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DYNAMICS OF AGGREGATION FORMATION IN JAPANESE BEETLES,

POPILLIA JAPONICA

by

Katelyn A. Kowles

THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE in BIOLOGICAL SCIENCES

In the Graduate School, Eastern Illinois University

Charleston, Illinois

2009

I hereby recommend that this thesis be accepted as fulfilling this part of the graduate

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Abstract

For most insect aggregations to form, they need to be started by an initial individual (the pioneer) and joined by later individuals (the joiners). Pioneers and joiners may differ with regard to characteristics such as sex and body size. We carried out three field experiments to examine the characteristics of Japanese beetles, *Popillia japonica*, pioneering and joining aggregations on host plants. Individual beetles were captured as they arrived on uninhabited grape plants, as well as plants designed to simulate aggregations with model beetles and feeding damage. For all experiments and treatments, the beetles arriving were significantly female-biased, with pioneer females having higher egg loads than females in aggregations. Females pioneering later in the day had higher egg loads than those arriving earlier. Male beetles found on uninhabited plants were smaller and arrived earlier in the day than males in the aggregation area of the experiment. These results suggest that female Japanese beetles are typically the initiators of aggregations (i.e. the pioneers) and males are joining later in the process, and that females with fewer eggs and males with larger body sizes are more likely to join aggregations. We use these patterns to hypothesize on the different uses of aggregations by male and female Japanese beetles.

Dedication

This thesis is dedicated to my parents, Joseph and Dorothea Kowles.

Acknowledgements

First and foremost, I thank my advisor Dr. Paul Switzer for his support and guidance over the past two years. The successful completion of my Master's degree would not have been possible without him. I also thank my committee members, Dr. Eric Bollinger and Dr. Ann Fritz for their support.

I thank my friend and field assistant, Anders Grau, for his help the past two summers with extensive field work. Additionally, this project would not have been successful without the help of Ryan Cumming, Eric David, Steve Malehorn, Dr. Scott Meiners, Daniel Quinn, Dr. Annalisa Switzer, and Dr. Wes Whiteside.

This project was supported by the National Research Initiative of the USDA Cooperative State Research, Education, and Extension Service, grant #2005-35302-16364, to PVS.

Table of Contents

Abstract	3
Dedication	4
Acknowledgements	5
Chapter I.....	7
Host Plant Selection in Insects	7
Japanese Beetle Biology.....	24
Chapter II	46
Introduction	46
Methods	49
Results	54
Discussion	59
Literature Cited	67
List of Figures	73

Chapter I

Host Plant Selection in Insects

Locating appropriate host plants is crucial for phytophagous insects to survive and reproduce. How they locate these host plants is not exactly known at every step, but it is usually divided into two stages: host plant finding and host plant acceptance. While these maybe divided into two separate stages they are not always readily distinguishable in the field.

Both host plant finding and acceptance, or rejection, by an insect are based on its response to the plants' physical and chemical features. The first features encountered by an insect are chemical in the form of plant volatiles attracting the insect from a distance. With the immense variability among host plants insects need to be able to locate their hosts. To do this they use a combination of visual, tactile, olfactory and gustatory cues.

Olfaction

Olfaction is a major component of an insect's sensory system, especially since plant volatiles are the first things that attract them to their host. They can smell a wide range of scents and can probably sense any plant whether it is a host for them or not. In a test with four related grasshopper species and host and nonhost plants, all four species were able to smell and responded similarly to the plants (White & Chapman 1990).

Experiments with the diamondback moth, *Plutella xylostella*, were conducted to test the role of olfactory and visual stimuli in host plant selection (Couty *et al.* 2006). Using olfactory cues alone, the female moths were able to discriminate between host and non-host plants and preferred host plants. This result shows that females have a clear attraction to host plant volatiles, and that olfaction plays a crucial part in host plant

selection for diamondback moths. In a different test that incorporated visual cues they found that the shape and color of host plants did play a minor role but olfactory cues dominated the process of host plant location (Couty *et al.* 2006).

Attraction

The different chemicals in plants that influence host selection by insects are categorized by how they affect insect behavior. Attractants cause insects to make oriented movements towards the stimulus, while repellants cause insects to make oriented movements away from the stimulus. When it comes to eating, a feeding stimulant is a chemical that elicits feeding. A deterrent is a chemical that inhibits feeding (Bernays & Chapman 1994). For example, the tobacco budworm, *Heliothis virescens* and cabbage looper, *Trichoplusia ni* are attracted to the odors of host plants in wind tunnels (Mitchell *et al.* 1991, Tingle & Mitchell 1992).

Several orders of phytophagous insects have a root-feeding phase of their life and the mechanisms by which they locate roots to feed on are variable. Johnson and Gregory (2006) reviewed 78 studies on root-feeding insects and host selection. Soil dwelling insects rely on semiochemicals alone to locate hosts because they cannot utilize visual cues underground. The semiochemicals are most likely in the form of secondary plant metabolites released from the roots of which 80% have attractant properties. There is a high level of specificity associated with these compounds for no single chemical was common to more than two species of insects (Johnson & Gregory 2006).

Plant Chemicals

While there are many host specific attractants, plants also produce a common range of chemicals that cause a general attraction called “green leaf volatiles” (Bernays &

Chapman 1994). Green leaf volatiles are typically a combination of chemicals common to many different species of plants. They include saturated and monosaturated six-carbon aldehydes, alcohols and esters. Green leaf volatiles can attract insects to host plants alone, or they may work together or against insect pheromones to increase or reduce attractiveness, respectively (Ruther 2000).

Plant volatiles from damaged plants induced by feeding insects can be used as olfactory signals for other insects to locate hosts (Williams *et al.* 2005). This is common among phytophagous insects and in many cases damaged plants are more attractive than undamaged ones. The attraction to green leaf volatiles was tested with the European cockchafer beetle, *Melolontha melolontha* L. with damaged and undamaged leaves. Using mechanically damaged leaves, the volatiles of the host plant *Fagus sylvatica* attracted the most male beetles out of any hosts tested. Volatiles from undamaged *F. sylvatica* leaves were not attractive to any males (Reinecke *et al.* 2002).

An experiment was done with female Colorado potato beetles, *Leptinotarsa decemlineata* (Say) to test their attraction to damaged and undamaged potato plants (Landolt *et al.* 1999). They found that significantly more beetles moved upwind toward plants with feeding damaged from conspecific larvae than plants with no damage. However, potato plants that had been damaged mechanically were not any more attractive to females than undamaged plants. These data show that the plant volatiles emitted from potato plants need some interaction through feeding conspecifics before they become attractive to the beetle (Landolt *et al.* 1999). In laboratory studies conducted with an olfactometer, it was found that the cottonwood leaf beetle, *Chrysomela scripta*, is attracted to volatiles given off from conspecifics feeding on plants. The plants

attractiveness to the beetle was increased once it sustained conspecific feeding damage (Kendrick & Raffa 2006).

Vision

Visual stimuli are important factors in host location for insects. However there is so much variation even within a single plant species that visual attraction usually occurs in conjunction with olfactory attraction. Factors that may be important include leaf shape, size and color. For example apple maggot flies, *Rhagoletis pomonella*, are attracted to yellow rectangles in the lab but not red, black or white rectangles; conversely, apple maggot flies are less attracted to yellow spheres than they are red and black spheres (Prokopy 1968). This indicates the insects are differentiating between foliage and fruit and their colors and shapes.

In laboratory and field studies one of the most attractive colors to flying insects is yellow. Black bean aphids, *Aphis fabae*, prefer to land on yellow leaves and more are caught in yellow traps (Bernays & Chapman 1994). In laboratory studies, females of the grass yellow butterfly, *Eurema hecabe*, landed on and oviposited significantly more on leaves colored yellow-green compared to other colors when given the choice (Hirota & Kato 2001). While these studies show insects' strong, clear attraction to yellow it is not completely obvious why.

Plant odors are clearly the major factor in host location; however a study with monophagous leaf beetles, *Altica engstroemi*, showed that they use solely visual cues (Stenberg & Ericson 2007). According to olfactometer tests, the beetles were never attracted to plant odors, damaged or undamaged, even when starved for 24 hours prior. However, a significant number of beetles were attracted to host plants in the field using

visual cues alone. Whether they were using the shape or color of the plant, or some combination of both was not able to be determined (Stenberg & Ericson 2007).

In the pine weevil, *Hylobius abietis*, visual stimuli are at least as important as olfactory stimuli in locating an undamaged host plant (Bjorklund *et al.* 2005). Weevil traps that have just visual or just olfactory stimuli catch more than traps with nothing, and ones that have both visual and olfactory stimuli together catch even more weevils. This implies that there is an additive effect between the two stimuli, but the exact interaction linking them is unknown (Bjorklund *et al.* 2005).

Host Plant Acceptance

Feeding stimulants, or phagostimulants, are usually in the form of nutrients and if present in high enough concentrations the insect will feed on the host. The main nutrients with phagostimulatory power are sugars, especially sucrose and fructose (Bernays & Chapman 1994).

For root-feeding insects once they locate and touch their host the chemicals involved are either phagostimulants or deterrents. In the 78 studies Johnson and Gregory (2006) reviewed the phagostimulants were mostly primary plant compounds and almost half (48%) of them were sugars. The deterrents were mainly secondary plant compounds, half (50%) of which were isoflavonoids. Various combinations of these chemicals may be crucial for root-feeding insects distinguishing hosts from non-hosts and even between different parts of the root (Johnson & Gregory 2006).

When an insect decides to reject a host it is usually because of plant secondary compounds acting as deterrents. Most species of plants have deterrents for the majority of insects. Many orders including Coleoptera, Lepidoptera, and Hymenoptera with

species ranging from monophagous to polyphagous have been shown to be deterred from a high percentage of plants tested (Jermy 1966). However, because of their specificity oligophagous insects are deterred by a higher number of plants than polyphagous insects. Even plants that have deterrents are acceptable to some species. These species typically feed frequently on plants with deterrents and have adapted to them.

Experience

Once an insect gains experience with a host plant it may vary its response. One example is habituation, with a potential benefit being chemical deterrents which can help an insect eat a plant that was once unacceptable. For example, tortoise beetles, *Cassida nebulosa*, adults rarely eat spinach because it contains feeding deterrents and typically feed on their host *Chenopodium album* var. *centrorubrum*. However, adults that were reared on spinach have a decreased response to the feeding deterrent compared to those reared on their usual host. Also, adults that were reared on leaves of *C. album* var. *centrorubrum* treated with the feeding deterrent found in spinach ate significantly more spinach than adults that were reared on leaves of *C. album* var. *centrorubrum* with no deterrent. These results suggest that the beetles had habituated to the deterrents found in spinach early in life and were able eat it as adults (Nagasawa & Matsuda 2007).

Another example of learning is associative learning; a particular type of negative associative learning is called food aversion learning where an insect learns to relate a noxious effect after eating a certain food. In a lab experiment the grasshopper *Schistocerca Americana* was given a novel plant then injected with a toxin to make it sick. When given this choice of plant again, it ate little or none compared to other plants

(Lee & Bernays 1990). This study shows that these grasshoppers had acquired food aversion learning toward that type of plant.

Associative learning has also been applied in host plant selection specifically in the case of the insect parasitoid *Aphidius ervi* Haliday (Hymenoptera, Brachonidae) (Guerrieri *et al.* 1997). Females use olfactory cues from a host-plant complex to orient themselves while flying (Guerrieri *et al.* 1993). After adult female parasitoids oviposit on aphids they demonstrate a drastic increase in their oriented flights to plants uninfested with aphids. Also, after oviposition they are reactive to plant volatiles that they did not respond to beforehand (Guerrieri *et al.* 1997).

Pioneering

In a group of animals the first individual to start the aggregation is known as the pioneer; the other individuals to join the aggregation after the initial colonizer are called joiners. The information on pioneer-joiner relationships is scarce, but there are some other analogous relationships that occur in insects and vertebrates.

Producer Scrounger

An analogous relationship to a pioneer-joiner is producer-scrounger. A producer is an individual who initially invests in a resource, while a scrounger exploits that resource provided by the producer (Barnard & Sibly 1981). In this model, the producer is similar to a pioneer since it is the first to a resource, and the scrounger is similar to a joiner since it comes later. This system works for scroungers because they lower the cost of acquiring a resource because they let the producers invest the energy to obtain the resource, and then steal the results (Barnard & Sibly 1981). In a general producer-scrounger model the benefits to scroungers increase with the number of producers

because they have more opportunities to take over resources. The model is frequency-dependent, so scroungers will do better than producers if scroungers are rare, but worse off if scroungers are more common (Barta & Giraldeau 1998).

Protandry

Protandry is a second relationship occurring in nature analogous to pioneer-joiner. It is the tendency for males to emerge before females and can occur daily or seasonally (Thornhill & Alcock 1983). In this relationship the pioneers are the males that emerge first in the day or season, and the joiners are those that emerge later on. It is standard in a number of insect orders, and common in several others.

Thornhill and Alcock (1983) describe four hypotheses that support protandry. The first one is to prevent inbreeding within a species. The second one is increase sexual selection, by removing males that are unfit before the reproductive process. The third theory for protandry is to decrease the death of females before they reproduce by allowing them to fertilize immediately after emergence. This theory is based on a major assumption that females mate right after reaching adulthood, so males will modify their emergence to this rather than when females are receptive. The last theory supporting protandry is that it increases the reproductive success of males by giving the early emerging males more access to females than the late emerging ones (Thornhill & Alcock 1983).

There is lots of support for protandry across different insect groups. Krombein (1967) looked at more than 100 different species of Hymenopterans and found that males were always on the outer cells of nests and females were in the inner cells. In these cases, males would have to emerge first because if they didn't, the females would kill their

brothers as they ate their way through the nest to get out. Different species of mosquitoes have been shown to be protandrous, specifically the pitcher-plant mosquito, *Wyeomyia smithii*. In this species the males' reproductive success is based on not only the timing of his emergence relative to the female's emergence, but also to other males' emergence (Holzapfel & Bradshaw 2002). The brimstone butterfly, *Gonepteryx rhamni*, also exhibits protandry with the males emerging on average 21 days before the females and reaching their peak abundance eight days before the females reach their peak abundance (Wiklund *et al.* 1996).

A phenomenon that has been compared to protandry is male pioneering, which is when males are active each day before females (Harari *et al.* 2000). This was studied in the beetle *Maladera matrida* Argaman (Coleoptera: Scarabaeidae) where the males emerge each night a few minutes before the females to feed and mate, and the feeding induced plant volatiles attract receptive females. It was found that the males that emerge earlier have a higher reproductive success than those that emerge later (Harari *et al.* 2000).

Protogyny

Protogyny is the later emergence of males, and earlier emergence of females (Thornhill & Alcock 1983). While this is a relatively rare phenomenon in nature, some instances have been documented among different orders of insects. In this case, the females that are emerging early are analogous to the pioneers and the females that emerge later in the day or season are analogous to the joiners. Protogyny can occur if females mate multiple times, becoming receptive shortly after oviposition, and if males are able to displace sperm from a female's earlier mates (Thornhill & Alcock 1983).

Mosquitoes of the species *Aedes taeniorhynchus* exhibit protogynous behavior and it was shown that later emerging males had greater reproductive success compared with males of other mosquitoes that are not protogynous (Nielsen & Nielsen 1953). In the two-spot ladybird, *Adalia bipunctata* (L.) females mate multiple times, and sexual competition for females is unlikely suggesting that both sexes should emerge simultaneously. However, in one study it was shown that females exhibited significant protogyny (Hemptinne *et al.* 2001).

Pioneering a New Host Plant

There are several reasons why an insect may leave a host plant and go start an aggregation at a new one, including seeking out novel food resources, safety from natural enemies, reduction of interspecific competition and effects from the plant that directly influence the insect.

Gross *et al.* (2004) looked at the role of natural enemies in leaf beetles, *Chrysomela lapponica*, pioneering a different host plant. *C. lapponica* populations feed on willow, but several have switched to birch. Predators and parasitoids are found on both plants, but instances of parasitism were significantly higher on willow plants and certain predators specializing on leaf beetles were only found on willows. These data show that the higher cases of parasitism and predators on the willow plants caused the beetles to switch hosts to birch (Gross *et al.* 2004).

Interspecific competition may cause some insects to leave one host and go seek a new one. Many studies have shown that plant eating insects may directly or indirectly alter plant suitability, however the level of these interactions may vary according to the insect and host (Gross *et al.* 2007). A review of 193 pair-wise interactions found that

interspecific competition occurred in 76% of interactions and was common in most feeding guilds, including sap feeders, wood and stem borers, and seed and fruit feeders (Denno *et al.* 1995). The likelihood of competition increased if the insects were related, introduced, sessile, aggregative or fed on discrete resources. For example, the leaf beetle *C. lapponica* feeds on willows and birches but has to compete with another species of beetle at willow plants, *Phratora vitellinae* (Gross *et al.* 2007).

Aggregation

It is very common for animals to aggregate into groups. It is a widespread phenomenon occurring in vertebrates and invertebrates and especially arthropods (Allee 1927). On the most basic level aggregations are divided into two different types, heterotypic and homotypic. Heterotypic aggregations contain more than one species, while homotypic aggregations have just one, and both types are extremely common (Allee 1927).

Ways to Form Aggregations

While we cannot be entirely sure about how and why animals are forming aggregations there are some basic manners of forming them. The first way is through a common tropism that each animal in the cluster is attracted to. A tropism is a directed movement by an organism in response to a particular stimulus. Some examples of tropisms include chemotropism, hydrotropism, phototropism, and thermotropism. Another way of forming an aggregation is by the basic method of trial and error, or simply random movements. This could occur if there is a limited amount of space available or no stimulus to direct the organism. A third mechanism of aggregation formation is by the animal's own choice to be in a group. They may decide to join their

conspecifics and form a group because of the benefits they receive from being in an aggregation (Allee 1927).

Chemotropic movement is when an organism or group of organisms responds towards a chemical stimulus: a specific type of a chemotropism is a pheromone. Pheromone mediated aggregation is a common phenomenon among insects and other animals as well (Wertheim *et al.* 2005). This is a mode of chemically controlled communication where the signals conveyed are specifically referred to as infochemicals or semiochemicals. These intraspecific chemical signals that induce groups are called aggregation pheromones, and they play a crucial role in insect ecology. Wertheim *et al.* (2005) define aggregation pheromones as released compounds causing aggregative behavior in conspecifics of both sexes or the same sex as the emitter. In the most general sense, aggregation pheromones function to form a group that serves for protection reproduction, feeding or a combination of those. In a survey they conducted it was found that over 300 species of nonsocial arthropods in 51 families in 12 orders use aggregation pheromones. And in most of these species the chemical composition of the pheromone is known. While there is a large diversity in the mechanisms insects use to form groups, similarities still exist in the ecological conditions in which the aggregation pheromones are being used (Wertheim *et al.* 2005).

Evidence of an aggregation pheromone produced by the red-headed ash borer, *Neodytus acuminatus acuminatus*, was recently reported (Lacey *et al.* 2004). This is the first report of an aggregation pheromone in the Cerambycid beetles, and in this species the males are the ones that produce the pheromone attracting both sexes. In this case it may be adaptive for males to attract males, even when competing for female mates if

more males have greater success in attracting females to their aggregation (Lacey *et al.* 2004). Recent studies also show support for an aggregation pheromone in the order Thysanoptera in the Western flower thrips, *Frankliniella occidentalis*. In this species it is also the males that produce the pheromone and attract both males and females (Hamilton *et al.* 2005).

In many cases insects forming aggregations by responding to pheromones are also responding to feeding induced host plant volatiles. The Brazilian soybean stalk weevil, *Sternus subsignatus* Boehman (Coleoptera: Curculionidae) forms aggregations and in order to mediate these groups the males emit a pheromone that attracts both sexes. However, both sexes are also attracted to host plant volatiles and this attraction is increased when the male produced pheromone is added. The actual structure of the compound released by the male changes depending on whether not they have access to their host plant (Ambrogi & Zarbin 2008). In a similar instance mites, *Caloglyphus polyphyllae* (Acari: Acaridae) exhibit different responses to a female and male produced pheromone depending on whether or not they are feeding (Shimizu *et al.* 2001). The pheromone is evenly distributed across both sexes. The pheromone produced is β -acaridial and when males are feeding the compound acts as a sex pheromone attracting males. However, to unfeeding, unmating mites β -acaridial acts as an aggregation pheromone for both sexes (Shimizu *et al.* 2001).

Many species of bark beetles use aggregation pheromones to attract large numbers of conspecifics in order to attack host trees. However this can create unwanted intraspecific competition for the beetles for food and space if they overcolonize. Different species have adapted particular functions to minimize competition, but still get

the most out of their aggregations. For example, *Ips paraconfusus* Lanier have sex-specific responses to aggregation pheromones which allow them to regulate the density of their colony (Byers 1983). The males of this species are the pioneers who first colonize a host tree and they emit an aggregation pheromone once they do. In a study using male infested pine logs, Byers (1983) found that more than four times as many females as males were found at the source of the pheromone. An equal ratio of males to females was found several meters downwind from the pheromone source. These data show that females were attracted directly to the males initially colonizing the host, while males settled on nearby uncolonized locations (Byers 1983).

Costs

Forming an aggregation can result in both costs and benefits for the animal. In most cases the benefits clearly outweigh the costs otherwise the aggregations wouldn't be formed. One of the main costs of existing in a group is competing for limited resources, namely food, shelter, and access to mates.

Even for social animals that are accustomed to living in aggregations there are costs associated with group formation. Colony aggregation was studied in the social wasp, *Polistes-annularis* in terms of its costs and benefits (Strassmann 1991). It was found that while they received protection in the group, they experienced increased reproductive competition. Those colonies on the edge of the aggregation produced less offspring than those on the inside (Strassmann 1991).

In many cases being in an aggregation can give animals increased access to mates, but in some instances this is not always beneficial. Campbell (2005) looked at the effects of multiple matings on the rice weevil, *Sitophilus oryzae* L. He found that compared with

a single mating with one male, continuous exposure to one male decreased a female's overall longevity and decreased average progeny size. Also, exposure to five males compared to one male significantly reduced a female's survival and the number and survival of her offspring (Campbell 2005).

For animals that forage in a group there is always competition for food. A specific type is shadow competition where sedentary foragers that are closer to the food source reduce the foraging success of individuals that are farther away (Wilson 1974). This was tested in the burrowing spider, *Seothyra henscheli*, looking at those foraging in aggregations or in solitary and those at the edge of aggregations compared to the inside (Lubin *et al.* 2001). The results showed that spiders in high density clusters grew less and were at a foraging disadvantage compared to solitary spiders. Also, the spiders in the outer positions of groups grew more than spiders in inner positions, supporting the shadow competition hypothesis (Lubin *et al.* 2001).

Benefits

One of the main benefits that animals receive from being in an aggregation is protection from predators and parasites. Another common advantage is acquisition and choice of mates. Animals also receive protection from various abiotic factors when they are in a group. Food comes more readily and in greater amounts in many cases when organisms are grouped. In addition, animals are able to obtain resources more efficiently while expending less energy individually. In some instances by being in an aggregation, animals can reduce interspecific competition. Lastly, when females lay their eggs in a group there is increased larval survival when they remain aggregated.

One of the most obvious benefits that animals gain from being in an aggregation is protection from predators. Specific types of protection include the dilution effect where the risk of being eaten is reduced as the number of animals in the group increases. Another type is the confusion effect where predators are unable to visually locate a single prey goal because there are too many (Parrish & Edelstein-Keshet 1999). Even prey that may not have any defense mechanisms may gain protection from being in group. For example, the aphid *Aphis varians* is defenseless against its predator the ladybug, but when it forms large aggregations the risk of being eaten is lowered (Turchin & Kareiva 1989). Experiments with butterfly larvae of the bordered patch butterfly, *Chlosyne lacinia*, show that larger aggregations are better protected from attack from airborne and solitary predators and parasitoids than those in smaller aggregations (Clark & Faeth 1997). Also, the yellow-rumped cacique, *Cacicus cela*, nests together and mobs as a group. In doing so, they avoid many avian predators and the effectiveness of their mobbing is positively correlated with the size of the group (Robinson 1985).

An example of the dilution effect is shown in bark beetles, which aggregate in order to overcome host plant defenses, were found to have diluted predator effects with increased colonization densities in studies with pine engravers, *Ips pini* (Aukema & Raffa 2004). The confusion effect is exhibited by schooling fish that are able to aggregate in large numbers and confuse a potential attacking predator (Turchin & Kareiva 1989).

Aggregations can help protect animals from various abiotic factors, such as temperature, wind, and sunlight (Allee 1927). Isopods, which are aquatic and terrestrial crustaceans, are better able to maintain their water levels when they are in aggregations (Allee 1927). In an environment that is full of moisture, they will absorb water less

slowly than solitary individuals, and in a dry environment they will lose water more slowly than solitary individuals. Through aggregating and maintaining stable water levels in their body, isopods can increase their lifespan (Allee 1927). Aggregations can also help animals conserve or protect themselves from heat. An example of this is the poikilothermal honeybees which group together in large clusters and generate heat when the temperature reaches a certain low (Allee 1927). In addition, social termites are able to thermoregulate their hive when they form large aggregations (Parrish & Edelstein-Keshet 1999).

When animals aggregate they are able to gain information they would not otherwise have if they were solitary individuals that can ultimately give them access to resources including food (Parrish & Edelstein-Keshet 1999). Being part of an aggregation allows them to input and analyze more information than if they were on their own, assisting them in the process of food location. For plant eating insects, feeding as a group enables them to better overcome plant defenses that might not be possible for lone individuals. For example, larvae of the bordered patch butterfly, *Chlosyne lacinia*, feed on sunflower leaves that are leathery and have spiky trichomes. For a single larva to overcome the toughness of this plant would be near impossible, but as a group they are able to feed (Clark & Faeth 1997).

For females ovipositing, it is beneficial to them to do so in a group because this increases the chances of larval survival. Grouped larvae are less likely to die when they are in aggregations compared to solitary individuals (Hunter 2000). In studies with *C. lacinia*, there was reduced desiccation at the egg stage when the eggs were grouped in a larger aggregation. Also, larvae developed faster and survived better when they were in

larger groups compared to smaller ones (Clark & Faeth 1997). In the Baltimore checkerspot butterfly, *Euphydryas phaeton*, larval aggregations of a moderate size had the least amount of parasitism compared to smaller aggregations (Stamp 1981).

Japanese Beetle Biology

The Japanese beetle, *Popillia japonica*, is an invasive species that first appeared in New Jersey in 1916 from Japan where it had minimal pest status. Since then Japanese beetles have become a major pest in United States agricultural and residential areas (Fleming 1972). It is now one of the most devastating insect pests in the eastern United States, feeding on more than 300 species of plants (Potter & Held 2002).

Feeding Preference

Adult Japanese beetles have a wide dietary range feeding on leaves, fruits and flowers of more than 300 plant species in 79 families (Potter & Held 2002). They will feed on fruits, garden crops, field crops, ornamental garden plants, ornamental shrubs and vines, shade and ornamental trees and some non-economic plants. Beetle grubs eat roots of garden plants, ornamental plants and grasses. Some of the most severely damaged plants include *Acer*, *Malus*, *Polygonum*, *Prunus*, *Ulmus*, *Vitis*, and *Tilia* genera. Japanese beetles will skeletonize leaves, feeding on the upper surface of plants and eating the tissue. If they are eating flowers they will usually eat the entire petal since the veins aren't too tough (Fleming 1969).

The previous lists of preferred hosts are based on feeding damage and do not give any information on how the host plants influence the beetle's feeding (Ladd 1987). Ladd looked at the influence of 45 favored host plants on the feeding behavior of the Japanese

beetle in the lab. Instead of looking at feeding damage alone, Ladd collected and weighed fecal pellets from the beetles as an indication of how much foliage they were eating. He used sassafras, *Sassafras albidum*, as a standard and compared it to different plants listed by Fleming (1969) as heavily or moderately fed upon. Only three species of plants were fed upon more than the sassafras; roses (*Rosa* sp.), European grape (*Vitis vinifera* L.), and red raspberry (*Rubus idaeus* L.). Roses and grape plants are considered heavily fed upon, as is sassafras, while red raspberry is only moderately fed upon (Fleming 1969). Five species of plants classified by Fleming as moderately fed upon induced feeding comparable to that of sassafras. However six species of plants considered heavily fed upon were significantly lower than sassafras in inducing feeding in Japanese beetles. While some of these results coincide with previous studies there are some differences and it indicates the variability in the beetle's host range (Ladd 1987).

Olfaction

Odor is probably the most important factor in the Japanese beetle's selection of a host plant (Fleming 1972). This was further shown by Ahmad (1982) when he concluded that olfaction not only played a role in host location but in host preference as well. He observed that beetles with antennectomies had reduced abilities to recognize and locate host plants, and also reduced levels of feeding. These data imply that there is an incorporation of smell and taste inputs in order for the beetle to achieve maximum feeding, which was not possible with the loss of antennae (Ahmad 1982).

Since the Japanese beetle was first found in the United States in 1916 and achieved pest status shortly thereafter, numerous chemical attractants have been tested. Screening tests showed that the beetles are attracted to a wide variety of unrelated odors,

probably because of its wide host range. However, the scent of fermenting fruit either on the ground or on a plant is a powerful attractant for the Japanese beetle (Fleming 1969). Langford *et al* (1943) isolated several attractants from apple, peach, saffron and rose plants, all known hosts, and found that they usually occur in combination with each other. The attractants are acetic acid, benzaldehyde, caproic acid, citral, citronellol, eugenol, geraniol, linalool, phenyl ethyl alcohol and valeric acid (Langford *et al* 1943). From 1928-1941, the standard attractant used for Japanese beetles at laboratories was a 10:1 mixture of technical geraniol and U.S.P eugenol (Fleming 1969).

Ladd *et al* (1976) found a highly attractive lure in a 3:7 mixture of phenethyl propionate (PEP) and eugenol. Tumlinson *et al* (1977) identified a synthetic sex pheromone (R,Z)-5-(1-decenyl)dihydro-2(3H)-furanone, named Japonilure, that attracts males in the same way the sex pheromones from live females do. Klein *et al* (1981) measured the Japanese beetle's response to each of the two attractants, and also a combination of the two. They found that while the synthetic sex attractant caught more males, the combination of the two mixtures caught significantly more beetles, both males and females, than either lure alone (Klein *et al* 1981). Ladd *et al* (1981) found that when the PEP and eugenol was mixed with geraniol in a 3:7:3 mixture it became even more attractive. Also, when Japonilure was added to the trap the attractiveness was significantly increased (Ladd *et al* 1981).

It was found that mixtures of chemicals were more attractive to beetles than expected than their components alone. For example bay oil has a relative attractiveness of 19% and pimenta oil has a relative attractiveness of 54%. When the two are combined in a 1:1 mixture they're expected to have an attractiveness of 37%, but instead it was

higher at 44% (Fleming 1969). In addition, the proportion of the components of the mixture is an important factor that modifies its attractiveness. For example, when anethole and caprioc acid are combined in a 9:1 mixture they only have a 12% attractiveness, but when it is changed to a 1:1 mixture the attractiveness increases to 58% (Fleming 1969).

While beetles have a strong attraction to fruit and floral scents (Fleming 1972) it was shown that they are attracted to a wide variety of volatiles with high complexity (Loughrin *et al.* 1998). They looked at 17 different compounds released by damaged and undamaged leaves and the beetle's level of attraction to each. The compounds included oxygenated terpenoids, terpene hydrocarbons, aliphatic esters and phenylpropanoids, all of which are structurally different. More beetles were caught with increasing numbers of compounds in the volatile blends, and increasing levels of complexity in the blends (Loughrin *et al.* 1998).

Many insects are attracted to herbivory induced volatiles and can exploit these as aggregation kairomones. Whether or not Japanese beetles are attracted to volatiles from damaged leaves was tested using crabapple leaves, *Malus x domestica* (Loughrin *et al.* 1995). They looked at the response to artificial damage, fresh feeding damage and overnight feeding damage. Results showed that crabapple leaves with overnight Japanese beetle feeding damage were significantly more attractive than undamaged leaves. But leaves with artificial damage or fresh feeding damage were not any more attractive than undamaged leaves. They concluded that leaves with overnight feeding damage give off a complex mixture of volatiles including aliphatic compounds, phenylpropanoid-derived compounds and terpenoids, while freshly damaged leaves give off a more simple mixture

of volatiles and mainly green leaf odors. It is these herbivory-induced volatiles that may be the best as to which host plants are the most suitable for beetles (Loughrin *et al.* 1995). Similar results were found with clonal grape plants, *Vitis labrusca* L., where the beetle's attractiveness was tested on undamaged vines, undamaged vines with non-feeding beetles, vines with fresh feeding damage and vines with overnight feeding damage (Loughrin *et al.* 1996). The highest number of beetles was attracted to vines with overnight feeding damage. Also, during the peak period of emission the volatile emissions from the overnight feeding damaged plants was about 65 times higher than from undamaged plants (Loughrin *et al.* 1996).

Studies clearly indicate plants that have sustained overnight feeding damage are more attractive than freshly damaged plants. However, whether or not this increased level of attractiveness was due to how long the plant had been damaged or what time of day the beetles were feeding was still unknown. Loughrin *et al.* (1997) looked at the volatile compounds released from grape plants, *V. labrusca* L., at different times of the day and found that the majority of the compounds followed a diurnal release. The peak emission period for the volatiles was between 12:00-15:00 and the period of lowest emission was between 00:00-03:00. Since feeding damage by Japanese beetles immediately produced high levels of volatiles that didn't drastically differ from that day to the next, it seems that herbivory induced volatiles are not dependent on how long the plant has been damaged. Instead they are more dependent on the time of day that the beetles are feeding (Loughrin *et al.* 1997).

For many insects when non-hosts are surrounding their hosts the volatiles create a mixture that no longer makes their host attractive, called non-host interference. This was

tested with Japanese beetles and a preferred host, roses, by interplanting with rue (*Ruta graveolens* L.), zonal geranium (*Pelargonium x hortorum* Bailey), and garlic chives (*Allium schoenoparum* L) (Held *et al.* 2003). None of the three treatments reduced the numbers of beetles on the roses, and the presence of geraniums significantly increased the number of beetles. The same study also looked at odor alone by using non-host volatiles including crushed red pepper (*Capsicum frutescens* L.), fennel seeds (*Foeniculum vulgare* Miller), crushed spearmint (*Mentha picata* L.), cedar shavings (*Juniperus* sp.), osage orange fruits [*Maclura pomifera* (Raif) Schneid.] and fleshy ginkgo seeds (*Ginkgo biloba* L.). Roses surrounded by fennel seeds, cedar shavings, crushed red pepper, and osage orange fruits had significantly more beetles than control plants. These results are most likely due to the fact that Japanese beetles are strongly attracted to complex blends of volatiles, even if they are non-hosts (Held *et al.* 2003).

Visual Cues

After the beetles use olfactory cues to locate their host plants, they use visual cues to locate and possibly accept hosts. One of the main factors affecting a Japanese beetle's decision is based on the height of the plant. This was tested using traps baited with the lures methyl cyclohexanepropionate eugenol (9:1) or phenethyl butyrate-eugenol (9:1) placed at different heights (Ladd & Jurimas 1972). They found that the traps were significantly more attractive when placed at 22 inches or 44 inches above the ground compared to ground level. Ladd & Klein (1982) did a similar experiment and tested traps ranging from ground level to 112 centimeters off the ground. They found that the most attractive height range was 28-56 cm above the ground (Ladd & Klein 1982). Alm *et al* (1996) tested standard Trece traps at ground level, 13cm and 90cm above the ground and

measured the number of Japanese beetles collected. The traps at 13cm caught significantly more beetles than those at ground level or at 90cm (Alm *et al.* 1996).

While it was clearly shown that Japanese have a certain attraction to height when being captured in traps, when they are feeding regardless of height they will still start at the top of a plant and work their way down (Fleming 1972). Potter *et al.* (1996) studied why beetles do this, looking at their aggregative behavior and also the nutritional value in the different levels of plant height. When they compared the upper canopy leaves that beetles initiate their attack on with leaves from other levels, there was no significant nutritional difference based on sugar or nitrogen levels. Regardless of this fact, in the field beetles still caused significantly more damage to the upper level of leaves compared to the lower level or inner shade leaves. Also, when they tested the height of plants, beetles caused significantly more damage to plants at 3.6 meters than those at 1.6m or at ground level. These results indicate that the height of plants affects where the beetles initially start to feed, and this may be due to visual cues that they receive from the host (Potter *et al.* 1996).

It is known that Japanese beetles prefer to feed on plants that are in direct sunlight and that these plants suffer more damage than those in the shade (Fleming 1972). Rowe & Potter (2000) looked specifically at rose plants and the effects that shade had on the characteristics of leaves. They also looked at the beetle's preference and fecundity when given leaves from sun or shaded conditions. The results showed that when given the option beetles significantly preferred leaves that were grown in sunlight over leaves grown in shade. After 14 days on either sun-grown or shade-grown leaves there were no significant differences in the beetle's survival or fecundity. When they looked at the

chemical components of the leaves, those grown in the sun had higher sugar concentrations. Considering the phagostimulatory power of sugars for Japanese beetles these could be the reason beetles prefer plants grown in the sun (Rowe & Potter 2000).

Deterrents

While the Japanese beetle is a highly polyphagous species there are some plants that are resistance to their attack. Fleming (1972) observed that the Bradford pear, *Pyrus calleryana*, is not a favored host of the Japanese beetle. The reason this species of pear is resistant was studied, and it was found that if the foliage was frozen and then thawed they became suitable for eating (Keathley *et al.* 1999). They observed that leaves that had been frozen turned brown after thawing, and it was in these brown areas of leaf tissue where feeding was the greatest. This led to the conclusion that enzymes are involved in damaged and browned pears becoming more suitable (Keathley *et al.* 1999).

Toxicity

Only two plants that the Japanese beetle readily eats have been shown to have toxic effects, the bottlebrush buckeye, *Aesculus parviflora*, and the geranium, *Pelargonium domesticum* (Fleming 1972, Ballou 1929). Japanese beetles will eat the bottlebrush buckeye, but if they eat enough they become paralyzed and die (Fleming 1972). They beetles also readily eat geranium and become paralyzed when they do. Most of them recover within 24 hours, but 35% of them die within four days (Ballou 1929). The flowers of the geranium are more toxic than the foliage, and the toxic effects are enhanced when the plants are exposed to sunlight.

Experience

Given that Japanese beetles will eat geranium plants, *P. hortorum*, even though they result in paralysis Potter & Held (1999) tested whether or not beetles could learn to avoid the toxic effects of the plant. When given a choice of either geranium or linden leaves, *Tilia cordata*, which is a normal host for the beetles, they strongly prefer geranium. Experienced beetles that had eaten the geranium and been through several bouts of paralysis still ate the geranium over the linden. After doing this for two weeks, the beetles' fecundity was reduced by more than ten times. These results show that Japanese beetles are not able to exhibit food aversion learning, possibly because of neurological damage from the toxicity or because of the strong positive feedback they receive from the taste of the geranium (Potter & Held 1999).

Pioneering

Very little information is known about the process of aggregation formation in Japanese beetles, especially how it is started. The pioneer beetles are the ones going to host plants and beginning the large feeding aggregations, however information on which these beetles are is scarce. In order to possibly gain some insight into beetle pioneering it may be helpful to look at their emergence patterns and sex ratios in feeding aggregations.

Japanese beetles have been shown to exhibit both protandry and protogyny. However they continually show behaviors synonymous with protogyny. Protandry is less likely to occur if a female's latest mate is able to displace sperm from previous mates, and also if females oviposit and then become receptive to mating again afterwards (Thornhill & Alcock 1983). These are all characteristics Japanese beetles continually shown throughout their lifetime. In a study looking at emergence patterns it was found that a specific population of Japanese beetles was protogynous, with females emerging

significantly earlier than males (Van Timmeren *et al.* 2000). The median emergence day for females was day 22 and for males it was day 27. It was also found that in lab studies females waited about a week after mating before oviposition, which does not support protandry.

Early in the season, Goonewardene *et al.* (unpublished data) observed a predominance of male beetles and later in the season found females to be more dominant. In one study (Goonewardene *et al.* 1973) they looked at sex ratios and emergence patterns of overwintered and nonoverwintered larvae. In both cases, more males emerged prior to females and the sex ratio was not influenced by collection time or the type of laboratory culture used.

In a similar study, Reginere *et al.* (1981) looked at the effects of temperature on the development of Japanese beetle grubs and measured their maturation periods. They found that the average days of adult maturation across all temperatures for males was 8.8 days and for females was 10.5 days. This suggests that males are emerging earlier than females, and then are attracted to the sites that females emerge from (Reginere *et al.* 1981).

In one study it was found that the overall sex ratios on food plants was significantly male biased, and that for single beetles the sex ratio was male biased as well (Switzer *et al.* 2001). These ratios varied significantly among days but not during any measured time periods. On the other hand, Iwabuchi and Takahashi (1983) found that the sex ratio of females to males was 1:1.7 in an aggregation formed using male and female beetles tethered as attractants.

Aggregation

Japanese beetles form two types of aggregations: one contains numerous males surrounding a female attempting to mate, and the other contains both males and females on a food plant.

Aggregations containing several males trying to copulate with a female are referred to as beetle balls, and can have anywhere from 25-200 males (Fleming 1972). These aggregations are formed because of a volatile sex pheromone emitted by the newly emerged, unmated females (Ladd 1970). It was found that this pheromone is produced in the abdomen, and production is stopped as soon as the female mates for the first time.

The second type of aggregation can contain hundreds to thousands of beetles of both sexes feeding on the same host plant. Iwabuchi and Takahashi (1983) looked at the process of aggregation formation in Japanese beetles and the role played by existing beetles in the group. In order to study this they measured the number of flying beetles attracted to tethered males and females separately, not removing any of the attracted beetles until the end of the experiment. They also measured the number of beetles attracted to tethered beetle models, using dummies or solely ether extracts of males and females as olfactory cues. The results showed that when male and female beetles were tethered they both attracted flying beetles, with most beetles attempting to copulate. This shows that the majority of the attracted beetles were males. They also found that beetles of both sexes tethered on leaves attracted mostly males, but the females were more attractive. The male and female beetle extracts mixed with ether used solely as olfactory cues also attracted mostly males. From this study, it was stated that both sexes of beetles attracted males and since the females used in the experiment had already mated, it was not the female sex pheromone they were attracted to. Iwabuchi and Takahashi concluded

that it is the formed occupants on host plants that play a crucial role in male participation in aggregation formation.

Since then several more studies have been conducted that looked at the cues involved in beetle aggregation formation. The current research suggest that Japanese beetles are attracted to feeding aggregations by the volatiles released from host plants after conspecific feeding (Loughrin *et al.* 1995, Loughrin *et al.* 1996). These studies found that beetles were significantly more attracted to crabapple leaves and grape vines with overnight Japanese beetle feeding damage or artificial damage. These herbivory-induced plant volatiles act as aggregation kairomones, aiding in host plant location or mate finding (Loughrin *et al.* 1995).

The goal of this thesis is to examine aggregation formation in Japanese beetles, specifically looking at which beetles are coming first (pioneers) and which beetles are joining later in the formation process.

Literature Cited

- Ahmad, S. 1982.** Host location by the Japanese beetle: Evidence for a key role for olfaction in a highly polyphagous insect. *The Journal of Experimental Zoology*, 220: 117-120.
- Allee, W.C. 1927.** Animal aggregations. *The Quarterly Review of Biology*, 2: 367-398.
- Alm, S.R., T. Yeh, C.G. Dawson, and M.G. Klein. 1996.** Evaluation of trapped beetle repellancy, trap height, and string pheromone dispensers on Japanese beetle captures (Coleoptera: Scarabaeidae). *Environmental Entomology*, 25: 1274-1278.
- Ambrogi, B.G. and H.G. Zarbin. 2008.** Aggregation pheromone in *Sternechus subsignatus* (Coleoptera: Curculionidae): Olfactory behaviour and temporal pattern of emission. *Journal of Applied Entomology*, 132: 54-58.
- Aukema, B.H. and K.F. Raffa. 2004.** Does aggregation benefit bark beetles by diluting predation? Links between a group-colonisation strategy and the absence of emergent multiple predator effects. *Ecological Entomology*, 29: 129-138.
- Ballou, C.H. 1929.** Effects of geranium on the Japanese beetle. *Journal of Economic Entomology*, 22: 289-293.
- Barnard, C.J. and R.M. Sibly. 1981.** Producers and scroungers: A general model and its application to captive flocks of house sparrows *Animal Behaviour*, 29: 543-550.
- Barrows, E.M. and G. Gordh. 1978.** Sexual behavior in the Japanese beetle, *Popillia beetle*, and comparative notes on sexual behavior of other scarabs (Coleoptera: Scarabaeidae). *Behavioral Biology*, 23: 341-354.
- Bernays, E.A. and R.F. Chapman. 1993.** Host-plant selection by phytophagous insects Chapman & Hall, New York.

- Bjorklund, N., G. Nordlander, and H. Bylund. 2005.** Olfactory and visual stimuli used in orientation to conifer seedlings by the pine weevil, *Hylobius abietis*. *Physiological Entomology*, 30: 225-231.
- Byers, J.A. 1983.** Sex-specific responses to aggregation pheromone: Regulation of colonization density in the bark beetle *Ips paraconfusus*. *Journal of Chemical Ecology*, 9: 129-142.
- Campbell, J.F. 2005.** Fitness consequences of multiple mating on female *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Environmental Entomology*, 34: 833-843.
- Clark, B.R. and S.H. Faeth. 1997.** The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*, 22: 408-415.
- Couty, A., H. Van Emden, J.N. Perry, J. Hardie, J.A. Pickett, and L.J. Wadhams. 2006.** The roles of olfaction and vision in host-plant finding by the diamondback moth, *Plutella xylostella*. *Physiological Entomology*, 31: 134-145.
- Denno, R.F., M.S. McClure, and J.R. Ott. 1995.** Interspecific interactions in phytophagous insects: Competition reexamined and resurrected. *Annual Reviews in Entomology*, 40: 297-331.
- Fleming, W.E. 1969.** Attractants for the Japanese beetle. United States Department of Agriculture Technical Bulletin No. 1399.
- Fleming, W.E. 1972.** Biology of the Japanese beetle. United States Department of Agriculture Technical Bulletin No. 1449.
- Goonewardene, H.F., R.G. Bingham, and B.G. Townshend. 1973.** Pattern of emergence of adult Japanese beetles from laboratory cultures. *Journal of Economic Entomology*, 66: 1356.

- Gross, J., N.E. Fatouros, S. Neuvonen, and M. Hilker. 2004.** The importance of specialist natural enemies for *Chrysomela lapponica* in pioneering a new host plant. *Ecological Entomology*, 29: 584-593.
- Gross, J., N.E. Fatouros, S. Neuvonen, and M. Hilker. 2007.** The role of competitors for *Chrysomela lapponica*, a north Eurasian willow pest, in pioneering a new host plant *Journal of Pest Science*, 80: 139-143.
- Guerrieri, E., F. Pennacchio, and E. Tremblay. 1993.** Flight behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in response to plant and host volatiles. *European Journal of Entomology*, 90: 415-421.
- Guerrieri, E., F. Pennacchio, and E. Tremblay. 1997.** Effect of adult experience on in-flight orientation to plant and plant-host complex volatiles in *Aphidius ervi* Haliday (Hymenoptera, Braconidae). *Biological Control*, 10: 159-165.
- Hamilton, J.G.C., D.R. Hall, and W.D.J. Kirk. 2005.** Identification of male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31: 1369-1379.
- Harari, A.R., D. Ben-Yakir, and D. Rosen. 1994.** Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). *Journal of Chemical Ecology*, 20: 361-371.
- Held, D.W., P. Gonsiska, and D.A. Potter. 2003.** Evaluating companion planting and non-host masking odors for protecting roses from the Japanese beetle (Coleoptera: Scarabaeidae). *Horticultural Entomology*, 96: 81-87.

- Hemptinne, J.-L., A.F.G. Dixon, and B. Adam. 2001.** Do males and females differ of the two-spot ladybird, *Adalia bipunctata* (L.), differ in when they mature sexually? *Journal of Insect Behavior*, 14: 411-419.
- Hirota, T. and Y. Kato. 2001.** Influence of visual stimuli on host location in the butterfly, *Eurema hecabe*. *Entomologia Experimentalis et Applicata*, 101: 199-206.
- Holzapel, C.M. and W.E. Bradshaw. 2002.** Protandry: the relationship between emergence time and male fitness in the pitcher-plant mosquito. *Ecology*, 83: 607-611.
- Hunter, A.F. 2000.** Gregariousness and repellent defences in the survival of phytophagous insects. *Oikos*, 91: 213-224.
- Iwabuchi, K. and J. Takahashi. 1983.** Aggregative distribution pattern of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and the role of former occupants in the formation of an aggregation. *Japanese Society of Applied Entomology and Zoology* 18: 324-329.
- Jermy, T. 1966.** Feeding inhibitors and food preference in chewing phytophagous insects. *Entomologia Experimentalis et Applicata*, 9: 1-12.
- Johnson, S.N. and P.J. Gregory. 2006.** Chemically-mediated host-plant location and selection by root-feeding insects. *Physiological Entomology*, 31: 1-13.
- Keathley, C.P., D.A. Potter, and R.L. Houtz. 1999.** Freezing-altered palatability of Bradford pear to Japanese beetle: Evidence for decompartmentalization and enzymatic degradation of feeding deterrents. *Entomologia Experimentalis et Applicata*, 90: 49-59.

- Kendrick, A.P. and K. Raffa, F. 2006.** Sources of insect and plant volatiles attractive to cottonwood leave beetles feeding on hybrid poplar *Journal of Chemical Ecology*, 32: 2585-2594.
- Krombein, K. 1967.** Trap-nesting wasps and bees and their associates. *Journal of the New York Entomological Society*, 75: 108.
- Lacey, E.S., M.D. Ginzel, J.G. Millar, and L.M. Hanks. 2004.** Male-produced aggregation pheromone of the cerambycid beetle *Neoclytus acuminatus acuminatus*. *Journal of Chemical Ecology*, 30: 1493-1507.
- Lacey, L.A., J.J. Amaral, J. Coupland, and M.G. Klein. 1994.** The influence of climatic factors on the flight activity of the Japanese beetle (Coleoptera: Scarabaeidae): Implications for use of a microbial control agent. *Biological Control*, 4: 298-303.
- Ladd, T.L., Jr. 1969.** Sex attraction in the Japanese beetle. *Journal of Economic Entomology*, 63: 905-908.
- Ladd, T.L., Jr. 1987.** Japanese beetle (Coleoptera: Scarabaeidae): Influence of favored food plants on feeding response. *Journal of Economic Entomology*, 80: 1014-1017.
- Ladd, T.L., Jr. and J.P. Jurimas. 1972.** Influence of trap height on captures of Japanese beetles *Journal of Economic Entomology*, 65: 407-408.
- Ladd, T.L., Jr. and M.G. Klein. 1982.** Japanese beetle (Coleoptera: Scarabaeidae): Effect of trap height on captures. *Journal of Economic Entomology*, 75: 746-747.
- Ladd, T.L., Jr., M.G. Klein, and J.H. Tumlinson. 1981.** Phenethyl propionate + eugenol + geranoil (3-7-3) and Japonilure - A highly effective joint lure for

Japanese beetles (Coleoptera, Scarabaeidae). *Journal of Economic Entomology*, 74: 665-667.

Ladd, T.L., Jr., T.P. McGovern, M. Beroza, C.R. Buriff, and M.G. Klein. 1976.

Japanese beetles-(Coleoptera:Scarabaeidae) attractancy of phenethyl propionate and eugenol (3-7) and synthetic eugenol. *Journal of Economic Entomology*, 69: 468-470.

Landolt, P.J., J.H. Tumlinson, and D.H. Alborn. 1999. Attraction of Colorado potato beetle (Coleoptera:Chrysomelidae) to damaged and chemically induced potato plants. *Environmental Entomology*, 28: 973-978.

Langford, G.S., M.H. Muma, and E.N. Cory. 1943. Attractiveness of certain plant constituents to the Japanese beetle. *Journal of Economic Entomology*, 36: 248-252.

Lee, J.C. and E.A. Bernays. 1990. Food tastes and toxic effects - Associative learning by the polyphagous grasshopper *Schistocerca americana* (Drury) (Orthoptera, Acrididae). *Animal Behaviour*, 39: 163-173.

Loughrin, J.H., Potter, Daniel A., Hamilton-Kemp, Thomas R. & Byers, Matthew E. 1997. Diurnal emission of volatile compounds by Japanese beetle-damaged grape leaves. *Phytochemistry*, 45: 919-923.

Loughrin, J.H., Potter, Daniel A. & Hamilton-Kemp, Thomas R. 1998. Attraction of Japanese beetles (Coleoptera: Scarabaeidae) to host plant volatiles in field trapping experiments. *Physiological and Chemical Ecology*, 27: 395-400.

- Loughrin, J.H., D.A. Potter, and T.R. Hamilton-Kemp. 1995.** Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *Journal of Chemical Ecology*, 21: 1457-1467.
- Loughrin, J.H., D.A. Potter, T.R. Hamilton-Kemp, and M.E. Byers. 1996.** Role of feeding-induced plant volatiles in aggregative behavior of the Japanese beetle (Coleoptera: Scarabaeidae). *Physiological and Chemical Ecology*, 25: 1188-1191.
- Lubin, Y., J.R. Henschel, and M. Baker, B. 2001.** Costs of aggregation: Shadow competition in a sit-and-wait predator. *Oikos*, 95: 59-68.
- Mitchell, E.R., F.C. Tingle, and R.R. Heath. 1991.** Flight activity of *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae) with reference to host-plant volatiles. *Journal of Chemical Ecology*, 17: 259-266.
- Nagasawa, A. and K. Matsuda. 2007.** Habituation by a tortoise beetle to the deterrents of spinach. *Journal of Applied Entomology*, 131: 716-721.
- Nielsen, H.T. and E.T. Nielsen. 1953.** Field observations on the habits of *Aedes taeniorhynchus*. *Ecology*, 34: 141-156.
- Parrish, J.K. and L. Edelstein-Keshet. 1999.** Complexity, pattern, and evolutionary trade-offs in animal aggregation. *Science*, 284: 99-101.
- Potter, D.A. and D.W. Held. 1999.** Absence of food-aversion learning by a polyphagous scarab, *Popillia japonica*, following intoxication by geranium, *Pelargonium x hortorum*. *Entomologia Experimentalis et Applicata*, 91: 83-88.
- Potter, D.A. and D.W. Held. 2002.** Biology and management of the Japanese beetle. *Annual Review of Entomology*, 47: 175-205.

- Potter, D.A., J.H. Loughrin, W.J. Rowe II, and T.R. Hamilton-Kemp. 1996.** Why do Japanese beetles defoliate trees from the top down? *Entomologia Experimentalis et Applicata*, 80: 209-212.
- Prokopy, R.J. 1968.** Visual responses of apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae): Orchard studies *Entomologia Experimentalis et Applicata*, 11: 403-422.
- Regniere, J., R.J. Rabb, and R.E. Stinner. 1981.** *Popillia japonica*: Simulation of temperature-dependent development of the immatures, and prediction of adult emergence. *Environmental Entomology*, 10: 290-296.
- Reinecke, A., J. Ruther, T. Tolasch, W. Francke, and M. Hillker. 2002.** Alcoholism in cockchafers: Orientation of male *Melolontha melolontha* towards green leaf alcohols. *Naturwissenschaften*, 89: 265-269.
- Robinson, S.K. 1985.** Coloniality in the yellow-rumped cacique as a defense against nest predators. *The Auk*, 102: 506-519.
- Rowe II, W.J. and D.A. Potter. 2000.** Shading effects on susceptibility of *Rosa* spp. to defoliation by *Popillia japonica* (Coleoptera: Scarabaeidae). *Environmental Entomology*, 29: 502-508.
- Ruther, J. 2000.** Retention index database for identification of general leaf volatiles in plants coupled by capillary gas chromatography-mass spectrometry. *Journal of Chromatography A* 890: 313-319.
- Shimizu, N., N. Mori, and Y. Kuwahara. 2001.** Aggregation pheromone activity of the female sex pheromone, *B*-acardial, in *Caloglyphus polyphyllae* (Acari: Acaridae). *Bioscience, Biotechnology, and Biochemistry*, 65: 1724-1728.

- Stamp, N.E. 1981.** Effect of group size on parasitism in a natural population of the baltimore checkerspot *Euphydryas phaeton* *Oecologia*, 49: 201-206.
- Stenberg, J.A. and L. Ericson. 2007.** Visual cues override olfactory cues in the host-finding process of the monophagous leaf beetle *Altica engstroemi*. *Entomologia Experimentalis et Applicata*, 125: 81-88.
- Strassmann, J.E. 1991.** Costs and benefits of colony aggregation in the social wasp, *Polistes-Annularis*. *Behavioral Ecology* 2: 204-209.
- Switzer, P.V., K. Escajeda, and K.C. Kruse. 2001.** Pairing patterns in Japanese beetles (*Popillia japonica* Newman): effects of sex ratio and time of day. *Journal of Insect Behavior*, 14: 713-723.
- Thornhill, R. and J. Alcock. 1983.** *The Evolution of Insect Mating Systems*
iUniverse.com, Inc., San Jose.
- Tingle, F.C. and E.R. Mitchell. 1992.** Attraction of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) to volatiles from extracts of cotton flowers. *Journal of Chemical Ecology*, 18: 907-914.
- Tumlinson, J.H., M.G. Klein, R.E. Doolittle, T.L. Ladd, and A.T. Proveaux. 1977.** Identification of the female Japanese beetle sex pheromone: Inhibition of male response by an enantiomer. *Science*, 197: 789-792.
- Turchin, P. and P. Kareiva. 1989.** Aggregation in *Aphis Variens*: An effective strategy for reducing predation risk. *Ecology* 70: 1008-1016.
- Van Timmeren, S.J., P.V. Switzer, and K.C. Kruse. 2000.** Emergence and reproductive patterns in the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). *Journal of the Kansas Entomological Society*, 74: 17-27.

- Wertheim, B., E.-J.A. van Baalen, M. Dicke, and L.E.M. Vet. 2005.** Pheromone-mediated aggregation in nonsocial arthropods: An evolutionary ecological perspective. *Annual Reviews in Entomology*, 50: 321-346.
- White, P.R. and R.F. Chapman. 1990.** Olfactory sensitivity of gomphocerine grasshoppers to the odours of host and non-host plants. *Entomologia Experimentalis et Applicata*, 55: 205-212.
- Wiklund, C., V. Lindfors, and J. Forsberg. 1996.** Early male emergence and reproductive phenology of the adult overwintering butterfly *Gonepteryx rhamni* in Sweden. *Oikos*, 75: 227-240.
- Williams, L., C. Rodriguez-Saona, P.W. Pare, and S.J. Crafts-Brandner. 2005.** The piercing-sucking herbivores *Lygus hesperus* and *Nezara viridula* induce volatile emissions in plants. *Archives of Insect Biochemistry and Physiology*, 58: 84-96.
- Wilson, D.S. 1974.** Prey capture and competition in the ant lion. *Biotropica*, 6: 187-193.
- Zhao, Y.X. and L. Kang. 2003.** Olfactory responses of the leafminer *Liriomyza sativae* (Dipt., Agromyzidae) to the odours of host and non-host plants. *Journal of Applied Entomology*, 127: 80-84.

Chapter II

Dynamics of aggregation formation in Japanese beetles, *Popillia japonica*

Introduction

Insects of many species assemble together to form aggregations (Allee 1927). An insect aggregation can serve many purposes including mating, sleeping, feeding or reducing the risk of predation (Harari *et al.* 1994, Clark & Faeth 1997, Alcock 1998, Aukema & Raffa 2004). For any aggregation to form, insects need an initial colonizer of the group that is willing to go to uninhabited areas (hereafter referred to as the ‘pioneer’). The characteristics of pioneers may differ from those individuals arriving later (hereafter called ‘joiners’) (Pitman & Vite 1969, Smyth & Hoffman 2003). Distinguishing the characteristics of these pioneers and joiners is important in learning about the dynamics of any aggregation. However, it is especially important to identify characteristics for insect pests that form large feeding aggregations and consequently cause extensive damage to plants.

One such insect pest is the Japanese beetle, *Popillia japonica*. The Japanese beetle first appeared in the United States in New Jersey in 1916. Since then, the Japanese beetle has become one of the most devastating insect pests of agricultural and horticultural plants in the eastern United States (Potter & Held 2002). Their pest status is due in part to their generalist nature, feeding on more than 300 different plant species, as well as their ability to form large aggregations of hundreds to thousands of beetles on a single plant (Smith & Hadley 1926, Fleming 1972, Potter & Held 2002).

Japanese beetles locate a wide variety of host plants using a combination of olfactory and visual cues, but odor is probably the most important component (Fleming 1972, Ahmad 1982, Held & Potter 2004). While not much is known about this process, studies show that Japanese beetles make between species host plant decisions based on complex blends of plant volatiles (Held *et al.* 2003) and within species host plant decisions based on the height of the plant and its location in sunlight or shade, preferring plants in sunlight and exhibiting top-down feeding (Smith & Hadley 1926, Fleming 1972, Alm *et al.* 1996, Potter *et al.* 1996).

Japanese beetles form two types of aggregations: relatively brief aggregations containing numerous males surrounding a female attempting to mate (Ladd 1970), and longer-term aggregations on a food plant. In this study, we focus on these food plant aggregations, which contain both males and mated females (Smith & Hadley 1926, Fleming 1972). Japanese beetles are attracted to feeding aggregations by the volatiles released from host plants after conspecific feeding. These herbivory-induced plant volatiles may act as aggregation kairomones, aiding in host plant location or mate finding by Japanese beetles (Loughrin *et al.* 1995, Loughrin *et al.* 1996).

Little work has been done examining the characteristics of pioneers and joiners in Japanese beetles. Smith and Hadley (1926) observed the first 10 beetles arriving on uninfested plants finding that there were no sex biases in the first beetles starting the infestation. However, it was unclear whether these plants had feeding damage prior to the experiment. Iwabuchi and Takahashi (1983) tethered beetles and found that both sexes were attracted, but again, it is unclear to what extent feeding damage played a role. Furthermore, both Smith and Hadley (1926) and Iwabuchi and Takahashi (1983), did not

compare characteristics by sex to test for the existence of more subtle differences among pioneers and joiners.

This study examines the characteristics of pioneer versus joiner beetles in feeding aggregation formation by Japanese beetles. Specifically, the objectives of this study are to establish which beetles are initiating aggregations on host plants, determine if host plant selection by beetles is affected by the presence of existing aggregations of beetles, and to explore any role plant kairomones may have in pioneering by Japanese beetles.

Methods

A series of related field experiments were conducted in the summer of 2008, on campus at Eastern Illinois University, in Coles County, IL. All experiments used grape plants (*Vitis labrusca* 'Niagra'); grape plants are a preferred host plant of Japanese beetles (Fleming 1972) and have been used in previous investigations of aggregation in this species (Loughrin *et al.* 1996, Loughrin *et al.* 1997). Grapes were individually potted in plastic pots (0.265 m diameter, 0.25 m height) in a mixture of sand and Premier Pro-Mix BX growing medium. Plants were given Scotts Osmocote Classic Controlled Release Fertilizer (18-6-12) and watered as necessary. Between experiments we removed Japanese beetles from the plants multiple times per day, to minimize feeding damage on the leaves. All grape plants had similar numbers of leaves and any damaged leaves were removed prior to use in an experiment. Grape plants were randomized with respect to treatment in each experiment.

All experiments were completed in two mowed grass fields, each of which had some small, mixed species of trees on the north and south. Additional landmark characteristics were a soybean field to the east and a road to the west of both fields. The grape plants were generally at least 7 meters from the nearest tree, although one corner of the array in experiments 2 and 3 (see below) was 3 meters from a tree. However, none of the closest trees were host plants for Japanese beetles (Fleming 1972).

At the end of each experiment, all beetles captured were sexed using foreleg morphology (Smith & Hadley 1926), size was determined using their maximum pronotum width (Van Timmeren 2000), and the females were dissected to obtain mature egg counts (Saeki *et al.* 2005).

Below I detail three experiments that were conducted to compare the characteristics of pioneer and joiner beetles.

Experiment 1: Pioneers Only

In order to establish which beetles were beginning aggregations, individual beetles were removed as they landed on a small array of uninhabited grape plants. Data were collected for 10 days between July 8-July 18, 2008. Weather on these days was relatively consistent (average high: 30.1°C, low: 20.7 °C, wind: 1.56 m/s). Grape plants were arranged in a 5 x 5 array, with 0.3 m between pots in each column and 1.5 m between pots in each row. Each night before an experimental day all of the beetles were removed from the plants between 2000-2200 hr. During the day plants were watched continuously from 0700-1700 hr and beetles were captured and individually bagged as they landed. In order to maximize the chances that the landing beetle was going to stay in that location, individuals were not collected until they remained on the plant for at least one minute.

To provide a comparison to beetles in aggregations, on each day of the experiment beetles were also collected from existing aggregations on rose (*Rosa* sp.) plants elsewhere on campus. This was done between 1330-1400 hr each day, and a random subsample of the beetles collected were sexed, measured and the females dissected for mature egg counts.

Experiment 2 Pioneers and Aggregations

In a similar experiment, we gave beetles the “option” of going to uninhabited host plants or host plants in which beetles had been allowed to settle naturally over the course

of the experiment. Data were collected August 5-August 8, 2008 continuously between 0900-1700 hr (average high: 28.1°C, low: 19.3 °C, wind: 1.52 m/s). For this study, 96 plants were arranged in a 12 x 8 array with 3.5 m between each plant in each row and column. The 24 plants in the northeast corner of the plot were designated as ‘aggregation plants’ and beetles were allowed to aggregate there without any disturbances for 4 days. The remaining 72 plants were watched continuously and beetles were individually captured and bagged as in the previous experiment.

Experiment 3: Plants with Model Beetles and Feeding Damage

To determine which beetles were arriving to chemical and visual cues that simulated an aggregation, an experiment was conducted that incorporated controlled feeding damage and dead beetles glued to leaves as models; the model beetles served to attract other beetles as was done in previous experiments (Iwabuchi & Takahashi 1983). Data were collected on four consecutive days July 31-August 3, 2008 with observations taken from 0900-1700 hr (average high: 39.2°C, low: 19.7 °C, wind: 1.21 m/s).

In this experiment, 96 grape plants were arranged in a 12 x 8 array with each plant 3.5 m apart in rows and columns. I replicated 4 experimental treatments: control, leaves with overnight feeding damage, leaves with 5 model beetles (1 pair, 2 single males and 1 single female consistent with the typical sex ratio on food plants [Switzer *et al.* 2001]) and leaves with overnight feeding damage and 5 model beetles (1 pair, 2 single males and 1 single female). Each of the 4 treatments was given a number (control = 1, feeding damage = 2, model beetles = 3, feeding damage and model beetles = 4). A Latin square was used to assign each of the treatments to a plant on the first experimental day. On

consecutive days the numbers were increased by one so at no time adjacent plants had the same treatment.

The leaf used for the treatment was standardized across treatments by using a similarly sized leaf that was southward facing, at the top of plant, and between 1.4-1.5 m off the ground. I marked control leaves with a small dot using black permanent marker. Elmer's Craft Bond Ultra Stix Clear glue was used to attach the dead beetles to the leaves in a lifelike position.

To cause feeding damage, at 1700 hr the day before an experimental day, we enclosed 30 live beetles (18 males and 12 females, in accordance with the typical sex ratio in a feeding aggregation [Switzer *et al.* 2001]) in a mesh pouch, 0.25 m x 0.14 m, surrounding a leaf. The pouch was tied at the top using a plastic electrical tie so that no beetles could escape. The bagged beetles were all removed at 0800 hr the next morning, kept in small plastic containers with wet paper towels for water, and given no food until they were used again that evening. If necessary, new beetles were added to replace dead or escaped beetles to maintain the feeding groups.

Each day a section of 32 plants (4 x 8) was used for the experiment while the remaining 64 plants were left untouched. Throughout the duration of the experiment beetles were kept off these 64 plants to avoid the formation of any aggregations or feeding damage. The following day, the next section of 32 plants was used for the experiment. Because we ran the experiment 4 days, we had to reuse the plants from the first day on the fourth day. However, all damaged leaves had been clipped from these plants to prevent kairomone release (Loughrin *et al.* 1995, Loughrin *et al.* 1996), no

beetles had been allowed to aggregate on these plants during the interim, and plants in this section were not given the same treatment the second time they were used.

Individual beetles were captured and bagged, as in previous experiments.

Additionally, the beetles' proximity to the 'treatment' leaf was noted. If they landed directly on the treatment leaf, the distance was recorded as 0; if they landed on another leaf, the distance from the tip of the leaf to the tip of the treatment leaf was measured.

At 1700 hours all of the treatment leaves, including the control leaves, were removed from the plants. Once all of the old leaves were removed, leaves were selected on plants for the next day's experiment and feeding beetles in mesh bags were attached.

Statistical Analyses

All data were analyzed using SAS (version 9.1). For each experiment, correlation and regression tests were used to examine possible temporal patterns. All of the times were converted to minutes beginning at 0700 or 0900 hr (whenever data collection began for that experiment) and stopped at 1700 hr. To calculate sex ratios, the number of male beetles was divided by the total number of beetles.

For comparisons among treatments, data were analyzed using the ANOVA tests. When the assumptions of normality were not met (e.g. with egg loads and distances), however, the nonparametric Kruskal-Wallis test was used. In some cases the Kruskal-Wallis tests were followed up by a Mann-Whitney U test to compare pair wise differences between treatments at the $\alpha = 0.05$ significance level.

Results

Experiment 1: Pioneers Only

The overall sex ratio of beetles landing on uninhabited grape plants was significantly female-biased with a proportion of males of 0.31 ($N = 290$; $\chi^2_1 = 41.72$, $P < 0.0001$). The sex ratio of the beetles in existing aggregations on the nearby rose plants did not differ from 1:1 at 0.48 ($N = 2586$, $\chi^2_1 = 2.86$, $P = 0.091$).

Body size was not significantly related to the time of day that beetles were arriving on the plants for either males or females (Males: $N = 90$; $r_s = -0.09$, $P = 0.38$; Females: $N = 200$; $r_s = 0.08$, $P = 0.28$). However females showed a significant positive correlation between the size of their egg load and the time of day (Fig.1; $r_s = 0.16$, $P = 0.02$).

When we compared the beetles pioneering on the grape plants to beetles in aggregations on rose plants, we found no significant difference with respect to male or female size (Males: Pioneers: $N = 90$; 6.21 ± 0.04 mm, Roses: $N=72$; 6.19 ± 0.04 , $F_{1,160} = 0.12$, $P = 0.73$; Females: Pioneers; $N = 200$; 6.79 ± 0.03 , Roses; $N = 180$; 6.81 ± 0.03 , $F_{1,378} = 0.13$, $P = 0.72$). However, there was a nonsignificant trend for females in rose aggregations to have fewer mature eggs than pioneers on grapes (Pioneers: $N = 200$; 4.26 ± 0.49 eggs, Roses: $N = 180$; 1.78 ± 0.21 eggs, Kruskal-Wallis: $\chi^2_{1,378} = 3.16$, $P = 0.08$).

Experiment 2: Pioneers and Aggregations

The beetles coming into the uninhabited plant section of grapes had a significantly female-biased sex ratio of 0.23 ($N = 270$; $\chi^2_1 = 78.95$, $P < 0.0001$). The sex ratio of the

beetles in the aggregation area did not differ from 1:1 at 0.56 ($N = 191$, $\chi^2_1 = 2.31$, $P = 0.13$).

Larger males were more likely to be found in the aggregation area than on the uninhabited grape plants (Pioneers: $N = 62$; 6.03 ± 0.04 mm, Aggregation area: $N = 106$; 6.19 ± 0.03 mm, $F_{1,166} = 8.93$, $P = 0.0032$). There was no difference in the size of females arriving at the uninhabited grape plants and those in the aggregation area (Pioneers: $N = 208$; 6.69 ± 0.02 mm, Aggregation area: $N = 85$; 6.68 ± 0.04 mm, $F_{1,291} = 0.04$, $P = 0.85$). Egg loads of females in the aggregation area did not differ significantly from females that were pioneers on uninhabited plants (Pioneers: $N = 208$; 1.51 ± 0.23 eggs, Aggregation area: $N = 85$; 0.80 ± 0.16 eggs, Kruskal-Wallis: $\chi^2_{1,291} = 0.02$, $P = 0.89$). As with the female pioneers in the previous experiment, egg loads were significantly correlated with the time of day a female arrived ($r_s = 0.19$, $P = 0.005$).

Within the aggregation area, beetles on leaves by themselves tended to be smaller than beetles in aggregations on leaves, but this pattern was not significant for either males or females (Males: Single: $N = 10$; 6.04 ± 0.14 mm, Aggregation: $N = 96$; 6.21 ± 0.034 mm, $F_{1,104} = 1.96$, $P = 0.17$; Females: Single: $N = 14$; 6.52 ± 0.08 mm, Aggregation: $N = 71$; 6.71 ± 0.04 mm, $F_{1,83} = 3.19$, $P = 0.08$). Also, female egg loads did not vary between solitary beetles and those in groups within the aggregation area (Single: $N = 14$; 0.79 ± 0.41 eggs, Aggregation: $N = 71$; 0.80 ± 0.17 eggs, Kruskal-Wallis: $\chi^2_{1,83} = 0.10$, $P = 0.75$).

Experiment 3: Plants with Model Beetles and Feeding Damage

The overall sex ratio for all treatments was significantly female-biased at 0.37 (N = 373; $\chi^2_1 = 27.35$, $P < 0.0001$). For each treatment, the sex ratio was also female-biased (Control: N = 39; sex ratio = 0.36, $\chi^2_1 = 3.10$, $P = 0.078$, Feeding damage: N = 92; sex ratio = 0.34, $\chi^2_1 = 9.78$, $P = 0.0018$, Models only: N = 58; sex ratio = 0.36, $\chi^2_1 = 4.41$, $P = 0.04$, Models and feeding damage: N = 184; sex ratio = 0.38, $\chi^2_1 = 10.52$, $P = 0.0012$).

More beetles landed on plants that had both models and feeding damage compared to the other three treatments (Fig. 2). This pattern is significant whether all beetles are combined (N = 373; Kruskal-Wallis: $\chi^2_{3,124} = 40.43$, $P < 0.0001$) or when sexes are analyzed separately (Males: N = 136; Kruskal-Wallis: $\chi^2_{3,124} = 20.76$, $P = 0.0001$; Females: N = 237; Kruskal-Wallis: $\chi^2_{3,124} = 39.18$, $P < 0.0001$).

To investigate whether treatments differed in how early they attracted beetles within a day, we compared the average time of arrival among treatments. We found no significant difference among treatments for either males or females (Males: Control: N = 14; 274.93 ± 35.67 minutes after 0900 hr, Feeding damage: N = 31; 219.71 ± 18.62 minutes, Models only: N = 21; 255.71 ± 26.28 minutes, Models and feeding damage: N = 70; 243.23 ± 12.76 minutes, Kruskal-Wallis: $\chi^2_{3,132} = 4.47$, $P = 0.22$; Females: Control: N = 25; 232.44 ± 25.75 minutes, Feeding damage: N = 61; 197.70 ± 12.57 minutes, Models only: N = 37; 218.22 ± 20.91 minutes, Models and feeding damage: N = 114; 240.95 ± 9.66 minutes, Kruskal-Wallis: $\chi^2_{3,233} = 6.28$, $P = 0.10$).

Males showed a significant correlation between the time they arrived and their size, with relatively larger males arriving later in the day (Fig. 3; $r_s = 0.21$, $P = 0.015$).

There were no significant correlations between the time at which female beetles arrived to plants and their size ($r_s = 0.0018$, $P = 0.98$) or their egg load ($r_s = -0.039$, $P = 0.55$).

Body size of arriving beetles did not significantly differ between treatments for males or females (Males: Control: $N = 14$; 6.03 ± 0.10 mm, Feeding damage: $N = 31$; 6.10 ± 0.06 mm, Models only: $N = 21$; 6.09 ± 0.073 mm, Models and feeding damage: $N = 70$; 6.19 ± 0.04 mm, $F_{3,132} = 1.47$, $P = 0.23$; Females: Control: $N = 25$; 6.72 ± 0.07 mm, Feeding damage: $N = 61$; 6.81 ± 0.05 mm, Models only: $N = 37$; 6.73 ± 0.06 mm, Models and feeding damage: $N = 114$; 6.68 ± 0.03 mm, $F_{3,233} = 1.43$, $P = 0.23$).

We found significant patterns among treatments in settlement distance, egg loads, and body size. The average distance beetles settled from the treatment leaf differed among treatments for both males and females (Fig. 4), with the shortest distance occurring in the treatment that had both feeding damage and model beetles (Males: Kruskal-Wallis: $\chi^2_{3,132} = 19.73$, $P = 0.0002$; Females: Kruskal-Wallis: $\chi^2_{3,233} = 11.69$, $P = 0.0085$). Female egg load differed among treatments; females arriving at plants with both feeding damage and model beetles had the lowest average number of eggs (Fig. 5; Kruskal-Wallis: $\chi^2_{3,233} = 10.98$, $P = 0.012$). For non-control treatments, males landing on treatment leaves were significantly larger than males landing on other leaves on the plant (Treatment leaf: $N = 27$; 6.33 ± 0.06 mm, Other leaves: $N = 109$; 6.09 ± 0.03 , $F_{1,134} = 12.51$, $P = 0.0006$). However, female size did not differ between arrivals on treatment leaves and other leaves on the plant (Treatment leaf: $N = 36$; 6.71 ± 0.06 mm, Other leaves: $N = 201$; 6.73 ± 0.03 mm, $F_{1,235} = 0.09$, $P = 0.77$). Females found on treatment leaves had smaller egg loads than females found on other leaves on the plants, although this difference was of borderline statistical significance (Treatment leaf: $N = 36$; $0.72 \pm$

0.35 eggs, Other leaves: N = 201; 1.88 ± 0.31 eggs, Kruskal-Wallis: $\chi^2_{1,235} = 3.37$, P = 0.07).

Discussion

In our three field experiments, we found significant patterns in the sex ratios of the arriving beetles, the egg loads of the females that were arriving, and the time of day in which they were arriving. These patterns suggest that aggregation formation and maintenance by Japanese beetles is not a random process, but rather settlement is relatively structured.

Pioneers versus Joiners

Females

In all three experiments, we found significantly-biased female sex ratios for the beetles that were pioneering the uninhabited plants, with all sex ratios being less than 0.4. Sex ratios from aggregations in our experiment were not significantly different from 1:1, and previous studies have reported sex ratios in feeding aggregations to be male-biased (e.g. Smith and Hadley 1926; Switzer *et al.* 2001). These patterns indicate that female beetles are more likely to arrive at uninhabited host plants than males, and that they may be the sex that initiates aggregations. Consistent with our results, Tigreros and Switzer (unpublished data) found that solitary beetles in rose flowers tended to be females, whereas unpaired beetles in aggregations in a flower tended to be male.

In addition to pioneering aggregations, female beetles seem to be joining them at later stages of the formation process. Our third experiment was designed to mimic actual aggregations with visual and chemical cues. Therefore the beetles arriving at these plants would not be pioneers, but rather joiners coming into existing aggregations. For all treatments in experiment 3, the beetles arriving were female-biased, suggesting that

female beetles are not only initiating aggregations (experiments 1 & 2) but joining them later on in the formation process as well. Essentially, more females overall were arriving at food plants.

However, because feeding aggregations are male-biased (Smith & Hadley 1926, Switzer *et al.* 2001; this study), males must be accumulating at aggregations more than females. One way a male-bias could be created is if females tend to leave an aggregation after a relatively shorter period of time than males. Some support exists for this idea; Tigreros and Switzer (2009) followed marked pairs of beetles in the field and found that females left food patches earlier than males. This difference in residence by females may be because females do not need to be in aggregations or can get what they need relatively quickly. As a result, females arrive and leave at a faster rate than males and may tend to colonize more locations.

In a study based on trap captures in different habitats, Regniere *et al.* (1983) concluded that gravid females were the most mobile. Likewise, we found that egg load affected female behavior. Although females in general were both pioneers and joiners, some females, particularly those with relatively low egg loads, appeared to be more likely to join aggregations than others. We found that females in existing aggregations on rose plants (mostly joiners) had lower egg loads than pioneers on grape plants (experiment 1). In addition, the females found in the aggregation area had lower egg loads than the females in the uninhabited plants (experiment 2). The females landing on plants with model beetles and treatment leaves had lower egg loads than females landing on plants without model beetles (experiment 3). Taken in combination, these results suggest that

females with lower egg loads are looking to be near conspecifics and are more likely to be joiners.

At least two explanations may explain the effect of egg load on female behavior. First, females with lower egg loads may be those that have just oviposited. When ovipositing, females are in the ground and are unable to eat. Consequently, these females may be under pressure to locate food relatively quickly and thus may use the cues provided by feeding aggregations to find a suitable host plant (Loughrin *et al.* 1996). Second, high and low egg load females may have different mating strategies. Male Japanese beetles seem to prefer larger females, which may have more and larger eggs (Switzer *et al.* 2001, Saeki *et al.* 2005, Switzer *et al.* 2008). Thus, if females with relatively low egg loads do need to remate (e.g. to replenish or increase the quality of the sperm; Thornhill & Alcock 1983, Simmons 2001), they may need to go to aggregations in order to make it more likely that they will find a suitable male.

Although we found that females with low egg loads were more likely to join aggregations, Tigreros *et al.* (submitted) found that isolated females had relatively lower egg loads compared to those females already in existing aggregations. This difference could be resolved if the change takes place after female arrival. For example, Tigreros *et al.* (submitted) proposed that males may be searching for females with higher egg loads (and thus forming aggregations with them). Another possibility is that the species and/or distribution of host plant plays a role in the dynamics of aggregations; Tigreros *et al.* (submitted) was conducted in a soybean (*Glycine max*) field whereas this study used more spatially-separated grape plants. Future studies in which aggregations are monitored continuously would help distinguish these possibilities (but see below).

Males

Although similar proportions of males pioneered (experiments 1 and 2) and joined (experiment 3), additional evidence indicates that males may be actively searching for aggregations. For example, in experiment 3, males in general landed significantly closer to leaves with model beetles on them compared to leaves without. Furthermore, this joining behavior may depend on their body size. We found that males in the aggregation area were larger than males pioneering the uninhabited plants (experiment 2), and males landing directly on treatment leaves with aggregation cues of model beetles and feeding damage were significantly larger than males landing on other parts of the plant (experiment 3).

A possible reason for this pattern in male size may be due to the increased competitiveness of larger males. On food plants, males attempt to take a female from another male while that male is either copulating with her or during post-copulatory mate guarding. In these cases, if a takeover is successful the challenger male is significantly larger than the resident male (Kruse & Switzer 2007). Therefore, it may be easier for larger males to secure mates within an aggregation compared to smaller males, and this competitive difference may lead to differences in the mate searching behavior of different sized males. Such sized-based differences in mating behavior are common in many insects (reviewed in Thornhill & Alcock 1983). In the case of Japanese beetles, smaller, less competitive males may be more likely to search for more isolated females.

In summary, our data on pioneers and joiners have led us to the following, working hypothesis on the use of aggregations by female and male Japanese beetles. Previous studies have speculated as to the different functions of aggregations for the two

sexes (Iwabuchi & Takahashi 1983, Loughrin *et al.* 1996), however identifying these differences is difficult. Based on our results, we propose that females do not necessarily need an aggregation; instead, they are likely looking for host plants, and differences in the characteristics of pioneer and joining females (e.g. in egg loads) reflect differences in their urgency to find food relative to the potential benefit to avoid unnecessary interactions with males. If true, this suggests that these joining females will be more sensitive to cues (chemical and visual) from aggregations and it implies that the pioneering females are not “intending” to initiate an aggregation. Males, in contrast, may primarily use aggregations as a mating rendezvous, and the differences in male joining behavior reflect differences in their competitive ability.

Time of Arrival

We found that females pioneering host plants later in the day tended to have larger egg loads, with the highest egg loads for arriving females occurring between 1200-1500 hr. This was one of the strongest and most consistent patterns across the experiments; however, we can only speculate as to why this pattern is occurring. While there are arriving females with low egg counts all day, the females with higher egg counts arriving later in the day seem to be driving the pattern.

One possible explanation for this could be the oviposition patterns of female Japanese beetles. Females have been reported to leave food plants in the afternoon to deposit eggs in the soil and may remain there until the next morning or for several days (Fleming 1972). Tigreros and Switzer (2009) found that females would leave food plants in the middle of the day, perhaps to oviposit. Moreover, females on food plants had

higher egg loads in the morning than females in the afternoon and evening, indicating that females with higher egg loads may have left sometime in the early afternoon (Tigreros unpublished data). Thus, the females we found with relatively high egg loads may be shifting locations prior to oviposition.

Another plausible reason for the temporal pattern in egg load could be the relative physiological costs of flying incurred by females carrying different numbers of eggs. Insect flight is an extremely demanding chemical process utilizing carbohydrates, lipids, and fat body reserves; all these are used at the cost of egg production (Wheeler 1996, Lorenz 2007). The result of these costs in many migratory insects is that migratory activity is reduced in reproducing females, a phenomenon called flight-oogenesis syndrome (Rankin *et al.* 1994, Isaacs & Byrne 1998, Oliveira *et al.* 2006, Lorenz 2007). Furthermore, in Japanese beetles, flight is temperature-dependent, with flight being more likely at higher temperatures (Fleming 1972, Kreuger & Potter 2001). So, for Japanese beetles, the energy expenditure needed for high egg production and flying, coupled with changes in daytime temperatures, may result in females with relatively more eggs remaining on food plants until later in the day.

Importance of Feeding Damage

Feeding-induced kairomone release has been shown to be extremely important for aggregation formation in Japanese beetles (Loughrin *et al.* 1995, Loughrin *et al.* 1996, Loughrin *et al.* 1997). We incorporated overnight feeding damage by Japanese beetles and model beetles to simulate conditions similar to an actual aggregation (experiment 3). The results showed that the highest numbers of beetles, males and females, were attracted

to plants that had both of these stimuli. Moreover, beetles were landing closest in proximity to leaves that had both model beetles and feeding damage. This suggests that an incorporation of both plant kairomones and the presence of beetles may be necessary to maximize aggregation formation. Nonetheless, the treatment with just feeding damage brought in significantly more beetles than the plants with models only, indicating that plant kairomones play a stronger role in attracting beetles to aggregations than do visual components. Typically, most insects locate host plants from a distance through plant volatiles then once they get closer use visual cues (Bernays & Chapman 1993) and this was most likely the sequence Japanese beetles followed when locating our grape plants.

Future directions and Management Implications

Our study illustrated strong patterns in aggregation formation in Japanese beetles, suggesting that the process is not entirely random. Single beetles landing on uninhabited plants were most likely to be females with higher egg loads, and beetles joining aggregations tended to be females with lower eggs or relatively larger males. When interpreting these patterns, we made some important assumptions that need to be taken into account. For example, we considered each lone beetle that landed on our grapes to be a “pioneer”. However, we did not wait and see if these pioneers initiated an aggregation, and some of these pioneer beetles may have stayed solitary. Additionally, we assumed that all beetles in existing aggregations were joiners, even though the original pioneer may have still been in the group. Clearly, studies using marked beetles that observe aggregation formation from the onset would help us further understand the dynamics of aggregations in this species. However, such studies are surprisingly difficult

to conduct on Japanese beetles in the field. Beetles are very sensitive to disturbance, particularly at high temperatures (Kreuger & Potter 2001), and males and females are difficult to distinguish without handling them. Thus marking and following the behavior of individually marked beetles over time is challenging (Tigreros & Switzer 2009). Fortunately, studies such as ours can still provide valuable information as to what behavioral mechanisms might or might not be involved during aggregation formation in Japanese beetles. For example, our results have some important implications for some of the methods commonly used to control Japanese beetles. First, because females seem likely to be the beetles initiating aggregations, management strategies focused on these females, or in breaking the link between these females and those beetles joining later, are likely to be fruitful. For instance, removing beetles from plants by hand is often suggested for control on a small scale. Although the effectiveness of hand-removal is unclear (Vitullo & Sadof 2007, Switzer unpublished data), our study indicates that removing pioneers may help reduce aggregation formation in some instances, by removing the chemical and visual stimuli provided by feeding beetles. Second, our results help explain patterns in beetle capture with lure-based traps. Switzer *et al.* (2009) found that females that were caught in the trap or around the trap had lower egg loads than females not attracted to the trap and females from control areas. If these traps are interpreted as “aggregations” by Japanese beetles, the results of our study suggest that this trapping pattern may be explained by the likelihood of females with different egg loads to go to aggregation sites.

Literature Cited

- Ahmad, S. 1982.** Host location by the Japanese beetle: evidence for a key role for olfaction in a highly polyphagous insect. *The Journal of Experimental Zoology*, 220: 117-120.
- Alcock, J. 1998.** Sleeping aggregations of the bee *Idiomelissodes duplocincta* (Cockerell) (Hymenoptera: Anthophorini) and their possible function. *Journal of the Kansas Entomological Society*, 71: 74-84.
- Allee, W.C. 1927.** Animal Aggregations. *The Quarterly Review of Biology*, 2: 367-398.
- Alm, S.R., T. Yeh, C.G. Dawson, and M.G. Klein. 1996.** Evaluation of trapped beetle repellancy, trap height, and string pheromone dispensers on Japanese beetle captures (Coleoptera: Scarabaeidae). *Environmental Entomology*, 25: 1274-1278.
- Aukema, B.H. and K.F. Raffa. 2004.** Does aggregation benefit bark beetles by diluting predation? Links between a group-colonisation strategy and the absence of emergent multiple predator effects. *Ecological Entomology*, 29: 129-138.
- Barrows, E.M. and G. Gordh. 1978.** Sexual behavior in the Japanese beetle, *Popillia japonica*, and comparative notes on sexual behavior of other scarabs (Coleoptera: Scarabaeidae). *Behavioral Biology*, 23: 341-354.
- Bernays, E.A. and R.F. Chapman. 1993.** Host-plant selection by phytophagous insects
Chapman & Hall, New York.
- Clark, B.R. and S.H. Faeth. 1997.** The consequences of larval aggregation in the butterfly *Chlosyne lacinia*. *Ecological Entomology*, 22: 408-415.
- Fleming, W.E. 1972.** Biology of the Japanese beetle. United States Department of

Agriculture Technical Bulletin No. 1449.

- Harari, A.R., D. Ben-Yakir, and D. Rosen. 1994.** Mechanism of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). *Journal of Chemical Ecology*, 20: 361-371.
- Held, D.W., P. Gonsiska, and D.A. Potter. 2003.** Evaluating companion planting and non-host masking odors for protecting roses from the Japanese beetle (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, 96: 81-87.
- Held, D.W. and D.A. Potter. 2004.** Floral affinity and benefits of dietary mixing with flowers for a polyphagous scarab, *Popillia japonica* Newman. *Oecologia*, 140: 312-320.
- Isaacs, R. and D.N. Byrne. 1998.** Aerial distribution, flight behaviour and eggload: their inter-relationship during dispersal by the sweetpotato whitefly. *Journal of Animal Ecology*, 67: 741-750.
- Iwabuchi, K. and J. Takahashi. 1983.** Aggregative distribution pattern of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and the role of former occupants in the formation of an aggregation. *Applied Entomology and Zoology*, 18: 324-329.
- Kreuger, B. and D.A. Potter. 2001.** Diel feeding activity and thermoregulation by Japanese beetles (Coleoptera: Scarabaeidae) within host plant canopies. *Environmental Entomology*, 30: 172-180.
- Kruse, K.C. and P.V. Switzer. 2007.** Physical contests for females in the Japanese beetle, *Popillia japonica*. *Journal of Insect Science*, 7.
- Ladd, T.L. 1966.** Egg viability and longevity of Japanese beetles treated with tepa,

- apholate and metepa. *Journal of Economic Entomology*, 59: 422-425.
- Ladd, T.L. 1970.** Sex attraction in the Japanese beetle. *Journal of Economic Entomology*, 63: 905-908.
- Ladd, T.L. 1987.** Influence of food, age, and mating on production of fertile eggs by Japanese beetles (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, 80: 93-95.
- Latty, T.M. and M.L. Reid. 2009.** First in line or first in time? Effects of settlement order and arrival date on reproduction in a group-living beetle *Dendroctonus ponderosae*. *Journal of Animal Ecology*, 78: 549-555.
- Lorenz, M.W. 2007.** Oogenesis-flight syndrome in crickets: Age-dependent egg production, flight performance, and biochemical composition of the flight muscles in adult female *Gryllus bimaculatus*. *Journal of insect Physiology*, 53: 819-832.
- Loughrin, J.H., D.A. Potter, and T.R. Hamilton-Kemp. 1995.** Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *Journal of Chemical Ecology*, 21: 1457-1467.
- Loughrin, J.H., D.A. Potter, T.R. Hamilton-Kemp, and M.E. Byers. 1996.** Role of feeding-induced plant volatiles in aggregative behavior of the Japanese beetle (Coleoptera: Scarabaeidae). *Environmental Entomology*, 25: 1188-1191.
- Loughrin, J.H., D.A. Potter, T.R. Hamilton-Kemp, and M.E. Byers. 1997.** Diurnal emission of volatile compounds by Japanese beetle-damaged grape leaves. *Phytochemistry*, 45: 919-923.
- Oliveira, G.A., D.L. Baptista, H. Guimaraes-Motta, I.C. Almeida, H. Masuda, and**

- G.C. Atella. 2006.** Flight-oogenesis syndrome in a blood-sucking bug: Biochemical aspects of lipid metabolism. *Archives of Insect Biochemistry and Physiology*, 62: 164-175.
- Pitman, G.B. and J.P. Vite. 1969.** Aggregation behavior of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) in response to chemical messengers. *The Canadian Entomologist*, 101: 143-149.
- Potter, D.A. and D.W. Held. 2002.** Biology and management of the Japanese beetle. *Annual Review of Entomology*, 47: 175-205.
- Potter, D.A., J.H. Loughrin, W.J. Rowe, II, and T.R. Hamilton-Kemp. 1996.** Why do Japanese beetles defoliate trees from the top down? *Entomologia Experimentalis et Applicata*, 80: 209-212.
- Rankin, M.A., E.N. Hampton, and K.R. Summy. 1994.** Investigations of the oogenesis-flight syndrome in *Anthonomus grandis* (Coleoptera: Curculionidae) using tethered flight tests *Journal of Insect Behavior*, 7: 795-810.
- Saeki, Y., K.C. Kruse, and P.V. Switzer. 2005.** Male preference for large females and female reproductive condition in the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). *Journal of the Kansas Entomological Society*, 78: 13-19.
- Simmons, L.W. 2001.** Sperm competition and its evolutionary consequences in the insects Princeton University Press, Princeton, NJ.
- Smith, L.B. and C.H. Hadley. 1926.** The Japanese beetle. United States Department of Agriculture Department Circular 363.
- Smyth, R.R. and M.P. Hoffmann. 2003.** A male-produced aggregation pheromone

facilitating *Acalymma vittatum* [F.] (Coleoptera: Chrysomelidae) early-season host plant colonization. *Journal of Insect Behavior*, 16: 347-359.

Switzer, P.V., P.C. Enstrom, and C.A. Schoenick. 2009. Behavioral explanations underlying the lack of trap effectiveness for small-scale management of Japanese beetles (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, 102: 934-940.

Switzer, P.V., P.C. Enstrom, and C.A. Schoenick. 2008. Environmental conditions affect sperm competition risk in Japanese beetles (Coleoptera: Scarabaeidae). *Annals of the Entomological Society of America*, 101: 1154-1161.

Switzer, P.V., K. Escajeda, and K.C. Kruse. 2001. Pairing patterns in Japanese beetles (*Popillia japonica* Newman): Effects of sex ratio and time of day. *Journal of Insect Behavior*, 14: 713-724.

Thornhill, R. and J. Alcock 1983. The evolution of insect mating systems. Harvard College, San Jose.

Tigreros, N. and P.V. Switzer. 2008. Effects of food deprivation, body size and egg load on the mating behavior of female Japanese beetles, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). *Ethology, Ecology and Evolution*, 20: 89-99.

Tigreros, N. and P.V. Switzer. 2009. Observations of multiple mating under field conditions for Japanese beetles (*Popillia japonica* Newman) (Coleoptera: Scarabaeidae). *Journal of the Kansas Entomological Society*, (in press).

Van Timmeren, S.J., P.V. Switzer, and K.C. Kruse. 2000. Emergence and

reproductive patterns in the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). Journal of the Kansas Entomological Society, 74: 17-27.

Vitullo, J.M. and C.S. Sadof. 2007. Effects of pesticide applications and cultural controls on efficacy of control for adult Japanese beetles (Coleoptera: Scarabaeidae) on roses. Journal of Economic Entomology, 100: 95-102.

Wheeler, D. 1996. The role of nourishment in oogenesis. Annual Review of Entomology, 41: 407-431.

Yasui, H., M. Fukaya, S. Wakamura, T. Akino, T. Yasuda, A. Kobayashi, and N.

Arakaki. 2007. Aggregation of the black chafer *Holotrichia loochooana loochooana* (Sawada) (Coleoptera: Scarabaeidae): Function of female pheromone and possible adaptive significance. Applied Entomology and Zoology, 42: 507-515.

List of Figures

Figure 1. Female egg count throughout the day from 0700-1700 hr for pioneers arriving at uninhabited grape plants (experiment #1). The line represents a best fit regression.

Figure 2. Average number of beetles (both male and female) arriving per replicate of each treatment for our experiment incorporating models and feeding damage (experiment #3). The number above the bar represents sample size and treatments with the same letter (within a sex) are not significantly different at $\alpha= 0.05$ in post-hoc tests.

Figure 3. Male body size of arriving pioneers in relation to time of day beginning at 0900 hr for our experiment incorporating models and feeding damage (experiment #3). The represents the best fit regression.

Figure 4. The average distance (\pm SE) to the treatment leaf for arriving male and female beetles for our experiment incorporating models and feeding damage (experiment #3). The number above the bar represents sample size; treatments with the same letter were not significantly different at the $\alpha= 0.05$ level in post-hoc tests.

Figure 5. The mean (\pm SE) number of eggs for females arriving at each treatment in our experiment incorporating models and feeding damage (experiment #3). The number above the bar represents sample size; treatments with the same letter were not significantly different at the $\alpha= 0.05$ level in post-hoc tests.

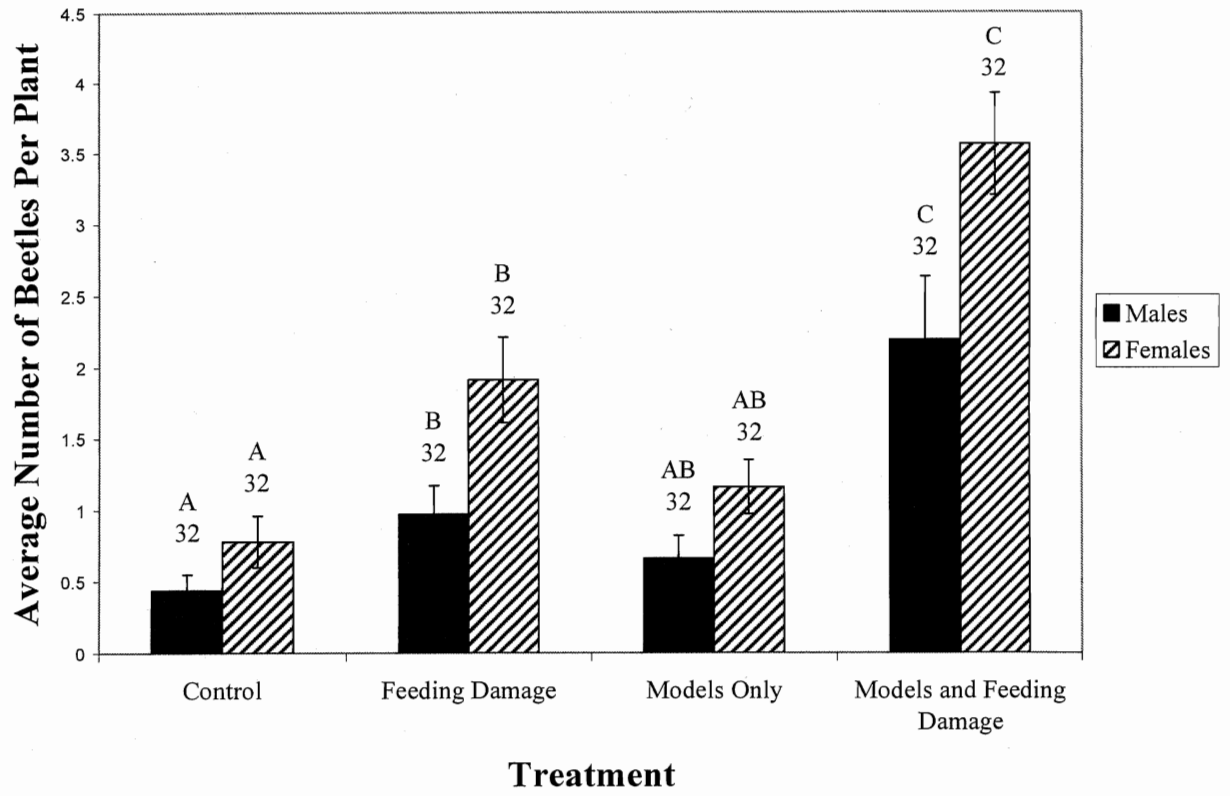


Figure 2

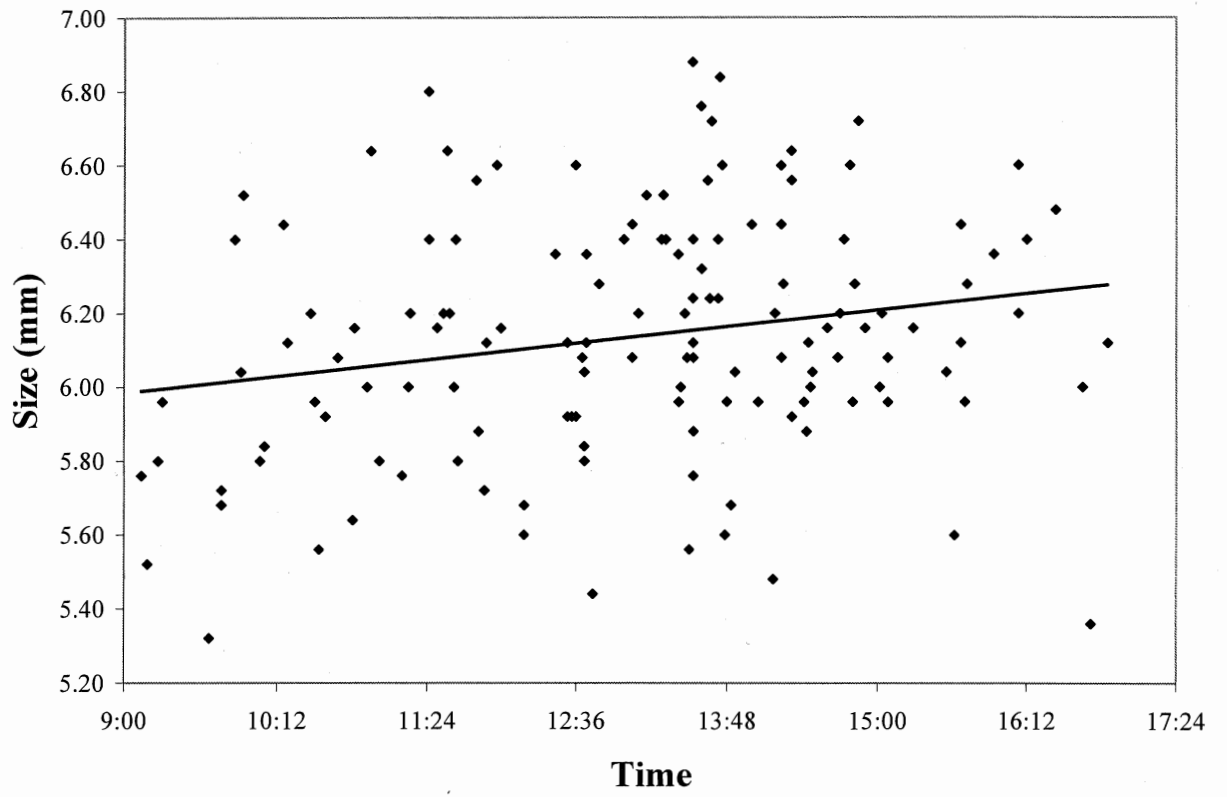


Figure 3

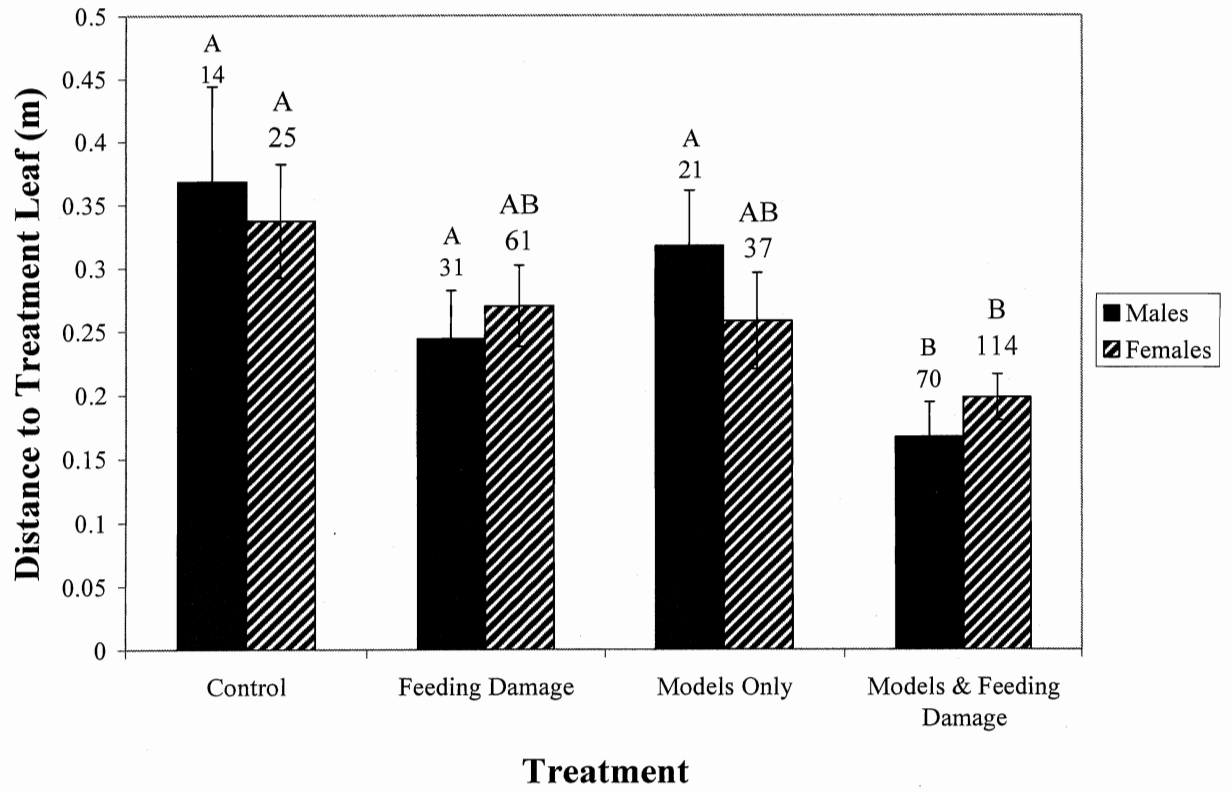


Figure 4

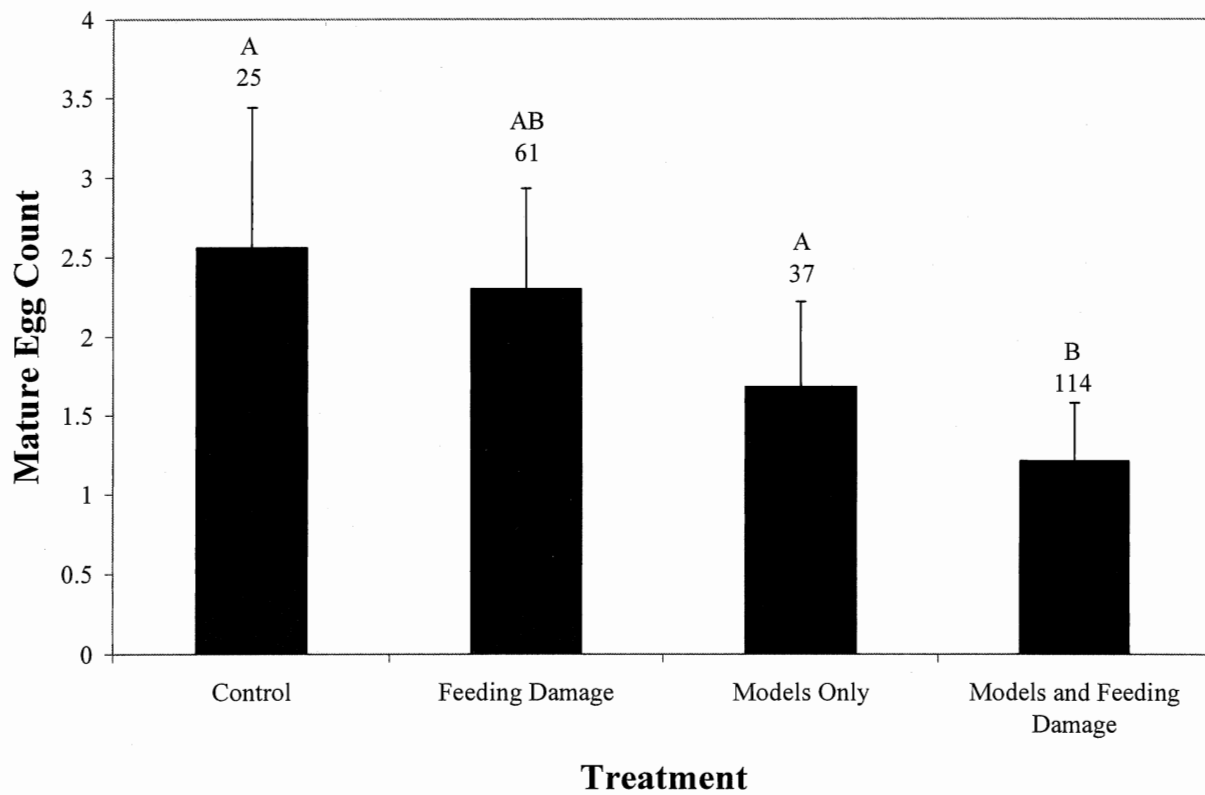


Figure 5