CHARACTERISTICS OF RHIZOCTONIA SPP. ISOLATED FROM WHEAT AND PEANUT AND THEIR PATHOGENICITY AND VIRULENCE ON HARD RED WINTER WHEAT

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1997

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2000.

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ACKNOWLEDGMENTS

I wish to express my heartfelt gratitude and appreciation to Dr. Robert Hunger, my major advisor, for his guidance, patience and support all through the duration of my study. I would like to thank Dr. Larry Singleton for his support and guidance throughout the course of my research. He has been such a support and guide at all times. It was a real pleasure working with Dr. Hunger and Dr. Singleton.

I would like to gratefully acknowledge Dr. Hassan Melouk and Dr. Eugene Krenzer for their valuable input and guidance which kept me on the right track and made the successful completion of this thesis possible. I would like to thank Dr. Mark Payton specially for his valuable guidance and help with the statistical analysis of the data. I would also like to thank Dr. Kenneth Conway for kindly providing the anastomosis tester isolates necessary for the study and for all his help and valuable guidance.

Craig Siegerist deserves special mention as a person who has been a great help throughout with my field studies and various other aspects of this research. Heartfelt gratitude is extended to the faculty, staff and my fellow students in the Department of Entomology and Plant Pathology, for their kind support and help. I also wish to thank Jennifer Barker, Emily Cline and Tina Coker for their help and friendship.

Finally, I would like to express my heartfelt gratitude towards my parents, my sister for their help, encouragement and constant support. I would like to thank my husband, Anil for his encouragement, patience and loving support.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER 3

CHAPTER 4

Figure **Page**

Figure **Page**

CHAPTER 1

INTRODUCTION

Wheat and peanut are two important agricultural commodities in Oklahoma. Wheat, which provides forage for cattle and grain for flour, is usually planted on 5-7 million acres each year in Oklahoma. Peanuts, which usually occupy about 80,000 acres in Oklahoma, are primarily used for human consumption, but are also used for seed, animal feed and oil. In 1999, Oklahoma ranked second in the United States for production of winter wheat and seventh in the production of peanuts with a production of 150,500,000 bu ($ 354 million) of wheat and 189,600,000 lb ($53 million) of peanuts (Oklahoma Agricultural Statistics, 1999).

Wheat varieties grown in North America are divided into five basic classes, *viz.* hard red winter (HRW), hard red spring (HRS), white (winter or spring), soft red winter (SRW) and durum (Cook et al, 1991). HRW wheat cultivars account for most of the wheat grown in the United States, and are mainly grown throughout the southern and central U.S., and into the northern Great Plains. HRS wheat cultivars are grown in the northern Great Plains, the northern corn belt, the inland Pacific Northwest, and under irrigation in California. HRS wheats have a high protein content. Flour milled from HRW and HRS wheats have high baking strength and baking quality and is primarily used to bake bread. White wheat is grown in the Northwest and the Pacific Northwest states, British Columbia, Ontario and Quebec and recently has had an incipient production in the central Great Plains. Flours milled from white wheats are used in cake

and pastry manufacture. SRW wheat, which is primarily grown in the eastern and south-eastern United States, is high yielding, but has a relatively low protein content. SRW wheat is milled to produce general purpose flours, which are used for making cakes, crackers and cookies. Durum wheat, which is grown under dryland conditions in the northern Great Plains and under irrigation in Arizona and southern California, is mainly used to make noodles and pastas.

Georgia (42%), Texas (16%), Alabama (14%), North Carolina (9%), Virginia (6%), Oklahoma (5%) and Florida (5%) account for approximately 97 percent of the peanut production in the United States (url: http://www.peanut-shellers.org/Facts/Types/types.html). Peanuts are divided into four basic types depending on their size and flavor *viz.* Runner, Spanish, Virginia and Valencia. Runner varieties account for about 75% of the total U.S. production and are grown mainly in Georgia, Alabama, Florida, Texas and Oklahoma. Runner varieties are mostly used for peanut butter. Virginias are grown mainly in southeastern Virginia and northeastern North Carolina, and account for about 21 percent of the total U.S. production. Virginia peanuts have the largest kernels and are sold primarily as salted shelled peanuts. Spanish varieties have smaller kernels and a higher oil content, and are predominantly used in peanut candy and for salted nuts and peanut butter. Spanish varieties are primarily grown in Oklahoma and Texas, and account for 4% of U. S. production. Valencia varieties, which are grown mainly in New Mexico and account for less than 1% of peanut production in the U.S, are used as boiled peanuts or roasted and sold as shelled peanuts.

Numerous diseases pose significant restraints to the production of wheat and peanuts in Oklahoma. Although foliar diseases such as wheat leaf rust can reduce grain

yield and quality, root diseases also significantly affect wheat cultivation in the southern Great Plains and in Oklahoma. Root rot diseases are of great economic importance because a healthy root system is important for wheat to tiller, and to yield high quality grain. The impact of these diseases on tillering and grain formation is the greatest when infection occurs during the early stages of plant growth and development. *Bipolaris sorokiniana* alone or in combination with *Fusarium* causes common root rot or dryland root rot, which is a problem in the Great Plains (Cook et al, 1991). In Canada, this disease is estimated to decrease wheat yield by 5.7%. *Fusarium* root rot may be caused by *F. graminearum*, *F. culmorum*, and rarely by *F. avenaceum*. Root diseases favored by wet soils are Take-all (*Gaeumannomyces graminis*). Rhizoctonia root rot (*Rhizoctonia solani*) and Pythium root rot (*Pythium* spp.).

Root diseases also can significantly affect peanut production. This is especially true for *R. solani*, which is associated with commercial peanut production and can attack any part of the plant at any stage of growth (Brenneman, 1996). *Rhizoctonia* alone, or in association with other pathogens like *Pythium, Rhizopus, Fusarium* and *Aspergillus* may cause seed decay or seedling damping-off. Winter wheat is often grown as a dual-purpose crop in the southern Great Plains, which generates additional income from cattle that are pastured on winter wheat (Krenzer, 1994). When wheat is grown as a dual-purpose crop in Oklahoma, it is planted in late August or September. In contrast, the optimum planting time for grain production is early to late October. Early planting favors root rots due to warm temperatures and high rainfall, which favor colonization of the roots by the pathogens.

Wind and water erosion are a major concern for Oklahoma winter wheat producers (Epplin et al, 1991). Hence, a reduced tillage production system is often employed in Oklahoma. However, Rhizoctonia root rot is frequently a serious problem when direct drilled, no-till or reduced till farming systems are employed (Mazzola et al, 1996).

Various rotations of wheat with other crops are followed in North America to facilitate production and to reduce diseases (Cook et al, 1991). These include (1) 4-6 year rotations of alfalfa, corn, oats, and wheat in the Great Lakes region, (2) double cropping with soybeans and 2 or 3 year rotations with peanuts, cotton, soybeans and corn in the southeastern states, and (3) alternating wheat and fallow on a two year rotation such as is followed in the Pacific Northwest and the northern Great Plains. Similar rotations of wheat and peanut are occasionally used in south-central and southwestern Oklahoma, and the potential to rotate wheat with soybeans or sorghum exists through out Oklahoma. However, in Oklahoma, monoculture of wheat is usually followed and these rotation options have not become a standard practice.

As with most root diseases, the incidence and severity of root diseases caused by *Rhizoctonia* spp. are significantly affected by many factors including planting date and cropping sequence. Therefore, the purpose of my research was to investigate the pathogenicity of *Rhizoctonia* spp. isolated from wheat and peanut, which will provide information on the affect that rotation of these crops has on the incidence of wheat root rot caused by *Rhizoctonia* spp.

CHAPTER 2

LITERATURE REVIEW

The Pathogens

Rhizoctonia, which is a soilborne, non-obligate fungus (Papavizas, 1970), belongs to the class Basidiomycetes (Parmeter et al, 1970) and is considered to commonly cause post-emergence damping-off (Hillocks, 1992). Members of this genus are either binucleate or multinucleate (Parmeter et al. 1970). Multinucleate *Rhizoctonia* have 3-28 nuclei per cell and have thicker hyphae when compared to binucleate *Rhizoctonia*. Multinucleate species include *R. solani, R. zeae* and *R. oryzae*. Binucleate species include *R. cerealis*.

***Rhizoctonia solani* (Teleomorphs: *Thenatephorus cucumeris, Pellicularia* spp)**

R. solani has been described by Parmeter et al (1970) and by Carling et al (1992) as having brownish, rapidly growing hyphae that branch near the distal septum of cells in the young advancing hyphae. Other characteristics include constriction of branch hyphae at the point of origin, formation of a septum in the branch near the point of origin, and production of uniformly textured sclerotia. Rhizomorphs and clamp connections are absent.

R. solani has many strains that differ in host range, virulence, optimum temperature for pathogenicity, etc (Baker, 1970). Thus, *R. solani* is a collective species, comprised of many related but genetically isolated groups (Anderson, 1982; Carling et al,

1994). Based on hyphal anastomosis, *R. solani* can be divided into smaller, more homogeneous groups. Anastomosis occurs between hyphae of isolates within the same anastomosis group (AG). As of 1994, twelve anastomosis groups of *R. solani* have been reported, including groups 1 to 11 and B 1 (Carling et al, 1994).

***Rhizoctonia cerealis* (Teleomorphs: *Ceratobasidium gramineum*, *C. cereale*, *Corticium gramineum*)**

R. cerealis forms cream colored colonies on potato dextrose agar, and has a slower growth rate compared to *R. solani* (O'Sullivan et al, 1991). *R. cerealis* can be differentiated from *R. solani* by looking at the number of nuclei per cell, by host specificity and by hyphal anastomosis. Among the binucleate *Rhizoctonia* spp. that form *Ceratobasidium* teleomorphs, seven anastomosis groups have been identified (Burpee et al. 1980). *R. cerealis* belongs to the group AG-D, and is capable of fusion with members of AG-D and CAG-1 (Sneh et al, 1994).

***Rhizoctonia* diseases - Wheat**

R. cerealis causes sharp eye-spot and *R. solani* causes root rot in wheat. Sharp eye-spot is characterized by elliptical lesions that develop on the base of the stem of young and mature plants (Clarkson et al, 1983). These lesions have sharply defined dark brown borders and a cream center. In advanced cases, the center may decompose leaving a shot hole. The disease may lead to lodging and premature ripening that results in white heads. Grain yield is reduced due to sterile heads and grain quality is affected as a result of the formation of small, shriveled grain.

Rhizoctonia solani (AG-4) was first reported to cause sharp eye-spot in wheat, but more recently the causal pathogen of this disease has been reported to be *Rhizoctonia*

cerealis (Lipps et al, 1982). Diseased plants usually occur in circular patches, and in the most acute form of the root rot, plants become stunted and chlorotic (Mazzola et al, 1996) and produce little or no grain. Under favorable conditions, these patches may coalesce; however, if the disease pressure is low, individual stunted plants may be seen interspersed within rows of productive plants.

***Rhizoctonia diseases* - Peanut**

Pod rot or limb rot is an important disease of peanut caused by *R. solani*. *R. solani* causes damping-off of the seedlings (Melouk et al, 1995). Dry, sunken cankers on the hypocotyl may be formed due to infection by *R. solani*. The vines may also become infected later in the growing season and as the disease progresses, the cankers expand and girdle the vine.

The symptoms of *Rhizoctonia* limb rot include the appearance of light, tan lesions with dark borders on the stem that develop where the lower limbs are damaged or in contact with the soil. (Brenneman et al, 1996). Girdling of the stem may cause loss of pods. Sometimes, the fungus may cause rotting of the pods, with no effect on other parts of the crop. In severe cases both the shell and the seed may rot. If the disease is not severe, only superficial brown or dark brown cankers appear. However, a prevalence of cool, wet weather favors pathogen growth and facilitates development of *Rhizoctonia*-induced seedling diseases (Sturgeon et al, 1986). Thus, peanut pod rot is an important root disease of peanuts in Oklahoma.

Factors favoring *Rhizoctonia*-induced disease incidence:

Various anastomosis groups of *R. solani* have different temperature optima for pathogenicity (Baker, 1970). For example, studies conducted on sugar beet showed that

pre-emergence damping-off caused by *R. solani* AG-4 was more severe at 16-30 C, as compared to AG-2, which was more virulent at lower (10-15 C) temperatures (O'Sullivan et al, 1991). In a study conducted on potato, isolates of AG-3 caused the most damage to sprouts at 10 C, and lesser damage at 15.5 C and 21.1 C (Carling et al, 1990). In hard red winter wheat, temperature greatly influenced Rhizoctonia root rot caused by AG-4 by decreasing seedling emergence as the temperature increased from 15 to 35 C (Mathieson, 1991).

Soil moisture is another factor that can affect root rot incidence. In a study conducted by Bateman (1961), he reported that under controlled experimental conditions, soil moisture influenced the development of Rhizoctonia root and stem rots in poinsettia. Four different soil moisture values, 36, 60, 70 and 87 percent moisture holding capacity (MHC) were tested. Rhizoctonia root and stem rots were affected similarly by soil moisture conditions. The disease was most severe at the lower moisture levels (36% MHC) and was very low at the highest moisture level (80% MHC). There existed an almost linear inverse relationship between disease development and soil moisture content between 36 and 80% MHC. It was concluded that the soil moisture affects soil aeration, which might suppress the growth of the pathogen.

Rhizoctonia diseases are generally more severe when crops are planted by direct drilling into the crop residue as compared to crops planted following conventional cultivation practices that greatly reduce or eliminate crop residue (Mazzola et al, 1996). Incidence and severity of *Rhizoctonia* diseases is inversely correlated to the intensity and depth of cultivation (Pumphrey et al, 1987). This is due to the reduced propagule size and reduced inoculum potential, which results from the fragmentation of plant debris

harboring the fungus (Rovira, 1986). These factors result in increased exposure of the pathogen to microbial antagonists. Thus, adjusting the intensity and depth of cultivation might help in managing *Rhizoctonia* diseases.

Other factors that influence the incidence and severity of Rhizoctonia root rot include the presence of volunteer wheat and weeds, and soil fertility. Volunteer wheat and weeds can serve as hosts of *R. solani*. However, timely applications of herbicides can eliminate volunteer wheat and weeds, and thereby reduce the incidence of *Rhizoctonia* root rot in direct-drilled fields (Mazzola et al, 1996). Deficiencies in nitrogen, zinc or calcium or excessive nitrogen also favor the disease (MacNish et al, 1996); however, soil tests and proper application of fertilizers can help to eliminate this factor.

Control of *Rhizoctonia*-induced diseases

Several methods have been used to control *Rhizoctonia*-induced diseases, including improved cultural practices, soil disinfestation, fungicides, herbicides for weed control and breeding for resistance. However, none of these methods provide complete control, or are economically feasible.

Rhizoctonia root diseases are usually less severe in organic or low input cropping systems (limited or no synthetic external inputs in the form of pesticides and fertilizers) as compared to conventional cropping systems (Van Bruggen et al, 1996). This reduction may be due to longer rotations, regular applications of organic amendments and reduced pesticide usage. Organic amendments are assumed to increase the general level of microbial activity resulting in increased competition and/or antagonism in the rhizosphere.

Soil disinfestation is another method that has been used to control *Rhizoctonia*-induced diseases. This method, which includes steaming soil, soil fumigation or soil solarization, eliminates pathogenic agents in the soil before a crop is planted. Pullman et al (1981) reported that in cotton fields, soil solarization reduced the population densities of *Verticilliun dahliae*, *Pythium* spp, *Thielaviopsis basicola* and *R. solani*. Employing soil solarization by tarping moist soil with transparent polyethylene plastic during the hot summer months was effective in reducing soil population densities of the four pathogens. As of now, no major undesirable disturbance on the soil's biological balance is known to be caused by solarization. This method can be integrated with other methods for disease management and used as an additional option for control.

Soil fumigation is effective in controlling many soilborne pathogens including *Rhizoctonia*. However, re-infestation of the soil may occur in fumigated soils. Strashnow et al (1985) reported that biological control of *R. solani* with *Trichoderma harzianum* could be integrated with soil fumigation using methyl bromide in beans and carrots. The biocontrol agent, *T. harzianum* tolerated higher doses of methyl bromide than *R. solani*. On addition of *Trichoderma* to the soil soon after the fumigation or before fumigation, the biocontrol agent multiplied in the soil and reduced the inoculum density of *R. solani* before it attacked the host. Thus, in the field and greenhouse, a combination of *Trichoderma* and a reduced dose of methyl bromide (200 kg/ha) significantly controlled the disease incidence. Unfortunately, using soil disinfestation to control wheat diseases is not feasible because of the low value of the commodity in relation to the cost of the control.

Fungicides can provide a means for controlling *Rhizoctonia*-induced diseases in

many cases. Fungicides can be applied as seed or soil treatments, or as foliar applications. Tolclofos-methyl and pencycuron are the most common fungicides used to control *Rhizoctonia* (Kataria et al, 1996). Tolclofos-methyl controls almost all types of *Rhizoctonia* diseases on a number of crop species. Triazole fungicides have also been used for the control of *Rhizoctonia* diseases. Beta–methoxyacrylates can also control *Rhizoctonia* diseases with low amounts and this compound exhibits slow systemic mobility in the plants, which is a very desirable property for successful long-term control of *Rhizoctonia* diseases. Effective control of *Rhizoctonia*-induced diseases can be achieved through integration of control using fungicides with other disease control measures.

Timely applications of herbicides for control of weeds, which serve as hosts for *Rhizoctonia* can indirectly reduce severity of *Rhizoctonia* diseases. Application of glyphosate or paraquat at least 2-3 weeks before planting significantly reduced Rhizoctonia root rot in direct-drilled wheat (Roget et al, 1987). The longer interval between planting and herbicide application allows additional time for saprophytic micro-organisms to displace *Rhizoctonia* in the dying roots of treated plants.

Resistant germplasm is the most effective and environmentally safe way to manage diseases like the Rhizoctonia root rots (Panella et al, 1996). A few cultivars of soft winter wheat have been released, including the cultivar Agripro Clemens, which exhibited a moderate level of resistance to *Rhizoctonia*. Agripro Clemens is an awnless, full-season maturing variety and has an exceptionally good milling quality and very good baking quality (Miskin et al, 1995). In 1993, 'Georgia Browne', a runner type peanut cultivar with resistance to limb rot was released (Branch, 1994). Thus, development and

use of resistant germplasm in combination with crop rotation and other cultural practices might prove to be a feasible solution for management of *Rhizoctonia* diseases.

Control of *Rhizoctonia*-induced diseases in wheat

Early planting (prior to October 01) of wheat greatly favors disease incidence and severity (Mathieson, 1991). This is especially true when wet and mild winters are accompanied by drought stress in the spring because these conditions favor the colonization of wheat root by the pathogen in the fall and winter and expression of the disease in the spring. Higher soil temperatures during early planting date may also favor certain anastomosis groups of *Rhizoctonia*. For example, in a study conducted by Mathieson (1991), he concluded that temperature greatly affected the activity of *Rhizoctonia solani* (AG-4). In this study, the emergence of wheat seedlings decreased as the temperature increased form 15-35 C. Hence, he suggested that planting wheat later in the year, when soil temperatures are cooler may be beneficial for control of *Rhizoctonia* (AG-4).

Rhizoctonia diseases are often more severe when wheat is planted by direct drilling than when planted following conventional tillage practices (Rovira et al, 1986). Increasing the depth and intensity of soil disturbance under the seed placement zone while drilling increases wheat yield (Jarvis et al, 1986). Direct drilling with narrow sowing points that disturbs the soil below the seed also reduces disease incidence in wheat (Roget et al, 1996). In direct-drilled fields, the inoculum is concentrated in the top 10 cm of the soil profile (Mazzola et al, 1996). Hence, deep cultivation prevents the plant from coming in direct contact with the pathogen until the seedlings are well established and have developed an extensive root system.

Balanced plant nutrition is another factor that facilitates control of *Rhizoctonia*-induced diseases. In a study conducted by MacNish (1985), it was shown that application of nitrogen (N) reduced Rhizoctonia bare patch, although sources of N (ammonium sulfate, sodium nitrate or urea) did not differ significantly in their effect. Application of calcium to calcium deficient soils and zinc to zinc deficient fields reduced Rhizoctonia bare patch and root rot (MacNish et al, 1996).

Control of *Rhizoctonia*-induced diseases in Peanut

As in wheat, planting date is an important factor affecting *Rhizoctonia* disease in peanut. Since low temperature favors *Rhizoctonia* diseases on peanut, late planting when soil temperatures are higher (16-30 C) helps reduce disease incidence. Also, early planting of peanut in cool soils delays peanut emergence, which favors Rhizoctonia limb rot development (Melouk et al, 1995).

Due to the weakening of pegs, infected pods are left in the soil during harvest, which then can serve as a source of inoculum. Movement of soil or crop debris can spread the disease. The viability of the pathogen declines after 6 months, although it can survive in colonized pods in soil for at least two years (Baird et al, 1993). Deep burial of shells (approximately 30 cm deep) using a mould board plough, helps reduce disease incidence (Bell et al, 1984).

Breeding for resistance is an efficient way to control Rhizoctonia limb rot of peanut. However, efforts to breed crops with resistance to *R. solani* have not been very successful. Spanish varieties are more tolerant of pod rot when compared to Florunner, especially when properly fertilized (Sturgeon et al, 1986). The cultivar Georgia Browne (GA T-2741) showed reduced Rhizoctonia limb rot (Branch and Brenneman, 1993).

'Virginia 81-B' is also partially resistant to limb rot (Barnes et al, 1990).

Irrigation is another factor that can be adjusted to control disease incidence. Due to extensive irrigation, plant growth becomes excessive and thus the sub canopy environment will be more conducive to disease development (Melouk et al, 1995). Hence, watering should be done only when necessary to replenish soil moisture. Fewer applications and reduced application of water late in the season are recommended to aid in control (Brenneman, 1996).

Some feeding insects and nematodes were found to have an impact on the severity of the disease (Csinos et al, 1997). Hence the soil should be analyzed for nematode populations and if feasible, control measures should be taken.

Crop rotation is another factor that can affect the incidence and severity of *Rhizoctonia* root rots. Rotations of wheat with other crops are followed in North America to facilitate production, to reduce diseases, (Cook et al, 1991) to prevent soil erosion and to allow for the rotation of herbicides. For example, peas and lentils are grown in rotation with wheat in the Pacific Northwest of the United States (Cook et al, 1991), to take advantage of nitrogen fixation. This rotation increases the microbial biomass and diversity, which limits the inoculum potential of *R. solani*. Reductions in root rot also have been attributed to the sparse tap root systems of peas and lentils, which provide less inoculum for a subsequent cereal crop. In contrast, the fibrous roots of cereals provide a larger inoculum source.

Rotations of wheat with either peanut or cotton are occasionally followed in southwestern Oklahoma. In these scenarios, wheat is grown for two years followed by peanuts. After the peanuts are harvested, wheat is planted late in the fall (*circa*

November) for the first year and used only for grain production. The next year, wheat is planted in late August or early September with the fall and winter growth being used as forage for cattle. Grain is then harvested in late May or early June. Peanuts are again planted in the fourth year. However, the affects of rotating these crops on the incidence and severity of root rot disease caused by *Rhizoctonia* spp. is not documented. Hence the objectives of this study were to:

1) describe three isolates of *Rhizoctonia* obtained from Oklahoma; one isolate of *R. cerealis* (RC) from wheat and two of *R. solani* one each from wheat (RSW) and peanut (RSP), for their colony characteristics on different media, and their hyphal width and hyphal extension on PDSA [Potato Dextrose Agar (1/5 strength PDA, Difco) amended with streptomycin sulfate (0.3 g/L)].
2) determine the pathogenicity and virulence of RC, RSW and RSP on six cultivars of hard red winter wheat under controlled conditions.
3) determine the optimum temperature and inoculum level for disease incidence for RC, RSW and RSP under controlled conditions, and
4) conduct a field study to determine the pathogenicity and virulence of RC, RSW and RSP, on the wheat cultivar 2137.

CHAPTER 3

CHARACTERISTICS OF RHIZOCTONIA SPP. ISOLATED FROM WHEAT AND PEANUT IN OKLAHOMA

ABSTRACT

Root diseases induced by *Rhizoctonia* spp. significantly affect wheat and peanut production in Oklahoma, where various rotations of wheat with either peanut or cotton are occasionally followed. However, the effect of rotating wheat and peanuts on the incidence and severity of *Rhizoctonia*-induced diseases is not well documented. The first step towards documenting the effect of wheat peanut rotations on Rhizoctonia root rots would be the characterization of isolates of *Rhizoctonia* from wheat and peanut. Hence, three isolates of *Rhizoctonia* spp. were obtained from Oklahoma, one isolate of *R. cerealis* (RC) from sharp eye spot lesions on wheat, and two isolates of *R. solani*, one each from diseased wheat roots (RSW) and diseased peanut pods (RSP). Colony appearance of RC, RSW and RSP were described on Oatmeal Agar (OA), Potato Dextrose Marmite Agar (PDMA) and Potato Dextrose Streptomycin Agar (PDSA) following incubation in the dark at 20 C for 14 days. RC, RSW and RSP were clearly distinct from each other on OA, PDMA and PDSA. RC produced cream-colored sclerotia on OA, but did not produce sclerotia on PDMA and PDSA. RSW did not produce sclerotia or show colony zonation on OA, PDMA or PDSA. RSW produced profuse white tufts of aerial hyphae on OA, PDMA and PDSA. RSP produced dark brown colored sclerotial crust and exhibited colony zonation on OA, PDMA and PDSA. Hyphal widths of RC, RSW and RSP were measured on PDSA after 72 hours of incubation at 20 C. The hyphal widths were the lowest for RC and the highest for RSP. Hyphal extension of RC, RSW and RSP were determined at seven temperatures ranging from 10 C to 30 C on PDSA. RC and RSW exhibited similar hyphal extension at all temperatures except 20 and 22.5 C, where RSW had a higher hyphal extension than RC. RSP exhibited higher hyphal extension than RC and RSW at all temperatures except 10 and 15 C. RC had its highest hyphal extension at 20, 22.5 and 25 C. RSW had the highest hyphal

extension at 20 and 22.5 C and RSP had the highest hyphal extension at 25 and 27.5 C. Based on these observations, growth of RSP as measured by hyphal extension appears to be favored by higher temperatures ($\geq$ 22.5 C).

INTRODUCTION

Numerous diseases pose significant restraints to the production of wheat and peanut in Oklahoma. Although many of these affect the foliage and fruit, diseases induced by soilborne pathogens also significantly damage these two commodities. For example, in wheat, *R. cerealis* causes sharp eyespot and *R. solani* causes Rhizoctonia root rot and in peanut *R. solani* attacks the pods causing peanut pod rot.

Rhizoctonia is a soilborne fungus with a wide host range, causing pre- and post-emergence damping-off in a number of crops (Baker, 1970). Isolates of *Rhizoctonia* are assigned to various anastomosis groups (AG) by pairing them against testers (Carling et al, 1994). Anastomosis occurs only between isolates that are vegetatively compatible. Therefore, each AG is genetically independent. *R. solani* is a complex species divided into various anastomosis groups. As of 1994, 12 anastomosis groups of *R. solani* have been identified, which include groups 1 to 11 and AG-BI. *R. cerealis* belongs to the anastomosis group CAG-1.

Fungicide response (Kataria et al, 1991), temperature optima (Carling et al, 1990) and host range (Baker, 1970) of each AG may be specific. Hence a fungicide that is labeled for use against *R. solani* may not be effective against all anastomosis groups of the fungus. For example, triadimenol seed treatment on wheat is effective against *R. solani* AG-4, but not against *R. solani* AG-8 (Mazzola et al, 1996). Hence, the development of effective seed treatments against *Rhizoctonia solani*-induced diseases on cereals may be difficult as each AG might have a different fungicide response. Hence, it

is important to identify the AG of *Rhizoctonia* isolates when determining fungicide treatments against this pathogen.

Determining the temperature optima for traits such as growth and pathogenicity of *Rhizoctonia* isolates also can facilitate making management decisions involving factors such as planting date. For example, O'Sullivan et al (1991) determined the optimum temperatures for growth and pathogenicity of *R. solani* isolates from several AGs and of *R. cerealis* on sugar beet in Ireland. In his studies, *R. solani* isolates of AG-2, AG-4, AG-5, an unidentified group, and *R. cerealis* were obtained. *R. solani* AG- 2 isolates were more widely distributed, virulent at relatively low temperatures, and hence would appear more likely to cause damping-off of sugar beet in Ireland where most crops are sown by mid April. However, 60% of the isolates of AG-2 had low virulence on sugar beet seedlings. Hence, he concluded that the potential of AG-2 to cause a serious problem in the field was limited. Isolates of AG-4, AG-5, and the unidentified group had a higher temperature requirement and a limited distribution. Hence these groups (AG-4 and AG-5 and unidentified) were unlikely to cause severe disease incidence in crops sown in March or April. *R. cerealis* required a relatively lower temperature to cause damping-off and could be a threat to early-sown crops. However, *R. cerealis* accounted for only 22% of the *Rhizoctonia* isolates collected in the study. Hence, this study demonstrates the importance of characterizing the isolates of *Rhizoctonia* present in the field before determining the optimum planting dates for crops.

Anastomosis groups *of R. solani* may also exhibit host specificity (Baker, 1970). For example, AG-3 and AG-5 are mostly associated with potatoes (Carling et al, 1992). Anastomosis groups associated with wheat include AG 2-1, 2-2, 4, 5, 8, etc (Mazzola et

al, 1996). The most common *R. solani* AG associated with peanut is AG-4. AG-1 also infects peanut, causing a foliar blight in certain genotypes of peanut (Brenneman et al, 1996). In crop rotations involving different crops, it is important to characterize the isolates of *Rhizoctonia* spp. present in the field to determine whether the fungus would be pathogenic to the succeeding crop.

Various rotations of wheat with either peanut or cotton are followed in Oklahoma to facilitate production. One such rotational scenario is to produce wheat for two years followed by peanuts. After the peanuts are harvested, wheat is planted late in the fall (*circa* November) for the first year and used only for grain production. The next year, wheat is planted in late August or early September with the fall and winter growth being used as forage for cattle and the grain being harvested in late May or early June. Peanuts are again planted in the fourth year. However, the effects of rotating wheat and peanuts on the incidence and severity of root rot diseases induced by *Rhizoctonia* spp. is not well documented. The first step towards documenting the effect of wheat peanut rotations on *Rhizoctonia* root rots would be the characterization of isolates of *Rhizoctonia* isolated from wheat and peanut. Hence, an isolate of *R. cerealis* obtained from sharp eye spot lesions of wheat and two isolates of *R. solani*, one each from wheat and peanut were characterized, for their colony characteristics on different media. The hyphal width and hyphal extension on PDSA [Potato Dextrose Agar (1/5 strength PDA, Difco) amended with streptomycin sulfate (0.3 g/L)], were also determined.

MATERIALS AND METHODS

Isolates

Two isolates of *R. solani*. one each from peanut and wheat, and an isolate of *R. cerealis*

from wheat were characterized. One *R. solani* isolate (#24) was obtained in 1998 from roots of wheat plants showing root rot symptoms growing near Tipton, OK by Dr. Larry Singleton (Associate Professor, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK) and was designated as RSW (*R. solani* from wheat). The *R. solani* isolate from peanut (#23) was obtained from Dr. Hassan Melouk (USDA Research Scientist, Stillwater, OK), and was originally isolated from peanut pods showing symptoms of pod rot collected from Hughes county, OK in 1982. This isolate was designated as RSP (*R. solani* from peanut) and was tentatively identified as belonging to AG-4 (Filonow et al, 1988). *R. cerealis* isolate (#5) was isolated in 1995 from a sharp eye spot lesion present on a wheat stem (variety 2180) growing near Haskell, OK by Dr. Larry Singleton and was designated as isolate RC (*R. cerealis*). The isolates were maintained at 22-25 C on PDSA [Potato Dextrose Agar (1/5 strength PDA, Difco, Detroit, Michigan, USA) amended with streptomycin sulfate (0.3 g/L)] and were transferred to fresh plates once every three to four days. The transfer plugs (0.5 cm) were excised from the actively growing outer margin of growth with a sterilized cork borer (0.5-cm). For long-term storage, the isolates were maintained at 20-22 C on PDA slants and were transferred twice or thrice a year to fresh slants.

Colony characteristics

Mycelial plugs (5mm) of RC, RSW and RSP were placed on Potato Dextrose Marmite Agar (PDMA), Oatmeal Agar (OA) and PDSA. The plates were incubated for two weeks in the dark at 20 C. The plates were then observed for colony color, sclerotial formation, zonation and aerial mycelium. Three replications (three plates) were inoculated with each isolate, and the experiment was conducted thrice.

Hyphal width

To measure the width of the hyphae, 5 mm mycelial plugs of the isolates were centrally placed on PDSA in a petri dish. Hyphal width was measured after 72 hours of incubation at 20 C. The width of fifteen mature cells about half way from the center and the outer margin of growth of each culture were measured. There were three replications (three plates) of each isolate.

Hyphal extension

To determine the hyphal extension, 5 mm mycelial plugs of the isolates were centrally placed on PDSA in a 9 cm petri dish. The diameters of three replicate cultures were measured after 24, 48 and 72 hrs. The cultures were kept at seven different temperatures in incubators [10 C (Curtin Maatheson scientific, Model C 1213), 15 C (Percival Scientific, model I-35 LL), 20 C (Curtin Maatheson scientific, Model C 1213), 22.5 C (Precision Scientific, Model 815), 25 C (Fisher Scientific, model 655 D), 27.5 C (Percival Scientific, model I-36 LL), 30 C (Fisher Scientific, model 655 D)] and the hyphal extension was measured. The experiment was repeated thrice. The data was analyzed using proc mixed (SAS, SAS Institute Inc., Cary, NC).

RESULTS

Colony characteristics

On Oatmeal Agar

RC, RSW and RSP were clearly distinct in appearance from each other on OA. RC produced a white-colored mycelium and showed no zonation. Aerial hyphae were sparse, and cream-colored sclerotia were produced randomly throughout the colony (Fig 1 A). RSW produced a white-colored mycelium, showed no zonation and produced profuse

white tufts of aerial hyphae on OA, which was less profuse on PDMA (Fig 1 B). Sclerotia were absent. RSP produced a light-brown-colored mycelium early in its growth stage, which later turned dark brown (Fig 1 C). This isolate exhibited a concentric pattern of growth and produced a dark-brown-colored sclerotial crust towards the center of the colony at first, which then spread through out the colony in the later stages of growth.

On Potato Dextrose Marmite Agar

RC, RSW and RSP were clearly distinct from each other on PDMA. RC was light buff in color and showed no zonation. The isolate produced no sclerotia or aerial hyphae (Fig 1 D). RSW had a light buff-colored mycelium and showed uniform growth with no zonation (Fig 1 E). This isolate produced very profuse, white tufts of aerial hyphae. Sclerotia were absent. RSP produced a light-brown to brown mycelium early in its growth stage, which later turned dark brown to black color (Fig 1 F). Aerial growth was sparse and the aerial hyphae were light-brown-colored. The isolate exhibited a concentric growth pattern. Small brown to black colored sclerotia were formed towards the center.

On Potato Dextrose Streptomycin Agar

RC, RSW and RSP were clearly distinct from each other on PDSA. RC produced a whitish mycelium with very sparse aerial hyphae. There was no sclerotial formation and the isolate exhibited uniform growth. RSW produced a light buff-colored mycelium with white tufts of aerial hyphae and no sclerotial formation. Tufts of hyphae were very profuse, but were more profuse on PDMA. RSP produced a light brown-colored mycelium in the early stages of growth, which later turned dark brown. The isolate

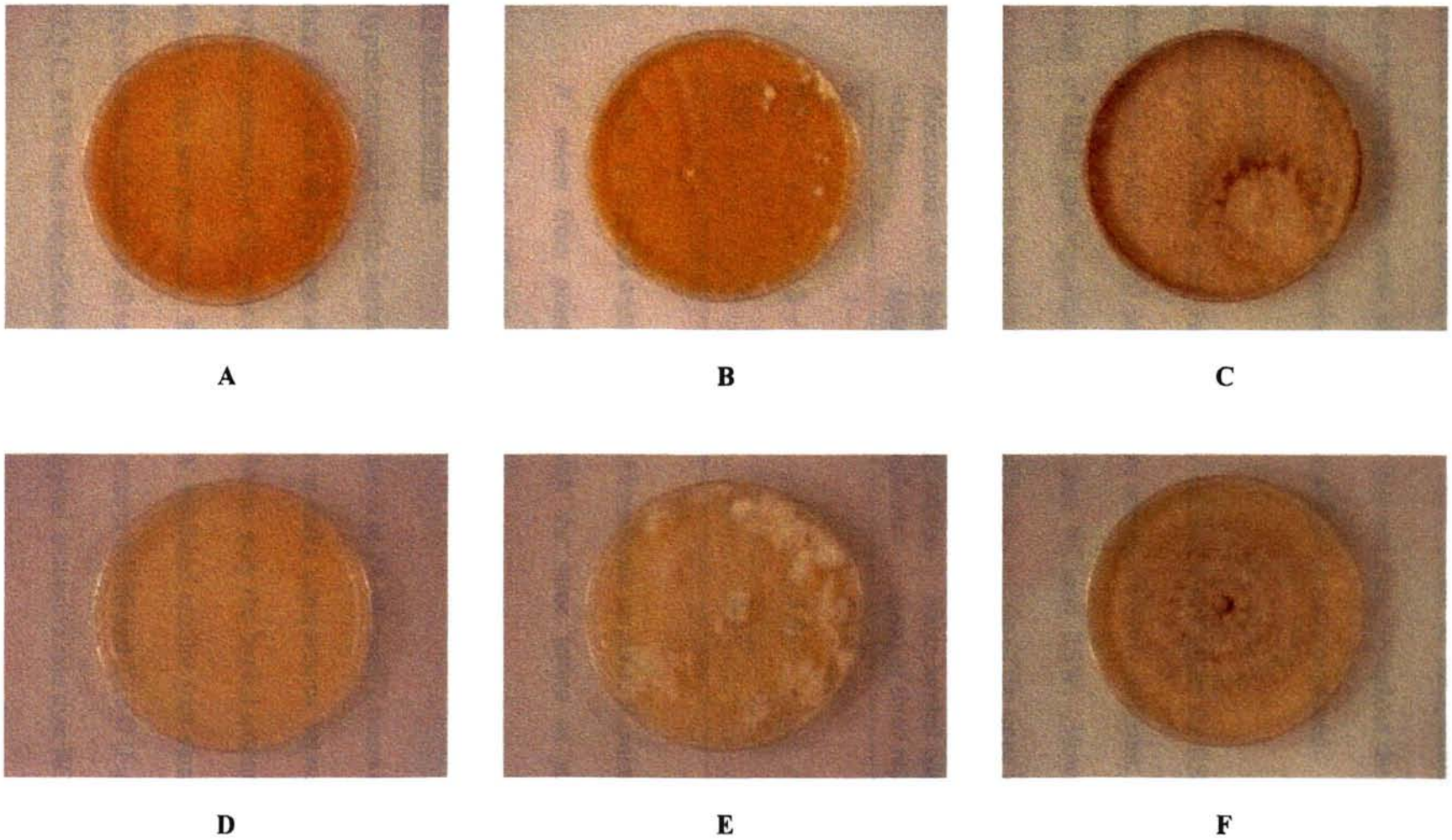

Fig 1. Appearance of *Rhizoctonia cerealis* (**RC**), *R.solani* from wheat (**RSW**) and *R. solani* from peanut (**RSP**) on Oatmeal agar (**OA**) and potato dextrose marmite agar (**PDMA**) after incubation in the dark for 14 days at 20C
A, **B** and **C** - RC, RSW and RSP respectively, on OA
D, **E** and **F** - RC, RSW and RSP, respectively, on PDMA

exhibited concentric pattern of growth. Dark brown to black-colored micro-sclerotia were produced in the center of the colony first and then throughout the colony in the later stages of growth. Light brown-colored aerial hyphae were produced.

Hyphal width

Mean hyphal widths were the lowest for RC and the highest for RSP (Table 1). The mean hyphal widths of RC and RSW were not significantly different. The mean hyphal width of RSP was significantly higher than RC and RSW. However, there was a considerable overlap between the ranges of hyphal widths of the three isolates.

Rhizoctonia isolate	Range of hyphal widths (μm) of three replicates	Mean hyphal widths (μm) of three replicates
RC	2 – 4.5	3.17[a]
RSW	3 – 4.5	3.81[a]
RSP	4 – 7.5	5.37[b]

Table. 1. Hyphal widths (μm) of *Rhizoctonia cerealis*, *R. solani* from wheat and *R. solani* from peanut. Data with the same letters as superscript are not significantly different.

Hyphal extension

Hyphal extension of the isolates was measured at 24, 48 and 72 hours of incubation. The data at 24 and 48 hrs were not sufficient to make comparisons between isolates, as the hyphal extension of RC was very low. Hence, only the hyphal extension measurements after 72 hrs are presented. RC and RSW exhibited similar (not significantly different) hyphal extension rates at all temperatures except 10, 20 and 22.5 C (Fig 2). At 10, 20 and 22.5 C, RSW had a significantly higher hyphal extension rate than RC. Comparing RC

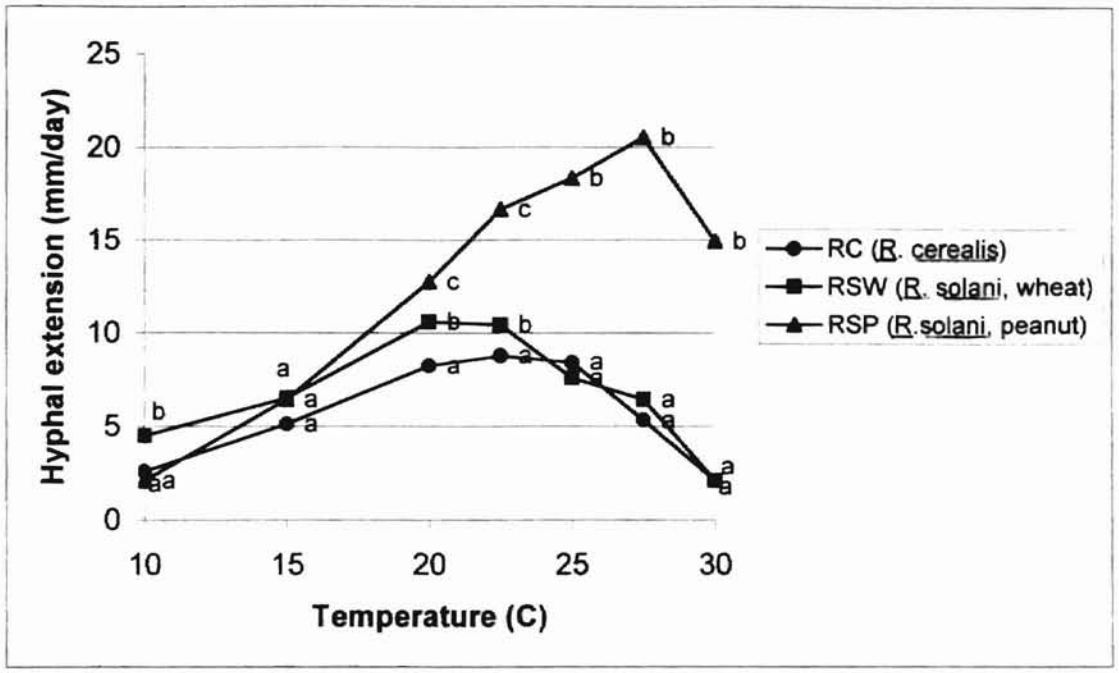

Fig.2. Hyphal extension rate of *Rhizoctonia* isolates on potato dextrose agar [(1/5 strength PDA, Difco) amended with streptomycin sulfate (0.3 g/L)], at temperatures between 10 and 30 C. Letters compare isolates within each temperature. Data points followed by the same letter are not significantly different at $P \leq 0.05$.

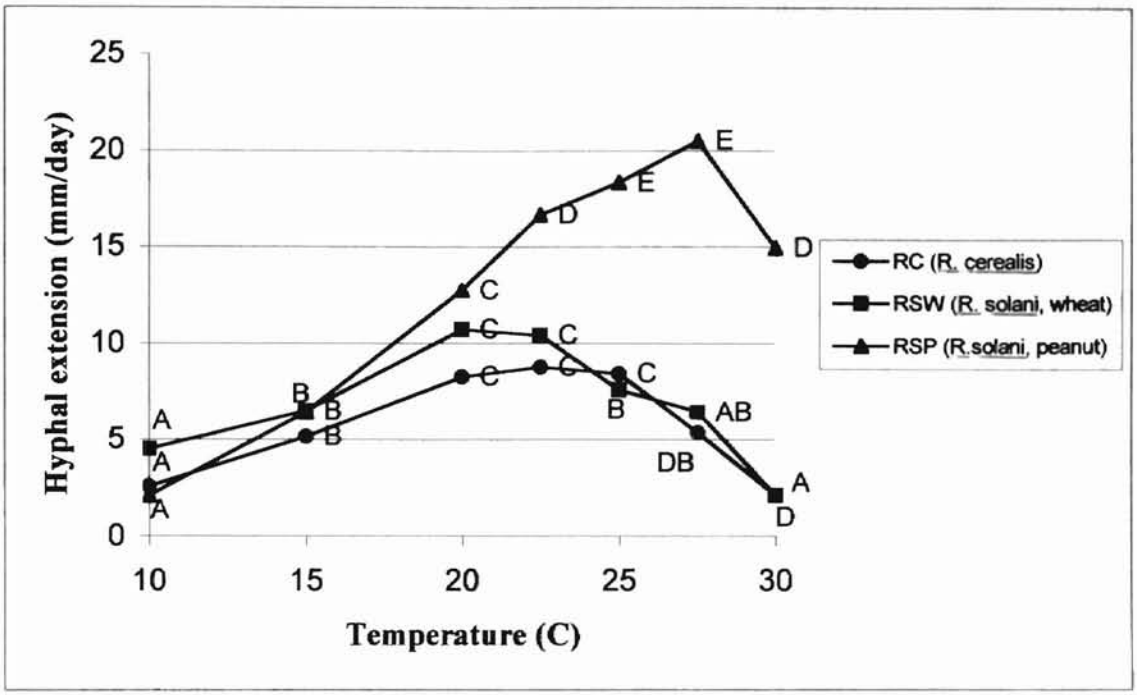

Fig.3. Hyphal extension rate of *Rhizoctonia* isolates potato dextrose agar [(1/5 strength PDA, Difco) amended with streptomycin sulfate (0.3 g/L)], at temperatures between 10 and 30 C. Letters compare each isolate across all temperatures. Data points followed by the same letter are not significantly different at $P \leq 0.05$.

across all temperatures, RC had its highest hyphal extension rate at 20, 22.5 and 25 C and its lowest hyphal extension rate at 30 C, followed by 10 C (Fig 3). Comparing RSW across all temperatures, RSW had its highest rate of hyphal extension at 20 and 22.5 C and its lowest hyphal extension rate at 10 and 30 C (Fig 3). RSP exhibited a significantly higher hyphal extension rate than RC and RSW at all temperatures except at 10 and 15 C (Fig 2). Comparing RSP across all temperatures, RSP had its highest rate of hyphal extension at 25 and 27.5 C and its lowest hyphal extension rate at 10 C, followed by 15 C (Fig 3).

DISCUSSION

The colony characteristics of RC on OA and PDMA were similar to that of an isolate of RC on these two media (OA and PDMA) in a study conducted on sugarbeets (O'Sullivan, 1991). However, the colony characteristics of RSP (which was tentatively identified as AG-4) were not similar to that of an isolate of AG-4 that was described by O' Sullivan (1991). The existence of variation in the appearance of different isolates within the same anastomosis group was reported by Sherwood (1969) in his study on different anastomosis groups (AG 1, 2, 3 and 4) of *R. solani*. In his study, he concluded that it was better to use anastomosis grouping than the morphologic-physiologic grouping, as the anastomosis behavior was a better measure of genetic relatedness, and morphologic expression may easily be influenced by environment.

There was a considerable overlap between the ranges of hyphal widths. This observation on the overlap of the range of hyphal widths between the isolates of different anastomosis groups is consistent with observations reported by Sherwood (1969). In his study, even though the group means of the hyphal width of the representative isolates of

each anastomosis group were significantly different, the ranges of hyphal widths of all groups overlapped. Hence he concluded that the hyphal width was only of secondary value in classifying isolates.

The hyphal extension rates of RC and RSW were similar at all temperatures except 10, 20 and 22.5 C. However, the hyphal extension rate of RSP was significantly higher than RC and RSW at all temperatures except 10 and 15 C. The hyphal extension rate of RSP was not significantly higher than the other two isolates (RC and RSW) at lower temperatures (10 and 15 C). However, the hyphal extension rate of RSP was much higher than that of RC and RSW at temperatures $\geq$ 22.5 C.

Based on the observations from this study, RC had the highest hyphal extension rate between 20 and 25 C and RSP (tentatively identified as AG-4) had the highest hyphal extension rate at 25 and 27.5 C. This observation is consistent with the study conducted on sugarbeets by O'Sullivan et al (1991), where the optimum temperature for the hyphal extension of *R. cerealis* was 22.5 C and that of an isolate of AG-4 was 27.5 C.

Based on the observations from this study, RSP appears to be favored by higher temperatures ($\geq$ 22.5 C). This is logical since peanut is a warm season crop and is cultivated in Oklahoma from May through October. Temperature frequently exceeds 35 C for many days during these months, which would provide the pressure to select for strains of *Rhizoctonia* spp. that have high optimum temperatures for many traits. In contrast, RSW and RC were isolates from wheat, which is grown from September through May. Temperatures during these months rarely exceed 35 C, which would select for isolates of *Rhizoctonia* spp with greatest growth at cooler temperatures. The fact that RSP prefers higher temperature might be important in choosing a planting date for wheat

in wheat-peanut rotations. Since RSP was isolated from peanut and is pathogenic to both peanut and wheat, this isolate might be a major problem in wheat-peanut rotations. A late planting date for wheat might be more suited for fields that are infested with RSP if wheat-peanut rotations are followed as the lower temperatures might reduce the virulence of the pathogen.

CHAPTER 4

PATHOGENICITY AND VIRULENCE OF RHIZOCTONIA SPP. ISOLATED FROM WHEAT AND PEANUT ON HARD RED WINTER WHEAT

ABSTRACT

Root diseases caused by *Rhizoctonia* spp. are a serious problem affecting wheat production in Oklahoma. Various factors including temperature and inoculum density affect the incidence and severity of *Rhizoctonia*-induced diseases in wheat. The affect of temperature on three isolates of *Rhizoctonia* spp. obtained from Oklahoma [one isolate of *R. cerealis* (RC) from sharp eye spot lesions on wheat, and two isolates of *R. solani*, one each from wheat roots (RSW) and peanut pods (RSP)] was studied. Pathogenicity and virulence of these isolates were also studied over a range on inoculum levels (10, 20 and 30 infected seeds) and also on different cultivars of hard red winter wheat. In each experiment ten healthy seeds were planted in small pots and inoculated with ten infected seeds (10, 20 or 30 inoculated seeds were used in the experiment with different inoculum levels). Stand counts (seedling emergence/pot) were recorded 14 days after planting. The plants were harvested after 14 days and rated for disease severity on a range from 1-6 (1- healthy seedlings; 6- seeds that did not emerge). Plant heights and fresh shoot and root weights were measured. Based on stand counts, disease severity rating, fresh shoot weight and fresh root weight, RSP was more virulent at higher temperatures ($\geq$ 25 C). Also, RSP was consistently the most virulent isolate at all temperatures as compared to RC and RSW. RSP was more virulent at 30 C than 10 C at all inoculum levels (10, 20 and 30 infected seeds). RC, RSW and RSP did not show any significant difference in virulence when increased levels of inoculum were tested at 10 or 30 C. Based on stand counts, disease severity rating, and root and shoot weights, TAM 101 appeared partially resistant to RSP at both 10 C and 30 C. However, TAM 101 seeds had been treated with Arasan Red (Thiram), which may have protected the seeds form the disease. The results suggest that RSP is more virulent than RC and RSW and is favored by higher temperatures.

INTRODUCTION

Root diseases caused by soilborne pathogens are a serious problem affecting wheat production in Oklahoma. *Rhizoctonia* is a soilborne fungus consisting of several species, some of which reduces emergence of wheat seedlings by causing pre- and post-emergence damping-off. The binucleate species of *Rhizoctonia*, *R. cerealis*, causes sharp eye spot and the multinucleate species, *R. solani*, causes Rhizoctonia root rot of wheat. Sharp eye spot is indicated by elliptical lesions on the stem base of both young and mature plants. Severe infection may result in white heads (Clarkson et al, 1983). Root rots induced by *R. solani* are indicated by the occurrence of diseased plants in circular patches. These plants become stunted and chlorotic in the most acute form of the disease, and white heads appear as a result of *R. solani* infection.

Various factors including temperature greatly influence the incidence and severity of *Rhizoctonia*-induced diseases. Studies conducted on different anastomosis groups of *R. solani* affecting various crops including potato (Carling et al, 1990), sugar beet (O`Sullivan et al, 1991) and hard red winter wheat (Mathieson, 1991) have all shown that temperature affects disease severity rating. For example, studies conducted on sugar beet showed that pre-emergence damping-off caused by *R. solani* AG-4 was more severe at 16-30 C, as compared to that caused by AG-2, which was more virulent at lower (10-15 C) temperatures (O'Sullivan et al, 1991). In a study conducted to determine the effect of temperature on Rhizoctonia root rot (AG-4) in hard red winter wheat, emergence of seedlings decreased significantly as temperature increased from 15 to 35 C (Mathieson, 1991). Hence, planting wheat later in the year when soil temperatures are cooler might help reduce the incidence of Rhizoctonia root rot (AG-4).

Inoculum density also can influence the severity of *Rhizoctonia*-induced diseases. The affects of inoculum density on the incidence of *Rhizoctonia*-induced root rots have been studied on various crops including canola (Yitbarek et al, 1988), cabbage (Keinath, 1995) and dry beans (Van Bruggen et al, 1986). For example, Yitbarek et al (1988) studied the effect of temperature and inoculum density on pre-emergence damping-off in canola. The effect of *R. solani* isolates of AG-4 and AG 2-1 on emergence of canola was tested at six different inoculum densities [0, 1,400, 2,800, 5,700, 11,400 and 22,800 viable propagules/L soil-free growth medium (VP/L SFM)] and at six different temperature regimes (7-8, 7-12, 7-18, 12-18, 19-25, and 26-35 C night-day air temperature regimes). The isolate of AG 2-1 caused more pre-emergence damping-off than the isolate of AG-4 at all inoculum levels tested in the 7-8 C and 7-12 C temperature regime. The isolate of AG 2-1 caused more pre-emergence damping-off at the 7-18 C regime than the isolate of AG-4 at the lower inoculum densities (< 5,700 VP/L SFM). The isolate of AG-4 caused more pre-emergence damping-off than the isolate of AG 2-1 at all inoculum densities in the 26-35 C regime and at the lower inoculum densities (< 5,700 VP/L SFM) in the 12-18 C and 19-25 C regimes. Both isolates caused similar levels of pre-emergence damping-off at the higher inoculum densities (11,400 and 22,800 VP/L SFM) in the 7-18, 12-18 and 19-25 C regimes. Thus, increasing the inoculum density results in increased disease severity rating, until a plateau is reached (Yitbarek et al, 1988). These studies, which demonstrate the affect of inoculum density on disease incidence and severity, demonstrate the importance of determining the response of specific crops to different AG groups of *Rhizoctonia* spp.

Determining the effects of temperature and inoculum density on the incidence

and severity of Rhizoctonia root rot is important for wheat producers in Oklahoma, where hard red winter wheat is planted for both grain and forage (Krenzer, 1994). For forage production, wheat is planted early (late August or September) when soil temperatures are higher. In contrast, the optimum planting time for grain production is early to late October, when the soil temperatures are much cooler. Since various species and anastomosis groups of *Rhizoctonia* have different temperature optima for pathogenicity, it is important to determine the effect of temperature and inoculum density on the incidence of *Rhizoctonia* induced diseases. Hence the main objective of this study was to determine the effects of temperature on disease induced by three isolates of *Rhizoctonia*. Pathogenicity and virulence of these isolates were also studied over a range of inoculum densities and also on different cultivars of hard red winter wheat.

MATERIALS AND METHODS

Isolates

Two isolates of *R. solani*, one each from peanut and wheat, and an isolate of *R. cerealis* from wheat were characterized. One *R. solani* isolate (#24) was obtained in 1998 from roots of wheat plants showing root rot symptoms growing near Tipton, OK by Dr. Larry Singleton (Associate Professor, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK) and was designated as RSW (*R. solani* from wheat). The *R. solani* isolate from peanut (#23) was obtained from Dr. Hassan Melouk (USDA Research Scientist, Stillwater, OK), and was originally isolated from peanut pods showing symptoms of pod rot collected from Hughes county, OK in 1982. This isolate was designated as RSP (*R. solani* from peanut) and was tentatively identified as belonging to AG-4 (Filonow et al, 1988). *R. cerealis* isolate (#5) was isolated in 1995

from a sharp eye spot lesion present on a wheat stem (variety 2180) growing near Haskell, OK by Dr. Larry Singleton and was designated as isolate RC (*R. cerealis*). The isolates were maintained at 22-25 C on PDSA [Potato Dextrose Agar (1/5 strength PDA. Difco, Detroit, Michigan, USA) amended with streptomycin sulfate (0.3 g/L)] and were transferred to fresh plates once every three to four days. The transfer plugs (0.5 cm) were excised from the actively growing outer margin of growth with a sterilized cork borer (0.5 cm). For long-term storage, the isolates were maintained at 20-22 C on PDA slants and were transferred twice or thrice a year to fresh slants.

Inoculum preparation

In a 250-ml Erlenmeyer flask, 23 ml of water was added to 25 g of wheat seeds, which were then sterilized by autoclaving for 20 minutes (121 C, 15 psi). The flasks were kept overnight at room temperature (20-25 C) and then inoculated aseptically in a laminar-flow transfer hood with a *Rhizoctonia* isolate. The isolates were grown on PDSA. Sterilized wheat seeds in the Erlenmeyer flasks were inoculated by adding three plugs (0.5 cm taken from the actively growing outer fringe of growth) of each isolate into separate flasks. The flasks were then kept at room temperature (20–25 C) for 7-8 days by which time hyphal growth had covered the wheat seeds. To avoid clumping of the seeds, flasks were shaken vigorously each day. Ten seeds from each flask were then plated on to PDSA to ensure that the culture used to inoculate the wheat seeds was not contaminated.

Pathogenicity at different temperatures

Ten certified wheat seeds of the hard red winter wheat 2137 were planted in a row in 8 x 8 cm plastic pots at a depth of ~ 1.5 cm. Seeds were planted in non-sterile Redi-earth

Peat-lite mix (Scotts-Sierra Horticultural Products Company, 14111 Scottslawn Rd, Marysville, OH 43041), which is a potting mixture consisting of vermiculite and Canadian sphagnum moss. Inoculum was prepared as described previously and the healthy seeds were inoculated with each of the *Rhizoctonia* isolates at a ratio of 1:1 (10 healthy: 10 inoculated seeds). The inoculated seeds were evenly distributed along the row, over the healthy seeds. The pots were kept in the incubators at seven temperatures (C) [10 (Curtin Maatheson scientific, Model C 1213), 15 (Percival Scientific, model I-35 LL), 20 (Curtin Maatheson scientific, Model C 1213), 22.5 (Precision Scientific, Model 815), 25 (Fisher Scientific, model 655 D), 27.5 (Percival Scientific, model I-36 LL), 30 (Fisher Scientific, model 655 D)], in the dark. The seedlings were removed from the incubator four days after planting, when the seedlings at 30 C began emerging. The pots were then kept at room temperature (20 – 25 C), under supplemental light, [(17.2 μ E S^{-1} m^{-2} at pot height (6")] for ten more days. All combinations of three isolates (RC, RSW and RSP) and the uninoculated control and seven temperatures (10, 15, 20, 22.5, 25, 27.5, 30 C) resulted in 28 treatments. Each treatment had three replications. The experiment was repeated thrice. Seedling emergence was recorded 7 and 14 days after planting (DAP). Individual plant heights also were measured. The seedlings were gently removed from the pots 14 DAP and the soil was washed from the roots under a stream of tap water. The stem and the leaf sheath were then examined and rated for damage from *Rhizoctonia* (disease severity rating) on a scale ranging from 1 to 6 (1 = healthy: no discoloration of the leaf sheath or stem; 2 = slight discoloration on the leaf sheath or stem; 3 = distinct eyespots on the leaf sheath or the stem; 4 = rotting at the base of the stem or of the whole plant; 5 = post- emergence damping-off or yellowing; 6

= no emergence). After grading, the roots and shoots were separated just above the seed and the fresh root and shoot weights were determined. A small portion of the leaf sheath was then placed on to PDA amended with streptomycin sulfate. In all cases *Rhizoctonia* was re-isolated. Analysis of variance was performed on all data using the proc mixed procedure (Statistical Analysis System, SAS Institute Inc., Cary, NC).

Pathogenicity as affected by inoculum level

As described previously, ten certified wheat seeds were planted in plastic pots and inoculated with each of the *Rhizoctonia* isolates. Three inoculum levels (10, 20 or 30 infected seeds) and a control (0 infected seeds) were tested in these experiments that were conducted at 10 and 30 C. The pots were then placed in incubators (Percival Scientific, model I-35 LL and I-36 LL) with 16:8 photoperiod (21.2 and 36.35 μ E S^{-1} m^{-2}), for 14 days. Seedling emergence and disease severity rating were measured as described previously. All combinations of the three isolates (RC, RSW and RSP), three inoculum levels (10, 20, 30 infected seeds) and control resulted in ten treatments for each temperature (10, 30 C). Each treatment had three replications and the experiment was repeated twice. However, the disease severity was rated in the three replicates of only one experiment. Analysis of variance was performed on all data using the proc mixed procedure (Statistical Analysis System, SAS Institute Inc., Cary, NC).

Pathogenicity on hard red winter wheat cultivars

Six hard red winter wheat cultivars, 2137, 2174, Custer, Jagger, Tonkawa and TAM-101 were used in this study. 2137, 2174, Custer, Jagger and Tonkawa were selected as they represent the commonly cultivated varieties in Oklahoma and TAM 101 was selected because of its consistent use in root rot trials over the past fifteen years in Oklahoma.

Ten certified wheat seeds of the six wheat cultivars were inoculated as described previously with each of the *Rhizoctonia* isolates at a ratio of 1:1. The pots were then incubated at either 15 or 30 C (Percival Scientific, model I-35 LL and I-36 LL) with 16:8 photo period (21.2 and 36.35 μ E S^{-1} m^{-2}) for 14 days. Seedling emergence and root and shoot weights were measured and the seedlings were rated for disease severity as previously described. There were 24 treatments at each temperature, three replications for each treatment, and the experiment was repeated thrice. Analysis of variance was performed on all data using the proc mixed procedure (Statistical Analysis System, SAS Institute Inc., Cary, NC).

RESULTS

Pathogenicity at different temperatures

Temperature and *Rhizoctonia* isolate significantly affected seedling emergence as measured by stand counts, and there was a significant interaction between the two factors. RC did not significantly reduce stand counts as compared to the uninoculated control at any temperature (Fig.1). There was no significant difference in the stand counts of RC-inoculated seedlings across temperatures. RSW did not significantly reduce stand counts as compared to the uninoculated control at any temperature (Fig 1). There was no significant difference in the stand counts of RSW-inoculated seedlings across temperatures. RSP significantly reduced stand counts as compared to the uninoculated control at all temperatures (Fig. 1). There was a significant difference in the stand counts of RSP-inoculated seedlings across temperatures and the stand counts were the lowest at 30 C followed by 27.5 C and 25 C (Fig 1).

Temperature and *Rhizoctonia* isolate significantly affected disease severity rating

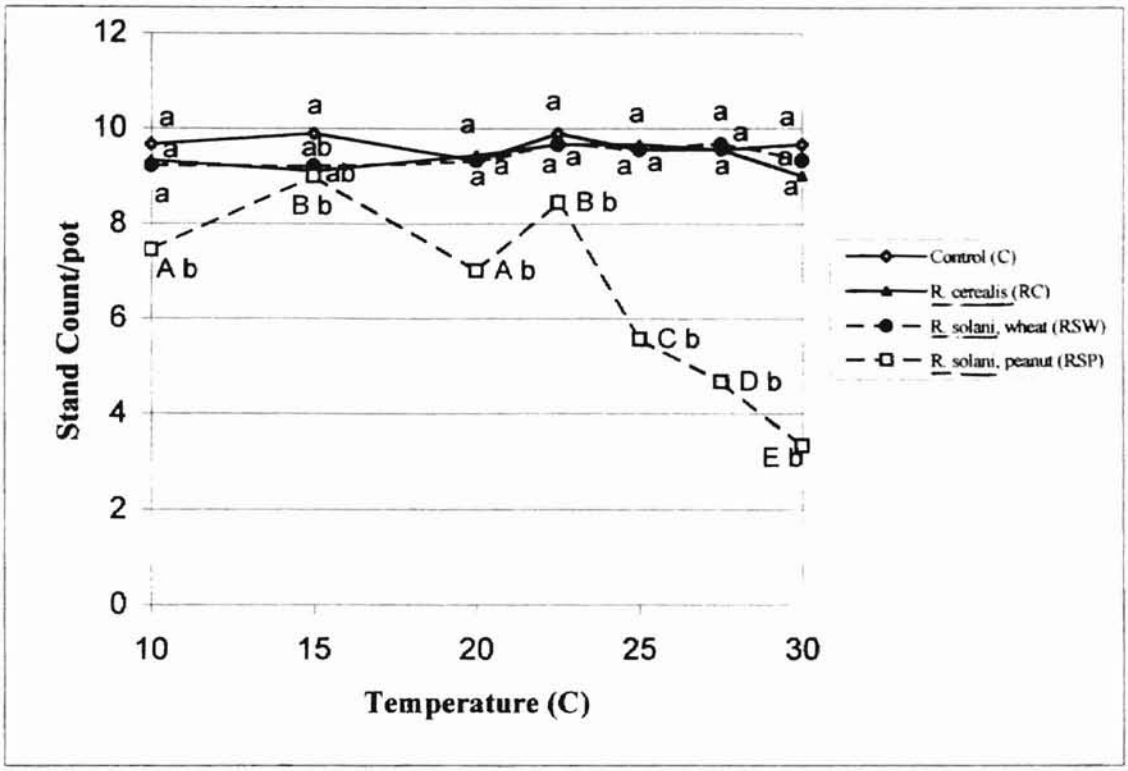

Fig.1. Effect of temperature on the stand count of wheat seedlings after 14 days following inoculation with *Rhizoctonia* isolates. Lower case letters compare stand counts as influenced by isolates within a temperature. Data points followed by the same letter are not significantly different at $P \leq 0.05$. Uppercase letters compare stand counts as influenced by RSP across temperatures. Data points followed by the same letter are not significantly different at $P \leq 0.05$.

and there was a significant interaction between the two factors. The disease severity rating was the lowest for control seedlings at all temperatures (Fig 2). RC-inoculated seedlings received a disease severity rating significantly higher than the uninoculated control only at 15 C and 30 C (Fig 2). There was no significant difference in the disease severity ratings of RC-inoculated seedlings across temperatures. Disease severity ratings of RSW-inoculated seedlings were significantly higher than the uninoculated control at all temperatures (Fig 2). There was no significant difference in the disease severity ratings of RSW-inoculated seedlings across temperatures. RSP-inoculated seedlings received the highest disease severity rating as compared to the control, RC and RSW-inoculated plants at all temperatures (Fig. 2). Comparing RSP across temperatures, RSP-inoculated seedlings received the highest disease severity rating at 30 C, followed by 27.5 and then by 25 C (Fig 2).

Plant height was significantly affected by temperature and *Rhizoctonia* isolate, and there was a significant interaction between the two factors. Control seedlings were tallest at all temperatures except 27.5 C (Fig 3). Comparing seedling height of control seedlings across all temperatures, seedlings at 25 C were significantly taller than control seedlings at all other temperatures except 22.5 C (Fig 4). Compared to the control, RC significantly reduced seedling height only at 15 C and 25 C (Fig 3). Comparing seedling height of RC-inoculated seedlings across temperatures, seedlings were significantly taller at 25 C than at 10 C and 15 C and 20 C (Fig 4). Compared to the control, RSW significantly reduced plant height only at 15 C (Fig 3). Comparing seedling height of RSW-inoculated seedlings across temperatures, seedlings were the tallest at 25 C (Fig 4),

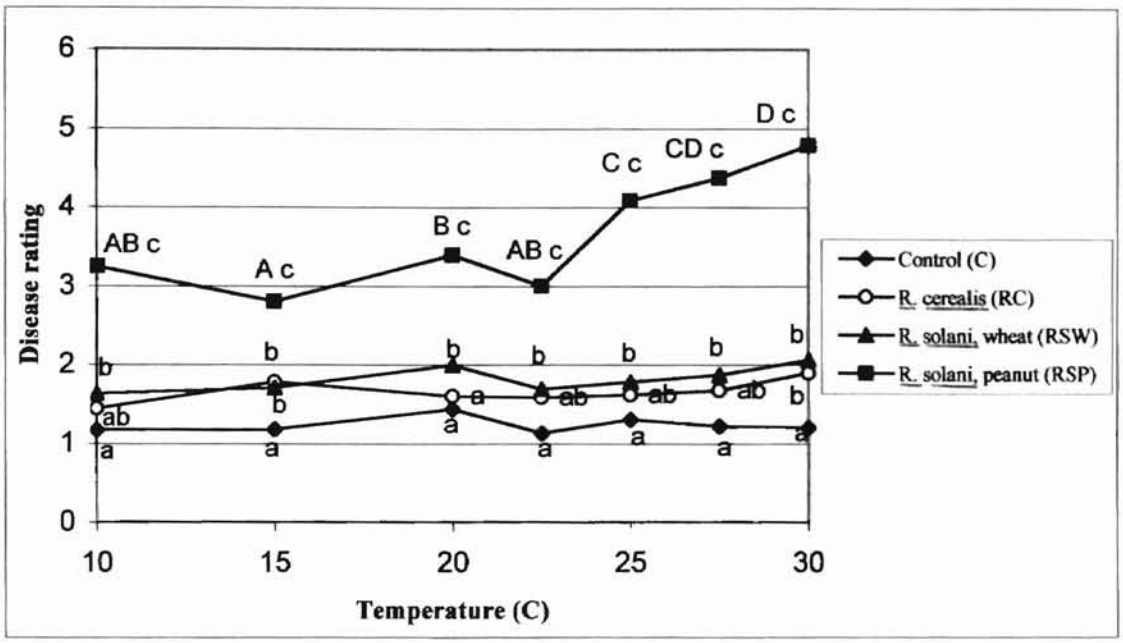

Fig.2. Effect of temperature on disease severity rating of wheat seedlings after 14 days following inoculation with *Rhizoctonia* isolates. Lower case letters compare the disease rating as influenced by isolates within a temperature and uppercase letters compare disease rating as influenced by RSP across temperatures ($P \leq 0.05$). Disease rating was based on a scale from 1-6, where 1 = healthy seedlings; 2 = slight discoloration on the leaf sheath or stem; 3 = distinct eyespots; 4 = rotting at the base of the stem or the whole seedling; 5 = post-emergence damping-off or yellowing; 6 = no emergence. Data points followed by the same letter are not significantly different at $P \leq 0.05$.

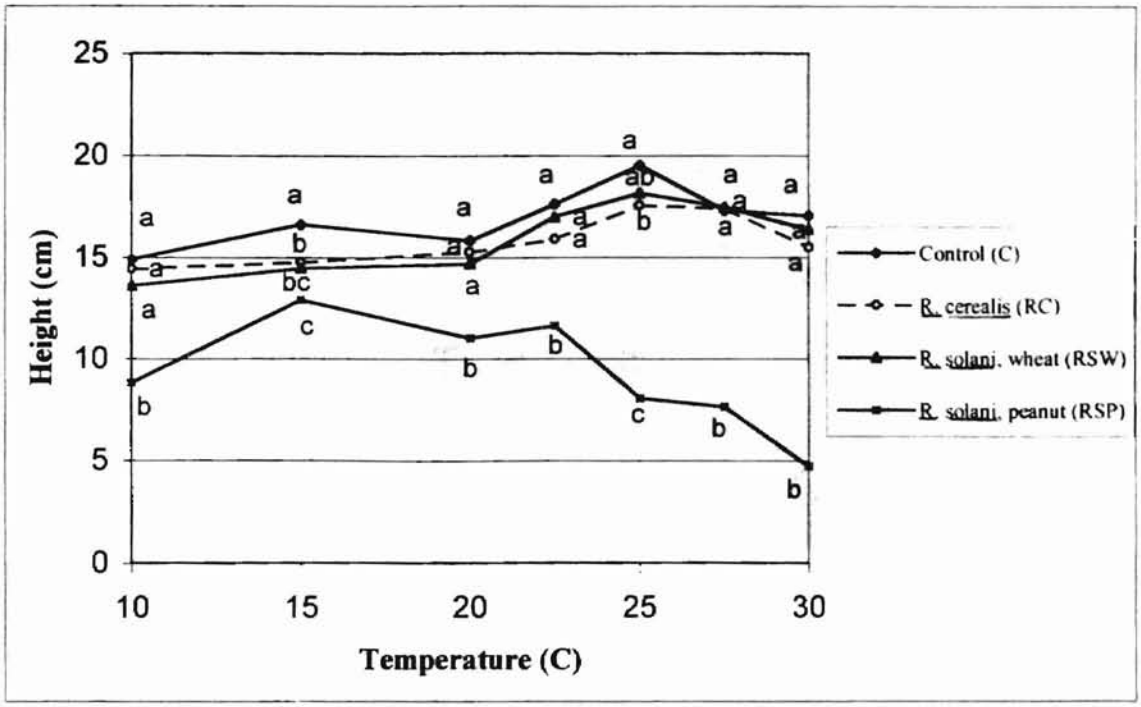

Fig.3. Effect of temperature on the height of wheat seedlings following inoculation with *Rhizoctonia* isolates. Letters compare differences in plant height as influenced by isolates within a temperature. Data points followed by the same letter are not significantly different at $P \leq 0.05$.

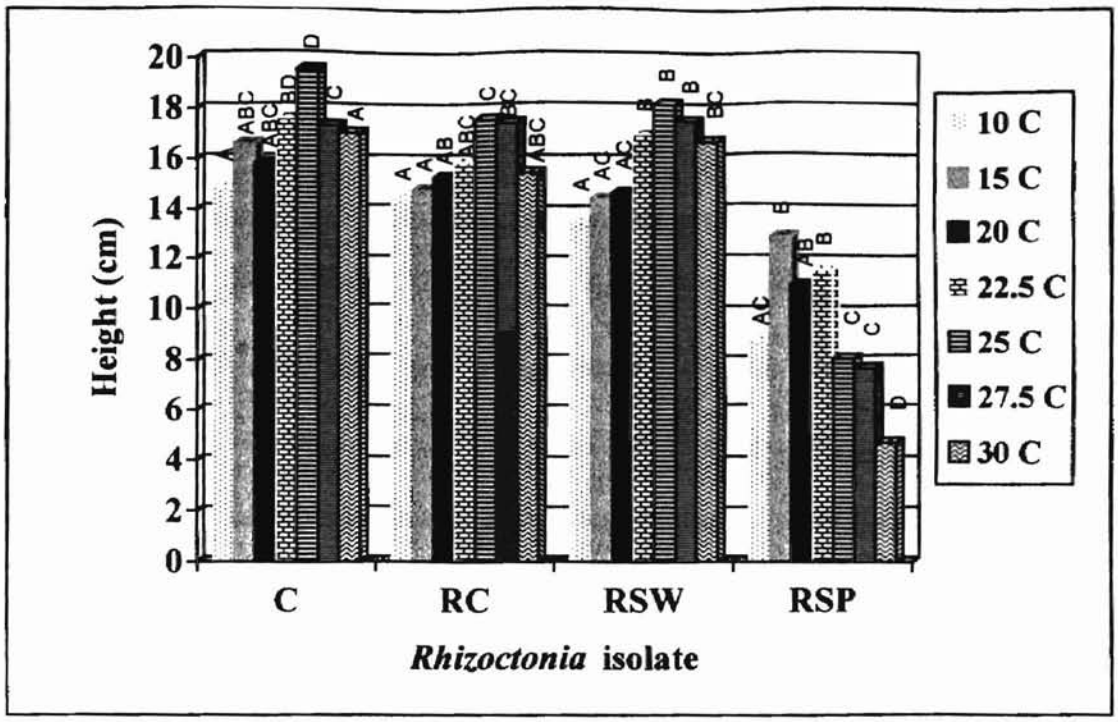

Fig.4. Effect of temperature on the height of wheat seedlings following inoculation with *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Letters compare differences in seedling heights as influenced by isolates across temperatures. Bars with the same letter are not significantly different at $P \leq 0.05$.

which was not statistically different from the seedlings at 22.5, 27.5 or 30 C. RSP-inoculated seedlings were significantly shorter than the uninoculated control at all temperatures (Fig 3). Comparing seedling height of RSP-inoculated seedlings across temperatures, seedlings had the lowest plant heights at temperatures $\geq$ 25 C (Fig 4).

Fresh shoot weight was significantly affected by *Rhizoctonia* isolate but not by temperature. However, there was a significant interaction between the two factors. Control seedlings had the highest shoot weight at all temperatures (Fig 5). Comparing shoot weight of control seedlings across temperatures, shoot weight at 25 C was significantly higher than the shoot weight at all other temperatures except 15 C (Fig 6). RC significantly reduced shoot weight as compared to the uninoculated control at all temperatures except 27.5 C and 30 C (Fig. 5). Comparing the shoot weight of RC-inoculated seedlings across temperatures, seedlings at 25 C had the highest shoot weight (Fig 6). Compared to the control, RSW significantly reduced shoot weight at all temperatures except 22.5, 27.5 and 30 C (Fig 5). Comparing the shoot weight of RSW-inoculated seedlings across temperatures, RSW-inoculated seedlings at 25 C was significantly higher than the shoot weights at 10 C, 15 C and 20 C (Fig 6). RSP reduced shoot weight significantly as compared to the control at all temperatures (Fig. 5). Comparing fresh shoot weight of RSP-inoculated seedlings across temperatures, shoot weight was the lowest at 30 C, with weights at 10, 25 and 27.5 C lower than at 15, 20 and 22.5 C (Fig 6).

Fresh root weight was significantly affected by *Rhizoctonia* isolate but not by temperature. There was a significant interaction between the two factors. In comparison to the control, RC did not significantly reduce root weight at any temperature (Fig. 7,

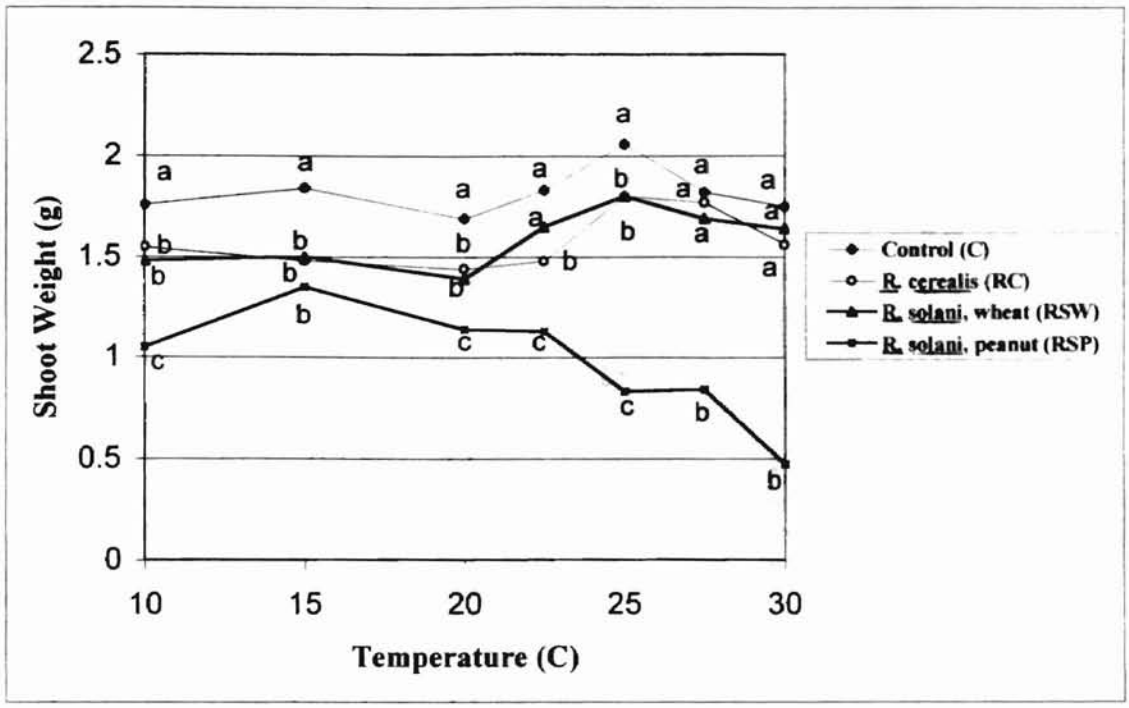

Fig.5. Effect of temperature on the fresh shoot weight of wheat seedlings following inoculation with *Rhizoctonia* isolates. Letters compare fresh shoot weights as influenced by isolates within a temperature ($P \leq 0.05$). Data points followed by the same letter are not significantly different at $P \leq 0.05$.

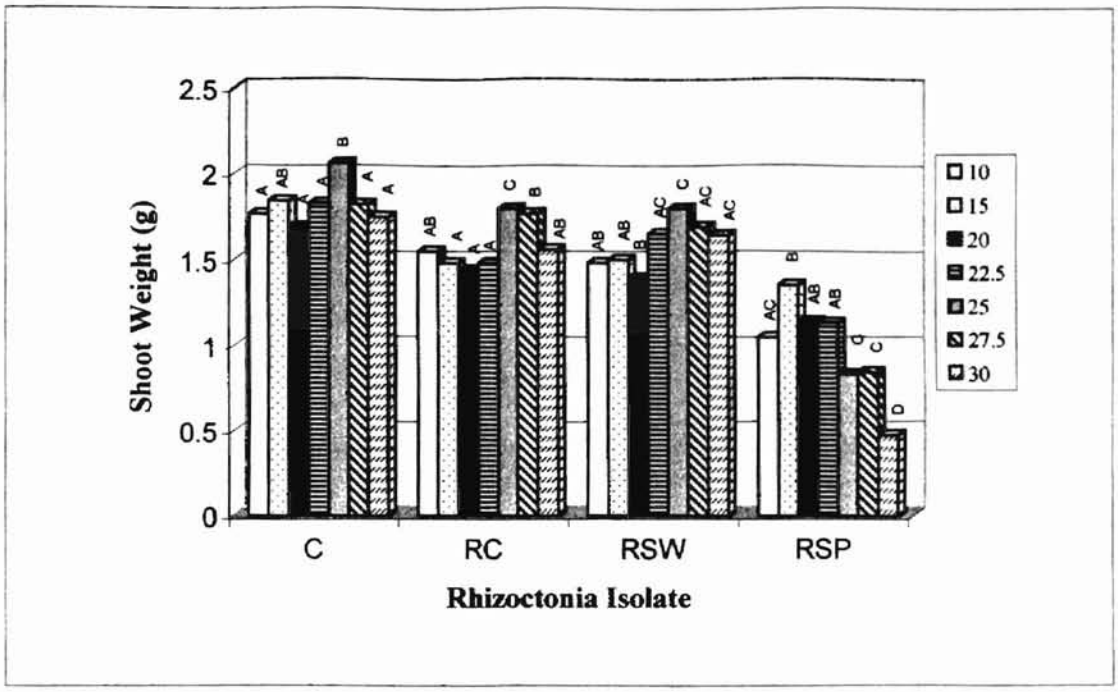

Fig.6. Effect of temperature on the fresh shoot weight of wheat seedlings following inoculation with *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Letters compare fresh shoot weights as influenced by isolates across temperatures. Bars with the same letter are not significantly different at $P \leq 0.05$.

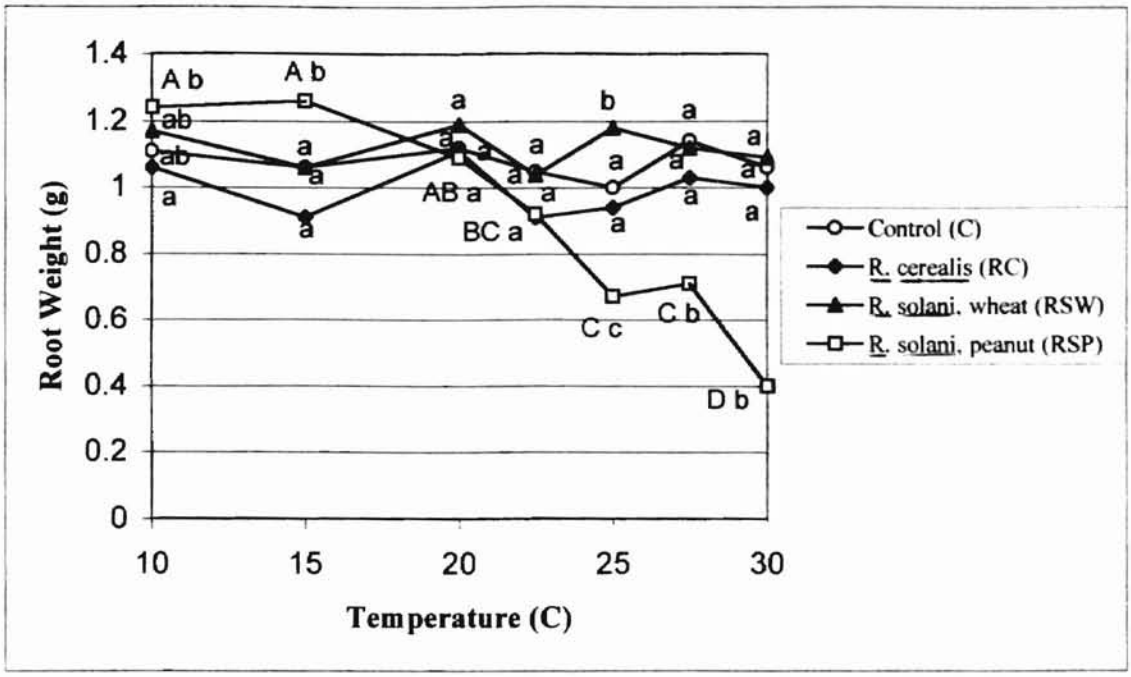

Fig.7. Effect of temperature on the fresh root weight of wheat seedlings following inoculation with *Rhizoctonia* isolates. Lower case letters compare fresh root weights as influenced by isolates within a temperature. Data points followed by the same letter are not significantly different at $P \leq 0.05$. Upper case letters compare fresh root weights as influenced by RSP across all temperatures. Data points followed by the same letter are not significantly different at $P \leq 0.05$.

lower case letters) and there was no significant difference in the root weights of RC-inoculated seedlings across temperatures. In comparison to the control, RSW did not significantly reduce root weights at any temperature (Fig 7, lower case letters) and there was no significant difference in the root weights of RSW-inoculated seedlings across temperatures. RSP significantly reduced root weights as compared to the uninoculated control at temperatures $\geq$ 25 C (Fig 7, lower case letters). Comparing root weight of RSP-inoculated seedlings across temperatures, seedlings had significantly lower root weights at temperatures $\geq$ 25 C (Fig 7, uppercase letters).

Pathogenicity as affected by inoculum level

Stand count was significantly affected by *Rhizoctonia* isolate but not by temperature and there was a significant interaction between *Rhizoctonia* isolate and temperature. RC and RSW did not significantly reduce stand counts as compared to the control at any inoculum level at 10 C or 30 C (Fig 8 and 9). At the same inoculum level, the stand counts of RC and RSW-inoculated seedlings were not significantly different across temperatures and hence the data is not presented. RSP did not significantly reduce stand counts as compared to the uninoculated control at any inoculum level at 10 C (Fig 8). However, at 30 C, RSP significantly reduced stand counts as compared to the uninoculated control at all inoculum levels (Fig 9). At all inoculum levels, the stand counts of RSP-inoculated seedlings were significantly lower at 30 than at 10 C (Fig 10).

Disease severity rating was significantly affected by *Rhizoctonia* isolate. There was no significant difference between inoculum levels in the disease severity rating within RC and RSW and RSP-inoculated seedlings at either temperature. At 10 C, RC and RSW-inoculated seedlings received disease severity ratings similar (not significantly

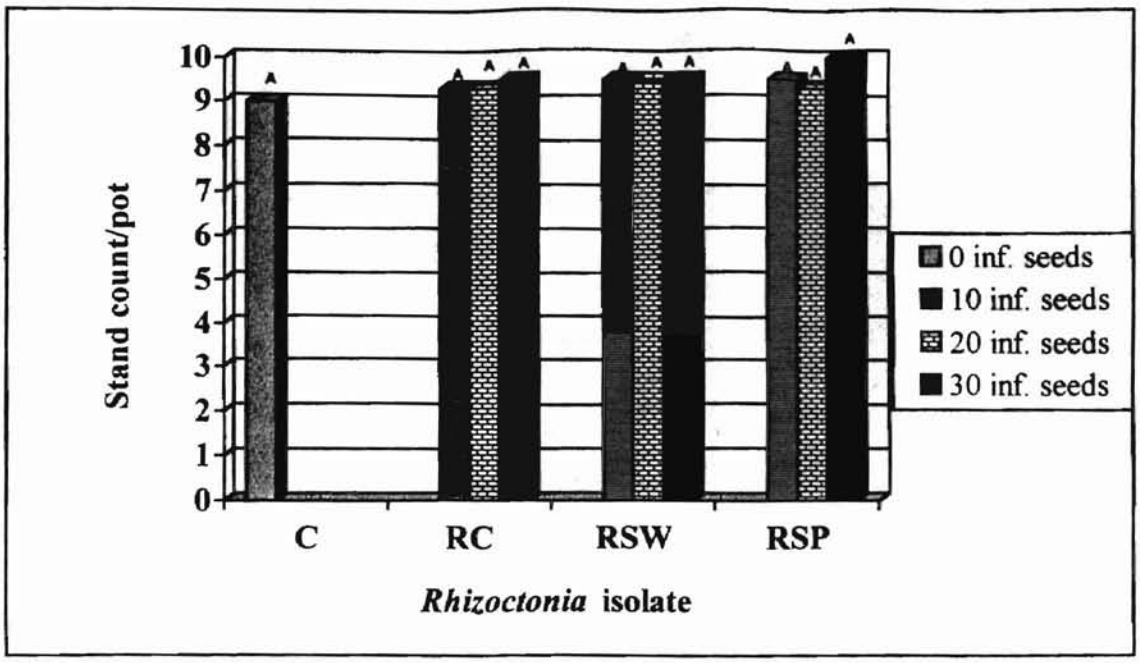

Fig 8. Effect of inoculum levels (0, 10, 20 and 30 infected seeds) on the stand counts of wheat seedlings after 14 days following inoculation with *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP), at 10 C. Letters compare stand counts as influenced by *Rhizoctonia* isolates at three inoculum levels. Bars with the same letter are not significantly different at $P \leq 0.05$.

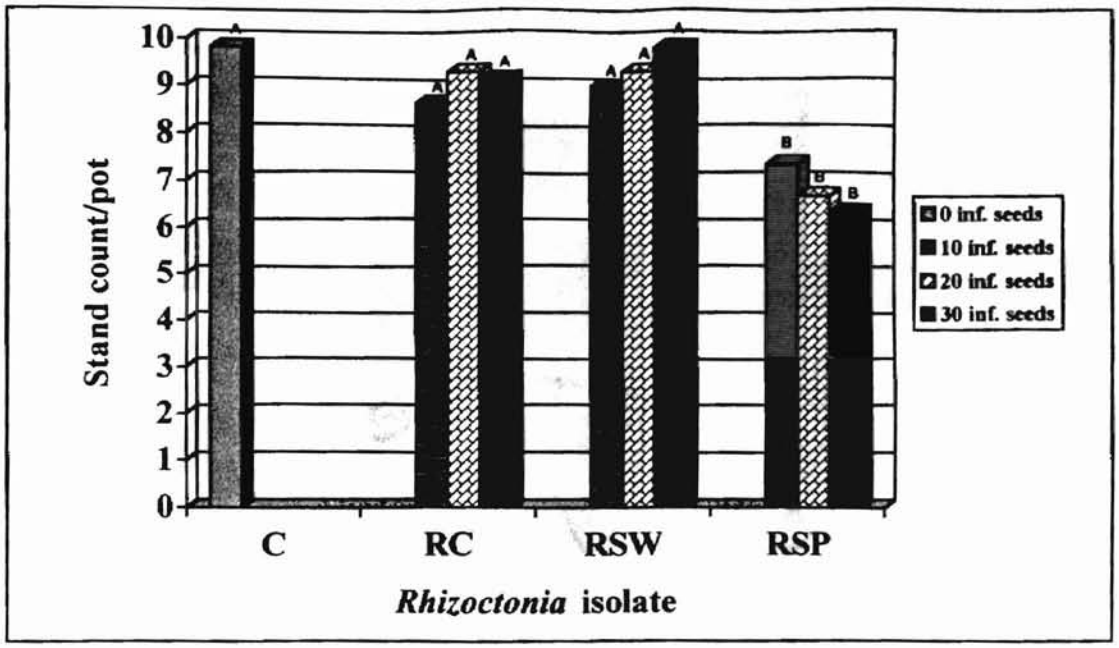

Fig 9. Effect of inoculum levels (0, 10, 20 and 30 infected seeds) on the stand counts of wheat seedlings after 14 days following inoculation with *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP), at 30 C. Letters compare stand counts as influenced by *Rhizoctonia* isolates at three inoculum levels. Bars with the same letter are not significantly different at $P \leq 0.05$.

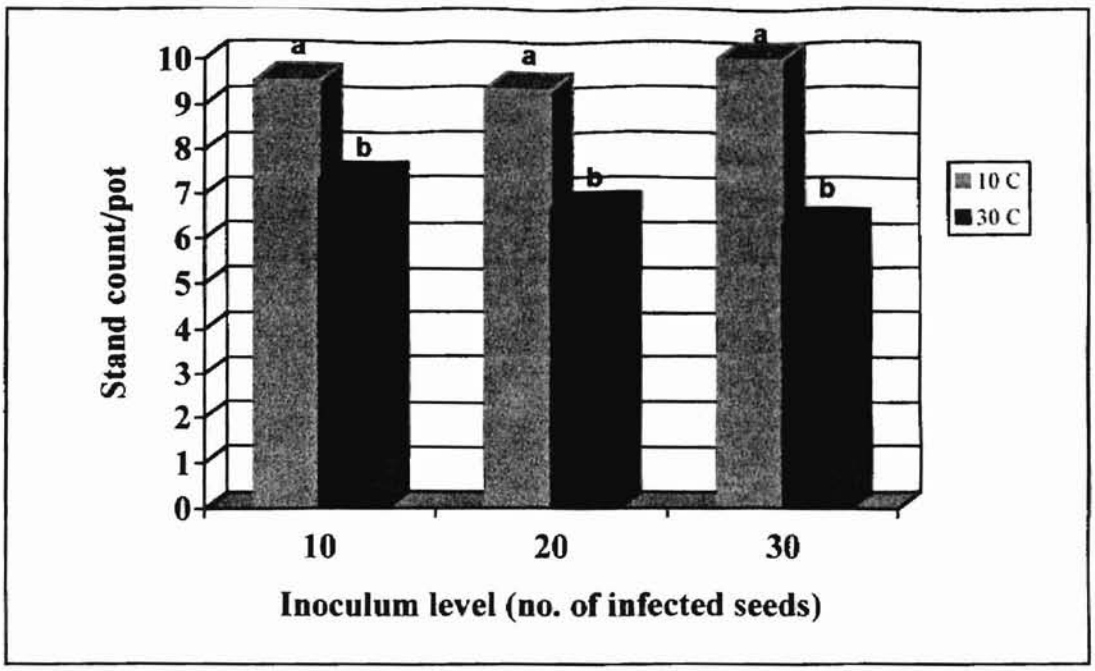

Fig 10. Effect of temperature (10 and 30 C) on the stand counts of wheat seedlings after 14 days following inoculation with three inoculum levels (10, 20, 30 infected seeds) of *Rhizoctonia solani* from peanut. Letters compare stand counts as influenced by temperature at each inoculum level. Bars with the same letter are not significantly different at $P \leq 0.05$.

different) to the control at all the three (10, 20 and 30 infected seeds) inoculum levels (Fig 11). However, at 30 C, RC and RSW-inoculated seedlings received significantly higher disease severity rating as compared to the control at all the three inoculum levels (Fig 12). RSP-inoculated seedlings received significantly higher disease severity rating as compared to the uninoculated control at all the three inoculum levels at both 10 and 30 C (Fig 11 and 12). At inoculum level of 30 infected seeds, the disease severity rating for RSP-inoculated seedlings was significantly higher at 30 C than at 10 C. There was no significant difference in the disease severity rating of RC and RSW and RSP-inoculated seedlings across the three inoculum levels at either temperature.

Pathogenicity on hard red winter wheat cultivars

At 15 C, wheat variety significantly affected the stand counts, where as *Rhizoctonia* isolate had no significant effect on stand counts. There was also no significant interaction between variety and isolate. Averaging across isolates to compare the varieties, stand counts showed only very slight statistical differences ($p \leq 0.05$), with wheat cultivars 2137 and Jagger having significantly lower stand counts than 2174 and TAM 101 (data not shown).

The disease severity rating was significantly affected by *Rhizoctonia* isolate and variety, and there was a significant interaction between the two factors. Control seedlings of all varieties received the lowest disease severity rating (Fig.13). Comparing control seedlings across all varieties, seedlings of 2174 and TAM 101 received significantly lower disease severity rating than Custer (Fig.14). RC-inoculated seedlings of all varieties received significantly higher disease severity rating as compared to the uninoculated control (Fig 13). RC-inoculated seedlings of all varieties received similar

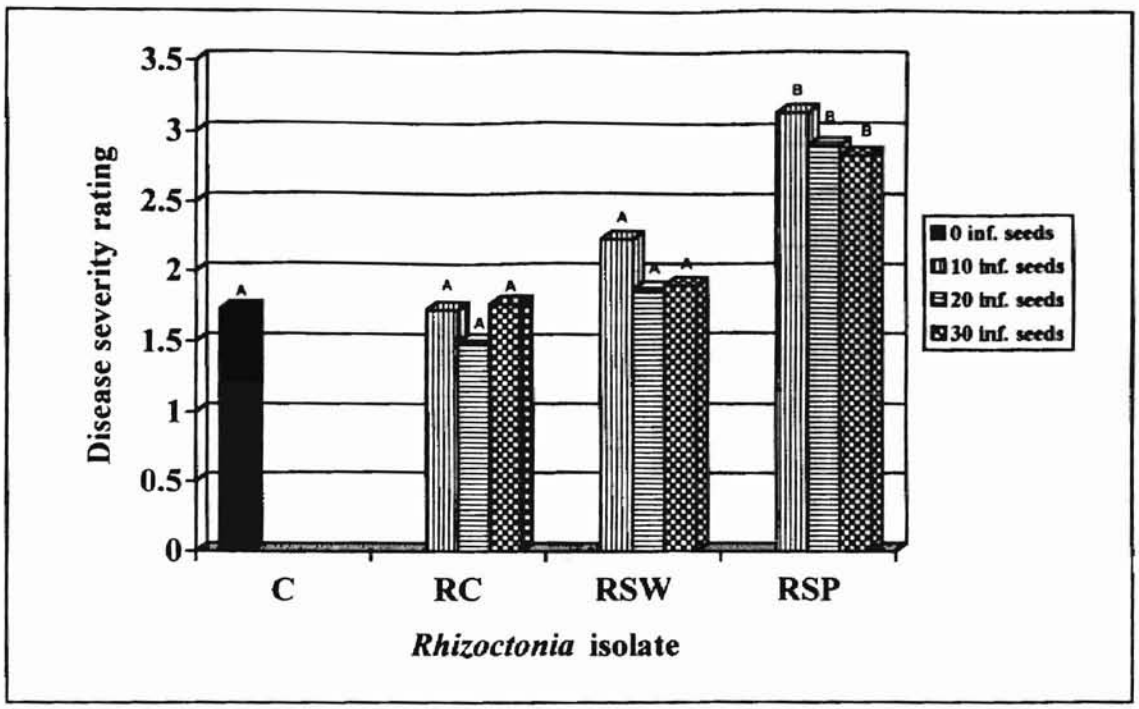

Fig 11. Effect of inoculum levels (0, 10, 20 and 30 infected seeds) on the disease severity rating of wheat seedlings following inoculation with *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP), at 10 C. Letters compare disease severity as influenced by *Rhizoctonia* isolates at the three inoculum levels. Bars with the same letter are not significantly different at $P \leq 0.05$.

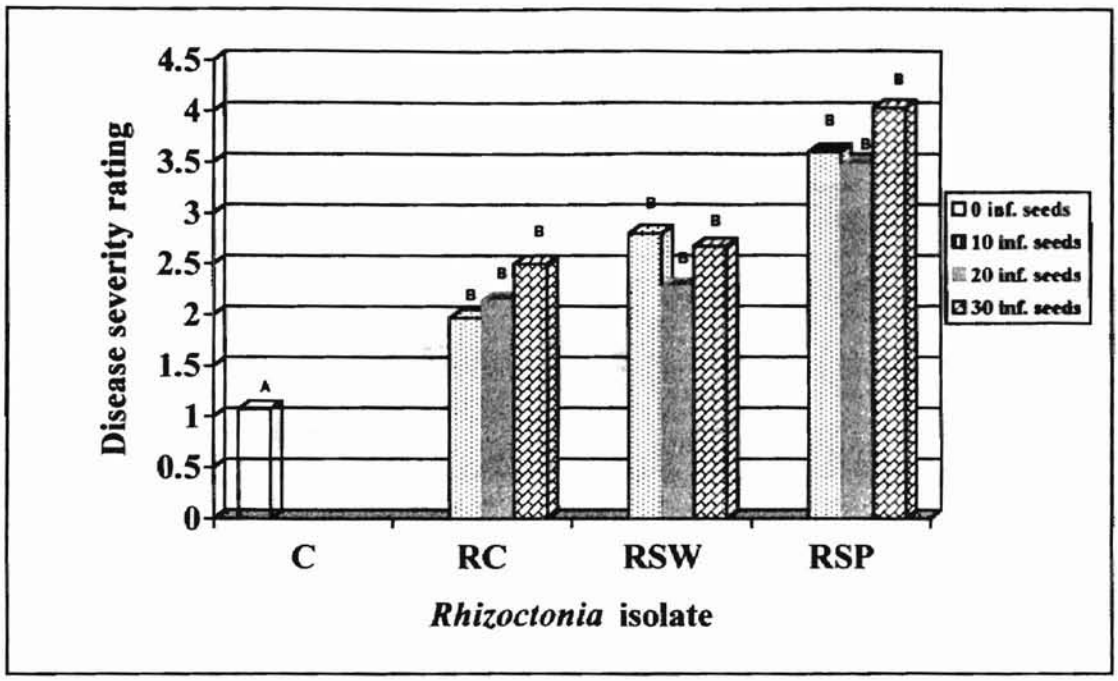

Fig 12. Effect of inoculum levels (0, 10, 20 and 30 infected seeds) on the disease severity rating of wheat seedlings following inoculation with *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP), at 30 C. Letters compare disease severity as influenced by *Rhizoctonia* isolates at the three inoculum levels. Bars with the same letter are not significantly different at $P \leq 0.05$.

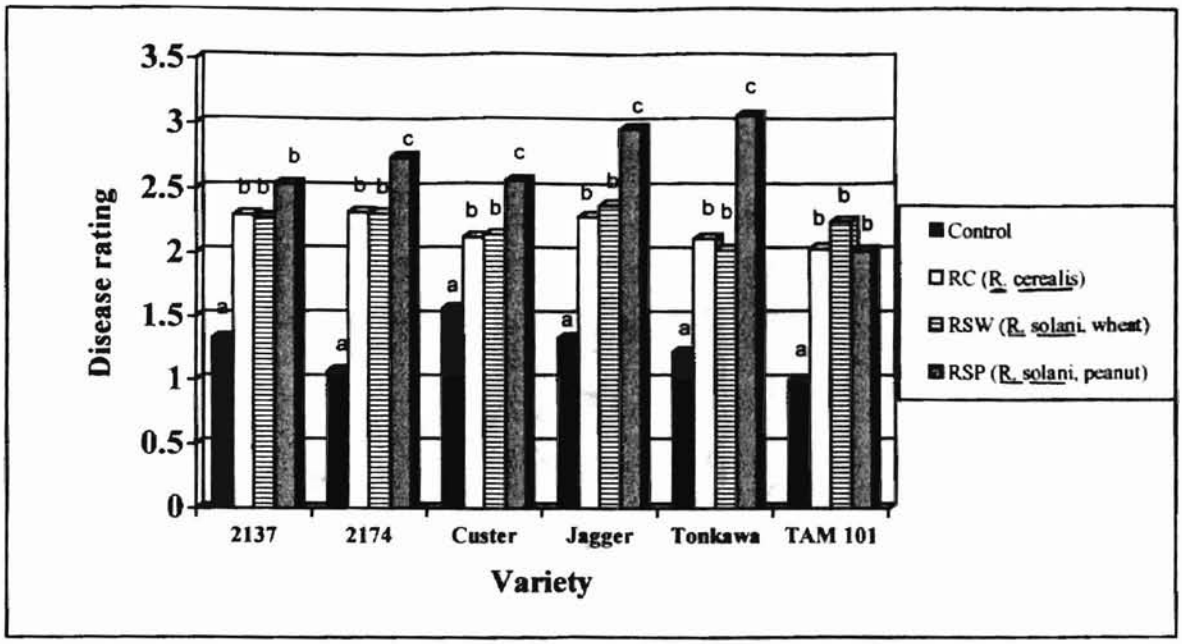

Fig. 13. Effect of *Rhizoctonia* isolates on the disease severity rating of hard red winter wheat varieties at 15 C. Letters compare disease rating as influenced by isolates within each variety ($P \leq 0.05$). Disease rating was based on a scale from 1-6, where 1 = healthy seedlings; 2 = slight discoloration on the leaf sheath or stem; 3 = distinct eyespots; 4 = rotting at the base of the stem or the whole seedling; 5 = post-emergence damping-off or yellowing; 6= no emergence

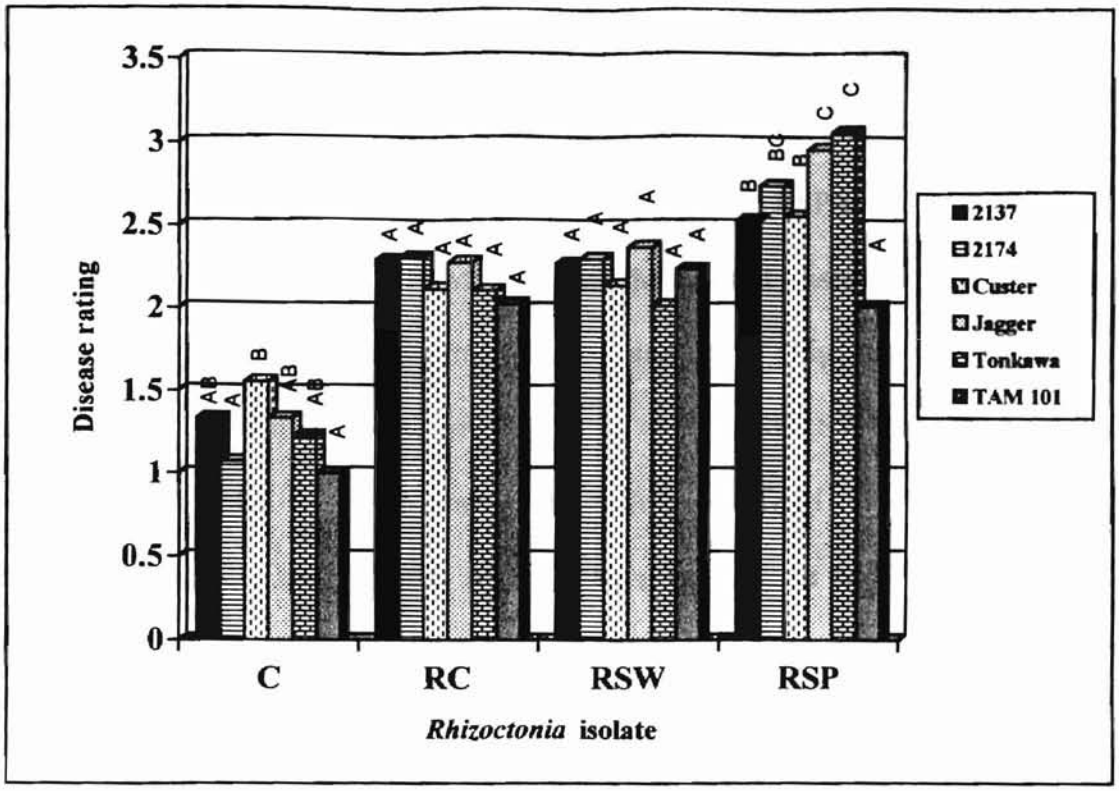

Fig.14. Effect of *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on disease severity rating of hard red winter wheat varieties at 15 C. Letters compare disease rating as influenced by isolates across varieties. Bars with the same letter are not significantly different at $P \leq 0.05$. Disease rating was based on a scale from 1-6, where 1= healthy seedlings; 2 = slight discoloration on the leaf sheath or stem; 3 =distinct eyespots; 4 = rotting at the base of the stem or the whole seedling; 5 = post-emergence damping-off or yellowing; 6 = no emergence.

(not significantly different) disease severity rating (Fig 14). RSW-inoculated seedlings of all varieties received significantly higher disease severity rating as compared to the uninoculated control (Fig 13). RSW-inoculated seedlings of all varieties received similar (not significantly different) disease severity rating (Fig 14). RSP-inoculated seedlings of all varieties except 2137 and TAM 101 had the highest disease severity rating as compared to an uninoculated control and RC and RSW (Fig 13). RSP-inoculated seedlings received similar disease severity rating as RC and RSW-inoculated seedlings for 2137 and TAM 101. RSP-inoculated seedlings of TAM 101, 2137 and Custer received significantly lower disease severity rating than Jagger and Tonkawa (Fig 14). Among the RSP-inoculated seedlings, TAM 101 received the lowest disease severity rating (Fig 14).

Fresh shoot weight was significantly affected by both isolate and variety, and there was a significant interaction between the two factors. Comparing control seedlings across all varieties, seedlings of 2137 and Custer had significantly lower shoot weights than 2174, Tonkawa and TAM 101 (Fig.16). RC-inoculated seedlings of all varieties except Custer had significantly lower shoot weights than the uninoculated control seedlings (Fig 15). Comparing RC-inoculated seedlings across all varieties, seedlings of 2137 had significantly lower stand counts than the RC-inoculated seedlings of all the other varieties (Fig 16). RSW-inoculated seedlings of all varieties except Custer had significantly lower shoot weights than the uninoculated control seedlings (Fig 15). Comparing RSW-inoculated seedlings across all varieties, seedlings of 2137 and Tonkawa had significantly lower shoot weights than the RSW-inoculated seedlings of all the other varieties (Fig 16). As compared to the uninoculated control, RSP did not

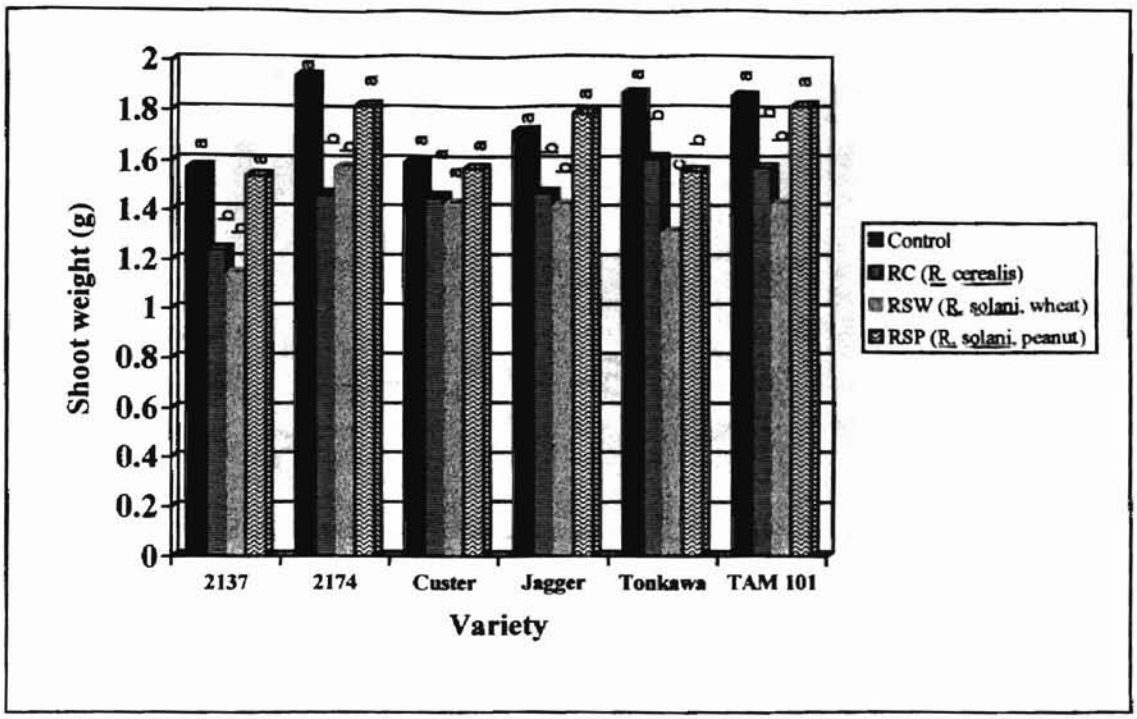

Fig. 15. Effect of *Rhizoctonia* isolates on the fresh shoot weight of hard red winter wheat varieties at 15 C. Letters compare fresh shoot weights as influenced by isolates within varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

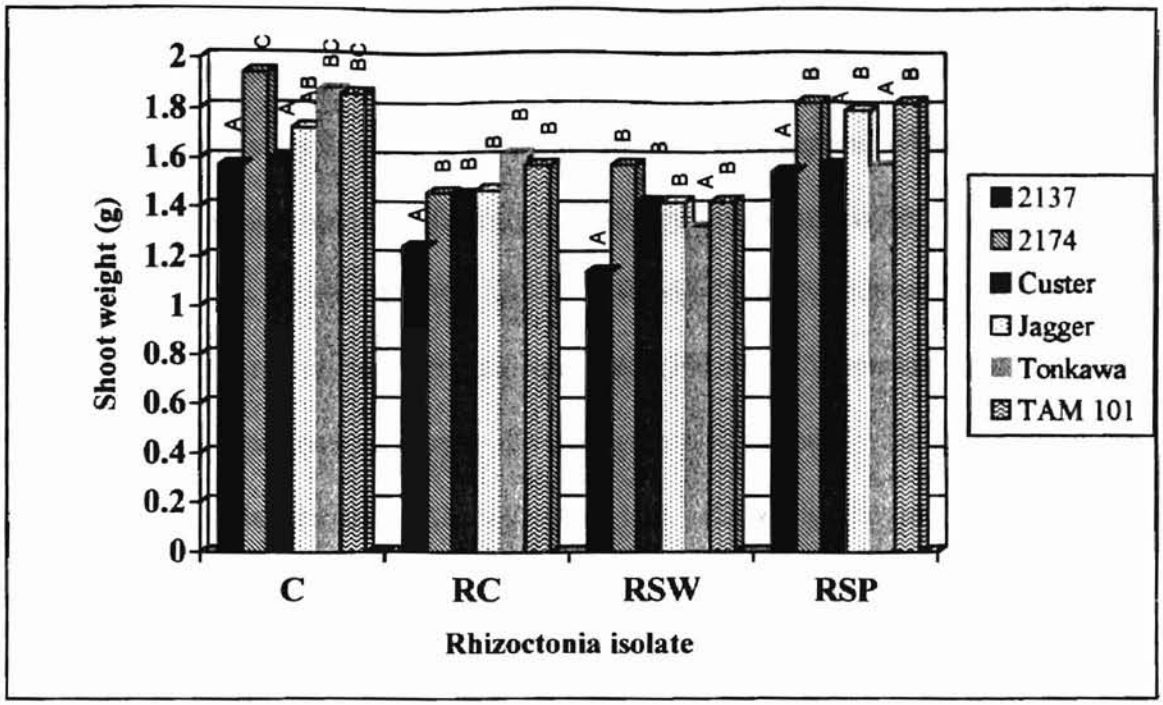

Fig.16. Effect of *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on the fresh shoot weight of hard red winter wheat varieties at 15 C. Letters compare fresh shoot weights as influenced by each isolate across varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

significantly reduce shoot weights of any of the wheat varieties except Tonkawa (Fig 15). Comparing RSP-inoculated seedlings across all varieties, seedlings of 2137, Custer and Tonkawa had significantly lower shoot weights than 2174, Jagger and TAM 101 (Fig 16).

Fresh root weight was significantly affected by isolate and variety and there was significant interaction between the two factors. RC significantly reduced root weight of only Custer as compared to the uninoculated control (Fig.17). Comparing RC-inoculated seedlings across all varieties, seedlings of Tonkawa had significantly higher root weights than 2137, Custer, Jagger and TAM 101 (Fig 18). RSW significantly reduced root weights of all varieties except 2137 as compared to an uninoculated control (Fig 17). Comparing RSW-inoculated seedlings across all varieties, seedlings of Custer had significantly higher root weights than Jagger (Fig 18). RSP significantly reduced root weight of only Tonkawa and Custer as compared to an uninoculated control (Fig 17). RSP-inoculated seedlings of 2137 had significantly higher root weight as compared to the control (Fig 17). Comparing RSP-inoculated seedlings across all varieties, seedlings of 2137 had significantly higher root weights than Custer, Jagger and Tonkawa (Fig 18).

At 30 C, *Rhizoctonia* isolate and variety had a significant effect on stand counts and there was significant interaction between the two factors. RC did not reduce stand counts of any of the varieties as compared to an uninoculated control (Fig.19). Comparing RC-inoculated seedlings across all varieties, the stand counts of seedlings were not significantly different across varieties (Fig.20). RSW did not reduce the stand counts of any of the varieties as compared to an uninoculated control (Fig.19). Comparing RSW-inoculated seedlings across all varieties, the stand counts of seedlings were not significantly different across varieties (Fig.20). RSP significantly reduced stand

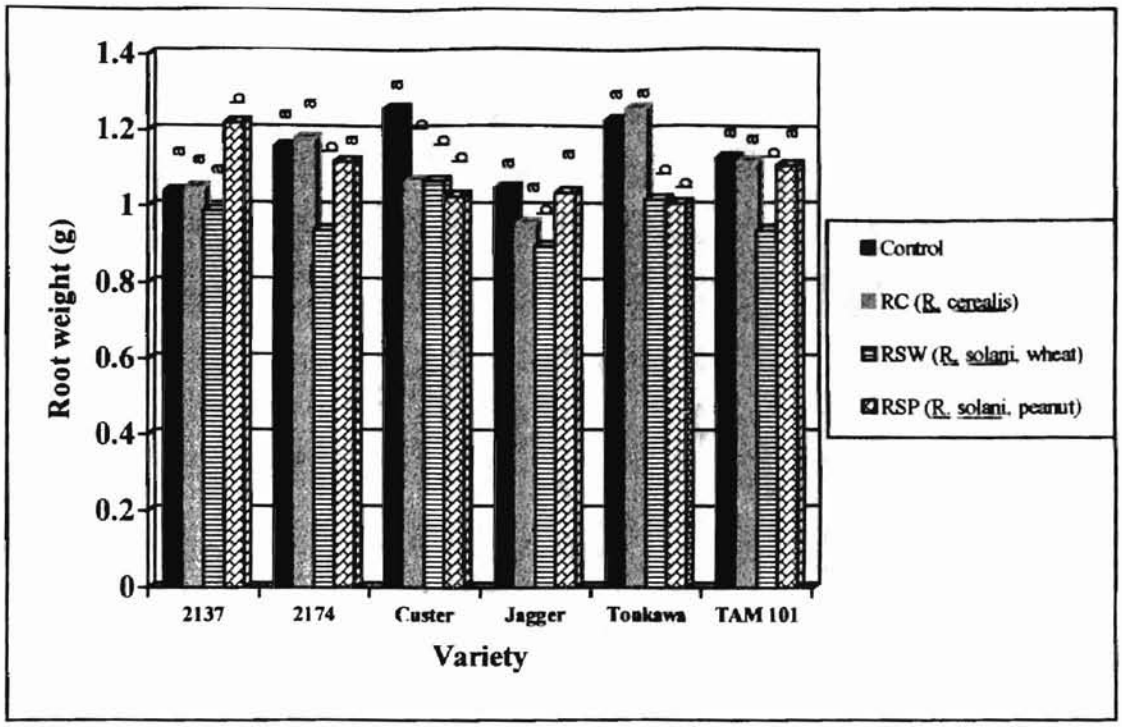

Fig. 17. Effect of *Rhizoctonia* isolates on the fresh root weights of hard red winter wheat varieties at 15 C. Letters compare root weights as influenced by isolates within varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

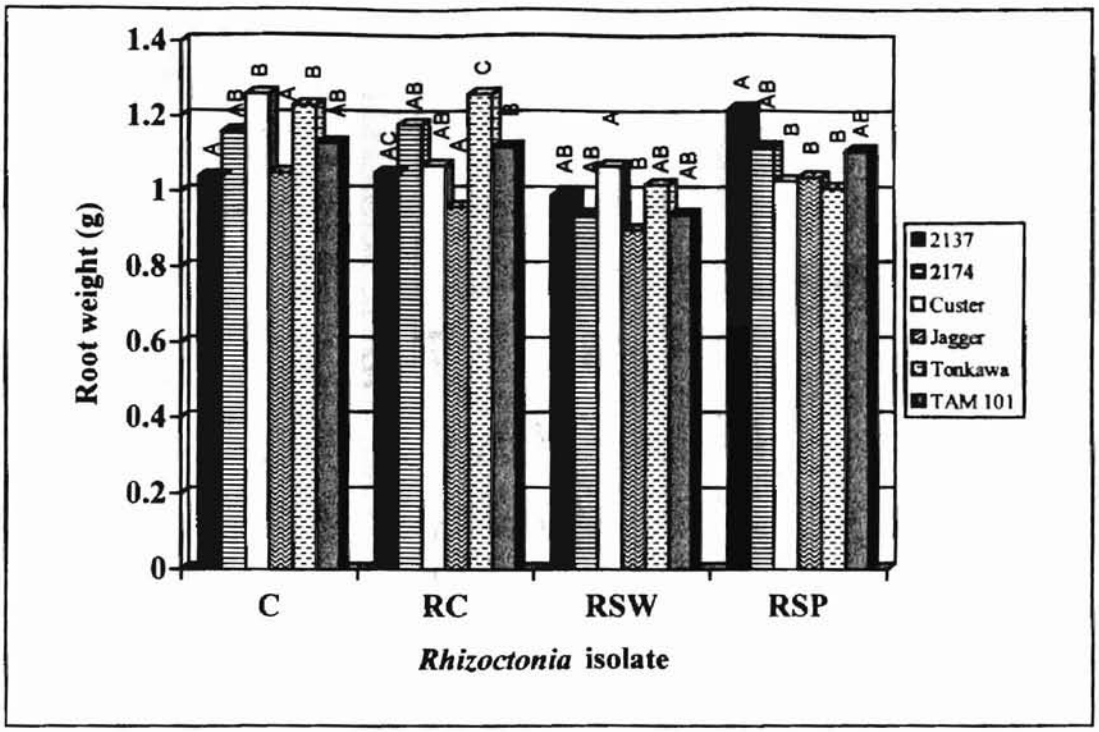

Fig.18. Effect of *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on fresh root weights of hard red winter wheat varieties at 15 C. Letters compare root weights as influenced by each isolate across varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

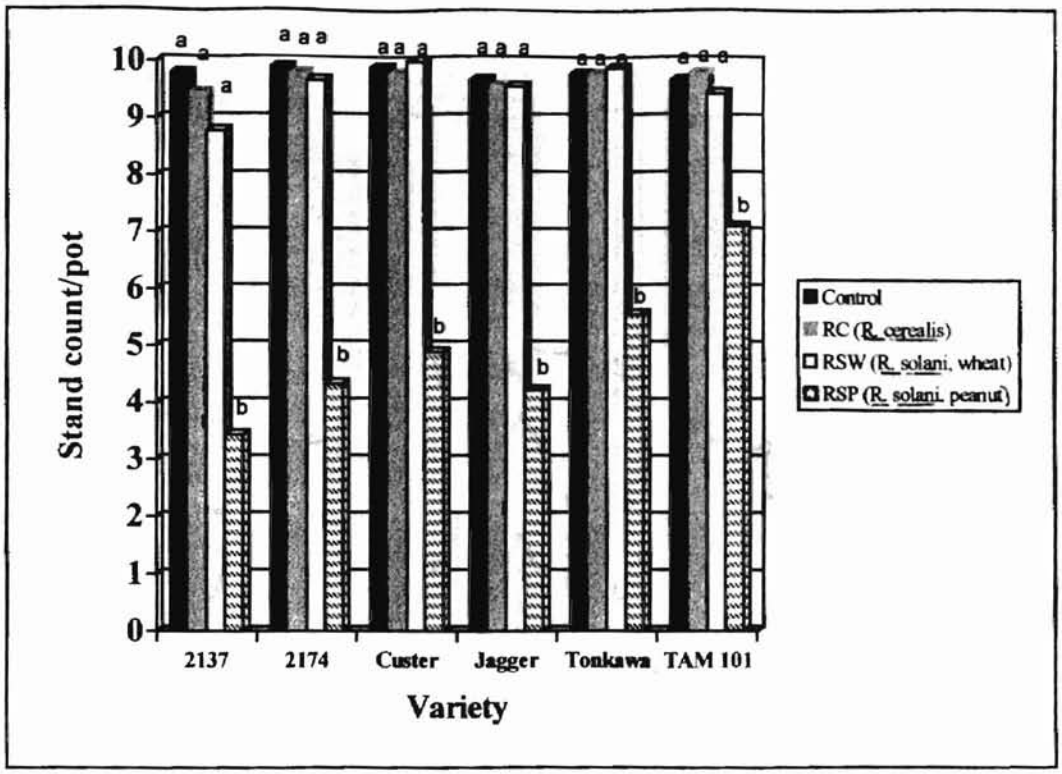

Fig. 19. Effect of *Rhizoctonia* isolates on the stand counts of hard red winter wheat varieties after 14 days at 30 C. Letters compare stand counts as influenced by isolates within varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

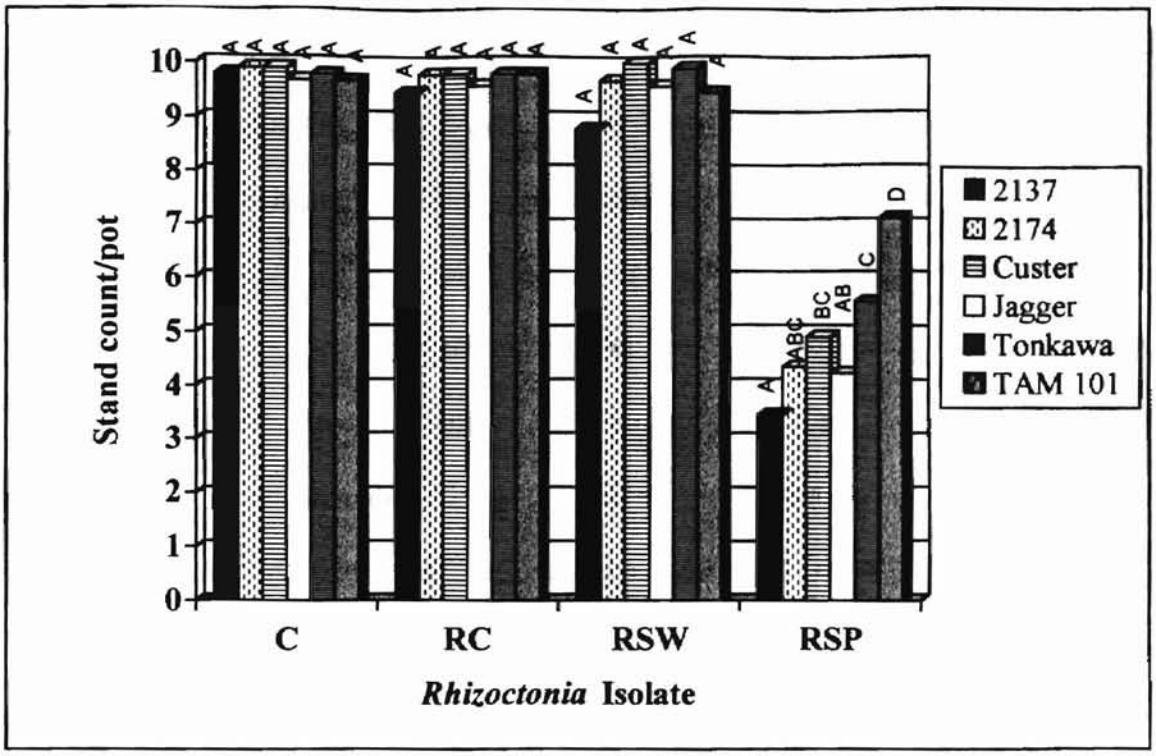

Fig.20. Effect of *Rhizoctonia* isolates *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on the stand counts of hard red winter wheat varieties after 14 days at 30 C. Letters compare stand counts as influenced by isolates across varieties. Bars with the same letter are not significantly different at P≤0.05.

counts of all varieties as compared to an uninoculated control (Fig 19). Comparing RSP-inoculated seedlings across all varieties, seedlings of 2137 had significantly lower stand counts than Custer, Tonkawa and TAM 101 (Fig 20). RSP-inoculated seedlings of TAM 101 had significantly higher stand counts as compared to all the other varieties.

Rhizoctonia isolate and variety had a significant effect on the disease severity rating and there was significant interaction between the two factors. RC-inoculated seedlings of all varieties received similar (not significantly different) disease index rating as compared to an uninoculated control (Fig. 21). Comparing RC-inoculated seedlings across all varieties, seedlings of all varieties received similar (not significantly different) disease severity rating (Fig.22). RSW-inoculated seedlings of all varieties received significantly higher disease severity rating than the uninoculated control seedlings (Fig 21). Comparing RSW-inoculated seedlings across all varieties, seedlings of all varieties received a similar (not significantly different) disease severity rating (Fig.22). RSP-inoculated seedlings of all varieties received the highest disease severity rating (Fig.21). Comparing RSP-inoculated seedlings across all varieties, seedlings of 2137 and 2174 received higher disease severity rating than Custer, Tonkawa and TAM 101 (Fig 22). RSP-inoculated seedlings of TAM 101 received the lowest disease severity rating as compared to the RSP-inoculated seedlings of all the other varieties (Fig 22).

Fresh shoot weight was significantly affected by *Rhizoctonia* isolate and wheat variety. There was no significant interaction between the two factors. RC did not reduce shoot weight of any variety significantly as compared to an uninoculated control (Fig 23). Comparing RC-inoculated seedlings across all varieties, seedlings of Custer, Tonkawa and TAM 101 had significantly higher shoot weights than 2137 (Fig.24). RSW did not

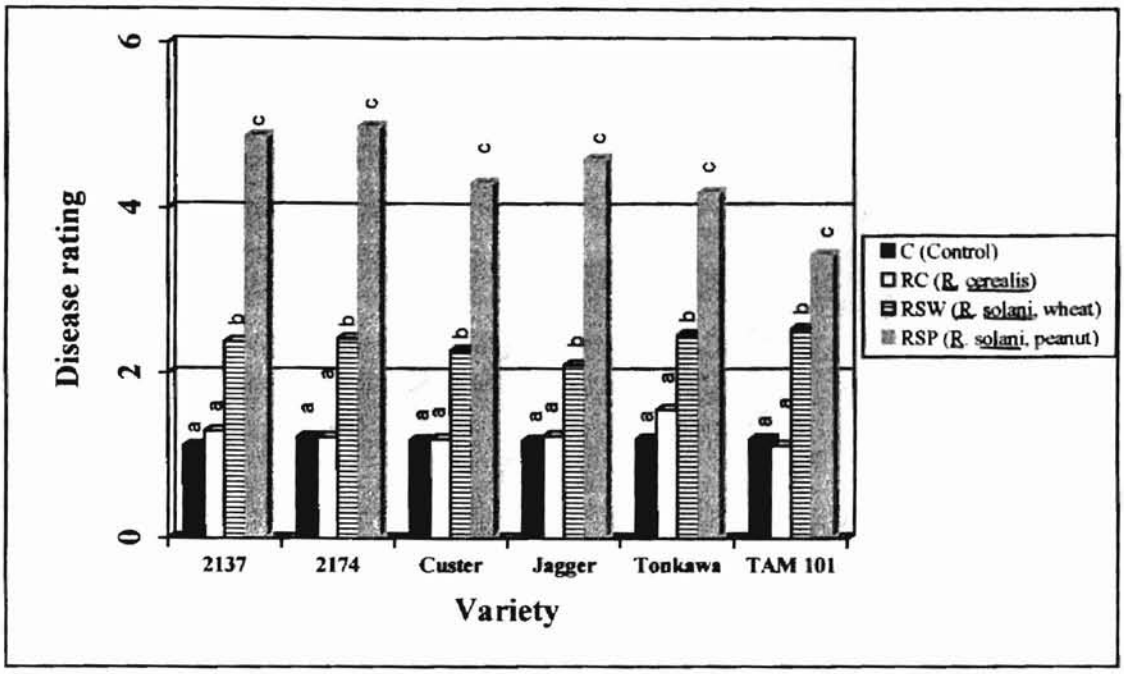

Fig. 21. Effect of *Rhizoctonia* isolates on the disease rating of hard red winter wheat varieties at 30 C. Letters compare disease rating as influenced by isolates within varieties. Bars with the same letter are not significantly different at $P \leq 0.05$. Disease rating was based on a scale from 1-6, where 1 = healthy seedlings; 2 = slight discoloration on the leaf sheath or stem; 3 =distinct eyespots; 4 = rotting at the base of the stem or the whole seedling; 5 = post-emergence damping-off or yellowing; 6 = no emergence.

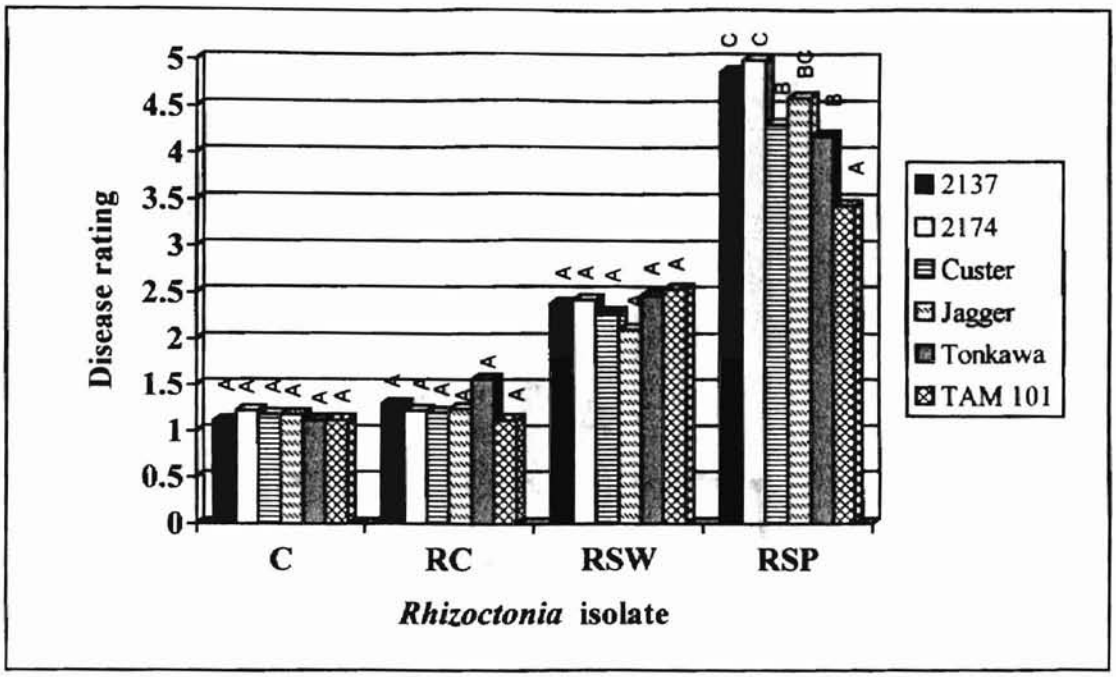

Fig.22. Effect of *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on disease rating of hard red winter wheat varieties at 30 C. Letters compare disease rating as influenced by isolates across varieties. Bars with the same letter are not significantly different at $P \leq 0.05$. Disease rating was based on a scale from 1-6. where 1 = healthy seedlings; 2 = slight discoloration on the leaf sheath or stem; 3 = distinct eyespots; 4 = rotting at the base of the stem or the whole seedling; 5 = post-emergence damping-off or yellowing; 6 = no emergence.

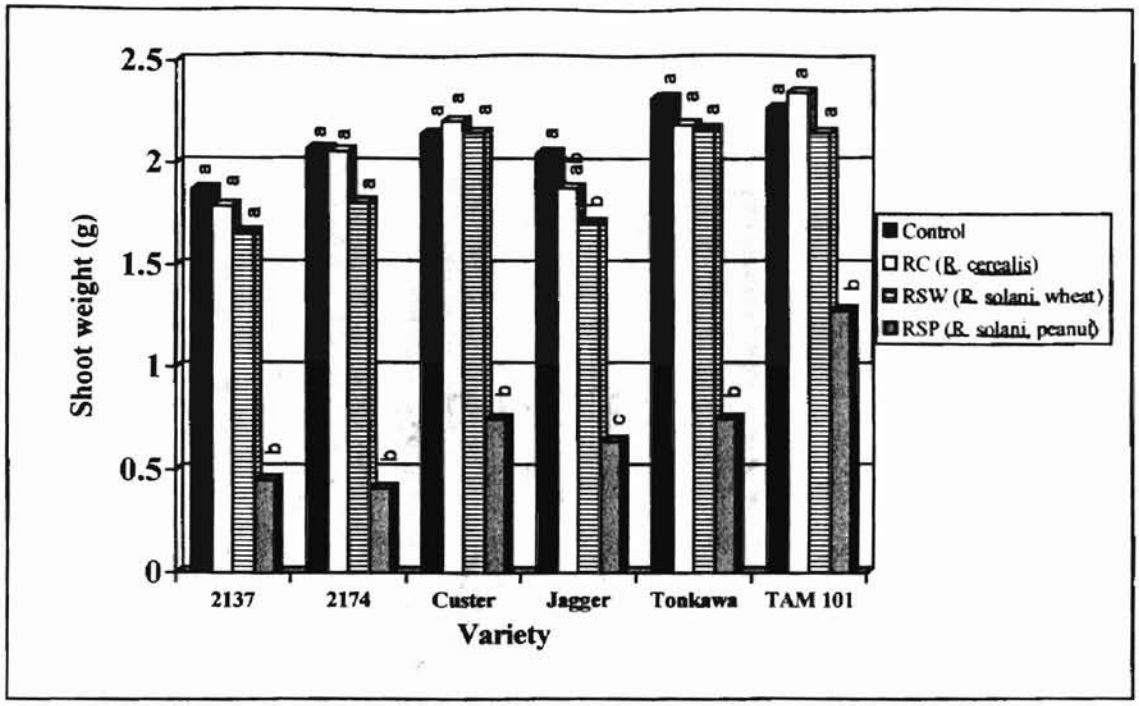

Fig. 23. Effect of *Rhizoctonia* isolates on the fresh shoot weights of hard red winter wheat varieties at 30 C. Letters compare shoot weights as influenced by isolates within each variety. Bars with the same letter are not significantly different at $P \leq 0.05$.

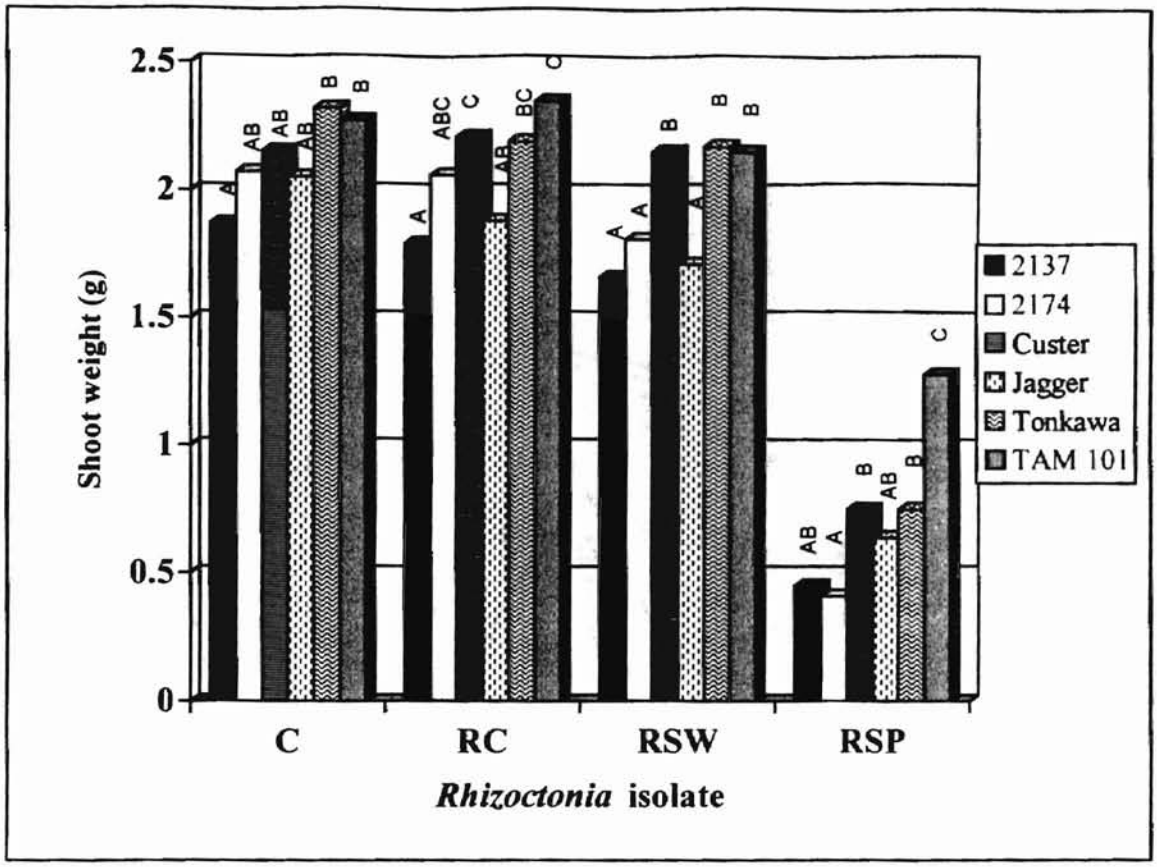

Fig.24. Effect of *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on fresh shoot weights of hard red winter wheat varieties at 30 C. Letters compare shoot weights as influenced by each isolate across varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

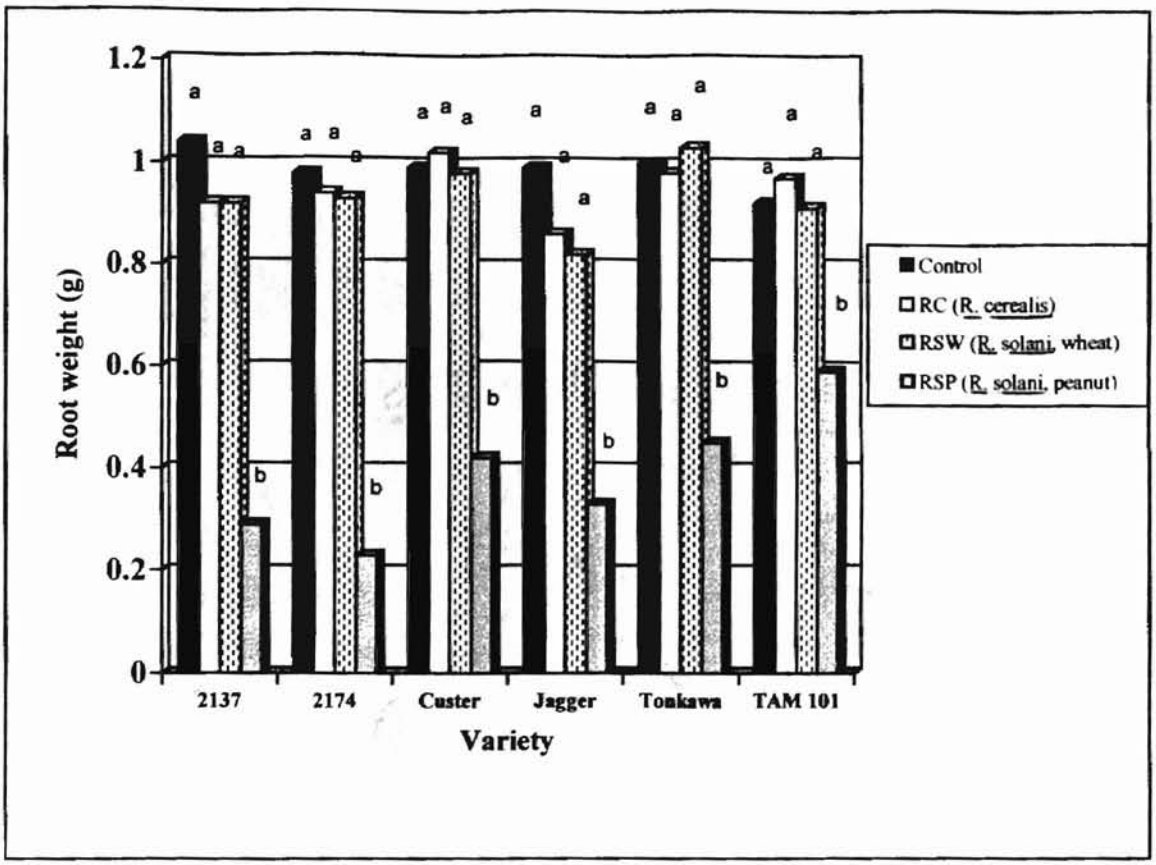

Fig. 25. Effect of *Rhizoctonia* isolates on the fresh root weights of hard red winter wheat varieties at 30 C. Letters compare root weights as influenced by isolates within each variety. Bars with the same letter are not significantly different at $P \leq 0.05$.

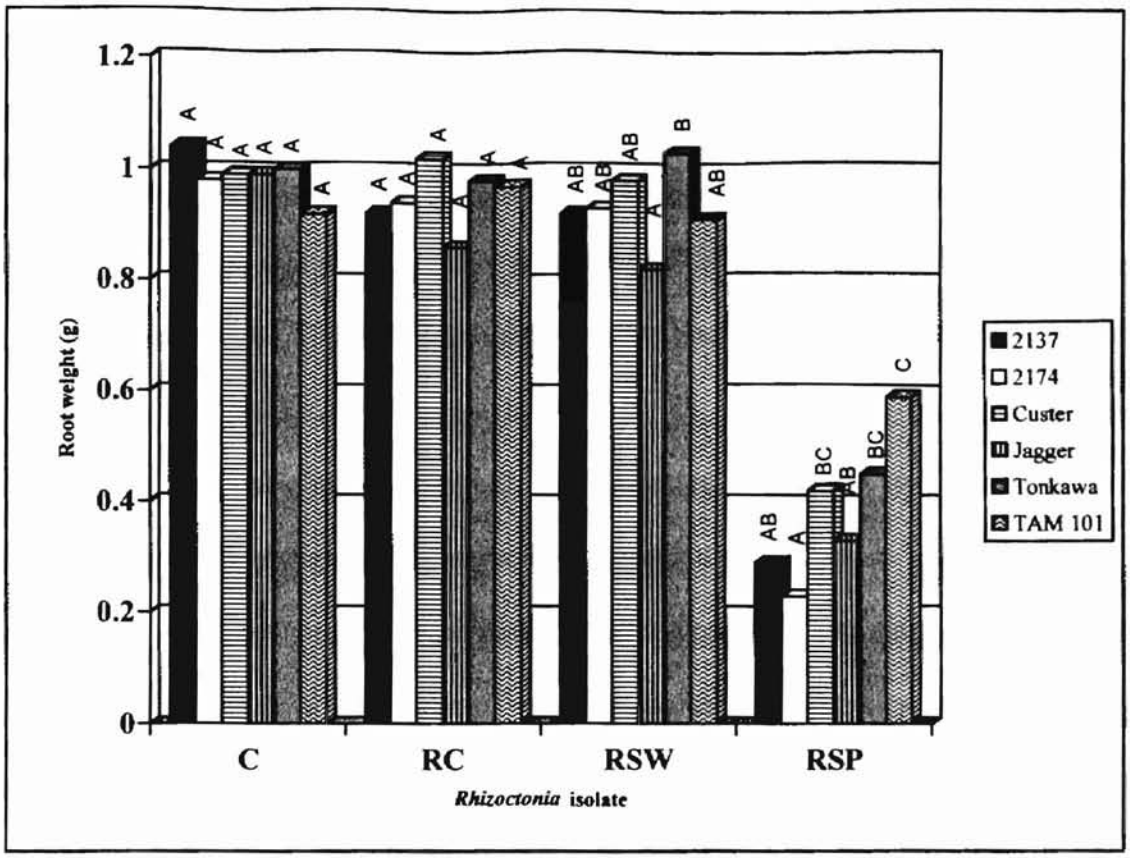

Fig.26. Effect of *R. cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on the fresh root weight of hard red winter wheat varieties at 30 C. Letters compare root weight as influenced by each isolate across varieties. Bars with the same letter are not significantly different at $P \leq 0.05$.

significantly reduce shoot weight of any variety except Jagger, as compared to an uninoculated control (Fig.23). Comparing RSW-inoculated seedlings across all varieties, seedlings of Custer, Tonkawa and TAM 101 had significantly higher shoot weight than 2137, 2174 and Jagger (Fig 24). RSP significantly reduced shoot weight of all varieties as compared to the control (Fig 23). Comparing RSP-inoculated seedlings across all varieties, seedlings of TAM 101 had the highest shoot weight, whereas 2174 had the lowest shoot weight (Fig.24)

Fresh root weights were significantly affected by the *Rhizoctonia* isolate and variety. There was no significant interaction between the two factors. RC did not reduce root weights of any variety significantly, as compared to an uninoculated control (Fig.25). Comparing RC-inoculated seedlings across all varieties, root weights of seedlings were not significantly different across varieties (Fig.26). RSW did not reduce the root weight of any variety significantly, as compared to an uninoculated control (Fig.25). Comparing RSW-inoculated seedlings across all varieties, seedlings of Jagger had significantly lower root weights than Tonkawa (Fig 26). In contrast, RSP significantly reduced the root weights of all varieties as compared to an uninoculated control (Fig 25). Comparing RSP-inoculated seedlings across all varieties, seedlings of 2174 had significantly lower root weights than TAM 101 (Fig 26).

DISCUSSION

Based on stand counts, disease severity rating and plant height, RSP was consistently the most virulent isolate at all temperatures as compared to RC and RSW. Also, based on stand counts, disease severity rating, plant height and root weight, RSP was more virulent at temperatures $\geq$ 25 C. *i.e.* RSP was more virulent at higher

temperatures.

In the experiments conducted with the various inoculum levels and varieties, RSP did not reduce stand counts at the lower temperature (10 C), although RSP-inoculated seedlings received equally high disease severity rating at low (10 C) and high (30 C) temperatures. In the study conducted at different temperatures, however, RSP reduced stand counts at the lower temperatures (10, 15, 20 and 22.5 C). One reason for this difference might be that in the experiment conducted at different temperatures, the pots were removed from the incubators four days after planting and were all kept at room temperature for 10 days. Hence, RSP might have received the higher temperature required for pathogenicity. However, in the experiments conducted with different inoculum levels and varieties, the pots were kept in the incubators for 14 days and hence, the seedlings received the same temperature (10 C or 15 C) through out the growing period.

In the study conducted with various inoculum levels, the stand counts of RC and RSW-inoculated seedlings were not significantly different at 10 C and 30 C. However, the stand counts of RSP-inoculated seedlings were significantly lower at 30 C than at 10 C. Thus, RSP was more virulent at 30 C than at 10 C, at all inoculum levels. This observation that RSP (tentatively identified as belonging to AG-4) is favored by higher temperatures is consistent with the study conducted by Mathieson (1991) to determine the effect of temperature on Rhizoctonia root rot (AG-4) in hard red winter wheat. In Mathieson's study, the emergence of wheat seedlings decreased significantly as temperature increased from 15 to 35 C. This result is also in accordance with the results described in the previous chapter, where the hyphal extension rate of RSP was higher at

temperatures $\geq$ 22.5C.

Based on stand counts, disease severity rating, and root and shoot weights, TAM 101 was more resistant to RSP at both 10 and 30 C. However, this may be due to the fact that the seeds of TAM 101 had been treated with Arasan Red (Thiram). Although most of the chemical was washed off, it may have protected the seeds from disease and promoted seedling emergence.

RC, RSW or RSP did not show any significant difference in virulence when increasing levels of inoculum were tested at either temperature (the only exception was the difference in the disease severity rating for RSP at inoculum level 30). This is in accordance with the study conducted by Yitbareck et al (1988), where the disease incidence increased when the inoculum levels increased, until a plateau was reached. In this study, the inoculum levels tested might have been higher than that required to produce the maximum disease, after which the increase in inoculum density has no effect on the disease severity rating.

The fact that higher soil temperatures favor RSP is important to the wheat producers in Oklahoma in making decisions about the planting date. In Oklahoma, wheat is planted both early (late August to September) when the soil temperatures are higher and late (early to late October) when the soil temperatures are lower. Hence, if RSP is present in a wheat field, it may be beneficial to plant wheat during the late planting date when the soil temperatures are lower.

CHAPTER 5

PATHOGENICITY AND VIRULENCE OF RHIZOCTONIA SPP. ISOLATED FROM WHEAT AND PEANUT ON WHEAT IN FIELD TESTS

ABSTRACT

Rhizoctonia-induced diseases are significantly affected by many factors including planting dates and cropping sequence. In Oklahoma, hard red winter wheat is planted both early (late August to September) and late (early to late October) for the dual purpose of forage for beef and grain for flour. Also, various rotations of wheat with either peanut or cotton are occasionally followed in Oklahoma. However, the effect of planting dates and wheat-peanut rotation on *Rhizoctonia* diseases is not well documented. Hence, a small scale field study was conducted to determine the affect of one isolate of *R. cerealis* (RC) from sharp eye spot lesions on wheat, and two isolates of *R. solani*, one each from diseased wheat roots (RSW) and diseased peanut pods (RSP), on hard red winter wheat variety 2137. Early (04 Sep) and late (23 Oct) planting dates and soil fumigation were also incorporated into the study. The experiment was conducted in 1998-99 and 1999-2000 as a split-plot arrangement in a randomized complete block design, with four replications. 100 healthy seeds were planted (1.9 cm deep, 1 seed/cm) in each of the five rows in each micro-plot (2.4 x 2.4 m) and 100 infected seeds were distributed evenly over the healthy seeds. Stand counts (seedling emergence/ft) were measured 11 and 21 days after planting (DAP) in the early planted wheat and 12, 20 and 29 DAP in the late planted wheat. In 1998-99, RC and RSW significantly reduced stand counts only in the late-planting date. RSP significantly reduced stand counts in both early and late planting dates. RC and RSW caused significant post-emergence damping-off in the late-planting date when the soil temperatures were lower, where as RSP caused significant post-emergence damping-off in the early planting date, when the soil temperatures were higher. Although *Rhizoctonia* isolates caused significant damping-off of seedlings, this reduction was not reflected in the fertile head count (number of fertile

heads/ft) or yield (yield/row). The disease expression in the field was also comparatively low. The number of diseased tillers (tillers showing a distinct lesion) was the highest in RSW-inoculated micro-plots followed by RC-inoculated micro-plots for both early and late planting dates. There was a difference in the effect of isolates on stand counts in 1998-99 and 1999-2000, which may be due to the difference in soil temperatures. The results suggest that soil temperatures influence the pathogenicity and virulence of RC, RSW and RSP. Since RSP was isolated from peanut and is pathogenic to wheat, RSP might be a major problem when wheat follows peanut in a crop rotation.

INTRODUCTION

Hard red winter wheat is an important crop in Oklahoma, where it is planted for the dual purpose of forage for beef cattle and grain for flour. To maximize forage production, wheat is planted in late August or September. In contrast, the optimum planting time for grain production ranges from early to late October. Early planting favors *Rhizoctonia*-induced root rots, especially when high temperatures are accompanied by high rainfall in the fall followed by drought stress in the spring. These conditions favor the colonization of wheat roots by the pathogen in the fall and expression of the disease during the next spring.

Studies conducted on various crops including wheat (Mathieson, 1991), sugar beet (O'Sullivan et al, 1991), potato (Carling et al, 1990) and canola (Yitbarek et al, 1988) have shown that temperature greatly influences the incidence and severity of *Rhizoctonia*-induced diseases. For example, Mathieson (1991) studied the effect of different temperatures (15, 20, 25, 30 and 35 C) on the development of Rhizoctonia root rot [anastomosis group (AG)-4] of wheat. In infested soils, emergence decreased as temperature increased from 15 to 35 C. He concluded that in fields infested *with R. solani* (AG-4) wheat should be planted late when soil temperatures were cooler to help

reduce root rot severity. However, Mathieson also indicated that isolates of *R. solani* from other AGs may have different temperature optima for pathogenicity.

Field studies conducted by Teo et al (1988) and Yitbarek et al (1988) on canola demonstrated that soil temperature greatly influenced the virulence of isolates of AG-4 and AG-2-1. Planting canola in early May before soil warmed to 15 C favored pre- and post-emergence damping-off caused by an isolate of AG-2-1 (Teo et al, 1988). However, disease due to an isolate of AG-4 increased as the temperature warmed to 20 C (Teo et al, 1988). Yitbarek concluded from his study (Yitbarek et al 1988) and another study conducted by himself (Yitbarek et al, 1987) that the increased virulence of isolates of AG-2-1 over isolates of AG-4 at relatively low soil temperatures and of isolates of AG-4 over isolates of AG-2-1 at relatively high soil temperatures, was reflected in the frequency at which the isolates of these anastomosis groups were isolated from different seedlings and mature plants in Saskatchewan. The isolates of AG-2-1 were exclusively obtained from seedlings, whereas isolates of AG-4 were obtained five times more frequently from mature plants, than from seedlings. Thus soil temperature can significantly affect the development of *Rhizoctonia*-induced disease and also impacts the pathogenicity and virulence of the isolates of different anastomosis groups of *Rhizoctonia* spp.

Crop rotation is another factor that can affect the incidence and severity of *Rhizoctonia* root rots. Rotations of wheat with other crops are followed in North America to facilitate production, to reduce diseases (Cook et al, 1991), to prevent soil erosion, and to allow for the rotation of herbicides. For example, peas and lentils are grown in rotation with wheat in the Pacific Northwest of the United States, to take

advantage of the nitrogen fixation. This rotation increases the microbial biomass and diversity, which limits the inoculum potential of *R. solani* (Smiley et al, 1994). Reductions in root rot also have been attributed to the sparse tap root systems of peas and lentils, which provide less inoculum for a subsequent cereal crop. In contrast, the fibrous roots of cereals provide a larger inoculum source.

Rotations of wheat with either peanut or cotton are occasionally followed in southwestern Oklahoma. In these scenarios, wheat is grown for two years followed by peanuts. After the peanuts are harvested, wheat is planted late in the fall (*circa* November) for the first year and used only for grain production. The next year, wheat is planted in late August or early September with the fall and winter growth being used as forage for cattle, with grain being harvested in late May or early June. Peanuts are again planted in the fourth year. However, the affects of rotating these crops on the incidence and severity of root rot disease caused by *Rhizoctonia* spp. is not documented.

The primary objective of this study was to determine the affect of two isolates of *R. solani* (one from wheat and one from peanut) and one isolate of *R. cerealis* from wheat on hard red winter wheat in small-scale field trials. Early and late planting dates, which may affect Rhizoctonia root rot via soil temperature, were incorporated into this study to simulate planting practices followed in Oklahoma. Soil fumigation also was used to attempt to separate the affects of the different *Rhizoctonia* isolates from indigenous soil microorganisms.

MATERIALS AND METHODS

Isolates

Two isolates of *R. solani*, one each from peanut and wheat, and an isolate of *R. cerealis*

from wheat were characterized. One *R. solani* isolate (#24) was obtained in 1998 from roots of wheat plants showing root rot symptoms growing near Tipton, OK by Dr. Larry Singleton (Associate Professor, Dept. of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK) and was designated as RSW (*R. solani* from wheat). The *R. solani* isolate from peanut (#23) was obtained from Dr. Hassan Melouk (USDA Research Scientist, Stillwater, OK), and was originally isolated from peanut pods showing symptoms of pod rot collected from Hughes county, OK in 1982. This isolate was designated as RSP (*R. solani* from peanut) and was tentatively identified as belonging to AG-4 (Filonow et al, 1988). *R. cerealis* isolate (#5) was isolated 1995 from a sharp eye spot lesion present on a wheat stem (variety 2180) growing near Haskell, OK by Dr. Larry Singleton and was designated as isolate RC (*R. cerealis*). The isolates were maintained at 22-25 C on Potato Dextrose Agar PDSA (1/5 strength PDA, Difco, Detroit, Michigan, USA) amended with streptomycin sulfate (0.3 g/L) and were transferred to fresh plates once every three to four days. The transfer plugs (0.5 cm) were excised from the actively growing outer margin of growth with a sterilized cork borer (0.5 cm). For long-term storage, the isolates were maintained at 20-22 C on PDA slants and were transferred twice or thrice a year to fresh slants.

Inoculum preparation

In a 250-ml Erlenmeyer flask, 23 ml of water was added to 25 g of wheat seeds, which were then sterilized by autoclaving for 20 minutes (121 C, 15 psi). The flasks were kept overnight at room temperature (20-25 C) and then inoculated aseptically in a laminar-flow transfer hood with a *Rhizoctonia* isolate. The isolates were grown on PDA (1/5 strength, Difco) amended with streptomycin sulfate (0.3 g/L). Sterilized wheat seeds in

the Erlenmeyer flasks were inoculated by adding three plugs (0.5 cm taken from the actively growing outer fringe of growth) of each isolate into separate flasks. The flasks were then kept at room temperature (20–25 C) for 7-8 days by which time hyphal growth had covered the wheat seeds. To avoid clumping of the seeds, flasks were shaken vigorously each day. Ten seeds from each flask were then plated on to PDSA to ensure that the culture used to inoculate the wheat seeds was not contaminated.

Field trials

Sixty-four micro-plots (2.4-m x 2.4-m) constructed with rail road ties were planted at two dates with the hard red winter wheat cultivar 2137, near Stillwater, Oklahoma in fall, 1998. Before planting, soil in the micro-plots was randomly tested to determine the fertility requirements [Nitrogen (N), Phosphorus (P) and Potassium (K)], soil texture and pH. Results indicate that the soil was a sandy loam. Lime was added as needed to the soil in the micro-plots to raise the pH to 5.5-6.0. Fertilizer requirements were calculated based on a wheat yield goal of 50 bu/acre. N, P and K fertilizers were broadcasted over the micro-plots as needed based on the recommendations. Generally, fumigated micro-plots required 75 lbs of N/acre and non-fumigated micro-plots required 45 lbs of N/acre. An additional 30 lb N for 1000 lb of dry matter removed/acre was added.

Five, 1.2-m rows were planted in each micro-plot, with 100 healthy seeds planted in each row at a depth of ~1.9 cm. The experiment was performed as a split-plot arrangement in a randomized complete block design, with four replications. Planting date was the main plot factor. The factorial combination of fumigation and isolate serve as the split-unit factor. The micro-plots were watered until damp and tilled. Half of the micro-plots were fumigated with methyl bromide (1.5 pound can/plot). Fumigation was

conducted a week before the early planting date and the micro-plots were left covered overnight with plastic sheets. Micro-plots used in the late planting were left covered until about week before planting to limit introduction of other microorganisms. Healthy seeds planted in each row were inoculated with one of the three *Rhizoctonia* isolates, RC, RSW or RSP. Wheat seed inoculum, which was prepared as explained previously, was used at a ratio of 1:1 (100 inoculated seeds with 100 healthy seeds in each row). The inoculated seeds were distributed evenly beside the healthy seeds. In control micro-plots, autoclaved wheat seeds were used instead of inoculated seeds. There were two planting dates, 04 Sep (early) and 23 Oct (late). The experiment was repeated in 1999, the only difference being in the planting dates, which were 10 Sep and 14 Oct. The soil temperature was monitored twice everyday, one in the morning and one in the afternoon at a depth of two inches (Appendix A and B).

The micro-plots were sprayed as needed with Cygon 2 E (8 oz/acre in 21 gallons of water), approximately one, three and five months after the first planting date, to control insect pests. The micro-plots were also sprayed with Quadris 2.08 SC (9.2 oz/acre in 20 gallons of water), about seven months after planting, to control foliar fungal diseases. Glean (0.3 oz/acre in 21 gallons of water) was sprayed approximately two weeks after the early and late planting dates to control weeds. Weeds were also eliminated as needed by hoeing.

Data measurements

All data, except yield (yield was measured/row) was collected on one foot in each of the inner three rows of each plot. Stand counts (number of seedlings/foot of row) were determined 11 and 21 days after panting (DAP) for early-planted wheat and 12, 20 and 29

DAP for late planted wheat. Forage was cut from the plants about 10 cm above the soil surface, dried in an oven at 50 C for five days, and the dry matter in grams was measured. Forage cuttings were taken from the early-planted wheat 61, 84, 103 and 168 DAP. From the late planted wheat, Forage was cut 119 DAP and the dry matter yield was measured in grams.

All data, except forage cuttings, were collected in a similar manner in the second year's experiment (1999-2000). Only a single forage cutting was taken 31 DAP from the early planted wheat in 1999-2000, because the winter was severe at the time for the second forage cutting and there was not enough vegetative growth on the wheat plants to take further forage cuttings. For the same reason, no forage cuttings were taken from the late-planted wheat during the second year.

The number of fertile heads/ft was counted before plants were harvested. Plants were harvested by hand from each of the three data rows and the total yield per row was determined, as were test weight and thousand kernel weight.

After harvest, one or two plants were randomly collected from one of the rows in each plot. Fifteen tillers were randomly selected and the lowest internode was rated for disease. The rating scale was from 1 to 8 (1 = healthy internode; 2 = slight discoloration; 3 = no clear lesions; 4 = lesions < 1 cm long or dead internode; 5 = lesions 1 - 2 cm long; 6 = lesions 2 - 3 cm long; 7 = lesions 3 - 4 cm long; 8 = lesions > 4 cm long). Number of tillers in each plot having a rating ≥ 4 was recorded and was used in a chi square test.

Analysis of variance was performed on all data except disease incidence data using SAS PROC MIXED procedure (Statistical Analysis System, SAS Institute Inc., Cary, NC). For the disease incidence data, log linear models were fit using SAS PROC

CATMOD to assess the relationship of disease incidence with planting date, fumigation and isolate and related interaction. Significant interactions of the independent variables were detected with disease incidence, so frequency tables demonstrating the relationship of each factor to disease were created for all levels of the other independent factors using PROC FREQ. For example, how isolates affected disease incidence was assessed for each level of fumigation and planting date.

RESULTS

Data measurements

In 1998-99, *Rhizoctonia* isolate, fumigation and sampling date significantly affected stand counts. There was a significant two-way interaction between planting date and isolate, planting date and sampling date, and isolate and sampling date. There was a significant three-way interaction between planting date, isolate and sampling date. RC and RSW did not cause significant pre-emergence damping-off [as determined by comparing the first stand count (SC 1) of the control wheat plants taken 11 DAP to the SC 1 of RC and RSW- inoculated wheat] in early-planted fumigated or non-fumigated micro-plots (data not shown). Also, RC and RSW did not significantly reduce stand counts (SC 2 taken 21 DAP) as compared to the uninoculated control in fumigated micro-plots in the early-planted wheat (Fig 1). Similar results were observed in the non-fumigated micro-plots also and hence that data is not presented. RC and RSW did not cause significant post-emergence damping-off [as determined by comparing the second stand count (SC 2) of the control wheat plants taken 21 DAP to the SC 2 of RC and RSW- inoculated wheat] in early-planted fumigated or non-fumigated wheat (Fig 2 and 3). RSP did not cause significant pre-emergence damping-off [as determined by

comparing the first stand count (SC 1) of the control wheat plants taken 11 DAP to the SC 1 of RSP- inoculated wheat] in early-planted fumigated or non-fumigated micro-plots (data not shown). RSP significantly reduced the stand counts (SC 2, taken 21 DAP) as compared to an uninoculated control in the early-planted fumigated wheat (Fig 1). Similar results were observed in the non-fumigated micro-plots also and hence that data is not presented. RSP caused significant post-emergence damping-off [as determined by comparing the second stand count (SC 2) of the control wheat plants taken 21 DAP to the SC 2 of RSP- inoculated wheat] in fumigated micro-plots, but not in non-fumigated micro-plots (Fig 2 and 3).

RC and RSW caused significant pre-emergence damping-off [as determined by comparing the first stand count (SC 1) of the control wheat plants taken 12 DAP to the SC 1 of RC and RSW- inoculated wheat] in late-planted fumigated micro-plots (data not shown). However, in the non-fumigated micro-plots, RC and RSW did not cause significant pre-emergence damping-off. RC and RSW significantly reduced stand counts (SC 3, taken 29 DAP) as compared to the uninoculated control in late-planted wheat in fumigated micro-plots (Fig 4). Similar results were observed in the non-fumigated micro-plots and hence that data is not presented. In late-planted wheat, RC and RSW caused significant post-emergence damping-off [as determined by comparing the third stand count (SC 3) of the control wheat plants taken 29 DAP to the SC 3 of RC and RSW- inoculated wheat] in both fumigated and non-fumigated micro-plots (Fig 5 and 6). RSP caused significant pre-emergence damping-off in late-planted fumigated and non-fumigated plots (data not shown). RSP significantly reduced the stand counts (SC 3, taken 29 DAP) as compared to an uninoculated control in late-planted wheat in the

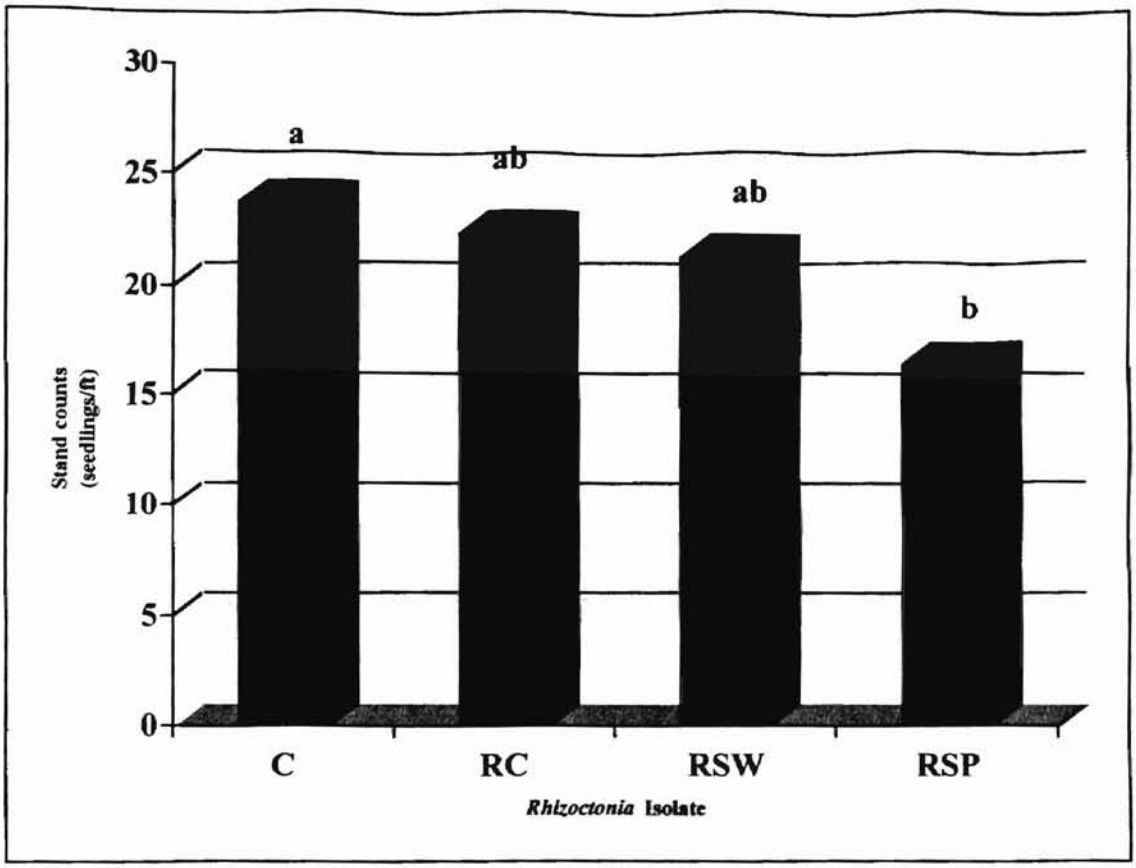

Fig 1. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts of fumigated, early-planted wheat 21 days after planting (1998-99). Bars with the same letters were not significantly different at $P \leq 0.05$.

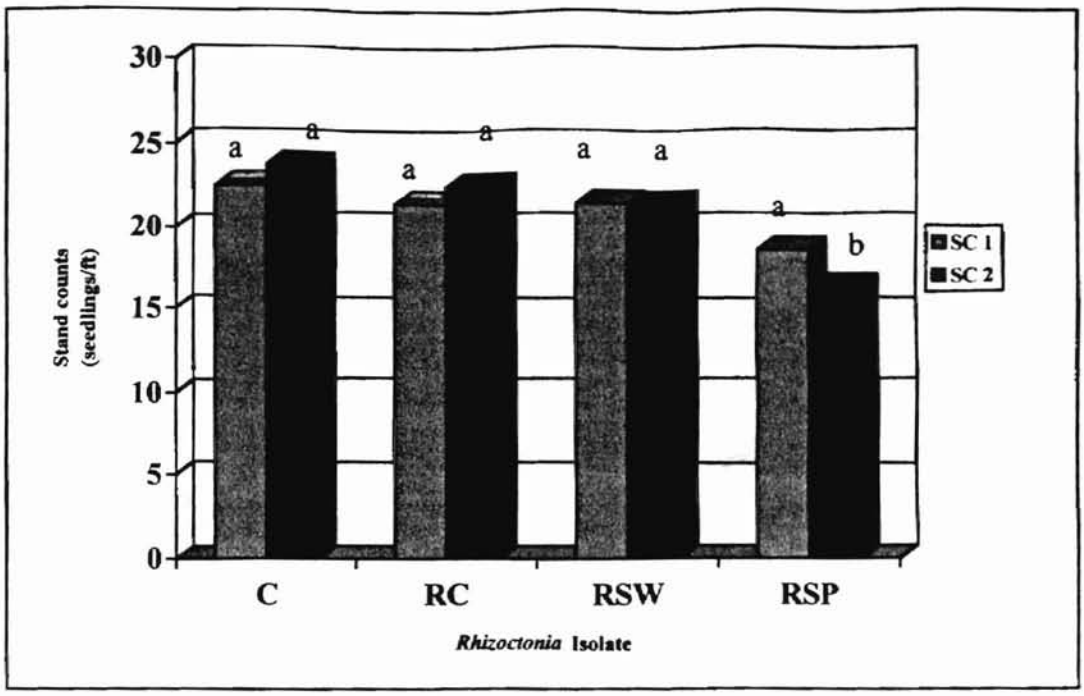

Fig 2. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of fumigated, early planted wheat 11 (SC 1) and 21 (SC 2) days after planting (1998-99). Letters compare SC 1 and SC 2 within isolates and bars with the same letters are not significantly different at $P \leq 0.05$.

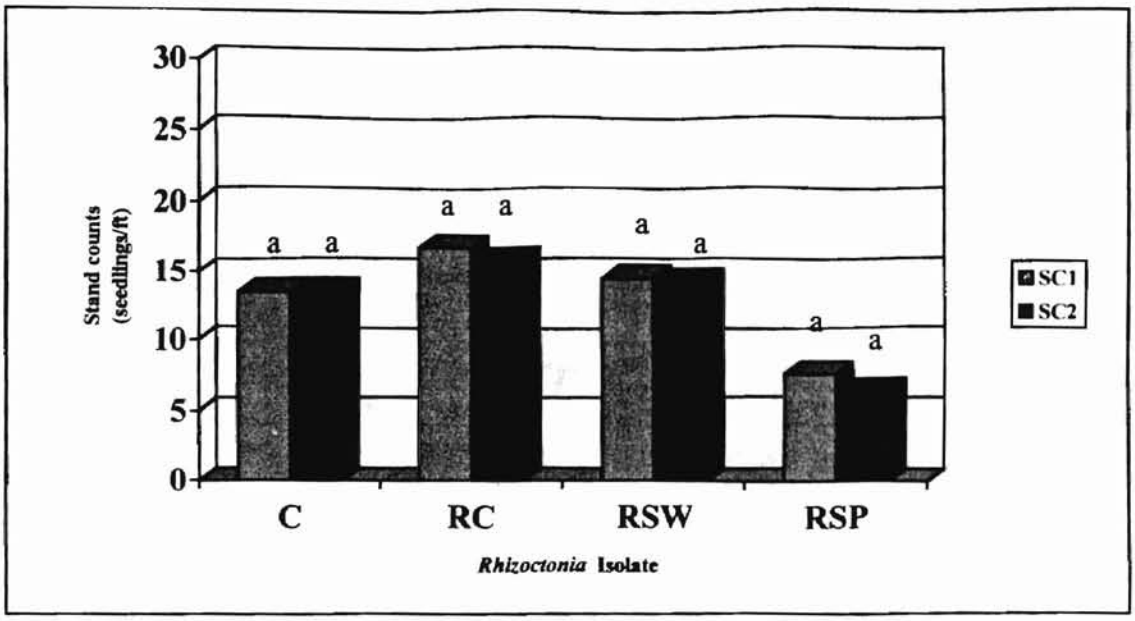

Fig 3. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of non-fumigated, early planted wheat 11 (SC 1) and 21 (SC 2) days after planting (1998-99). Letters compare SC 1 and SC 2 within isolates and bars with the same letters are not significantly different at $P \leq 0.05$.

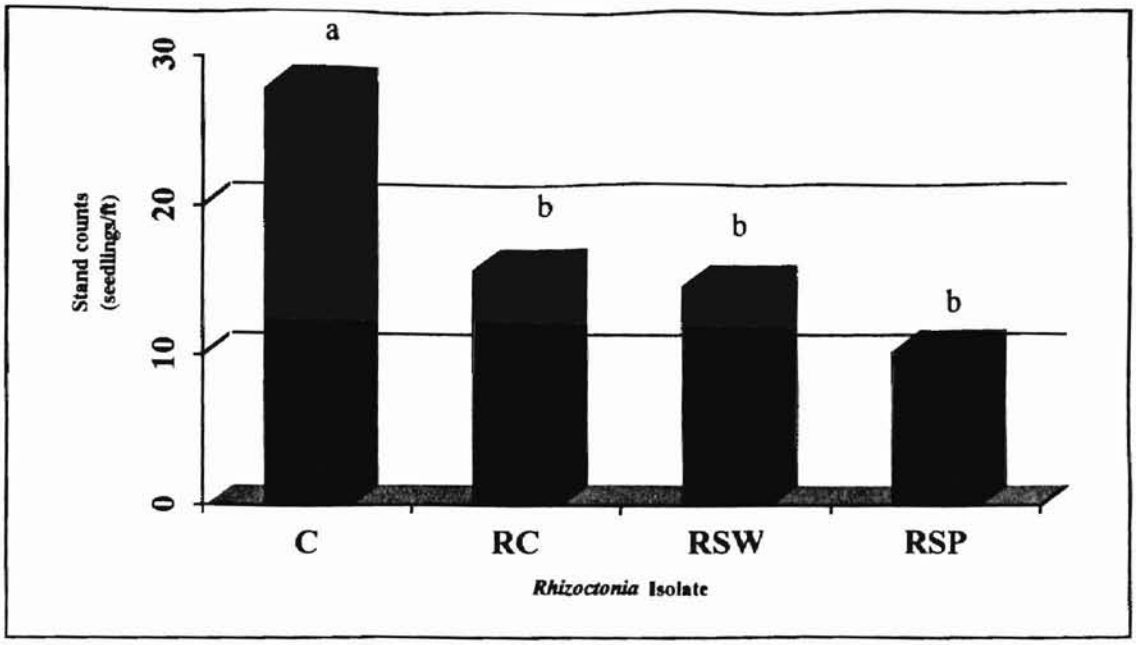

Fig 4. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of fumigated, late planted wheat 29 days after planting (stand count 3) in 1998-99. Bars with the same letters were not significantly different at $P \leq 0.05$.

fumigated micro-plots (Fig 4). Similar observations were made in the non-fumigated micro-plots and hence that data is not presented. However, RSP did not cause significant post-emergence damping-off in fumigated or non-fumigated micro-plots in the late planting date (Fig 5 and 6).

Planting date and fumigation significantly affected forage yield with significant two-way interactions between planting date and fumigation. The forage dry matter yield from fumigated micro-plots was significantly higher than the dry matter yield from non-fumigated micro-plots for all isolates and the control (Fig 7, lower case letters). In early-planted wheat, RC and RSW did not significantly reduce forage yield as compared to an uninoculated control in fumigated or non-fumigated micro-plots (Fig 7, upper case letters). However, in early-planted wheat, RSP significantly reduced the forage yield as compared to the uninoculated control in fumigated micro-plots, but not in non-fumigated micro-plots (Fig 7, upper case letters).

For early and late-planted wheat, there was no significant effect of isolates or fumigation on the number of fertile heads. The yield was also not significantly affected by isolates, planting dates, or by fumigation. The test weights were not significantly affected by planting date, fumigation or isolate. However, thousand kernel weight was significantly affected by planting date. In RC and RSP-inoculated fumigated micro-plots, the thousand kernel weights were significantly higher in late-planted than in early-planted wheat (data not shown).

Comparing the percentage of diseased tillers across planting dates, within each isolate, the percentage of diseased tillers was similar (not significantly different) in the early and late planting dates in the fumigated control (Fig 8). However in the non-

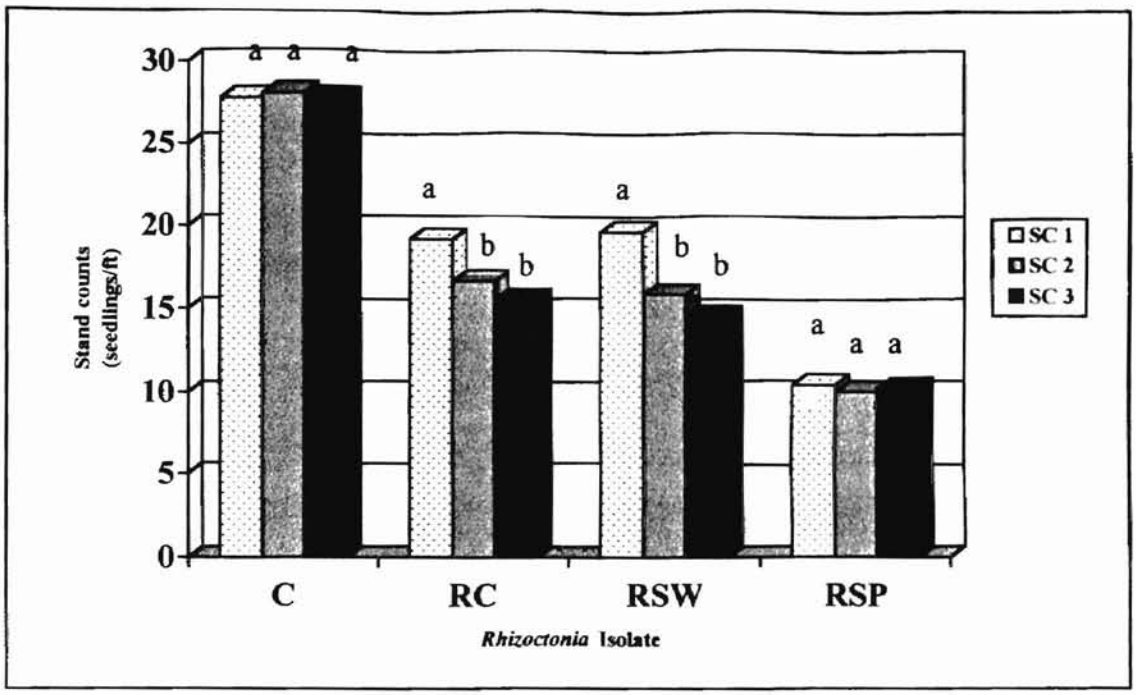

Fig 5. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of fumigated, late planted wheat after 12 (SC 1), 20 (SC 2) and 29 (SC 3) days after planting (1998-99). Letters compare SC 1, SC 2 and SC 3 within isolates and bars with the same letters are not significantly different at $P \leq 0.05$.

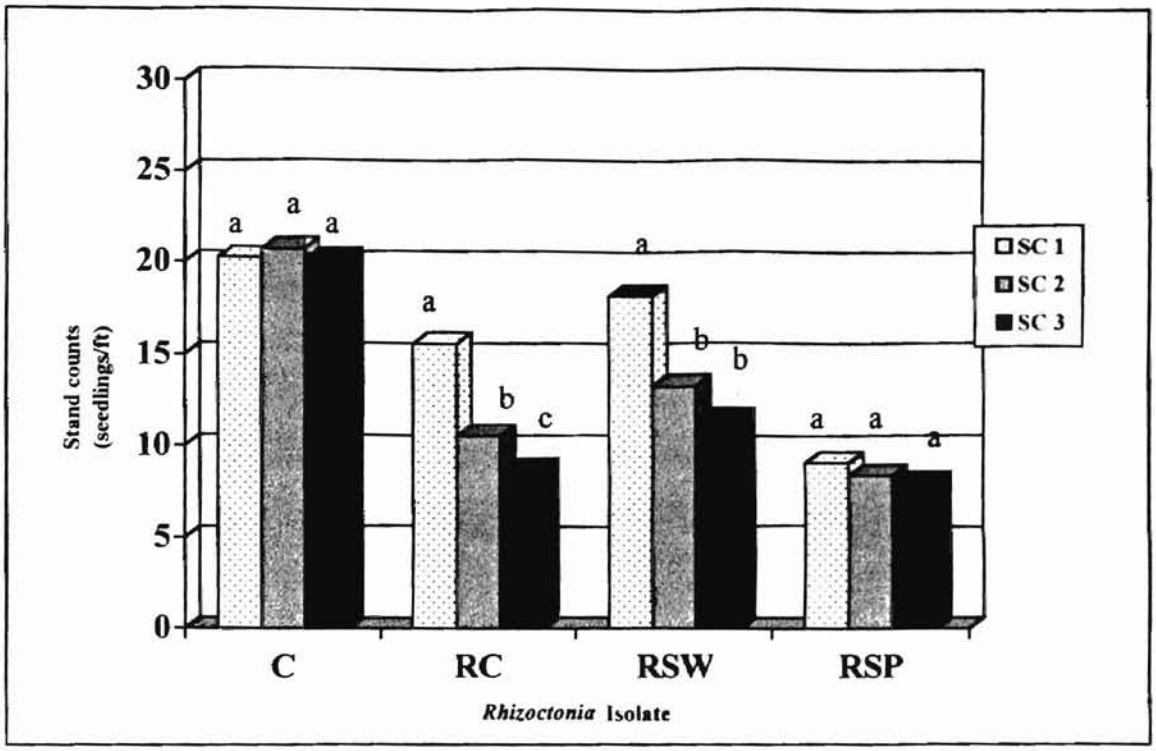

Fig 6. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of non-fumigated, late planted wheat after 12 (SC 1), 20 (SC 2) and 29 (SC 3) days after planting in 1998-99. Letters compare SC 1, SC 2 and SC 3 within isolates and bars with the same letters are not significantly different at $P \leq 0.05$.

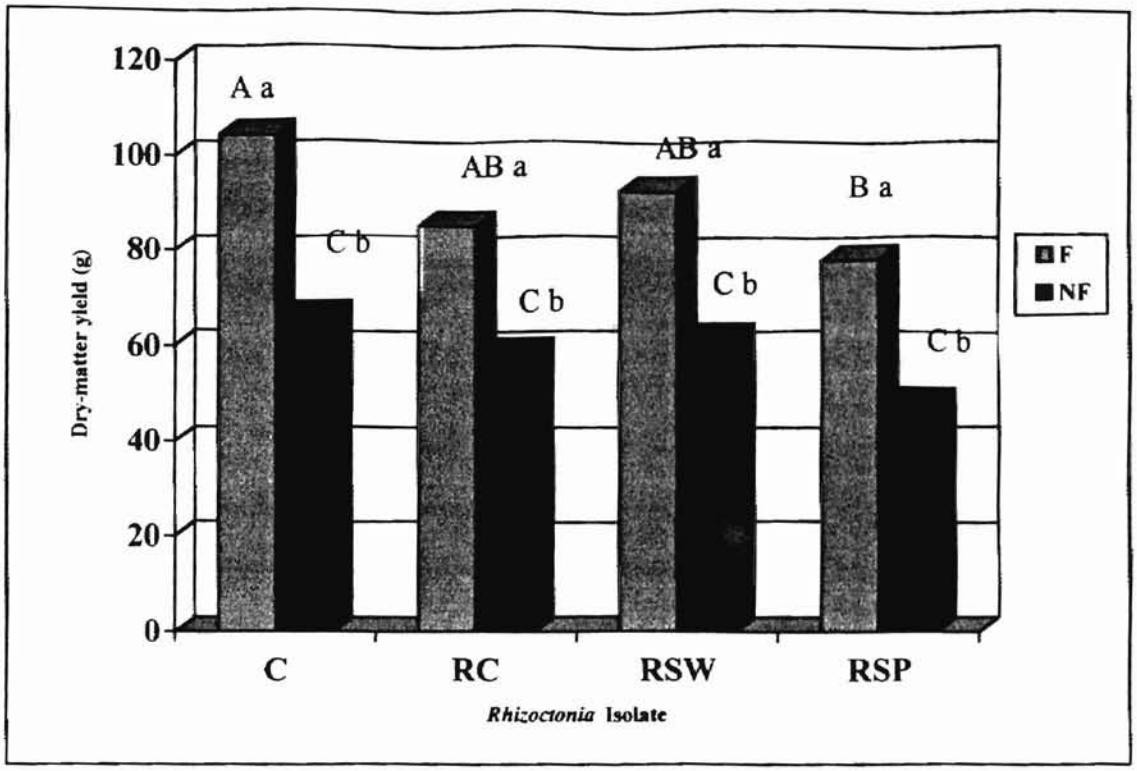

Fig 7. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on forage yield of fumigated, early planted wheat (1998-99). Small case letters compare dry matter yield, as influenced by fumigation within each isolate and uppercase letters compare dry matter yield within fumigation across isolates. Bars with the same letters were not significantly different at $P \leq 0.05$.

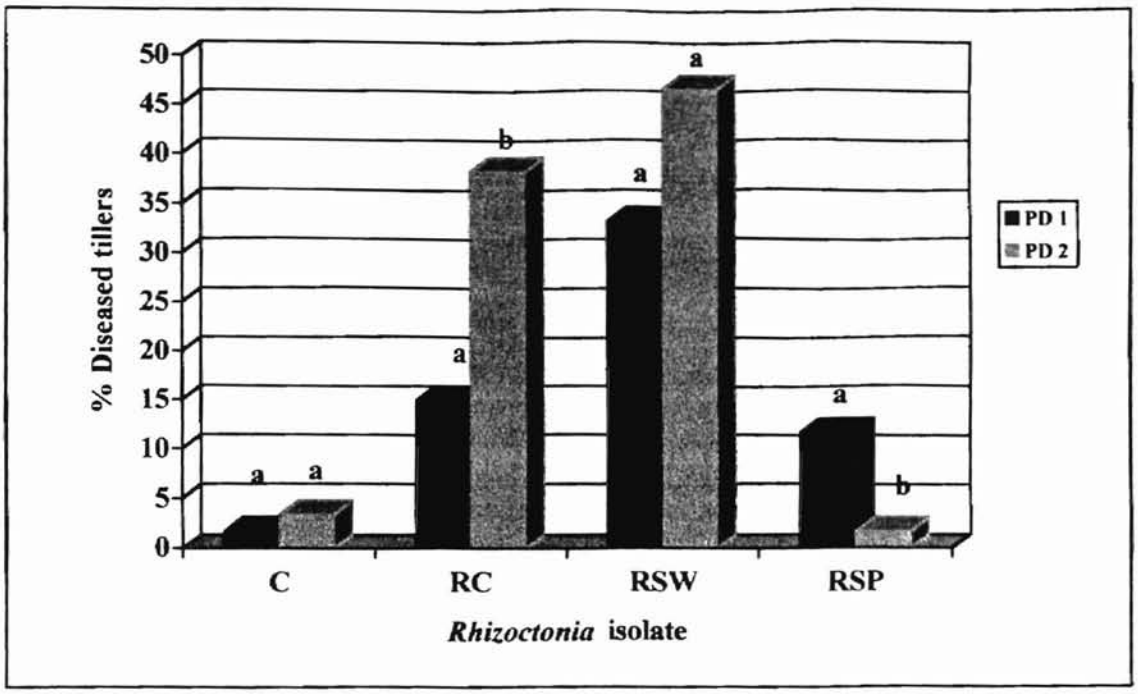

Fig 8. Percentage of tillers with a disease rating ≥ 4 on the first internode of wheat plants planted in fumigated micro-plots, during early and late planting dates [PD 1 (04 Sep), PD 2 (23 Oct)] in 1998-99, and inoculated with *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Letters compare the percentage of diseased tillers as influenced by planting dates within each isolate. Bars with the same letter are not significantly different at $P \leq 0.05$.

fumigated control micro-plots, the percentage of diseased tillers was significantly higher in the late planting date than in the early plating date (Fig 9). In RC-inoculated fumigated and non-fumigated micro-plots, the percentage of diseased tillers was higher in the late planting date than in the early planting date (Fig 8 and 9). The percentage of diseased tillers in the early and late planting dates were similar (not significantly different) in the RSW-inoculated fumigated micro-plots (Fig 8). However, the percentage of diseased tillers was significantly higher in the late planting date than in the early planting date in the RSW-inoculated non-fumigated micro-plots (Fig 9). In RSP-inoculated fumigated micro-plots, the percentage of diseased tillers was significantly higher in the early planting date than in the late planting date (Fig 8). However, in RSP-inoculated non-fumigated micro-plots, the percentage of diseased tillers was similar in the early and late planting dates (Fig 9).

Comparing the disease incidence across isolates within each planting date, in the early planted fumigated wheat, the percentage of diseased tillers was the lowest in the control plots, RC and RSP-inoculated wheat had a similar (not significantly different) percentage of diseased tillers and RSW-inoculated wheat had the highest percentage of diseased tillers (Fig 10). In the late-planted fumigated wheat, control and RSP-inoculated wheat had a similar (not significantly different) percentage of diseased tillers (Fig 10). RC and RSW-inoculated wheat had a similar (not significantly different) percentage of diseased tillers (Fig 10). In the early and late-planted non-fumigated wheat, control and RSP-inoculated seedlings had a lower percentage of diseased tillers compared to RC and RSW (Fig 11). Control and RSP-inoculated wheat had a similar (not significantly different) percentage of diseased tillers, and RC and RSW-inoculated seedlings had a

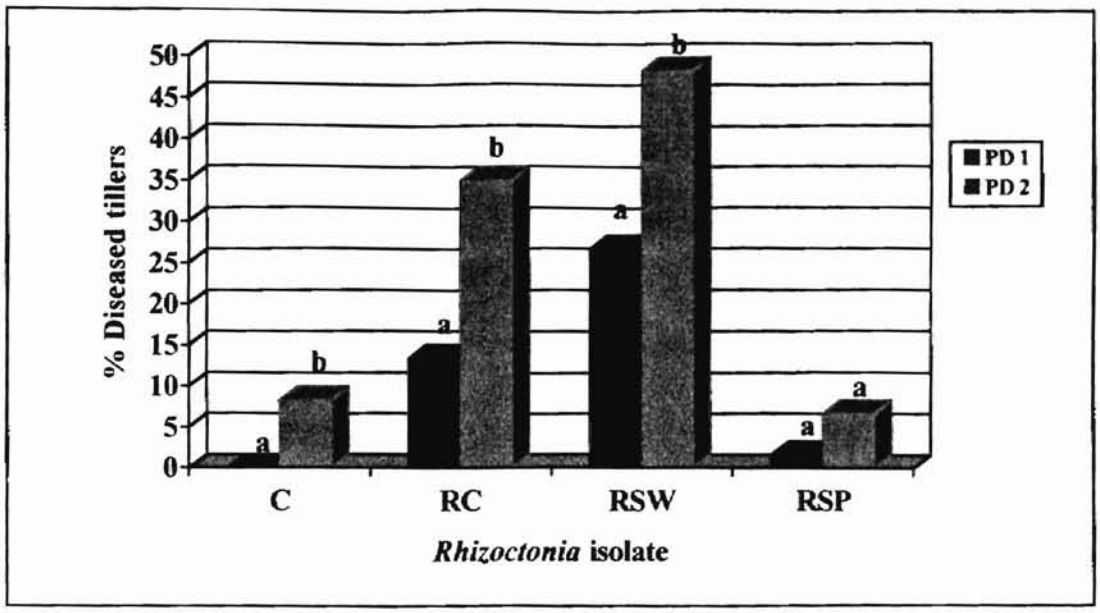

Fig 9. Percentage of tillers with a disease rating ≥ 4 on the first internode of wheat plants planted in non-fumigated micro-plots, during early and late planting dates [PD 1 (04 Sep), PD 2 (23 Oct)] in 1998-99, and inoculated with *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Letters compare the percentage of diseased tillers as influenced by planting dates within each isolate. Bars with the same letter are not significantly different at $P \leq 0.05$.

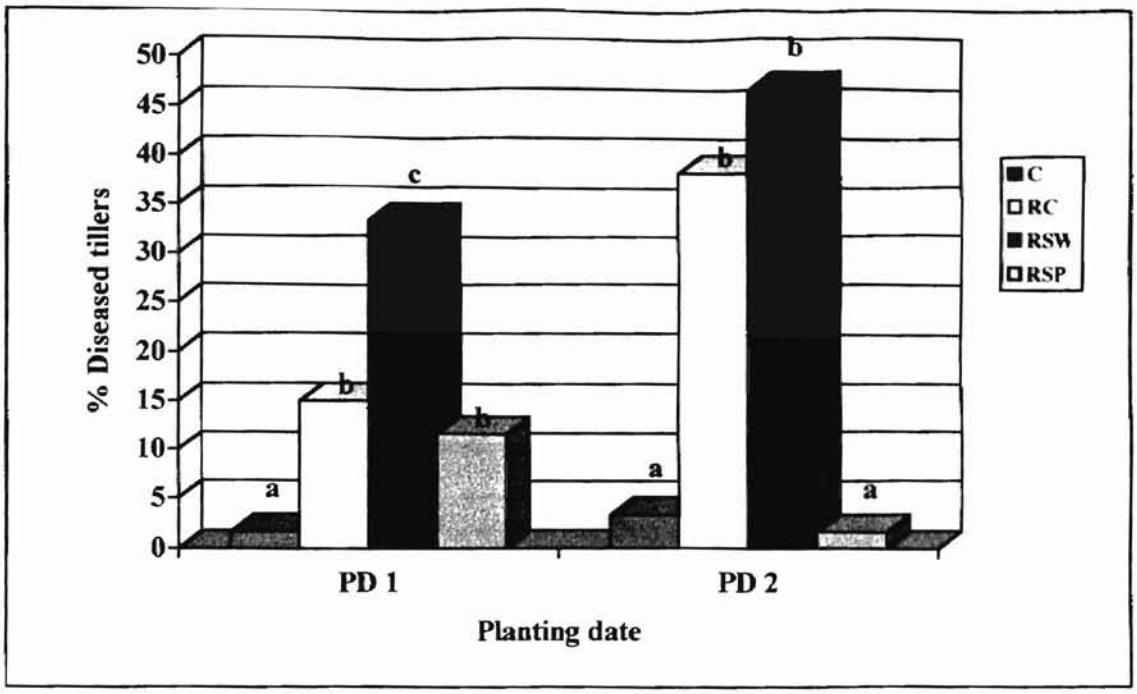

Fig 10. Percentage of tillers with a disease rating ≥ 4 on the first internode of wheat plants planted in fumigated micro-plots, during early and late planting dates [PD 1 (04 Sep), PD 2 (23 Oct)] in 1998-99, and inoculated with *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Letters compare the percentage of diseased tillers across all isolates within each planting date. Bars with the same letter are not significantly different at $P \leq 0.05$.

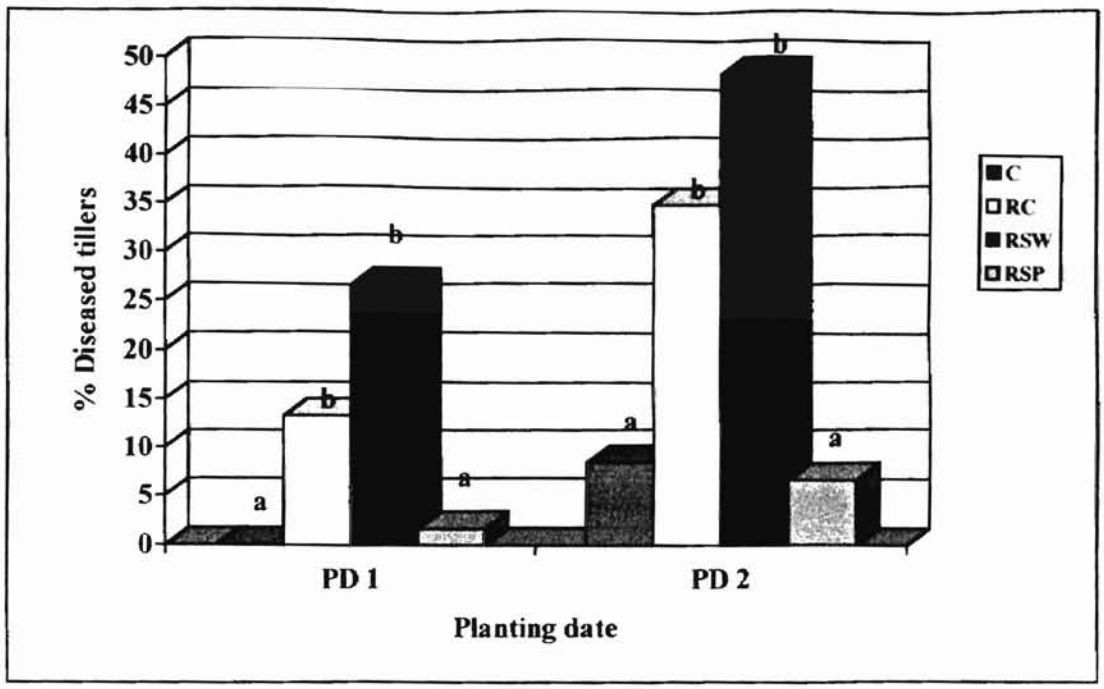

Fig 11. Percentage of tillers with a disease rating ≥ 4 on the first internode of wheat plants planted in non-fumigated micro-plots, during early and late planting dates [PD 1 (04 Sep), PD 2 (23 Oct)] in 1998-99, and inoculated with *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP). Letters compare the percentage of diseased tillers across all isolates within each planting date. Bars with the same letter are not significantly different at $P \leq 0.05$.

similar (not significantly different) percentage of diseased tillers (Fig 11).

In the second year's field experiment, there was no significant effect of any of the variables on stand count and there was no significant interaction between any variables in the early-planted wheat. However there was a significant effect of fumigation and sampling date on stand count and there was no significant interaction between these variables in the late planting date. None of the isolates reduced the stand counts significantly in the early or late planting date. In fumigated and non-fumigated micro-plots, there was no significant post-emergence damping-off caused by RC, RSW or RSP in the early planting date and hence that data is not presented. In the late-planted RC-inoculated wheat, there was a significant reduction (post-emergence damping-off) in the third stand count as compared to the second stand count in both fumigated and non-fumigated micro-plots (Fig 12 and 13). However, RSW and RSP did not cause any significant post-emergence damping-off.

Fumigation had a significant effect on the forage dry matter yield in 1999-2000. In the control micro-plots and in the RC and RSW-inoculated micro-plots, the forage yield was significantly higher from fumigated as compared to the non-fumigated micro-plots. In RSP-inoculated micro-plots, there was no significant difference between forage yields from fumigated and non-fumigated micro-plots. None of the isolates reduced forage yield significantly as compared to the control in fumigated or non-fumigated micro-plots.

DISCUSSION

In 1998-99, there was significant post-emergence damping-off in RC and RSW-inoculated wheat in the late planting date, when the soil temperature was lower. In

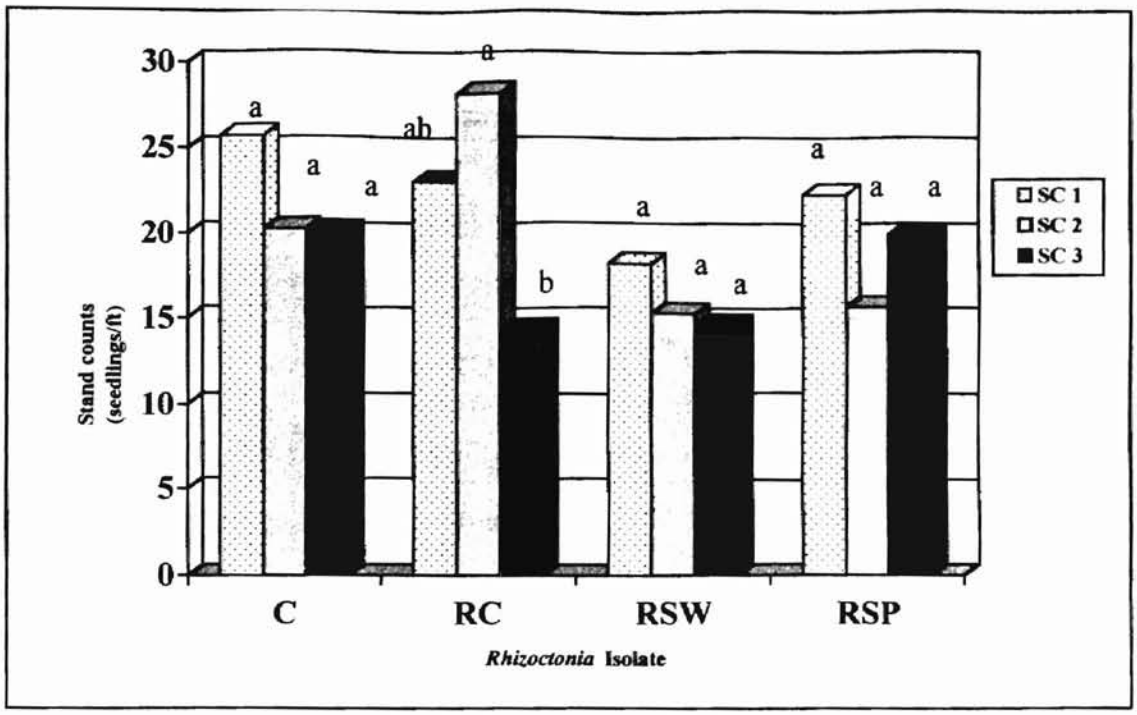

Fig 12. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of fumigated, late planted wheat after 12 (SC 1), 20 (SC 2) and 29 (SC 3) days after planting in 1999-2000. Letters compare SC 1, SC 2 and SC 3 within isolates and bars with the same letters are not significantly different at $P \leq 0.05$.

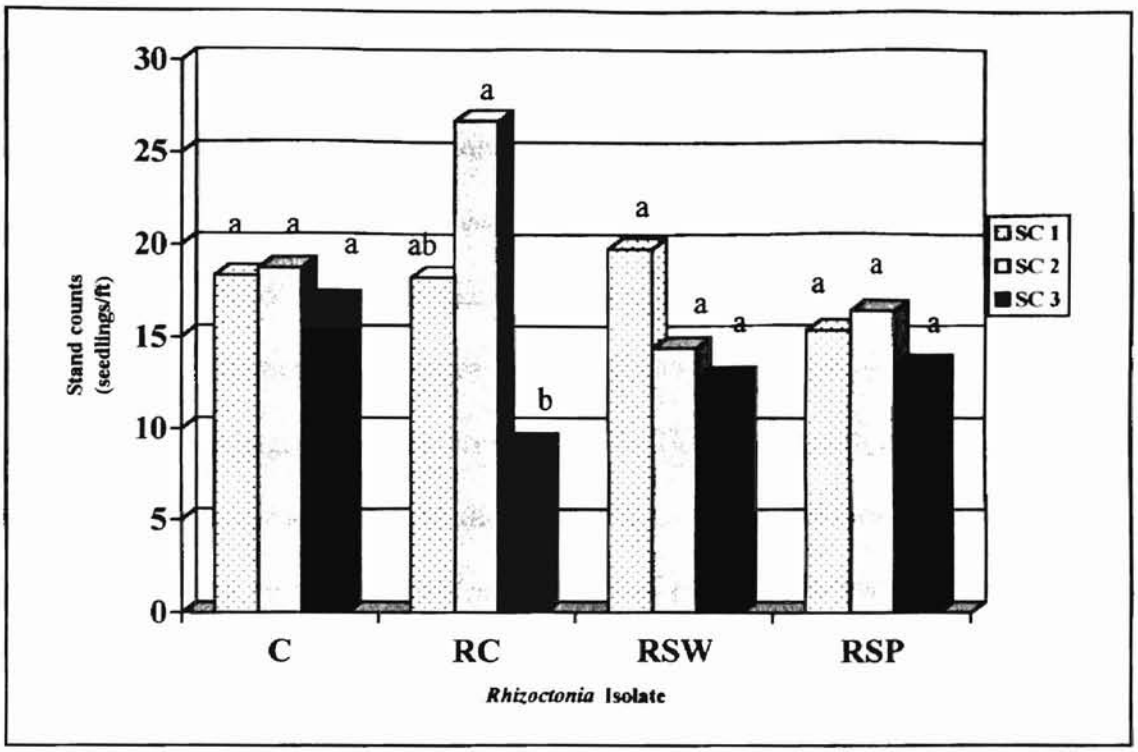

Fig 13. Effect of *Rhizoctonia cerealis* (RC), *R. solani* from wheat (RSW) and *R. solani* from peanut (RSP) on stand counts (SC) of non-fumigated, late planted wheat after 12 (SC 1), 20 (SC 2) and 29 (SC 3) days after planting in 1999-2000. Letters compare SC 1, SC 2 and SC 3 within isolates and bars with the same letters are not significantly different at $P \leq 0.05$.

contrast, there was significant post-emergence damping-off in RSP-inoculated micro-plots in the early-planted wheat when the soil temperature was higher. Thus, RSP (which was tentatively identified as belonging to AG-4) was also more virulent during the early planting date when the soil temperature was higher (~ 102 F). This result corresponds to the studies described in the previous chapters, where RSP had the greater hyphal extension rate at higher temperatures (≥ 22.5 C) and was more virulent in the growth chamber studies at higher temperatures (≥ 25 C). This observation is also in accordance with the study conducted by Mathieson (1991), which demonstrates the effect of temperature on the virulence of *Rhizoctonia solani* (AG-4). In his study, emergence of wheat decreased as temperature increased from 15 to 35 C in infested soils. He concluded that in fields infested with *R. solani* (AG-4), wheat should be planted late when soil temperatures were cooler to help reduce root rot severity.

In the second year's field experiment, additional inoculum was added to the micro-plots. This might have resulted in a higher inoculum level in the second year's experiment, which may have contributed to higher disease expression. However, results of a study (described in the previous chapter) conducted in the growth chambers using different inoculum levels of the pathogen suggest that the virulence of the pathogens remained the same when ten, twenty or thirty inoculated seeds were used. Accordingly, there was no increase in disease expression in the second year's field study when compared to the first year's field study.

There was a difference in the effect of various isolates on stand counts in the first and second year. This difference might be due to the difference in soil temperatures (Appendix A and B). In 1998-99, the soil temperature was very high (102 F) during the

early planting date. The temperature remained high (above 80 F), until the last week of September. The high soil temperature might have favored RSP and significantly reduced seedling emergence in the early planting date. During the late planting date, the soil temperature was much lower (below 70 F). RC and RSW reduced the stand counts significantly during the late planting date, when the soil temperatures were lower. Another reason for RSP being favored by higher temperatures may be that RSP was isolated from peanut, which is a warm season crop. RC and RSW were isolated from wheat, which may be the reason RC and RSW are favored by lower temperatures.

In the year 1999-2000, the summer was mild and the soil temperature was lower (71 F) during the early planting date. The temperature remained low, below 75 F until about the second week of October. In contrast to the first year, the soil temperature was much higher (81 F) during the late planting date than the early planting date. The temperature remained above 75 F for a few days after planting. Lower temperatures during the early planting date might be a reason that there was no significant damping-off in RSP-inoculated micro-plots. Also, the RSP inoculum used for the early-planted wheat was contaminated with *Fusarium* spp., which might have affected the pathogenicity of the isolate. Higher temperatures during the late planting date might be a reason for the absence of post-emergence damping-off in RSW-inoculated micro-plots.

In the first years field experiment, there was significant pre and post emergence damping off by RSP in the early planting date (higher soil temperatures) and significant post emergence damping-off caused by RC and RSW in the late planting date (lower soil temperatures). However, there was no significant pre or post emergence damping off by either RC or RSW in the growth chamber studies at any temperature. The difference in

the disease severity in the field and growth chamber studies might be because various other factors in the field influence disease severity. These factors include soil fertility, soil pH, variation in soil temperatures during the growing period etc.

The number of diseased tillers (first internode) was the highest in the RSW-inoculated micro-plots followed by RC-inoculated micro-plots for both planting dates in fumigated and non-fumigated plots. Although RSP was the most virulent isolate as far as stand counts and forage yield was concerned, RSP-inoculated micro-plots had the least number of diseased tillers. As expected, the number of diseased tillers was higher in the late planting date for RC and RSW-inoculated plots in the non-fumigated micro-plots. Also, RSP-inoculated micro-plots had higher number of diseased tillers in the early planting date than in the late planting date in the fumigated plots.

Although the *Rhizoctonia* isolates caused significant damping-off of seedlings, this reduction was not reflected in the fertile head count or yield. This might be because of the ability of the wheat plants to compensate and produce more tillers. Another reason might be the change in the soil temperatures later in the season. The decrease in soil temperatures after the early planting date might be detrimental to RSP and it might not be able to cause as much damage on the wheat plant.

All favorable conditions for severe disease expression existed in the micro-plots. However, the actual disease expression was low in the micro-plots as compared to the actual fields. One reason for the reduced disease expression might be that the wheat was planted following conventional tillage practices. Hence, there was no residue left on the soil for the fungus to use as an additional nutrient source. This is consistent with the study conducted by Pumphrey et al (1987), where he concluded that Rhizoctonia root rots

are generally lower in wheat planted following conventional tillage. Another reason for the lower disease expression might be the absence of a severe drought stress during the spring in which the experiments were conducted.

A high level of disease expression is important in the field to precisely study the effect of the three isolates on wheat. In order to increase the disease expression in the micro-plots, instead of using a single isolate of *Rhizoctonia* to inoculate the healthy seedlings in the micro-plots, a combination of isolates could be used, which is usually the situation in the field. According to the first year's field study, warmer soil temperatures favored RSP and cooler soil temperatures favored RC and RSW. Hence, if a combination of these isolates were used to inoculate the healthy seedlings, at least one of the isolates will be pathogenic to wheat throughout the growing period and might even reduce the yield of the wheat plants. Also, since soil pH affects the severity of *Rhizoctonia*-induced diseases, raising the pH above 6.0 might also increase the disease severity. Soil fertility also affects *Rhizoctonia*-induced diseases. Deficiencies in N, Ca and Zn, or excessive N increase Rhizoctonia root rots (MacNish et al, 1996). Hence, adjusting the soil fertility, especially adjusting the N content in the soil, may help increase the disease severity.

The fact that RSP prefers higher temperature might be important in choosing a planting date for wheat in wheat-peanut rotations. Since RSP was isolated from peanut and is pathogenic to wheat, this isolate might be a major problem in wheat-peanut rotations. A late planting date for wheat might be more suited for fields that are infested with RSP if wheat-peanut rotations are followed as the lower temperatures might reduce the virulence of the pathogen.

Appendix – A

Field Temperatures (F) measured (2 inches below soil surface), between 9:30 AM and 11:30 AM (Fall 1998)

Date	Temp (F)
9/8/98	69
9/9/98	63
9/10/98	53
9/11/98	72
9/14/98	67
9/15/98	65
9/16/98	66
9/17/98	64
9//18/98	67
9/21/98	65
9/22/98	59
9/23/98	58
9/24/98	62
9/25/98	84
9/28/98	67
9/29/98	66
9/30/98	75
10/1/98	74
10/2/98	63
10/5/98	58
10/6/98	53
10/7/98	51
10/9/98	45
10/12/98	57
10/13/98	54
10/14/98	52
10/15/98	51
10/16/98	64
10/19/98	42
10/20/98	47
10/21/98	57
10/22/98	36

Date	Temp (F)
10/23/98	57
10/26/98	68
10/27/98	61
11/2/98	50
11/3/98	47
11/4/98	40
11/5/98	39
11/6/98	41
11/9/98	48
11/10/98	50
11/12/98	47
11/13/98	45
11/17/98	52
11/18/98	57
11/19/98	45
11/23/98	54
11/25/98	56
11/30/98	57
12/01/98	58
12/02/98	56
12/03/98	55
12/04/98	56
12/07/98	42
12/08/98	45
12/09/98	43
12/10/98	42
12/11/98	44
12/14/98	41
12/15/98	49
12/16/98	44
12/17/98	29

Field Temperatures (F) measured (2 inches below soil surface), between 2:00 PM and 3:30 PM (Fall 1998)

Date	Temp (F)
9/4/98	107
9/8/98	102
9/9/98	101
9/10/98	92
9/11/98	93
9/14/98	94
9/15/98	82
9/16/98	85
9/17/98	87
9//18/98	84
9/21/98	91
9/22/98	61
9/23/98	71
9/24/98	79
9/28/98	86
9/29/98	89
9/30/98	82
10/5/98	59
10/6/98	57

Date	Temp (F)
10/7/98	71
10/8/98	73
10/9/98	72
10/12/98	73
10/13/98	74
10/15/98	74
10/19/98	78
10/20/98	54
10/21/98	64
10/22/98	60
10/23/98	64
10/28/98	70
10/29/98	73
10/30/98	65
11/11/98	54
11/16/98	69
11/20/98	56
11/24/98	59

Appendix B

Field Temperatures (F) measured (2 inches below soil surface), between 9:30 AM and 11:30 AM (Fall 1999)

Date	Temp (F)
9/10/99	67
9/13/99	53
9/14/99	48
9/15/99	59
9/16/99	55
9/17/99	57
9/20/99	52
9/21/99	55
9/22/99	51
9/23/99	44
9/24/99	47
9/27/99	45
9/28/99	52
9/29/99	40
9/30/99	39
10/01/99	49
10/04/99	50
10/05/99	48
10/06/99	49
10/07/99	51
10/08/99	54
10/11/99	56
10/12/99	58

Date	Temp (F)
10/13/99	56
10/14/99	55
10/15/99	55
10/18/99	53
10/19/99	51
10/20/99	31
10/21/99	38
10/22/99	56
10/25/99	51
10/26/99	42
10/27/99	44
10/28/99	47
11/01/99	45
11/02/99	48
11/03/99	38
11/04/99	39
11/05/99	51
11/08/99	48
11/09/99	51
11/10/99	49
11/11/99	50
11/12/99	51

Field Temperatures (F) measured (2 inches below soil surface), between 2:00 PM and 3:30 PM (Fall 1999)

Date	Temp (F)
9/13/99	69
9/14/99	71
9/15/99	72
9/16/99	74
9/17/99	71
9/20/99	62
9/21/99	73
9/22/99	74
9/23/99	71
9/24/99	72
9/27/99	57
9/28/99	53
9/29/99	65
9/30/99	68
10/01/99	69
10/04/99	71
10/05/99	70
10/06/99	71
10/07/99	69
10/08/99	62
10/11/99	74
10/12/99	78

Date	Temp (F)
10/13/99	76
10/14/99	81
10/15/99	80
10/18/99	77
10/19/99	73
10/20/99	63
10/21/99	65
10/22/99	64
10/25/99	74
10/26/99	75
10/27/99	74
10/29/99	76
11/01/99	73
11/02/99	76
11/03/99	56
11/04/99	68
11/05/99	69
11/08/99	70
11/09/99	69
11/10/99	70
11/11/99	71
11/12/99	72

Appendix C

CONCLUSION

A major drawback of this study was that the results from the experiments conducted in the controlled environment did not correspond to the results from the field. In this study, the *Rhizoctonia* isolates RC and RSW caused significant reduction in stand counts and caused post-emergence damping off in the field during the late planting date, when the soil temperatures were lower (below 70 F). However, RC and RSW did not reduce stand counts significantly even at lower temperatures (10 C to 20 C) in the controlled environment experiments. One reason for this difference might be the difference in the soil fertility, soil moisture, etc. that affect the incidence and severity of *Rhizoctonia* diseases. Also, field conditions may be better simulated if the plants were grown in a greenhouse and harvested at maturity and the fertile head count, yield, disease incidence etc were also rated, instead of rating 14 day old seedlings in the controlled environment experiments.

A better grading system to assess the disease incidence in the field and in the controlled environment has to be devised. The grading system used in the field did not consider the severity of the lesions in that the rating was the same for lesions that girdled the stem and those that did not. Small lesions that girdle the stem might affect the plant more than large lesions that do not girdle the stem.

The different isolates of *Rhizoctonia* spp. used in the study, especially RSP had specific temperature optima for pathogenicity and virulence. Thus, this study shows the importance of characterizing the *Rhizoctonia* spp. isolates present in a field in order to be able to recommend effective control measures (including planting dates, fungicide

applications) against *Rhizoctonia*-induced diseases.

Appendix D

Determination of anastomosis groups

R. solani has many strains that differ in host range, virulence, optimum temperature for pathogenicity, etc (Baker, 1970). Thus, *R. solani* is a collective species, comprised of many related but genetically isolated groups (Carling et al, 1994). Based on hyphal anastomosis, *R. solani* can be divided into smaller, more homogeneous groups. Anastomosis occurs between hyphae of isolates within the same anastomosis group (AG). As of 1994, twelve anastomosis groups of *R. solani* have been reported, including groups 1 to 11 and B 1.

Among the binucleate *Rhizoctonia* spp. that form *Ceratobasidium* teleomorphs, seven anastomosis groups have been identified (O'Sullivan et al, 1991). *R. cerealis* belongs to the group AG-D, and is capable of fusion with members of AG-D and CAG-1 (Sneh et al, 1994). Fungicide response (Kataria et al, 1991), temperature optima (Carling et al, 1990) and host range (Baker, 1970) of each AG may be specific. Hence determination of anastomosis groups may be important in recommending effective control measures against *Rhizoctonia*-induced diseases.

Determination of anastomosis groups was made by pairing mycelial plugs (0.5 cm) of tester isolates belonging to known anastomosis groups with each unknown isolate. The tests were conducted on 7.5 x 2.5 cm glass slides containing ~ 2 ml of 2% water agar. The mycelial plugs were taken from actively growing outer margins of growth on PDSA with a sterilized cork borer (0.5-cm). The slides were incubated at 22 C until the hyphae growing from each plug began to overlap (48-72 hrs). The overlapping hyphae were then stained with lacto-fuchsin red, covered with a cover slip and observed under a

light microscope for hyphal anastomosis. There are four categories of anastomosis reaction; C 0- No reaction; C 1- No fusion or cell death; C 2- Wall fusion and cell death, but no cytoplasmic fusion; C 3- Wall and cytoplasmic fusion. C 3 indicates a close relationship and indicates the same AG, the same vegetatively compatible population and possibly the same individual. C 2 represents related isolates that are in the same AG, but different vegetatively compatible population. C 1 occurs within highly heterogenous AGs including AG 2, 8 or B1, or between AG s where bridging relationship exists. C 0 indicates different AGs. Anastomosis groups are usually assigned based on C 2 reaction. *R. solani* from peanut was paired against tester isolates of AG-1, AG-2, AG-6 (obtained from Dr. Conway, Professor, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK) and RSW. The reactions observed were either C 0 or C 1. There was no C 2 reaction observed in any of these pairings. Hence it was concluded that RSP did not belong to AG-1, AG-2, AG-6 and also that RSP and RSW belong to different anastomosis groups. RSP was also anastomosed with tester isolates of AG-4 (obtained from Dr. Conway) and a few C 2 reactions were observed. However, the number of C 2 reactions observed was not sufficient to assign RSP to AG-4.

Appendix E

Determination of thiamine requirement

Different anastomosis groups of *R. solani* have different requirements for thiamine (Ogoshi et al, 1979). Some AG of *R. solani* are thiamine autotrophic where as others are thiamine auxotrophic. Thiamine autotrophic anastomosis groups include AGs 1, 3, 4, 6, and 7, AG 2-1 etc. AGs 5, and B1, AG 2-2 III B, 2-2 IV etc. are thiamine auxotrophic. Hence the thiamine requirement of RC, RSW and RSP were determined to help characterize the three isolates.

As described by MacNish et al (1994), a mycelial disc of RC, RSW and RSP was placed into Czapek solution agar with or without 10^{-5} M thiamine hydrochloride. To prepare this medium, Czapek solution agar was prepared and filter sterilized thiamine hydrochloride (.0034g/L) was added to it. Tester isolates of AG-2-2-IV and AG-5, which are auxotrophic, and tester isolates of AG-2-Type 1 and AG-1, which are autotrophic were used as controls. The plates were placed in the dark at 21 C for 2 weeks. The culture was then placed in a beaker with 100 ml of water and heated in a water bath to melt the agar. Vaccum filteration was employed to separate the mycelium from the contents of the beaker. The mycelium was dried in an oven at 70 C for 24 hours and weighed. Isolates were considered thiamine autotrophic if the ratio of the mycelial weight when grown on media without thiamine over the weight of mycelium grown on media with thiamine was 1.5 or less. If the ratio was 1.6 or greater, the isolates were considered auxotrophic. There were three replicates of each isolate and the experiment was conducted only once. However, some of the plates were contaminated and hence there were only single plates of some isolates.

As expected, the ratio of the mycelial weight when grown on media without thiamine over the weight of mycelium grown on media with thiamine for AG-5 (which is thiamine auxotrophic) was 2.566. However the same ratio for AG-2 2 IV was 0.869, while the expected ratio was 1.5 or higher. The ratio of the mycelial weight when grown on media without thiamine over the weight of mycelium grown on media with thiamine for AG-1 (which is thiamine autotrophic) was 1.178. The ratio of the mycelial weight when grown on media without thiamine over the weight of mycelium grown on media with thiamine for AG 2 Type 1 (which is thiamine autotrophic) was 1.038. For RC, RSW and RSP, the ratio of the mycelial weight when grown on media without thiamine over the weight of mycelium grown on media with thiamine were 0.958, 1.085 and 0.893 respectively. This observation might suggest that RC, RSW and RSP are thiamine autotrophic. However a definitive conclusion cannot be drawn from this experiment, as there were only single culture plates of some of the isolates.

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