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Host plant preference of *Lygus hesperus* exposed to three desert-adapted industrial crops

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Abstract

The desert-adapted crops vernonia (*Centropalpus pauciflorus*), lesquerella (*Physaria fendleri*), and camelina (*Camelina sativa*) are being grown in the arid southwestern USA as potential feedstock for biofuel and/or other environmentally friendly products. A plant feeding choice test was conducted to determine the relative attractiveness of these three “new” crops to a possible insect pest, *Lygus hesperus* Knight. Adult *L. hesperus* were readily observed feeding or resting on the flowering structures of each plant type, but they were seen most often on vernonia and least often on camelina. *Lygus hesperus* readily deposited their eggs on each plant species, but again, the greatest amount of egg deposition was found on vernonia and the least on camelina. These studies indicate that *L. hesperus* might pose a threat to the production of these new crops. Moreover, the commercial expansion of these crops could significantly alter the population dynamics of the existing arthropod community. New challenges for managing this pest during regional crop production changes are discussed.

Keywords: *Centropalpus pauciflorus*, *Parthenium argretatum*, *Physaria fendleri*, Choice test, Oviposition preference, Feeding preference

1. Introduction

Several desert-adapted (*i.e.*, low water and fertilizer input) industrial crops including vernonia (*Centropalus pauciflorus* (Willd.)), lesquerella (*Physaria fendleri* (A. Gray) O'Kane & Al-Shehbaz), and camelina (*Camelina sativa* (L.) Crantz) are being grown on a relatively small scale in the Southwestern USA. Vernonia is a short season crop that is cultivated in the spring and summer months. It yields high quantities of epoxy fatty acids that are useful in the reformulation of oil-based paints to reduce the emission of volatile organic compounds that contribute to the production of smog (Perdue et al., 1986). Other potential markets for the epoxy fatty acids include eco-friendly plasticizers, additives in polyvinyl chloride, polymer blends and coatings, cosmetic, and pharmaceutical applications (Shimelis et al., 2013). The unique structure of vernolic acid may have a much wider use than epoxidized oils at about half the cost of soybean and linseed oils (Carlson and Chang, 1985). Lesquerella is a perennial mustard that is cultivated during winter and spring months (Wang et al., 2010). It is being developed as an oilseed crop for use in making eco-friendly lubricants, resins, waxes, nylons, plastics, and cosmetics (Dierig et al., 1993). Lesquerella oil can also be used for high-performance biodiesel or as an additive to diesel fuels to lessen engine damage while lowering vehicle emissions such as hydrocarbons, carbon monoxide, and particulate matter (Goodrum and Geller, 2005; Moser et al., 2008). Typically grown in the spring, camelina is a short season oil seed crop that is undergoing resurgent interest due to its potential as a biofuel feedstock (Moser and Vaughn, 2010), livestock feed (Colombini et al., 2014), and salmon feed (Hixson et al., 2014). It also yields seeds that have high concentrations of unsaturated Omega 3 fatty acids that compare favorably with other vegetable oils (Wittkop et al., 2009).

The development of these desert-adapted crops offers many exciting new possibilities as biofuel feedstocks, as well as potentially providing nutritional and medical products. However, their commercial development and integration into the existing agro-ecosystem, as either replacement for conventional crops (*i.e.*, cotton or alfalfa) or as additions to a producer's cropping system, will undoubtedly influence the structure, dynamics, and function of the extant arthropod community. Of particular interest are the potential impact of arthropods on these new crops and the changes in arthropod community dynamics that may exacerbate or ameliorate pest problems in current conventional crops grown in the region.

Lygus spp. (primarily *L. hesperus* Knight) are regarded as a major threat to cotton and many other crops grown in the arid regions of the USA (Leigh, 1976; Leigh et al., 1988; Mauney and Henneberry, 1984; Strong, 1970). *Lygus*' status as a major pest is due to several factors. First, *Lygus* spp. are known to feed on >150 different host plants including cotton, alfalfa, canola, and many fruits, vegetables, ornamentals and weeds (Schwartz and Footitt, 1998; Young, 1986; Wheeler, 2000). Second, *Lygus* spp. often feed directly on the plant's young fruit, shoot tips, and seeds which cause fruit deformity or dislodgement (Mauney and Henneberry, 1984; Strong, 1970; Swezey et al., 2007). Third, *Lygus* bug is multivoltine, capable of having five generations per year. Fourth, they cryptically overwinter as adults and undergo a reproductive diapause. In the early spring, they become highly active and seek flowering and fruiting host plants. They tend to colonize early flowering host plants in the spring and then disperse *en masse* to a wide variety of crops (*i.e.*, cotton) over the summer (Sevacherian and Stern, 1975). These highly nomadic and opportunistic feeders are capable of rapidly infesting a wide variety of crops throughout the year. Finally, their pest status is exacerbated by the fact that growers have very few tactics, outside of insecticides, available for *Lygus* spp. control. Unfortunately, some of these

insecticides are broad-spectrum in activity and disruptive to the environment in general and the natural enemy and pollinator complexes in particular. Given these characteristics, *Lygus* spp. are poised to negate some of the major gains made in integrated pest management (IPM) over the past quarter century.

Vernonia, lesquerella, and camelina appear to be attractive to *L. hesperus* and other arthropods (*i.e.*, other herbivore pests, natural enemies and pollinators) that are commonly encountered in cotton and alfalfa. However, the relative preference of *L. hesperus* to these three new crops is unknown. Therefore, the goal of this study was to characterize and quantify the feeding and oviposition preferences of *L. hesperus* to these three industrial crops. To this end, we conducted *L. hesperus* feeding and oviposition host plant choice tests in arenas containing flowering vernonia, lesquerella, and camelina plants.

2. Materials and methods

2.1 Test plants and insects

Individual vernonia, lesquerella and camelina plants were grown in a 0.5-gallon pots containing a standard soil mixture. The plants were maintained in a greenhouse at 18 °C (night) to 30 °C (day) and 30% relative humidity (RH). Plants were watered as needed. A 1:1 mixture of all-purpose Scotts Miracle-Gro Excel (21-5-20) and Cal-mag Miracle-Gro® Professional (15-5-15) was applied (250 ml/plant) at a rate of 1% shortly after the seeded plants emerge. All plants were approximately the same size and flowering during the host preference feeding choice bioassays. The adult *L. hesperus* used in all the bioassays were obtained from colony reared on an established artificial diet (Debolt, 1982).

2.2 Host plant feeding preference test

A feeding choice study was conducted in enclosed arenas that contained a single vernonia, lesquerella, and camelina plant. Each arena was a 61-cm tall x 35.5-cm diameter clear cylindrical cage (Figure 1). The arenas were covered with a fine organdy mesh fabric to facilitate air exchange. The arenas were erected within a 15 x 20-m air conditioned greenhouse at the U.S. Arid-Land Agriculture Research Center, Maricopa, Arizona, USA. The greenhouse was maintained at a 35:25 °C day:night cycle and at 30% RH. Ten cages were erected on each of three different dates (30 April, 7 May and 14 May, 2010), which served as blocks in the experimental design. The arenas were put on benches at chest height to facilitate the direct focal observations. The arrangement of plants within each cage was randomized. For each cage, 3 to 5 day-old adult *L. hesperus* ($n = 50$ per cage) were released at 14:00 at an approximate sex ratio of 1:1. The number and location (*i.e.*, main stem, petioles, leaves, and flowering structures) of *L. hesperus* on each plant were then recorded for the next four consecutive days at 08:00, 11:00 and 14:00 h.

2.3 Statistical analysis

We fitted a series of mixed linear models to assess the host plant preference of *L. hesperus*. A value of 0.5 was added to each recorded insect count to eliminate mathematical errors (*i.e.*, taking the log transformation of zero insect counts) encountered during the process of identifying the optimal model. To ensure that the data adhered to the statistical assumptions made for the mixed linear model, the Box-Cox procedure (Box and Cox, 1964) was conducted, and Studentized deleted residuals (Kutner et al., 2004) were examined from models that accounted for plant preference, block, day, and time of day. Upon completion of these diagnostic measures, it was determined that the inverse transformation (*i.e.*, the reciprocal of insect counts) was

optimal, and that there were no outlying observations. Subsequently, the following model was fitted to the data:

$$(i) Y_{ijklm} = \mu + Plant_Species_i + Block_j + Day(Block)_{k(j)} + Time(Day)_{l(k)} + \varepsilon_{ijklm}, (i)$$

Where Y_{ijklm} is an individual observation, μ is the grand mean, $Plant_Species_i$ is the fixed effect of i^{th} plant species, $Block_j$ is the random effect of j^{th} block, $Day(Block)_{k(j)}$ is the random effect of the k^{th} day nested within the j^{th} block, $Time(Day)_{l(k)}$ is the random effect of the l^{th} time of day in which insect counts were recorded within the k^{th} day, and ε_{ijklm} is the random error term.

Significant differences in the mean visitation rate on each plant species were identified by the Tukey multiple-comparison procedure (Tukey, 1953).

2.4 Host plant oviposition preference test

At the conclusion of each four day feeding choice experiment, the plants were cut at their base and frozen at -20 °C. Freezing the plants served two purposes: (1) it arrested egg development and (2) it facilitated the process of locating cryptically concealed eggs embedded in the plant tissue (*i.e.*, frozen eggs are easier to spot than viable eggs, JRH, pers. obs.). After the plants had been frozen, the number and location (*i.e.*, main stem, petioles, leaves, and flowering structures) of the eggs deposited on each host plant was identified by focal observation using a 10x dissecting microscope.

2.5 Statistical analysis

A similar statistical analysis to that conducted for the host plant feeding preference study was conducted for the oviposition choice tests. Briefly, a value of 0.5 was added to all egg counts, the data were subsequently subjected to the Box-Cox procedure (Box and Cox, 1964),

and Studentized deleted residuals (Kutner et al., 2004) were used to screen for outliers. Compared to the host plant feeding preference data, the only major difference was that these procedures were conducted using models that accounted only for plant and block effects. Subsequently, the egg counts were transformed to the -0.15^{th} power to ensure that the data adhered to the statistical assumptions of the mixed model. After these diagnostic measures had been completed, the following model was fitted to the data:

$$Y_{ijk} = \mu + \text{Plant_Species}_i + \text{Block}_j + \varepsilon_{ijk}, \text{ (ii)}$$

Where Y_{ijk} is an individual observation, μ is the grand mean, Plant_Species_i is the fixed effect of i^{th} plant species, Block_j is the random effect of j^{th} block, and ε_{ijk} is the random error term. Significant differences in the mean visitation rate on each plant species were identified by the Tukey multiple-comparison procedure (Tukey, 1953).

3. Results

3.1 Host plant feeding preference test

There were highly significant differences in host plant preference exhibited by adult *L. hesperus* in the feeding arenas on each of the three plant species examined ($F = 166.36$; $P < 0.001$). Specifically, *L. hesperus* were observed most often on vernonia and least often on camelina (Figure 2A). The location of adult *L. hesperus* on each of the plant species were very similar. Specifically, the bugs were observed on the flowering structures > leaf tissue > petiole > main stem for each of the plant types (Figure 2B).

3.2 Host plant oviposition preference test

There were highly significant differences in the number of eggs deposited on each plant type ($F = 73.96$; $P < 0.001$). Female *L. hesperus* strongly preferred vernonia > lesquerella > camelina (Figure 3A). In general, the *L. hesperus* oviposition patterns on each plant species were similar. Specifically, *L. hesperus* eggs were found on the petiole (except for vernonia) > flowering structures > leaf tissue > main stem (Figure 3B).

4. Discussion

Lygus hesperus and other members of the *Lygus* spp. complex feed and reproduce on a broad range of valuable crops (Scott, 1977). *Lygus* spp. are serious pests because they feed preferentially on plant reproductive tissue and cause damage with polygalacturonase, a salivary enzyme (Shackel et al., 2002). Examples of feeding damage to crops include boll damage and abscission in cotton (Mauney and Henneberry, 1978); flower “blasting”, reduced seed viability, and seed shed in alfalfa and other seed crops (Tingey and Pillemer, 1977); and fruit deformity (known as catfacing) in strawberry (Swezey et al., 2007). This feeding choice study and a no-choice plant feeding study (Naranjo and Stefanek, 2012) indicate that *L. hesperus* focus their feeding activity on the reproductive tissues of vernonia, lesquerella, and camelina.

Historically, cotton is a major summer crop grown in arid regions of the USA. *Lygus* spp. (primarily *L. hesperus*), whitefly (*Bemisia tabaci*), and pink bollworm (*Pectinophora gossypiella*) are regarded as the three most destructive cotton pests in this region. There has been substantial progress made over the past quarter century using the IPM approach to reduce cotton damage caused by whitefly and pink bollworm. This is due, in large part, to the integration of insect growth regulators for control of whitefly and *Bt* cotton for control of pink bollworm. In

addition, cultural control tactics (*e.g.*, strategic planting of cotton refuges for *Bt* resistance management) and biological control tactics (*e.g.*, better conservation of natural enemies) have played key roles in the IPM of whitefly and pink bollworm. Unfortunately, there has not been as much progress toward establishing an effective IPM program for managing *L. hesperus* in cotton and in other arid-land crops. This is due in large part to the use of broad spectrum insecticides used to control *Lygus* spp. and the nomadic behavior of this polyphagous pest.

Although there has been progress toward the IPM of two of the major cotton pests, the total cotton production acreage in California and Arizona continues to decline each year (Blake, 2015) due to lower prices, continued drought, and increased competition to produce higher valued crops. The drop in cotton acreage will require development of alternative high cash and low input crops, and therefore increased production of vernaonia, lesquerella, and camelina is highly likely in the near future. The introduction of new crops will undoubtedly change the agricultural landscape and the arthropod population dynamics. Such a drastic change may exacerbate or ameliorate crop pest problems in the region. For example, while *L. hesperus* is not as attracted to lesquerella and camelina as vernaonia, their phenologies are very different from vernaonia's. Specifically, lesquerella and camelina are planted in the fall and winter and flower in the spring. Conversely, vernaonia is planted in the late spring and blooms during the summer. As such, a significant increase in the production of lesquerella and camelina might provide an ideal overwintering habitat (refuge) for *Lygus* spp. that could later disperse into vulnerable summer crops (*e.g.*, cotton).

Trap cropping has proven to be an effective cultural control tactic for managing *Lygus* spp. in organically grown strawberry fields. For example, some strawberry producers in California dedicate two percent of their farm space for strategic plantings of alfalfa (*e.g.*, one row of alfalfa

for every 50 rows of strawberry). Alfalfa (especially when it is blooming) is much more attractive to *Lygus* spp. than strawberry (Swezey et al., 2007). This cultural control tactic lures *Lygus* spp. away from the valuable strawberry crop and provides a favorable habitat for natural enemies and pollinators (Swezey et al. 2007, 2013, 2014). Data from this study indicate that *L. hesperus* exhibits a strong attraction towards vernonia's flowering structures. **If vernonia can attract *L. hesperus* during the summer and withstand an invasion, the crop could provide valuable ecological services to the entire agroecosystem.** Specifically, the vernonia could serve as a lure (trap crop) for *L. hesperus* and as a refuge for natural enemies and pollinators.

Whether these desert-adapted crops go into widespread production remains uncertain. If so, this study reveals that *L. hesperus* might pose a threat to the production of vernonia, lesquerella and camelina and could alter the population dynamics of the entire arthropod community.

Further studies are needed to determine the extent of damage caused by *L. hesperus* on seed yield and quality relative to pest density. In addition, studies are needed to determine the effect of the phenology of each crop on the surrounding agroecosystem. Such basic agronomic information will be essential for the successful stewardship of these crops into commercial production.

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Figure captions

Figure 1. A *Lygus hesperus* feeding/oviposition choice arena containing a flowering camelina, lesquerella, and vernonia plant.

Figure 2. (A) The average ($\pm$ 95% confidence interval) number of *Lygus hesperus* observed on each host plant in the feeding preference arenas ($n = 360$ plants). **(B)** The frequency of occurrence of *L. hesperus* on the various plant structures.

Figure 3. (A) The average ($\pm$ 95% confidence interval) number of *Lygus hesperus* eggs deposited onto each host plant in the oviposition preference arenas ($n = 30$ plants). **(B)** The frequency of occurrence of *L. hesperus* eggs on the various plant structures.

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Figure 1

Figure 2

Figure 3