Preferences of *Lygus pratensis* (Hemiptera: Miridae) for Four Potential Trap Crop Plants[1]

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Abstract The mirid, *Lygus pratensis* (L.) (Hemiptera: Miridae), is a major pest of cotton (*Gossypium* spp.) in Xinjiang Uyghur Autonomous Region, China. In this study, we aimed to reveal the preferences of *L. pratensis* toward four potential trap crop plants for use in controlling this pest. The population densities of *L. pratensis* on five host plants were investigated by visual observation, and the behavioral responses of *L. pratensis* to the five host plants were determined by Y-type olfactory assays and laboratory selectivity assays. The results showed significant differences in host preferences in the field. The average numbers of adults on the five host plants are in the following order, from high to low: *Lepidium latifolium* (L.), *Kochia prostrata* (L.) Schrad, *Chenopodium glaucum* (L.), *Medicago sativa* (L.), and *Gossypium* spp. Laboratory selectivity assays found that *K. prostrata* had the highest number of adults at 5 min, but *L. latifolium* had the highest number of adults from 1.5 to 4.5 h. The Y-tube olfactometer assays found no significant evidence for selection of *L. pratensis* between the volatiles of *M. sativa* and pure air, but did for the other four plants. In conclusion, the blooming stage of *L. latifolium* was the most attractive to *L. pratensis* in the field and is, thus, a potential trap crop plant for *L. pratensis*.

Key Words host preference, selectivity, trap plants

Lygus pratensis (L.) (Hemiptera: Miridae) is the dominant cotton (*Gossypium* spp.) pest in southern Xinjiang, China. Nymphs and adults feed on plant sap and induce abscission of cotton buds and bolls, resulting in 23∼98% abscission of flower buds and major yield losses that can reach more than 50% if not controlled in time (Gou et al. 2018). In recent years, the rapid development and widespread planting of transgenic cotton resistant to lepidopteran pests has led to reduced use of insecticides in cotton fields. Among other factors, this has enabled *L. pratensis* to become an increasingly important pest in Xinjiang cotton fields (Lu et al. 2010; Wu et al. 2002; Yang et al. 2004). Research on *L. pratensis* has mainly focused on its biological characteristics and control technology (Gou et al. 2019; Yang et al. 2017). Insecticides are still a crucial control tactic for *L. pratensis*. However, chemical pesticides risk killing the natural enemies of *L. pratensis* and destroying the ecological balance that prevents pest resurgence and can also impact human

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health. Alternative forms of control are necessary for integrated *L. pratensis* management on cotton.

One such environmentally friendly alternative is the use of trap plants that attract the pest more strongly than does the crop. For example, corn (*Zea mays* L.) planted around tomato *(Solanum* spp.) fields effectively attracts *Helicoverpa zea* (Boddie) and minimizes herbivory on tomatoes (Rhino et al. 2014; Agrawal 1988). Trap plants can be combined with predicted population dynamics to control pests by using insecticides on the trap plants or by removing the trap refugia and pests together during a critical developmental period (Johnston and Martini 2020). Trap plants have effectively controlled other mirid bug species. *Lygus rugulipennis* Poppius can be trapped by *Medicago sativa* (L.) and *Helianthus annuus* (L.) (Accinelli et al. 2005; Ondiaka et al. 2016). *Medicago sativa* is also used as a trap plant for *Lygus hesperus* Knight in cotton fields and organically grown strawberry (*Fragaria* spp.) fields in the United States (Godfrey and Leigh 1994; Swezey et al. 2007). This study endeavored to identify a suitable trap plant for *L. pratensis* on cotton by investigating its preferences for different host plants in the field.

The attractiveness of the host plant to pests is one of the criteria for evaluating the suitability of trap plants (Fougere et al. 2020). Higher population densities of insects on specific plant species among several different hosts suggest the insects have a preference. In addition, insect attraction to plant volatiles in the lab often correlates with selectivity in the field (Cao et al. 2019). Plant volatile odors are important chemical signals for insects to locate the hosts (Schoonhoven et al. 2005; Visser 1986). The Y-tube olfactometer is widely used in selectivity research of insects because it accurately reflects the preferences of insects (Chen et al. 2016; DeVries et al. 2019; Marler and Marler 2018). This study combined Y-olfactometer assays with field studies to confirm the role of volatile odors in *L. pratensis* selectivity.

At present, few publications cover trap plants for *L. pratensis* and only for a small range of crop species. A prior study examined behavioral responses and population dynamics of *L. pratensis* to five host plants in southern Xinjiang and concluded that safflower (*Carthamus tinctorius* L. can be used as a trap crop for *L. pratensis* (Wang et al. 2020a). In this study, four potential trap plants were compared to cotton. All of them are common plants in the surrounding fields in Northern Xinjiang and were previously identified as potential hosts for *L. pratensis* (Cao et al. 2017*)*. This study, therefore, was undertaken to determine the preferences of *L. pratensis* for these different hosts to serve as a theoretical basis for the selection of trap plants and the development of chemical attractants for *L. pratensis* in the field.

Materials and Methods

Insect collection and rearing. *Lygus pratensis* was originally collected from *Chenopodium glaucum* (L.) growing at Tarim University (81°17′E, 40°32′N; Alaer City, Xinjiang, China) in May 2020. The colony established from this collection was maintained continuously with pods of green bean, *Phaseolus vulgaris* (L.) (Rosales: Phaseolus) over three generations in nylon mesh (60 mesh) containers (30 × 30 × 30 cm) at 25 ± 0.5°C, 70 ± 5% relative humidity, and 16:8 L:D photoperiod in the laboratory. Green bean pods as the food sources and spawning substrates were purchased from the market and sprayed with 5% sodium hypochlorite for disinfection and sterilization, then washed with distilled water, dried, and cut into 2-cm-long lengths. A 10% honey solution was provided on degreased cotton to supplement nutrition (Gou et al. 2019). After eggs were laid, the plant was moved to a separate box and maintained at the same conditions until the first nymphs emerged. Newly hatched nymphs were placed individually in plastic tubes (diameter = 2.5 cm, height = 8.5 cm) that had their tops covered with fine mesh for ventilation. Nymphs were fed with pods of *P. vulgaris* until the first day of emergence and then transferred to a new, 30 × 30 × 30 cm, 60-mesh nylon container. The newly emerged *L. pratensis* adults were maintained continuously with pods of green beans for 3 d and used for laboratory selectivity assays and Y-tube olfactometer assays.

Culturing of host plants. Five species of host plants were planted on the campus of Tarim University: cotton, *Gossypium* spp. (Malvales: Malvaceae); oak-leaved goosefoot, *Chenopodium glaucum* (L.) (Centrospermae: Chenopodiaceae); alfalfa, *Medicago sativa* (L.) (Rosales: Leguminosae); forage kochia, *Kochia prostrata* (L.) Schrad (Centrospermae: Chenopodiaceae); and perennial pepper-weed, *Lepidium latifolium* (L.) (Brassicales: Brassicaceae). Seeds of cotton variety XLZ37 were provided from the Chinese Academy of Agriculture. Other host seeds were collected from wild plants on the campus of Tarim University in 2019. Each plant was cultured in three, 1 × 5 m plots on 10 April, with three seeds sowed every 15 cm. The soil type was loam, and all host plants were routinely irrigated once every 2 wk and maintained without applying chemical insecticides and fertilizer.

Field population dynamics investigation. During the summers of 2019 and 2020, from 15 May to 13 August, the plants were inspected every 7 d for a total of 14 investigations each year. During the investigation, two 1 × 1-m transects from each plot were randomly selected and covered with a 1 × 1 × 1-m nylon mesh cage. The plants within each cage could be shaken and beaten, causing the insects to jump onto the nylon mesh, and then the number of *L. pratensis* nymphs and adults in each section were counted visually.

Laboratory selectivity assay. To study the feeding selectivity of *L. pratensis* in the laboratory, 50 g of upper branches in the flowering and budding phase of the four trap plants that had been grown for 3 mo were cut, wrapped with wet cotton, and randomly positioned in the four corners of a cage (30 × 30 × 30 cm). Fifty *L. pratensis* adults (25 male, 25 female) that had been starved for 6 h were chilled briefly and released in the center of cage. They were allowed to move freely. The number of *L. pratensis* on different hosts was recorded first after 5 min, then at 1.5, 3, and 4.5 h after the start of the assay by the visual checking method. The number of test insects that had made a choice and were physically on each host plant as well as those that did not make a choice was recorded. Two people observed at the same time to ensure that all 50 insects were counted. Three replicates were performed.

Y-tube olfactometer assay. We used previously published Y-tube olfactometer assembly and test methods (Sun et al. 2017). The tested plants had been grown for 3 mo and were in the flowering and budding phase. These were sprayed with 5% sodium hypochlorite and then washed with distilled water. Branches (20 cm) including flowers and buds were cut, and the cut end was wrapped with moist cotton towels and enclosed inside a 48 × 65-cm plastic shopping bag to prevent wilting. These branches were later placed in a 500-ml washing flask as the odor sources. The Y-tube olfactometer was made of transparent glass with an internal diameter of

3 cm, the base was connected to two sidearms with an angle of 60° between them, and all three arms were 15 cm in length. The release tube of the adults was connected to the base. At the end of each sidearm was a natural wooden cork into which a glass tube was inserted and connected to the washing flask. A QC-1B atmospheric sampler was placed at the base of the Y-tube to pull air through the device, and the airflow was maintained at 500 ml/min. All glassware and the atmospheric sampler were provided by the Beijing Municipal Institute of Labour Protection. The airflow was filtered by activated carbon and humidified by distilled water before entering the washing flask of the odor source and then finally entering the Y-tube olfactometer. Adult *L. pratensis* were loaded individually into the base of the Y-tube. Their olfactory responses were observed after 5 min. If an adult climbed a third of the way up a sidearm and stayed for 15 s, it was determined to have chosen the odor of that arm. If the adult made no choice after 5 min, this was judged as no response. Each Y-tube was cleaned with ethanol and distilled water and allowed to dry before being reused. After 10 adults were observed, the Y-tube was rotated 180 degrees to avoid position effects. After 30 adults were observed, the Y-tube was changed. No individual *L. pratensis* was used more than once. The two treatment comparisons included (1) five flowering host plants versus pure air, and (2) five flowering host plants versus flowering cotton plants. Each treatment was repeated for three sets of 30 individuals.

Data analysis. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, Version 25, Chicago, IL). The Y-tube olfactometer bioassay data were analyzed using chi-square (χ^2) tests against the null hypothesis, which was that the number of *L. pratensis* adults reaching the end of either olfactometer arm had a 50:50 distribution. The chi-square and significant difference level values were calculated after excluding nonresponding individuals. Two-way analysis of variance followed by the Tukey honest significant difference multiple comparisons were used to analyze the effects of time of survey, host plants, and their interaction on population density of *L. pratensis* (adults or nymphs).

Results

Field population dynamics of *L. pratensis* on five species of host plants. In 2019, among all five hosts, the average number of nymphs on *L. latifolium* was the highest, but it had no significant difference with that on *C. glaucum*. The plants ranked from highest to lowest by average number of nymphs overall in 2019 were *L. latifolium*, *C. glaucum*, *K. prostrata*, *M. sativa*, and *Gossypium* spp. (Table 1). The infestation of *L. pratensis* nymphs was the highest on *C. glaucum* in mid-May 2019 ($F = 55.59$; df $= 4, 15$; $P < 0.001$). From June to mid-July, the number of nymphs found on *C. glaucum* and *L. latifolium* reached their peak (mean ± standard error, 26.4 ± 1.49 and 24.8 ± 1.01). From mid-July onward, the number of nymphs on *K. prostrata* was highest followed by *C. glaucum* and then *L. latifolium* ($F = 124.53$; df $=$ 4, 75; $P < 0.001$). The number of nymphs on *M. sativa* was low and stable. Time of survey ($F = 29.58$; df $= 13, 210$; $P < 0.001$), host plants ($F = 238.27$; df $= 4, 210$; $P < 0.001$), and their interaction ($F = 15.83$; df $= 52, 210$; $P < 0.001$) had a significant impact on the population density of *L. pratensis* nymphs (Fig. 1).

Table 1. Mean ± standard error number of *L. pratensis* adults and nymphs on five host plants.

Year	**Stage**	***Chenopodium glaucum***	***Medicago sativa***	***Kochia prostrata***	***Lepidium latifolium***	***Gossypium* spp.**	***F***	**df**	***P***
2019	Nymphs	19.2 ± 0.8ab*	12.7 ± 0.6c	15.1 ± 0.9bc	20.8 ± 2.0a	1.3 ± 0.2d	238.3	4, 210	<0.001
2019	Adults	19.2 ± 0.7b	10.5 ± 0.5c	20.6 ± 1.5b	27.5 ± 2.4a	1.4 ± 0.2d	244.3	4, 210	<0.001
2020	Nymphs	19.3 ± 0.9a	11.9 ± 0.8b	15.9 ± 1.2ab	20.9 ± 2.4a	1.9 ± 0.2c	254.7	4, 210	<0.001
2020	Adults	18.0 ± 0.7b	10.7 ± 0.8c	20.8 ± 1.5ab	26.2 ± 2.6a	1.8 ± 0.2d	240.5	4, 210	<0.001

* Means within a row followed by different lowercase letters are significantly different ($P < 0.05$) by the Tukey honest significant difference test.

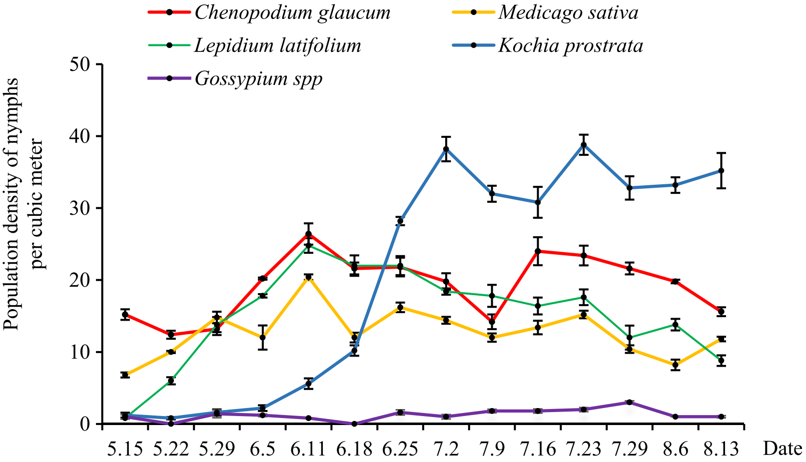

Fig. 1. Population dynamics of *L. pratensis* nymphs on five host plants in 2019 (± standard deviation = bars about each mean).

In 2020, the average number of *L. pratensis* nymphs on *C. glaucum* and *L. latifolium* was higher than on the other three host plants, though not significantly different from *K. prostrata*. The ranking was the same as in 2019 (Table 1). The number of *L. pratensis* nymphs on *C. glaucum* and *L. latifolium* remained high until July. The number of nymphs on *K. prostrata* was higher than on the other 4 host plants from mid-July onward. Time of survey ($F = 31.33$; df = 13, 47; $P < 0.001$), host plants ($F = 254.66$; df = 4, 210; $P < 0.001$), and their interaction ($F = 32.76$; df = 52, 210; $P < 0.001$) had a significant impact on the population density of *L. pratensis* nymphs (Fig. 2).

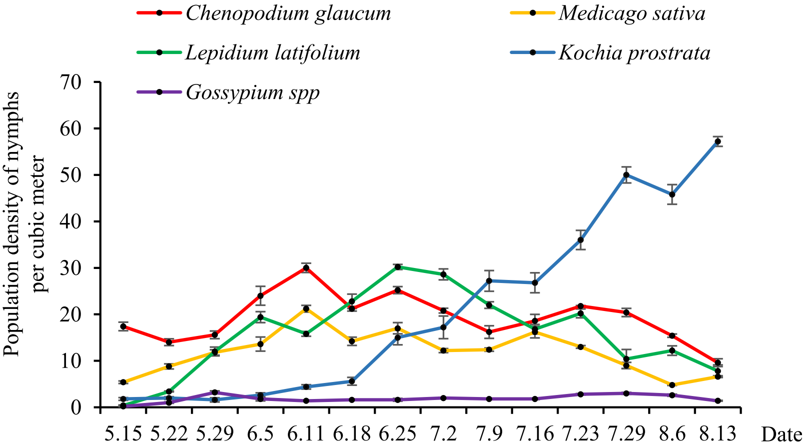

Fig. 2. Population dynamics of *L. pratensis* nymphs on five host plants in 2020 (± standard deviation = bars about each mean).

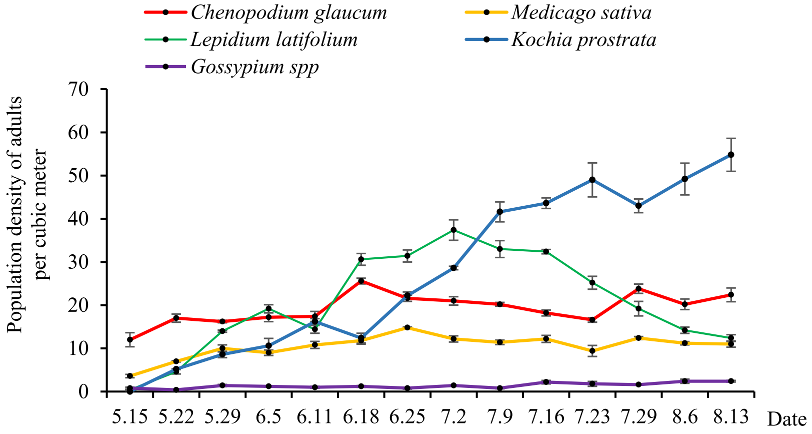

Fig. 3. Population dynamics of *L. pratensis* adults on five host plants in 2019 (± standard deviation = bars about each mean).

In 2019, the average number of adults on *L. latifolium* was the highest and was significantly higher than that on *M. sativa*, but had no significant difference with that on *C. glaucum*. The host plants ranked by average number of adult *L. pratensis* from high to low were *L. latifolium*, *K. prostrata*, *C. glaucum, M. sativa*, and *Gossypium* spp. (Table 1). At the beginning of June 2019, the number of adults on *L. latifolium* was higher than on other plants. In early July, it reached a peak (mean ± standard error, 37.4 ± 2.37). The infestation of adult *L. pratensis* on *C. glaucum* was always higher than that on *M. sativa*. The number of adult *L. pratensis* on *K. prostrata* outnumbered those on the other three hosts from early July onward. Time of survey ($F = 30.82$; df = 13, 47; $P < 0.001$), host plants ($F = 244.34$; df = 4, 210; $P < 0.001$), and their interaction ($F = 32.76$; df = 14; $P < 0.001$) had a significant impact on the density of *L. pratensis* adults (Fig. 3).

The rankings of the average numbers of adult *L. pratensis* on the five host plants in 2020 were consistent with the results in 2019. The average number of adult *L. pratensis* on *K. prostrata* was significantly different than on *L. latifolium* in 2019, but not in 2020 (Table 1). *Chenopodium glaucum* was the most attractive host plant from May to early June in 2020, *L. latifolium* was the most attractive host plant from early June to early July, and *K. prostrata* was the most attractive from mid-July to mid-August. Time of survey ($F = 27.88$; df = 13, 47; $P < 0.001$), host plants ($F = 240.53$; df = 4, 210; $P < 0.001$), and their interaction ($F = 19.97$; df = 14; $P < 0.001$) had a significant impact on the density of *L. pratensis* adults (Fig. 4).

Selectivity of *L. pratensis* adults on four host plants. In the laboratory experiments of selectivity, the response of *L. pratensis* was significantly influenced by the host plants ($F = 36.74$; df = 3, 47; $P < 0.001$). The number of adult *L. pratensis* on *K. prostrata* was the highest (37%) after 5 min, but gradually decreased. After 1.5 h, the number on *K. prostrata* was lower than on *L. latifolium* but higher than on *C. glaucum* or *M. sativa*. *Lepidium latifolium* had the most adults after 1.5 h, with significant differences compared to other hosts after 3 and 4.5 h.

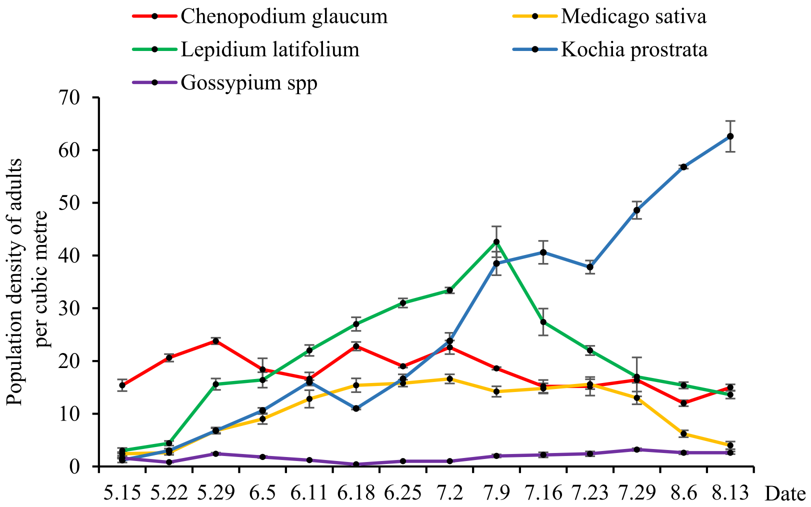

Fig. 4. Population dynamics of *L. pratensis* adults on five host plants in 2020 (± standard deviation = bars about each mean).

The number of adult *L. pratensis* on *M. sativa* was significantly lower than that on other hosts (Fig. 5).

Olfactory responses of *L. pratensis* adults on five host plants. Adult *L. pratensis* responses to volatiles from the host plants are shown in Figure 6. The

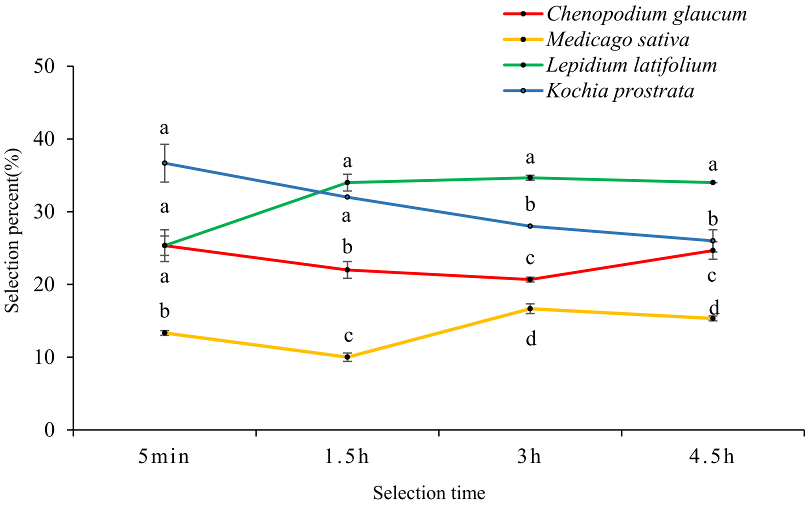

Fig. 5. Selectivity of *L. pratensis* adults on four host plants (± standard deviation = bars about each mean). Means within a graph line marked with the same lowercase letter are not significantly different (*P* > 0.05l, Tukey honest significant difference).

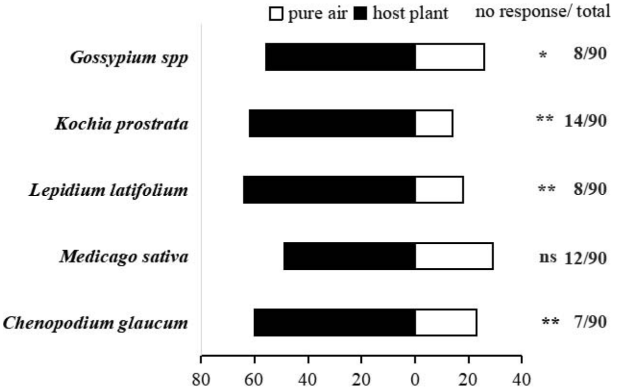

Fig. 6. Olfactory responses of *L. pratensis* adults on five host plants (ns indicates no significance, *$P < 0.05$), **$P < 0.01$). *Gossypium* spp.: $\chi^2 = 5.92$, $P = 0.015$; *Kochia prostrata*: $\chi^2 = 17.94$, $P < 0.001$; *Lepidium latifolium*: $\chi^2 = 14.54$, $P < 0.001$; *Medicago sativa*: $\chi^2 = 2.79$, $P = 0.095$; *Chenopodium glaucum*: $\chi^2 = 9.00$, $P = 0.003$.

adults significantly preferred volatile orders from all plants except *M. sativa* relative to the control of pure air. The volatile odors from *C. glaucum*, *L. latifolium*, and *K. prostrata* had a high level of significant difference compared to the control. The plants ranked in attractiveness, relative to the control from high to low, were *L. latifolium*, *K. prostrata*, *C. glaucum, Gossypium* spp., and *M. sativa* (Fig. 6). In pairwise comparisons to *Gossypium* spp. volatiles, the volatile odors from *C. glaucum*, *L. latifolium*, and *K. prostrata* were significantly more attractive, while the volatile odors from *M. sativa* exhibited no significant difference (Fig. 7).

Discussion

Previous studies have shown that *L. pratensis* has a preference for flowers and tender plants (Dong et al. 2013; Lu et al. 2009); therefore, plants that have tender growth or are flowering should be more attractive to *L. pratensis*. Furthermore, the use of plants as trap crops should also coincide with the peak time of pests feeding on economic crops in order to maximize the trapping effect and minimize economic harm (Hokkanen 1991). Our results show that, from the middle of May onward, the number of *L. pratensis* adults and nymphs was highest on the plant species in our study, as adults gradually emerged in mid-March. Peak occurrence of *L. pratensis* in the cotton field was from mid-June to mid-July (Zhang et al. 2018).

The results of the field population density investigation showed that *K. prostrata* was more attractive to *L. pratensis* than were other host plants after July. This result may be due to the appearance of flowers and buds on *K. prostrata* from June to July. *Kochia prostrata* is one of the most important plants for windbreak and erosion control in Xinjiang. Wang et al. (2020a) also found that *K. prostrata* is highly attractive to *L. pratensis*, but concluded that its most attractive time does not coincide with the peak population period of *L. pratensis* in cotton fields.

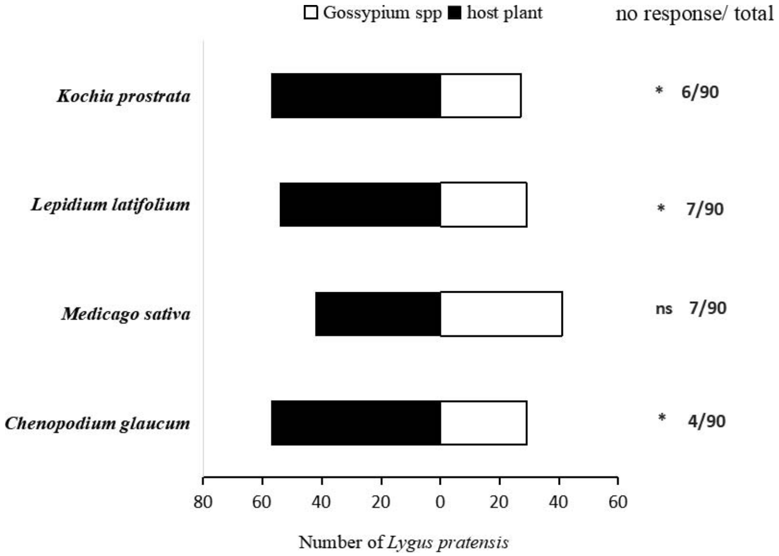

Fig. 7. Olfactory responses of *L. pratensis* adults for four host plants versus *Gossypium* spp. (ns indicates no significance, $^*P < 0.05$). *Kochia prostrata*: $\chi^2 = 5.00$, $P = 0.025$; *Lepidium latifolium*: $\chi^2 = 4.001$, $P = 0.045$; *Medicago sativa*: $\chi^2 = 0.006$, $P = 0.937$; *Chenopodium glaucum*: $\chi^2 = 4.78$, $P = 0.029$.

Chenopodium glaucum and *L. latifolium* are common weed plants. Their period of budding and flowering is from May to October, which coincides with the peak population period of *L. pratensis*. *Chenopodium glaucum* was one of the main weed hosts for oviposition by the first generation of adults. From June to mid-July, the attractiveness of *C. glaucum* to *L. pratensis* was not statistically different from that of *L. latifolium*. As a potential trap plant, *C. glaucum* has the same problem as *K. prostrata*. The attraction of *L. latifolium* to *L. pratensis* was highest in June and July, and the attraction was lower than that of *C. glaucum* and *K. prostrata* in the remainder of the study period. This reduced the risk of dissemination of *L. pratensis*. Therefore, we concluded that *L. latifolium* was the most suitable host for trapping *L. pratensis* in the field during the pest's peak population period of June and July.

In the field population dynamics investigation, *C. glaucum*, *L. latifolium*, and *K. prostrata* were more attractive to *L. pratensis* than was cotton, but at different times. This enables selective use of trap plants according to season. For example, *C. glaucum* was the most attractive plant to *L. pratensis* in May, thus the application of insecticides to *C. glaucum* at this time can reduce the number of first-generation nymphs. *Kochia prostrata* was the most attractive plant after July, thus pesticide application on this trap plant should be considered to reduce the *L. pratensis* overwintering population. Furthermore, *L. latifolium* was the most suitable trap plant because its time of greatest attraction coincided with the peak population period of *L. pratensis* in cotton fields. Flowering of *C. glaucum* occurred from mid-May until June, while flowering of *L. latifolium* and *M. sativa* was from June to July, cotton in

July, and *K. prostrata* after mid-July, which correlated with the population dynamics of *L. pratensis*. This indicated that the flowering of these plants had a great influence on their attractiveness and potential as trap plants to *L. pratensis*.

Other factors need to be addressed to properly use trap plants. Their planting area is important, as a small planting area is insufficiently attractive (Lu 2009). Trap plants also must be chemically controlled in a timely manner, as the risk of pest outbreak and spread will increase if the trap plants are not controlled (Badenesperez et al. 2005; Castle 2006). To align trap plant flowering period with pest dynamics, sowing dates should also be considered (Wang et al. 2020b). As attractant plants, weed plants have the advantages of strong adaptability and reduced manpower and material resources, but as trap plants they should be planted with a reasonable amount of space between them and the main crop so as not to cause weed pest problems or earlier infestation of insect pests due to the traps attracting pests to the crop (Yan et al. 2021). All of our trap plants were several times more attractive to the pests than was the original crop, posing a risk of early infestation. Further studies will be conducted with *L. latifolium* to confirm whether or not it can cause early infestation of cotton and how to mitigate this problem.

In conclusion, our study compared the attractiveness of four potential trapping plants and cotton to *L. pratensis*. Among the selected plants, *C. glaucum*, *L. latifolium*, and *K. prostrata* were most attractive to *L. pratensis*. *Lhenopodium latifolium* is the most suitable for use as a trap plant in cotton fields because its most attractive time coincides with the peak population period of *L. pratensis* in cotton fields. To further determine the best way of trapping *L. pratensis* in the field, relevant field studies can be conducted with different hosts examining factors such as scale, planting method, and application time.

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