

Pest Management

Effects of Agronomic Practices on *Lygus* spp. (Hemiptera: Miridae) Population Dynamics in Quinoa

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Abstract

Crop diversification often promotes farm sustainability. However, proper management of newly introduced crops is difficult when pests are unknown. Characterizing herbivore dynamics on new crops, and how they respond to agronomic factors, is crucial for integrated pest management. Here we explored factors affecting *Lygus* spp. (Hemiptera: Miridae) herbivores in quinoa crops of Washington State. Quinoa is a newly introduced crop for North America that has multiple varieties and a range of agronomic practices used for cultivation. Through arthropod surveys and discussions with growers, we determined that *Lygus* spp. was the most abundant insect herbivore and likely contributed to low quinoa yields in previous seasons. We assessed how different varieties (Pison and QQ74), irrigation regimes (present and not), and planting methods (direct-seeded and transplanted) affected *Lygus* population dynamics. *Lygus* phenology was correlated with timing of quinoa seed-set in July and August, corresponding to a period when quinoa is most susceptible to *Lygus*. Both irrigation and planting manipulations had significant effects on *Lygus* abundance. Irrigation reduced *Lygus* abundance compared with nonirrigated plots in 2018. Planting method had a significant effect on *Lygus* populations in both 2017 and 2018, but effects differed among years. Variety had a significant effect on *Lygus* abundance, but only in nonirrigated plots. Overall, our study shows that *Lygus* is a common insect herbivore in quinoa, and careful selection of variety, planting method, and irrigation regime may be key components of effective control in seasons where *Lygus* abundance is high.

Key words: integrated pest management, direct-seeding, irrigation effect, crop variety, *Lygus*

Diversification, where novel crops are introduced to agroecosystems, is a key tactic to increase farm sustainability by lengthening crop rotations, enriching soil, and breaking up pest and disease cycles (Lin 2011, Waha et al. 2018). However, when new crops are introduced, they may have unforeseen impacts on pest populations, and growers must consider how diversification affects pests that may occur in the system (Bebber et al. 2019). Moreover, agronomic practices that are used to cultivate a new crop may mediate pest population dynamics in ways that either promote or suppress pests (Pedigo and Rice 2009). Understanding interactions between crop cultivation practices and pest populations is critical for cultivating novel crops (Altieri 2004).

The distribution and abundance of crop pests is often indirectly regulated by agronomic practices such as irrigation, planting

date, and fertilization regime (Pedigo and Rice 2009, Geiger et al. 2010, Brévault and Clouvel 2019). For example, irrigation practices can increase *Lygus* bug (Hemiptera: Miridae) populations in cotton (Asiimwe et al. 2014). More direct pest control methods, like planting resistant cultivars, also can alleviate yield losses in many crops, including amaranth (Ogedegbe and Ezeh 2015), sorghum (Szczepaniec 2018), soybean (Ragsdale et al. 2011, McCarville et al. 2014), and wheat (Smith et al. 1991, Hawley et al. 2003). Understanding how management practices affect insect herbivore population dynamics is thus critical to mediating pests and for developing integrated pest management (IPM) strategies (Pedigo and Rice 2009).

Quinoa (*Chenopodium quinoa* Willd) (Caryophyllales: Amaranthaceae) is a relatively new crop to North America, where

growers are adopting it as an economically viable option for rotations due to its popularity as a health food and its resilience to drought and high salinity soils (Peterson and Murphy 2015, Hinojosa et al. 2019b). The native region of quinoa is in the Andes of Peru and Bolivia, where it has been grown for over 6,000 yr (Bruno and Whitehead 2003, Ruiz et al. 2014), though its wild tetraploid relative *Chenopodium berlandieri* Moquin-Tandon (Caryophyllales: Amaranthaceae) is found across North America and was formerly a food crop (Jarvis et al. 2017). In Peru, key quinoa pests include stem boring Lepidopterans in the families Gelechiidae and Notonectidae (Rasmussen et al. 2001). Quinoa was introduced to the United States and Canada over the last few decades, and herbivore complexes are relatively unknown given that it is not widely cultivated (Ruiz et al. 2014). A single study from Colorado showed most pests of quinoa are generalists that feed on other plants in the family Amaranthaceae like sugar beet (*Beta vulgaris* Linnaeus) (Caryophyllales: Amaranthaceae) and lambsquarter (*Chenopodium album* Linnaeus) (Caryophyllales: Amaranthaceae) (Cranshaw et al. 1990). Although herbivore observations have been made by growers in the Pacific Northwest (Robinson 1986, Oelke et al. 1992), no surveys have been conducted in the last three decades. As little overlap occurs in pests of North and South America (Peterson and Murphy 2015), it is possible that quinoa has unique herbivores in the Pacific Northwest United States. Our study thus assessed quinoa pests in eastern Washington State, an area characterized by high growing season temperatures, low precipitation, and low humidity (Hinojosa et al. 2019b), distinct climatic conditions compared with other regions where quinoa pests have been studied.

The pests of quinoa may be affected by agronomic practices. Quinoa is a broadly adapted crop that is resilient to many environmental conditions; it has excellent drought resistance, thrives in soils across a wide pH range, and tolerates light frost and late rains (Oelke et al. 1992). With its rise in popularity over the last few decades as a highly nutritious food and ability to be cultivated in a range of environments, quinoa production has spread worldwide (Ruiz et al. 2014, Bazile et al. 2016, Murphy et al. 2016). Cultivars introduced outside of their native range are exposed to different growing conditions, including climate and agronomic techniques, that can differ significantly from conditions present in its native range (Danielsen et al. 2003, Ruiz et al. 2014, Hinojosa et al. 2018). For example, although quinoa is typically tolerant to drought (Aguilar and Jacobsen 2003, Peterson and Murphy 2015), irrigation can reduce heat stress and increase yields (Walters et al. 2016, Hinojosa et al. 2019a). Yet these responses of quinoa to irrigation or heat stress can vary across cultivars that have been developed in United States breeding programs (Murphy et al. 2018, Hinojosa et al. 2019b). Recent work has also compared direct seeding to transplanting seedlings sown in a greenhouse (Ludvigson et al. 2019). Although quinoa is more commonly grown directly from seed, studies have shown that transplanted quinoa has earlier maturity and higher yields (Ludvigson et al. 2019). However, the effects of variation in such agronomic practices on pest complexes in quinoa are relatively unknown.

Here we addressed knowledge gaps in how agronomic practices affect insect herbivores in quinoa. We surveyed plots grown with various agronomic practices in eastern Washington State and spoke to growers in the area and identified the numerically dominant insect herbivores to be *Lygus* spp. We then used manipulative field experiments to test how variation in irrigation, quinoa variety, and planting method affected *Lygus* abundance across two seasons (2017 and 2018). Our objective was to determine the

main factors mediating *Lygus* abundance, which could provide the foundation to develop an IPM program for this emerging crop.

Materials and Methods

Field Trials

Our experiment was conducted at the Spillman Agronomy Farm in Pullman, WA, in both 2017 and 2018. Quinoa is grown in a relatively limited geographic scope in eastern WA; thus, our plot locations were representative of the area. We leveraged current work being conducted on research farms to run a concurrent manipulative field experiment that assessed the effects of irrigation (present or not), planting method (direct seed or transplant), and variety (Pison or QQ74) on *Lygus* in quinoa. Plots were laid out in a randomized complete block design, with irrigation applied to whole plots, planting method as the subplot, and variety as the sub-subplot (Fig. 1). There were four blocks in each of 2 yr (2017 and 2018), each of which had 8, 2.8 m² subplots (four with each planting method), with four replicates of each of variety in each subplot (Fig. 1). The dimensions of plots were based on typical practices for quinoa variety trials in eastern Washington State (Hinojosa et al. 2019b). There was a 0.7-m buffer between each sub-subplot, and a 3-m buffer between irrigated and nonirrigated main plots. This design approximates a common garden design, as insects could move between the plots. To account for potential edge effects, experimental plots were located within a larger field of quinoa.

For irrigated plots, drip irrigation was installed at the whole plot level. Drip lines were placed between each row of quinoa within plots so each plant could receive water. Water was supplied twice per week from planting until fruit development, according to Hinojosa et al. (2019b). The total water amount delivered per plot was between 230 and 250 mm in both 2017 and 2018. Nonirrigated plots were not watered. The total precipitation from the beginning of the year to the end of the field season (1 January–1 September) in 2017 was 30 cm and in 2018 was 23 cm. Two varieties of quinoa, Pison and QQ74, were chosen for heat and drought tolerance (Peterson and Murphy 2015, Hinojosa et al. 2019b). Although both varieties were shown to grow well and produce yield in the region (Hinojosa et al. 2019b), no studies have been done to determine pest resistance of these specific varieties. Two hundred and forty plants of each were sown in potting mix (Sunshine LC1 Grower Mix, Sun Gro Horticulture, Agawam,

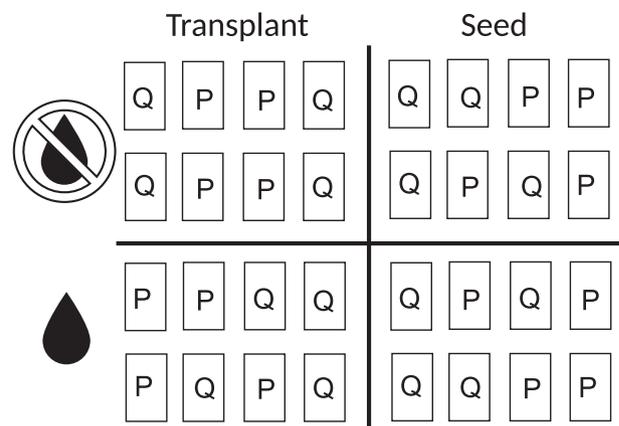


Fig. 1. Diagram showing the layout of treatments within the field plots. Irrigation was applied to whole plots, planting method (transplant or direct seed) as the subplot, and variety (P = Pison, Q = QQ74) as the sub-subplot.

MA) in 10 cm³ pots on 15 April 2017 and 14 April 2018 in greenhouses (16:8 h light:dark, 21–24°C:16–18°C light:dark). Five to 10 seeds were planted per pot and thinned to one plant 10 d after seeding. Half the plots were hand transplanted on 11 May 2017 and 24 May 2018. There were 30 transplants in each plot with 30 cm between plants, which is the standard seeding rate for quinoa variety trials in the area. The other half of the plots were directly seeded with 5 g of seed per plot on 9 May 2017 and 9 May 2018. One month after planting, all direct-seeded plots were thinned to 30 plants per plot to match the density of transplanted plots. All plots were weeded weekly. As *Lygus* feed on developing seeds, causing seed abortion and a loss of yield in seed crops (Olson and Wilson 1990), seed yield from all plots was recorded at the end of the 2017 and 2018 seasons.

Insect Monitoring

Initial visual observations were taken in the plots in June 2017 to provide baseline data on the abundance of potential pests in quinoa. Using this information, and observations by researchers conducting quinoa breeding trials in the area, we focused our study on *Lygus* bugs (*Lygus* spp.), although we observed cowpea aphid (*Aphis craccivora* Koch) (Hemiptera: Aphididae) at low density. *Lygus* was the most abundant insect herbivore and was the main potential pest of concern for researchers conducting breeding trials who hypothesized that high *Lygus* populations severely reduced quinoa yield for several seasons prior to our study (Hinojosa et al. 2019b). Sampling involved visually scanning 10 randomly selected plants per plot and counting adult *Lygus* spp. found. Nymphs were also observed but not counted due to their being difficult to reliably distinguish between other mirid nymphs present. Visual observations were used instead of sweep netting to avoid damaging the quinoa seed head for future yield calculations. We acknowledge potential limitations with using visual only observations and recognize that other methods may have altered counts. These observations were taken weekly, with randomly selected plants each week from 20 June to 28 August in 2017 and 29 June to 30 August in 2018. Quinoa plants were beginning to reach the flowering stage (anthesis) by the date of the first observations, and seed was set by the date of the last observations. We collected ≈30 additional *Lygus* using sweep nets on nonsample plants in both years at three points during the season (May, July, and August), which were identified to species using morphological characteristics to assess species present (Mueller et al. 2003).

Temperature Data

Data on daily temperature at the Spillman field site each year were obtained from Washington State University AgWeatherNet from a weather station within the field site. To calculate accumulated degree days each week, we used the rectangular method (Herms 2004):

$$DD = \left(\frac{T_{\max} + T_{\min}}{2} \right) - T_{\text{base}}$$

where DD is the measure of daily heat accumulation, T_{\max} is the average daily maximum temperature, T_{\min} is the average daily minimum temperature, and T_{base} is the lower developmental threshold of the insect. The lower developmental threshold value used for *Lygus* spp. was 10°C (Spurgeon and Cooper 2012). Accumulated degree days, the total heat accumulated from T_0 , were calculated in each year using a biofix date of 1 January (D'Auria et al. 2016). Data on the *Lygus* abundance per week were plotted based on accumulated degree days to identify periods of peak *Lygus* density within each growing season (Weninger et al. 2017). Previous studies on

quinoa phenology show that degree day models can predict time to anthesis, suggesting that these models can be used to time planting to avoid quinoa being most susceptible to insect damage when *Lygus* populations are highest (Geerts et al. 2008).

Statistical Analysis

Analyses were conducted in R v. 3.6.2 (R Core Team 2020). Generalized linear mixed models (GLMM) were used to evaluate treatment and variety effects on *Lygus* abundance. GLMMs were fit using the lme4 package (Bates et al. 2015), with *Lygus* abundance fit with a Poisson distribution for count data (Long 1997). The full GLMM used *Lygus* abundance as the dependent variable, with treatments and year as fixed effects (Bolker et al. 2009). In analyses, we investigated the interaction between year and each treatment effects (irrigation × year, planting method × year, variety × year) and a fully crossed interaction term for irrigation × planting method × variety. This allowed us to determine whether treatments were context dependent based on timing or different manipulations. *Lygus* abundance varied consistently over the course of the season (Fig. 2a), thus cumulative degree days were used as a covariate in the GLMM. Finally, since this is a repeated-measures design (10 repeated samples of 64 unique plots), we used plots as a random effect. Critical values and significance tests (*P*-values) were based on analysis of deviance test in the car package (Fox and Weisberg 2011) while the emmeans package (Lenth 2016) was used to plot estimates of the mean and estimates of the standard error based on the fitted GLMM as well as run post-hoc tests (Tukey's HSD).

Results

Pest Complex and Seasonal Distribution

We captured two species of *Lygus*, *Lygus hesperus* Knight (Western tarnished plant bug) and *Lygus elisus* Van Duzee (pale legume bug). Overall, *L. hesperus* was the numerically dominant insect herbivore species, making up ≈70% of individuals. In both years, the temporal window of *Lygus* appearance was narrow, with adult *Lygus* being observed from late June to mid-August (Fig. 2a). There was a 3-wk peak abundance from 21 July to 3 August in both years (Fig. 2a). This period coincided with the end of bloom and initial seed set (E.C.O., personal observations), which is when quinoa is most susceptible to *Lygus* damage (Schwartz and Footitt 1992). In 2017, only variety Pison produced seed, with irrigated plots procuring an average of 233.75 kg/ha and nonirrigated plots producing an average of 59.65 kg/ha. Variety QQ74 produced no seed. In 2018, none of the plots produced any measurable yield. During this period, the peak *Lygus* abundance was about 40 adults per sub-subplot. A model showing the percent adult *Lygus* counts over the season by degree days is useful regarding *Lygus* occurrence (Fig. 2b). In 2017, the *Lygus* population was highest centered around a peak at 4,200 degree days and low the rest of the season, whereas in 2018, the population was more evenly spread across the season and had a lower peak population at 4,600 degree days. Peak densities were similar across years, with approximately 50 *Lygus* per plot in 2017 and 40 per plot in 2018.

Effects of Irrigation, Planting Method, and Variety on *Lygus* Populations

Total adult *Lygus* abundance on quinoa was affected by both experimental manipulations and host variety, but effects of treatments varied among years (Table 1). Irrigated plots had reduced *Lygus* populations by 23% compared with nonirrigated plots in 2018

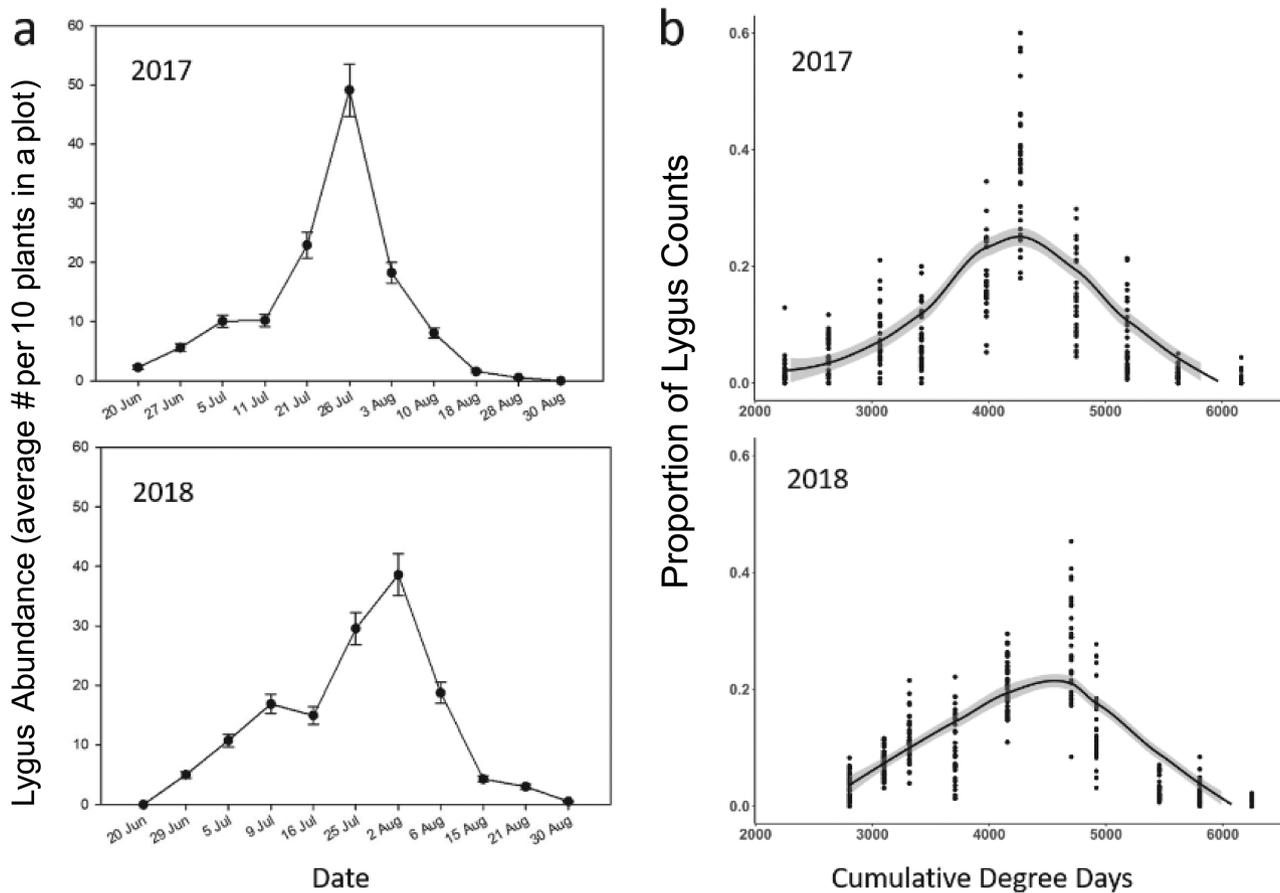


Fig. 2. (a) Average *Lygus* abundance throughout the 2017 and 2018 seasons and (b) the relative number of *Lygus* counts in each week for 2017 and 2018. Shown are observed values (points) and an estimated logistic model fit through the data.

(irrigation \times year, $\chi^2 = 5.00$, $P = 0.025$), but this difference was not observed in 2017 (Tukey's HSD, Fig. 3). Planting method had a significant effect on *Lygus* populations in both 2017 and 2018, but effects differed among years (planting method \times year, $\chi^2 = 44.23$, $P < 0.001$). In 2017 as direct-seeded plots had 23% higher *Lygus* numbers, whereas in 2018, direct-seeded plots had 40% reduced *Lygus* numbers compared with transplanted plots (Tukey's HSD, Fig. 4). Variety had a significant effect on *Lygus* abundance, but only in nonirrigated plots (irrigation \times variety, $\chi^2 = 5.91$, $P = 0.015$, Fig. 5), where QQ74 had 30% fewer *Lygus* than Pison (Tukey's HSD).

Discussion

Variation in environmental conditions affects how insects interact with their host plants (Price et al. 2012). Agronomic practices are a source of environmental variation that affects herbivores, and characterizing these effects is a key component of integrated pest management (IPM). Quinoa is gaining popularity across the world, yet relatively few studies have looked at the population dynamics and complex of pests on the crop. Here we show that in eastern Washington USA, an area potentially suited for quinoa cultivation given its arid climate and relatively moderate summer temperatures (Peterson and Murphy 2015), that *Lygus* spp. were the most numerically abundant insect herbivore on quinoa, although cowpea aphid was also present. *Lygus* dynamics were fairly consistent across years. In addition to low humidity, *Lygus* may have contributed to low quinoa yields, with little to no quinoa yield observed in either 2017

or 2018 (Hinojosa et al. 2019b). We cannot rule out that the two varieties performed poorly due to factors unrelated to *Lygus*, however. As we were unable to get reliable yield measurements, we were unable to draw more direct relationships between *Lygus* density and quinoa yields, but the high *Lygus* density and direct feeding damage observed suggests *Lygus* are a pest of quinoa.

Taking a broader perspective of quinoa pests from other regions, several major pests have been described that we did not observe in large numbers. For example, in previous studies from Colorado, major pests of quinoa were a defoliating lepidopteran, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae), the aphids *Hayhurstia atriplicis* Linnaeus (Hemiptera: Aphididae) and *Macrosiphum euphorbiae* Thomas (Hemiptera: Aphididae), and several seed feeding mirids, primarily *Lygus* (Cranshaw et al. 1990). Similarly, we show that aphids and *Lygus* bugs are the most common insect herbivores found on quinoa in eastern Washington. *Lygus* was the numerically dominant insect, with an average peak abundance of 40 adults per 10 plants. Although there are no established economic thresholds for *Lygus* in quinoa, thresholds in other crops can provide perspective on these densities. Economic thresholds for *Lygus* are 8–15 adults per 100 sweeps in cotton, depending on the cotton growth stage (Ellsworth 2001, Musser et al. 2009), 15 adults per 10 sweeps in canola (Wise and Lamb 1998), and 8–10 per sweep in alfalfa (Natwick and Lopez 2008). Although our data were based on visual observations and not sweep netting, it is likely that the peak densities observed (40 adults per plant) would correlate to values at or above these thresholds, especially for cotton and canola. The fact that we observed little to no marketable yield also suggests that

Lygus likely negatively affected quinoa growth at the densities we observed.

Lygus are highly mobile (Kelton 1955, Natwick and Lopez 2008) and feed on hundreds of different host species (Jackson et al.

Table 1. GLMM table for effects of treatments, year, and statistical interactions on *Lygus* abundance (average number of *Lygus* on 10 plants per plot)

Analysis of deviance table (type II Wald χ^2 tests)			
Factor	χ^2	df	P-value
Year	4.771	1	0.029
Irrigation	6.791	1	0.009
Planting method	1.488	1	0.22
Variety	7.36	1	0.007
Cumulative degree days	97.72	1	<0.001
Year \times irrigation	6.282	1	0.012
Year \times planting method	44.24	1	<0.001
Year \times variety	0.068	1	0.079
Irrigation \times planting method	0.458	1	0.5
Irrigation \times variety	5.919	1	0.015
Planting method \times variety	0.120	1	0.73
Irrigation \times Planting method \times variety	0.576	1	0.45

To prevent overfitting, the only interaction terms selected were year and treatment two-way interaction terms and three-way treatment interaction terms. For this repeated-measures analysis, 'plot' was treated as a random effect ($\sigma^2 = 0.027$). Statistically significant main effects and interactions shown in bold. Obs. = 640, plots = 64 (random effect), random effect variance = 0.027.

1995). In quinoa, *Lygus* feed on leaves and reproductive structures, causing seed abortion and major yield loss (Schwartz and Footitt 1992, Jackson et al. 1995). In eastern Washington, it is thought that *Lygus* adults overwinter in alfalfa fields and move to other crops in spring and summer, and *Lygus* can also feed and move into crops from weeds like common lambsquarter (Kelton 1955). In cotton, *Lygus* often move from seed and forage alfalfa to cotton (Carrière et al. 2006). *Lygus* movement between crops complicates management, as *Lygus* likely move into quinoa from other crops throughout a season. However, we observed *Lygus* was only highly abundant during a 3-wk period following the end of bloom and preceding initial seed set, and growers may only need to use insecticide sprays in this period. As quinoa is most attractive to *Lygus* during bloom and initial seed set (Schwartz and Footitt 1992), growers may limit yield loss by targeting sprays to these growth stages.

We found that nonirrigated plots had more *Lygus* than irrigated plots in 2018, but not in 2017. This lack of irrigation effect in 2017 may be due in part to higher precipitation levels: the beginning of the year through the end of the growing season (1 January–1 September) had 35% more inches of precipitation in 2017 (12.15 in) than the same period in 2018 (9.37 in). Irrigation effects on herbivores may also be mediated by plant defense chemicals. Quinoa seeds have a seed coat containing saponins, secondary metabolites that provide a chemical defense and pest deterrent (Valoy et al. 2015, Medina-Meza et al. 2016). Moreover, the saponin content of seeds increases when quinoa plants are well irrigated (Gómez-Caravaca et al. 2012, Pulvento et al. 2012). Although it remains to be tested, variation in saponin content may be at least partially responsible for why we observed the most *Lygus* in nonirrigated plots. Irrigation has also been

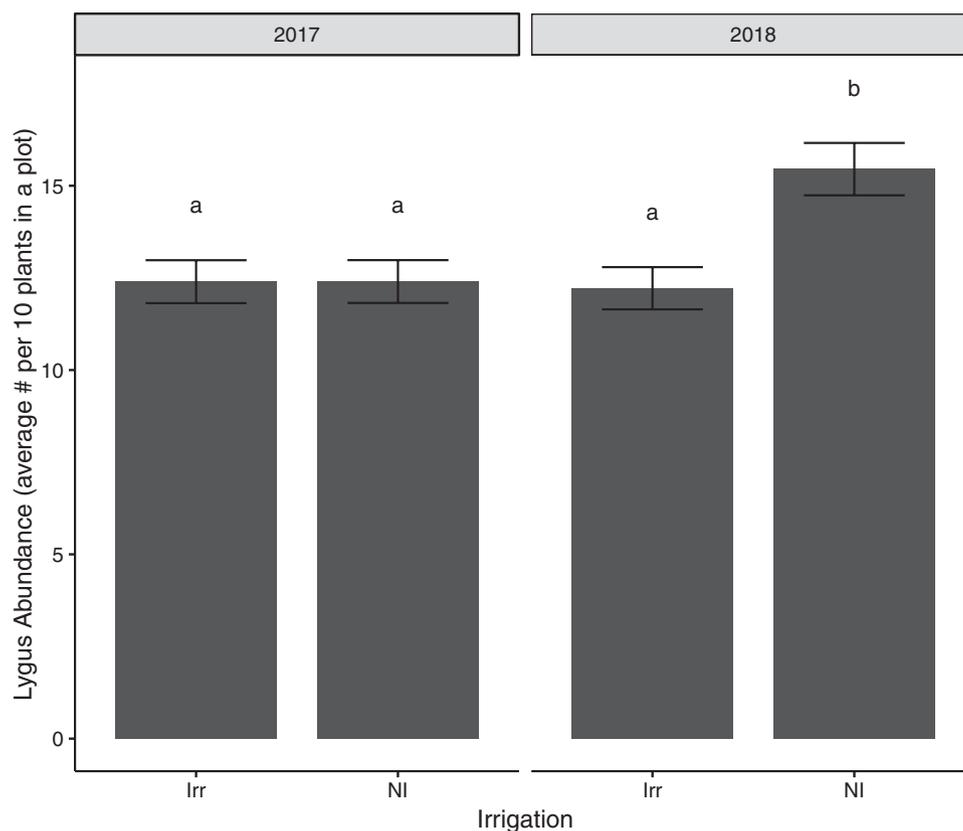


Fig. 3. Average *Lygus* abundance by irrigation in 2017 and 2018 (Irr: irrigated plots; NI: non-irrigated plots). Bar height indicates estimated means based on generalized linear mixed models, whereas error bars represent estimated standard error associated with those means. Bars not connected by the same letter are significantly different (Tukey's HSD).

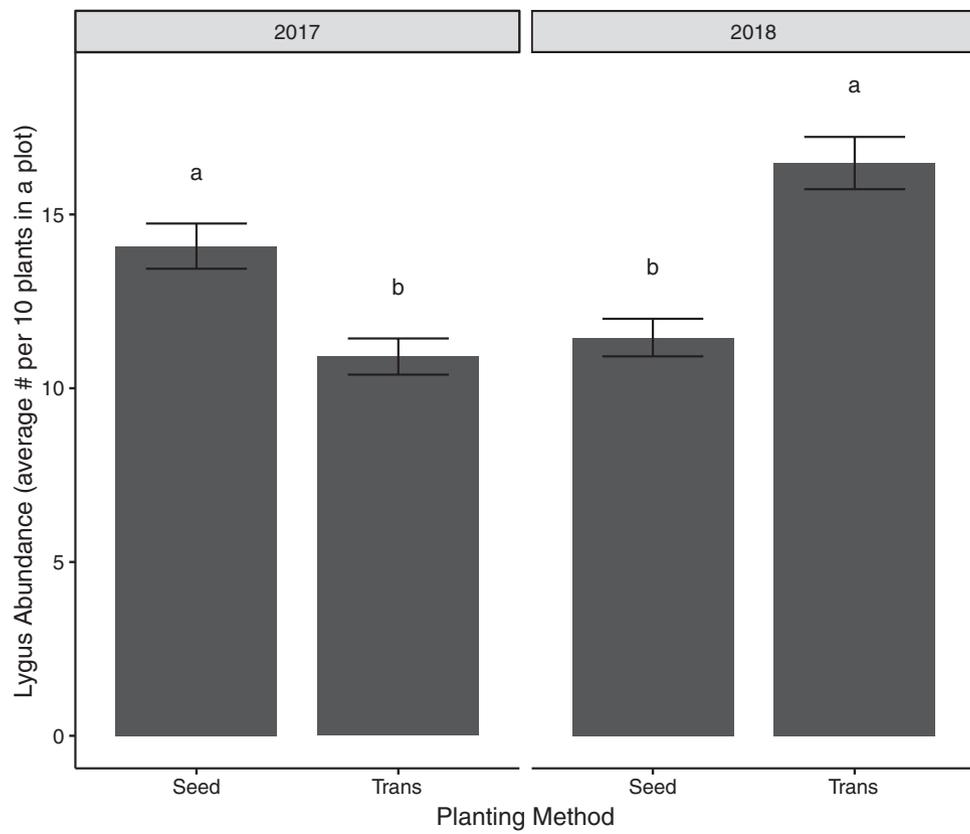


Fig. 4. Average *Lygus* abundance by planting method in 2017 and 2018 (Seed: direct seeded plots; Trans: Transplanted plots). Bar height indicates estimated means based on generalized linear mixed models, whereas error bars represent estimated standard error associated with those means. Bars not connected by the same letter are significantly different (Tukey's HSD).

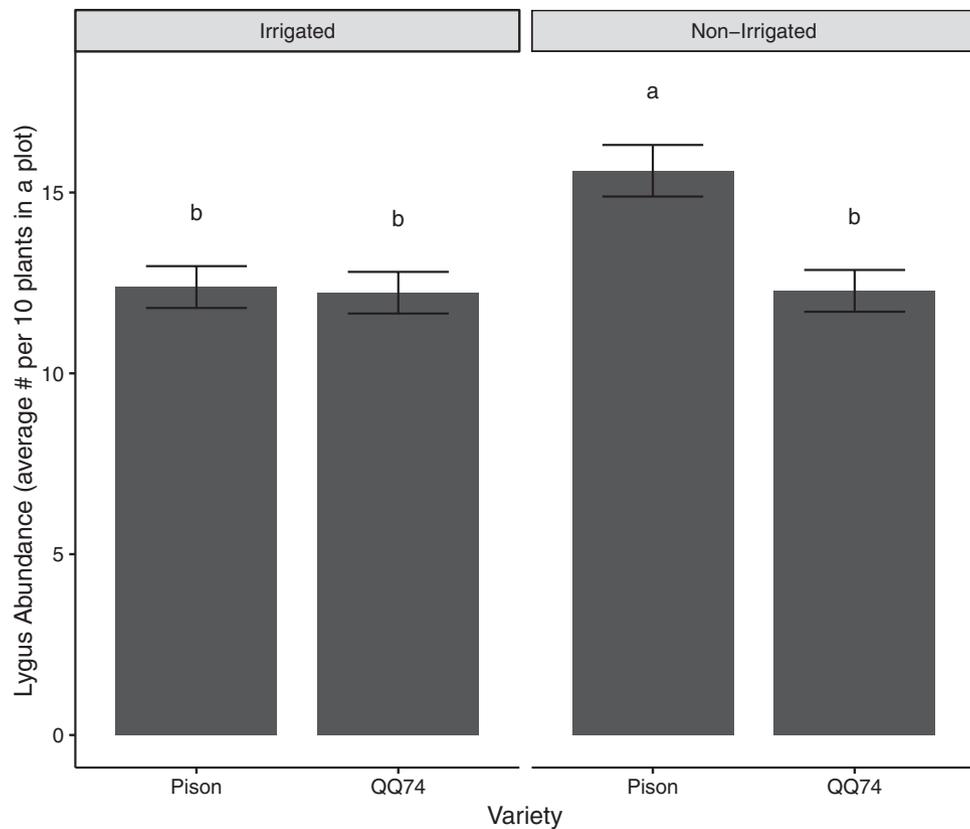


Fig. 5. Average *Lygus* abundance by variety among irrigation treatments. Bar height indicates estimated means based on generalized linear mixed models, whereas error bars represent estimated standard error associated with those means. Bars not connected by the same letter are significantly different (Tukey's HSD).

found to reduce pest populations in other crops, such as European corn borer (*Ostrinia nubilalis* Hübner) (Lepidoptera: Crambidae) in maize (Sarajlić et al. 2017).

Differences in *Lygus* populations were also observed due to planting method, but effects were not consistent across years. In 2018, direct-seeded plots had fewer *Lygus* than transplanted plots. In 2018, we observed issues with seed germination (possibly due to the use of old seeds), and direct-seeded plots matured relatively slowly compared with transplants. By the time *Lygus* had reached their peak abundances in late July, many direct-seeded plots had not reached anthesis, when quinoa is most attractive to *Lygus* as a host plant (Schwartz and Footitt 1992). Growers may thus be able to alter planting date as a cultural strategy to control *Lygus* by limiting overlap between susceptible plant life stages and periods when *Lygus* are active in quinoa fields. This is similar to other crop systems where manipulation of planting date has been shown to affect the level of insect pest-induced damage in other crop systems such as flea beetles in canola and corn earworm in maize (Doddall and Stevenson 2005, Bajwa and Kogan 2009, Rusch et al. 2010).

In agroecosystems, there are many common agronomy practices that can be used together. Finding the right combination of practices is key for producing high yielding and high-quality crops and preventing pest outbreaks. Understanding how combinations of practices work to affect pests is crucial for developing IPM strategies. Our work here shows that growers may be able to achieve effective *Lygus* management in the future by understanding the role of variety, irrigation, and planting methods on *Lygus* populations. By choosing varieties that have higher saponin content or growing particular varieties in irrigated systems to promote plant chemical defenses, growers may limit damage. Manipulation of planting date and knowledge of *Lygus* seasonal dynamics may also help limit feeding periods and peak outbreak densities. Although we know that *Lygus* is a pest of quinoa, future research should continue to focus on how *Lygus* affect yields so economic thresholds for this pest can be determined. Overall, this information can provide a foundation for effective *Lygus* control in quinoa and promote the sustainable and profitably production of this newly emerging North American crop.

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