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Biology and Influence of *Pterotylenchus cecidogenus* on *Desmodium ovalifolium*

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Abstract: Pterotylenchus cecidogenus did not inhibit germination of *Desmodium ovalifolium* but did reduce survival of seedlings and root and stem growth. Stem gall formation was associated with nematode population densities which were positively correlated with plant age at time of inoculation. Nematode population densities increased 100 times in 52 days following inoculation.

Key words: stem gall nematode, population dynamics, host-parasite relationship.

Until December 1981 *Desmodium ovalifolium* Wall. (CIAT accession No. 350) appeared to be the most promising legume for use in tropical pastures in association with the aggressive stoloniferous *Brachiaria* spp. (1). However, galls caused by the nematode *Pterotylenchus cecidogenus* Siddiqi & Lenné (4) were detected on stems of *D. ovalifolium* at several locations in Colombia (3). This nematode also destroys cortical and vascular tissue in stems resulting in plant dieback and death. My objective was to study aspects of the host-parasite relationships of *P. cecidogenus* parasitizing *D. ovalifolium.*

Materials and Methods

Soil was collected from under native savanna at the Instituto Colombiano Agropecuario-Centro Internacional de Agricultura Tropical (ICA-CIAT) Research Station, Carimagua in the Llanos Orientales of Colombia. Untreated soil allowed adequate root nodulation of *D. ovalifolium* (CIAT accession No. 350), the plant used in all experiments. In the final four experiments, seedlings were germinated at 24 C in 9-cm petri dishes containing filter paper, 2 ml tap water, and about 50 seeds and watered as necessary for 1 week. Seedlings were planted approximately 2 cm deep in savanna soil in 20-cm-d plastic pots. Plant age was calculated from sowing time. Pots were arranged randomly, and treatments were replicated 10 times except the second experiment which had six replications. Nematodes for inoculum were extracted from galls using a mistifier (5). Following each test, where necessary, plants were blended in a Waring blender at high speed for 15 seconds and nematodes extracted in modified Baerman funnels.

Effect of P. cecidogenus on germination of seed of D. ovalifolium

1) Fifty uniform seeds of *D. ovalifolium* were placed on sterile filter paper in 9-cm-d petri dishes. Five replicate dishes arranged randomly were infested with 0, 100, or 500 motile *P. cecidogenus* juveniles and females in 2 ml water and stored in the dark at 24 C.

2) Fifty uniform seeds were sown uniformly over a 3-cm-d circle 5 mm deep in each of five 12-cm-d pots. Nine drops of aqueous 2.5% methyl cellulose containing 0, 100, 200, or 800 motile juveniles and females were placed in a 3 × 3 pattern to cover the seed area. Seeds were covered with beakers to prevent drying, and pots were placed in a greenhouse at 22–26 C.

Effect of P. cecidogenus on survival of seedling D. ovalifolium

Stems of 7-day-old plants were wounded at soil level with six strokes of a scalpel blade just deep enough to break the cuticle. Plants were inoculated at the stem base with 0, 25, 50, 75, 100, 200, 500, or 1,000 juveniles and females in 1 ml water. Beakers were placed over plants for 1 week following inoculation to reduce evaporation. Visible green leaves per plant were counted at 13, 22, 35, and 41 days after inoculation. After 57 days, a plant was considered alive if any green material was visible. The number of galled plants was recorded for each nematode treatment.

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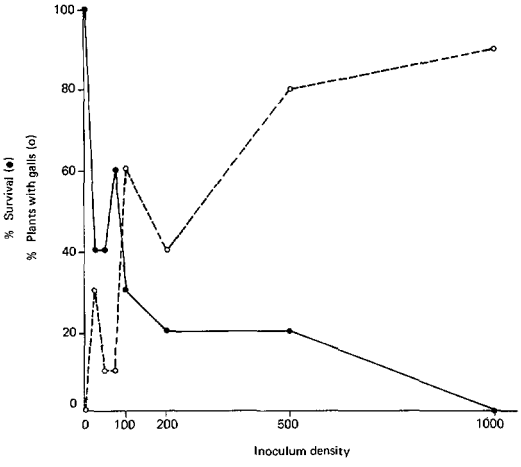

FIG. 1. Seedling survival (●) and number of galled plants (○) of *Desmodium ovalifolium* inoculated with different numbers of *Pterotylenchus cecidogenus*.

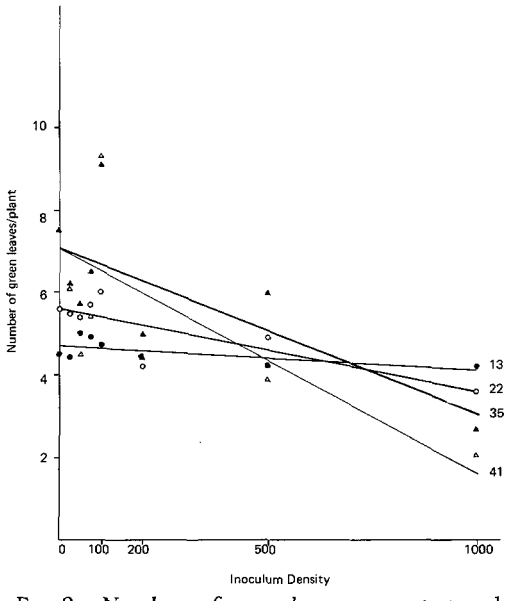

FIG. 2. Numbers of green leaves present at each of four sampling times on plants of *Desmodium ovalifolium* inoculated with different numbers of *Pterotylenchus cecidogenus*. Straight lines (with corresponding number of days after inoculation) are calculated regression lines: ● = 13, ○ = 22, ▲ = 35, △ = 41 days after inoculation.

Effect of P. cecidogenus on growth of D. ovalifolium

Two-week-old plants were inoculated, at the bases without wounding, with 0 or 70 motile juveniles and females in one drop of aqueous 2.5% methyl cellulose. After 40 and 80 days, 10 plants from each treatment were harvested and roots were washed free of soil. The following measurements were taken: stem height from soil to apex; root length, using a modified line intersect method (6); number of leaves; and shoot and root dry weights. The shoot : root ratio was also calculated.

Effect of plant age and inoculum density on nematode penetration and reproduction

Plants 0, 1, 2, 4, and 8 weeks old were inoculated, at the bases without wounding, with 75 motile juveniles and females in one drop of aqueous 2.5% methyl cellulose. After 7 and 45 days, 10 plants in each treatment were harvested and nematodes were extracted and counted to assess their penetration and reproduction capacities. If plants had developed galls, nematodes were extracted from the gall tissues separately.

Two-week-old plants were inoculated, without wounding, with 1, 2, 5, 10, 20, 50, 100, or 200 motile juveniles and females in one drop of aqueous 2.5% methyl cellulose. After 7 and 52 days, 10 plants in each treatment were harvested and nematodes extracted and counted to assess penetration and reproduction.

TABLE 1. Growth of *Desmodium ovalifolium* (means of 10 plants) measured 40 and 80 days after inoculation with 70 juveniles and females of *Pterotylenchus cecidogenus*.

Days after inoculation	Treatment†	Stem length (cm)	Root length (cm)	No. leaves	Root dry wt. (mg)	Shoot dry wt. (mg)	Shoot : root ratio
40	C	1.8	54.5	8	7	30	4.46
	I	1.3**	32.5*	5*	5	18**	3.20
80	C	3.1	84.6	18	98	59	0.64
	I‡	3.1	67.3	17	50	84	3.21

Difference due to inoculation significant, * $P < 0.05$ and ** $P < 0.01$, using Student *t*-test.
† C = noninoculated, I = inoculated with nematodes.
‡ Four plants died. Values are means of measurements on six live plants.

Results

All stages, except males, of *P. cecidogenus* were found in all stem galls examined.

Germination of *D. ovalifolium* seeds in pot and petri dish tests was not affected by *P. cecidogenus* at any inoculum density. However, inoculum density was negatively correlated ($r = -0.833$, $P < 0.01$) with seedling survival and positively correlated with gall frequency ($r = 0.694$, $P < 0.05$) (Fig. 1). Survival percentage and gall frequency were negatively correlated ($r = -0.833$, $P < 0.01$). Increasing inoculum density also suppressed progressively the number of green leaves remaining on plants after day 13 ($r = -0.66$, NS; $r = -0.83$, $P < 0.01$; $r = -0.74$, $P < 0.05$; $r = -0.69$, $P < 0.05$, respectively, at 13, 22, 35, and 41 days) (Fig. 2). Between days 35 and 41 the number of leaves on noninoculated plants did not change but inoculated plants continued to lose leaves. Other symptoms of *P. cecidogenus* infection included chlorosis and folding of leaves inwards along the midvein and downwards at the petiole.

Effect of P. cecidogenus on growth of D. ovalifolium

Stem and root growth were suppressed 40 days after inoculation with *P. cecidogenus* (Table 1). Root dry weight and shoot weight to root weight ratio, however, were not affected. Eighty days after inoculation, four of the inoculated plants had died. No differences in growth among the remaining live plants, infected or not with nematodes, were observed.

Effect of plant age and inoculum density on nematode penetration and reproduction

No correlation existed between plant age at time of inoculation and the numbers of nematodes inside plants at 7 days after inoculation. The numbers of nematodes per plant 7 days after inoculation at 0, 1, 2, 4, and 8 weeks of age were 1, 148, 66, 96, and 194, respectively. The numbers of nematodes per whole plant and nematodes per gall 45 days after inoculation were correlated with plant age at inoculation ($r = 0.89$, $P < 0.05$ and $r = 0.90$, $P < 0.05$, respectively) (Fig. 3).

The numbers of nematodes recovered per plant at 7 and 52 days after inoculation were correlated with initial inoculum density ($r = 0.96$, $P < 0.001$ and $r = 0.74$, $P < 0.05$, respectively) (Fig. 4). Slopes of lines relating the number of nematodes per plant to inoculum density show that the number of nematodes per plant 52 days after inoculation (slope = 2.36) was about 100 times that recorded at 7 days after inoculation (slope = 0.027).

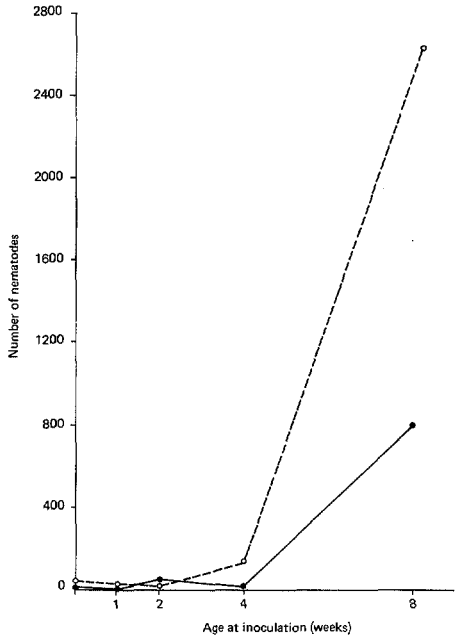

FIG. 3. Numbers of *Pterotylenchus cecidogenus* per whole plant (●) and per gall (○) 45 days after inoculation of *Desmodium ovalifolium* plants of different ages.

Discussion

Pterotylenchus cecidogenus did not affect germination of *D. ovalifolium* seed under

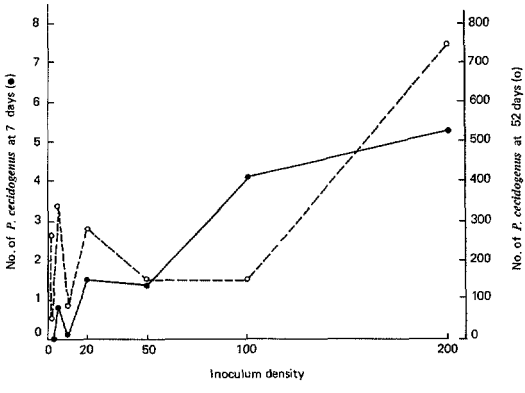

FIG. 4. Penetration and reproduction of *Pterotylenchus cecidogenus* on *Desmodium ovalifolium* following inoculation with different numbers of nematodes and measured 7 (●) and 52 (○) days after inoculation.

the conditions of my experiments. Inoculation of seedlings with nematodes caused galls, chlorosis, necrosis, leaf folding, and death. The negative correlation between survival and galling of plants following inoculation suggests that plant death was due to galling and subsequent tissue breakdown and loss of function of the vascular tissue.

Both shoot and root growth were suppressed by nematode infection 40 days after inoculation but not after 80 days. At 80 days, roots of noninoculated plants had filled the space available in the pots. Therefore, root growth and, possibly, shoot growth of these plants ceased some time before the second sampling.

Increases in the numbers of nematodes produced on plants of increasing age when inoculated with nematodes were related to increased numbers of penetration sites and amounts of plant tissue with increased plant size. Penetration by nematodes was not restricted to the early seedling stage. Penetration of 12-week-old plant material has been observed (unpubl.). Furthermore, artificial wounding was not essential for penetration by the nematode.

Few nematodes (less than 1% of the total number of nematodes) occurred in nongalled plants or in nongalled areas of plants with galls. To accelerate counting of nematode populations at least in this accession of *D. ovalifolium*, only galls need be examined.

The numbers of nematodes per plant at 7 and 52 days after inoculation at each inoculum density were described by linear regressions and their correlation coefficients were significant. Therefore, the increase in numbers of nematodes per plant at 52 days with increasing inoculum density was probably due more to increased penetration than to an effect on reproduction.

Pterotylenchus cecidogenus may be native to the Carimagua region of Colombia, as it has been found on the native *D. barbatum* in undisturbed savanna (unpubl.). Infestations in other regions may be native, but the nematode could have been introduced on seed from Carimagua.

The use of *D. ovalifolium* in pastures in the Llanos is threatened unless this nematode can be controlled. In one trial (J. M. Lenné, unpubl.), burning of pastures suppressed for 3 months the number of plants with galls. After 3 months, however, no significant differences in numbers of galled plants occurred between burned and unburned plots. Many seeds of *D. ovalifolium* were found 5 cm deep in the soil and seeds deeper than 2 cm are not affected by burning (2). The seed pod may be one source of nematode infestation (X. X. Aranda, unpubl.), so burning may reduce the nematode population. Surviving nematodes then reproduce on newly germinated plants until they reach the same population as in unburned areas. Chemical control on improved pastures in the Llanos of Colombia probably is not economically feasible, and short rotations are not possible for perennial pastures. Resistance or tolerance appears to be the most suitable form of control.

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