

Biology of Brinjal Shoot and Fruit Borer (*Leucinodes orbonalis* Guenee) and screening of various genotypes for resistance

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Abstract

Present investigations were conducted in many areas of Himalayan region during 2018-19. The Shoot and fruit borer, *Leucinodes orbonalis* Guenee were found most destructive pest of brinjal. The biological of pest were studied under laboratory conditions and various parameters were determined. The number of eggs laid, egg length, breadth and fecundity were 195, 0.78mm, 0.50mm and 217, respectively. The pre-oviposition, oviposition, incubation, larval and pupal periods were 1.15, 4.5, 3.95, 9.75 and 6.71 days, respectively. Longevity of adult females was higher than male with means values 5.56 and 3.61 days, respectively. Both sexes were different in their body lengths, breadths and wing span with mean sex ratio of 0.47. Life cycle was completed in 31.3 days. The genotypes screened against *L. orbonalis* showed varied responses. Maximum of the genotypes screened were categorized as resistant. Higher damages were recorded on fruits compared to shoots. Two varieties Pusa Purple Cluster and Pusa Purple Long suffers high level of fruit damage and were categorized as highly susceptible and susceptible on fruit weight basis and number basis, respectively.

Keywords: Brinjal, Shoot and fruit borer, weight, Number, *Leucinodes orbonalis*

1. Introduction

Brinjal also called egg plant or aubergine (*Solanum melongena* L.) belongs to genus *Solanum* of nightshade family Solanaceae (Tsao and Lo 2006). It is an essential solanaceous crop grown throughout in world and has been described as native to India, where it continues to grow wild (Abhishek and Dwivedi 2020, Dhaliwal and Aggarwal 2021, Trujillo 2003). The contribution to total cultivation and yield is higher from Asia with China ranks first followed by India in global production. Globally, the cultivation is more than 1600000 ha yielding around 50 million MT with 90% production contributed by major five countries. China shearing 58% of total global output followed by India with 25% (FAO, 2012). The average production in India is 11045.47 thousand tonnes with Bihar contributing by 23.69% of total national production (National Horticulture Board 2018-19). In India there are many issues which are contributing to reduced yield and among them the insect pest is one of the major and important factors. In brinjal crop a total of 26 pests have been observed (Vavai 1970, Dar *et al.* 2015) and among them the brinjal shoot and fruit borer *Leucinodes orbonalis* Guenee is most devastating pest (Hung *et al.* 2020, Kar *et al.* 2020) and may cause a loss of upto 100% if not controlled (Hussain *et al.* 2002). The *L. orbonalis* is a monophagous insect pest and is very important constraint owing to its internal feeding habit. By nature, it is internal soft tissue feeder damages tender shoots and fruits from nursery till harvesting (Jat *et al.* 2020). Pest lays eggs singly on tender parts and after hatching the first instar stage larvae puncture inside the fruit to feed on soft pulp. It has five instar stages, pupal and final adult stages. Females are bigger, swollen abdomen and higher wing span. Generally, female sex dominates in population. The damage is first observed on shoots and later attacks the fruits commencing from flowering stage till harvesting. The mean fruit infestation caused by this pest is upto 90.86% (Rahman 1997). Generally the wild genotypes exhibit high level of resistance against *L. orbonalis* and among them the most prominent are *anomalum*, *gilo*, *incanum*, *indicum*, *integrifolium*,

khasianum, *sisymbriifolium* and *xanthocarpum* possessing high level of resistance against the pest (Behera and Singh 2002). The resistance due to wild genotypes is either by lack of attractants or by plant repellent effect to borer (Tlak Gajger and Dar 2021). The interspecific progenies of brinjal were screened for resistance to shoot and fruit borer and the progeny of F8 generation were found less damaged (Ghosh *et al.* 2011). Similarly, a wide range of susceptibility was recorded worldwide. In present investigation we are studying the biology of pest and screened 10 prominent genotypes from India to evaluate resistance level against shoot and fruit borer attack.

2. Materials and Methods

The present studies were done using the different varieties of brinjal (*Solanum melongena*) from Himachal Pradesh. The research area is situated between 32° 22'40 to 33° 12, 40 north latitude and 75° 47'55 to 79° 04'22 east longitude. In altitudes the experimental areas range from 350m to 6,975m above the mean sea level in the lap of Himalayas. The laboratories facilities were taken from New Delhi-India during 2018-19.

Culture of *L. orbonalis*

The infested fruits were gathered from the unsprayed brinjal field and a batch of 50 infested fruits were taken and kept in a plastic rearing cage of size 96 x 26 | 680 um Aperture with dimensions W24.5 x D24.5 x H24.5 cm. The cage was monitored daily till the emergence of the adult. A total of five pairs of day-old adults were caught gently and later introduced into white ovipositional cage of size 92 cm x 70 cm x 106 cm. The oviposition cage is already put with tender shoots and fresh small fruits of brinjal plant.

For studying the biology of the *L. orbonalis*, the rearing of the *L. orbonalis* was done under laboratory conditions to obtain mass culture of larvae of different instar stages. Infested fruits collected from unsprayed brinjal field were kept in glass Petri dish of size 20x20cm. The Petri dish have layer of cut pieces of paper covered with a piece of thin muslin cloth and tightened with strong rubber band. The fresh and tender brinjal twigs and fruit cuttings as food were changed on every alternate day and the fecal pellets excreted by larvae were cleared to maintain proper hygienic conditions during the study period. Days passed and consumption of food continues and finally the full grown 5th instar larvae channel out from surface through an exit bore that is bigger than entry hole. The larvae spun cocoon and pupates in paper folds or periphery of muslin cloth. The pupae were collected, counted and kept in separate glass Petri dish under laboratory conditions. After weeks time the eclosion of pairs of moth occurs and events were monitored; five pairs of male and female adult moths were transferred into separate glass chimneys provided with sugar solutions. The chimneys were containing black paper strips for egg laying which occurs in second night of emergence when 86-90% of mating has occurred in first night of emergence. Further, the mating process, oviposition and adult emergence occurred actively in first night and were recorded. The adult emergence occurred near sunset whileas mating occurs in late night of first day of emergence. The adult moths were fed with 10-20% sugar solution. The soaked cotton in sugar solution was kept inside glass chimneys for overnight and replaced five hours. The upper broad end of the each glass chimney was closed by muslin cloth and rubber band. The mating orientation and events were monitored carefully during night hours that varied with time and copulation event (will be discussed in detail in next article). The eggs obtained from gravid female moth were shifted to Petri dishes of size 20 × 20 cm along with egg bearing substratum paper strips. Eggs were counted and kept for hatching; later the hatching larvae were kept separated for one more generation on brinjal fruit and fresh succulent twigs. Investigation was initiated with eggs harvested from later generation of the moths and later were utilized for detailed study. In total ten petridish were taken and in each fresh eggs were kept for biological studies under laboratory conditions. The 1st instar larvae of mean age 0-12 hours after hatching were transferred to glass petri dishes of size 20 × 30 cm. The fresh food slices (shoot, fruit, twigs) were immediately put in petridish for feeding. The foods in petridish were changes regularly to prevent disease development and also to maintain hygienic conditions. Fecal pellets were cleaned after every two hours. Weather data were recorded during rearing, and favorable conditions were maintained.

Studies on various biological stages:

Egg

The normal egg count laid by female of *L. orbonalis* under natural conditions is 52-250. In open the eggs were laid singly on tender parts, however in the present experiment the eggs were deposited on walls of glass chimneys which were later gently removed with the support of camel hair brush. Eggs on walls of glass were also laid singly. The females after copulation lay eggs within the period ranging from 2 -5 days after emergence. The eggs were white to creamy and flat in appearance and incubation period of egg batch, pre-oviposition, fecundity, oviposition period, egg hatching period, egg hatching and egg length and breadth was recorded.

Biology of Brinjal Shoot and Fruit Borer (*Leucinodes orbonalis* Guenee) and screening of various genotypes for resistance

Larvae

Within one day after egg laying the larvae hatches out and begins to search for the food. Larvae went under five moultings of different durations and the fully grown 5th instar larvae were pink coloured, stout in look, with brown head. We recorded various observations on larvae after first hatching. Five petridish were taken and maintained with food like soft leaves and tender buds (in three petridish), new fruit buds and stem cuttings (two petridish). All feeding recordings were done instar wise and food wise. The time duration from hatching till initiation of pupation were considered as larval period. Larval body has warts all around and through them small microscopic hairs protrudes out on skin. The larval period consumes approximately 10-12 days, and on maturity it exit out from host tissues and subsequently form pupae in petridish, however under open conditions it pupates inside crop debris. The larval periods were recorded during the studies and their growth rate was determined by Dyar's rule.

Pupa

The pupation period is concealed stage of the larvae, and generally larvae pupation occurs inside a grey tough silky cocoon. In pupal stage the pest undergoes overwintering. Under laboratory conditions when larvae pupate inside petridish, it was kept undisturbed till adult emergence. On emergence the moth were separated out into pairs. The duration of pupal period lasts from pupal formation till adult eclosion. Pupal period was recorded.

Adult

Various adult characteristics were recorded:

Adult moth is nocturnal in behaviour and under either situation (open or laboratory) it starts emerging from the pupae just after sunset. The adult emergence continues till sun rise and no adult emerged in day time. We observe emergence at two times in mid-night and in morning, and percentage was recorded. The longevity of adults was recorded from moths kept in glass chimneys provided with 10-20% sugar solution as immediate food. The time period from eclosion to death period was taken as longevity of adult moth, irrespective of gender. Insect sexes were separated based on variation in abdomen, specially projected in females with bigger size. Sex ratio was recorded. Life cycle (egg to adult), total developmental period, longevity, mating, moth lengths, breadth and wing span were recorded.

Screening of genotypes against *Leucinodes orbonalis*

Screening of genotypes was done under field conditions. The seedlings of 10 promising brinjal genotypes were transplanted in plots of size 3.5 x 2.25 m during 1st June 2019 following Randomized Block Design with three replications and ten treatments. The plant to plant and row to row distance of 65 and 40 cm, respectively. The plots have clay loam soil texture. In total 25 plants from different genotypes per replication were tagged at random and damage on the basis of shoot, fruit weight and number were recorded commencing from first picking (40 DAT) till harvest of the crop.

Per cent infestation by weight (g)=	$\frac{\text{Weight of infested fruit}}{\text{Total weight of fruits taken}} \times 100$
Per cent infestation by number (n) =	$\frac{\text{Number of fruits damaged}}{\text{Total number of fruits taken}} \times 100$
Per cent incidence (shoot) =	$\frac{\text{Number of shoots damaged}}{\text{Total number of shoots taken}} \times 100$

On the basis of mean per cent damage due to *L. orbonalis* the severity of infestation was graded as under.

Fruit and shoot damage (%)	Grade	Rating
0	Highly resistant (immune)	0
1-3	Resistant	1
4-7	Fairly Resistant	2
8-11	Tolerant	3
12-15	Susceptible	4
>16	Highly susceptible	5

Statistical analysis:

The data collected were subjected to Analysis of Variance (ANOVA). Latest softwares were used for the analysis and graphic representation of the data. Tukey's test was used to draw significance of damage by *L. orbonalis* to different brinjal genotypes under field conditions.

3.Results and Discussion

Brinjal shoot and fruit borer belongs to order Lepidoptera and group endopterygota is holometabolic with distinct egg, multiple instars, pupa and adult stages. In present investigation the biological features were studied in laboratory for two generations using brinjal slices from tender twigs, stems, fruits and small flowers as food source. We represented mean data and standard error of variance of both years together (**Table-1 & Fig-1, 3**). During course of studies we observed that in months March to May the temperature ranges from 21.5-24 to 26.5-32°C; while as the mean relative humidity from morning to evening were in range of 70 to 90 per cent, respectively. The eggs of *L. orbonalis* were creamy to white in colour once laid. The eggs gradually turned to orange and finally to black before hatching. The eggs are laid singly scattered on black paper, varied in shape from oval to elongated and are glued to surface once laid. Biology of *L. orbonalis* were studied in detail by Hung *et al.* (2020) found that eggs are 1.0 – 1.2mm in length and are oval to elliptical in shape. The observed incubation period varied from 3.20 to 3.75 days in course of two generations (**Table-1**) with an average duration of 3.95 days. Rahman (2006) in an experiment found that incubation period of brinjal borer eggs ranges from 3-5 and 7-8 days in summer and winter, respectively. Further, Rahman (2006) also observed that eggs hatch from creamy white to a dark white larvae 1st instar larvae. During the experiment we found that oviposition period under laboratory conditions ranged from 2 to 5 days (**Fig-1**) in separate oviposition glass chimneys. In one of our trail we recorded only 2 days as oviposition period. But the mean oviposition period was in the range of observations made by other authors. Therefore, the confirmation was drawn from Kumar *et al.* (2011) and Bindu *et al.* (2013) who found that oviposition period range from 3 to 6 days (\bar{x} =4.5 days) under controlled conditions. The first instar larva also called as newly hatched larva was dirty white to white in colour, glabrous, bare and exposed. The body colour of the newly hatched larvae changed from whitish to dark pinkish with slightly lustrous appearance. After completing the four instar stages it moults finally into 5th full grown larva. All the larvae has different durations, sizes and head width (**Fig-2**). The mean of total larval duration was 9.75 days in for both generations. However, the average duration is less than measured from field conditions. It was supported by many researchers who recorded that the larval duration varied from 9 to 18 days (Pal *et al.* 2003, Maravi *et al.* 2013) under controlled rearing conditions.

The pupation period is concealed stage of the larvae, and generally larvae pupation occurs inside a grey tough silky cocoon. In pupal stage the pest undergoes overwintering. Under laboratory conditions when larvae pupate inside petridish, it was kept undisturbed till adult emergence. On emergence the moth were separated out into pairs. The duration of pupal period lasts from pupal formation till adult eclosion and ranged from 4.5-8.7days with mean duration of 6.71 days (**Table-1**). Inside glass jar, soil, muslin cloth is also desirable substratum for pupation. However the pupation was also recorded to occur inside fruit and other residues of the crop. The coloration changes from pinkish to dark brown. Object with blunt anterior end with conical tapering look posteriorly. The pupae have distinct body divisions and pair of spiracles on each abdominal segment. The average pupal duration recorded was 6.71 days. Like other biological parameters the pupal period also varied and last from 6.65-6.71 days depending on various climatic conditions and crop stages (Alam *et al.* 2003). The results were supported by Maravi *et al.* (2013) and Onekutu *et al.* (2013) who found that pupal period extends from 7 to 12 days under controlled conditions. The wing span of males and females were in range of 2.05-2.09 to 2.10-2.14 respectively. The wings are larger in females compared to males. However, in both sexes the wings were whitish with reddish tinge; head and abdomen are blackish brown. In both sexes the forewings have brown and black markings; while as hind wings look dirty white with brownish to black dots and angled margin. The abdomen of females is bigger, swollen, tapering and pointed towards end and looks ovate. In contrast, abdomen in males is small, thinner, and cylindrical with blunt at apical end having whitish hairy structure. During the experiment we observed that adult longevity extend from 2.35-4.75 and 3.98-7.09 days (**Table-1**), respectively in males and females. The longevity of female moth was large compared to males. The total life cycle of the shoot and fruit borer *L. orbonalis* varied from 25 to 35 days with mean value of 31.3 days (**Table-1**). However, some researchers recorded even higher total life cycle duration that varied from 19 to 44 days (Maravi *et al.* (2013) and Onekutu *et al.* (2013). After the pupation, the moth that emerged out were collected, reared and distinguished based on their sexes. The moths were kept in plastic jars provided concentrated sugar solution. We found that population of female moth were higher, so is the dominance inside and outside laboratory conditions. Sex ratio (Male/Female) was calculated and it varied from 0.35-0.55 with an average of 0.47 (**Table-1**). These observations were confirmed by Pal *et al.* (2003) Maravi *et al.* (2013) and Onekutu *et al.* (2013) that under laboratory conditions the sex ratio (M/F) extends from 1:1.07 to 1:2. We found that maximum mating occurred during first half of the night of emergence (**Fig-3**)

Performances of various genotypes against brinjal shoot borer damage were given in table (**Table-2**). The mean shoot infestation was less and maximum of genotypes were categorized as resistant except Pusa Purple Cluster and 'Pusa Purple Long which were categorized as fairly resistant. Similarly, on fruit number basis and weight basis the damage was high; and resistance scale was drawn (**Table-2**). The grading of various genotypes

Biology of Brinjal Shoot and Fruit Borer (*Leucinodes orbonalis* Guenee) and screening of various genotypes for resistance

were done and among all ten genotypes evaluated the maximum of genotype were found fairly resistant, while as Pusa Purple Cluster and 'Pusa Purple Long showed susceptibility on fruit number damage basis whileas as same genotypes showed highly susceptibility on fruit weight damage basis. None of the genotype evaluated were found immune to pest infestation. Dar and Mir (2016) and Dar *et al.* (2017a, b, c) evaluated various genotypes against *L. orbonalis* under field conditions and found the similar results.

4. Summery and Conclusion

The present studies on biology and screening of some brinjal cultivars against *Leucinodes orbonalis* were done in the Himalayan region of Himachal Pradesh during 2018-19. Various biological aspects like egg, larvae, pupa and adults characteristics were determined under laboratory conditions. Ten genotypes were screened against *L. orbonalis* and different resistance responses were received under field conditions.

Table 1: Morphological and Biological parameters of brinjal shoot and fruit borer under laboratory conditions (N=5)

Parameter	2018-19 Range	Mean± SE
Egg length (mm)	0.75-0.79	0.78±0.08
Egg breadth (mm)	0.48-0.51	0.50±0.10
Number of eggs laid	178-270	195±5.00
Pre-oviposition period (days)	1.09-1.18	1.15±0.30
Fecundity (eggs/female)	176-245	217±0.12
Egg hatching (%)	79-85	83.5±3.22
Overall egg hatching (%)	81-98	90.3±4.12
Pupal period (days)	4.5-8.7	6.71±0.39
Life cycle (egg to adult) (days)	25-35	31.3±5.32
Incubation period (days)	3.20-3.75	3.95±0.76
Longevity of adult (days)		
Male	2.35-4.75	3.61±0.11
Female	3.98-7.09	5.56±0.21
Sex ratio (male/female)	0.35-0.55	0.47±0.09
Adult male Length (cm)	1.38-1.41	1.41±0.02
Adult male breadth (cm)	0.31-0.33	0.33±0.01
Adult female length (cm)	1.41-1.43	1.42±0.10
Adult female breadth (cm)	0.45-0.47	0.47±0.04
Male wing span (cm)	2.05-2.09	2.08±0.24
Female wing span (cm)	2.10-2.14	2.13±0.19

Table-2: Screening and categorization of brinjal genotypes against shoot and fruit borer (*L. orbonalis*) on the basis of damage percentage in shoot, fruit weight and fruit number under field conditions during 2018-19

S. No	Name of variety	Shoot damage (%)	Category (shoot damage basis)	Fruit damage (%)				Rank (on the basis of fruit weight damage %)
				Weight basis	Category (weight damage basis)	Number basis	Category (number damage basis)	
1	Pusa Purple Cluster	5.95 ^d	FR**	19.60 ^e	HS**	14.50 ^d	S**	5
2	Punjab Barsati	2.03 ^b	R**	7.80 ^c	FR	5.75 ^b	FR	2
3	Long Green	1.25 ^a	R	4.45 ^a	FR	3.78 ^a	R	2
4	'Pusa Purple Long	4.35 ^c	FR	16.01 ^d	HS	13.25 ^c	S	5
5	Arka Nidhl	2.45 ^b	R	5.57 ^b	FR	4.23 ^a	FR	2
6	Neelkanth	2.38 ^b	R	6.45 ^b	FR	4.97 ^b	FR	2
7	SM 6-7	1.01 ^a	R	3.76 ^a	R	2.98 ^a	R	1
8	Local long	1.57 ^a	R	3.45 ^a	R	2.65 ^a	R	1
9	Hisar Shyamal	2.32 ^b	R	7.75 ^c	FR	6.78 ^b	FR	2
10	Ganesh	1.40 ^a	R	3.51 ^a	R	2.90 ^a	R	1
Statistics		P=≤0.045		p=≤0.031		p=≤0.030		Σ=23
Tukey's test		CD=1.01		CD=2.03		CD=2.10		N=10
*Superscriptions in the numerical values of infestation determines the statistical significance at 0.05% **R=Resistant , FR=Fairly Resistant, S=Susceptible, HS=Highly susceptible								

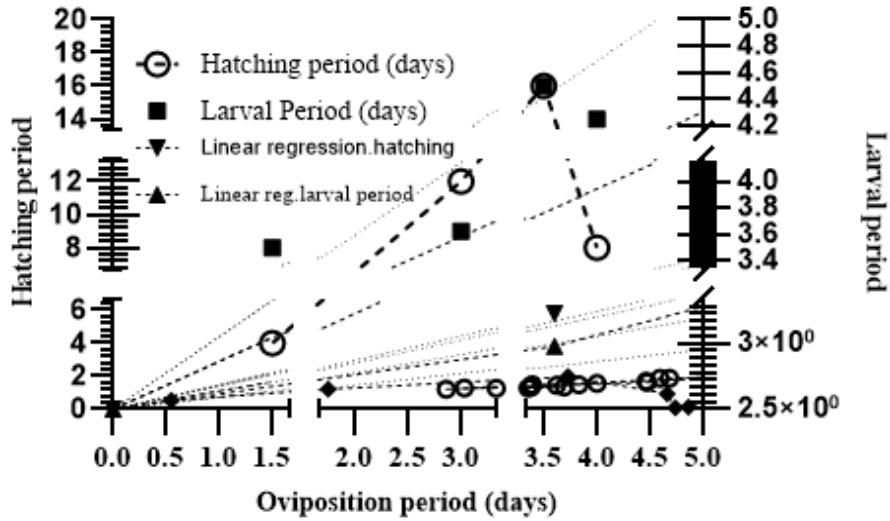


Fig 1: Mean value (N=5, d.f =4) of variation of biological characteristics of *L. orbonalis* under laboratory conditions.

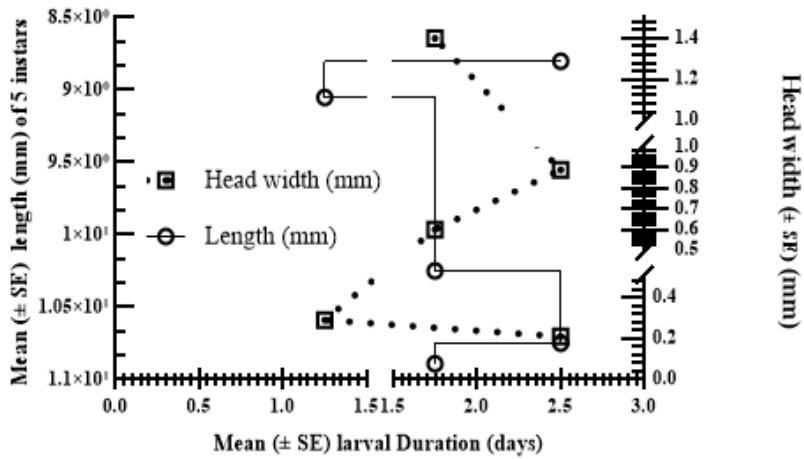


Fig 2: Mean (±SE) value (N=5, d.f =4) of variation of morphological characteristics of *L. orbonalis* under laboratory conditions.

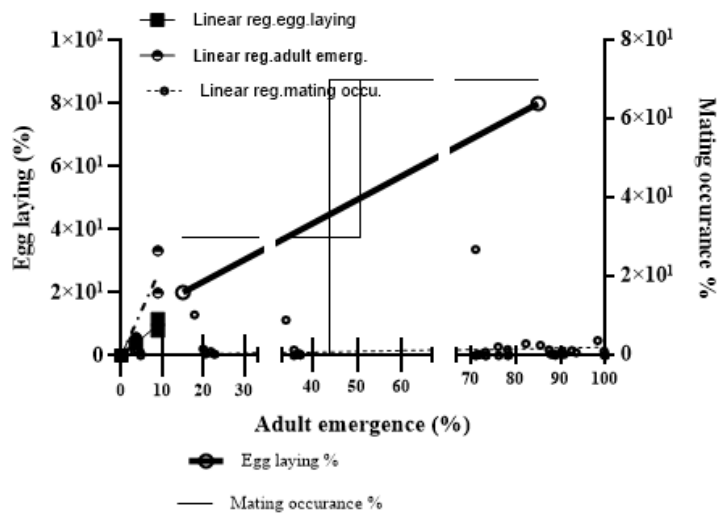


Fig 3: Adult emergence (%), mating event occurrences (%) and egg lying (%) of *L. orbonalis* under controlled conditions..

Biology of Brinjal Shoot and Fruit Borer (*Leucinodes orbonalis* Guenee) and screening of various genotypes for resistance

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