Sampling strategies for detection of density-dependent parasitism of soil-borne nematodes by nematophagous fungi

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SUMMARY

Spatial and temporal relationships between numbers of the plant-parasitic nematode *Criconemella xenoplax* and the extent of parasitism by the nematophagous fungus *Hirsutella rhossiliensis* were measured in 20-cm-diameter plots in a peach orchard. The percentage of *C. xenoplax* parasitized was positively correlated with *C. xenoplax* density when data from all plots were included in the analysis. Parasitism was not correlated with density when data from within plots, sampled five times over 6 months, were analyzed. Thus, spatial but not temporal sampling indicated a relationship between numbers of hosts and parasitism. Numbers of nematodes and the extent of fungal parasitism were relatively constant within each plot. A proportion of all vermiform stages of *C. xenoplax* was parasitized by *H. rhossiliensis.*

RÉSUMÉ

Stratégie d'échantillonnage pour la détection du parasitisme par les champignons en relation avec la densité des nématodes vivant dans le sol

Les rapports spatiaux et temporels entre la valeur des populations du nématode phytoparasite *Criconemella xenoplax* et celle du parasitisme par le champignon nématophage *Hirsutella rhossiliensis* ont été évalués sur des parcelles de 20 cm de diamètre dans un verger de pêchers. Le pourcentage de *C. xenoplax* parasités est corrélé positivement à la densité du nématode si les chiffres relatifs à l'ensemble des parcelles sont pris en compte. Cette corrélation n'apparaît pas dans les chiffres correspondant aux cinq prélèvement effectués, en six mois, sur la même parcelle. Ainsi, un échantillonnage réparti dans l'espace démontre la relation entre nombre d'hôtes et taux de parasitisme, ce qui n'est pas le cas d'un échantillonnage réparti dans le temps. Le nombre des nématodes et le taux du parasitisme par le champignon sont relativement constants dans chaque parcelle. Une certaine proportion de chacun des stades de *C. xenoplax* est parasitée par *H. rhossiliensis.*

Researchers generally have considered density dependence as a desirable characteristic in the interaction of parasitic biological control agents and their hosts; host-specific parasites are thought to have little effect on host populations at low host densities and large effect at high host densities, and thus provide stable and long-term regulation of the host population (Stirling, 1988).

Density-dependent parasitism is used in two different contexts. The term spatial density-dependent parasitism is used when highly motile parasites, such as parasitoid wasps, aggregate in areas of high host density, resulting in a greater *per capita* probability of parasitism in these areas than in areas of low host density. Spatial density-dependent parasitism involves the rapid redistribution of parasites in space (Hassell, 1966; Brown, 1989). The term temporal density-dependent parasitism is used when changes in host density and parasitism occur over time. The parasite could be motile and exhibit spatial density dependence or the parasite could be relatively immotile. In the latter case, increases in parasitism in response to host density reflect increased reproduction of the parasite rather than aggregation. In temporal density-dependent parasitism, change in parasite density occurs over generations of the parasite (Hassell, 1966; Anderson & May, 1981; Brown, 1989).

The probability of a soil-borne nematode host being parasitized is likely to be temporally dependent on nematode density when the parasite requires the nematode as a substrate for production of spores or other infective propagules (Perry, 1978). An increase in numbers of nematodes should result in an increase in numbers of spores which in turn should increase the probability that a nematode will contact a spore. Although the spatial distribution of spores and nematodes is important, temporal rather than spatial density-depependent parasitism is the appropriate context for studying this interaction.

Temporal density dependence is studied by period-

ically sampling the same populations of interacting parasites and hosts. Two factors make investigation of temporal density-dependent parasitism of soil-borne nematodes difficult. First, the appropriate universe or patch size for studying the dynamics is small because the hosts and parasites are microscopic and move slowly; the patch size should reflect the range of the interacting organisms (Eastburn & Butler, 1988; Wiens, 1989). Second, soil sampling is destructive. Thus, the patch is removed or at least greatly disturbed with each sampling (assuming that the patches are small).

Jaffee, Gaspard and Ferris (1989) studied parasitism of the plant-parasitic nematode *Criconemella xenoplax* (Raski) Luc & Raski by *Hirsutella rhossiliensis* Minter & Brady (a fungus that obligately parasitizes nematodes) in three mature peach orchards in California. The authors recognized the problem of repeated sampling of small patches and attempted to make inferences on temporal density dependence based on spatial sampling. They assumed that samples collected at one time from separate patches in the same region of an apparently uniform orchard provided similar data with respect to density dependence as would samples from one patch collected through time. The purpose of the present study was to test this assumption.

Materials and methods

Fifteen trees were selected in one area (about 0.07 hectares) of a 10-year-old peach orchard (78 % sand, 13 % silt, 9 % clay, < 1.0 % organic matter, and pH = 4.4) in Livingston, CA. This was the same area and orchard designated " orchard M " in the previous study (Jaffee, Gaspard & Ferris, 1989). The soil contained large numbers of *C. xenoplax*, many parasitized by *H. rhossiliensis*. A 20-cm-diameter plot was established at each tree by inserting a stake into the soil 80 cm from the trunk and equidistant between the irrigation furrows which had been formed on two sides of the tree. Spatial sampling previously indicated a positive relationship between parasitism and host density at this position (Jaffee, Gaspard & Ferris, 1989).

The plots were sampled on 13 October 1987 (month 0) and again 1, 2, 4, and 6 months later. Samples were collected with a Veihmeyer tube (2 cm diameter, 10 to 55 cm depth, one core/plot) about 5 cm from the stake and from a specified location within each plot on each sample date to prevent resampling of the same location. Soil temperature at 30-40 cm was 19.0, 14.5, 10.5, 11.0, and 17.0 °C at 0, 1, 2, 4 and 6 months, respectively. Soil moisture was not measured, but samples were always moist because of fall and spring irrigation and winter rain.

Soil samples were stored overnight at 10 °C, and nematodes were extracted by elutriation and centrifugation (Jenkins, 1964; Byrd *et al.*, 1976). Each nematode suspension (10 ml) was treated with 0.5 % NaOCl for 30 sec and rinsed, and aliquots were spread on water agar containing 200 mg streptomycin sulfate/l (Jaffee *et al.*, 1988). After 5 days at 22 ± 2 °C, non-parasitized and parasitized *C. xenoplax* (as evidenced by sporulation of *H. rhossiliensis*) were counted by stage of nematode development. Other nematodes were counted as non-parasitized or parasitized but were not identified to genus or stage. Other parasites of *C. xenoplax* were not observed. Counts were corrected for extraction efficiency of *C. xenoplax* (0.44, 0.47, 0.51, and 0.69 for juvenile stages J2, J3, J4, and adults, respectively).

SAS GLM procedures (Anon., 1985) were used for all statistical analyses. The relationship between the percentage of *C. xenoplax* parasitized and population density of *C. xenoplax* was examined in four analyses. In analysis 1, parasitism was regressed on density for samples collected on month 0. In analysis 2, parasitism was regressed on density for samples from all sample times but time was removed as a source of variance; the independent variables were density, time, and density × time. These two analyses measured the spatial (among plot) relationship between parasitism and density. In analysis 3, parasitism was regressed on density for samples from all sample times but plot was removed as a source of variance; the independent variables were density, plot, and density × plot. Analysis 3 was a direct measure of temporal density-dependent parasitism because any measured variation in parasitism and density occurred over time (within plots) rather than in space. Analysis 4 was similar to analysis 3, but parasitism was regressed on the host density from the previous sample period, thus permitting detection of delayed density dependence. In analyses 1-3, data from samples containing no *C. xenoplax* were excluded. Number of observations (n) was 14 in analysis 1, 71 in analyses 2 and 3, and 60 in analysis 4.

The constancy of numbers of *C. xenoplax* and parasitized nematodes, and percent parasitism (transformed data) was examined by analysis of covariance; the independent variables were plot and time.

Results

The percentage of *H. rhossiliensis*-parasitized *C. xenoplax* was correlated with numbers of *C. xenoplax* among samples distributed in space at month 0 (analysis 1) (Fig. 1). Parasitism also was correlated with numbers of nematodes ($r^2 = 0.35$; $P = 0.002$; slope = 0.005) in all plots for all sampling periods when time but not plot was removed as a source of variance (analysis 2). In contrast, data collected through time within plots did not reveal density dependence ($P = 0.25$ for analysis 3 and 0.69 for analysis 4). In other words, parasitism was not related to nematode population density when plot was removed as a source of variance.

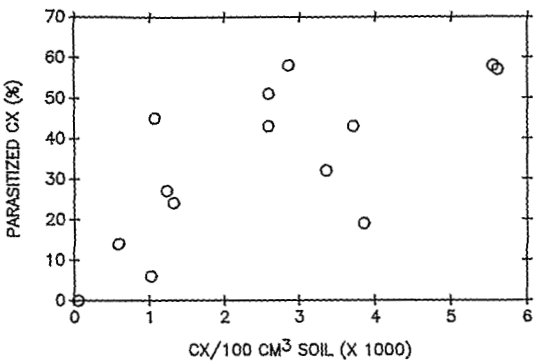

Fig. 1. Relationship between the percentage of *Criconemella xenoplax* (CX) parasitized by *Hirsutella rhossiliensis* and numbers of *C. xenoplax* in fourteen plots at month 0. [One sample that did not contain CX was not included in the figure or in the analysis. $r^2 = 0.49$ ($P = 0.005$) according to linear regression analysis.]

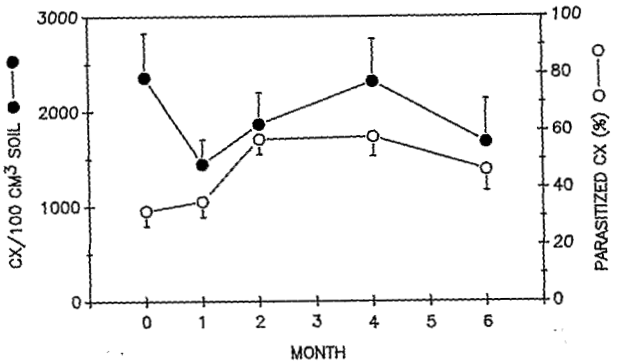

Fig. 2. Mean numbers of *Criconemella xenoplax* (●) and percentages of *C. xenophax* parasitized by *Hirsutella rhossiliensis* (○) in fifteen plots. (Vertical bars indicate one standard error).

Mean values of nematode population density and parasitism were relatively constant through time (Fig. 2). This constancy also occurred within plots (Fig. 3). Variability among plots accounted for most of the variance in the numbers of *C. xenoplax* and in the numbers of parasitized *C. xenoplax* (Table 1).

Table 1

Percentage of total variation in *Criconemella xenoplax*/100 cm³ soil (Cx/100 cm³), *Hirsutella rhossiliensis*-parasitized *C. xenoplax*/100 cm³ soil (Hr/100 cm³), and % *C. xenoplax* parasitized (% Hr) as explained by plot, time, and error.

Factor	% of total variation		
	Cx/100 cm³	Hr/100 cm³	% Hr
Plot	85.5	86.1	41.1
Time	0.5	0.0	6.2
Error	14.0	13.9	52.7

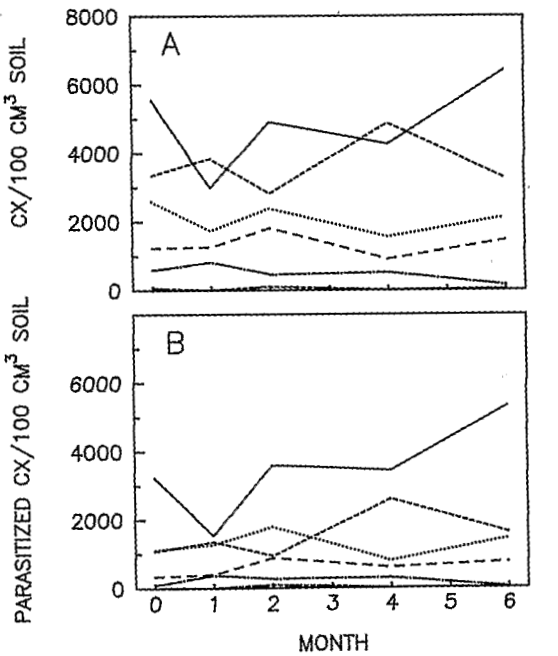

Fig. 3. Constancy of numbers of nematodes and parasitism in six plots. A : Numbers of *Criconemella xenoplax* (CX); B : Numbers of *C. xenoplax* parasitized by *Hirsutella rhossiliensis*. (Each line represents one plot, and data for each plot on each date were from one soil core.)

The age structure of *C. xenoplax* did not change ($P > 0.05$) through time; J2, J3, J4, and females represented 6, 20, 35, and 39 % of the population, respectively. The percentage of each stage parasitized by *H. rhossiliensis* over all dates was 56, 52, 46, and 42 for J2, J3, J4, and females, respectively. Fewer than 5 % of the nematodes parasitized by *H. rhossiliensis* were species other than *C. xenoplax*.

Discussion

Spatial but not temporal sampling indicated a positive relationship between parasitism and host density. The failure to detect temporal density dependence in the present study could be due to *i)* insufficient variation in parasitism and host density within plots (one cannot measure how variables change with respect to each other if the variables are not changing), *ii)* an insufficient number of sampling periods (a longer time series would have been useful but it was not possible to collect more

than five cores from the 20 cm-plots without sampling previously sampled locations), and *iii)* the possible existence of density-independent parasitism.

Because spatial and temporal sampling gave different results, the usefulness of the spatial analysis for understanding temporal density dependence is questionable. One might speculate that the plots in the present study were basically similar, varying primarily in food supply (roots) for the nematode; plots with many roots supported high nematode densities and thus high levels of parasitism. Each plot would therefore indicate the equilibrium level of parasitism at a specific host density, and the existence of a density-dependent relationship would be supported. The plots, however, could have differed in other, unknown ways.

Valuable information can be obtained by studying epidemics as they occur in the field, but the data presented here and before (Jaffee, Gaspard & Ferris, 1989) have provided little insight into within-plot dynamics or the rates and relationships that drive those dynamics. We suggest that within-plot dynamics should be investigated by repeated sampling of replicate plots under controlled conditions. Repeated sampling is facilitated in the laboratory or greenhouse where replications of a specific plot (containing known numbers of the nematode and the fungus) can be established and periodically sampled (sacrificed) many times, and where other variables can be controlled. In contrast, plots in the present study were not true replicates, number of samples per plot was limited by plot size, and parasitism and host density within plots appeared to be at equilibrium.

The dynamic relationship between parasitism and host density could be examined with mathematical models if essential parameters (e.g., rates at which nematodes contact spores, become infected, and produce new spores; spore distribution and persistence; rates of nematode reproduction and mortality) were quantified. The ability of the fungus to suppress nematode population density might be indicated by such models but should be demonstrated by comparison of numbers of nematodes in plots with and without the fungus.

Although the epidemiology of *H. rhossiliensis* in *C. xenoplax* populations remains to be elucidated, the fungus was not effectively controlling *C. xenoplax* in the study site. The mean nematode density was far above economic injury levels, high levels of parasitism tended to occur only at extremely high nematode densities, and there was no sign of an impending decline in numbers of nematodes in plots with high levels of parasitism (Fig. 3).

The constancy of parasitism and numbers of nematodes at the plot level explains the constancy previously described at the orchard level (Jaffee, Gaspard & Ferris, 1989). The basis for the constancy at the plot level is unknown, but constancy was not due to insufficient time for turn over of individual nematodes. Parasitized nematodes, especially juveniles, are degraded rapidly and thus disappear rapidly from the soil (Jaffee *et al.*, 1988).

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