To: Lisa James

From: S. Patricia Stock, Assistant Professor
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Reference: ACRC Final Research Project Report
Project Title: Evaluation of entomopathogenic nematodes for the control of eye-gnats and reduction of the citrus nematode populations in Yuma Co

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Background and Objectives:

Research conducted in PI Stock's laboratory (2003 ACRC funded project) showed that the entomopathogenic nematode (EPN) *Steinernema riobrave* can cause reduction in the number of citrus nematode females per root and egg production when citrus-infected seedlings are exposed to this nematode. These preliminary stimulated consideration of EPN both for control of eye-gnats and citrus nematodes. EPN are very effective in controlling soil inhabiting-stages of various insect pests. At present many agricultural pests such as mole crickets and citrus weevils are successfully controlled by EPN in Florida and other states. Moreover, the EPN *S. feltiae* successfully control fungus-gnats in greenhouses. These studies clearly demonstrate, that given the right conditions and pathogen-host matching, EPN offer an environmentally-safe alternative for controlling agricultural and urban pests. With this background information our goal in this project was to consider commercially available EPN, *Steinernema riobrave* to test its performance in controlling citrus nematode populations in the field and also to assess the virulence of this nematode in controlling eye-gnat populations.

Results:

We have conducted one field trial using one entomopathogenic nematode species, *S. riobrave* (biocontrol agent), for control of citrus nematode. The nematodes are formulated as water-dissolved clay and were provided by Becker-Underwood™.
We chose the orange orchard across the Yuma Ag Center to conduct these trials, as the grower agreed on the use of this field for our essays. The orchard was sampled for accounting of citrus nematode populations, prior to the application of the nematode commercial formulation (See Table 1). The sampling was conducted last March. Number of egg masses (in the roots) and juveniles (in the soil) were accounted. *S. riobrave* was applied in June 2006 in three concentrations: 54, 108 and 532 nematodes/ cm^2. Effect of *S. riobrave* on citrus nematode populations was estimated at (See table 1). This evaluation consisted on collection soil and root samples to estimate level of citrus nematode infestation at the collection time. Scoring of citrus nematode populations was done on 3 and 4 months after application of S. riobrave.
Similarly eye gnat populations were recorded prior application of S. riobrave at two different periods

Citrus Nematode & Gnat Plot - Citrus Nematode Counts (Pre and post *S. riobrave* application)

Block # and EPN treatment	Row & Tree #	Pretreatment Citrus Nema J2 Counts 13 March '06	Post-treatment Citrus Nema J2 Counts 20 Sept. '06	Post-treatment Citrus Nema J2 Count 27 Oct. '06
Block 1 Treatment: 108 S. riobrave/cm^2	5 - 2	128.27	77.78	96.00
	5 - 3	114.02	47.39	56.18
	6 - 2	229.48	60.82	38.31
	6 - 3	717.65	8.68	182.41
Block 2 Treatment: 532 S. riobrave/cm^2	5 - 5	250.52	23.43	43.86
	5 - 6	368.98	115.48	359.18
	6 - 5	48.84	160.43	113.70
	6 - 6	308.68	125.52	111.11
Block 3 Treatment: 54 S. riobrave/cm^2	5 - 8	255.46	9.45	111.55
	5 - 9	80.20	35.90	45.77
	6 - 8	146.08	21.66	89.80
	6 - 9	40.28	112.22	487.05
Block 4 Treatment: 0 S. riobrave applied CONTROL	5 - 11	129.18	21.90	11.40
	5 - 12	366.26	39.89	4.63
	6 - 11	19.23	43.76	117.99
	6 - 12	42.93	7.62	36.74
Block 5 Treatment: 0 S. riobrave applied CONTROL	8 - 2	220.89	1.92	303.03
	8 - 3	521.74	0.00	250.45
	9 - 2	684.21	50.14	36.70
	9 - 3	291.26	78.87	276.03
Block 6 Treatment: 108 S. riobrave/cm^2	8 - 5	183.14	2.68	0.00
	8 - 6	275.41	5.88	123.52
	9 - 5	285.51	45.64	333.33
	9 - 6	450.87	157.52	598.80

Block 7 Treatment: 532 S. riobrave/cm^2	8 - 8	221.56	31.17	68.13
	8 - 9	196.86	22.99	28.67
	9 - 8	53.81	95.67	39.27
	9 - 9	276.28	52.93	52.57
Block 8 Treatment: 54 S. riobrave/cm^2	8 - 11	592.38	27.80	27.50
	8 - 12	101.01	2.34	117.65
	9 - 11	500.73	23.67	207.32
	9 - 12	715.71	28.17	225.99
Block 9 Treatment: 532 S. riobrave/cm^2	11 - 2	495.70	94.19	236.99
	11 - 3	612.15	46.51	458.02
	12 - 2	445.90	65.40	300.81
	12 - 3	484.32	235.92	498.46
Block 10 Treatment: 54 S. riobrave/cm^2	11 - 5	846.93	32.39	18.69
	11 - 6	766.13	6.33	9.05
	12 - 5	264.78	33.00	303.37
	12 - 6	604.17	34.04	135.59
Block 11 0 S. riobrave applied CONTROL	11 - 8	310.65	250.64	120.72
	11 - 9	555.19	420.28	770.00
	12 - 8	457.48	410.40	504.50
	12 - 9	254.68	176.87	64.00
Block 12 Treatment: 108 S. riobrave/cm^2	11 - 11	186.05	45.02	147.29
	11 - 12	581.12	89.31	41.74
	12 - 11	640.72	74.07	259.11
	12 - 12	278.12	161.51	246.91
Block 13 Treatment: 54 S. riobrave/cm^2	14 - 2	794.87	107.38	2758.62
	14 - 3	456.01	192.44	704.00
	15 - 2	648.87	207.02	156.90
	15 - 3	390.92	64.52	164.38
Block 14 0 S. riobrave	14 - 5	647.98	395.40	598.75
	14 - 6	538.50	77.80	180.59

applied CONTROL	15 - 5	623.55	135.28	517.80
	15 - 6	2318.37	413.79	360.36
Block 15 Treatment: 108 S. riobrave/cm^2	14 - 8	426.27	327.27	242.91
	14 - 9	103.49	18.02	44.59
	15 - 8	493.33	185.51	311.85
	15 - 9	1569.74	93.65	86.96
Block 16 Treatment: 532 S. riobrave/cm^2	14 - 11	447.89	19.30	309.46
	14 - 12	278.61	16.67	77.33
	15 - 11	938.07	132.53	221.50
	15 - 12	712.73	58.95	185.76

In yellow are indicated significant reductions accounted in citrus nematode populations ($P < 0.05$) after *S. riobrave* applications

Eye-Gnat population levels at pre-treatment and post-treatment with *S. riobrave*

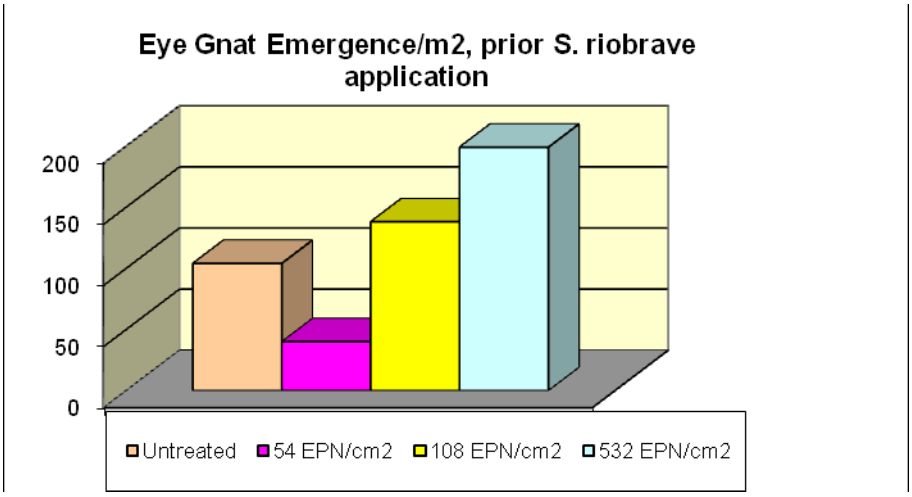

Figure A

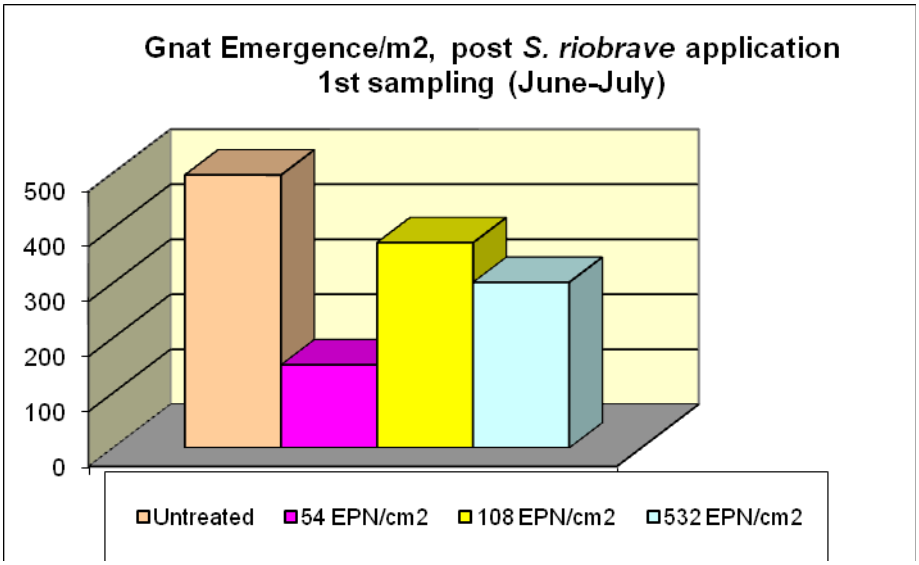

Figure B

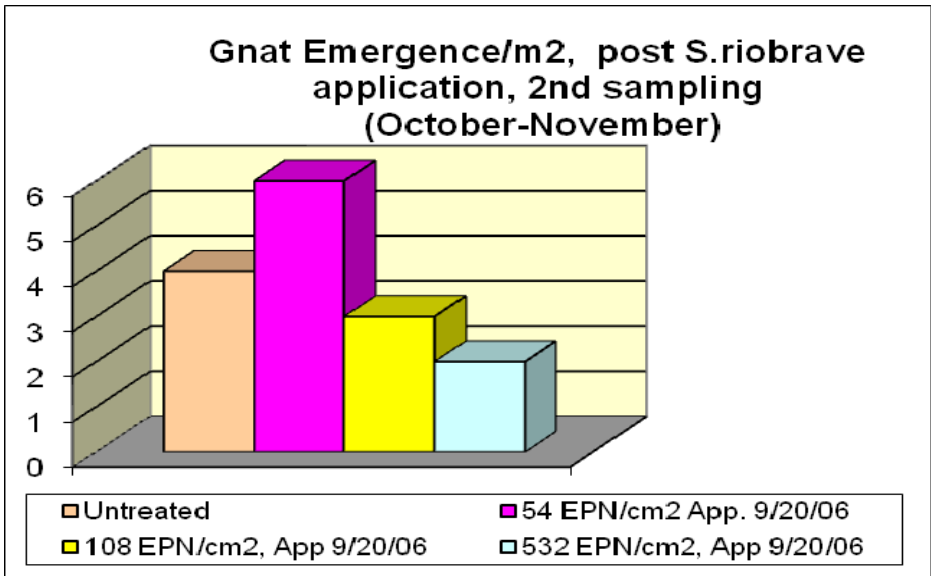

Figure C

Difficulties encountered:

In our original proposal we plan to do to applications of the EPN S. riobarave. Unfortunately the owner of the orchard where we run the first trial decided to get rid of this orchard by the end of November 2006. We hoped to select another orchard but this was not possible because Becker-Underwood, the company that provided us with the nematode formulation, did not have product availbale for a second trial.

Conclusions:

In spite of the difficulties encuountered at the techical levels, our data indicates that S. riobrave shows potential for controlling citrus nematode populations in the field. We

believe the best way of applying these nematodes should be improved, considering perhaps the option of nematode-infected insect cadavers. This technique has been proven to be more successful than the acqueous suspensions for controlling plant parasitic nematode populations.

With respect to the control of eye-gnats, the data indicates no control of these insects by the tested nematodes. We speculate these insects may not be susceptible to the infection by these nematodes. Moerover, the small size of the immature stages of these insects my also be a reason for the lack of success of these nematodes in controlling eye-gnat populations.