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# Augmentation and Aldicarb Treatment of Nematodes in Selected Sugarcane Weed Habitats[1]

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*Abstract:* In a single experiment, field-grown Louisiana sugarcane was augmented with phytoparasitic nematodes, treated with aldicarb, or left untreated in both weedy and weed-free habitats to study interactions among nematodes, weeds, sugarcane, and sugarcane free amino acid titers. Aldicarb reduced three of the six phytoparasitic nematode genera at various times during the two growing seasons and was associated with 17% more free proline in the sugarcane. Nematode augmentation resulted in higher field populations of *Meloidogyne* spp. Free cysteine, histidine, proline, and serine concentrations in sugarcane were lower where nematodes were added. Densities of *Tylenchorhynchus annulatus* and total phytoparasitic nematodes were lower in weedy habitats compared to weed-free conditions. Sixteen of the 17 sugarcane free amino acids were significantly lower in weed-free areas. It is suggested that further research be conducted on the relationship of plant stresses to free amino acid levels to better understand plant-mediated interactions among crop pests.

*Key words:* aldicarb, amino acid, *Meloidogyne*, nematode, *Saccharum*, sugarcane, *Tylenchorhynchus annulatus*, weed.

Sugarcane, an interspecific hybrid of *Saccharum* spp., hosts at least 14 phytoparasitic nematode genera, with species of *Meloidogyne, Pratylenchus, Trichodorus,* and *Tylenchorhynchus* considered the major pests (5,19). Aldicarb, a plant systemic (23) oxime carbamate that persists in soil for about 10 weeks (8,20), can improve sugarcane yields (4), but it failed to consistently diminish phytoparasitic nematode populations in several crops (7,23,25), including sugarcane (26). Aldicarb use has been associated with reductions of predaceous arthropods and greater injury by the sugarcane borer *Diatraea saccharalis* F. to Louisiana sugarcane (20). Recent sugarcane research has emphasized the potential contribution of annual weeds to insect and nematode pest suppression (21).

Host-plant free amino acid (FAA) titers may change with stress and appear to be linked with resistance to some pests (27), including nematodes (17). Sugarcane mosaic virus infection and weed-induced stress have been associated with FAA accumulations in sugarcane; correlations were detected for certain nematode taxa and sugarcane FAA levels (22). Our objective was to examine the effects of a systemic nematicide and augmented nematode populations on the nematode community and on FAA levels in weedy and weed-free sugarcane.

## Materials and Methods

The experiment was conducted in 2.5 ha of sugarcane (cv. CP 74-383) planted in 1985 on 1.8-m row centers in Assumption Parish, Louisiana. The plant and first ratoon crops were studied. Soil (Commerce loam; 24% clay, 68% silt, 8% sand) moisture content varied from saturation to occasional topsoil desiccation.

The experiment was a randomized complete block design replicated six times with a split-plot arrangement of treatments. Whole plots (0.2 ha each) were either weedy (W) or weed-free (WF). Weedy plots were spot-treated with dicamba (9.6 g a.i./liter) to select for grass species as possible sugarcane nematode hosts. Dicot weeds that did emerge were removed by hand. In early May, weed-free plots were sprayed with a tank mix of metribuzin (1.3 kg a.i./ha) and dicamba + 2,4-D (1.3 and 1.1 kg a.i./

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TABLE 1. Inoculum levels of six phytoparasitic nematode genera used to augment nematode densities in sugarcane plots, 1985 and 1986.

| Nematode genus | Nematodes/250 cm³ soil (± SE) 1985 | 1986 |
|---|---|---|
| *Criconemella* | 71 ± 52 | 46 ± 15 |
| *Meloidogyne* | 1,080 ± 492 | 867 ± 379 |
| *Paratrichodorus* | 44 ± 23 | 46 ± 16 |
| *Pratylenchus* | 159 ± 72 | 0 |
| *Rotylenchulus* | 10,268 ± 5,347 | 93 ± 93 |
| *Helicotylenchus* | 628 ± 137 | 78 ± 78 |
| Total phytoparasitic | 12,250 ± 4,255 | 1,131 ± 441 |

ha, respectively) from a tractor-mounted spray boom.

Subplots, 0.5 ha each, were treated with granular aldicarb (A) at 0.08 kg a.i./ha immediately incorporated into the soil to a depth of about 20 cm using tractor-drawn chisels, left as untreated controls (C), or augmented with phytoparasitic nematodes (+). Nematodes for the augmented plots were collected 6 months in advance from sugarcane fields and raised in a greenhouse on root-knot nematode susceptible tomato, *Lycopersicon esculentum* Mill. (cv. Rutgers), planted in steam-sterilized soil (25% sand). The nematodes were manually distributed along the cane row tops at a rate of about 1,200 liters of infested greenhouse soil per nematode subplot in May of each year (Table 1). Thus, at the subplot level, six habitats were created: W+, WC, WA, WF+, WFC, and WFA.

Total weed biomass was measured by taking five random 0.5-m² quadrats of foliage clipped to the soil surface from each subplot in June, August, and September of each year. Two of the five quadrat samples were also used to determine differences in species composition. All samples were dried for 48 hours at 94 C and then weighed.

Nematodes were collected from 2-cm-d soil cores to a depth of 25 cm. Two 18-core subsamples were taken within 8 cm of randomly selected cane stalks from each of the subplots in late spring, August, and October of 1985 and 1986. The 250-cm³ soil subsamples were enclosed in plastic-lined paper bags and transported to the Mississippi Cooperative Extension Service Laboratory for nematode extraction by elutriation, sugar-flotation centrifugation (16), identification, and counting.

In late October 1986, the basal 10 cm of four sugarcane stalks were cut from each subplot, sealed in plastic bags, and placed on ice. Ten ml of cane juice was pressed from each of the cuttings, and the four juice samples within each subplot were combined. A 1-ml aliquot was filtered from each four-stalk juice solution using 0.45-μm membrane filters mounted on plastic syringes. The filtrate was stored at −100 C until FAA concentrations were measured using a Waters Pico-Tag amino acid analysis system (Millipore, Milford, MA) that hydrolyzed the sugarcane juice with HCl and derivatized the hydrolyzate with phenylisothiocyanate to produce phenylthiocarbamyl-amino acids. FAA were analyzed by reversed-phase high-performance liquid chromatography (HPLC) with a Waters 510 solvent delivery system (Millipore, Milford, MA) pumping Buffer A (140 mM sodium acetate and 6% acetonitrile) and Buffer B (60% acetonitrile in water) at 1 ml/minute on a convex gradient through a Waters Pico Tag $C_{18}$ column (Millipore, Milford, MA; 3.9 × 150 mm) at 40 C. Absorbance at 254 nm was monitored with a Waters Lambda Max variable wavelength detector (Millipore, Milford, MA). Identification and quantification of PTC-amino acids were achieved by calibrating with a standard mixture of amino acids. All FAA were accurately quantified, except serine, which was not readily distinguished from glucosamine. Analysis of variance (13) was used to delineate effects of the weed habitats, nematode augmentation, and aldicarb treatment on nematode populations and FAA accumulations.

## RESULTS

The most common nematodes collected in each of the six habitats were *Criconemella curvata* (Raski) Luc and Raski, *C. onoensis* (Luc) Luc and Raski, *Helicotylenchus dihystera* (Cobb) Sher, *H. pseudorobustus* (Stein-

er) Golden, *Meloidogyne incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *Paratrichodorus minor* (Colbran) Siddiqi, *Pratylenchus zeae* Graham, and *Tylenchorhynchus annulatus* (Cassidy) Golden. Total densities of nonphytoparasitic nematodes were also measured (Table 2). *Hoplolaimus columbus* Sher and *Rotylenchulus reniformis* Linford and Oliveira composed less than 1% of the total phytoparasitic nematode populations. Because nematode populations may respond to changing soil conditions (e.g., moisture), we did not compare nematode abundances across time.

On three sampling dates, *Meloidogyne* spp. numbers were higher ($P < 0.05$) in the augmented plots than in control plots, but *Criconemella* spp., *Helicotylenchus* spp., *P. minor,* and total phytoparasitic nematode levels were significantly lower in the augmented plots in the first sampling of the ratoon crop. Free cysteine in sugarcane extract was 22% lower ($P < 0.05$) in plots where nematode populations had been supplemented; free histidine, proline, and serine were also significantly reduced (Table 3). A weed-nematode interaction was detected ($P < 0.05$) for arginine.

Compared to the control systems, aldicarb significantly reduced *Criconemella* spp., *Helicotylenchus* spp., *P. minor, P. zeae,* and total phytoparasitic nematodes at various sampling times (Table 2). Free proline in the controls was 17% ($P < 0.05$) lower than in the aldicarb-treated plots (Table 3). Significant interactions between the weed and nematode treatment factors were detected for arginine, cysteine, and proline.

Weeds in weedy habitats included *Brachyaria platyphylla* Nash, *Digitaria sanguinalis* Scop., *Echinochloa* spp., *Cynodon dactylon* Pers., *Panicum dichotomiflorum* Michx., and *Cyperus esculentus* L. (Table 4). Differences ($P < 0.05$) in the relative biomass of each weed species were not detected among the three treatments.

Weed growth was not associated with altered nematode populations until August of 1986, when *T. annulatus* and total phytoparasitic nematode levels were lower ($P < 0.05$) than in weed-free plots. In September, *T. annulatus* and total phytoparasitic nematode populations were lower ($P < 0.05$) in weedy habitats (Table 2). In the presence of weed competition, 16 of the 17 sugarcane FAA were significantly reduced (Table 3).

## Discussion

Augmentation of nematodes did not result in higher populations until nearly one full growing season had elapsed. Because most of the augmented nematodes were *M. incognita, M. javanica,* and *R. reniformis* (Table 1), it was not unexpected that *Meloidogyne* spp. populations were enhanced in the augmented habitats. The greenhouse-raised *R. reniformis* inoculum populations appear to have been favored by the tomato host, but not necessarily by sugarcane (3). We speculate that the occasionally low *Criconemella* spp., *Helicotylenchus* spp., *P. minor,* and total phytoparasitic nematode levels resulted from interspecific competition for available root space (3).

Infestations of augmented nematodes, especially *Meloidogyne* spp., in October 1986 were associated with significantly lower accumulations of free cysteine, histidine, proline, and serine than were the controls. The weed-nematode interaction between the six treatment combinations indicated that arginine levels were higher in WFC and WFA sugarcane than in weed-stressed and (or) nematode-stressed plants. Because FAA analyses were only done for one of the two years, FAA results, although preliminary, should be regarded as possibly indicative of trends. We suggest that *Criconemella* spp., *Helicotylenchus* spp., *T. annulatus,* and total phytoparasitic nematode levels may be related to changes in specific FAA accumulations. Host FAA concentrations are altered in other nematode-plant interactions (9–11, 15, 17, 18). Changes induced by sugarcane mosaic virus and weed stress in free cysteine are correlated ($r = 0.59$, $P < 0.001$) with *T. annulatus* populations (22). Further research on FAA relationships to plant stress may reveal a method by which to compare relative levels of plant stress.

TABLE 2. Effects of nematode augmentation, aldicarb treatment, and weed habitat on densities of nematode genera in Louisiana sugarcane, 1985–86.

| Nematode genus | Date | Number of nematodes/250 ml soil | | | | | | Orthogonal contrasts† | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | W+ | WC | WA | WF+ | WFC | WFA | W vs. WF | + vs. C | A vs. C |
| *Criconemella* | 14 June 1985 | 136 | 134 | 85 | 127 | 220 | 362 | — | — | — |
| | 7 Aug. 1985 | 231 | 373 | 196 | 214 | 130 | 135 | — | — | — |
| | 7 Oct. 1985 | 382 | 525 | 439 | 587 | 744 | 660 | a | — | — |
| | 28 May 1986 | 368 | 582 | 307 | 376 | 701 | 324 | — | ** | * |
| | 7 Aug. 1986 | 310 | 728 | 429 | 681 | 1,095 | 680 | — | — | — |
| | 7 Oct. 1986 | 229 | 818 | 676 | 747 | 1,032 | 916 | — | a | — |
| *Helicotylenchus* | 14 June 1985 | 12 | 0 | 8 | 87 | 7 | 46 | — | — | — |
| | 7 Aug. 1985 | 59 | 66 | 16 | 8 | 30 | 6 | — | — | * |
| | 7 Oct. 1985 | 9 | 0 | 0 | 0 | 53 | 4 | — | — | * |
| | 28 May 1986 | 10 | 32 | 0 | 15 | 58 | 52 | — | * | — |
| | 7 Aug. 1986 | 81 | 20 | 0 | 7 | 32 | 0 | — | — | — |
| | 7 Oct. 1986 | 25 | 45 | 30 | 41 | 52 | 0 | — | — | — |
| *Meloidogyne* | 14 June 1985 | 0 | 526 | 11 | 0 | 0 | 0 | — | — | — |
| | 7 Aug. 1985 | 56 | 0 | 8 | 0 | 0 | 0 | — | — | — |
| | 7 Oct. 1985 | 84 | 0 | 6 | 173 | 69 | 0 | — | * | — |
| | 28 May 1986 | 0 | 0 | 21 | 0 | 0 | 0 | — | — | — |
| | 7 Aug. 1986 | 37 | 0 | 0 | 52 | 0 | 23 | — | ** | — |
| | 7 Oct. 1986 | 32 | 77 | 53 | 387 | 0 | 14 | — | * | — |
| *Paratrichodorus* | 14 June 1985 | 0 | 14 | 8 | 0 | 0 | 13 | — | — | — |
| | 7 Aug. 1985 | 22 | 20 | 11 | 0 | 12 | 8 | — | — | — |
| | 7 Oct. 1985 | 156 | 55 | 88 | 194 | 58 | 21 | — | a | — |
| | 28 May 1986 | 22 | 0 | 50 | 7 | 40 | 13 | — | ** | — |
| | 7 Aug. 1986 | 155 | 138 | 16 | 251 | 122 | 0 | — | — | * |
| | 7 Oct. 1986 | 133 | 177 | 120 | 204 | 180 | 40 | — | — | * |
| *Pratylenchus* | 14 June 1985 | 20 | 43 | 79 | 31 | 54 | 108 | — | — | — |
| | 7 Aug. 1985 | 91 | 131 | 60 | 44 | 33 | 47 | — | — | — |
| | 7 Oct. 1985 | 182 | 102 | 20 | 108 | 64 | 15 | — | — | — |
| | 28 May 1986 | 126 | 117 | 21 | 70 | 94 | 56 | — | — | — |
| | 7 Aug. 1986 | 0 | 231 | 330 | 33 | 224 | 142 | — | — | * |
| | 7 Oct. 1986 | 106 | 234 | 162 | 211 | 222 | 342 | — | — | — |
| *Tylenchorhynchus* | 14 June 1985 | 164 | 14 | 187 | 170 | 16 | 193 | — | — | — |
| | 7 Aug. 1985 | 354 | 127 | 135 | 408 | 253 | 116 | — | — | — |
| | 7 Oct. 1985 | 420 | 0 | 0 | 91 | 0 | 0 | — | a | — |
| | 28 May 1986 | 0 | 0 | 0 | 0 | 0 | 0 | — | — | — |
| | 7 Aug. 1986 | 102 | 354 | 226 | 1,937 | 1,130 | 430 | *** | — | — |
| | 7 Oct. 1986 | 339 | 230 | 721 | 580 | 1,433 | 611 | * | — | — |
| Total phytoparasitic | 14 June 1985 | 332 | 731 | 378 | 415 | 297 | 722 | — | — | — |
| | 7 Aug. 1985 | 813 | 717 | 426 | 674 | 458 | 312 | — | — | — |
| | 7 Oct. 1985 | 1,233 | 682 | 553 | 1,153 | 988 | 700 | — | — | — |
| | 28 May 1986 | 526 | 731 | 399 | 458 | 893 | 445 | — | * | * |
| | 7 Aug. 1986 | 685 | 1,471 | 1,001 | 2,961 | 2,603 | 1,275 | ** | — | * |
| | 7 Oct. 1986 | 844 | 1,581 | 1,762 | 2,170 | 2,919 | 1,923 | * | — | * |
| Total nonphytoparasitic | 14 June 1985 | 1,097 | 1,681 | 1,233 | 1,252 | 1,030 | 3,512 | — | — | — |
| | 7 Aug. 1985 | 298 | 486 | 362 | 321 | 193 | 294 | — | — | — |
| | 7 Oct. 1985 | 1,214 | 804 | 1,287 | 1,483 | 1,075 | 909 | — | — | — |
| | 28 May 1986 | 922 | 762 | 653 | 788 | 774 | 457 | — | — | — |
| | 7 Aug. 1986 | 1,632 | 1,656 | 880 | 981 | 976 | 601 | — | — | — |
| | 7 Oct. 1986 | 1,013 | 1,318 | 1,181 | 1,172 | 1,306 | 710 | — | — | — |

Data are means of six replications. W+ = weedy, nematodes added; WC = weedy, control; WA = weedy, aldicarb; WF+ = weed-free, nematodes added; WFC = weed-free, control; WFA = weed-free, aldicarb.

†— = not significant; a = $P < 0.07$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$.

TABLE 3. Effects of nematode augmentation, aldicarb treatment, and weedy habitat on sugarcane-free amino acid concentrations in preharvest basal internodes, 1986.

| Free amino acid | Concentration (nmol/10 μl juice) | | | | | | Orthogonal contrasts‡ | | |
|---|---|---|---|---|---|---|---|---|---|
| | W+ | WC | WA | WF+ | WFC | WFA | W vs. WF | + vs. C | A vs. C |
| Alanine | 343.4 | 372.6 | 501.1 | 749.6 | 796.5 | 692.2 | *** | — | — |
| Arginine | 160.5 | 138.4 | 142.0 | 216.9 | 306.3 | 316.0 | *** | — | — |
| Asparagine | 1,245.1 | 1,236.4 | 1,158.0 | 1,715.0 | 1,835.5 | 2,265.8 | *** | — | — |
| Cysteine | 5.2 | 9.3 | 18.9 | 80.6 | 101.4 | 96.5 | *** | * | — |
| Glutamic acid | 207.1 | 213.8 | 209.2 | 190.8 | 216.3 | 250.4 | — | — | — |
| Glycine | 858.6 | 681.4 | 685.1 | 929.8 | 1,042.2 | 1,582.2 | ** | — | — |
| Histidine | 47.0 | 66.1 | 52.0 | 99.9 | 120.2 | 119.2 | *** | * | — |
| Isoleucine | 57.0 | 73.8 | 67.4 | 112.1 | 117.5 | 122.7 | *** | — | — |
| Leucine | 22.5 | 39.8 | 32.5 | 68.8 | 66.8 | 72.2 | ** | — | — |
| Lysine | 11.0 | 12.9 | 14.6 | 26.8 | 26.6 | 33.6 | ** | — | — |
| Methionine | 8.5 | 13.8 | 16.7 | 26.8 | 24.7 | 33.0 | * | — | — |
| Phenylalanine | 20.7 | 28.0 | 25.4 | 37.6 | 45.5 | 39.2 | * | — | — |
| Proline | 511.6 | 650.2 | 997.4 | 1,045.2 | 1,167.8 | 1,196.9 | *** | * | * |
| Serine† | 642.2 | 763.6 | 596.5 | 890.6 | 1,516.3 | 1,433.9 | *** | * | — |
| Threonine | 80.0 | 77.6 | 74.0 | 141.4 | 156.8 | 188.4 | *** | — | — |
| Tyrosine | 492.8 | 462.4 | 467.2 | 386.4 | 407.9 | 419.8 | ** | — | — |
| Valine | 139.1 | 155.8 | 144.1 | 241.0 | 244.5 | 273.6 | *** | — | — |
| Total† | 4,711.0 | 4,996.0 | 5,201.9 | 6,959.3 | 8,192.8 | 9,135.4 | *** | — | — |
| Total-serine† | 4,068.8 | 4,232.3 | 4,605.3 | 6,068.7 | 6,676.5 | 7,701.7 | *** | — | — |

Data are means of six replications. W+ = weedy, nematodes added; WC = weedy, control; WA = weedy, aldicarb; WF+ = weed-free, nematodes added; WFC = weed-free, control; WFA = weed-free, aldicarb.

† Because serine comigrated with glucosamine during HPLC analysis, results for serine and total free amino acids may not be accurate.

‡— = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.005$.

Although aldicarb has been reported to increase sugarcane yields (4), it failed to provide long-season control of phytoparasitic nematodes in our, and other, sugarcane studies (27). Aldicarb may merely impede nematode migration in the soil instead of causing mortality (14,15,24). The increased free proline levels in aldicarb-treated cane could be a result of reduced phytoparasitic nematode populations, or an effect of the aldicarb itself.

Baird and Bernard (1) found that nematode population trends in soybean/wheat cropping systems require nearly one full

TABLE 4. Relative biomass of annual monocot weed genera and absolute total weed biomass in weedy nematode-augmented, control, and aldicarb-treated sugarcane habitats averaged over 1985 and 1986.

| Treatment | Month | Percentage of total weed biomass | | | | | | Total biomass (g/0.5 m²) |
|---|---|---|---|---|---|---|---|---|
| | | *Panicum* | *Brachy-aria* | *Digi-taria* | *Echino-chloa* | *Cynodon* | *Cyperus* | |
| Nematode-augmented | June | 1.8 | 5.8 | 85.4 | 2.7 | 4.2 | 0.6 | 73 |
| | Aug. | 0.6 | 4.1 | 91.8 | 0.4 | 3.3 | 0.0 | 88 |
| | Sept. | 2.4 | 1.0 | 83.8 | 0.4 | 9.5 | 0.0 | 36 |
| Control | June | 7.2 | 6.2 | 75.9 | 5.6 | 4.6 | 0.4 | 69 |
| | Aug. | 9.9 | 7.0 | 78.6 | 1.3 | 4.8 | 0.0 | 119 |
| | Sept. | 0.6 | 1.3 | 64.0 | 4.1 | 14.8 | 0.0 | 43 |
| Aldicarb-treated | June | 7.1 | 4.3 | 80.9 | 2.5 | 5.2 | 0.0 | 76 |
| | Aug. | 11.0 | 1.5 | 74.0 | 3.2 | 9.5 | 0.8 | 94 |
| | Sept. | 0.4 | 3.8 | 90.6 | 2.8 | 2.5 | 0.0 | 38 |

Significant ($P < 0.05$) differences not detected among treatments within months. Biomass of individual genera was determined using six replicates of two 0.5-m² quadrats per 0.02-ha subplot; total weed biomass was determined using six replicates of five 0.5-m² quadrats per subplot.

season to become apparent. Differences between phytoparasitic nematode populations in the two sugarcane weed regimes were not detected until midway through the ratoon season. Of the six phytoparasitic nematode groups encountered, *Criconemella* spp., *T. annulatus,* and total phytoparasitic nematodes were lower in the weedy plots. Although McSorley and Campbell (18) found that weed growth resulted in intensified *Pratylenchus brachyurus* and *R. reniformis* densities on avocado roots and that weeds can host many injurious nematodes (3), other studies have indicated that certain plants, including *Melilotus vulgare* (6), and *Digitaria decumbens* (12), may be antagonistic to nematode populations. We suggest that the different nematode species were variously suited to the different weed species.

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