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Author(s): David M. Lowenstein and Emily S. Minor
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Effect of Number of *Bombus impatiens* (Hymenoptera: Apidae) Visits on Eggplant Yield

DAVID M. LOWENSTEIN[1,2] AND EMILY S. MINOR[1,3]

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ABSTRACT Eggplant (*Solanum melongena* L.) is a crop with perfect flowers capable of self-pollination. Insect pollination enhances fruit set, but little is known about how pollination success varies by number of visits from bumble bees. To quantify the efficiency of bumble bees at pollinating eggplants, we allowed 1, 2, 6, and 12 bumble bees (*Bombus impatiens* Cresson) to visit eggplant flowers and compared percentage of flowers that set fruit, fruit weight, and seed set after 3 wk. We compared yield from these visit numbers to eggplant flowers that were left open for unlimited visitation. Eggplant flowers set the most fruit from open-pollination and 12 visits. Larger, seedier fruits were formed in open-pollinated flowers. However, fruit characteristics in the 12 visit treatment were similar to lower visitation frequencies. We confirm *B. impatiens* as an efficient eggplant pollinator and document the greatest benefit from 12 bumble bee visits and open-pollinated flowers. To maintain effective eggplant pollination, local conditions must be conducive for bumble bee colony establishment and repeated pollen foraging trips.

KEY WORDS eggplant, *Solanum melongena*, pollination, bumble bee

Insect pollinators enhance fruit set of many vegetable and fruit crops (Klein et al. 2007). Eggplant is among the crops that benefits from insect pollination (Amoako and Yeboah-Gyan 1991, Free 1993). Eggplant pollen is dispersed through vibration of the anthers by wind or insect pollination. Few visitors are attracted to eggplant flowers, because the flowers do not produce nectar. Furthermore, many bees are inefficient at eggplant pollination, although honey bees and several wild bee species are known to increase yield (Levin 1989, Free 1993, Gemmill-Herren and Ochieng 2008). Several species of bumble bees are used for commercial pollination of Solanaceous crops (Velthius and van Doorn 2006), and bumble bees are one of the few pollinators in the United States that effectively buzz pollinate eggplant flowers. Studies report up to a 22% increase in eggplant fruit set by adding bumble bees to greenhouses (Abak et al. 1995, Abak et al. 2000). While pollination efficiency has been measured in several Solanaceous crops (Free 1993), no study has yet quantified the effect of visitation frequency on eggplant fruit characteristics.

In the eastern and central United States, eggplant flowers are primarily visited by *B. impatiens* Cresson 1863, a common, generalist bumble bee (Matteson and Langellotto 2009). The inability of other pollinators in our study system to buzz pollinate prevents suitable vibration of eggplant anthers by species not in the genus *Bombus*. Reported declines in certain bumble bee species (Cameron et al. 2011) will make each individual pollinator visit especially crucial in outdoor areas where few bumble bees are present.

We investigated eggplant fruit and seed set from sequential bumble bee visits to individual flowers until the occurrence of the 12th visitor. We hypothesized that a greater number of visits to an eggplant flower would lead to a heavier and seedier fruit. As bees visited flowers, we recorded the cumulative duration of each visit, as this may reflect the amount of pollen transfer in other Solanaceous crops (Jarlan et al. 1997, Palma et al. 2008) as well as fruit characteristics in other crops (Gingras et al. 1999). Information about bumble bees' contribution to pollination will be useful to greenhouse and outdoor growers who are considering supplementing mechanical or self-pollination with insect pollinators.

Materials and Methods

We used 70 eggplant plants (*Solanum melongena* var. 'Black Beauty') grown from seed and transplanted into two-gallon pots. We standardized the floral display such that each plant had a maximum of three open flowers during the experiment. Bees had access to eggplant flowers within 48 h after anthesis. All data were collected on sunny to partly sunny days in the hours of 10 a.m.–3 p.m. between 26 August and 11 September 2013. This experiment occurred outside of the University of Illinois at Chicago greenhouse, located ~2 km from the city center of Chicago, IL.

Visits to Flowers. *B. impatiens* is the dominant bumble bee in the two largest eastern US cities (Matteson et al. 2008, Lowenstein et al. 2014). We restricted

[1] Department of Biological Sciences, University of Illinois at Chicago, 845 W. Taylor St. (M/C 066), Chicago, IL 60607.
[2] Corresponding author, e-mail: dlowen2@uic.edu.
[3] Institute for Environmental Science and Policy, University of Illinois at Chicago, 2121 West Taylor Street (MC 673), Chicago, IL 60612.

floral visits to *B. impatiens* due to the concern that larger bumble bee species in the vicinity, including *Bombus fervidus* (F. 1798), would cause nominal differences in pollen deposition. We randomly assigned treatments to flowers with a single visit ($N = 21$), two visits, ($N = 25$), six visits ($N = 27$), and 12 visits ($N = 24$). Multiple treatments could be assigned to different flowers on the same plant. All subsequent visits to a flower occurred within 30 min from the initial visit. We also included an open pollinated treatment ($N = 8$), in which pollinators had unrestricted access to eggplant flowers for 3 d. An observer recorded the duration of each visit, in seconds, for all treatments, except open-pollinated. Flowers were covered with a fine mesh bag shortly before opening, so bumble bees had access to 1–2-d-old unvisited flowers. Flowers were covered again after data collection until each flower was no longer receptive to bee visits. Open pollinated treatments remained unbagged after the experiment. We removed any new flowers that formed after experimental flowers were visited by bumble bees. Three weeks after the initial visit to a flower, we collected data on percentage of flowers in each treatment that set fruit, fruit weight, and the number of seeds from each developing fruit.

Data Analysis. We performed a G-test of independence to evaluate if the proportion of blooms that matured into an eggplant fruit ("fruit set") varied by number of bumble bee visits (1, 2, 6, 12, open-pollination). We applied a Bonferroni correction to compensate for type I error when making multiple post hoc analyses. To analyze if the number of bumble bee visits affected seed set and fruit weight, we used ANOVA with plant as a blocking factor. We used Tukey's HSD to investigate pairwise comparisons in the presence of significant differences for the ANOVA. We used Pearson's correlation coefficient (r) to identify if fruit weight and seed set were correlated. Finally, we generated the cumulative duration of visits to each flower by summing the duration of each visit and related this measure to fruit weight and seed number using linear regression.

Results

Eggplant fruits matured in all treatments (Fig. 1), and there was a significant difference in fruit production by number of visits to a flower ($G = 32.65$, $df = 4$, $P < 0.01$). Post hoc tests identified that open-pollinated flowers set significantly more fruit compared with flowers with 1, 2, 6, or 12 visitors ($G = 20.44$, $df = 1$, $P < 0.01$). Additionally, flowers visited by 12 bees set significantly more fruit than flowers visited by 2 bees ($G = 14.4$, $df = 1$, $P = 0.01$). Seed set was significantly greater in open-pollinated eggplants than all other treatments ($F = 16.3$, $df = 4$, $P = 0.03$; Fig. 2). Of the plants that produced fruit, eggplant fruit weight significantly differed between treatments ($F = 10.5$, $df = 4$, $P = 0.01$), with no significant effect by block ($F = 0.26$, $df = 26$, $P = 0.90$). Post hoc tests indicated the only significant difference in fruit weight occurred between open-pollinated flowers and flowers with 1–12 bumble bee visits (Fig. 2). The number of seeds per fruit was

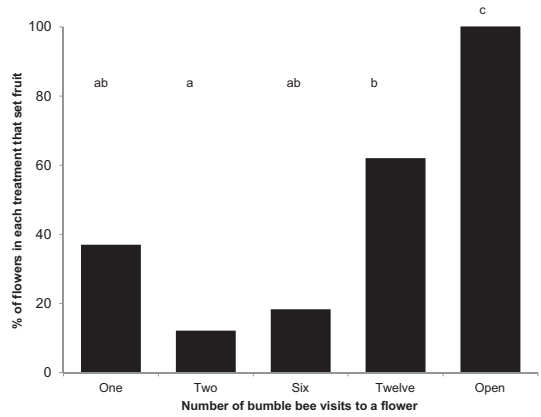

Fig. 1. Percentage of flowers in each treatment that matured into eggplant fruits. Open-pollinated eggplants shown in comparison with treatments where number of bumble bee visits were measured. Bars followed by different letters indicate significant differences ($P < 0.05$; G-test of independence).

significantly correlated with fruit weight ($r = 0.85$, $P < 0.01$).

On average, a bumble bee visit to a flower (±SD) lasted 10.6 ± 7.4 s. There was no significant difference between the duration of the first visit and any subsequent visits ($F = 0.56$, $df = 11$, $P = 0.34$). There was no significant effect of cumulative duration of visits on eggplant weight ($R^2 = 0.04$, $P = 0.91$) or on eggplant seed set ($R^2 = 0.05$, $P = 0.96$).

Discussion

Our results indicated that open-pollinated flowers set fruit in 100% of instances, and that eggplant may require at least 12 bumble bee visits for optimal fruit set. We determined that a single bumble bee visit to an eggplant flower is capable, though not always successful, of producing a fruit. Greater numbers of pollinator visits enhance yield in other vegetable crops dependent on insect pollination (Gingras et al. 1999, Artz et al. 2011). In eggplant, fruit set increased from 37 to 62% when 12 bumble bees rather than a single bee visited a flower. However, flowers with 12 visits did not produce significantly larger or seedier fruits than flowers with fewer visitors. The significantly higher fruit weight in open-pollinated flowers suggests that eggplant fruits can grow to a more marketable size after more than 12 bumble bee visits or through a combination of over 12 visits and wind or self-pollination.

As bees visited the same flower for the second and later visits, any reduction in available pollen did not change the duration of their visits. It is possible that some of the bees making the 2nd, 6th, or 12th visit collected pollen superficially on the surface of the anthers without buzz-pollinating, which might explain why these treatments did not result in significantly more fruit set, seed set, or heavier fruits than the single visit treatment. On average, bumble bees visit up to five

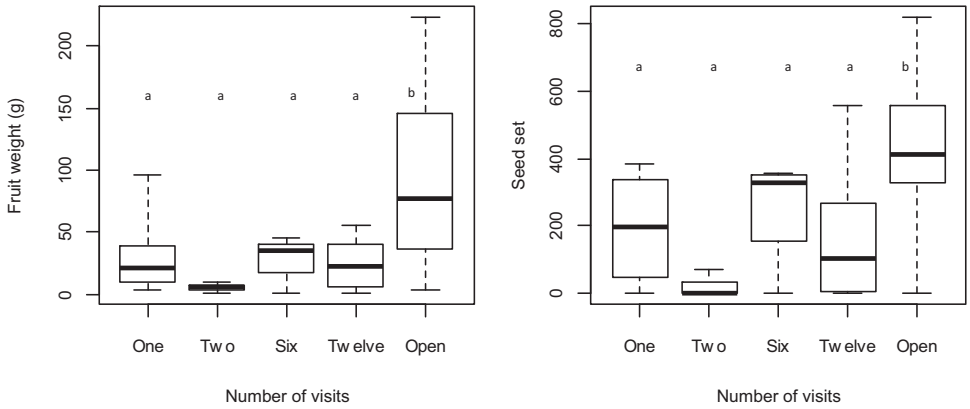

Fig. 2. Box and whisker plot of eggplant fruit weight and seed set arranged by the number of bumble bee visits to flowers. The box extends from the 25th to 75th percentiles, with the bold horizontal line within the box indicating the median value. The whiskers (dashed lines) beyond the box extend to the minimum and maximum values. Bars followed by different letters indicate significant differences ($P < 0.05$; ANOVA; Tukey's HSD test).

eggplant flowers per minute (Abak et al. 1995), and bees could have spent an insufficient length of time on flowers for buzz pollination. In our study, *B. impatiens* remained on flowers for less time compared with another Solanaceous crop, tomato (Palma et al. 2008).

Insect pollination is known to enhance eggplant fruit production (Free 1993, Abak et al. 1995), but we documented that many pollinator visits are required for a noticeable increase in seed set or fruit weight. Several factors could be responsible for the similarity in seed set and weight between treatments. Flowers with an intermediate number of visits (2, 6, or 12) could have had greater fruit set if the visits were spaced over a longer time period, allowing the anthers to release more pollen. However, waiting longer for subsequent visits to outdoor eggplants could make it difficult to separate the effects of insects from wind pollination, which has comparable seed set to insect pollination (Gemmill-Herren and Ochieng 2008). Rao (1980) suggests 2-d-old eggplant stigmas have maximum receptivity, and delaying bumble bee visits may have further increased seed set and fruit weight. Eggplants' heterostyly affects fruit set, and longer styled flowers are more likely to set fruit (Sekara and Bieniasz 2008). Because we did not record style length, we cannot rule out the effects of heterostyly as a confounding variable in this experiment. Finally, using multiple flowers on the same plant could have caused the plant to allocate resources in a manner that reduced fruit size and number of seeds per fruit. Heterostyly, assignment of treatments, or stigma receptivity altered the relative effects of each treatment. We suggest that future researchers may want to allow more time between subsequent visits to test whether this increases fruit or seed set in the treatments with intermediate numbers of visits.

Greater than 50% fruit set after 12 visits is encouraging news for outdoor eggplant growers in a region where bumble bees are in decline. For growers considering using solely mechanical vibration to pollinate flowers, we demonstrate that allowing more bumble bee visits to flowers will lead to greater yield. Bees quickly learn to collect pollen on consecutive foraging bouts (Raine and Chittka 2006) and will visit eggplant flowers multiple times. While we did not find a significant difference in seed set or fruit set between treatments with 6 or 12 visits, we identified a pattern of increasing fruit set in flowers with 12 visits and open-pollinated flowers. Further work is needed to analyze the effect of multiple visits on fruit characteristics when visits occur over a longer period of time and with a single treatment flower per plant.

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