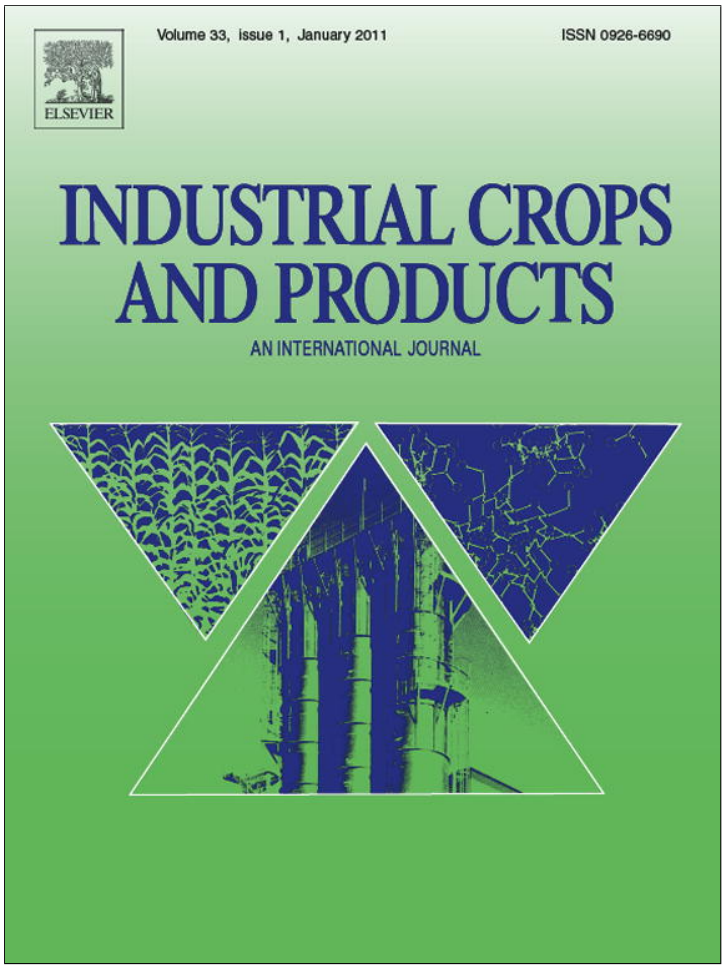

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Short communication

Feeding behavior of a potential insect pest, *Lygus hesperus*, on four new industrial crops for the arid southwestern USA☆

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ABSTRACT

Camelina (*Camelina sativa*), guayule (*Parthenium argentatum*), lesquerella (*Physaria fendleri*), and vernonia (*Centrapalus pauciflorus* [formerly *Vernonia galamensis*]) are either under limited commercial production or being developed for production in the southwestern USA. Insect pests are a potential economic threat to all these new crops, with *Lygus hesperus*, the western tarnished plant bug, among the most prominent due to its regional abundance and propensity to feed on reproductive plant tissue. The objectives of this study were to establish baseline data on the feeding behavior and potential impact of *L. hesperus* on camelina, guayule, lesquerella and vernonia. Behavioral observations of adult females and males, and nymphs of this insect were made in the laboratory. Insects spent ≈35% of their time either probing (=tasting) or feeding on various reproductive and vegetative tissues of guayule, lesquerella or vernonia, but only 20% on camelina. When insects did probe and feed they preferred reproductive tissues, primarily flowers and siliques/achenes, and there were differences in these behaviors relative to crop but not generally to insect stage or sex. Insects probed and fed more on flower tissue of guayule and vernonia compared with camelina and lesquerella, and more on siliques of lesquerella compared with achenes of vernonia. When probing and feeding on vegetative tissue, there was generally a preference for stems compared with leaves in all crops except guayule. Results show that *L. hesperus* will readily feed on the economically important tissues of all crops, and although research has shown that this feeding did not consistently affect lesquerella yield, further work will be needed to determine if such feeding poses a risk to commercial production of camelina, guayule or vernonia.

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1. Introduction

The changing landscape in southwestern USA agriculture due to urbanization, water shortages and shifting commodity demands on the world market has led to a growing interest in developing alternative crops that might open new markets and allow for more efficient use of limited natural resources (Dierig et al., 2011). Among the industrial crops being developed or under limited commercial production are camelina (*Camelina sativa*), guayule (*Parthenium argentatum*), lesquerella (*Physaria fendleri*), and vernonia (*Centrapalus pauciflorus* [formerly *Vernonia galamensis*]). Camelina is cultivated as a short season winter/spring annual in this region and can tolerate marginal soils and low agronomic input. It is enjoying renewed interest as a biofuel feedstock and a source of unsaturated omega 3 fatty acids (Budin et al., 1995; Fröhlich and Rice, 2005). Guayule is commercially produced as a perennial on a limited scale and is a source of highly desirable hypoallergenic latex (Nakayama, 2005; Ray et al., 2005). The bagasse of guayule may also represent a significant biofuel feedstock (Nakayama, 2005; Boateng et al., 2009). Lesquerella, like camelina, is cultivated as a winter/spring annual and its seeds are a rich source of hydroxy fatty acids useful in a number of industrial applications (Dierig et al., 2011). Vernonia is a short season annual rich in epoxy fatty acids that have numerous industrial uses (Carlson and Chang, 1985; Perdue et al., 1986). Seeds of lesquerella and camelina (both Brassicaceae) are contained in pod-like structures called siliques. Vernonia and guayule (both Asteraceae) 'seeds' are technically referred to as achenes.

Local surveys indicated that all these crops have several potential insect pests, the most prominent being plant bugs, *Lygus* spp. (Naranjo et al., 2008, 2011; unpubl. data). *Lygus* spp. cause economic damage to a wide array of agronomic and horticultural crops in the USA (Wheeler, 2001) by feeding primarily on reproductive plant tissue. Thus, this insect has the potential to negatively affect the production of all these new crops. The objectives of this study were to establish baseline data on the feeding behavior and potential impact of *Lygu hesperus*, the primary *Lygus* sp. in the western USA, on camelina, guayule, lesquerella and vernonia under controlled laboratory conditions.

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2. Materials and methods

2.1. Plants

All plant materials were collected from the field during their respective growing seasons at the University of Arizona, Maricopa Agricultural Center farm from 2007 to 2011. Lesquerella and camelina were cultivated as winter/spring annuals, vernonia as a summer annual and guayule as a perennial. Branches of each plant (≈15 cm in length), consisting of stems, leaves, floral buds, flowers, and siliques or achenes, were collected from the field, placed immediately in 7.6 cm long water picks (Aquapics, Floral Supply Syndicate, Camarillo, CA, USA) filled with distilled water, and returned to the laboratory.

2.2. Insects

Lygus hesperus were obtained from a laboratory colony maintained at the USDA-ARS, Maricopa, AZ, USA. The colony was reared using an artificial diet (Debolt, 1982) presented in a Parafilm M (Pechiney Plastic Packaging, Chicago, IL, USA) packet supplemented with water (cotton wicks inside water-filled glass vials) and green beans (early instar nymphs only). The colony was reared in a controlled room at 27 °C and 50% RH with a 14:10 L:D cycle. The colony was regularly infused with wild stock collected from alfalfa, cotton and lesquerella.

2.3. Behavioral observations

Individual plant branches in water picks containing 5–10 of each of the reproductive structures (buds, flowers and siliques/achenes) were pushed into a small block of floral foam (Floral Supply Syndicate, Camarillo, CA, USA) and placed within a clear cylindrical cage (21.6 cm height by 8 cm diameter) constructed from a sheet of acetate and capped with an 10 cm diameter plastic Petri dish lid. A single insect was introduced into the cage with the plant branch and observed for 30 min at room temperature (ca. 25 °C) with ambient overhead lighting. In a factorial design, three insect treatments were conducted for each of the four plant species (7–15 day old females, males, or newly emerged 5th instar nymphs of unknown sex) for a total of 12 treatments. Each treatment was replicated ca. 30 times using 6 individual observers. Preliminary observations were used to determine 14 insect behaviors: probing floral buds, flowers, siliques/achenes, leaves or stems, feeding on floral buds, flowers, siliques/achenes, leaves or stems, and four nominal behaviors (walking, resting, grooming, or ovipositing [by adult females]). Probing was characterized by brief stabs of the insect's mouthparts into plant tissue (=tasting), while feeding resulted in prolonged insertion (>5 s) of the sucking mouthparts. On guayule it was difficult to discern flowers from achenes due to the small size of both structures and non-uniform ripening. Thus, only "flowers" were observed on this plant. The Observer® V5.0 (Noldus Information Technology, Wageningen, The Netherlands) was used to record the timing and duration of each behavior over the 30 min assay period.

2.4. Analysis

Output from The Observer was compiled to estimate the total time that each insect spent performing each of the 14 behaviors over the 30 min assay period. These total times were then converted to proportions of the total 30 min devoted to each behavior. Proc Mixed (SAS Institute, Cary, NC, USA) was used to conduct two-way ANOVAs to estimate the effect of insect stage (adult female, adult male, nymph) and crop type on the proportion of time spent doing various behaviors. The first analysis pooled the 14 behaviors into 5 larger classes (probing reproductive tissue, feeding on reproductive tissue, probing vegetative tissue, feeding on vegetative tissue and nominal behaviors). A second analysis pooled probing and feeding behaviors into 5 classes (probing and/or feeding on floral buds, flowers, siliques/achenes, leaves or stems). For this second analysis, two separate two-way ANOVAs were conducted to (1) estimate the effects of insect stage and crop type for each behavior and (2) estimate the effects of stage and behavior within each crop. Tukey tests were used to compare means across treatments. Proportional data were transformed by arcsine $\sqrt{x}$ to achieve normality and homogeneity of variance prior to analyses, but untransformed data are presented.

3. Results

Insects spent the majority of their time engaged in nominal behaviors such as grooming, walking, resting and, infrequently, ovipositing (for adult females). This behavior did not vary by insect stage ($P > 0.05$) but did vary by crop type ($^{***}P < 0.001$) with insects spending more time (≈80%) in such behaviors on camelina compared with lesquerella, guayule or vernonia (≈63–65%) (Fig. 1). The interactions between crop and insect type (stage or sex) were generally not significant for nominal behavior or any feeding or probing behaviors ($P > 0.05$) indicating that insects spent proportionally the same time on each behavior for each plant regardless of developmental stage or sex. Crop type did not affect insect behavior when probing or feeding on vegetative plant parts (leaves or stems, $P > 0.05$, Fig. 1), but insect type did influence probing behavior on vegetative tissue with adults ($\bar{x}$ prop. = 0.025) engaging in this behavior more frequently than nymphs ($\bar{x}$ prop. = 0.01) ($^{***}P < 0.001$). Crop type, but not insect type influenced probing and feeding on reproductive plant tissue ($^{***}P < 0.001$) with insects on camelina spending less time engaged in these behaviors compared with the other three crops (Fig. 1). Overall, *L. hesperus*, regardless of stage or sex, spent more time probing, and especially feeding, on reproductive plant tissue when engaged in non-nominal behaviors.

Insects generally probed and fed disproportionately on different plant tissues depending on crop type (Fig. 2). Insect type ($^{***}P < 0.001$) but not crop type ($P > 0.05$) influenced the time spent probing and feeding on bud tissue, with nymphs ($\bar{x}$ prop. = 0.072) engaging in this behavior about five times more frequently than adults ($\bar{x}$ prop. = 0.015). For all remaining plant tissues there were differences with regard to crop type ($^{**}P < 0.01$–$^{***}P < 0.001$) (Fig. 2), but effects due to insect type only for stem tissue ($^{*}P < 0.05$), where nymphs ($\bar{x}$ prop. = 0.049) probed and fed less than adults ($\bar{x}$ prop. = 0.079). Insects probed and fed more on flowers of guayule and vernonia compared with camelina and lesquerella, and more on siliques of lesquerella compared with achenes of vernonia; camelina and lesquerella did not differ. For vegetative tissue, insects probed and fed more on leaves of guayule compared with all other crops and more on stems of lesquerella than guayule (Fig. 2). There were difference in probing and feeding on different plant tissues within each crop ($^{***}P < 0.001$) but insect type only affected behavior on vernonia ($^{**}P < 0.01$). Here adult females ($\bar{x}$ prop. = 0.097) preferred stems relative to adult males and nymphs ($\bar{x}$ prop. = 0.062). In general, insects probed and fed more on reproductive tissues with a preference for flowers or siliques/achenes. When probing and feeding on vegetative tissue, there was generally a preference for stems compared with leaves in all crops except guayule (Fig. 2).

4. Discussion

The results here are consistent with the known behavior of *L. hesperus* and other members of the genus as bud, flower and fruit

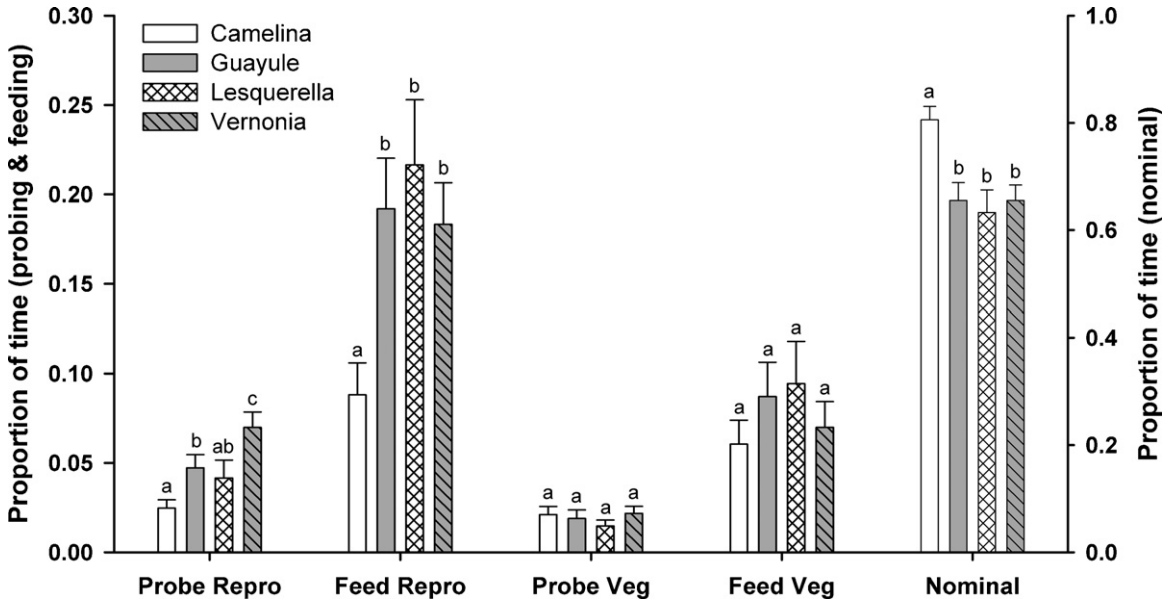
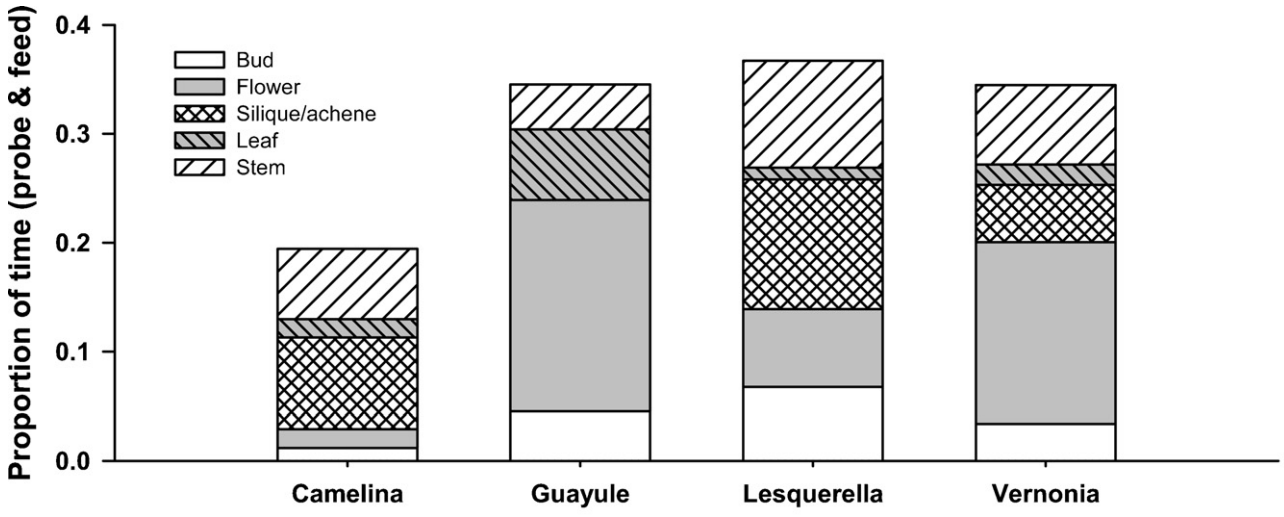


Fig. 1. Mean proportion of time (±SEM) *Lygus hesperus* were engaged in various probing, feeding and nominal behaviors on four new crops pooled over three insect stages (adult females, adult males, nymphs of unknown sex), Maricopa, AZ. Bars within a behavioral class denoted with different letters are statistically significant ($P < 0.05$, Tukey test). Repro = reproductive tissues, Veg = vegetative tissues. Note the difference in scale for nominal behaviors.

feeders (Wheeler, 2001). In key crops such as cotton in the desert southwestern USA, they feed on various reproductive tissues, but do the most damage by feeding on floral buds, causing them to abort and directly affecting cotton lint yield (Leigh et al., 1988). In this study, *L. hesperus* exhibited differential behavior depending on crop and, to a more limited extend, on developmental stage and sex. One of the most striking patterns was the low level of probing and feeding on camelina compared with the other crops. Camelina has been shown to express resistance to certain pathogens and insects (Sharma et al., 2002; Henderson et al., 2004) due to various anti-nutritional compounds (e.g., Matthäus and Zubr, 2000) and this is consistent with the patterns observed. In contrast, guayule is



	Camelina	Guayule	Lesq	Vernonia
Bud	a	a	a	a
Flower	a	b	a	b
Sil/ach	ab	-	b	a
Leaf	a	b	a	a
Stem	ab	a	b	ab

	Bud	Flower	Sil/ach	Leaf	Stem
Camelina	a	a	b	a	b
Guayule	a	b	-	a	a
Lesquerella	ab	ab	c	a	bc
Vernonia	ab	c	ab	a	b

Fig. 2. Mean proportion of time *Lygus hesperus* were engaged in probing and/or feeding behaviors on various plant tissues for four new crops pooled over three insect stages (adult females, adult males, nymphs of unknown sex), Maricopa, AZ. Tables below indicate difference for each behavior among crops and differences among behavior within each crop (letters within rows; $P < 0.05$; Tukey test).

known to contain a number of compounds with insecticidal properties (Nakayama, 2005), yet *Lygus* spp. and many other arthropods flourish and reproduce in this crop (Naranjo et al., 2008) and *L. hesperus* readily fed upon multiple tissues of this crop. Although insects consistently probed and fed on reproductive tissue there were differences in the particular tissues involved among crops. On lesquerella and camelina, *L. hesperus* exhibited more probing and feeding behavior on siliques than flowers, while the opposite was true for vernonia. It is not clear what the ultimate damage and yield consequences would be relative to the different reproductive tissues impacted by feeding. Camelina, lesquerella and vernonia are all oil-seed crops and thus damage to any of the structures contributing to seed development could be potentially important. This is less the case for guayule as the primary product, latex, is extracted from the stems and roots. Damage to reproductive tissue would probably only be important in seed and seedling production. The relatively small amount of feeding on leaves and stems in this crop by *L. hesperus* would likely be inconsequential. In a four year study, Naranjo et al. (2011) showed that *Lygus* spp. readily damage reproductive tissues of lesquerella in the field but yield effects were only noted when the crop was produced under less than ideal agronomic conditions and, thus, less able to compensate for this damage. Further work will be needed to determine if *Lygus* spp. feeding poses a risk to commercial production of camelina, guayule or vernonia.

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