Ceylon J. Sci. (Bio. Sci.) 22, No. 1, 1992

INSTAR DETERMINATION AND LARVAL DISTRIBUTION IN BRINJAL SHOOT AND FRUIT BORER, *Leucinoides orbonalis* (Guen.)

W. R. MANOHARIE SANDANAYAKE * AND JAYANTHI P. EDIRISINGHE

Department of Zoology, University of Sri Jayawardenapura, Nugegoda, Sri Lanka.

Correspondence: Dr. J. P. Edirisinghe, Department of Zoology, University of Peradeniya, Peradeniya, Sri Lanka.

ABSTRACT

Larval instars of *Leucinodes orbonalis* Guen. reared on *Solanum melingena* (L.) were determined by measuring the width of the intact as well as the shed larval head capsules. Larvae were separable into five, distinct, non-overlapping instars by cluster analysis (of both intact and shed head capsule measurements) and by frequency distribution (of only the shed head capsule measurements). Larvae were found to shed their head capsules five times during a total larval period of 11-16 days. The mean widths of the shed head capsules corresponding to the five larval instars were 0.2, 0.36, 0.64, 0.95 and 1.46 mm respectively. The stadial periods of the five instars were 3.5, 1.6, 1.8, 3.2 and 2.0 days respectively. First instar larvae were confined to flower-buds and flowers whilst the second instars were present in all the susceptible parts of the plant. Third to fourth instar larvae were confined to the shoots and fruits while final instar larvae fed exclusively on the fruits. The size of the entrance hole made by a larva entering the plant was found to be a good indicator of its instar status.

---

* Present address: Natural Resources, Energy and Science Authority of Sri Lanka, 47/5, Maitland Place, Colombo 7, Sri Lanka.

INTRODUCTION

Leucinodes orbonalis (Guen.) (Lepidoptera:Pyralidae) which is a pest of *Solanum melongena* L. (Solanaceae) causes serious crop losses ranging from 15-70% in all the brinjal producing areas of the world. Early larval instars of *L. orbonalis* feed exclusively on flower buds, flowers and shoots of *S. melongena*, while later instars bore into fruits reducing their marketable value and, in extreme cases, making them unfit for human consumption. In Sri Lanka, control of this pest has largely been through the use of insecticides whilst resistant cultivars are being developed. The role of parasitoids in the control of *L. orbonalis* is also being investigated. Sandanayake (1987) recorded three larval parasitoids of *L. orbonalis* from different parts of the country.

Although, information is available on the life history of *L. orbonalis* from work done in India (Lal and Ahamad, 1965; Atwal and Verma, 1972; Nyar *et al.*, 1976; Yang, 1982: Mehto *et al.*, 1983) there appears to be a paucity and considerable variation on the information on larval development, particularly on the number of larval instars and their stadial periods. Moreover, for a study of larval parasitoids this information must be available. The objective of this study was to determine the number of larval instars and the stadial periods of *L. orbonalis* reared on *S. melongena*. The distribution of the different larval instars on the plant was also investigated during the study.

MATERIAL AND METHODS

The technique of rearing *L. orbonalis* on fruits of *S. melongena* is described in detail by Sandanayake (1987). Larvae that hatched out from eggs laid by laboratory reared and mated females were maintained on fruits of the susceptible brinjal cultivar 'purple lenairi.' Mean temperature and humidity in the laboratory were 29± 2°C and 69±5% RH, during the study.

Instar determination

Larval instar were determined by two methods. In the first, larvae that hatched out from a batch of 311 eggs laid by a single female were used. Two hundred newly hatched larvae selected at random were reared individually on brinjal slices (2 cm thick). Each day, seven larvae were removed, etherized and the width of their head capsules measured under a stereomicroscope (10 x 4). Larvae once measured were discarded. This procedure was continued until pupation. A total of 133 larvae were measured in this manner.

In the second method a batch of 100 newly hatched larvae were reared on brinjal slices (1 larva/slice) until pupation. The frass produced by the larvae and the fruit slices on which they were reared were examined daily for any shed larval head capsules. These were collected and measured as previously. The dates on which consecutive head capsules were shed by individual larvae were noted to determine the stadial period.

Larval distribution

The distribution of larvae on the host plant was studied in a field plot bearing 100 three-month-old plants of the susceptible brinjal cultivar 'purple lenairi.' Plants were spaced at 75 cm between and 90 cm within rows. The usual agricultural practices were followed except that no insecticides were used. Fifty plants

selected at random were uprooted and used in the study. The different parts of the plant such as shoots, buds, flowers and fruits, showing signs of borer damage (withering, die-back, frass) were removed, carefully cut open with a sharp razor blade and examined for larvae. Instar determination were based on head capsule widths.

Larval entrance holes

The entrance-holes bored by larvae that entered the different parts of the plant were measured and the larvae responsible for the damage were removed and their head capsules measured as previously.

RESULTS

In the first method, where the intact head capsules of larvae were measured, the widths ranged from 0.18 - 1.8 mm. When these width were plotted against larval frequency, the histogram obtained did not indicate clear-cut groups, especially between the first and second instars (Fig. 1). However, when cluster analysis (Chatfied and Collins, 1980) was used, head capsule widths were clearly separable into 5 non-overlapping groups of clusters (Fig. 2). The mean head capsule width of each of these cluster groups was calculated using all the data contributed to a particular cluster group (Table 1). The head capsule increments differed significantly from one group to another ($t = 8.0$, $P < 0.05$), the values ranging from

Table 1. Head capsule widths (intact) and stadial periods of *L. orbonalis* larval instars.

Larval instar (n)	Head capsule width (mm)			Stadial period (days)	
	Range	Mean ± S.D.	Increment	Mean ± S.D.	Range
First (21)	0.18-0.21	0.19 ± 0.01	-	1.5 ± 0.05	(1-2)
Second (20)	0.24-0.38	0.33 ± 0.04	0.14	1.9 ± 0.8	(1-2)
Third (24)	0.45-0.68	0.62 ± 0.06	0.29	2.2 ± 0.98	(1-3)
Fourth (4)	0.82-1.05	0.96 ± 0.08	0.34	1.9 ± 0.81	(1-3)
Fifth (44)	1.20-1.80	1.45 ± 0.15	0.49	3.5 ± 1.73	(1-6)
			t=8.0	t=14.40	

t = x/s/n * Means $P < 0.05$

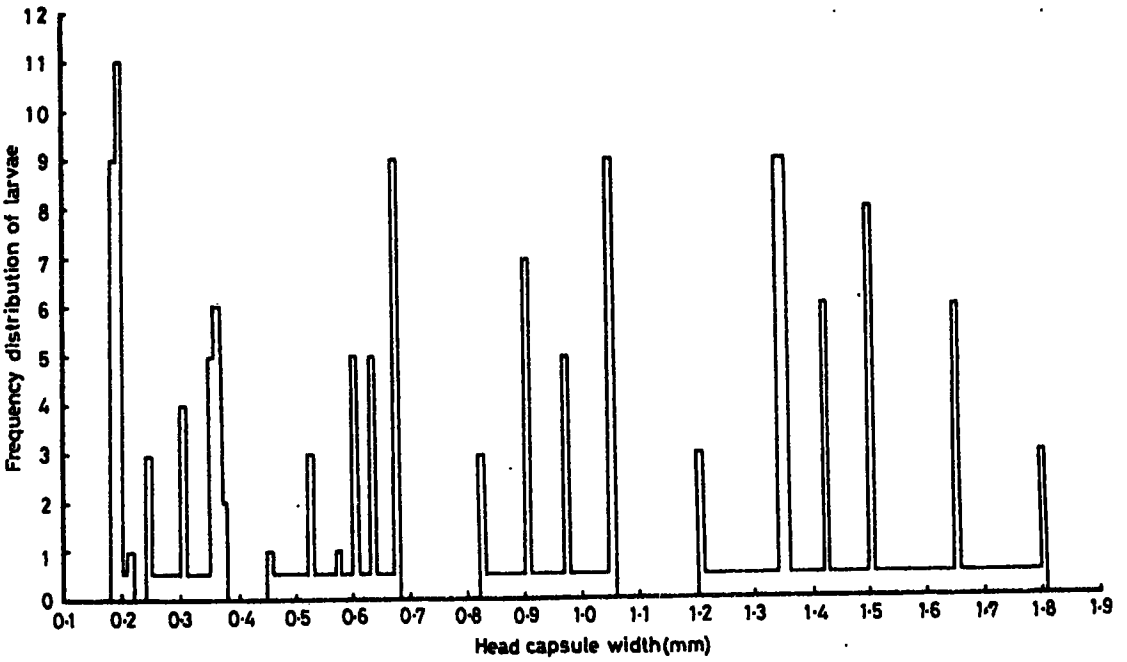

Fig. 1. Frequency Distribution of Intact Larval Head Capsule Widths of *L. orbonalis*

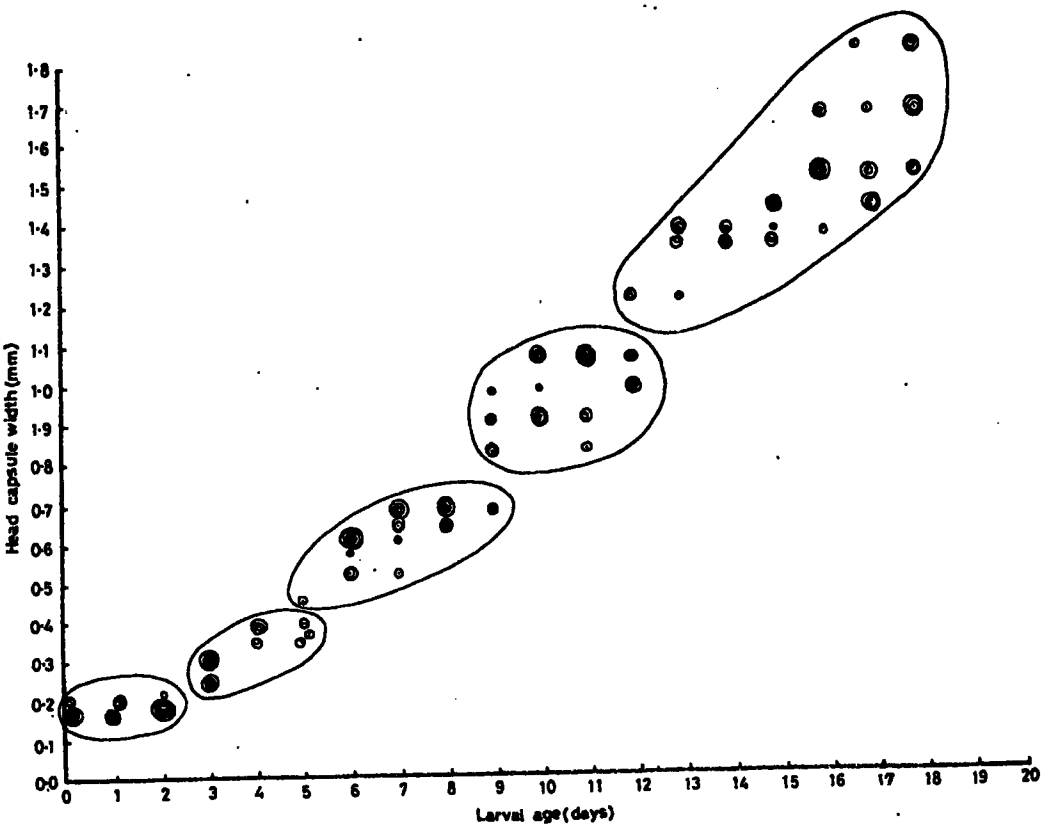

Fig. 2. Cluster Diagram of Intact Larval Head Capsule Widths *vs.* the Age of *L. orbonalis*

Table 2. Conformity test for L. orbonalis larval instars to Dyar's rule (Intact head capsule width measured in mm)

Larval instar	Observed mean	Calculated mean	Coef. of variation (%)	Difference	$X_n + 1/X_n$ ratio increase
I	0.19	-	5.26	-	-
II	0.33	0.36	11.76	0.03	1.74
III	0.62	0.63	9.67	0.01	1.88
IV	0.96	1.18	8.33	0.22	1.55
V	1.45	1.83	10.42	0.38	1.5
					Av = 1.66

$t = d/s/n = 3.66$, $P < 0.05$

0.14 - 0.49 mm. The head capsule widths for the different larval groups conformed to Dyar's (1890) rule: the width of the head capsule increases in geometric progression in successive instar by a ratio of about 1:4 (Table 2). The stadial periods of these larvae belonging to the cluster groups I - V were 1.5, 1.9, 2.2, 1.9 and 3.5 days respectively. These stadial periods too were significantly different (Table 1).

When shed head capsules of larvae were measured, their widths were found to range from 0.19 - 1.67 mm (Table 3). When these head capsules widths were plotted against larval frequency the histogram obtained showed five distinct, non-overlapping groups representing 5 larval instars (Fig. 3). Close examination of the batch of larvae under study revealed that they shed their head capsules 5 times during their entire larval period which ranged from 11-16 days. The head capsules were shed at different intervals giving mean stadial periods of 3.5, 1.6, 1.8, 3.2 and 2.0 days respectively for the 1st to 5th instar larvae respectively.

When the same data were subjected to cluster analysis, five markedly separable cluster groups were obtained (Fig. 4). The mean widths of the shed head capsules and their increase from one instar to another in given in Table 3. These increments too were significantly different ($t = 8.67$, $P < 0.05$) with the head capsule widths of different larval groups conforming to Dyar's rule (Table 4).

Of the different parts of the *S. melongena* plant, fruits suffered the highest damage and the buds, the least (Table 5). Some of the damaged buds, flowers and shoots did not contain any larvae at the time of examination, whilst the maximum of larvae present per damaged part was 2, except in fruits which contained up to 4 larvae. First instar larvae were confined to buds and flowers only while the 2nd instar larvae were distributed in all the different parts of the host plant (Table 5). Third to 4th instars were found mostly in shoots and fruits, while the 5th instars were confined to fruits alone. Of a total of 395

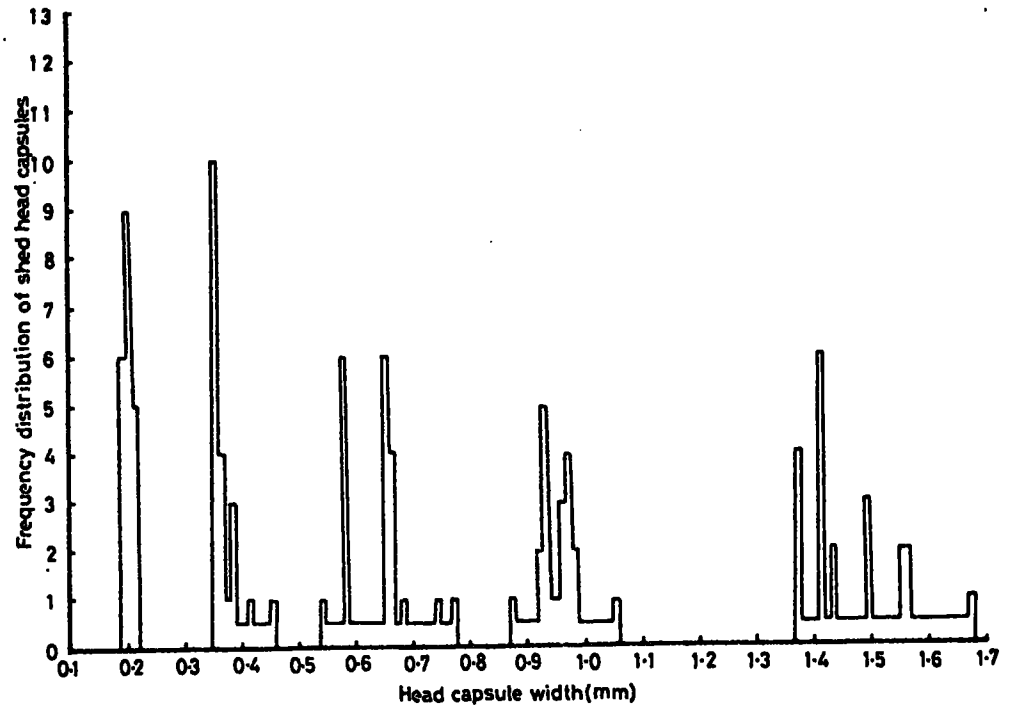

Fig. 3. Frequency Distribution of Shed Larval Head Capsule Widths of *L. orbonalis*

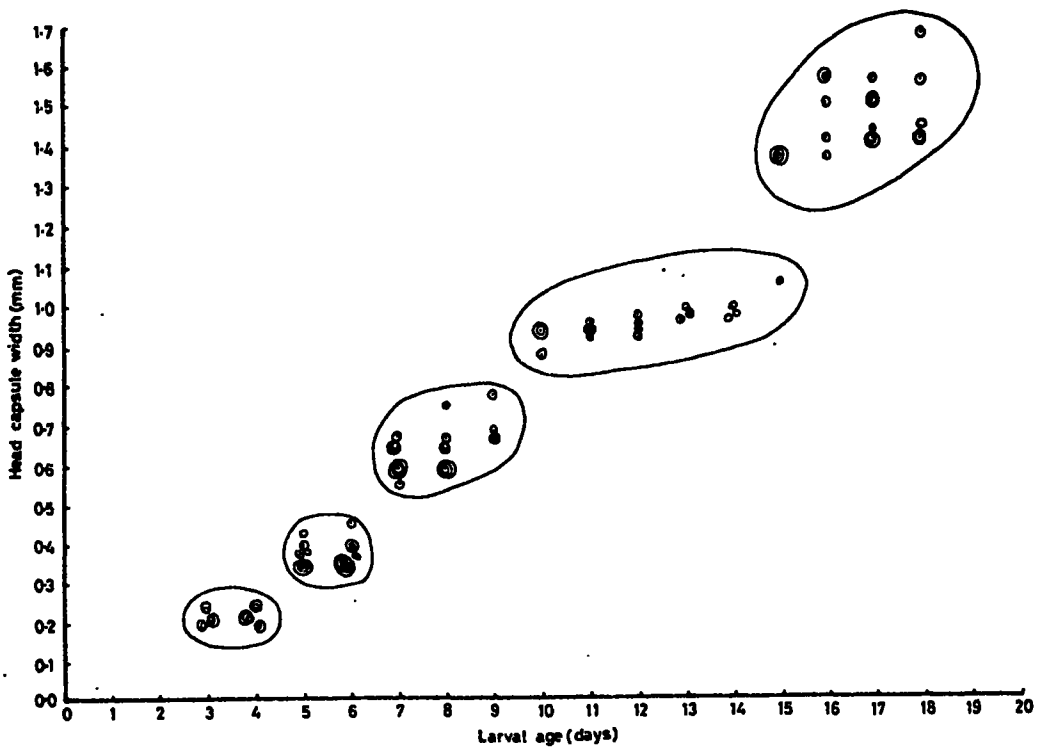

Fig. 4. Cluster Diagram of Shed Larval Head Capsule Widths *vs.* the age of *L. orbonalis* Larvae

Table 3. Head capsule widths (shed) and stadial periods of *L. orbonalis* larval instars

Larval instar (n)	Head capsule width (mm)			Stadial period (days)	
	Range	Mean ± S.D.	Increment	Mean ± S.D.	Range
First (20)	0.19-0.22	0.20 ± 0.01	-	3.5	(3-4)
Second (20)	0.35-0.45	0.36 ± 0.03	0.16	1.6	(1-3)
Third (20)	0.54-0.77	0.64 ± 0.06	0.28	1.8	(1-3)
Fourth (20)	0.87-1.05	0.95 ± 0.03	0.31	3.2	(1-5)
Fifth (20)	1.36-1.67	1.46 ± 0.08	0.51	2.0	(1-3)
			t=8.67*	t=13.71*	

t = x/s/n * Means P < 0.05

larvae collected from the different parts of the plant, mature fruits contained the majority of the larvae, while buds contained the least. Overmature fruits (yellowish in color) were not subjected to fresh attacks at all. Fruits were observed to be most susceptible to larval attack when they were 2 weeks old from petal fall. When the host plant bore no fruits, damage to shoots was observed to be high. When infested shoots bearing larvae began to wither and die and the fruits to rot, larvae were seen to move out and bore into fresh shoots and fruits on the same plant.

The size of the entrance hole bored by larvae entering the plant was found to give a clue to their instar. As such, entrance holes bored by first to fifth instars had a mean diameter of 0.25, 0.40, 0.75, 1.0 and 1.5 mm respectively (n = 25).

DISCUSSION

Several workers have reported that the frequency distribution of head capsule measurements does not always show clear-cut differences between instars, thus leading to incorrect estimation (Kishi 1981; McNeil, 1978). In the present study too the frequency separation was not very clear-cut when intact head capsule widths were considered. However, with shed head capsules, five distinctly separable non-overlapping groups were obtained suggesting five larval instars. Recovery of 5 shed head capsules from each of the individually reared larvae further confirms five larval instars for *L. orbanalis*. Although the increases in the head capsule widths for successive instars were variable, the head capsule measurements conformed to Dyar's (1890) rule. Dyar (1890) observed that the width of the head capsule increases in a regular geometrical progression in successive instars by a ratio of about 1:4. In *L. orbonalis*, the rate

Table 4. Conformity test for L. orbonalis larval instars to Dyar's rule (Shed head capsule width measured in mm).

Larval instar	Observed mean	Calculated mean	Coef. of variation (%)	Difference	X_{n+1}/X_{nnn} ratio increase
I	0.2	-	5.0	-	-
II	0.36	0.3	8.33	0.06	1.8
III	0.64	0.54	9.37	0.1	1.78
IV	0.95	0.96	3.16	-0.01	1.48
V	1.46	1.42	5.48	0.04	1.54
					Av = 1.65

$t = d/s/n = 4.15$ $P < 0.05$

of increase of the head capsule widths from one instar to another is sufficient to differentiate between stadial increments but not large enough to deviate from Dyar's rule.

Alghali (1985) working on *Chilo partellus* Swinhoe (Lepidoptera:Pyralidae) used analysis of variance, Duncan's multiple range test and scatter diagrams to determine the number of larval instars. However,

Table 5. Incidence and distribution of L. orbonalis larval instars on S. melongena

Part of plant (n)	% damaged parts (n)	% larvae/plant part (n)	No. of Larvae/Instar				
			First	Second	Third	Fourth	Fifth
Bud (207)	9.1 % (19)	5 % (20)	6	14	0	0	0
Flower (184)	19.5 % (36)	9 % (36)	8	16	12	0	0
Shoot (1065)	9.3 % (100)	25 % (98)	0	22	48	28	0
Young fruit	65.3 % (147)	26 % (96)	0 (103)	24	38	32	9
Mature fruit	68.3% (177)	35% (12)	0 (138)	29	42	36	31

none of these methods revealed a clear-cut separation of larvae into instars. Cluster analysis of intact head capsule width measurements gives a better separation (both visually and statistically) of instar groups, especially when shed head capsules are inaccessible.

The size of the entrance hole bored by larvae gave a good indication of the larval instar responsible for the damage. Hence, the diameter of the entrance-hole is a convenient measure of the instar inhabiting the internal tissues.

The spatial distribution of *L. orbanalis* larvae within the host plant (based on total larval counts, irrespective of their instars) has been described as a negative binomial by Shukla (1986). The present study, however, concentrates on the distribution of larval instars with respect to the different parts of the host plant, which reflects the pattern of movement of larvae on the host plant. Larvae, on hatching, settle on the softest parts of the plant, flower buds and flowers, and bore into their calyxes. The finding that the different instars are confined to specific parts of the host plant indicates that larval distribution and movement within the plant are determined by the hardiness of the plant part together with the availability of edible tissue. The first factor confines the first instar larvae with weak mandibles to the softest parts of the plant. The second factor has a direct relevance to the age and voracity of larvae. The very voracious final instar larvae with well developed mandibles being therefore confined to fruits, the part that bears sufficient edible tissue and has a hard epicarp. One of the plant characteristics associated with resistant *S. melongena* varieties includes fruits with hard epicarp (Panda *et al.*, 1971). Therefore, it is envisaged that growing of such varieties together with the use of natural enemies of *L. orbanalis* would enable farmers to overcome heavy dependance on chemical pesticides.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Natural Resources, Energy and Science Authority of Sri Lanka for providing financial support. Advice given by Dr. R. O. Thattill, Faculty of Agriculture, University of Peradeniya, with the statistical analysis is gratefully appreciated. We are thankful to Dr. F. P. Amerasinghe, Department of Zoology, University of Peradeniya for his comments on the manuscript.

REFERENCES

Alighali, M. A. 1985. Larval instar determination for *Chilo partellus* Swinhoe (Lepidoptera:Pyralidae) reared on sorghum. *Insect. Sci. Applic.*, 6 : 677-79.

Atwal, A. S. and Verma, N. D. 1972. Development of *Leucinods orbonalis* Guen (Lepidoptera: Pyraustidae) in relation to different levels of temperature and humidity. *Indian J. Agric. Sci.*, 42 : 849-854.

Chatfied, C. and Collins, A. J. 1980. Introduction to multivariate analysis. Chapman and Hall Ltd. 246 pp.

Dyar, H. G. 1890. The number of moults of Lepidopterous larvae. *Psyche. Camb.*, 5 : 420-422.

Kishi, Y. 1981. Reconsideration of the method to measure the larval instar by use of the frequency distribution of head capsule widths or lengths. *Can. Ent.*, 103 :1011-1015.

Lal, B. S. and Ahamad, S. Q. 1965. The biology and control of brinjal (egg plant) fruit and shoot borer, *Lencinodes orbonalis*. *J. Econ. Entomol.* **58** : 448-451

McNeil, J. N. 1978. The number of larval stages of *Thymelicus lineola* (Lepidoptera:Hesperiidae) in Eastern Canada. *Can. Ent.* **110** : 1293-1295.

Mehto, D. N., Sing, K. M., Sing, R. N. and Prasad, D. 1983. Biology of brinjal fruit and shoot borer *Leucinodes orbonalis* Guen. *Bull. Ent.*, **24** (2) : 112-115.

Nyar, K. K., Ananthakrishnan, T. N. and David, B. V. 1976. General and Applied Entomology. Tata Mcgraw Hill Publishing Co. Ltd.

Panda, N., Mahapatra, A. and Sahoo, M. 1971. Field evaluation of some brinjal shoot and fruit borer, *Leucinodes orbonalis* Guen. *Indian J. Agric. Sci.* **41**, 597-601.

Sandanayake, M. 1987. Biology of the brinjal shoot fruit borer, *Leucinodes orbonalis* (Guen.) and its larval parasite *Trathala flavoorbitalis* (Cam.). M. Sc. Thesis. University of Sri Jayewardenapura, Nugegoda, Sri Lanka, pp. 301.

Shukla, R. P. 1986. Spatial pattern of egg plant shoot and fruit borer, *Leucinodes orbonalis* Guen. (Lepidoptera:Pyraustidae) larvae. *Insect Sci. Applic.*, **7**: 611-615.

Yang, Z. Q. 1982. A preliminary study of *Leucinodes orbanalis* Guen. *Kunchong Zhisi*, 19: **20-22.**