RESEARCH NOTE—NOTA DE INVESTIGACION

INTERACTIONS BETWEEN *PRATYLENCHUS PENETRANS* AND *FUSARIUM AVENACEUM* IN RED CLOVER[†]

Xixuan Jin[‡], J. B. Kotcon, and J. B. Morton

Division of Plant and Soil Sciences, 401 Brooks Hall, West Virginia University, Morgantown, WV 26505-6057, U.S.A.

Accepted:
1.IV.1991
Aceptado:

RESUMEN

Jin, X., J. B. Kotcon, y J. B. Morton. 1991. Interacciones entre *Pratylenchus penetrans* y *Fusarium avenaceum* en trébol rojo. Nematrópica 21:

Plantas de trébol rojo, crecido por 45 días en 160 cm^3 de suelo esterilizado con vapor, fueron inoculados con 0, 1, o 5 *Pratylenchus penetrans*/cm^3 de suelo y con 0, 3.6×10^5, o 3.6×10^6 unidades formadoras de colonias de *Fusarium avenaceum*/cm^3 en todas las combinaciones. El peso seco de partes aéreas fue reducido significativamente por inoculación con ambos parásitos, pero los efectos de ellos en combinación fueron aditivos. Infección de las raíces por *F. avenaceum* aumentó con el incremento de densidad de inóculo de *F. avenaceum* y de *P. penetrans*. La densidad final de la población de nematodos en el suelo y en las raíces aumentó con el incremento de densidad de inóculo de ambos parásitos y la interacción entre los parásitos aumentó significativamente las densidades finales del nematodo.

Palabras clave: interacción, *Fusarium avenaceum*, nematodo lesionador, *Pratylenchus penetrans*, trébol rojo, *Trifolium pratense*.

Nematode feeding injury enhances infection by *Fusarium* spp. and development of red clover (*Trifolium pratense* L.) root rot (3,7). Most studies on the interaction between *Fusarium* and *Pratylenchus* spp. in legume root rot complexes have been conducted on alfalfa, where yields were reduced when soil was infested with both *P. penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven and propagules of *F. tricinctum* (Corda) Sacc. (7). Little is known about the interaction between *P. penetrans* and *F. avenaceum* (Corda ex Fr.) Sacc. in red clover roots. This experiment was designed to examine the interaction between *P. penetrans* and *F. avenaceum* in red clover roots at different inoculum densities under greenhouse conditions.

[†]Published with the approval of the Director of the West Virginia Agricultural and Forestry Experiment Station as Scientific Article #2159. This research was supported with funds appropriated under the Hatch Act.

[‡]Present address of the first author: Department of Horticultural Sciences, New York State Agriculture Experiment Station, Cornell University, Geneva, NY 14456, U.S.A.

Fusarium avenaceum (isolate #959) was highly virulent on red clover roots in a seedling bioassay (9). To produce inoculum, this isolate was grown in 0.95-L Mason jars containing 500 cm^3 of perlite moistened with 150 ml of potato broth amended with 2% sucrose. After 15 days growth at 25 C, the medium, which was covered with hyphae of *F. avenaceum*, was finely ground in a Wiley Mill (Model #3), air-dried for 48 hours, and stored at 4 C until used as inoculum. The average propagule density was estimated, via dilution plating, to be 3.6×10^6 colony forming units (CFU) per cm^3 of the medium. Noninfested medium consisting of perlite amended only with 2% sucrose potato broth served as a control.

Pratylenchus penetrans were collected from *Festuca arundinacea* Schreb. in a peach orchard near Kearneysville, West Virginia, U.S.A. The nematodes were extracted by the shaker technique (1) and counted under a dissecting microscope.

Soil collected from a grass-legume pasture was passed through a 4-mm-pore sieve and steamed at 100 C for two 1-hour periods separated by a 24-hour cooling interval. Fertilizer amendments (70, 150, and 40 mg/kg, nitrogen, phosphorus, and potassium, respectively) were mixed with the bulked soil after steaming. The steamed soil was infested with *F. avenaceum* and *P. penetrans* at inoculum densities of 0, 3.6×10^4, and 3.6×10^5 *Fusarium* CFU/cm^3, and 0, 1, and 5 nematodes/cm^3 soil in factorial combinations. Sterilized plastic Cone-tainers (Ray Leach Nurseries, Canby, Oregon 97013, U.S.A.) were filled with 160 cm^3 steamed soil, and seeded with surface-sterilized Kenstar red clover seeds. Treatments were replicated five times. Seedlings were thinned to three per Cone-tainer when the first true leaf appeared. Plants were grown in the greenhouse at 25 ± 2 C with a 12-hour daily photoperiod (169 $\mu E\ m^{-2}\ sec^{-1}$ fluorescent lighting) for 45 days. At harvest, soil attached to the roots was washed away with tap water, and shoots were oven dried and weighed. Nematodes were extracted from the roots by incubating for 3 days using the shaker technique (1) and from soil by centrifugal-flotation (5). After nematode extraction, roots were collected and surface-sterilized in a 1.6% NaOCl solution for 2–3 min. Fifteen randomly selected root segments (about 0.5 cm long) from each replicate were placed on PDA supplemented with 6 mg/L streptomycin sulfate and 50 mg/L chlorotetracycline for 5 days at 25 C. Frequency of infection by *F. avenaceum* was determined from the percentage of root segments colonized. Data were subjected to analysis of variance.

Red clover shoot dry weight decreased with increasing amounts of inoculum of both organisms (Table 1). Differences in shoot dry weight were significant among nematode levels ($P \leq 0.01$) and among *Fusarium* levels ($P \leq 0.05$). Shoot dry weight was lowest when soil was co-infested at the highest inoculum densities (5 *P. penetrans* and 3.6×10^5 *F. av-*

Table 1. Effects of *Pratylenchus penetrans* and *Fusarium avenaceum* at different inoculum densities on dry weight of red clover shoots, frequency of *Fusarium* infection, and number of nematodes recovered from rhizosphere soil and red clover roots after 45 days of growth.

Pratylenchus inoculum density (nematodes/cm³)	*Fusarium* inoculum density (CFU/cm³)	Red clover shoot dry weight[w] (mg/pot)	*Fusarium* infection[x] (%)	No. of nematodes extracted From roots[y] (per pot)	No. of nematodes extracted From soil[z] (per pot)
0	0	308	0	0	0
0	3.6×10^4	312	13	0	0
0	3.6×10^5	260	18	0	0
1	0	256	0	18	76
1	3.6×10^4	282	21	23	73
1	3.6×10^5	243	24	28	97
5	0	255	0	42	671
5	3.6×10^4	238	28	48	691
5	3.6×10^5	195	32	69	860

Data are means of five replicates.
[w]Differences among *P. penetrans* levels and among *F. avenaceum* levels were significant at $P \leq 0.01$ and $P \leq 0.05$, respectively. There was no *P. penetrans* × *F. avenaceum* interaction.
[x]Differences among *P. penetrans* levels and among *F. avenaceum* levels were significant at $P \leq 0.01$. There was no *P. penetrans* × *F. avenaceum* interaction.
[y]Differences among *P. penetrans* levels and among *F. avenaceum* levels were significant at $P \leq 0.01$. The *P. penetrans* × *F. avenaceum* interactions was significant at $P \leq 0.05$.
[z]Differences among *P. penetrans* levels and among *F. avenaceum* levels were significant at $P \leq 0.01$. The *P. penetrans* × *F. avenaceum* interaction was significant at $P \leq 0.01$.

enaceum CFU/cm³ soil). However, the two-way interaction between these two pathogens on plant growth was not significant. Infection of red clover roots by *F. avenaceum* increased ($P \leq 0.01$) with increasing inoculum density of the fungus (Table 1). The presence of *P. penetrans* also promoted ($P \leq 0.01$) infection by *F. avenaceum*. The interaction of inoculum levels of these two organisms, however, did not influence infection by *F. avenaceum*. Greater numbers of nematodes were found in the rhizosphere soil than in roots (Table 1). Numbers of *P. penetrans* recovered increased significantly with increasing initial inoculum densities of nematodes ($P \leq 0.01$ for soil and for roots) and *F. avenaceum* ($P \leq 0.01$ for soil and for roots). The interaction between *P. penetrans* and *F. avenaceum* inoculum density affected nematode population densities recovered from soil ($P \leq 0.01$) and roots ($P \leq 0.05$).

Mauza and Webster (8) reported that *F. oxysporum* Schlet. and *P. penetrans* caused a synergistic suppression of alfalfa growth. In this study, however, the growth reduction of red clover caused by *F. avenaceum* and *P. penetrans* was additive because the interaction between these two pathogens on plant growth was not significant (11). Infection by *F. avenaceum* reduced red clover growth in Cone-tainers but not the growth of red clover in pots or field tests (6). Cone-tainers provide a

controlled environment for studies of host and pathogen interactions; however, physiological stresses associated with confinement of roots may have indirectly enhanced inhibitory effects of fungal infection on shoot development. Pathogenicity of *P. penetrans* to red clover was evidenced at a density of one nematode/cm^3 soil in the Cone-tainers. It is uncertain, however, if these results extend to field situations.

Fusarium avenaceum is considered to be an opportunistic pathogen (6), but hyphae can penetrate roots and colonize cortical and vascular tissue, either directly or through wounds (2,9,10). Wounds from *P. penetrans* may create an avenue for infection by *F. avenaceum*, or may alter the physiology of host-pathogen interactions to favor fungal colonization of the root cortex (10).

The enhanced recovery of *P. penetrans* from soil and roots of *Fusarium*-infected plants suggests that nematode reproduction may be stimulated by the fungus. Similar stimulation of *P. minyus* Sher & Allen reproduction was observed in peppermint plants infected by *Verticillium dahliae* (Kleb.) f. *menthae* Nelson (4). Changes in host physiology induced by the fungus might be responsible for the increase in nematode reproduction.

LITERATURE CITED

1. BIRD, G. W. 1971. Influence of incubation solution on the rate of recovery of *Pratylenchus brachyurus* from cotton roots. Journal of Nematology 3:378–385.
2. CHI, C. C., W. R. CHILDERS, and E. W. HANSON. 1964. Penetration and subsequent development of three *Fusarium* species in alfalfa and red clover. Phytopathology 54:434–437.
3. ELLIOTT, E. S., R. E. BALDWIN, and R. B. CARROLL. 1969. Root rots of alfalfa and red clover. West Virginia University Agricultural Experimental Station Bulletin 585T. Morgantown, West Virginia, U.S.A.
4. FAULKNER, L. R., and C. B. SKOTLAND. 1965. Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint. Phytopathology 55:583–586.
5. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.
6. JIN, X. 1988. Role of *Fusarium acuminatum* and *Fusarium avenaceum* in red clover root decline complex. Ph.D. Dissertation. West Virginia University, Morgantown, West Virginia, U.S.A.
7. LEATH, K. T., F. L. LUKEZIC, H. W. CRITTENDEN, E. S. ELLIOTT, P. M. HALISKI, F. L. HOWARD, and S. A. OTAZESKI. 1971. The Fusarium root rot complex of selected forage legumes in the Northeast. The Pennsylvania State University, College of Agriculture, Agricultural Experiment Station Bulletin 777. University Park, Pennsylvania, U.S.A.
8. MAUZA, B. E., and J. M. WEBSTER. 1982. Suppression of alfalfa growth by concommitant populations of *Pratylenchus penetrans* and two *Fusarium* species. Journal of Nematology 14:364–367.
9. STUTZ, J. C., and K. T. LEATH. 1983. Virulence differences between *Fusarium roseum* "Acuminatum" and *F. roseum* "Avenaceum" in red clover. Phytopathology 73:1648–1651.

10. STUTZ, J. C., K. T. LEATH, and W. A. KENDALL. 1985. Wound-related modifications of penetration, development, and root rot by *Fusarium roseum* in forage legumes. Phytopathology 75:920–924.
11. WALLACE, H. R. 1983. Interactions between nematodes and other factors on plants. Journal of Nematology 15:221–227.

Received for publication:
25.IX.1990
Recibido para publicar: