Bee Contribution to Partridge Pea (Chamaecrista fasciculata) Pollination in Florida

Author(s): Joshua W. Campbell, John H. Irvin, and James D. Ellis
Source: The American Midland Naturalist, 179(1):86-93.
Published By: University of Notre Dame
https://doi.org/10.1674/0003-0031-179.1.86
URL: http://www.bioone.org/doi/full/10.1674/0003-0031-179.1.86

Am. Midl. Nat. (2018) 179:86–93

Bee Contribution to Partridge Pea (Chamaecrista fasciculata) Pollination in Florida

JOSHUA W. CAMPBELL[1], JOHN H. IRVIN and JAMES D. ELLIS

University of Florida, Entomology and Nematology Department, Steinmetz Hall, Natural Area Dr., Gainesville 32611

ABSTRACT.— Partridge pea (*Chamaecrista fasciculata*) is a common annual legume that has many important uses for humans and wildlife. During the growing season, partridge pea produces numerous flowers that are visited by many bee and wasp species. However, which bees/wasps act as the principle pollinators of partridge pea has not been explored thoroughly. We used mesh cages with different hole sizes (2.5, 0.64, 0.32, and 0.16 cm^2) to screen out different sized pollinators and collected data on pod length, seed number, and percentage of pods formed per flower number (pod set). We also collected partridge pea flower insect visitors and estimated pollen loads on their bodies to determine the amount of pollen available for pollination services. Overall, partridge peas in cages with larger holes produced longer pods and more seeds compared to those in cages with smaller holes. However, no significant difference in pod set was observed among plants grown in the various size cages. Thirteen flower visitors were observed to visit open partridge pea flowers. The most common insect visitors were *Bombus* spp., *Agapostemon splendens*, and *Apis mellifera*. *Apis mellifera* contained significantly less pollen on its body compared to that on all other common bees. Likely pollinators of partridge pea in Florida are larger bees such as *Xylocopa* and *Bombus* and possibly *Agapostemon splendens*, a smaller halictid bee.

Introduction

Partridge pea, *Chamaecrista fasciculata* (Michx.) Greene, is a herbaceous annual legume that inhabits prairies, open fields, or cut-overs throughout the eastern half of the United States (USDA-NRCS). In north-central Florida, flowering begins early summer and continues until late fall. Partridge pea flowers are perfect and contain ten stamens, nine of which are covered by a cucullus (rigid upper petal) that may act as a pollinator guide (Wolfe and Estes, 1992). Individual flowers bloom for approximately 1 d and plants may produce hundreds of flowers during a growing season. Once flowers have been pollinated, bean pods form and seeds mature within them. Partridge pea has been shown to have many beneficial uses, including ethnobotanical (Sturtevant, 1954; Hamel and Chiltoskey, 1975), aiding in the augmentation of mole cricket parasitoids (Portman *et al.*, 2010), possession of antimicrobial properties (Borchardt *et al.*, 2008), and as a main food source for bobwhite quail and other wildlife which eat the seeds (Morris, 2012).

Flowers do not produce nectar but do produce large quantities of pollen that attract many bee species. Nectar is produced through extrafloral nectaries on the stems below the flowers. This may protect the plant from insect herbivores by attracting ants which aid in defense (Kelly, 1986) though they do not defend against seed predators (Ruhren, 2003).

Partridge pea is dependent on pollen transfer and this is primarily accomplished by bees (Robertson, 1890; Thorp and Estes, 1975). Pollen is released through terminal pores in the anthers and may require "milking" or buzz pollination by large bodied bees (Thorp and Estes, 1975; Fenster, 1995). Bumble bees (*Bombus* spp.), the eastern carpenter bee (*Xylocopa virginica* L.), several species of halictids, the honey bee (*Apis mellifera* L.), the mining bee

[1] Corresponding author: e-mail: joshuacampbell@ufl.edu

(*Svastra atripes* Cresson), and the leaf-cutter bee (*Megachile brevis* Say) were found to be common visitors of partridge pea flowers (Robertson, 1890; Hardin *et al.*, 1972; Thorp and Estes, 1975; Wolfe and Estes, 1992; Williams *et al.*, 2015). Thorp and Estes (1975) suspected halictids are probable pollen thieves and do not contribute to partridge pea pollination given they are incapable of buzz pollination. We collected data on flower visitors to partridge pea to gain a better understanding of partridge pea pollination in Florida. We tested the hypothesis that only larger bodied bees can serve as pollinators through the use of different sized cages that excluded different sized pollinators.

METHODS

FIELD SITES

Individual seeds of partridge pea (Wildflower Seed and Plant Growers Association-Crescent City, Florida) were planted in pots in May 2016 at the University of Florida Bee Biology Unit (29.626835N, –82.356050W). Once seeds sprouted and plants reached ~25 cm, six to eight plants were transferred to three individual outside plots (1.12 m^2) in July 2016. A fourth plot, established from seed the year before, was also used. All plants within these plots were used for the cage study. Numerous (30+) honey bee colonies are kept at this site and were located within 50 m of the partridge pea plots. Additionally, surveys of insect visitors to partridge pea flowers were conducted at the University of Florida (UF) Natural Teaching Laboratory (NATL) (29.634029N, –82.368893W) where a large (~2–3 ha) wild population of partridge pea was established. No known managed honey bee colonies were found within NATL.

CAGE STUDY

Hardware cloths of different mesh sizes were fashioned into cylindrical cages (35.6 cm length and 20.3 cm diameter, Fig. 1). A square window was cut out so that a side panel could be installed over it and held in place with magnets (Fig. 1). A circular hole was cut into one end of the cage and fitted with a fine nylon mesh to allow the distal ends of branches to be inserted into the cage without damaging them.

Cages were placed at the UF Bee Unit before the plants bloomed and remained on the plant until it stopped flowering. We used cages made from four different mesh sizes in order to exclude pollinators of different sizes. The four mesh sizes were: (1) large- 2.5 cm^2, (2) medium- 0.64 cm^2, (3) small- 0.32 cm^2, and (4) control- 0.16 cm^2. The large mesh size was designed to allow all pollinators access to partridge pea flowers, whereas the control excluded all but extremely small pollinators from the partridge pea flowers. The two intermediate sized meshes excluded correspondingly larger pollinators that could otherwise access the partridge pea flowers.

Flower counts for each cage were taken daily from June–November, 2016 for the established plot and July–November, 2016 for the three plots planted from seed. Seed pods were collected once they matured and turned brown. Seed pod lengths were measured with calipers and the number of seeds (mature and immature) per pod counted. A seed was considered mature if it appeared similar to seeds used for our plantings and measured a minimum of 3 mm diameter. In addition to mature seeds, some seeds were very small (<1 mm diameter) and were considered to be immature.

VIDEO CAMERA USE

At the beginning of the experiment, Sony HD Handycams (Sony HDR-CX240) were used to film the caged plants. The camcorders were used to confirm pollinator visits to the flowers

FIG. 1.—An example of a cylindrical small cage (35.6 cm length and 20.3 cm diameter) containing a partridge pea stem that was used for this study. A nylon mesh was affixed to the bottom of the mesh cage so that plant stems could be enclosed within cages without breaking them. Small black circles on the pictured cage are magnets holding a panel. The panel could be removed and mature seed pods gathered without having to take the entire stem out of cage

within the cages and that cages (except the smallest cage size) would not impede pollinator visitation. From 15–20 June 2016, 28 h of video were recorded on eight different days. All video was recorded between 0800–1600. The recordings were made various times each day [*e.g.*, early morning (0800–1000), midday (1000–1400), and afternoon (1400–1600)] so that all visiting pollinators could be filmed.

INSECT COLLECTION AND POLLEN REMOVAL

Pollinators were collected from partridge pea at UF NATL in order to avoid affecting bee behavior or population in the experimental plots. NATL is located approximately 3 km away from the site of the cage study. On 10 different days during the summer of 2016, a

researcher walked slowly for approximately 30 min within areas that contained flowering partridge pea and collected flower visitors. Insects that were observed visiting partridge pea flowers were captured with a sweep net and placed in individual vials. Insects were stored in a freezer for future identification and pollen analyses. We measured distance between the outer edge of the tegula of commonly collected (3+) insect visitors to provide a relative measure of body size. Three bees of each species were measured and averaged.

Pollen was washed off each insect and the amount of partridge pea pollen quantified. To harvest pollen, each insect was placed in a micro-centrifuge tube containing ethyl alcohol (95%). The tube was agitated with a vortex and inspected to see that all pollen was washed from its body. The vial also was washed with alcohol to remove any remaining pollen, which was added to the micro-centrifuge tube. Washing insects with ethyl alcohol has been shown to be a sufficient technique for removing pollen (Jones, 2012). The remaining alcohol-pollen solution was centrifuged for 10 min at 3000 rpm. The supernatant was decanted down to 0.5 ml. To count pollen the sample was dissociated with the vortex and a 10 *u*l aliquot (N = 3) was taken and placed on a hemocytometer slide. Pollen from the sample was compared to a reference slide of partridge pea pollen for identification purposes. Partridge pea pollen and foreign pollen were recorded and pollen counts were averaged over the three samples. Prior to the pollen wash, pollen from the corbicula of honey bees and bumble bees was removed and placed in separate vials as this pollen would not be available for partridge pea pollination. Due to relatively low amounts of pollen recovered from honey bee bodies, pollen from honey bee corbicula was spread on a slide and a minimum of 300 pollen grains were counted to determine percentage of partridge pea pollen. To obtain the number of pollen grains per sample the following formula was used: (# of pollen grains counted/ hemocytometer volume) multiplied by the total volume of sample.

STATISTICAL ANALYSIS

A Kruskal-Wallis one-way nonparametric analysis of variance test was used to determine if pod length, immature and mature seed numbers per pod, total number of flowers per cage size, and the amount of partridge pea pollen found on bee bodies varied by cage mesh size (Statistix 9.0 Analytical Software, Tallahassee, Florida, U.S.A.). Kruskal-Wallis tests were used after square-root and logarithmic transformation applied to the data failed to eliminate heteroscedasticity. We used a GLM to conduct one-way ANOVAs with cage sizes as an independent variable and percent of pods formed per bloomed flowers and total pods formed as dependent variables. A linear regression was performed to determine the correlation between pod length and number of mature seeds per pod.

RESULTS

CAGE STUDY

Overall, 258 seed pods were gathered from the caged plants. Mature seed pods were collected between August and November, 2016. Pod length was significantly longer for plants in medium and large cages than in small and control cages ($z = 2.638$, $P < 0.0001$) (Table 1). The numbers of mature seeds per pod in large cages were significantly different from those in small cages but not the other cage sizes ($z = 2.638$, $P < 0.0001$) (Table 1). The numbers of immature seeds per pod were significantly less in the large and small cages compared to medium cages but not the control cages ($z = 2.638$, $P < 0.0001$) (Table 1). The number of pods formed per flower (pod set) was not significantly different among the treatments ($F = 1.87$, $df = 3, 18$, $P = 0.18$) nor were the total number of flowers that bloomed

TABLE 1.—Average pod length (mm), immature seeds/pod, mature seeds/pod, % pod set, and mean # of flowers/cage (±SE) from plants in each size of cage. Sample size (N) is the number of seed pods collected from plants in the different sized cages. Within a column, different letters indicate a significant difference among the treatment groups (P = 0.05)

Cage Size	Pod Length (mm)	Immature Seeds/Pod	Mature Seeds/Pod	% Pods/Flower (Pod Set)	Mean # Flowers/Cage
Control (N = 35)	27.9 (2.0)^{b}	3.2 (0.7)^{ab}	1.7 (0.6)^{ab}	7.9 (4.3)^{a}	122 (53.5)^{a}
Small (N = 68)	30.6 (1.4)^{b}	1.4 (0.4)^{b}	0.9 (0.3)^{b}	16.7 (5.4)^{a}	109 (30.9)^{a}
Medium (N = 39)	41.5 (2.3)^{a}	5.1 (0.9)^{a}	1.6 (0.4)^{ab}	11.3 (5.3)^{a}	76.4 (19.1)^{a}
Large (N = 116)	39.3 (1.2)^{a}	2.3 (0.3)^{b}	4.0 (0.5)^{a}	23.6 (5.8)^{a}	83.6 (21.8)^{a}

within the cages ($z = 0.6057$, P = 0.9) (Table 1). The total number of pods and total number of seeds formed among the cage treatments were higher in cages with larger holes but were not significantly different (F = 1.91, df = 3, 17, P = 0.17; $z = 0.467$, P = 0.9, respectively). A regression analysis showed the number of mature seeds contained within a pod is weakly positively correlated with seed pod length ($R^2 = 0.36$, P < 0.0001, Fig. 2).

INSECT COLLECTION AND POLLEN ANALYSIS

Overall, we collected 78 individuals, representing 13 species of potential pollinators of various sizes, from partridge pea flowers in NATL (Table 2). The most common visitors were *Bombus* spp., *A. mellifera*, and *A. splendens*. All individuals collected were female. Individual *X. micans* contained significantly more pollen on their bodies than did *A. splendens* or *A. mellifera* (Table 3). *Apis mellifera* bodies contained significantly less pollen (without including corbicular pollen) than all other bee species. All corbicula from *A. mellifera* contained partridge pea pollen averaging 89% partridge pea pollen. Based on our video and observations, pollinator activity was highest during the early morning hours and largely

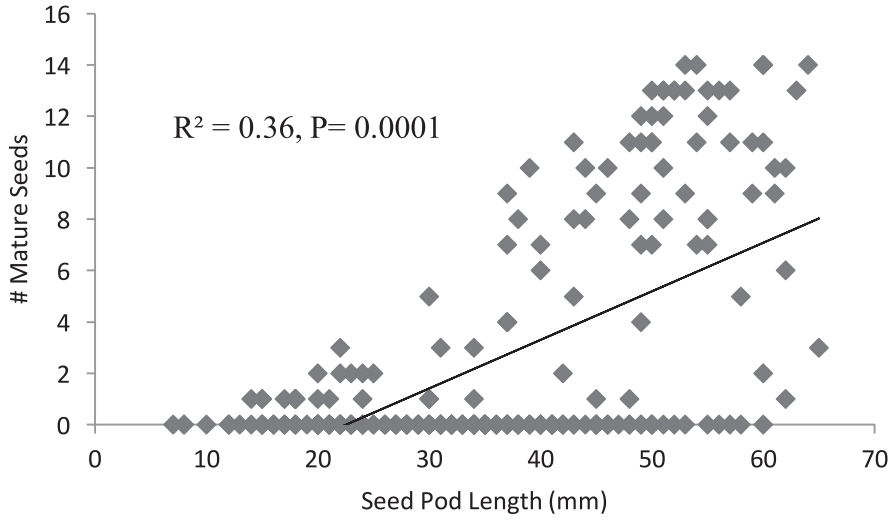

FIG. 2.—Regression of the # of mature seeds per pod and seed pod length

TABLE 2.—List of all flower visitors collected in NATL and observed visiting partridge pea flowers within our cage treatments located at the UF Bee Unit and average outer tegula distance (mm) (±SE) of partridge pea flower visitors collected on the 10 sampling days during summer 2016. Outer tegula distance is only included for species in which a minimum of three specimens were captured

		# Collected	# Observed on Video	Outer Tegula Distance (mm)
Bees				
Apidae	*Apis mellifera*	13	5	3.9 (0.03)
	Bombus impatiens	16		5.8 (0.1)
	Bombus pensylvanicus	8	4	6.5 (0.4)
	Nomada fervida	1		
	Svastra atripes	7		4.9 (0.2)
	Xylocopa micans	6	2	7.0 (0.04)
Halictidae	*Agapostemon splendens*	16	23	3.6 (0.02)
	Augochlora pura	1		
	Lasioglossum apopkensis	1		
	Lasioglossum reticulatum	1		
	Lasioglossum tarponense	3		1.4 (0.07)
	Lasioglossum spp.		4	
Megachilidae	*Megachile mendica*	4		4.5 (0.08)
	Megachile spp.		3	
Wasps				
Mutillidae	*Dasymutilla* sp.	1		
Vespidae	*Polistes exlamans*	1		

stopped by late morning/early afternoon. Despite this abrupt change in flower visitation rate, extrafloral nectaries continued to attract bees and wasps throughout the day. Our video showed bees of body sizes corresponding to the custom mesh size of the cages were not impeded or discouraged from entering the cages. Oftentimes bees landed on the mesh and walked through, prior to flying to the flower. At other times they simply flew right through the cage mesh. Overall 41 individual bees were observed entering the cages (Table 2). No bees were observed entering our control cages and only *A. splendens* and *Lasioglossum* spp. entered the small cage size. *Agapostemon splendens* and *A. mellifera* both utilized flowers within medium and large cages. *Bombus penslyvanicus* was only observed entering the large cage size.

DISCUSSION

We observed many different sizes of bees and wasps within the experimental plots and at NATL. Presumably, insect visitation to flowers stopped by late morning/early afternoon because pollen quantities had been depleted and flowers contained few rewards. Extrafloral

TABLE 3.—Mean (±SE) # of partridge pea pollen grains per bee body. Mean numbers with the same letter are not significantly different (P = 0.05)

Agapostemon splendens (N = 16)	139,271 (78,742)[b]
Apis mellifera (N = 13)	2,821 (809)[c]
Bombus impatiens (N = 16)	210,104 (76,609)[ab]
Bombus pensylvanicus (N = 8)	121,905 (42,792)[ab]
Svastra atripes (N = 7)	295,952 (215,841)[ab]
Xylocopa micans (N = 6)	856,389 (256,508)[a]

nectaries continued to attract insects throughout the day, likely to attract ants for purposes of protection from herbivores. Despite some researchers suggesting buzz pollination must occur in order to obtain high seed set (Thorp and Estes, 1975), our medium sized cage excluded bumble bees and other large bees but plants contained within these cages exhibited similar seed set and pod length as those in the large cage. The most common bee that our video documented or we visually observed entering the medium sized cage was *A. splendens*; a bee that does not buzz pollinate. Among the metallic green halictids, *Agapostemon* is relatively large. Although *A. splendens* pollen loads did differ significantly compared to those of *X. micans*, pollen loads were not significantly different from those of *Bombus* spp.; one of the main proposed pollinators of partridge pea (*e.g.*, Thorp and Estes, 1975). Therefore, it is possible that not all halictids act as pollen thieves and *Agapostemon* may contribute to pollination services of partridge pea.

Wolfe and Estes (1992) found if the cucullus is removed, fruit set is highly reduced in the field but not if flowers were hand pollinated, suggesting the cucullus may act a pollinator guide. Despite this finding, the flower style is not enclosed and could be pollinated by any direct contact with an insect that has partridge pea pollen on its body. Based on our pollen counts, numerous bee species could be potential pollen vectors. Although *Bombus* spp. (and others) may buzz pollinate the apically opening anthers (Thorpe and Estes, 1975), this behavior may not be necessary in order to obtain substantial partridge pea pollen and for successful pollination to occur.

Pod set per flower was not significantly different among plants housed in the various sized cages. However, numerous pods formed during the later portion of the study (late Oct/Nov) were shriveled and contained small seeds with few-to-no mature seeds. This may be been caused by other environmental factors that can cause a decrease in seed/pod production (Lee and Bazzaz, 1982; Fenster, 1991). Despite no significant differences in total number of flowers per cage, pod set, and total seeds, plants housed in the large cages produced many more pods and mature seeds compared to control cages suggesting the importance of insect pollen vectors. Our control cages would have excluded most bees and other commonly known pollinators. However, smaller insects such as small *Lasioglossum* bees and thrips could still have been able to visit flowers within the control cages. Thrip pollination has been documented in other plant species (Eliyahu *et al.,* 2015) and could be one explanation for some mature seeds forming in the control cages. Self-pollination can also occur in partridge pea (Etterson, 2004) and could have also contributed to some of the pollination success in controls and other cage sizes.

Our cage study occurred within an apiary where numerous (30+) honey bee colonies were located. Despite this large number of nearby honey bee colonies, honey bees were not a common partridge pea visitor. Within NATL our flower visitor collection site, 13 honey bees were collected while they visited open partridge pea flowers. Honey bees contained significantly less pollen available on their bodies for pollination services than any other bee species collected. That said, the majority of their corbicular pollen was comprised of partridge pea. Honey bees may be adept at collecting partridge pea pollen but very efficient at transferring it to their corbicula compared to other bees. Therefore, honey bees could be considered a pollen thief of partridge pea.

CONCLUSIONS

Overall, we documented numerous bees and wasps visiting partridge pea flowers. All of the common native bee species visiting partridge pea flowers were found to be carrying large quantities of partridge pea pollen. Therefore, buzz pollination (or "milking" of the anthers)

may not be required for bee access to partridge pea pollen or for successful pollination of the plant species.

Acknowledgments.—The authors acknowledge funding provided by the United States Department of Agriculture -National Institute for Food and Agriculture Specialty Crop Research Initiative (http://nifa.usda.gov), from project 2012-01534: Developing Sustainable Pollination Strategies for U.S Specialty Crops and Syngenta as part of the *Operation Pollinator* initiative. We thank Rob Jean for bee identification and Anna Moskovitz for lab assistance.

LITERATURE CITED

BORCHARDT, J. R., D. L. WYSE, C. C. SHEAFFER, K. L. KAUPPI, R. G. FULCHER, N. J. EHLKE, D. D. BIESBOER, AND R. F. BEY. 2008. Antimicrobial activity of native and naturalized plants of Minnesota and Wisconsin. *J. Med. Plants*, **2**:98–110.

ELIYAHU, D., A.C. MCCALL, M. LAUCK, A. TRAKHTENBROT, AND J.L. BRONSTEIN. 2015. Minute pollinators: the role of thrips (Thysanoptera) as pollinators of point leaf manzanita, *Arctostaphylos pungens* (Ericaceae). *J. Poll. Ecol.*, **16**, 64–71

ETTERSON, J.R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution*, **58**, 1446–1458.

FENSTER, C. B. 1995. Mirror image flowers and their effect on outcrossing rate in *Chamaecrista fasciculata* (Leguminosae). *Am. J. Bot.*, **82**:46–50.

HAMEL, P. B. AND M. U. CHILTOSKEY. 1975. Cherokee Plants and Their Uses – A 400 Year History. Herald Publishing Co., Sylva, N.C. 71 p.

HARDIN, J. W., G. DOERKSEN, D. HERNDON, M. HOBSON, AND F. THOMAS. 1972. Pollination ecology and floral biology of four weedy genera in Southern Oklahoma. *Southwest Nat.*, **16**:403–412.

JONES, G.D. 2012. Pollen extraction from insects. *Palynology*, **36**:86–109.

KELLY, C. A. 1986. Extrafloral nectaries: ants, herbivores and fecundity in *Cassia fasciculata*. *Oecologia*, **69**:600–605.

LEE, T. D. AND F. A. BAZZAZ. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology*, **63**:1363–1373.

MORRIS, J.B. 2012. Showy partridge pea [*Chamaecrista fasciculata* (Michx.) Greene] with potential for cultivation as a multi-functional species in the United States. *Genet. Resour. Crop. Evol.*, **59**:1577–1581.

PORTMAN, S.L., J. H. FRANK, R. MCSORLEY, AND N. C. LEPPLA. 2010. Nectar-seeking and host-seeking by *Larra bicolor* (Hymenoptera: Crabronidae), a parasitoid of *Scapteriscus* mole crickets (Orthoptera: Gryllotalpidae). *Environ. Entomol.*, **39**:939–943.

ROBERTSON, C. 1890. Flowers and insects. V. *Bot. Gaz.*, **15**:199–204.

RUHREN, S. 2003. Seed predators are undeterred by nectar-feeding ants on *Chamaecrista nicititans* (Caesalpineaceae). *Plant Ecol.*, **166**:189–198.

STURTEVANT, W. 1954. The Mikasuki Seminole: Medical beliefs and practices. Dissertation, Yale University, New Haven, C.T.

THORP, R. W. AND J. R. ESTES. 1975. Intrafloral behavior of bees on flowers of *Cassia fasciculata*. *J. Kans. Entomol. Soc.*, **48**:175–184.

USDA-NRCS Plant guide: Partridge pea *Chamaecrista fasciculata* (Michx.) Greene. (https://plants.usda.gov/plantguide/pdf/pg_chfa2.pdf)

WILLIAMS, N. M., K. L. WARD, N. POPE, R. ISAACS, J. WILSON, E. A. MAY, J. ELLIS, J. DANIELS, A. PENCE, K. ULLMANN, AND J. PETERS. 2015. Native wildflower plantings support wild bee abundance and diversity in agricultural landscapes across the United States. *Ecol. Appl.*, **25**:2119–2131.

WOLFE, A.D. AND J. R. ESTES. 1992. Pollination and the function of floral parts in *Chamaecrista fasciculata* (Fabaceae). *Am. J. Bot.*, **79**:314–317.

SUBMITTED 4 APRIL 2017 ACCEPTED 14 SEPTEMBER 2017