## Ecology of Nematodes Under Influence of Cucurbita spp. and Different Fertilizer Types<sup>1</sup>

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Abstract: In a field study conducted in Georgia, cucurbit plants with high (*Cucurbita andreana*) and low (*Cucurbita maxima*) concentrations of cucurbitacins were used in combination with two types of fertilizers to investigate their effects on the community of soil nematodes. Ecological measures of soil nematode community structure such as total nematode abundance, number of genera, trophic diversity, trophic group proportions, fungivore/bacterivore ratio, and modified maturity index were assessed and compared among treatments. In general, poultry manure (an organic source of nitrogen) and synthetic fertilizer (a nonorganic source of nitrogen) did not differ in their effects on the nematode communities throughout one growing season. Few differences between the two plant species were found for any of the nematode community measurements. Bacterial- and fungal-feeding nematodes were the most abundant trophic groups, averaging 60% and 20% of the nematode community, respectively. Trophic diversity, nematode maturity index, and fungivore/bacterivore values were lowest at the beginning and highest at the end of the experiment.

Key words: Cucurbita andreana, cucurbitacins, Cucurbita maxima, ecology, fertilizer, nematode, secondary plant metabolite.

Damage caused by plant-parasitic nematodes can limit crop production (1). Conventional strategies for management of these pests involve chemical soil treatments and resistant cultivars (5). In recent years, the application of nematicides in agriculture has been questioned due to their toxicity to humans and wildlife (16). Organic amendments have been used for centuries to improve soil fertility and crop yield (25). Other studies suggest that organic amendments can also be used as nematicidal agents (15,19,22). Although much is known about the effects of organic amendments on plant-parasitic nematodes (7,19, 22), little information is available on their effects on free-living nematodes (7,9,15), and even less on the entire nematode community.

Nematode populations contribute to both decomposition processes and nutrient cycling (26). Free-living nematodes may have an indirect beneficial effect on plant growth. Grazing by bacterial- and fungal-feeding nematodes stimulate decomposition and nitrogen mineralization and thus enhances the level of nutrients available to the plant (3,13,33).

Poultry manure is an animal waste which, in areas of extensive poultry production, is a potential cause of non-point water pollution and ground water contamination with nitrates (27). Conversely, chicken litter is considered a valuable organic soil fertilizer rich in all the macro and micronutrients necessary for plant growth (29). Utilization of poultry litter might offer an inexpensive alternative for both soil fertilization and nematode control (22) and at the same time provide an environmentally safe method of chicken waste disposal.

Cucurbits, like other cultivated plants, are susceptible to damage by plantparasitic nematodes (6). *Cucurbita* spp., particularly wild species, are characterized by the presence of highly bitter and toxic triterpenoid compounds, cucurbitacins. Cucurbitacins are present throughout the whole plant, but their concentrations vary with age, plant organ, plant species and environmental conditions (23). High levels of cucurbitacins are found in roots and fruits. Cucurbitacins are barely detectable

Received for publication 24 April 1995.

<sup>&</sup>lt;sup>1</sup> This research was supported by the Andrew Mellon Foundation allocated to the Institute of Ecology at the University of Georgia, Athens, GA. <sup>2</sup> Graduate Student and Professor, Institute of Ecology,

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We thank C. A. Hoffman and C. R. Carroll for their invaluable suggestions in analysing and interpretation of the data.

in cultivated *Cucurbita* species, in contrast to their wild relatives (23). In aboveground systems, cucurbitacins act as natural deterrents of herbivores and pathogens (18,23). If their activity in the belowground system is similar, cucurbitacins could serve as natural nematicides.

We examined the effects of two fertilizer types (organic chicken manure and synthetic fertilizer) on soil nematode community responses. Further, we compared the effects of a wild cucurbit with high concentrations of cucurbitacins and a cultivated cucurbit with low levels of cucurbitacins on nematode population densities and community structure.

## MATERIALS AND METHODS

The research was conducted in a grower's field near Watkinsville, Oconee County, Georgia on typic rhodic Kanhapludult soil with sandy loam texture and pH 5.75. Following cultivation in spring 1993, four split-blocks (5 m by 10 m) were established. Blocks were separated from each other by 3-m-wide strip covered with vegetation. Two of the blocks had been under Cucurbita pepo for last two years and the other two had a cover of fescue (Festuca spp.). Following the recommendations of the Soil Testing Laboratory at the University of Georgia, Athens, Georgia, 110 kg/ ha of nitrogen in the form of either chicken manure (the organic form: N, 12.5  $g/kg; NH_4^+, 0.04 g/kg; and [NO_2^-]$ +NO<sub>3</sub><sup>-</sup>], 0.33 g/kg [27]) or granular synthetic fertilizer (NH<sub>4</sub>NO<sub>3</sub>) were applied to every half-block alternatively. Because the chicken litter also contained phosphorus and potassium (N:P:K ratio 3:2:2), equivalent amounts (73.33 kg/ha) of P and K as  $P_{2}O_{5}$  and  $K_{2}O_{5}$ , respectively, were applied to the half-blocks treated with synthetic nitrogen fertilizer. Within each half-block, four treatments were established, as follows: Cucurbita maxima grown alone (a winter squash, cv. Buttercup, low cucurbitacin level); Cucurbita andreana grown alone (wild relative, high cucurbitacin level; seeds obtained from the Thomas Whitaker

Cucurbit Germplasm Collection, Davis, California); C. maxima and C. andreana grown together; and bare fallow (no cucurbits). Within each treatment area (2.5 by 2.5 m) nine mounds (25-cm-d, 10 cm high, 45 cm apart) were hand-formed. Four seeds were planted per mound on 14 May, except for the bare fallow treatment. After plants reached the two-leaf stage, the mounds were reduced to five per treatment, and plants were thinned to two per mound. Plots were watered to field capacity with trickle irrigation for two hours daily. Weed cloth was used to prevent weed growth between mounds. Weeds were also removed by hand when necessary. At the end of the growing season, the plant stems were cut off and removed.

Two soil cores (5-cm-d and 12 cm deep) per treatment were taken on eight occasions, as follows: 8 May (just before fertilization), 14 May (at planting), 17 June (plant thinning), 2 July (mound reduction), 29 July, 18 August (shoot cutting), 19 August, and 26 August. The cores were taken near the plant stem, composited, and mixed. Nematodes were extracted from soil placed in Baermann funnels (24) for 48 hours. To minimize the thickness of the soil layer due to small diameter of the funnels (3 cm), a 7–9 g subsample of soil was used for extraction.

All nematodes were fixed with 4% formalin and counted and identified to either genus or family, if genus level was not possible, on an inverted microscope. For statistical analysis, nematode numbers were converted to numbers per 10 g dry soil and transformed to  $\log (x + 1)$  values. Nematodes were assigned to trophic groups (32) and the proportions of each feeding group were determined. The following ecological indices were calculated: richness, the number of taxa per sample (10); trophic diversity,  $T = 1/\sum p_i^2$ , where  $p_i$  is the proportion of the *i*th trophic group in the nematode community (10); fungivore/bacterivore ratio (10,17); and modified maturity index,  $\Sigma MI = \Sigma v_i p_i$ , where  $v_i$  is the *c*-*p* value assigned by Bongers (2) of the *i*th genus and  $p_i$  the proportion of the genus in the nematode community (30). The *c-p* values describe the nematode life strategies, and range from 1 (for colonizers) to 5 (for persisters) (2). Data were subjected to analysis of variance and means were compared by Fisher's protected LSD at P = 0.05.

## **RESULTS AND DISCUSSION**

Twenty-three nematode families and 23 genera were identified (Table 1). Rhabditidae, Cephalobidae and Aphelenchoididae were the most abundant families. Within treatments, the number of taxa ranged from 4 to 14 (Fig. 1), and seemed to be low compared to other studies (10,17,31). On 2 July and later, plots with synthetic fertilizer appeared to have a greater range in richness than plots with chicken litter, but these trends among treatments were not significant ( $P \leq 0.05$ ). Richness tended to be the lowest in treatments without plants,



FIG. 1. Richness in treatments with synthetic (top) and organic (bottom) fertilizers. CM = C. maxima, Cam = C. and reana and C. maxima together, Ca = C. and reana, NP = no plant.

which may indicate lack of diverse food resources. Richness can reflect environmental quality. In productive systems, richness tends to remain high. In contrast, as eco-

Trophic group	Family	Genus	<i>c-p</i> value <sup>a</sup>
Bacterivores	Alaimidae	Alaimus	4
	Cephalobidae	Acrobeles	2
		Acrobeloides	2
		Eucephalobus	2
		Heterocephalobus	2
	Leptolaimidae	Chronogaster	3
	Odontolaimudae	Odontolaimus	3
	Panagrolaimidae	Panagrolaimus	1
	Plectidae	Plectus	2
		Wilsonema	2
	Prismatolaimidae	Prismatolaimus	3
	Rhabditidae	b	1
Fungivores	Anguinidae		2
8	Aphelenchidae	Aphelenchus	2
	Aphelenchiodidae	Aphelenchoides	2
	Diphtherphoridae	Diphtherophora	3
	Leptonchidae	Leptonchus	4
Omnivores	Dorylaimidae		4
Plant-parasites	Criconematidae	Criconemella	3
L.	Hoplolaimidae	Helicotylenchus	3
	Longidoridae	Xiphinema	5
	Paratylenchidae	Paratylenchus	2
	Trichodoridae	Trichodorus	4
	Tylenchidae	Tylenchus	2
Algal feeders	Achromadoridae	Áchromadora	3
Predators	Mononchidae		4
	Trypilidae	Trypila	3

TABLE 1. Classification by trophic group, family, genus, and *c-p* value of nematodes identified in *Cucurbita* spp. field plots in Georgia in 1993.

<sup>a</sup> Values taken from Bongers (2).

<sup>b</sup> Not identified to genus.

system degradation proceeds (due to habitat alteration, over-exploitation, pollution, etc.), the number of species declines (20). In soil ecosystems, loss of taxa may affect structure of food chains and webs, and ultimately decomposition and nutrient mineralization (8).

Total numbers of nematodes ranged from 39 to 692/10 g dry soil. Similar nematode densities were found throughout all treatments, including bare fallow (data not shown). We suspect that weeds, despite being regularly removed, played a key role in supporting relatively high nematode numbers.

Bacterial-feeding and fungal-feeding nematodes were always the most abundant trophic groups, averaging 60 and 20% of the nematode community, respectively. These proportions appear typical compared with some other agricultural sites (10,17). Bare fallow plots had a higher proportion of bacterivores (up to 98%) than plots with C. maxima alone (55%) or C. maxima and C. andreana grown together (50%) on 18 and 26 August under organic fertilizer, and C. maxima (20%) on 18 August under synthetic fertilizer ( $P \le 0.05$ ) (Fig. 2). These differences, however, diminished at later sampling dates. In general, fertilizers did not differ in their effects on densities of bacterial- and fungal-feeding groups. Predominance of bac-



FIG. 2. Proportion of bacterivorous nematodes in treatments with synthetic (top) and organic (bottom) fertilizers. Cm = C. maxima, Cam = C. andreana and C. maxima together, Ca = C. andreana, NP = no plant.



FIG. 3. Proportion of plant-parasitic nematodes in treatments with synthetic (top) and organic (bottom) fertilizer. Cm = C. maxima, Cam = C. andreana and C. maxima together, Ca = C. andreana, NP = no plant.

terivorous nematodes may indicate higher population levels of bacteria than fungi, and thus a bacterial rather than fungal pathway of decomposition and nutrient mineralization (11). The lack of a significant fertilizer effect here suggests that chicken litter and synthetic amendments are not much different in terms of nutrient release and nutrient availability for the plant growth on a short time scale.

Omnivorous nematodes made up 0-20% of the soil nematode community. Although densities of omnivores usually appeared highest in all treatments with C. maxima and C. andreana grown together and lowest in treatments without cucurbits (data not shown), the differences were not significant. Usually, the proportion of plant-parasitic nematodes was lower (0-10%) than that of other trophic groups. This value was somewhat low compared with sites in Michigan (25%) (10), Netherlands (above 10%) (4), South Australia (above 40%) (31), and Florida (30%) (17). Under both fertilizers, treatments without cucurbits tended to have the lowest densities of plant-parasitic nematodes, perhaps due to continuous weeding, but no difference was found (Fig. 3). On 18 August, in plots with synthetic fertilizers, C. maxima supported more plant-parasitic nematodes than in plots with chicken manure ( $P \leq$ 0.05), but by the end of the experiment



FIG. 4. Trophic diversity in treatments with synthetic (top) and organic (bottom) fertilizer. Cm = C. maxima, Cam = C. and reana and C. maxima together, Ca = C. and reana, NP = no plant.

this difference disappeared. Although plant feeders tended to be less abundant in treatments with chicken litter than in treatments with synthetic fertilizer, the trend was not significant. Densities of algal feeding nematodes varied neither with plant species nor fertilizer type, and were only 2–3% of the nematode community (data not shown). Predators were not common in any of the treatments and in most cases they were totally absent, possibly due to subsample size or sampling method.

Trophic diversity index (T) describes trophic group distribution. Communities dominated by one trophic group (regardless of trophic type) are characterized by low values of T and vice-versa. The average values of T in our study were comparable to values obtained by other researchers: Neher and Campbell (21), 2.54; Freckman and Ettema (10), 2.94; Yeates and Bird (31), 2.56; and McSorley and Frederick (17), 2.45. The dominance of bacterialfeeding nematodes explains low values of T, especially in treatments without plants (Fig. 4). The plant species present did not affect trophic diversity index. Although trophic diversity seemed to be more variable in plots treated with synthetic fertilizers on 19 August, the differences were not significant.

The ratio of fungivores to bacterivores (F/B) reflects the structure of the micro-

flora community (34). Bacteria and fungi are the primary decomposers directly affecting nutrient cycling and nutrient supply to plants (13). The rate of decomposition and mineralization is directly related to composition of microflora (12). Bacteria-based food webs exhibit higher decomposition rates than fungi-based webs. In our soils, F/B ranged from 0.15 to 2.30 (Fig. 5), and was within the range obtained in other studies (10,17,31). In general, however, F/B oscillated around 0.5 suggesting that bacteria were probably the predominant food source for nematodes. Thus, both plant species and fertilization, regardless of nitrogen source, promoted bacterial-based food webs and probably comparable nutrient cycling rates.

The modified maturity index ( $\Sigma MI$ ) is a measure based on the composition of the nematode community, and can reflect the degree of disturbance of the soil ecosystem (2).  $\Sigma MI$  was about 1.65 for treatments under both fertilizer types at the beginning of the experiment (Fig. 6). At that time, the most common families were Rhabditidae and Panagrolaimidae. These nematodes fall into the group of the lowest values on the *c-p* scale. During the later part of the season (29 July, 18 August, and 19 August),  $\Sigma MI$  was higher in the *C. maxima* and *C. andreana* treatments in plots treated



FIG. 5. Ratio of fungivorous to bacterivorous nematodes (F/B) in treatments with synthetic (top) and organic (bottom) fertilizer. Cm = C. maxima, Cam = C. andreana and C. maxima together, Ca = C. andreana, NP = no plant.



FIG. 6.  $\Sigma$ MI in treatments with chemical (a) and organic (b) fertilizer. Cm = C. maxima, Cam = C. andreana and C. maxima together, Ca = C. andreana, NP = no plant.

with synthetic fertilizer, than in plots treated with chicken manure ( $P \le 0.05$ ). Nematode families dominating those communities were Cephalobidae (Acrobeloides, Heterocephalobus) and Aphelenchoididae (Aphelenchoides); however, occasional treatment differences in  $\Sigma MI$  did not persist. The average values of  $\Sigma MI$  were comparable to those obtained in studies in Florida (17), Poland (28), South Australia (31).

In conclusion, no consistent differences between nematode community responses to either type of fertilizer or plant species were observed. It is likely that the ecological indices we used were not sensitive enough to changes in the nematode community on such a short time scale, especially in relation to fertilizer type; however, it seems, that chicken manure has similar effects to those of synthetic fertilizer in the short term. More research is necessary to determine the effects of chicken litter in long-term applications. Although not significant, we observed occasional suppression of plant-parasitic nematodes in plots treated with chicken litter. Similar favorable effects of chicken litter were found by Mian and Rodríguez-Kábana (19), and Kaplan and Noe (14). We suspect that the low numbers of plant-parasites caused difficulty in detecting differences in the nematode community between plant species. The lack of clear patterns might have resulted from low numbers of replicates, cores per sample, or amount of soil used for nematode extraction. Perhaps other factors were involved also, such as extremely high temperatures, drought, or insect grazing of above-ground plant parts. It would be valuable to investigate these interactions under conditions of higher levels of plant-parasitic nematodes.

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