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EFFECT OF INOCULUM DENSITY OF *DRECHSLERA SOROKINIANA* AND *FUSARIUM AVENACEUM* ON THE INCIDENCE OF WHEAT ROOT ROT IN OKLAHOMA

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Abstract

Inoculum density of *Dreschslera sorokiniana* and *Fusarium avenaceum* and the incidence of wheat root rot disease on hard red winter wheat (*Triticum aestivum*) cv. 'Danne' were evaluated in the glass house. Percentage recovery of *D. sorokiniana* from subcrown internodes ranged from 48 to 97% as the inoculum density increased from 1 to 1000 conidia per g of soil and plateaued between 250 to 1000 conidia per g of soil. Percentage recovery of *F. avenaceum* from subcrown internodes ranged from 41 to 57% as inoculum density increased from 1 to 1000 macroconidia per g of soil, plateaued between 100 and 500 macroconidia per g of soil, and finally declined to 45% at 100 macroconidia per g of soil. Disease severity rating of the subcrown internodes based on percentage lesions covering the surface areas of the internodes followed similar trend for each of the pathogens.

Introduction

Drechslera sorokiniana (Sacc.) Subram and Jain (*Helminthosporium sativum* Pammel, King & Bakke) and *Fusarium* species are principal causal agents of wheat root rot disease in Oklahoma. Primary inoculum of the pathogens consist of mycelium and conidia of *D. sorokiniana* and mycelium, chlamydospores, and macro and microconidia of *Fusarium* species. The pathogens attack any part of both wheat seedlings and mature wheat plants. Infected plants usually produce premature 'wheat heads' and shrivelled seeds (Cook, 1980; Wiese, 1977).

Ledingham & Chinn (1955) assayed soil samples collected from 47 fields for conidial populations of *D. sorokiniana* and found spore populations ranging from less than 10 to over 250 viable conidia per g of soil. Chinn *et al.* (1962) examined spores of *D. sorokiniana* from 200 soil samples collected from 100 fields within a radius of 236 Km of Saskatoon, Canada and found conidiospores of *D. sorokiniana* ranging from less than 10 to about 900 per g of soil. Cook (1980) reported that dilution plate counts of soil had 1,500 propagules per g of soil of *F. culmorum* in the Harrington field. Cook (1968) noted that about 100 propagules of *F. culmorum* per g of soil is enough to cause damage if conditions for disease are favourable. Wiese (1977) also reported that under favourable environmental conditions, the threashold population of *F. culmorum* necessary to cause a

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detectable yield reduction is ahout 100 chlamydospores per g of soil. This study was undertaken to determine the effects of different levels of inoculum densities of *D. sorokiniana* and *F. avenaceum* each alone and in combination on the incidence of wheat root rot.

Materials and Methods

Production of inoculum: Isolate 47 of *D. sorokiniana* was obtained from a wheat field near Custer, Oklahoma and isolate 34 of *F. avenaceum* was obtained from a wheat field near Ceary, Oklahoma. Pathogenicity studies of different isolates of the two pathogens (Umechuruba & Singleton, unpublished), indicated that these two isolates were more virulent than other isolates. Single spore cultures of *D. sorokiniana* and *F. avenaceum* were obtained and conidial inoculum of each pathogen was prepared with conidia produced by mycelium growing on oat kernels (oat kernel: water, 2:1, v/v) in 250 ml Erlenmeyer flasks. The oat kernels prepared were autocalved for 90 min at 1.1 atm, cooled, inoculated with each fungal spores and incubaed at 25°C for 14 days. Conidial spore suspensions of each pathogen were obtained by flooding flasks with sterile distilled water, agitating and filtering through sterile cheese cloth. Clear conidial spore suspensions were obtained by repeated flooding of the flasks with sterile distilled water and decanting the upper liquid layer after spores had settled at the bottom of the flasks. The number of conidia of *D. sorokiniana* were counted in a nematode counting dish under a stereoscope. The macroconidia concentration of *F. avenaceum* was determined by direct microscopic count of a 0.01 ml aliquot using a haemocytometer.

Inoculation. To known weights of sterile Lincoln sandy soil (94.9% sand and 3% clay) known volumes of condial suspension of each pathogen were added such that conidial populations of 1, 10, 100, 250, 500, and 1000 conidia or macroconidia per g of soil on a dry weight basis were obtained. Each inoculated soil was mixed thoroughly in an electric Bucket Mixer No. 7658711 (McMaster-Carr Supply Company, Chicago, Illinois 60680, U.S.A.) for 30 min to obtain uniform distribution of the conidia in the soil, and water added while mixing to raise the water content of the soil to field capacity (7.5%). The Lincoln sand was previously treated with methyl bromide, air-dried and passed through a 2.0 mm mesh sieve. The inoculated soils were then put in platic pots (11.0 x 14.5 cm). Soil temperature in the pots was controlled at 25°C (± 2C) using the Oklahoma State University Soil Temperature Control System developed by Dr. C.C. Russell of the Department of Plant Pathology, Oklahoma State University, Stillwater, Oklahoma. This system consits of "U" – shaped coated copper heat – sinks inserted in the soil. Desired temperature controlled water obtained from a temperature control water-bath was pumped through the heat-sinks to obtain uniform soil temperature throughout the pots. The heat-sink-temperature control water-bath is a modification of the "Cornell Temperature Control Baths" (Ferris *et al.*, 1955).

Certified seeds of hard, red winter wheat cv. 'Danne' were planted in the conidium-inoculated soil in pots. Five seeds were planted in each pot at 5 cm depth. Control pots contained uninoculated soil. A complete randomized design was used in the study. There were 5 replications for each fungal inoculum-density-level treatment and the experiment was triplicated. The soils were kept fairly moist throughtout the entire study period and fertilized (15-30-15; 4 g/L). The experiment was terminated after 9 weeks. Subcrown internodes with lesions covering 50 to 100%, 25 to 49%, 12.5 to 24% and 0 to 12% of their surface areas were classified as severe, moderate, slight and healthy, respectively. The diseased-rated subcrown internodes were clipped off the plants, surface sterilized by agitating them for 2 min., in 25% sodium hypochlorite (Clorox) solution followed by a rinse in sterile water and plated 5 pieces per Petri dish on modified Czapek-Dextrose Agar (CDA) (Stack, 1977) for isolation of *D. sorokiniana* and on acidified potato dextrose agar (APDA) medium amended with 0.05 g chloromycin and 0.05 streptomycin sulfate per liter for isolation of *F. avenaceum.* Plated subcrown internodes were incubated at room temperature (26-28°C) for 7 days after which colony counts were made to determine percentage recovery of the pathogens from the subcrown internodes.

The effects of the *D. sorokiniana* and *F. avenaceum* each alone, and the two combined on 'Danne' wheat seedlings using three inoculum density of 100, 250 and 500 conidia/macroconidia per g of soil for *D. sorokiniana* and *F. avenaceum,* respectively was also determined. The experiments were repeated three times.

Results

Drechslera sorokiniana inoculum density study: Mean percentage recovery of isolate 47 of *D. sorokiniana* from subcrown internodes of 9 week old 'Danne' wheat seedlings grwon in soil inoculated with conida of *D. sorokiniana* @ 0, 1, 10, 100, 250 and 1000 conidia per g of soil was 16, 52, 48, 77, 91, 97 and 97, respectively. The results showed that *D. sorokiniana* infected the plants at each inoculum density level, percentage infection increasing linearly with rising inoculum density.

Results also showed that mean percentage recovery of *D. sorokiniana* from plants inoculated @ 1 and 10 conidia per g of soil (52% vs. 48%, respectively) were not significantly different. Similarly, mean percentage recovery of the pathogen in palnts inoculated @ 250, 500 and 1000 conidia per g of soil (91%, 97% and 97%, respectively) were not significantly different. They were however, significantly different at $P = 0.01$ from mean percentage recovery of the pathogen from plants inoculated @ 1 and 10 conidia per g of soil. Mean percentage recovery of *D. sorokiniana* from plants inolulated @ 100 conidia per g of soil (77%) was significantly differnt at $P = 0.01$ from that of all the other treatments.

Disease severity/inoculum density rating results also showed similar trend. Percentage disease severity rating of the subcrown internodes rose from 2 to 80% as the inoculum density levels increased from 1 to 1000 conidia per g of soil. Also, the disease severity rating attained a broad peak in plants inoculated @ 250 to 100 conidia per g of soil.

Fusarium avenaceum inoculum density study: The plants were infected at each inoculum density studied. The mean percentage recovery of *F. avenaceum* from the subcrown internodes of seedlings in soils inoculated at 0, 1, 10, 100, 250, 500, 1000, respectively, was 9, 41, 49, 57, 56, 51 and 48%. The values obtained from all the inoculated soils were not significantly different at P = 0.1. Each was, however, significantly different at P = 0.01 from that of the control. Mean percentage disease severity rating of the subcrown internodes of seedling in the inoculated soils showed that 50-85% were healthy, 8 to 25% slightly infected, 8 to 20% moderately infected and 0 to 12% severely infected.

D. sorokiniana, F. avenaceum, each alone and in combination: Comparative study of plants inoculated with either *D. sorokiniana* or *F. avenaceum* alone and inoculated with both using three inoculum density levels (100, 250 and 500 conidia for *D. sorokiniana* and macroconidia for *F. avenaceum*) showed that *D. sorokiniana* ability to attack the plants did not differ significantly in all the inoculum density levels used whether it was inoculated alone or in combination with *F. avenaceum* (Fig. 1). This was not the case

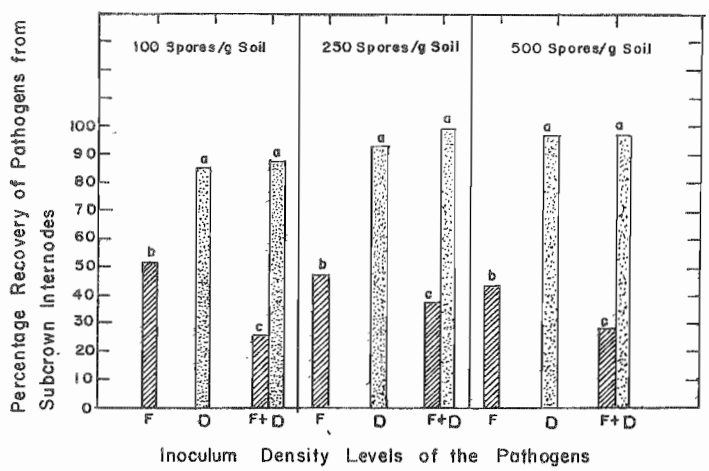

Fig. 1. Percentage recovery of isolate 47 of *Drechslera sorokiniana* and isolate 34 of *Fusarium avenaceum* from subcrown internodes of wheat cv. "Danne" inoculated at different inoculum density levels (100, 250 and 500 conidia/marcoconidia per g. soil) and grown for 9 weeks under controlled soil temperature of 25C ± 2C. Bars having the same letters within each inoculum density level are not significantly different (P = 0.01) according to Duncan multiple test range. F = *Fusarium avenaceum*, D = *Drechslera sorokiniana*. Mean of three replicates.

with *F. avenaceum*. *F. avenaceum* ability to attack the plants when inoculated alone was not significantly different in all the inoculum density levels but differed significantly when in combination with *D. sorokiniana* (Fig. 1). Also, there were no significant differences in all the inoculum density levels used when the pathogens were combined. Inoculum density levels/disease severity rating of the subcrown internodes (Fig. 2) showed no additive effects when the two pathogens were combined. The effects of each pathogen alone seemed to be average of the effects of both pathogens together (Fig. 2).

Discussion

Ledingham & Chinn (1955) found spore population of *D. sorokiniana* ranging from less than 10 to over 250 viable conidia per g of soil in soil samples collected from 47 wheat fields. Chinn *et al.*, (1962) found conidiospores of *D. sorokiniana* to number from less than 10 to 900 per g of soil. Findings of Chinn *et al.* (1962) suggest that conidia population of *D. sorokiniana* can range from 1 to 900 conidia per g of soil in wheat fields depending on conditions of soil environment and that the fungal conidial population may vary seasonally.

Inoculum disnity of *D. sorokiniana* used in this study was, therefore, typical of what can be found in the field. The information obtained in this study can be helpful in host-

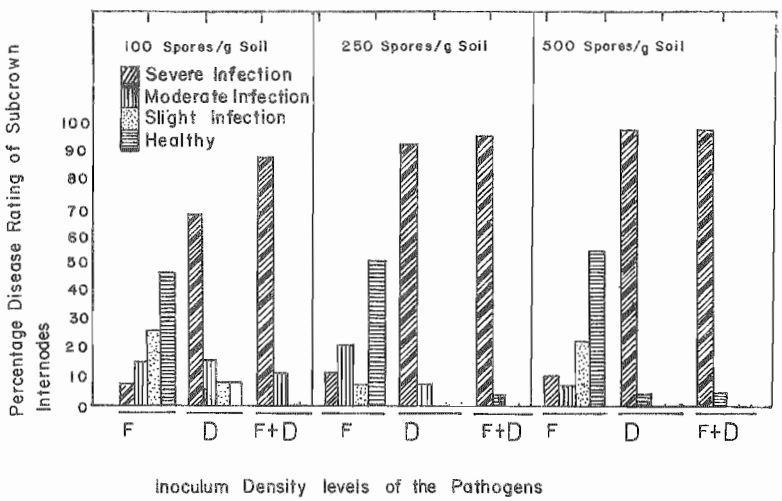

Fig. 2. Percentage disease rating of subcrown internode of wheat cv. "Danne" as caused by isolate 47 of *Drechslera sorokiniana* and isolate 34 of *Fusarium avenaceum* at different inoculum dinsity levels (100, 250 and 500 conidia/macroconidia respectively) and grown for 9 weeks under controlled soil temperatue of 25C ± 2C. F = *Fusarium avenaceum*, D = *Drechslera sorokiniana*. Mean of three replicates.

resistance screening trials and in determining when to apply other control measures against the pathogen since the inoculum density of the pathogen which can cause economic injury to host plants is already established; thereby saving unnecessary waste of time and money in the disease control.

A decline in percentage of recovery of *F. avenaceum* to 48% at inoculum density of 1000 macroconidia per g of soil seems to suggest antagonism as described by Van der Plank (1975). That is, there is a competitive displacement among the spores for limited infection sites on host plant as the pathogen population density reaches 1000 macroconidia per g of soil. It is also possible that at this level of pathogen infestation, the plants are not resistant to the pathogen, but are stimulated by it as reported by Yarwood (1980). Cool (1968) and Wiese (1977) reported that 100 propagules of *Fusarium* spp. per g of soil are sufficient to cause damage if conditions for disease are favourable. But results of this study showed *F. avenaceum* macroconidia applied @ one macroconidium per g of soil can cause severe infection which can cause detectable yield reduction. Maximum severe infections were obtained in plants inoculated at inoculum density range of 100 to 500 macroconidia per g of soil. Less severe infection occurred on plants inoculated @ 1000 macroconidia per g of soil. This seems to suggest that soil heavily infested with the pathogen (1000 and above propagules per g of soil) may be beneficial to the plants. Results also indentified the inoculum density level of *F. avenaceum* capable of causing detectable economic injury on host plant.

Existence of antagonism between the two pathogens (*D. sorokiniana* and *F. avenaceum*) when inoculated together as reported in the literature (Boosalis 1962; Ledingham 1942; Van der Plank 1975, Young 1943) was noted (Fig. 1). Chinn *et al* (1962) in a comparative study of the role of spore population of *D. sorokiniana* and *Fusarium* spp., in infected field and glasshouse with naturally infected soils were able to isolate *D. sorokiniana* from seedlings of 94 soil samples used and disease ratings ranged from 1 to 46 with a mean of 28 and isolates of *Fusarium* spp., ranged from 0 to 26 with a mean of 10. Chinn *et al.* (1962) findings are in agreement with the results of this study because greater percentage recovery of *D. sorokiniana* was obtained in each inoculum density level used than *F. avenaceum* when both pathogen were combined (Fig. 1). Similar trend of results were obtained with respect to disease severity rating when the two pathogens were combined (Fig. 2).

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