions as follows: Slight = no discoloration to pinpoint lesions, moderate = extended linear lesions not surrounding the circumference, and severe = extended linear to encircling lesions (Ledingham et al. 1973). A disease rating (DR) was calculated for each plot (Grey and Mathre 1984).

The percentage occurrence (%) of F. culmorum and C. sativus was determined on plants collected for disease evaluation. The subcrown internode tissue was excised from a minimum of 20 plants from each of the ten spring barleys, thoroughly rinsed with cold tap water to remove soil, and surface sterilized with 0.5% NaOCl for 5 min. The mycoflora was isolated on acidified potato dextrose agar. Mycological examinations of plant subcrown internode tissue was used to estimate the frequency of F. culmorum and C. sativus from plants per cultivar and treatment. The mean frequency across all cultivars was representative of the occurrence of fungal species in the treatment. The fusaria were identified according to Nelson et al. (1983).

Results

Line-Source Sprinkler Study

Growth Moisture. The accumulative rainfall received by the dryland regime in 1986 and 1987 was 214 and 209 mm, respectively (Fig. 1). Rainfall distribution differed between growing seasons during the first 42 days after planting. The first 21 days received an accumulative

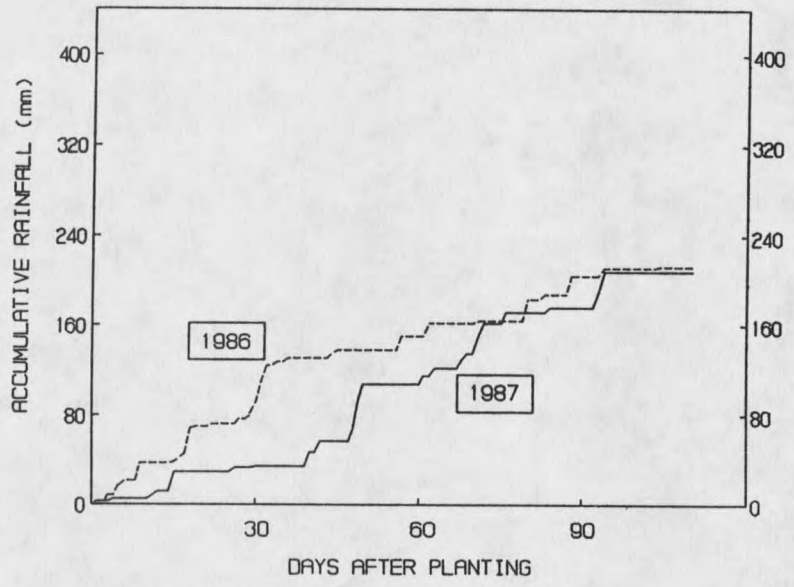

Figure 1. Accumulative rainfall received in the dryland regime from date of planting to harvest of Clark barley in 1986 and 1987 at Huntley, MT.

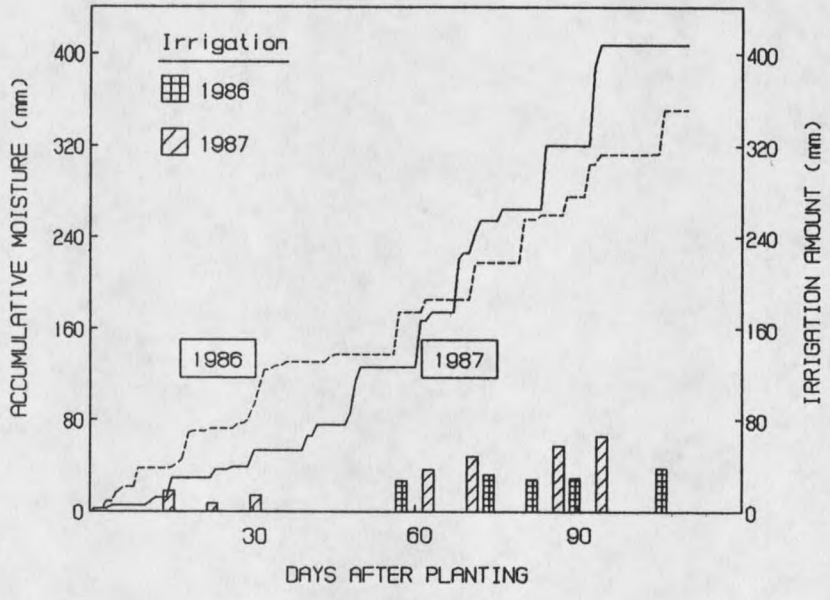

Figure 2. Accumulative moisture, including rainfall and irrigation water, and the irrigation amount received in the high moisture regime from date of planting to harvest of Clark barley in 1986 and 1987 at Huntley, MT.

rainfall of 73 and 31 mm in 1986 and 1987, respectively. By day 42 or boot stage (GS 39-42) accumulative rainfall in 1986 and 1987 was 140 and 60 mm, respectively.

Irrigation in 1986 was first applied on day 53 and, in 1987, on day 15. Even with irrigation, the high moisture regime in 1987 received less accumulative moisture than the high moisture regime in 1986 during the first 30 days after planting (Fig. 2). The 1986 dryland and high moisture regimes and the 1987 high moisture regime received the same accumulative moisture by day 48 (140 mm). By comparison, the 1987 dryland regime had received only 64 mm of accumulative moisture by day 48 (Fig. 1).

Canopy Temperature. The temperature differential between the crop canopy and ambient air averaged over three plant stages (booting, milk, and soft dough) in the 1987 dryland, medium, and high moisture regimes was +1.72°C, -0.33°C, and -1.77°C, respectively (data not shown). There was no difference in temperature differential with inoculation by either pathogen at the growth stages measured. In 1986, inoculation had no effect on air temperature differential measured at milk stage.

Plant Emergence. Infested oat kernel inoculum of *C. sativus* reduced plant emergence across moisture regimes compared with the control in 1986 and 1987 (Tables 1 and 2). Macroconidial inoculum of *F. culmorum* applied to the healthy seed caused an overall reduction in plant emergence in 1986 (Table 1) but not in 1987 (Table 2).

Grain Yield and Yield Components. Grain yield was increased sequentially by application of irrigation water from dryland to high

Table 1. Plant emergence, mean grain yield, yield components, and disease rating of Clark barley inoculated with Fusarium culmorum or Cochliobolus sativus and grown under varied moisture regimes in 1986 at Huntley, MT.

Moisture regime (MR)	Growth moisture (mm)	Plant emergence (no m^{-2})		Grain yield (kg ha^{-1})		Harvestable tillers (no m^{-2})		Kernel wt (g)		Disease[+] rating	
		Control	Inoc	Control	Inoc	Control	Inoc	Control	Inoc	Control	Inoc
F. culmorum											
Dryland	214	162	129	2622	2252	578 *	450	27.2 *	29.5	64	73
Low	249	153	132	2492	2361	617	550	32.9 *	34.9	57	72
Medium	308	159 *	112	2510	2388	588 *	465	37.5	38.7	57	75
High	352	134	102	3065	3442	568 *	486	43.7	45.3	62	57
MR mean		152 *	119	2672	2611	588 *	488	35.3 *	37.1	60	69
C. sativus											
Dryland	214	149 *	68	2420	2279	550 *	397	29.2 *	35.2	54	65
Low	249	123 *	62	2550	2386	526 *	400	33.0 *	38.5	55	67
Medium	308	149 *	70	2529	2431	602 *	495	38.1 *	41.5	56	70
High	352	145 *	56	3314 *	3813	543 *	433	43.8 *	47.6	59	58
MR mean		142 *	64	2703	2727	555 *	431	36.0 *	40.7	56	65
Overall mean		147 *	92	2688	2669	572 *	460	35.7 *	38.9	58	67

Table 1. (Continued)

Moisture regime (MR)	Plant emergence (no m^{-2})	Grain yield (kg ha^{-1})	Harvestable tillers (no m^{-2})	Kernel wt (g)	Disease+ rating
MR LSD‡					
Dryland	35	382	95	2.0	25
Low	22	264	83	1.6	18
Medium	34	144	99	2.3	19
High	42	420	68	2.2	22
Statistical tests§					
Ctrl X Inoc = Method	0.00	NS	0.00	0.00	NS
Fc X Cs = Pathogen	0.00	NS	0.01	0.00	NS
Method X Pathogen	0.01	NS	NS	0.00	NS
MRs	NS	0.01	NS	0.00	NS
Method X MRs	NS	0.02	NS	NS	NS
Pathogen X MRs	NS	NS	0.04	NS	NS
Method X Path X MRs	NS	NS	NS	NS	NS

+ Disease Rating, 0 = Healthy to 100 = Severe.
‡ LSD ($P<0.05$) = least significant difference comparison of control and inoculated; noted by *.
§ Wilks' likelihood ratio criteria test; NS = nonsignificant ($P>0.05$).

Table 2. Plant emergence, mean grain yield, yield components, and disease rating of Clark barley inoculated with Fusarium culmorum or Cochliobolus sativus and grown under varied moisture regimes in 1987 at Huntley, MT.

Moisture regime (MR)	Growth moisture (mm)	Plant emergence (no m^{-2})		Grain yield (kg ha^{-1})		Harvestable tillers (no m^{-2})		Kernel wt (g)		Disease[+] rating	
		Control	Inoc	Control	Inoc	Control	Inoc	Control	Inoc	Control	Inoc
F. culmorum											
Dryland	209	138	139	1730	1616	349	349	36.4	35.9	52 *	71
Medium	302	138	139	2743	2693	457	444	44.6	44.1	54 *	73
High	400	142	140	3369	3537	557	528	46.1	46.4	49 *	66
MR mean		139	139	2614	2615	454	440	42.4	42.2	52 *	70
C. sativus											
Dryland	209	141 *	87	1674 *	1397	299	264	36.4	37.2	49	62
Medium	302	143 *	75	2706	2607	489 *	413	44.4	44.8	43	39
High	400	121 *	87	3670	3640	595 *	507	46.6	47.1	41	46
MR mean		135 *	83	2683	2548	461 *	395	42.5	43.1	44	49
Overall mean		137 *	110	2649	2582	458 *	418	42.5	42.7	48 *	60

Table 2. (Continued)

Moisture regime (MR)	Plant emergence (no m^{-2})	Grain yield (kg ha^{-1})	Harvestable tillers (no m^{-2})	Kernel wt (g)	Disease[+] rating
MR LSD‡					
Dryland	18	238	71	1.9	15
Medium	23	155	68	1.0	16
High	24	196	71	1.6	9
Statistical tests[§]					
Ctrl X Inoc = Method	0.00	NS	0.03	NS	0.00
Fc X Cs = Pathogen	0.00	NS	NS	NS	0.00
Method X Pathogen	0.00	NS	NS	NS	0.00
MRs	NS	0.00	0.00	0.00	NS
Method X MRs	NS	NS	NS	NS	NS
Pathogen X MRs	NS	0.04	0.05	NS	NS
Method X Path. X MRs	NS	NS	NS	NS	NS

+ Disease Rating, 0 = Healthy to 100 = Severe.
‡ LSD ($P<0.05$) = least significant difference comparison of control and inoculated; noted by *.
§ Wilks' likelihood ratio criteria test; NS = nonsignificant ($P>0.05$).

moisture regimes in 1986 and 1987 (Tables 1 and 2). The differential between grain yields in dryland and the high moisture regime was smaller in 1986 than in 1987 (1016 and 2340 kg ha^{-1}, respectively).

Grain yield was not significantly reduced by inoculation with C. sativus and F. culmorum compared with the controls in 1986 (Table 1). However, grain yield was increased by inoculation with C. sativus in the high moisture regime ($P<0.02$). Inoculation with either pathogen reduced the number of harvestable tillers, with a greater reduction by C. sativus than by F. culmorum ($P<0.01$). Kernel weight was heavier in inoculated plots compared with the controls, except with F. culmorum in the medium and high moisture regimes ($P<0.01$). Inoculation with C. sativus resulted in higher kernel weights than with F. culmorum ($P<0.00$).

Grain yield was reduced by inoculation with C. sativus in the dryland regime compared with the control in 1987 ($P<0.04$) (Table 2). Harvestable tillers were not affected by inoculation with F. culmorum compared to the controls. Harvestable tillers were reduced by inoculation with C. sativus in the medium and high moisture regimes but not in the dryland regime ($P<0.05$). Kernel weights of plants were unaffected by inoculation with C. sativus and F. culmorum.

Disease Evaluation. The DR of plants at the soft dough stage was not affected by the amount of growth moisture in either 1986 or 1987 (Tables 1 and 2). The DR was similar in plots inoculated with C. sativus and uninoculated treatments. In 1987, inoculation with

F. culmorum resulted in greater disease severity than inoculation with C. sativus and the controls ($P<0.01$) (Table 2).

Location Study

Growth Moisture. The growth moisture received in Willow Creek, Huntley, and Bozeman, was 163 mm, 198 mm, and 240 mm, respectively. Frequent high winds and medium water holding capacity soil (Montagne et al. 1980) at the Willow Creek location placed additional drought stress on the plants. Growth moisture and very high water holding capacity soil at Bozeman placed no moisture stress on the plants while at Huntley, early season rainfall and very high water holding capacity soil moderated the stress on the plants from elevated air temperatures.

Plant Emergence and Harvestable Tillers. Infection from infested oat kernels of C. sativus reduced overall plant emergence and harvestable tillers in all locations compared with the control. Macroconidial inoculum of F. culmorum applied to healthy seed caused no overall reduction in plant emergence (Table 3).

Grain Yield. Grain yield was sequentially greater at the Willow Creek, Huntley, and Bozeman locations, and was related to moisture available for growth (Table 3). Grain yield was reduced by inoculation with C. sativus as compared with the control under drought conditions at Willow Creek. However, grain yield was increased by inoculation with C. sativus under conditions of no moisture stress at Bozeman. Grain yield was unaffected by inoculation with C. sativus at Huntley or by inoculation with F. culmorum at the three locations.

Table 3. Plant emergence, mean grain yield, harvestable tillers, and disease rating combined over ten spring barleys inoculated with Fusarium culmorum or Cochliobolus sativus grown at three Montana locations in 1986.

Treatment	Plant emergence (no m^{-2})			Grain yield (kg ha^{-1})			Harvestable tillers (no m^{-2})			Disease+ rating		
	BZ‡	HT	WC	BZ	HT	WC	BZ	HT	WC	BZ	HT	WC
F. culmorum												
Control	161	171	142	4043	2996	2213	490	488	238	24	53	54
Inoculated	169	166	143	4395	2918	2148	505	486	234	24	54	60
C. sativus												
Control	158	139	131	4085	3043	2383	500	476	285	26	49	64
Inoculated	110	98	98	4662	3041	2127	425	407	191	24	49	66
Mean	150	144	130	4296	3000	2218	480	464	239	25	51	61
Treatment LSD§	11	7	10	515	215	176	27	53	33	10	5	10

+ Disease rating, 0 = Healthy to 100 = Severe.
‡ Locations; BZ = Bozeman, HT = Huntley, and WC = Willow Creek, MT.
§ LSD ($P < 0.05$) = least significant difference among control and inoculated treatments.

Disease Evaluation. The disease rating ranged from a slight reaction at Bozeman to severe reaction at Willow Creek. The relative ranking of spring barleys to CRR, from low to severe disease rating, was consistent across locations (Kendall's degree of concordance 0.84) (Table 4). Bowman was in the set containing the single best entry for low disease severity at all locations, Robust and CC XLV at two locations, and Lewis, Piroline, and Melvin at one location. On the other hand, Gallatin and Steptoe, with relatively severe disease ratings, were rejected from the set containing the single best entry in all locations. Glenn had a differential disease reaction to CRR and was rejected from the set possessing low disease when inoculated with C. sativus as compared with F. culmorum.

Selection of the set containing the single best entry for high grain yield was not consistent with a low disease rating (Table 5). Lewis, Gallatin, and Steptoe were among the set in at least one treatment at all locations and Bowman, Piroline, and Melvin in at least one treatment at two locations. Lewis, Bowman, Piroline, and Melvin were rejected from the set by inoculation with C. sativus but not with F. culmorum, suggesting low yield tolerance to C. sativus. On the other hand, Steptoe was included in the set with high grain yield following inoculation with C. sativus at Bozeman and Huntley. Selection for low disease rating and high grain yield was identified in Bowman.

Frequency of isolating C. sativus from infected plants was higher at Huntley and Willow Creek than at Bozeman (Table 6). The number of plants infected with C. sativus was not affected by inoculation with

Table 4. Common root rot disease rating of ten spring barleys inoculated with Fusarium culmorum or Cochliobolus sativus from three Montana locations in 1986.

Spring barley	Bozeman		Huntley		Willow Creek	
	F. culmorum	C. sativus	F. culmorum	C. sativus	F. culmorum	C. sativus
	Disease rating+					
Bowman	11 B‡	10 B	25 B	27 B	47 B	61 B
Robust	26 B	19 B	42	42	43 B	50 B
Glenn	24 B	14 B	54	43	57 B	67
CC XLV	21 B	28 B	60	51	52 B	61 B
Melvin	19 B	25 B	41	47	66	82
CC XLIII	26 B	27 B	56	56	69	60 B
Lewis	23 B	29 B	65	55	55	68
Steptoe	33	42	54	47	65	68
Gallatin	32	35	56	56	65	66
Piroline	26 B	27 B	81	69	5	67
LSD§	15.8		9.7		12.8	

+ Mean of inoculated and control treatments; Disease rating, 0 = Healthy to 100 = Severe.
‡ The set of spring barleys, denoted by B, contains the single best entry with probability 0.95.
§ LSD ($P < 0.05$) = least significant difference among cultivars.

Table 5. Mean grain yield of ten spring barleys inoculated with Fusarium culmorum or Cochliobolus sativus from three Montana locations in 1986.

Spring barley	Bozeman		Huntley		Willow Creek	
	F. culmorum	C. sativus	F. culmorum	C. sativus	F. culmorum	C. sativus
	kgha^{-1}+					
Lewis	5139 B‡	5553 B	3482 B	3238	2668 B	2573 B
Gallatin	4820 B	4729	3540 B	3853 B	2639 B	2299 B
Steptoe	4127	4837 B	2973	3640 B	2479 B	1992
Bowman	4818 B	4795 B	3400 B	3019	2056	1896
Piroline	3717	4471	3144 B	3355	2441 B	2400 B
CC XLV	4160	4475	2611	3050	1984	1961
Melvin	4046	3745	3304 B	2853	2484 B	2263 B
Robust	4220	4139	2739	2654	2163 B	2171 B
Glenn	4332	3914	2807	2756	1846	1973
CC XLIII	2008	3040	2054	2060	2198 B	1853
LSD§	634		367		417	

+ Mean of inoculated and control treatments.
‡ The set of spring barleys, denoted by B, contains the single best entry with probability 0.95.
§ LSD ($P < 0.05$) = least significant difference among cultivars.

Table 6. Mean percentage occurrence of fungal species isolated from infected spring barleys[+] inoculated with Fusarium culmorum and Cochliobolus sativus at three Montana locations in 1986.

	Bozeman		Huntley		Willow Creek	
Spring barley	F. culmorum	C. sativus	F. culmorum	C. sativus	F. culmorum	C. sativus
	----------	----------	----------	----------	----------	----------
			%			
Fusarium culmorum						
Control	14 a[‡]	6 a	17 a	72 b	26 a	41 a
Inoculated	39 b	6 a	53 b	38 a	56 b	29 a
Cochliobolus sativus						
Control	5 x	19 x	6 x	80 x	7 x	51 x
Inoculated	2 x	29 x	8 x	74 x	7 x	68 x
Mean	15	15	21	66	24	47
Treatment LSD[§]	19.5	23.0	20.1	21.9	23.2	23.3

+ Subcrown internode tissue from 20 plants for each of the ten spring barleys sampled during the soft dough growth stage.

‡ Comparison of column means between control and inoculated for F. culmorum or C. sativus at each location followed by the same letter are not significantly different at $P < 0.05$.

§ LSD ($P < 0.05$) = least significant difference for treatment and location.

infested oat kernels. The recovery of F. culmorum from infected plants was increased with inoculation of seed with a macroconidial suspension of this fungus in all locations. The proportion of C. sativus infected plants at Huntley was decreased with macroconidial inoculum of F. culmorum. Frequency of field occurrence of fungal species by tissue isolation (Table 6) can distinguish between infected plants that was not possible by a disease rating based on symptoms (Table 3).

Discussion

A field of barley represents a population of individual plants that often are differentially affected by CRR and may show a range in symptoms from slight to severe. The grain yield from such a population reflects the summation of individual plant yield. Although many researchers have studied the various factors influencing the severity of CRR (Conner and Atkinson 1989; Conner et al. 1987; Duczek et al. 1985), most have focused on individual plant effects (Ledingham et al. 1973; Verma et al. 1974) and not on the interaction of these factors as they affect the population of plants. The role of moisture in affecting both the host and the pathogen, as well as their interaction, is a key factor in this disease (McKinney 1923; Bailey et al. 1989).

Water is a major limitation to barley production in the Northern Great Plains. Periods of water deficits in this environment can reduce grain yield and affect yield components differentially depending on the timing of stress during plant development. A prolonged drought early in plant development can have a large effect on subsequent water use and yield (Day et al. 1978). In my line-source sprinkler study, the

total rainfall was similar during the growing seasons, but 1986 had a moist spring and 1987 a spring drought. This variation in distribution offered an opportunity to study the effects of early season water deficit and CRR during pre-anthesis development in barley.

Water deficits during different plant growth stages affect the development of yield components (Day et al. 1978). The lack of early season rainfall in 1987 limited the development of harvestable tillers in the dryland regime, whereas, in 1986, there were no differences among moisture regimes because of the early season rainfall. Infection by C. sativus during stem elongation and resultant tiller death affected the development of harvestable tillers analogous to a water deficit. Moisture during subsequent plant development produced an increase in the weight of surviving kernels with no loss in grain yield. However, when there was a combination of moisture deficit and CRR caused by C. sativus, as in the 1987 dryland regime and in the drought location at Willow Creek, a reduction in grain yield did occur.

When moisture is not limiting during plant development as in the irrigated regimes and in the high moisture location at Bozeman, infection by C. sativus did not result in reduced yields. Plants were able to compensate for reduction in plant emergence and harvestable tillers, resulting in greater yields as compared with controls, which had higher plant density. Under dryland conditions where moisture was limiting early in the season, the plants did not compensate for stand reduction due to CRR. The importance of healthy seedlings to increased grain yield is illustrated by tests with barley treated with imazilil systemic fungicide. In southern Idaho, commercial plantings of

imazilil treated barley had increased grain yield and decreased disease severity by CRR as compared to nontreated barley (Hermann et al. 1990). However, in Canada, research plots of barley treated with various seed treatment fungicides had reduced disease severity, but no increase in grain yield (Piening et al. 1983). Chemical protectants applied to the seed may provide a measure of disease control in the seedling stage but the grain yield response will depend on moisture received during subsequent plant development.

Plant canopy temperature is an indicator of water use. An increase in leaf temperature is related to a decrease in transpiration cooling as a result of stomatal closure (Chaudhuri et al. 1986). Differences in plant canopy temperatures were detectable between dryland and irrigated moisture regimes, but not from inoculation with C. sativus or F. culmorum. Measurements of canopy temperature made from booting to soft dough stages may have been past the critical period of moisture stress caused by disease.

A high level of residual soil inoculum was present at the Huntley and Willow Creek locations based on the frequency of C. sativus isolations from infected plants and the moderate to severe disease ratings. In the same field at Huntley, there were no differences in disease ratings as related to the soil moisture gradient established by the line-source sprinkler system. It is probable that temperature can influence the rate of lesion development and therefore disease severity once moisture is not limiting (Bailey et al. 1989).

In spite of the differences in disease severity between locations, the relative ranking of spring barleys to CRR was consistent across

locations, as also reported by Duczek (1984). A quantitative measure associated with the set of spring barleys containing the single best entry could be useful in decisions to select or reject a entry from multiple tests. In our study it was possible to identify Bowman as a member of the set possessing low disease rating at all locations and high grain yield at two locations. Differential reaction and yield tolerance to CRR were identified by examining the entrants included in the set by the treatments of inoculation with C. sativus and F. culmorum.

Variability in barley responses to CRR have been explained by changes in cropping practices (Sturz and Bernier 1989), soil inoculum (Duczek et al. 1985), cultivar differences (Duczek 1984), plant stage (Verma et al. 1974), and climatic factors (Sallans 1948; Bailey et al. 1989). The primary limitation to crop production on the Northern Great Plains is the availability of soil moisture. Both the quantity and the distribution of moisture during crop development will affect the responses of plants to CRR. The ability of barley to compensate for the effects of CRR during a period in plant development will depend upon the availability of moisture. Yield reductions appear to only occur if the seedlings are severely diseased during a prolonged spring drought and when subsequent moisture is not sufficient to allow the plants to compensate for early season infection.

THE INFLUENCE OF TEMPERATURE ON COLONIZATION OF SPRING BARLEYS BY VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI

Introduction

Mycorrhizae are symbiotic associations between roots and fungi found in most plant species and play a role in plant nutrition. Three main types of mycorrhizae are distinguished anatomically. Ecto-mycorrhizae, occur mainly in woody plants, form a sheath around the root, penetrate, and grow between cortical cells. Endomycorrhizae, including vesicular arbuscular, ericoid, and orchid mycorrhizae, penetrate the living cells within the root. The third type is ectendomycorrhizae, which has characteristics of both ecto and endomycorrhizae. In all mycorrhizae the mycelium grows out from the root and effectively increases the soil explored by the plant for nutrients (Hayman 1983). The main benefit conferred by vesicular arbuscular mycorrhizal (VAM) fungi on their host plants is stimulation of growth from the improved phosphate uptake from soil by mycorrhizal plant roots. VAM fungi are ubiquitous in most soils existing as resting spores and/or infected root fragments. Germinated resting spores or hyphae contact plant roots and penetrate the cortical cells of the fine root hairs. Individual mycorrhizal cells are not killed and are characterized by two fungal structures, arbuscules and vesicles (Hayman 1983). The arbuscule is a fine branched structure through which nutrients and plant carbohydrates are transported. It is by the

identification of either arbuscules or vesicles in the plant roots, or by the extension of hyphae intracellularly to the root surface and as extramatrical hyphae extending into the soil, that a mycorrhizal association can be confirmed (Hayman 1983). The degree of exchange between the cortical cells of the host root and the fungal endophyte depends largely on the amount of exchange surface and the inherent efficiency of the endophyte in acquiring water and nutrients (Menge 1983).

Phosphorus uptake in an annual crop may be significantly affected only if arbuscular colonization by VAM fungi in the young, active regions of the root system is well established shortly after seedling emergence (Jakobsen and Nielsen 1983). Infection of the seedling and a well-developed external mycelium can be of beneficial value to the host even with less than 10% of the root colonized by mycorrhizae (Sanders et al. 1977). In temperate climates, fall sown cereals may have substantial levels of infection during the autumn, but this early infection may undergo a decline in the proportion of colonized mycorrhizal roots during the winter (Dodd and Jeffries 1986, 1989). In North America, the peak infection period in winter wheat is late spring and early summer because of early season low soil temperatures (Hetrick et al. 1984). Spring sown wheat and barley also experienced a delay in root infection when planted early but there was a rapid increase in rate of colonization in summer (Black and Tinker 1979, Buwalda et al. 1985). Root colonization studies on winter cereals sown early in the fall and on rates of development of infection in spring cereals would

suggest that soil temperature may be a limiting factor in penetration and spread by VAM fungi in temperate climates.

VAM fungi exhibit physiological adaptation to the environment. VAM fungal isolates from tropical climates had a higher optimum germination temperature than those from temperate climates (Safir et al. 1990, Schenck et al. 1975). In wheat there was almost no mycorrhizal colonization at 10°C, with a maximum at 20 and 25°C (Hetrick and Bloom 1984). Beneficial plant growth responses to mycorrhizal colonization are temperature dependent and may even be reduced as compared with nonmycorrhizal plants due to low temperature stress (Smith and Roncadori 1986). However, the benefits from early root colonization in the spring may be an advantage to plants that are not normally colonized until later in the season, such as cereals (Hetrick et al. 1984).

Unlike the obligate biotrophic fungi that colonize the shoots of plants, there is no known case of specificity in any mycorrhizal association such that a single fungal strain is restricted to a single host genotype (Beringer et al. 1987). However, in a study on succession of fungi associated with a change from native vegetation to monoculture agriculture, the changes in the incidence of VA mycorrhizae were due to host-plant species rather than soil type (Schenck and Kinloch 1980). There also appears to be species and strain differences in the rate of root colonization and in the extent of plant growth response as a result of infection. Inoculation of barley with VAM fungi at the time of planting in fields with soil phosphorus at 10 mg kg^{-1} (Olsen technique) doubled the weight of heads. The addition of

phosphate increased yield by threefold but phosphate plus one of the VAM inoculants further increased yield by 35% (Clarke and Mosse 1981). In Danish agricultural soils, there is often a reduced intensity of infection by indigenous VAM fungi with high phosphate, but the shoot-P level was the same in low and high fertility plots. In general, reduced soil-P levels is balanced by a higher mycorrhizal infection and greater efficiency of P uptake (Jensen and Jakobsen 1980). In glasshouse experiments with sterile soil, the addition of different Glomus spp. increased barley grain yield by 56% but the increased growth was not related to mycorrhizal intensity (Jensen 1982). The strain of VAM fungi utilized as an inoculant may not matter in situations of acute P deficiency but under fertile soil conditions the selection of VAM fungi may improve symbiotic efficiency and plant growth responses (Clarke and Mosse 1981).

The mycorrhizae associated with different cultivars of a plant species have been examined for wheat (Azcon and Ocampo 1981, Young et al. 1985), maize (Hall 1978), and cowpea (Rajapaske et al. 1989). However, studies of mycorrhizal infection of differing host genotypes under field conditions are complicated by variable inoculum, fertility, and moisture (Abbott and Robson 1982). The comparison of mycorrhizae associated with host genotypes initially may have to be approached under conditions where VAM fungal species composition, inoculum level, nutrition, growing conditions, and stage of plant growth can be controlled.

The purpose of this study was to determine if soil temperature is a factor in the ability of VAM fungi, selected from agricultural soils

in Montana or Syria, to form mycorrhizae in barley. The second purpose was to determine if barley genotypes, developed for adaptation to the semi-arid conditions in Montana or Syria, are a factor in the development of mycorrhizae.

Materials and Methods

Pasteurized Soil

A soil was prepared with high aeration and low organic phosphorus for the production of VAM fungi. Soil was collected at a depth of 3-6 m from the C1ca horizon of Amsterdam, silty clay loam (fine-silty, mixed Typic Cryoboroll) on the Arthur H. Post Field Laboratory, near Bozeman, MT. Soil was tumbled with washed river sand (1:8 soil:sand v:v), passed through a 1 cm sieve, heated with aerated steam to 60°C for 1 hr, air dried to field capacity, sieved to produce uniformity of soil structure, and stored in sealed tubs. Soil analysis was as follows: Olsen P 1-2 $\mu g\ g^{-1}$, pH 8.2, an electrical conductivity 0.66 mmhos cm^{-1}, organic matter <0.1%, and bulk density 1.72 g cc^{-1}. This pasteurized soil was utilized for producing inoculum, as a diluent for inoculum, and for various experiments.

VAM Fungi

VAM fungi were obtained from agricultural soils with a crop history of small grains-fallow at 19 locations in Montana and propagated on barley or sudangrass in the Plant Growth Center, Montana State University. Glomus macrocarpum Tul. and Tul was the predominant fungus at four locations with different soil types in Montana,

including: Rosebud, Edgar fine-loamy (Edgar Ustollic Camborthids); Belgrade, Amsterdam fine silty loam (Amsterdam typic Cryoborolls); Great Falls, Rothiemay fine-loamy (Aridic Calciborolls); and Norris, Varney silt-loam (Aridic Argiborolls). A single spore pot culture of G. macrocarpum was established from each of four soils. Inoculum of Montana VAM fungi from Rosebud, MT, comprised of G. macrocarpum and a nonidentified Glomus spp., was used for experiments based on high spore concentration as compared with inoculum originating from the other three locations.

VAM fungi were also obtained from four agricultural soils near Aleppo, Syria and propagated on barley in the plastic house at the International Center for Agriculture in the Dry Areas (ICARDA), Tel Hadya, Syria. The four locations had a crop history of barley-legume rotation but differed in amounts of annual precipitation. The locations included: Breda, low rainfall; Boueidar, moderate rainfall; Tel Hadya, high rainfall; and Hegla, low rainfall and high salinity. Under permission of a USDA-APHIS quarantine permit, VAM spores extracted from pot culture inoculum in Syria were propagated in the Plant Growth Center at MSU. Inoculum of VAM fungi from Boueidar, Syria, comprised of G. hoi, G. geosporum, and G. fasciculatum, was used in experiments based on high spore concentration as compared with the pot culture inoculum from the other three locations.

Sudangrass (Sorghum vulgare var sudanese Hitch. [cultivar] Piper) was used as a host for production of VAM inoculum. Seed was sown on a mixture of VAM inoculum and pasteurized soil (1:3, inoculum:pasteurized soil) in a plastic pot (11 cm X 11 cm, 650 ml) and covered with sand to

prevent cross contamination of soil by spores during watering. Repotting was done at 10-12 wk intervals to maintain actively growing cultures. Emerged seedlings were fertilized once with Peter's liquid fertilizer solution (20-20-20) (Peter's Fertilizer Products, Fogelsville, PA 18051) and at two week intervals with Peter's "no phosphorus" liquid fertilizer solution (25-0-25) with microelements. Metalaxyl (0.5 ml per liter a.i.) was applied as a soil drench to control seedling blight caused by Pythium spp. and plants were alternatively sprayed with insecticidal soap (20 ml per liter) and an organophosphate pesticide (0.6-1.2 g per liter a.i.) for thrip or aphid control.

Inoculum Preparation

Spores of VAM fungi were collected from pot cultures for initiation of single spore cultures, identification, and estimation of spore density. Spores were extracted from pot culture by washing root pieces and soil through a 250 micron sieve and collected on a 45 micron sieve. Soil and spores retained on the fine sieve were further clarified in a sucrose solution (60% w/v). Suspended spores and fine silt were passed through a 250 micron sieve and collected on a 37 micron sieve. Spores retained on the small sieve were rinsed, suspended in water, and collected with a tapered glass pipette. Sudangrass seedlings were inoculated by placing one or more spores on the root, transplanted to pasteurized soil, and maintained as a pot culture. Spores were identified in water, Melzer's reagent, and a

mounting medium (polyvinyl alcohol 24-32 centipoise 8.33 g, lactic acid 50 ml, glycerine 5 ml, water 50 ml).

Spore identification and concentration of VAM fungal species in the Montana VAM or Syrian VAM were determined before the plants were inoculated to insure a high inoculum level of the VAM inoculant. Root and soil inoculum was wet sieved and retained on the fine screen without sucrose clarification to avoid additional loss of spores. Spore concentration was determined using a Howard Mold Counting Chamber. Inoculum of Montana VAM fungi consisted of G. macrocarpum 90% and a nonidentified Glomus spp. 10% for a total Glomus spore concentration of 36 spores g^{-1} of soil. Inoculum of Syria VAM fungi consisted of G. hoi 78%, G. geosporum 11%, and G. fasciculatum 11% for a total Glomus spore concentration of 66 spores g^{-1} of soil.

Inoculum of VAM consisted of infected root pieces plus associated spores in soil. Water was withheld from the pot culture for one week prior to harvest for the soil to dry. Plant shoot growth was discarded and plant roots cut into 2-3 mm pieces. Roots and soil were mixed with pasteurized soil for five min in a rotatory twin-shell blender. The effect of inoculum potential on mycorrhizal colonization was determined from original inoculum blended with pasteurized soil in concentrations of 50%, 25%, 12.5%, and 6.25% (v/v). To insure uniform soil structure and density, noninfested pasteurized soil was also tumbled in the blender. Mixed soils were placed in sealed containers and stored at 4°C.

Mycorrhiza Evaluation

Stained plant roots were examined microscopically to detect mycorrhiza infection. Roots were sandwiched between the top and bottom of a plastic petri dish (100 X 15 mm) in a layer of water and placed on a circular (9 cm diam) white card with one cm square grids. Roots were illuminated using fiber optics and examined with a stereoscopic microscope using a range of magnifications from 10.5 to 63.0 x.

A mycorrhizal infection index, with categories from 0 to 5, was used to assess the stage of mycorrhizal infection and extent of cortical tissue colonized. Root tissue within a one cm square area was assigned to a mycorrhizal infection category as follows: 0 = no sign of VAM spore or root piece inoculum; 1 = VAM spores or root piece inoculum present; 2 = germinated VAM spores, preinfection hyphae, or runner hyphae on the root surface; 3 = infection point, extramatrical hyphae, or minimum arbuscule development; 4 = arbuscule development less than 25% of root cortex and extramatrical hyphae; 5 = arbuscule development greater than 25% of root cortex and extensive extramatrical hyphal development, or intraradical vesicles. Plant roots transecting a minimum of 12 grids were examined for each plant. Plants with a positive infection had a mycorrhiza in a 3, 4, or 5 category. Plants with a negative infection had a mycorrhiza in a 0, 1, or 2 category. Percentage of positive mycorrhiza infection was calculated based on the number of positive plants in a set of samples for each treatment.

A mycorrhizal rating was determined based on the stage of infection and proportion of root colonized. Mycorrhizal infection category values, 2, 3, 4, and 5, were replaced by pretransformed

category values, 15, 50, 85, and 100, respectively. The preformed scale reflected the increased proportion of root cortex colonized by arbuscules. The mycorrhizal rating for each plant was calculated from the sum of the number of grids in each pretransformed category divided by total number of grids examined multiplied by 100.

Experimental Design and Protocol

Plastic columnar containers (16.5 cm X 1.2 cm) (Ray Leach "Cone-tainer" Nursery, Canby, OR 97019 USA) were used to grow barley. Container drain holes were plugged with nonabsorbent cotton and filled with pasteurized soil (70 g). Each container received a layer of VAM inoculum or noninfested pasteurized soil (10 g), misted with a fine spray to prevent excessive drying, covered, and stored at 4°C for 2-3 days before planting.

Spring barleys, chosen for adaptability to semi-arid conditions in Montana or the Middle East, included: Clark (CI5857) two-rowed cultivar, Steptoe (CI15229) six-rowed cultivar, Harmal two-rowed (improved cultivar selected from Arabi Abiad), and Rihane six-rowed (improved cultivar selected from Arabi Aswad). Arabi Abiad white seeded and Arabi Aswad black seeded are widely grown Syrian landraces (Ceccarelli et al. 1987). Seed was surface sterilized in 70% ethanol (3 min) and rinsed with water. One seed was placed on the soil in each container and covered with sand.

Plants were grown under controlled conditions in the Plant Growth Center at MSU, Bozeman, MT in two experiments conducted during the spring and fall of 1989. Containers were inoculated with VAM fungi on

6 April and 15 October, planted on 8 April and 17 October, containers placed in soil incubators on 18 April and 20 October, and plants harvested on 6 June and 6 December. In the spring experiment, containers were planted with nongerminated seed and placed in the soil incubators once the plants emerged or ten days after planting. In the fall experiment, containers were planted with germinated seed to insure uniform plant emergence and placed in the soil incubators two days after planting. Fertilizer application and pesticide control were as described for pot culture maintenance.

Plant shoot dry weight and growth stage (GS Zadoks) (Tottman and Broad 1987) were determined at harvest. In the spring experiment, plants were harvested at 9 wks at various growth stages (see Table 7). In the fall experiment, plants were harvested at 7 wks when Harmal was at late booting development (GS 49) and Clark was at early booting development (GS 42). Plant shoots were dried in a forced air oven (49°C) for 48 hours.

Plant roots were cleared and stained for detection of mycorrhiza according to Koske and Gemma (1989). In brief, plant roots were rinsed free of soil and a 3 cm section excised 2 cm below the crown, and placed in a tissue capsule (HistoPrep 38 mm X 10 mm). Root tissue remained in the individual capsule during clearing, staining, and storage. Root tissue was cleared in 10% potassium hydroxide (121°C for 12 min), rinsed with water, soaked in 1% hydrochloric acid (1-4 hr), stained with 0.05% trypan blue (50 mg trypan blue in 100 mls 1:2:1 v/v/v/ lactic acid:glycerol:water) at room temperature (20-30 min), and stored in acidified 5% glycerol solution at 4°C. Excision of a root

portion 2 cm below the plant crown reduced the variability associated with the age and location of the root in the container.

Soil Temperature

Plants were grown in 'incubators' to provide the soil temperatures that were cool, temperate and warm. Plant foliage in the three soil incubators was subject to the same ambient air temperature and light intensity in the Plant Growth Center. Three soil incubators were constructed as rectangular frames (0.80 m X 1.75 m) from wood lumber (3.8 cm X 12.7 cm) with styrofoam insulation sheets (5.1 cm thick) above and below the frames. Heavy black plastic sheet lined the incubator along the side and bottom. Additional insulation was placed on top of the frames and along the sides of the container racks. Holes in the bottom allowed excess water to drain from the frames. Two adjoining container racks (each 31 cm x 60 cm) were placed in the center of the frame. A rectangle (62 cm x 60 cm) was cut from the top insulation to fit over the racks. Aluminum foil was placed over the container rack to cover triangle holes and block air convection.

Soil temperature was mediated by three methods in the soil incubators. For the warm soil, air was heated by a plant propagating pad (61 cm x 91 cm) (Pro-grow Supply Corp.) on the base of the frame and regulated by a thermostat with a sensor in one of the soil containers. Temperate soil was equilibrated with the ambient air in the Plant Growth Center but was generally 2 to 3°C cooler. Cool soil was chilled by a refrigeration water cooler connected to brass tubing (50 m length) coiled along the base of the frame. A pump circulated the

water coolant through the tubing in the refrigeration system. In the spring experiment, cooling capacity was limited by the small diameter brass tube (0.45 cm o.d.) but was increased (0.95 cm o.d.) to handle a larger volume of coolant for the fall experiment. Foliage ambient air temperature and soil temperatures at a depth of 9 cm in a container in each incubator were recorded hourly through the course of experiments.

A split plot design was utilized with cultivars as main plots. The spring experiment contained four cultivars and eight treatments that included, inoculation with Montana VAM at four inoculum concentrations, and inoculation with Syrian VAM at four inoculum concentrations. VAM inoculum was mixed with pasteurized soil to provide VAM inoculum concentrations of 50%, 25%, 12.5%, and 6.25% of the original VAM pot culture. The fall experiment contained two cultivars and the same eight treatments as in spring experiment, except for the substitution of a noninfested control in place of the 6.25% concentration. In both experiments, each treatment contained six samples or containers. The individual containers were organized in groups of 50, with four groups to a container rack. Each group included treatments and samples randomized for a total of 48 containers and two additional noninfested control containers. The experiments were identical for the three soil temperatures.

Statistical Analyses

Analysis of variance with the SAS GLM procedure (1988) was used to compare effects of cultivar and treatments on plant shoot dry weight, shoot dry weight of plants inoculated with VAM as compared with shoot

dry weight of noninfested controls, mycorrhizal rating, and incidence of positive mycorrhizal plants for each soil temperature. Plant shoot dry weight, mycorrhizal rating, and incidence of positive mycorrhizal plants for noninfested control treatments lacked mycorrhizal infection and were removed from analysis of variance because of non-homogeneous variance. Experimental error was partitioned from residual sums of squares and used in F-ratio tests with cultivar, isolate, and inoculum concentrations. Least significant difference (LSD) comparison test was used to contrast inoculum concentrations and cultivar means. Percentage of positive mycorrhiza plant data was angular transformed to approximate a normal distribution prior to analysis. Actual percentage means are reported. Number of infectious propagules per container was estimated by most probable number (MPN) technique, based on number of positive mycorrhiza plants in a set of samples as each inoculum concentration. A MPN program for personal computers (Singleton, L. L., Dept. Plant Pathology, Oklahoma State Univ., Stillwater, OK 74078 USA) was used to estimate MPN (Pfender et al. 1981).

Results

Soil Temperature

Soil temperatures in the three incubators fluctuated daily between a high temperature at 13:00 to 15:00 hr and a low temperature at 1:00 to 3:00 hrs. Plant ambient air temperature in both experiments was 20 to 23°C but cool, temperate, and warm soil temperatures were higher in the spring experiment as compared with corresponding soil temperatures in the fall experiment (Fig. 3 and 4). Plant Growth Center

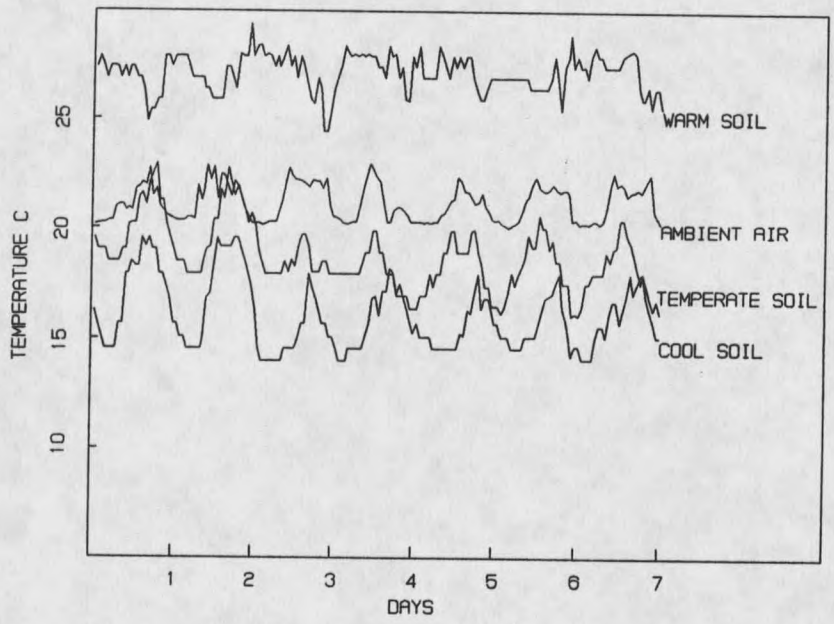

Figure 3. Daily patterns of ambient air temperature and soil temperatures of barleys inoculated with VAM fungi and grown in three soil incubators in the Plant Growth Center during the spring of 1989.

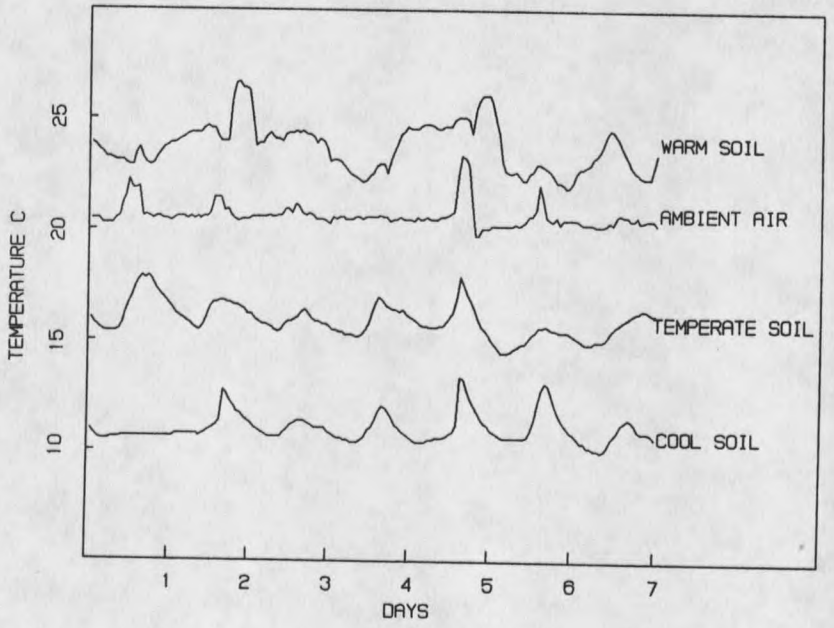

Figure 4. Daily patterns of ambient air temperature and soil temperatures of barleys inoculated with VAM fungi and grown in three soil incubators in the Plant Growth Center during the fall of 1989.

temperatures in spring are warmer than in fall because of higher light intensity and warmer outside air temperatures. Cool soil in the fall experiment was 11 to 13°C because of an improvement in cooling capacity of the refrigeration system. However, the temperature was 15 to 18°C for both the cool soil in the spring experiment and temperate soil in the fall experiment. Warm soil temperature was 25 to 28°C in the spring experiment and 23 to 26°C in the fall experiment.

Plant Growth

Plant growth was slightly stunted and uniculm in the containers. Plant roots filled the soil volume of the container except with warm soil in the fall experiment. In the spring experiment, plants were grown for ten days and were at single leaf development when placed in the incubators. The root systems of plants grown at all soil temperatures filled the soil volume. In the fall experiment, plants were placed in the incubators before emergence in order to maintain a specified soil temperature from planting to harvest. In the warm soil, heat stress during seedling growth affected later development and roots failed to completely fill the soil volume at harvest.

In both experiments, plants grown in temperate soil had higher shoot dry weight than plants grown in cool and warm soils (Tables 7 and 8). In the spring experiment, plants of all cultivars differed in growth stage and shoot dry weight when grown in three soil temperatures (Table 7). In the fall experiment, Harmal had lower shoot dry weight than Clark when grown in cool soil (Table 8).

Table 7. Number of plants, growth stage, and plant dry weight of spring barleys inoculated with vesicular arbuscular mycorrhizal fungi from Montana or Syria at four inoculum concentrations and grown at three soil temperatures in the Plant Growth Center during spring 1989

	Soil Temperature								
	Cool			Temperate			Warm		
	Plant (no)	Growth[+] stage	Drywt (mg)	Plant (no)	Growth stage	Drywt (mg)	Plant (no)	Growth stage	Drywt (mg)
Cultivar									
Clark	88	43	199	91	49	255	91	46	256
Harmal	88	68	224	93	69	262	86	69	255
Steptoe	96	55	237	93	64	332	90	49	210
Rihane	95	42	145	96	44	181	91	34	173
VAM fungi									
Montana	182	52	205	184	57	264	178	50	226
Syria	185	52	198	189	56	251	180	49	221
Inoculum concentration									
50%	89	52	198	90	57	260	87	49	223
25%	93	51	190	95	56	247	89	50	219
12.5%	90	51	200	93	57	259	89	50	232
6.25%	95	53	215	95	57	262	93	48	219
Mean	367	52	201	358	56	257	373	49	223
Control	15	53	208	14	57	272	16	56	279
LSD‡									
Cultivar		.5	15		10	28		13	32
VAM fungi		NS	NS		NS	NS		NS	NS
InocConc		NS	14		NS	NS		NS	NS

+ Zadoks decimal code (Tottman and Broad 1987).

‡ LSD ($P<0.05$) = least significant difference;
NS ($P>0.05$) = nonsignificant.

Table 8. Number of plants and plant dry weight of spring barleys inoculated with vesicular arbuscular mycorrhizal fungi from Montana or Syria at three inoculum concentrations and grown at three soil temperatures in the Plant Growth Center during fall 1989

	Soil Temperature								
	Cool			Temperate			Warm		
	Plant (no)	Ctrl[+] (%)	Drywt (mg)	Plant (no)	Ctrl (%)	Drywt (mg)	Plant (no)	Ctrl (%)	Drywt (mg)
Cultivar									
Clark	138	95	93	141	94	108	136	103	89
Harmal	140	95	87	143	103	98	141	92	80
VAM fungi									
Montana	140	96	90	142	97	102	138	97	84
Syria	138	94	89	142	101	105	139	99	85
Inoculum concentration									
50%	93	97	91	95	101	106	93	101	87
25%	92	95	90	93	95	99	90	97	84
12.5%	93	94	89	96	100	105	94	95	82
Mean	278	95	90	284	99	103	277	98	84
Control	93	--	94	95	--	105	96	--	86
LSD‡									
Cultivar		NS	*		NS	NS		NS	NS
VAM fungi		NS	NS		NS	NS		NS	NS
InocConc			NS			NS			NS

+ Percentage dry weight of plant inoculated with VAM fungi as compared with noninfested check.

‡ LSD ($P<0.05$) = least significant difference, noted by *;
NS ($P>0.05$) = nonsignificant.

Inoculation of barley with VAM fungi from Montana or Syria had little effect on plant growth stage and shoot dry weight. In the spring experiment and in cool soil, plants inoculated with VAM fungi at a 25% concentration had reduced dry weight as compared to plants inoculated with a 6.25% concentration (Table 7). Mycorrhizal infection may reduce shoot dry weight when plants are grown at low temperature stress. Differences in shoot dry weight among cultivars may be related to differences in plant growth stage (Table 7). In the fall experiment, one-quarter of the plants were grown in noninfested soil to compare with plants inoculated with VAM fungi at three inoculum concentrations. However, no differences in dry weight at boot stage of development were associated with inoculation by VAM fungi (Table 8).

Mycorrhizal Evaluation

Plants were assessed on the incidence of mycorrhizal plants and a mycorrhizal rating. Evidence of early mycorrhizal development was the appearance of hyphae with a swelling or infection cushion on the root surface and an arbuscule within the cortical cell. More advanced mycorrhizal development appeared as amorphous blue staining of cortical cells and extensive external hyphae. Placement of inoculum below the seed resulted in high infection. The 3 cm root portion excised 2 cm below the plant crown had a higher proportion of mycorrhizae than root portions further from the crown. Common soil organisms, Mucor and Chaetomium spp., colonized less than 1% of the plants. The number of mycorrhizal plants grown in noninfested soil that developed mycorrhizae was less than 1%. Mycorrhizal contamination from pasteurized soil or

between adjacent containers was not a factor in both experiments (Tables 9 and 10).

Spring Experiment 1989. The incidence of mycorrhizal plants was lower in cool soils (19%) than in temperate soils (38%) or warm soils (60%) (Table 9). In warm soil the number of mycorrhizal plants was greater with inoculation by VAM fungi from Syria (74%) than from Montana (47%). In cool and temperate soils the number of mycorrhizal plants decreased as inoculum of either Montana or Syria VAM was diluted with pasteurized soil. In cool soil at 16°C, there were an equal number of Montana and Syria VAM plants. In warm soil at 26°C, the number of mycorrhizal plants was the same in each inoculum concentration of Syria VAM but there were fewer plants at the 6.25% concentration as compared with more concentrated inoculum of Montana VAM (Table 9).

Mycorrhizal rating combines the proportion of root colonized with an intensity of arbuscule development. In warm soil, a greater proportion of the root was colonized with VAM fungi from Syria than from Montana (Table 9). Furthermore, inoculum concentration had no effect on root colonization with VAM fungi from Syria in warm soil (Table 9). This would suggest that once infection has occurred the rate of colonization is faster in warm soil and is not limited by inoculum concentration.

Warm soil differentiated genotypes for mycorrhizal infection. In warm soil, the mycorrhizal ratings of Clark and Harmal were greater than Steptoe and Rihane (Table 9). Furthermore, both Clark and Harmal

Table 9. Mycorrhizal rating and frequency of mycorrhizal spring barleys inoculated with vesicular arbuscular mycorrhizal fungi from Montana or Syria at four inoculum concentrations and grown at three soil temperatures in the Plant Growth Center during spring 1989.

	Soil Temperature					
	Cool		Temperate		Warm	
	Mycorrhizal		Mycorrhizal		Mycorrhizal	
	Rating+	Plants‡	Rating	Plants	Rating	Plants
	----------%					
Cultivar						
Clark	3.5	15	8.6	29	26.0	76
Harmal	4.7	22	10.6	41	28.5	64
Steptoe	3.1	21	10.1	49	22.4	51
Rihane	3.4	19	9.2	35	14.3	50
VAM fungi						
Montana	3.6	21	10.9	37	12.2	47
Syria	3.8	18	8.3	40	33.4	74
Inoculum concentration						
50%	5.0	28	13.7	62	26.5	73
25%	5.2	23	13.3	44	21.1	62
12.5%	3.2	14	8.9	30	23.9	60
6.25%	1.5	13	2.6	19	19.8	48
Mean	3.7	19	9.6	38	22.8	60
Control	0.2	01	0.1	01	0.1	01
LSD§						
Cultivar	NS	NS	NS	NS	1.0	16
VAM fungi	NS	NS	NS	NS	*	*
InocConc	2.9	11	5.0	16	NS	11
Isol X Inoc	NS	NS	*	NS	NS	*

+ Mycorrhizal rating 0 to 100; 0 = no mycorrhiza, 100 = all roots mycorrhizae.

‡ Percentage of mycorrhizal plants in the set of samples.

§ LSD ($P<0.05$) = least significant difference, noted by *; NS ($P>0.05$) = nonsignificant.

Table 10. Mycorrhizal rating and frequency of mycorrhizal spring barleys inoculated with vesicular arbuscular mycorrhizal fungi from Montana or Syria at three inoculum concentrations and grown at three soil temperatures in the Plant Growth Center during fall 1989.

	Soil Temperature					
	Cool		Temperate		Warm	
	Mycorrhizal		Mycorrhizal		Mycorrhizal	
	Rating+	Plants‡	Rating	Plants	Rating	Plants
	----------	----------	%	----------	----------	----------
Cultivar						
Clark	8.5	48	13.5	61	5.2	28
Harmal	11.0	56	15.3	75	6.2	42
VAM fungi						
Montana	12.5	69	17.0	72	5.7	34
Syria	6.3	36	11.8	65	5.8	36
Inoculum concentration						
50%	12.8	68	20.2	82	7.2	44
25%	9.5	51	14.0	68	6.1	38
12.5%	6.0	38	9.0	54	4.0	23
Mean	9.4	52	14.4	68	5.8	35
Control	0.5	2.1	0.1	1.0	0.1	1.0
LSD§						
Cultivar	NS	NS	NS	NS	NS	NS
VAM fungi	*	*	*	NS	NS	NS
InocConc	2.4	22	4.7	11	NS	15
Isol X Inoc	NS	NS	NS	NS	NS	NS

+ Mycorrhizal rating 0 to 100; 0 = no mycorrhiza, 100 = all roots mycorrhizae.

‡ Percentage of mycorrhizal plants in the set of samples.

§ LSD ($P<0.05$) = least significant difference, noted by *; NS ($P>0.05$) = nonsignificant.

had significantly greater shoot dry weights than Steptoe or Rihane in warm soil (Table 7). In warm soil, an increased mycorrhizal rating was associated with growth stimulation of Clark or Harmal as compared with Steptoe or Rihane (Tables 7 and 9). However, differences in plant growth stage may in part explain differences in shoot dry weights (Table 7).

Fall Experiment 1989. Mycorrhizal infection of plants was temperature dependent in the cool and temperate soils. Mycorrhizal infection of plants in warm soil did occur but was highly variable possibly due to high temperature stress during seedling development (Table 10). Number of mycorrhizal plants and mycorrhizal rating were greater in temperate soil as compared with cool soil (Table 10). Mycorrhizal infection decreased with addition of pasteurized soil to inoculum of VAM fungi from Montana or Syria. In cool soil, the incidence of mycorrhizal plants inoculated with VAM fungi from Montana was greater than from Syria. In contrast to the spring experiment, in cool soil at 11°C, the number of and proportion of root colonized in Montana VAM plants was greater than Syria VAM plants. In temperate soil at 16°C, a greater amount of root was colonized by VAM fungi from Montana (17%) than from Syria (11.8%) (Table 10).

Inoculum Potential

Inoculum potential is an estimate of the number of infectious propagules for a specific quantity as opposed to inoculum density which is an estimate of propagule concentration without concern for the infectivity of inoculum. Environmental conditions or host and

endophyte interactions that might delay the infection process, such as temperature or stage of host development, will affect the estimate of inoculum potential (Pfender et al. 1981). The MPN estimate averaged for both experiments was 3 infectious propagules per 10 g root-soil inoculum (Table 11) whereas, the spore concentration was 513 spores per 10 g root-soil inoculum. The infectivity ratio of inoculum potential to spore concentration was 0.5% or at least 1 out of 200 spores was infectious in the root-soil inoculum.

Most probable number values were affected by temperature, source of VAM fungi, and barley genotypes. In the spring experiment, the stage of host development varied from boot to early dough (GS 34 to 69) and may in part explain the lack of differences in MPN between cultivars and VAM fungi (Table 11). In the fall experiment, barley plants were examined during boot development (GS 40 to 49) and were less variable between treatments than in the spring treatment. Inoculum potential of VAM fungi from Montana was 5.4 and VAM fungi from Syria was 1.9 in cool soil (Table 11). Difference between inoculum potential of VAM fungi from Montana and Syria was much less in temperate soil (Table 11). The inoculum potential was higher with Harmal as a host for infection than with Clark except when soil temperature did not favor the physiological adaptation of the VAM fungi. For instance, VAM fungi from Montana distinguished genotypes in cool and temperate soils but not in warm soil. On the other hand, VAM fungi from Syria distinguished genotypes in warm and temperate soils but not in cool soil (Table 11).

Table 11. Inoculum potential, estimated by most probable number, of vesicular arbuscular mycorrhizal fungi from Montana or Syria towards spring barleys and grown at three soil temperatures in the Plant Growth Center during the spring and fall 1989.

	Soil Temperature					
	Cool		Temperate		Warm	
	VAM fungi		VAM fungi		VAM fungi	
	Montana	Syria	Montana	Syria	Montana	Syria
	(MPN)+					
Spring experiment						
Cultivar						
Clark	0.5	0.8	0.9	1.2	2.9	3.8
Harmal	0.6	0.9	1.9	2.6	3.8	3.1
Steptoe	1.0	0.5	3.5	2.0	1.3	1.7
Rihane	0.5	0.8	1.5	1.9	2.2	2.0
Mean	0.7	0.8	2.0	1.9	2.6	2.7
Fall experiment						
Cultivar						
Clark	4.4	1.7	4.1	3.7	1.4	1.1
Harmal	6.3	2.1	7.6	5.2	1.8	2.3
Mean	5.4	1.9	5.9	4.5	1.6	1.7
LSD‡						
Spring experiment			Fall experiment			
Temperature	0.7		Temperature	1.1		
Cultivar	NS		Cultivar	0.9		
VAM fungi	NS		VAM fungi	0.9		
Temp X Cult	*		Temp X Isol	*		

+ MPN = Most probable number estimate for the number of infectious propagules per 10 g inoculum.

‡ LSD ($P<0.05$) = least significant difference, noted by *; NS ($P>0.05$) = nonsignificant.

VAM Fungi Identification

Glomus hoi Berch and Trappe spores constituted the greater proportion of Glomus spore type in the VAM inoculum obtained from Boueidar, Syria. The following information was used to identify the fungus according to Schenck and Perez (1988). Spores are borne singly in the soil, globose 67 x 90 micron, and light brown. The spore wall is composed of two distinct, separate layers; outer layer is a unit wall, yellow-brown in transmitted light, 2-4 micron thick, with an outer surface that sloughs off; inner layer is hyaline, membranous separating from the outer wall. Subtending hypha single, 4.5 micron diameter, slightly flared toward the point of attachment to the spore where it is 7-8 micron diameter with a single wall 2.4 micron thick. Hypha attachment is not septate or constricted. Pore in subtending hypha occluded by a fine, curved septum below its point of attachment to the spore. In addition to G. hoi, G. geosporum (Nicolson and Gerdemann) Walker and possibly G. fasciculatum (Thaxter) Gerdemann and Trappe emend. Walker and Koske were extracted from the Syria VAM pot culture but in a much lower amount.

Glomus macrocarpum Tul. & Tul was the predominant spore type in the VAM inoculum obtained from Rosebud, MT. Spores 105-140 micron, globose, and yellow-brown. Spore composed of two walls; outer wall is hyaline and not always on all spores, staining slight pink in Melzer's reagent; inner wall is laminated, yellow-brown in transmitted light. Subtending hypha, 15-18 micron diameter, single, persistent, often extending 200 micron beyond point of attachment with a single wall 5-7 micron thick and hyaline with Melzer's reagent.

Discussion

The wide range of vesicular arbuscular mycorrhizal fungi found in naturally formed ecosystems suggests that a diversity of species have adapted to similar ecological factors (Molina et al. 1978). In contrast, monoculture crops have acted selectively on indigenous endophytes and have reduced the diversity in an agriculture soil (Schenck and Kinloch 1980). However, the co-adaptation of an annual crop, such as barley, and spore forming endophytes in agricultural soils to environmental stress may have selected for superior host and endophyte combinations. Barley was one of the first domesticated crops in the Fertile Crescent of Syria and Jordan (Ceccarelli et al. 1984). In these environments, stress such as drought, cold, heat and salinity are common. Syrian barley landraces or selections from landraces are widely grown and are considered dependable by farmers for high yields, especially where environmental stress is the yield-limiting factor. In the semi-arid conditions of North America, barley has also been developed for high yield and yield stability over a multitude of environments. In cooperation with ICARDA research staff, I had the opportunity to compare the co-adaptation of host and endophyte. This was done by utilizing barley selected from Syrian landraces, barley adapted to semi-arid conditions in Montana, and the associated VAM fungi collected from Syrian and Montana agricultural soils. These would seem to represent a host-endophyte association from relatively recent agriculture in Montana to an association that could be traced

back to the domestication of barley from Hordeum spontaneum in the Fertile Crescent of Syria and Jordan (Harlan 1979).

Temperatures can have an effect on early mycorrhizal colonization of fall and spring sown cereals (Buwalda et al. 1985). Soil temperatures are warmer in Syria when barley is sown in the fall and during regrowth in the spring than in Montana when barley is planted in the spring. A beneficial growth response by the host would require rapid colonization of the seedling by heat tolerant VAM fungi in Syria and cold tolerant VAM fungi in Montana. Mycorrhizae developed at a range of temperatures from 10 to 28°C but a greater proportion of colonized roots occurred at warm temperatures. In addition, in soils at 26°C a growth stimulation was associated with an increased proportion of the root colonized by mycorrhizae in Clark and Harmal as compared with Steptoe or Rihane. VAM fungi from Montana, primarily Glomus macrocarpum, were more tolerant of cool soils of 10 to 13°C, whereas, VAM fungi from Syria, primarily G. hoi, were more tolerant of warm soils of 25 to 27°C. Differences in optimum temperatures for mycorrhizal colonization among Glomus spp. have also been reported in wheat (Hetrick et al. 1984) and in cotton (Smith and Roncadori 1986). Optimum infection of winter wheat with G. epigaeum occurred at 25°C but not at 10°C. Glomus spp. stimulated cotton growth at 24 to 36°C, with G. ambisporum more efficient as a symbiont at 36°C than either G. intraradices or G. margarita. The wide range of temperatures indicates a variability for temperature tolerance among different species of Glomus.

The formation of an arbuscular mycorrhiza is the culmination of the infection process and a specific reaction between the host and

endophyte (Hayman 1983). Presence of an arbuscule is, therefore, an identifiable 'marker' of an established symbiosis between the cortical and fungal cells. In my studies, a distinctive arbuscule was required for a plant to be counted as mycorrhizal. By counting the number of mycorrhizal plants it was possible to distinguish the response of barley cultivars to VAM fungi and it was correlated with inoculum density or temperature.

In order to determine the effect of temperature on spread of mycorrhiza after infection had occurred, the proportion and intensity of colonized root was calculated as a mycorrhizal rating. In a warm soil at 26 to 28°C, the proportion of colonized root was the same in a concentrated or diluted inoculum. In a cool soil at 10 to 13°C, the proportion of colonized root was greater with VAM fungi from Montana than from Syria. In warmer soils at 16 to 18°C, there were no differences between the VAM fungi. Once a mycorrhiza has established, further spread is temperature dependent. In an environment with sudden rises in temperature, colonization rate may be more important to the success of a host and endophyte association then the minimum required temperature for infection.

A major limitation in assessment of mycorrhizal roots and colonization is the time and effort for microscopic examination. On the average, 40 plants could be observed in one hour with a maximum of 2 hrs in one day before fatigue affected evaluation of mycorrhiza. A decision was made to use observations from a greater number of plants with less time spent to examine the roots rather than from a limited number of plants with an extensive examination of the roots. This

allowed the design of a MPN experiment to quantify the effects of temperature or barley genotypes on inoculum potential of VAM fungi from Montana or Syria. A MPN experiment consisted of multiple samples at each inoculum concentration and a positive or negative mycorrhizal evaluation. A greater number of infectious propagules were detected with Harmal as a host rather than Clark. Under the conditions of the MPN, Harmal would be more susceptible to mycorrhizal colonization than Clark at several temperatures.

The inclusion of a determination of mycorrhizal colonization into a breeding program could pose the questions of mycorrhizal colonization and mycorrhizal efficiency. Efficiency includes a stimulation of growth response that may be correlated with the extent of colonization. Mycorrhizal colonization is a specific reaction between the host and endophyte. Barley genotypes could be screened for ability to form a mycorrhiza with a selection made at the lowest inoculum concentration where a positive mycorrhiza can be identified. A modification of the protocol utilized in this study would include placement of plants inoculated with VAM fungi at a temperature to delay mycorrhizal colonization but still allow root growth (5 to 9°C). The cool temperature can also improve spore germination and synchronization (Safir et al. 1989). When plant roots have filled the soil volume, the plants are grown at temperature conditions optimum for mycorrhizal colonization by the VAM fungi chosen as inoculant. Mycorrhizal colonization would be determined at boot development in half the plants, and the remainder grown to maturity for determination of mycorrhizal efficiency as compared with noninfested control plants.

Studies on the benefit of mycorrhizae to agriculture have emphasized the identification of species or strains with desired characteristics and the exploitation of those strains as inoculants (Abbott and Robson 1982, Hetrick et al. 1984, Menge 1983). However, the major problems of VAM inoculum production and dissemination must be first addressed before this goal is met (Baltruschat 1987). Utilization of barley genotypes with ability for mycorrhizal colonization may favor the natural selection of VAM fungi with increased spore production, rapid colonization of roots, and stimulated growth responses in the host.

THE INTERACTION OF VESICULAR ARBUSCULAR MYCORRHIZAE AND COMMON ROOT ROT (*Cochliobolus sativus*) IN BARLEY

Introduction

Vesicular arbuscular mycorrhizae (VAM) are soil-borne fungi that form a symbiotic relationship with the roots of a wide range of plants. Growth responses in mycorrhizal plants, which can be shown to be similar to those obtained by application of phosphorus fertilizers, vary with the level of soil phosphorus available to both the plant root and the mycorrhizal fungus. In natural soil environments, mycorrhizae are not a substitute for phosphorus fertilizer, but rather improve its utilization efficiency. In addition, a mycorrhizal association is thought to benefit nutrient uptake and water use during periods of moisture stress (Hayman 1983). This symbiosis may be enhanced by localized placement of effective inoculum near the plant roots, and by selecting superior VAM strains and hosts (Abbott and Robson 1982).

The role of indigenous soil pathogens in dryland barley cultivation needs to be investigated. In particular, the effect of common root rot (a term used to designate a group of diseases of wheat and barley characterized by necrosis of lower leaf sheaths, culms, crowns, subcrown internodes, and roots) on VAM should be studied. Cochliobolus sativus (Ito and Kurib.) Drechsl. ex Dastur. and Fusarium spp. are primary pathogens isolated from necrotic root tissue of barley

collected from countries of North Africa and the Middle East as well as from the Northern Great Plains of North America. Seed-borne and soil-borne inocula of these fungi are largely responsible for reduced yields under low moisture conditions during the growing season (Grey and Mathre 1984).

The purpose of this work was to determine the effects of VAM fungi and/or Cochliobolus sativus on two barley cultivars grown under simulated low moisture conditions with high and low phosphorus-fertilizer regimes at the International Center for Agricultural Research in the Dry Areas (ICARDA).

Materials and Methods

Chickpea (Cicer arietinum L.) plants were inoculated with spores of Glomus spp. collected from field soils at Tel Hadya, Syria. The resultant pot cultures were harvested at plant maturity and used as a source of mycorrhizal inoculum (courtesy of E. Weber, Food Legumes Improvement Program, ICARDA). To inoculate the plants, a mixture of soil-root VAM inoculum was layered on the soil surface (100 ml), sown with seed, and covered with a 6-8 cm layer of soil.

Common root rot inoculum was originally isolated from discolored subcrown internode tissue of barley plants collected from the ICARDA nursery at Boueidar, Syria. Actively growing C. sativus cultures on corn meal agar were rinsed with sterile distilled water to collect detached conidia. The suspension was adjusted to a concentration of 10^3 conidia per milliliter, and 25 ml were poured on the soil surface

both at planting and at plant emergence to insure infection. Four treatments were initiated: (i) VAM and C. sativus together, (ii) VAM alone, (iii) C. sativus alone, and (iv) no addition of either fungal inocula. Treatments received either phosphorus fertilizer or no additional fertilizer.

Plants were grown in columnar containers (1.0 m x 0.15 m) cut from a polyvinyl chloride (PVC) irrigation pipe (Ellis et al. 1985). Each container was placed vertically with the lower end resting in a 6-liter pail. A greenhouse soil mix (soil:sand, 2:1) was steam pasteurized and 22 kg added to each container. Half of the containers contained soil mix fertilized with triple superphosphate (46% a.i.) at a rate of 0.4 g kg^{-1} soil mix, and the rest contained a soil mix without supplemental fertilizer. The soil was brought to field capacity by filling the pails with water and allowing capillary action to saturate the soil. The containers were placed under a plastic cover with evaporative cooler ventilation. Average night/day temperature was 18/25°C until boot stage (Zadoks 38-42), and 25/33°C thereafter until harvest.

Seeds of two spring, two-rowed barley (Hordeum vulgare L.) cultivars, WI 2291 (Waite Institute, Australia) and Harmal (Union x CI 03576 x Coho), free of chemical treatments, were provided by ICARDA's Cereal Improvement Program. These two varieties have shown good performance under dry conditions in West Asia (WI 2291) and North Africa (Harmal). Five seeds in each container were planted on 1 April and thinned to 3 plants on 15 April. One month after emergence, 50 ml of potassium nitrate (70 mg kg^{-1}) were added to each container. Water

was maintained in the pails until initiation of early boot stage (Zadoks 40-42), then the plants were stressed by discontinuing water addition to the pails. Watering from the top of the containers was to be discontinued after plant boot stage, but there was uneven water usage between treatments due to differences in plant biomass. A marginal amount of water was applied to the pails to even the water usage between treatments until plant maturity. The experimental design was a randomized complete block with three replications. Data were collected at maturity on plant dry weight and grain weight.

The subcrown internode tissue from each plant was surface sterilized with 0.5% NaOCl for five min and plated on acidified potato dextrose agar (2% w/v). The number of plants with subcrown internode tissue yielding C. sativus was determined.

Results and Discussion

The 1-m-long columnar container was adequate to grow spring barley to maturity in spite of high temperatures (>30°C) during grain development. However, a low percentage of sterility among Harmal florets (<10%) was noted in the VAM-phosphorus fertilizer treatment.

The consistent isolation of C. sativus from subcrown internode plant tissue taken from the non-inoculated treatments (Table 12) suggests that there was a residual level of background soil inoculum in spite of treating the soil with heat. Inoculation with C. sativus increased the percentage recovery by 1-2 fold in WI 2291 as compared to the non-inoculated treatments, but had no effect or only minor increase

Table 12. The frequency (%) of Cochliobolus sativus recovered from subcrown internode tissue of two barley cultivars grown at ICARDA.

Cultivar	- VAM		+ VAM		Mean
	C. sativus		*C. sativus*		
	-	+	-	+	
WI 2291	22	62	28	50	42
Harmal	24	39	28	29	30
Mean	23	50	28	40	

in Harmal. Phosphorus fertilization had no effect on the recovery of C. sativus. The frequency of C. sativus recovered from the subcrown internode tissue in Harmal was lower than in WI 2291 (30% and 42%, respectively).

Without adding C. sativus, the grain yield of WI 2291 was increased by VAM from 31.9 g to 46.6 g. with supplemental phosphorus, but there was no increase without phosphorus (Table 13). This would confirm that a VAM symbiosis with a barley plant requires a minimum level of available phosphorus for beneficial growth responses to result (Hayman 1983). However, when both VAM inoculum and phosphorus fertilizer were applied, C. sativus inoculation significantly reduced the yield of WI 2291 to a level similar to that of a non-fertilized treatment, and negated any benefits provided by VAM inoculation. A similar effect of C. sativus inoculation was observed on plant dry weight.

Table 13. The effect of Cochliobolus sativus and vesicular arbuscular mycorrhizae (VAM) fungi on grain yield and plant dry weight (g/3 plants) of two barley cultivars grown with and without phosphorus fertilizer at ICARDA.

	- VAM		+ VAM		LSD (P<0.05)
	C. sativus		*C. sativus*		
	-	+	-	+	
WI 2291					
Grain yield					
No phosphorus	26.0	25.0	23.2	31.7	19.4
Phosphorus	31.9	28.7	46.6	24.8	
Dry weight					
No phosphorus	34.6	48.9	33.8	43.6	23.3
Phosphorus	66.7	42.0	63.7	39.3	
Harmal					
Grain yield					
No phosphorus	30.4	21.3	31.1	35.0	NS
Phosphorus	27.1	37.1	31.1	34.3	
Dry weight					
No phosphorus	40.8	40.7	61.1	50.4	21.1
Phosphorus	46.8	61.9	33.4	65.4	

In contrast to WI 2291, Harmal showed no response in grain yield to the addition of phosphorus fertilizer, VAM, or C. sativus (Table 13). With VAM inoculation only, Harmal's plant dry weight significantly decreased in response to phosphorus application, but increased when both VAM and C. sativus inoculation accompanied phosphorus addition (Table 13). This increase in plant dry weight and the lower frequency of C. sativus recovered from Harmal plant tissue

suggest that Harmal may exhibit a higher level of tolerance to C. sativus infection than WI 2291.

It appears that the beneficial effects of a VAM symbiosis may be negated by infection with soil pathogens such as C. sativus. Results from this study should be confirmed by field experiments. Detailed experiments with varying levels of both VAM and C. sativus inoculum and sampling during growth stage would aid in elucidating the complex interactions among soil-borne organisms.

SUMMARY

In field studies on common root rot of barley, the primary reduction in crop yield over 2 years and 3 locations was concomitant with stand reduction and moisture stress during seedling development. Disease ratings and yield losses are often poorly correlated because of the crops' ability to compensate. Stunted seedlings can often outgrow the effects of infection and under favorable conditions regain the growth initially lost. Since moisture predictions are unreliable, at best, in the semi-arid agricultural regions of North America, the importance of seedling establishment for a healthy crop cannot be underestimated. Use of seed protectants, either biological or chemical, may be justified as an insurance against seedling blight. Information on rainfall amount and timing during specific growth stages is helpful in understanding the plant response to seedling infection. Breeding programs to improve seedling blight resistance of barley would make an immediate impact on seedling establishment. Finally, for barley breeders the criterium of yield stability in locations with moisture stress may allow selection for tolerance to both the seedling and mature phases of the disease.

The discovery of VAM fungi for rapid root colonization, as influenced by soil temperature at time of planting, could lead to establishment of seedlings with improved phosphorus nutrition. In my study, mycorrhizal colonization of barleys was influenced by soil

temperature and was related to the physiological adaptation of the VAM fungi. The native VAM fungi from Syria or Montana were adapted to soil temperatures common to the regions from which they were collected. The deployment of non-indigenous VAM fungal isolates may fail to produce enhanced root colonization. Selection of VAM fungi for root colonization may be best accomplished by screening indigenous isolates from the intended sites where the crop is to be grown.

In this study, barley cultivars were differentiated by the amount of root colonized or the number of infectious propagules. The barley cultivars Harmal and Clark had higher mycorrhizal colonization than Steptoe and Rihane. In addition, when grown in warm soil at 26°C, Harmal and Clark had higher shoot dry weights than Steptoe or Rihane. The associated high mycorrhizal colonization with shoot dry weight may indicate a mycorrhizal efficiency in warm soil. The deployment of cultivars with high mycorrhizal colonization may be correlated with yield stability under stress conditions.

The allocation of greater resources to improve the development of VAM fungi or the promoting effect of mycorrhizae on plant growth in intensive agriculture is not realistic. But with a change to 'integrated agriculture', with reduced inputs and rotations to alternative crops, the frequency of occurrence, as well as the importance of plant mycorrhizae can be expected to increase. Since VAM are obligate parasites, the inoculum potential of soils is related to the rotation of crops and the soil fertility. The inoculum potential can decrease with rotation to non-host crops, such as sugar beets or canola, or with

host crops grown under high fertility. Increased presence of mycorrhizae will lead to more efficient use of available soil phosphorus and to decreased predisposition to many stress factors, including shortage of minor elements, drought stress, and biotic stresses, i.e. plant disease. The protective effect against biotic stress by mycorrhizae can be explained by exclusion and site competition. Thus, mycorrhizae may be a possible biological protective agent. However, the deployment of VAM fungi in integrated agriculture may not be necessary since they are favored by low inputs and diversity of crops. Factors that create the best conditions for VAM fungi are synonymous with ecologically sound agriculture.

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