# DISEASE INTERACTION OF *PRATYLENCHUS NEGLECTUS* AND *FUSARIUM* SPP. ON WINTER WHEAT WITH A COMPARISON OF NEMATODE ROOT INCUBATION TECHNIQUES

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# CHAPTER I

## INTRODUCTION

In recent years, there has been a growing recognition of the interaction of plant parasitic nematodes with fungal pathogens in the development of certain diseases. In Oklahoma, the association of the root lesion nematode *Pratylenchus neglectus* (Rensch) Filipjev and Schuurmans Stekhoven and several pathogenic fungus species in the rhizosphere of winter wheat (*Triticum aestivum* L. em Thell.) poses the opportunity for nematode-fungus disease interactions on this crop. Greenhouse and field studies have demonstrated disease interactions between *P. neglectus* and some of its fungal associates in Oklahoma (49), but the nematode's interaction with commonly occurring *Fusarium* spp., which are relatively weak pathogens of wheat in Oklahoma (58), has not previously been investigated. The literature shows each of these genera to be common interactants in disease complexes (4, 6, 7, 8, 15, 18, 21, 22, 26, 30, 31, 33, 40, 42, 44). This study was designed to determine the nature of the association of two *Fusarium spp.*, *F. roseum* f. *cerealis* (LK) Snyder and Hansen 'Acuminatum' and *F. solani* (Mart.) Sacc. with *P. neglectus* on wheat under both greenhouse and field conditions. This

included evaluation of the influence of fungal infection of the host plant upon the reproduction of P. neglectus.

Moisture stress has been reported to influence the disease interaction of nematodes and fungi (40). The pathogenicity of Fusarium spp. has been shown to increase under extremely dry soil moisture conditions (13, 14, 56, 58). Determination of the effect of moisture stress upon the interaction of P. neglectus and Fusarium spp. in Oklahoma was also an objective of this study.

The final part of this study concerned the use of nematode extraction by root incubation to determine the influence of root mass and the root weight to water volume ratio upon nematode recovery. By observing a variety of comparisons, the most efficient root incubation technique could be identified. This would insure that the maximum number of nematodes were recovered from the roots, an important consideration not only in the precision of estimation of the influence of experimental conditions on populations, but also in the collection of inoculum for research.

# CHAPTER II

# LITERATURE REVIEW

## Nematode-Fungus Disease Complexes

In nature, plants are often exposed to a combination of microorganisms which are pathogenic or potentially pathogenic. This is especially true in soil, which provides an excellent medium for disease complex activity. A significant portion of soil microflora consists of a variety of plant parasitic nematodes and plant pathogenic fungi whose frequent disease interactions have been documented by Powell (45, 46) in his review of the subject. The majority of the nematode-fungus disease complexes may be divided into two types, (1) the vascular wilt diseases and (2) the root rots and seedling diseases.

### Vascular Wilt Diseases

The most commonly reported organisms involved in nematode-fungus complexes are the Fusarium wilt fungi and root knot nematodes (Meloidogyne spp.). Several studies have shown Meloidogyne spp. capable of negating varietal wilt resistance in a wide range of hosts, particularly cotton (Gossypium hirsutum L.) and tomato (Lycopersicon

esculentum Mill.) (22, 31). Other genera of nematodes known to interact with Fusarium spp. include a sedentary semiendoparasite, Heterodera or cyst nematode on soybean (Glycine max (L.) Merr.) (48), the migratory ecto-to endoparasitic nematode, Hoplolaimus uniforimus Thorne in early yellowing of peas (Pisum sativum L.) (26), and the ectoparasitic sting nematode, Belanolaimus longicaudatus on cotton (15). The species of Fusarium most commonly associated in these complexes is Fusarium oxysporum Schl. em. Snyder and Hansen.

Verticillium wilt is another vascular wilt disease often influenced by the presence of plant parasitic nematodes, the dominant species being those of the root-lesion nematode, Pratylenchus. Much of the work with this type of disease complex has involved eggplant (Solanum melongena L.), and peppermint (Mentha piperita L.) as hosts (16, 17, 18, 32, 39). Synergistic relationships between the nematodes and fungi were reported in these studies. Either pathogen alone was usually capable of causing disease, but the damage was greatest with concomitant infection. In more recent studies, Pratylenchus penetrans (Cobb) Filipjev and Schuurmans Stekhoven was found to increases the incidence of Verticillium wilt in strawberry (33) and potato (Solanum tuberosum L.) (7, 8, 30). The nematode increased the incidence of strawberry wilt even though conditions were not conducive to symptom expression (33).

## Root Rots

Nematode-fungus complexes which result in root decay have not received the attention given to those that produce wilting as the primary symptom. The more subtle root rot complexes, however, are thought to be of more practical significance (46).

Meloidogyne spp. play an important role in root rot complexes. In most cases interaction is attributed to the infection courts for decay organisms provided during rupturing of the roots by egg masses and subsequent larval invasions (45, 46). The ability of root knot nematodes to predispose to invasion and extensive decay by other organisms seems to apply to whatever fungus happens to be present in the rhizosphere.

Mountain (37) and Benedict and Mountain (3, 38) published some of the earliest reports of nematode influence on a root disease. They reported that Pratylenchus neglectus (Rensch) Filipjev and Schuurmans Stekhoven interacted with Rhizoctonia solani Kuhn in a root rot of winter wheat. The combined effect of the fungus and nematode was almost twice as great as that of either pathogen alone. Pratylenchus scribneri and F. moniliforme also reduced the fresh weight of corn (Zea mays L.) significantly more when together than when either was alone (42). Similar root-rot complexes involving the interaction

of various nematode and fungi have recently been reported on cantaloup (Cucumis melonis L.) (10) and soybean (9, 20).

## Root Rot Complex of Wheat

Wheat root rot in Oklahoma has been reported to be a disease complex involving various combinations of the fungi Biploris sorokiana (Sacc. in Sorok) Shoemaker (Syn.) (Helminthosporium sativum Pammel, King, and Bakke), Fusarium spp., and R. solani with the nematode, P. neglectus (58). Bipolaris sorkiana is the most commonly reported cause of root and crown rot of winter wheat, followed by cultivars of F. roseum f. cerealis (LK.) Snyder and Hansen, especially Graminearum, Culmorum, and Avenaceum (19). In Oklahoma, Fusarium species have consistently been isolated in close association with P. neglectus. Both pathogens have a history of nematode-fungus disease complex activity and thus were chosen as components of the present study.

### Fusarium spp.

The genus Fusarium was erected by Link in 1809 for species with fusiform, nonseptate spores borne on a stroma, but the presence of fusoid macroconidia with a foot cell bearing some kind of heel is now accepted as the most definite character (5). There are one thousand or more published names within the genus but Booth (5) recognized approximately fifty species and varieties. In other revisions of the genus, Snyder and Hansen (52, 53, 54)

reduced all fusaria to nine species. Later Snyder et al. (55) introduced the cultivar concept for morphologically different strains of F. roseum and several other species. Strains of F. roseum which were pathogenic to cereal were designated as forma cerealis.

Fusarium roseum f. sp. cerealis 'Culmorum' has been identified as the predominant cultivar associated with root rots of cereals in the Pacific Northwest (12). Oswald (41), using the system of classification published by Wollenweber and Reinking in Die Fusarien (60) found F. graminearum Schw. and F. culmorum (W. G. Sm.) Sacc. to be the most pathogenic Fusarium sp. in his study of California cereal root rots. Fusarium culmorum was also the most commonly isolated species from root-rotted specimens of wheat in Manitoba (24).

The cultivar, 'Culmorum', has been reported to exist in the soil as single, double, and clumps of chlamydospores free or embedded in organic matter (12) and to be favored by a dry soil condition (13, 14, 56). Crowns of cereal plants are entered via infected crown roots and wounds made by crown root emergence four to six weeks after planting. Comparatively, a low incidence of infection was found to occur in the seminal roots and subcrown internodes (12).

## Pratylenchus neglectus

Pratylenchus spp. have been reported as widespread and serious parasites of wheat (3, 4, 21, 37, 38), but reports of wheat infestation by P. neglectus in North America are restricted to Ontario and Oklahoma. P. neglectus has been reported to be an important pathogen of a large variety of hosts and also participates in several nematode-fungus interactions, frequently resulting in synergism (3, 4, 16, 17, 18, 37, 38). It should be noted that the nematode involved in these studies appears in the literature as Pratylenchus minyus, orginally described by Sher and Allen (51), but Loof, in 1960 (28), reported that P. minyus and P. neglectus were conspecific. Seinhorst (50) rejected this synonymy, but Loof reinstated it in 1978 (29).

Members of this genus are migratory endoparasites, feeding on cell contents as they force their way through the root tissues. Linford (27) found that Pratylenchus pratensis (de Man) Filipjev was attracted to the piliferous region behind the elongation zone of roots and particularly to fresh wounds where the nematodes assembled in masses. Observation on the feeding of Pratylenchus thornei Sher and Allen revealed that the cortex of wheat roots was often destroyed, followed by sloughing of the epidermis (2). The wounds formed by nematode penetration have long been assumed to be responsible for increased susceptiblity to subsequent disease because they provide ready portals of entry (46).

In many cases, however, more complex mechanisms such as physiological changes within the host seem to be involved.

## Mechanisms of Nematode-Fungus Disease Interaction

The mechanism of interaction between nematodes and fungi has not been identified for many disease complexes. However, observations on the role of individual pathogens and on physiological changes in the host have provided evidence of the types of mechanisms involved.

### Attractions

A study involving Meloidogyne incognita (Kofoid and White) Chitwood and R. solani on tomato showed that a severe root rot developed when both pathogens were present together, but not when either pathogen occurred alone (59). Roots of tomatoes inoculated with R. solani alone also developed a severe rot when they received an application of root exudates from M. incognita-infected roots. When the root leachates were removed from infected roots by drip irrigation, root rot did not develop. The authors concluded that the root exudates were responsible for attracting the fungus to the nematode galls.

### Predispostion

Meloidogyne spp. influence the suitability of host tissues for invasion and for support of growth of fungi (34,

44, 46, 47, 59). Van Gundy et al. (59) found that 3 to 4 weeks after nematode infection, the nutritional balance on the surface of tomato roots favored a parasitic rather than a saprophytic development of R. solani. The fungus exhibited luxuriant growth within gall tissue and then moved into surrounding xylem elements of the roots, inducing tyloses and vascular browning of stem tissue. Host resistance of the root had apparently been weakened by nematode parasitism.

The most common perception of the nematode's role in nematode-fungus disease interactions is limited to the provision of fungal infection courts due to mechanical cell damage resulting from nematode feeding and penetration (4, 16, 32, 39). However, the influence of discrete infection loci has been studied using a split root technique with Fusarium wilt of tomato (5) and tobacco (34), and Verticillium wilt of peppermint (16). Faulkner et al. (17) found that P. neglectus influenced the length of the incubation period of Verticillium dahliae (Kleb.) f. menthae Nelson and the incidence and severity of wilt even when each pathogen parasitized separate root systems of the same plant. Similar results were reported by Bowman and Bloom (6) who found that infection by M. incognita appeared to change the physiology of the entire plant, making it more susceptible to wilt by F. oxysporum f. lycopersici. Moorman et al. (35) found that M. incognita did not induce a systemic alteration of wilt resistance in tobacco.

## Nematode Reproduction

Populations of sedentary nematodes are generally depressed as a result of interactions with fungi, but reproduction of migratory forms, especially root lesion nematodes (*Pratylenchus* spp.), is often increased when fungi are present (46). McKeen and Mountain (32, 39) reported that the level of inoculum of *Verticillium albo-atrum* Reinke and Berth affected the rate of reproduction of *P. penetrans*. Significantly higher numbers of nematodes were recovered from roots in the presence of than in the absence of *Verticillium* except at the higher inoculum levels of the fungus. The nematode population in turn influenced the incidence of wilt in eggplant. Faulkner and Skotland found that reproduction of *P. neglectus* was enhanced by *Verticillium dahliae* (Kleb.) f. *menthae* Nelson in Verticillium wilt of peppermint (18). They also noted that indole acetic acid was produced by *V. dahliae* and postulated that increases in nematode population might be traced to this product or to some physiological change induced in the host.

## Factors Influencing Nematode-Fungus Disease Interaction

It can be stated that disease conditions result from a combination of factors, both biotic and abiotic. The influence of all these factors as well as the role of each

individual pathogen must be considered before the impact of disease interaction can be anticipated.

## Soil Moisture and Temperature

Soil temperature plays an important role in Verticillium wilt of peppermint (16) and Fusarium wilt of tomato (36). The optimum soil temperature for disease development was reported to be 24°C (75°F) for plants inoculated with *V. dahliae* alone, and 27°C (81°F) for plants inoculated with both *V. dahliae* and *P. neglectus*. Final populations of *P. neglectus* were greatest at a soil temperature of 24°C (75°F) when both organisms were present, and 30°C (86°F) when the nematode alone was present. This suggests that conditions that were more favorable for the nematode were also optimum for development of disease symptoms. Similarly, the optimum soil temperature for Fusarium wilt of tomato was decreased in the presence of *M. incognita* (35).

Soil temperature also influenced a root rot of winter wheat in southwestern Ontario involving *R. solani* and *P. neglectus* (2). A soil temperature of 32°C (90°F) was discovered to be optimum for both disease severity and nematode reproduction.

Pathogenicity tests of *Fusarium* spp. on wheat have revealed that the intensity of infection increases with an increase in soil temperature, but that soil moisture does not have a significant influence (24). Also, the effect of

soil temperature on the host appeared to be more important than its direct effect on the fungus. Other tests with Fusarium spp. have shown that the greatest damage to wheat plants occurs under extremely dry soil moisture conditions (13, 56). Experimental drought conditions also significantly increased the symptoms of charcoal rot of sorghum involving an association of Pratylenchus hexincisus Taylor and Jenkins and Macrophomina phaseoli (Maubl.) Ashby (40).

The combined influence of soil temperature and moisture was considered responsible for differences in disease development on different planting dates of winter wheat and barley (19). The highest instance of root rot occurred with the earliest planting dates.

## Infection Timing and Locus

In most studies involving nematode-fungus interactions, both pathogens were added simultaneously. However, studies involving M. incognita have shown that plants are maximally predisposed to Fusarium infection only after the nematodes have been in contact with the plants for three to four weeks (44, 45). Similar studies of disease complexes involving M. incognita and either R. solani or Pythium ultimum on tobacco (Nicotiana spp.) (34, 47) also showed that the greatest damage resulted when plants had been invaded by the nematode three to four weeks prior to fungal infection.

Very little necrosis occurred when either of the fungi and the nematodes were added simultaneously.

Inoculum Rates

McKeen and Mountain (32, 39) found that at low and intermediate levels of Verticillium inoculum, P. penetrans increased wilt symptoms in eggplant. At high levels of fungus inoculum, V. albo-atrum appeared to compete with the nematode for available nutrients.

In Verticillium wilt of peppermint (18), successively higher rates of P. neglectus induced successively greater reductions in plant dry weights. All nematode inoculum rates increased both the incidence and severity of wilt symptoms.

# CHAPTER III

## MATERIALS AND METHODS

### Greenhouse Interaction Study

An isolate of root-lesion nematode identified as *Pratylenchus neglectus* (Rensch) Filipjev and Schuurmans Stekhoven (Syn. *P. minyus* Sher and Allen) and two isolates of *Fusarium* spp. identified as *Fusarium roseum* F. *cerealis* (LK) Snyder and Hansen 'Acuminatum' and *F. solani* (Mart.) Sacc. were inoculated to wheat, each alone and in all pathogen combinations, and evaluated for their disease interactions under greenhouse conditions. These pathogens were collected from soil and diseased wheat plants on the Cormack farm (South 1/2, Section 21, Township 20N Range 4E of the Indian Meridian), Alfalfa County, Oklahoma. The nematode inoculum rate used in this study was based upon natural field population levels encountered at this site during pre-plant sampling for 1979-80 field trials. Nematodes were extracted from 100 ml sub-samples of soil using a modification of the Christie-Perry technique (11), described by Alby (1) as the Oklahoma State University (OSU) tub technique. Inoculum rates for the fungi were determined from serial soil dilutions as described by Johnson and Curl

(23) using a modified PCNB agar medium (43). After approximately one week, Fusarium colonies were identified and counted, and numbers of propagules per gram of dry soil were determined for each of the two species isolated.

Inoculum of P. neglectus was obtained from the roots of infected wheat plants collected from the Cormack field location. Washed root masses were incubated in 500 ml Erlenmeyer flasks of water. The incubation unit was aerated by forcing air through the root mass and water during the incubation period. At weekly intervals, emergent nematodes were collected by pouring the contents of each flask through nested 30 mesh and 400 mesh screens. The roots, which remained on the 30 mesh screen, were returned to the flask. Each flask was then refilled with water and the roots incubated for another week. The contents of the 400 mesh screen were processed using the OSU tub technique (1). Only freshly extracted nematodes obtained from subsequent week's root incubation were inoculated to stock colonies to be used later in "Root Incubation Studies".

Conidial inoculum of the Fusarium isolates was prepared in the manner described by Umechuruba (58). Inoculum consisted of macroconidia in the case of the F. roseum isolated and macro and microconidia in the case of F. solani isolate. The conidial concentration of each isolate was determined by direct observational count of 0.01 ml aliquots using a microscope and hemacytometer.

Air-dried, methyl bromide-treated Lincoln fine sand (LFS) was passed through a 30 mesh screen and placed in an electric cement mixer. With the mixer in operation, sufficient spore suspension was added to achieve the desired inoculum concentration. After inoculation, the soil was mixed for an additional 30 minutes to insure uniform conidial distribution. During the post-inoculation mixing, additional sterile distilled water was added to the soil to bring it to field capacity (7.5% by weight). Soil to be used in non-inoculated control and nematode alone treatments was mixed prior to the fungus inoculated treatments and handled in the same manner with the exception that only sterile water was added. The mixer was thoroughly disinfested by washing with chlorox and water between batches of inoculated soil.

The fungus inoculum level used in this study was 400 conidia per gram of soil on a dry weight basis. Treatments that involved the inoculation of both fungi in combination received only 200 conidia per gram of soil for each fungal isolate, so that the overall inoculum rate of 400 conidia per gram of soil was maintained.

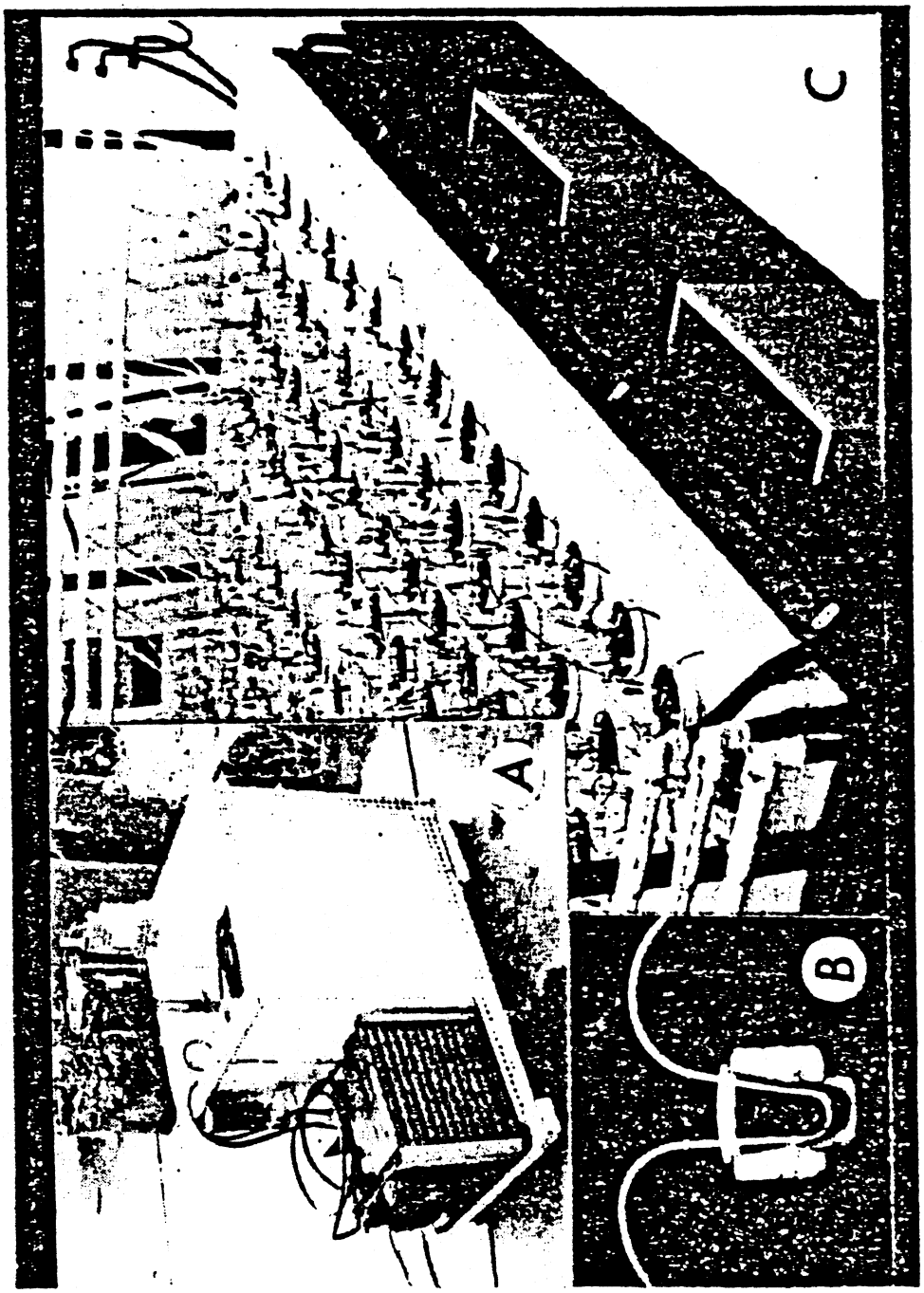
Conidia-infested soil of each of the Fusarium isolates alone and both in combination, as well as sterile soil for the control and nematode alone treatments was placed in 900 ml plastic pots (11 X 14.5 cm). Four seeds of the hard winter wheat cultivar 'TAM 101' (C.I. 15324) were planted in each pot at a depth of 5 cm. Control of the soil

temperature in the pots at 25° C (±2° C) was obtained by circulating temperature controlled water through "U"-shaped copper tubing heat sinks coated with marine epoxy to reduce the possibility of copper toxicity to the plants (Fig. 1B). Each pot contained a heat sink connected to the heat sinks of adjacent pots by plastic tubing forming a circulation loop as in Figure 1C. The temperature of the water was controlled in tanks equipped with refrigeration units (Fig. 1A). Thermometers were inserted in the water tank and in several pots of soil to monitor the uniformity of the temperature control method. The temperature control system also involved sheets of styrofoam in which holes had been cut to hold and insulate the pots of soil (Fig. 1C).

Those pots which were to be inoculated with nematodes each received a suspension containing approximately 1500 *P. neglectus* adult females and larvae. The nematode suspension was pipetted into a small hole at the center of the pot and the hole was filled with sterile LFS. Sterile water was pipetted by the same procedure into those pots that did not receive nematode inoculum.

A completely randomized design with eight treatments and six replications was used in this study. Treatments consisted of an uninoculated control, *P. neglectus* (Pn), *F.roseum* (Fr), *F. solani* (Fs), and the combinations Pn + Fr, Pn + Fs, Fr + Fs, and Pn + Fr + Fs.

Figure 1. Illustrations of the Soil Temperature Control System. A) Water Refrigeration Unit. B) Copper Tubing Heat Sink. C) Complete Temperature Control Unit Showing Circulation Loop.


A
B
C

Plants were watered as needed and were fertilized during the fourth week of the test with Peter's water soluble fertilizer (15-20-15) at the rate of 50 ml per pot.

Forage yields were taken on a per pot basis 65 days after planting. All plants were clipped 5 cm above above the soil surface level and fresh forage weights were obtained with a Mettler Analytical Balance (nearest 0.001 g). The forage was then oven-dried (24 hrs; 105° C), equilibrated for 12 hrs and again weighed on the Mettler Analytical Balance (nearest 0.001 g).

After 142 days, the experiment was terminated. The plants were clipped at the soil surface to obtain fresh and dry forage weights in the manner previously described. The roots were separated into seminal and adventitious (crown) and fresh weights were measured for each on a Mettler Analytical Balance (nearest 0.001 g). The roots were then placed separately in the root incubation units to be processed at weekly intervals for three weeks as described previously. After removal of the roots, the soil in each pot was mixed and a 100 ml aliquot processed for nematodes using the OSU tub technique (1).

Disease severity of the seminal and adventitious roots was evaluated based on the amount of discoloration as follows: 0 = no discoloration; 1 = 1-25% of roots discolored; 2 = 26-50% of roots discolored; 3 = 51-75% of roots discolored; 4 = 76-100% discoloration. Subcrown iternodes were clipped from all plants, surface sterilized

in a chlorox-ethanol solution (1:1 V/V) for 30 seconds followed by a rinse in sterile water, and plated on modified PCNB agar medium (43). After incubation at room temperature (approx. 25° C) for one week, counts were made to determine the percentage recovery of the fungal pathogens from infected internodes. All data were recorded on a per pot basis.

## Field Experiments

Field soil was collected from the Perkins Agricultural Experiment Station, Payne County, Oklahoma and uniformly mixed in an electric cement mixer. Mechanical analysis showed the soil to be a sandy loam with the following composition: 64.5% sand; 16.5% silt; 19.0% clay. Further analysis revealed that no significant plant parasite nematodes were present in the soil and, although Fusarium spp. were recovered in soil solutions, the Fusarium isolates to be used in this study were absent. Therefore, the unsterilized field soil was used for the following experiments.

Aside from an uninoculated control, treatments consisted of P. neglectus (Pn), F. roseum (Fr), and F. solani (Fs), each alone, and the combination treatments Pn + Fr, Pn + Fs, Fr + Fs, and Pn + Fr + Fs. As in the Greenhouse Interaction Study, inoculum rates of 1500 nematodes per pot and/or 400 conidia per gram of soil were

used in treatments receiving a single pathogen or when combinations involved a single fungus species. Treatments Fr + Fs and Pn + Fr + Fs were inoculated at the rate of 200 conidia per gram of soil per fungus species so that the total inoculum potential remained at 400 conidia per gram of soil for all fungus inoculated treatments.

For each inoculum treatment, 20 plastic pots (11 X 14.5 cm) containing 800 ml each of treated soil were planted with four seeds of TAM 101 wheat per pot and placed outside in a sheltered area. All plants were watered as needed and fertilized (Peter's water soluble, 15-20-15) during the fourth week of the test.

One month after initiation of the study, randomly selected pots of each treatment were transplanted to the Perkins field station. Two identical studies each consisting of a randomized complete block design with eight treatments and six replications were constructed one for obtaining grain yield, the other be destructively sampled for forage and root yield.

A tractor mounted post-hole digger was used to dig 48 holes (11 X 76 cm) for each study at the field station. The holes were lined with tubes of saran screen (32 mesh, 11 X 92 cm) to contain the roots of transplanted seedlings allowing easy recovery of the roots without inhabiting diffusion of substances (i.e. water and minerals) from the field soil surrounding the tubes. Field soil was added to nearly fill each hole, after which the contents of

individual pots were installed and the soil was brought to its original level. All treatments were watered as needed and sprayed periodically with the insecticide Malathion for control of aphids.

A companion grain yield study consisting of a randomized complete block design with eight treatments and four replications was arranged in the greenhouse from remaining original pot treatments.

The forage yield experiment was terminated 187 days following treatment inoculation. Plants and soil were harvested by simply pulling the saran tubes from the ground. The tubes, along with their contained plants, plant roots, and soil were wrapped in plastic and returned to the lab. The plants were washed, blotted dry, and separated into seminal and adventitious roots, and stem and leaf tissue. Fresh and dry forage weights and fresh root weights were measured as described under the previous Greenhouse Interaction Study. In addition, seminal and adventitious roots were measured for longest root lengths (cm) per plant. The roots were then evaluated for amount of discoloration and incubated for nematodes as previously described. Subcrown internodes were also treated as before.

The field grain yield experiment was terminated 221 days after treatment inoculation. The following data were taken: number of surviving plants, number of tillers and heads on a per 'pot' basis, and grain yield on a per 'pot'

and per plant basis. To determine grain yield, heads were collected from each treatment, counted, and hand threshed. The grain was air dried for one week and weighed on a Mettler Analytical Balance (nearest 0.001 g).

The greenhouse grain yield study was terminated 257 days after original inoculation. Data were collected in the same manner as described for the field grain yield experiment. In addition, nematode populations were determined by processing 100 ml soil sub-samples and counting the extracted nematodes.

## Drouth Stress Experiment

Lincoln fine sand (LFS) was inoculated with *P. neglectus* (Pn), *F. roseum* (Fr) and the combination Pn + Fr as described for the Greenhouse Interaction Study. Inoculum rates were 3750 nematodes per pot and/or 400 macroconidia per gram of soil. The control received no pathogen treatments. The number of treatments were doubled by duplicating each treatment so that two moisture regimes could be applied. Ten seeds of the wheat variety TAM 101 were planted in each plastic pot (11 X 14.5 cm) and the pots were arranged in a randomized complete block design with eight treatments and four replications. Soil moisture probes were buried 5 cm from the botton of each pot and soil temperature was controlled with the system previously described.

When all seeds had germinated (approx. 1 wk), the moisture stress treatments were initiated. The soil moisture of each pot was monitored every 24 hours with a Wescor, Inc. Dew Point Microvoltmeter (model No. HR-33T). Pots in the low moisture stress series were watered to saturation whenever the soil moisture reached -5(±3) bars of pressure, while pots in the high moisture stress series were watered to saturation whenever the soil moisture reached -20(±) bars of pressure. These cycles were maintained until the experiment was terminated, 40 days after inoculation.

Root fresh weights and forage fresh and dry weights were measured at termination of the experiment as described for the Greenhouse Interaction Study. The roots were incubated for nematodes and the subcrown internodes were plated on agar also as described previously. In addition, the percent lesion of each subcrown internode was recorded as well as a subcrown lesion coloration rating based on the following scale: 0 = no discoloration; 1 = light brown; 2 = brown; 3 = dark brown; 4 = light black (gray); 5 = black.

## Root Incubation Studies

### Effect of Root Quantity

Wheat root systems were collected from *P. neglectus* greenhouse stock colonies (see p. 15). Seminal and adventitious roots were chopped into segments and uniformly mixed together. This root bulk was separated into 1.0 g,

2.5 g, and 5.0 g units (as determined by a Mettler Balance) and placed in 100 ml of water in 100 ml plastic centrifuge tubes, 250 ml of water in 250 ml Erlenmeyer flasks, and 500 ml of water in 500 ml Erlenmeyer flasks, respectively. These containers were then arranged in a randomized complete block design with three treatments and ten replications, and the roots incubated with aeration for three weeks. The roots were processed at weekly intervals in the same manner as previously described for obtaining inoculum.

Effect of Root/Water Ratio

Wheat root systems were collected directly from the Cormack field location and returned to the lab where only the seminal roots were clipped, chopped into segments, and mixed as before. These roots were divided into 0.5 g, 1.0 g, and 2.0 g units, all of which were placed in 100 ml of water in 100 ml plastic centrifuge tubes to be incubated for two weeks. A randomized complete block design with three treatments and ten replications was used. The contents of the tubes were processed at weekly intervals in the same manner as before.

# CHAPTER IV

## RESULTS AND DISCUSSION

### Greenhouse Interaction Study

Average 65-day fresh and dry forage weights for treatments inoculated with *P. neglectus* (Pn), *F. roseum* (Fr), and *F. solani* (Fs), each alone and in all pathogen combinations are compared with those of the control in Table I. Although the control outyielded all the inoculated treatments except Pn + Fr which yielded 100% of control, there were no statistically significant differences between fresh forage weights. The lowest fresh forage weight occurred with the nematode alone at 90% of control. The Pn + Fr, Pn + Fs, and Pn + Fr + Fs treatments all outyielded the treatments inoculated with nematodes alone or fungi alone. This data suggests an initial inhibition or competition between the nematodes and fungi involved in this study.

The average dry forage weight for plants inoculated with *P. neglectus* and *F. roseum* together was again 100% of control, but the combination Pn + Fs, which yielded the lowest dry forage weight (91% of control), was significantly different from the control ($P=0.05$). This approached an additive effect of the nematode (5% reduction) and the

TABLE I

INFLUENCE OF *PRATYLENCHUS NEGLECTUS*, *FUSARIUM ROSEUM* 'ACUMINATUM', AND *FUSARIUM SOLANI* EACH ALONE AND IN COMBINATION ON FORAGE YIELD OF TAM 101 WHEAT PLANTS UNDER GREENHOUSE CONDITIONS 65 DAYS AFTER INOCULATION[a]

| Inoculum Treatment[b] | Average Forage Fresh Wt g | % of Control | Average Forage Fresh Wt g | % of Control |
|---|---|---|---|---|
| 1) Control | 1.271 | 100 | 0.495 | 100 |
| 2) Pn | 1.148 | 90 | 0.469 | 95 |
| 3) Fr | 1.160 | 91 | 0.471 | 95 |
| 4) Pn + Fr | 1.270 | 100 | 0.496 | 100 |
| 5) Fs | 1.181 | 93 | 0.464 | 94 |
| 6) Pn + Fs | 1.218 | 96 | 0.451 | 91 |
| 7) 1/2 Fr + 1/2 Fs | 1.184 | 93 | 0.458 | 93 |
| 8) Pn + 1/2 Fr + 1/2 Fs | 1.196 | 94 | 0.455 | 92 |
| LSD 10% | NS | | 0.034 | |
| 5% | NS | | 0.041 | |
| 1% | NS | | NS | |
| CV% | 13.435 | | 7.384 | |

[a]Averages are means of six replicates on a per pot basis.

[b]Pn = *Pratylenchus neglectus*, Fr = *Fusarium roseum*, Fs = *Fusarium solani*; inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both.

fungus (6% reduction). The combinations Fr + Fs and Pn + Fr + Fs also produced significant (P=Ø.1Ø) reductions in dry forage weight from that of the control. The presence of the nematode did not significantly reduce dry forage weight over the combination of both fungi.

Average terminal fresh root weights and fresh and dry forage weights at 142 days are listed in Table II, and indicate a completely different trend from that observed at 65 days. The combined effects of P. neglectus and F. roseum were synergistic at the termination of this experiment, resulting in the lowest weights for each type of plant data taken. The nematode alone caused a 5% reduction in root fresh weight, and 7% and 8% reductions in forage fresh and dry weights, respectively. Plants inoculated with F. roseum alone exhibited a fresh root weight reduction of 4% and forage fresh and dry weight reductions of 4% and 3%, respectively. However plants from the treatment Pn and Fr showed a 25% reduction in fresh root weight, a 14% reduction in fresh forage weight, and a 16% reduction in dry forage weight, all significantly different from non-inoculated controls (P=Ø.1Ø). Average seminal and adventitious root disease severity ratings (Table II) also indicate a synergistic interaction between the two pathogens. The increased amount of tissue discoloration that occurs with the combination Pn + Fr is unlikely to be simply an additive effect since the nematode alone has not been observed to cause discoloration of wheat root tissue. However, due to

TABLE II

INFLUENCE OF *PRATYLENCHUS NEGLECTUS*, *FUSARIUM ROSEUM* 'ACUMINATUM', AND *FUSARIUM SOLANI* EACH ALONE AND IN COMBINATION ON GROWTH OF TAM 101 WHEAT PLANTS UNDER GREENHOUSE CONDITIONS 142 DAYS AFTER INOCULATION[a]

| Inoculum Treatment[b] | Avg Root Fresh Wt g | % of Control | Avg Forage Fresh Wt g | % of Control | Avg Forage Dry Wt g | % of Control | Avg Disease Index[c] Seminal Roots | Avg Disease Index[c] Adventitious Roots |
|---|---|---|---|---|---|---|---|---|
| 1) Control | 5.383 | 100 | 1.971 | 100 | 1.106 | 100 | 1.0 | 0.8 |
| 2) Pn | 5.088 | 95 | 1.838 | 93 | 1.018 | 92 | 1.2 | 0.7 |
| 3) Fr | 5.143 | 96 | 1.893 | 96 | 1.074 | 97 | 0.8 | 1.0 |
| 4) Pn + Fr | 4.032 | 75 | 1.698 | 86 | 0.929 | 84 | 2.2 | 1.5 |
| 5) Fs | 4.619 | 86 | 1.846 | 94 | 1.042 | 94 | 1.0 | 0.8 |
| 6) Pn + Fs | 4.236 | 79 | 1.936 | 98 | 0.976 | 88 | 1.3 | 0.7 |
| 7) 1/2 Fr + 1/2 Fs | 5.425 | 101 | 2.008 | 102 | 1.055 | 95 | 1.0 | 0.7 |
| 8) Pn + 1/2 Fr + 1/2 Fs | 5.494 | 102 | 1.980 | 100 | 1.106 | 100 | 1.2 | 1.0 |
| LSD 10% | 1.046 | | 0.270 | | 0.119 | | 0.60 | 0.49 |
| 5% | 1.257 | | NS | | 0.143 | | 0.72 | 0.59 |
| 1% | NS | | NS | | NS | | 0.97 | 0.79 |
| CV% | 21.759 | | 14.611 | | 11.725 | | 50.920 | 56.013 |

[a]Averages are means of six replicates on a per pot basis.

TABLE II (Continued)

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[b]Pn = *Pratylenchus neglectus*, Fr = *Fusarium roseum*, Fs = *Fusarium solani*; inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both.

[c]Based on amount of necrosis where 0 = no necrosis and 4 = 100% necrosis.

background contamination, as indicated by the disease ratings of the control and Pn treatments, these results are inconclusive. Background fungal contamination other than that by the Fusarium spp. used in the test was not measured, but was assumed to be a uniform treatment effect.

The results in Table II also indicate that an additive interaction occurred between P. neglectus and F. solani in this experiment. A 21% reduction in fresh root weight and a 12% reduction in dry forage weight was obtained from plants inoculated with the combination Pn + Fs, while the Fs treatment yields were reduced by 14% and 6%, respectively. Fresh forage weights, however, failed to show this response. The Pn + Fs treatment exhibited a high moisture content and thus there were no statistically significant differences between the fresh forage weights of the control, Pn, Fs, and Pn + Fs treatments. There were likewise no statistically significant differences between the average seminal and adventitious root disease severity ratings of these treatments.

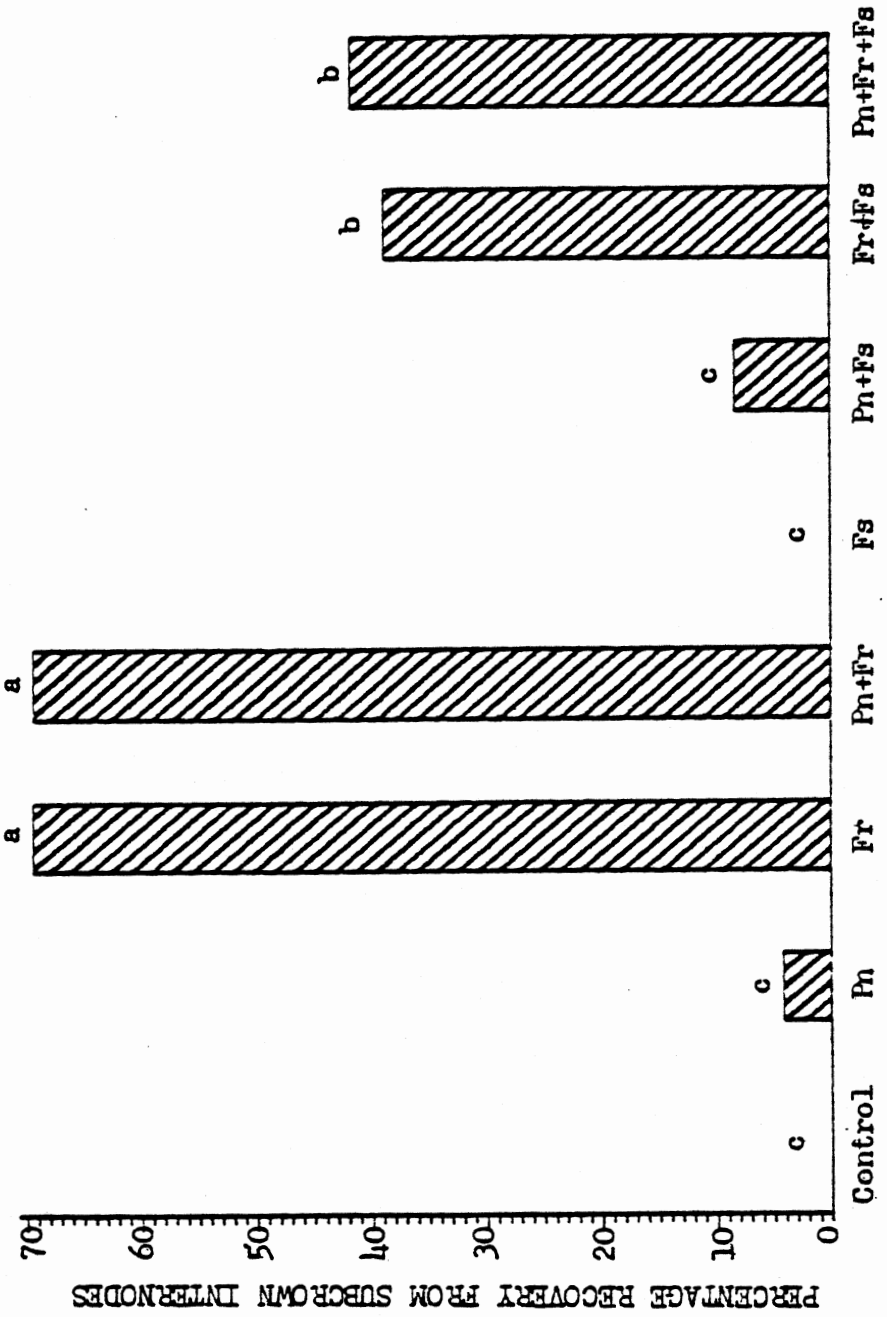
Neither the Fr + Fs combination nor the Pn + Fs combination reduced fresh root or fresh forage yields. A reduction in dry forage weight did occur with the Fr + Fs treatment, but it was not statistically significant. There were no significant differences between the control and Pn + Fr + Fs treatment in either dry forage weight or average root disease severity ratings.

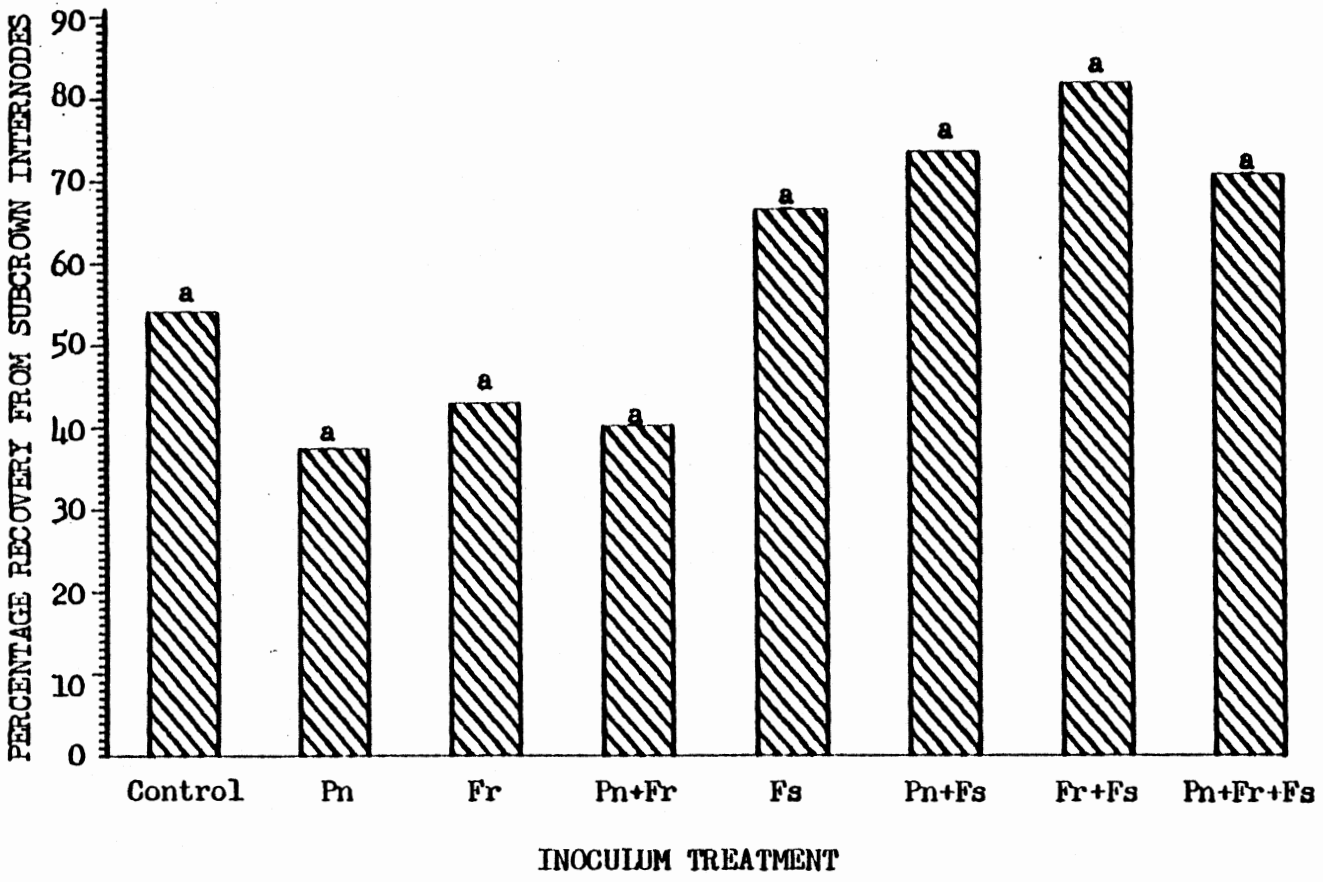
The failure of the Fr + Fs and Pn + Fr + Fs treatments to cause significant damage to the wheat plants in this experiment might be attributed to reduced infection resulting from the decreased inoculation rate (1/2) of the individual fungal components and/or the occurrence of antagonism or competition between the fungi and between the nematode and fungi when all three occur simultaneously.

Figure 2 shows the percent of infected subcrown internodes from which F. roseum was recovered. As expected, treatments that were inoculated with this fungus all showed significantly higher percent recoveries that uninoculated treatments. Recovery from Fr and Pn + Fr treatments was also significantly greater by statistical comparison than that from Fr + Fs and Pn + Fr + Fs treatments. This might also be due to the reduced inoculum rate of each fungus in the combination treatments or to inhibition or competition between the fungi.

The percent recovery of F. solani is depicted in Figure 3. A high rate of contamination, ranging from 38% to 54%, occurred in the uninoculated treatments. Splashing during watering, together with the rapid growth of F. solani may explain the spread of this fungus to uninoculated pots. The influence of this contamination cannot be assessed. It could have occurred at any period during the experiment. It is possible that the true interaction of P. neglectus and F. solani could have been 'masked' due to this contamination. In any case, it is noteworthy that plant data indicates that

Figure 2. Percentage Recovery of *Fusarium roseum* From Subcrown Internodes of Wheat Cultivar 'TAM 101' for Each Treatment Under Greenhouse Conditions. Bars having the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.
Pn = *Pratylenchus neglectus*.
Fr = *Fusarium roseum*.
Fs = *Fusarium solani*.
Inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both. Mean of six replicates. CV = 45.01%


PERCENTAGE RECOVERY FROM SUBCROWN INTERNODES
0
10
20
30
40
50
60
70
c
c
a
a
c
b
b
Control
Pn
Fr
Pn+Fr
Fs
Pn+Fs
Fr+Fs
Pn+Fr+Fs
INOCULUM TREATMENT


PERCENTAGE RECOVERY FROM SUBCROWN INTERNODES
90
80
70
60
50
40
30
20
10
0
a
a
a
a
a
a
a
a
Control
Pn
Fr
Pn+Fr
Fs
Pn+Fs
Fr+Fs
Pn+Fr+Fs
INOCULUM TREATMENT

the stronger interaction occurred between the nematode and the weaker fungal pathogen (F. roseum). No effect of the nematode upon fungal recovery was measured for any treatment.

Average numbers of P. neglectus recovered from nematode inoculated treatments at termination of the experiment are shown in Table III. Numbers of nematode recovered as contaminants in uninoculated treatments were insignificant, ranging from 92 to 133 total nematodes per pot. Statistically significant differences between the numbers of nematodes recovered from the nematode inoculated treatments were found only with soil populations. Numbers of nematodes per gram of roots and total nematodes per pot were not significantly different between treatments. However, the treatment Pn + Fr + Fs did yield the lowest total nematode population level. This suggests a slight suppression of nematode reproduction or survival. The Pn + Fr treatment yielded the highest population level of all the treatments. Although this occurrence was not statistically significant, it is supported by precedent for enhanced nematode reproduction during nematode-fungus interaction on other hosts (19, 32, 39). The fact that much higher numbers of P. neglectus were observed per gram of seminal than adventitious (crown) roots is also supported by previous observation (25).

It is apparent from these results that the synergistic interaction implicated between P. neglectus and F. roseum in

TABLE III

AVERAGE NUMBERS OF *PRATYLENCHUS NEGLECTUS* RECOVERED FROM NEMATODE INOCULATED TREATMENTS AT TERMINATION OF GREENHOUSE PATHOGENICITY EXPERIMENT[a]

| Inoculum Treatment[b] | Nemas per 100 ml of Soil | Nemas per gram of Seminal Roots | Nemas per gram of Adventitious Roots | Total Nemas[c] |
|---|---|---|---|---|
| Nematodes alone | 399 | 4601 | 2818 | 18,066 |
| Nematodes + *F. roseum* | 572 | 4149 | 3850 | 18,702 |
| Nematodes + *F. solani* | 411 | 5436 | 2996 | 17,269 |
| Nematodes + *F. roseum* + *F. solani* | 172 | 4404 | 2796 | 15,011 |
| LSD 5% | 293 | NS | NS | NS |
| 1% | 393 | NS | NS | NS |
| CV% | 64.24 | 56.96 | 63.86 | 46.67 |

[a]Averages are means of six replicates.

[b]1500 nematodes were originally added to each pot.

[c]Total nemas = (Nemas/100 ml soil * 9) + (nemas/g seminal roots * seminal root wt (g)) + (nemas/g adventitious roots * adventitious root wt (g)).

this study is not based to a significant degree upon an enhanced nematode reproduction rate or increased fungal infection. Further studies are needed to determine if this synergism results from a physiological change in the host.

## Field Experiments

### Root and Forage Yield

Average root and forage yields of field transplanted wheat plants inoculated with *P. neglectus* (Pn), *F. roseum* (Fr), and *F. solani* (Fs) each alone and in all pathogen combinations are recorded in Tables IV and V. The yields obtained by all parameters measured were highest in the Pn and Pn + Fr treatments and lowest in the Fr + Fs and Pn + Fr Fs treatments. These results are in direct conflict with the results of the greenhouse study and probably reflect the effects of biotic and abiotic interactions.

Average total fresh root weight for the treatments Pn and Fr were 210% of control and 90% of control, respectively while the Pn + Fr treatment yielded 148% of the fresh root weight of the control (Table IV). Average seminal root lengths for the same treatments were similar, but the combination Pn + Fr yielded a 30% reduction in adventitious root length from that of the control. The validity of this difference is questionable, however, as it is not statistically significant and may simply be due to variation. Average total dry forage weights (Table V)

TABLE IV

AVERAGE ROOT YIELD OF 'TAM 101' WHEAT PLANTS GROWN UNDER FIELD CONDITIONS FOR 187 DAYS FOLLOWING TREATMENT INOCULATIONS[a]

| Inoculum Treatment[b] | Avg Seminal Root Ln[c] cm | % of Control | Avg Adv Root Ln[c] cm | % of Control | Total Avg Root Fresh Wt g | % of Control | Avg Disease Index[d] Seminal Roots | Avg Disease Index[d] Adventitious Roots |
|---|---|---|---|---|---|---|---|---|
| 1) Control | 18.0 | 100 | 14.1 | 100 | 0.557 | 100 | 1.0 | 1.0 |
| 2) Pn | 22.3 | 124 | 13.7 | 95 | 1.170 | 210 | 1.5 | 1.0 |
| 3) Fr | 17.6 | 98 | 12.5 | 87 | 0.501 | 90 | 1.0 | 0.8 |
| 4) Pn + Fr | 22.0 | 122 | 10.1 | 70 | 0.827 | 148 | 1.2 | 0.6 |
| 5) Fs | 17.7 | 98 | 10.3 | 72 | 0.532 | 96 | 1.0 | 1.2 |
| 6) Pn + Fs | 20.5 | 114 | 9.6 | 67 | 0.623 | 112 | 1.2 | 0.8 |
| 7) 1/2 Fr + 1/2 Fs | 16.6 | 92 | 8.4 | 58 | 0.387 | 69 | 1.4 | 1.0 |
| 8) Pn + 1/2 Fr + Fs | 17.2 | 96 | 8.5 | 59 | 0.211 | 38 | 1.0 | 0.3 |
| LSD 10% | NS | | 5.6 | | 0.662 | | 0.5 | 0.4 |
| 5% | NS | | NS | | 0.795 | | NS | 0.5 |
| CV% | 33.2 | | 46.5 | | 103.838 | | 39.5 | 46.4 |

[a]Averages are means of five replicates on a per pot basis.

TABLE IV (Continued)

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[b]Pn = *Pratylenchus neglectus*, Fr = *Fusarium roseum*, Fs = *Fusarium solani*; inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both.

[c]Root lengths are averages of the longest seminal and adventitious roots for each plant.

[d]Based on amount of necrosis where 0 = no necrosis and 4 = 100% necrosis.

TABLE V

AVERAGE FORAGE YIELD OF 'TAM 101' WHEAT PLANTS GROWN UNDER FIELD CONDITIONS FOR 187 DAYS FOLLOWING TREATMENT INOCULATIONS[a]

| Inoculum Treatment[b] | Avg No. Surviving Plants | Avg No. Tillers | Avg Leaf Dry Wt g | % of Control | Avg Stem Dry Wt g | % of Control | Avg Total Dry Forage Wt g | % of Control |
|---|---|---|---|---|---|---|---|---|
| 1) Control | 2.6 | 6.8 | 0.463 | 100 | 0.677 | 100 | 1.139 | 100 |
| 2) Pn | 3.0 | 9.0 | 0.758 | 164 | 1.755 | 259 | 2.513 | 100 |
| 3) Fr | 3.0 | 5.0 | 0.330 | 71 | 0.755 | 112 | 1.084 | 95 |
| 4) Pn + Fr | 3.4 | 9.0 | 0.840 | 181 | 1.518 | 224 | 2.358 | 207 |
| 5) Fs | 3.8 | 6.6 | 0.444 | 96 | 0.905 | 134 | 1.349 | 118 |
| 6) Pn + Fs | 2.8 | 5.2 | 0.325 | 70 | 0.850 | 126 | 1.175 | 103 |
| 7) 1/2 Fr + 1/2 Fs | 3.0 | 3.8 | 0.269 | 58 | 0.317 | 47 | 0.586 | 51 |
| 8) Pn + 1/2 Fr + 1/2 Fs | 2.2 | 4.0 | 0.225 | 49 | 0.270 | 40 | 0.495 | 43 |
| LSD 10% | 1.1 | 3.8 | 0.489 | | 0.923 | | 1.382 | |
| 5% | 1.3 | 4.6 | 0.588 | | 1.109 | | 1.660 | |
| CV% | 35.1 | 58.3 | 100.641 | | 98.855 | | 97.336 | |

[a]Averages are means of five replicates on a per pot basis.

[b]Pn = *Pratylenchus neglectus*, Fr = *Fusarium roseum*, Fs = *Fusarium solani*; inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both.

resembled fresh root weights in that the treatments Pn, Fr, and Pn + Fr yielded 221%, 95%, and 207% of control, respectively. Average numbers of tillers and average leaf and stem dry weights also conformed to the pattern obtained from total root and forage yields. Statistical analysis of these data revealed that none of the differences observed between the treatments; Pn, Fr, Pn + Fr, and the control were significantly different.

Average root and forage yields likewise revealed no statistically significant differences between controls and the treatments; Pn, Fs, and Pn + Fs. The treatments Fr + Fs, and Pn + Fr + Fs yielded the lowest average root and forage yields observed, but these were only significantly different from the treatment Pn, which exhibited the highest yields.

Figure 4 shows the percent recovery of F. roseum from infected subcrown internodes. All treatments inoculated with this fungus, with the exception of the Pn + Fr + Fs treatment, were significantly greater (P=0.05) than treatments not inoculated with F. roseum, exhibiting recoveries of 35% to 38%. Percent recovery of F. solani is shown in Figure 5. Although all treatments inoculated with F. solani showed higher recoveries than uninoculated treatments only the treatment Pn + Fs was significantly greater (P=0.05) than all the treatments without F. solani. Background contamination included several additional Fusarium species from the field soil which appeared to be

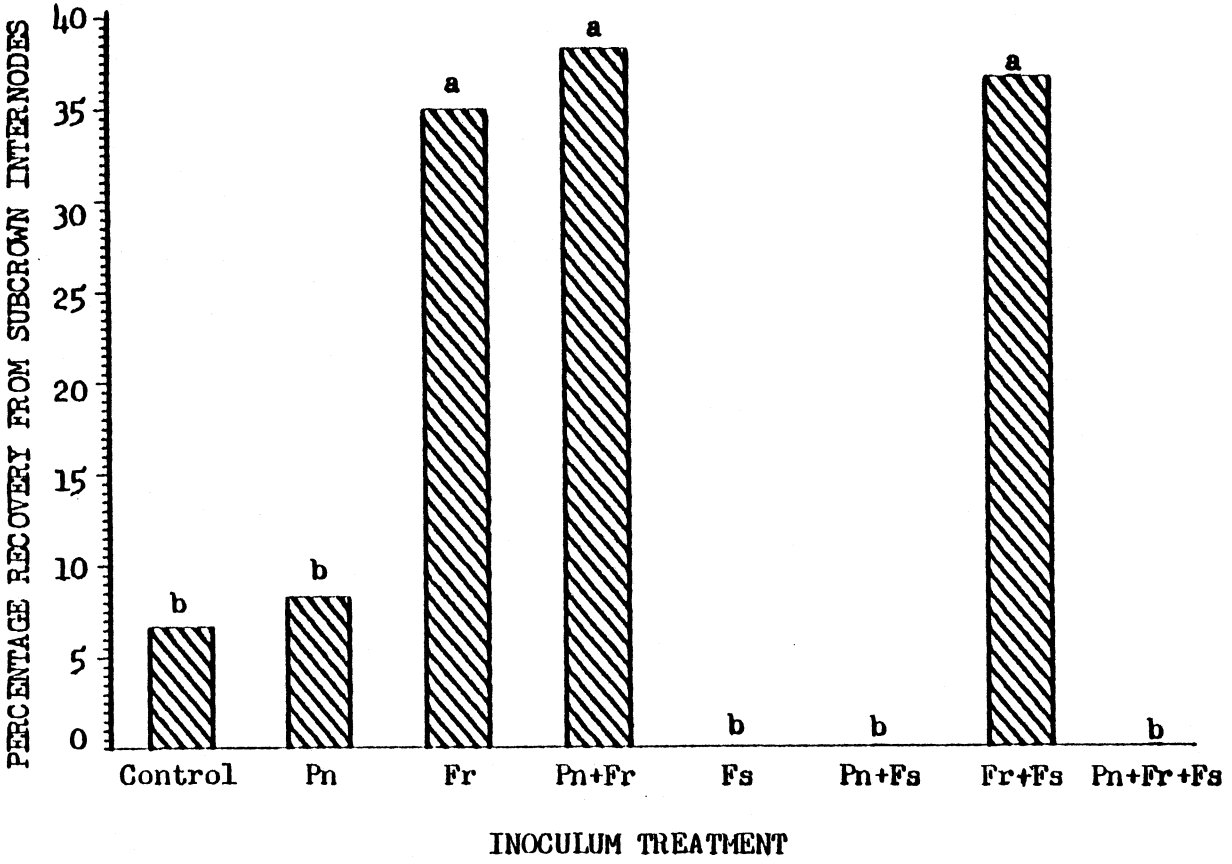
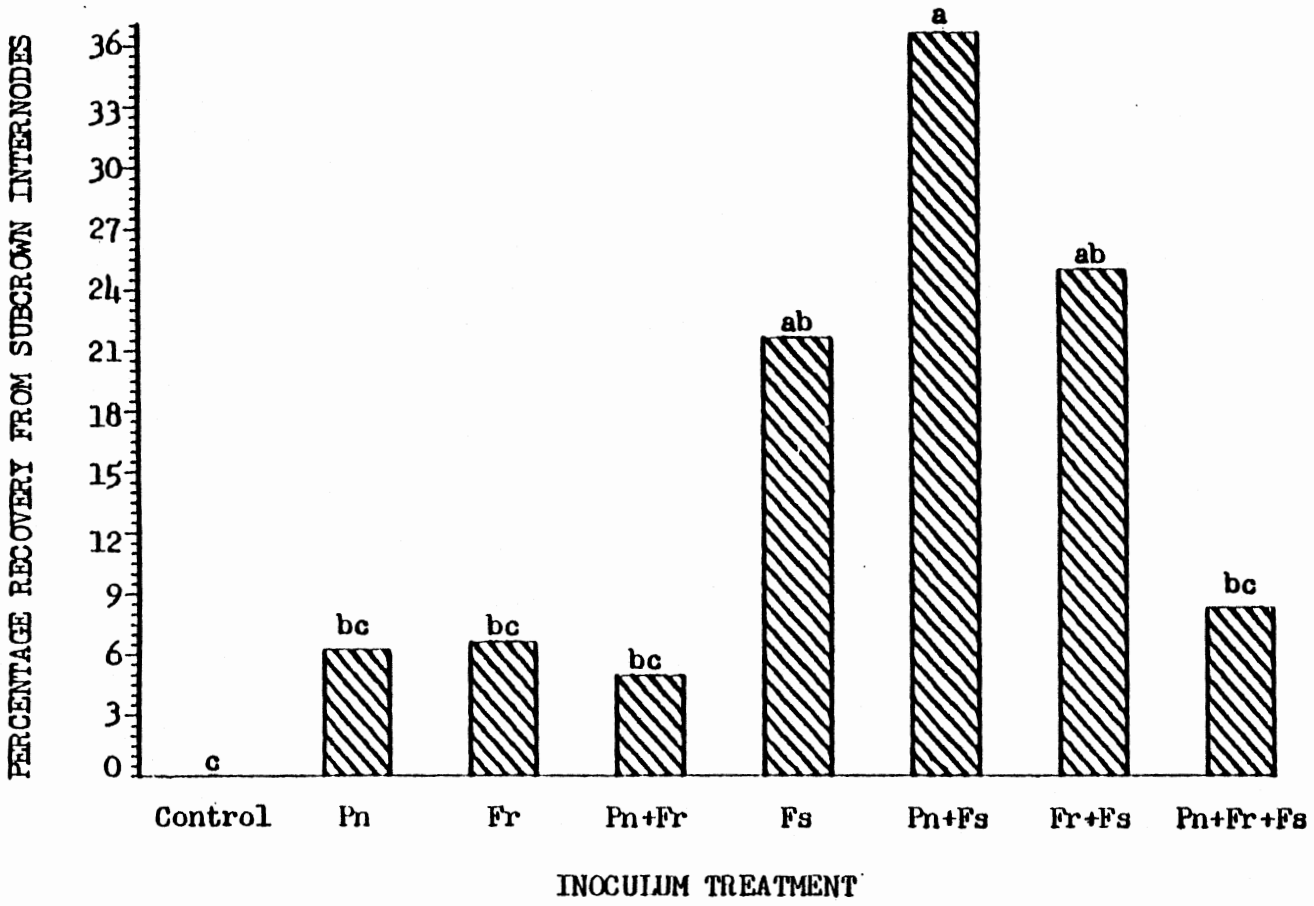

PERCENTAGE RECOVERY FROM SUBCROWN INTERNODES
40
35
30
25
20
15
10
5
0
a
a
a
b
b
b
b
b
Control
Pn
Fr
Pn+Fr
Fs
Pn+Fs
Fr+Fs
Pn+Fr+Fs
INOCULUM TREATMENT

Figure 5. Percentage Recovery of *Fusarium solani* From Subcrown Internodes of Wheat Cultivar 'TAM 101' for Each Treatment Under Field Conditions. Bars having the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.
Pn = *Pratylenchus neglectus*.
Fr = *Fusarium roseum*.
Fs = *Fusarium solani*.
Inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both. Mean of five replicates. CV = 109.72%


PERCENTAGE RECOVERY FROM SUBCROWN INTERNODES
0
3
6
9
12
15
18
21
24
27
30
33
36
c
bc
bc
bc
ab
a
ab
bc
Control
Pn
Fr
Pn+Fr
Fs
Pn+Fs
Fr+Fs
Pn+Fr+Fs
INOCULUM TREATMENT

uniformly distributed across all treatments. It should be noted that the percent recoveries of F. roseum and F. solani do not explain the plant root and forage yields discussed previously.

Average numbers of P. neglectus recovered from all treatments are listed in Table VI. Total nematode counts were low across all treatments, with no statistically significant differences between nematode inoculated vs. uninoculated treatments. The combination treatment Pn + Fr + Fs did yield significantly higher numbers of nematodes per gram of seminal roots than any of the nematode uninoculated treatments (P=Ø.1Ø). The treatment Pn + Fr significantly outyielded all other treatments (P=Ø.Ø5) in numbers of nematodes per gram of adventitious roots. The data also revealed that the nematode-fungus combination treatments all yielded higher numbers of nematodes per gram of seminal roots and higher total nematode populations than the treatment inoculated with nematodes alone. This substantiates the observation that enhanced nematode reproduction occurred under greenhouse conditions for the treatment Pn + Fr. However, these data, like the fungal recovery data, do not explain the results obtained from plant measurements.

Although the results of this study were unexpected, they may be due to the predominating influence of abiotic conditions. For example, in this study the reduced area of

TABLE VI

AVERAGE NUMBERS OF *PRATYLENCHUS NEGLECTUS* RECOVERED FROM NEMATODE INOCULATED TREATMENTS AT TERMINATION OF FIELD PATHOGENICITY EXPERIMENT[a]

| Inoculum Treatment[b] | Nemas per 100 ml of Soil | Nemas per gram of Seminal Roots | Nemas per gram of Adventitious Roots | Total Nemas[c] |
|---|---|---|---|---|
| **Nematode Inoculated Treatments** | | | | |
| Nematodes alone | 0 | 66 | 3 | 13 |
| Nematodes + *F. roseum* | 1 | 114 | 15 | 27 |
| Nematodes + *F. solani* | 0 | 193 | 0 | 28 |
| Nematodes + *F. roseum* + *F. solani* | 0 | 272 | 0 | 22 |
| **Non-Nematode Inoculated Treatments** | | | | |
| Control | 0 | 7 | 0 | 1 |
| *F. roseum* | 1 | 9 | 0 | 2 |
| *F. solani* | 0 | 61 | 0 | 12 |
| *F. roseum* + *F. solani* | 0 | 0 | 0 | 0 |
| LSD 10% | 1 | 192 | 7 | NS |
| 5% | 1 | 231 | 8 | NS |
| CV% | 455.13 | 208.44 | 252.58 | 213.93 |

[a]Averages are means of five replicates.

[b]1500 nematodes were originally added to each pot.

[c]Total nemas = (nemas/100 ml soil) + (nemas/g seminal roots * seminal root wt (g)) + (nemas/g adventitious roots * adventitios root wt (g)).

inoculated plants would be expected to correspondingly reduce the transpiration area (and rate) of the plants transplanting. This reduction in transpiration rate may have increased the survival potential of the plants under the moisture stress conditions which prevailed during this study.

Grain Yield

No significant differences occurred in the average total seed weight per treatment (Table VII) of field grown wheat plants. However, every treatment inoculated with nematodes and/or fungi outyielded the control, with the combination Pn + Fr + Fs yielding the highest grain weight (221% of control). Results were similar when average seed weights were derived on a per plant basis. The Pn + Fs treatment produced the highest grain yield on a per plant basis (278% of control) and was significantly different from the control, Pn, and Fs treatments (P=Ø.1Ø). The Pn + Fs treatment also had the lowest average number of surviving plants and was the only treatment that was significantly different from the control (P=Ø.1Ø). No treatment differed statistically from the control in the average number of tillers and heads produced per treatment, although significant differences did occur between the pathogen treatments.

The results of this experiment parallel those of the previously discussed field experiment and tend to reinforce

TABLE VII

GRAIN YIELD OF TAM 101 WHEAT PLANTS GROWN UNDER FIELD CONDITIONS AS INFLUENCED BY *PRATYLENCHUS NEGLECTUS* AND *FUSARIUM* SPP. 221 DAYS AFTER TREATMENT[a]

| Inoculum Treatment[b] | Avg No. Surviving Plants | Avg No. Tillers per Trt | Avg No. Heads per Trt | Avg Seed Wt[c] per Trt g | % of Control | Avg Seed Wt[c] per Plant g | % of Control |
|---|---|---|---|---|---|---|---|
| 1) Control | 3.5 | 4.7 | 2.8 | 0.3180 | 100 | 0.0941 | 100 |
| 2) Pn | 3.6 | 4.4 | 3.2 | 0.3799 | 119 | 0.0950 | 101 |
| 3) Fr | 2.7 | 5.2 | 2.8 | 0.6152 | 193 | 0.1789 | 190 |
| 4) Pn + Fr | 3.2 | 4.5 | 2.3 | 0.4486 | 141 | 0.1394 | 148 |
| 5) Fs | 3.1 | 3.4 | 2.9 | 0.3676 | 116 | 0.0989 | 105 |
| 6) Pn + Fs | 2.3 | 4.4 | 3.2 | 0.5657 | 178 | 0.2612 | 278 |
| 7) 1/2 Fr + 1/2 Fs | 3.3 | 5.3 | 2.8 | 0.5536 | 174 | 0.1498 | 159 |
| 8) Pn + 1/2 Fr + 1/2 Fs | 3.5 | 6.3 | 3.8 | 0.7041 | 221 | 0.2024 | 214 |
| LSD 10% | 1.2 | 2.1 | 1.5 | NS | | 0.1422 | |
| 5% | NS | 2.5 | NS | NS | | NS | |
| CV% | 38.5 | 44.9 | 52.6 | 94.9047 | | 97.3981 | |

[a]Averages are means of six replicates.

[b]Pn = *Pratylenchus neglectus*, Fr = *Fusarium roseum*, Fs = *Fusarium solani*; inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both.

[c]Seed weights were taken after seeds were air-dried for one week.

the conclusion that, although much of the data is obviously aberrant due to variation, abiotic factors where a major influence.

Table VIII summarizes the grain yields obtained from wheat plants grown under greenhouse conditions, but in pots with field soil. Many of the data patterns observed in the previous field experiments were repeated in this test. The combination treatment Pn + Fr exhibited the highest average seed weights per treatment and per plant, but these were not significantly different from the control. However, the combination Pn + Fs exhibited the lowest average seed weight per treatment and per plant, but still was not significantly different from the control in either instance. Average seed weights per plant for the treatments; Pn, Fs, and Pn + Fs were reduced from the control by 14%, 27%, and 35%, respectively. This approaches the additive interaction observed between these two pathogens in the previous greenhouse experiment, although this conclusion is not confirmed by the average seed weights per treatment. No significant differences occurred between the average seed weights of the treatments; Fr + Fs, Pn + Fr + Fs, and the control. The average numbers of plants, tillers, and heads per treatment showed low numbers of each of these data points for the control treatment. This may partly explain the relatively low yields encountered with the control in this study.

TABLE VIII

GRAIN YIELD OF 'TAM 101' WHEAT PLANTS GROWN UNDER GREENHOUSE CONDITIONS AS INFLUENCED BY *PRATYLENCHUS NEGLECTUS* AND *FUSARIUM* SPP. 257 DAYS AFTER TREATMENT[a]

| Inoculum Treatment[b] | Nemas per 100 ml Soil | Avg No. Surviving Plants | Avg No. Tillers per Trt | Avg No. Heads per Trt | Avg Seed Wt[c] per Trt g | % of Control | Avg Seed Wt[c] per Plant g | % of Control |
|---|---|---|---|---|---|---|---|---|
| 1) Control | 8 | 2.3 | 2.5 | 2.3 | 0.5979 | 100 | 0.2451 | 100 |
| 2) Pn | 240 | 3.5 | 3.8 | 3.5 | 0.7632 | 128 | 0.2101 | 86 |
| 3) Fr | 0 | 3.0 | 3.5 | 2.5 | 0.5337 | 89 | 0.1779 | 73 |
| 4) Pn + Fr | 112 | 2.8 | 3.0 | 2.8 | 0.9389 | 157 | 0.3536 | 144 |
| 5) Fs | 0 | 3.7 | 4.0 | 3.7 | 0.6363 | 106 | 0.1794 | 73 |
| 6) Pn + Fs | 167 | 3.3 | 4.3 | 2.5 | 0.5004 | 84 | 0.1583 | 65 |
| 7) 1/2 Fr + 1/2 Fs | 3 | 3.0 | 3.5 | 2.3 | 0.7249 | 121 | 0.2416 | 99 |
| 8) Pn + 1/2 Fr + 1/2 Fs | 43 | 2.5 | 3.8 | 2.0 | 0.5923 | 99 | 0.3191 | 130 |
| LSD 10% | 95 | 0.7 | 1.3 | 1.0 | 0.3516 | | 0.1393 | |
| 5% | 114 | 0.9 | 1.6 | 1.2 | 0.4223 | | 0.1674 | |
| 1% | 153 | 1.2 | NS | 1.6 | NS | | NS | |
| CV% | 110.75 | 20.7 | 31.6 | 31.5 | 44.4604 | | 49.1023 | |

[a]Averages are means of four replicates.

TABLE VIII (Continued)

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[b]Pn = *Pratylenchus neglectus*, Fr = *Fusarium roseum*, Fs = *Fusarium solani*; inoculum levels were 1500 nematodes per pot or 400 fungal propagules per gram of soil or both.

[c]Seed weights were taken after seeds were air-dried for one week.

Final nematode populations were measured for this study by extracting the nematodes from 100 sub-samples and are included in Table VIII for comparison with nematode recovery in the field experiment discussed previously. Nematode counts were low (43 to 240 nemas/100 ml of soil in nematode inoculated treatments), considering the fact that all nematodes should have evacuated the roots by this time, but were considerably higher than the populations encountered under field conditions (possibly due to dispersal in the field). All nematode inoculated treatments except Pn + Fr + Fs exhibited significantly higher nematode populations than uninoculated treatments (P=0.10). The treatment Pn showed a higher nematode count than the other nematode inoculated treatments, while the treatment Pn + Fr + Fs showed the lowest count. The fact that low populations of nematodes were recovered from studies using field soil instead of sterilized soil suggests that a factor in the field soil may have inhibited nematode reproduction. Further research is needed to verify this hypothesis.

The data collected in this study were highly variable and the results which appear aberrant when compared to the more carefully controlled greenhouse study, may be due to this variation. It seems likely that both moisture stress variation due to field soil characteristics, and background fungal populations, which could not be quantified with the procedures used, contributed to the erratic nature of the data obtained.

## Drouth Stress Experiment

The effects of high and low moisture stress upon the disease interaction of *P. neglectus* (Pn) and *F. roseum* (Fr) on wheat is shown in Table IX. In the low moisture stress series, the treatments Pn and Fr each reduced the average fresh root weight by 2%, while the combination Pn + Fr yielded 109% of the average fresh root weight of the control. Fresh forage weights for the treatments; Pn, Fr, and Pn + Fr were reduced by 7%, 17%, and 4%, respectively. Average dry forage weights showed similar reductions. Fresh and dry forage weights for the treatment Fr were significantly different from those of the control ($P=0.05$).

In the high moisture stress series, the treatments; Pn, Fr, and Pn + Fr stimulated root growth over that of the control by 6%, 2%, and 8%, respectively. Average fresh forage weights, however, were reduced 5%, 11%, and 8% by the treatments; Pn, Fr, and Pn + Fr, respectively. Similar reductions occurred with the dry forage weights. Statistically significant differences in root and forage weights were not found between treatments under the high moisture stress regime.

Average fresh root weight was increased by the treatment Pn + Fr, under both moisture stress series. This suggests that a competitive interaction occurred between *P. neglectus* and *F. roseum* under the conditions of this test. Each pathogen alone caused a reduction in the fresh and dry

TABLE IX

EFFECT OF *PRATYLENCHUS NEGLECTUS* AND *FUSARIUM ROSEUM* 'ACUMINATUM' EACH ALONE AND IN COMBINATION ON GROWTH OF 'TAM 101' WHEAT PLANTS AS INFLUENCED BY SOIL MOISTURE UNDER GREENHOUSE CONDITIONS[a]

| Inoculum Treatment[b] | Avg Root Fresh Wt g | % of Low Stress Control | Avg Forage Fresh Wt g | % of Low Stress Control | Avg Forage Dry Wt g | % of Low Stress Control | Avg % Lesion per Subcrown | Avg Color Index per Plant[c] |
|---|---|---|---|---|---|---|---|---|
| | | | Low Moisture Stress Series[d] | | | | | |
| 1) Control | 3.762 | 100 | 0.603 | 100 | 0.545 | 100 | 13.1 | 0.9 |
| 2) Pn | 3.675 | 98 | 0.562 | 93 | 0.502 | 92 | 8.1 | 0.4 |
| 3) Fr | 3.674 | 98 | 0.503 | 83 | 0.448 | 82 | 12.9 | 0.7 |
| 4) Pn + Fr | 4.119 | 109 | 0.580 | 96 | 0.522 | 96 | 10.1 | 0.4 |
| | | | High Moisture Stress Series[e] | | | | | |
| 1) Control | 2.472 | 66 | 0.494 | 82 | 0.454 | 83 | 18.1 | 1.3 |
| 2) Pn | 2.630 | 70 | 0.471 | 78 | 0.427 | 78 | 11.4 | 0.8 |
| 3) Fr | 2.526 | 67 | 0.441 | 73 | 0.403 | 74 | 15.5 | 1.1 |
| 4) Pn + Fr | 2.678 | 71 | 0.456 | 76 | 0.414 | 76 | 9.7 | 0.5 |
| LSD 5% | 0.643 | | 0.083 | | 0.074 | | 6.6 | 0.6 |
| 1% | 0.863 | | 0.112 | | 0.100 | | 8.9 | 0.8 |
| CV% | 14.030 | | 11.287 | | 11.145 | | 37.3 | 57.9 |

TABLE IX (Continued)

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[a]Experiment was terminated after 40 days; averages are means of four replicates on a per pot basis; 10 seeds were planted per pot.

[b]Pn = Pratylenchus neglectus, Fr = Fusarium roseum; inoculum levels were 3750 nematodes per pot or 400 propagules per gram of soil or both.

[c]Based on subcrown lesion coloration where 0 = no discoloration and 5 = black.

[d]Low series consisted of an alternating cycle of soil saturation and -5 ± 3 bars.

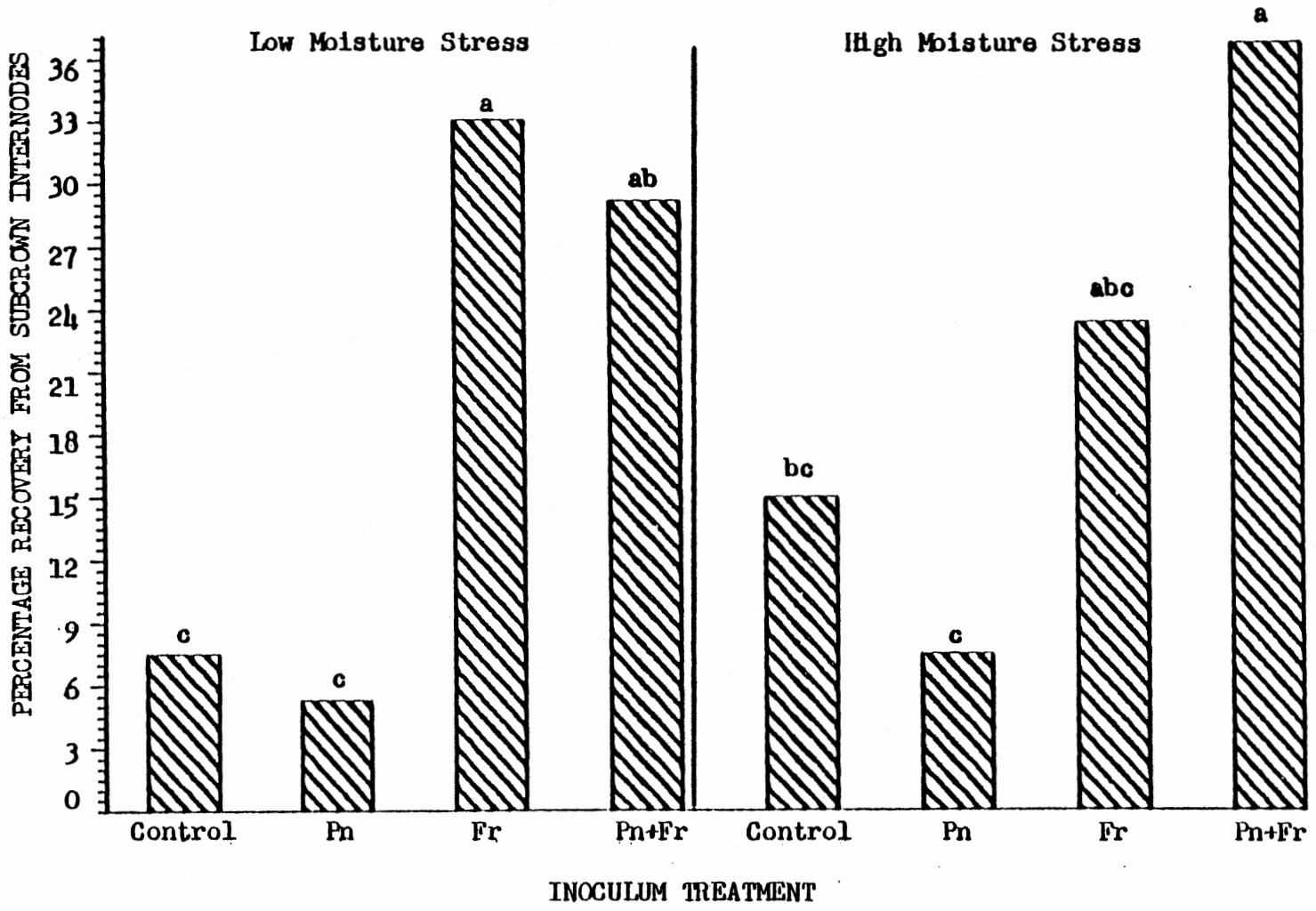
[e]High series consisted of an alternating cycle of soil saturation and -20 ± 5 bars.

forage weights under both moisture regimes, but the combined effect of both pathogens was less than additive in each case. This again indicates a competitive interaction between the nematode and fungus. However, the results obtained from the interaction Pn + Fr might instead be due to limited substrate as a result of moisture stress, such that the true interaction may have been "masked" by the more severe influence of the moisture stress.

Average lesion percentages per subcrown internode and average lesion color indexes per plant are included in Table IX. These data indicate that a relatively high rate of background fungal contamination may have existed since treatments inoculated with F. roseum exhibited lower lesion percentages and color indexes than the uninoculated control. It should be noted, however, that all the lesion percentages and color indexes are actually quite low, which may be a result of the short test duration (40 days) or an inhibition of disease expression due to moisture stress. The latter explanation is unlikely, as the higher moisture stress series exhibited the higher lesion percentages and color indexes.

Percent recovery of F. roseum from infected subcrown internodes is shown in Figure 6. All treatments originally inoculated with the fungus were observed to have higher percent recoveries (23%-37%) than those of the uninoculated treatments (5%-15%), but only the treatment Fr in the low moisture stress series and the combination Pn + Fr in the

Figure 6. Percentage Recovery of Fusarium roseum From Subcrown Internodes of Wheat Cultivar 'TAM 101' for Each Treatment Under Moisture Stress Conditions. Bars having the same letter are not significantly different (P=0.05) according to Duncan's multiple range test. Low moisture stress series consisted of an alternating cycle of soil saturation and -5 ± bars. High moisture stress series consisted of an alternating cycle of soil saturation and -20 ± bars.
Pn = Pratylenchus neglectus.
Fr = Fusarium roseum.
Inoculum levels were 3750 nematodes per pot or 400 fungal propagules per gram of soil or both. Mean of four replicates. CV = 57.70%


Low Moisture Stress
High Moisture Stress
PERCENTAGE RECOVERY FROM SUBCROWN INTERNODES
0
3
6
9
12
15
18
21
24
27
30
33
36
c
c
a
ab
bc
c
abc
a
Control
Pn
Fr
Pn+Fr
Control
Pn
Fr
Pn+Fr
INOCULUM TREATMENT

high moisture stress series were significantly different from all uninoculated treatments (P=Ø.Ø5). Contamination of F. roseum in uninoculated treatments is likely to have resulted from splashing during watering.

Table X shows the average numbers of P. neglectus recovered from nematode inoculated treatments at termination of the experiment. Nematode contamination in uninoculated treatments was negligible. Statistical analysis revealed no significant differences between the final total nematode population of the treatment Pn and that of the treatment Pn + Fr in either moisture stress series. The low moisture stress treatment Pn + Fr did yield a significantly higher total nematode population than either nematode inoculated treatment in the high moisture stress series (P=Ø.1Ø). The treatment Pn also yielded significantly higher numbers of nematodes per gram of roots under both moisture regimes than did the treatment Pn + Fr (P=Ø.1Ø). These differences appear to be a function of the differences in root mass among treatments. For instance, the reduced total nematode populations observed in the high moisture stress series (Table X) coincide with reduced root yields (limited substrate) for the same treatments (Table IX) as compared with those of the low moisture stress series.

The results of this experiment indicate that P. neglectus and F. roseum may have been competitive under the stress conditions of this study. Previous researchers have reported that Fusarium spp. cause the greatest damage

TABLE X

AVERAGE NUMBERS OF *PRATYLENCHUS NEGLECTUS* RECOVERED FROM NEMATODE INOCULATED TREATMENTS AT TERMINATION OF SOIL MOISTURE EXPERIMENT[a]

| Inoculum Treatments[b] | Nemas per 100 ml of Soil | Nemas per gram of Roots[c] | Total Nemas[d] |
|---|---|---|---|
| Low Moisture Stress Series[e] | | | |
| Nematodes alone | 16 | 168 | 748 |
| Nematodes + *F. roseum* | 28 | 134 | 759 |
| High Moisture Stress Series[f] | | | |
| Nematodes alone | 18 | 176 | 607 |
| Nemaotdes + *F. roseum* | 29 | 136 | 608 |
| LSD 10% | 13 | 34 | 149 |
| 5% | NS | 41 | NS |
| CV% | 95.43 | 37.05 | 36.64 |

[a]Averages are means of four replicates.

[b]Inoculum levels were 3750 nematodes per pot or 400 fungal propagules per gram of soil or both.

[c]Only seminal roots wee present at termination of experiment.

[d]Total nemas = (nemas/100 ml * 8) + (nemas/g roots * root wt (g)).

[e]Low series consisted of an alternating cycle of soil saturation and -5 ± bars.

[f]High series consisted of an alternating cycle of soil saturation and -20 ± 5 bars.

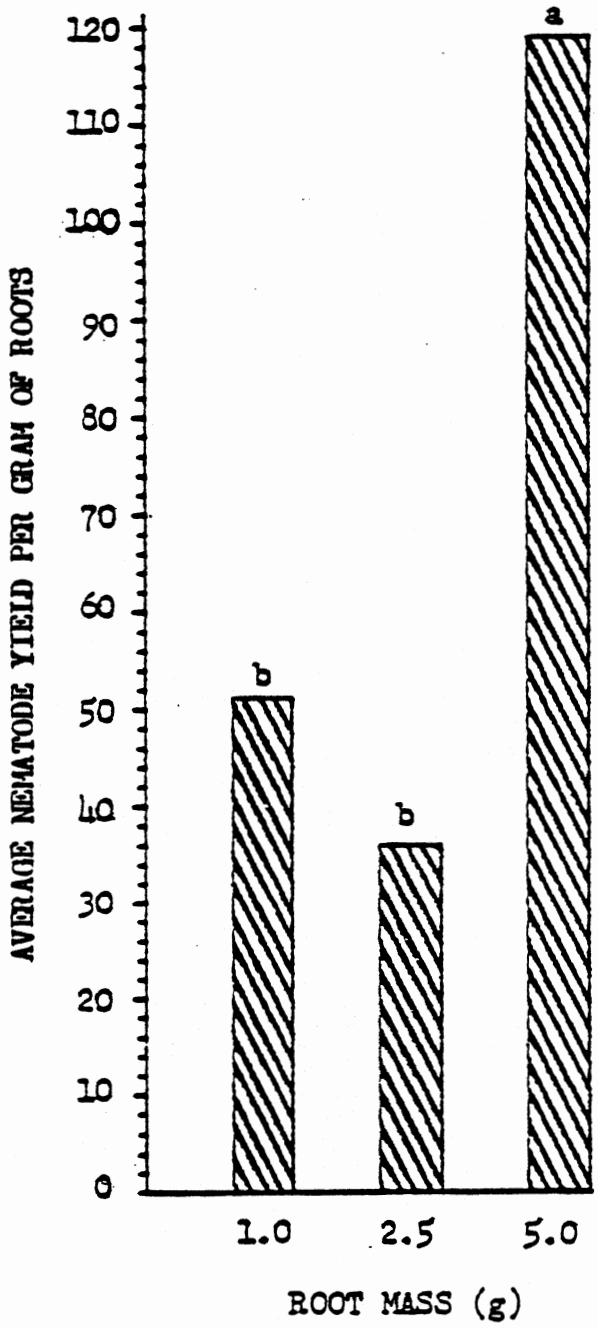
to wheat plants under extremely dry soil moisture conditions (13, 58), and this was substantiated by the present study. However, short test duration and excessive moisture stress may have inhibited the complete interaction development between the pathogens and their host plants.

## Root Incubation Studies

The effect of root quantity on extraction of *P. neglectus* by root incubation is depicted in Figure 7. The incubation of 5 g of roots significantly outyielded (P=Ø.Ø5) the other two treatments in numbers of nematodes recovered per gram of roots. This is an unexpected result as all treatments involved the incubation of 1 g of roots per each 1ØØ ml of water, and since there was not a significant difference between the numbers of nematodes extracted per gram of roots when 1 g vs. 2-1/2 g of roots were incubated. The data indicates that a critical mass of roots may be required for optimum nematode recovery. However, this result may have been caused by an inadvertent bias due to experimental error in chopping of the roots. For example, the 1 g and 2-1/2 g root samples received more chopping for weight adjustment and thus suffered more root disturbance than did the 5 g root samples.

No significant differences were observed between treatments in the percent of total nematodes recovered during each week's root incubation. This indicates that

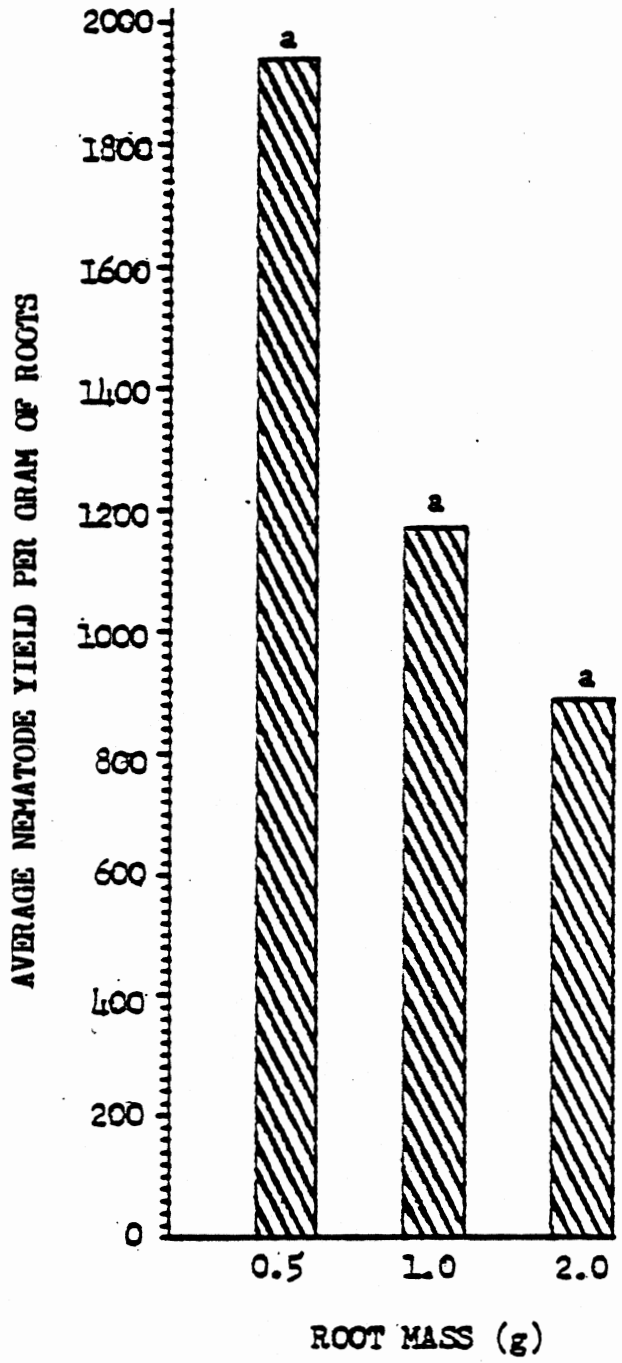
Figure 7. Average Nematode Yield Per Gram of Roots as Influenced by Root Mass. Each root mass was incubated in 100 ml water per each 1 g roots. Bars having the same letter are not significantly different (P=0.05) according to Duncan's multiple range test. Mean of ten replicates. CV = 83.44%


AVERAGE NEMATODE YIELD PER GRAM OF ROOTS
120
110
100
90
80
70
60
50
40
30
20
10
0
a
b
b
1.0
2.5
5.0
ROOT MASS (g)

root mass has no influence on nematode extraction by root incubation as long as the ratio of root mass to water volume is maintained at 1:100.

Figure 8 depicts the results obtained from a second root incubation test which was conducted to determine the effects of different ratios of root mass to water volume on nematode recovery, with 1 g of roots in 100 ml of water as the standard. Although none of the treatments were significantly different, statistically, in the numbers of nematodes recovered on a per gram of root basis, the highest recovery rate was produced when 0.5 g of roots were incubated in 100 ml of water and the lowest recovery rate was produced when 2 g of roots were incubated in 100 ml of water. The standard yielded an intermediate level of nematodes per gram of roots. These results indicate that nematode recovery is reduced when the ratio of root mass to water volume exceeds 1:100 and that the most efficient recovery occurs when the root mass to water volume ratio is less than 1:100.

Figure 8. Average Nematode Yield Per Gram of Roots as Influenced by the Ratio of Root Mass (g) to Water Volume (ml). Each root mass was incubated in 100 ml water. Bars having the same letter are not significantly different (P=0.05) according to Duncan's multiple range test. Mean of ten replicates. CV = 106.08%.


AVERAGE NEMATODE YIELD PER GRAM OF ROOTS
2000
1800
1600
1400
1200
1000
800
600
400
200
0
a
a
a
0.5
1.0
2.0
ROOT MASS (g)

# CHAPTER V

# SUMMARY AND CONCLUSIONS

## Summary

*Pratylenchus neglectus* (Pn), *F. roseum* 'Acuminatum' (Fr) and *F. solani* (Fs), each alone and in all pathogen combinations were inoculated to wheat in a greenhouse study and two companion field experiments (one for forage yield, the other for grain yield). In addition, an experiment was conducted in which the above pathogens were inoculated to wheat grown in field soil, but under greenhouse conditions. Pathogen treatments consisted of 400 propagules (conidia) per gram of soil or 1500 nematodes per pot or both. Controls received no pathogen treatments. The treatments were compared on the basis of plant forage and root weights, grain yield, final nematode populations, and percentage recovery of *Fusarium* isolates from subcrown internodes. From these data, the nematode-fungus combinations were evaluated for the type of interaction.

The results of the greenhouse study indicated the existence of a synergistic interaction between *P. neglectus* and *F. roseum* and an additive interaction between *P. neglectus* and *F. solani*. The combination treatments

Fr + Fs and Pn + Fr + Fs did not exhibit any significant differences in plant data from the control. Fusarium solani appeared to have an inhibitory effect upon the percent recovery of F. roseum from subcrown internodes when both fungi were present in combination, indicating the existence of a competitive interaction between the two Fusarium species. No significant differences in final nematode populations occurred between nematode inoculated treatments. However, seminal roots consistently yielded more nematodes per gram of root weight than adventitious (crown) roots.

Root and forage yields of plants grown under field conditions revealed no significant differences between the inoculated treatments and the uninoculated control. This result recurred in field and greenhouse grain yields. In each experiment, however, the highest yields were obtained from inoculated treatments. Fungal contamination and low final nematode populations confounded these results and hindered the identification of any pathogen interaction.

A greenhouse experiment was designed to evaluate the effect of soil moisture stress upon the interaction of P. neglectus and F. roseum. Inoculated and uninoculated treatments were subjected to low (- ) and high (- ) moisture stress series for one month. Under both moisture regimes, plant data showed that the nematode-fungus combination produced less than an additive effect and in most instances produced higher yields than treatments inoculated with either the nematode alone or the fungus alone. Percent

recovery of F. roseum from subcrown internodes did not appear to be influenced by moisture stress. Final nematode populations were, however, significantly lower in the high moisture stress series than in the low stress series. This may be explained by the fact that total root weights were significantly decreased under the high stress series, thus reducing the amount of roots available to support nematode reproduction. Numbers of nematodes were not reduced on a per gram of root weight basis by increased moisture stress.

The influences of root mass and root weight to water volume ratio on nematode extraction efficiency by root incubation were determined. The results of the root mass study revealed that nematode recovery per gram of root increased significantly with the largest root mass. The root/water ratio study revealed that nematode recovery per gram of root increased as the root weight to water volume ratio decreased.

## Conclusions

1. Greenhouse grown wheat plants revealed a synergistic disease interaction between P. neglectus and F. roseum 'Acuminatum' in sterile Lincoln fine sand (LFS).

2. The disease interaction of P. neglectus and F. solani was additive under greenhouse conditions.

3. Competitive interactions occurred between F. roseum + F. solani and between P. neglectus + F. roseum + F.

*solani* under greenhouse conditions.

4. Final nematode populations were not significantly influenced by fungal interactants, but nematodes per gram of roots were consistently higher for seminal roots than for adventitious roots.

5. Results of experiments in which non-sterilized field soil was used produced a suppression of nematode populations and an over-all inhibition of disease expression.

6. Disease expression by wheat plants inoculated with *P. neglectus* and/or *F. roseum* was greater with increased moisture stress, but, due to the predominating influence of the moisture stress treatments, the interaction of the two pathogens was less than additive.

7. Nematode recovery on a per gram of root basis was increased by the incubation of increased root mass.

8. As the ratio of root weight to water volume decreased, nematode recovery per gram of root increased.

## Recommendations for Future Research

The non-sterilized field soil observed to suppress disease expression and nematode reproduction in this study, indicates the presence of a suppressive factor, possibly biotic, in the soil. Further studies are needed to document this phenomenon and to identify the factor(s) responsible.

Further research should be initiated to elucidate the combined influences of soil moisture and soil temperature

upon the nematode-fungus interactions observed in this study. Likewise, the effect of plant nutrition upon the expression of disease interaction should be considered.

The mechanisms involved in the synergistic disease interaction observed in this study also need to be identified. This includes the possibility of physiological interaction at discrete infection loci. The importance of factors such as predisposition and infection court provision needs to be determined.

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