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BENEFITS OF CROSS-POLLINATION IN VEGETABLE SOYBEAN EDAMAME

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Abstract—Dependence on cross-pollination varies widely among wild and cultivated plant species. Even among crops that are less dependent on outcrossing, such as soybean (*Glycine max* L.), cross-pollination can improve fruit quality and commercial value. There is a growing body of literature regarding the role of insect pollination in soybean; however, there is a knowledge gap on the intersection between the reproductive system of soybean and its pollination ecology. To address this gap, we first sought to characterize the reproductive system of vegetable soybean (edamame) in terms of benefits and reliance on outcrossing using three traditional experimental pollination scenarios in field conditions: open pollination, automatic selfing (pollinator-exclusion), and hand cross-pollination (controlled crossing). We also tested whether proximity to floral supplements planted on one edge of the field affected its reproductive outputs, and surveyed the floral visitors of the crop. Overall, we found a significant increase in fruit weight among open-pollinated plants compared to those in the automatic selfing treatment, with this effect accentuated with proximity to the flower strip. Despite open pollinated flowers having 30% higher flower abortions rates compared to automatic selfing, the average number of developed seeds per fruit was similar among these treatments, with open-pollination having a greater proportion of commercial grade-A fruits. Additionally, grade-A fruits in open-pollination and hand cross-pollination treatments were similar in weight, both of which were significantly heavier than those in the automatic selfing treatment. Although edamame can automatically self, our results suggest that reproductive outputs including fruit weight and number of commercial grade-A fruits are positively affected by cross-pollination and proximity to floral supplements.

Keywords—Reproductive system, flower strip, plant mating system, floral supplementation, crop

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most widely cultivated crops worldwide with an economic value expected to reach upwards of $200 billion by 2025 (Voora et al. 2020). A record high 87 million acres were planted in the US in 2021, and soybean acreage is expected to increase by 35% in 2026 (Lee et al. 2016). Despite its economic importance, our understanding of the reproductive biology (e.g., pollination, ability to outcross) of soybean is unresolved. Soybean is considered an autogamous crop, with anther dehiscence and stigma receptivity occurring before anthesis (Free 1993; Roubik 1995; Carlson & Lersten 2004). Its floral morphology is also thought to enforce self-pollination, with a relatively low natural outcrossing rate (i.e., 0.04-6.3%; Ray et al. 2003). However, research suggests that soybeans modestly benefit from outcrossing (Klein et al. 2007), and that yield dependency on pollinators varies along latitudes (Cunha et al. 2023) and varieties (Erickson 1975; Garibaldi et al. 2021).

Cross-pollination in non-cleistogamous soybean varieties is facilitated by insects (Erickson et al. 1978; Rust et al. 1980; Milfont et al. 2013). Many insect groups have been observed visiting soybean flowers, including Hymenoptera, Diptera, Coleoptera and Thysanoptera (Rust et al. 1980; Ray et al. 2003; Zhao et al. 2009; Santos et al. 2013; Gill & O'Neal 2015; Levenson et al. 2022). To date, managed bees including honey bees (*Apis mellifera*) and alfalfa leafcutting bees (*Megachile rotundata*) are the most commonly studied pollinators in the soybean agroecosystem, with more recent studies focusing on wild bees (Gill & O'Neal 2015; Levenson et al. 2022). Many of these studies found that visitation by managed bees was associated with increases in the reproductive output of the crop, including yield, seed-set, and fruit weight (Palmer et al. 2001; Garibaldi et al. 2021), presumably through the deposition of outcrossed pollen or improved pollen distribution on the stigmatic surface (Blettler et al. 2018). Specifically, it was found that in 64% of soybean pollination studies, bee visitation was associated with increased yields (Garibaldi et al. 2021), while Cunha et al. (2023) identified this contribution to change along latitudinal gradients. Further, a recent study found that enhancing floral diversification through the addition of flower strips adjacent to soybean fields increased seed weight and flower visitations by wild and managed bees (Levenson et al. 2022).

From a biological and evolutionary perspective, it is not unexpected that soybean benefits from outcrossing more than historically assumed (reviewed in Oguro et al. 2019; Garibaldi et al. 2021). For example, soybean flowers have anatomical floral features that are characteristic of entomophilous flowers, such as well-developed nectaries, UV nectar guides, a tongue channel, tongue guides, and a nectary ridge with stomates that emit volatiles (Palmer et al. 2001). Research has also shown that soybean's annual and perennial wild relatives have an outcrossing rate of up to 19%, indicating that outcrossing is evolutionarily present in the lineage (Fujita et al. 1997).

The majority of soybean pollination research has largely been pollinator-focused, exploring bee community composition (Gill & O'Neal 2015; Wheelock & O'Neal 2016; Levenson et al. 2022), landscape ecology (Santos et al. 2013; Zelaya et al. 2018; Huais et al. 2020), or changes in reproductive output through field-based pollinator-exclusion (i.e., plants are bagged to prevent visitation from floral visitors) and/or pollinator-addition experiments (i.e., pollinators were added to caged enclosures of soybean plants) (Garibaldi et al. 2021; Santone et al. 2022). However, to our knowledge the reproductive system of soybeans has not been thoroughly studied. For instance, traditional reproductive tests, such as those that use controlled hand-pollination, have not been formally used to evaluate the reproductive outputs of soybean. Controlled pollination is a technique commonly used to quantify self-compatibility and/or the relative success of self-pollination versus outcrossing (Kearns & Inouye 1993). This is also a method commonly used by soybean breeders to develop hybrid crosses with desired genetic traits (Talukdar & Shivakumar 2012) and can provide insights into the timing of stigma receptivity. In this work, we aim to implement these methods on soybeans to better characterize its reproductive system and biology.

Vegetable soybean (edamame; *Glycine max*) was used as the model crop for this study. Edamame is a soybean variety widely cultivated in Asia which is also gaining popularity in the USA as a speciality vegetable crop, providing farmers with high net-returns and overall increased consumer consumption (Binder 2010). Although edamame and grain soybean are the same species, edamame differs from the latter in that its fruits are harvested immaturely (growth stage R6) as a food-grade soybean (Li et al. 2023). Edamame is sold fresh with weight and seed-set being important determinants of its market value, and only 2- and 3-seeded fruit (i.e., commercial grade-A) considered marketable (Konovsky et al. 2020). Given that edamame and grain soybean are the same species, the knowledge gained from this study is applicable to both. Using edamame as a model, the objectives of this study are: i) to assess the reproductive strategy of vegetable soybean (edamame) through the analysis of reproductive output metrics under the three pollination treatments (i.e., hand cross-pollination, automatic selfing, and open pollination), and ii) to evaluate reproductive output effects of the plant's proximity to flower strips.

MATERIALS AND METHODS

FIELD SET-UP

Field experiments were carried out at the University of Maryland Central Maryland Research and Education Center (CMREC) (39.012674, -76.825628) during the 2021 growing season (May-September). The average high and low temperatures during the experiment were 30.5 °C and 19.7 °C, with a total rainfall of 25.9cm. The experimental setup consisted of one 15 m x 15 m plot with 16 rows of edamame, (*Glycine max;* variety Midori Giant, Wannamaker Seeds, Inc), interspaced at 76 cm, and planted with a seeding rate of 175,000/ha. This variety was selected because it is a common commercial variety used in the USA Mid-Atlantic (Li et al. 2023; Wyenandt & Vuuren 2022). The plot size was defined taking into consideration available space and funding, and the field sizes usually used by edamame farmers in the region. Edamame was planted on May 21, 2021 and the experiment was conducted between July 5, 2021 and August 31, 2021, which spanned different plant developmental stages, including initial bloom (growth stages R1 to R2) and harvestable fruit production (growth stage R6; edamame is harvested immature as a food-grade soybean) (McClure 2022). A 15 m-long by 3.5 m-wide flower strip (Maryland Upland Native Wildflower Seed Mix, Ernst Seeds; Table S1) was planted at one end of the edamame plot (at 76 cm from the first edamame row) on March 15, 2021.

TREATMENTS AND DATA SAMPLING

The experiment consisted of three pollination treatments: i) hand cross-pollination, where pollen from a donor plant was deposited onto the stigma of a receiving plant; ii) automatic selfing, where flowers were bagged, left unmanipulated, and floral visitors excluded; iii) open pollination, where flowers were left unmanipulated and exposed to floral visitors. For each pollination treatment, we recorded floral abortion rate, seed-set, fruit weight, and, for the hand-pollination treatment, time of day. Up to five flowers per plant were selected for pollination tests with one treatment applied to each plant. Although several flowers were selected per plant, no more than one was retained per raceme, with all remaining flowers of that raceme removed with fine-tipped forceps to avoid intranodal competition (Fehr et al. 1980). Each manipulated flower was tagged with the date, time of day (for hand cross-pollination only), plant number, row number, and pollination treatment.

The hand cross-pollination treatment followed the protocol by Fehr et al. (1980). We marked 174 flowers from a total of 101 plants (1.7 flowers per plant on average). Soybean stigmas become receptive ~24 hours prior to anthesis (Fehr et al. 1980; Free 1993; Roubik 1995; Carlson & Lersten 2004). Because anthers dehisce on the day of anthesis, to avoid self-pollination, flowers serving as pollen recipients were emasculated between 16 and 24 hours prior to opening, and pollinated with pollen from freshly-opened flowers from another plant (i.e., pollen donors; Fig. S1 & S2). Pollen recipients were emasculated with clean forceps (Fig. S3), and pollen from up to three pollen-donors was brushed against their stigma immediately after emasculation and until pollen was clearly visible on the stigma. The plant was then immediately covered with a 1-gallon mesh paint strainer (200 MICRO, HDX) to isolate it from floral visitors until the onset of fruit development/abortion (about 10 days), after which the bag was removed to reduce its potential effects on fruit development. Because it is not understood exactly when stigma receptivity occurs, hand cross-pollination was carried out between 06:00 and 16:00. This allowed covering a wide range of stigma receptivity times, and could provide cues for specific time windows to focus on in future stigma receptivity studies. However, as the day progressed, we observed that less pollen became available from pollen donors, thus, reducing the number of possible afternoon hand cross-pollination events. For the automatic selfing treatment, prior to anthesis we marked 258 flowers (Fig. S1) on 65 plants (3.9 flowers per plant), removed all other flowers developing on the same raceme, and covered the plant with a 1-gallon mesh paint strainer (200 MICRO, HDX) to isolate it from floral visitors. After either fruit onset or flower abortion (about 10 days after bagging), bags were removed to let the fruits continue to develop until harvest. For the open-pollination treatment, prior to anthesis we marked 277 flowers (Fig. S1-S3) on 60 plants (4.6 flowers per plant). These flowers were left open and accessible to visitors and, consequently, to natural cross-pollination.

To test for the potential effect of distance to flower strips on reproductive outputs, all treatments were randomly distributed across the field at different distances from the flower strip. It was not possible to choose regularly spaced plants for the treatments because plants preferably had to have three to five buds within 24 hours of opening to be included in a treatment on a given day, which made such a selection challenging and not always possible for the hand cross-pollination treatment.

Once fruits reached the R6 growth stage that corresponds to edamame harvest (August), they were hand-harvested, bagged and left in cool conditions to avoid desiccation, and weighed fresh within eight hours using a digital scale (Mettler AJ 100). Seed-set was recorded upon recording fresh weight. To obtain dry weight, after fresh weighing, all fruits were dried in an oven at 55˚C for five days, and stored at room temperature (~20˚C) until weighed.

Although characterizing floral visitor composition was not an aim of the present study, we collected such data, which allowed us to interpret our results in an ecological context. To do this, visitor collections were conducted on two sunny days during the edamame flowering period: July 11, 2021 and July 12, 2021. Two 15-minute collections were conducted per day, one in the morning (between 8:00-11:59) and the other in the afternoon (between 12:00-16:00). All insects seen interacting with the reproductive parts of the edamame flowers were hand-netted while walking throughout the field. Collected specimens were immediately stored in 70% ethanol for later genus-level identification using taxonomic keys (Goulet et al. 1993; Schuh et al. 2010).

STATISTICAL ANALYSES

Four response variables that relate to the reproductive output of the plant were measured: average fruit weight (g), seed-set (number of mature seeds per fruit), the proportion and weight of commercial grade-A fruit and floral abortion rate (number of aborted flowers/number of tagged flowers). Fruit weight is the individual weight of each fruit before (fresh weight) and after (dry weight) drying. Fruits that had dried on the plant at the time of harvest were excluded from the weight analyses (six fruits from six unique plants). The effect of the treatment on fruit weight was measured with a linear mixed model (LMM) (Zuur et al. 2009) with treatment, distance (m) to the flower strip and their interaction as fixed effects, and the individual plant as a random factor. The effects of the treatment on proportion of commercial grade-A fruit and floral abortion rate were quantified with generalized linear mixed models (GLMM) (Zuur et al., 2009) with treatment, distance to the flower strip and their interaction as fixed effects, and the individual plant as a random factor. A binomial distribution was used to account for the error distribution of the data. The *DHARMa* R package (Hartig & Lohse 2022) was used to visually assess model residuals and test data uniformity (Kolmogorov-Smirnov test), dispersion, and outliers. The Akaike information criterion (AIC) was used to compare models, choosing the model with the lowest AIC value. An alpha level of 0.05 was used throughout. All models were built with R package *lme4* (Bates et al. 2015).

RESULTS

Given that the results of dry weight (Fig. 1-2; Table S2-S3) are similar to those of fresh weight (Fig. S4-S5; Table S4), only those for dry weight are reported here. Our LMMs of the dry weights of commercial grade-A fruits (Table S3; Fig. S6) indicate fruits produced through hand cross-pollination (estimate: 0.09, t = 2.04, $P = 0.04$) and open-pollination treatments (estimate: 0.09, t = 3.5, $P < 0.001$) are significantly heavier than those produced through automatic selfing, but not different from each other. The LMMs for dry weight data (Table S2) indicate that the weight of fruits from the open-pollination treatment are significantly higher than those obtained in the automatic selfing (estimate: -0.189, t = -3.78, $P < 0.001$; 20% heavier;) and the hand cross-pollination (estimate: -0.215; t = -2.38; $P = 0.017$; 10% heavier) treatments (Fig. 1), but that there is no significant difference in weights obtained in the automatic selfing and hand cross-pollination treatments. Our models also identify a significant effect of distance to the flower strip only for fruits from open-pollinated flowers, with it decreasing with distance from the flower strip (estimate: -0.017, t = -2.95, $P = 0.003$; Fig. 2).

In total, 640 developed seeds were recorded across all treatments (284 seeds in open pollination; 289 seeds in automatic selfing; 67 seeds in hand cross-pollination). Seed-set ranged from zero to three seeds per fruit, with the average seed-set not differing significantly between treatments (open pollination: 2.0 +/- 0.6; automatic selfing: 1.8 +/- 0.6; hand cross-pollination: 1.7 +/- 0.7; Table S5). The GLMMs on the proportion of commercial grade-A fruit (seed-set > 1; Table S6) per treatment indicate that the proportion is significantly higher in open-pollination treatments than in both the

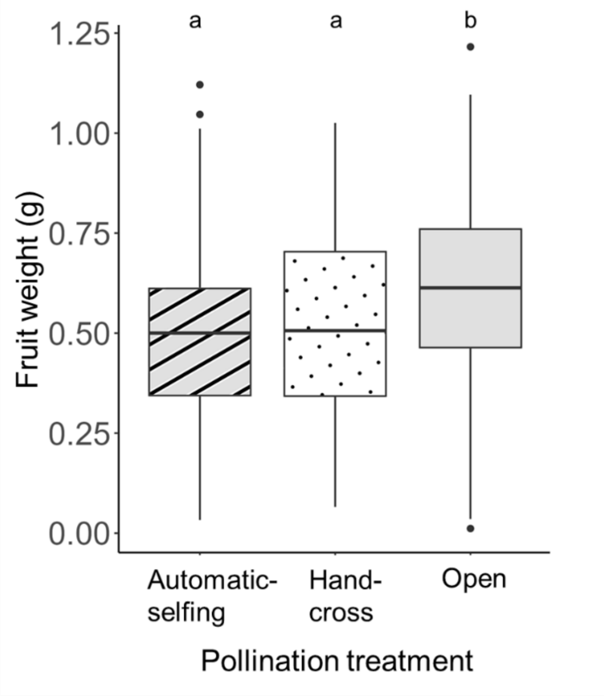

Figure 1. Average individual dry fruit weight (g) per pollination treatment. Different letters denote treatment groups that are significantly different from each other (*P* < 0.05).

automatic selfing (estimate: 0.6, z=2.03, p=0.04) and hand cross-pollination (estimate: -1.047; z = -2.692, *P* = 0.007) treatments, while proportions of the latter two are not significantly different from each other (Fig. 3). The GLMMs do not recover any significant effect of the distance to the flower strip (Table S6) on the proportion of grade-A fruit.

A total of 709 flowers on 226 plants were analysed, of which 367 developed fruits and 342 aborted. Flowers in the hand cross-pollination (134 aborted flowers/174 tagged flowers; 77%) had the most floral abortions, followed by those in the open pollination (122 aborted flowers/277 tagged flowers; 44%) and the automatic selfing treatments (86 aborted flowers/258 tagged flowers; 33%; Fig. 4; Table S7). The GLMM also identified a small significant effect of distance from the flower strip on floral abortion (estimate: 0.07, z = 2.6, *P* = 0.009).

We collected 94 individual insects visiting edamame flowers. Hymenoptera (55%) and Coleoptera (38%) comprised the majority of insects collected. Among these two orders, 75% of Hymenoptera were collected in the morning

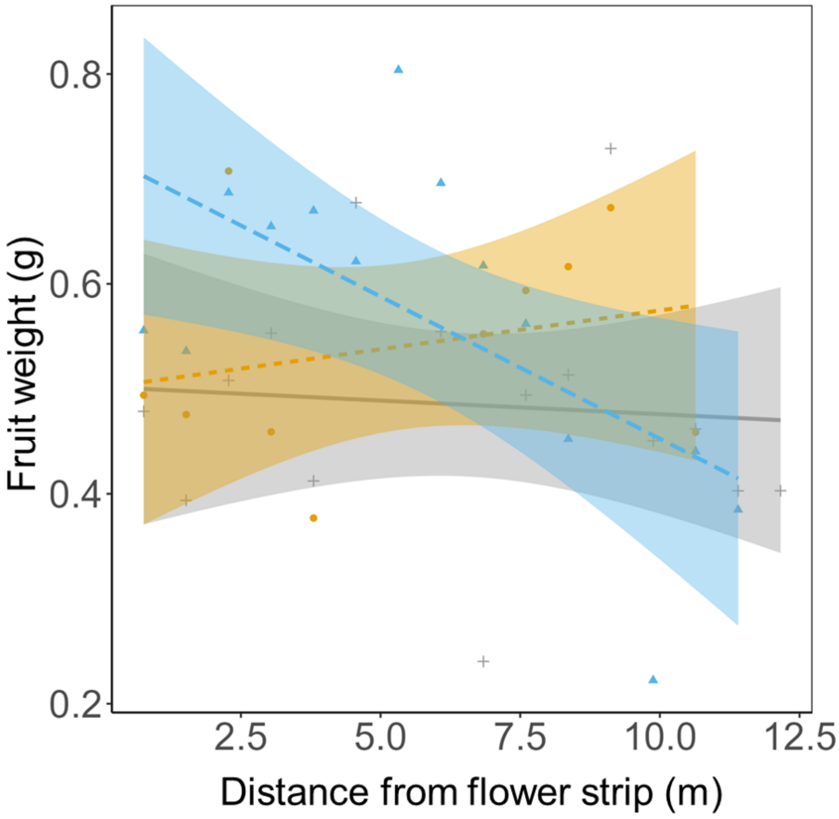

Figure 2. Average individual dry fruit weight per pollination treatment as a function of distance to the flower strip. Colours indicate the pollination treatment. Gray: automatic selfing; orange: hand cross-pollination; blue: open pollination.

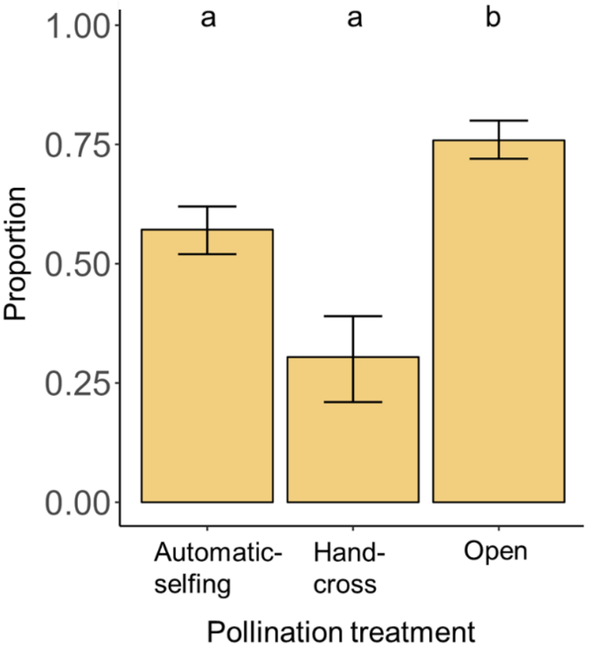

Figure 3. Proportion of commercial grade-A fruit per pollination treatment. Different letters denote treatment groups that are significantly different from each other (*P* < 0.05); error bars represent standard errors for each pollination treatment proportion.

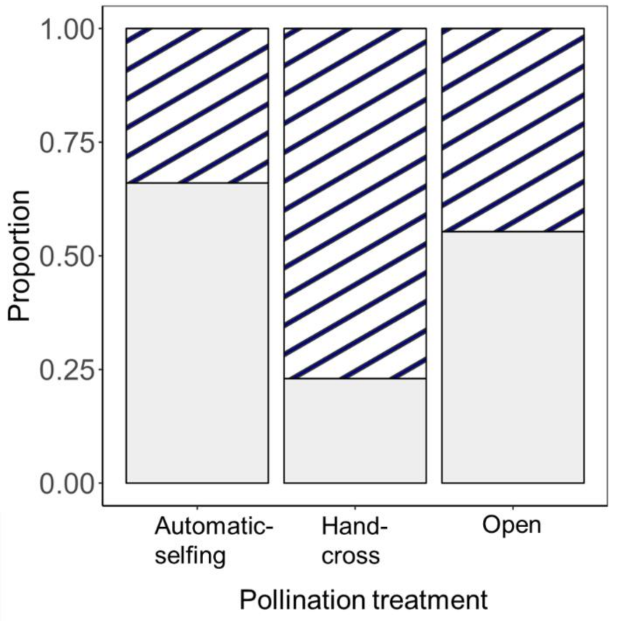

Figure 4. Proportion of aborted flowers (striped) and developed fruits (solid) per pollination treatment. Proportions in all treatments are statistically significant from each other. Flowers in the hand cross-pollination (*N* = 134/174) had the most floral abortions, followed by those in the open pollination (*N* = 122/277) and the automatic-selfing treatments (*N* = 86/258).

(before 12:00), while 66% of Coleoptera were collected in the afternoon. *Bombus* was the most abundant genus (26%), followed by *Epicauta* (22%), *Popillia* (14%), *Apis* (10%), *Melissodes* (7%) and Megachilidae bees (5%).

DISCUSSION

OVERVIEW

The primary objectives of this study were to characterize the reproductive strategy of vegetable soybean (edamame) and to evaluate the effect of flower strips on their reproductive output. Using traditional reproductive techniques, our results indicate that although edamame can self-pollinate, it benefits from cross-pollination and likely from floral visitation in measurable and market-relevant ways. Specifically, fruits from open-pollinated flowers were on average 17% heavier than those from the automatic selfing and hand cross-pollination treatments. In addition, a higher proportion of commercial grade-A fruits was recorded in the open-pollination than in the automatic selfing and hand cross-pollination treatments. Interestingly, however, grade-A fruits obtained in the open-pollination and hand cross-pollination treatments were similar in weight, and significantly heavier than those produced through automatic selfing, indicating a positive effect of cross-pollination on marketable fruit weight. Finally, plants in the open-pollination treatment that were closer to the flower strip had greater fruit weight, a pattern not found in the other treatments, indicating that nearby floral habitats can increase yield, likely through enhanced floral visitation and/or biological control. These results are also compatible with results from prior work on agronomic soybean (Levenson et al. 2022). Overall, we show that both cross-pollination and the presence of more diverse habitats in proximity to edamame fields can increase market-relevant reproductive outputs in this crop.

EDAMAME BENEFITS FROM CROSS-POLLINATION

Soybeans are effective selfers. For decades it has been assumed that they do not benefit from cross-pollination or pollinator visits in terms of market-relevant metrics and, although this is true for cleistogamous varieties, research findings have suggested that this may not apply to all varieties and environmental conditions (e.g., see Garibaldi et al. 2021, Cunha et al. 2023). Although edamame can automatically self, our results indicate that reproductive output metrics, including fruit weight and number/weight of commercial grade-A fruits, are positively affected by cross-pollination and likely insect visitation. These findings align with previous studies on soybean yield (Garibaldi et al. 2021) and corroborate our existing biological knowledge regarding the floral morphology, physiology, and pollinator interactions of soybean.

Soybeans display high rates of floral abortion, often between 32% and 84% (Panthee 2010; Van Roekel et al. 2015). We observed an overall abortion rate of 48%, with these values significantly higher among open- and hand cross-pollinated flowers, compared to those within the automatic selfing pollination treatment. Many factors can lead to floral abortions in soybeans, most notably heat and water stress, low soil fertility (Van Roekel et al. 2015), and intranodal flower competition (Egli & Bruening 2006; Panthee 2010). Given that treatments were done within the same plot, the abiotic stressors can be assumed to be similar across treatments, and therefore, it is likely that biotic factors (e.g., floral visits, herbivory) contributed to the differential abortion rates across treatments. In our study, open-pollinated flowers were vulnerable to damage by pollinators and herbivorous predation (Singh & Emden 1979; Justus et al. 2022). On this, Coleoptera are common herbivores (Turnipseed & Kogan 1976) and were among the most common visitors of edamame flowers in our field plot, specifically *Epicauta* and *Popillia*, which together comprised 36% of floral visitors. This, along with the very small significant and positive effect of distance to the flower strip on the proportion of floral abortions (see below), suggests that floral supplements can lead to reductions in this metric. Future studies should explore this pattern in more detail.

Likewise, floral manipulation by researchers and floral visitors could have also led to increases in floral abortions through damage to the stigma, especially considering that soybean flowers are very delicate and abort with minor injuries to the pistil (Panthee 2010; Talukdar & Shivakumar 2012). This would explain the high abortion rates seen in the hand cross-pollination treatment and, to a lower extent, the open-pollination treatment. Although these results would indicate a trade-off between cross-pollination and floral abortion, it is possible that edamame yield would not be affected. For example, it is common for plants to produce floral surpluses and selectively abort inferior fruit (Brown & McNeil 2006). Regarding this, we did not observe a significant difference in the average number of developed seeds per fruit among treatments. This floral over-production may allow for the selection of optimal fruit/seed size and quality through selective abortion, and/or provide an assurance policy for lost flowers or fruits (Burd 1998). Our results suggest that floral abortions in edamame could be reproductively compensated by the development of heavier fruits with larger seed-sets. Interestingly, grade-A fruits were significantly heavier in both open- and hand cross-pollination, compared to the selfing treatment. This indicates that although more abortions were present in the open- and hand-pollination treatments, this was compensated with more abundant fruits (in the open pollination treatment) of higher market value (both treatments). Compared to other studies, this could also explain the higher soybean yields obtained by Levenson et al. (2022) in fields closer to floral habitats. To resolve this uncertainty, we suggest that future studies also evaluate yield at larger (field-level) spatial scales.

FLOWER STRIPS INCREASE REPRODUCTIVE OUTPUTS AND MARKET-RELEVANT METRICS IN EDAMAME

A common management practice intended to increase pollinator abundance and diversity in agricultural settings is the incorporation of managed floral resources (Haaland et al. 2011; Kovács-Hostyánszki et al. 2017). These floral additions increase (pollinator) biodiversity by offering nesting and food resources (Haaland et al. 2011; Albrecht et al. 2021) and are often correlated with increases in yield and crop quality (Pufal et al. 2017). Despite these general increases in yield, these effects are often limited to rows adjacent to the flower habitats and do not extend into the field interior (Blaauw & Isaacs 2014; Albrecht et al. 2020). In the case of soybean production, because pollinators had been considered irrelevant to seed-set, these practices have not been recommended as soybean cultivation practices. However, recent evidence shows that implementing pollinator management strategies, such as flower habitats, into soybean production can improve some yield metrics (Levenson et al. 2022). Despite the smaller field size in our study, our results agree with previous works, suggesting that floral strips positively affect edamame fruit weight in manners that are likely to also improve marketability and farmer income.

Specifically, the improved reproductive output in open-pollinated plants from rows closer to the flower strip may be the result of pollinator and/or biological control spill-over from it. For example, the flower strip could have acted as a biodiversity magnet, with flowers in rows closer to the strip benefitting from greater beneficial insect visitation (Blitzer et al. 2012). This conclusion is further supported in that fruit weight in neither the automatic selfing nor hand-cross pollination treatments showed this trend, suggesting that the effect of distance from the flower strip is likely due to changes in the biotic rather than the abiotic conditions of the field, and driven by the flower strip supplement. These results indicate that flower strips have the potential to increase reproductive outputs in edamame, but that this effect remains restricted to rows closer to the supplemented area. Future work should investigate this pattern more thoroughly, using plot replication and an experimental setup built around flower strip effect testing (e.g., comparing plots with and without flower strips), and potentially testing the ability of in-field floral resources (e.g., intercropping) to extend these benefits further into the field. In addition, to confirm the effect of these strips on biodiversity, insect surveys should be conducted within and nearby the floral additions, and evaluate their ability to deposit cross-pollen and/or improved pollen distribution on the stigmatic surface.

TOWARDS AN IMPROVED UNDERSTANDING OF THE REPRODUCTIVE BIOLOGY OF SOYBEANS

Although not the main goal of this work, our study provides insights into the timing of floral receptivity in soybean and gives pointers to protocol modifications for future studies. To serve future research(ers), we summarize these findings below.

Soybean flowers open in the morning (Fehr et al. 1980) and fertilization occurs within 10-15 hours post-anthesis, shortly before the flower senesces (Johnson & Bernard 1962). Previous research in soybeans demonstrated that time of day is a primary factor affecting floral volatile production (Robacker et al. 1988), pollen production (Fehr et al. 1980), and nectar productivity, with nectar and pollen peaking prior to 11:00 and decreasing as the day progresses (Severson & Erickson 1984). Further, one study showed that insect visitation in soybean fields peaks between 10:00 and 11:00 (Pando et al., 2019). Such temporal overlap of pollen and nectar production, volatile emissions and stigma receptivity is a well-known pattern in plants, coupling floral rewards and pollinator attraction when the stigma is receptive (Willmer 2011), which in turn leads to higher floral visitation by insects. Agreeing with this body of knowledge, we also collected more flower-visiting Hymenoptera (predominantly *Bombus*) in the morning than in the afternoon. These observations coincide with a study in the southern USA, which found that most floral visitation occurred during the mid-morning hours on the day of flowering (Peterson et al. 1992). In this context, our observed reduction in the amount of pollen from flower donors in the hand cross-pollination treatment as the day progressed, coupled with data from the insect survey, suggests that edamame floral receptivity peaks in the morning. Based on these observations, we suggest that future stigma-receptivity studies should aim to understand its synchronicity with other pollination-related traits in edamame and other soybean varieties.

Observations we obtained from our use of hand cross-pollination protocols also allows us to recommend protocol modifications in future works. Standard breeder hand-pollination protocols such as the one used here requires hand-crosses to be conducted about 24 hours prior to the flower opening (Fehr et al. 1980; Talukdar & Shivakumar 2012). However, stigma receptivity tends to be the lowest at the bud stage and increases closer to anthesis (Kaur et al. 2005). Given the high abortion rates observed in our hand cross-pollination, it is not unlikely that at least some of these hand-pollinated flowers received pollen before their stigmas were receptive (Kaur et al. 2005), leading to inflated abortion rates compared to those seen in open-pollinated flowers. This result was probably further marked by the fact that our hand-pollinated flowers did not have the opportunity to receive self-pollen. While open-pollinated flowers likely received both self- and outcrossed pollen, hand cross-pollinated ones had been emasculated and thus their pollen was not available when the stigma was receptive (Fehr et al. 1980). Integrating what we observed and learned from this work, and to reduce inflated abortion rates in future studies, we suggest an updated protocol for future hand-pollination experiments. First, we recommend performing most hand-crosses in the morning of anthesis, when the stigma is the most likely to be receptive. Second, we strongly encourage the use of extreme caution when manipulating flowers, given their high fragility.

CONCLUSION

Soybeans have been historically considered a pollinator-independent and self-pollinating crop, although a body of research suggests otherwise for many varieties and ecological conditions (Garibaldi et al. 2021; Levenson et al. 2022; Palmer et al. 2001; Santone et al. 2022). There are gaps in the general knowledge of the reproductive biology of soybean and its pollination ecology. In this study, we show that although edamame (vegetable soybean) can automatically self, it benefits from cross-pollination, with this effect accentuated with proximity to flower habitats (e.g., flower strips) neighbouring the field. Beyond characterizing the reproductive strategy of edamame, this study also provides insights into the reproductive biology of the crop, along with methodological improvements for future research, particularly on the timing of hand-pollination to reduce inflated abortion rates. Overall, our results provide support to the idea that edamame reproductive outputs benefit from floral visitors and pollen movement between flowers. Additionally, the higher weight of commercial grade-A fruit in both the open and hand cross-pollinations treatments further supports the idea that soybeans may benefit more from outcrossing than traditionally assumed, with positive market-relevant effects.

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AUTHOR CONTRIBUTION

Concept and design KCE, AE, & CH, data collection KCE & JE, data analysis KCE, writing KCE, AE, JEH & CH, edits and approval for publication KCE & AE.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

DATA AVAILABILITY STATEMENT

The data used to write this article are available at https://github.com/kciolae/BenefitsCrossPollEd.EvansEtAl.JPE.

APPENDICES

Additional supporting information may be found in the online version of this article:

Table S1. Species composition of the flower strip

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