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Araştırma Makalesi/Research Article

Observation of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuide) Infestation on *Gladiolus grandiflorus* (*Iridaceae*) in Çanakkale

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Abstract

Helicoverpa armigera (Hübner), the cotton bollworm, is the serious and commonly known pest in agricultural areas in the world. In this study, *H. armigera* was reared at 23±2°C, 65% RH and 16:8 h L:D in the laboratory on leaves and flowers of *Gladiolus grandiflorus*. The eggs were laid singly on the upperside of the *G. grandiflorus* leaves. There were 5 larval stages based on molts and head capsule measurements. The development of larvae was completed in an average of 16.75 ± 4.13 days and the survival rate was 64.1%. In this study, developmental period of larval stages, adult fecundity and pupal sex differentiation were determined on the ornamental host of the pest.
Keywords: *Helicoverpa armigera*, Cotton bollworm, *Gladiolus grandiflorus*, Rearing

Öz

Çanakkale'de *Gladiolus grandiflorus* (*Iridaceae*) üzerinde *Helicoverpa armigera* Hübner (Lepidoptera: Noctuide) 'nın Gözlenmesi

Yeşil kurt, *Helicoverpa armigera* (Hübner), dünyadaki tarım alanlarında yaygın olarak görülen önemli bir zararlıdır. Bu çalışmada, *H. armigera* laboratuvarda kontrollü koşullarda, 23±2°C, 65% oransal nem ve 16:8 fotoperiyotta, *Gladiolus grandiflorus*'un yaprak ve çiçeklerinde yetiştirildi. Baş kapsül ölçümleri ve deri değiştirme sayısına göre 5 larva dönemi olduğu belirlendi. Larva gelişme süresi 16,75 ± 4,13 gün ve canlı kalma oranı % 64,1 olarak tespit edildi. Çalışmada süs bitkisi konukçusu üzerinde, larva dönemlerinin gelişme süresi, dişilerin bıraktığı yumurta sayısı ve pupa döneminde cinsiyet tayini belirlendi.
Anahtar Kelimeler: *Helicoverpa armigera*, yeşil kurt, *Gladiolus grandiflorus*, yetiştirme

Introduction

H. armigera Hübner (Lepidoptera:Noctuidae) is an important pest and well-known polyghagous insect in agricultural areas in worldwide (Liu et al., 2004; Krinski and Godoy, 2015; Mironidis and Savopoulou-Soultani, 2008). It is commonly known as the cotton bollworm, the old world bollworm, scarce bordered straw worm, tomato fruit worm, the gram pod borer, tobacco budworm, corn earworm, African cotton bollworm, and American bollworm (Begemann and Schoeman, 1999; Krinski and Godoy, 2015). It is widely distributed in many countries in Europe, Asia, Africa, Australia, and most recently in South America (Bueno and Sosa-Gomez, 2014; Murua et al., 2004). Damage is caused by larvae and has been reported more than 172 plant species from 40 families, including cultivated and uncultivated plants, belonging to Solanaceae, Fabaceae, Asteraceae, Malvaceae and Poaceae (Krinski and Godoy, 2015; Liu et al., 2004; Venette et al., 2003). It has also caused ecomonical losses to agricultural crops such as cotton, maize, tomato, legumes, tobacco, sorghum, sunflower, soybean, and fruit trees (Moral-Garcia, 2006; Krinski and Godoy, 2015; Venette et al., 2003). In addition, ornamental plants such as *Dianthus* spp., *Gerbera* spp., *Rosa* spp., *Pelargonium* spp.,*Chrysanthemum* spp. are potential hosts for the cotton bollworm (Demirözer, 2012; Fitt, 1989 ; Multani and Sohi, 2002; Venette et al., 2003; Pal and Sarkar, 2009).

Females lay eggs singly on the leaves and flowers. The larvae feed on various parts of the plants, causing damage to reproductive structures, and reduce yields by lowering overall crop values (Krinski and Godoy, 2015). The cotton bollworm can survive in various habitats and easily adapt to enviromental changes, aided by high mobility and fecundity, polyphagy, and facultative diapause (Mironidis and Savopoulou-Soultani, 2008). Control is dependent on chemical applications, but it has already developed resistance to many insecticides (Mabbett et al., 1980; Maelzer and Zalucki, 1999).

Gladiolus (*Gladiolus* spp.), is an important ornamental plant, has high economical profits, and is widely cultivated by many countries. Gladiolus are ranked 8th in the world's cut flower trade, and were sold at 73 million branch domestic and international markets in the Netherlands in 2007 (Anonymus, 2009). Over 10.5 million branch of Gladiolus produced in Turkey, in 2014 (Anonymus, 2015). The gladiolus corm is not a real tuber that is produced by the stem of the plant as storage of food under the soil. The plant, which has 10-18 pieces of florets in a spike, blooms between April and September. In general, it varies from 40 to 150 cm length depending on climate conditions and varieties (Verlag, 1985). The origin of the plant is in tropical regions of South Africa, and has been known as the corn lily for more than 2000 years in Anatolia. There are about 250 species of Gladiolus that have naturally spread in a small area of England, Central Europe, Canary Islands and West Asia (Mengüç, 1996). According to Geelhear (1990), Gladioluses can be divided into 4 main group; a) *Gladiolus grandiflorus*: giant flowered varieties (generally have 1 m and more plant height, 10-15 florets), b) *Gladiolus primulinus*: (generally have yellow flowers and 6-12 florets), c) Butterfly shaped varieties: (*G. romosus* x *G. tristis* hybrids, more rickety and earlier than *G. grandiflorus*), d) Dwarf varieties: (*G. cardinalis* x *G. tristis* hybrids, 40-45 cm length and much earlier).

The larval host plant has an important role in insect development, survival and reproduction. According to Zalucki et al. (1986), determination of a host plant requires rearing insects to the adult stage on that specific plant, collecting pupae on the plant from field, and testing the fertility of the consecutive adults. There are several studies on the cotton bollworm such as life table parameters on various cultivated plants (Liu et al., 2004; Twine, 1978; Liu et al., 2004; Czepak et al., 2013; Storer et al., 2001; Mironidis and Savopoulou-Soultani, 2008; Stavridis et al., 2004; Mironidis et al., 2010; Krinski and Godoy, 2015, Liu et al., 2010) geographic distributions (Kriticos et al., 2015; Murua et al., 2016) rearing on different artificial diets in the laboratory (Jha et al., 2014; Assemi et al., 2012; Abbasi et al., 2007; Barbosa et al., 2016) genetic diversity (Asokan et al., 2012; Arneodo et al., 2015; Behere et al., 2008; Mastrangelo et al., 2014; Kranthi et al., 2006;) and pesticide resistances (Konuş and Karaağaç, 2014; Karaağaç et al., 2013; Kumar et al., 2009). Although, the cotton bollworm is a serious pest especially on cotton, maize, tomato and tobacco, the infestation status and survival of the pest on ornamental plants are limited to host reports (Demirözer, 2012; Pal and Sarkar, 2009). The biology and life cycle of the cotton boll worm on *Gladious grandiflorus* has not been conducted.

The aim of the present study was to report the infestation of *H. armigera* on *G.s grandiflorus* and provide biological data by rearing the cotton bollworm on *G. grandiflorus* for a generation and testing the host suitability in the laboratory condition.

Materials and Methods

The surveys were conducted at Çanakkale Onsekiz Mart University, Dardanos Campus in Çanakkale, Turkey. The study area, having 4 sets of 4 plants in a 1x1 m experimental plot, was indicated to the red rectangular in Figure 1. *Gladiolus grandiflorus* leaves and the upper third of the plants were visually checked out the presence of *H. armigera* eggs or larvae (Figure 2). The collected individuals were brought to the laboratory. Pesticides were not applied during the study. The colony of *H. armigera* was established in late July 2016 from larvae collected from *G. grandiflorus* at Çanakkale Onsekiz Mart University, Dardanos Campus (Figure 1 and 2).

The neonate larvae were reared until the third instar in the freshly cut *G. grandiflorus* covered with a collapsible nylon cloth cages that were 50 cm in length and 20 cm in width (Figure 3). The plants were changed every two-day until the third instar. After the third instar, larvae were reared individually due to cannabalism (Kakimoto et al., 2003; Liu et al. 2004) until pupation. Larvae were monitored daily for molting and survivorship. Mature larvae pupated in sterilized moist soil. The sexes of pupae were seperated by examining externally last abdominal segments as other lepidopterans (Genc, 2005; Genc, 2015; Genc, 2016). Emerging individuals were identified according to the characteristic wing patterns (Pogue, 2004). Adults were allowed to mate in adult screen cages (45cm length x 45cm width x 45cm high) and fed with a honey or sugar solution (10%). Biological stages were monitored and photographed from the beginning of egg laying to adult emergence with an Olympus SZX9 stereozoom microscope. All experiments were in controlled laboratory conditions, at 23±2°C, 65% RH and 16:8h (light: dark) photoperiod. Observations were made on the number of oviposition stings, the number of pupae, and the adult emergence. Data were analyzed according to one way ANOVA. The means were compared by Duncan's multiple-range test. Significance was accepted with $p \leq 0.05$.

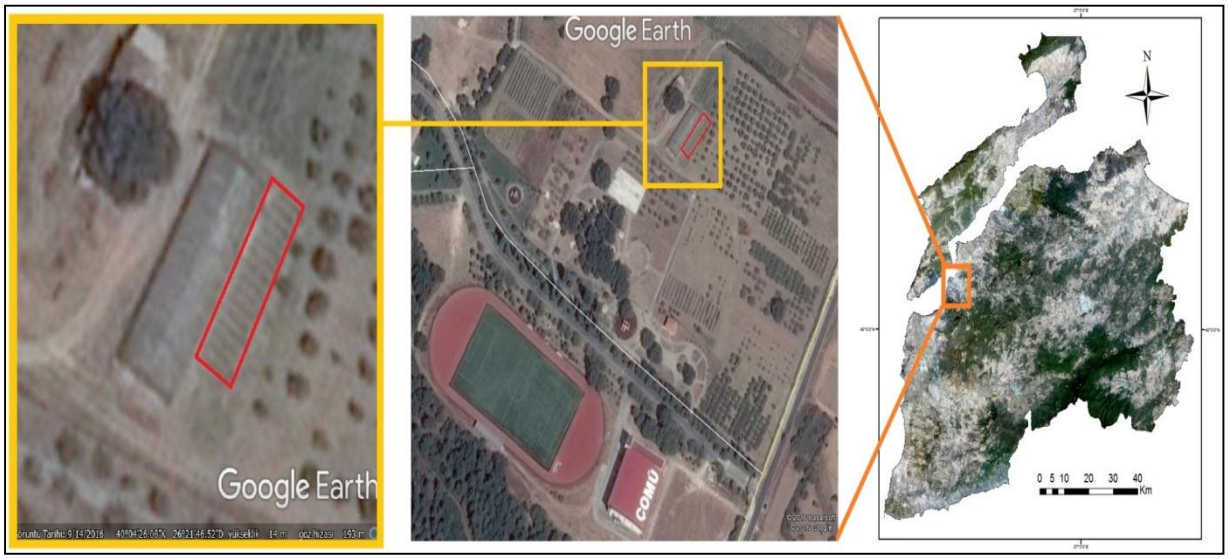

Figure 1. Experimental plot (red rectangular), Campus of Dardanos, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

Figure 2. A close view of the experimental plot, *Gladiolus* sp. varieties: White prosperity, Peter pears, Red Balance and Priscilla (A, B, and C).

Figure 3. Freshly cut *Gladiolus grandiflorus* (A) and a view of collapsible nylon cage (B).

Results and Discussion

Eggs

The larvae of the cotton bollworm completed their developments from egg to adult emergence on *G. grandiflorus* in the laboratory. Eggs were laid individually on leaves and fresh shoots of *G. grandiflorus* (Figure 4A). Eggs are spherical with a flattened base and about 0.25 ± 0.01 mm in wide and 0.30 ± 0.01 mm in long (n=50) with several longitudinal ridges. Newly laid eggs were creamy white in color and as the embryo developed darker patches showed up on the anterior side of the eggs (Figure 4B). Just before hatching, the head of first instar became clear through the chorion (Figure 4C). The mean period of egg development was about 3.50 ± 0.70 days at 23±2°C with 73.75% viability in the laboratory.

Figure 4. Egg stage of *Helicoverpa armigera*, (A) newly laid egg, (B) 2-day old egg, C) egg before hatching.

Larvae

Neonate larva made an exit hole through the eggshell to emerge (Figure 5). The first instar was pale cream, and the pronotum had a visible brown patch with dorsally distributed brown-black spots. The thoracic legs were brown (Figure 5). The first instar was about 1.81 ± 0.05 mm in length and 0.13 ± 0.01 mm in width. The duration of the first instar was about 3.81 ± 0.54 days in the laboratory. In general, larval survival was higher in early instars. The cotton bollworm larvae reared on *G. grandiflorus* molted five times based on the measurements of the head capsule width. The sizes of the head capsules were 0.225 ± 0.001 mm for the first instar, 0.412 ± 0.001 mm for the second instar, 0.603 ± 0.007 mm for the third instar, 0.931 ± 0.053 mm for the fourth instar and 1.587 ± 0.082 mm for the fifth instar. Varians analysis of head capsule width indicated significant differences (p<0.05). Mean comparison also revealed significant differences among all the cotton bollworm instars. Thus, we concluded that the cotton bollworm had five instar on *G. grandiflorus* in the laboratory.

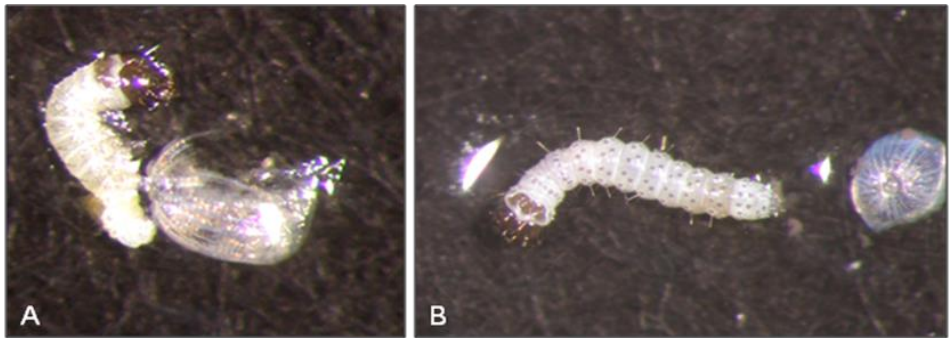

Figure 5. Newly hatched larva of *Helicoverpa armigera* with the eggshell (A and B).

The second instar was light brown, the head and anal shields were yellowish brown. The newly molted second instar was shown in Figure 6A. The third instar was typically brown in color and had characteristic larval appereance of the cotton bollworm (Figure 6B.). The fourth and fifth instars were differrent combinations of greenish, yellow to brown in color and usually darker in later stages (Figure 6C.). Cannabalism was an important behaviour to consider when being reared in the laboratory (Figure 6D.). The mature larva or last instar was about 25.96 ± 0.37 mm long and 2.40 ± 0.71 mm wide. The duration of the mature larva was 6.33 ± 1.57 days in the laboratory conditions. The overall larval development period was 16.75 ± 4.13 days**.** The larval survivorship was 64.1% on *G. grandiflorus* in the laboratory. The larvae initially feed on the leaves, and then on flower buds, flowers and florets in the spike. The second instar appeared to have a preference for feeding on the florets of *G. grandiflorus* (Figure 7A). Typically the second instar bored into the floret and inserted its head, with the rest of the body outside (Figure 7B. and 7C.).

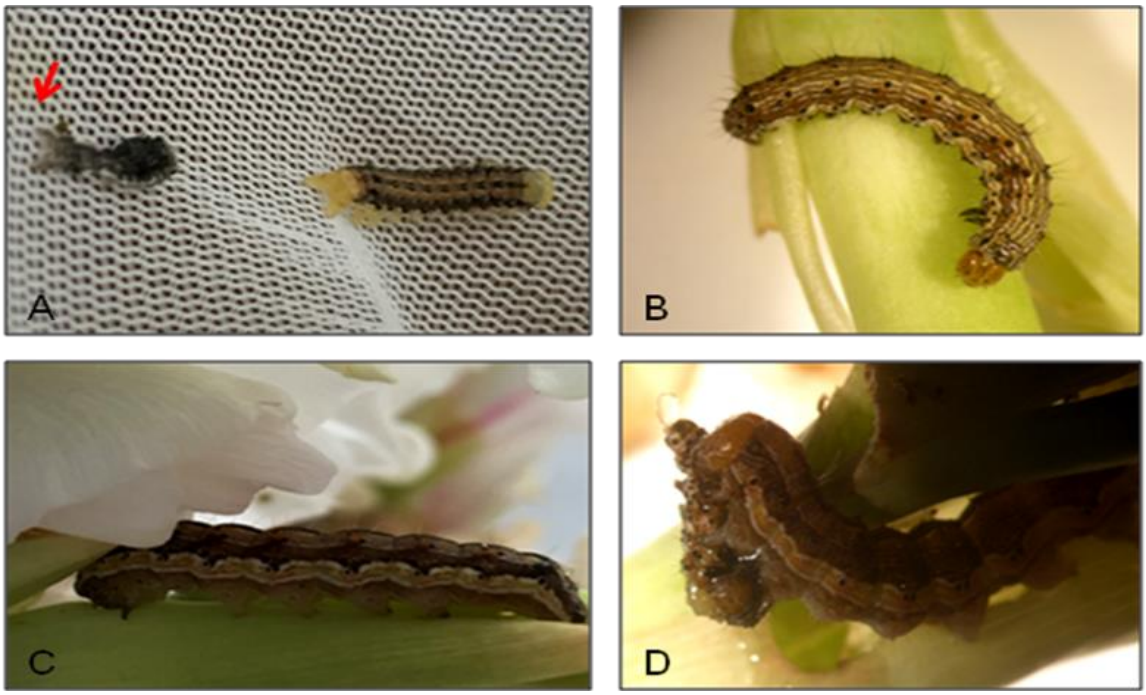

Figure 6. Different larval stages of Helicoverpa armigera. A) newly molted larva with exuvia, B) third instar, C) fifth instar and D) cannibalism behaviour of late instar.

Figure 7. The larva penetrates through florets of *Gladiolus grandiflorus* (A), (B), (C).

Pupae

When the larvae were fully grown, and they were placed on sterilized soil for pupation. They tunnelled 6-7 cm into soil (Figure 8). The pupae were initially soft, smooth surfaced and light brown in color (Figure 8B. and 8C.). The measurement of female pupae was about 2.96 ± 0.41 mm in wide and 9.893 ± 1.18 mm in long, and weighed 0.279 ± 0.02 g. Male pupae were about 2.62 ± 0.13 mm in wide and 9.152 ± 0.56 mm in long, and weighed 0.270 ± 0.04 g. The duration of pupal stadium was about 14.25 ± 2.5 days. Pupal emergence was 75.3%. The pupae of the cotton bollworm were examined under the stereozoom microscope to distinguish the male and female (Figure 9, 9A.). The female genital opening or suture was located in the middle of 8th abdominal segments whereas the male genital opening was shown at the 9th abdominal segment (Figure 9B.).

Figure 8. Mature larvae of *Helicoverpa armigera* (A) and pupal chamber (B) and pupal appearance with exuvia (C).

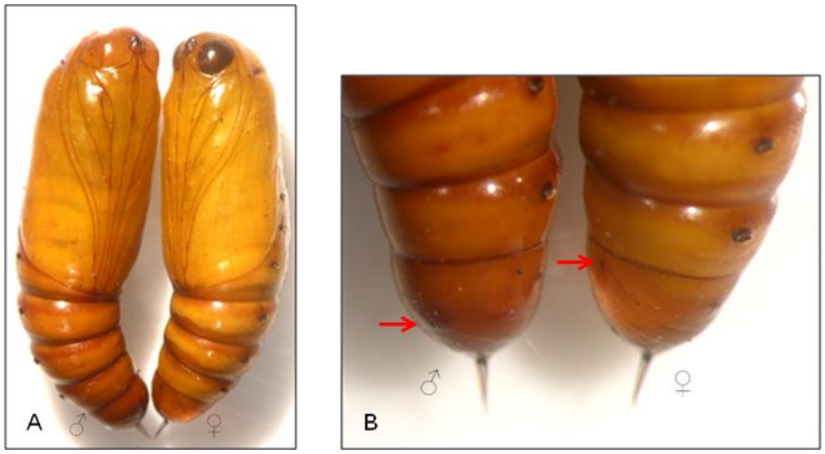

Figure 9. Pupal stage of *Helicoverpa armigera* A) a view of male and female pupae, B) close up ventral view of pupae. Genital sutures indicated with red arrow.

Adults

The cotton bollworm is a typical noctuid moth with a wingspan about 29.28 ± 2.15 mm (Figure 10). Adult appereance is variable, depending mostly on larval host. The wings are light brown to orange brown in color. The middle of the forewings has a dark kidney-colored marking. The hindwings are lighter colored with a creamy white patch (Figure 10A) in the dark marking on the outer margin. This is a key chracteristic view for this species. The ventral part of the adults is shown in Figure 10B, also having similar appareance. A female laid about 1030 eggs in her life span in the laboratory.

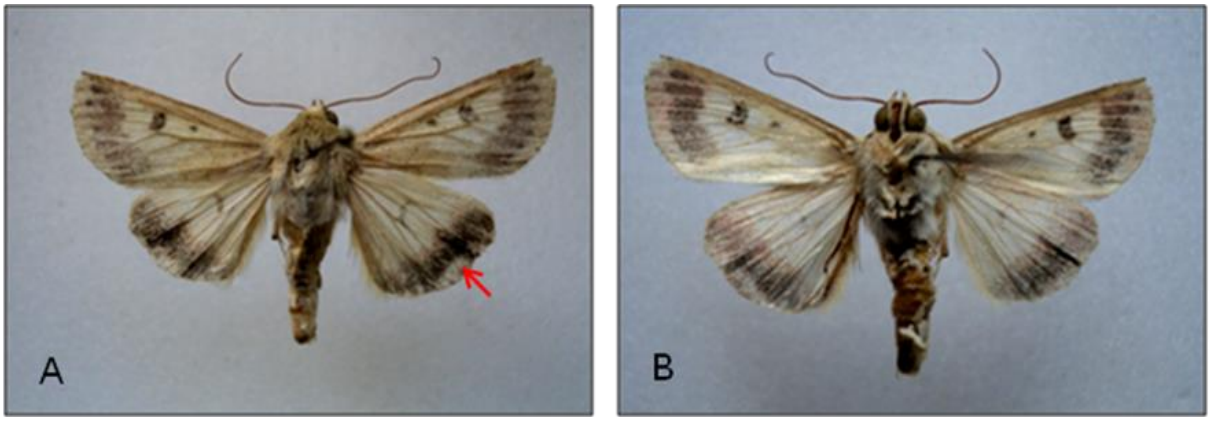

Figure 10. Adult stage of *Helicoverpa armigera* A) Dorsal view, a pale patch in the dark marking on the hindwings, B) Ventral view.

Damage of *Helicoverpa armigera* on *Gladiolus grandiflorus* in the laboratory

Early larval stages of the cotton bollworm feeding on the plant surface were easily monitored and detected. Larvae fed on *Gladiolus grandiflorus* leaves, flower buds and flowers (Figure 11). Feeding damage resulted in various shaped holes into the reproductive structure. The entry holes were large and circular at the base of the florets. Larval damages increased after the third instar. Sometimes plants had to be dissected to confirm the presence of larvae. Larvae preferred to feed on flowers buds and flowers, but adults laid eggs only on leaves. Feeding can be extensive causing reducing market value and yield loss. Presence of frass was generally seen in places indicating larval feeding and damaged holes with or without larvae were seen (Figure 11).

Many insects, mites and other pests attack ornamental plants causing economical problems and limitation of their commercial production. Information on pest in that specific agro-ecosystem is crucial to develop optimal pest management strategies (Sohi and Singh, 1995). However, such the information on ornamental plants are limited by pest records and surveillance reports (Pal and Sarkar, 2009; Demirözer, 2012). Therefore, infestation and laboratory rearing of the cotton bollworm were described.

This study reports that *Helicoverpa armigera* succesfully develops, survives and reproduces on *Gladiolus grandiflorus*. We observed occurence and infestation of different larval stages in the field conditions. We determined the cotton bollworm larvae had 5 stages based on molts and head capsule measurements on *G. grandiflorus* in the laboratory. Several studies have shown significant differences in head capsule width of cotton bollworm larvae (Hardwick, 1965; Kirkpatrick, 1961; Twine, 1978; Stavrides et al. 2004). They assumed that this variation might be due to the larval food either on a host plant or an artificial diet, population density, temperature or humidity. Kumar et al (2009) observed 5 instar whereas Twine (1978) reported 5 to 7 instars, Barbosa et al. (2016) stated 3 to 7 instars, Jha et al. (2014) observed 6 instars on an artificial diet, and Liu et al (2004) reported 7 instars depending on the host. Similar to our observations were reported by Liu et al. (2004) as the bollworm had fewer instars on appropriate host.

Our observations showed that the cotton bollworm develop on leaves, flower buds and flowers of *G. grandiflorus*. The larval developmental period was about 16.75 ± 4.13 days in controlled laboratory conditions. Barbosa et al. (2016) reported 12.7 ± 0.3 days and Mironidis and Savopoulou-Soultani (2008) observed at 25 ºC, 15.52 ± 0.26 days on artificial diet. The pupal development period was 14.25 ± 2.5 days in our study which was similar to 12.35 ± 0.17 days by Mironidis and Savopoulou-Soultani (2008). Our study shows that *G.grandiflorus* is an appropriate larval host for the cotton

bollworm in the field and needs to be considered for further research in pest management and genetic diversity.

In general, pupal differentiation can be identified by a slit on the ventral part of the eighth and ninth abdominal segments (Genc, 2005; Posada et al., 2011; Genc, 2015). The pupal key characters of the cotton bollworm were shown here and similar to other lepidopteran species.

Figure 11. Larvae of *Helicoverpa armigera* feeding inside the flower buds and flowers of *Gladiolus grandiflorus* and larval entry holes.

Conclusion

In this study, we investigated the cotton bollworm on *Gladiolus grandiflorus* in the controlled laboratory conditions. The immature stages were demostrated and photographed. Adults were fertile and laid eggs on *G.grandiflorus*. Meanwhile, this study showed that *G.grandiflorus* is an appropriate larval host for the cotton bollworm in the field and need to be considered for further research in pest management and genetic diversity.

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