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Why is management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee, difficult? an examination into the pest's unique feeding behavioral biology

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Abstract

Brinjal is the second highest consumed vegetable in India, produced with a yield of 8-9 million tons annually. *Leucinodes orbonalis* Guenee (Lepidoptera: Crambidae) commonly called brinjal shoot and fruit borer (BSFB) being a major pest of brinjal causes heavy loss to the crop productivity. The inadequate information about the special feeding behavior of BSFB has led to its management challenging and difficult. The present study was taken up to report the unique feeding behavior of BSFB. Freshly hatched first instar neonate larvae of BSFB were released on different reproductive parts of brinjal as a host plant. Details of feeding behavioral dynamics were studied by cross-examining individual larval feeding. Three feeding behavioral patterns viz., “homing” “vein tunneling” and “compel feeding” were found to be key factor of initial stages of host-insect larval interactions that represent one of the weakest links in the developmental cycle of BSFB and that will be useful in the strategic development of effective pesticides.

Keywords: *Leucinodes orbonalis*, feeding behavior, vascular tissue, homing.

1. Introduction

Brinjal (eggplant, aubergine, *Solanum melongena* L.) is an important vegetable crop of the world and India ranks second, after China, in the global production of brinjal grown in approximately 5.65 lakh hectares with a production of 90 to 95 lakh tonnes, constituting nearly 8% of the total vegetable production in the country [2, 5]. However, the yield and marketable quality of this crop is severely affected by brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* Guenee (Lepidoptera: Pyraustidae), causes significant losses to the tune of 70%. Brinjal receives very high doses of chemical pesticide applications. Nearly 90% of the Indian farmers apply chemical pesticides in brinjal cultivation and the number of pesticide sprays range from 10 to 28 thus spending a significant part of the cost of cultivation mainly towards chemical pesticide-based plant protection. Ironically, the brinjal farmers in India are resource-poor and mainly belong to small, medium and marginal groups, who depend on this crop as an important source of income. This contradictory and opposing scenario of poor farmers extensively spending on chemical control of BSFB is a serious concern which demands urgent intervention by modern biology to mitigate the problem of BSFB. However, management of this pest has long been considered problematic due to several reasons including apparent absence of resistant genetic sources in the global germplasm and lack of effective pesticides specifically directed towards management of BSFB. Chemical pesticide management of BSFB has long been vexingly difficult due to several reasons. BSFB is purportedly believed to be a monophagous pest, feeding exclusively on brinjal in the absence of any “choice” of compatible wild and related feral host plants [9]. The serious issue to be examined is the nature of insect feeding biology on brinjal. As soon as they hatch, the freshly hatched first instar neonate larvae of BSFB enter internal tissues of the plant through rapid scraping and accelerated feeding. Once inside the tissues, the larvae make themselves inaccessible to outer environment by plugging the entry points using their fecal matter, simultaneously causing wilting and dieback in shoots (thus reducing fruit bearing capacity) and damaged young fruits [10]. Actively feeding larvae of BSFB contaminate the fruits with their tunnels and frass, inviting secondary infection and severely reducing marketable quality. This entitles the pest as one of the voracious internal feeders. Consequently, the larvae become inaccessible to most contact pesticides including the latest molecules like indoxacarb and chlorantraniliprole (Rynaxypyr®)

forcing the grower to apply higher doses of contact or systemic insecticides. With so profound repercussions emanating from BSFB feeding, surprisingly, important research details of the nature of the feeding behavior and interaction of BSFB with its host are lacking. In this paper, we carefully examine the nature of the feeding mechanisms of BSFB, especially of the freshly hatched first instar neonate larvae, and identify critical weak points in this regime of insect stage that will be useful for developing effective strategies in the management of BSFB.

Experiments were conducted by examining both the natural infestation as well as field challenge inoculation of different infective larval stages of BSFB obtained from *in vitro* reared cultures [3, 6, 7]. For developing cultures of BSFB, a method standardized in our lab was used. Primary infested brinjal fruits were collected from field samples and cut open partially to ascertain the presence and active feeding of later instar larvae. The fruits were maintained on sand beds allowing the completion of larval stages. Well developed larvae came out of the bored fruits and enter the sand for pupation. Pupae were collected and maintained in insect cages till the emergence of adult moths. Male and female adult moths were immediately separated and each pair of male and female moths were placed in glass test tubes (8-10 cms dia x 20 cms ht) containing supported cotton swabs dipped in a feeding solution mix of honey, glucose, multivitamin and multivitamin syrup and streptomycin. The tubes were covered with black cotton cloth to facilitate aeration and egg laying. Eggs were allowed to hatch naturally. Freshly hatched first instar neonate larvae were used for challenge inoculation onto brinjal plants

maintained in the insect proof net houses. Using fine camel hair brush, the larvae were transferred onto growing meristematic tips, leaf petiolar junctions and young, developing fruits. Observations were recorded at timely intervals 3 days post challenge inoculation. Any visible symptom like bored holes with/without extruding excreta, wilting and drooping of the apical shoot meristem, scraping of tissues and presence of actively feeding larvae and their completion of life cycle, was taken as susceptibility to BSFB while absence of these symptoms even after prolonged periods of more than 10 days was considered as the expression of resistance. Challenging was done using first instar neonate larvae of *L. orbonalis*. Symptoms of typical insect damage on host plants were recorded. Details of feeding behavioral dynamics were studied by interrogating individual larval feeding.

As soon as they hatch, first instar neonate larvae of BSFB were found to actively search for host plants. This was indicated by their differential movement behaviors; random movements on the black cotton cloth covered tubes which do not contain any brinjal tissues and immediate scraping as soon as the neonates were placed on to various host plant parts including leaf lamellae, shoot apical meristem and calyx region of the young, developing fruits. Careful examination of the movements and feeding behavior of the larvae indicated that the neonates tend to migrate towards leaf veins and more specifically leaf midribs irrespective of their initial placement on the leaf lamellae (Figure 1 a-d).



Fig 1: Vein tunneling and feeding behaviour of brinjal shoot and fruit borer, *L. orbonalis*. *a*, Extensive feeding of leaf lamellar tissue. *b*, Homing in onto veins by the first instar young larva and initiation of vein tunneling. *c*, Fully cross-cut leaf midrib due to feeding and homing. *d*, Close up of the insect larva extensively feeding in the region of junction of leaf lamella and veins (arrow indicates larval position).

The neonates burrow the leaf tissue during this process rather than simple and mechanistic scraping. Once the neonates reach their destination, they burrow deep inside the midribs. On shoots, neonates were found to prefer leaf petioles and junction between leaf petiole and stems. Neonates were also found to attack on other parts of the plant like flower buds and

young developing fruits. On developing shoot meristems, the neonates preferably burrow deep inside the main shoot apical meristems as these meristems offer a resourceful niche. Irrespective of the site of entry, neonates were found to plug the entry holes with fresh excreta (Figure 2).



Fig 2: Tunneling, internal feeding and establishment of BSFB on different parts. *a*, Extensive meristem damage and fecal matter (meristem tip partially cleared to show the feeding larva). *b*, Infestation on leaf petiolar region. *c*, Infestation on young floral bud. *d*, Typical voracious feeding on internal fruit tissues by larva with extensive feces plugged at entry hole.

In our knowledge, these three feeding behavioral patterns viz., “homing” towards leaf venation and leaf midrib, corresponding “vein tunneling” feeding behavior and a shift in the feeding behavior of neonates from external feeders to compel internal feeders, have not been reported previously elsewhere [1]. A thorough understanding of the neonate feeding biology *vis-à-vis* host-pest interaction is important in addressing the management of insect pests like BSFB where such information is not available. However, on the contrary, most research is concentrated on the older instars as they are easy to handle and observe, conveniently disregarding first instar neonates.

Young leaf veins penetrate and spread throughout the mesophyll layers of a leaf. Veins comprise of vascular tissue, xylem and phloem, and connect vascular tissue of the stem to the photosynthetic cells of the mesophyll, *via* the petiole. While xylem cells mainly transport water and dissolved minerals from the roots to the leaves, phloem cells mainly transport photosynthates made in leaves and chlorophyllous cells to the sink. Effectively, vascular tissue or any origin represents a nutrient-rich and self-sustaining niche for BSFB larvae, while young developing fruits also offer same advantages. In our observations, larvae often were found not to stay long on leaf laminae but were found to start scraping leaf tissues in search of veins. The hungry freshly hatched first instar neonate larvae receive their first and crucial doses of food when they start scraping and feeding on epidermal tissues, before they apply their innate behavior of scouting for vascular tissues of midribs, petioles, shoot apical meristems, stems and young fruits. Besides leaf lamellar veins and midribs, the insect larvae attack growing shoot meristems and leaf petioles also. The first instar larvae bore into the meristems after homing in and establish inside the growing region. This not only stunts and affects the host but also makes the entire plant even unproductive if the infestation is at a younger and vegetative period of the host. Similar

observations on feeding behavior were observed previously on related and feral brinjal species [8].

Combination of the pest’s dedicated monophagous diet and atypical homing and vein tunneling behavior marks it unique among the members of the family Lepidoptera. This evolutionary feeding behavior of the insect pest also enables it to directly face the challenges of chemical pesticides and thereby significantly increasing the chances of either adaptation or targeted selection of resistant alleles in the pest population. Lepidopteran neonates face the daunting task of a paraphernalia of plant defense mechanisms including surface characters and internal tissue characters, apart from mounting biochemical plant defenses, enemies (in the form of predators, pathogens and parasitoids) and hostile climate to survive. In spite of these vagaries, first instar neonates are the most destructive and problematic stage, more so in the case of BSFB. It is now apparent that management of BSFB is difficult because the susceptible first instar neonates are exposed open on plant surface very briefly before they are safely cocooned inside the plant tissues. The first few hours of the neonates immediately after hatching are their most susceptible period and this window represents an opportunity for taking up effective integrated pest management. To this end, our findings regarding homing, vein tunneling and feeding behavioral shift observed in the neonates of BSFB represent key component of initial stages of host-insect larval interactions that represent one of the weakest links in the developmental cycle of BSFB and that will be useful in the strategic development of effective pesticides.

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