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### **Benefits of Cross-Pollination in Vegetable Soybean Edamame**

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#### **APPENDIX I**

Our supplemental material includes a table of the flower species present in the flower strip, tables with all GLMM and LMM analyses, three images related to the hand-pollination protocol and the results from the fresh weight analyses.

Table S1. Species composition of the flower strip (Maryland Upland Native Wildflower Seed Mix from Ernst Seeds; ERNMX-172). An Asterix (\*) following the species name indicates that the species bloomed.

Table S2. Results from the selected best linear mixed model (LMM) for dry fruit weights per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

Table S3. Results from the selected best linear mixed model (LMM) for dry weights of commercial grade-A fruits per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

Table S4. Results from the selected best linear mixed model (LMM) for fresh fruit weights per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

Table S5. Results from the selected best linear mixed model (LMM) for average seed-set per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

Table S6. Results from the selected best generalized linear mixed model (GLMM) for the proportion of commercial grade-A fruit per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

Table S7. Results from the selected best generalized linear mixed model (GLMM) for the proportion of abortions per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

Figure S1: A cluster of edamame flowers at different blooming stages: A – bud opening within 24 hours which served as pollen recipients and tagged flowers; B – open flower which served as pollen donors; C and D – bud several days prior to opening.

Figure S2: For hand-pollination flowers, sepals and petals were removed from buds expected to open within 24 hours. Immediately after preparation, pollen from pollen-donor(s) was brushed against the stigma of the pollen-recipient by hand.

Figure S3: Flower shortly after opening which served as pollen donor for the hand-pollination treatments.

Figure S4. Average individual fresh fruit weight (g) per pollination treatment. Different letters denote treatment groups that are significantly different from each other ( $p < 0.05$ ). Fruits harvested from the open-pollination treatment were heavier than fruits in the automatic-selfing treatment.

Figure S5. Average individual fresh fruit weight (g) per pollination treatment and distance to the flower strip. The LMM identified a significant effect of distance to the flower strip on the fruit weight of open-pollinated flowers only, with it decreasing with distance.

Figure S6. Dry (left) and fresh (right) fruit weights per pollination treatment for commercial grade-A fruits. Colours correspond to the number of seeds per fruit. Our LMMs indicate that fruits from the hand- and open-pollination treatments weighed significantly more than those produced through automatic selfing.

Table S1. Species composition of the flower strip (Maryland Upland Native Wildflower Seed Mix from Ernst Seeds; ERNMX-172). An Asterix (\*) following the species name indicates that the species bloomed.

| <b>Plant Species</b>  |
|---|
| <i>Schizachyrium scoparium</i> , (little bluestem, PA Ecotype) *  |
| <i>Elymus virginicus</i> (Virginia wildrye, PA Ecotype) *         |
| <i>Rudbeckia hirta</i> (Blackeyed Susan) *                        |
| <i>Asclepias tuberosa</i> (Butterfly Milkweed, PA Ecotype) *      |
| <i>Chamaecrista fasciculata</i> (Partridge Pea, PA Ecotype) *     |
| <i>Eragrostis spectabilis</i> (Purple Lovegrass, RI Ecotype) *    |
| <i>Lespedeza virginica</i> (Slender Lespedeza, VA Ecotype) *      |
| <i>Senna hebecarpa</i> (Wild Senna, VA & WV Ecotype) *            |
| <i>Penstemon digitalis</i> (Tall White Beardtongue, PA Ecotype)   |
| <i>Aster novae-angliae</i> (New England Aster, PA Ecotype) *      |
| <i>Aster pilosus</i> (Heath Aster, PA Ecotype) *                  |
| <i>Chamaecrista nictitans</i> (Sensitive Pea, NC Ecotype) *       |
| <i>Monarda fistulosa</i> (Wild Bergamot, PA Ecotype) *            |
| <i>Pycnanthemum tenuifolium</i> (Narrowleaf Mountainmint)         |
| <i>Solidago bicolor</i> (White Goldenrod, PA Ecotype) *           |
| <i>Solidago nemoralis</i> (Gray Goldenrod, PA Ecotype) *          |
| <i>Penstemon laevigatus</i> (Appalachian Beardtongue, PA Ecotype) |
| <i>Pycnanthemum incanum</i> (Hoary Mountainmint, MD Ecotype)      |
| <i>Penstemon hirsutus</i> (Hairy Beardtongue)                     |

Table S2. Results from the selected best linear mixed model (LMM) for dry fruit weights per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

|                                       | Estimate      | Std. Error   | t value       | Pr(> t )         |
|---------------------------------------|---------------|--------------|---------------|------------------|
| Automatic selfing (intercept)         | <b>0.493</b>  | <b>0.036</b> | <b>13.632</b> | <b>&lt;0.001</b> |
| Hand cross pollination                | -0.026        | 0.091        | -0.290        | 0.772            |
| Open pollination                      | <b>0.189</b>  | <b>0.050</b> | <b>3.781</b>  | <b>&lt;0.001</b> |
| Distance (m)                          | -0.001        | 0.005        | -0.270        | 0.787            |
| Hand cross pollination * Distance (m) | 0.011         | 0.013        | 0.873         | 0.383            |
| Open pollination * Distance (m)       | <b>-0.017</b> | <b>0.006</b> | <b>-2.951</b> | <b>0.003</b>     |

|                                       | Estimate      | Std. Error   | t value       | Pr(> t )         |
|---------------------------------------|---------------|--------------|---------------|------------------|
| Open pollination (intercept)          | <b>0.682</b>  | <b>0.034</b> | <b>19.71</b>  | <b>&lt;0.001</b> |
| Automatic selfing                     | <b>-0.189</b> | <b>0.050</b> | <b>-3.781</b> | <b>&lt;0.001</b> |
| Hand cross pollination                | <b>-0.215</b> | <b>0.090</b> | <b>-2.38</b>  | <b>0.017</b>     |
| Distance (m)                          | <b>-0.017</b> | <b>0.005</b> | <b>-2.951</b> | <b>0.003</b>     |
| Automatic selfing * Distance (m)      | -0.001        | 0.005        | -0.27         | 0.786            |
| Hand cross pollination * Distance (m) | 0.011         | 0.012        | 0.873         | 0.382            |

Table S3. Results from the selected best linear mixed model (LMM) for dry weights of commercial grade-A fruits per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

|                               | Estimate     | Std. Error   | t-value       | p-value          |
|-------------------------------|--------------|--------------|---------------|------------------|
| Automatic selfing (intercept) | <b>0.599</b> | <b>0.018</b> | <b>33.735</b> | <b>&lt;0.001</b> |
| Hand cross pollination        | <b>0.084</b> | <b>0.041</b> | <b>2.046</b>  | <b>0.043</b>     |
| Open pollination              | <b>0.087</b> | <b>0.025</b> | <b>3.523</b>  | <b>0.0006</b>    |

|                              | Estimate      | Std. Error   | t-value       | p-value          |
|------------------------------|---------------|--------------|---------------|------------------|
| Open pollination (intercept) | <b>0.686</b>  | <b>0.017</b> | <b>39.897</b> | <b>&lt;0.001</b> |
| Automatic selfing            | <b>-0.087</b> | <b>0.024</b> | <b>-3.523</b> | <b>&lt;0.001</b> |
| Hand cross pollination       | -0.002        | 0.04         | -0.071        | 0.942            |

Table S4. Results from the selected best linear mixed model (LMM) for fresh fruit weights per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

|                                       | Estimate      | Std. Error   | t value       | Pr(> t )         |
|---------------------------------------|---------------|--------------|---------------|------------------|
| Automatic selfing (intercept)         | <b>1.907</b>  | <b>0.137</b> | <b>13.921</b> | <b>&lt;0.001</b> |
| Hand cross pollination                | -0.158        | 0.345        | -0.457        | 0.648            |
| Open pollination                      | <b>0.636</b>  | <b>0.190</b> | <b>3.338</b>  | <b>&lt;0.001</b> |
| Distance (m)                          | -0.006        | 0.020        | -0.332        | 0.740            |
| Hand cross pollination * Distance (m) | 0.054         | 0.048        | 1.115         | 0.265            |
| Open pollination * Distance (m)       | <b>-0.050</b> | <b>0.023</b> | <b>-2.191</b> | <b>0.029</b>     |

|                                       | Estimate      | Std. Error   | t value       | Pr(> t )         |
|---------------------------------------|---------------|--------------|---------------|------------------|
| Open pollination (intercept)          | <b>2.541</b>  | <b>0.132</b> | <b>19.194</b> | <b>&lt;0.001</b> |
| Automatic selfing                     | <b>-0.635</b> | <b>0.19</b>  | <b>-3.338</b> | <b>&lt;0.001</b> |
| Hand cross pollination                | <b>-0.793</b> | <b>0.343</b> | <b>-2.31</b>  | <b>0.021</b>     |
| Distance (m)                          | <b>-0.05</b>  | <b>0.022</b> | <b>-2.19</b>  | <b>0.029</b>     |
| Automatic selfing * Distance (m)      | -0.006        | 0.019        | -0.331        | 0.74             |
| Hand cross pollination * Distance (m) | 0.054         | 0.048        | 1.1153        | 0.265            |

Table S5. Results from the selected best linear mixed model (LMM) for average seed-set per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

|                               | Estimate     | Std. Error   | z value       | Pr(> z )         |
|-------------------------------|--------------|--------------|---------------|------------------|
| Automatic selfing (intercept) | <b>1.695</b> | <b>0.096</b> | <b>17.635</b> | <b>&lt;0.001</b> |
| Hand cross pollination        | -0.008       | 0.134        | -0.043        | 0.965            |
| Open pollination              | 0.137        | 0.085        | 1.608         | 0.109            |
| Distance (m)                  | -0.002       | 0.012        | -0.194        | 0.846            |

|                              | Estimate     | Std. Error   | z value       | Pr(> z )         |
|------------------------------|--------------|--------------|---------------|------------------|
| Open pollination (intercept) | <b>1.832</b> | <b>0.086</b> | <b>21.071</b> | <b>&lt;0.001</b> |
| Automatic selfing            | -0.137       | 0.085        | -1.608        | 0.109            |
| Hand cross pollination       | -0.143       | 0.135        | -1.057        | 0.291            |
| Distance (m)                 | -0.002       | 0.012        | -0.194        | 0.846            |

Table S6. Results from the selected best generalized linear mixed model (GLMM) for the proportion of commercial grade-A fruit per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

|                               | Estimate     | Std. Error   | z value      | Pr(> z )     |
|-------------------------------|--------------|--------------|--------------|--------------|
| Automatic selfing (intercept) | <b>0.924</b> | <b>0.299</b> | <b>3.082</b> | <b>0.002</b> |
| Hand cross pollination        | -0.489       | 0.369        | -1.325       | 0.185        |
| Open pollination              | <b>0.559</b> | <b>0.276</b> | <b>2.023</b> | <b>0.043</b> |
| Distance (m)                  | -0.012       | 0.039        | -0.315       | 0.753        |

|                              | Estimate      | Std. Error   | z value       | Pr(> z )         |
|------------------------------|---------------|--------------|---------------|------------------|
| Open pollination (intercept) | <b>1.483</b>  | <b>0.288</b> | <b>5.136</b>  | <b>&lt;0.001</b> |
| Automatic-selfing            | <b>-0.559</b> | <b>0.276</b> | <b>-2.022</b> | <b>0.043</b>     |
| Hand cross pollination       | <b>-1.047</b> | <b>0.389</b> | <b>-2.692</b> | <b>0.007</b>     |
| Distance (m)                 | -0.012        | 0.039        | -0.315        | 0.752            |



Table S7. Results from the selected best generalized linear mixed model (GLMM) for the proportion of abortions per treatment. Significant values are bolded. Top: analysis setting automatic selfing as the intercept. Bottom: analysis setting open pollination as the intercept.

|                               | Estimate      | Std. Error   | z value       | Pr(> z )         |
|-------------------------------|---------------|--------------|---------------|------------------|
| Automatic selfing (intercept) | <b>-1.181</b> | <b>0.238</b> | <b>-4.970</b> | <b>&lt;0.001</b> |
| Hand cross pollination        | <b>2.084</b>  | <b>0.256</b> | <b>8.146</b>  | <b>&lt;0.001</b> |
| Open pollination              | <b>0.539</b>  | <b>0.205</b> | <b>2.636</b>  | <b>0.008</b>     |
| Distance (m)                  | <b>0.071</b>  | <b>0.027</b> | <b>2.594</b>  | <b>0.009</b>     |

|                              | Estimate      | Std. Error   | z value       | Pr(> z )         |
|------------------------------|---------------|--------------|---------------|------------------|
| Open pollination (intercept) | <b>-0.642</b> | <b>0.205</b> | <b>-3.125</b> | <b>0.001</b>     |
| Automatic selfing            | <b>-0.539</b> | <b>0.204</b> | <b>-2.636</b> | <b>0.008</b>     |
| Hand cross pollination       | <b>1.544</b>  | <b>0.243</b> | <b>6.351</b>  | <b>&lt;0.001</b> |
| Distance (m)                 | <b>0.071</b>  | <b>0.027</b> | <b>2.594</b>  | <b>0.009</b>     |



Figure S1. A cluster of edamame flowers at different blooming stages (A-D): A – flower bud expected to open within 24 hours were selected as pollen recipients; B –open flower which served as pollen donors; C and D – flower buds several days prior to opening.



Figure S2. Flower shortly after opening which served as pollen donor for the hand-pollination treatments.



Figure S3. For hand-pollination flowers, sepals and petals were removed from buds expected to open within 24 hours. Immediately after preparation, the anthers from up to three pollen-donor(s) was brushed against the stigma of the pollen-recipient with forceps. Pollen was visible on the stigma of each pollen-recipient.

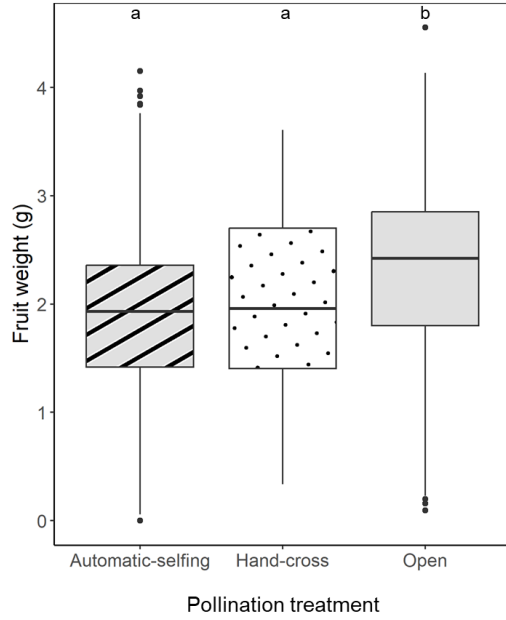


Figure S4. Average individual fresh fruit weight (g) per pollination treatment. Different letters denote treatment groups that are significantly different from each other ( $p < 0.05$ ). Fruits harvested from the open-pollination treatment were heavier than fruits in the automatic-selfing treatment.

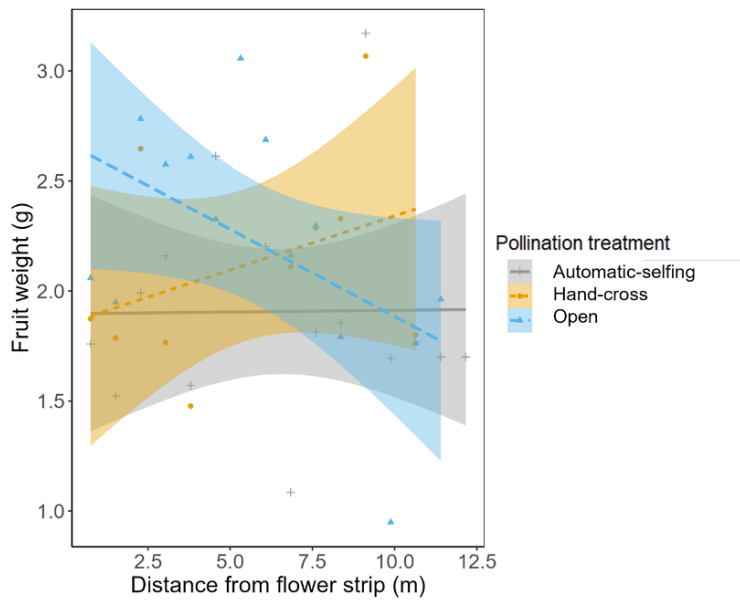


Figure S5. Average individual fresh fruit weight (g) per pollination treatment and distance to the flower strip. The LMM identified a significant effect of distance to the flower strip on the fruit weight of open-pollinated flowers only, with it decreasing with distance.

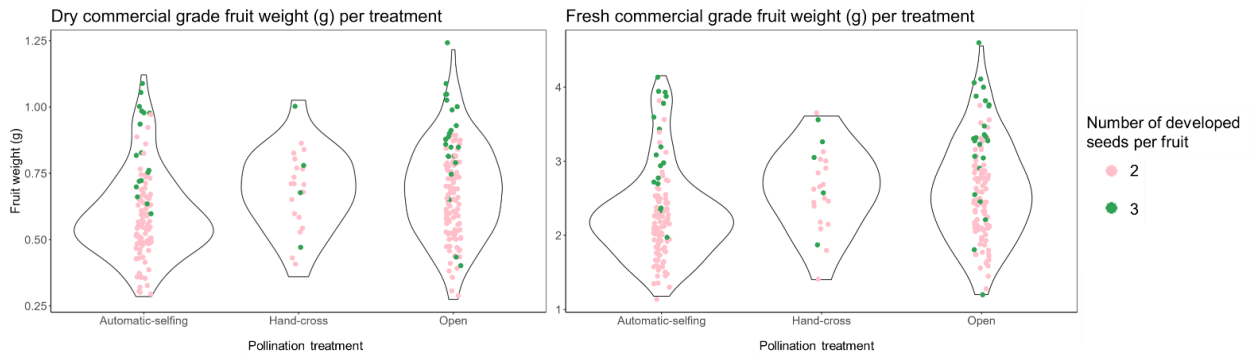


Figure S6. Dry (left) and fresh (right) fruit weights per pollination treatment for commercial grade-A fruits. Colors correspond to the number of seeds per fruit. Our LMMs indicate that fruits from the hand- and open-pollination treatments weighed significantly more than those produced through automatic selfing.