An investigation into mouse-ear cress (*Arabidopsis thaliana*) flowering schedule and pollination mechanism

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Abstract: *Arabidopsis thaliana*, a model organism in several fields of biology, is a largely self-pollinating species in the laboratory. Outside of the laboratory, there have been reports of insect visitors on flowers of *A. thaliana* and reported rates of outcrossing. In North America, however, the pollination ecology of *A. thaliana* is largely unknown. In this experiment performed at Pierce Cedar Creek Institute in Hastings, Michigan, an artificial garden of *A. thaliana* was observed in a natural setting for possible wind and pollinator-mediated pollination. To test for outcrossing we added plants to the garden that contained a gene coding for resistance to the antibiotic kanamycin. At the end of the experiment the seeds from the wild-type plants were tested for kanamycin resistance to determine if outcrossing had occurred between wild-type and kanamycin resistant plants. *A. thaliana* flowers were found to have a very high rate of insect visitation, especially from members of the family Syrphidae. Nevertheless, plating the seeds collected from the wild-type plants on a tested kanamycin media did not reveal any signs of successful outcrossing. Under these artificial conditions pollination was not evident. Future studies will utilize plants native to the region.

Introduction: *Arabidopsis thaliana*, a weed from the Brassicaceae family, is a model organism that is appreciated in the laboratory for its small size, short life cycle, small genome, and its ability to self-pollinate. The latter of these trademark qualities is plastic in a non-laboratory setting. *A. thaliana plants* have been shown to be susceptible to outcrossing during of a stage in flower development in which the flower is open, but the anthers of the flower are not able to deposit pollen on the stigma of the same flower (Tan *et al*., 2005). Multiple studies have shown a small outcrossing rate in *A. thaliana* populations (Abbott & Gomes, 1989; Bergelson *et al*., 1998; Fedorenko *et al*., 2001). A follow-up study in Germany gave evidence that *A. thaliana* is subjected to natural insect visitation (Hoffmann *et* al., 2003) and another recent study in China provided evidence of wind pollination (Tan *et al*., 2005). After being introduced to North America by human action (Hoffmann, 2005), the nonnative species (*A.* thaliana) may have adapted a new pollination ecology. The goal of this project was to determine if there were flower visitors in wild populations of *A. thaliana* in southwest Michigan and, if so, whether or not they were successful pollinators. However, when the project began at the end of May, the natural populations of *A. thaliana* had already begun to produce seeds. To determine the frequency of flower visits, observations of an artificial garden were made from sunrise until sunset. In this study we introduced a genetic resistance to the antibiotic kanamycin in *A. thaliana* plants that grew adjacent to wild-type plants in order to determine through analysis of the F1 generation whether or not outcrossing was occurring. The study was conducted at Pierce Cedar Creek Institute in Hastings, Michigan.

Materials and Methods:

Choosing A Location

As in many other ecological studies, location was the key to success. For this project, the location of the artificial garden needed to be a natural habitat of *Arabidopsis thaliana* (dry, sandy soil) in order to reasonably assume an interaction between the plant and its flower visitors. After locating several possible garden locations, they were each characterized by their insect and plant diversity. The location with the highest insect and plant diversity was chosen.

Artificial Garden

There were two types of *A. thaliana* used in this experiment. The first type, RS76, contained a gene coding for resistance to the antibiotic kanamycin. The second type of *A. thaliana* was Columbia wild-type. Both RS-76 and Columbia wild-type plants were planted in pots in a laboratory at Valparaiso University in Valparaiso, Indiana and were transported to Pierce Cedar Creek Institute in Hastings, Michigan. Upon arrival, the plants were cut slightly above the rosette at the base to maximize the time that the flowers were exposed to their natural environments. There were six wings of plants with each wing containing 3 pots in an arrangement established in an earlier study (Tan *et al*., 2005) (see Figure 1). Each pot contained approximately five plants. The wings were labeled A-F, and the pots were labeled 1-3 from the innermost plants outward. The plants were labeled to establish the probability of wind pollination based upon measurements of wind direction. The pots were left at the designated location above ground.

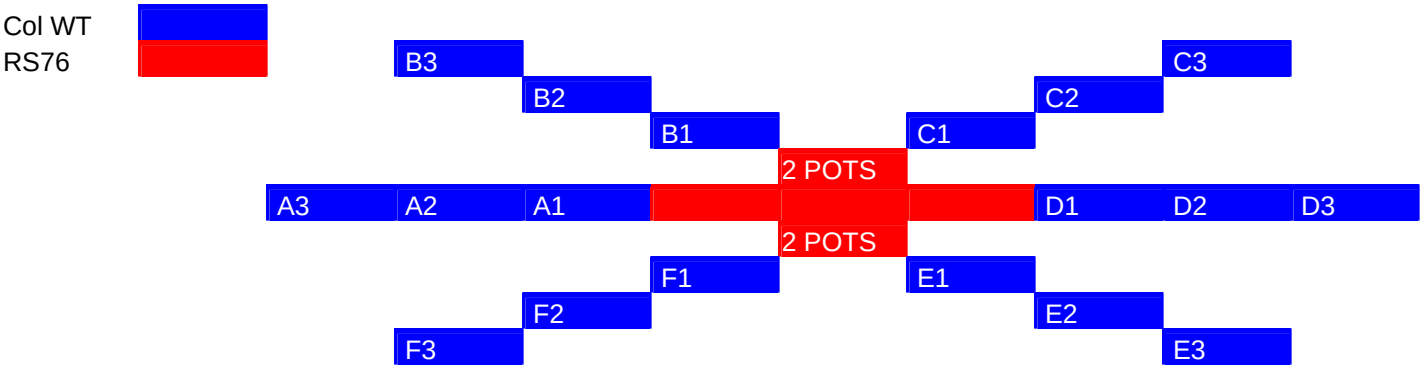

Figure 1. This figure depicts the arrangement of the artificial garden containing both types of *Arabidopsis thaliana* plants. The red squares represent kanamycin-resistant plants while the blue squares represent Columbia wild-type plants. The plants were labeled to establish the probability of wind pollination based upon measurements of wind direction. Each colored space indicates one pot unless indicated otherwise; each pot contained approximately five plants.

The Observation

Because the garden was entirely above ground, the pots were watered each day to prevent dehydration. At the appearance of flowers, the observation began. The flowers were observed for pollinators from sunrise (approximately 6 A.M. E.T.) until sunset (approximately 9 P.M. E.T.) over the course of three days from 6/18/07-6/20/07 (see Table 1). In order to be counted as a possible pollinator, it was necessary for an organism to make contact with the flower of an *A. thaliana* plant. To allow greater accuracy in data collection, only possible pollinator visits to plants in wing "D" were recorded. The prevailing weather conditions were also noted on an hourly basis during observations.

Checking For Outcrossing

Once the Col WT seeds had matured, they were collected and sent back to Valparaiso University in Valparaiso, Indiana. At the laboratory the seeds were sterilized using chlorine gas, and 50 uM kanamycin MS (Murashige & Skoog salts) plates were prepared and tested. Seeds with the gene for kanamycin resistance (RS-76) were able to grow on the plates, while those without the gene (Col WT) could not. This method was adopted to determine if outcrossing had occurred. The

number of seeds collected from each pot was recorded and the seeds were plated out. The number of seeds that germinated on the media and the number of those seeds testing positive for kanamycin were recorded. If any of the seeds collected from the Col WT plants tested positive by growing on the media beyond germination, it would signify an exchange of genetic information via sexual recombination between the RS-76 plants and the Col WT plants. Such a finding would strongly support the hypothesis that *A. thaliana* is not entirely a self-pollinating plant, but rather that it is subjected to cross-pollination in the wild.

Results:

Flower Visitors

During observations of the artificial garden at Pierce Cedar Creek Institute in Hastings, Michigan, visitors were recorded on *Arabidopsis thaliana* flowers. The cumulative total of insect visitations during the 15-hour observation can be seen in Figure 2. From these observations, the dominant insect visitor was identified as a member of the Family Syrphidae. The time of day in which the most visits were observed was also recorded along with the immediate weather conditions corresponding to those specific one-hour segments (see Table 1). The time of day with the most visits was from 9 A.M. E.T. until 12 P.M. E.T., with the hour stretching from 10 A.M. E.T. until 11 A.M. E.T. having the largest number of visitations.

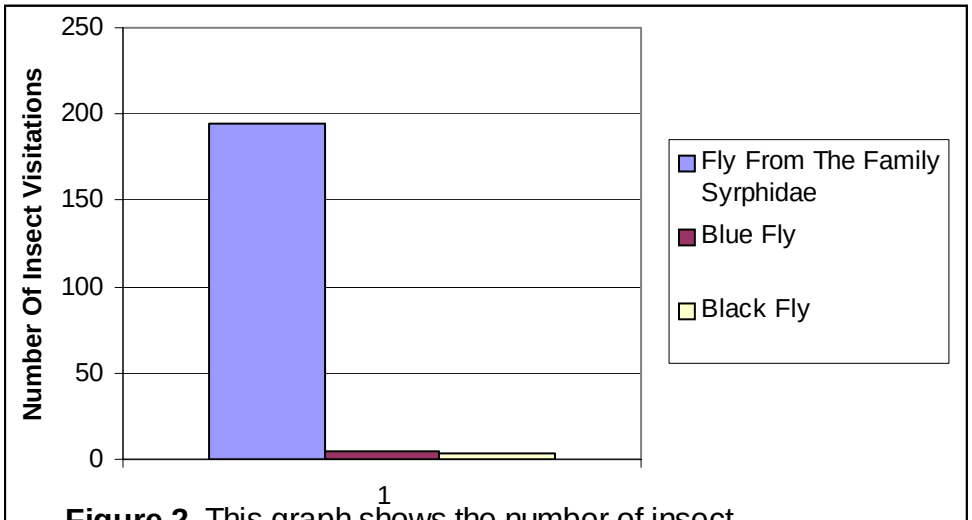

Figure 2. This graph shows the number of insect visitations on *Arabidopsis thaliana* flowers over several days spanning the hours from sunrise to sunset (approximately 6 A.M. E.T. to 9 P.M. E.T.).

Date	Time Of Day	Number Of Insect Visitations	Prevailing Weather Conditions
6/18/2007	6 A.M. -- 7 A.M.	0	Foggy, Cool
6/18/2007	7 A.M. -- 8 A.M.	4	Overcast,Cool
6/18/2007	8 A.M. -- 9 A.M.	15	Overcast, Cool
6/18/2007	9 A.M. -- 10 A.M.	38	Cloudy, Warm
6/18/2007	10 A.M. -- 11 A.M.	58	Sunny, Warm
6/18/2007	11 A.M. -- 12 P.M.	26	Sunny, Warm
6/18/2007	12 P.M. -- 1 P.M.	12	Sunny, Light Wind
6/18/2007	1 P.M. -- 2 P.M.	0	Sunny, Hot
6/19/2007	2 P.M. -- 3 P.M.	11	Sunny, Cool
6/19/2007	3 P.M. -- 4 P.M.	8	Sunny, Light Wind
6/19/2007	4 P.M. -- 5 P.M.	14	Sunny, Light Wind
6/19/2007	5 P.M. -- 6 P.M.	6	Sunny, Light Wind
6/20/2007	6 P.M. -- 7 P.M.	2	Clear, Warm
6/20/2007	7 P.M. -- 8 P.M.	0	Cloudy, Warm
6/20/2007	8 P.M. -- 9 P.M.	0	Cloudy, Cool

Table 1. This table records the number of insect visitations on the “D” wing of *Arabidopsis thaliana* plants in an artificial garden at Pierce Cedar Creek Institute in Hastings, Michigan. The data is per one-hour segment; the segments were observed over the course of several days, spanning the hours from sunrise until sunset (approximately 6 A.M. E.T. until 9 P.M. E.T.).

Pot Number	Number Of Seeds Collected	Number Of Seeds Germinated	Number Of Seeds With Kanmycin Resistance
A1	1491	576	0
A2	1359	424	0
A3	0	0	0
B1	2140	723	0
B2	0	0	0
B3	0	0	0
C1	1764	424	0
C2	1012	200	0
C3	0	0	0
D1	1341	493	0
D2	1869	632	0
D3	911	344	0
E1	108	24	0
E2	0	0	0
E3	320	116	0
F1	2804	948	0
F2	1327	568	0
F3	0	0	0

Table 2. For each pot of Columbia Wild-Type *Arabidopsis thaliana* plants in the artificial garden, this table records: the number of seeds collected, the number of seeds that germinated on a kanamycin media, and the number of those seeds that tested positive for kanamycin resistance by exhibiting growth beyond germination.

Outcrossing Rate

The number of seeds collected from each pot, the number of seeds that germinated on the media and the number of those seeds testing positive for kanamycin are shown in Table 2. None of the seeds that were plated on a kanamycin media showed any resistance to the antibiotic.

Wind Pollination

There was a regular wind blowing from between wings "B" and "C" to between wings "E" and "F". The direction and intensity of the winds did not have an effect on the frequency of *Arabidopsis thaliana* outcrossing in the wild. Without evidence of cross-pollination of *A. thaliana* in the wild there is no indication of wind as a means of pollen dispersal leading to fertilization.

Discussion:

Flower Visitors

The primary visitors of *Arabidopsis thaliana* plants at Pierce Cedar Creek Institute in Hastings, Michigan were members of the Family Syrphidae. This family of visitors constituted almost 96% of all recorded flower visits. The hours between sunset and sunrise were not observed for flower visits, as there were no means readily available to accurately quantify flower visitation without skewing the results by providing an unnatural source of light necessary for observation to occur. Members of the Family Syrphidae have been known to visit *A. thaliana* in other countries including China (Tan *et al.*, 2005), and Germany (Hoffmann *et al.*, 2003), and it appears that these findings are also applicable in southwest Michigan.

Outcrossing

The number and frequency of visitors on *Arabidopsis thaliana* flowers was surprisingly high considering the evidence that outcrossing did not occur between plants in the wild. Contrary to other studies reporting small outcrossing rates less than one percent (Abbott & Gomes, 1989; Fedorenko *et al.*, 2001; Tan *et al*., 2005) we do not have any data that supports those claims. There is a possibility that our flowers were simply not pollinated during their window of opportunity in which the anther of a flower is incapable of depositing pollen on the stigma of that same flower. With such a small reported outcrossing rate, a larger garden would have a greater chance of revealing those research findings, and it will be a variable to consider in the future.

Future Research Questions

Although flower visitors were recorded, there was no indication of pollen transfer between kanamycin-resistant plants and Columbia wild-type plants. It is possible that the pollinators are incapable of delivering pollen. Future experiments will focus on the physical transfer of pollen between plants and insect visitors. The frequency of *A. thaliana* flower visitors transferring

pollen could have been underrepresented due to the small window of time in which outcrossing can occur.

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