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Damage Potential and Reproduction of *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* on Alyceclover[1]

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Abstract: Alyceclover (*Alysicarpus* spp.) is an annual, high-quality leguminous forage, suitable for production under tropical and subtropical climates where the husbandry of conventional leguminous forages, *Trifolium* spp., is uneconomical. The damage potential and reproduction of *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* on alyceclover were studied under greenhouse conditions, using sand and sandy clay loam soil materials, respectively. Both nematode species reproduced on alyceclover, but only *B. longicaudatus* was pathogenic. Symptoms of *B. longicaudatus* damage were suppression of shoot yield, limited root system, stunting, incipient wilting, and occasional seedling mortality. In one experiment, the threshold-damage density was three nematodes/100 cm^3 sand, whereas in the other experiment it was zero nematodes.

Key words: *Alysicarpus* spp., damage threshold, lance nematode, leguminous forage, nematode, sting nematode, susceptible host, tolerant host.

Traditional perennial leguminous forages, *Trifolium* spp., are not adapted to the high temperatures of subtropical and tropical regions of the world (2), whereas alyceclover (*Alysicarpus* spp.), a leguminous forage with few reported economic pests, is adapted to subtropical and tropical conditions, including much of the southeastern United States (2). Breeding and selection for forage quality and early maturity at the University of Florida, Gainesville, culminated with the 1985 release of an early-maturing *A. vaginalis* (L.) DC genotype FL-5 (3). However, this cultivar is susceptible to damage by the root-knot nematodes, particularly *Meloidogyne incognita* (Kofoid & White) Chitwood, which is predominant in much of the southeastern United States (3). Screening for resistance to *Meloidogyne* spp. using genotypes with established forage quality showed that a late-maturing *A. ovalifolius* (Schumach) J. Leon genotype FL-3 possessed some resistance to *M. incognita,* resulting in its 1989 release as a line resistant to root-knot nematodes (1).

During seed increase of alyceclovers FL-1, FL-2, and FL-3, it was observed that plant growth of all three genotypes was unthrifty in certain portions of a field (11). Subsequent surveys showed that population densities of the sting nematode, *Belonolaimus longicaudatus* Rau, and the lance nematode, *Hoplolaimus galeatus* (Cobb) Filipjev & Schuurmans Stekhoven, were high in portions of the field with sand and sandy clay loam soils, respectively (11). Inverse relationships between *B. longicaudatus* population densities and alyceclover yield over two growing seasons were consistent with pathogenicity, whereas *H. galeatus* population densities were directly related to yield (11). However, the effects of sand content on both nematode levels and crop yield did not allow a definitive conclusion that either nematode adversely affected alyceclover yield (11). The two parasites are widely distributed in parts of the southeastern United States (10,14). *Belonolaimus longicaudatus* is a serious pathogen to certain fruit and aesthetic trees, vegetables, and field crops in this region (14). The damage potential of either nematode to alyceclover has not been studied. Because alyceclover has the potential of being a summer forage in the southeastern United States, this study was initiated to test the damage potential of *B. longicaudatus* and *H. galeatus* to *M. incognita*-resistant cultivar FL-3 in the greenhouse.

Materials and Methods

Seeds of FL-3 were planted in polystyrene trays on steamed fine sand (91%

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sand, 3% silt, and 6% clay; 1.2% OM, pH 6.0) collected from alyceclover field plots with high population levels of *B. longicaudatus.* Five seedlings at the four-leaf stage were transplanted into 15-cm-d clay pots, each containing 550 cm^3 of this soil. Seedlings were selected for uniformity and thinned to two plants/pot 1 week after transplanting.

The *B. longicaudatus* population for inoculum was mass-reared on bermudagrass, *Cynodon dactylon* (L.) Pers., in the greenhouse. Nematodes for inoculum were separated from soil a day after thinning the test plants by the Baermann method (16) and grouped in a geometric series (0, 4, 16, 64, and 256 specimens/vial) in 10 ml water. To insure that both nematode sexes were present in the lower initial population densities (Pi) 4 and 16, 2 and 4 males, respectively, were included; the balance were female. The control plants were inoculated with nematode inoculum filtrate (25-μm-pore sieve) to establish consistency in case any microorganisms were associated with this pathogen.

Seedlings were inoculated by pouring nematodes in the 10 ml of water into a 5-cm-deep hole between the two seedlings. Each infestation level was replicated five times, and pots were arranged in a completely randomized design on the greenhouse bench. Greenhouse temperatures averaged 31 C (28–35 C) maximum and 26 C (24–27 C) minimum. Seedlings were irrigated daily with 100 ml of tap water and fertilized weekly with 100 ml of liquid fertilizer, prepared by dissolving 4 g of 0:10:20 ($N:P_2O_5:K_2O$) in 1 liter water.

Six weeks after inoculation, shoots were excised at the soil surface, weighed, and measured for length. Pot contents were spread in a large plastic container, roots were collected and rinsed in water, and excess water was removed by pressing the roots between paper towels prior to weighing. Severed withered rootlets found in the soil were not weighed. The nematodes were extracted from a representative subsample of 100 cm^3 soil by sieving and centrifugation (7), and counts were reported as specimens/550 cm^3 sand. The experiment was repeated once.

The *H. galeatus* and *B. longicaudatus* experiments were conducted concurrently in the same greenhouse. Seeds of FL-3 were planted in polystyrene trays on steamed sandy clay loam (74% sand, 22% clay, and 4% silt; 1.2% OM, pH 6.0) collected from alyceclover field plots with high population levels of *H. galeatus.* The nematodes for inoculum were collected from the same plots and separated from soil using the Baermann tray method (16). Other materials, methods, and experimental conditions were as described for *B. longicaudatus.* The experiment also was repeated once.

The damage potential of each nematode to FL-3 was tested by linear regression (9) of yield variables on the transformed nematode Pi values, $\log_{10}(Pi + 1.0)$, whereas host status to either nematode was tested by regressing $\log_{10}$ (Pf + 1.0) on $\log_{10}$ (Pi + 1.0). Shoot yield and untransformed Pi of *B. longicaudatus* were fitted to Seinhorst's (17) equation:

$$Y = m + (1 - m)z^{(P-T)} \text{ for } P > T,$$

where Y = relative yield, the yield at a given Pi level divided by the yield in the absence of nematodes; m = minimum yield, the average plant growth at very high nematode population densities; z = the slope-determining variable, the proportion of plant undamaged in the presence of parasitism by a single nematode; P = nematode population density per unit of soil or root; and T = the tolerance limit, the nematode population density below which yield suppression is unmeasurable. Estimates for m, T, and z were derived from the least sums of squared residuals using a computer algorithm (6).

Results and Discussion

Fresh shoot or root weights and plant heights were negatively correlated with $\log_{10}$ (Pi + 1.0) of *B. longicaudatus* levels (Table 1). The strongest correlations occurred between nematode Pi and shoot weights, followed by those for plant heights and root weights.

TABLE 1. Linear regression models relating fresh shoot and root weights (g) and shoot heights (cm) of alyceclover FL-3 to $\log_{10}$ (Pi + 1.0) of *Belonolaimus longicaudatus* on sand.

Experiment 1		Experiment 2	
Model	r	Model	r
Shoot = 1.129 − 0.417x	−0.89**	Shoot = 17.081 − 5.459x	−0.85**
Root = 0.847 − 0.320x	−0.88**	Root = 1.142 − 0.389x	−0.56*
Height = 10.620 − 2.942x	−0.81**	Height = 20.043 − 5.735x	−0.71**

Data are means of five replicates.
** Significant at $P \leq 0.05$, *$P \leq 0.10$.

Belonolaimus longicaudatus was damaging to alyceclover FL-3 under the conditions of these studies. The Seinhorst model (17) suggested the T values (tolerance limit) of zero and three *B. longicaudatus* nematodes/100 cm^3 sand for the two experiments, with minimum yields (m) of 0.5 and 0.6 (Fig. 1A). Similar low T values (1–2 *B. longicaudatus* nematodes/100 cm^3 soil) were reported for peanut, cotton, corn, and soybean (16). These low T values reflect the assertion that this nematode is an aggressive plant pathogen (14). The pathogenic symptoms on FL-3 included suppression of yield and root systems, stunting, occasional seedling mortality, and incipient wilting.

Incipient wilting, which preceded seedling mortality, was common at Pi = 256 nematodes. Because some seedlings could not recover from the wilting stress over a period of time, the duration of the studies was limited to 8 weeks after planting, which is half the growing time for FL-3 under field conditions. Soils infested with this pathogen contained numerous severed withered rootlets. Because numbers of similar rootlets were negligible in non-infested soils, this observation may confirm Christie's (4) finding that this parasite girdles the root tips during feeding, which eventually break off at maturation. The latter may partly explain the restricted root system, which in turn clarifies the stunted growth, suppressed shoot yield, and (or) incipient wilting observed in these studies.

Hoplolaimus galeatus did not damage alyceclover under the conditions of these studies (data not shown). Measurable yield suppression due to *H. galeatus* feeding was not detected on cotton (8), corn (13), and some vegetables (12,15), although the plants supported high population levels of this parasite.

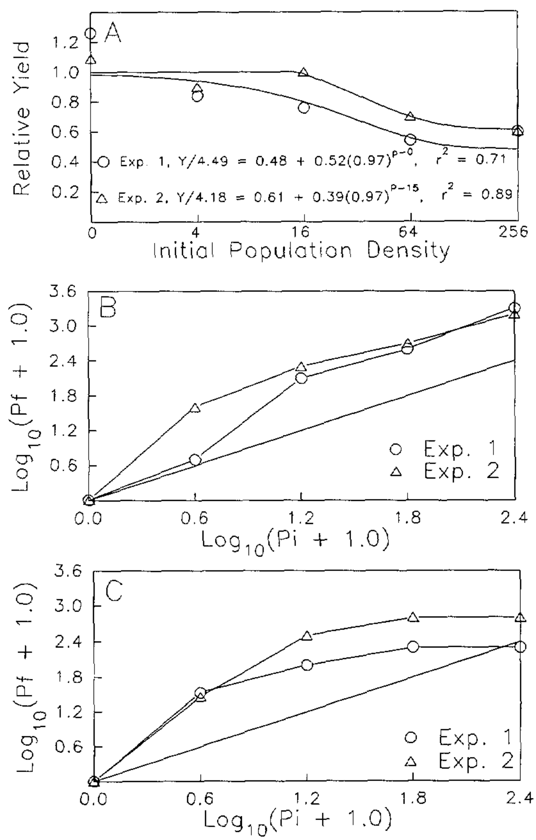

FIG. 1. Relationships between initial population densities (nematodes/550 cm^3 sand) and final densities or yield on alyceclover FL-3 in two greenhouse experiments. A) Initial density of *Belonolaimus longicaudatus* and relative yield (fresh shoot weight) of alyceclover. B) Initial and final densities of *B. longicaudatus*. C) Initial and final densities of *Hoplolaimus galeatus*.

At Pi > 0, the Pf for *B. longicaudatus* (Fig. 1B) and *H. galeatus* (Fig. 1C) were above the maintenance line, Pf = Pi (19), except for *H. galeatus* at Pi = 256 nematodes in Experiment 1. The final population density curves, $\log_{10}$ (Pf + 1.0), versus the initial population density curves, $\log_{10}$ (Pi + 1.0), for *B. longicaudatus* remained roughly parallel to the maintenance line as Pi increased within the inoculum range, duration, and conditions of these studies. In *H. galeatus,* the two curves increased to a "ceiling level" (18,20), and, in Experiment 1, eventually transected the maintenance line at the equilibrium point, "E" (18,20). For both nematode species, the Pf versus Pi curves for experiments 1 and 2 have more or less equal slopes, which represent the maximum reproduction rate, "a" (20).

In *B. longicaudatus,* "E" may be very high on alyceclover, because it was not attained under the conditions of our studies. Because the higher Pi levels of *B. longicaudatus* resulted in seedling mortality before "E" was reached, this situation may suggest an unadaptability in the host–parasite interaction of alyceclover and *B. longicaudatus.*

The apparently high "E" and "a" values for *B. longicaudatus* and *H. galeatus* on FL-3 suggest that alyceclover is a good host to both nematode species. However, because alyceclover did not incur damage while sustaining population densities of *H. galeatus,* it may be designated a tolerant host (5). Conversely, alyceclover is a susceptible host to *B. longicaudatus* because population densities were sustained at the expense of yield loss (5).

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