Diet Quality Mediated Effects on *Manduca sexta* and its Specialist Parasitoid, *Cotesia congregata*, Over a Gradient of Host Plants

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Introduction:

Herbivores tend to eat toxic and seemingly unhealthy plants. These toxic plants cause the herbivores to have reduced fitness. At the same time the secondary metabolites can give the herbivore defenses against its predators and parasites. This trade off between the plant and its predator is the result of coevolution. The plant's defense mechanism against the herbivore becomes the herbivore's defense. This can be seen in the monarch butterfly and the milkweed. The plant produces cardenolides to fend off herbivores and the caterpillar sequesters the toxin and uses it as its own defense against predators (Price et al, 2011). Another example of this tradeoff can be seen with *Manduca sexta* and Solanaceae plants. These plants contain the allelochemical nicotine. The caterpillar is able to feed on the plant despite the fact that the plant causes delayed growth. Research has shown that lower concentrations of nicotine have little to no effect on the caterpillar (Hunter, 2003). The caterpillar uses the nicotine to help defend itself against parasites, predators and pathogens. *Manduca sexta* doesn't sequester the toxin but rather it detoxifies the nicotine by excreting it and traces of the toxin stay in the hemolymph (Price et al, 2011). *Manduca sexta* uses the nicotine to help fight off the parasitoid *Cotesia congregata*. Research done by Barbosa et al (1991) suggests that nicotine is directly toxic for parasitoids. It is expected that plants with higher nicotine concentrations will have less successful parasitism rates, longer developmental time and overall smaller relative fitness of the wasp (Barbosa, 1991; Gunasena, 1990). This study takes a look at why the caterpillar prefers to eat a plant that is bad for it, and how the parasitoid performance is changed over a gradient of the host's diet. I believed that the caterpillar would perform worst on the tobacco and the wasps would perform the best on the lower nicotine plants. A comparison of wasp performance on tobacco and tomato has not been done before.

Methods:

I planted three species of nicotine containing Solanaceae plants: flowering tobacco (*Nicotiana alata*) with low nicotine concentrations, tomato (*Solanum lycopersicum*) with low concentrations of nicotine, and smoking tobacco (*Nicotiana tabacum*) with high nicotine concentrations. I germinated seeds with micro germination mix. At 4 weeks the plants were transplanted into six-inch pots and supplemented with fertilizer water. At 6 weeks I put ten neonates on each plant, four plants of each variety, placed in random order in the greenhouse. At the fourth instar the caterpillar was stung. To sting the caterpillar I held it in the cage of wasps and let a female sting the caterpillar for five

seconds or less twice. The caterpillar was then put back on the plant where they were allowed to feed. When the plant reached poor health or was completely consumed a new plant of the same age replaced it. The caterpillar was collected when the wasps spun cocoons. The cocoons were then counted, and then the wasps emerged as adults and died. Then the wasps were separated by sex and counted again. The wasps were then measured and massed. To measure the wasp to determine performance, both hind tibias were measured in millimeters by using the Leica Las EZ software program for basic measurements. For mass and measurement I used a random subset of five.
Performance Assay: Neonates were reared on the plants for eight days then massed for performance data.

Results:
I ran five ANOVA tests on herbivore performance, wasp sex ratio, wasp tibia length, wasp mass, and total cocoon number to determine if the different plants had different effects on performance (tables). Male wasp weight on flowering tobacco was significantly different than the male weight on tomato and smoking tobacco (Fig 1). Female wasp weight followed the trend in the male weight (Fig 1). Wasp tibia length for male and female both were not significantly different which is the opposite from the expected outcome. The female wasp tibia length was larger than the male tibia length (Fig 2). The tomato plant had the larger female tibia lengths (Fig 2). The smoking tobacco produced more female wasps than male wasps, which is the opposite of the sex ratio seen on the tomato and the flowering tobacco (Fig 4). Flowering tobacco produced the highest number of males (Fig 3). The flowering tobacco had higher wasp egression (Fig 5). Tobacco had lower average cocoon, total wasps, and egressed wasps (Fig 5). Tobacco also had a slightly higher average dead wasp total than the other plants (Fig 5). Flowering tobacco and tomato shared similar wasp response with total wasps, cocoon totals, male and female totals and dead wasps (Fig 5). The performance assay showed that the caterpillars performed best on the tobacco with highest nicotine concentration and poorest on the lower nicotine concentration. The caterpillars performed better on the tomato than the flowering tobacco (Fig. 6).

Discussion:
Manduca sexta has coevolved with the smoking tobacco plant, which can explain why it performed better on it than the lower nicotine plants. A study done by Pavan (2013) shows the coevolution between *Manduca sexta* and the tobacco plant in regards to nicotine and plant defensive chemicals can become the caterpillar’s defenses. The wasps performed better on the tomato and flowering tobacco because they are less toxic and provided better quality of hosts. This can explain why the sex ratios are so high, because the tobacco has poor resources for the wasp so the wasp puts more resources into producing females. With better host quality, a wasp will produce more males. The male

wasp weight is higher for the smoking tobacco than the flowering tobacco indicating that the smoking tobacco is a better nutrient quality host. The reasoning behind why the tobacco produced fewer cocoons is unknown, that could be due to fewer eggs being laid, or a lower survival rate. It is unknown why the differences of the wasp tibia length are not significant between the different plants. The use of real plants instead of diet adds more unknown variables and the plant's other chemicals and defenses could be the cause of the trends shown. With more replication the differences in the sex ratio can be further explored. *Manduca sexta* is a common garden pest and this study shows that maintaining a high wasp density can help control the pest problem on low nicotine plants such as tomato or pepper. For higher nicotine plants like smoking tobacco wasps can be used for control but they are not as effective and will require other control methods.

Works Cited

Barbosa et al. 1991. Influence of Plants Allelochemicals on the Tobacco Hornworm and its Parasitoid, *Cotesia congregata*. Ecology, Vol. 72, No. 5, pp. 1567-1575.

Gunasena et al. 1990. Effects of Nicotine on Growth, Development, and Survival of the Tobacco Budworm (Lepidoptera: Noctuidae) and the Parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). Journal of Economic Entomology, Vol. 83, No. 5, pp. 1777-1782.

Hunter et al. 2003. Effects of Plant Quality on the Population Ecology of Parasitoids. Agricultural and Forest Entomology, Vol. 5, No. 1, pp. 1-8.

Pavan et al. 2013. Natural history-driven, plant-mediated RNAi-based study reveals *CYP6B46*'s Role in a Nicotine-Mediated Antipredator Herbivore Defense. Proceedings of the National Academy of Sciences, Vol. 111, No. 4, pp. 1245-1252.

Price et al. 2011. Insect Ecology, Behavior, Populations and Communities. Cambridge University Press, New York, New York, U.S.A. pp. 493.

Van Nouhuys et al. 2008. Population Dynamics and Sex Ratio of a Parasitoid Altered by Fungal-Infected Diet of Host Butterfly. Proceedings of the Royal Society B: Biological Sciences, Vol. 275, No. 1636, pp. 787-795.

Figures

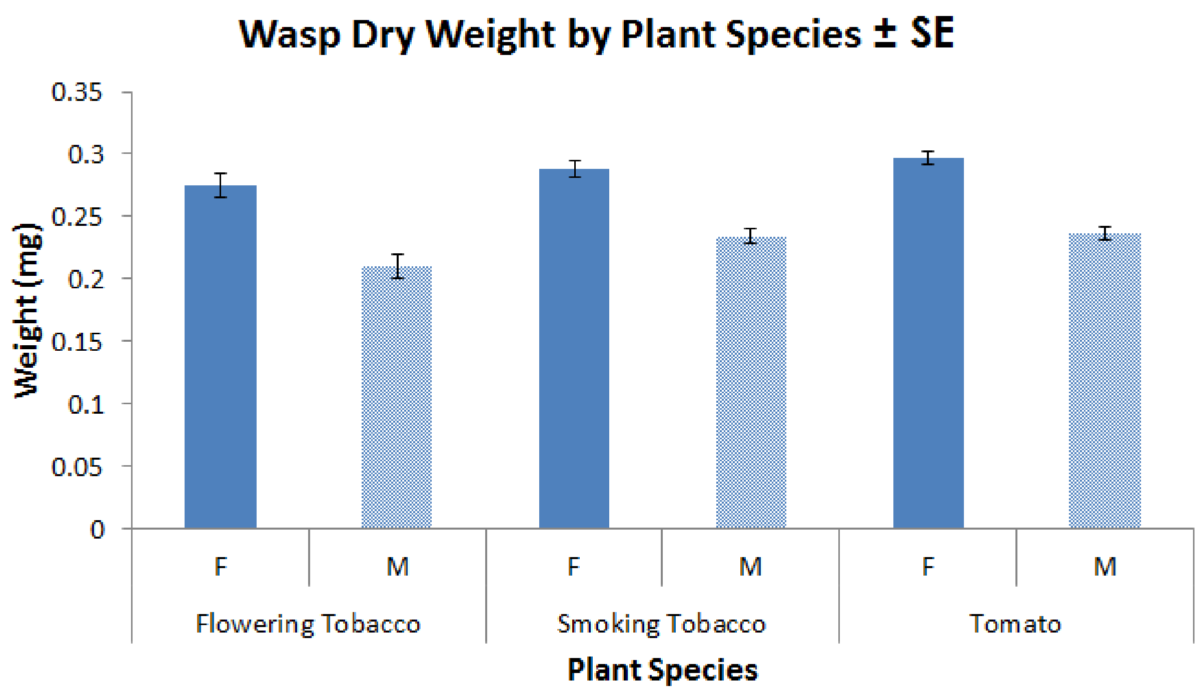


Fig 1

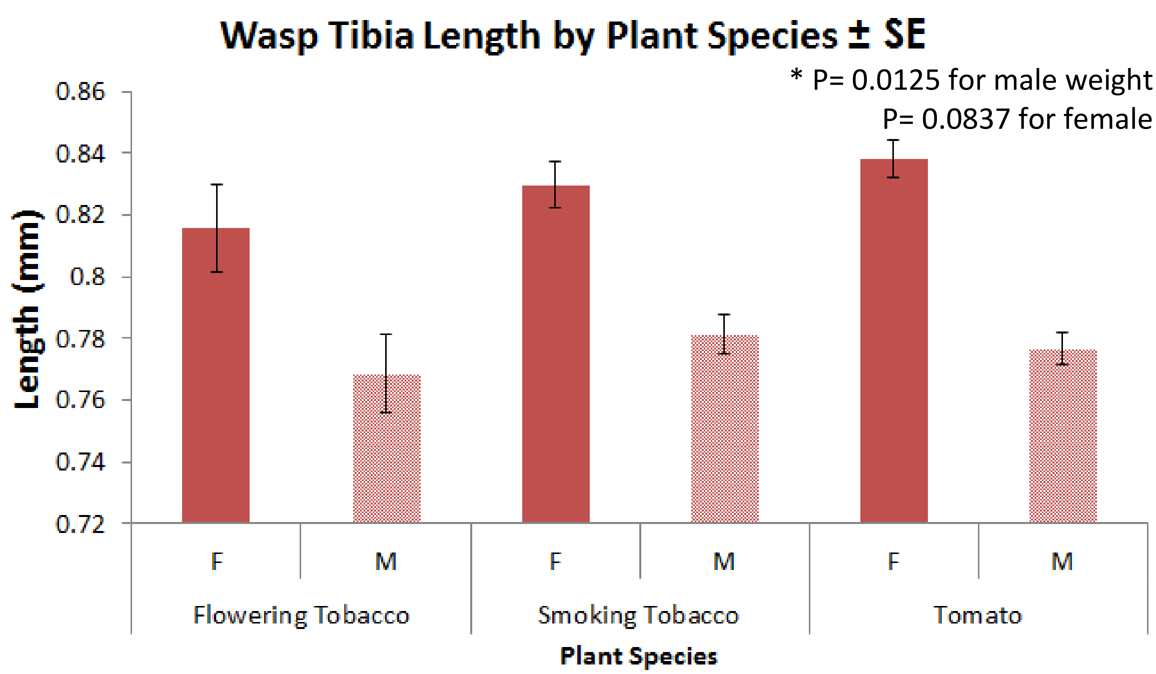
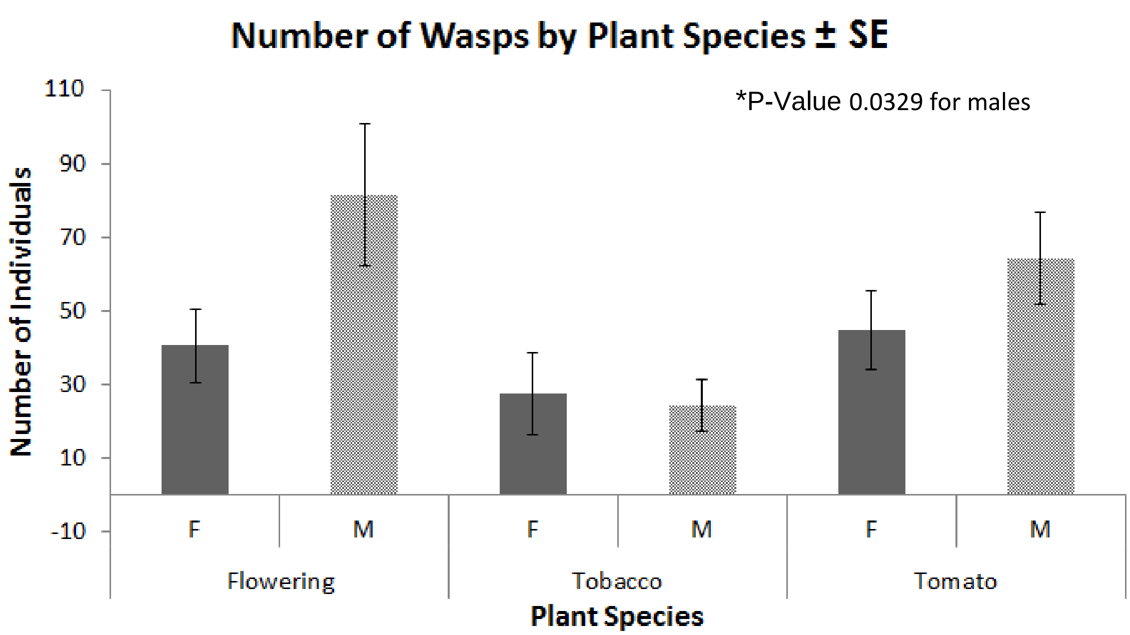

Wasp Tibia Length by Plant Species ± SE
* P= 0.0125 for male weight
P= 0.0837 for female
Length (mm)
0.86
0.84
0.82
0.8
0.78
0.76
0.74
0.72
F
M
F
M
F
M
Flowering Tobacco
Smoking Tobacco
Tomato
Plant Species

Fig 2



Number of Wasps by Plant Species ± SE
*P-Value 0.0329 for males
Number of Individuals
110
90
70
50
30
10
-10
F
M
F
M
F
M
Flowering
Tobacco
Tomato
Plant Species

Fig 3

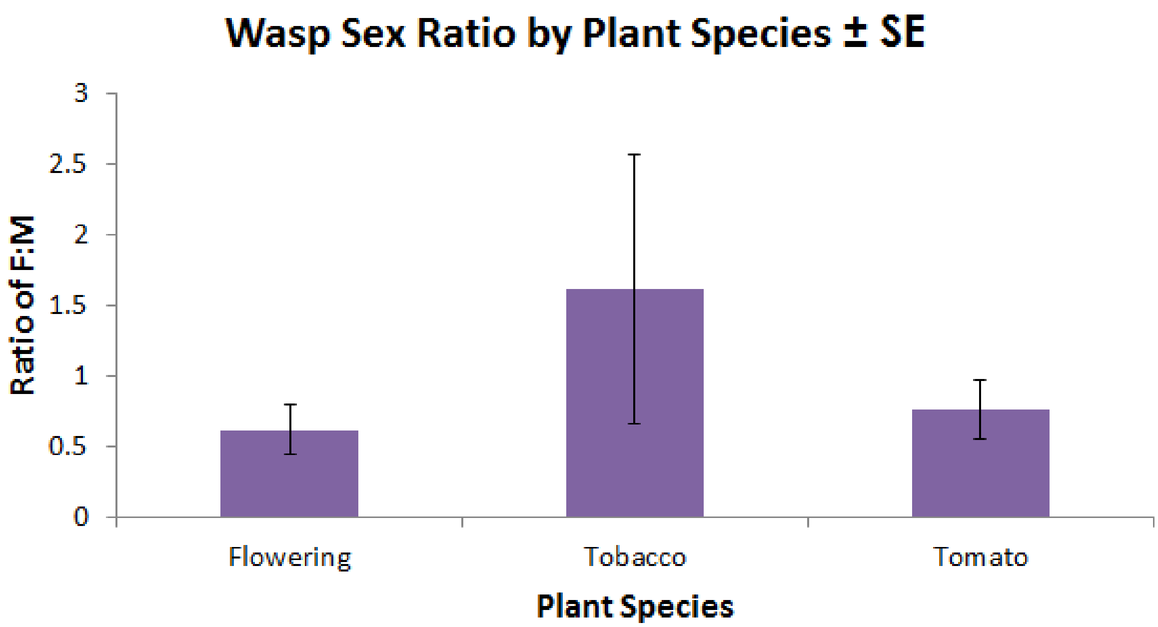

Wasp Sex Ratio by Plant Species ± SE
3
2.5
2
1.5
1
0.5
0
Ratio of F:M
Flowering
Tobacco
Tomato
Plant Species

Fig 4

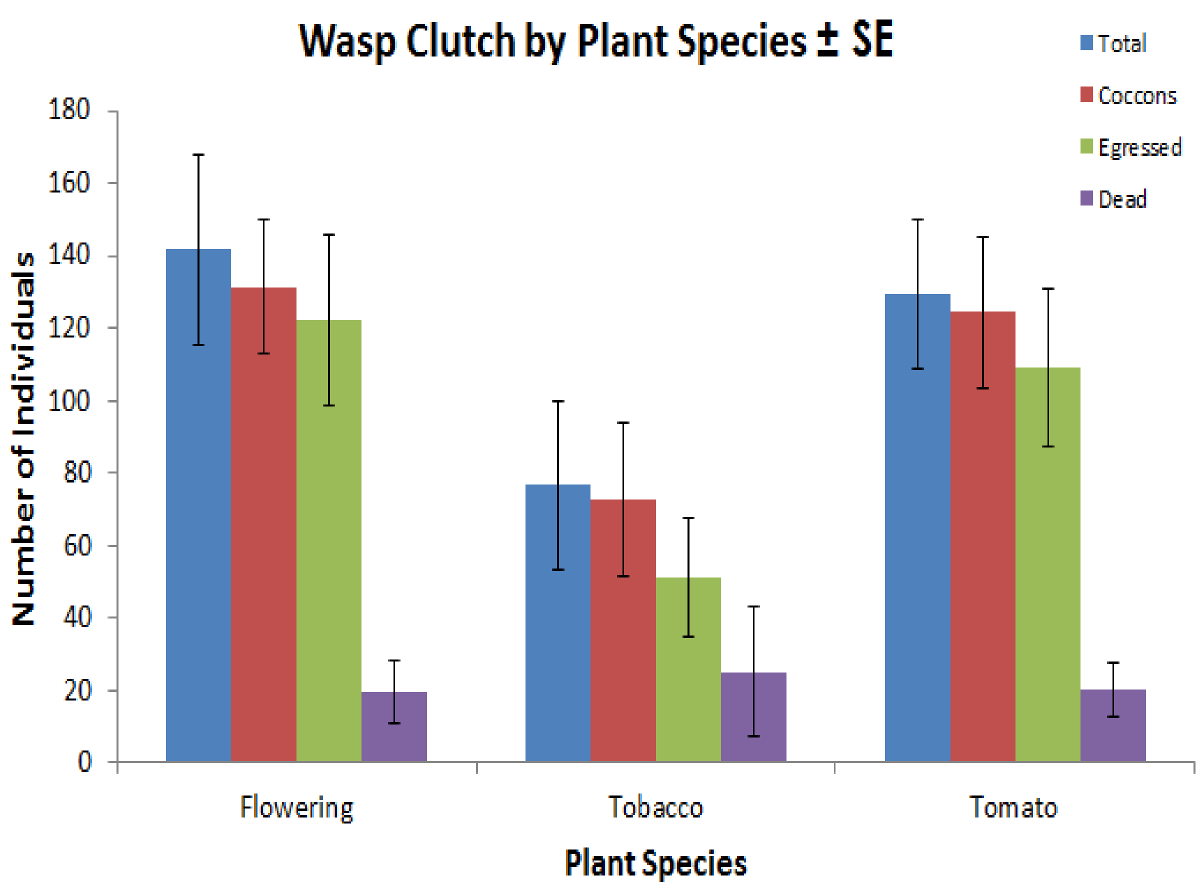

Wasp Clutch by Plant Species ± SE
Total
Coccons
Egressed
Dead
Number of Individuals
180
160
140
120
100
80
60
40
20
0
Flowering
Tobacco
Tomato
Plant Species

Fig 5

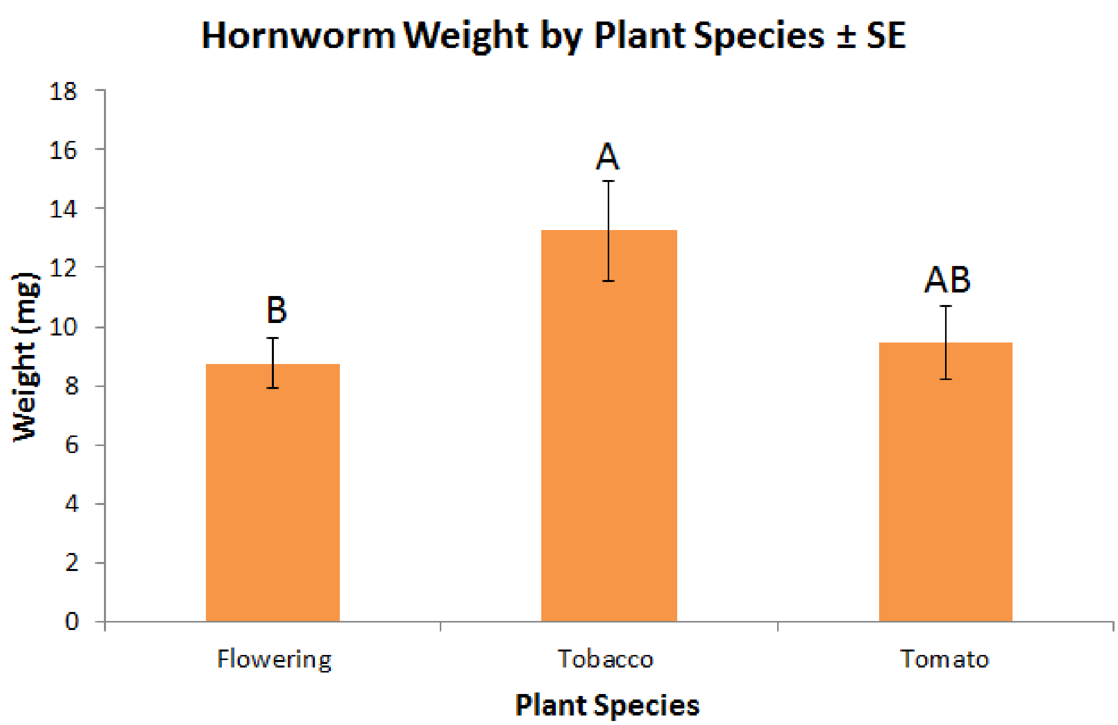

Hornworm Weight by Plant Species ± SE
Weight (mg)
18
16
14
12
10
8
6
4
2
0
B
A
AB
Flowering
Tobacco
Tomato
Plant Species

Fig 6

Tables

Anova Male Weight					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	0.01296524	0.006483	4.5613	0.0125
Error	110	0.15633526	0.001421		
C. Total	112	0.1693005			

Table 1

Anova Male Leg Size					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	0.00230806	0.001154	0.5712	0.5665
Error	110	0.2222424	0.00202		
C. Total	112	0.22455046			

Table 2

Anova Female Weight					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	0.00706005	0.00353	2.5461	0.0837
Error	95	0.13171151	0.001386		
C. Total	97	0.13877156			

Table 3

Anova Female Leg Size					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	0.00745749	0.003729	1.4349	0.2432
Error	95	0.24686211	0.002599		
C. Total	97	0.2543196			

Table 4

Sex Ratio					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	4.979225	2.48961	0.867	0.432
Error	26	74.655814	2.87138		
C. Total	28	79.635038			

Table 5

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Total Cocoons					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	17908.71	8954.35	1.8118	0.1834
Error	26	128496.53	4942.17		
C. Total	28	146405.24			

Table 6

Total Wasps					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	20002.83	10001.4	1.8685	0.1745
Error	26	139170.62	5352.7		
C. Total	28	159173.45			

Table 7

Males					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	13151.943	6575.97	3.9045	0.0329
Error	26	43789.022	1684.19		
C. Total	28	56940.966			

Table 8

Females					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	1659.66	829.83	0.6177	0.5469
Error	26	34926.133	1343.31		
C. Total	28	36585.793			

Table 9

Dead Wasps					
Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	167.396	83.7	0.0602	0.9417
Error	26	36161.156	1390.81		
C. Total	28	36328.552			

Table 10

Analysis of Variance for Caterpillar Performance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Plant	2	293.3743	146.687	3.7247	0.0293
Error	66	2599.2584	39.383		
C. Total	68	2892.6327			

Table 11