

What are parasitoid wasps visiting *Helianthella quinquenervis* targeting, and could they influence plant fitness and reproduction?

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Introduction and General Plan of Study

Abstract (generated by ChatGPT)

****Abstract****

****Background**:** Insect herbivory imposes significant challenges on plant health, leading to the evolution of both direct and induced defenses in plants. Among these, induced defenses are particularly adaptive, activating in response to damage and potentially recruiting natural enemies such as parasitoid wasps. This study examines the impact of two distinct classes of parasitoids—idiobionts and koinobionts—on the fitness of *Helianthella quinquenervis*, a perennial aster, by investigating their effects on seed-predators and the resulting plant damage.

****Methods**:** We conducted a field study in a Rocky Mountain meadow to evaluate the impact of parasitoid wasps on *Helianthella quinquenervis*. Three groups of flowers were studied: (1) flowers exposed to specific parasitoids, (2) flowers exposed to ambient parasitoid activity, and (3) control flowers protected from parasitoid exposure. Flower heads were analyzed for seed-predator presence and damage, and the developmental stages of parasitoid targets were documented.

****Results**:** ANOVA revealed significant differences in larval mass among treatment groups ($p = 0.0242$), with Group 1 showing lower larval mass compared to other groups. However, proportional area damage did not significantly differ across treatments ($p = 0.542$), indicating that parasitoid presence did not consistently translate into measurable changes in seed-predator damage. Pearson's correlation confirmed a strong relationship between larval mass and capitulum damage ($r = 0.7597$, $p < 0.001$).

****Discussion**:** The results suggest that parasitoid effects on seed-predators are variable and dependent on the parasitoid class and developmental stage of the target.

Although parasitoids affected larval mass, no conclusive evidence was found to support a consistent protective or destructive impact on *Helianthella quinquenervis*.

The study highlights the need for longer-term and more controlled experiments to better understand the complex interactions within this tri-trophic system and their implications for plant fitness and pest management.

Background

Insects are the most threatening herbivores in nature and plants have developed both direct and induced defenses against them (Chen, 2008) [Tan et al. 2020]. Induced defenses are only activated when the plant is under attack and could be a cost-saving strategy as opposed to maintaining constant defenses (Huot, Yao, Montgomery, & He, 2014) [Tan et al. 2020]. This could mean recruiting natural predators or parasitoid insects (Kessler & Baldwin, 2001; Mumm & Dicke, 2010) [Tan et al. 2020], which may improve plant reproduction and fitness (Gómez & Zamora, 1994; Hoballah & Turlings, 2001; Kessler & Baldwin, 2004; Pashalidou et al., 2015; van Loon, de Boer, & Dicke, 2003) [Tan et al. 2020]. Recruitment of parasitoids by herbivore-damaged plants has been documented in many systems (Mumm & Dicke, 2010) [Tan et al. 2020], and may benefit plant fitness due to their relatively low impacts on beneficial insects and reduction of herbivore populations that damage plant tissues (Gols et al., 2015; Gómez & Zamora, 1994; Romero & Koricheva, 2011; Turlings & Erb, 2018) [Tan et al. 2020].

Herbivorous insects can cause harm to agricultural and forest ecosystems. Phytophagous insects on land undergo periodic outbreaks in North America and Europe, destroying millions of hectares of forest every year (McManus et al. 1992; Li et al. 2015) [Abdala-Roberts et al., 2019, p. 2151]. Outbreaks can be affected both by changes in host plant resistance and nutritional quality (Turchin et al. 1991) [Abdala-Roberts et al., 2019, p. 2151], and by the loss of natural enemies such as parasitoids, predators, and pathogens that would keep herbivorous insect populations regulated (Turchin et al. 1999) [Abdala-Roberts et al., 2019, p. 2151]. Algal blooms in aquatic systems can be caused by eutrophication and natural enemy suppression of herbivorous zooplankton as well (Carpenter et al. 1985; Micheli 1999) [Abdala-Roberts et al., 2019, p. 2151]. For these reasons, parasitoids are often used as agents in biological pest control. The introduction of these natural enemies is meant to reduce the population density through environmentally friendly means and reduce the use of pesticides (Singh, 2023, p. 1).

There are two major wasp parasitoid classes that have different consequences for downstream plant fitness. The first of these, idiobionts, are typically ectoparasitoids whose eggs are oviposited outside of the host, often in an enclosed environment such as flower heads

or fruit. Host development usually ceases upon parasitism by an idiobiont, typically via paralysis (Otto and Mackauer, 1998, p. 353). Idiobionts moreover tend to be generalists, not targeting any one species specifically. Idiobionts have the potential to benefit the plant target of their host depending on the developmental stage of that host. Of all the stages (ovum, larva, pupa), larvae possess the greatest potential to do damage because they are motile. Eggs and pupae are unable to move among fruits and accrue mass on their own

The other type of wasp parasitoid, the koinobiont, is usually a species-specific endoparasitoid which oviposits directly into a host, wherein its larvae then develop (Wharton et al. 2000, Wang et al. 2003) [Wang and Messing, 2004, p. 1313] Koinobiont parasitism allows for further development and mobility of the host, allowing them to continue to grow (Slansky 1986; Godfray 1994; Beckage & Gelman 2004) [Xi et al., 2015, p. 1104]. In fact, koinobionts are known to alter host appetite, causing hosts to increase in mass as a way of boosting parasitoid fitness (Rahman 1970; Espirito-Santo, Faria & Fernandes 2004; Smallegange et al. 2008) [Xi et al., 2015, p. 1104]. For example, the caterpillar *Trichoplusia ni* increased its leaf consumption when parasitized by wasps (Ode, Harvey, Reichelt, Gershenzon, & Gols, 2016) [Tan et al. 2020]. Corn Earworm, *Helicoverpa zea*, when parasitized by *Microplitis croceipes*, had reduced elicitor activity in their saliva, reducing expression of tomato defenses and increasing parasitized caterpillar growth, thereby improving fitness of parasitoid offspring developing inside the caterpillar (Tan et al. 2018) [Tan et al. 2020]. By inducing an increased appetite in their hosts, koinobionts confer a negative effect on the target plant of their hosts, two trophic levels down.

Top-down trophic cascades have traditionally been understood through examples in which predators (secondary consumers) suppress herbivores (primary consumers), indirectly benefiting producers at the bottom of the food chain. (Hairston, Smith & Slobodkin 1960; Shurin et al. 2002) [Xi et al., 2015, p. 1109]. A textbook example is the explosion of pacific purple sea urchin (*Strongylocentrotus purpuratus*) populations due to the decline in the population of their natural predator, the sea otter (*Enhydra lutris*). This resulted in sea urchins overgrazing on giant kelp (*Macrocystis pyrifera*), destroying kelp forests along the coast of California (Estes and Palmisano 1974; Szpak et al. 2013) [Biology LibreTexts]. Only when the sea otters' population increased did the giant kelp population improve, two trophic levels below the otters.

In contrast to predator-induced trophic cascades, this study investigates the significance of parasitoids' indirect effects on plants. By inducing the appetite of their herbivorous hosts, koinobiont parasitoids can have a negative downstream effect (Xi et al., 2015, p. 1110), while idiobionts may have positive (if they target active larvae) or negative (if they target immobile pupae or eggs) effects on producers). This tri-trophic system represents

atypical (or underrepresented) trophic cascades where the sign of the indirect effect a secondary consumer has on the producer is variable, and possibly species-specific.

We investigated the tri-trophic system occurring on *Helianthella quinquenervis*, a perennial aster occurring throughout the Rocky Mountains. *Helianthella* is the target of multiple pre-dispersal seed-predators including tephritid flies (*Neotephritis finalis* and *Trupanea nigricornis*), an agromyzid fly (*Melanagromyza* sp.), and a phycitid moth (*Homeosoma* sp.) (Inouye and Taylor, 1979, p. 2). At our site, *Helianthella* is mostly predated on by the tephritid flies and some *Melanagromyza* flies.

The seed predators of *Helianthella* are themselves targets of parasitoid wasps, at least 3 species of which (Braconidae and Chalcidoidea) have been observed ovipositing into the flower heads by RMBL scientists. These wasps appear to be natural enemies of the seed-predators of *Helianthella*.

With multiple species of predators targeting *H. quinquenervis* and multiple species of parasitoids potentially targeting those predators, the parasitoid-induced trophic cascade may depend on parasitoid species, seed predator species, and/or the interaction between the two. Here we attempt to answer the following questions:

- (1) What is the identity and developmental stage of the targets of parasitoid wasp oviposition?
- (2) Do parasitoids of *H. quinquenervis*'s seed predators provide a seed-protecting, seed-destroying, or neutral effect on the flowers through their interactions with developing flies?

Specific Methodologies

Site and Study Species

The proposed study site lies in a vast, continuous meadow (>80,000 m²) on the Gothic-Baldy Saddle downslope of Mt. Baldy, often referred to as Washington Gulch. Pre-existing site boundaries established by Principal Investigator (PI) Sears encompass a roughly 35,000 m² eastern portion of the meadow, ranging from ~10,700 to 11,000 ft above sea level. Among other asters and montane wildflowers, *H. quinquenervis* is patchily distributed throughout the larger meadow. At this site, both *Neotephritis finalis* and *Trupanea nigricornis* have been observed causing damage to *H. quinquenervis*, and at least three species of braconid and chalcidoid wasps oviposit on open flowers.

To meet our objectives, a sample of 142 *Helianthella quinquenervis* were located amongst the site, recorded via GPS, and bagged just before their buds opened to prevent uncontrolled parasitism from occurring (parasitoid visits generally appear to occur after flowers have opened). At this stage, a large percentage of the flower heads already contained seed-predator offspring according to observations at Rocky Mountain Biological Laboratory.

56 of the flowers (Group 1) were introduced to female chalcidoid wasps. 56 flowers (Group 2) were exposed to ambient rates of parasitoid visitation for a week. 30 flowers (Control) remained bagged for the entire season to prevent parasitism. All flower heads were collected, damage to them assessed, and classified as Parasitized (P), Not Parasitized (NP), or Free of Seed-Predators (F). Analyses were conducted using R Studio.

Identification and Collection

Before the sample *Helianthella* flowers opened, researchers engaged in observation of *Helianthella* with the intention of netting and or trapping adult insect visitors for the purposes of identification and building a stock of wasps to introduce to sample Group 1. Flower head samples from previous years containing seed predators were also used for identification of parasitoids and seed-predators. Morphospecies, proxies for species based on morphology, were used for identification purposes where species identification was difficult. One morpho species of chalcidoid wasp was selected for use in Group 1 based on its prevalence and that it was observed ovipositing on *Helianthella*.

Capitulum Damage Assessment

Each flower's capitulum, the dense flat cluster of small flowers or florets typical of the Asteraceae, was measured for damage done by seed-predator larvae by area and by larval mass for a smaller portion of the sample (101 capitula,). Masses of capitula and larvae were measured using a scale. The proportion of area damaged to total area was measured using the program ImageJ.

Group 1

The 56 plants in Group 1 were introduced to a female of one of the 3 wasp species for 24 hours (56 flowers for each morpho species of wasp: A, B, C). After 3 days, the wasps were removed, and the samples collected. Upon retrieval they were dissected to inspect them

for targets of parasitism. Offspring present were identified by morphology including size, mouth parts, and coloration. Targets of parasitism could be identified by rearing all insects in the samples, but time did not allow for this. Many capitula were inspected under a microscope. Individual capitula were numbered and assessed for damage.

The experiment on Group 1 answers the following questions: (1) Do parasitoids of *H. quinquenervis*'s seed-predators provide a seed-protecting, seed-destroying, or neutral effect on the flowers through their interactions with developing flies? (2) What species is the target of the parasitoid wasps' oviposition into the flower? (3) What is the developmental stage of the target?

Concurrent species of wasps and seed-predators present in the capitula were recorded to determine if there are species-specific targets.

Group 2

The flower heads of Group 2 were exposed to ambient rates of parasitoid visitation for a week and then collected. Upon retrieval, damage to capitula was assessed.

The experiment on Group 2 answers the following questions: (1) Do parasitoids of *H. quinquenervis*'s seed-predators provide a seed-protecting, seed-destroying, or neutral effect on the flowers through their interactions with developing flies?

Control

The Control group of 30 flowers remained bagged for the duration of the experiment to prevent parasitism. This group was to provide baseline data for *Helianthella*'s capitulum size and mass and for non-parasitized larval mass. Analysis began once the capitula were collected 3 days later.

Results

All analysis was conducted in R Studio. Shapiro-Wilk Normality Tests were conducted on all numeric data and all were found to be parametric, or distributed normally.

ANOVA on Larval Mass across Treatments

Treatment groups 1, 2, and control had mean larval masses of 0.01054g, 0.0315g, and 0.01545g respectively. (One way ANOVA: p-value = 0.0242, F-value = 3.868) (see Table 2)

ANOVA on Proportional Area Damage across Treatments

The mean proportion of area damaged for groups 1, 2, and control were 0.06641, 0.06218, and 0.08865 respectively (One way ANOVA: p-value = 0.542, F-value = 0.613) (See Table 3)

Pearson's Correlational Test between larval mass and damaged area

A Pearson's test revealed that larval mass and damaged capitulum area were correlated by an estimate of 0.7596661 (p-value = $2.2e-16$, .95 CI = (0.6626025 to 0.8316339))

Discussion

Question 1

An overwhelming amount of samples contained *Trupanea nigricornis* offspring. If present, no parasitoids had yet emerged. A staggering amount of samples also contained pupae or casings. Few samples contained larvae that were still feeding.

From inspection of Sears' capitula samples, it seems likely that the chalcidoid wasps target *Trupanea*, but the results of this study were inconclusive. In the interest of answering this question, I would recommend that future research be conducted with a longer timeline, so as to complete the rearing of any insects within the samples.

Question 2

For the ANOVA run on larval mass, the p-value for treatments is 0.0242, which is less than the commonly used significance level of 0.05. This indicates that there is a statistically significant difference in larval mass among the treatment groups. The F-value of 3.868 suggests that the variance in larval mass per capita explained by the treatment factor is significantly greater than the residual variance.

However, for the ANOVA test run on proportional area damage, the p-value for the treatment factor was 0.542 indicating that the differences between the mean proportional area damage between the treatment groups are not statistically significant. The F-value of 0.613 was quite low, suggesting that the treatment factor does not explain a significant proportion of the variability in the dependent variable compared to the residual variability.

Xi et al. found a 52-68% difference in the proportion of seeds damaged by parasitized seed-predators vs non-parasitized seed-predators in some species of Asteraceae. For our study, no significant difference in damaged area as a proportion or area was found between treatment groups. As such, it remains unclear whether parasitoids of *H. quinquenervis*'s seed predators provide a seed-protecting, seed-destroying, or neutral effect on the flowers through their interactions with developing fly larvae. This lack of difference could be due to flaws in experimental design, specifically where the duration of the experiment, the timing of parasitism, and controlled environments are concerned.

This likely means either our treatment was ineffective. The larvae in Group 1 may have not been targeted by the chalcidoid wasps, or the time from parasitoid oviposition to assessment of damage may not have been long enough. A more controlled environment where oviposition could be more easily confirmed and where wasps could be reared for use in treatments could greatly benefit a similar experiment in the future. A change of site to a lower elevation might improve the chances of parasitism as well if not extending the length of time of the study.

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Tables and Figures

Predicted Short-Term Parasitoid Trophic Cascades

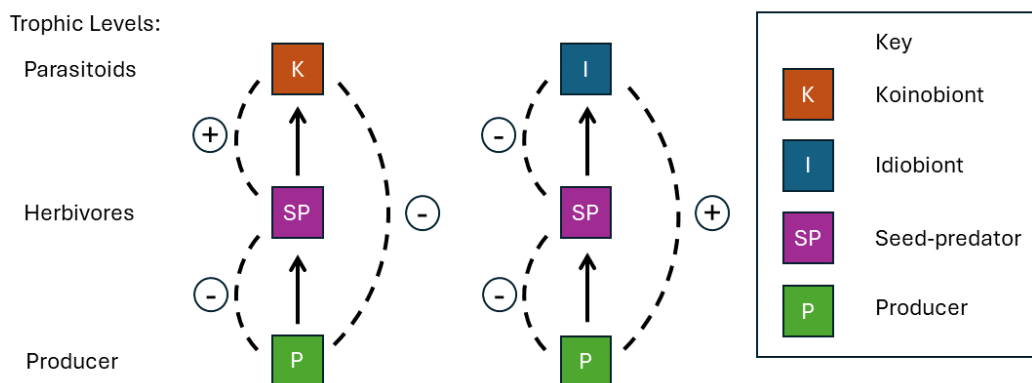


Fig. 1 Predicted Short-Term Parasitoid Trophic Cascades

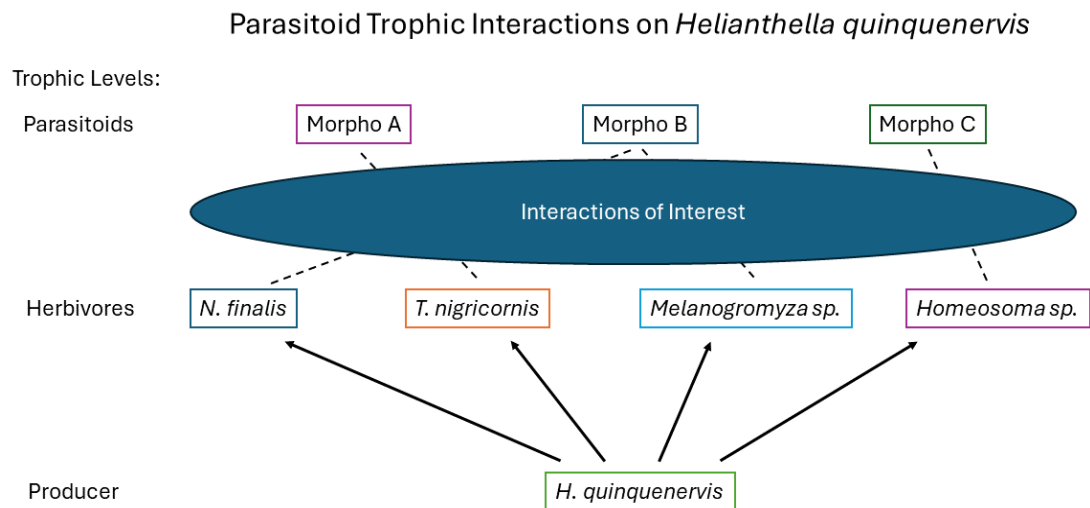


Fig. 2 Parasitoid Trophic Interactions on *Helianthella quinquenervis*
 Capitulum Area Damage as a function of Larval Mass

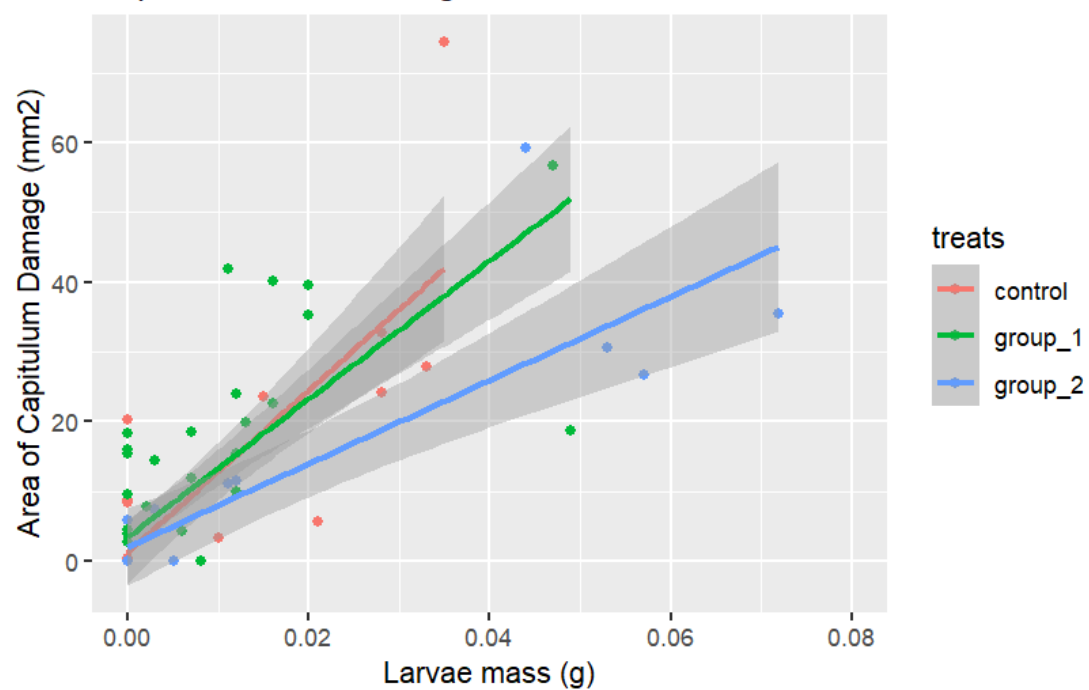


Table 1 Capitulum Area Damage as a function of Larval Mass

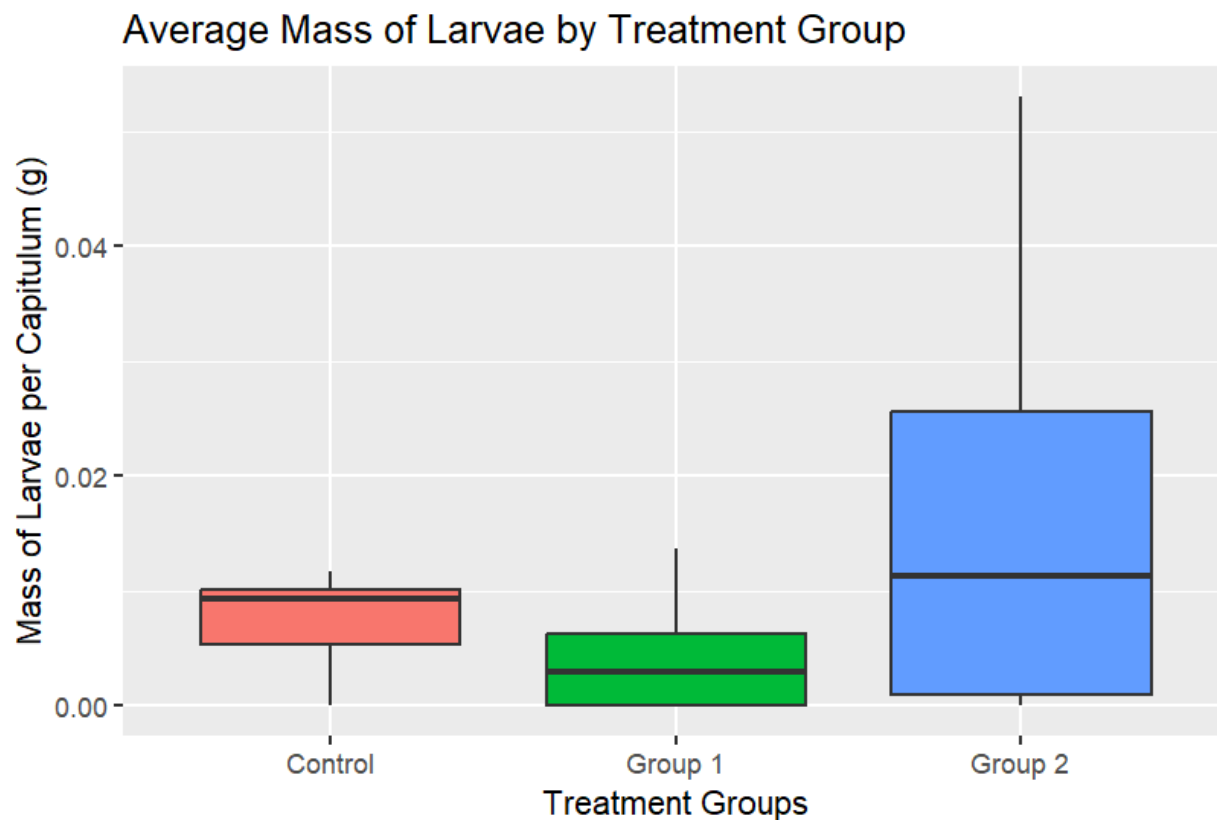


Table 2 Average Mass of Larvae by Treatment Group

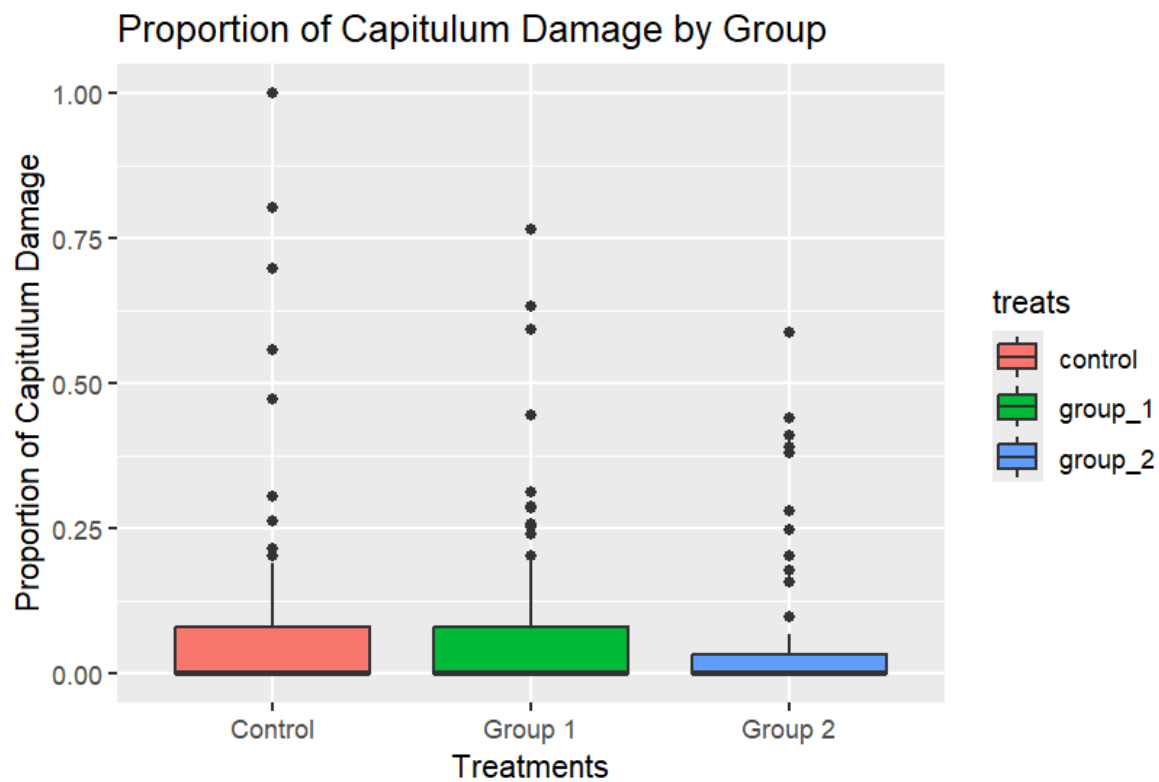


Table 3 Proportion of Capitulum Damage by Group

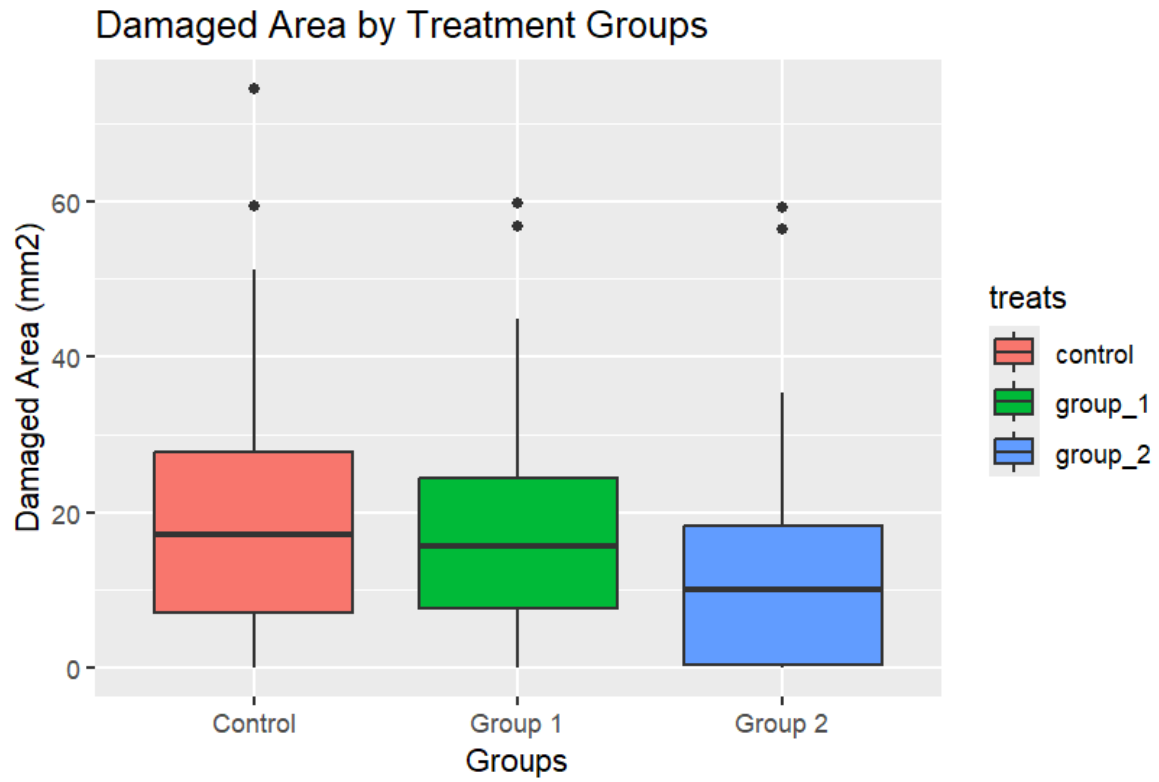


Table 4 Damaged Area by Treatment Groups