Comparison of Resistant and Susceptible Cowpea Plants to Cowpea Aphid (Koch) (Hemiptera: Aphididae)

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Abstract: Aphids are one of the most devastating agricultural pests to multiple plants. Plant transformations are being considered as a population control method. This long-term research aims to characterize the molecular mechanism underlying plant-aphid interaction between the cowpea plant and the cowpea aphid. A resistant cowpea line (IT98K-205-8) and a susceptible cowpea line (IT98K-589-2) were observed to analyze how effective a resistant cowpea is in comparison to a susceptible one. Each line was planted, analyzed and scored. Both lines, resistant and susceptible resulted in significantly different results in both room 127 and room 124. Line 205 showed a lower population of aphids starting at day one while line 589-2 population began to decrease on the third day. As seen in this experiment, the overall result was that the resistant line was significantly lower in progeny production than that of the susceptible plant. The survival of the aphid population also decreased on each leaf from day one. Resistant cowpea plants are potential to reducing the aphid population. Additional research should go into manufacture of cowpea plants that are resistant to aphids to benefit crop production for farmers around the world.

Keywords: cowpea, plant, transformation, *Aphis craccivora*, resistance

Aphids are one of the most devastating agricultural pests that cause major yield losses. They are the most common group of virus vectors and can transmit two major viruses as they feed on plant phloem (Fouad 2016). The cowpea aphid, *Aphis* is known to inject a powerful toxin into the cowpea that can stunt or kill the plant (DAF 2010). Meanwhile, their feeding produces a honeydew excretion that causes a growth of sooty mold on the cowpea plants that reduce their chances of photosynthesis (DAF 2010).

Application of synthetic pesticides is the primary practice for insect pest control in the United States approximating to over one billion pounds of pesticides used per year and 5.6 billion pounds used worldwide (Alavanja 2009). This approach is not only costly, but also unfriendly to the environment and may be one of the reasons for the extinction of other beneficial insects as they are exposed to the chemicals through their everyday pollinating activities (Como et al. 2017).

Other approaches such as biological control are time consuming and the introduction of new species into an environment has been proven, in many cases, to be controversial (Lacey 2015). A balance of environmental, social, and economic systems is now what ecologists and farmers are aiming for (Boussemart 2016). With this, plant transformation methods are being

considered. Plant transformation is the approach whereby an organism's DNA is inserted into the genome of a species of interest creating a transgenic plant for both research and agriculture (Cornell University 2017). Identifying the host plant defense genes involved in plant-aphid interaction will help best design pest management strategies. This long-term research aims to characterize the molecular mechanism underlying plant-aphid interaction.

Methods

Planting the Cowpea Seeds. Both resistant (IT98K-205-8) and susceptible (IT98K-589-2) cowpea seeds were planted in a growth chamber. The plants were kept under a ~25ºC and 12:12 L:D photoperiod making sure all plants were located on the same shelf under the same heat and humidity levels. Once the cowpea plants grew their first trifoliate, six different trifoliate were chosen from each line. They were chosen to resemble uniformity throughout the line by looking at similar size, shape and color to ensure they were genetically close to each other. Each cowpea leaf petiole was place in a half cut 15 mL conical centrifuge tube (Falcon, Corning, NY) to hold water for the leaf and sealed with parafilm (Bemis, Sheboygan Falls, WI) to prevent any leakage.

Fig. 1. The sealed trifoliate leaves ready for a five-day aphid screening.

Age synchronizing cowpea Aphids. Four days prior to adding the aphids to the selected leaves, 250 aphids were chosen from the kept aphid culture in the lab. They were brushed off the culture's HEB cowpea plants (HEB, San Antonio, TX) using a small camel's hair brush (Fisherbrand, Waltham, MA) and placed on newly non-infested HEB trifoliate leaves. The trifoliate leaves were kept in separate petri dishes (Fisherbrand, Waltham, MA). Fifty aphids were placed on each of these leaves. After 24 hours, the adults were removed leaving only progeny that were the same age to receive the most accurate data. The progeny were given 48 hours to grow before transfer.

Transfer of Cowpea Aphids. After the 48 hours, four of the growing progeny were placed on each line selected leaves. The progeny individuals were chosen based on their similar size and color for accurate data. Each leaf was placed in a petri dish (Fisherbrand, Waltham, MA) to prevent

aphids from escaping. Three petri dishes from each line were placed in two different laboratory rooms to challenge the issues of variation in temperature and heat and to make sure conditions did not favor one line over the other. Each day for five days, the infested cowpea leaves were observed and documented looking for newly emerged progeny and survival rates for each line. The data was then analyzed using one-way ANOVA as well as the post-hoc Tukey HSD test.

Results

Both lines, resistant and susceptible, resulted in significantly different results in both room 127 and room 124 ($p=0.0196$ and $p=0.0483$), respectively. The average progeny and survival rates for each day were totaled (Figure 2). The progeny for line 589-2 resulted in an average of approximately 45.7 and 52.3 newly emerged progeny while line 205 resulted in 15 and 0.33 total averages for newly emerged progeny. The 589-2 line had a significantly larger progeny average than that of line 205 as the p-values tested all resulted to less than 0.05 for each separate room experiment (Table 1).

Table 1. Lines 589-2 and 205 emerged progeny data were averaged per day; averages were totaled for a comparison of progeny averages and p-values against each line in each room under laboratory conditions.

Room	Line	Average Total	ANOVA	Tukey
Room 127	589-2	45.67	0.0196	0.0196
	205	15.00		
Room 124	589-2	52.33	0.0483	0.0483
	205	0.33		

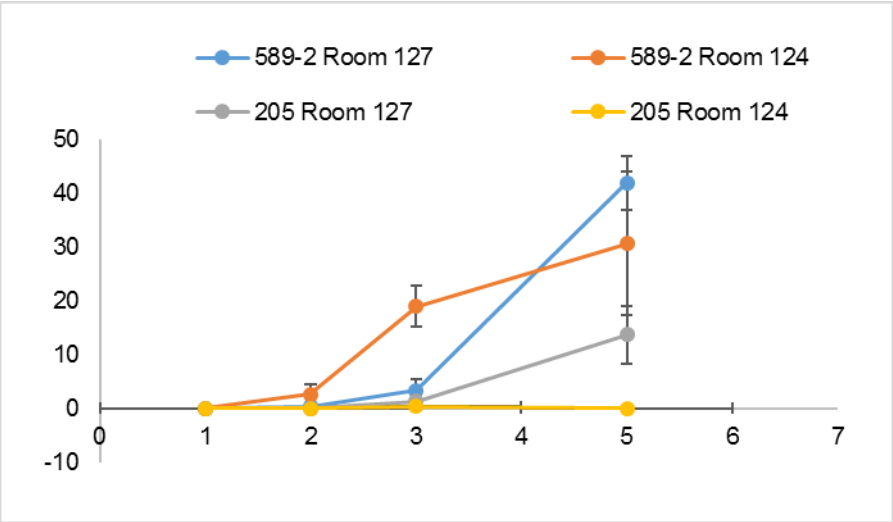

Fig. 2. The daily progeny averages were totaled and graphed along with their designated standard errors.

Survival averages per day were also recorded for each line and each room along with their standard errors (Figure 3). Line 589-2 in room 124 showed a survival average less than 100% towards day three. Line 205 population began to decrease after the first day of observations. Room 127 showed no significant difference between both lines (p=0.0924) yet the lines in room 124 had a significant difference of (P=0.0057) (Table 2).

Table 2. Survival average percentages taken daily for each line and each room along with respective p-values under laboratory conditions.

		Survival Rates				ANOVA	Tukey
	Line	Day 1	Day 2	Day 3	Day 5		
Room 127	589-2	100%	100%	96%	100%	0.0924	0.0924
	205	79%	58%	54%	29%		
Room 124	589-2	100%	100%	92%	75%	0.0057	0.0057
	205	67%	25%	17%	0%		

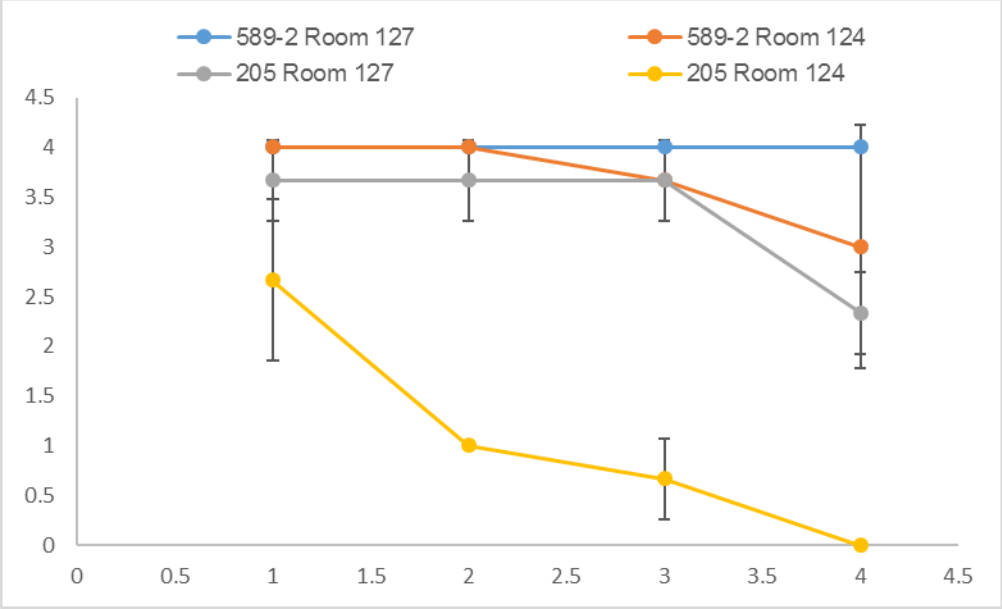

Fig. 3. A graphical representation of survival per day for lines 589-2 and 205 along with their standard error bars.

Discussion

Controlling the aphid population is currently an issue that is being taken care of using environmentally harmful and costly methods. With their parthenogenetic reproduction, an aphid population can go from zero to a few hundred in one day. The viruses they vector such as the broad bean mosaic virus, groundnut rosette virus, and cucumber mosaic virus are costly to farmers around the world (DEEDI 2009). The honeydew excretion alone is detrimental to plants overtime causing soot to occur (DAF 2010).

As seen in this experiment, the overall result was that the resistant line was significantly lower in progeny production than that of the susceptible plant. The survival of the aphid population also decreased on each leaf from day one. This is caused by the aphids not wanting to feed on the resistant plants that they find not enticing. Many of the progeny that died, starved to death and many of the living progeny ate too little to gain the needed nutrients to grow into adults and produce their own progeny. The two rooms showed a similar pattern in progeny emergence and survival rate, yet room 127 was warmer possibly increasing the development of the aphids causing a greater difference for each test.

Possibly limitations to accurate results could have been caused by the petri dishes that were used. Insects were quick to escape the plants as they could fit through the crevices of the petri dish top and bottom plates. The escaped aphids were counted as deceased for the experiment as we assumed they escaped because they did not find the cowpea plant they were placed on appetizing. We assumed it was a sign of resistance. It would be best to recreate this experiment with better means of holding the cowpea leaves with the cowpea aphids to minimize escaping individuals.

Plant transformation methods are the new tool of research that could potentially be the answer to control the aphid population. More research should go into making cowpea plants resistant to aphids to benefit crop production for farmers around the world.

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