An Evaluation of Biological Agents for Control of Citrus Nematode and Liohippelates Eye Gnat[1]

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Abstract

Biological agents have been employed in a series of experiments to evaluate their efficacy in control of the citrus nematode and Liohippelates eye gnat in the Yuma mesa area. Steinernema riobravis, an entomopathological nematode, considered climatically adapted to western Arizona temperatures, was selected from commercially available sources. Three field trials and a greenhouse study utilizing the nematodes at population rates of one and two billion juveniles per acre were each unsuccessful in reducing the nematode or insect pest. Poor viability and survival were attributed to the negative results in each of the investigations. The microbial nematicide, DiTera, which was included in two limited trials, was found to be highly effective in suppression of citrus nematode populations infecting Yuma citrus. These results have prompted Abbott Laboratories, manufactures of the product; to establish two demonstration plots in the Yuma mesa area.

Introduction

Since the cancellation of DBCP in the late 1970's, citrus growers here and throughout the world have been left without adequate protection against the citrus nematode, *Tylenchulus semipenetrans*. Trials to find an alternative for the compound have involved both chemical and biological agents, all with disappointing results. Either the method or chemical is cost prohibitive, environmentally unacceptable, or there is inadequate reduction of the multiple generations of nematodes that occur in the Yuma growing area

An insect nuisance pest also makes its home in the citrus soil of Yuma. The Liohippelates eye gnat, *Liohippelates* collusor, is quite annoying to homeowners and those who work in the citrus groves. They are annoying by flying into the eyes and can cause irritation and allergies. The small insect breeds in the disked soil in the citrus groves where chemical control, to date, has not proven satisfactory.

A single control procedure that would provide satisfactory and economic control of both the citrus nematode and the Liohippelates eye gnat is highly desirable. An environmentally friendly, biological control agent that could become established for a prolonged period of time and effectively control both pests economically would be ideal.

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Preliminary studies have been reported (1) using an obligate parasitic nematode for eye gnat control. Of the fifty-five described species in this group of entomopathological nematodes five or six are now commercially available (2). Various species of plant parasitic nematodes have also been found to be attacked by this group of parasites. *Steinernema riobravis* was discovered in the hot arid soils of lower Texas. In tests it has been demonstrated to infect both insects and nematodes. Because of its adaptability to climatic conditions similar to those of the Yuma area, the species was selected as the lead candidate for the evaluation of eye gnat and citrus nematode investigations.

In addition to the parasitic nematode, a recently discovered microbial produced nematicide was introduced in this study. It has not exhibited insect activity and was therefore not expected to have any affect on eye gnat populations. The active toxic ingredient in DiTera is produced by a common species of saprophytic soil fungus with cosmopolitan distribution (3). It is nontoxic to warm-blooded animals and has been given EPA exempt status from requirements of a tolerance on all agricultural commodities. It has been tested for control of the burrowing nematode attacking citrus with promising results.

The commercial availability of these biological agents and the encouraging results from reports of other researchers has prompted the selection of *Steinernema riobravis* and the experimental nematicide DiTera of Abbott Laboratories for evaluation in the control of citrus nematode and the Liohippelates eye gnat in Yuma citrus soil.

Materials and Methods

To evaluate the efficacy of *S. riobravis* in control of citrus nematode and the eye grant in Yuma citrus soil, three field trials were established. A trial under controlled greenhouse conditions was carried out utilizing known populations of pests and parasitic nematodes. DiTera, the microbial nematicide, was incorporated in one of the trials with the parasitic nematodes and applied alone in a fourth field trial in a commercial orange grove on the Yuma mesa.

Trial # 1.

In the first trial, a commercial, mature, bearing orchard of Minneola tangelos, located on the Yuma mesa (Kiva #6, Blk. 5) was selected for the study. Randomized plots were treated with two population levels of *S. riobravis* employing two delivery systems. The first and second treatments consisted of one and two billion nematodes per acre respectively and were applied by hand under each of the treated trees. The desired numbers of nematodes were mixed in four gallons of water and applied by hand to the soil surface beneath the tree from trunk to the skirt line. The treated area was immediately rototilled to a depth of six inches and flood irrigated within 24 hours of treatment. The same numbers of nematodes were used in additional treatments but delivered in approximately three-acre inches of flood irrigation water to soil that had been pre-irrigated. Each of the four treatments consisted of three replications with four trees in each replication. The same number of untreated trees served as controls for each treatment (Fig. 1). In the irrigation delivery treatments, trees were randomly selected from the treated area at varying distances from the point of application. Soil samples were collected from four sides of each of the trees, amalgamated, mixed, then 100 cc of the soil was placed on each Baermann funnel for extraction of nematodes.

Eye gnat populations were monitored using a cone-shaped modified boll weevil screened trap, one-meter square at the base. These were place in each of the treated areas. The trapped gnats were counted at various periods of time following treatment by removing the entrapment jar and visually counting the contained gnats.

Populations of citrus nematode and parasitic *S. riobravis* were determined by removing soil samples from four locations from beneath each tree, mixing the amalgamated samples and removing 100cc for placement on Baermann funnels. After four days, the water was drained from the funnels and the populations of nematodes contained therein were determined by aid of a dissecting microscope.

Trial # 2

Poor survival of the parasitic nematodes in the first trial resulted in a second trial in the same citrus groove that consisted of a single treatment of two billion *S. riobravis* per acre equivalent in flood irrigation water. Viability of the parasitic nematodes at time of application was approximately 85%. Sampling of pests and parasites followed the same procedure as in the first trial of the water run treatment. Gnats were collected on seven different dates over a five-month period from the May 30, 1996, application. Soil samples were removed two weeks after application and the nematode populations determined as in the first trial.

Trial #3

The poor survival of *S. riobravis* in the second trial was indicated by the low populations of that species recovered in all of the samples regardless of tree position in the treated area. With a pre-application viability of approximately 85%, the results indicated the nematode was unable to survive under the conditions of the trial. To insure that nematode prey was available for the parasite, a trial was established in the greenhouse under controlled conditions of temperature and with known populations of citrus nematodes available as food source. Treatments consisted of 1) 500 citrus nematodes, 2) 1000 *S. riobravis* and 3) 500 citrus nemas and 1000 *S. riobravis* per container of 500 cc of soil. Each treatment was replicated ten times and the entire contents of each container was screened for nematodes using the gravity wet screening technique in an effort to better determine the exact number of nematodes and their survival rate. Populations were counted using magnification provided by a dissecting microscope. Two containers of the ten per treatment were read on each of five dates following treatment. Efforts to establish populations of eye gnat for similar controlled trials were unsuccessful as the colonies continued to die before the nymphs had opportunity to mature.

Trial # 4

The fourth trial was established on the Yuma Mesa Agriculture Center The area selected was in the northeast corner of Block 10 planted to old Marsh grapefruit consisting of a single row per treatment with twelve trees each. Soil samples were taken from four trees randomly selected from each row. Treatments consisted of a single population level (2 billion) as in the first trial and also a single 200-gallon per acre treatment of the microbial nematocide, DiTera. Nematodes and the nematicide were both applied in approximately three-acre inches equivalent per acre of irrigation water, following a pre-irrigation, to thoroughly moisten the soil. Samples were taken as previously described and nematodes were extracted by the wet-sieve gravity screening method in an effort to better determine the survival of the nematode population. The eye gnat populations were not monitored in this trial.

Trial # 5

The fifth trial was a limited single application of DiTera at the rate of 200 gallons of the commercial material applied in the flood irrigation water. The commercial orange grove of Jayson Parriconi of the Yuma mesa was chosen for this trial as the citrus nematode population was found to be unusually high for the time of year; and, the application could be made with minimum effort to include the maximum number of trees in the treated area. The application was made on October 1, 1996, and samples were randomly taken from treated trees on the seventh of the same month and again on January 7, 1997.

Results and Discussion

The disappointing results obtained in all trials (Tables 1 through 6) with the entomopathological nematode *S. riobravis* could only be attributed to their very poor viability as received from the company that produced them. Discussion with representatives from Biosys indicated that our procedures in reviving the worms from the crytobiotic state were in error in that we had failed to aerate the cultures prior to their application. It was also suggested that even though there was no movement after hydration and before application, that upon entrance into the soil, they would be revived to a life state and begin to infest any prey encountered

To evaluate the validity of the supplied information, the greenhouse study was set up using new cultures of the same parasitic species of nematode as well as following all suggestions made by the company representatives. Results from this trial were similar to those previously described (Tables 3 and 4). The initial 85% viable *S. riobravis* juveniles were soon found to rapidly die. There appeared to be no indication of citrus nematode mortality as they continued to survive for the duration of the study even without a food source.

The differences between treated and non-treated eye gnat populations (Tables 2 and 4) in the first two trials cannot be attributed to the parasitic *S. riobravis*. The survival and longevity of the nematode was the same where gnats were trapped as in the treatments involving the citrus nematode. Since there was an observed reduction in the gnat populations in the parasitic nematode treatments, one may wonder if the inert carrier in which the nematode was supplied could have an affect on the eye gnat populations. It will be interesting to test this theory under controlled laboratory conditions with established populations of nematodes and treatments with the inert carrier. Such trials will begin as soon as the cultures of eye gnats are established.

DiTera, in its present formulation, has proven to have promising control potential for citrus nematode in the Yuma mesa soils (Table 7). While the trials in this report were limited due to both time and available material, the results warrant further investigation. Abbott Laboratories has been sufficiently impressed to commit resources to conduct at least two, and probably three, demonstration trials in citrus on the Yuma mesa. These will be monitored closely to evaluate their efficacy and compare the results with those obtained in our reported investigations.

References

(1) Sumner, C.P. 1996. Use of beneficial nematodes for eye gnat and citrus nematode control. The Arizona Citrus Newsletter. Vol. 3, No. 4, pp 1-2.

(2) Smart, G.C. 1995. Entomopathogenic nematodes for the biological control of insects. Jr of Nematology. Supp. Vol. 27, No. 4, pp 529-534.

(3) Anonymous 1996. DiTera, Biological Nematicide. Technical Bulletin. Abbott Laboratories. pp 1-27.

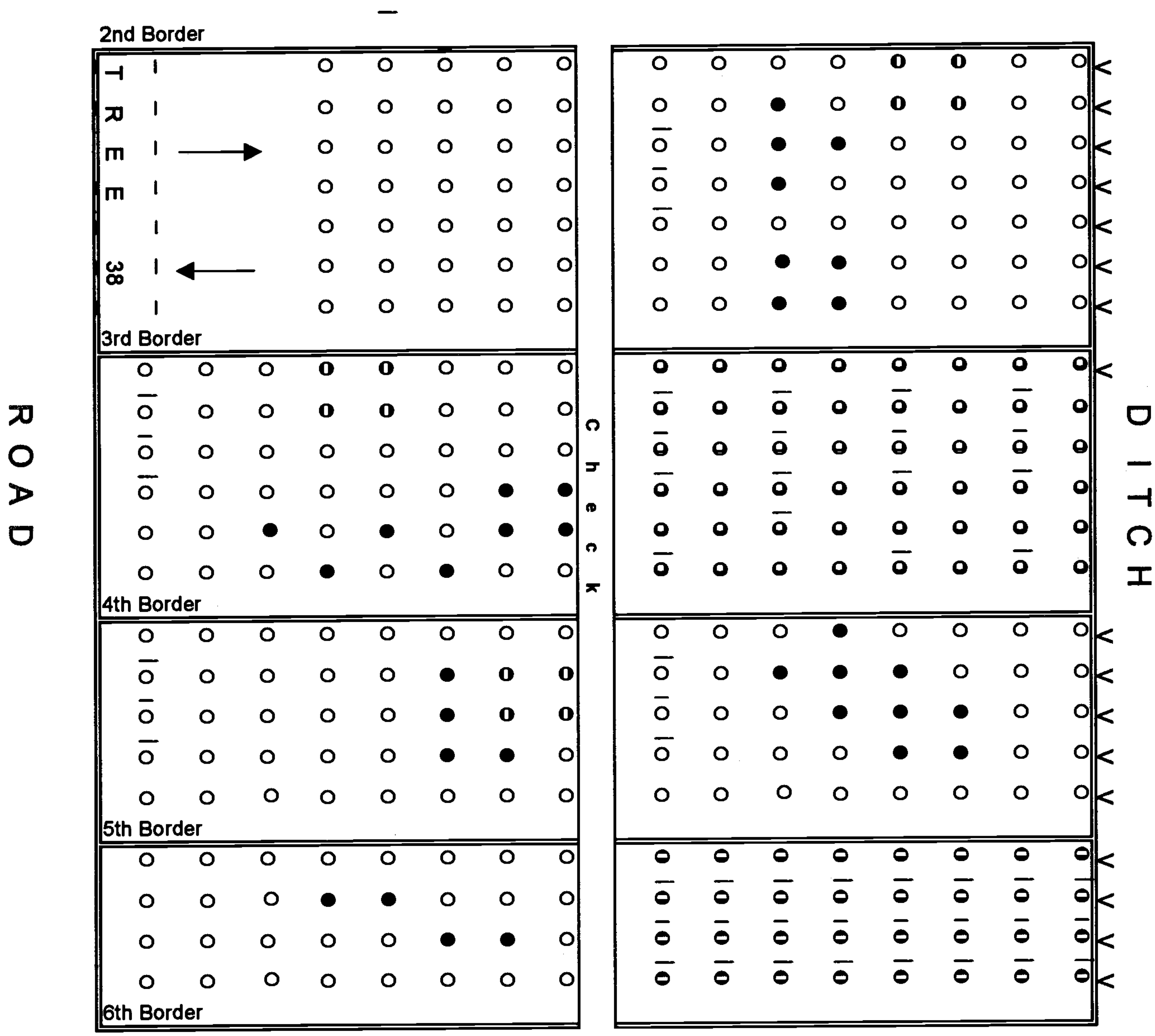
Figure 1

↑ N

C.V. Spencer, Inc. -- Yuma, Arizona

Minneola Tangelos, Kiva #6 Block 5

Biocontrol Trial of Citrus Nema and Eye Gnat - 1996



Legend:

< IRRIGATION TURNOUT

A) ● 2 BILLION ROTO TILLED

B) ⁰ 1 BILLION ROTO TILLED

C) ° 2 BILLION WATER RUN

D) ⁻ 1 BILLION WATER RUN

E) ● NON TREATED CONTROL

(Oranges) ↓

Table 1. Nematodes recovered following application [1] (Trial 1)

Treatment	22 April 1996			5 May 1996		
	Citrus Nema	*S. riobravis*	Other spp.	Citrus Nema	*S. riobravis*	Other spp.
A) 1 Billion S. riobravis/A (water run)	**31[2]**	**0**	**69**	**46**	**0**	**61**
B) 2 Billion S. riobravis/A (water run)	**9**	**5**	**104**	**128**	**7**	**19**
C) 1 Billion S. riobravis/A (roto tilled)[3]	**46**	**2**	**14**	**31**	**4**	**69**
D) 2 Billion S. riobravis/A (roto tilled)	**19**	**0**	**23**	**14**	**0**	**30**
E) Non Treated Control[4]	**28**	**0**	**46**	**32**	**0**	**17**

(1) Application of *S. riobravis* 15 April 1996.
(2) All numbers represent number per 100 cc soil averaged from 4 trees.
(3) Roto tilled soil following direct application of nemas to soil.
(4) Control replications and roto tilled treatment irrigated after applicaiton.

Table 2. Eye gnat captured following Treatment [1] (Trial 1)

Treatment	10 May 1996	16 May 1996
A) 1 Billion (water run)	**0[2]**	**1**
B) 2 Billion (water run)	**8**	**3**
C) 1 Billion (roto tilled)[3]	**7**	**1**
D) 2 Billion (roto tilled)	**0**	**4**
E) Non Treated Control	**0**	**0**

(1) Application 15 April 1996.
(2) All numbers represent average total number per/cage of 4-6 replications.

Table 3. Nematodes recovered following treatment [1] (Trial 2)

Tree Number	15 June 1996 (2 Billion/A) Citrus Nema	*S. riobravis*	Other spp.	Control Citrus Nema	*S. riobravis*	Other spp.
1[3]	19[2]	0	12	6	0	46
2	146	15	71	148	0	19
3	36	0	28	91	0	21
4	15	0	42	11	0	50
5	0	0	6	25	0	47
6	167	5	15	62	0	38
7	2	0	3	11	0	24
8	0	0	89	5	0	17
9	Missing	0	0	27	0	52
10	14	2	8	181	0	23

(1) *S. riobravis* applied 2 Billion/A in flood irrigation, 30 May 1996.
(2) Average number/100 cc soil from each sampled tree.
(3) Trees 1-4 - 1/3 distance of row from point of application.
Trees 5-7 - 1/2 distance of row from point of application.
Trees 8-10 - 2/3 distance of row from point of application.

Table 4. Eye gnat captured following Treatment [1] (Trial 2)

Sample Date	Non Treated Control	2 Billion *S. riobravis* /A
20-Jun	0[2]	0.6[2]
27-Jun	5[2]	1.2[2]
4-Jul	46[2]	17.7[2]
15-Oct	0.9[3]	0.2[3]
22-Oct	0.9[3]	0.1[3]
15-Nov	0.1[3]	1.7[3]
21-Nov	0[3]	1.3[3]

(1) Application 30 May 1996.
(2) Ave. number/10 reps before discing.
(3) Ave. number/8 reps after discing.

Table 5. Nematodes recovered following treatment.

(Trial 3 - Greenhouse)

Treatment[(1)]	12-Oct	19-Oct	30-Oct	5-Nov	12-Nov
1) Citrus nematode	306[(2)]	249	261	92	21
2) *S. riobravis*	117[(3)]	203	46	5	0
3) Citrus nema & *S. riobravis*	406 + 501	208 + 62	201 + 12	111 + 2	41 + 0

(1) (a) Citrus nema 500/pot (500cc soil); Greenhouse trial.
(b) *S. riobravis* 1,000/pot
(c) Citrus nema & *S. riobravis* 500 + 1,000.
All nemas added to pots, 5 Oct. 1996.

(2) Total number nemas recovered from 500 cc soil; gravity screening. Numbers are average of 5 pots.

(3) Number of *S. riobravis* are viable recoveries only.

Table 6. Nematodes recovered following treatment [(1)] (Trial 4)

	15 June 1996[(2)]			15 August 1996			6 September 1996			7 January 1997		
Treatments[(3)]	A	B	C(4)	A	B	C[(4)]	A	B	C(4)	A	B	C(4)
DiTera 200 gal/A	23	0	61	19	0	94	67	0	36	124	0	19
S. riobravis	317	7	49	107	0	28	115	0	62	674	0	33
Non Treated Control	0	0	0	98	0	35	181	0	46	896	0	0

(1) Treated 27 May 1996 - Blk 10; Yuma Mesa Agriculture Center.

(2) Sample dates.

(3) A = Citrus nema
B = *S. riobravis*
C = Other spp.

(4) Treatments made in approximately 2.5 acre inches of irrigation water.

Table 7. Nematodes recovered following treatment [1]
(Trial 5)

Treatment	21 October 1996	7 January 1997
DiTera 200 gal/A	89[2]	125
Non Treated Control	(5000)[3]	1288

[1] Applied 7 October 1996; Jayson Parricone Rch, Yuma Mesa.
[2] Average per 4 trees.
[3] Nemas recovered prior to treatment from 100 cc soil.