Steinernematid Nematode Infections of Pink Bollworm Larvae in Field Tests

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Abstract

Under field conditions, pink bollworm (PBW), Pectinophora gossypiella (Saunders), larvae were exposed to soil samples from plots treated with Steinernema riobravis at the rate of 5 nematodes per cm^2 of soil surface. Larval mortalities were 50% on the day of treatment and 2.5% on day 90 following treatment with irrigations about every 14 to 21 days,. Larval mortality percentages after exposure to soil samples from plots treated with S. carpocapsae at the rate of 5 per cm^2 of soil surface were 32.5, 15.3, 5.3 and 2.5 for the day of treatment and day 1, 7, and 15 following treatment, respectively. No further mortality occurred in bioassays conducted up to 90 days following treatment. With plots treated with 25 nematodes per cm^2 of soil surface, PBW larval mortalities ranged from 100% on the day of treatment to 7.5% on day 63 following treatment with S. riobravis and 92.5% on the day of treatment to 5% on day 7 following treatment with S. carpocapsae. Percentages of larval mortality after exposure to soil samples from plots treated with S. riobravis increased after each irrigation, but did not increase after exposure to soil samples from plots treated with S. carpocapsae.

Introduction

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), last stage larvae have been reported highly susceptible to the Kapow strain of *Steinernema carpocapsae* (Weiser) (Lindegren et al. 1992, 1993c) and *S. riobravis* Cabanillas, Poinar and Raulston (Henneberry et al. 1996). *S. riobravis* is also highly rated in relation to host searching efficiency (Lindegren et al. 1993b). PBW larvae develop in cotton squares or bolls tunnel out of the fruiting forms in the last larval stage to pupate in or on the soil (Butler and Henneberry 1976). This behavioral characteristic may offer an opportunity to consider biological control with nematodes during the growing season. Thus, persistence of a candidate soil inhabiting natural enemy would be an important factor in determining biological control of PBW. The urgent need for biological components of integrated management systems for PBW prompted us to compare the persistence of *S. carpocapsae* and *S. riobravis* in treated soil in the field as a factor in determining PBW biological control potential.

Materials and Methods

PBW larvae and pupae used in the studies were obtained from the laboratory culture at the USDA-ARS, Western Cotton Research Laboratory, Phoenix, AZ. Rearing methods were as described by Bartlett and Wolf (1985). *S. carpocapsae* and *S. riobravis* nematodes were reared using the *in vivo* method of Lindegren et al. (1993a).

Experiments 1 and 2 were conducted to determine the persistence of *S. riobravis* and *S. carpocapsae* nematodes under field conditions at Phoenix, AZ. The experiments were conducted in 27.4 m^2 plots arranged in randomized block designs with four replications. In experiment 1, treatments were *S. riobravis* or *S. carpocapsae* at the rate of five nematodes per cm^2 of soil surface. In experiment 2, treatments were with *S. riobravis* and *S. carpocapsae* at the rate of 25 nematodes per cm^2 of soil surface. In both experiments, all plots were furrow irrigated 1 day prior to treatment; nematodes were applied to the plots in 8 liters of water with a sprinkling can 1 day later. Control plots were treated with 8 liters of water alone. To simulate a typical Arizona cotton irrigation schedule, all plots were irrigated every 22-23 days for March through May in Experiment 1 and every 14 days in all other cases after nematode treatment. There were six irrigations in Experiment 1 and five irrigations in Experiment 2. Morning (0730 h) and midafternoon (1430 h) soil temperatures at 2.5 - 5.0 cm depths were taken with pocket dial thermometers (VWR, Phoenix, AZ) five days a week for the duration of each experiment. Soil samples from each plot, in each experiment, were taken on the day of treatment (Day 0), the day following treatment (Day 1) and at approximate 7-day intervals thereafter for 90 days in Experiment 1 and 63 days for Experiment 2. Soil was collected in 5-cm deep circular aluminum cylinders with a volume of 90.5 cm^3. All soil in each sample for each plot was spread evenly in individual 25 x 150 mm petri dishes and moistened with 3 ml of water. Ten last instar PBW larvae were released on the soil surfaces in each petri dish. Petri dishes with soil and larvae were held at 26.7° C in constant temperature boxes for 72 h, when dead and living larvae were recorded in each dish.

Results

During 28 March to 27 June average morning soil temperatures ranged from 62 to 81° F and midday temperatures from 84 to 109° F (Table 1). PBW larval mortality after exposure to soil from untreated control plots over the 90 days of the experiment, averaged 0.2 ± 0.2%. Larval mortality after exposure to soil samples treated with *S. riobravis* nematodes (5 per cm^2 of soil surface) ranged from 50 to 60% for the first 7 days following treatment. Percentage mortalities decreased to 28 and 10% on days 15 and 22 following treatment, respectively, and increased on day 28, 7 days after irrigation. Similar increases in PBW larval mortality occurred after exposure to soil samples 12, 5, and 4 days after irrigations on 10 and 24 May and 7 June. In contrast, PBW larval mortality after exposure to soil samples from plots treated with *S. carpocapsae* nematodes (5 per cm^2 of soil surface) was 33% on the day of treatment and decreased to 2.5% on day 15 following treatment. No further mortality occurred with larvae exposed to soil samples on days 22-90 following treatment.

From 25 April to 27 June, average morning and midday soil temperatures ranged from 60-84° F and 75-112° F, respectively (Table 1). Average PBW larvae mortality after exposure to soil from untreated plots was 0.5%. Mortality of PBW larvae exposed to soil samples from plots treated with 25 *S. riobravis* nematodes per cm^2 of soil surface ranged from 70 to 100% for the first 7 days following treatment. Mortality increased followed irrigations. Larval mortalities of PBW larvae exposed to soil from plots treated with 25 *S. carpocapsae*/cm^2 of soil surface were 92.5, 80.0, 5.0, and 0.0%, respectively, for soil samples on the day of treatment and 1, 7 and 15 days following treatment. No larval mortality occurred thereafter when larvae were exposed to soil samples taken on day 22 through 63 following treatment, irrespective of irrigation scheduling.

Discussion

The role of moisture in entomopathogenic nematode activity is well known (Poinar 1979), and the rate of nematode desiccation appears to play an important role in survival. Simons and Poinar (1973) showed that prolonged drying, as may occur under soil conditions, resulted in immobile, collapsed or twisted appearance, but *S. carpocapsae* readily revived when immersed in water. The phenomena of an exceptional *S. riobravis* survival mechanism in the soil was suggested from the results of our field tests where some PBW larval infection occurred for as long as 63-90 days as compared to 7-15 days for *S. carpocapsae*. Further, increasing levels of PBW larval infection in relation to irrigations indicated that gradual drying out of the soil environment between irrigations induced the gradual nematode desiccation and survival mechanisms discussed by Poinar (1979).

Both steinernematid nematode species used in our studies have a place in PBW integrated pest management programs. Additional field testing is being conducted to determine the most effective time, application rates and most efficacious nematode species in relation to PBW and cotton plant growth phenology. Gouge et al. (1996) reported significant reductions in PBW boll infestations and 19% increased cotton yield in cotton fields treated with *S. riobravis* compared with untreated fields. Thus, implementation of *S. riobravis* as a biocontrol agent for PBW control in cotton appears feasible.

References

Bartlett, A. C., and W. W. Wolf. 1985. *Pectinophora gossypiella*, pp. 415-430. *In* Sengh-Pond and R. F. More [eds.]. Handbook of Insect Rearing, Elsevier, Amsterdam.

Butler, G. D., Jr., and T. J. Henneberry. 1976. Biology, behavior and effects of larvae of pink bollworm in cotton flowers. Environ. Entomol. 5:970-972.

Gouge, D. H., L. L. Reaves, M. M. Stoltman, J. R. VanBerkum, R. A. Burke, L. J. Forlow Jech, and T. J. Henneberry. 1996. Control of pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) larvae using the entomopathogenic nematodes *Steinernema riobravis* (Cabanillas, Poinar and Raulston) (Rhabditida: Steinernematidae), pp. 1078-1082. *In* P. Dugger and D. Richter (eds.) Proc. Beltwide Cotton Conferences, Nashville, TN, National Cotton Council, Memphis, TN.

Henneberry, T. J., L. Forlow Jech, R. A. Burke, and J. E. Lindegren. 1996. Temperature effects on infection and mortality of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) larvae by entomopathogenic nematode species. Environ. Entomol. 25: 179-183.

Lindegren, J. E., K. A. Valero, and B. E. Mackey. 1993a. Simple *in vivo* production and storage methods for *Steinernema carpocapsae* infective juveniles. J. Nematol. 25: 193-197.

Lindegren, J. E., K. A. Valero and L. J. Forlow Jech. 1993b. Host searching response of various entomopathogenic nematodes: sand barrier bioassay, pp. 1044-1045. *In* Proc. Beltwide Cotton Prod. and Res. Conf. 1993, National Cotton Council, Memphis, Tenn.

Lindegren, J. E., K. F. Meyer, T. J. Henneberry, P. V. Vail, L. J. Forlow Jech, and K. A. Valero. 1993c. Susceptibility of pink bollworm (Lepidoptera: Gelechiidae) soil associated stages to the entomopathogenic nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Southwestern Entomol. 18: 113-120.

Lindegren, J. E., T. J. Henneberry, and L. Forlow Jech. 1992. Mortality response of pink bollworm to the entomopathogenic nematode *Steinernema carpocapsae*, pp. 930-931. *In* Proc. Beltwide Cotton Prod. and Res. Conf. 992, National Cotton Council, Memphis, TN.

Poinar, G. O., Jr. 1979. Nematodes for biological control of insects, pp. 128-159. CRC Press, Inc., Boca Raton, Florida.

Simons, W. R., and G. O. Poinar, Jr. 1973. The ability of *Neoaplectana carpocapsae* (Steinernematide: Nematoidea) to survive extended periods of desiccation. J. Invertebr. Pathol. 22:228-230.

Table 1. Mean (± SE)[a] Percentages of Pink Bollworm Larval Mortality After Exposure to Soil Samples from Irrigated[b] Untreated Field Plots or Field Plots Treated with *S riobravis* or *S. carpocapsae* at the Rate of 5 per cm^2 of Soil Surface for Experiment 1 and 25 per cm^2 for Experiment 2.

Days After		Average		Percent Mortality (± S.E.)		
Nematode	Last	Weekly Temps.		Treatment		
treatment	irrigation	0730 h	1430 h	Untreated	*S. riobravis*	*S. carpocapsae*
				Experiment 1 (March 28-June 27)		
0	1	66	84	0.0 ± 0.0 i	50.0 ± 12.3 a-c	32.5 ± 4.8 b-d
1	2	66	84	0.0 ± 0.0 i	60.0 ± 7.1 a	15.3 ± 6.4 d-h
7	8	68	90	0.0 ± 0.0 i	55.3 ± 20.5 a	5.3 ± 3.1 g-i
15	16	71	96	2.5 ± 2.5 hi	27.5 ± 6.3 c-e	2.5 ± 2.5 h-i
22	1	68	93	0.0 ± 0.0 i	10.0 ± 4.1 e-i	0.0 ± 0.0 i
28	7	62	85	0.0 ± 0.0 i	57.5 ± 13.2 ab	0.0 ± 0.0 i
35	14	72	103	0.0 ± 0.0 i	25.0 ± 12.6 d-f	0.0 ± 0.0 i
43	1	66	87	0.0 ± 0.0 i	12.8 ± 9.4 f-i	0.0 ± 0.0 i
48	5	73	97	0.0 ± 0.0 i	5.0 ± 5.0 g-i	0.0 ± 0.0 i
56	12	74	85	0.0 ± 0.0 i	20.0 ± 13.5 d-g	0.0 ± 0.0 i
63	5	81	107	0.0 ± 0.0 i	30.3 ± 23.4 cd	0.0 ± 0.0 i
69	11	71	100	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i
76	4	80	109	0.0 ± 0.0 i	20.0 ± 14.1 hi	0.0 ± 0.0 i
83	11	78	96	0.0 ± 0.0 i	2.5 ± 2.5 i	0.0 ± 0.0 i
90	4	---	----	0.0 ± 0.0 i	2.5 ± 2.5 i	0.0 ± 0.0 I
Seasonal Means:		---	----	0.2 ± 0.2 C	25.2 ± 39 A	3.7 ± 1.4 B
				Experiment 2 (April 25-June 7)		
0	1	60	75	0.0 ± 0.0 e	100.0 ± 0.0 a	92.5 ± 4.8 ab
1	2	60	75	2.5 ± 2.5 de	100.0 ± 0.0 a	80.0 ± 7.1 bc
7	8	71	103	0.0 ± 0.0 e	70.0 ± 2.4 c	5.0 ± 5.0 de
15	2	66	85	0.0 ± 0.0 e	10.0 ± 5.8 de	0.0 ± 0.0 e
21	8	75	103	0.0 ± 0.0 e	67.5 ± 17.9 c	0.0 ± 0.0 e
28	1	72	82	0.0 ± 0.0 e	82.2 ± 7.4 bc	0.0 ± 0.0 e
36	8	84	112	2.5 ± 2.5 de	20.0 ± 14.1 d	0.0 ± 0.0 e
42	1	71	98	0.0 ± 0.0 e	20.0 ± 16.8 d	0.0 ± 0.0 e
49	8	80	107	0.0 ± 0.0 e	7.5 ± 4.8 de	0.0 ± 0.0 e
56	1	78	93	0.0 ± 0.0 e	17.5 ± 6.3 d	0.0 ± 0.0 e
63	8	---	----	0.0 ± 0.0 e	7.5 ± 2.5 de	0.0 ± 0.0 e
Seasonal Means:		---	----	0.5 ± 0.3 C	45.7 ± 6.4 A	16.1 ± 5.1 B

[a] Means of 4 replications. Means in a row or column not followed by the same letter are significantly different. Seasonal means in a row not followed by the same capital letter are significantly different. In each $P \leq 0.05$, method of least significant differences.

[b] Irrigations of 0.3 m of water/0.4 ha on 3/28, 4/19, 5/10, 5/24, 6/7, and 6/21 for experiment 7 and 0.3 m of water/0.4 ha on 4/25, 5/9, 5/23, 6/16, and 6/20 for experiment 8.