

Foraging Behavior of *Oxycarenus hyalinipennis* (Lygaeidae: Hemiptera) Toward Different Cotton Fruiting Structures and Two Major Volatile Compounds

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Abstract

The dusky cotton bug *Oxycarenus hyalinipennis* has become a significant pest of cotton crops worldwide, especially in Pakistan. Fundamental studies of *O. hyalinipennis* are crucial for developing its integrated management plan. Therefore, basic studies regarding early detection, behavior, and host preference of *O. hyalinipennis* among fruiting cotton parts, which is vital for designing effective control measures, still need to be completed. Considering the importance of *O. hyalinipennis*, current study aimed to evaluate the preference for the fruiting part of cotton by *O. hyalinipennis* under both laboratory and field conditions and to report the impact of three different components e.g., β -carene (90%), heptanoic acid ($\geq 80\%$, FCC, FG), and *p*-xylene ($\geq 96.0\%$ (GC) against the adults of *O. hyalinipennis*, these components were selected based on the previous literatures that reported most prominent components of cotton fruiting parts. The results showed that early planting enhanced the population of *O. hyalinipennis* in cotton (Bt-CIM-554) compared to mid-May cotton planting. Overall, the data show that early planting of Bt cotton enhances the population of dusky cotton bugs. According to field studies and choice tests, the open boll of cotton (Bt-CIM-554) was preferred by *O. hyalinipennis* compared to other fruiting parts. The Y-tube olfactometer choice assay results indicated that β -carene was the most attractive component for adults of *O. hyalinipennis*, followed by *p*-xylene and heptanoic acid. Our study showed that β -carene and *p*-xylene have potential for use as novel attractants for the early detection and management of *O. hyalinipennis*.

Introduction

Gossypium hirsutum L. cotton, particularly Bt-cotton, is a well-known main crop that is a significant source of income for people worldwide, including Pakistan (Ahmad et al., 2020; Arshad et al., 2021). Recently, Bt-cotton production in Pakistan has decreased due to a number of factors that lower cotton yields (Arshad et al., 2021). Among them, insect pests are one of the most critical factors responsible for yield reduction. However, previous literature revealed that Bt cotton was effective against cotton bollworms, which are pests of cotton, except for a few thrips species, and that its management typically relies on synthetic pesticides (Jaleel et al., 2014; Razaq et al., 2019). The indiscriminate application of chemicals has challenged human health, the environment, and bioagents (Poudel et al. 2020). Additionally, chemicals have made insects less susceptible to insecticides, leading to their reappearance and outbreaks due to unreliable chemical techniques (Jaleel et al., 2020; Lei et al., 2021; Saeed et al., 2016). However, the dusky cotton bug *Oxycarenus hyalinipennis* (Costa) (Lygaeidae: Hemiptera) is currently the most dangerous pest among sucking pests of cotton crops.

The *O. hyalinipennis* is distributed worldwide, including in Saudi Arabia (El-Hawagry et al., 2013; USDA, 2010), Pakistan (Ullah et al., 2016), Egypt (El-Rahim et al., 2015), India (Sammaiah et al., 2012), the USA (Nagoshi et al., 2012), and the Bahamas (Smith and Brambila, 2008). The *O. hyalinipennis* is an important pest of the Malvaceae family, although it has a wide host range, including avocado, okra, corn, figs, apple, dates, grapes, guava, peach, pomegranate, pineapple, moringa, chilies, and ornamental plants (Naeem-Ullah et al., 2020; Saeed et al., 2022). This pest is now recognized as a significant pest of Pakistan's cotton crop (Hafeez et al., 2021), as well as other nations, such as China, the Turks & Caicos Islands, the Bahamas, and Cayman (Colmenárez et al., 2020; Stocks and Peña, 2013). Adults and nymphs influence the quality, quantity, and ability of cotton seeds to extract oil (Rind et al., 2021). Both nymphs and adults of *O. hyalinipennis* cause damage by feeding on the squares of cotton plants, which results in the shedding and yellowing of the squares (Rajendran et al., 2018). The dusky cotton bug population parameters may be affected by many factors, such as early sowing and excessive insecticide use (Rocha-Munive et al., 2018).

The *O. hyalinipennis* exhibits various behaviors on various host plants, and the host plant influences the selection pressure for biological factors in insects (Ananthakrishnan et al., 1982; Atakan et al., 2021). Female *O. hyalinipennis* must choose an appropriate host before oviposition to foster the growth of their young ones (Sarmad et al., 2020). The skin, smell, size, color, and form of the fruit, twigs, and foliage characteristics may impact how insect pests choose their hosts (Prokopy and Owens, 1983). Usually, *O. hyalinipennis* prefers to lay eggs on soft or new bolls (Khan and Naveed, 2017). *O. hyalinipennis* feeds on the inner side of the bolls and inside the open bolls. The nutritional level of cotton bolls can impact the development and survival of their progeny (Faghani et al., 2022). Additionally, the levels of toxins in fruits, latex (Makkar et al., 1997), and resin vary, particularly in Anacardiaceae plants, which may impact immature plants (Rattanapun et al., 2009).

To develop an effective management strategy against insect pests, it is necessary to understand basic pest biology (Jaleel et al., 2018a), including feeding behavior (Atakan et al., 2021). Furthermore, data on the preference of *O. hyalinipennis* for cotton fruiting parts still need to be included. However, little is known about the effect of planting date on *O. hyalinipennis* infection of cotton in Pakistan. *O. hyalinipennis* lives on fruiting parts of cotton, including squares, flowers, immature bolls, and mature bolls. However, there is a lack of knowledge about the preference of fruiting parts under field conditions. The objectives of this study were to assess fruiting part preference or within-plant distribution of *O. hyalinipennis* and to compare the impacts of early planting (mid-April) and normal planting (mid-May) of cotton at the population level. Further to determine the preference of *O. hyalinipennis* for fruiting parts under both field and laboratory conditions. Present study could aid in our understanding of this problem and could result in the development of more efficient techniques for eradicating it. This research advances our knowledge of this pest and may help us develop more efficient management strategies.

Materials and Methods

Experimental site

The field tests were conducted at an experimental location at the Central Cotton Research Institute (CCRI), Multan (30.120 N and 71.280 E), in southern Punjab, Pakistan, under semiarid climatic conditions on silt loam soils.

Experimental design and planting dates

The experiment was conducted by using a randomized complete block design with three replicates. Cotton seeds, i.e., Bt. CIM-599 plants were obtained from CCRI's Cotton Breeding Section. Multans were planted in mid-April (15th April) and mid-May (15th May) during year 1 and year 2, respectively. The bed and furrow methods were used for planting. The dimensions of each treatment were 7.31 m × 12.19 row-to-row and plant-to-plant, and the distances were 0.83 m and 0.25 m, respectively. Cotton seeds were manually dipped into irrigated furrows and treated with 10 ml of sulfuric acid per kilogram of seed. Irrigation was again applied 72 hours after dibbling. Subsequent irrigations were applied according to the following recommendations. In the case of fertilizer, 60 kg/ha of phosphorus fertilizer (diammonium phosphate containing 16% N and 48% P) was applied at the time of seedbed preparation, while 100 kg of nitrogen fertilizer (urea 46% N) was applied to the crop at the seedbed preparation, flowering, and boll formation stages at three equal doses. A no-insecticide approach was used to manage *O. hyalinipennis* during both years to monitor its peak density. When the population/damage of other sucking pests (jassid and whitefly) and bollworms approached the economic threshold level (ETL), insecticides

were ineffective or had low toxicity against *O. hyalinipennis* viz. Acetamiprid Mospilon (20WP), spirotetramat + biopower (Movento 240SC), nitenpyram (pyramid 10SL), bifenthrin (Talstar 10 EC) and gamma-cyhalothrin (Proaxis 60 CS) were selected and applied as recommended by CCRI, Multan.

Sampling of *O. hyalinipennis*

Every week in the early morning, *O. hyalinipennis* nymphs and adults were counted from all the reproductive components, including the square, flower, immature/small boll, mature boll, and open boll (as shown in Fig. 1.) of randomly selected plants (n = 15, 5 plants/replicate) from each treatment. Counting was performed visually on the randomly selected plants after the first appearance of *O. hyalinipennis* (Iqbal et al., 2018).

Collection of *O. hyalinipennis* for laboratory study

The Central Cotton Research Institute (CCRI), Multan, Punjab, Pakistan, kept a colony of dusky cotton bugs in its entomology section lab. Nymphs and adults of the dusky cotton bug were collected from the abovementioned cotton crops cultivated in CCRI during the second year of the study. The organized individuals of *O. hyalinipennis* were shifted into plastic jars 14 × 24 cm, covered with muslin cloth and then placed under controlled laboratory conditions. These populations were fed *Bt*-CIM-599 (provided by cotton in the Breeding Section of CCRI, Multan, Pakistan) for two generations to maintain population homogeneity and prepare them for laboratory research.

Preliminary study regarding the biology of *O. hyalinipennis*

Eighty eggs of *O. hyalinipennis* were collected from the laboratory culture, and 20 eggs were separated under the microscope for each stage of flower development: square, immature bolls, and mature bolls (Fig. 1). We cannot record the complete life cycle or biological parameters of *O. hyalinipennis* on flowers, squares, immature bolls, or mature bolls. All populations of *O. hyalinipennis* died before entering the 3rd nymphal stage. Preliminary data on the biology of *O. hyalinipennis* can be obtained on demand.

Choice test among different fruiting parts of cotton

The square/bud flower, immature/small boll, mature boll, and open boll were picked from the experimental cotton field. We washed these fruiting parts, dried them under laboratory conditions, and then transferred them to cages (60 × 60 × 60 cm). Fruiting parts were placed randomly in the cage. The 40 newly emerged adults of *O. hyalinipennis* were released into the cage for the preference trial. After 24 hrs, the preference data were recorded. Four replications were performed.

Olfactometer bioassay

The behavioral responses of *O. hyalinipennis* to the most prominent volatile components were determined with a Y-tube olfactometer. The Y-shaped glass tube olfactometer was chosen as described in the study of (Jaleel et al., 2019). Volatile components, e.g., β -carene (90%), heptanoic acid ($\geq 80\%$, FCC, FG), and *p*-xylene ($\geq 96.0\%$ (GC) were purchased from Alfa Aesar, Beijing, China. The structural formulae are shown in Fig. 2. The following treatments were used: (1) β -carene, (2) heptanoic acid, (3) *p*-xylene, (4) β -carene + heptanoic acid, (5) β -carene + *p*-xylene, and (6) heptanoic acid + *p*-xylene (7) β -carene + heptanoic acid + *p*-xylene.

For each treatment, three different concentrations of acetone (40%) were used: 10%, 5%, and 1% (v/v). A mixture of volatile substances was produced at a 1:1:1 (v/v) ratio. Each treatment in one arm was contrasted with the acetone-only control arm. For this experiment, 100 l of each treatment solution was applied to a piece of filter paper (2.0 × 0.5 cm) set on a small glass holder near the Y-tube's air inlet.

A total of 80 *O. hyalinipennis* adults were selected and used per treatment. The *O. hyalinipennis* that did not move within 15 min were considered nonresponders. After the test of 5 *O. hyalinipennis*, the Y-tube or odor sources were switched to avoid contamination by *O. hyalinipennis* or the test order. The tubes were washed with 80% alcohol and placed in an oven at 250°C for 20 mins. The experiment proceeded with new odor sources, clean tubes, and *O. hyalinipennis*. Each *O. hyalinipennis* adult was used only once. Each experiment was replicated eight times.

Statistical analysis

One-way ANOVA was used for analyzing the number of *O. hyalinipennis* in both experiments, e.g., field and choice tests in the laboratory. The χ^2 test was used to compare the differences between early- and late-sowing *Bt*-CIM-554 for the population of dusky cotton bugs. Afterward, the mean *O. hyalinipennis* density/10 reproductive parts were calculated. The impacts of the treatments on *O. hyalinipennis* densities were compared using repeated measures analysis of variance (ANOVA). Least significant difference (LSD) tests were used to separate means showing considerable treatment effects (Westfall et al., 2011). The difference between the two treatments was analyzed by the chi-squared (χ^2) test in SPSS Statistics 15.0 (SPSS Inc., Chicago, IL, USA) with the expected value ($\alpha = 0.05$). Non-responder females were not included in the statistical analysis.

Results

Population dynamics on different fruit parts of cotton

The population density of *O. hyalinipennis* was greatest in the early planting of *Bt* cotton, and among the fruiting parts of cotton, only open bolls were preferred by *O. hyalinipennis* (Fig. 2). During 2021, *O. hyalinipennis* appeared in July with a fluctuating trend throughout the season, and its peak intensity occurred in October on both early and normal sown cotton. Among the fruiting parts, DCB preferentially harbored and multiplied in open bolls, while a minor DCB population was observed in flowers on both sowing dates. However, its maximum population was observed in mid-April sown cotton (early planting) compared to mid-May sown cotton (normal planting) (Fig. 2A, C, E, G & I).

In both early and normally sown cotton, *O. hyalinipennis* appeared in July, with peak intensity occurring in October of 2022. Similar to the previous year, *O. hyalinipennis* preferred to harbor and multiply in open bolls, and the maximum population was observed in Mid-April sown cotton (early planting) compared to Mid-May sown cotton (normal planting) (Fig. 2B, D, F, H & J). However, the *O. hyalinipennis* population was greater in 2022 than in 2021 (Fig. 2).

Effect of cotton sowing time on the density of *O. hyalinipennis*

Similarly, in both years, data have shown that early planting of cotton enhances the population of *O. hyalinipennis* (Table 1). In both years, only open bolls were preferred by *O. hyalinipennis* (Fig. 3).

Table 1
Comparative population fluctuations of *O. hyalinipennis* on different fruiting parts of Bt-CIM-554 at different planting times under field conditions

Different fruiting parts of cotton	Year 1		Year 2	
	Early	Normal	Early	Normal
Flower	0.22 ± 0.07	0.07 ± 0.01	1.81 ± 0.24	0.5 ± 0.07
Square	0.75 ± 0.02	0.63 ± 0.12	4.82 ± 0.17	2.74 ± 0.32
Small boll	0.81 ± 0.14	0.44 ± 0.05	4.11 ± 0.19	2.08 ± 0.21
Mature boll	1.97 ± 0.12	0.9 ± 0.15	5.0 ± 0.28	4.48 ± 0.62
Open boll	42.78 ± 4.44	13.13 ± 0.88	43.19 ± 1.75	15.25 ± 1.59

Preference of *O. hyalinipennis* on different fruit parts of cotton

Laboratory studies have shown that among the preferred fruiting parts of cotton crops, open bolls were more common and preferred after 24 hrs, when *O. hyalinipennis* was released into the cage (Fig. 4).

Effect of components on *O. hyalinipennis*

All three factors as dusky cotton bug, volatile components, concentrations, and interactions between the dusky cotton bug and volatile components, significantly influenced the response.

The remaining possible interactions, e.g., interactions between (1) dusky cotton bugs and concentrations, (2) volatile components and concentrations, and interactions among dusky cotton bugs, volatile components, and concentrations, had no significant effects on the response to the volatile components.

The effects of β -carene at all concentrations (10%, 5%, and 1%) on dusky cotton bugs were 68.75%, 61.25%, and 46.25%, respectively, while that of the control was 22.5%. This means that there was a significant difference between the treated and untreated arms. The effects of heptanoic acid at all concentrations (10%, 5%, and 1%) on the dusky cotton bugs were 56.25%, 51.25% and 40%, respectively, while that of the control was 27.5%. This means that there was a significant difference between the treated and untreated arms. The effects of *p*-xylene at all concentrations (10%, 5%, and 1%) on dusky cotton bugs were 53.75%, 50%, and 36.25%, respectively, while that of the control was 35%. This means that there was no significant difference between the 1% treated and untreated arms.

The effects of the mixture of volatile compounds at all concentrations (10%, 5%, and 1%) on the dusky cotton bug were 60%, 58.5% and 33.7%, respectively, while that on the control was 22.5%. This means that there was a significant difference in the 10% and 5% treatment groups compared to the control group, but the 1% treatment group had little difference. The effects of the mixture of volatile compounds at all concentrations (10%, 5%, and 1%) on the dusky cotton bug were 80%, 66.25% and 53.75%, respectively, while that on the control was 17.5%. This means that there was a large difference between the treated and nontreated arms. The effects of the mixture of volatile compounds at all concentrations (10%, 5%, and 1%) on the dusky cotton bug were 52.2%, 48.75%, and 32.5%, respectively, while that on the control was 33.7%. This means that there was little difference between the treated and nontreated arms. In comparison to the 1% mixture, the control mixture had a greater percentage of infection. The effects of the mixture of volatile compounds at all concentrations (10%, 5%, and 1%) on the dusky

cotton bugs were 62.5%, 47.5%, and 42.5%, respectively, while that on the control was 31.25%. This means that there was a difference between the treated and nontreated arms, but not a large difference (Fig. 5). The response percentage was remarkably high for the mixture of β -carene + *p*-xylene (80%). At the same time, the smallest difference was observed in the mixture of heptanoic acid + *p*-xylene compared with the other volatile components and their combinations (Fig. 5).

Discussion

The number of cultivated genetically engineered cotton plants carrying *Bacillus thuringiensis* genes, also known as Bt cotton, continues to increase quickly on a global scale (Naranjo, 2011). In Pakistan, the broader adoption of Bt cotton has led to a shift to an earlier sowing period and a marked reduction in the use of insecticidal applications against bollworms (Jaleel et al., 2014). The number of minor pests previously suppressed by broad-spectrum insecticides used against bollworms has increased, and these pests have become economically important to cotton in Pakistan (Catarino et al., 2015). *O. hyalinipennis* is a minor pest that was first identified before the introduction of Bt cotton and has since become a major pest (Babu and Meghwal, 2014). It is a cosmopolitan and polyphagous insect that consumes a variety of foods, including avocado, corn, apple, dates, grapes, peaches, okra, pineapple, pomegranate, guava, moringa, mango, lemon, okra, and chilies (Ananthakrishnan et al., 1982; Dimetry, 1971; Ram and Chopra, 1984).

Adult females of all insects must select a host before oviposition to lay their eggs (Xu et al., 2012). The olfactory and visual senses of *Neoceratitis cyanescens* play a significant role in the selection of suitable hosts during the host selection period (Brevault and Quilici, 2010). The adult females of *Toxotrypana curvicauda* often respond differently to their hosts depending on the release ratio of volatiles from the host plant and the toughness of their skin (Landolt et al., 1992). Furthermore, the adult females of *O. hyalinipennis* prefer to feed on more suitable hosts when a variety of potential hosts are offered to them (Dimetry, 1971). The toughness of the skin of bolls has a major impact on how much DOB can harm cotton (Rattanapun et al., 2009). Previous studies reported that the boll hardness tended to increase in response to female insects (Jaleel et al., 2018b). In this investigation, *O. hyalinipennis* visited and pierced open fruit more frequently. This might be because the skin is softer than the other studied fruiting bodies (Fig. 3). *O. hyalinipennis* does not prefer to oviposit on the hard skin/pericarp of fruits (Dimetry, 1971).

The *O. hyalinipennis* nymphs and adults both consume the oil and moisture found in mature cotton plant seeds, leaves, and stems (Atakan et al., 2021). The estimated losses in terms of seed cotton yield, seed weight, and oil content were greater than 6.8%, 32%, and 6%, respectively. This results in both qualitative and quantitative losses (Hafeez et al., 2021). Moreover, reductions in cotton seed germination or viability (up to 32.13%), lint discoloration (slightly dark yellow), and lint staining (pink color owing to bugs being crushed during the ginning process) cause considerable economic losses (Sammaiah et al., 2012).

Oviposition behavior in bugs, similar to that in other insects, is mediated by cues emitted from the end of the ripening stage (Alagarmalai et al., 2009). The *O. hyalinipennis* prefers to oviposit eggs onto open bolls than in other stages (Atakan et al., 2021). Oviposition may be influenced by a variety of additional parameters, including host hardness, ripening phases, and host availability (Singer, 1986). The carene has also been reported in the open bolls of cotton (Tamura et al., 2000). Jaleel et al. (2019) reported that carene is a strong attractant for flies. Based on these results, we concluded that open or cracked bolls of cotton could be used as bait commodities to attract

O. hyalinipennis. This study could be useful for future work regarding the host preference of *O. hyalinipennis*, as well as for field infestation data.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

RS, SIAS, MZK, MAG, RA and WJ wrote the main manuscript text. RS, SIAS, and WJ prepared all figures. All authors reviewed the manuscript.

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Data Availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Figures



Figure 1

Different fruit parts of cotton crops.

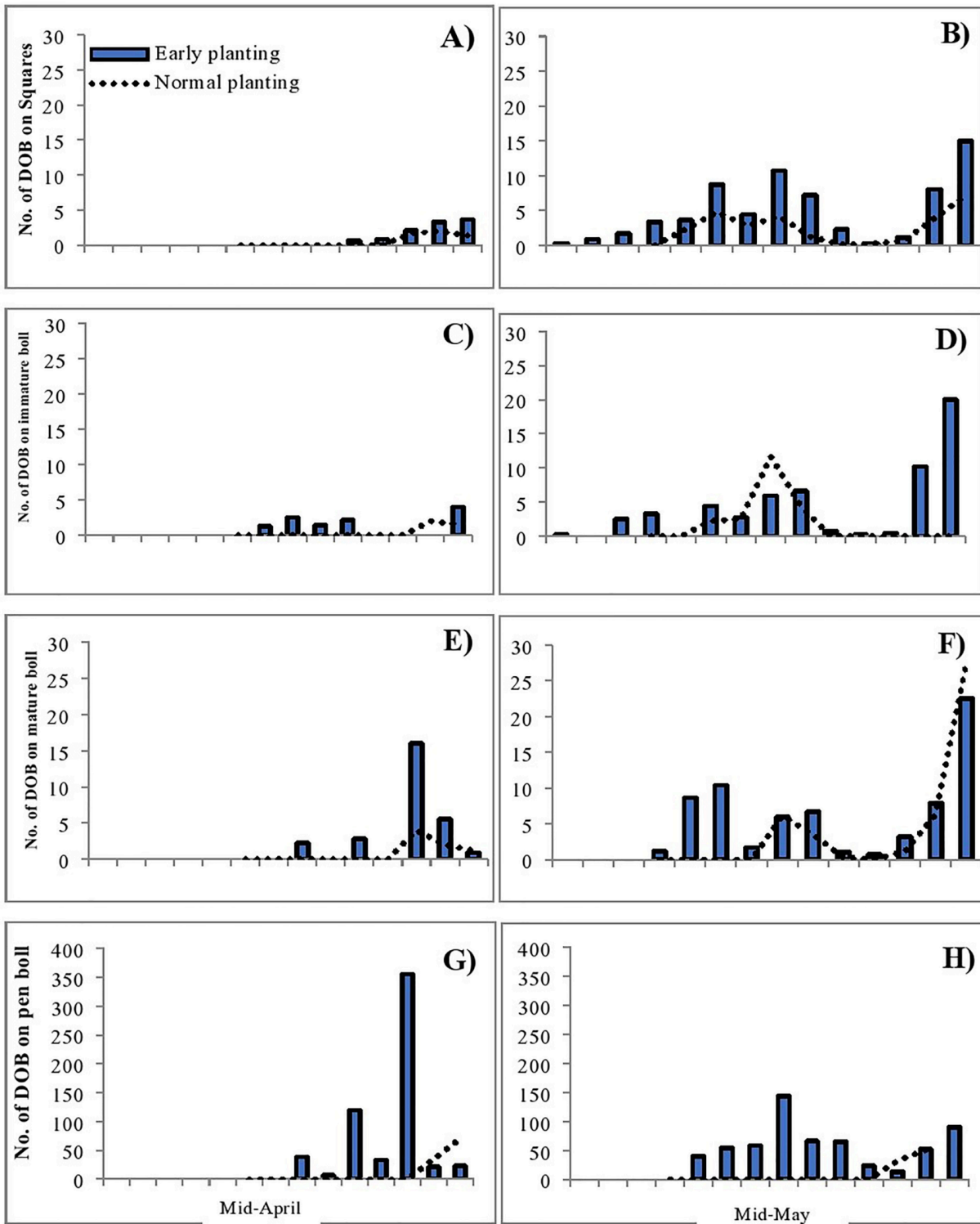


Figure 2

Seasonal trend in the density of *O. hyalinipennis* on the fruiting parts of the transgenic (Bt. CIM-554) cotton plants at different planting times under spray conditions. DOB: Dusky cotton bug. **(A)** Squares during 2014 and **(B)** 2015; **(C)** small bolls during 2014 and **(D)** 2015; **(E)** mature bolls during 2014 and **(F)** 2015; **(G)** open bolls during 2014 and **(H)** 2015.

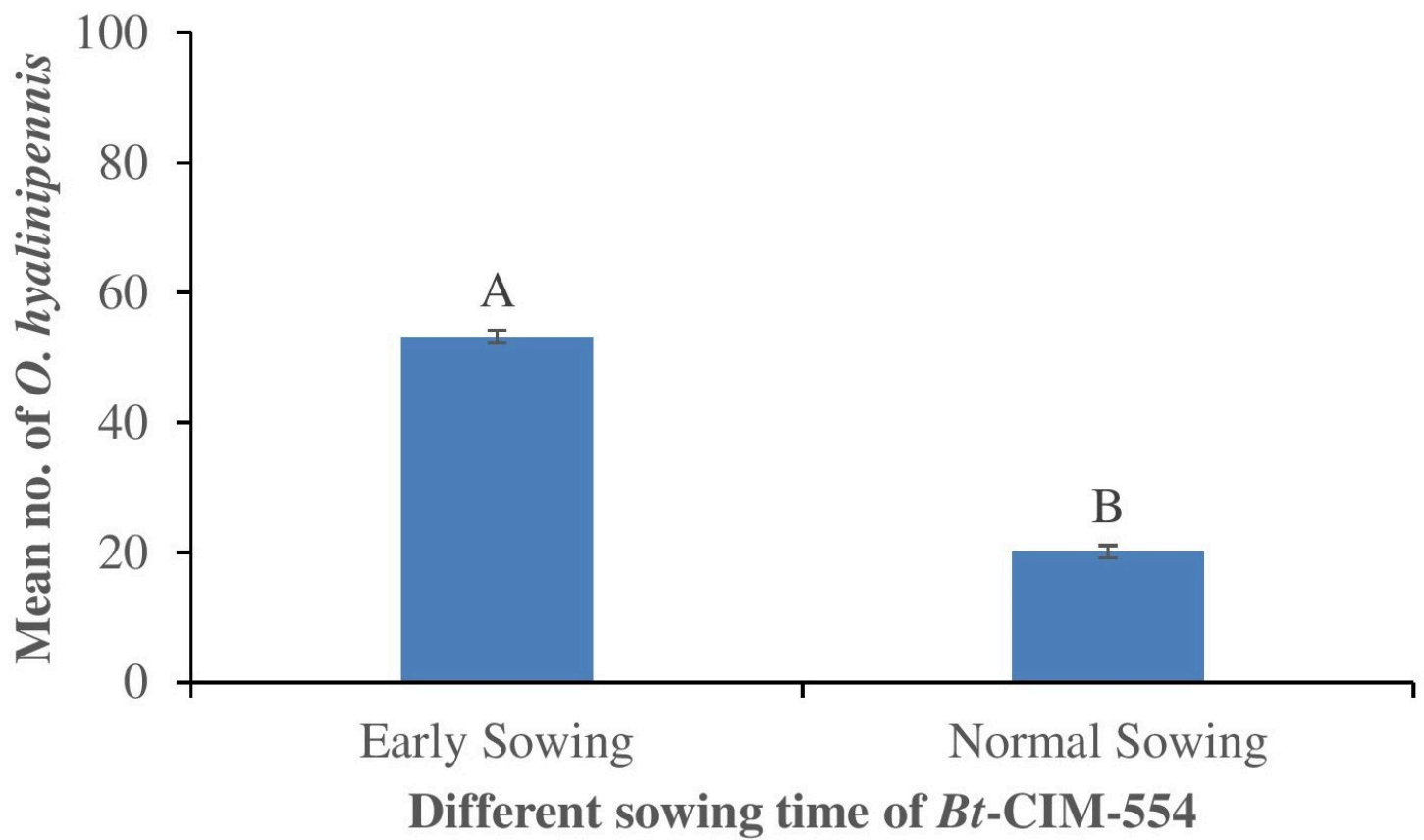


Figure 3

Comparative effect of planting time on *O. hyalinipennis* density (data pooled for fruiting parts and years). The χ^2 test was used to compare the differences between early and late sowing for the population of dusky cotton bugs. The two different letters represent differences between early and normal sowing plants.

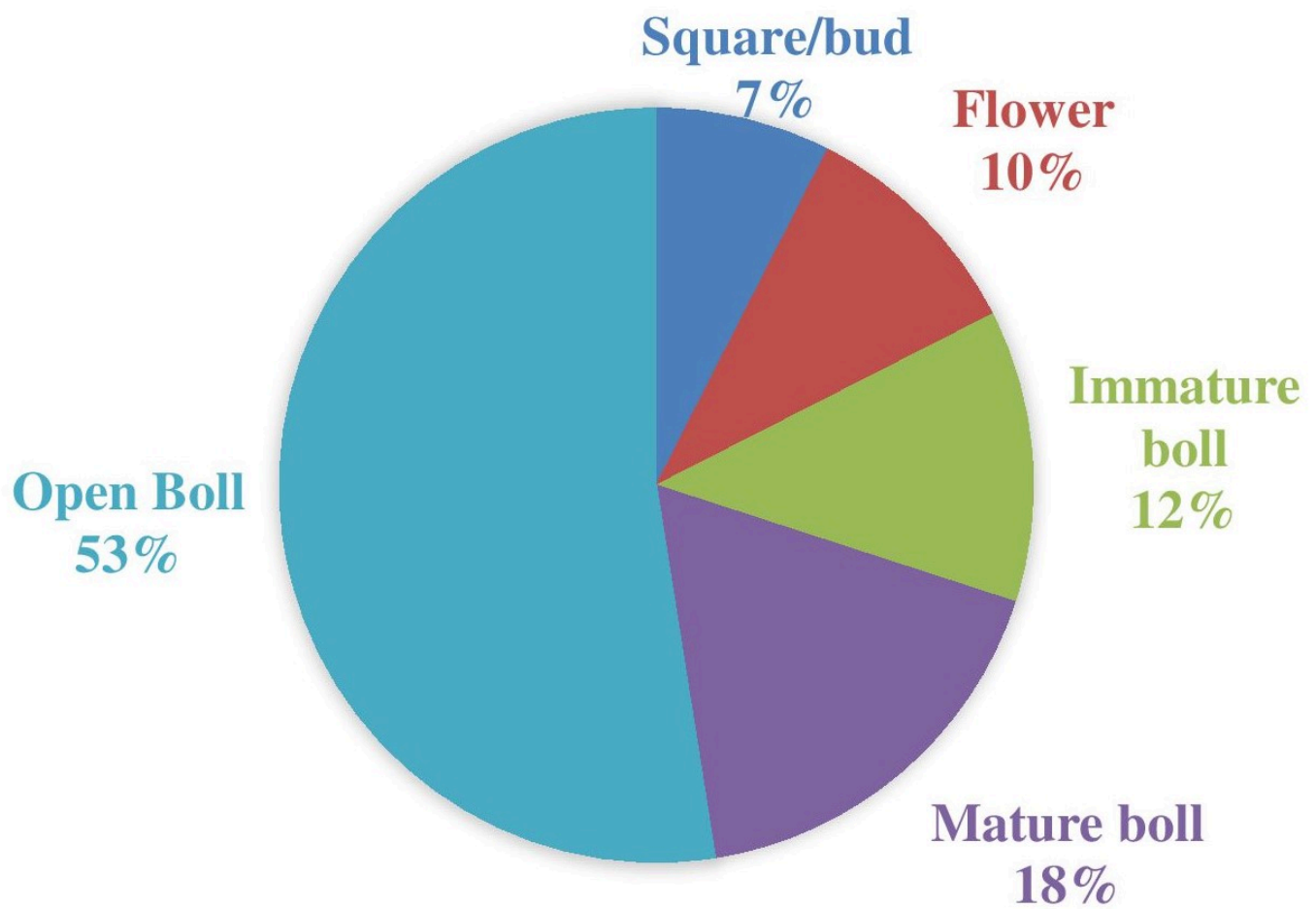


Figure 4

Preference (%) of *O. hyalinipennis* on different fruiting parts of *Bt*-CIM-554 under laboratory conditions.

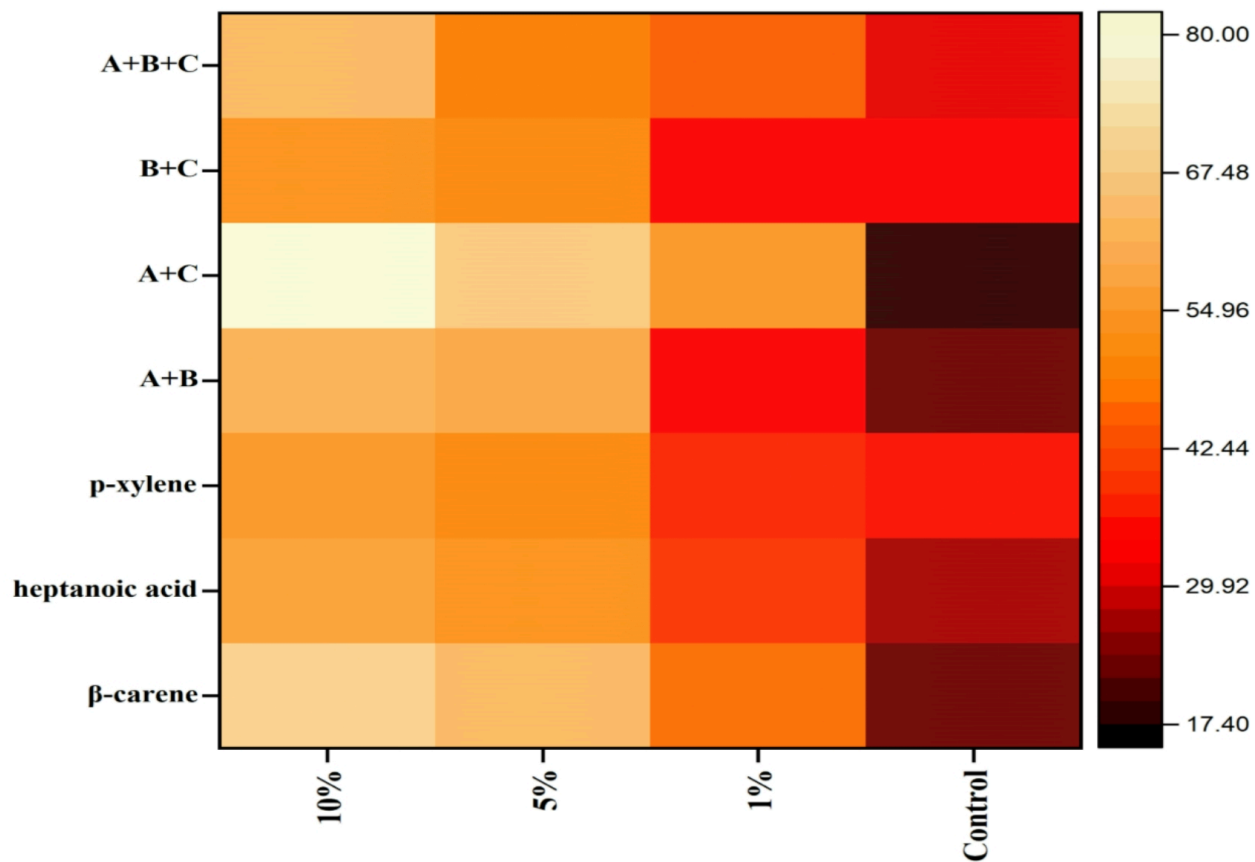


Figure 5

Attraction of *O. hyalinipennis* adults to three different concentrations (10%, 5%, and 1%) of β -carene, heptanoic acid, and *p*-xylene in a Y-tube olfactometer.