Journal of Nematology 17(2):162–165. 1985.

Studies on the Host Range, Biology, and Pathogenicity of *Punctodera punctata* Infecting Turfgrasses[1]

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Abstract: Punctodera punctata completed its life cycle on *Poa annua* (annual bluegrass), *P. pratensis* (Merion Kentucky bluegrass), *Lolium perenne* (perennial ryegrass), and *Festuca rubra rubra* (spreading fescue). Minimum time for completion of a life cycle from second-stage juvenile to mature brown cyst was 40 days at 22–28 C. Inoculation by single juveniles indicated that reproduction was most likely by amphimixis. Infestation levels of 50 or 500 juveniles/250 cm³ soil did not affect top dry weight, root dry weight, or total dry weight of *Poa annua*.

Key words: cyst nematode, *Poa annua, P. pratensis, Lolium perenne, Festuca rubra rubra,* pathogenicity, life cycle, reproduction.

Punctodera punctata (Thorne, 1928) Mulvey and Stone, 1976, the grass cyst nematode, was described by Thorne (10) from specimens infecting wheat roots in Saskatchewan, Canada. During routine soil inspections of potato fields for the golden nematode in 1949, Chitwood (3) discovered *P. punctata* in Pembina County, North Dakota, the first record of this species in the United States.

Subsequent reports from Michigan (2), Texas (5), Minnesota (9), and Quebec (1) listed the cool-season turfgrasses *Poa annua, P. pratensis,* and *Agrostis palustris* as hosts for this nematode. According to records of the United States Department of Agriculture Plant Quarantine Division, *P. punctata* has been intercepted from 29 countries since 1949 (6). Wheeler (11) also reported this nematode had been intercepted from France and England. Recently *P. punctata* was found associated with golf course greens and fairways in New Jersey (7,8).

Despite its world-wide occurrence, information concerning the host range, biology, and pathogenicity of *P. punctata* is sparse. The objectives of this study were to acquire information on the host range, life cycle, mode of reproduction, and pathogenicity of a New Jersey population of *P. punctata* found infecting annual and perennial turfgrasses.

MATERIALS AND METHODS

Cysts were extracted from 250-cm³ samples of soil throughout this study by the centrifugal-flotation method (4). Cysts were collected from nested 250- and 149-μm-pore sieves no longer than 2 days before use and stored in distilled water at 4 C. Inoculum of *P. punctata* was obtained di-

Received for publication 30 July 1984.

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The authors thank Dr. C. R. Funk for supplying grass seed, Drs. R. T. Robbins and R. D. Riggs for reviewing the manuscript, and Ms. Carol A. Midkiff for technical assistance.

rectly from the field during early spring. Mature brown cysts were dissected to release eggs and juveniles.

Host range: Forty-five plant species representing 18 families were seeded in heat pasteurized loam soil (3:1, loam:sand) in 7.5-cm-d clay pots which were then placed inside 15.0-cm-d peat pots filled with moist vermiculite. When seedlings were approximately 2 weeks old, an aqueous suspension containing 250–500 eggs and second-stage juveniles of *P. punctata* was added to each of three pots of each plant species tested. Greenhouse temperature was 24–30 C, and plants were illuminated with fluorescent and incandescent lights for 12 hours a day. Plants were fertilized with a 20:20:20 N:P:K solution every 2 weeks.

Life cycle: Forty Cone-Tainers® (R. Leach Cone-Tainer Nursery, Canby, Oregon) with a volume of 17.8 cm^3 (7.0 $in.^3$) were filled with pasteurized loam soil, seeded with Merion Kentucky bluegrass (*Poa pratensis*), and placed in sand-filled containers on 3 March 1983. When seedlings emerged after 20 days, suspensions containing approximately 250–500 eggs and juveniles of *P. punctata* were pipetted into a hole (0.5 cm d × 5.0 cm deep) in the soil at the center of each Cone-Tainer. The Cone-Tainers were then placed on greenhouse benches at 22–28 C. Plants were fertilized as above and periodically cut to a height of 2.5 cm with hand shears.

To determine the degree of nematode development, nematodes were recovered using a 38-μm-pore sieve at various intervals.

Nematode reproduction: Fifty Cone-Tainers without drain holes were filled with pasteurized loam-sand soil mix and seeded with Merion Kentucky bluegrass. When a dense stand of grass emerged, after 30 days, a single juvenile was pipetted into a hole (0.5 cm d × 5.0 cm deep) in the soil at the center of each Cone-Tainer. Cysts were extracted from the soil and about 75 days later were placed in water and dissected to determine egg production and juvenile development.

Pathogenicity: Eighteen 7.5-cm-d clay pots were filled with 250 cm^3 loam soil (3:1, loam:sand) and placed inside 15.5-cm-d peat pots filled with moist vermiculite. Inoculation sites were made by depressing and leaving 55 × 1.5-mm specimen bottles in the center of each pot between three planted stolons of *Poa annua*. Forty days after planting, the bottles were removed, inocula consisting of nematodes suspended in water were added, and the depressions filled with soil mix. Treatments consisting of a water control, 50 second-stage juveniles per pot, or 500 juveniles per pot were replicated six times in a randomized complete block design on a greenhouse bench. Pots were fertilized with a solution 20:20:20 N:P:K every 2 weeks. Greenhouse temperature was 23–30 C. Leaves, stems, and seedheads were removed 120 days after inoculation. Roots were washed free of soil and cysts were collected as described earlier. The roots and above-ground plant parts were oven dried at 85 C for 3 days and weighed. Numbers of nematodes and plant weights were statistically analyzed by the Student-Newman-Keuls Test.

Results and Discussion

Host range: Punctodera punctata reproduced exclusively in plants of the Gramineae (Table 1). *Poa annua* was the most suitable host based upon reproduction followed by *P. pratensis, Lolium perenne,* and *Festuca rubra rubra. Poa* spp. were previously listed as hosts of *P. punctata* by Baker (1) and Horne (5). Gramineous species not supporting reproduction were *Secale cereale* (rye), *Avena sativa* (oats), *Sorghum* cv. Bird-A (sorghum), *Hordeum vulgare* (barley), *Triticum aestivum* cv. Sulivan (wheat), *Agrostis palustris* cv. Penn Eagle (creeping bentgrass), *A. palustris* cv. Seaside (creeping bentgrass), *Agrostis alba* (Red top), *Festuca elatior* cv. Revel (tall fescue), *Festuca longifolia* cv. Reliant (hard fescue), *Festuca rubra cummutata* cv. Shadow (Chewings fescue), *Festuca ovina glauca* cv. Aries (sheep's fescue), *Stenotaphrum secundatum* (St. Augustine grass), *Zoysia japonica* cv. Meyer (zoysiagrass), *Echinochloa colunum* (barnyard grass), *Zea mays* cv. Quicksilver (corn), and *Zea mexicanus* (teosinte). Nongramineous species of plants not supporting reproduction were *Amaranthus retroflexus* (pigweed), *Opuntia* sp. (prickly pear), *Cleome spinosa* cv. Pink Queen (cleome), *Chenopodium amaranticolor* (lambsquarters), *Beta vulgaris* cv. Early Wonder (beet), *Dianthus deltoides* (carnation), *Alyssum* sp. cv. Sweet (alyssum), *Dichondra repens* (dichondra), *Cucurbita maxima* cv. Early Prolific (squash), *Euphor-*

TABLE 1. Gramineous host plants of *Punctodera punctata*, means of three replicates.

Plant	Binomial	Reproduction*
Annual bluegrass	*Poa annua*	+++
Kentucky bluegrass cv. Merion	*Poa pratensis*	++
Kentucky bluegrass cv. Touchdown	*P. pratensis*	++
Perennial ryegrass cv. Citation	*Lolium perenne*	+
Perennial ryegrass cv. Diplomat	*L. perenne*	+
Spreading fescue	*Festuca rubra rubra*	+

* Reproduction key: +++ = 35 cysts or more; ++ = 25–34 cysts; + = 1–24 cysts.

bia sp. cv. Polychroma (spurge), *Trifolium repens* cv. Ladino (white clover), *Lespedeza striata* cv. Kobe (lespedeza), *Medicago sativa* cv. Saranac (alfalfa), *Pisum sativa* cv. Little Marvel (garden pea), *Glycine max* cv. Williams (soybean), *Humulus lupulus* (hops), *Rumex crispus* (sour dock), *Salix babylonica* (willow), *Lycopersicon esculentum* cv. Rutgers (tomato), *Solanum tuberosum* (potato), *Nicotiana tabacum* cv. NC-95 (tobacco), *Solanum melongena* cv. Black Beauty (eggplant), and *Daucus carota* (Queen Ann's lace). Contrary to our data, Baker (1) reported creeping bentgrass as a host. Wheat, barley, and oats were also shown to be hosts of *P. punctata* by others (5,10), suggesting possible difference among populations of the nematode or that the cultivars of these plants tested were resistant.

Life cycle: Punctodera punctata developed from second-stage juveniles to immature white females in 21 days (Table 2). Eggs were first detected at 40 days when cysts were light brown. After 40 days eggs, second-stage juveniles and males were detected.

TABLE 2. Development of *Punctodera punctata* on Merion Kentucky bluegrass in a greenhouse at 22–28 C.

Days	White females	Mature brown cysts	Males	Eggs (containing J2)
21	4	–	–	–
25	1	–	–	–
29	4	–	–	–
34	0	–	–	–
40	12	2	+	+
45	4	1	–	–
49	16	3	+	+
55	10	9	+	+
62	7	9	+	+
74	8	8	+	+
89	0	19	+	+
96	0	22	+	+
108	0	24	+	+

* Each tube inoculated 23 March 1983 with 250–500 eggs and juveniles. + = present; – = absent.

At no time were egg sacs or gelatinous matrices observed on mature cysts as reported by Thorne (10). Eggs dissected from newly matured cysts containing second-stage juveniles did not hatch and were apparently in diapause. In addition, cysts extracted from soil obtained from various greens and fairways after December contained eggs that hatched, but before this period, eggs from newly matured cysts would not hatch. Our observations concur with those of Horne (5) regarding egg hatching and suggest that a single generation occurs each year.

Nematode reproduction: Five white, swollen females were recovered after 75 days from the 50 Cone-Tainers containing *Poa pratensis* each inoculated with a single *P. punctata* juvenile. Microscopic examination of ruptured females, revealed no signs of embryogenesis or juvenile development. The failure to produce offspring from single juvenile inoculations and the abundance of males found in the field (48–60/150 cm^3 soil) April and May 1983 suggest that this nematode probably reproduces by amphimixis.

Pathogenicity: Inoculations with *P. punctata* on the most susceptible host in our tests, *Poa annua*, had no ($P = 0.05$) effect on plant top dry weight, root dry weight, or total dry weight despite reproduction by this nematode. Infection by moderate numbers of *P. punctata* appeared to cause little damage to *Poa annua*.

LITERATURE CITED

1. Baker, A. D. 1957. Notes on some nematodes in Canada, 1956. Canadian Insect Pest Review 34: 120–122.

2. Brzeski, M. W., C. W. Laughlin, J. M. Vargas, Jr., D. K. McCallu, and M. K. Hanna. 1971. Occur-

rence of cyst nematodes (*Heterodera* spp.) in Michigan. Plant Disease Reporter 55:399.

3. Chitwood, B. G. 1949. Cyst forming *Heterodera* encountered in soil sampling. Plant Disease Reporter 33:130–131.

4. Dunn, R. A. 1969. Extraction of cysts of *Heterodera* species from soil by centrifugation in high density solutions. Journal of Nematology 1:7 (Abstr.).

5. Horne, C. W. 1965. The taxonomic status, morphology and biology of a cyst nematode (Nematoda:Heteroderidae) found attacking *Poa annua* L. Ph.D. dissertation, Texas A&M University, College Station, Texas (Diss. Abstr. 65-12, 287).

6. Horne, C. W., and W. H. Thames, Jr. 1966. Notes on occurrence and distribution of *Heterodera punctata.* Plant Disease Reporter 50:869–871.

7. Radice, A. D., and P. M. Halisky. 1983. Nematodes parasitic on turfgrasses in New Jersey. New Jersey Academy Science 28:25 (Abstr.).

8. Radice, A. D., R. F. Myers, and P. M. Halisky. 1983. The grass cyst nematode in New Jersey. Journal of Nematology 15:4–8 (Abstr.).

9. Spears, J. F. 1956. Occurrence of the grass cyst nematode, *Heterodera punctata*, and *Heterodera cacti* group cysts in North Dakota and Minnesota. Plant Disease Reporter 40:583–584.

10. Thorne, G. 1928. *Heterodera punctata* n. sp. a nematode parasite on wheat roots from Saskatchewan. Scientific Agriculture 8:707–710.

11. Wheeler, W. H. 1949. Interceptions of the genus *Heterodera* in foreign soil. Plant Disease Reporter 33:446.