The Great Carrot Showdown: A Study on the Egg-Laying Preferences (Yellowstone, Danvers, Resistafly, and Cosmic Purple varieties) by Female Carrot Weevil Jenna Mason

Introduction

Carrots are a go-to healthy snack for all ages. Based on the 2023 wholesale value "the approximate price range for the US carrot is between US\$1.15 per kilogram and US\$0.52 per pound (US Carrots Prices, 2023)."

"Carrot production can be a profitable market for small-scale farmers and resource-poor growers, as carrots are short-duration crops and higher produce can be obtained per unit area. In terms of area, production and market value, carrots are among the top ten most economically important vegetable crops in the world (Sharma et al., 2020)."

Carrots come in a variety of shapes, sizes, flavors, and colors. There are 4 main carrot categories: imperator, danvers, nantes, and chantenay. Each variety has its preferred growing conditions. The rise in carrot popularity happened after 1986 when the market was introduced to the "baby-cut" carrot. Carrots are "a member of the Apiaceae family, which also includes celery, dill, and cilantro." It is a cool-seasonal crop that is planted directly into the ground. They are high in essential nutrients such as "beta carotene, vitamin K, potassium, minerals, and dietary fiber (Carrots, 2021)."

Unfortunately, the food that is grown for our survival also provides resources for pests to flourish. Carrot weevil, *Listronotus oregonensis* (LeConte, 1857) (Curculionidae), is a predominant pest in carrot fields (hence their name). Carrot weevil damage is highly significant, "as these specialty crops are valued at \$1.3 billion annually in the United States." Carrot weevils go through complete metamorphosis (egg, instar, pupa, adult) and have two generations per year. They can easily be found from mid-May until harvest season, on any accessible carrot plant (outside, inside, storage). Adults will feed on the carrot's leaves and petioles, preventing the plant from successfully growing, thus decreasing its quality. After mating, females deposit eggs into the carrot's petioles or near the crown. When the eggs hatch, larvae will chew through the tissue and head towards the roots. This stage is the most destructive because it causes major internal damage, causing quality reduction. Larvae will move to the next carrot after it has finished feeding on the carrot's roots it hatched from. Lastly, larvae will pupate below the soil's surface and into an adult, restarting the life cycle (Justus & Long, 2019).

Signs of Infestation

To identify carrot weevil damage first scout for the adults, they will be on the outside of the carrot plant. Adult carrot weevils are small (approx. 1/6" long). An adult can be identified by their brown coloration and light brown or copper scales (Fig.1). Female carrot weevils lay their eggs in the carrot's petioles or near the crown (Fig.2). To identify an egg mass, look for an oval black dot (anal secretion) on the plant. Larvae are more difficult to scout, as they are feeding into the plant (Fig. 3). Larvae "are legless, cream colored, and have a light brown head capsule (Justus & Long, 2019)."

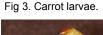
For a more in depth description about the basic biology of the carrot weevil, I recommend the publication titled "Biology and Management of the Carrot Weevil (Coleoptera: Curculionidae) in North America" researchers Emily J. Justus and Elizabeth Y. Long.

Fig 1. Carrot weevil, *Listronotus oregonensis*.

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Fig 2. Oviposition scars on petiole of carrot plant.







Research Question

Luckily, as a growing society, we have come up with solutions to combat these pests. One integrated pest management strategy is to use a resistant (but still delicious) carrot variety. For this experiment we wanted to test if female carrot weevils have an oviposition variety preference. When presented with either Yellowstone, Cosmic Purple, Danvers, or Resistafly carrot varieties (varieties included were based on results of field trial in 2021 - prior to joining the laboratory)

The first experiment simulated the carrots' natural growth in the fields and focused on the location of where the females laid their eggs in the carrots. The second experiment examined if weevils showed a preference when only presented with carrot on the volatiles of two varieties: Danvers and Resistafly.

Methods

Experiment #1

The first experiment was to determine the oviposition preferences of female carrot weevils, given the varieties Yellowstone, Cosmic Purple, Danvers, and Resistafly. A randomized complete block design was used (with 5 experimental replicants) Materials included: plastic plant pots (17.8 cm tall x 16.5 inches diameter), a variety of each carrot (Yellowstone, Cosmic Purple, Danvers, and Resistafly), netting bags with zippers to prevent the weevils from escaping, bamboo sticks to hold up the nets, rubber bands to secure the mesh and pot together, sorted 1:2 male and female weevil ratio, non-toxic powder dust to easily find the weevils, and weigh boats.

The experiment was conducted with carrots that were planted the first full week of July 2022. Adults (reared in the lab and were between two weeks and 1 month old) were sexed and dusted with a non-toxic Flo powder before assembling the entire experiment. The experiment had 5 replicates, each replicant group (1-5) was randomized to have a total of 12 carrot plants with two carrot varieties (treatment) per pot.

For each pot, a bamboo stick was placed in the middle of the pot closest to the rim with the mesh bag over the entire pot. Then secured to the bottom with rubber bands. A weigh boat was placed in the pot between the two carrot varieties, each pot got a set of 1 male and 2 female. The experiment ran for 3 days. If no egg scars were present after 3 days, plants were watered and checked for eggs scars after an additional 3 days. After the extended days, it was time to measure the plant and count the number of eggs. The total number of egg scars and the number of eggs on each carrot plant was recorded. Lastly, each carrot plant was measured from the crown to the tallest leaf tip and the number of stems were recorded.

Experiment #2

For the second experiment, we examined egg-laying and feeding preferences of female carrot weevils on Danvers and Resistafly carrot varieties. We conducted a randomized complete block design for each replicant group (Danvers or Resistafly being on the left or right side). This experiment required 10 large glass petri dishes (15 cm), adult males and females pairs for 10 reps (20 total), carrots of each variety for 10 reps (20 total), a scale, a ruler, parafilm, sterilized razer (to cut the carrot squares), weigh boat, cleaning solution for petri dish. There were 10 replicates, each replicant group had 2 carrot squares (Resistafly or Danvers) per petri dish.

The petri dishes were washed with 1% Alconoz and baked at 100 degrees Celsius for 24 h to ensure there was no residue from previous experiments. The carrots were washed and dried. Two male and two female weevils (total of 20) were used in each replicant group. Prior to the experiment, they were separated from each other and the colony to starve for 24 hours. The next day was assembly, a small carrot (<2cm long by 1cm high) rectangle was cut from the Resistafly and Danvers carrots. Each variety had its own sterilized razor to expose the surface of the carrot and to prevent contamination from the other variety. After cutting and weighing the individual rectangle, it was immediately placed on the petri dish far enough apart, but not touching the glass. Two pairs of carrot weevils (2 males and 2 females) were placed in a weigh boat located in the middle of both carrot varieties. The petri dish was covered with the lid and wrapped parafilm to prevent escaping. After 48 h, slices were weighed (to the nearest gram) to compare the differences in the amount of feeding. Additionally, the number of egg scars and eggs on each slice was recorded.

Statistical Analysis

T-Test

A t-test was used to determine if there is any significant difference between the two mean groups and their relationship. Experiment #1 and Experiment #2 were both subjected to a t-test with a P-value for significance of <0.05.

Results & Discussion

The purpose of Experiment #1 study was to determine if the female carrot weevil had an oviposition preference, given the varieties Yellowstone, Cosmic Purple, Danvers, and Resistafly.

There was no significant difference in the number of eggs laid for each pair that was compared to one another between all 4 varieties (Fig. 4). The pair Resistafly vs Danvers was removed from the comparison because 0 presence of egg scars and eggs laid. Trends might indicate that Resistafly and Yellowstone had the highest average of eggs laid (Fig. 4). However, due to tremendous variability it depended on the variety it was paired with, but none were significant. Reasons for no ovipositional differences could be a non preference for varieties, the influence of the variety they were raised on or mating inconsistencies resulting in ovipositional differences. All these factors should be considered in future experiments

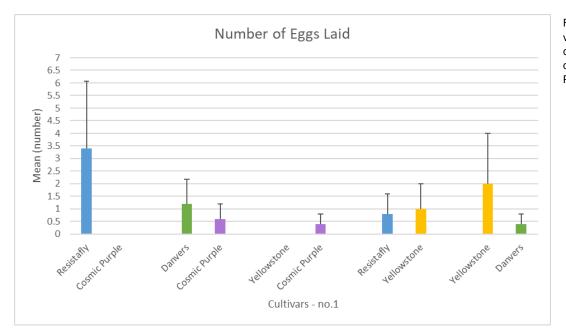


Fig 4. Number of eggs laid per variety in any of the 5 pairwise comparisons. No significant differences were found (T-test P0.05), (X±SEM). There was no significant difference in the number of egg scars for any of the pairs that were compared between all 4 varieties. An egg scar represents a successful oviposition attempt by the female and within that scar, at least one egg is found. Variation between variaties for ovipositional scarring is most likely the cause for the lack of significance (Fig. 5). Like Experiment #1, this variation might be due to the mating success of females, their age, or other environmental differences: lab temperature, carrot age, light cycle, or previous host preference.

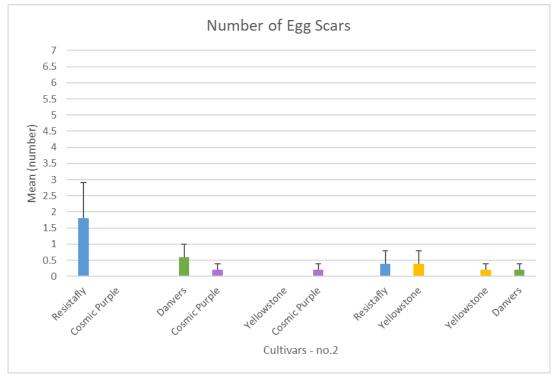


Fig 5. Number of egg scars per variety with T-test analysis for the 5 pairwise comparisons (P0.05), (X±SEM).

Cultivar Comparison	Mean no.eggs cultivar #1 (X ± SE)	T-Value	P-Value	Mean no.egg scars cultivar #2 (X ± SE)	T-Value	P-Value
Resistafly vs Cosmic Purple	0 ± 3.4	1.27	0.272	0 ± 1.8	1.616	0.181
Danvers vs Cosmic Purple	0.6 ± 1.2	0.526	0.614	0.2 ± 0.6	0.894	0.405
Yellowstone vs Cosmic Purple	0 ± 0.4	-1	0.373	0 ± 0.2	-1	0.373
Resistafly vs Yellowstone	0.8 ± 1	-0.156	0.879	± 0.4	0	1
Yellowstone vs Danvers	0.4 ± 2	0.784	0.476	± 0.2	0	1
Resistafly vs Danvers	± 0	N/A	N/A	± 0	N/A	N/A

Table 1. T-test statistics for the number of eggs laid per cultivar in the 6 pairwise comparisons.

The purpose of Experiment #2 study was to determine the egg-laying and feeding preferences of female carrot weevils on carrot varieties Danvers and Resistafly.

There was no significant difference in the number of grams of carrot eaten when comparing the two varieties: Resistafly and Danvers (Fig. 6). However, when we examine the number of eggs laid on the same two varieties, although not significantly different due to the high number of treatment groups where no eggs were laid. There was a trend towards more eggs laid in the Danvers variety (Fig. 7). The number of eggs laid in Experiment #2 on these cultivars (rs and d), was less than Experiment #1. Again, the high number of replicates with the number of eggs laid, indicates more work is needed to understand what conditions a carrot

weevil will oviposit in the laboratory. Factors such as mating success, time since mating, lab environmental conditions, carrot shape, size, presentation, and time on cultivar, all could be critical to this experimental design. Selecting and raising fertile, mated, or ready to reproduce may be flawed and thus a change in methods might reduce variation. This confirms that this experiment should be repeated to decrease variation and control for the number of zeros.

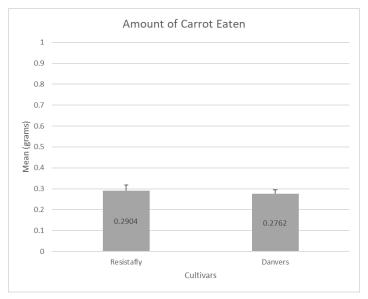


Fig 6. Amount of carrot eaten(g) per cultivar (X±SEM).

Cultivar	Resistafly	Danvers	
Mean amount eaten (X±SE)	0.29	0.276	
T-Value	0.5	99	
P-Value	0.557		
Mean number of eggs laid (X±SE)	0.2	0.4	
T-Value	-0.5	547	
P-Value	0.5	91	

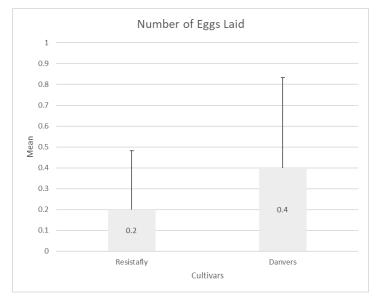


Fig 7. Number of eggs laid per cultivar (X±SEM).

Table 2. T-test statistics for the number of eggs laid and the amount of carrot consumed for the cultivars Resistafly vs Danvers.

Conclusion

Overall, there was no significant difference between the number of eggs laid, number of oviposition attempts (scars), and feeding preference in any of the cultivar pairings presents to carrot weevils. Significant additional work is needed to remove variation and understand the reproductive biology and ovipositional preference of this important pest.

Future Research

The carrot weevils used in the study were difficult to work with. There were several potential factors that might have affected the experiment's outcome. One, the age of the weevils. I would consider doing 2 to 3 different age groups (virgin - allowing a present period to mate, 1 to 6 months younger and older than 6 months). Insects generally have a consistent

pattern of oviposition. Knowing when peak oviposition occurs would be critical to understanding oviposition preference. This could help determine which age the weevils might produce more or less eggs. Two, the temperature of the laboratory, the light cycle, and lengthen the feeding time. It could be that the laboratory temperature was not ideal to mate in (21 degrees Celsius). Higher temperatures (such as field conditions) could promote the carrot weevils' mating behavior. The light cycle of the room could be too long or short. It is known in the field that shorter photoperiods reduce oviposition. Matching ideal photoperiods, rather than one that might be inducing termination of oviposition (since larvae might not complete development before seasonal changes occur). It could be that the weevils were not given enough time to reproduce or feed long enough on the cultivars. If repeated, increase the hours/day to weeks. Three, increasing the male to female ratio to each treatment group. By offering more male carrot weevils, the female's mating success could increase as there are more options and competition from the males. Lastly, it could be the original carrot host the weevils emerged on influenced their adult ovipositional preference. The colony was given the root of the carrot (store bought, probably Danvers variety). This could cause them to have a preference because they already know that this variety is suitable for their offspring.

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Data tables

Experiment #1

	Resistafly	Cosmic Purple		Danvers	Cosmic Purple		Yellowstone	Cosmic Purple
Mean	3.4	0	Mean	1.2	0.6	Mean	0	0.4
Variance	35.8	0	Variance	4.7	1.8	Variance	0	0.8
Observations	5	5	Observations	5	5	Observations	5	5
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	4		df	7		df	4	
t Stat	1.2706397		t Stat	0.52623481		t Stat	-1	
P(T<=t) one-tail	0.136365		P(T<=t) one-tail	0.30749034		P(T<=t) one-tail	0.186950483	
t Critical one-tail	2.1318468		t Critical one-tail	1.89457861		t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.27273		P(T<=t) two-tail	0.61498068		P(T<=t) two-tail	0.373900966	
t Critical two-tail	2.7764451		t Critical two-tail	2.36462425		t Critical two-tail	2.776445105	
Std Dev	5.9833101	0	Std Dev	2.16794834	1.341640786	Std Dev	0	0.894427191
Std Err	2.6758176	0	Std Err	0.96953597	0.6	Std Err	0	0.4
Pvalue	0.272		Pvalue	0.614		Pvalue	0.373	

	Resistafly	Yellowstone		Yellowstone	Danvers
Mean	0.8	1	Mean	2	0.4
Variance	3.2	5	Variance	20	0.8
Observations	5	5	Observations	5	5
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	8		df	4	
t Stat	-0.1562		t Stat	0.7844645	
P(T<=t) one-tail	0.43988		P(T<=t) one-tail	0.2383103	
t Critical one-tail	1.85955		t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.87977		P(T<=t) two-tail	0.4766207	
t Critical two-tail	2.306		t Critical two-tail	2.7764451	
Std Dev	1.78885	2.236067977	Std Dev	4.472136	0.894427191
Std Err	0.8	1	Std Err	2	0.4
Pvalue	0.879		Pvalue	0.476	

Table 3. A T-test was done for the number of eggs laid in each treatment pair to use to compare to the other treatment groups.

	Resistafly	Cosmic Purple
Mean	1.8	0
Variance	6.2	0
Observations	5	5
Hypothesized Mean Difference	0	
df	4	
t Stat	1.61644772	
P(T<=t) one-tail	0.09065171	
t Critical one-tail	2.13184679	
P(T<=t) two-tail	0.18130341	
t Critical two-tail	2.77644511	
Std Dev	2.48997992	0
Std Err	1.11355287	0
Pvalue	0.181	

	Danvers	Cosmic Purple
Mean	0.6	0
Variance	0.8	0
Observations	5	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.894427191	
P(T<=t) one-tail	0.202770402	
t Critical one-tail	1.943180281	
P(T<=t) two-tail	0.405540804	
t Critical two-tail	2.446911851	
Std Dev	0.894427191	0.4472135
Std Err	0.4	C
Pvalue	0.405	

	Yellowstone	Cosmic Purple
Mean	0	0.2
Variance	0	0.2
Observations	5	5
Hypothesized Mean Difference	0	
df	4	
t Stat	-1	
P(T<=t) one-tail	0.186950483	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.373900966	
t Critical two-tail	2.776445105	
Std Dev	0	0.447213595
Std Err	0	0.2
Pvalue	0.373	

	Resistafly	Yellowstone		Yellowstone	Danvers
Mean	0.4	0.4	Mean	0.2	0.2
Variance	0.8	0.8	Variance	0.2	0.2
Observations	5	5	Observations	5	5
Hypothesized Mean Difference	0		Hypothesized Mean Difference	0	
df	8		df	8	
t Stat	0		t Stat	0	
P(T<=t) one-tail	0.5		P(T<=t) one-tail	0.5	
t Critical one-tail	1.859548		t Critical one-tail	1.85954804	
P(T<=t) two-tail	1		P(T<=t) two-tail	1	
t Critical two-tail	2.3060041		t Critical two-tail	2.30600414	
Std Dev	0.8944272	0.894427191	Std Dev	0.4472136	0.447213595
Std Err	0.4	0.4	Std Err	0.2	0.2
Pvalue	1		Pvalue	1	

Table 4. A t-test was done for the number of egg scars in the each treatment pair to use to compare to the other treatment groups

Experiment #2

	Resistafly	Danvers
Mean	0.2904	0.2762
Variance	0.0037196	0.0018964
Observations	10	10
Hypothesized Mean Difference	0	
df	16	
t Stat	0.59920413	
P(T<=t) one-tail	0.27871339	
t Critical one-tail	1.74588368	
P(T<=t) two-tail	0.55742678	
t Critical two-tail	2.1199053	
Std Dev	0.06098852	0.04354768
Std Err	0.0272749	0.01947511
Pvalue	0.557	

Table 5. A t-test was done for the amount of carrot eaten in Resistafly vs Danvers

	Resistafly	Danvers
Mean	0.2	0.4
Variance	0.4	0.93333333
Observations	10	10
Hypothesized Mean Difference	0	
df	16	
t Stat	-0.54772256	
P(T<=t) one-tail	0.29572105	
t Critical one-tail	1.74588368	
P(T<=t) two-tail	0.5914421	
t Critical two-tail	2.1199053	
Std Dev	0.63245553	0.96609178
Std Err	0.28284271	0.43204938
Pvalue	0.591	

Table 6. A t-test was done for the number of eggs laid in Resistafly vs Danvers