



Behavioral studies of pomegranate fruit borer, *Deudorix isocrates fabricious* (Lepidoptera: Lycinidae)

Ravindrakumar D Patil

Department of Zoology, Arts, Science and Commerce College, Ozar, Nashik, Maharashtra, India

Abstract

Deudorix isocrates is one of the serious pests of Pomegranate, *Punica granatum*. The present study was undertaken since behavioral understanding of pests has crucial significance in the Pest Management. The present study dealt with larval behavior like searching period, selection of site to enter in the fruit, increase in size of entrance hole, selection of sites for pupation and adult behavior with respect to oviposition and fecundity. It was found that rarely the gravid female lays egg on flower, twigs and underside of leaves. The calyx end of fruit is the most preferred site of egg laying (68.65%) than other parts like lower half (11.56%), stalk base (8.31%), upper half (7.49%) and middle region (3.97%) of the fruit. After hatching the most of the neonate larvae (32%) consumed 16 to 30 minutes of period to search suitable site to enter in the fruit. the searching period is ranged between less than 15 minutes (6%) to more than 4 hours (2%) in the rare cases. The lower half of the fruit is the most suitable site for larval entry (57.77%) while the stalk base region of the fruit was rejected for larval entry (0%). The larvae cut the fruit rind to increase diameter of entrance hole from early to late instar stages. It was found 0.84 mm in first instar, 1.43 mm in second instar, 2.68 mm in third instar and 5.76 mm in fourth instar stage of larva. While studying pupation sites it was revealed that the maximum number of larvae (62.60%) pupated inside the fruit, a least number (1.56%) of larvae pupated on the fruit surface. 2.43% of the larvae pupated inside the calyx while 33.39% of the larvae pupated somewhere else.

Keywords: *Deudorix Isocrates*, pomegranate, pest, behavior

Introduction

It is well known that larvae of butterflies and moths are the serious pest of various crops and are responsible for considerable economic loss. Hence, the lepidopteran pests became “forefront object” of bioecological studies. In the pest ecology the behavioral study has a greater significance to understand adaptive features in lepidopteran pests and their host plant specificity (Chardonnet *et al.*, 2014) [8]. Understanding of behavior of the pest is also important to design Pest Management Strategies (PMS) and Insect Resistant Management (IRM) (Spangler and Calvin, 2001; Paula *et al.*, 2012; Pannuti, 2019) [31, 25, 24]. Mostly the behavioral studies on various species of lepidopteran pests have been carried out with respect to ovipositional behavior, feeding and searching behavior of larva (Lietal., 2015; Rajapakshe and Walter, 2007; Lakshmipathy, 2000, Dolek *et al.*, 2013) [18, 11]. *Deudorix* is one of the lepidopteran genera (formerly known 'Virachola') includes some pest species which attack wide range of host plants, mostly the fruit plants. *D. isocrates* is one of the polyphagous pest species of this genus. It attacks wide range of host plants like guava, aonla, ber, citrus, tamarind, apple, sapota apart from the pomegranate (Singh and Singh, 2009; Balikai *et al.*, 2011; Chhetry *et al.*, 2015; Arya and Dubey, 2017; Gundappa *et al.*, 2017; Muthiah and Indragandhi, 2021) [3, 10, 1, 13]. However, it appeared as the most destructive pest of Pomegranate, *Punica granatum* (Gupta and Dubey, 2005, Kumar, 2010) [13] causing 65-70% yield loss worldwide (Kumar *et al.*, 2017). It is distributed all over India and common in Maharashtra (Riteshkumar, 2020). In India it causes 40-90% yield loss of pomegranate (Wadhi and Batra, 1969; Nair, 1978) [33] consequently results in to remarkable economic loss of the pomegranate growers. In Maharashtra and Karnataka, the incidence of *D. isocrates* on pomegranate has been reported throughout the year with varying degrees of intensity (Shevale and Khaire, 1999). The considerable research work has been carried out on bionomics, pest incidences on various host plants, population dynamics, and management of *D. isocrates* but its behavioral aspects are not studied extensively. The present study has importance to add a knowledge in behavioral understanding of this pest which will help to suggest and plan the pest management practices against its attack on pomegranate.

Materials and Methods

The present work was undertaken at the pomegranate farms nearby villages of Baramati, District Pune from Maharashtra state of India. The behavioral study of *D. isocrates* was undertaken with respect to site of oviposition, searching behavior of newly hatched larva for entry site, site of larval entry into the fruit, relative size of entrance hole in accordance with larval growth and site of pupation. Field observations were made to study the site of oviposition, site of larval entry into the fruit while studies on searching behavior of newly

hatched larva for entry site and relative size of entrance hole in accordance with larval growth were undertaken in laboratory.

Site of oviposition

The plant parts of pomegranate such as leaves, twigs and fruits were screened to know oviposition sites during field survey. When it came to notice that most of the eggs were deposited on fruits, special attention was given to check the regions of fruit surface preferred by female to lay the eggs. Five different regions of the fruit such as, inside of the calyx, lower half portion, middle portion, upper half portion and base of the fruit were taken into consideration. Total 1107 observations were recorded and analyzed in the form of specific region of the fruit and the number of eggs deposited on it. The percentage of eggs deposited on each specific region of fruit was calculated to find the most preferred site of oviposition

Searching behavior of larva for entry site

The fruits along with eggs were plucked and brought to the laboratory. The partial rind of the fruit bearing egg was cut carefully and placed in glass petri dish (9.5 cm dia.). The precaution was taken to avoid fungal growth on the fruit rind and the egg by keeping experimental *set* always clean. The eggs were observed regularly till hatching. The time of hatching was noted. The newly hatched larva was gently lifted by camel hairbrush and placed on the fresh pomegranate fruit so as to allow it to enter into the fruit. Separate fresh fruit was provided for each larva and observed critically till it started boring the fruit rind. The period between hatching and commencement of boring the rind was referred to as searching period. Total 50 observations were recorded for further analysis.

Site of larval entry into the fruit

The larva enters into the fruit by making an entrance hole. Location of entrance hole on specific region of the fruit was taken as a clue of larval entry site. Considering the entrance hole as the site of larval entry, total 1113 entrance holes were observed to note site of larval entry into the fruit from five different regions of the fruit such as inside of the calyx, lower half portion, middle portion, upper half portion and base of the fruit. The observations were analyzed in the form of specific region of the fruit and number of the entrance holes observed on it. The percentage of entrance holes corresponding to the specific regions was calculated so as to know the choice of larva of specific region to get entry into the fruit.

Relative size of entrance hole in accordance with larval growth

To find out relative size of entrance hole in accordance with larval growth, the fruits with live infestation were collected from the field and brought to the laboratory. The live infestation was identified by fresh excreta oozing out from entrance hole. From each infested fruit the diameter of entrance hole was measured and the fruit was dissected to report instar stage of larva inside it. The same procedure was repeated till getting 50 observations of each larval instar stage. The observations were summarized in the form of larval instar stage and average diameter of entrance hole.

Site of pupation

The pest-infested fruits were marked in the field. The marked fruits were observed daily to check the larva pupated on the fruit surface and inside the calyx. Sites of pupation on the fruit surface and inside calyx were easy to locate and recorded accordingly. During this survey it was noticed that the infested fruits with dried excreta at entrance hole are tied to the twig by the web of silk thread accommodate the pupa inside the fruit. Such fruit were collected from the field during the survey, brought to the laboratory and dissected open to confirm the presence of pupa inside the fruit. When the fruit was found without pupa inside it was treated as the pupation site is elsewhere from the fruit. Such 575 fruits were observed to record various pupation sites. The data was presented in the form number and percent of pupae were found on the particular site.

Results

The females of *D. isocrates* preferred the fruit to lay eggs than leaves and twigs of pomegranate trees. The oviposition site selected by female on the fruit was also not one and the same. Majority of the eggs (68.65 %) were laid inside the calyx while least number (3.97%) of eggs were laid on middle region of the fruit. These results suggested that the preferable oviposition site is inside of the calyx; while the least preferable site is the middle region of the fruit.

After hatching larvae spent varying time period in crawling on the fruit surface to search entry site on fruit rind. It is referred here as searching period. The searching period was reported from less than 15 minutes up to more than 4hrs. but maximum number (32%) of larvae spent 16 to 30 minutes to search entry site.

Majority of the larvae (57.77%) preferred lower half of the fruit for entry, 0.35% of the larvae preferred calyx as entry site even though significant number of eggs were deposited inside calyx. Stalk base was totally avoided for entry into the fruit though a few eggs were laid on that region. The upper half and the mid region accounted for 20.66% and 21.20% respectively for larval entry into the fruit. This implies that larvae prefer these regions equally for entry into the fruits.

In present study it was observed that at the beginning newly hatched larva entered the rind of fruit by continuous chewing and cutting the rind till it entered in the fruit completely. The larva made a narrow tunnel always heading towards inside of the fruit. In later days of instar to instar, the larva turned and heading outwards it cut the rind time to time. This activity resulted into gradual increase in size of the entrance hole. According to present investigation the average size of entrance whole was found 0.84 mm in first instar, 1.43 mm in second instar, 2.68 mm in third instar and 5.76 mm in fourth instar stage of larva.

The field studies on pupation sites of *D. isocrates* revealed that with varying number the larvae of *D. isocrates* pupated on the fruit surface, inside calyx and inside the fruit. The maximum number of larvae (62.60%) pupated inside the fruit, a least number (1.56%) of larvae pupated on the fruit surface. 2.43% of the larvae pupated inside the calyx while 33.39% of the larvae pupated somewhere else from the fruit. These places were not known and hence were not recorded. These observations imply that most preferable site of pupation for larvae of *D. isocrates* is the inner portion of pomegranate fruit.

Discussion

D. isocrates female lays eggs singly, some times in a pair and rarely in the small clutch on flowers, leaves and young fruit surface including calyx cup. This general ovipositional behavior has been reported by earlier workers (Balikai *et al.*, 2011; Bhut *et al.*, 2013; Kumar *et al.*, 2017; Bharti *et al.*, 2021; Kaushal *et al.*, 2021) [3]. also. In present investigation it was found that leaves and flowers were rarely preferred for oviposition and mostly the fruits of varying stages were used to deposit eggs. The calyx end of fruit is the highly preferred site of egg laying than other parts like lower half, stalk base, upper half and middle region of the fruit. The similar ovipositional behavior of female *D. isocrates* has been reported earlier (Shevale, 1997) by comparing egg laying from different portions of fruit surface including calyx end. An appropriate site of egg laying is the part of parental care (Mani, 1971) [20]. Deposition of eggs on the most protected site is a strategy for escaping egg predation (Baguette and Schtickzelle, 2003) [5]. Accordingly in case of *D. isocrates* "appropriate" site of oviposition is found to be inside of the calyx where the eggs get protected from predation as a parental care. This finding is helpful to monitor pest attack during early stage at egg laying before fruit damage caused by early instar larva. The results also suggest paper bagging of fruits as a preventive measure against the pest attack through which we can avoid egg laying on the fruits. The paper bagging against this infestation is recommended by earlier work (Kumar and Kamala Jayanthi, 2018; Riteshkumar, 2020) [17] so as to minimize pest attack. In case of *D. isocrates* this control method reduces pest attack up to 10% against 90% in open fruits (Bagle, 2011).

After hatching the 1st instar larva spent some time on fruit surface (Kaushal *et al.*, 2021) to find suitable site to enter into the fruit. This period is treated here as a 'searching period' during which the larva crawls on the fruit rind. On getting suitable site of entry, it cuts the rind (Kumar *et al.*, 2017) with its mandibular mouth parts and makes a fine hole; the entrance hole. The larva continues to cut the fruit rind till it reaches inside the fruit. The present study revealed that the maximum number of larvae (32%) consumed 16 to 30 minutes as a searching period. The searching period of *D. isocrates* larva has been reported previously as 16 to 20 minutes (Shevale, 1997). Finding of present study is nearly similar to previous report. This result of present study reveals that for a very short time period the larvae remain outside before entering into the fruit. Therefore, efficient control can be achieved by properly timing sprays of pesticides when neonate larvae are still on the fruit (Kaushal *et al.*, 2021).

The present study on larval entry sites revealed that the most preferred site for larval entry into the fruit is the lower half of the fruit. The larvae stalk base of fruit to enter into the fruit while under heavy infestation rarely the larval entry was found through calyx cup. According to previous reports on *D. livia* (Awadallah *et al.*, 1970) and *D. isocrates* larvae (Shevale, 1997) shows the same behavior. Their observations are same as that of present study. It appears that the larva usually rejects stalk base and calyx since the rind of the fruit in these regions is comparatively thicker than general surface of the fruit. The selection of lower half of larval entry may be the adaptation of growing larva to drop out its excreta on the ground instead on the fruit surface. Due to which the larva tries to keep infested fruit clean inside as well as outside. Furthermore, the present results also show that the larval entry site is different than oviposition site.

Necessarily, the larva increases diameter of entrance hole by cutting the fruit rind throughout the larval period. The size of entrance hole increases from 0.4 mm up to 7.5 mm. This behavior of *D. isocrates* larva is also reported from the aonla fruit, *Emblica officinalis* (Srivastava and Jain, 1973) and noted the dimensions of larval tunnel. The size of entrance hole was proportionate to larval size and growth. According to earlier report from pomegranate the increase in size of entrance hole is in the range of 0.41 mm, on the first day of 1st larval instar to 7.11 mm on fifteenth day of larval period, at the prepupation (Shevale, 1997). This result is supporting our findings. The present study also showed that on an average the diameter of the entrance whole is 0.84 mm, 1.43 mm, 2.68 mm and 5.76 mm during 1st, 2nd, 3rd and 4th instar stage of larva, respectively. There is no report available on size of entrance hole in accordance with the larval instar stage. The increased size of the entrance hole helps the sequential larval instar stage to drop out their excreta outside the fruit and also helps the imago to come out of the fruit, when it ecloses.

The pupation takes place preferably within the infested fruit of pomegranate. However, outer surface, calyx end and elsewhere from the fruit are other pupation sites selected rarely. The observations of present study are supported by reports of the earlier research work on pupation of *D. isocrates* (Kumar *et al.*, 2017; Bharti *et al.*, 2021; Riteshkumar, 2020, Kaushal *et al.*, 2021). This behavior appears common in other species of *Deudorix* (Mould, 1976). While studying the pupation site one more interesting behavior of last instar larva was found that

before prepupation the larva comes out from the fruit and spin the silken web on stalk base portion of infested fruit to tie it with the twig. It prevents fruit fall and protects the pupa inside it. These findings suggest the characteristic adaptation of the pest to protect pupa against predation by concealing it inside the infested fruit and avoiding fruit fall as it is tied to twig by silken web.

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