Vertical Distribution in Soil, Persistence, and Efficacy Against Citrus Root Weevil (Coleoptera: Curculionidae) of Two Species of Entomogenous Nematodes (Rhabditida: Steinernematidae; Heterorhabditidae)

L. W. DUNCAN AND C. W. MCCOY

University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850

Environ. Entomol. 25(1): 174–178 (1996)

ABSTRACT Experiments were conducted in a 6-yr-old citrus orchard to test the efficacy of different formulations of the nematodes *Steinernema riobravis* (Cabanillas, Poinar & Raulston) and *Heterorhabditis bacteriophora* (Poinar) against a citrus root weevil, *Diaprepes abbreviatus* (L.), and to measure nematode persistence at different soil depths. One hour after application, >55% of both nematode species that were recovered in soil samples were at a depth of 0–1 cm and 33% of both species were recovered between depths of 3 and 15 cm. Numbers of *S. riobravis* at depths of 3–15 cm remained constant during the following 7 d, but numbers of *H. bacteriophora* declined 64%. During the same period, the numbers of both species recovered from depths of 0–1 cm declined by >99%. The data suggest that the lack of persistence reported for these nematodes in Florida citrus orchards is related to their vertical distribution in soil following application. Nine weeks after application, the numbers of *D. abbreviatus* at soil depths of 0–45 cm were reduced by 77 and 90%, respectively, in plots treated with liquid or granular formulations of *S. riobravis*. Neither formulation of *H. bacteriophora* affected the population density of *D. abbreviatus*.

KEY WORDS *Heterorhabditis bacteriophora*, *Steinernema riobravis*, efficacy, persistence, spatial patterns, vertical distribution

CONTROL METHODS THAT are currently available to manage citrus root weevil, *Diaprepes abbreviatus* (L.), in Florida citrus orchards often are ineffective. The weevils can kill citrus trees and frequently reduce fruit production in orchards to nonprofitable levels (McCoy 1995). Adult weevils feed on young leaves, but the significant damage to trees is caused by the feeding of all larval stages on fibrous roots of citrus and on the cortex of major roots (Wolcott 1936, Woodruff 1985). Adult weevils emerge from puparia and exit soil throughout the year, thereby reducing the effectiveness of managing weevils exclusively through control of adults.

Current approaches to managing this pest focus on pesticide sprays to reduce adult weevils, combined with methods to control larvae in soil (Knapp 1994). No chemical pesticides are approved for larval control, and the potential for groundwater contamination in Florida's sandy soils makes chemical control problematic. Three species of entomogenous nematodes, *Steinernema carpocapsae* (Weiser), *S. riobravis* (Cabanillas, Poinar & Raulston), and *Heterorhabditis bacteriophora* (Poinar), have demonstrated varying levels of efficacy for the control of larval stages of *D. abbreviatus*. *S. carpocapsae* was commercially available for several years to manage rootweevil larvae, but the efficacy of these nematodes was not demonstrated in recent field (Duncan et al. 1995b) or laboratory (Schroeder 1994) trials. *S. riobravis* became commercially available to citrus growers in Florida in mid-1994. Despite low survival of *S. riobravis* and *H. bacteriophora* in 2 Florida citrus orchards, some formulations of both nematodes reduced counts of weevil larvae in soil by 57–93% (Duncan et al. 1995b). Higher recovery of *H. bacteriophora* from soil beneath tree canopies than in the unshaded soil between tree rows (Duncan et al. 1995b) suggests that nematodes remaining near the soil surface after application are most vulnerable to mortality by factors such as ultraviolet radiation (Gaugler and Boush 1978) or low soil moisture (Duncan et al. 1995a).

In this article, we report results of 2 studies conducted in a central Florida citrus orchard. The efficacy of liquid and granular formulations of *S. riobravis* and *H. bacteriophora* against *D. abbreviatus* was measured. The vertical distribution of both nematode species following application also was measured to help determine whether low survivorship of these nematodes in Florida citrus or-

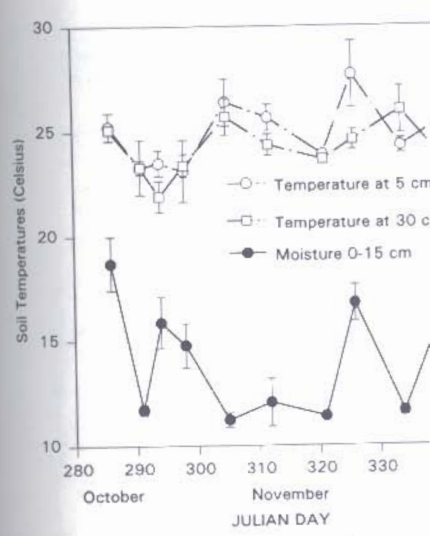

Fig. 1. Soil moisture and soil tempe canopies of 6-yr-old orange trees during tr persistence and efficacy against *D. abbrev* cies of entomogenous nematodes.

chards is related to factors that va depth.

Materials and Methods

Two experiments were conducted ly in a *D. abbreviatus*-infested orcha Hamlin orange trees on Carrizo citra The soil type was astatula fine sand silt:clay; field capacity, 6% H_2O). Tr gated using 1 microjet emitter per tre raked from beneath tree canopies ment, and trees were pre-irrigated w water in a circular area (1.2-m radiu were applied on 13 October 1994 1300 hours, under cloudy skies.

Nematode Efficacy and Persi treatments were applied in a random block design to trees of comparable Liquid and water-soluble granular f *S. riobravis* (Biosys, Palo Alto, CA) *iophora* (Ecogen, Langford, PA) wer to 10 trees, and 10 trees served as trols. The granular formulation of commercially available under the tr vector 355. Two million nematodes applied in 7.5 liters of water in th area (90 by 90 cm) of the tree using Plots were irrigated with 38 liters c nematode application and for 1 h t ment. Trees were irrigated 2–3 tim ing the experiment, and soil moistu temperature were monitored perio

Nematode population densities a 15 cm beneath each tree were mon 0, 7, and 14 after treatment. Soil sar cessing were according to previo

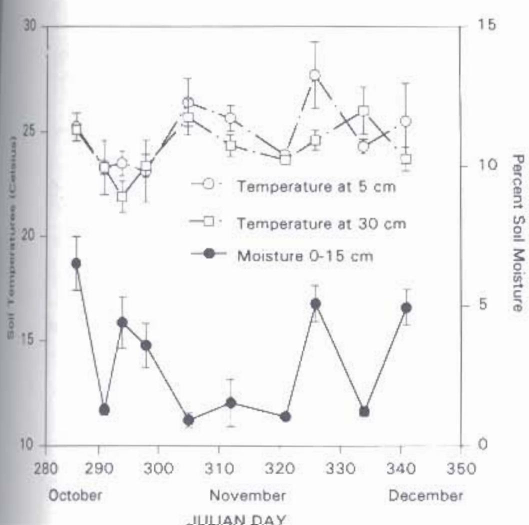

Fig. 1. Soil moisture and soil temperature beneath canopies of 6-yr-old orange trees during trials to measure persistence and efficacy against *D. abbreviatus* of 2 species of entomogenous nematodes.

chards is related to factors that vary with soil depth.

Materials and Methods

Two experiments were conducted simultaneously in a *D. abbreviatus*-infested orchard of 6-yr-old Hamlin orange trees on Carrizo citrange rootstock. The soil type was astatula fine sand (92:2:6 sand:silt:clay; field capacity, 6% H_2O). Trees were irrigated using 1 microjet emitter per tree. Debris was raked from beneath tree canopies before treatment, and trees were pre-irrigated with 38 liters of water in a circular area (1.2-m radius). Treatments were applied on 13 October 1994 during 0900–1300 hours, under cloudy skies.

Nematode Efficacy and Persistence. Five treatments were applied in a randomized complete block design to trees of comparable size and vigor. Liquid and water-soluble granular formulations of *S. riobravis* (Biosys, Palo Alto, CA) and *H. bacteriophora* (Ecogen, Langford, PA) were each applied to 10 trees, and 10 trees served as untreated controls. The granular formulation of *S. riobravis* is commercially available under the trade name Biovector 355. Two million nematodes per tree were applied in 7.5 liters of water in the undercanopy area (90 by 90 cm) of the tree using watering cans. Plots were irrigated with 38 liters of water during nematode application and for 1 h following treatment. Trees were irrigated 2–3 times weekly during the experiment, and soil moisture and ambient temperature were monitored periodically (Fig. 1).

Nematode population densities at a depth of 0–15 cm beneath each tree were monitored on days 0, 7, and 14 after treatment. Soil sampling and processing were according to previously described methods (Duncan et al. 1995a), except that 1 rather than 2 soil samples per plot were collected. Nematode identification was based only on gross morphology at 40× magnification using an inverted compound microscope. The experiment was terminated 9 wk after treatment. Trees were uprooted and soil beneath trees and in the crown area of the root system was sampled to recover larvae of *D. abbreviatus* as previously described (Duncan et al. 1995a). Briefly, 2 containers of soil (18.75-liter capacity) were obtained from each of 4 quadrants 60–90 cm from the spot previously occupied by the tree trunk. One container at each quadrant was filled with soil at depths of 0–15 cm and the other with soil at depths of 15–45 cm. Larvae were separated from soil by sieving and counted. Larvae were also washed from the crown of the root system onto a plastic tarpaulin and counted. Counts of *D. abbreviatus* were subjected to analysis of variance (ANOVA), and differences between means were separated using the Tukey honestly significant difference test.

Vertical Distribution and Persistence of Nematodes in Soil. Two trees were selected adjacent to the experiment described above. One tree was treated with the granular formulation of *S. riobravis* and the other with the granular formulation of *H. bacteriophora*. Treatments were applied and trees maintained as in the adjacent experiment. Soil beneath each tree was sampled at 1 h and 2 and 7 d after treatment to estimate the vertical distribution and persistence of each nematode species. Four samples were obtained from each tree at each sampling date. Each sample consisted of 6 cores (2–cm diameter) of soil at depths of 0–1, 1–3, and 3–15 cm. Cores were obtained randomly from the irrigated zone, beneath tree canopies, at distances of 30–60 cm from the tree trunk. Soil was transported to the laboratory, where it was mixed and nematodes were extracted from 10 cm^3 subsamples by a modification of the centrifugal flotation method (Jenkins 1964). Soil was mixed with 1 M sucrose and centrifuged 4,000 × *g* for 30 s, after which nematodes were collected on a 500-mesh sieve. Counts of nematodes from 10 cm^3 of soil were adjusted to reflect numbers of nematodes per sample and numbers of nematodes per 100 cm^3 of soil.

Results

Nematode Efficacy and Persistence. Numbers of *D. abbreviatus* recovered from both soil depths in plots treated with either formulation of *S. riobravis* declined ($P = 0.05$) compared with numbers in control plots (Fig. 2). In soil at depths of 0–45 cm, the liquid and granular formulations of *S. riobravis* reduced *D. abbreviatus* by 77 and 90%, respectively. Compared with control trees, no differences were detected in the relatively small numbers of *D. abbreviatus* recovered from crowns of trees treated with either formulation of *S. rio-*

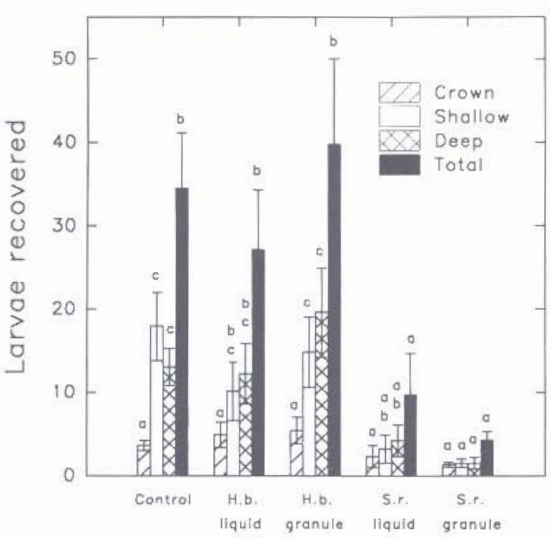

Fig. 2. Efficacy of liquid and granular formulations of *S. riobravis* (S.r.) and *H. bacteriophora* (H.b.) against *D. abbreviatus*. Weevil larvae were washed from uprooted root scaffold (crown), and were recovered from soil samples taken at depths of 0–15 cm (shallow) and 15–45 cm (deep) beneath tree canopy. Means for a given sample location with the same letter are not different ($P = 0.05$) according to the Tukey HSD test.

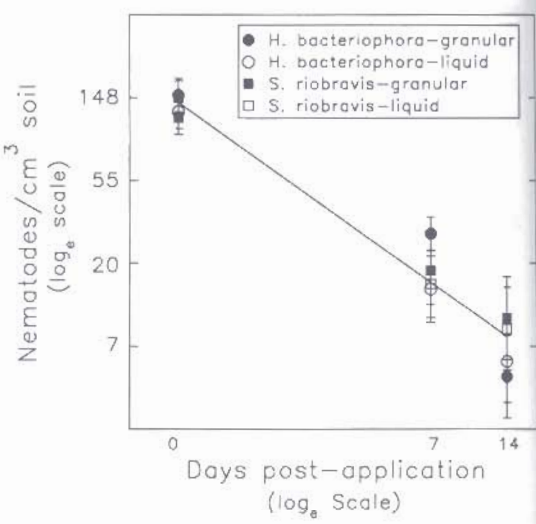

Fig. 3. Persistence of 3rd-stage larvae of *S. riobravis* and *H. bacteriophora* during 2 wk in soil beneath canopies of 6-yr-old Hamlin orange trees on Carrizo citrange rootstock.

bravis. Neither formulation of *H. bacteriophora* affected recovery of *D. abbreviatus* from the soil or crowns of trees.

The numbers of *S. riobravis* from the granular formulation recovered at 7 and 14 d, after application were 17 and 7% as numerous, respectively, as on day 0 (Fig. 3). The corresponding comparisons of the liquid formulation of *S. riobravis* were 16 and 8%. Twenty-three percent and 14% as many *H. bacteriophora* from the granular formulation were recovered on days 7 and 14 respectively, after application as on day 0. The corresponding comparisons for the liquid formulation of *H. bacteriophora* were 16 and 7%.

Vertical Distribution and Persistence of Nematodes in Soil. The background level of steinernematid–heterorhabditid nematodes in control plots was estimated for each sample date at 2.5 nematodes per 100 cm^3 of soil (SE = 1.3). One hour following application, the majority of both nematode species recovered were at depths of <1 cm soil (Fig. 4A). One-third of the nematodes of both species recovered were found below a depth of 3 cm. Based on total nematode recovery, the proportions of *S. riobravis* recovered at depths of 0–1, 1–3, and 3–15 cm were 0.56, 0.11, and 0.33, respectively. The corresponding proportions for *H. bacteriophora* (0.60, 0.07, and 0.33) were very similar to those of *S. riobravis*. During 7 d following application, the numbers of both species recovered from soil depths of <1 cm declined by >99%. Numbers of *S. riobravis* at 1–3 cm declined by 69%, but no change was detected in numbers of nematodes recovered below 3 cm. Numbers of *H. bacteriophora* recovered from depths of 1–3 and 3–15 cm declined during 7 d by 76 and 64%, respectively.

Discussion

Steinernema riobravis demonstrated good potential as a biocontrol agent of *D. abbreviatus* in the sandy soils of central Florida citrus orchards. The commercial formulation (granules) of *S. riobravis* provided ≈90% control of weevil larvae, 9 wk after application, a level comparable with that found previously for weevil larvae in the crown and throughout the soil profile (Duncan et al. 1995b). It is likely that the nematode can be applied effectively and economically through microirrigation systems or through herbicide applicators to distribute nematodes in a manner similar to that obtained in this study. Moreover, the cost of a single application of nematodes is currently less than those to apply registered biocides for control of soilborne nematodes, insects, and fungi in citrus.

Despite its potential as a management tool, key questions require further investigation to use *S. riobravis* effectively in a program to manage *D. abbreviatus* in Florida citrus. There are no reports of nematode performance against *D. abbreviatus* in heavier soils common in the coastal and interior flatwoods regions of the state. Thus, the influence of soil texture on nematode survival and efficacy (Kung et al. 1990) requires further study in the field. The persistence of efficacy against *D. abbreviatus* is unknown, so that optimum timing and frequency of treatments cannot be determined accurately at present. Similarly, accurate determination of the influence of storage time on the efficacy

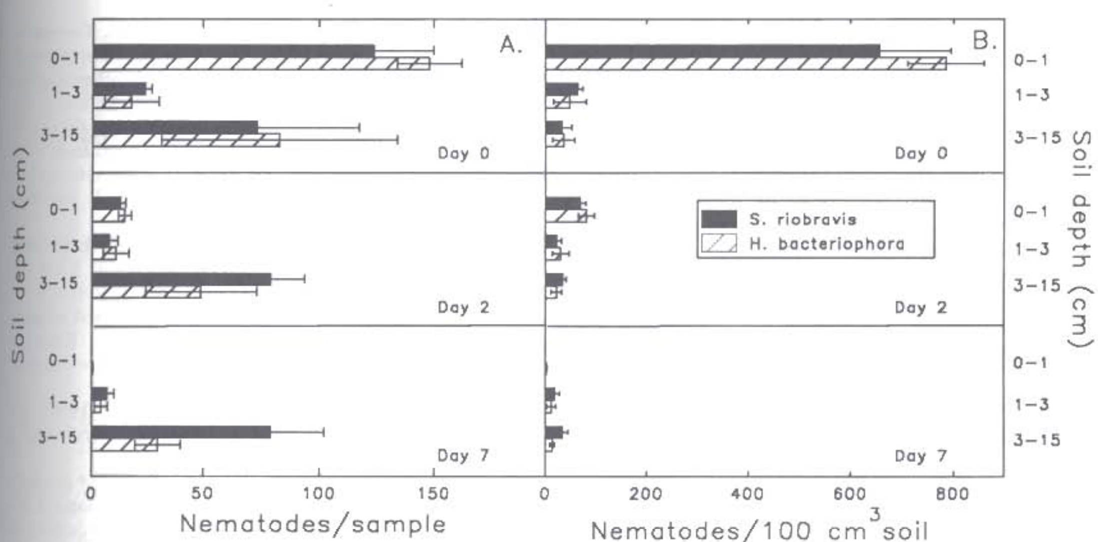

Fig. 4. Persistence of 3rd stage larvae of *S. riobravis* and *H. bacteriophora* at 3 soil depths during 7 d beneath canopies of 6-yr-old Hamlin orange trees on Carrizo citrange rootstocks.

of the present formulation is needed. These and other published data (Duncan et al. 1995b, Schroeder 1994) were derived using freshly formulated nematodes, whereas, commercial recommendations permit storage of the granules for 30 d. Finally, the effect of managing rootweevil larvae on tree growth and yield has not been determined.

The reason for lack of performance by *H. bacteriophora* in this study is unknown. Previously, liquid formulations of *H. bacteriophora* reduced population densities of *D. abbreviatus* larvae (Duncan et al. 1995b) and adults emerging from soil (Schroeder 1990, Downing et al. 1991). Differences in study conditions or the quality of nematode inoculum are possible reasons for the variability in experimental results.

The persistence of both nematode species was somewhat higher than previously reported under similar conditions. Fourteen days following application, population densities had declined to levels attained in 1 wk in several previous studies (Duncan et al. 1995b). The reasons for this discrepancy are unknown. Application of nematodes during microjet irrigation may enhance survivorship initially, in contrast to previous studies in which plots were irrigated following nematode application.

The vertical distribution in soil of these nematodes following application suggests that lack of persistence of entomogenous nematodes in Florida citrus may be caused by mortality related to factors associated with depth in soil. Migration of nematodes from the treatment area may account for some of the reduction in population density over time. However, few *H. bacteriophora* were recovered 1 m beyond the treatment zone under similar conditions in a previous study (Duncan et al. 1995b). Similarly, it is not possible to infer from these data how many nematodes migrated more deeply in soil following the initial measurements on day 0. Population density at subsequent times did not increase in the deeper horizons, suggesting that downward migration was negligible or that mortality was high at all soil depths. Moreover, *S. riobravis* exhibited negative geotropic migration in soil in the laboratory (Duncan et al. 1995a). Published results suggest that soil moisture may have affected the persistence of these nematodes. Soil moisture ranged between 1.5 and 6.0% during the first 7 d following nematode application. Survivorship of *S. riobravis* in vitro increased in sandy soil as soil moisture increased from 0.5 to 2.0% and declined as soil moisture increased from 2.0 to 12.0% (Duncan et al. 1995a). Those data suggest that anhydrobiotic survival of *S. riobravis* may commonly occur in Florida's sandy soils, particularly near the soil surface where evaporation can quickly reduce soil water potential. However, if moisture depletion occurs very rapidly, as it can at the soil surface, nematodes cannot complete metabolic changes necessary to enter an anhydrobiotic state (Womersley 1990). Alternatively, if soil desiccation during the daytime is followed by rehydration from rainfall or dew at night, alternating cycles of anhydrobiotic-related metabolic events in the nematodes may reduce their anhydrobiotic capability (Duncan 1986). Consequently, high mortality in the large numbers of nematodes remaining near the soil surface may result from rapid or repeated desiccation in addition to factors such as exposure to ultraviolet radiation (Gaugler and Boush 1978). Survival of nematodes during 7 d in vitro in sandy soil at moisture levels of 4–8% was ≈50% (Duncan et al. 1995a). The overall survival rate measured in the current study at 7 d after application (16–23%) could have occurred if 1/2 of the 33% of the nematodes found at >3 cm depth survived, whereas most nematodes nearer the soil surface died.

In summary, 2 formulations of *S. riobravis* effectively reduced numbers of *D. abbreviatus* larvae in soil compared with controls, whereas neither formulation of *H. bacteriophora* affected numbers of weevils. Rapid reduction in numbers of both nematode species following application to soil may be caused partly by a propensity for large numbers of nematodes to remain near the soil surface. The use of *S. riobravis* to manage *D. abbreviatus* in Florida citrus appears promising depending upon 3 criteria: (1) the persistence of economically feasible weevil control caused either by nematode recycling or repeated nematode applications, (2) tree and yield responses to these levels of control, and (3) the quality and storage capacity of commercial formulations of *S. riobravis*.

Acknowledgments

The authors are grateful for the assistance of Jerome Fojtik, Denise Dunn, Karin Crosby, Steven Rogers, Dan Lacey, and Mark Bryan. This is Florida Agricultural Experiment Station Journal Series No. R-04418.

References Cited

Downing, A. S., C. G. Erickson, and M. J. Kraus. 1991. Field evaluations of entomopathogenic nematodes against citrus root weevils (Coleoptera: Curculionidae) in Florida citrus. Fla. Entomol. 74: 584–586.

Duncan, L. W. 1986. Effects of bare fallow on plant-parasitic nematodes in the Sahelian zone of Senegal. Rev. Nematol. 9: 75–81.

Duncan, L. W., D. C. Dunn, and C. W. McCoy. 1995a. Spatial patterns of entomopathogenic nematodes in microcosms: implications for laboratory experiments. J. Nematol. (in press).

Duncan, L. W., C. W. McCoy, and A. C. Terranova. 1995b. Sample size and persistence of entomogenous nematodes in sandy soils and their efficacy against the larvae of *Diaprepes abbreviatus* in Florida. J. Nematol. (in press).

Gaugler, R., and G. M. Boush. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoaplectana carpocapsae*. J. Invertebr. Pathol. 32: 291.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48: 692.

Knapp, J. L. 1994. 1994 Florida citrus pest management guide SP-43. Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, University of Florida, Gainesville.

Kung, S. P., R. Gaugler, and H. K. Kaya. 1990. Soil type and entomopathogenic nematode persistence. J. Invertebr. Pathol. 55: 401–406.

McCoy, C. W. 1995. Past and current IPM strategies to combat the spread of *Diaprepes abbreviatus* (L.) in Florida citrus. Proc. Caribbean Food Crops Soc. 30: 247–256.

Schroeder, W. J. 1990. Suppression of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) adult emergence with soil application of entomopathogenic nematodes (Nematoda: Rhabditida). Fla. Entomol. 73: 680–683.

1994. Comparison of two steinernematid species for control of the root weevil *Diaprepes abbreviatus*. J. Nematol. 26: 360–362.

Wolcott, G. N. 1936. The life history of *Diaprepes abbreviatus* at Rio Piedras, Puerto Rico. J. Agric. Univ. PR 20: 883–914.

Womersley, C. Z. 1990. Dehydration survival and anhydrobiotic potential, pp. 117–137. *In* R. Gaugler and H. K. Kaya [eds.], Entomopathogenic nematodes in biological control. CRC, Boca Raton, FL.

Woodruff, R. E. 1985. Citrus weevils in Florida and the West Indies: preliminary report on systematics, biology, and distribution (Coleoptera: Curculionidae). Fla. Entomol. 68: 370–379.

Received for publication 13 March 1995; accepted 15 September 1995.

Temperature Ef
Pectinophora gossyp
by Two Ento

T. J. HENNEBERRY, L. F

Western Cotton Research Labora

ABSTRACT *Steinernema*
Pectinophora gossypiella (Sa
peratures >21.0°C, mortalit
occur until 2–6 d later. In s
mortality by *S. riobravis* ar
from 21.0 to 32.2°C. The ac
S. riobravis. Temperatures c
in decreased numbers of ne
of dead larvae with dead or

KEY WORDS *Steinerner*
pathogenic nematodes

TEMPERATURE HAS BEEN reported
environmental parameter in surviva
tion, development, and reproduct
pathogenic nematodes (reviews b
Woodring and Kaya 1988). In lab
Lindegren et al. (1993c) reported
tality of pink bollworm, *Pectinop*
(Saunders), larvae from *Steinerne*
(Weiser) infection at temperatur
21.1 to 32.2°C. Lower (47.2%) pink
mortality occurred at 15.6°C. Raul
reported an indigenous *Steinerner*
ing prepupae and pupae of the co
icoverpa zea (Boddie), and fall arm
tera frugiperda (J. E. Smith), in
Valley, Texas. Cabanillas et al. (19
described it as *S. riobravis* Cab
Raulston. In preliminary studies,
bollworm larvae exceeded 90%
ranging from 19 to 38°C (unpubli
tionally, Lindegren et al. (1993b)
riobravis was the most efficacious
ogenic nematode species in host
greater wax moth, *Galleria mello*
field, the higher temperature tol
was more effective against pink b
temperatures ranging from 26.5 to
pocapsae (Lindegren et al. 1994)

This article reports the results of resea
proprietary product does not constitute an
ommendation for its use by USDA.

[1]Horticultural Crops Research Laborat
South Peach Avenue, Fresno, CA 93727