Population Growth Patterns of Four Species of Aphelenchoides on Fungi

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Abstract: Qualitative and quantitative differences in population growth patterns of *Aphelenchoides rutgersi* from Florida, *A. sacchari* from Jamaica, *A. dactylocercus* from Great Britain, and *A. cibolensis* from New Mexico were assessed on 28 species of fungi. The patterns of population growth of *A. rutgersi* and *A. sacchari* were statistically similar although not identical, and they differed considerably from those of *A. dactylocercus* and *A. cibolensis*. It is suggested that *A. rutgersi* and *A. sacchari*, from Florida and Jamaica respectively, may be more closely related to each other than to either *A. dactylocercus* or *A. cibolensis*. *Key Words: Aphelenchoides rutgersi, A. sacchari, A. dactylocercus, A. cibolensis,* population, fungi, host range, taxonomy, evolution.

The nematode genus *Aphelenchoides* is a diverse group which includes plant-parasitic, mycophagous, and predatory forms (16). Many species are cosmopolitan and morphologically similar, so their taxonomic study is difficult. Estimates of the number of *Aphelenchoides* species have varied considerably (6, 7, 10, 15, 16).

In a comparative study of several isolates of *Aphelenchoides sacchari* (8) from Florida and Jamaica, Hooper and Myers (9) designated the new species *rutgersi* for the Florida isolate and retained the name *sacchari* for the Jamaica form. Their justification for the new species was its tail shape and the length of the post-vulval sac in the female. A related species from the southwestern United States, *A. cibolensis,* may be distinguished from *A. sacchari* by its shorter body, much shorter posterior uterine sac, and angular tail with spine-like mucro (14). Another species, *A. dactylocercus,* was originally isolated from soil around Sitka spruce trees at Rothamsted Agricultural Station, Great Britain (8). It may be distinguished from *A. cibolensis* by its longer tail, shape of tail terminus and mucro, and by the position of the excretory pore in relation to nerve ring and median bulb (14). Moreover, *A. dactylocercus* differs from the three other species by its more pointed tail and greater length. Hooper and Myers (9) indicated that *A. rutgersi* might be a variant of *A. dactylocercus.*

Christie and Crossman (4) reported that distinctions could be made among several plant parasitic Aphelenchoides on the basis of their growth on *Alternaria citri.* This paper compares the growth of *A. rutgersi, A. sacchari, A. dactylocercus,* and *A. cibolensis* on five strains of the fungus *Pyrenochaeta terrestris* Hansen and on 27 species of other fungi. The study was undertaken to determine whether the growth patterns of the four nematode species were similar to each other, or if distinctions between them, which might be useful for clarifying their taxonomic and evolutionary relationships, could be made on the basis of growth patterns.

MATERIALS AND METHODS

The four species of *Aphelenchoides* used are *A. rutgersi* Hooper and Myers, originally isolated from soil around citrus roots in Florida (9); *A. sacchari* Hooper, originally isolated from sugar cane soil in Jamaica (8); *A. dactylocercus* Hooper, from Rothamsted, Great Britain (8); and *A. cibolensis,* Riffle, from New Mexico (14). All were maintained in the laboratory on *Pyrenochaeta terrestris,* strain STD, grown on potato dextrose agar (Difco, Detroit, Michigan).

Monoxenic inocula containing larvae and adult female nematodes were cultured with the test fungi as follows. Parthenogenetic females and larvae were harvested from 8- to 10-week-old monoxenic cultures growing on *P. terrestris* STD and separated from the culture medium by the method of Myers (12). A section of the monoxenic

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culture containing the nematode, *P. terrestris* cells, and agar were transferred into 1.2% water agar in petri plates. After 3 h, when the nematodes had migrated throughout the surrounding agar, they were extracted from it by means of the test tube method of Petriello and Myers (13).

A 1-cm diam disc of the test fungus, covered with a small piece of sterile filter paper, was placed in the center of potato-dextrose agar in a petri plate and allowed to grow for three days. Fifty nematodes, in approximately 0.2 ml of liquid, and isolated in the manner described previously, were pipetted onto the filter paper. Six replicate growth plates were employed for each fungus-nematode pair. After inoculation, the plates were incubated at 25 C for 8 weeks. The nematodes were then harvested (12) and fixed in 5% formalin, and the total population was determined. Sterile precautions were observed throughout the entire incubation procedure.

In Experiment 1, the nematodes were grown on five strains of the imperfect fungus *Pyrenochaeta terrestris* (STD, F51, F52, F53, F54). In Experiment 2, the 28 species of fungi obtained from the Department of Plant Pathology, Rutgers University were utilized (Table 1).

After the experiments were completed, the number of nematodes on each plate was converted into its logarithm, and means and standard errors were computed. The mean log number (MLN) of nematodes on the fungal test species was subjected to analysis of variance. The factors in the analysis were nematodes (4 species), fungi (5 subspecies and 28 species in Experiments 1 and 2, respectively), and 6 replicates per nematode-fungus pair. The nematode-fungus interaction term is of the greatest interest since its significance implies that the pattern of population growth on the fungi is different for the nematodes, that is, that their fungal host-range specificities differ. To determine which of the four nematode species had similar patterns of population growth, Pearson product-moment and Kendall rank-order correlation coefficients (r and τ) were computed between all pairs of MLN's from the 28 fungi plus *P. terrestris* STD. Kendall's τ measures the degree to which the two sets of MLN's lie in the same rank order across the 28 fungi, and its use is appropriate for making qualitative comparisons between patterns of population growth.

MLN's were also adjusted to allow for the possibility that some fungi are better food sources for the nematodes than others. The adjustment was made by averaging the MLN's from all four nematodes on a given fungus, and subtracting the average MLN from the MLN obtained for an individual nematode on the same fungus. Since the growth data are expressed as logarithms, there is a resulting ratio of the MLN for the individual nematode to the average growth of all four nematodes on a given fungus. These ratios are called specific-to-average growth ratios, and they will be positive or negative according to whether the individual nematode grows better or worse than average on the given fungus. Computations were performed on computer by using the BMD08v analysis of variance program (1) and other statistical programs. "Nematode growth" is used to replace the longer phrase "increase in numbers of nematodes in the population," so that "growth" refers to population increase and not to an irreversible increase in individual size or weight.

RESULTS

The nematode species differed in their ability to increase on the strains of *P. terrestris* (Fig. 1). When numbers of nematodes were averaged for all five test strains, *A. rutgersi* grew best. Successively lower numbers occurred with *A. sacchari*, *A. cibolensis*, and *A. dactylocercus*. The growth of *A. dactylocercus* was less than average ($P < 0.01$). The fungi also differed in their ability to support nematode reproduction ($P < .001$). There were differences in population growth patterns among the four nematode species (for the nematode-fungus interaction term, $P < 0.001$). *A. rutgersi* and *A. sacchari* grew about equally well on all but strain F54; on this, *A. rutgersi* grew better than *A. sacchari* ($P < 0.01$). *Aphelenchoides cibolensis* grew best on F52 and relatively poorly on STD. This pattern of population growth differed from those of *A. rutgersi* and *A. sacchari*.

Differences were also detected among the MLN's of the four nematode species on

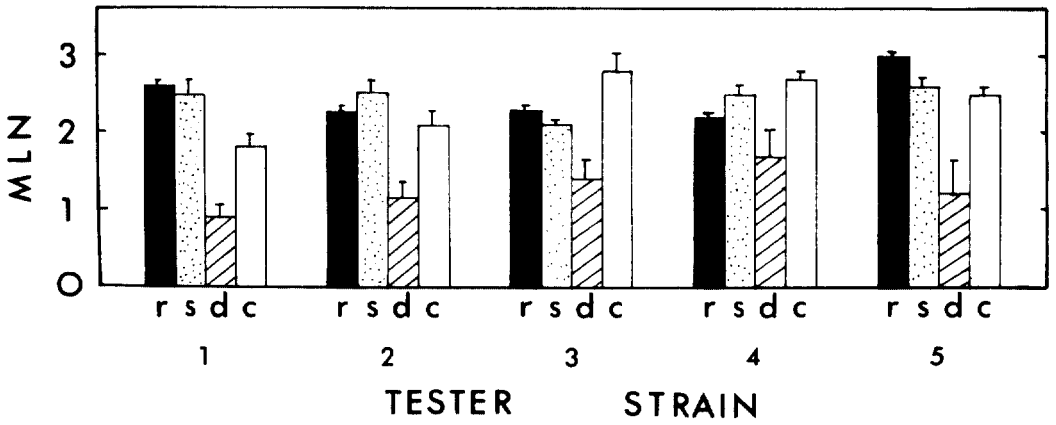

FIG. 1. Growth of *Aphelenchoides* species on five strains of *Pyrenochaeta terrestris*. Abbreviations: MLN = mean log number; r = *A. rutgersi*; s = *A. sacchari*; d = *A. dactylocercus*; c = *A. cibolensis*. Test strains: 1: *P. terrestris* STD; 2: *P. terrestris* F51; 3: *P. terrestris* F52; 4: *P. terrestris* F53; 5: *P. terrestis* F54. Error bars indicate standard errors of the means. For nematodes, $P = 0.001$; for fungi, $P = 0.001$.

the 28 fungi (Table 1, Fig. 2). When numbers of nematodes were averaged across all fungi, *A. sacchari* grew best; next in order, were *A. cibolensis, A. rutgersi,* and *A. dactylocercus.* In contrast to its growth in Experiment 1, the growth of *A. dactylocercus,* although low, was not significantly different from average in Experiment 2.

As in Experiment 1, the ability of a given fungus to support population growth was nematode-species specific (for the nematode-fungus interaction term, $P < .001$). Again, *A. rutgersi* and *A. sacchari* had similar patterns of population growth. There was no difference between the MLN's of *A. rutgersi* and *A. sacchari* when their mean numbers for the 28 fungi were computed. Moreover, test fungi that supported the growth of *A. rutgersi* populations also tended to support the growth of *A. sacchari* populations, and conversely (Fig. 2).

The degree of similarity between the fungal host-ranges of these nematode species can be assessed by computing correlation coefficients between their MLN's on the test fungi. For the MLN's of *A. rutgersi* and *A. sacchari,* product-moment and rank-order correlation coefficients were positive and statistically significant ($r = 0.626$, $P < 0.001$; $\tau = 0.416$; $P < 0.001$), an implication that, if *A. rutgersi* populations grew well on a certain test fungus, then *A. sacchari* was also likely to grow well on it, and conversely. The correlation was not due to similarities on one or two fungi, but was, instead, general (Fig. 3). Moreover, the correlations between the MLN's of *A. rutgersi* and *A. sacchari* were larger than those between the MLN's of *A. rutgersi* and *A. dactylocercus* ($r = 0.361$; $\tau = 0.241$; NS). There was a small but statistically significant correlation between the MLN's of *A. dactylocercus* and *A. cibolensis* ($r = .425$; $\tau = .270$; $P < 0.05$).

The differences in reproduction rates of *A. rutgersi* and *A. sacchari,* in comparison to those of *A. dactylocercus* and *A. cibolensis,* can be seen most clearly for fungi on which the former pair grew poorly (Fig. 1; Table 1). *A. dactylocercus* and *A. cibolensis* grew reasonably well on fungi #20, #21, #25, and #28, whereas these fungi do not support extensive population growth of *A. rutgersi* or of *A. sacchari.* Although there were test fungi on which *A. rutgersi* and *A. sacchari* did not grow equally well (#2, #8, #20, and #23 in Fig. 1 and Table 1), *A. rutgersi* and *A. sacchari* had similar patterns of population growth on the 28 fungi, and resembled each other more than they resembled either *A. dactylocercus* or *A. cibolensis.*

Since the fungi used in Experiment 2 differed among themselves in average ability to support nematode growth (Table 1), the ability of *A. rutgersi* and *A. sacchari* to grow well on the same set of test fungi might mean only that this group of fungi is a particularly well-suited food for nematodes in general. The observed MLN's were therefore adjusted for differences in the average abilities of the test fungi to

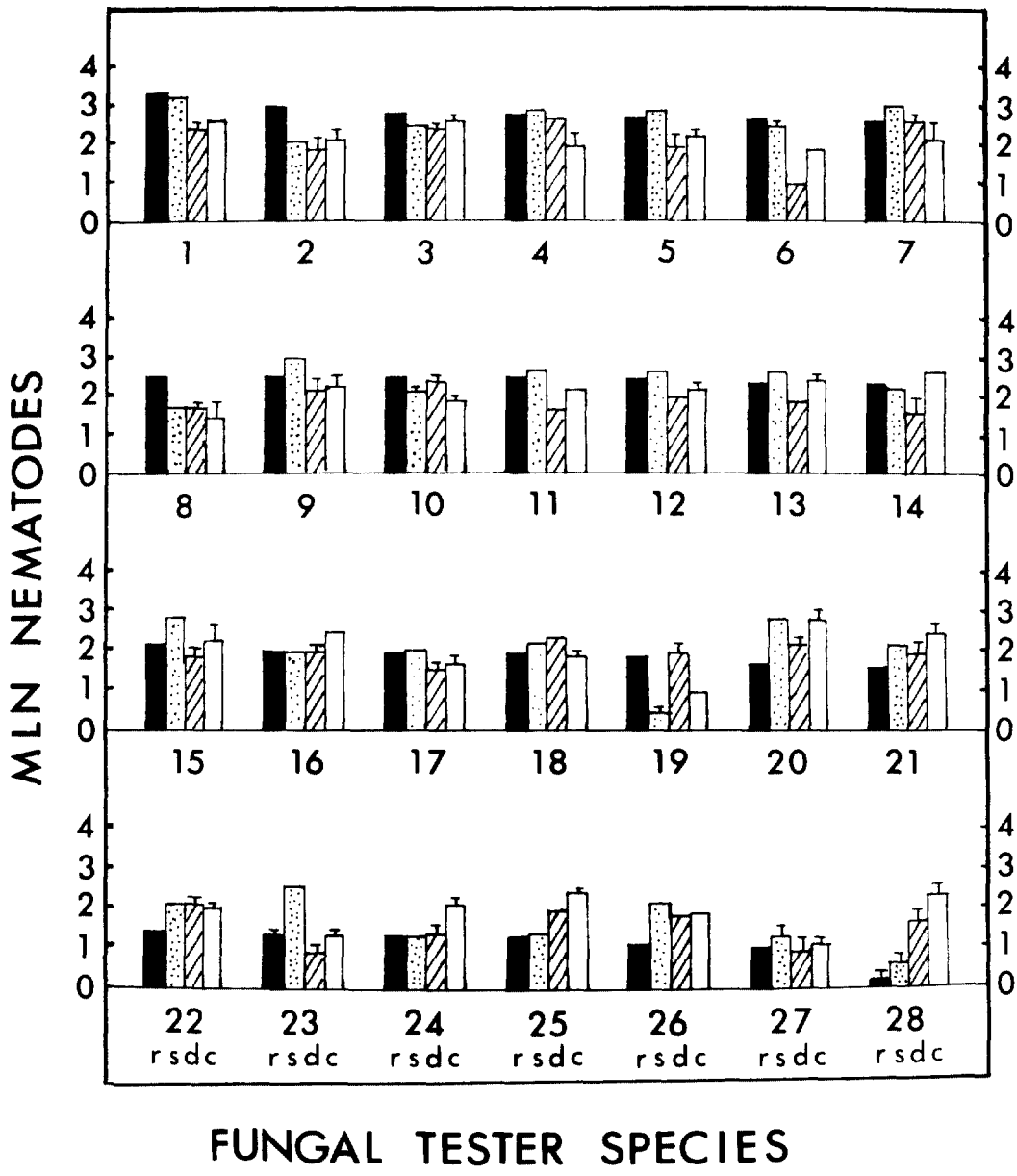

FIG. 2. Growth of *Aphelenchoides* species on 28 species of test fungi. Abbreviations: MLN = mean log number; r = *A. rutgersi;* s = *A. sacchari;* d = *A. dactylocercus;* c = *A. cibolensis,* test fungi identified in Table 1. Error bars indicate standard errors of the means. For nematodes, $P = 0.001$; for fungi, $P = 0.001$.

support population growth by converting them to specific-to-average growth ratios and computing rank-order correlations between them for the four nematode species. The correlation between specific-to-average growth ratios for *A. rutgersi* and *A. sacchari* was not significant ($\tau = -.032$, NS), an indication that these species did not behave identically on the test fungi. However, specific-to-average growth ratios of *A. rutgersi* and *A. sacchari* were each significantly negatively correlated with the ratios for *A. dactylocercus* and *A. cibolensis* ($\tau = -0.280$; -0.458; -0.423; $-.215$; for all, $P < 0.05$). Fungi best able to support the growth of *A. rutgersi* and *A. sacchari* were least able to support growth of *A. cibolensis* and *A. dactylocercus.*

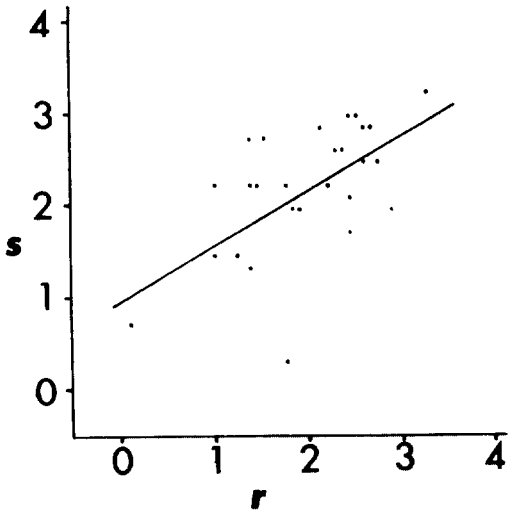

FIG. 3. Scatter plots of mean log number (MLN) of *Aphelenchoides rutgersi* (abscissa) and *Aphelenchoides sacchari* (ordinate). For each fungus, the MLN obtained on it with *A. rutgersi* is plotted against the MLN obtained on the same fungus with *A. sacchari*. The line shown is the least squares regression line.

DISCUSSION

The patterns of population growth of *A. rutgersi* and *A. sacchari* were quite similar. Moreover, the similarity appears not to be due to similarities in the average ability of the test fungi to support nematode growth. These results tend to suggest that *A. rutgersi* and *A. sacchari* are relatively related forms. *A. rutgersi* and *A. sacchari* were isolated from regions of the Caribbean (Florida and Jamaica; 8, 9) and their growth patterns were more similar than could be expected from chance alone. On evolutionary grounds, it seems plausible that they might be more closely related to each other than to either *A. dactylocercus,* from inland Great Britain (8), or to *A. cibolensis,* from New Mexico (14). This possibility is supported by the observation that the fungal host-ranges of *A. rutgersi* and *A. sacchari* differed systematically from those of *A. dactylocercus* and *A. cibolensis.*

Evidence from a variety of plants and

TABLE 1. Test fungi and their ability to support population growth of *Aphelenchoides* species.

Number (for fungi)	Fungi	Ability to support nematode growth[a]				
		r	s	d	c	mean
1	*Pestalotia sp.*	1	1	5	4	1
2	*Rhizoctonia solani*	2	20	20	18	13
3	*Gloeosporium sp.*	3	13	4	3	3
4	*Monilia americana*	4	5	1	19	4
5	*Cladosporium cucumerinum*	5	4	16	12	6
6	*Pyrenochaeta terrestris STD*	6	12	27	22	18
7	*Diaporthe sp.*	7	2	2	14	2
8	*Verticillium albo-atrum*	8	23	17	25	20
9	*Sclerotinia homeocarpa*	9	3	8	10	5
10	*Fusarium roseum*	10	19	6	21	14
11	*Aspergillus nidulans*	11	10	23	15	11
12	*Cunninghamella sp.*	12	9	13	13	9
13	*Epicoccum sp.*	13	11	15	8	8
14	*Rhizopus stolonifer*	14	14	22	2	12
15	*Alternaria olereaceae*	15	6	19	11	10
16	*Pythium debaryanum*	16	21	10	5	15
17	*Trichoderma koningii*	17	22	24	24	22
18	*Thielaviopsis sp.*	18	15	3	20	16
19	*Rhodotorula sp.*	19	28	12	28	26
20	*Botrytis sp.*	20	7	7	1	7
21	*Curvularia sp.*	21	16	14	7	17
22	*Graphium sp.*	22	17	11	17	19
23	*Phycomyces blakesleeanus*	23	8	28	26	24
24	*Cytospora sp.*	24	26	25	16	25
25	*Phytophthora cinnamoni*	25	24	9	6	21
26	*Helminthosporium sativum*	26	18	18	23	23
27	*Sordaria fimicola*	27	25	26	27	28
28	*Gliocladium roseum*	28	27	21	9	29

[a]Arranged in rank order according to ability to support *A. rutgersi* growth. Abbreviations: r = *A. rutgersi;* s = *A. sacchari;* d = *A. dactylocercus;* c = *A. cibolensis.*

animal species shows that geographically separated subpopulations of closely related forms will display physiological differences without their necessarily being clear morphological differences between them (5, 11). If it is assumed that the differences in fungal host-ranges found here represent differences in nutritional, physiological, or other characteristics of the nematodes, it can be suggested that *A. rutgersi* and *A. sacchari* are genetically related species that have not yet diverged greatly from one another. Since, however, their host-ranges are not identical, Hooper and Myers's (9) description of a new species for the former species receives support from us, although we feel that their suggestion that *A. rutgersi* is a variant of *A. dactylocercus* seems less likely.

Christie and Crossman (4) also reported differences in fungal host ranges for *A. besseyi* and *A. fragariae,* polyphagous plant-parasitic forms from the southeastern and northeastern United States. These nematode species also differ in temperature sensitivity, the more northern *A. fragariae* being less resistant to heat than the southern *A. besseyi* (3), as well in their survival abilities when they are transferred from one region to the other (2). Hence, selectively physiological variation associated with geographic distance is not unknown in the genus, and physiological differentiation in relation to natural hosts of members of the genus *Aphelenchoides* may contribute to evolution of the genus. We speculate that *A. rutgersi* and *A. sacchari* might represent geographically separated but physiologically related species in a single allopatric group physiologically distant from either *A. dactylocercus* or *A. cibolensis.* Verification or rejection of this speculation would require physiological examination of other members of the genus *Aphelenchoides* from the Caribbean and the southern United States.

The differences in host-ranges found here must represent physiological differences among the nematodes, but it is unclear if they reflect different nutritional requirements of the nematodes, the presence of cell walls more difficult for some nematodes to digest than others, or differences in the ability of the nematodes to ingest different fungi. These and other factors probably contribute to the differences in the growth patterns observed. However, without more information about these differences or their physiological bases, the host-range data are best treated as purely formal measures of similarity and dissimilarity between the nematodes. The data do not eliminate the possibility that fungi low in the rank-order actively inhibit the reproduction of the nematodes.

The determination of fungal host-range specification has value for making distinctions among the four nematodes studied, despite the as yet purely formal nature of the host-range descriptions. Host-range studies may therefore provide a useful way to supplement morphological studies of speciation in the genus *Aphelenchoides.*

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