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Density-Dependent Parasitism of the Soil-Borne Nematode *Criconemella xenoplax* by the Nematophagous Fungus *Hirsutella rhossiliensis*

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Abstract. Spatial sampling was used to investigate temporal density-dependent parasitism of the plant-parasitic nematode *Criconemella xenoplax* by *Hirsutella rhossiliensis* in three peach orchards on eight sample dates. The patches of soil in which the nematode and fungus interacted were assumed to possess similar density-dependent dynamics and to be small, independent, and asynchronous. Furthermore, sampling of separate patches was assumed to provide similar information with respect to density dependence as would temporal (repeated) sampling of the same patch. Percent parasitism was dependent on the number of *C. xenoplax*/100 cm^3 soil ($P = 0.0001$). The slope was unaffected by orchard or date but ranged from 0.0001 to 0.0043 depending on distance from the irrigation furrow. The relative shallowness of the slope and the large variation in percent parasitism not explained by nematode density suggest that *H. rhossiliensis* is a weak regulator of *C. xenoplax* population density.

Introduction

The probability of a host being attacked by a parasite is often thought to be dependent on host density [1, 3]. When hosts are rare, encounters between hosts and parasites are unlikely, and the parasite has little effect on host population density. When hosts are abundant, parasite reproduction or aggregation results in temporal or spatial increases in density. As parasite density increases, encounters become frequent, and the parasite can limit host population growth. Density-dependent suppression of hosts by parasites is defined as regulation [4]. Determination of the nature of regulation increases understanding of the parasite's potential to suppress the host population and provides information on the stability of host and parasite numbers [1–4].

Regulation of soil-borne nematodes by fungal and bacterial parasites is poorly understood. Linford et al. [15] implied that nematode-trapping fungi regulated the plant-parasitic nematode, *Meloidogyne* sp., but parasitism was not quantified. Subsequently, Cooke [7] showed that parasitism of nematodes by nematode-trapping fungi was unrelated to host numbers. Perry [16] included regulation by obligate fungal parasites in a model describing the population dynamics of the plant-parasitic nematode *Heterodera avenae*. Density-dependent para-

sitism was suggested in the interaction of the nematode *Meloidogyne* sp. and the bacterial parasite *Pasteuria penetrans* in sugarcane fields [18] and in the interaction of the nematode *Criconemella xenoplax* and unidentified fungi in vineyards [5]. Gray [9] described strong regulation of bacterial-feeding nematodes by fungal parasites, but the system involved activated sludge and not soil. The liquid nature of the system permitted sampling through time of an apparently uniformly distributed, well-defined population.

The soil-borne nematode *Criconemella xenoplax* Raski (Luc and Raski) is a serious pest of peach trees and other *Prunus* spp. All stages other than the egg are vermiform and motile in the soil (movement probably limited to less than 5 mm/day) and feed only on host roots. Generations overlap, and the age structure is stable throughout the year in California peach orchards (H. Ferris, unpublished data). The life cycle requires about 30 days at 20°C [19]. One hundred *C. xenoplax*/100 cm^3 soil is considered the "economic injury level"; if populations are above this level, pesticide treatment is recommended.

The fungus *Hirsutella rhossiliensis* Minter and Brady parasitizes and is frequently associated with *C. xenoplax* [10]. All vermiform stages of the nematode are susceptible to the fungus. *H. rhossiliensis* produces nonmotile spores that adhere to and initiate infection of passing nematodes. At 20–25°C, the fungus kills the nematode within 72 hours and sporulates from the cadaver shortly thereafter [11]. Parasitized nematodes disappear from soil in about 15 days, but the rate of degradation varies with soil temperature and nematode life stage [13]. The relative density of *H. rhossiliensis* spores is highly correlated with the number of *H. rhossiliensis*-parasitized *C. xenoplax* in peach orchard soils (T. M. McInnis and B. A. Jaffee, unpublished data). The fungus parasitizes certain species of nematodes other than *C. xenoplax* but has no saprophytic activity in the presence of other soil organisms [12].

Our unpublished observations suggest that the level of parasitism of *C. xenoplax* by *H. rhossiliensis* depends on nematode population density. Because the presence or absence of density-dependent parasitism could affect the utility of this fungus as a biological control agent [2], we would like to determine if and how parasitism is affected by host nematode density.

The most direct way to detect and characterize temporal density-dependent parasitism within a population is to quantify parasitism and host density through time. Because of extremely limited mobility of soil nematodes and fungal parasites, the volume of soil (patch) occupied by interacting nematodes and fungi is probably limited. We assume that these patches are approximately 700 cm^3 (the volume collected by our sampling tool). Repeated sampling of these small patches is difficult because soil sampling is destructive and a significant portion of the patch and population is removed or at least disturbed with each sample.

In this study, we used spatial sampling to make inferences on temporal density-dependent parasitism of *C. xenoplax* by *H. rhossiliensis*. We assumed that (1) a peach orchard contained many similar but independent and asynchronous populations of *C. xenoplax*, (2) these populations occurred in patches of 700 cm^3 of soil, and (3) samples from separate populations collected at one time in the same area provided similar data as would samples from one population collected through time.

Materials and Methods

Selection of Orchards and Trees

Orchards G, C, and M (located in Merced County, CA and containing 8- to 12-year-old cling peach trees on "Nemaguard" rootstock) were selected because *C. xenoplax* and *H. rhossiliensis* were present [13]. Fifteen healthy trees in a limited area (about 270 m^2) in each orchard were selected. The growers disked the orchards to control weeds and irrigated along furrows formed on two sides of each tree about 80 cm from the trunk. Soil characteristics for these orchards have been described [13].

Collection of Soil Samples

A sample was collected from one of eight locations around each of the 15 trees in each orchard at 6-week intervals for 1 year (giving eight sampling dates). The locations were 60–70 cm from the trunk and at 45° radial intervals. Locations equidistant between the furrows (at 0 or 180°) were designated "position 1," those adjacent to the furrows (90 or 270°) were designated "position 3," and those intermediate (45, 135, 225, or 315°) were designated "position 2." Locations sampled on a particular day were randomly selected, but each location for a particular tree was sampled only once throughout the eight sample dates, and the same location was not sampled more than twice per date per orchard. The same combination of trees (1–15) and locations was used for all three orchards on each date.

Samples were collected with a 5 cm-diameter soil auger. Each sample consisted of approximately 670 cm^3 of soil taken from a depth of 33–66 cm (the top 33 cm were discarded). This depth was selected because it is less subject to mechanical mixing and to fluctuations in temperature and moisture than are surface layers. On each date, all 45 samples were collected within 4 hours, stored in polyethylene bags in ice chests, returned to the laboratory, and a 500 cm^3 subsample was removed from each sample and stored overnight at 10°C. In April and June 1987, the root material from each subsample was collected on a sieve (420 μm) during elutriation (separation based on density and size), dried, and weighed.

Extraction of Nematodes from Soil

One day after collection, nematodes were extracted from soil by elutriation [6] and centrifugation [14]. The elutriator was adjusted to collect 20% of the subsample; thus, the extract contained nematodes from 100 cm^3 soil. Extracts were placed in 30 ml vials and stored overnight at 10°C. Extraction efficiencies were measured (0.44, 0.47, 0.51, and 0.69 for juvenile stages J2, J3 and J4 and for adults, respectively), and nematode counts were adjusted accordingly.

Determination of Percent Parasitism of C. xenoplax *by* H. rhossiliensis

Eighteen hours after extraction, aliquots of each extract were spread onto streptomycin-amended water agar plates, as described elsewhere [13]. Briefly, the extract in each vial was reduced to 10 ml, treated with NaOCl, rinsed, adjusted to 10 ml, and a 333 μl aliquot was spread onto each of three Petri plates. After incubation at 22 ± 2°C for 4.5–5.5 days, the plates were examined with a dissecting microscope at 20–60×. The number of *C. xenoplax* (J2, J3, J4, and adults) parasitized or not parasitized by *H. rhossiliensis* was determined. Also counted were instances when *H. rhossiliensis* grew from other nematodes, mites, or from unidentified debris.

Table 1. Variability in the percentage of *Criconemella xenoplax* parasitized by *Hirsutella rhossiliensis* as influenced by *C. xenoplax* density (Cx), orchard, position, and date

Source	Sum of squares	df	*P*
Cx	6,131	1	0.0001
Orchard	8,241	2	0.0001
Position	96	2	0.7756
Date	621	7	0.8577
Cx * orchard	75	2	0.8199
Cx * position	2,512	2	0.0015
Cx * date	1,532	7	0.3286

Statistical analysis

The relationship between percent parasitism and nematode density was examined with analysis of covariance [17]. The dependent variable was the percentage of *C. xenoplax* parasitized by *H. rhossiliensis*, and the principal independent variable was *C. xenoplax*/100 cm³ soil. Orchard, date, position, and interactions between nematode density and position, orchard, and date were independent covariables. The significance of the independent variables was determined using a "Type III" analysis. In two of 360 samples, *C. xenoplax* was not present; these observations were excluded from the analysis. Slopes of the relationship between percent parasitism and nematode density were determined and compared by the "estimate" and "contrast" options of the General Linear Model.

Least-square means for nematode density, parasitism, and root density (by orchard and position) were compared by the "pdiff" option. This method is not conservative and has error properties similar to LSD [17].

Results

The density of *C. xenoplax* was a highly significant factor in the Type III analysis (Table 1). The model R^2 was 0.45. A model based on *C. xenoplax* density alone would explain 16% of the total sum of squares. The main effect of orchard and the interaction of nematode density * position were also significant. The other main effects (date and position) and interactions (density * orchard, density * date) in the model were not significant.

The significant interaction between density and position indicated that the regression slope of parasitism on density differed among positions. Nonsignificant interactions were removed from the model statement before the estimate and contrast options were executed. The slopes for position 1 (furthest from the furrow), 2 (intermediate), and 3 (closest to the furrow) were 0.0043, 0.0027, and 0.0001, respectively (Fig. 1). Slopes in position 1 and 2 were greater than the slope in position 3 ($P < 0.02$) but did not differ significantly from each other ($P = 0.08$). Intercepts ranged from 1 to 29% depending on the orchard and date. The estimated intercept (averaged across orchards and dates) was 13.7%.

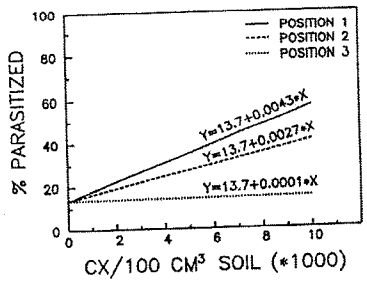

Fig. 1. Regressions of percentage of *Hirsutella rhossiliensis*-parasitized nematodes on *Criconemella xenoplax* (Cx) density as affected by distance from the irrigation furrow. Position 3 was closest to the furrow, position 1 was farthest away, and position 2 was intermediate. Regressions were averaged across orchards and dates.

Table 2. *Criconemella xenoplax*/100 cm³ soil, % *Hirsutella rhossiliensis*-parasitized *C. xenoplax*, and root density as influenced by orchard

Orchard	Nematode density	% Parasitism	Root density
G	1,760 a	15 b	1.1 a
C	1,660 a	11 a	1.5 a
M	2,550 b	30 c	2.7 b

Values for nematode density and parasitism are the means of 120 observations. Values for root density (g dry roots/500 cm³ soil) are the means of 30 observations. Means in a column followed by the same letter are not significantly different

Root density was greater ($P < 0.02$) in position 1 (2.3 g) than in positions 2 (1.6 g) or 3 (1.4 g/500 cm³ soil). Root density was greater in orchard M than in orchards G and C (Table 2).

Mean nematode density and parasitism were greater in orchard M than in orchards G or C (Table 2). These variables were relatively constant through time in all orchards but appeared to oscillate in orchard M (Fig. 2).

In orchard C (but not G or M), trees tended to have constant nematode densities and parasitism over all dates (data not shown). Mean percent parasitism in orchard C was related to mean nematode density ($r = 0.63$, $P = 0.01$) and root density ($r = 0.43$, $P = 0.10$) by tree.

Other fungal parasites of *C. xenoplax* were not observed, but *H. rhossiliensis* sporulated from other nematodes (in 26% of the samples), soil mites (in 1% of the samples), or from unidentified debris (in 2.5% of the samples). Sporulation from nematodes other than *C. xenoplax* involved fewer than 50 nematodes per 100 cm³ soil in 90% of the cases. Sporulation from mites or debris involved only one or two instances in any sample. When debris supporting sporulation of *H. rhossiliensis* was examined carefully at higher magnification, a parasitized *C. xenoplax* was observed in about 95% of the instances.

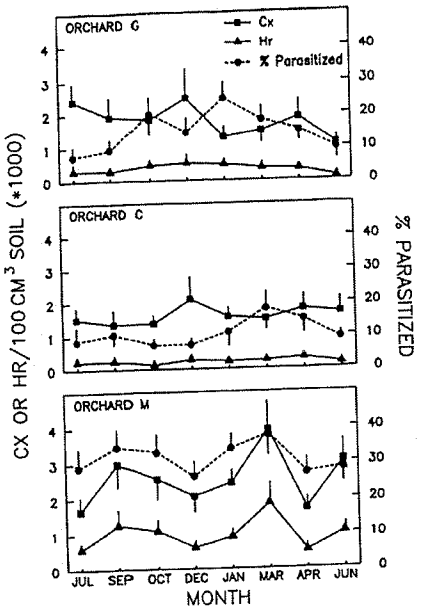

Fig. 2. Densities of *Criconemella xenoplax* (Cx) and *Hirsutella rhossiliensis*-parasitized *C. xenoplax* (Hr) and percent parasitized nematodes in three orchards on eight dates, July 1986 to June 1987. Data points are the means of 15 samples. Vertical bars = 1 SEM.

Discussion

Temporal density-dependent parasitism of soil-borne nematodes by obligate fungal parasites is not an unexpected phenomenon. The rate of transmission of inocula among hosts is likely to increase with increasing host density [3], and an increased rate of transmission should contribute to an increased proportion of parasitized hosts. In the present study, spatial sampling indicated that parasitism of *C. xenoplax* by *H. rhossiliensis* was density dependent. However, the regression slope of parasitism on density was relatively shallow. Furthermore, nematode density explained only a small portion of variation in percent parasitism. Apparently, the fungus does not strongly regulate nematode population density.

This study has a number of limitations. First, our assumption that the different patches within an orchard are similar but asynchronous may be incorrect; the density-dependent relationship may differ among patches, as is the case with patches in different positions with respect to the irrigation furrow (Fig. 1). Second, the estimation of "percent parasitism" can be misleading if host generations overlap and birth and death rates of host and parasite are dynamic

and unmeasured [8]. Third, the host range of *H. rhossiliensis* suggests that our analysis should have been based on all susceptible nematodes, not just *C. xenoplax*. However, few nematodes other than *C. xenoplax* occurred and these were infrequently parasitized. Fourth, our estimation of patch size was based on the size of our sampling tool; more information on patch size is needed. Finally, temporal sampling of the same patch is needed to confirm our inferences. Such sampling will require better definition of the patch and less disruptive sampling techniques.

The slope of the regression of percent parasitism on nematode density was affected by position of the patch relative to the irrigation furrow. This result was unexpected. Soil near the irrigation furrow might differ from soil away from the furrow in several respects, the most obvious being water potential. *H. rhossiliensis* does not sporulate when submerged [11]. The position near the furrow might remain saturated longer than the position away from the furrow, but the orchards in question are very sandy and well drained, and it is unlikely that the soil would remain saturated long enough to inhibit sporulation of the fungus. In addition to water, the furrow sides of the tree also receive more cultivation and heavy equipment traffic. Mechanical disturbance is detrimental to the fungus. Disturbance removes spores from the phialides and such spores are not infective (T. M. McInnis and B. A. Jaffee, unpublished data). However, mechanical disturbance should be minimal at the sampling depth of 33–66 cm. Compaction of the soil from farm equipment might change a number of parameters that affect transmission of fungal spores, including rate of nematode movement. The fungus depends on the nematode for spread through the soil, and increased compaction would decrease nematode motility.

Mean root density was positively correlated with mean nematode density and mean level of parasitism in the comparison of orchards (orchard M had more roots, more nematodes, and higher parasitism) and also in the comparison of trees in orchard C. Thus, food supply may have a greater effect than fungal parasitism on nematode density. The condition of high host numbers and high percent infection in orchard M is consistent with weak regulation by the parasite and a high intrinsic rate of increase by the host [3]. Low pathogenicity is another possible explanation but is not supported by laboratory data.

High root density could also increase numbers of bacterial-feeding nematodes because of increased root exudation. If the bacterial-feeding nematodes were susceptible to *H. rhossiliensis*, the total density of susceptible nematodes would be relatively high. Density-dependent parasitism would then result in a higher percentage of parasitized *C. xenoplax* than if the additional suscepts were absent. This scenario was first proposed by Linford et al. [15] to explain the suppression of nematode pests in pineapple soils amended with organic matter. However, B. A. Jaffee (unpublished data) recently found that fewer than 5% of the nematodes other than *C. xenoplax* in orchard M were parasitized by *H. rhossiliensis*.

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