

Bugs Carry Pollen Too: Pollination Efficiency of Plant Bug *Pseudatomoscelis seriatus* (Hemiptera: Miridae) Visiting Cotton Flowers

Authors: Garcia, Loriann, Gould, Jason, and Eubanks, Micky

Source: Florida Entomologist, 106(2) : 122-128

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.106.0209>

The BioOne Digital Library (<https://bioone.org/>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<https://bioone.org/subscribe>), the BioOne Complete Archive (<https://bioone.org/archive>), and the BioOne eBooks program offerings ESA eBook Collection (<https://bioone.org/esa-ebooks>) and CSIRO Publishing BioSelect Collection (<https://bioone.org/csiro-ebooks>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Bugs carry pollen too: pollination efficiency of plant bug *Pseudatomoscelis seriatus* (Hemiptera: Miridae) visiting cotton flowers

Loriann Garcia^{1,*}, Jason Gould², and Micky Eubanks²

Abstract

Pollinators for the fiber crop cotton are underused despite evidence that cross-pollination can increase yields. In addition, existing research largely ignores the potential of insects other than bees (Hymenoptera: Apoidea: Anthophilia) to provide pollination services for cotton. We observed plant bugs, cotton fleahoppers, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), visiting flowers of upland cotton, *Gossypium hirsutum* L. (Malvaceae) and carrying cotton pollen grains on their bodies. We hypothesized that fleahoppers might contribute to cross-pollination of cotton as they forage among flowers. To test this hypothesis, we examined *P. seriatus* flower visitation frequency, cotton pollen load, pollen analog dispersal, and cross-pollination capacity. We found that cotton fleahoppers visited 21% of flowers observed in our field site and that they deposited a pollen analog on 12.5% of the flowers accessible in a field cage. However, individual cotton fleahoppers are likely too small to carry enough grains to fertilize self-sterile cotton flowers, because field collected cotton fleahoppers carried approximately 25 pollen grains per insect, which is less than what is needed for cotton flowers to set fruit. Overall, we found that cotton fleahoppers were unable to stimulate cotton fruit development in self-sterile flowers. Nevertheless, we predict that cotton fleahoppers may contribute to cross-pollination of cotton within a community of pollinators, and that they may pollinate their wild host plants which have smaller or clustered flowers. We encourage researchers to continue to investigate non-bee pollinators in wild and agroecosystems.

Key Words: pollinators; agroecosystem; *Gossypium hirsutum*; conservation; plant-insect interactions; sustainability

Resumen

Los polinizadores para el cultivo de fibra de algodón están infrautilizados a pesar de la evidencia de que la polinización cruzada puede aumentar los rendimientos. Además, la investigación existente ignora en gran medida el potencial de los insectos distintos a las abejas (Hymenoptera: Apoidea: Anthophilia) para proporcionar servicios de polinización para el algodón. Observamos chinches de plantas, saltahojas del algodón, *Pseudatomoscelis seriatus* (Reuter) (Hemiptera: Miridae), visitando flores de algodón americano (upland), *Gossypium hirsutum* L. (Malvaceae) y llevando granos de polen de algodón sobre sus cuerpos. Presumimos que los saltahojas podrían contribuir a la polinización cruzada del algodón mientras se alimentan entre las flores. Para probar esta hipótesis, examinamos la frecuencia de visitas de flores de *P. seriatus*, la carga de polen de algodón, la dispersión de análogos de polen y la capacidad de polinización cruzada. Encontramos que los saltamontes del algodón visitaron el 21% de las flores observadas en nuestro sitio de campo y que depositaron un análogo de polen en el 12,5% de las flores accesibles en una jaula de campo. Sin embargo, es probable que los saltahojas de algodón individualmente sean demasiado pequeños para transportar suficientes granos para fertilizar las flores de algodón autoestériles, porque los saltahojas de algodón recolectados en el campo transportaban aproximadamente 25 granos de polen por insecto, que es menos de lo que se necesita para que las flores de algodón produzcan frutos. En general, encontramos que los saltahojas del algodón no pudieron estimular el desarrollo del fruto del algodón en flores autoestériles. Sin embargo, predecimos que los saltahojas del algodón pueden contribuir a la polinización cruzada del algodón dentro de una comunidad de polinizadores, y que pueden polinizar sus plantas hospedantes silvestres que tienen flores más pequeñas o agrupadas. Alentamos a los investigadores a continuar investigando polinizadores que no sean abejas en ecosistemas silvestres y agroecosistemas.

Palabras Clave: polinizadores; agroecosistema; *Gossypium hirsutum*; conservación; interacciones planta-insecto; sostenibilidad

Given insect pollinators frequently have positive impacts on crop yields, the economic benefits of conspicuous pollinators such as managed Western honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), are investigated often (Klein et al. 2007; Khalifa et al. 2021). However, increased global concern for Western honey bee health and management sustainability has brought increased attention to wild insect pollinators, such as bumble bees *Bombus* spp. Latreille (Hymenoptera: Apidae) and flies (Diptera) (Garibaldi et al. 2013; Orford et al. 2015; Rader et al. 2016). Notably, it has been found for many crops that pollinator

diversity contributes more to increasing yields than pollinator abundance (Hoehn et al. 2008; Brittain et al. 2013; Rogers et al. 2014). This is because pollination services by multiple species can be synergistic, increasing cross-pollination among flowers due to pollinator variation in preference for flower height, foraging times, and behaviors for manipulating the pollen they carry (Hoehn et al. 2008; Rogers et al. 2014). For example, Cusser et al. (2021) found that butterflies (Lepidoptera) and flies (Diptera) visited flowers spatially and temporally separated

¹Austin College, Sherman, Texas 75090, USA; Email: lgarcia@austincollege.edu (L. G.)

²Texas A&M University, College Station, Texas 77843, USA; Email: ambago@gmail.com (J. G.), m-eubanks@tam.u.edu (M. E.)

*Corresponding author; E-mail: lgarcia@austincollege.edu

from bees and ultimately contributed about one-third of pollinator mediated yield in upland cotton, *Gossypium hirsutum* L. (Malvaceae).

Overall, a better understanding of pollination services provided by diverse pollinators is expected to aid development of sustainable agricultural practices (Garratt et al. 2014; Dainese et al. 2019). This is particularly true for cotton production where pollination services remain underused despite findings that ambient pollinators contribute upwards of 20% of the final yield (Pires et al. 2014; Cusser et al. 2016, 2021; Muhammad et al. 2020). Upland cotton, *G. hirsutum*, is a major crop in the southern US; cotton was valued at \$7 billion in 2019 (USDA-ERS 2022). Pollinators may have been overlooked historically in cotton agriculture because cotton is self-pollinating and cross-pollination is not necessary for cotton to develop fruit (McGregor 1976). It also is likely that pollination by insects in cotton fields has been limited in the past due to high pesticide use (Muhammad et al. 2020). Nevertheless, pollinators may become increasingly important contributors to improving cotton production, especially now that pesticide use has been significantly reduced in many regions (Free 1993; Ward & Ward 2001; Pires et al. 2014; Cusser et al. 2016). For example, Pires et al. (2014) observed a positive correlation between bee species richness and cotton-seed yield. Additionally, manipulative studies show introduced honey bees can increase seed-cotton yield by 16.5 to 24.5% (McGregor et al. 1955; Rhodes 2002) and introduced bumble bees can increase seed-cotton yield by 17.0 to 46.6% (Saeed et al. 2012).

One area where information regarding pollination services in cotton remains sparse is the capacity for less conspicuous insects to be pollinators. For instance, in addition to Hymenoptera, Diptera, and Lepidoptera, insects such as beetles (Coleoptera), thrips (Thysanoptera), and true bugs (Hemiptera) are known to be pollinators in other systems (Ananthakrishnan 1982; Kevan & Baker 1983; Young 1986; Willemstein 1987; Wheeler 2001). The only published research the authors could locate on this subject in cotton was by Pierre and Hofs (2010); they reported that flower beetles, *Astylus atromaculatus* (Blanchard) (Coleoptera: Melyridae) were unexpected, but efficient pollinators of cotton in South Africa. *Astylus atromaculatus* frequently visited flowers and carried a similar pollen load as Western honey bees (Pierre & Hofs 2010). Although these beetles also are considered pests of cotton seeds and seedlings, they could provide some benefit to crop production once the plant reaches the flowering stage by cross-fertilizing flowers (McGregor 1976; Pierre & Hofs 2010). Cotton flowers are only open for 1 d for pollination before withering, and so opportunities to cross-pollinate with viable pollen grains are limited (Stewart et al. 2010). An improved understanding of which insects are visiting cotton flowers and are capable of cross-pollinating (i.e., Cusser et al. 2016, 2021) is needed to maximize opportunities for increasing cotton yield worldwide without increasing land-use for agriculture (Muhammad et al. 2020).

We observed that plant bugs, cotton fleahoppers, *Pseudatomoscelis seriatus* Reuter (Hemiptera: Miridae), are frequent flower visitors of cotton, *G. hirsutum* in Texas. Cotton fleahoppers are native to the southwestern US and are considered early season pests of cotton because they feed on flower buds and cause them to abscise (Stewart & Sterling 1989). Once the cotton is in its flowering stage, however, fleahoppers can be seen dusted with pollen while foraging within cotton flowers (L. G., personal observation). These fleahoppers likely are visiting flowers to feed on pollen grains (Burden et al. 1989; Wheeler 2001), but because fleahoppers are very mobile insects (Reinhard 1926), we hypothesized that fleahoppers might contribute to cross-pollination of cotton as they forage among flowers while carrying pollen grains.

Herein we present the results of our investigation into the pollination abilities of the cotton fleahopper. We quantified how frequently fleahoppers visited cotton flowers and how many cotton pollen grains

they carried on their bodies (i.e., their pollen load). We also used fluorescent powder as a pollen analog to estimate fleahopper dispersal among flowers and pollen deposition behaviors. Finally, we determined their pollination efficiency by measuring the capacity of cotton fleahoppers to pollinate self-sterile flowers.

Materials and Methods

STUDY SITE

Seeds of cotton cultivar *G. hirsutum* 'Deltapine 174 RF' were planted in a conventionally managed 11 ha field at the Texas A&M Field Laboratory (30.531730°N, 96.414343°W) in Burleson County, Texas, USA. Irrigation was applied to the field approximately every 2 wk. Glyphosate was used to control weeds but no insecticide was used at this field site. Fleahopper flower visitation, pollen load, dispersal experiments were conducted in Jul to Aug 2013, and fleahopper pollination experiments were conducted in the same field in May to Oct 2014.

FLEAHOPPER FLOWER VISITING FREQUENCY

Observations of fleahoppers at flowers were conducted using an instantaneous scan sampling technique, whereby we recorded the number of cotton fleahoppers at the moment we approached the focal sample flower (Altmann 1974). Forty randomly chosen flowers per d were observed on 4 d: 9 Jul, 25 Jul, 30 Jul, and 7 Aug 2013 (160 total flowers observed). Cotton flowers are only open and available for pollination for 1 d, approximately 9:00 AM to 5:00 PM each d (L. G., personal observation). The number of adult fleahoppers at each flower were observed at 3 time points each d (11:00 AM, 1:00 PM, and 3:00 PM) for a total of 480 observations. Up to 2 observers were able to check focal flowers quickly, within 15 min of the interval start. The weather on these sample dates were mostly sunny with daily temperatures ranging between 25 °C and 39 °C. We determined how observation date and time affected the average number of fleahoppers per flower using repeated measures ANOVA, with each flower as a subject error term (RStudio, Version 1.2.1335, RStudio, Inc., Boston, Massachusetts, USA). Data are reported as mean number of fleahoppers per flower \pm 1 standard deviation.

FLEAHOPPER POLLEN LOAD

Pollen load was counted from 32 fleahoppers collected while foraging freely in cotton flowers on 2 Aug 2013 between 3:00 PM and 5:00 PM. Weather on this date was mostly sunny with temperatures between 25 °C and 38 °C. Fleahoppers were frozen until grains could be counted under a microscope at 80 \times magnification. To prepare the samples for the microscope, we removed pollen grains from fleahoppers, dyed the grains, and mounted them to a slide to be counted. To make the dye solution, 1 g of Safranin O (Sigma-Aldrich, Saint Louis, Missouri, USA) was mixed with 100 mL of 50% EtOH (Jones 2012). First, we placed the fleahopper into a microcentrifuge tube (Fisher Scientific, Waltham, Massachusetts, USA) with 40 μ L of the dye solution. We then vortexed the sample for 1 min to dislodge pollen grains from the fleahopper body and then centrifuged the sample at 10,000 gs for 15 s. We used a pipette to transfer the liquid solution containing pollen grains 1 drop at a time to a glass microscope slide sitting on a hot plate set at 70 °C, waiting for the EtOH to evaporate between adding drops. Once all the EtOH evaporated, only dyed pollen remained on the slide. To complete slide preparation, a small amount of glycerin (Sigma-Aldrich, Saint Louis, Missouri, USA) was added, and the sample was covered with a cover slip and sealed by painting the edges of the cover slip with

clear nail polish. We used pollen grain counts to calculate the average number of pollen grains per flea hopper \pm 1 standard deviation as well as the median number of pollen grains per flea hopper.

FLEAHOPPER DISPERSAL AND POLLEN DEPOSITION

To estimate flea hopper dispersal among flowers while carrying a pollen load, we released fleahoppers dusted with a fluorescent powder (Bioquip Products Luminous Powder, Rancho Dominguez, California, USA) into field cages in our study site with flowering cotton plants. Florescent powder is an appropriate pollen analog for studying pollination efficiency in many systems (Adler & Irwin 2006) and has been used in mark-recapture studies of other mirids (Stern & Mueller 1968; Bancroft 2005). Our preliminary testing demonstrated that dusted fleahoppers can survive in the laboratory for at least 24 h and can transfer the fluorescent powder to excised cotton stigmas inside a vial (data not shown). Fleahoppers used in this experiment were collected from nearby feral fields of *Solanum elaeagnifolium* Cav. (Solanaceae), silverleaf nightshade, and maintained in the laboratory with organic green beans (*Phaseolus vulgaris* L.; Fabaceae) as a food source until use in experiments. On the morning of 2 Aug 2013, we dusted fleahoppers with fluorescent powder and at 12:00 PM we released 24 dusted fleahoppers each into ten $1.8 \times 1.8 \times 1.8$ m Lumite field cages (BioQuip, Rancho Dominguez, California, USA). Field cages contained, on average, 8 flowering cotton plants and 14.5 flowers (range: 10–20 flowers per cage). Fleahoppers were released onto the tops of plants nearest to the 4 corners of the cage (6 fleahoppers per corner). Weather on this date was mostly sunny with a temperatures between 25 °C and 38 °C. We recorded the number of flowers visited in the field cages using instantaneous scan sampling of all flowers (145 total flowers across all cages) at 2:00 PM and again at 4:00 PM. This data was used to determine the percentage of flowers in each cage visited by cotton fleahoppers at 2 and 4 h after release. At 5:00 PM we harvested all the flowers in each field cage and stored them in plastic bags. Flowers were kept refrigerated until their stigmas, petals, and anthers could be examined for fluorescent powder deposits under a dissecting microscope (Zeiss, Dublin, California, USA) using a UV light, to determine the proportion of floral organs with powder left behind by foraging fleahoppers.

POLLINATION EFFICIENCY

The capacity of cotton fleahoppers to pollinate flowers was determined by measuring seed number, seed weight, and lint weight of self-sterile flowers visited by fleahoppers carrying cotton pollen grains. Seed number, seed weight, and lint weight are known to be correlated (i.e., Saeed et al. 2012), but we chose to observe each separately given their relative usefulness for considering cotton's ecology and evolution (i.e., seed number and weight) or for cotton's economic value (i.e., lint weight). Flower treatments in this experiment were: (1) no pollination, (2) self-pollination, (3) flea hopper pollination, and (4) ambient pollination (control). On 3 dates, 5, 6, and 13 Aug 2014 flowers in our field were prepped for each treatment between 7:00 AM and 9:00 AM. We chose only the flowers opened closest to the main stem (i.e., the first fruiting position on a fruiting branch) because flowers at different positions on branches produce different sized fruits (Bednars & Roberts 2001), but otherwise flower selection was on randomly chosen plants in our plot and only 1 flower per plant was used. Plot edges were avoided. Organza bags (8 cm²) (Uline, Pleasant Prairie, Wisconsin, USA) were used for insect enclosure or exclusion. For the no pollination treatment, between 8 and 10 flowers per treatment d were emasculated by submerging the

floral organs in water for 1 min and then bagged to prevent pollinator visitation (total $N = 23$). Water destroys cotton pollen grains and is a proven emasculation tool (Burke 2002). For the self-pollination treatment, 10 flowers per treatment d were bagged to prevent ambient pollinator visits (total $N = 30$). For the flea hopper pollination treatment, 10 to 15 flowers per treatment d were emasculated as described above and once the water had dried, 2 adult fleahoppers carrying cotton pollen were added to bags enclosing the flower (total $N = 34$). Emasculation was conducted on these flowers to prevent confounding flea hopper pollination with self-pollination in our results (Macfarlane et al. 2018). These fleahoppers were collected from nearby silverleaf nightshade (*S. elaeagnifolium*) fields of the d before the experiment and fed organic green beans (*P. vulgaris*) in the laboratory until the d of the experiment (Breene et al. 1989). Before the start of the experiment, fleahoppers were placed in vials with excised cotton stigmas and were allowed forage freely on anthers for 1 h to pick up pollen prior to transfer to bagged emasculated flowers. Fleahoppers remained in the bagged flowers until 5:00 PM and we assume bags prevented flower visitation by ambient pollinators. Finally, for ambient pollination (control) we tagged and allowed 10 to 15 flowers per treatment d to self or out-cross as they would naturally with ambient pollination visitation (i.e., no bags used; total $N = 38$). At the end of each d, flowers from all treatments were placed inside the mesh bags to protect developing fruit from future herbivory. At maturity (i.e., full open bolls) we were able to collect 10 fruits from emasculated flowers, 16 fruits from flea hopper pollinated flowers, 24 fruits from self-crossed flowers, and 31 fruits from ambient pollinated flowers. Reduced target sample sizes are due to fruit abscission. Cotton naturally abscises flowers and fruit with insufficient pollination or damage (Heitholt 1993). Fruits were processed in a gin to separate lint from seeds and average seed number, seed weight, and lint weight per treatment group were compared using an ANOVA model. Gins are mechanical machines that use rotating saws to separate cotton fiber from seeds in mature fruits (i.e., "to gin cotton"). Treatment date was included as a block factor analysis of each dependent variable and treatment means comparison tests were performed using Tukey's HSD ($\alpha = 0.05$) (GraphPad Prism9, Version 9.4.0, GraphPad Software LLC, San Diego, California, USA). Means are reported \pm 1 standard error (SE).

Results

FLEAHOPPER FLOWER VISITING FREQUENCY

Fleahoppers frequently visited flowers at our study site. Overall, 1 to 3 adult fleahoppers per flower were observed during 21% (100 of 477) of all scan sampling time points. At 11:00 AM, 16.9% (27 of 160) of flowers had 1 or more fleahoppers, with an average of 0.23 ± 0.56 per flower. At 1:00 PM, 22% (35 of 159) of flowers had 1 or more fleahoppers, with an average of 0.26 ± 0.52 per flower. At 3:00 PM, 24% (38 of 158) of flowers had 1 or more fleahoppers, with an average of 0.28 ± 0.54 per flower. Observation date and time had no effect on flea hopper flower visiting frequency (date: $F = 1.142$; $df = 3$, 468; $P = 0.332$; time: $F = 0.767$; $df = 1$, 468; $P = 0.382$).

FLEAHOPPER POLLEN LOAD

The majority of fleahoppers collected (27 of 32; 85%) were carrying pollen grains (Fig. 1). On average, fleahoppers carried 25.1 ± 40.8 pollen grains per insect (median: 5.5 pollen grains). The maximum number of pollen grains observed per flea hopper was 163 pollen grains.

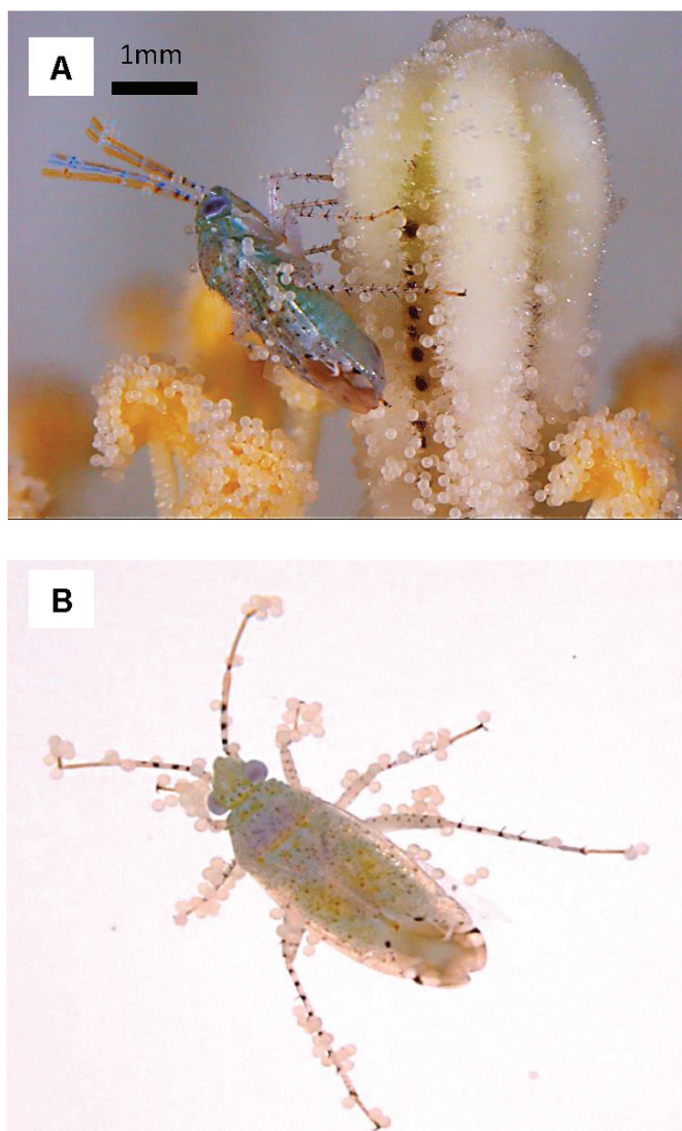


Fig. 1. Photographs of a cotton flea hopper, *Pseudatomoscelis seriatus*, taken under a dissecting microscope (A) foraging on a cotton, *Gossypium hirsutum*, stigma, and (B) carrying cotton pollen grains.

FLEAHOPPER DISPERSAL AND POLLEN DEPOSITION

At 2:00 PM, 19% (28 of 145) of flowers in the field cages had flea hopper visitors and at 4:00 PM, 23.5% (34 of 145) of flowers had flea hopper visitors. Following flea hopper visitation we observed that 12.5% (18 of 145) of flowers had fluorescent powder on their floral organs. Half (50%) of the flowers with powder had powder on stigmas, 12.5% had powder on anthers, and 37.5% had powder on petals.

POLLINATION EFFICIENCY

Seed number, seed weight, and lint yield varied among treatments (Fig. 2). Control (ambient pollination) and self-pollinated flowers developed into fruits with significantly more seeds ($F = 32.54$; $df = 3,69$; $P < 0.0001$), heavier seed weight ($F = 29.29$; $df = 3,69$; $P < 0.0001$), and higher lint weight ($F = 24.14$; $df = 3,69$; $P < 0.0001$) than emasculated and flea hopper pollinated plants. Measurements from emasculated flowers and flea hopper pollinated plants, however, did not differ. Treatment date was not a significant source of variation for any dependent

variable; the effect of treatment date was as follows: seed number: $F = 0.6643$; $df = 2,69$; $P = 0.5179$; seed weight: $F = 0.4262$; $df = 2,69$; $P = 0.6547$; lint weight: $F = 0.6148$; $df = 2,69$; $P = 0.5437$.

Discussion

Cotton fleahoppers are clearly anthophilous (flower-loving) insects. We observed adult cotton fleahoppers foraging frequently in cotton flowers and with pollen grains attached to their legs, abdomen, and antennae. In addition, cotton fleahoppers were able to disperse among flowers inside field cages after being dusted with a pollen analog. In our experiment, however, we observed that plants visited by pollen loaded cotton fleahoppers produced a similar number and mass of seeds, as well as similar lint weight as emasculated flowers. In contrast, plants able to self-cross or experience ambient pollination produced more seeds, seed weight, and lint weight; this result is similar to that observed by others (Saeed et al. 2012; Cusser et al. 2016).

Inadequate pollination likely is explained in part by our observation that cotton fleahoppers carried a pollen load much smaller than optimal for upland cotton flower fertilization. Approximately 50 viable pollen grains are needed to fertilize a full-sized cotton fruit (McGregor 1976), but cotton fleahoppers carried a median pollen load of 5.5 pollen grains. Relatively small pollen loads likely are due to the small size of this flea hopper (3.0–4.0 mm in length) and their smooth bodies. In contrast, Western honey bees are exceptionally hairy, up to 4 times larger than cotton fleahoppers (12–15 mm), and they can carry around 500 cotton pollen grains (Pierre & Hofs 2010). Moreover, furry bumblebees are up to 7 times larger than cotton fleahoppers and can carry thousands of cotton pollen grains (Berger et al. 1988). We did not observe active pollen collecting behaviors by cotton fleahoppers, unlike that typical of bees collecting food for their nests and which are reliant only on floral resources. Fleahoppers, in contrast, are also omnivores, feeding on other small insects such as lepidopteran eggs (L. G., unpublished data).

Nevertheless, we predict that the small size of cotton fleahoppers would not prevent them from being pollinators of other host plants with smaller or composite (i.e., clustered) flowers, like those in the plant family Asteraceae or Apiaceae (Willemstein 1987; Wheeler 2001). Levin et al. (1967), for example, found that plant bugs, *Lygus hesperus* Knight (Hemiptera: Miridae), were an efficient pollinator of safflower, *Carthamus tinctorius* L. (Asteraceae). In their experiment, *L. hesperus* transferred pollen to 28% of self-sterile safflowers inside field cages (Levin et al. 1967). *Carthamus tinctorius* has a composite flower head that is 15 mm in width and pollen grains that are 52 to 67 μm , approximately half the size of cotton pollen grains (Smith 1996). Like *L. hesperus*, cotton fleahoppers frequent many other host plants with much smaller flowers than cotton, such as wholly croton, *Croton capitatus* Michx. (Euphorbiaceae), silverleaf nightshade; *S. elaeagnifolium*; and horsemint, *Monarda punctata* L. (Lamiaceae) (Esquivel & Esquivel 2009). Our study, however, is the only study of cotton flea hopper pollination. How cotton fleahoppers affect the reproduction of their wild hosts is unknown.

While individual cotton fleahoppers may not be important cotton pollinators, cotton fleahoppers could contribute to cotton cross-pollination as part of a community of flower visitors (Wheeler 2001; Parys et al. 2020). In the future, a treatment group in which pollen carrying cotton fleahoppers are released onto flowers also exposed to ambient pollinators may illuminate the possibility of community contribution (see for example: Cusser et al. 2016, 2021). In addition, our results are consistent with others who found that self-crossed flowers and ambient pollinated flowers produced similar yield outcomes (Saeed

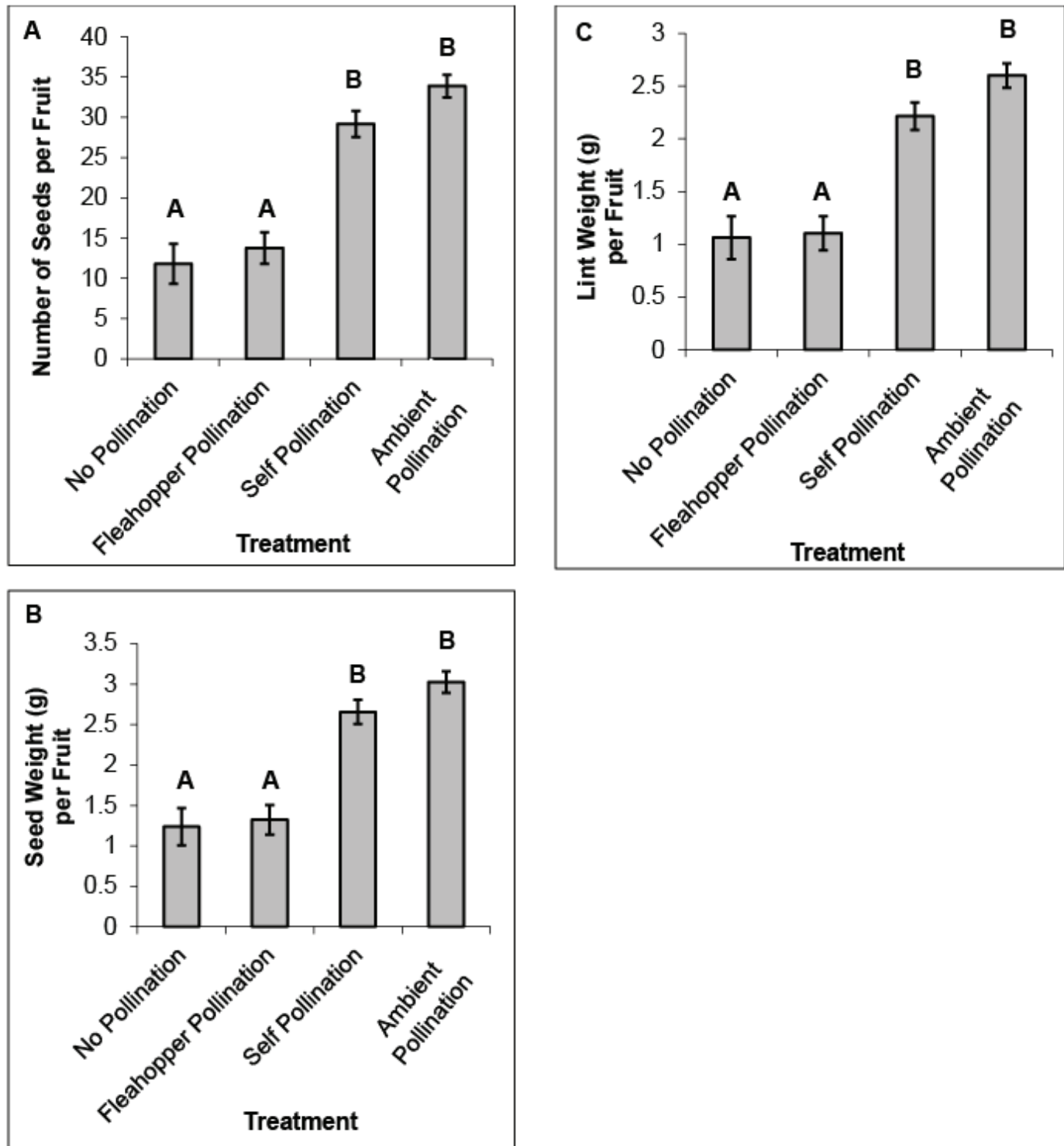


Fig. 2. Pollination of cotton, *Gossypium hirsutum*, by cotton fleahopper, *Pseudatomoscelis seriatus*: (A) number of seeds per fruit among pollination treatments; (B) seed mass per fruit among pollination treatments; (C) lint weight per fruit among pollination treatments. Bars represent treatment means and error bars represent \pm standard error of the mean. Treatment means listed with the same letter are not significantly different ($P > 0.05$).

et al 2012; Cusser et al. 2016). Other experiments, however, highlight that cotton is pollen limited. Cusser et al. (2016), for instance, found that hand pollinated flowers produced more seed-cotton weight than other treatment groups, including ambient pollination. Likewise, Saeed et al. (2012) found variety specific increases in seed-cotton with hand pollination compared to ambient pollination. These results alongside

findings of increased yield following pollinator augmentation (i.e., McGregor et al. 1955; Rhodes 2002; Saeed 2012), suggest that cotton is not only pollen limited if only able to self-cross but also pollinator limited within ambient pollinator communities (Rhodes 2002). Therefore, efforts to not only conserve diverse pollinator populations, but to increase their abundance in cotton fields may be paramount to realize

the economic benefits of pollination in cotton agroecosystems (Pires et al. 2014; Cusser et al. 2016; Muhammad et al. 2020).

Avenues of continued research in cotton pollination include: (1) documenting flower visitor diversity and pollinator efficiency in different agricultural cotton growing regions and in wild *G. hirsutum* populations (Mayes & Petrillo 2017; Parys et al. 2020); (2) observing effects of landscape management on ambient pollinator populations (Cusser et al. 2016; Dainese et al. 2019); (3) determining effects of genetically modified cotton traits on pollination services (Hofs et al. 2008; Niu et al. 2017, 2018); (4) investigating pollinators as mediators of gene flow between genetically modified cotton and wild cotton plants and the ultimate impacts of gene flow on wild cotton ecology and evolution (Vázquez-Barrios et al. 2021); (5) determining effects of cotton variety on yield outcomes for self and out-crossed flowers (Saeed et al. 2012; Stein et al. 2017); (6) observing the impacts of pollinator augmentation to cotton fields (Rhodes 2002, Saeed 2012); and (7) determining effects of nectar robbing by ants attracted to nectaries and aphids on pollinator visitation (Levan & Holway 2015). Overall, improving pollination services in cotton agriculture is expected to be a sustainable approach to increase yield without increasing the area of cultivated land (Aizen et al. 2009; Kevan et al. 2009).

To date, few authors have investigated the pollination abilities of plant bugs (Wheeler 2001). Plant bugs are inefficient pollinators for some plants (Bohart & Nye 1960; Lindsey 1984), but the notion they could be minor pollinators for others should not be disregarded without experimentation (Scott 1983; Wheeler 2001). We found that cotton fleahoppers can readily move and transfer a pollen analog to cotton stigmas and we suggest that they could contribute to cotton pollination as part of a community of pollinators, or pollinate some of their wild hosts. When studying pollinator communities, plant bugs should not be overlooked because bugs carry pollen too. Frequent flower visitors like plant bugs could contribute to the pollinator diversity that we rely on to increase crop yields.

Acknowledgments

I am grateful for the support of Eubanks laboratory members past and present for their intellectual and hands-on support for this study. My gratitude extends to Gretchen D. Jones (USDA-ARS) for methods consultation for staining cotton pollen grains for counting. This study was funded by a Texas A&M University Graduate Diversity Fellowship, a Texas A&M Regent's Fellowship, a J. H. Benedict Memorial Scholarship, USDA Agriculture and Food Research Initiative Grant 2012-67013-19346 and USDA SARE GS12-109.

References Cited

- Adler LS, Irwin RE. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. *Annals of Botany* 97: 141–150.
- Aizen MA, Garibaldi LA, Cunningham SA, Klein AM. 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany* 103: 1579–1588.
- Altmann J. 1974. Observational study of behavior: sampling methods. *Behaviour* 49: 227–266.
- Ananthakrishnan TN. 1982. Thrips and pollination biology. *Current Science* 51: 168–172.
- Bancroft JS. 2005. Dispersal and abundance of *Lygus hesperus* in field crops. *Environmental Entomology* 34: 1517–1523.
- Bednarz CW, Roberts PM. 2001. Spatial yield distribution in cotton following early-season floral bud removal. *Crop Science* 41: 1800–1808.
- Berger LA, Vaissière BE, Moffett JO, Merritt SJ. 1988. *Bombus* spp. (Hymenoptera: Apidae) as pollinators of male-sterile upland cotton on the Texas High Plains. *Environmental Entomology* 17: 789–794.
- Bohart GE, Nye WP. 1960. Insect pollinators of carrots in Utah. *Utah Agricultural Experiment Station Bulletin #214*. Utah Agricultural Experiment Station, Logan, Utah, USA.
- Breene R, Martin W, Sterling W. 1989. Rearing methods for the cotton fleahopper. *The Southwestern Entomologist* 14: 249–253.
- Brittain C, Williams N, Kremen C, Klein A-M. 2013. Synergistic effects of non-*Apis* bees and honey bees for pollination services. *Proceedings of the Royal Society B* 280: 20122767. <https://doi.org/10.1098/rspb.2012.2767>
- Burden BJ, Morgan PW, Sterling WL. 1989. Indole-acetic acid and the ethylene precursor, ACC, in the cotton fleahopper (Hemiptera: Miridae) and their role in cotton square abscission. *Annals of the Entomological Society of America* 82: 476–480.
- Burke JJ. 2002. Moisture sensitivity of cotton pollen: an emasculation tool for hybrid production. *Agronomy Journal* 94: 883–888.
- Cusser S, Haddad NM, Jha S. 2021. Unexpected functional complementarity from non-bee pollinators enhances cotton yield. *Agriculture, Ecosystems & Environment* 314: 107415. <https://doi.org/10.1016/j.agee.2021.107415>
- Cusser S, Neff JL, Jha S. 2016. Natural land cover drives pollinator abundance and richness, leading to reductions in pollen limitation in cotton agroecosystems. *Agriculture, Ecosystems and Environment* 226: 33–42.
- Dainese M, Martin EA, Aizen MA, Albrecht M, Bartomeus I, Bommarco R, Carvalho LG, Chaplin-Kramer R, Gagic V, Garibaldi LA, Ghazoul J, Grab H, Jonsson M, Karp DS, Kennedy CM, Kleijn D, Kremen C, Landis DA, Letourneau DK, Marini L, Poveda K, Rader R, Smith HG, Tscharnkte T, Andersson GKS, Badenhausser I, Baensch S, Bezerra ADM, Bianchi FJJA, Boreux V, Bretagnolle V, Caballero-Lopez B, Cavigliasso P, Četković A, Chacoff NP, Classen A, Cusser S, da Silva FD, Arjen De Groot G, Dudenhöffer JH, Ekroos J, Fijen T, Franck P, Freitas BM, Garratt MPD, Gratton C, Hipólito J, Holzschuh A, Hunt L, Iverson AL, Jha S, Keasar T, Kim TN, Kishinevsky M, Klatt BK, Klein AM, Krewenka KM, Krishnan S, Larsen AE, Lavigne C, Liere H, Maas B, Mallinger RE, Pachon EM, Martínez-Salinas A, Meehan TD, Mitchell MGE, Molina GAR, Nesper M, Nilsson L, O'Rourke ME, Peters MK, Plečaš M, Potts SG, Ramos D de L, Rosenheim JA, Rundlöf M, Rusch A, Sáez A, Scheper J, Schleuning M, Schmack JM, Sciligo AR, Seymour C, Stanley DA, Stewart R, Stout JC, Sutter L, Takada MB, Taki H, Tamburini G, Tschumi M, Viana BF, Westphal C, Willcox BK, Wratten SD, Yoshioka A, Zaragoza-Trello C, Zhang W, Zou Y, Steffan-Dewenter I. 2019. A global synthesis reveals biodiversity-mediated benefits for crop production. *Science Advances* 5. doi: 10.1126/sciadv.aax0121
- Esquivel JF, Esquivel SV. 2009. Identification of cotton fleahopper (Hemiptera: Miridae) host plants in central Texas and compendium of reported hosts in the United States. *Environmental Entomology* 38: 766–780.
- Free J. 1993. *Insect Pollination of Crops*. 2nd ed. Academic Press Inc., San Diego, California, USA.
- Garibaldi LA, Steffan-Dewenter I, Winfree R, Aizen MA, Bommarco R, Cunningham SA, Kremen C, Carvalho LG, Harder LD, Afik O, Bartomeus I, Benjamin F, Boreux V, Cariveau D, Chacoff NP, Dudenhöffer JH, Freitas BM, Ghazoul J, Greenleaf S, Hipólito J, Holzschuh A, Howlett B, Isaacs R, Javorek SK, Kennedy CM, Krewenka KM, Krishnan S, Mandelik Y, Mayfield MM, Motzke I, Munyuli T, Nault BA, Otieno M, Petersen J, Pisanty G, Potts SG, Rader R, Rickerts TH, Rundlöf M, Seymour CL, Schüepp C, Szentgyörgyi H, Taki H, Tscharnkte T, Vergara CH, Viana BF, Wanger TC, Westphal C, Williams N, Klein AM. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science* 339: 1608–1611.
- Garratt MPD, Coston DJ, Truslove CL, Lappage MG, Polce C, Dean R, Biesmeijer JC, Potts SG. 2014. The identity of crop pollinators helps target conservation for improved ecosystem services. *Biological Conservation* 169: 128–135.
- Heitholt JJ. 1993. Cotton boll retention and its relationship to lint yield. *Crop Science* 33: 486–490.
- Hoehn P, Tscharnkte T, Tylanakis JM, Steffan-Dewenter I. 2008. Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B* 275: 2283–91. doi:10.1098/rspb.2008.0405
- Hofs J-L, Schoeman AS, Pierre J. 2008. Diversity and abundance of flower-visiting insects in Bt and non-Bt cotton fields of Maputaland (KwaZulu Natal Province, South Africa). *International Journal of Tropical Insect Science* 28: 211–219.
- Jones GD. 2012. Pollen extraction from insects. *Palynology* 36: 86–109.
- Kevan PG, Baker HG. 1983. Insects as flower visitors and pollinators. *Annual Review of Entomology* 28: 407–453.
- Kevan PG, Clark EA, Thomas VG. 2009. Insect pollinators and sustainable agriculture. *American Journal of Alternative Agriculture* 5: 13–22.
- Khalifa SAM, Elshafiey EH, Shetaia AA, El-Wahed AAA, Algethami AF, Musharraf SG, Alajmi MF, Zhao C, Masry SHD, Abdel-Daim MM, Halabi MF, Kai G, al Naggar Y, Bishr M, Diab MAM, El-Seedi HR. 2021. Overview of bee pollination and its economic value for crop production. *Insects* 12: 688. doi: 10.3390/insects12080688

- Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B* 274: 303–313.
- Levan KE, Holway DA. 2015. Ant-aphid interactions increase ant floral visitation and reduce plant reproduction via decreased pollinator visitation. *Ecology* 96: 1620–1630.
- Levin M, Butler G, Rubis D. 1967. Pollination of safflower by insects other than honey bees. *Journal of Economic Entomology* 60: 1481–1482.
- Lindsey AH. 1984. Reproductive biology of Apiaceae I. Floral visitors to *Thaspium* and *Zizia* and their importance in pollination. *American Journal of Botany* 71: 375–387.
- Macfarlane RC, Davis AR, Roubik DW. 2018. Evaluating Pollinators, pp. 129–138 *In* Roubik DW [Ed.], *The Pollination of Cultivated Plants: A Compendium for Practitioners*. FAO—Food and Agriculture Organization of the United Nations, Rome, Italy.
- Mayes DM, Petrillo H. 2017. Cotton flower-visiting insects in small-scale farm fields in Mwachisompola, Zambia. *Journal of the Kansas Entomological Society* 90: 122–130.
- McGregor SE. 1976. *Insect Pollination of Cultivated Crop Plants*. USDA Handbook. USDA, Washington, DC, USA.
- McGregor SE, Rhyne C, Worley S, Todd FE. 1955. The role of honey bees in cotton pollination. *Agronomy Journal* 47: 23–25.
- Muhammad W, Ahmad M, Ahmad I. 2020. Pollination behavior of cotton crop and its management, pp. 163–175 *In* Ahmad S, Hasanuzzaman M [Eds.], *Cotton Production and Uses: Agronomy, Crop Protection, and Postharvest Technologies*, Springer, Singapore.
- Niu L, Ma W, Lei C, Jurat-Fuentes JL, Chen L. 2017. Herbicide and insect resistant Bt cotton pollen assessment finds no detrimental effects on adult honey bees. *Environmental Pollution* 230: 479–485.
- Niu L, Tian Z, Liu H, Zhou H, Ma W, Lei C, Chen L. 2018. Transgenic Bt cotton expressing Cry1Ac/Cry2Ab or Cry1Ac/EPSPS does not affect the plant bug *Adelphocoris suturalis* or the pollinating beetle *Haptoncus luteolus*. *Environmental Pollution* 234: 788–793.
- Orford KA, Vaughan IP, Memmott J. 2015. The forgotten flies: the importance of non-syrphid Diptera as pollinators. *Proceedings of the Royal Society B* 282: 20142934. doi: 10.1098/rspb.2014.2934
- Parys KA, Esquivel IL, Wright KW, Griswold T, Brewer MJ. 2020. Native pollinators (Hymenoptera: Anthophila) in cotton grown in the Gulf South, United States. *Agronomy* 10: 1–14.
- Pierre J, Hofs J-L. 2010. *Astylus atromaculatus* (Coleoptera: Melyridae): abundance and role in pollen dispersal in Bt and non-Bt cotton in South Africa. *Environmental Entomology* 39: 1523–1531.
- Pires VC, Silveira FA, Sujii ER, Torezani KRS, Rodrigues WA, Albuquerque FA, Rodrigues SMM, Salomão AN, Pires CSS. 2014. Importance of bee pollination for cotton production in conventional and organic farms in Brazil. *Journal of Pollination Ecology* 13: 151–160.
- Rader R, Bartomeus I, Garibaldi LA, Garratt MPD, Howlett BG, Winfree R, Cunningham SA, Mayfield MM, Arthur AD, Andersson GKS, Bommarco R, Brittain C, Carvalheiro LG, Chacoff NP, Entling MH, Foully B, Freitas BM, Gemmill-Herren B, Ghazoul J, Griffin SR, Gross CL, Herbertsson L, Herzog F, Hipólito J, Jaggar S, Jauker F, Klein AM, Kleijn D, Krishnan S, Lemos CQ, Lindström SAM, Mandelik Y, Monteiro VM, Nelson W, Nilsson L, Pattemore DE, Pereira NDO, Pisanty G, Potts SG, Reemer M, Rundlöf M, Sheffield CS, Scheper J, Schüepp C, Smith HG, Stanley DA, Stout JC, Szentgyörgyi H, Taki H, Vergara CH, Viana BF, Woyciechowski M. 2016. Non-bee insects are important contributors to global crop pollination. *Proceedings of the National Academy of Sciences of the USA* 113: 146–151.
- Reinhard HJ. 1926. Control of the cotton flea hopper in Texas. Circular #40. Youngblood B [Ed.]. Texas Agricultural Experiment Station, College Station, Texas, USA.
- Rhodes J. 2002. Cotton pollination by honeybees. *Australian Journal of Agricultural Research* 42: 513–518.
- Rogers SR, Tapy DR, Burrack HJ. 2014. Bee species diversity enhances productivity and stability in a perennial crop. *PLoS ONE* 9: e97307. <https://doi.org/10.1371/journal.pone.0097307>
- Saeed S, Sajjad A, Kwon YJ. 2012. Bumble bees (*Bombus terrestris*) can be the efficient pollinators of cotton crop. *Pakistan Entomologist* 31: 17–20.
- Scott DR. 1983. *Lygus hesperus* Knight (Hemiptera: Miridae) and *Daucus carota* L. (Umbelliflorae: Umbelliferae): an example of relationships between a polyphagous insect and one of its plant hosts. *Environmental Entomology* 12: 6–9.
- Smith JR. 1996. Safflower. The American Oil Chemists Society, Urbana, Illinois, USA.
- Stein K, Coulibaly D, Stenchly K, Goetze D, Porembski S, Lindner A, Konaté S, Linsenmair EK. 2017. Bee pollination increases yield quantity and quality of cash crops in Burkina Faso, West Africa. *Scientific Reports* 7: 17691. <https://doi.org/10.1038/s41598-017-17970-2>
- Stern VM, Mueller A. 1968. Techniques of marking insects with micronized fluorescent dust with especial emphasis on marking millions of *Lygus hesperus* for dispersal studies. *Journal of Economic Entomology* 61: 1232–1237.
- Stewart JM, Oosterhuis DM, Heitholt JJ, Mauney JR [Eds.]. 2010. *Physiology of Cotton*. First edition. Springer, Dordrecht, Netherlands.
- Stewart SD, Sterling WL. 1989. Susceptibility of cotton fruiting forms to insects, boll rot, and physical stress. *Journal of Economic Entomology* 82: 593–598.
- USDA-ERS. 2022. USDA Economic Research Service — Cotton Sector at a Glance. <https://www.ers.usda.gov/topics/crops/cotton-wool/cotton-sector-at-a-glance/> (last accessed 3 Mar 2023).
- Vázquez-Barrios V, Boege K, Sosa-Fuentes TG, Rojas P, Wegier A. 2021. Ongoing ecological and evolutionary consequences by the presence of transgenes in a wild cotton population. *Scientific Reports* 11: 1959. <https://doi.org/10.1038/s41598-021-81567-z>
- Ward RN, Ward KE. 2001. Impact of honey bee pollination activities on Bt cotton production in Northern Alabama. 2002 Beltwide Cotton Conferences, Atlanta, Georgia, USA, 8–12 Jan 2002.
- Wheeler AG. 2001. *Biology of the Plant Bugs*. Comstock Publishing Associates, Ithaca, New York, USA.
- Willemstein SC. 1987. *An evolutionary basis for pollination ecology*. Leiden University Press, Leiden, Netherlands.
- Young HJ. 1986. Beetle pollination of *Dieffenbachia longispatha* (Araceae). *American Journal of Botany* 73: 931–944.