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Effects of olfactory cues on the movement behavior of the predatory beetle *Calosoma wilcoxi*

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science,
at Virginia Commonwealth University.

by

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Abstract

EFFECTS OF OLFACTORY CUES ON THE MOVEMENT BEHAVIOR OF THE PREDATORY BEETLE *CALOSOMA WILCOXI*

By Kennesha Myrick-Bragg, M.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science,
at Virginia Commonwealth University.

Virginia Commonwealth University, 2016.

Major Director: Derek M Johnson, Assistant Professor, Department of Biology

Arthropod predators often use prey and conspecific cues to make foraging decisions. *Calosoma wilcoxi* (Leconte) is a voracious predatory beetle that specializes on lepidopteran larvae often found in the forest canopy, including the fall cankerworm. This study tested the hypothesis that *C. wilcoxi* uses olfactory cues to detect prey and conspecifics. A Y-tube olfactometer was used to test attractiveness to larvae, larval frass, conspecific cues, and volatiles from herbivore-damaged white oak leaves. *C. wilcoxi* did not preferentially choose the treatment in any of the experiments. There was no difference in mean time spent in the treatment or control arm for any of the cues assayed. The time to choose the treatment was significantly shorter in the female conspecific experiment only. I found no evidence that *C. wilcoxi* uses olfaction to locate prey; however, *C. wilcoxi* is attracted to conspecifics. *C. wilcoxi* may use conspecific cues to make informed foraging decisions.

Introduction

Predators face the challenge of searching for prey that vary in abundance both spatially and temporally. Using environmental information to locate prey can help mitigate those challenges (Dill 1983, Mitani 2004) and can ultimately increase fitness (Danchin et al. 2004, Page and Ryan 2005, KIELTY et al. 1996). The use of environmental cues has been observed in a broad range of predators, including barn owls, beetles, and spiders (Blamires et al. 2011, Page and Ryan 2005). These cues could be tactile, visual, acoustic, gustatory, olfactory, or some combination of these (Catania et al. 2008, Olberg et al. 2000, Payne 1971, Hansen 1983). In arthropod systems, location of feeding sites is commonly influenced by olfactory or visual cues from prey, resources utilized by prey, or other conspecifics (Hassel and Southwood 1978, Sternlicht 1973, Coolen et al. 2005).

In tri-trophic systems composed of plants, herbivores, and their predators, both constitutive and induced defense mechanisms are employed by many plant species (Amo et al. 2013). Plants have evolved numerous types of responses to herbivore attack; these include decreasing palatability, intoxication, and increased emission of plant volatiles (Amo et al. 2013, Pare and Tumlinson 1999). Plants typically release small amounts of volatiles, however when some plants are attacked by herbivores, the diversity of volatiles and rate of release increases. The release of plant volatiles can attract parasitoids and arthropod predators (Pare and Tumlinson 1999). Pearse et al. (2012) found evidence that a number of oak species, produce altered volatile profiles when mechanically damaged to simulate herbivory. Volatiles produced in the greatest quantities were those that have been shown to be highly bioactive in attraction of natural enemies (Pearse et al. 2012, Rose et al. 1998). Staudt and Lhoutellier (2007) investigated the effects of herbivore feeding on volatile production from holm oak trees; herbivore feeding induced new volatile emission at increased rates. In a recent study, the carabid beetle *Pterostichus melanarius* was shown to be

attracted to the volatile producing damaged leaves of prey host plants (Oster et al. 2014). *P. melanarius* was not attracted to the undamaged leaves, which did not emit any volatile organic compounds. This study demonstrated the importance of volatile production in predator attraction.

Natural enemies of several lepidopteran species use olfactory cues of larval frass to locate prey (Reddy et al. 2002, McCall et al. 1993). During lepidopteran outbreaks, larvae produce high volumes of frass, which rains onto the substrate below infested trees (Wesolowski and Rowinski 2005, Staudt and Lhoutellier 2007). Large frass inputs resulting from outbreaks of defoliating insects can impact plant-herbivore-predator trophic interactions (Barber and Marquis 2009, Lovett et al. 2002). *Dibrachys cavus*, a larval parasitoid, uses frass to discriminate between potential hosts (Chuche et al. 2006). To my knowledge, the role of larval frass in prey finding has never been studied in a forest defoliator system.

The use of social information has largely been attributed to colonial insects, however, recent studies demonstrate the benefits of social information in solitary insects (Coolen et al. 2005, Chittka and Leadbeater 2005). Social information can reduce the risk of predation, increase mating success, and reduce the risk of consuming unsuitable food (Danchin 2004, Jones et al. 2013). When predators are mobile and prey are relatively immobile, spatial distribution of predator and prey are expected to be positively correlated (Sih 1984). In a study that investigated wasp attraction to conspecifics and food, attraction was found to be density-dependent (D'adamo and Lozada 2005). Foraging wasps were more attracted to locations with greater numbers of conspecifics. The results of this study suggest that aggregations of conspecifics are indicative of a plentiful quality food source, thus, active predators may exploit conspecific pheromones to locate prey.

Beetles (Order Coleoptera) are the most diverse insect taxa, consisting of about 400,000 species worldwide. Beetles populate all major habitats, excluding marine and polar regions (Banerjee 2014). Many ground beetle species (Family Carabidae) are voracious predators, demonstrating an active foraging strategy. Active foragers have wide ranges and are frequently searching the environment for prey (Butler 2005). Although carabids are generally characterized by their use of random search patterns, a previous study has shown that these ground beetles use environmental cues to detect prey (Lovei and Sunderland 1996). Several beetle species are such successful active predators that they are used in integrated pest management (IPM) (Banerjee 2014). Coccinellid beetles are attracted to the honey dew and plant volatiles produced by aphids during feeding (Ninkovec et al. 2001). Both parasitoids and predators of several arthropod species have been shown to use the sex pheromones of their hosts to locate prey (Hassel and Southwood 1978, Symondson et al. 2002, Sternlicht 1973). Clerid beetles use the pheromones of their bark beetle prey to locate feeding patches (Hansen 1983). Coccinellids, along with other beneficial insects are used in pest management worldwide to regulate pest populations. Regulation of insect pest species through IPM is critical to sustainable, profitable, and productive agricultural practices (Koul and Cuperus 2007). Understanding the underlying mechanisms of how predators locate prey can help managers make informed decisions in complex systems.

The objective of this study was to evaluate the ability of the ground beetle *Calosoma wilcoxi* (Carabidae) to detect olfactory cues associated with a lepidopteran larva, *Alsophila pometeria* Harris, commonly known as the fall cankerworm (FCW), a host plant of the FCW, and conspecifics. I expected that *C. wilcoxi* would detect olfactory cues associated with larval frass, herbivore damaged leaves, larvae, and conspecifics, which would be evidenced by the beetles moving toward the odor in a laboratory setting.

Study System

Calosoma wilcoxi (LeConte), a North American ground beetle in the family Carabidae, is an avid predator that specializes on lepidopteran larvae often found in the forest canopy (Burgess and Collins 1917, Allen 1977, Evans 2014). *C. wilcoxi* can be found in Canada, and the eastern, southern, and central United States (Evans 2014). Little is known about the natural history of *Calosoma wilcoxi*, but members of the genus *Calosoma* generally emerge from hibernation from early spring to early summer. It is believed that *C. wilcoxi* adults feed for several weeks on the larvae of various species of Lepidoptera, then females lay eggs in the soil. Eggs hatch approximately one week later and larvae feed ravenously on lepidopteran larvae and pupae. Larvae enter pupation after approximately 28 days and emerge later as adults. Adults live up to 3 years (Burgess and Collins 1917).

C. wilcoxi adults have been observed climbing trees and co-occurring on branches while feeding on FCW (Derek Johnson, *pers. comm.*). The FCW is an herbivorous forest lepidopteran (Geometridae) that is native to eastern North America (Schneider 1980). In 2012-14, populations of FCW caused considerable forest defoliation in central Virginia, showing a preference for mature *Quercus alba* (white oak) stands. Oaks are both economically and ecologically valuable, having important uses in construction and playing a role in promoting biodiversity (Asaro and Chamberlin 2015). Oaks provide habitat and food for over 200 species of herbivores, as well as several species of insectivorous birds, rodents, ungulates, and bears (Wold and Marquis 1997, McShea et al. 2007). Considering the implications of the effects of insect outbreaks, understanding the factors that influence predator movement around the forest may prove both economically and ecologically beneficial.

Materials & Methods

Behavioral Assays

I tested orientation responses of *C. wilcoxi* to multiple FCW and white oak olfactory cues in six treatments: damaged leaves, frass, larvae, female beetles, male and female beetles together, and a mixed factor treatment. Damaged leaves, frass, and female beetle experiments were conducted in 2015. All six treatments were tested in 2016. Damaged white oak leaves had been fed on by FCW caterpillars for a period of 1-2 hours. In the experiments, I used a damaged leaf approximately 20cm² in area. The frass treatment used approximately 0.5g of frass collected from FCW rearing cups during a 24-hour period. In the larvae treatment, caterpillars were removed from leaves 1 hour prior to use in treatment to decrease the likelihood of frass production during trials. I also tested attraction to conspecifics. The female beetle treatment consisted of 2 females and the mixed sex treatment consisted of 1 male and 1 female beetle. Only male beetles were used in the Y-tube for the female experiment. Caterpillars, damaged leaves, and frass were also combined in the mixed factor treatment.

Olfactometer Design

I conducted the behavioral assays using a Y-tube olfactometer based on the design of Oster et al. (2014). There were differences in materials and methods between years 1 and 2. In 2015, the Y-tube was constructed using three plastic tubes, inner diameter (ID) 50.8mm, that were connected using a Y PVC joint (120° angle). In 2016, the Y-tube was a Pyrex Y-tube, ID 32mm, having two arms (90° angle), each connected to an Erlenmeyer flask using Vinyl-Flex tubing. We used glass Y-tubes in 2016 to increase visibility, as it was difficult to observe entry into an arm with the PVC joint in the previous year. Each Erlenmeyer flask was then connected to a 1.2 LPM flowmeter (Cole-Parmer), which was in turn connected to another Erlenmeyer flask containing a

water and carbon solution for purifying the air. The Erlenmeyer flasks containing the solution were each connected to 115V air pumps (Tetra) using airline tubing. The air pumps were used to propel air through the first flasks for air purification, then through the treatment/control flasks, and finally to the two arms of the Y-tube. A treatment stimulus was placed in one flask while the other flask acted as the control. The control for each treatment was odorless air. All stimulants were placed in sealed flasks at least 30 minutes prior to experiments to ensure concentration of cues. All tubing, with the exception of that connected to the arms of the Y-tube and the air pump, were connected to stainless steel dip tubes using metal cable ties and fit into one of two openings in a rubber stopper; one opening was fit with a dip tube that allowed inflow of air while the other opening allowed an outflow of air. The arms of the Y-tube and the opening of the air pump were closed with a rubber stopper having only one opening for air flow through the dip tubing. Y-tubes were oriented at an upward angle to mimic the slope of a tree trunk. To improve traction in 2015, texture was added to plastic tubing using sand paper, similarly, in 2016, traction was provided using tread strips. I used vapor trail testing to visualize airflow through the apparatus and ensure that there was no mixing of air beyond the juncture of the Y-tube. The vapor trail was produced by placing solid CO₂ (dry ice) in flasks with water and turning airflow on. Airflow was adjusted to 0.25 LPM at which rate there was no mixing beyond the juncture.

All trials were conducted in a dark room to mimic the beetle's natural foraging period. A lamp containing a red light was placed near the juncture of the Y-tube to eliminate effects of external light that may be directional and influence the beetle to walk in the direction of the Y-tube. The red light was used because *C. wilcoxi* is nocturnal and insects are unable to see in the red color spectrum. All beetles were sexed prior to the experiment. Each trial offered a choice between one of the six treatment stimuli and a control. The Y-tube was cleaned between each trial

with ethanol and allowed to dry completely before use in subsequent trials. Treatment arms were randomly assigned in each trial to control for bias. In 2015, a beetle was placed in a small plastic cup and allowed a 2-minute acclimation period. Prior to release, the airflow was turned on to 0.25 LPM in each arm. After 2 minutes the beetle was introduced into the Y-tube via an elbow PVC joint. In 2016, beetles were not allowed an acclimation period, due to beetles repeatedly flipping on their elytra in the holding cup observed in 2015. In 2015 beetles were allowed 15 minutes to make a choice; first choice (treatment or control) and time to choose was recorded. In 2016, because the majority of beetles in the previous year that made a choice made it within 5 minutes, beetles were allowed 5 minutes to make a choice; I recorded first choice and time spent in the treatment and control arms during each trial. Additionally, in 2016, beetle elytra were marked in order to record individual responses. A choice was recorded upon entry into an arm. If beetles did not move beyond the Y-juncture, response was recorded as no choice.

Insects

Adult beetles and FCW larvae were collected April-May of 2015 and 2016 from Rockwood Park (37.4526N, 77.5800W), Chesterfield County, VA, Forest Hill Park (37.5175N, 77.4722W) and Bryan Park (37.5889N, 77.4777W), Richmond VA, the Virginia Commonwealth University Rice Rivers Center (37.3306N, 77.2085W), Charles City County, VA, and one residential property (37.5285N, 77.5743W) in Bon-Air, VA. Larvae were reared on white oak leaves in paper containers with plastic lids. Beetles were housed in plastic containers that were filled with approximately 1 inch of organic garden soil. Beetles were kept on an 11:13 L:D photoperiod and fed FCW larvae every 48 hours. In 2016, previously infested white oak leaves were introduced into the containers during feeding sessions to encourage feeding associations. This practice was not included in the 2015 rearing protocol. Additionally, in 2016, Y-tubes were left in beetle

containers 24 hours prior to trials to desensitize beetles to tubes. This system is ephemeral and as such insect collection and behavioral assays were conducted within 3-4 weeks.

Statistical Analyses

I performed a one-tailed exact binomial test to determine significance in preference for treatment or control, analyzing the proportion of beetles that chose treatment for each experiment. First choice data from the frass, damaged leaf, and female treatments in 2015 and 2016 were combined because I found no difference in responses using the different mechanisms and protocols. A one-tailed paired t-test was used to determine whether the mean time spent in the treatment arm was significantly greater than the mean time spent in the control arm of the Y-tube. This test was used for each experiment except the mixed factor and mixed sex experiments. Because t-test assumptions were not met, a one-tailed Mann-Whitney test was used in the mixed factor and mixed sex experiments to test for differences in the mean time spent in treatment and control arms, respectively. The individual beetle was the unit of measure, thus, time was averaged for each individual beetle prior to analysis for all experiments. I used a generalized linear model to test for differences in the time to choose treatment or control in the frass, damaged leaf, and treatment experiments from 2015. A generalized linear model was used to test for the effect of sex on proportion of beetles choosing the treatment in each experiment. All analyses were run using the software R version 3.1.1.

Results

Beetles did not preferentially chose the stimulus in the caterpillar ($p=0.43$, 50 trials: 15 treatment, 13 control, 22 no choice), mixed factor ($p=0.30$, 20 trials: 7 treatment, 6 control, 7 no choice), damaged leaf ($p=0.15$, 90 trials: 27 treatment, 19 control, 44 no choice), female ($p=0.50$, 73 trials: 23 treatment, 22 control, 28 no choice), frass ($p=0.80$, 66 trials: 15 treatment, 19 control,

32 no choice), or mixed sex ($p=0.50$, 22 trials: 9 treatment, 6 control, 7 no choice) experiments (Fig. 1). Beetle responses varied among trials. Some beetles walked up the Y-tube in a behavior similar to natural foraging movement, others moved rapidly in an erratic fashion, while others moved very little or not at all from the release point. Beetles did not spend more time in either of the experimental arms in the caterpillar ($p=0.60$, $t_{11}=-0.25$), mixed factor ($p=0.15$, $V=32$), damaged leaf ($p=0.63$, $t_{11}=0.3306$), female ($p=0.79$, $t_7=-0.87$), frass ($p=0.25$, $t_{11}=0.69$), or mixed sex ($p=0.13$, $V=67$) experiments (Fig. 2). Beetles that moved beyond the Y juncture often entered an arm, moved to the terminal end, and then returned to investigate the other arm. Beetles that first choose the stimulus in the female experiment ($p<0.001$) chose considerably faster than those that first chose the control, but this was not reflected in the frass ($p=0.469$) and damaged leaf ($p=0.599$) experiments (Fig 3). Sex of the foraging beetle had no effect on the proportion of beetles choosing the treatment in any of the experiments (Fig. 4, Table 1).

Discussion

Insect predators commonly use olfactory cues to locate prey (Kielty et al. 1996). Aphids, ants, and honeybees release alarm pheromones when exposed to predator attack (Verheggen et al. 2010), and trail pheromones have been identified in trail-following lepidopteran larvae. In this study, I found no evidence that *C. wilcoxi* uses olfaction to locate FCW caterpillars. This is consistent with the idea that larval pheromones are not typically expected in primitively social lepidopteran larvae (Capinera 1980). Larval pheromones may be advantageous for colonial insects where aggregation proves beneficial to the colony, but for non-colonial insects like the FCW, the cost of emitting pheromones may outweigh the benefits (Verheggen et al. 2010, Capinera 1980); thus, unintentional olfactory cues are likely to be fewer and in lower concentrations in solitary species.

Host plant volatiles attract predators and parasitoids to encourage predation on herbivores (Pare and Tomlinson 1999); however, in this study there was no attraction to damaged leaves, nor to the combination of leaves and herbivores. Past olfaction studies have focused primarily on agriculturally important plant species. To my knowledge, there are no previous studies that investigated the relationship between volatiles and natural enemies of insect herbivores in oak species. Kielty et al. (1996) found that three species of carabid were attracted to aphid alarm pheromone, springtail, and plant odors. Maeda and Takabayashi (2001) showed a positive relationship between predator attractiveness and the magnitude of volatiles produced; volatile amount was similarly related to density of herbivores. There may be some distinct density of FCW feeding at which *C. wilcoxi* responds to volatiles. Whether the concentrations of volatiles in this study were too low to illicit a response from *C. wilcoxi* is unclear because there is a lack of information on olfaction sensitivity in *C. wilcoxi*.

In 2015 and 2016 *C. wilcoxi* adults emerged shortly after FCW larvae were observed feeding on leaves, and were undetectable soon after FCW larvae dropped to the soil to pupate (*Personal Observation*). Upon hatching, *C. wilcoxi* larvae feed on lepidopteran pupae (Burgess and Collins 1917). This suggests that the FCW and *C. wilcoxi* life stages are closely synchronized. Given this relationship between life cycles, it is reasonable to infer that *C. wilcoxi* may be specializing on the FCW. In the mixed factor experiment, I expected that caterpillar regurgitant would stimulate a desired response from *C. wilcoxi*. Caterpillar regurgitant may be exploited by predators of insect larvae, as regurgitant triggers the production of green leaf volatiles associated with predator attraction. It is consequently the interaction of the regurgitant with the damaged plant that indirectly illicit the predator response (Stowe et al. 1995). The FCW is known to feed on several hundred species of trees and shrubs (Asaro and Chamberlin 2015). If *C. wilcoxi* is

specializing on the FCW, volatiles associated with white oak may not be important, because the FCW is a generalist herbivore feeding on a wide range of woody species.

Natural enemies that target the larval stage of insect prey are often attracted to the frass of prey when frass is located close to developing larvae (Chuche et al. 2006, McCall 1993). *Microplitis croceipes* (Cresson), a parasitoid wasp, is attracted to the frass of several lepidopteran larvae that feed on cowpea and cotton plants (McCall 1993). Similarly, green lacewings use larval frass of the diamondback moth, whose host plant is cabbage, to detect larvae (Reddy et al. 2002). Cabbage, cotton, and cowpea foliage grow close to the soil, thus, the prey remain in close proximity to their frass. In this study, I found no evidence that *C.wilcoxi* is attracted to olfactory cues emitted by larval frass. Fall cankerworms feed in the forest canopy and frass falls to the forest floor, far from larvae feeding sites; thus, frass is less likely to be a reliable cue for locating fall cankerworm larvae. Moreover, *C. wilcoxi* is brightly colored green, making this species conspicuous to predators on the ground. Thus, it may be advantageous for *C. wilcoxi* to quickly ascend into and remain in the forest canopy, moving from tree to tree in search of prey, providing little opportunity to encounter FCW frass.

In both the female and mixed sex experiments, beetles did not preferentially choose the stimulus over the control. This response was the most unexpected because pheromones play a vital role in sexual communication in many arthropod species (Witzgall et al. 2010). However, the time it took a male beetle to choose a female beetle was significantly faster than time to choose the control. This is evidence that some males are responding to female sex pheromones. The combination of these two results may be due to some females not releasing pheromones in the choice trials. Beetles of the genus *Calosoma* can live for 3 or more years. As egg production is related to food supply, females may forgo mating in one year, if conditions are not favorable (Lövei

and Sunderland 1996). The laboratory feeding regime and living conditions may have affected female pheromone emission.

If *C. wilcoxi* is not responding to olfactory cues, then what is the mechanism of prey location? Carabids may rely on visual, tactile, or gustatory cues (Lövei and Sunderland 1996, Negro et al. 2008). During feeding sessions, beetles were observed passing larvae in their immediate vicinity many times without attempting to subdue them. These observations, along with what is known about the natural history of this nocturnal organism, suggest that vision is of minimal importance in prey detection for this species. Specifically, many species of carabid characteristically forage for prey by walking in random search patterns (Lövei and Sunderland 1996). This behavior is often associated with olfactory-tactile predators (Negro et al. 2008). Unlike many animals, arthropods do not taste in the oral cavity. Tiny sensilla cover the appendages of many insects. Insects rely on these sensilla for gustatory information that is perceived through contact (Chapman 2003). Thus, gustation and touch are inherently bound together. It may be that *C. wilcoxi* requires olfactory information combined with gustatory and tactile information to effectively locate prey.

First choice may not be the defining measure of preference in olfactometer experiments using highly mobile species (Kielty 1996). Members of the genus *Calosoma* are active searchers, and by definition are inclined to be in motion during feeding intervals. Additionally, the time spent in each arm may not be reliable, because beetles were not rewarded for choosing the treatment. In fact, in many of the trials, beetles searched all arms, including the stem of the Y-tube, after failing to locate the source of the stimulus in the treatment arm. Recording the time to choose for individuals that have positive experiences with the stimuli may be the best measure for active insects.

This study provides the first assessment of the mechanisms of prey finding of FCW by *C. wilcoxi*. The results of this study suggest that olfaction is not the key underlying factor in prey detection in FCW, yet, it is evident that olfactory information is important in locating conspecifics. Information acquired from conspecifics allows inexperienced individuals to make informed decisions about mating, predator avoidance, and feeding (Chittka and Leadbeater 2005, Coolen et al. 2005). It remains to be seen whether cues associated with conspecifics play a significant role in foraging behavior of *C. wilcoxi*. Future studies investigating levels of predator experience and the differences in behavior in and out of the presence of conspecifics are needed to determine the relationship of conspecifics in this host-herbivore-predator complex.

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Tables

Table 1. Results of generalized linear model for effect of sex on proportion of beetles choosing the experimental arm of the Y-tube. The experimental arm had one of the following treatments: (1) caterpillar, (2) mixed factor—caterpillar, herbivore damaged leaves, and herbivore frass combined, (3) herbivore damaged leaves, (4) herbivore frass, or (5) female and male beetle pair.

Treatment	Estimate	SE	z	P
Caterpillar	-1.07	0.84	-1.28	0.20
Mixed Factor	1.10	1.38	0.79	0.43
Damaged	-0.50	0.66	-0.76	0.45
Frass	0.15	0.73	0.21	0.83
Mixed Sex	-0.92	1.30	-0.70	0.48

Figures

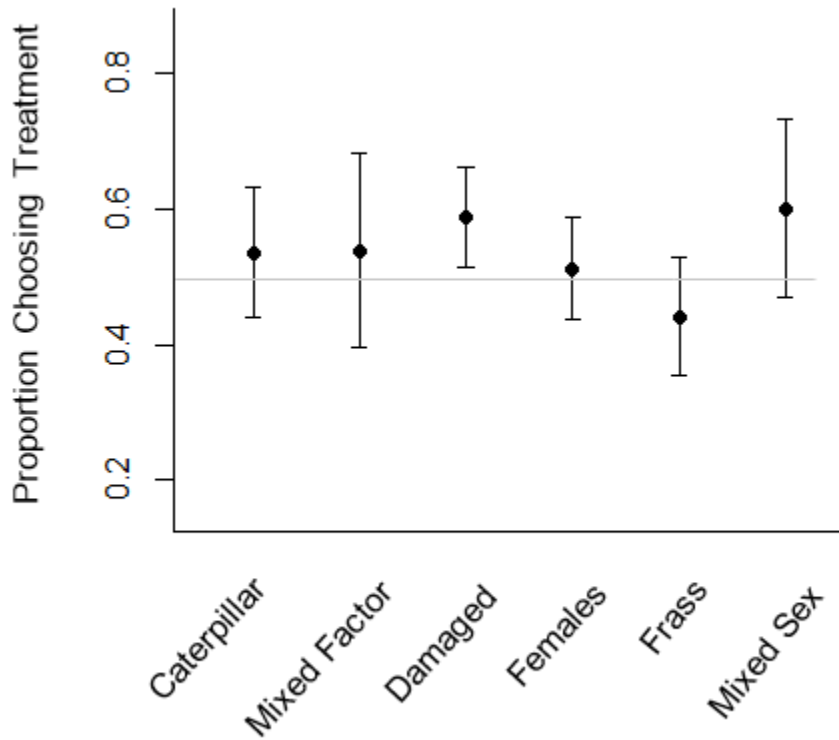


Figure 1. Proportion \pm SE of beetles that chose the experimental arm of the Y-tube. Error bars are based on the binomial distribution. The experimental arm had one of the following treatments: (1) caterpillar, (2) mixed factor—caterpillar, herbivore damaged leaves, and herbivore frass combined, (3) herbivore damaged leaves, (4) female beetle pair, (5) herbivore frass, or (6) female and male beetle pair. The gray line indicates the point where fifty percent of beetles chose the experimental arm of the Y-tube.

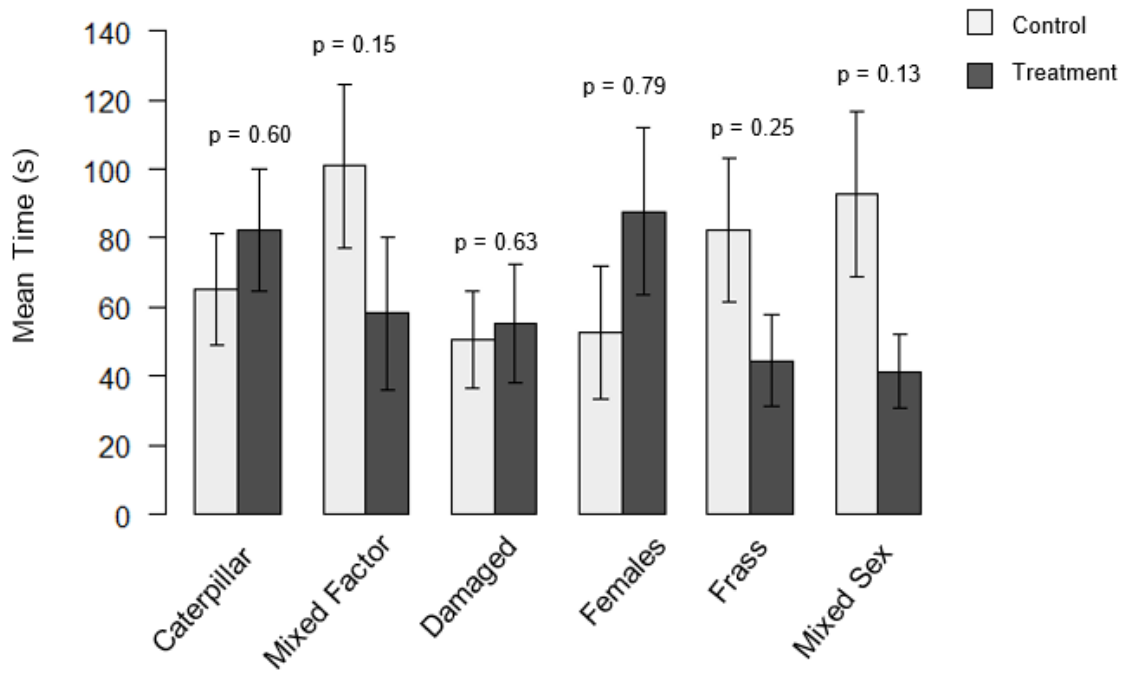


Figure 2. Mean \pm SE of the time beetles spent in the experimental and control arms of the Y-tube during five minute trials. The experimental arm had one of the following treatments: (1) caterpillar, (2) mixed factor—caterpillar, frass, and herbivore damaged leaves combined, (3) herbivore damaged leaves, (4) a female beetle pair, (5) herbivore frass, or (6) a male and female beetle pair.

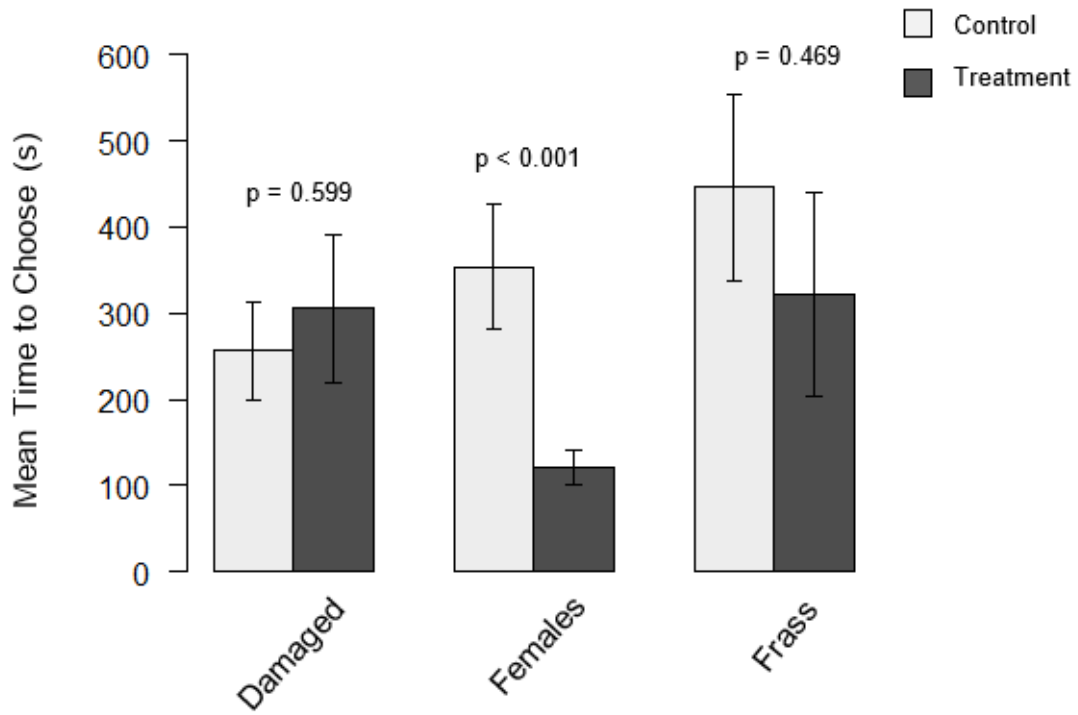


Figure 3. Mean \pm SE of the time it took beetles to choose the experimental or control arms of the Y-tube. The experimental arm had one of the following treatments: (1) herbivore damaged leaves, (2) a female beetle pair, or (3) herbivore frass.

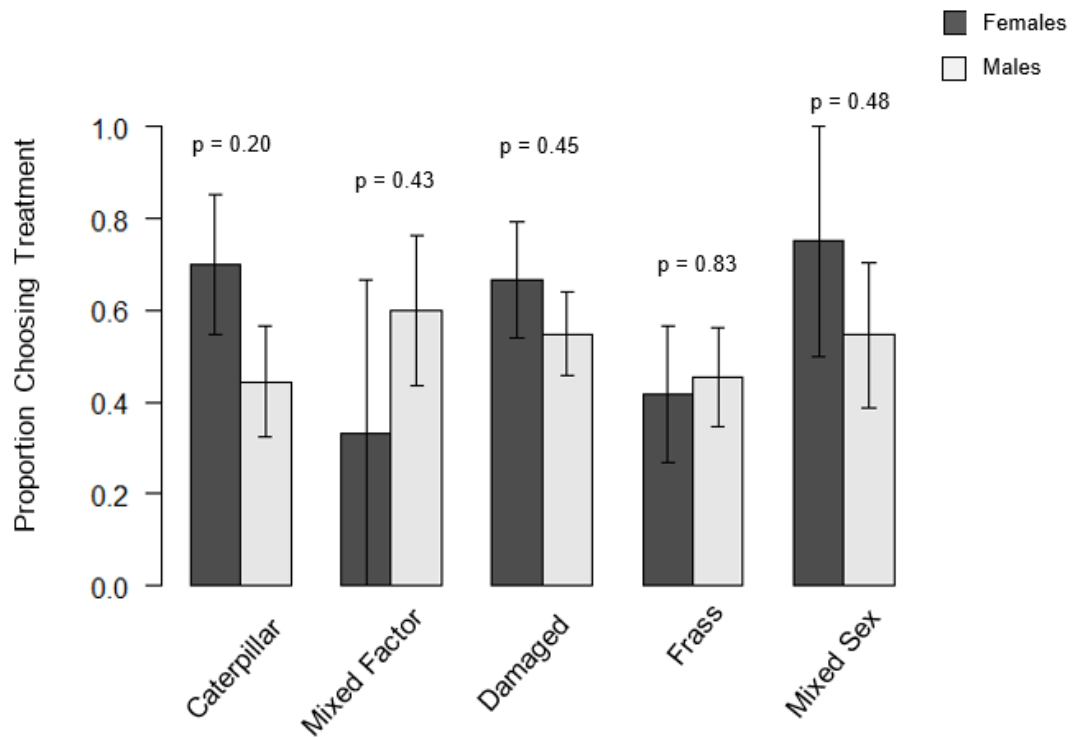


Figure 4. Proportion \pm SE of female and male beetles choosing the experimental arm of the Y-tube first. The experimental arm had one of the following treatments: (1) caterpillar, (2) mixed factor—caterpillar, herbivore damaged leaves, and herbivore frass combined, (3) herbivore damaged leaves, (4) herbivore frass, or (5) female and male beetle pair. Error bars are based on the binomial distribution.