Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 475-479

Received: 17-05-2018
Accepted: 21-06-2018

R Channakeshava
Assistant Professor, (Entomology), AICRP on Soybean, University of Agricultural Sciences, Dharwad, Karnataka, India

VT Sannaveerappanavar
Professor, Department of Agril. Entomology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Correspondence
R Channakeshava
Assistant Professor, (Entomology), AICRP on Soybean, University of Agricultural Sciences, Dharwad, Karnataka, India

Effect of host plants on the susceptibility of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae to *Hear*NPV

R Channakeshava and VT Sannaveerappanavar

Abstract
The influence of five host plants *viz.,* fieldbean, pigeonpea, chickpea, tomato and cotton on the activity of the nuclear polyhedrosis virus against the American bollworm, *Helicoverpa armigera* (Hbn.) and the effect of host plant surface on the activity of *Hear*NPV were studied. Median lethal time (LT_{50}) and Median lethal concentration (LC_{50}) in third instar larvae of *H. armigera* indicated that cotton and tomato plants had higher adverse effect on the virulence of the virus resulting in lower larval mortality followed by chickpea, pigeonpea and fieldbean. The yield of polyhedral occlusion bodies (POB's) per larva was highest from the larvae that fed on field bean foliage and was least from the larvae that fed on cotton. The LT_{50} values increased as the duration of contact between the *Hear*NPV and the leaf surface increased irrespective of host plants and doses of the virus.

Keywords: host plants, *Helicoverpa armigera*, *Hear*NPV, Karnataka, India

1. Introduction
Helicoverpa armigera (Hübner) is one of the most destructive polyphagous pests in India, which is commonly known as the cotton bollworm, tomato fruit borer and gram pod borer. With increasing problems due to insecticide resistance in *H. armigera*, microbial insecticides based on nucleopolyhedrovirus (*Hear*NPV) play an important role in the successful management of this pest. Though the nucleopolyhedrosis virus of *H. armigera* (*Hear*NPV) has been found to be very effective against the pest on a number of crops [8], its bioefficacy has been found to be highly variable with different geographical isolates showing differences in virulence, biological characters and DNA profiles. It has also been shown that the virulence of *Hear*NPV isolates is greatly influenced by the host plants [2]. Host plants can influence virus-insect interactions in many ways: plant architecture affects virus persistence, palatability modifies host mobility and virus acquisition, plant chemistry modulates infection in the gut and nutrient content determines host survival. The impact of plant phytochemicals, such as phenolics and terpenoids on host susceptibility has received most attention and numerous studies have shown that both mortality [3, 5] and speed of kill vary depending on plant type or allelochemicals. Finding the mechanisms behind the observed differences in plant mediated effects could provide a better framework for understanding and predicting plant-baculovirus interactions. Keeping all these things in view, the present studies were undertaken to study the influence of selected host plants on the virulence of *Hear*NPV to larvae of *H. armigera*.

2. Materials and Methods
The laboratory studies were carried out at Department of Agricultural Entomology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka and Bio-control Research Laboratories (BCRL), a division of Pest Control (India) Ltd., Sriramanahalli, Bengaluru (12^0 58^1 N and 77^0 38^1 E), Karnataka during 2015.

2.1 Establishment of *Helicoverpa armigera* laboratory culture
The establishment of culture began with collection of large number of late fifth instar larvae from the infested fields of chickpea and tomato plants during the flush seasons. Field collected larvae were reared individually in the laboratory in multicavity trays using semi-synthetic diet and were allowed to pupate in the cavity itself. Five days after pupation, the healthy pupae were collected and washed with 10 per cent formaldehyde and were rinsed in sterile distilled

water. The male and female pupae were kept in sterile plastic boxes for moth emergence. The newly emerged adults were paired and caged separately for egg laying. Eggs were collected daily on black muslin cloth and surface sterilized with 10 per cent formaldehyde. The sterilized eggs were transformed to sterile plastic boxes for incubation and hatching of neonates.

2.2 Host Plants

Five different host plants *viz.,* tomato (*Lycopersicon esculentum*), field bean (*Dolichos lablab*), chickpea (*Cicer arietinum*), cotton (*Gossypium hirsutum*) and pigeonpea (*Cajanus cajan*) were selected for the virus-host plant interaction studies. The host plants were grown separately in pots at greenhouse. Day and night temperatures within the greenhouse were maintained throughout the experiment.

2.3 Establishment of *Hear*NPV culture

Field collected early fifth instar *H. armigera* larvae were dosed with *Hear*NPV containing 1 x 10^7 polyhedral occlusion bodies (POBs) per ml. Aliquot of 10 µl of virus suspension was applied on the semi-synthetic diet dispensed in multicavity trays (each including 100 cavities). Larvae were released individually into each cavity of multicavity trays after inoculation and were incubated at a temperature of 25±1^{0}C. When the larvae exhausted the feed, fresh untreated diet was provided. The cadavers collected were homogenized using a grinder and the concentrate was diluted with distilled water. The homogenate was filtered through a sterile, double layered muslin cloth. The filtrate was spun for 3 minutes at 600 rpm to remove debris and larger particles. The supernatant was re-spun for 20 minutes at 5000 rpm to collect the pellet-containing polyhedra. The pellet was resuspended in distilled water and washed thrice for obtaining semi-purified inoculum. The standardization and enumeration of POBs were estimated with the help of a double ruled improved Neubauer haemocytometer of depth factor 0.1 mm under a phase contrast microscope.

2.4 Influence of host plants on the susceptibility of *H. armigera* larvae to *Hear*NPV

The larvae of *H. armigera* were reared on different host plants *viz.,* tomato, field bean, chickpea, cotton and pigeonpea. The newly emerged adults were paired at 1:1 ratio and caged separately for egg laying. When the eggs hatched (F_1 generation), neonate larvae were transferred on to the leaves of respective host plants and were placed separately in sterilized plastic tubs. Upto the end of second instar, larvae were reared en-mass on their respective host plants. When they moulted to third instar uniform sized larvae were selected, starved for a day and used in leaf disc bioassay. Serial dilutions of the NPV ranging from 5 x 10^4 to 1.6 x 10^1 POBs per ml (with five times reduction in each treatment) were prepared. An aliquot of 10 µl virus suspension was applied on each leaf disc (5mm diameter) of respective host plants using micropipette and the droplets were spread uniformly over the entire area of the discs using a sterile glass rod. The treated discs were transferred to individual sterilized glass vials and fed to the pre-starved third instar larvae. After 24 h of treatment, those larvae that consumed the entire discs were selected and returned back to respective plastic tubs containing untreated host plants for the remainder of the experiment. For comparison, semi-synthetic diet was also included as one of the treatments for all the viral concentrations (5 x 10^4 to 1.6 x 10^1 POBs per ml). Controls without virus treatment were maintained. Each treatment had 30 larvae replicated three times.

Observations were made on larval mortality at 24 h intervals from second day onwards till the tenth day to establish LT_{50} values. LT_{50} values were computed using the Statistical Package for Social Sciences (SPSS), version 10.0 for windows. Larval mortality in control was corrected using Abbott's correction formula [1].

The dead larvae found on respective host plants were collected separately and kept in amber coloured vials. The yield of the virus per larva was determined by the following formula:

$$\text{Yield/larva (POB)} = \frac{\text{POBs per ml} \quad \text{X Suspension volume (ml)}}{\text{Total number of cadavers}}$$

2.5 Interaction between leaf surfaces of host plants and *Hear*NPV

Another set of experiments were conducted separately to study the interaction between *Hear*NPV and host plant surface at different intervals of time. The larvae of *H. armigera* were reared on semi-synthetic diet up to pupation. The newly emerged adults were paired and caged separately for egg laying. When the eggs hatched (F_1 generation), the neonate larvae were reared on the semi-synthetic diet up to the end of 2nd instar. When they moulted to third instar, uniform sized larvae were selected and starved for a day before their use in bioassays.

An area of 10 mm diameter was marked with a marker pen on the upper surface of the leaves of 40 days old host plants grown in green house. Serial dilutions of *Hear*NPV ranging from 5 x 10^4 to 1.6 x 10^1 POBs per ml were prepared. An aliquot of 10 µl virus suspension was applied on the marked surface area of the leaf using a micropipette. The droplets were spread uniformly over the entire area of leaf using a sterile glass rod with polished and rounded tip. Then the treated leaves from the respective host plants were drawn randomly and leaf discs (5 mm diameter) were prepared at various intervals like 0, 24 and 48 h after treatment. Treated leaf discs were transferred to individual glass vials and fed to the pre-starved third instar larvae. After 24 h of treatment, those larvae that consumed the entire leaf discs were returned to untreated semi-synthetic diet for the remainder of the experiment.

Observations were recorded on larval mortality at 24 h intervals from the second day till the tenth day, to establish LT_{50} values. LT_{50} values were computed using the Statistical Package for Social Sciences (SPSS), version 10.0 for windows. Larval mortality in control was corrected using Abbott's correction formula.

3. Results and Discussion

3.1 Time-mortality response (LT_{50}) of *H. armigera* larvae reared on different host plants to *Hear*NPV

The LT_{50} values of *Hear*NPV to third instar *H. armigera* larvae dosed with field bean, pigeonpea & chickpea leaf discs were inversely proportional to the doses of the virus. The median lethal time of *Hear*NPV to *H. armigera* larvae ranged from 5.52 to 12.56 days, 5.88 to 13.05 days & 6.26 to 14.16 days in fieldbean, pigeonpea and chickpea, respectively (Table 1). The viral yield harvested from the virosed larvae was highest 4.64 x 10^8 POBs/larva, 3.83 x 10^8 POBs/larva & 3.12 x 10^8 POBs/larva when exposed to the virus at 5 x 10^4

POBs per ml and was least 1.62 x 10^8 POBs/larva, 1.23 x 10^8 POBs/larva & 0.77 x 10^8 POBs/larva when exposed to the virus at 1.6 x 10^1 POBs per ml in fieldbean, pigeonpea and chickpea, respectively.

Table 1: Probit analysis of time-mortality response of third instar larvae of *Helicoverpa armigera* to *Hear*NPV reared on field bean, pigeonpea & chickpea

Dose (POBs/ml)	Fieldbean			Pigeonpea			Chickpea		
	LT_{50} (days)	LT_{99} (days)	Yield (POBs/ larva)	LT_{50} (days)	LT_{99} (days)	Yield (POBs/ larva)	LT_{50} (days)	LT_{99} (days)	Yield (POBs/ larva)
5 x 10^4	5.52	12.86	4.64 x 10^8	5.88	14.57	3.83 x 10^8	6.26	16.76	3.12 x 10^8
1 x 10^4	6.23	16.68	4.11 x 10^8	6.83	18.51	3.37 x 10^8	7.12	21.07	2.42 x 10^8
2 x 10^3	8.05	25.26	3.76 x 10^8	8.34	24.92	2.64 x 10^8	8.78	27.75	1.79 x 10^8
4 x 10^2	9.46	34.04	2.82 x 10^8	10.20	34.13	2.01 x 10^8	11.00	35.02	1.13 x 10^8
8 x 10^1	10.48	38.86	1.91 x 10^8	11.51	39.18	1.59 x 10^8	13.28	39.33	0.92 x 10^8
1.6 x 10^1	12.56	42.23	1.62 x 10^8	13.05	43.42	1.23 x 10^8	14.16	45.03	0.77 x 10^8

The LT_{50} values of *Hear*NPV to *H. armigera* reared on tomato and cotton plants exposed to the virus on tomato and cotton leaves ranged from 7.07 to 14.92 days & 8.13 to 17.02 days at inoculum doses of 5 x 10^4 to 1.6 x 10^1 POBs per ml in tomato and cotton, respectively (Table 2). Whereas, the LT_{99} values ranged from 18.77 to 45.29 days & 23.16 to 55.43 days in tomato and cotton, respectively. The viral yield at different doses *Hear*NPV ranged from 2.19 x 10^8 to 0.16 x 10^8 POBs per larva & 3.69 x 10^7 to 0.94 x 10^7 POBs per larva in tomato and cotton, respectively.

Table 2: Probit analysis of time-mortality response of third instar larvae of *Helicoverpa armigera* to *Hear*NPV reared on tomato, cotton & semi-synthetic diet

Dose (POBs/ml)	Tomato			Cotton			Semi-synthetic diet		
	LT_{50} (days)	LT_{99} (days)	Yield (POBs/ larva)	LT_{50} (days)	LT_{99} (days)	Yield (POBs/ larva)	LT_{50} (days)	LT_{99} (days)	Yield (POBs/ larva)
5 x 10^4	7.07	18.77	2.19 x 10^8	8.13	23.16	3.69 x 10^7	4.58	6.75	2.01 x 10^9
1 x 10^4	8.32	24.39	1.67 x 10^8	9.26	28.10	2.78 x 10^7	4.93	8.22	1.78 x 10^9
2 x 10^3	9.38	28.12	1.08 x 10^8	11.09	35.78	2.06 x 10^7	5.30	9.68	1.61 x 10^9
4 x 10^2	11.38	36.48	0.63 x 10^8	12.10	39.42	1.63 x 10^7	6.11	14.30	1.14 x 10^9
8 x 10^1	14.04	44.39	0.35 x 10^8	15.36	47.10	1.39 x 10^7	8.87	32.52	0.85 x 10^9
1.6 x 10^1	14.92	45.29	0.16 x 10^8	17.02	55.43	0.94 x 10^7	10.06	35.51	0.53 x 10^9

The lowest LT_{50} value of 4.58 days was obtained with viral concentration of 5 x 10^4 POBs per ml for third instar larvae reared on semi-synthetic diet and a maximum of 10.06 days at a dose of 1.6 x 10^1 POBs per ml (Table 2). Compared to the LT_{50} values of *Hear*NPV obtained for larvae reared on different host plants, the LT_{50} values for larvae reared on synthetic diet did not vary much across the doses of the virus. Similarly, LT_{99} values ranged from 6.75 to 35.51 days across the doses of the virus. The highest viral yield obtained was (2.01 x 10^9 POBs per lava) at a dose of 5 x 10^4 POBs per ml and the lowest (0.53 x 10^9 POBs per larva) at 1.6 x 10^1 POBs per ml.

3.2 Dose-mortality (LC_{50}) response of *H. armigera* larvae reared on different host plants to *Hear*NPV

LC_{50} of *Hear*NPV was the lowest in case of larvae reared on semi-synthetic diet (0.02 POBs/mm^2) followed by those reared on field bean (0.04 POBs/mm^2) whereas, it was the highest with cotton (0.23 POBs/mm^2). Moderate levels of LC_{50} values (0.07 and 0.09 POBs/mm^2) were obtained for larvae reared on pigeonpea and chickpea, respectively. The LC_{50} of *Hear*NPV to larvae reared on tomato was also higher (0.11 POBs/mm^2). Based on the LC_{50} values, the larvae reared on synthetic diet were most susceptible to the *Hear*NPV followed by those reared on field bean, pigeonpea, chickpea, tomato and cotton (Table 3).

Table 3: Probit analysis of dose-mortality response of third instar larvae of *H. armigera* to *Hear*NPV reared on different host plants

Host	χ^2 (n-2)	Slope 'b'± S.E	LC_{50} (POBs/mm^2)	Fiducial limit (POBs/mm^2)	
				Lower	Upper
Field bean	1.27	0.87 ± 0.19	0.04	0.012	0.079
Pigeonpea	1.44	0.68 ± 0.17	0.07	0.009	0.106
Chickpea	1.62	0.63 ± 0.16	0.09	0.015	0.157
Tomato	2.58	0.51 ± 0.16	0.11	0.020	0.344
Cotton	4.19	0.44 ± 0.15	0.23	0.050	1.280
Semi-synthetic diet	1.21	0.79 ± 0.19	0.02	0.004	0.055

3.3 Interaction between leaf surfaces of host plants and *Hear*NPV

When the larvae were fed with leaf discs immediately after treatment ('0' hrs.), the LT_{50} values ranged from 5.01 to 7.40 days at the highest doses of 5 x 10^4 POBs/ml across the host plants (Table 4). The LT_{50} values of *Hear*NPV were higher for larvae that fed on tomato and cotton compared to those for larvae that acquired the virus through field bean, pigeonpea and chickpea. The same trend was observed across the doses of the *Hear*NPV.

Table 4: Probit analysis of time-mortality response of third instar larvae of *H. armigera* to *Hear*NPV reared on different host plants when exposed at '0' hours after treatment

Dose (POBs/ml)	LT_{50} values (days)				
	Field bean	Pigeonpea	Chickpea	Tomato	Cotton
5 x 10^4	5.01	5.33	5.93	6.83	7.40
1 x 10^4	5.66	6.19	6.85	8.17	8.54
2 x 10^3	7.10	7.55	8.56	9.42	10.89
4 x 10^2	9.42	9.97	10.39	11.22	12.11
8 x 10^1	11.22	11.38	11.62	13.87	14.92
1.6 x 10^1	12.10	12.52	12.76	14.92	17.02

When the larvae were fed with the leaf discs 24 hr after treatment with the virus, the LT_{50} values ranged from 5.23 to 8.27 days (@ 5 x 10^4 POBs/ml) with the larvae that fed on field bean, pigeonpea and chickpea recording lower values compared to those that fed on treated tomato and cotton leaf discs (Table 5). A similar trend was observed across the six concentrations tested.

Table 5: Probit analysis of time-mortality response of third instar larvae of *H. armigera* to *Hear*NPV reared on different host plants when exposed at '24' hours after treatment

Dose (POBs/ml)	LT_{50} values (days)				
	Field bean	Pigeonpea	Chickpea	Tomato	Cotton
5 x 10^4	5.23	5.54	6.60	7.62	8.27
1 x 10^4	5.90	6.65	7.83	9.01	9.78
2 x 10^3	7.37	7.63	9.31	10.89	11.73
4 x 10^2	9.92	10.44	10.57	12.07	12.77
8 x 10^1	11.61	12.11	12.57	14.72	16.38
1.6 x 10^1	13.01	13.47	13.64	17.02	19.45

Feeding of larvae with leaf discs of different host plants 48 hr after treatment resulted in slightly higher LT_{50} values ranging of 5.72 to 9.41 days (Table 6) at the highest dose of the virus (5 x 10^4 POBs/ml). In this assay, the medium lethal time of the virus was shorter for larvae that fed on field bean and pigeonpea (5.72 and 5.80 days, respectively) compared to LT_{50} values for the larvae that fed on chickpea, tomato and cotton (7.79 to 9.41 days). A similar trend was observed across the concentrations.

Table 6: Probit analysis of time-mortality response of third instar larvae of *H. armigera* to *Hear*NPV reared on different host plants when exposed at '48' hours after treatment

Dose (POBs/ml)	LT_{50} values (days)				
	Field bean	Pigeonpea	Chickpea	Tomato	Cotton
5 x 10^4	5.72	5.80	7.79	8.58	9.41
1 x 10^4	6.63	6.87	8.96	10.06	11.46
2 x 10^3	7.98	8.14	11.14	12.38	13.01
4 x 10^2	11.00	11.22	12.11	14.72	16.27
8 x 10^1	13.74	13.87	14.47	17.02	19.46
1.6 x 10^1	15.39	15.84	16.38	19.45	21.63

Irrespective of the host plants and doses of the virus, the LT_{50} values of *Hear*NPV increased as duration of contact between the virus and the leaf surface increased. However, the degree of variation in LT_{50} values across time intervals was minimum in the case of field bean and pigeonpea compared to the values in the case of chickpea, tomato and cotton. The host plants had profound influence on the susceptibility of *H. armigera* larvae to the virus. The larvae that fed on field bean and pigeonpea were more susceptible to *Hear*NPV compared to the larvae that fed on chickpea, tomato and cotton.

Earlier studies have indicated that ingestion of certain foliage could affect the integrity of the peritrophic membrane in the larval midgut, allowing better virion access to midgut cells [5]. Also phenols and tannins or their oxidative products in particular, have been implicated in inhibition of virus infection [4]. Cotton with higher levels of phenols and tannins produced the lowest larval mortality and yield of polyhedral bodies compared to other host plants [7] are in conformity with the present findings. Accordingly, the lower susceptibility of larvae reared on cotton and tomato to *Hear*NPV could be attributed to the inhibitory effects of higher contents of above phytochemicals and the enzyme in those plants. Conversely, the greater virulence of the virus against larvae reared on field bean and pigeonpea can be attributed to the lower levels of total phenols and peroxidase activity in those plants.

Larvae were reared on semi-synthetic diet and dosed on respective host plants at different intervals of time to test whether leaf surface conditions affected the viral activity before ingestion by the larvae. Irrespective of the duration of contact of virus particles with the leaf surface, the LT_{50} values of *Hear*NPV varied greatly across the host plants at any given dose of the virus. Generally, the LT_{50} values were higher for the larvae that fed on cotton and tomato compared to those for larvae that acquired the virus through field bean, pigeonpea and chickpea. Secondly, irrespective of the host plants and doses of the virus, the LT_{50} values increased as the duration of contact between the virus and the leaf surface increased. However, the extent of variation in LT_{50} values across time intervals was minimum in the case of field bean and pigeonpea compared to the values in the case of cotton, tomato and chickpea. These results clearly indicate that the leaf surfaces of cotton, tomato and to some extent, chickpea

have some adverse effect on the virus that reduces its virulence against the host larvae. The loss of virus infectivity within three days on cotton foliage was also reported by [6]. Cotton leaf surface was more detrimental to the *Hear*NPV compared to leaf surfaces of chickpea and sunflower [9]. In another study [10] it was observed that, very little activity of *Heliothis* NPV after 24 hr on cotton while the virus persisted on soybean and tomato upto 90 hr, even when the plants were shielded from sunlight, thus indicating that rapid inactivation was due to some factor other than UV light.

4. Conclusion

The present study revealed that host plants had profound influence on the susceptibility of *H. armigera* larvae to the virus. The larvae that fed on field bean and pigeonpea were more susceptible to *Hear*NPV compared to the larvae that fed on chickpea, tomato and cotton. This indicates, the leaf surfaces of cotton, tomato and to some extent, chickpea caused more detrimental effect on the virus that ultimately reduces its virulence against the host larvae.

5. Acknowledgements

Authors thank University of Agricultural Sciences, Bengaluru for encouragement and support. We thank the Bio-Control Research Laboratory (BCRL) and Dr. K. P. Jayanth, Vice-President of BCRL, Bengaluru for encouragement and facilities.

6. References

1. Abbott WS. A method of computing the effectiveness of an insecticide. J of Econ. Entomology. 1925; 18:265-267.
2. Battu GS, Arora R. Genetic diversity of baculoviruses-implications in insect pest management. In: Biotechnological Perspectives in Chemical Ecology of Insects, Oxford and IBH Publication Company, Pvt. Ltd., New Delhi, 1996, 170-200.
3. Farrar RR, Ridgway RL. Host plant effects on the activity of selected nuclear polyhedrosis viruses against the corn earworm and beet armyworm (Lepidoptera: Noctuidae). Environ. Entomology. 2000; 29:108-115.
4. Felton GN, Duffey SS, Vail PV, Kaya HK, Manning J. Interaction of nuclear polyhedrosis virus with catechols: potential incompatibility for host plant resistance against noctuid larvae. J of Chem. Ecol. 1987; 13:947-957.
5. Forschler BT, Young SY, Felton GW. Diet and the susceptibility of *Helicoverpa zea* (Noctuidae: Lepidoptera) to a nuclear polyhedrosis virus. Environ. Entomology. 1992; 21:1220-1223.
6. Ignoffo CM, Bradley JR, Gilliland FR, Harris FA, Falcon LA, Larson LV, *et al.* Field studies on stability of *Heliothis* nuclear polyhedrosis virus at various site throughout the cotton belt. Environ. Entomology. 1972; 2:388-390.
7. Keating ST, Yendol WG, Schultz JC. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a baculovirus and host plant foliage constituents. Environ. Entomology, 1988; 17:952-958.
8. Rabindra RJ, Jayaraj S. Microbial control of *Heliothis armigera*. In: Proc. of National Workshop, Tamil Nadu Agriculture University, Coimbatore, 1990, 154-164.
9. Rabindra RJ, Ballali CR, Ramanujan B. Biological options for insect pests and nematode management in pulses. In: Pulses in New Perspective, Indian Society of Pulses Research and Development, Kanpur, India, 2004, 400-425.
10. Young SY, Yearian WC. Persistence of *Heliothis* NPV on foliage of cotton, soybean and tomato. Environ. Entomology. 1974; 3:253-255.