POPULATION DYNAMICS AND PATHOGENICITY OF *Cylindrocladium crotalariae* ON SOYBEAN IN NORTH CAROLINA*

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ABSTRACT

Inoculum rates of 0.4 microsclerotia (ms)/g of soil or greater significantly suppressed seed yield of soybean cultivars Ransom and Forrest grown in microplots filled with Fuquay sandy loam soil, whereas in greenhouse tests, 6.4 ms/g of soil or greater were required to significantly supress seed yield of soybean cultivars Ransom, Forrest and Lee 74. Numbers of microsclerotia tended to be greatest in Norfolk sandy loam than in any of the other three soil types tested, whereas seed yield was lowest in Cecil clay loam regardless of soybean cultivars. In microplots, ms densities increased from planting to harvest when the initial numbers of ms/g of soil were 0.4 and 6.4, but numbers decreased when the initial inoculum density was 57.5 ms/g of soil. Microsclerotia densities declined considerably in all plots 3.5 months after harvest.

Key words: *Cylindrocladium crotalariae*, soybean, *Glycine max*.

RESUMO

Densidade de inóculo de 0,4 microescleródios (ms)/g de solo ou maiores suprimiu significativamente a produção de sementes das cultivares de soja Ransom e Forrest quando cultivadas em microparcelas contendo solo do tipo "Fuquay sandy loam" enquanto que, em experimentos de casa de vegetação, foi necessário uma densidade de 6,4 ms/g de solo, ou maior, para suprimir significativamente a produção nas cultivares Ransom, Forrest e Lee 74. O número de microescleródios apresentou uma tendência para ser maior no solo "Norfolk sandy loam" do que em qualquer outro dos três tipos de solo testados enquanto que, a produção de sementes foi menor no solo "Cecil clay loam" independentemente da cultivar de soja utilizada. Nas microparcelas, a densidade de microescleródios aumentou do plantio à colheita quando os números iniciais de ms/g de solo foram de 0,4 e 6,4, mas diminuiu quando a densidade de inóculo inicial foi de 57,5 ms/g de solo. A densidade de microescleródios diminuiu consideravelmente em todas as parcelas três meses e meio após a colheita.

Palavras chave: Cylindrocladium crotalariae, Glycine max.

INTRODUCTION

Cylindrocladium crotalariae Bell and Sobers causes a disease of peanuts and soybeans which is present in several North American southern states (BELL et al., 1973; GARREN, 1972; MORTON, 1972). The few studies of this disease on soybeans (KRIGSVOLD & GRIFFIN, 1975; KRIGSVOLD et al., 1977; PHIPPS &

BEUTE, 1976; ROWE et al. 1973) have delt primarily with the effects of cultural practices on the fungus and its population dynamics.

The effect of edaphic factors on the development of *C. crotalariae* is not well understood. *Clylindrocladium* black rot (CBR) has been reported to be more prevalent in finer textured soil in Georgia (BELL & SOBERS, 1966); but, there is no apparent relationship in North Carolina (ROWE et al., 1973) and Virginia (GARREN et al., 1972). Soil temperature of 25°C and moisture content near "field capacity" were optima for root infection and rot of peanuts (PHIPPS & BEUTE, 1977). Microsclerotia germination was adversely affected at low temperatures (ROTH & GRIFFIN, 1977).

The objectives of this research were to determine i) the effect of inoculum density on disease severity; ii) the influence of cultivars, soil type and inoculum density on the development of the fungus; and iii) the population dynamics of *C. crotalariae* microsclerotia.

MATERIALS AND METHODS

Inoculum preparation. *Cylindrocladium crotalariae* isolate C 64 (A.T.C.C. # 32368) was grown in a liquid medium (ROWE et al., 1974) for 6 weeks. Microsclerotia (ms) were freed from the mycelium by comminutation for 5 min in a blender and then poured through a serie of sieves. Ms retained by a 45 micron sieve were rinsed with water to remove mycelial fragments. Microsclerotia numbers were determined by suspending them in water, adding 1 ml subsamples to a 3-cm dish containing a gridded milipore filter on a filter pad and counted (PHIPPS et al., 1976). Microsclerotia were used as inoculum for all experiments.

Microplot experiment. Four inoculum densities and two varieties were studied in a 4 x 2 factorial experiment arranged in a randomized complete block design with four replications. Thyrty-two fiberglass frames (76-cm diam and 61-cm height) were placed 50-cm deep into a Fuquay sandy loam soil free of *C. crotalariae* at the Central Crops Research Station, Clayton, NC, USA. The soil was infested with 0.4, 6.4 or 57.5 viable ms/g of soil by thoroughly mixing the top 15-cm of soil. Non infested soil served as control. Each microplot was planted with 30 seeds of 'Forrest' or 'Ransom' soybeans (*Glycine max* (L.) Merrill.) inoculated with *Rhizobium japonicum*. Eight soil samples were taken at random with a 2.5-cm diam soil probe and composited one week after planting, 1 month after planting, at flowering, at harvest and 3.5 months after harvest. The composed samples were placed in polyethylene bags and sealed, transported to the laboratory, stored at room temperature for 14 days or less, mixed thoroughly, and assayed for ms (BYRD et al., 1976; PHIPPS et al., 1976). Seed production, weight of 100 seeds and the total plants per microplot were determined. Analysis of variance was used to test the significance of inoculum density, sampling time, yield, cultivar, and their interactions. Correlation coefficients were determined for ms number at the various sampling date vs. seed yield.

Greenhouse experiment. Four soil types, four inoculum densities and three cultivars were arranged in a 4 x 4 x 3 factorial experiment in a randomized complete block design with four replications. Treatments involving Fuquay sandy loam were replicated eight times. The soil types Fuquay sandy loam, Portsmouth fine sandy loam, Norfolk sandy loam, and Cecil clay loam were collected from the Central Crops Research Station (Clayton, NC), Tidewater Research Station (Plymouth, NC), Upper Coastal Plains Research Station (Rocky Mount, NC), and Research Farm Unit 2 (Raleigh, NC), respectively. The soils were

infested with 0.4, 6.4 and 57.5 viable ms/g of soil by pipeting suspended ms in water onto the soils. In a polyethylene bag the suspension was mixed thoroughly and placed in a 15-cm diam clay pot. Non infested autoclaved soil served as control. Seeds of cultivars Ransom, Lee 74 and Forrest were inoculated with *R. japonicum* and planted three per pot. Two weeks after, plants were thinned to one/plot.

Four replications of the Fuquay sandy loam were harvested at flowering. The remaining treatments were harvested at plant maturity. Seed weight and ms population density were determined (BYRD et al., 1976; PHIPPS et al., 1976). Tests of significance were performed for inoculum level, seed yield, final ms population, soil type, cultivar and their interactions.

RESULTS

Microplot experiment. 'Ransom' and 'Forrest' yielded more (P = 0.05) in the control plots than in the infested plots (Fig. 1). The yield of 'Ransom' was greater (P = 0.05) at 0.4 ms/g of soil than at higher inoculum densities. The yields of 'Ransom' were negatively correlated (r = -0.48) with numbers of ms/g of soil at harvest. Yield of 'Forrest' was not different between initial inoculum densities. 'Ransom' yielded more (P = 0.05) than 'Forrest' in the control, 0.4 and 57.5 ms/g of soil. There were no differences in 100 seed weight between treatments. Infection by *C. crotalariae* was confirmed by root observation.

Microsclerotia densities were not different between cultivars, thus means for cultivars were combined. Ms densities increased from initial populations of 0.4 and 6.4 ms/g of soil to their maximum at harvest, but was significantly greater compared to other sampling dates only for the latter. The number of ms continously declined from 57.5 to 2.6 ms/g of soil

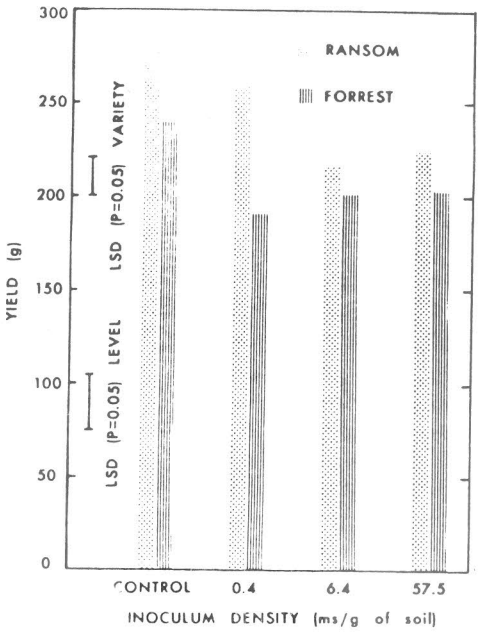

Figure 1. Average yield (g/plot) of 'Ransom' and 'Forrest' soybeans grown in Fuquay sandy loam soil in the control and in soils infested with 0.4, 6.4, and 57.5 ms/g of soil.

during the test period (Fig. 2). The numbers of ms/g of soil declined sharply during the winter at all inoculum densities.

Plant height was correlated (r = -0.49, P = 0.05) only with initial ms density for 'Forrest'. Total nitrogen in the seed was not different between treatments.

Greenhouse experiment. Seed weight and number of seed/plant were lower (P = 0.01) for all cultivars grown in Cecil clay loam than in the other soil types (Table 1). 'Lee 74' grown in Portsmouth fine sandy loam yielded more (P = 0.05) than in Fuquay sandy loam. 'Ransom' yielded less than 'Lee 74' and 'Forrest' (P = 0.05) in Norfolk sandy loam and less than 'Lee 74' in Portsmouth fine sandy loam. Seed-weights/plant and weight of 100 seeds averaged

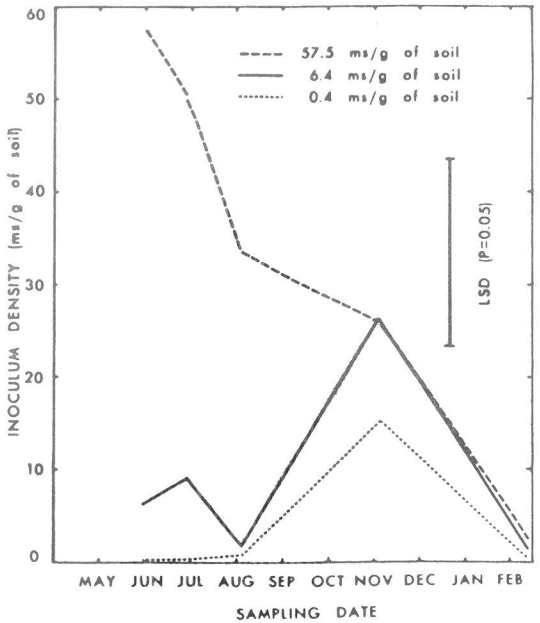

Figure 2. **Combined *Cylindrocladium crotalariae* ms/g of soil during the growing season at five different sampling times (planting, one month after planting, flowering, harvest, and 3.5 months after harvest).**

over soil types and cultivars were 5.32, 5.37, 4.18, and 4.52 g, and 11.8, 12.1, 9.6, and 10.7 g for the control, 0.4, 6.4 and 57.5 ms/g of soil, respectively. The differences between the control and 0.4 ms/g of soil were significant (P = 0.05) from 6.4 and 57.5 ms/g of soil for seed wt/plant and wt/100 seeds.

The number of seeds/plant were greater (P = 0.05) for 'Ransom' grown in Fuquay and Norfolk soils than for the Portsmouth soil (Table 1). There were fewer seeds/plant (P = 0.01) of 'Ransom' in the Norfolk and Portsmouth soils than of 'Forrest' and 'Lee 74'.

Microsclerotia density, measured at plant maturity, was not different between cultivars. The number of ms recovered increased with increasing initial inoculum density (Table 2). The greatest numbers of ms occurred in Norfolk sandy loam at the highest inoculum density (P = 0.05). Number of ms/g of soil at 6.4 and 57.5 ms/g of soil in Norfolk was different from Cecil and Fuquay (P = 0.01 for 6.4 ms/g of soil, and P = 0.05 for 57.5 ms/g of soil). The initial numbers of ms/g of soil were

Table 1. **Effect of *Cylindrocladium crotalariae* on seed weight and number of seed/plant of three *Glycine max* cultivars grown in four soil types(1).**

Soil type	Cultivars(2)					
	Forest		Ransom		Lee 74	
	weight(3) (g)	no. of seeds(3)	weight(3) (g)	no. of seeds(3)	weight(3) (g)	no. of seeds(3)
Cecil	1.6	16.1	3.2	18.3	1.6	17.8
Fuquay	5.7	55.4	5.1	50.5	5.7	54.0
Norfolk	6.4	60.4	5.0	48.1	6.4	61.9
Portsmouth	6.3	56.4	5.2	38.5	7.1	56.6

(1) Data are means of four replications.
(2) Since seeds weights and number of seeds among inoculum densities and the control were not different, means were combined over cultivars.
(3) LSD (.05) weights = 1.1.
no. of seeds = 9.1.

different (P = 0.05) between Portsmouth and Fuquay soils when initial inoculum was 6.4 ms/g of soil. Ms in the control was apparently from contamination during watering, since precautions were taken in setting up the experiment to avoid contamination.

DISCUSSION

A relatively low ms population density of *Cylindrocladium crotalariae* suppressed yield of soybean cultivars Ransom, Forrest and Lee 74. Only 0.4 ms/g of soil suppressed the yield of 'Ranson' by 10% and 'Forrest' by 30% in the microplot experiments; however, 6.4 ms/g of soil were required to suppress yields in the greenhouse. The greater amount of water stress under natural conditions may lower the damage threshold of soybean. Although the only significant correlation with number of ms/g of soil and yield was for the cultivar Ransom at harvest, number of ms at planting time were directly related to yield. This relationship need to be tested in the field under natural management to determine predictability. Determining ms in the soil may be essential in disease management because typical symptoms did not occur in this experiment, thereby reliance of symptom expression for determining distribution may not be adequate for systematically planning control strategies.

Knowledge of edaphic factors such as soil type and associated soil properties and their interaction with cultivars may be important in prediction of crop loss at certain ms inoculum densities. The highest final ms populations occurred in the Norfolk and Portsmouth soils; but, seed yield of cultivars Lee 74 and Forrest, were also highest in these soils. We can only speculate on factors involved since there is not a clear association of ms population density with soil type. However, the largest final ms density occurred in one of the course-textured soils, Norfolk sandy loam. Soil aeration may account for some of the differences, e.g., *Sclerotium rolfsii* on peanuts as suggested by DUBEY (1958). However, factors other than soil aeration are apparently involved because ms densities in Fuquay sandy loam soil were lower than in Cecil clay loam soil.

The low soybean yield in Cecil clay loam soil was probably due to several factors: i) conditions favorable for disease; ii) soil factors inherent to the specific soil type (CALDWELL et al., 1973); iii) irrigation regime which consisted of watering the pots every day keeping the Cecil clay loam soil at high water content during the experiment.

Table 2. Number of microsclerotia/g of soil of *Cylindrocladium crotalarie* on soybean at maturity as influenced by four soil types and four inoculum densities(1).

Soil type	Inoculum density (ms/g of soil)(2)			
	Control	0.4	6.4	57.5
Cecil	1.8	18.0	36.8	110.6
Fuquay	0.0	6.9	33.0	117.6
Norfolk	0.0	8.9	64.7	140.1
Portsmouth	1.4	12.1	57.5	122.2

(1) Data are means of four replications.
(2) LSD (.05) = 18.7.

Regardless of initial ms population density, the number of ms tended to approach a similar density by plant maturity. For example, ms densities at plant maturity were 15.5, 26.5, and 25.9 ms/g of soil for initial inoculum densities of 0.4, 6.4 and 57.5 ms/g of soil, respectively. The marked increase in ms populations of samples taken from flowering until harvesting could be due to increased host susceptibility or to more favorable climatic conditions for disease development, or both. PHIPPS & BEUTE (1977) reported that soil temperature of 25°C was optimal for *C. crotalariae* on peanut, whereas at higher temperatures, *C. crotalariae* developed poorly or not at all. The reason for the decrease of the ms population at 57.5 ms/g of soil is not clear. Perhaps ms densities were too high in the soybean rhizosphere for germination to occur or too few roots were available for colonization causing a high ms mortality.

Winter temperature played a significant role in the survival of the fungus. The overall ms density in the microplots dropped from 22.6 ms/g of soil at harvest to 1.5 ms/g of soil 3.5 months after harvest. This negative effect of low temperature was observed throughout North Carolina in 1977 (P. M. Phipps, personal communication). ROTH & GRIFFIN (1977) reported that ms germinability was adversely affected by low temperature. In 1976, PHIPPS & BEUTE (1976) found that ms densities at harvest and 3 months later were not significantly different from initial populations. In our study, however, *C. crotalariae* ms were probably adversely affected by the unusually low temperatures in January and February of 1977 (NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION, ENVIRONMENT DATA SERVICE, 1977).

The threshold densities of *Cylindrocladium crotalariae* ms could be 0.4 ms/g of soil or lower. Edaphic and climatic factors may modify this threshold density. More research is required before predictions of this disease by soil assays become possible.

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