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Pathogenicity of *Steinernema riobravis* against corn earworm, *Helicoverpa zea* (Boddie)

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Summary – The pathogenicity of a new entomopathogenic nematode species, *Steinernema riobravis* against prepupae and pupae of corn earworm, *Helicoverpa* (= *Heliothis*) *zea* (Boddie) found in soil samples of corn fields in the Lower Rio Grande Valley of Texas, was tested under laboratory conditions. Exposure to 10, 20, 40, 80, and 100 infective juveniles per prepupa in filter paper resulted in mortalities of 40, 55, 85, 90, and 100 % respectively. The LC_{50} of *S. riobravis* for *H. zea* prepupae was thirteen nematodes per prepupa. The nematodes multiplied similarly in prepupae and pupae of corn earworm with average nematode production per prepupa and pupa cadavers of 321 000 and 300 000, respectively. Production of nematodes was independent of concentration of infective juveniles from 5 to 100 per host. The overall yield of nematodes per prepupa and pupa was 311 000. The highest average yield of nematodes per insect cadaver was 375 000 which occurred at an exposure concentration of 40 infective juveniles per prepupa. These results indicate a high degree of infectivity and parthogenicity of *Steinernema riobravis* and its symbiotic *Xenorhabdus* bacterium to corn earworm and suggest it may have a great potential against prepupal and pupal stages of *H. zea*.

Résumé – ***Pathogénie de* Steinernema riobravis *envers la chenille des épis du maïs,* Helicoverpa zea *(Boddie)*** - La pathogénie de *Steinernema riobravis,* nouvelle espèce de nématode entomopathogène, a été testée au laboratoire contre les stades prépupes et pupes de la chenille des épis du maïs, *Helicoverpa* (= *Heliothis*) *zea* (Boddie). L'exposition de prépupes sur papier filtre à 10, 20, 40, 80 ou 100 juvéniles infestants provoque une mortalité de 40, 55, 85, 90 et 100 %, respectivement. La LC_{50} de *S. riobravis* pour le stade prépupe de *H. zea* est de treize nématodes par prépupe. Les nématodes se multiplient de la même manière dans les prépupes et les pupes avec une production moyenne de 321 000 et 300 000 nématodes par prépupe et pupe, respectivement. Entre 5 et 100 juvéniles par hôte, la reproduction du nématode est indépendante de la concentration initiale en juvéniles infestants. Le nombre total de nématodes produits par prépupe et pupe est de 311 000. Le plus grand nombre de nématodes produits par insecte est de 375 000 lorsque la concentration initiale en juvéniles infestants est de 40 par prépupe. Ces résultats indiquent, pour *Steinernema riobravis* et son symbionte bactérien *Xenorhabdus,* un degré élevé d'infestivité et de pathogénie envers la chenille des épis du maïs et suggèrent d'importantes potentialités en vue de la lutte contre les stades prépupe et pupe de cet insecte.

Key words : Biological control, entomopathogenic nematodes, *Steinernema riobravis,* corn earworm, *Helicoverpa zea.*

The genus *Helicoverpa* contains some of the most damaging insects to agriculture worldwide. In the Americas, *Helicoverpa* (= *Heliothis*) *zea* (Boddie) attacks a wide variety of cultivated crops and this insect is known by different common names such as corn earworm, cotton bollworm, tomato fruitworm, and soybean podworm. Corn is its preferred host in which *H. zea* larvae feed in the whorl, and on the tassels, silk, and grain, which often results in the introduction of secondary pests and/or molds.

The primary control strategy for *H. zea* is the application of insecticides that result in egg and larval mortality. Because of development of insect resistance to insecticides and concern about environmental damage resulting from chemical pesticides there is an increased interest in biological control. Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* possess most of the characteristics of an ideal biological control agent for insect control. The pathogenicity of entomopathogenic nematodes to *H. zea* has been demonstrated previously (Tanada & Reiner, 1962; Howell, 1979; Bong & Sikorowski, 1983; Bong, 1986; Richter & Fuxa, 1990). However, their infectivity is quite different depending on nematode species and developmental stage of the insects (Samsook & Sikora, 1981; Glazer & Navon, 1990). Differences in infectivity of *S. carpocapsae* to *H. zea* pupae due to pupal age were observed by Kaya and Hara (1981). At present, the use of these nematodes against prepupae or pupae stages of *H. zea* has been limited.

An entomopathogenic nematode recently described as *Steinernema riobravis* Cabanillas *et al.,* 1994 was isolated from soil samples in corn fields after harvest in the Lower Rio Grande Valley of Texas where it appears to be indigenous. Previous observations made in this area and the northeastern part of Tamaulipas, Mexico indicated that prepupae and pupae of corn earworm and fall armyworm were naturally infected by *Steinernema*

sp. nematodes in about 34 % and 24 % of the corn fields, respectively (Raulston *et al.*, 1992). This newly suspected pathogen seems to be the cause of insect mortality, but no previous reports exists to support this. Therefore, Koch's postulates should be used to verify the hypothesis that the isolated pathogen, the nematode and its symbiotic bacterium, is the cause of the septicemia- insect disease. If there is a pathogenic relationship, it is important to know how many nematodes will be necessary to kill at least 50 % or more of the soil borne stages of *H. zea*. The purposes of this study were to : *i)* determine the pathogenicity of this nematode against *H. zea* prepupae, *ii)* estimate the lethal concentrations, and *iii)* investigate the progeny production of infective juvenile nematodes per dead insect in response to nematodes concentration.

Materials and methods

Nematode extraction and culture

Steinernema riobravis was isolated from soil samples taken from corn plots after harvest in July 1990. The corn plots were located at the US Department of Agriculture South Farm in Weslaco, Texas.

When isolating nematodes from soil, a modified Bedding and Akhurst (1975) baiting technique for detecting steinernematid nematodes in soil was used. *H. zea* prepupae were used as trap hosts instead of wax moth (*Galleria mellonella* L.) larvae. Approximately 1 kg of a Hidalgo sandy clay loam soil type (47.9 % sand, 35.6 % clay, and 16.5 % silt), was collected at each sample site from the top 10-15 cm of soil. Five prepupae were placed at the bottom of a 30-cm diam ceramic pot, covered with moist soil excavated from the corn plots, and incubated at about 23 °C for 5 days. Dead prepupae were transferred to White traps (White, 1929) and infective juveniles (IJ) were collected 10-14 days after exposure to the soil sample. *S. riobravis* nematodes were cultured *in vivo* in the laboratory using *H. zea* prepupae as a susceptible host.

Following harvest, the nematodes were suspended in 50 ml of water and stored in 275-ml canted neck Corning tissue culture flasks at 10 °C. Nematodes were used for experiments within one week of harvesting.

H. zea rearing

H. zea were reared in the laboratory on artificial diet at 29.5 °C. Prepupae used in this test were collected 11 days post eclosion and weighed an average of 644 mg.

Pathogenicity tests

The Petri plate bioassay procedure was used for determining the pathogenicity of this nematode against *H. zea* prepupae. One prepupa was placed into a Petri dish (60 × 15 mm) containing different numbers (0, 1, 5, 10, 20, 40, 80, and 100) of infective juveniles. The infective juveniles were suspended in 0.5 ml of sterile distilled water, and distributed evenly onto a piece of 5.5 cm-diameter filter paper (Whatman No. 1) in the bottom of the Petri dish before placement of the prepupa. The Petri dishes were subsequently placed in a plastic bag and incubated in the dark at room temperature (23 ± 2 °C) for 5 days. There were four replicates for each nematode concentration, and this experiment was repeated five times under the same conditions (total of 20 insects per nematode concentration). After five days all dead insects were individually transferred to White trap dishes (White, 1929) and held an additional 9 days. The insects were then examined for the presence of nematode progeny and estimates of the number of infective juveniles were made from those insects containing nematodes. This entomopathogenic nematode was isolated again from infected insects for comparison with the nematode initially applied according to Koch's postulates.

The insect mortality data were analyzed by the SAS PROBIT procedure as indicated in the SAS Technical Report, (Anon., 1988 *a*) following Log_{10} transformation of the concentration value. A probit regression analysis and chi square goodness-of-fit test were computed to determine lethal concentration (LC) values and 95 % fiducial limits.

Method to estimate production of nematodes

Nematodes were extracted from each host 14 days after inoculation by transferring the dead insect to a 50-ml plastic centrifuge tube containing about 5 ml water, grinding it with a spatula, and agitating with a Vortex mixer for 1 min to facilitate release of nematodes from host tissue. The tube contents were washed through the 25-mesh sieve into a 2000 ml beaker. Nematodes were also washed from the " White trap " dishes and filter paper through a 25-mesh screen sieve and collected in the beaker. The total volume was adjusted with distilled water to 1000 ml and stirred on a magnetic stir-plate to maintain a homogenous aqueous suspension of nematodes. A 1-ml aliquot of the suspension was placed in each of three counting dishes and the average number of third-stage infective juveniles was estimated from counts made under a dissecting microscope. The mean production of nematodes per prepupa and pupa (dependent variable) was regressed against concentration of nematodes initially added per prepupa (independent variable) using linear and quadratic models (Anon., 1988 *b*). The coefficient of determination (R^2) and plots of standardized residuals *vs* predicted values from regression analysis were used to evaluate goodness of fit to a model.

Results

Pathogenicity tests indicated that *Steinernema riobravis* and its symbiotic bacterium were highly virulent against *H. zea* prepupae (Table 1). Nematode concen-

Table 1. *Effect of different concentrations of* Steinernema riobravis *on the mortality of* Helicoverpa zea *in vitro.*

Nematodes added/ prepupa	Dead insects *	Insect mortality %	Probit ** LC value	95 % Fiducial limits
1	1	5	2	1-3
5	4	20	5	2-7
10	8	40	10	7-13
-	-	50	13	9-18
20	11	55	15	11-21
40	17	85	48	33-84
80	18	90	65	43-125
100	20	100	-	-

* Based on 20 prepupae.

** The LC_{50} value computed by Probit analysis was added to this table. The probit regression model is *Probit* $Y = -2.1 + 1.8 \quad Log_{10} W$ ($P = 0.0001$, df = 6, and Sy.x = 0.078), where Y: insect mortality response, X: nematode concentration per prepupa.

tration differentially affected the insect mortality of *H. zea* prepupae ($P = 0.0001$).

One hundred percent mortality of *H. zea* prepupae was achieved with exposure to 100 infective juvenile nematodes (IJ) per prepupa (Table 1). At this concentration, 60 % died in the prepupal stage; however, 40 % of the prepupae continued development to the pupal stage prior to nematode-induced death. Concentrations of 10, 20, 40, and 80 nematodes per *H. zea* prepupa caused mortalities of 40, 55, 85, and 90 % respectively. The lowest mortalities (5 and 20 %) occurred when prepupae were exposed to only 1 or 5 nematodes per prepupa. No mortality occurred in the control treatments. The effective lethal concentration estimated to cause 50 % insect mortality (LC_{50}) was thirteen IJ nematodes (Table 1). The general response of *H. zea* mortality *(Y)*, as a function of nematode concentration *(X)* per prepupa was estimated by a Probit regression model :

$$Probit\ Y = -2.1 \pm 1.8\ Log_{10} X\ (P = 0.0001,\ df = 6 \text{ and } Sy{\cdot}x = 0.078).$$

Production of nematodes per cadaver (averaged over prepupa and pupa) was also affected by the concentration of nematodes to which they were exposed ($P = 0.001$). The highest average yield of nematodes per insect cadaver (3.75×10^5) occurred at an exposure concentration of 40 IJ nematodes/prepupa (Fig. 1). The lowest average yield of nematodes (2.52×10^5) was obtained at an exposure level of 5 nematodes/prepupa. The overall average production of nematodes per prepupa and pupa was 3.11×10^5. There was no significant difference in the production of nematodes from *H. zea* dying as prepupae (3.21×10^5 nematodes per prepupa) compared to those which continued development to the pupal stage prior to death (3.00×10^5 nematodes per pupa). The general response of nematode production *(Y)* per insect cadaver (averaged over prepupa and pupa) as a function of nematode concentration *(C)* was approximated by a quadratic response curve ($P = 0.001$) ($r^2 = 0.38$) (Fig. 1). The yield of third-stage infective juveniles per insect cadaver increased with an increase in nematode concentration (up to 40 nematodes per insect) then it started decreasing as the prepupae of corn earworm were exposed to increasing nematode concentrations. Although this significant relationship was low, it showed the tendency of nematode production in response to nematode concentration (Fig. 1).

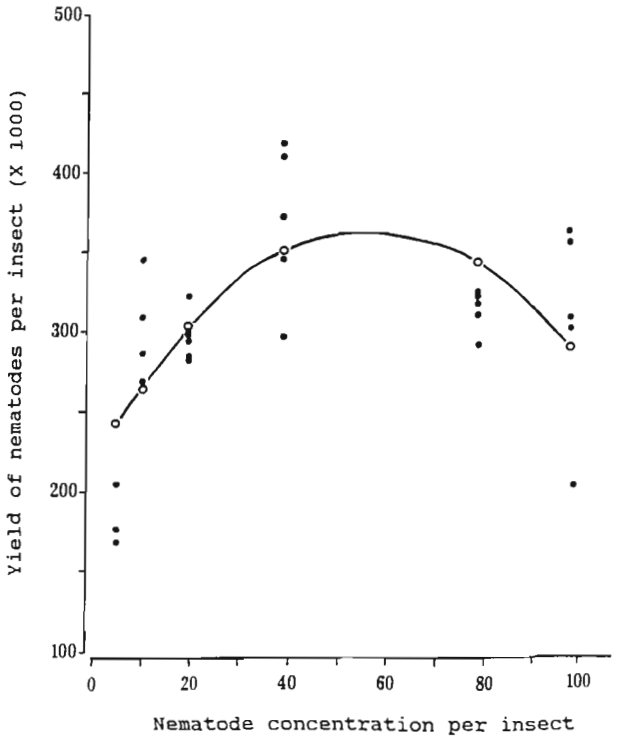

Fig. 1. *Yield of third-stage infective juveniles of* Steinernema riobravis *per prepupa and pupa of* Helicoverpa zea, *14 days after prepupae were exposed to different nematode concentrations* (● = *observed values;* ○ = *predicted values;* $\hat{Y} = 218.71 + 4.95\ C - 0.04\ C^2$).

Discussion

The results obtained with this new species of *Steinernema* collected from Texas corroborated previous findings on the pathogenicity of steinernematid nematodes to *H. zea*. However, the number of infective juveniles of *S. riobravis* required to achieve insect mortality is relatively low compared to several other examples. Complete mortality of *H. zea* was achieved with exposure to 100 IJ *S. riobravis* per prepupa in our experiments compared to 200 IJ of *S. carpocapsae* strain All (=

S. feltiae) for *H. armigera* (Glazer & Navon, 1990), 200 IJ of *S. carpocapsae* strain All (= *Neoaplectana carpocapsae*) for *Spodoptera exigua* prepupae (Kaya & Hara, 1980), 1000 IJ of *S. carpocapsae* strain DD-136 for *Spodoptera litura* larvae (Kondo, 1987), 1000 IJ of *S. carpocapsae* strain All per insect for *Heliothis virescens* F. larvae (Samsook & Sikora, 1981) and 4000 IJ of *S. carpocapsae* strain All for *S. frugiperda* (Landazabal *et al.*, 1973).

The references listed above indicate differences in the virulence of nematodes species and strains in infecting different insect pests. Bedding *et al.* (1983) suggested that a preliminary scan at a concentration of 100 nematodes per insect may help in selecting nematodes as potential control agents for a particular pest insect. Using this criterion, *S. riobravis* appears to be a promising candidate for controlling *H. zea* when applied to prepupal stages. Furthermore, the probit regression model can be of great value in screening entomopathogenic nematodes as potential candidates against *H. zea* prepupa. The significance of this model is that it describes not only the statistical relationship between the insect mortality response and the nematode concentration but it also provides a value for the slope which estimates the change in activity or pathogenicity per unit change in concentration of infective juvenile nematodes. The slope of the probit-mortality regression for this nematode was 1.8. This parameter may be useful in comparing the pathogenicity of this nematode with other steinernematids and for selecting potential nematode candidates for specific target insect pests.

Nematode production per dead insect is affected by the initial number of nematodes exposed to corn earworm prepupae. There is a nonlinear relationship between yield of third-stage juveniles and initial nematode concentration per insect. In our study, the high average yield of nematodes is achieved when corn earworm prepupa is exposed to 40 infective-stage juveniles. Dutky *et al.* (1964) indicated 160 000 nematodes of *Steinernema carpocapsae* (Weise) (DD-136 strain) per insect larvae to be a good yield. In our experiment, nematode yields from prepupae and pupae of *H. zea* averaged over 300 000 per insect (466 nematodes/mg of *H. zea* prepupa) as estimated at 14 days after nematode inoculation. We observed that nematode progeny production began 10 days after treatment and continued for at least 17 days after treatment. Therefore, our progeny production estimates which were made 14 days post treatment probably underestimated total nematode production. Dutky *et al.* (1964) reported that greater wax moth larvae, *Galleria mellonella* L., a host commonly used in nematode propagation, yielded up to 200 000 infective-stage nematode of *S. carpocapsae* (strain DD-136). They also reported that *G. mellonella* larvae of small, medium, and large sizes resulted in 1715, 1285, and 1110 nematodes/mg of larvae, respectively. From a biological control standpoint, the important aspect is the death of the target insect; however, nematode reproduction in its target insect must occur for a successful establishment of the nematode in the host's environment. In the present study, nematode reproduction occurred in the host insect; therefore, establishment and recycling could occur in the soil where conditions are favorable for nematode survival.

The use of laboratory pathogenicity biossays in our experiment has been relevant in showing consistently the pathogenic capability of *S. riobravis* to *H. zea* prepupa during the five times that this experiment was repeated. Although laboratory biossays do not provide an assurance of field efficacy, *S. riobravis* is well adapted to field conditions in this semi-arid region. For example, field evaluations on the efficacy of *S. riobravis* (TX) and *Steinernema carpocapsae* (All strain) applied to soil to control corn earworm prepupae and pupae resulted in a higher rate of insect mortality (89 – 100 %) due to *S. riobravis* compared to the failure of parasitism by *S. carpocapsae*. The soil temperature, 35 ± 4 °C at 5 cm. – deep, appears to be a significant factor for the success or failure of these entomopathogenic nematodes (Cabanillas & Raulston, unpubl.).

Generally, the use of entomopathogenic nematodes has been against the feeding stages of various insect pests. However, our results indicate that this nematode will kill prepupae and pupae of *H. zea* when the nematode is applied at the prepupal stage. Prepupal and pupal mortality resulting from infection by this nematode may be a significant factor in suppressing corn earworm populations. Thus, utilization of *S. riobravis* against these stages in the soil appears feasible for the control of the corn earworm. Further research is necessary to use *S. riobravis* more effectively as a potential biocontrol agent against corn earworm and other harmful crop insect pests.

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