INFLUENCE OF INOCULUM LEVEL AND TEMPERATURE ON PATHOGENICITY AND POPULATION DEVELOPMENT OF LESION NEMATODES ON SOYBEAN[1]

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ABSTRACT

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Greenhouse studies were conducted on the host-parasite relationships and the effects of temperature on pathogenicity and population development of lesion nematodes on soybean. Clark 63 soybean was a highly favorable host for *Pratylenchus alleni* and *P. scribneri*. The degree of damage was influenced by initial nematode density and temperature. At a mean temperature of 29° C, an initial inoculum level of 20,000 nematodes per pot significantly reduced shoot and root weights. Reductions in shoot and root weights were 45 and 49%, respectively, for *P. alleni* and 23 and 47% for *P. scribneri* when compared with noninoculated controls. Neither species affected plant weight at inoculum levels of 1,000 and 10,000 nematodes per pot. Soil temperature played a significant role in expression of damage by *P. scribneri* alone and only at relatively high constant temperatures. Stress on plants at 34° C, reduced tolerance to this thermophilic species resulting in considerable growth reduction. *Pratylenchus alleni* had no detectable effect on growth at 34° C, although its final numbers exceeded those of *P. scribneri* at this temperature. Both species reproduced between 22 and 34°, but population increase was least at 22° C. The optimum for population development of *P. alleni* was 30° C, while that for *P. scribneri* was between 30-34° C.

Additional key words: Glycine max, soybean production, tolerance limit, site of inoculation, seed yield, thermophilic, tolerance.

RESUMEN

Acosta, N. 1982. Influencia del nivel de inóculo y la temperatura en la patogenicidad y desarrollo poblacional de nematodos lesionadores en soya. Nematrópica 12:189-197

Se llevaron a cabo estudios de invernadero para determinar la relación hospedero-parásito y el efecto de temperatura sobre la patogenicidad y el desarrollo de una población de nematodos lesionadores en soya Clark 63. La variedad de soya Clark 63 fue un hospedero muy favorable para *Pratylenchus alleni* y *P. scribneri*. El grado de daño estuvo influenciado

por la densidad inicial de nematodos y por la temperatura. A una temperatura promedio de 29° C, un nivel de inóculo inicial de 20,000 nematodos por tiesto redujo significativamente el peso de la parte aérea y raíces de la planta. Las reducciones fueron de 45 y 49%, respectivamente con *P. alleni* y de 23 y 47% con *P. scribneri* cuando se compararon con los testigos sin inocular. Ninguna de las especies afectó el peso de las plantas a los niveles de inóculo de 1,000 y 10,000 nematodos por tiesto. La temperatura del suelo jugó un rol significativo en la expresión de daño por *P. scribneri* sólo y únicamente a temperaturas relativamente altas y constantes. El efecto de 34° C sobre las plantas redujo la tolerancia contra esta especie termofílica, resultando en una reducción del crecimiento. *Pratylenchus alleni* no mostró efecto sobre el crecimiento de plantas mantenidas a 34° C, aun cuando los números finales de nematodos excedieron a los de *P. scribneri* a la misma temperatura. Ambas especies se reprodujeron a temperaturas entre 22 y 34° C, pero el incremento poblacional fue menor a 22° C. La temperatura óptima para el desarrollo de *P. alleni* fue de 30° C, mientras que la de *P. scribneri* está entre 30 y 34° C.

Palabras claves adicionales: Glycine max, producción de soya, límite de tolerancia, punto de inoculación, producción de semillas, termofílico, tolerancia.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.], the prime world source of vegetable oil and protein for human and animal consumption (5), is a host for several species of lesion nematodes (8,9,10,11). Ferris and Bernard (10) found that populations of *Pratylenchus hexincisus* Taylor and Jenkins, *P. neglectus* (Rensch.) Filipv. and Schuurm-Stekh., *P. penetrans* (Cobb) Filipv. and Schuurm-Stekh, and *P. scribneri* Steiner tended to increase in fields of soybean in Illinois. *Pratylenchus alleni* Ferris and *P. scribneri* appear to be the two species most commonly encountered in soybeans in Illinois and the surrounding region (8). Lindsey and Cairns (12) found that *P. brachyurus* (Godfrey) Filipv. and Schuurm-Stekh. in the greenhouse reduced seed yield on soybean cultivar Hood. Ferris and Bernard (9) recorded a 25% root weight reduction caused by *P. alleni.* Dave (6) noted extensive decay of soybean roots induced by *P. scribneri.* Acosta *et al.* (4) found that cultivars Clark 63, Woodworth and Williams were highly susceptible to *P. scribneri* in the greenhouse in Illinois. Laboratory studies conducted by Acosta (1) to determine the distribution of *P. alleni* and *P. scribneri* in Clark 63 soybean roots showed that populations of both nematodes were more abundant in the first 10-15 cm of the root zone.

Histological examination of *P. alleni* infected roots and of *P. scribneri* infected roots 5,11,18, and 45 days after inoculation, showed specimens of *P. scribneri* located entirely within the cortex oriented longitudinally to the vascular cylinder, either outstretched in the same plane or coiled through several cells. Both nematode species caused extensive rupturing of cell walls, retraction and disappearance of cytoplasm and thickening of cell walls and

necrosis of cells surrounding feeding sites (2).

Temperature is an important factor in the seasonal fluctuations of plant growth and nematode numbers (14,16). Soil temperature also appears to play a complex role in the expression of nematode damage to plants. Lindsey and Cairns (12) found that the density of *P. brachyurus* populations in roots of several cultivars generally increased with temperature up to 29° C. Taylor *et al.* (15) showed that *P. penetrans* could reduce root weight of soybean seedlings when grown at 21° C. Dickerson (7) found that population increases of *P. scribneri* at constant temperatures over the range 20-37.5° C were correlated directly with temperature. A significant increase in *P. scribneri* populations was obtained raising the temperature from 25 to 35° C for either 3 or 9 days. Studies conducted by Acosta and Malek (3) demonstrated that the most rapid population increase of *P. alleni, P. brachyurus, P. coffeae* (Zimmermann) Filipv. and Schuurm-Stekh., *P. neglectus, P. scribneri,* and *P. zeae* Graham occurred at 30° C, whereas populations of *P. penetrans* and *P. vulnus* Allen & Jensen increased more rapidly at 25° C.

This research was undertaken to determine the pathogenic potential of *P. alleni* and *P. scribneri* on soybean and the effect of four constant temperature regimes on nematode population development.

MATERIALS AND METHODS

Pathogenicity study:

A pregerminated Clark 63 soybean seed was planted at inoculation time in each of forty-five 15-cm diameter clay pots, each containing 1,400 cm^3 of steam-sterilized Sparta loamy-fine sand (84.9% sand, 10.2% silt and 4.9% clay) with 0.4% organic matter and pH 4.3. Pots were either inoculated with *P. alleni* or *P. scribneri* or left uninoculated. Inoculum levels used were 1,000, 10,000, and 20,000 *P. alleni* or *P. scribneri* per pot, respectively, which had been increased on soybean plants in the greenhouse and extracted from roots in a mist chamber by the funnel-spray method of Oostenbrink (13). Three uninoculated controls were included: water free of nematodes decanted from a suspension of approximately 160,000 *P. alleni* that had settled for 12 h, water decanted from a suspension of around 200,000 *P. scribneri,* and distilled water. Inoculations were made by dispensing 25-ml aliquots of appropriate dilution of suspension into 6-cm holes in the soil. Five replications per treatment were included in a randomized complete block design. Plants were kept on a greenhouse bench for a 90 day period during which they were watered when necessary. No nodulating bacteria were added to the plants, but a 23-19-17 fertilizer solution was applied monthly. Soil temperatures ranged from 24-35° C ($\bar{x}$=29° C). Ninety days after inoculation, data on dry shoot and fresh root weight were recorded and nematode populations determined.

Temperature study:

Pregerminated soybeans were planted in 15-cm diameter plastic containers

(containers with drainholes placed inside another without drain holes containing stones to prevent flotations) filled with 1,400 cm^3 of steam-sterilized soil. Inoculum levels used were 1,000 and 10,000 *P. alleni* or *P. scribneri* per container, respectively. Uninoculated control plants were included. Five days after inoculation, four replications of each treatment were placed in constant temperature water baths (Esco Cabinet Co., West Chester, PA), maintained at 22,26,30, and 34°C. A fifth block was retained on a greenhouse bench, where soil temperature fluctuated from 26-37 C ($\bar{x}$=30°C). A split-plot, randomized complete block design was used in each temperature regime. Ninety days after inoculation final data were recorded as in the pathogenicity study.

RESULTS AND DISCUSSION

Pathogenicity study:

The highest inoculum level of *P. alleni* and *P. scribneri* (20,000 nematodes per pot) significantly reduced shoot and root weights of Clark 63 soybeans (Table 1). Reductions were 45 and 49%, respectively for *P. alleni* and 23 and 47% for *P. scribneri,* when compared to the decanted supernatant controls. A control treatment obtained by decanting nematodes from the suspension after setting for 12 h is less than perfect because some pathogenic organisms could have settled with the nematode. Lack of effect on growth by this treatment does suggest that no pathogenic organisms other than nematodes were present in large numbers in the inoculum. Neither species significantly affected plant weight at the low (1,000) and intermediate (10,000) inoculum levels under the experimental conditions. The highest and the lowest final numbers of both species were recovered from soil and roots of plants inoculated with the intermediate and lowest levels, respectively. *Pratylenchus alleni* increased 12-fold at these inoculum levels but about 2-fold at the high level (e.g., 4.4 x 10,100÷20,000). Those of *P. scribneri* increased 65-12-and-6-fold at low, intermediate and high levels, respectively.

Based on shoot weights, *P. alleni* appeared to be more pathogenic than *P. scribneri.* Using soybean cv. Lincoln, Ferris and Bernard (9) also obtained a reduction in root weight of plants infected with *P. alleni.* Seinhorst (14) stated that damage by nematodes, based on growth and yield of plants occurs only when nematode densities exceed the tolerance limit of the attacked plant and the limit undoubtedly varies with changes in the degree of stress from other environmental factors.

Although the relationship between inoculum level of *P. alleni* and degree of injury to soybean was not well defined, there was a trend toward increased damage with increased initial nematode density. Dave (6) showed that final populations of *P. scribneri* alone were proportional to initial densities at low and intermediate inoculum levels, but decreased at the high level, attributing this decrease to instraspecific competition.

Table 1. Effects of inoculum levels of *Pratylenchus alleni* and *P. scribneri* on soybean plants.

Inoculum level	Weight (g)[y]		Number (1000's) of nematodes/g fresh root
	Dry shoot	Fresh root	
Distilled water	7.1 a	9.4 a	0.0
P. alleni			
Decant[x]	7.1 a	8.3 ab	0.0
1,000	7.0 a	8.5 ab	1.8
10,000	6.8 a	8.5 ab	15.7
20,000	3.9 b	4.4 c	10.1
P. scribneri			
Decant	7.9 a	10.1 a	0.0
1,000	8.9 a	9.1 a	8.6
10,000	7.9 a	8.7 a	15.6
20,000	6.0 b	5.3 bc	22.2

[x]Water decanted from nematode suspension and free of nematodes; [y]Each value is the mean of five replications with one plant/pot; column means followed by unlike letters differ (P=0.05) according to Duncan's new multiple range test.

Temperature study:

No significant differences in shoot or root weights were found among plants inoculated with *P. alleni* under any of the temperature regimes. (Table 2 and 3). Average shoot weights of plants inoculated with 1,000 or 10,000 nematodes were consistently lower than those of noninoculated controls at all temperatures.

A significant difference in shoot weight of plants inoculated with *P. scribneri* and control plants was observed at 34° C (Table 2), and a similar reduction was also obtained in root weights of plants inoculated with 10,000

Table 2. Dry shoot weights (g) of soybean plants inoculated with *Pratylenchus alleni* (Pa) or *P. scribneri* (Ps)

Inoculum	Temperature (°C)[y]				
Level	22	26	30	34	26-37
0 (decant)[x]	16.5 a	15.2 a	18.2 a	13.0 a	20.8 a
1,000 Pa	14.1 a	14.5 a	15.6 a	11.0 ab	21.1 a
10,000 Pa	15.1 a	12.3 a	15.1 a	11.5 ab	18.9 a
1,000 Ps	14.9 a	13.5 a	13.6 a	9.2 b	26.9 a
10,000 Ps	18.5 a	15.4 a	11.9 a	4.6 c	21.8 a

[x]Water decanted from nematode suspension and free of nematodes. [y]Each value is the mean of four replications; column means followed by unlike letters differ (P=0.05) according to Duncan's new multiple range test.

Table 3. Fresh root weights (g) of soybean plants inoculated with *Pratylenchus alleni* (Pa) or *P. scribneri* (Ps).

Inoculum	Temperature (°C)[y]				
Level	22	26	30	34	26-37
0 (decant)[x]	28.9 a	20.1 a	23.9 a	26.0 a	30.5 a
1,000 Pa	22.6 a	23.8 a	21.9 a	24.1 a	25.8 a
10,000 Pa	27.6 a	18.3 a	21.0 a	21.9 a	32.1 a
1,000 Ps	28.8 a	17.1 a	21.0 a	21.9 a	32.1 a
10,000 Ps	31.9 a	21.0 a	16.8 a	12.8 b	33.8 a

[x]Water decanted from nematode suspension and free of nematodes. [y]Each value is the mean of four replications; column means followed by unlike letters differ (P=0.05) according to Duncan's new multiple range test.

Table 4. Numbers (1,000's) of *Pratylenchus alleni* (Pa) and *P. scribneri* (Ps) recovered from soil and roots of soybean

Inoculum	Temperature (°C)[x]				
Level	22	26	30	34	26-37
1,000 Pa	28.0 a	129.0 a	307.0 a	203.3 a	293.6 a
10,000 Pa	209.7 b	716.3 b	950.3 b	522.9 b	562.7 b
1,000 Ps	23.7 a	167.2 a	538.2 a	551.2 b	142.1 a
10,000 Ps	69.3 a	150.1 a	314.7 a	164.1 a	152.6 a

[x]Each value is the mean of four replications; column means followed by unlike letters differ (P=0.05) according to Duncan's new multiple range test.

specimens per pot at 34°C (Table 3).

Both species reproduced throughout the range of temperatures tested but population development was least at 22°C (Table 4). Final populations of *P. alleni* showed that 30°C is the approximate optimum temperature for repro duction of this species. These results are similar to those obtained by Acosta and Malek (3) in 1979 using various species of *Pratylenchus* at different temperature regimes. Nematode numbers in roots at the high initial density at 30°C averaged 239,812 per gram of dry roots, 90 days after inoculation.

The degree of population development of *P. scribneri* at the low inoculum level indicates that the optimum temperature for this species lies between 30-34°C, somewhat higher than that for *P. alleni.* Based on results obtained in 1979 (3), 35°C is somewhat high for population development of *P. scribneri.* At these temperatures, the total population increase at the low initial density was approximately 23 times that which occurred at the lowest temperature. In contrast to that of *P. alleni,* population development under fluctuating temperatures was less than at either 30 or 34°C. The average temperature in the containers was 30°C, but the median temperature probably was somewhat lower.

Under the otherwise generally favorable conditions for soybean growth in this experiment, only the upper extreme soil temperature played a significant role in expression of damage by lesion nematodes. The high constant temperature and its influence on plant growth in turn lowered the nematode population threshold for detectable damage, in the form of reduced plant weight, below that observed in the greenhouse pathogenicity test.

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