

Effects of mite parasitism, pesticide addition, and variable nitrogen content on plant feeding preference in the clear-winged grasshopper (*Camnula pellucida*)

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Abstract: High nitrogen levels in plant material have traditionally been preferred by many species of foraging grasshoppers. However, as more species of grasshoppers are experimented upon, nitrogen may not always have a universal effect on grasshoppers. Additionally, the introduction of numerous pesticides and noxious compounds into grasshopper habitat and how this affects preference shifts in foraging has also received little attention. This paper examines the role of low and high nitrogen levels and low and high pesticide levels in food preference for the clear winged grasshopper *Camnula pellucida*. Fertilizers and the naturally occurring noxious chemical pyrethrin were applied in various combinations to dandelion leaves to test for thresholds in food preference switching. Results arcsine-transformed from a Manly test and applied to a one-way ANOVA indicate that *C. pellucida* ideally forages on the highest nutrient, lowest pyrethrin containing leaves it can find ($F_{6,63}=2.347$, $p=0.041$); after this general threshold, the preferences become more ambiguous. Improved experimental design will likely determine the relative importances of nitrogen and pyrethrin alone and their interaction on grasshopper food preference. In addition, the effect of food preference as a manifestation of mite parasitism was tested. No significant relationship between the two was found, indicating that mite parasitism may exact long term costs, though day to day costs are relatively low.

Introduction:

Grasshoppers have persuasively been shown to prefer food sources high in nitrogen (Joern and Behmer 1998, Belovsky 1986). When fed artificial diets, grasshoppers will often readjust intake of nitrogenous compounds if given the choice (Simpson et al. 1988). In fact, depending on ambient nitrogen content, self regulation of nitrogen intake for certain grasshoppers can reach 90% of their intake (Defoliart and Fielding 2008). Interference with protein intake in grasshoppers has been shown to result in tissue resorption and symptoms resembling starvation (Olfert et al. 1992). Despite the fact that most grasshoppers have evolved to effectively process the most abundant food source in their surroundings, low-nitrogen grasses (Wyoming Agricultural Station Bulletin, September 1994), many grasshopper species incorporate some sort of more nitrogen rich food source in their diet, such as forbs. This is what is known as a mixed diet. Mixed diets do not feature equal proportions of their dietary components, however; grasshoppers eating a mixed diet will still exhibit nitrogen preference and often selectively feed on forbs rather than grasses for their elevated nitrogen content (Jonas and Joern 2008).

This preference for high nitrogen, however, is not universal or free of refutation: when

presented with fertilized plant material, *Ageneotettix deorum*, a grass- and sedge- mixed diet grasshopper, consumed more intermediately-fertilized plant material, not highly-fertilized plant material. Other grasshoppers exhibited inconsistent preferences and other mixed-diet grasshoppers did not change their foraging based on this fertilization (Heidorn and Joern 1987). Additionally, in an eight year study performed by Ritchie (2000), it appeared that mixed-feeding grasshoppers did not respond to a change in nitrogen content of nearby grass habitat. Furthermore, survivorship of *Phoetaliotes nebrascensis* has been witnessed to be highest on plant material of low nitrogen food content (Joern and Behmer 2002). Thus, a uniform explanation across species and food types is unlikely to be reached, placing importance on understanding each species' dynamic relationship with nitrogen to inform local biocontrol efforts.

Little to no research with regard to changing nitrogen content on food preference adjustments has been done on *Camnula pellucida*, a grasshopper species native to Grasshopper Nation (GN), a former agricultural field on University of Notre Dame Environmental Research Center (UNDERC) in Gogebic County, Michigan. *C. pellucida* is especially intriguing as a study organism because, despite the prevailing preference of grasshoppers to incorporate forbs as the majority of their mixed diet, *C. pellucida* often relegates forbs to fill only a small portion of its total diet. Additionally, previous work on *C. pellucida* contextualizes it within a disparate ecosystem in Canada (Pickford and Reigert 1964). Finally, no work has been done on fertilizing the food source that constitutes a minority of a mixed-feeder's diet; both the aforementioned Heidorn and Joern 1987 study and the Ritchie 2000 studies fertilized grasses, the food source that constitutes the majority of the studied grasshoppers' diets. *C. pellucida* is again an ideal candidate for a study that fertilizes forbs as well because forbs comprise a small percent of their diet (Wyoming Agricultural Experiment Station Bulletin 912, September 1994). This study will examine the effect of high and low nitrogen

quality of forbs on *C. pellucida* food preference, to help corroborate or refute claims of fertilization effects of one food type on mixed-feeders.

Jonas and Joern assert that because such a strong preference for nutrient ratios was exhibited by *Melanoplus bivitattis* in a 2008 study, nutrient plant composition may trump any effect of secondary defensive plant chemical production on foraging. However, the range of plant defense chemicals that have been empirically tested and not merely suggested remains rather small. Such a chemical that has known effects on grasshopper life history and is present in nature is pyrethrin, a collection of esters derived from the chrysanthemum *Chrysanthemum cinerariaefolium*. The chemical attacks the nervous system of insects (Jovetic and de Gooijer 1995). The chrysanthemum is abundant at GN, a former agricultural field on University of Notre Dame Environmental Research Center (UNDERC) property in Gogebic County, Michigan.). A preference study that presents plants of varying nutrient quality selectively treated with pyrethrins to *C. pellucida* would be a timely examination to further elucidate the intensity of grasshopper nitrogen preferences. Additionally, this study would examine if nitrogen preferences can be switched by the presence or absence of these chemicals, a valuable piece of information for biocontrol efforts.

Finally, in an effort to account for the specific characteristics of the GN system on nitrogen preference, this study will examine the effect of mite parasitism on nitrogen preference. Small red mites (*Eutromidium locustarum*) parasitize the wings and integuments of grasshoppers, surviving on grasshopper hemolymph. They have been demonstrated to dramatically alter grasshopper life histories, lowering fecundity through depressed egg production (Branson 2003). There have been no studies done in this region nor with *C. pellucida* that has attempted to relate food consumption and food preference to mite parasite number per grasshopper, though the mite is very prevalent and may have an effect on food preference. Estimates of grasshoppers infected with mites in this area

range from 70 to 80%, though analyses of population density have been conducted. Any sensitivity exhibited by mite parasitized *C. pellucida*, especially among the females, with regard to food preference or food consumption may more clearly elucidate how grasshoppers in this particular system make manifest their nitrogen preferences.

I hypothesize that the grasshoppers will exhibit feeding preferences. When the effects of fertilization alone are considered, grasshoppers will prefer forbs with higher nitrogen content. I predict that females will especially consume the fertilized food preferentially because egg generation is a nitrogen-intensive process (Joern and Behmer 1997). I predict that when pyrethrin is encountered without the effects of fertilization, grasshoppers will prefer forbs with less pyrethrin. I predict that when pyrethrin is encountered along with fertilization, pyrethrin presence will trump fertilization and *C. pellucida* will eat the leaf untreated with pyrethrin or will eat along the periphery of the leaf treated with pyrethrin. I predict that females with mite parasites will consume more high-nitrogen foods to combat the egg-atrophying effects of mite parasitism, and will especially show an aversion to pyrethrin treated forbs.

Materials and Methods:

Fertilization treatment: Ninety grams of Miracle-Gro® was applied to a 1m² plot of lawn grass. This application amount was enough to stimulate an average of X percent increase in biomass from a control site (Appendix 1) as well as a boost in soil nitrogen content from medium levels to high levels (Kevin Betone, unpublished data.) After at least one week had elapsed, dandelion leaves within the fertilized plot were harvested and placed in waterpicks. Dandelions were chosen because they are forbs and have been observed to be a preferred food of grasshoppers (Anthony Joern and Jennifer Slade, personal communication). The dandelions were weighed once turgor pressure had

reestablished, typically after 30 minutes to two hours in the waterpicks. An equivalent number of dandelion leaves were harvested from an area of lawn that was not fertilized and were also left in waterpicks and weighed after turgor pressure was reestablished.

Pyrethrin treatment: Half of the leaves from each plot were painted with one layer of .02% pyrethrin solution. Treated leaves were painted with pesticides using a cotton swab; at least a 1 mm margin was left on the treated leaves so that grasshoppers could sample the leaves, as is their tendency in laboratory feeding experiments (Anthony Joern, personal communication) and so that opportunity for a foraging preference was presented. All leaf weights were recorded and leaves were tested in seven different pairings in the grasshopper cages, in which the variables of fertilization/no fertilization and pyrethrin treatment/no pyrethrin treatment were the variables manipulated (Appendix 1).

Grasshopper preparation: Grasshoppers were hand-captured with plastic bags in GN. Once captured, the grasshoppers were kept in 4'x6'x13' plastic containers with holes drilled for air circulation. They were starved for between 20 and 30 hours. Once sufficiently food deprived, one grasshopper was placed in each cage and presented with a pair of leaves from one of a variety of treatment combinations (Appendix 1). If the grasshopper was a nymph it was given a stick to shed its skin on at the instance that it molted; if it was an adult it did not receive such a stick. After 24 hours had elapsed, the leaves were removed and weighed. If one or both of the leaves in the cage was a treated leaf, then the number of bites taken inside of the treated area and the number of bites taken outside of the treated area were denoted.

Statistical Analyses: All statistics were performed using SYSTAT. A Manly test (Manly 1993) was run in keeping with the following procedure: a proportion composed of plant material eaten divided by

the total amount present in the leaf given was generated for each of the leaves for all pairs of leaves. The proportion accorded to Type 2 (untreated leaves) was subtracted from Type 1 (treated leaves). These figures were arcsine transformed, and they were tested for normality using a Lilliefors probability estimate. Once the figures corresponded satisfactorily with a normality curve, they were compared to the control through a one-way ANOVA where pair type was the discrete variable.

To statistically test mite parasitism data, a one way ANOVA comparing the amount those females ate against the discrete variable of high or low parasite amounts was performed.

Results:

Preference Experiment: Grasshoppers did exhibit a significant feeding preference. After ensuring that the data were normal (Figure 1), the ratios of food consumed to food given for each paired consumption (Appendix 2) were examined using a one-way ANOVA. There was a significant difference between the consumption in at least two pairs among the consumption of the seven test pairs ($F_{6,63}=2.347$, $p=0.041$, Table 1). After performing a Tukey's Honestly Significant Difference test and a Bonferroni Test, only two test pairs showed enough disparity between their two values to be significantly different. The pair of the fertilized/untreated leaf paired with an unfertilized/treated leaf was significantly different from the pair featuring the untreated/fertilized leaf and the unfertilized/untreated leaf ($p=0.019$, Table 2) as well as significantly different from the pair featuring the treated/fertilized leaf and the treated/unfertilized leaf ($p=0.046$, Table 2). This signifies that within the first mentioned pair, there was such a preference in foraging that it was different from the pairs mentioned second and third, which featured almost no preference in foraging. After graphing the average consumption of fertilized/untreated leaf paired with an unfertilized/treated leaf, it was found that biologically the fertilized/untreated leaf pair is so highly

preferred over the unfertilized/treated leaf that it was this pair that caused the significant divergence from the control.

Mite Experiment: Mite amount did not have any statistically significant effect on consumption based on proportion ($F_{1,4}=2.942$, $p\text{-value}=0.161$). However, there was potential biological significance (Figure 4).

Discussion:

The four part hypothesis was somewhat supported by the results; the original and broadest hypothesis that grasshoppers would exercise a preference was supported, though none of the other specific hypotheses were supported with equally significant results. The support for this “presence-of-a-preference” hypothesis was nearly inevitable, because only two leaves were given in each cage. If either one was even slightly more consumed than the other, any amount removed was detectable and could be measured to contribute towards significance. Although this significant conclusion may not seem at its fundamental design to truly reflect a novel trend, it does assert at the very least that these *C. pellucida* are different from the grasshoppers that exhibited no preferences in the Heidorn and Joern 1987 study.

The support for the hypothesis that preference does occur in *C. pellucida* was due to one pair that featured an unfertilized/treated and a fertilized/untreated leaf. This combination gave the grasshoppers a choice between the leaf with the higher nitrogen content and the lower concentration of poison as a choice and the leaf with the lower nitrogen content and the higher concentration of poison. It should not be surprising, therefore, that four of the ten replicates of the fertilized/untreated leaf were over 59% consumed (Table 2), while seven of the ten replicates of the unfertilized/treated leaf were 0% consumed (Table 2). The disparity between these two values of

this pairing is enormous (Figures 3 and 4).

When the factors of fertilization and pyrethrin content were isolated, however, foraging did not significantly differ from the control (Figure 2). For example, in the experiment comparing a control pair to a pair of two pyrethrin-untreated leaves, one fertilized and the other unfertilized, the p-value was 0.902. This signifies that foragers did not selectively forage for nitrogen. Additionally, when two fertilized leaves, one treated with pyrethrins and the other not, were compared to a control pair, the p-value was 0.179. This may be marginally significant but still does not definitively posit a preference switch that is reliant solely on one of the two factors examined in this study. This signifies that the significant foraging witnessed for the unfertilized/treated + fertilized/untreated pair was due to an interaction of them both rather than the effects of a single powerful variable.

The specific prediction that pyrethrin content would be more important in determining leaf preference was not significantly demonstrated to be true, although the p-values between the several different pairs were marginally significant. For example, the Tukey's HSD test comparing a pair of untreated leaves, one fertilized and one unfertilized, to a pair of leaves in which the unfertilized leaf is treated with pesticides, then a marginally significant p-value of 0.063 results. This and other marginally significant values (Table 2) associated with changes in pyrethrins provides enough evidence to warrant further testing of secondary plant compounds as important regulators of food preference, potentially even overruling nutrient quality. However, these marginally significant values do not allow for the explicit support of that segment of the hypothesis, although the biological information is compelling.

Although I expected to see a more pronounced attraction to nitrogen content in leaves, though pairwise comparisons examining have little in the way of significance to provide. One

possible explanation of why nitrogen content was not more pronounced as a basis for selection draws on the physiology of *C. pellucida*. They are stimulated to bite into plants based on detectable sucrose content (Thornsteinson, 1960, as cited by Chapman and Joern 1995). Because sucrose content was never accounted for, there may be a lurking variable of sucrose that could have changed the results. At the very least, nitrogen content is not as apparent as sucrose content is: further testing might also explore other chemical messengers that to which *Cammula pellucida* respond.

Finally, in terms of mite parasitism concurrent effects on both feeding rate and egg production, the juvenile reproductive tracts of many of the adult female grasshoppers prevented a truly diagnostic survey of eggs from being made (Figure 5). Additionally, only six adult females were used in this study. A well conducted future study would incorporate more individuals and feed them with the food for a longer amount of time. However, a gradient was still observed despite the lack of a statistically significant output. Two of the three grasshoppers who ate markedly more food (26-36% as opposed to 0-3%) had no mites: if more replicates were performed it may be possible to achieve significance with this data.

In terms of experimental design, preference studies in grasshoppers face many hurdles on their path to amelioration. The current methods of food presentation, in dry bundles or in single blooms in waterpicks, both feature disadvantages that detract from the precision of the experiment. The waterpick method employed in this experiment featured many small faults that may have had a larger bearing on the experiment than desired. For instance, none of the leaves that were untouched weighed the same pre- and post- experiment-each one experienced at least some measure of water loss or even more bizarrely, water gain. Additionally, leaking from the waterpicks created mini-moats within the grasshopper cages that prevented them from accessing certain parts of the leaves. Also concerning the architecture of the cage, the presence of sticks absolutely altered the parts of

the leaves that were consumed. Because only juvenile grasshoppers were given sticks in their cages, adult grasshoppers often climbed on the water picks themselves and ate the leaves from such a vantage point; this explains why only about 8-10 leaves featured any substantial consumption of the crown. It would appear as though the inclusion of sticks would lead to a more balanced consumption of the leaf; however, in the juvenile grasshoppers, they simply ate the part of the leaf that they could reach from their perch on the stick. Thus, the positioning of a stick within the cage, the presence of a stick in a cage, or the lack of a stick in the cage has an inordinate effect on what parts of leaves are eaten and total consumption rate. Additionally, if the weight per bite could be calculated than a modified microcosmic version of “giving up density” on a pesticided leaf within the context of Optimal Foraging Theory could be applied to predict when grasshoppers will leave an area.

A major assumption of this experiment was that the dandelions from the fertilized plot actually differed in nitrogen content from those originating in unfertilized plots. Because no nitrogen analysis of the leaves were run, and use of soil data derived from different parts of property though the same soil type was used to determine nitrogen endowment, it is not entirely certain whether nitrogen really mattered. Although there was a very visible average length and weight difference between fertilized and unfertilized leaves, nitrogen content may not always be reflected by average weight and length. I believe that if nitrogen content had been more tightly regulated then a marginally significant or even significant effect of nitrogen would be noted, in corroboration with the Jonas and Joern 2008 study. Another major assumption of this study was that grasshoppers would sample leaves with their mandibles and those small indentations would serve as benchmarks of what was acceptable forage. However, grasshoppers rely on a series of aural, nasal, and oral cues to make a decision about food choice. Thus, this simple assumption removes the other “tasting”

possibilities from consideration; a future study should incorporate this by videotaping and measuring tarsal length of time on the leaf to measure initial “tasting” habits.

This study of pyrethrin and fertilizer induced preference switching raises many questions and fuels the increasingly ambiguous role of nitrogen as a basis for grasshopper food preference. Because pyrethrins and nitrogen were not shown to individually account for “why” a grasshopper bites a food source, further research into what does motivate that decision could be beneficial for developing new, responsible insecticides and implementing new forms of biocontrol. Additionally, learning these characteristics about previously unstudied species will further inform the literature and contribute to the scientific body of knowledge about insect herbivores.

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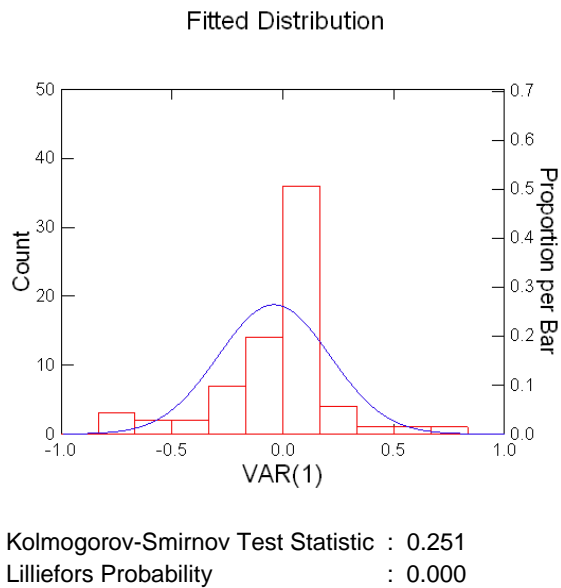


Figure 1: Verification of normality of proportional data. Raw amounts of consumption was divided by total number of leaf litter. After some subtraction the resulting number was arcsine transformed to test for normality.

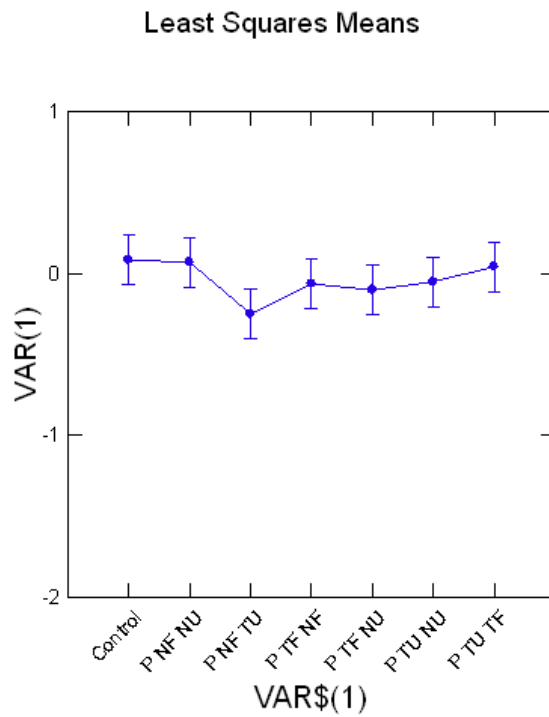


Figure 2: A graphical representation of food preference consumption differences based on different

pairings of pyrethrin and nitrogen amounts. The third data point is the only significant data point (calculated with a one-way ANOVA, $F_{6,63}=2.347$, $p=0.041$.) “N” signifies untreated with pyrethrin, “F” signals fertilized, “U” signals unfertilized, and “T” signifies treated with pyrethrin.

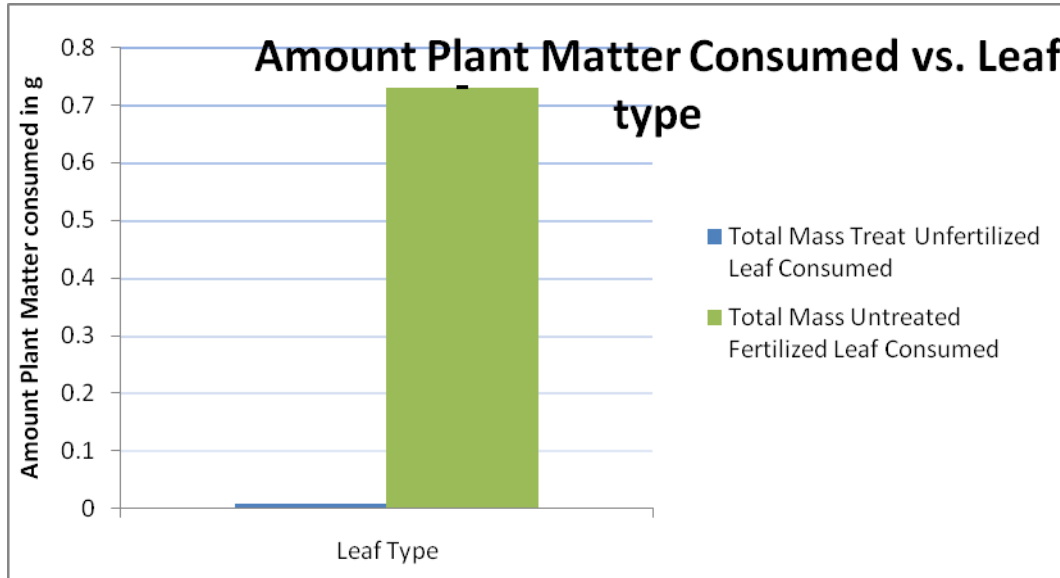


Figure 3: A graphical representation of the raw consumption disparity for the only significant data point calculated from the one way ANOVA comparing plant consumption to various combinations of pyrethrins and nitrogen content. In this graph, the fertilized leaf free of pyrethrin pesticide is significantly more consumed than an unfertilized leaf treated with the pyrethrin pesticide.

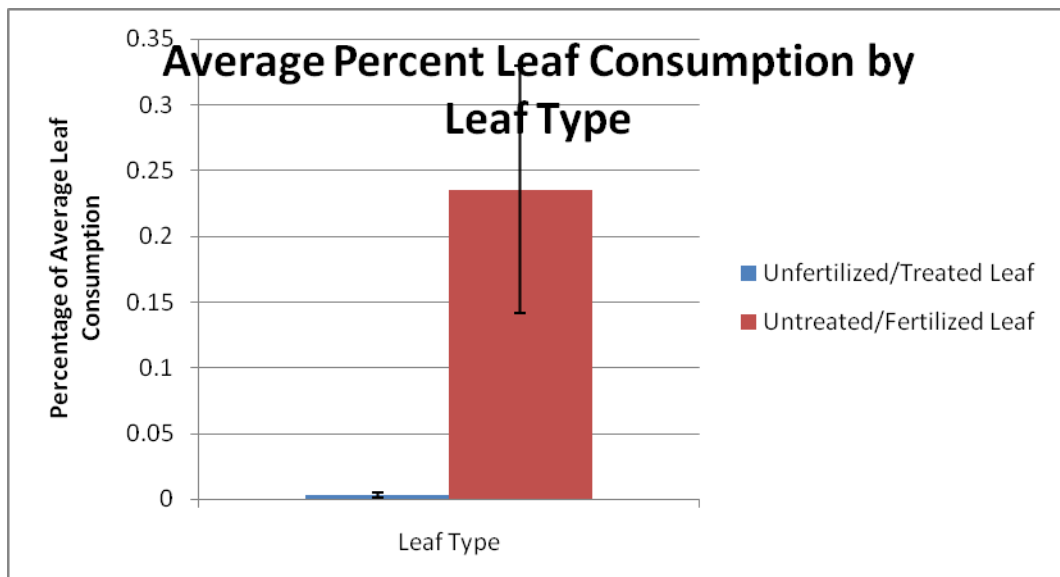


Figure 4: A graphical representation of the proportional consumption disparity for the only significant data point calculated from the one way ANOVA comparing plant consumption to various combinations of pyrethrins and nitrogen content. In this graph, the fertilized leaf that is

untreated with pyrethrin pesticides.

Least Squares Means

an unfertilized leaf treated with

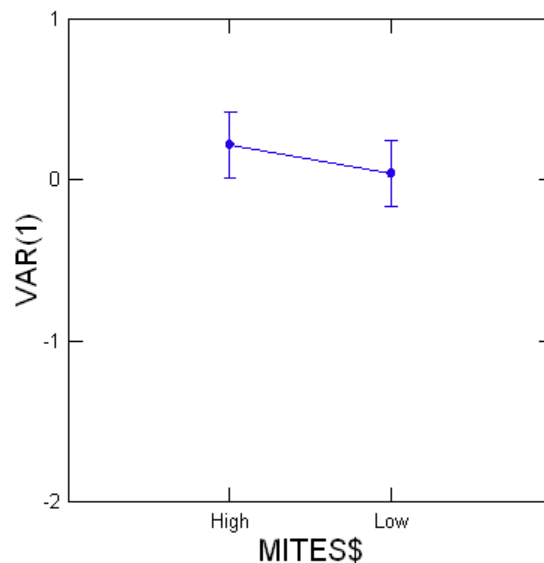


Figure 5: A graphical representation of mite number of food preference and consumption. This was calculated using six individuals, and though there is a biological gradient established, the results are not significant ($F_{1,4}=2.942$, $p\text{-value}=0.161$).

Table 1: One-way ANOVA output for arcsine transformed feeding proportions based on pair of forbs presented. Var\$(1) denotes the type of pair, of which there were 7.

Analysis of Variance					
Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
VAR\$(1)	0.821	6	0.137	2.347	0.041
Error	3.673	63	0.058		

Table 2: Tukey's HSD Pairwise Comparison Test for all proportions of food consumed, taken from Table 1. Significant differences between pairs are highlighted.

Tukey's Honestly-Significant-Difference Test					
VAR\$(1)(i)	VAR\$(1)(j)	Difference	p-Value	95% Confidence Interval	
				Lower	Upper
Control	P NF NU	0.013	1.000	-0.316	0.342
Control	P NF TU	0.332	0.046	0.003	0.661
Control	P TF NF	0.147	0.821	-0.182	0.476
Control	P TF NU	0.184	0.615	-0.145	0.513
Control	P TU NU	0.134	0.877	-0.195	0.463
Control	P TU TF	0.040	1.000	-0.288	0.369
P NF NU	P NF TU	0.319	0.063	-0.010	0.648
P NF NU	P TF NF	0.134	0.877	-0.195	0.462
P NF NU	P TF NU	0.171	0.694	-0.158	0.500
P NF NU	P TU NU	0.120	0.921	-0.208	0.449

Tukey's Honestly-Significant-Difference Test					
VAR\$(1)(i)	VAR\$(1)(j)	Difference	p-Value	95% Confidence Interval	
				Lower	Upper
P NF NU	P TU TF	0.027	1.000	-0.302	0.356
P NF TU	P TF NF	-0.185	0.608	-0.514	0.144
P NF TU	P TF NU	-0.148	0.816	-0.477	0.181
P NF TU	P TU NU	-0.198	0.529	-0.527	0.130
P NF TU	P TU TF	-0.292	0.115	-0.621	0.037
P TF NF	P TF NU	0.037	1.000	-0.291	0.366
P TF NF	P TU NU	-0.013	1.000	-0.342	0.316
P TF NF	P TU TF	-0.106	0.955	-0.435	0.223
P TF NU	P TU NU	-0.051	0.999	-0.379	0.278
P TF NU	P TU TF	-0.144	0.835	-0.473	0.185
P TU NU	P TU TF	-0.093	0.977	-0.422	0.236

Appendix 1:

Types of treatments performed:

Pesticided and fertilized leaf * unpesticided and unfertilized leaf

Unpesticided and fertilized leaf * pesticed and unfertilized leaf

Unpesticided and unfertilized leaf * unpesticided and unfertilized leaf

Pesticided and unfertilized leaf * unpesticided and unfertilized leaf

Pesticided and fertilized leaf * unpesticided and fertilized leaf

Pesticided and fertilized leaf * pesticed and unfertilized leaf

Unpesticided and fertilized leaf * unpesticided and unfertilized leaf

Appendix 2: Proportions of leaves eaten

-0.197006849	0	-0.49484	0.214397	-0.18964	-0.02972	0.24675	-
-0.1484633	-0.03678	0	0	0	0.060636	-0.0974	
0.074567652	0.0764	-0.09873	0.134506	-0.75534	-0.01997	0	
							-
0.013196864	-0.01112	0.034996	0	0.178539	0	0.02984	
0	0	0.019596	0.036429	0	0.008682	0	
0.369485146	0	0.029489	0.102096	-0.52861	-0.00482		
-0.392945836	-0.23517	0.093755	-0.08546	0.012678	-0.6985		
0.25342873	-0.268	0.080163	0	0.52196	-0.77425		

0.830438205	0.142182	-0.0114	-0.03465	0.003759	-0.63998
0.012674611	-0.28615	0	0	0.064814	-0.01744
-0.188735931	-0.03081	0.020568	0.303222	0.059699	0.003778

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