1-29-2015

Host Plant Feeding Preferences of the Adult Asiatic Garden Beetle, Maladera castanea Arrow (Coleoptera:Scarabaeidae)

Laura E. Eckman
University of Connecticut - Storrs, Laura.E.Eckman@gmail.com

Host Plant Feeding Preferences of the Adult Asiatic Garden Beetle, *Maladera castanea* Arrow (Coleoptera: Scarabaeidae)

Laura Eliana Eckman

B.S., Trinity College, 2009

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

At the

University of Connecticut

2015

APPROVAL PAGE

Master of Science Thesis

Host Plant Feeding Preferences of the Adult Asiatic Garden Beetle, *Maladera castanea* Arrow (Coleoptera: Scarabaeidae)

Presented by

Laura Eliana Eckman, B.S.

Major Advisor____
Ana Legrand

Associate Advisor____
Karl Guillard

Associate Advisor____
Julia Kuzovkina

University of Connecticut

2015

Acknowledgments

I would like to thank the following people: my major advisor, Dr. Ana Legrand, for guidance, inspiration, and support throughout my research endeavors; my associate advisor Dr. Karl Guillard for continued guidance with statistical analyses; and my associate advisor Dr. Julia Kuzovkina for guidance in choosing relevant plants for this study. I would also like to thank Dr. Ming-Hui Chen and Hee-Koung Joeng for assistance with statistical analyses, Dr. Mark Brand for assistance in locating landscape plants on campus, and Jason Bennett and Andrew Hirsbrunner for general assistance. I also owe a debt of gratitude to the University of Connecticut farm staff, particularly Steve Olsen, Todd Wright, Kyle Knox, and Geoffrey Vose, and the UConn greenhouse staff, particularly Nick Pettit, Bob Shabot, and Chris Claussen. I would also like to thank the various other people who have helped me with any aspect of my research, from purchasing supplies to pondering results. Every small contribution of time, effort, or interest has helped me to reach my goal of producing this thesis. Finally, I would like to acknowledge the generous funding provided by the USDA National Needs Fellowship.

Table of Contents

List of Tables

List of Figures

Abstract

The Asiatic garden beetle (AGB), *Maladera castanea* Arrow, is an invasive pest of crops, ornamentals, and turfgrass that has been minimally studied since the 1930s. Experiments were performed in 2011 and 2012 to investigate adult AGB feeding preferences and seasonality in Connecticut, with the goal of supporting informed planting and monitoring decisions. A common garden field experiment involved counting beetles on three cultivars each of basil, beet, carrot, eggplant, kohlrabi, parsnip, hot pepper, sweet pepper, and turnip. A no-choice laboratory experiment produced values of mass and area of leaf disks consumed. This included the same basil, beet, and kohlrabi varieties in 2011, and elderberry, arrowwood viburnum, green ash, red maple, sugar maple, and American sweetgum in 2012. Counts of beetles collected in light traps were performed throughout each field season.

Basil harbored the most AGBs in the field experiment in 2011 and 2012, and was most consumed in the laboratory experiment using edibles in 2012. However, the 2011 laboratory mass data showed that beets were more consumed than kohlrabi, and basil was consumed equally to beets and kohlrabi. In the 2011 field experiment, 'Mexican Spice' was preferred over 'Lemon' basil. Red maple was significantly more consumed than sugar maple in the laboratory study of ornamentals. In 2012, the first AGB adults were caught on June 20. Peak populations of adult AGBs in Connecticut occurred from mid-July to late August. This study has developed methods and outlined further lines of research on the AGB.

Chapter 1
Introduction

The Asiatic garden beetle

The Asiatic garden beetle (AGB), *Maladera castanea* Arrow (Coleoptera: Scarabaeidae), formerly known in the United States as the oriental garden beetle (Hallock, 1930), Japanese garden beetle (Britton, 1934), *Aserica castanea* (Hallock, 1930), and *Autoserica castanea* (Hallock, 1932), is a member of the white grub complex of the East Coast and northeastern United States. The white grub complex is known as the most damaging group of turfgrass insect pests in the region; furthermore, their damage is not limited to turf (Koppenhöfer et al., 2003). White grubs are root-feeding larvae of beetles in the scarab family, some of which also feed in the adult form (Koppenhöfer, 2010). The AGB feeds in both its larval and adult forms, the latter of which is nocturnal and eats both foliage and flowers. The AGB's generalist herbivorous habits, and the fact that it feeds in both life stages, can make it a serious pest of ornamental and crop plants in addition to turfgrass (Hallock, 1932; Heller, 1995). The AGB, like many white grub pests, is also an invasive species: it is foreign to the United States, considered harmful, and is slowly spreading across the country (Hallock, 1930; Held & Ray, 2009; ISAC, 2006; Skelley, 2012).

The AGB has been considered an economically significant pest in the northeastern United States and beyond (Hallock, 1929, 1930, 1931; Koppenhöfer et al., 2003), although reports of the significance of AGB feeding damage are varied. The AGB is not historically known to cause as much damage as other scarab pests, such as the Japanese beetle (*Popillia japonica*), oriental beetle (*Anomala orientalis*), or the European chafer (*Rhizotrogus majalis*) (Grewal et al., 2002; Potter, 1991). However, on ornamental plants and turfgrass in the

northeastern U.S. and central East Coast, the four scarabs may cause similar levels of damage (Koppenhöfer & Fuzy, 2003b). In corn fields in Michigan and Ohio, AGB grubs are even reported to be more damaging than other white grubs (Hammond, not dated; MacKellar, 2012). Regardless of ranking among scarabs, the AGB can be a significant pest of many plant types.

Distribution in the United States

The Asiatic garden beetle is native to northern China, Japan, North Korea, South Korea, and far east Russia, and was first collected in the United States in New Jersey in 1921 (Ahrens, 2007; Arrow, 1913; Hallock, 1932). By 1929, the AGB was described as economically damaging (Hallock, 1929), and by 1933, it was recorded in Connecticut, Delaware, Maryland, Massachusetts, New Jersey, New York, Pennsylvania, Rhode Island, South Carolina, Virginia, and Washington, D.C. (Britton, 1930; Hallock, 1930, 1932, 1933; Hamilton, 1929; Marlatt & Wallace, 1933; USDA Bur. of Ent., 1933). By 2009, the beetle was also present in Alabama, Georgia, Illinois, Indiana, Maine, Michigan, New Hampshire, North Carolina, Ohio, Vermont, and West Virginia, with unverified reportings in Kansas and Missouri (Brandenburg & Baker, 1994; Cappaert & Smitley, 2002; Estes & McLaughlin, 2010; Held & Ray, 2009; Nielsen, 1997; Pierce, 2009; Purdue Univ. CERIS, 2014; Shetlar & Niemczyk, 1999; Sideman, 2008; Skelley, 2012). The spread is continuing: the most recent report of AGB infestation was in Florida in 2012 (Skelley, 2012). The AGB has also been reported in the Canadian provinces of Québec in 1996 (Chantal, 2003) and Nova Scotia in 2003 (Cutler & Rogers, 2009). Table 1 and Figure 1 show the states in which the AGB is currently present, and Table 1 provides the years in which it was first detected in each state.

Table 1. States in which the Asiatic garden beetle is found, and the earliest year in which it is known to have been detected in each state. Presence in MO and KS is unverified. *Source explicitly claims that given year is the first record of AGB presence in the state.

State	**Year**	**Source**
New Jersey	1921*	Hallock, 1929
New York	1926	Hallock, 1930
Connecticut	1929*	Britton, 1930
Pennsylvania	1929	Hamilton, 1929
Virginia	1929	Britton, 1930
Delaware	1930	Hallock, 1930
Maryland	1930	Hallock, 1930
Massachusetts	1932	Hallock, 1933
Rhode Island	1933	USDA Bur. of Ent., 1933
South Carolina	1933	Marlatt & Wallace, 1933
Georgia	1978	Skelley, 2012
Ohio	1994	Brandenburg & Baker, 1994
Vermont	1997	Nielsen, 1997
New Hampshire	1999	Shetlar & Niemczyk, 1999
North Carolina	1999	Shetlar & Niemczyk, 1999
West Virginia	1999	Shetlar & Niemczyk, 1999
Michigan	2000	Cappaert & Smitley, 2002
Indiana	2006	Pierce, 2009
Missouri	2007	Purdue Univ. CERIS, 2014
Alabama	2008*	Held & Ray, 2009
Kansas	2008	Purdue Univ. CERIS, 2014
Maine	2008	Sideman, 2008
Illinois	2009	Estes & McLaughlin, 2010
Florida	2012*	Skelley, 2012

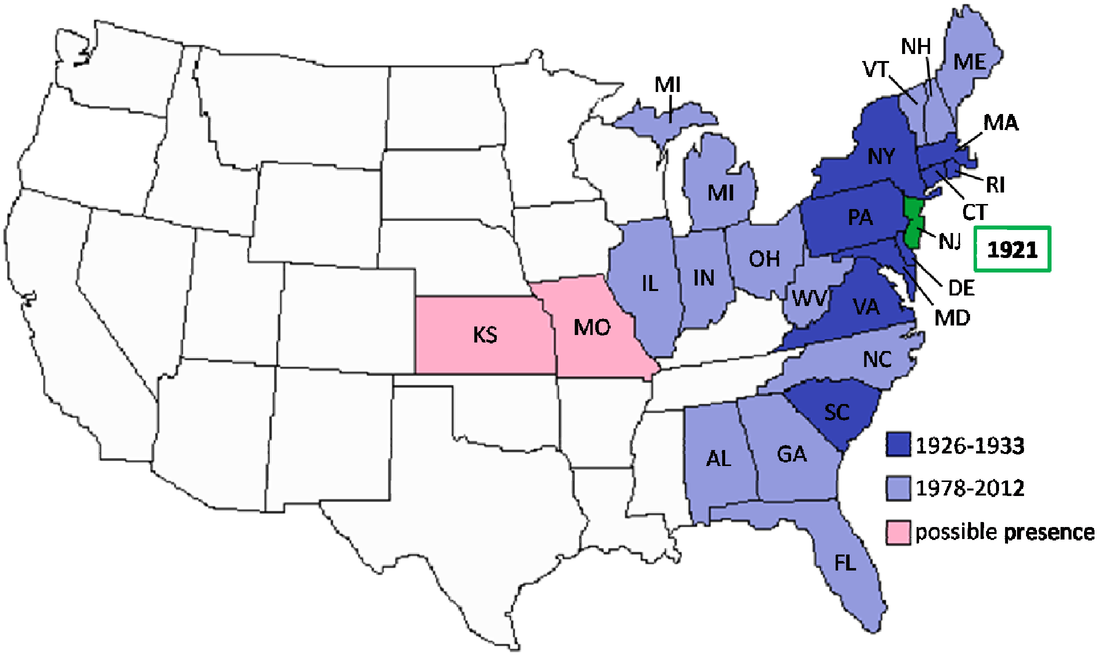

Figure 1. Distribution of the Asiatic garden beetle in the United States, by state, as of September 2014. Green represents the location of first discovery.

Life cycle

In the northeastern United States, the Asiatic garden beetle life cycle is a one-year process from hatching until death. The exact timing of the beetle's cycle is likely to vary somewhat with changes in climate, even within the Northeast. In the latitude of New York City, the beetle emerges from its underground egg as a small, white larva, or grub, between early July and late October. This first instar larva remains buried in soil up to 13 cm deep, feeding on young roots and decaying plant material. When it has eaten enough, each larva will molt to pass into its second instar. By mid-October, most larvae will have molted once more, reaching their third and final larval instar. At this time, the AGB larvae bury themselves from 15 to 30 cm beneath the soil and stop feeding, entering their winter dormancy (Hallock, 1932, 1936a; Hamilton, 1929).

The slow-developing or late-hatching larvae, about one quarter of the original larval population, do not reach the third instar before cold weather arrives. Some of these pass the winter as second instars, but first instar larvae do not appear to survive the winter. Around the middle of April, the surviving AGB larvae move back towards the soil surface to continue feeding at 13 cm deep or less. The third instar AGB is C-shaped, dirty white, and reaches 2 cm in length (Hallock, 1932). The AGB larva looks very similar to Japanese beetle, oriental beetle, and other scarab larvae. However, it is somewhat smaller and possesses some unique identifying characteristics. On its raster, the ventral side of its final body segment, the AGB has a Y-shaped anal slit. Although this is shared by some other larvae, it provides a positive identification when seen with the transverse curved row of spines nearby (Hallock, 1932; Brandenburg & Villani, 1995). The AGB larva also has uniquely enlarged stipes, white structures, part of the maxillae, which are frequently in motion (Brandenburg & Villani, 1995; Tashiro, 1987). Finally, the AGB is generally more vigorous than other grubs, running and wriggling more readily when caught (Legrand, pers. obs.).

After living as a larva for roughly ten months, the third instar AGB makes a pupal cell by compacting soil 4 to 10 cm below the surface. In New York City, this behavior begins in mid-June, and is seen until mid-July (Hallock, 1932). In New Jersey, it is seen from late May to early July (Hamilton, 1929). Inside the pupal cell, the AGB enters the prepupal stage, and remains apparently inactive for 4 days, after which it pupates fully. AGB pupae differ from those of many other scarabs in that they are not covered by the shed third instar skin, a trait that is useful for pupal identification. The adult AGB emerges from the pupal stage after 10 days, on average, and remains in the pupal skin for several more days while its exoskeleton hardens (Hallock, 1932, 1936a).

The adult AGB (Figure 2) is around 1 cm long, and 0.5 cm wide, somewhat smaller than the Japanese and oriental beetles (Hallock, 1932; Hamilton, 1929). The adult is an iridescent brown color with a darker head, mostly obscured by the pronotum when viewed from above. The adult AGB has spiny, hairy legs, and an abdomen that extends nearly two segments beyond its elytra, or wing covers, which have many shallow, longitudinal grooves (Britton, 1930; Eckman, pers. obs.; Hallock, 1932). The adult AGB can also be recognized by the presence of yellowish hairs on the head, around certain edges of the prothorax and elytra, on the ventral side of the thorax, and in a single, transverse row on the ventral surface of each abdominal segment (Eckman, pers. obs.; Hallock, 1932). There are also extremely short hairs on the surface of the elytra, but these can only be seen under magnification (~ 3x or higher) (Eckman, pers. obs.; Hamilton, 1929). Adult AGBs are easy to confuse with masked chafers, whose adult stage overlaps in time with that of the AGB in the northeastern United States. Masked chafers are a lighter, more yellowish brown color, less iridescent, and with a nearly black head when seen with the naked eye. The black head is actually a dark brown mask extending between the eyes. The masked chafer is somewhat smaller and thinner than the AGB, with less spiny legs, and less hair on the head (Eckman, pers. obs.).

Adult AGBs are seen from the last week of June to the last week of October in the latitude of New York City, with peak numbers occurring between July 15 and August 15. On average, AGBs live for one month as adults, but can survive for more than 100 days. Adult AGBs are nocturnal and fly only on nights with an average temperature of 21°C (70°F) or higher (Hallock, 1932, 1936a). Most feeding, mating, and egg-laying occurs during these periods; at other times, adults can usually be found in moist soil, often beneath a food plant (Hallock, 1930).

However, light feeding may occur at temperatures as low as 16°C (60°F). AGB adults feed inwards from the edges of leaves and petals (Hallock, 1936a).

Male AGBs tend to be more common at the beginning of the season than females. Males may also outnumber females at light traps that use 500-W daylight bulbs. Male beetles are thought to be responsible for nearly 70% of the damage caused by AGB adults (Hallock, 1936b). From early July to late October in New York City and late July to September in New Jersey, female AGBs deposit their small, round, white eggs in clusters of 1 to 19 between 1 and 10 cm below the soil surface. On average, each female deposits 60 eggs, with the maximum observed number being 178 eggs (Hallock, 1932; Hamilton, 1929). The preferred areas for oviposition are shady, moist, and cool areas such as weedy turf. Eggs are preferentially laid under tall, wide-leaved, or densely foliated weeds, particularly orange hawkweed (Hallock, 1936b). AGB eggs hatch about 10 days after they are deposited (Hallock, 1932).

Figure 2. Adult Asiatic garden beetle. Enlarged photo.

Asiatic garden beetle management

Control methods for the Asiatic garden beetle include chemical, biological, physical, and cultural management methods, or combinations of these. Most current control methods treat the

larval stages of the AGB, which is not an optimal solution because larvae live underground, and treatments must be performed at the proper times for maximum effectiveness and minimal waste (Vittum, 2011). Many larval insecticides also cause egg mortality, and there are some treatments applicable to adult beetles. Although the use of insecticides applied to soil is the traditional approach to white grub management, and generally the most effective, the AGB is not affected by some common chemicals labeled for use in the control of other white grubs such as those of the Japanese beetle. Several non-chemical treatments are also not as effective on AGBs as they are on other scarab grubs.

Among the chemical control methods tested for use on the AGB, the neonicotinoids acetamiprid and imidacloprid have been found ineffective against AGB larvae (Koppenhöfer et al., 2002; Koppenhöfer & Fuzy, 2003a; Morales-Rodriguez et al., 2010). Also ineffective are bifenthrin, a pyrethroid; carbaryl, a carbamate; and halofenozide, a molt-accelerator (Koppenhöfer et al., 2003; Koppenhöfer & Fuzy, 2003a). Bifenthrin- and halofenozide-treated turf even had higher numbers of third instar grubs than control turf in one experiment (Koppenhöfer et al., 2003). Conflicting results have been reported regarding the effectiveness of the neonicotinoid thiamethoxam against AGB larvae (Koppenhöfer et al., 2002; Koppenhöfer & Fuzy 2003a; Morales-Rodriguez et al., 2010).

Three chemical insecticides that, when used alone, can successfully control AGB eggs and young larvae include chlorantraniliprole, an anthranilic diamide; clothianidin, a neonicotinoid; and trichlorfon, an organophosphate (Koppenhöfer & Fuzy, 2003a; Morales-Rodriguez et al., 2010; Vittum, 2011). For the control of adult AGBs, carbaryl, a carbamate; imidacloprid, a neonicotinoid; and malathion, an organophosphate, may cause mortality if applied to the leaves of food plants (Australian Government, 2004; Metcalf & Metcalf, 1993).

The control of adult beetles is especially difficult, however, because AGB adults are strong fliers: when there is a large population nearby, beetles that are killed can quickly be replaced (Hallock, 1932, 1936b).

Entomopathogenic nematodes are another, biological, control option for white grubs. The nematodes *Heterorhabditis bacteriophora*; *H. zealandica*; another, unknown, species of *Heterorhabditis*; and *Chromonema heliothidis* have been shown to lightly or moderately control AGB larvae, although different strains of *H. bacteriophora* had different impacts on AGB population sizes (Khan et al., 1976; Koppenhöfer et al., 2002; Koppenhöfer et al., 2004; Koppenhöfer et al., 2006; Koppenhöfer & Fuzy, 2003a; Morales-Rodriguez et al., 2010). Another nematode, *Steinernema glaseri*, failed to control third instar AGBs in one study (Koppenhöfer & Fuzy, 2003a), although it was somewhat successful in another (Koppenhöfer et al., 2004). *S. scarabaei*, currently commercially unavailable, appears to provide very good larval AGB control. This nematode has been shown more effective on third than second instar AGBs (Koppenhöfer et al., 2004; Koppenhöfer & Fuzy, 2003a; Koppenhöfer & Fuzy, 2004; Morales-Rodriguez et al., 2010).

An additional potential biological control for white grubs is *Bacillus thuringiensis* (*Bt*; vars. *galleriae* and *tenebrionis*), which was effective in one trial against AGB larvae. However, *Bt* products for AGB treatment are currently commercially unavailable (Morales-Rodriguez et al., 2010). Furthermore, in an earlier set of five experiments, various formulations of Cry8Cal δ-endotoxin, produced by *Bt*, did not cause significant AGB mortality compared to controls (Bixby et al., 2007), so the effectiveness of *Bt* against AGB grubs is unclear. Another white grub biological control, the entomopathogenic fungus *Metarhizium anisopliae*, has been shown effective against AGB larvae. The fungus *Beauveria bassiana*, on the other hand, does not

appear effective for AGB control. The bacteria *Paenibacillus* (=*Bacillus*) *popilliae*, commonly known as the causal agent of milky disease, also seems not to harm AGB larvae (Morales-Rodriguez et al., 2010), although, in the past, it has been mentioned as slightly useful in their control (Capinera, 2001). Other biological products, spinosad and diatomaceous earth, appear effective in controlling AGB grubs, and azadirachtin can be used to control adult AGBs (Australian Government, 2004; Morales-Rodriguez et al., 2010).

Suggested physical methods of controlling AGB adults include the use of screening and row covers (Pundt & Smith, 2005). Light traps may also aid in the control of adult AGBs (Hallock, 1936c; Metcalf & Metcalf, 1993), and some AGBs have been found in western bean cutworm moth traps using RV anti-freeze as an attractant (MacKellar, 2012). For small gardens, beetles may be knocked by hand into soapy water (Raupp, 2014). In terms of cultural management, it may be useful to remove the AGB's preferred food plants, including weeds, from areas at high risk of AGB damage (Brandenburg & Villani, 1995). However, this may cause an increase in herbivory of cultivated plants during the first summer after removal (Hallock, 1934). Therefore, it may be useful to grow non-preferred plants for the first year of transition from an AGB-infested area to productive land that will include favored food plants (Metcalf & Metcalf, 1993). The second year of transition after the removal of preferred food plants should produce fewer AGBs than found in previous years (Hallock, 1934). In a preliminary experiment, Hallock (1936b) found that mulching strawberry beds with hay seemed to reduce AGB oviposition, and thus the number of larvae feeding on the strawberry roots. Mulching strawberries with hay has been recommended more recently by Metcalf and Metcalf (1993). Finally, it may be possible to reduce AGB numbers by limiting the watering of plants during the early, moisture-dependent life phases of the AGB, particularly the egg stage (Australian Government, 2004).

The state of management options for the AGB is not dire, nor is it ideal. Several cultural, physical, biological, and chemical control methods are available, at differing rates of effectiveness, cost, and required labor. Of these choices, traditional insecticides are still the major control method used for AGBs. When dealing with chemical pesticides, there is always the concern of negative environmental effects and the development of resistance. Additionally, chemical options may become limited due to the threat of continuing restrictions on turfgrass insecticide use (Koppenhöfer & Fuzy, 2003b). One major weakness in current AGB management options is the limited ability to effectively control adult beetles. Although grubs cause the most damage (Capinera, 2001), adult AGBs are also harmful. AGB adults can completely defoliate multiple rows of plants in one night under ideal conditions (Hallock, 1932). Adult AGBs are also important because adult females determine the location of future larvae through oviposition, or egg-laying.

Rationale for Asiatic garden beetle feeding preference studies

The Asiatic garden beetle is invasive in the United States, and is a recognized pest of turfgrass, ornamentals, and crop plants. AGB larvae are more difficult to control than are some other white grubs due to their lower susceptibility to chemicals and nematode infection. AGB adults are also particularly difficult to control due to their capacity to quickly repopulate areas where the former AGB occupants were killed. These reasons alone provide incentive to study the habits of the AGB, and to search for new management tactics. However, the AGB may be an even more important pest than is commonly recognized. First, because it is nocturnal, the AGB adult is infrequently observed feeding, and thus the damage it incurs is more likely to be erroneously attributed to other pests. Second, the AGB larva is similar in appearance to other

white grubs. For this reason, as well, the AGB may go unrecognized. Finally, it is likely that the AGB will become more of a problem as it spreads to warmer areas, and as the global climate changes.

In warmer climates, the amount of damage done by adult AGBs could dramatically increase (Hallock, 1936b), as the adults feed only when nighttime temperatures reach 21°C (70°F) and above (Hallock, 1932). Increased feeding due to longer and more frequent warm nighttime periods might provide the nutrition necessary to increase egg production and cause population sizes to expand. Hallock (1936b) also suggests that multiple generations may be produced every year in warmer climates with sufficient moisture, as he observed three generations per year when beetles were raised in the lab at 27°C (81°F). Problems of temperature-exacerbated AGB damage may even apply to the northeastern United States as the climate warms according to projected trends. In addition to increased AGB feeding and the production of multiple, perhaps larger, generations, increases in temperature may allow more AGB grubs and other pest insects to survive the winter. Increased temperatures may also decrease the effectiveness of some pesticides (Wolfe et al., 2008).

Another reason to pursue AGB research is the lack of study on this beetle, particularly in its adult form. Aside from a paper on the anatomy of the AGB eye by Meyer-Rochow and Gokan (1987), there has only been one recent peer-reviewed research article focusing exclusively on the habits of the AGB or its control, Koppenhöfer and Fuzy's 2003(a) investigation into biological and chemical control options for the larvae. The most detailed research on and descriptions of the AGB were mainly published by Harold Hallock in the 1930s, and had a strong observational emphasis in contrast to experimental data collection and quantitative analysis. Hallock did frequently focus on adult AGBs, but, since then, most of the little research that has been done has

only involved study of the grubs. Our understanding of the AGB is still incomplete, particularly in regard to adult beetles and their control. Further study of the AGB may lead to the discovery of information that could help in the development of improved control methods.

One method of controlling both adult and larval AGB damage is to discourage AGB presence in an area. This is best done with the adult beetle, as adult females determine egg placement, and thus grub location and the emergence sites for the next generation of adults. AGB larvae have been found to be more abundant in turf adjacent to favored adult food plants than in turf that is not near favored adult food plants (Hallock, 1936a). Information regarding adult feeding and oviposition preferences could be used to minimize the attractiveness of an area to the AGB for both feeding and reproduction. For example, if their identities were known, favored plants for feeding and oviposition could be removed from an area of managed plants, as suggested by Hallock (1934), Metcalf and Metcalf (1993), and Brandenburg and Villani (1995). On the other hand, it might be possible to use favored food plants as trap or perimeter crops to distract adult AGBs from managed plants or to kill them with targeted pesticide applications. This approach was shown to work, reducing the need for pesticide use by about 97%, in a butternut squash (*Cucurbita moschata* 'Waltham') production system using hubbard squash (*C. maxima* 'Blue Hubbard') or buttercup squash (*C. maxima* 'Burgess') as the perimeter trap crop for striped cucumber beetles (*Acalymma vittatum*, Coleoptera: Chrysomelidae) (Cavanagh et al., 2010). Favored plants could also be used for monitoring AGB populations. If identified, AGB-resistant plants could be recommended for use in areas with large AGB populations. Furthermore, they could form the basis of chemical ecology or plant breeding studies that could lead to the discovery of more AGB-resistant plants, their development, or even the creation of AGB repellents.

Quantitative information regarding the feeding preferences of the adult Japanese beetle has been obtained from controlled experiments. Planting recommendations are made using this information to avoid severe damage in areas with high Japanese beetle densities (USDA APHIS, 1998). A similar endeavor would be useful in providing information to potentially reduce AGB damage. Currently, a reliable comparison of AGB food plant preferences for potential use in these pest management applications is unavailable.

Hallock's publications from 1929 to 1936 still appear to be the main sources used in extension publications addressing the AGB. Hallock (1932) found that adult AGBs feed on many native and invasive wild plants, weeds, grasses, herbaceous and woody ornamentals, herbs, vegetables, and fruits, including fruit trees. Many of Hallock's papers do contain lists of the AGB adult's preferred food plants, but the evidence is apparently anecdotal and qualitative; no numerical figures are published, except from one small study (Hallock, 1936b) in which most plants were tested only once, producing unreliable results. Furthermore, Hallock's rankings of AGB adult food plants vary somewhat from year to year with little explanation and, at times, great contradiction. In 1936(a), Hallock published a final list of AGB adult food plants, and indicated that his previously published preference information should be ignored. To make the information from this final publication more accessible, and to add some preference information published by other authors, a list was compiled of published food plant preference rankings for the adult AGB (Appendix A). Rankings in Appendix A are mostly based on the final rankings by Hallock (1936a), although indications of preferred foods by other authors are also included. Appendix A is not intended to be a complete list of AGB food plants.

Hallock's feeding preference information, while useful, has two more drawbacks beyond age and lack of controlled, quantitative comparisons: the inability to compare AGB feeding

preferences within the category of plants that Hallock considers to be preferred, and the lack of preference information beyond the species level. Chandrasena et al. (2012) show that the Japanese beetle has significantly different preferences for different lines of soybean (*Glycine max*), which were already known to have different levels of resistance to the soybean aphid (*Aphis glycines*). Spicer et al. (1995) show that the Japanese beetle has significantly different preferences for several cultivars of crabapple (*Malus* spp.). AGBs could also have food preferences at the cultivar level, which would be useful to know when choosing plants. Improved feeding preference information could be very useful for making informed decisions regarding ideal plants to be maintained in areas of high AGB density. This study will compare some plants that are already listed as preferred by Hallock (1936a) to determine if more specific differences in preference level can be found. This study will also be the first to produce rigorously determined quantitative measures of adult AGB food plant preference, and the first to compare AGB preferences for different cultivars within the same species.

Objectives

The major objective of this study is to investigate the feeding preferences of the Asiatic garden beetle for foliage of several edible plants (vegetables and herbs) and landscape plants (woody shrubs and trees). A secondary objective is to observe the time period during which AGB adults are present in the vicinity of Storrs, Connecticut. A better understanding of this seasonal occurrence of AGBs should be useful in monitoring and management efforts for the pest in Connecticut. A better understanding of AGB adult feeding preferences can help indicate which plants are more resistant to AGB feeding, and thus suggest better choices for areas with large AGB populations. Particularly susceptible, or preferred, plant types could have the

potential to function as trap crops in the future, diverting attention away from the intended managed plants.

This study includes two components: field experiments to study feeding preferences and beetle seasonality in a natural setting, and laboratory experiments to study feeding preferences in a no-choice setting. The field experiments include counts of AGB adults on edible plants grown in a common garden in 2011 and 2012, as well as counts of adult AGBs caught in black light traps throughout the 2011 and 2012 field seasons. The laboratory portion of this study consists of no-choice feeding tests, involving the measurement of mass and area of leaf disks consumed by a single beetle when presented with no other food options. The laboratory tests focus on a subset of the edible plants used in the field experiments in 2011 and 2012, and also include landscape plants in 2012. This study builds on Hallock's work from the 1930s, improving the accuracy and precision of his published information, and is based on similar investigations into the food plant preferences of the related Japanese beetle (Ladd, 1987; Ladd, 1989; Miller & Ware, 1999; Spicer et al., 1995), the results of some of which are summarized in Held (2004).

Chapter 2
Field evaluation of adult Asiatic garden beetle plant preferences and seasonal occurrence

Introduction

The better a pest is understood, the easier it becomes to control. In the case of the Asiatic garden beetle (AGB), adult feeding preference is an area of study that has great potential to help improve management methods for the pest. As discussed in Chapter 1, knowledge of which plants are most and least likely to be attacked, and which are preferred over others, is valuable to the process of deciding which plants to grow in an area populated by a given pest. Information regarding pest feeding preferences can also help direct early monitoring and preventative control measures to plants most likely to be harmed by the pest, in this case the AGB.

Harold Hallock was concerned with adult AGB feeding preferences in the 1930s. He developed lists of AGB food plants and ranked them by preference (Appendix A), but it appears that most of his information was observational and therefore qualitative rather than quantitative, and did not come from a controlled setting in which plants could be equally compared to one another. For example, apparent preference for certain plants could be attributed to a higher presence of AGBs in the area where those plants were observed (Ladd, 1987). Hallock's plant preference rankings were also inconsistent between publications, indicating his attempts to produce useful and correct information, but also indicating that he may not have reached a final, solid understanding of order of preference, even for the plants in his final list in 1936(a).

The goal of the common garden field count experiment performed as part of this study was to investigate food plant preferences of the AGB in a controlled way, using quantitative measures while retaining a realistic setting. This experiment was partially based on one of Hallock's (1936b) experiments, performed in 1933 and 1934, which involved counting beetles at

night in a common garden, and employed the use of a field cage to keep AGBs contained. Improvements on Hallock's (1936b) experiment include multiple replications of each plant variety combination, the use of statistical analyses to test for significant differences between beetle numbers on different plant types, and more detailed reporting of experimental methods. A different combination of plants was used to avoid repeating Hallock's (1936b) work.

This study investigated AGB preferences for visiting different plants, which are likely to be correlated with AGB plant feeding preferences. Another study, involving direct measurements of feeding in a no-choice laboratory setting, is included in this thesis in part to verify that counts of beetles on plants in the field may represent interest in feeding on those plants. Results from this common garden field count experiment will contribute to our current understanding of adult AGB food plant preferences, in a more reliable and informative way than previously done.

Other information that may be important for improving AGB management strategies pertains to the insect's life cycle. Basic life cycle information has already been detailed by Hallock (1929, 1930, 1932, 1933, 1934, 1936a, 1936b) and Hamilton (1929) for New York and New Jersey, respectively. However, insect life cycles and habits can vary depending on climate, so information on seasonal occurrence specific to Connecticut could help improve management efforts in the area. The goal of the light trap portion of this field study was to document the seasonal occurrence of adult AGBs in the Storrs, Connecticut area.

Materials and methods

Common garden field count experiment

Twenty-seven cultivars of edible plants (Table 2) were grown in 2011 and 2012. Plants were from five families: Apiaceae (carrot and parsnip), Brassicaceae (kohlrabi and turnip),

Chenopodiaceae (beet), Lamiaceae (basil), and Solanaceae (eggplant and pepper). Each crop type except for basil (beet, carrot, eggplant, kohlrabi, parsnip, pepper, and turnip) has been reported by Hallock (1934, 1936b) to be heavily damaged by the adult Asiatic garden beetle, and beet, carrot, parsnip, pepper, and turnip were listed by Hallock (1934) as preferred foods for the adult AGB. However, Hallock (1936a) later included only carrot, red pepper, and turnip as preferred in his final published feeding preference list, which was mostly based on visual observations (included and expanded in Appendix A). Basil was reported by Pundt and Smith (2005) to be a favorite, presumably also due to visual observations, and was not ranked in comparison to other plants. Cultivars in this experiment were selected due to common use in Connecticut or unique characteristics such as leaf scent or color.

Plants were grown in a field at the University of Connecticut Plant Science Research and Education Facility in rows of 1.2 m x 8.5 m embossed black plastic mulch (Rain-Flo Irrigation Co., East Earl, PA) over 84B Paxton and Montauk fine sandy loam with a pH of 6.3 treated with 0.078 kg/m^2 of 15-15-15 N-P-K granulate fertilizer (Crop Production Services, Broadbrook, CT). A randomized complete block design was used, consisting of five blocks (Figures 3 & 4). Experimental units within blocks consisted of three individual plants of the same cultivar in a row. Each block contained three rows of nine experimental units each, with all plants 0.3 m apart, and with 0.3 m of space on the ends of each row. Rows were 0.6 m apart within blocks, and blocks were lined up 1.2 m apart in 2011 except for one block 0.9 m to the side. In 2012, blocks were lined up 1.5 m apart. Location of experimental units within blocks was randomly assigned using the PLAN procedure in SAS 9.2 (SAS Institute Inc., Cary, NC).

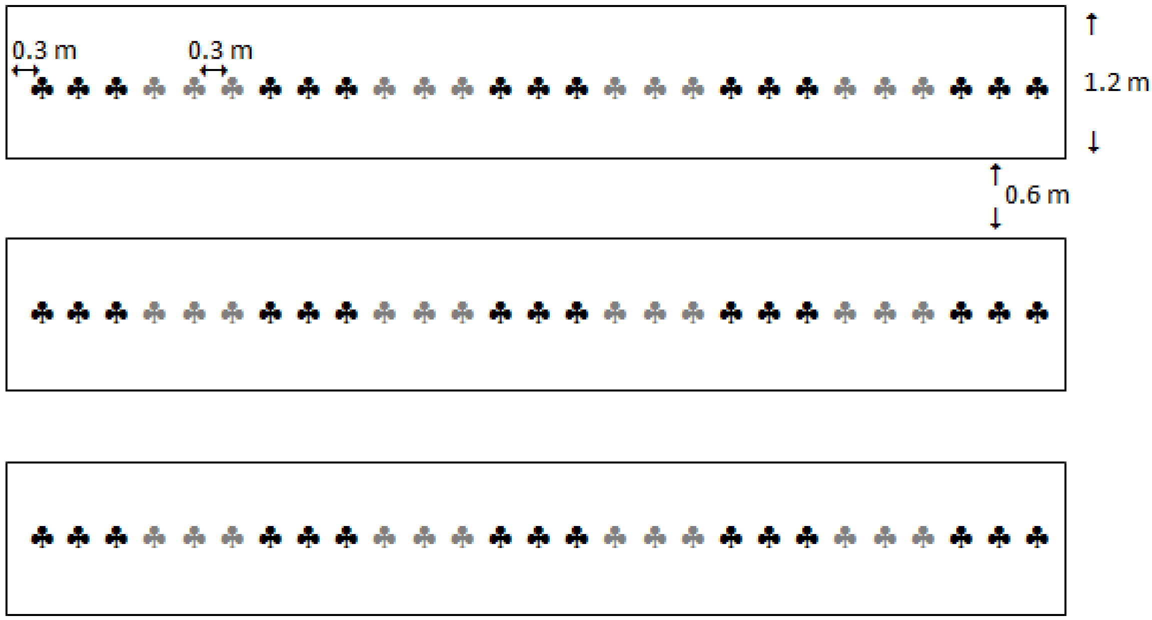

Figure 3. Setup of one block in the common garden field count experiment. Shading indicates that each experimental unit consisted of three individual plants of the same cultivar, for twenty-seven cultivars per block.

Figure 4. Newly transplanted cultivars in Block 1 in 2012.

Table 2. Cultivars used in the common garden field count experiment.

Plant type	**Seed source**
Basil, *Ocimum basilicum*	
'Italian Large Leaf'	NE SEED, Hartford, CT
'Mexican Spice'	John Scheepers Kitchen Garden Seeds, Bantam, CT
Basil, *Ocimum basilicum citriodorum*	
'Lemon'	John Scheepers Kitchen Garden Seeds, Bantam, CT
Beet, *Beta vulgaris*	
'Bull's Blood'	John Scheepers Kitchen Garden Seeds, Bantam, CT
'Chioggia'	Comstock, Ferre & Co., Wethersfield, CT
'Detroit Dark Red'	Comstock, Ferre & Co., Wethersfield, CT
Carrot, *Daucus carota sativus*	
'Danver's Half Long'	The Chas C. Hart Seed Co., Wethersfield, CT
'Imperator 58'	NE SEED, Hartford, CT
'Red Core Chantenay'	NE SEED, Hartford, CT
Eggplant, *Solanum melongea*	
'Black Beauty'	The Chas C. Hart Seed Co., Wethersfield, CT
'Long Purple'	The Chas C. Hart Seed Co., Wethersfield, CT
'Rhapsody Hybrid'	NE SEED, Hartford, CT
Kohlrabi, *Brassica oleracea gongylodes*	
'Kongo'	John Scheepers Kitchen Garden Seeds, Bantam, CT
'Purple Vienna'	NE SEED, Hartford, CT
'White Vienna'	The Chas C. Hart Seed Co., Wethersfield, CT
Parsnip, *Pastinaca sativa*	
'All American'	NE SEED, Hartford, CT
'Hollow Crown'	Comstock, Ferre & Co., Wethersfield, CT
'Panache'	John Scheepers Kitchen Garden Seeds, Bantam, CT
Pepper, hot, *Capsicum anuum*	
'Cayenne Long Thick'	The Chas C. Hart Seed Co., Wethersfield, CT
'Jalapeño'	The Chas C. Hart Seed Co., Wethersfield, CT
Pepper, hot, *Capsicum chinense*	
'Habanero Orange'	NE SEED, Hartford, CT
Pepper, sweet, *Capsicum anuum*	
'California Wonder'	The Chas C. Hart Seed Co., Wethersfield, CT
'Cubanelle'	NE SEED, Hartford, CT
'Sweet Banana'	NE SEED, Hartford, CT
Turnip, *Brassica rapa* var. *rapa*	
'Purple Top White Globe'	NE SEED, Hartford, CT
'Royal Crown Hybrid'	NE SEED, Hartford, CT
'Tokyo Cross Hybrid'	NE SEED, Hartford, CT

Plants were raised from seed in the University of Connecticut Floriculture Greenhouse (Storrs, CT) and transplanted to the field. Seedlings were grown in a 50% peat moss potting mix (Fafard 3B mix, Conrad Fafard, Agawam, MA) in 6-cell containers 12.5 cm long x 12.5 cm wide x 7 cm deep. In 2011, seeds were planted on May 16, except ‘Habanero Orange’ hot peppers, which were planted on May 20. Seedlings were transplanted to the field on June 27, except replacements for those that transplanted poorly were transplanted on July 15. In 2012, seeds were planted on April 30, and seedlings were transplanted to the field on June 22. In 2011, supplemental fertilizer was applied to plants in the field in the form of a diluted 20-20-20 N-P-K water soluble fertilizer (Peters Professional, Everris, Israeli Chemicals Ltd., Tel Aviv, Israel). In 2012, supplemental fertilizer was applied instead to seedlings in the greenhouse, also in the form of a diluted 20-20-20 N-P-K water soluble fertilizer (All Purpose Miracle-Gro, The Scotts Miracle-Gro Company, Marysville, OH). In 2011, some plants were sprayed with insecticidal soap (Safer Brand Insect Killing Soap with Seaweed Extract II, Woodstream Corporation, Lititz, PA) before and after transplanting to the field to reduce severe insect infestations, mostly of unidentified thrips, aphids and flea beetles. Most spraying was done on beet, eggplant, pepper, and turnip plants. Spraying was done at least seven days before AGB counts began. The soap breaks down within seven to ten days, so is likely to have had a minimal effect on AGB behavior. Seedlings in the greenhouse also spent time in mesh fabric cages to protect them from pests in 2011.

The number of adult Asiatic garden beetles on each plant was counted at night on July 20 and 27, and August 1, 8, and 10, 2011, and on July 11, 19, and 24, and August 6, 8, and 13, 2012, starting between 9 p.m. and 10 p.m. and ending between 11 p.m. and 1:15 a.m. Beetles were also counted on August 17, 2011, but this date was removed from analyses due to low

overall beetle numbers. The temperature ranged between 17.3°C and 23.2°C, with an average of 21.2°C during the sampling periods. An effort was made to perform counts on warm nights with starting temperatures of at least 21°C, the minimum temperature reported for AGB flight (Hallock, 1932), but not every date met this preference. Temperatures below 16°C were not recorded during beetle counts; this is the lowest temperature at which light feeding is said to occur (Hallock, 1936a). Experimental units consisting of only two plants were allowed in cases of plant death, poor health, or small size, but individual plant types were omitted from any block in which fewer than two acceptable plants of that type existed. Counts from all plants in an experimental unit were combined for statistical analyses.

Each year's count data were statistically analyzed using two separate repeated measures analyses of variance using the GLIMMIX procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Experimental unit size (two or three plants in a row) was included as a covariate. Within each analysis of variance, means were separated using the Tukey-Kramer test if a significant *P*-value was obtained at the $\alpha = 0.05$ level. Data were analyzed using best-fit models of the covariance structures, and a Poisson distribution. The 2011 data were analyzed using a variance components model to find differences between cultivars, and a compound symmetry model to find differences between crop types (basil, beet, carrot, etc.). The 2012 data were also analyzed separately to find differences between cultivars and crop types, using in each case a first order autoregressive model. The large number of zero values (observations of zero beetles on an experimental unit) made it infeasible to analyze interactions between date, block, and cultivar.

Cultivars in which all count values were zero were not included in the statistical analyses at the cultivar level because they tended to complicate the analyses due to their lack of variance. 'Detroit Dark Red' beets, 'White Vienna' kohlrabi, 'Habanero Orange' hot peppers, and 'All

American' parsnips were not included in the analysis of 2011 field count data at the cultivar level. All three cultivars of beets, 'Kongo' and 'White Vienna' kohlrabi, 'Cayenne Long Thick' hot peppers, 'All American' and 'Hollow Crown' parsnips, 'Sweet Banana' sweet peppers, and all three cultivars of turnips were not included in the analysis of 2012 field count data at the cultivar level. Beets and turnips were not included in the analysis of 2012 field count data at the crop type level, because all three cultivars of each had only zero values.

Asiatic garden beetle seasonal activity

Adult Asiatic garden beetles were collected using black light traps (Figure 5; Ellisco, Inc., Philadelphia, PA) for purposes of population monitoring and collecting for later use in laboratory experiments in 2011 and 2012. During the 2011 field season, beetles were collected using three black light traps: one located at the University of Connecticut's Storrs campus (Storrs, CT), the second at the University's Depot campus (Mansfield, CT), and the third at the University's Plant Science Research and Education Facility (Storrs, CT). All three traps were placed on turfgrass, and were in proximity to wooded areas. The Research Facility trap was also in proximity to various ornamental research plants. In 2012, the Depot campus location was repeated, a second trap was placed at a University farm property in Mansfield, CT, and a third, modified trap using white light was placed at the Research Facility near the 2011 site. The Mansfield farm trap was placed on turfgrass and was in proximity to a wooded area and a corn field. Traps were checked at irregular intervals from June 17 to September 6 in 2011, and from June 14 to September 13 in 2012, and adult AGBs were collected and counted. AGBs collected with black light traps were kept in the lab until needed for laboratory no-choice feeding experiments.

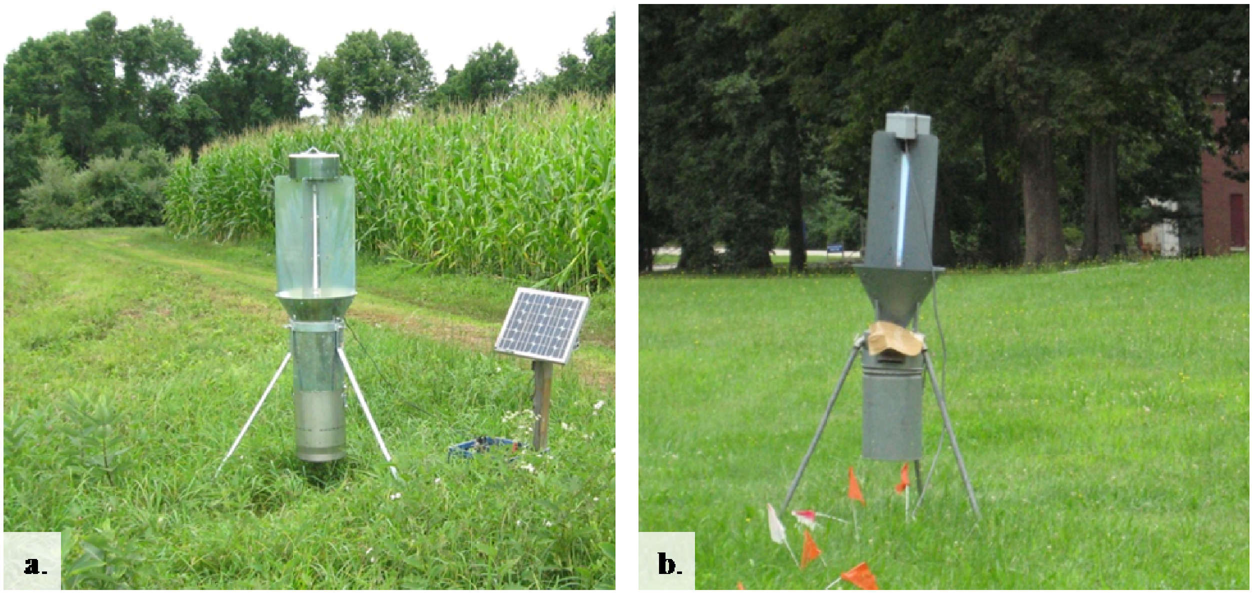

Figure 5. Black light traps. **a**. Mansfield farm location, 2012. **b**. Depot campus location, 2012.

Results

Common garden field count experiment

Analysis of variance on 2011 field count data to test for differences in the number of Asiatic garden beetles found on different cultivars showed that all three basil cultivars had significantly more beetles on them than all non-basil cultivars included in the statistical analyses (at $\alpha = 0.05$), except no difference was found between 'Lemon' basil and 'Hollow Crown' parsnip (Figure 6; $F = 24.57$; $df = 22, 86$; $P < 0.0001$). Among the basil cultivars, only 'Mexican Spice' and 'Lemon' basil were significantly different, with 'Mexican Spice' harboring more beetles. Analysis of 2011 field count data to test for differences in the number of beetles found on crops, including all three cultivars of each when possible, showed that basil had significantly more beetles on it than each of the other crop types tested (Figure 7; $F = 51.16$; $df = 8, 119$; $P < 0.0001$).

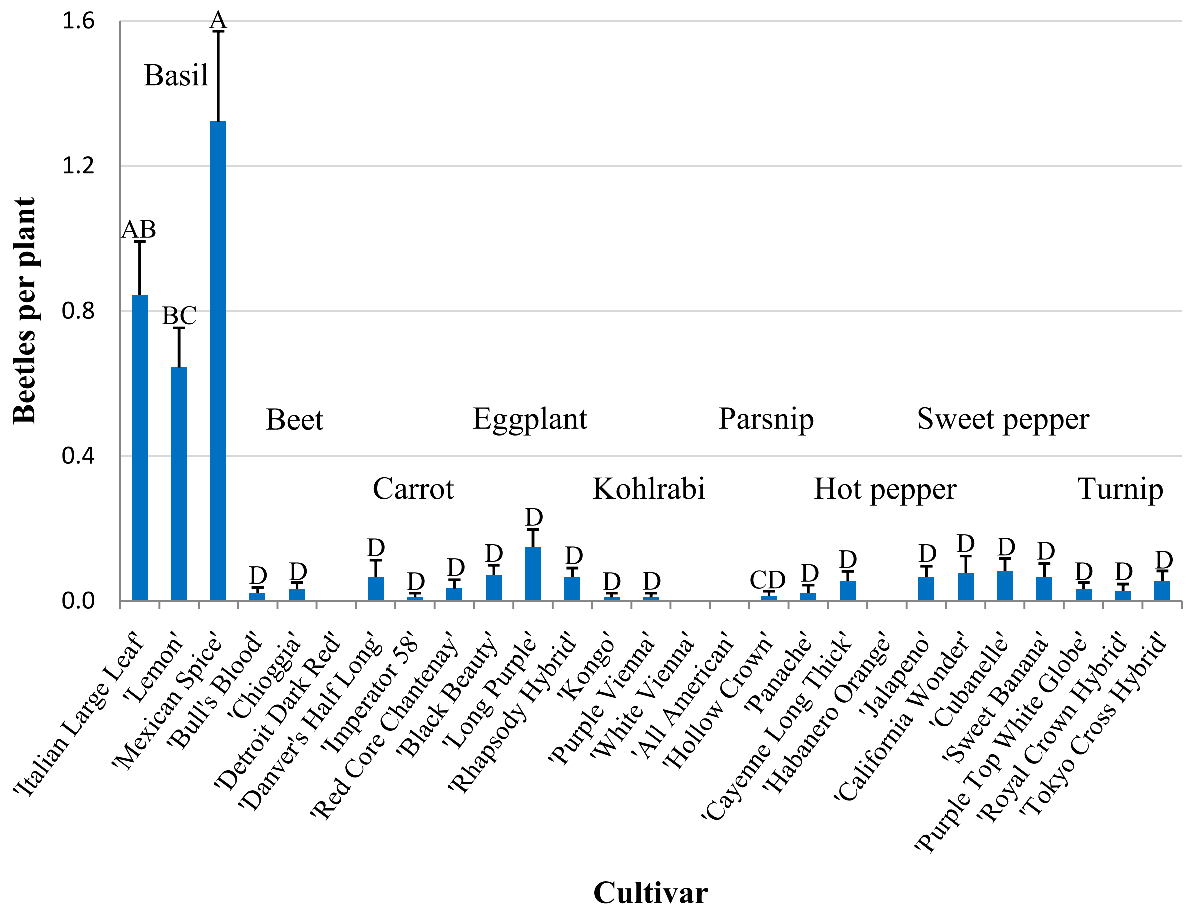

Figure 6. Common garden field count results at the cultivar level showing number of Asiatic garden beetles per plant in 2011 (mean + SE). AOV on raw count data; n ranges from 20 to 25; $P < 0.0001$. Columns with the same letter are not significantly different according to the Tukey-Kramer test. Absence of a column and letter indicates a cultivar for which all counts were zero; these were not included in the statistical analysis.

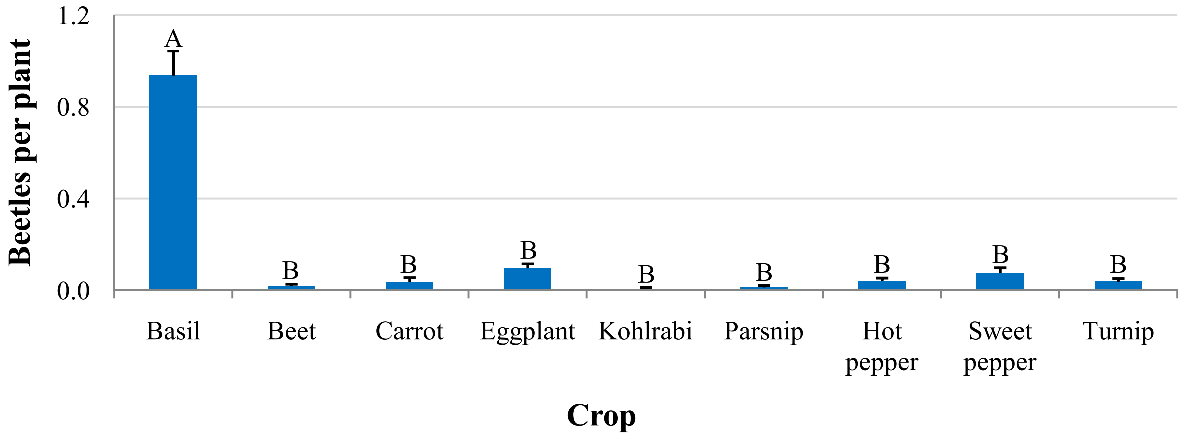

Figure 7. Common garden field count results at the crop level showing number of Asiatic garden beetles per plant in 2011 (mean + SE). AOV on raw count data; n ranges from 65 to 75; $P < 0.0001$. Columns with the same letter are not significantly different according to the Tukey-Kramer test.

Analysis of 2012 field count data to test for differences in the number of beetles found on cultivars showed that ‘Italian Large Leaf’ and ‘Mexican Spice’ basil had significantly more beetles on them than most non-basil cultivars included in the statistical analyses. No differences were found between either of these above basil cultivars and ‘Lemon’ basil, ‘Imperator 58’ carrot, ‘Panache’ parsnip, and ‘California Wonder’ and ‘Cubanelle’ sweet pepper (Figure 8; $F = 10.04$; $df = 13, 46$; $P < 0.0001$). No significant differences were found between basil cultivars. Analysis of 2012 field count data to test for differences in the number of beetles found on crops showed that basil had significantly more beetles on it than each of the other crop types tested (Figure 9; $F = 22.03$; $df = 6, 80$; $P < 0.0001$).

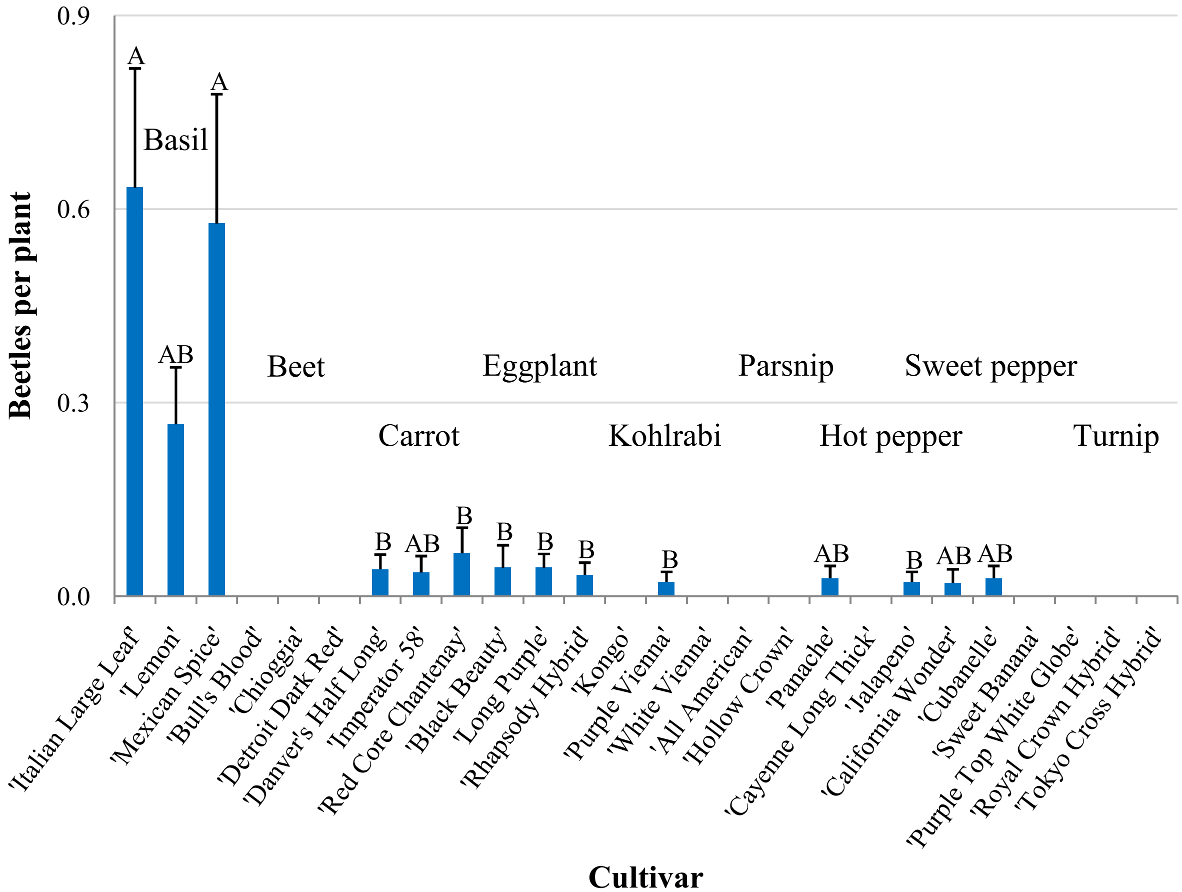

Figure 8. Common garden field count results at the cultivar level showing number of Asiatic garden beetles per plant in 2012 (mean + SE). AOV on raw count data; n ranges from 18 to 30; $P < 0.0001$. Columns with the same letter are not significantly different according to the Tukey-Kramer test. Absence of a column and letter indicates a cultivar for which all counts were zero; these were not included in the statistical analysis.

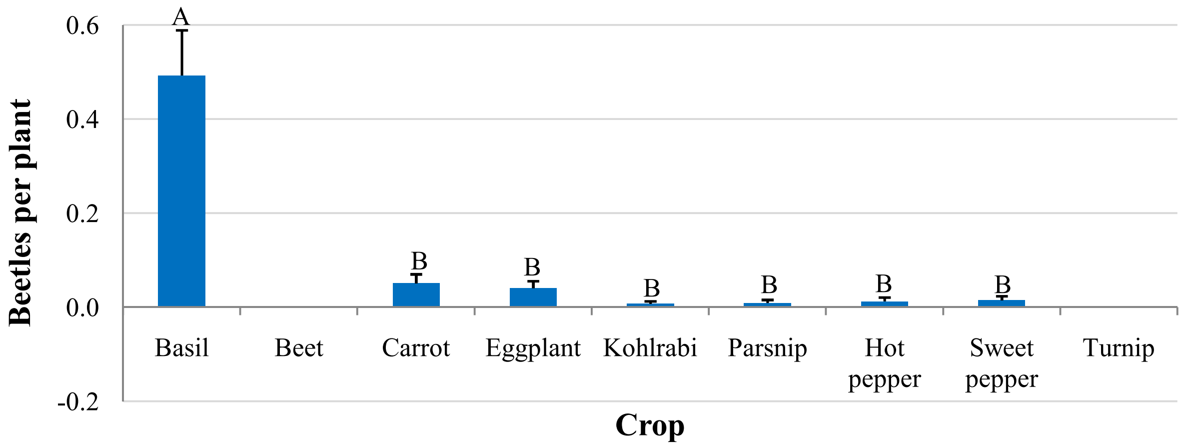

Figure 9. Common garden field count results at the crop level showing number of Asiatic garden beetles per plant in 2012 (mean + SE). AOV on raw count data; n ranges from 54 to 90; $P < 0.0001$. Columns with the same letter are not significantly different according to the Tukey-Kramer test. Absence of a column and letter indicates a crop type for which all counts were zero; these were not included in the statistical analysis.

Asiatic garden beetle seasonal activity

In 2012, the first Asiatic garden beetle adults of the season were caught on June 20. The majority of AGB adults were observed to occur before September in both 2011 and 2012. The cumulative number of adult AGBs caught over the course of the field season can be seen in Figure 10 for both 2011 and 2012, as recorded from the two main traps used in each year. The section of each curve with the highest slope is indicative of the period of highest AGB adult activity recorded using that trap. In 2011, peak population sizes occurred between July 22 and August 10. In 2012, peak population sizes occurred between July 10 and August 18.

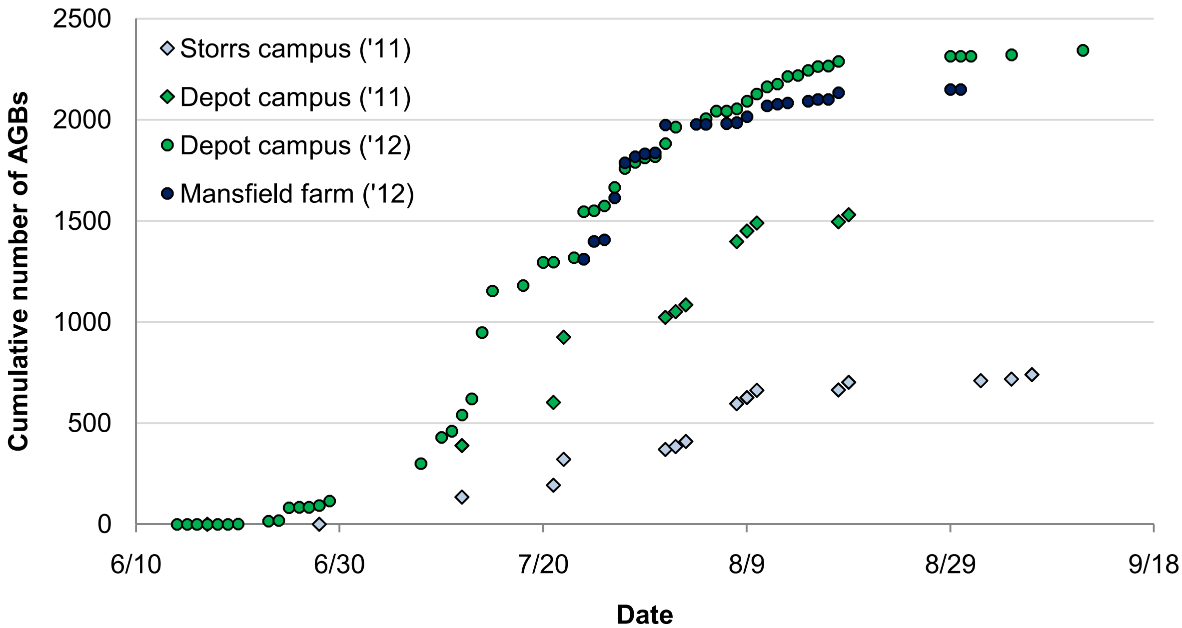

Figure 10. Cumulative number of Asiatic garden beetle adults caught during the 2011 and 2012 field seasons. Results are shown from two traps per season.

Discussion

Analyses of both 2011 and 2012 field count data indicated that basil had significantly more adult Asiatic garden beetles on it than the other crops tested, although some basil cultivars did not have significantly more beetles than some cultivars of other crops tested in each year. Interestingly, carrot, red pepper, and turnip, listed as the only preferred foods out of these tested crops in Hallock's (1936a) final AGB preference list, did not appear more preferred than any other crop types, and appeared less preferred than basil, which was more recently reported to be a favorite food plant of the AGB (Pundt & Smith, 2005), presumably based on visual observations, and not ranked in comparison to other plants. It is also interesting that there were so few beetles on crops other than basil, despite all experimental crop types being listed as heavily damaged by Hallock (1934, 1936b). Maybe basil acted as a distraction from the other plants, which might be visited by more AGBs in basil's absence. In 2011, 'Lemon' basil had

significantly fewer beetles on it than 'Mexican Spice' basil. In 2012, 'Lemon' basil did not have a significantly different number of beetles on it than any other cultivar, including the two other basil cultivars, despite having the maximum number of replications in this experiment. These results indicate that basil is the most favored of the tested plants for AGBs to visit, although 'Lemon' basil does not appear to have as strong an effect as the other two cultivars.

'Lemon' basil has a lemony citrus scent, 'Mexican Spice' basil has a spicy cinnamon scent, and 'Italian Large Leaf' basil has a more typical basil scent. Partial chemical compositions have been reported for the essential oils from basil cultivars that may be identical or similar to those used in this study. Juliani and Simon (2002) published the chemical composition of essential oils of 'Italian Large Leaf' basil, *Ocimum basilicum* 'Cinnamon', and *O. citriodorum* 'Sweet Dani Lemon'. 'Cinnamon' basil is the same as 'Mexican Spice' basil, and *O. citriodorum* 'Sweet Dani Lemon' may be similar to or the same as the 'Lemon' basil, sold as *O. basilicum citriodorum*, used in this experiment. Lachowicz et al. (1997) also published the chemical composition of essential oil of *O. basilicum* 'Cinnamon'. Nurzyńska-Wierdak (2013) published the chemical composition of essential oils of *O. basilicum* var. *cinnamon*, *O. basilicum* var. *citriodorum*, and *O. basilicum* 'Lemon', which may be similar to or the same as the 'Mexican Spice' and 'Lemon' cultivars used in this experiment. *O. basilicum* var. *citriodorum* and *O. basilicum* 'Lemon' had the same major essential oil components, at essentially the same ratios.

Combining information from these three chemical studies, it appears that the major components of 'Italian Large Leaf' basil essential oil are methylchavicol, comprising 45% of the oil; linalool, comprising 22%; and 1,8-cineole, comprising 8%. The major components of 'Mexican Spice'-type basil essential oil appear to be methylcinnamate, comprising 28-45% of the oil; linalool, comprising 13-27%; and methylchavicol, comprising 5-13%. The major

components of 'Lemon'-type basil essential oil appear to be geraniol, comprising 20-33% of the oil; and neral, comprising 16-26%. Of the three 'Lemon'-type basils, one is reported to contain 6% methylchavicol, 0% linalool, and 0% 1,8-cineole, and two are reported to contain 10% linalool, and do not have methylchavicol or 1,8-cineole reported as major components. Of the three 'Mexican Spice'-type basils, one is reported to contain 4% 1,8-cineole, another is reported to contain 1-2% 1,8-cineole, and the last is not reported to contain 1,8-cineole as a major component. Only the basils similar to 'Lemon' basil contained geraniol and neral (Juliani and Simon, 2002; Lachowicz et al., 1997; Nurzyńska-Wierdak, 2013).

It is possible that AGBs are less attracted to 'Lemon' basil than 'Italian Large Leaf' and 'Mexican Spice' basils due to the slightly lower levels of, or the absence of, linalool and methylchavicol, and the absence of 1,8-cineole. In this case, linalool, methylchavicol, and 1,8-cineole might serve as attractants for AGB adults. All three chemicals can serve as both attractants and deterrents, or even poisons, for different beetle species (Abd El-Aziz et al., 2006; Parra et al., 2009; Ruther & Mayer, 2005; Werner, 1972, 1995). Alternatively, 'Lemon' basil might be less preferred by AGBs due to possible deterrent effects of geraniol and neral, but this seems less likely, as, in 2011, 'Lemon' basil had significantly more beetles on it than every non-basil cultivar included in the statistical analyses, except for 'Hollow Crown' parsnip. Without testing the chemical compositions of essential oils from the particular plants used in this experiment, and conducting further AGB preference tests involving the manipulation of these chemicals, any chemical basis for preference of 'Italian Large Leaf' and 'Mexican Spice' basils to most other cultivars in this experiment is uncertain, although chemistry is likely to play a role.

Non-basil cultivars that were found not to have a significantly different number of beetles than basil cultivars did not have higher average numbers of beetles on them than some cultivars

that were found to have significantly fewer beetles than basil cultivars. It is likely that no differences were found in these cases due in part to lower numbers of replications for these cultivars than some others, due to removal of experimental units from the analyses when plants were too small, unhealthy, or dead. Replications were the number of times that beetles were counted on one cultivar, calculated as the number of blocks including that cultivar, each containing one experimental unit of two or three plants in a row, multiplied by the number of nights on which beetles were counted. In 2011, the number of replications for 'Hollow Crown' parsnip, the only cultivar included in the analyses but found not to have significantly fewer beetles than each cultivar of basil, was 20, while most other cultivars, including all three basil cultivars, had 25 replications. In 2012, 'Imperator 58' carrot had only 18 replications, and the other three cultivars that did not have significantly fewer beetles than 'Italian Large Leaf' and 'Mexican Spice' basils had only 24 replications, while most other varieties included in the statistical analyses, including all three basil cultivars, had 30 replications. The reduced number of replications for cultivars found not to have significantly fewer beetles than basil cultivars, combined with similar average beetle numbers compared to cultivars that were found to have significantly fewer beetles than basil cultivars, suggests that real differences may exist between attractiveness of these cultivars compared to the basil cultivars, but the number of replications was too low for the Tukey-Kramer test to confirm the presence of those differences.

Presumably, beetle presence on the experimental plants might be indicative of an interest in feeding on those plants, but this relation is not definitively known. Some AGBs were observed to be feeding during field counts, but the field counts were performed with limited time, so only beetle presence was carefully observed and recorded. It is possible that the beetles were attracted to basil plants for reasons other than feeding, such as resting, mating or ovipositing in the

vicinity. Indeed, some beetles that were counted appeared to be mating. Preferred mating sites for AGBs have not been studied, although the Japanese beetle, another generalist herbivorous scarab, is known to mate on its food plants: female Japanese beetles often continue to feed during mating (Fleming, 1972). This suggests that the presence of mating beetles on plants could indicate an interest in feeding on the same plants. AGBs are known to oviposit preferentially in moist, shaded soil, and it has been noted that they tend to place more eggs in soil nearby their favored food plants (Hallock, 1936a). Japanese beetles are also known to oviposit near their food plants (Fleming, 1972). This suggests that AGBs could be attracted to basil plants because they shade the ground below them, creating an optimal environment for egg placement. However, even if oviposition had been noted under basil plants, the choice of location could still have been related to feeding preferences for basil. Little or no research is available regarding adult generalist herbivorous insect use of food plants for resting to determine if correlations exist between favored resting plants and favored food plants. The claim that AGBs often spend their daytime hours beneath a food plant (Hallock, 1930), suggests that they spend a significant amount of time in proximity to their food plants, and therefore might rest on them even when they are not feeding. Factors that may have influenced the beetles' preference for landing on basil plants, other than innate preference for the experimental cultivars, include the abundance of basil in the field adjacent to the experimental field, and the fact that basil has the strongest scent of the experimental crops tested. Basil plants also tended to be larger and healthier compared with many other crop types in both years of the common garden field count experiment.

The results of the common garden field count experiment indicate that basil is by far the preferred experimental crop, although damage done to basil plants by AGBs did not appear overwhelming to the plants. Low damage levels on basil could indicate that beetles did not feed

on it, or that the beetle population was not large enough to cause severe damage. Indeed, all cultivars in this experiment had on average fewer than two beetles per plant at any given time, while Hallock's (1932) statements suggest that beetle infestation can be much greater. Basil might even exhibit high levels of compensatory growth in response to herbivory. For example, seedlings of the wild plant *Brosimum alicastrum* that had 50% of their leaves removed were shown to have heights, numbers of leaves, leaf areas, and relative growth rates of plant biomass that were not significantly different from those of control plants after two years, and an increased predicted probability of survival over two years compared to control plants (Ballina-Gómez et al., 2008). The no-choice laboratory portion of this study will help determine if the observed field preference for basil is related to a preference for feeding on basil. The no-choice laboratory feeding study provides an opportunity to further investigate some of the same crop types and cultivars used in the common garden field experiment. This is important not only to verify that beetle presence on plants is due to interest in feeding on those plants, but also because the strong preference for basil in this multiple-choice field study may have masked AGB preferences for other plants.

Possible improvements to the field count portion of this study include counting beetles earlier in the evening rather than later at night, as AGBs are known to start feeding at dusk and to stop feeding as the evening temperature falls or when daylight arrives (Hallock, 1936a). In this study, the total number of beetles counted in each block tended to decrease over the course of the night, as did the temperature, suggesting that more beetles might have been present had the counts started earlier, which would have provided more useful information. It is also possible that performing AGB counts earlier in the field season would provide more useful information regarding AGB feeding preference, as male AGBs are more common earlier in the season, and

are thought responsible for nearly 70% of AGB adult feeding damage (Hallock, 1936b). However, field counts were done during the period from July 10 to August 18, shown in the light trap portion of this experiment to have the highest beetle population, so a version of this experiment in which counts are done earlier might encounter difficulties with low total numbers of AGB adults. Finally, a version of this field count experiment in which plants are grown in field cages enclosing a certain number of AGB adults could be useful to perform, because it would allow for a larger concentration of beetles than found in this experiment, and would also allow feeding damage estimates to be recorded for each plant type, as it could be assumed that most feeding damage was due to the enclosed AGBs, because other pests would be excluded by the cages. Hallock (1936b) did perform several similar caged field count experiments, although they did not involve multiple replications of each combination of plant types.

The results of the light trap portion of this field study indicate that the AGB life cycle in Connecticut is similar to that in New York, with most adults flying in July and August (July 15 – August 15 in New York according to Hallock [1932], and July 10 – August 18 in Connecticut in this study). The first date when adults were observed in this study, June 20 in 2012, is also in close agreement with the June 23 and June 24 dates given by Hamilton (1929) for New Jersey from 1927-1929, and the last week of June time period mentioned by Hallock (1932) for New York. It is possible that the June 20 first adult AGB catch date from 2012 is slightly early for Connecticut due to a particularly warm winter that year, but the results of this study suggest that, in areas around Connecticut where the AGB is a problem, it would be advisable to begin monitoring efforts in mid-June.

The results of this experiment indicate that AGBs may have similar life cycle timing in Connecticut, New York, and New Jersey, at least regarding the time period of adult activity, with

adults emerging at the end of June and being most active in July and August. In monitoring efforts, it is important to remember that this beetle is nocturnal and can best be found by digging around the base of damaged plants in the daytime, looking at leaves at night with a flashlight, or using a black light trap. Based on observations made during the common garden field count study, AGBs are most active (present on plants, rather than underground) directly after dark, with numbers strongly declining less than four hours after 9 p.m. Indeed, Hallock (1936a) stated that beetles began feeding at dusk. Beetles seemed to become less abundant in the common garden counts toward the middle of August, perhaps somewhat earlier than was noted from the light trap counts. This could be due to factors beyond typical AGB seasonal behavior, for example it is possible that counts were performed on nights with uncharacteristically low beetle numbers, perhaps due to low temperatures for the beetles, or beetles may have been active, but visiting younger plants elsewhere as those in the experimental field aged. Counts from light traps are a more reliable measure of AGB activity than counts on plants because light trap counts can be done more quickly, and therefore more often, and the light traps themselves do not change much over time, in contrast to plants.

Chapter 3
No-choice laboratory feeding study for the adult Asiatic garden beetle

Introduction

Laboratory studies of insect feeding tendencies can provide a different perspective on host plant feeding preferences than field counts of the same insects on the same plants. To supplement the field studies described in Chapter 2, which involved counts of adult Asiatic garden beetles (AGBs) in a common garden containing multiple plant types, no-choice laboratory feeding studies were performed in which beetles had one choice of food, and feeding preference was investigated using two direct measurements of feeding: mass and area consumed. Counts of AGBs on plants in the field indicate the species' ability to locate plants, its preference for visiting different plant types, and its possible use of the plants as food sources. This laboratory no-choice feeding experiment provided quantitative evidence of beetle feeding beyond their presence on a plant, and was used to determine potential feeding levels of AGBs on different plant types without the added complications of varying plant sizes, the need for the beetles to locate plants, and the beetles having a choice of plant types, all of which were present in the field experiments. The laboratory setting of these no-choice experiments also permitted better control of the beetles' numbers, hunger levels, and their environmental conditions. The no-choice aspect of these laboratory tests prevented the most favored plant type from attracting the majority of the beetles and therefore obstructing information about AGB preferences for less favored plants, as may have occurred in the case of the three basil cultivars in the field study.

Many laboratory feeding preference studies have been performed using the Japanese beetle, another scarab that feeds in the adult form. The methods used in this experiment are based on some of the Japanese beetle research. Two main categories of laboratory feeding preference

research for the Japanese beetle include multiple-choice and no-choice tests. For this AGB research, a no-choice experiment was chosen to balance the field count experiment in which beetles had multiple choices of host plant. Multiple-choice tests are more natural and comparative, but no-choice tests show the absolute potential to feed on a given plant, which can be more useful in understanding risks to plants that are not grown in the experimental groupings.

Many no-choice laboratory feeding tests for Japanese beetles involve the presentation of single circular leaf pieces to single beetles in closed petri dishes, including studies by Ladd (1987, 1989) and Spicer et al. (1995). Risch (1985) has detailed the risks associated with using cut leaf pieces in feeding preference experiments, particularly experiments involving generalist feeders such as the AGB. These risks are related to the chemical changes that occur in damaged plant parts, and Risch (1985) has shown that preference results can vary between tests using cut leaf disks, whole leaves removed from plants, and whole plants. Risch (1985) claims that there is a greater difference in results between whole leaves and leaf disks than between whole leaves and whole plants, which indicates that the use of leaf disks may be the worst choice. However, Risch's (1985) results also indicate that the use of leaf disks might lead to greater statistical significance between preferences for different plant varieties, which could be useful if the differences are real. Risch (1985) also states that insect pests naturally encounter both damaged and undamaged plant parts, which means that leaf disk tests are not necessarily useless; Risch (1985) therefore recommends the use of several methods to determine feeding preferences. This AGB study includes both the field portion involving whole plants and the laboratory portion involving cut leaf pieces in part to minimize the risks presented by Risch (1985). Together, the field and laboratory portions of this AGB study will contribute to a better understanding of adult AGB feeding preferences.

Materials and methods

Adult Asiatic garden beetles collected with black light traps (as described in Chapter 2, page 24) were kept in the lab until needed for no-choice feeding experiments. Beetles were kept in 236 mL plastic deli containers (Solo Cup Company, Urbana, Illinois) with moist sponge pieces or moist cotton balls and paper towel. In 2011, beetles were fed leaves of wild plants, including bittersweet, black walnut, and wineberry. In 2012, beetles were fed carrot pieces for a more consistent and more time-efficient diet. Dishes were kept in incubators (Low Temperature Illuminated Incubator 818, Thermo Electron Corporation, Marietta, OH) set to 15:9 h L:D, 26.7°C:18.3°C in 2011. In 2012, the incubators were set to a constant 22.2°C, with no light. The 2011 light and temperature cycle was intended to match Connecticut's climate, and was changed in 2012 to reflect the fact that AGBs might not experience daytime high temperatures or light, as they bury themselves underground until nighttime. Additionally, it was thought that beetles would be more active during tests if experimental temperatures did not drop below 21°C, the minimum temperature required for flight (Hallock, 1932).

Nine cultivars of edible plants (Table 3) were used in the no-choice laboratory experiments in 2011 and 2012. In 2012, six types of landscape plants (Table 3) were also tested. Edible plants used were the three cultivars each of basil, beet, and kohlrabi tested in the common garden field experiment. These were chosen because basil was the crop of interest in the field count study, consistently having the highest numbers of AGBs, and beet and kohlrabi were the crops with the most variation in leaf color between different cultivars. Spicer et al. (1995) showed that Japanese beetles fed more on cultivars of crabapples (*Malus* spp.) with red leaves or green leaves that began growth as red leaves than on cultivars with consistently green leaves, while Rowe et al. (2002) later found that Japanese beetles often, but not always, preferred

purple-leaved cultivars over green-leaved cultivars of several woody landscape plants. Rowe et al. (2002), however, also showed that Japanese beetles were more attracted to imitation trees with green paint than those with purple paint. Like Japanese beetles, AGBs might have a leaf color preference. Landscape plants used were common, native plants that were growing at the University of Connecticut. Viburnum was of particular interest, as it is known to be a preferred food plant of the adult AGB (Hallock, 1933, 1936a, 1936b).

Table 3. Plants used in the no-choice laboratory experiment.

Edible plants	**Seed source**
Basil, *Ocimum basilicum*	
‘Italian Large Leaf’	NE SEED, Hartford, CT
‘Mexican Spice’	John Scheepers Kitchen Garden Seeds, Bantam, CT
Basil, *Ocimum basilicum citriodorum*	
‘Lemon’	John Scheepers Kitchen Garden Seeds, Bantam, CT
Beet, *Beta vulgaris*	
‘Bull's Blood’	John Scheepers Kitchen Garden Seeds, Bantam, CT
‘Chioggia’	Comstock, Ferre & Co., Wethersfield, CT
‘Detroit Dark Red’	Comstock, Ferre & Co., Wethersfield, CT
Kohlrabi, *Brassica oleracea gongylodes*	
‘Kongo’	John Scheepers Kitchen Garden Seeds, Bantam, CT
‘Purple Vienna’	NE SEED, Hartford, CT
‘White Vienna’	The Chas C. Hart Seed Co., Wethersfield, CT

Landscape plants	**Plant source**
Shrub	
Elderberry, *Sambucus canadensis*	UConn Plant Sci. Research Facility, potted
Arrowwood viburnum, *Viburnum dentatum*	UConn Plant Sci. Research Facility, potted
Tree	
Green ash, *Fraxinus pennsylvanica*	UConn Storrs campus, landscape
Red maple, *Acer rubrum*	UConn Storrs campus, landscape
Sugar maple, *Acer saccharum*	UConn Storrs campus, landscape
American sweetgum, *Liquidambar styraciflua*	UConn Storrs campus, landscape

Edible plants were grown from seed in a 50% peat moss potting mix (Fafard 3B mix, Conrad Fafard, Agawam, MA) in the University of Connecticut Floriculture Greenhouse (Storrs, CT). In 2011, seeds were planted on May 16 in 6-cell containers 12.5 cm long x 12.5 cm wide x 7 cm deep, and seedlings were transplanted to larger, 140 mm top diameter, pots on July 6, when a 14-14-14 N-P-K slow-release fertilizer (Osmocote Classic 3-4 month, Everris, Israeli Chemicals Ltd., Tel Aviv, Israel) was added to the potting mix. In 2011, plants spent time in mesh fabric cages to protect them from pests, and some were sprayed with insecticidal soap (Safer Brand Insect Killing Soap with Seaweed Extract II, Woodstream Corporation, Lititz, PA) to reduce severe insect infestations, mostly of unidentified thrips, aphids and flea beetles. Spraying was done at least twelve days before lab tests began. The soap breaks down within seven to ten days, so is likely to have had a minimal effect on AGB behavior, and no effect on AGB survival. In 2012, seeds were planted on June 4 in 140 mm top diameter pots to avoid the need to transplant. Insecticidal soap and supplemental fertilizer were not used in 2012 due to University insecticide regulations and the perceived lack of need for fertilizer. Landscape plants were from the University of Connecticut's Storrs campus (Storrs, CT) and the University's Plant Science Research and Education Facility (Storrs, CT).

Leaves were collected up to three days prior to testing dates, and were stored cold in sealed plastic bags with moist sponge pieces. In 2011, leaves were collected from eight individuals of each edible plant type tested so that one or two replicates per type originated from the same individual plant. In 2012, leaves were collected from seventeen individuals of each edible plant type so that only one replicate originated from each individual plant. Leaves were collected from nine individuals of each landscape species tested so that one or two replicates per plant type originated from the same individual plant. When possible, leaves were taken from

different branches of landscape plants. Two circles, a control and an experimental circle, were cut from each leaf using a piece of copper pipe, except in 2011, when some paired circles were cut from different basil leaves from the same plant due to small leaf size, and in 2012, when all paired viburnum circles were cut from opposite leaves due to small leaf size. Ash and elderberry circles were cut from the same leaflet. In 2011, circles were 2.2 cm in diameter. In 2012, circles were 1.7 cm in diameter. In most replicates in both years, excess leaf material was provided beyond what was consumed.

In 2011, one experimental trial was conducted, with 14-16 intended replications per plant type. In 2012, two trials were conducted, the first with 9-10 intended replications, and the second with 5-7 intended replications, forming 14-17 total intended replications. On test starting dates (August 22, 2011; August 1 and 18, 2012), leaf circles were cut, weighed on an analytical balance (Mettler Toledo AG104, Mettler-Toledo, LLC., Columbus, OH), and scanned to digital images (HP Scanjet 5590, Hewlett-Packard, Palo Alto, CA). Each leaf circle was placed on a 9 cm diameter filter paper (Whatman No. 1, Whatman, Maidstone, Kent, United Kingdom) moistened with 1 mL distilled water in a closed, upside-down 8.5 cm diameter polystyrene petri dish (Fisherbrand, Houston, TX). One unsexed adult AGB, starved for at least 24 hours, was added to each experimental petri dish. Starving insects before feeding tests is commonly done to ensure a general willingness to feed. Control leaf circles were not exposed to beetles. Petri dishes were stacked in trays in completely randomized designs created using the PLAN procedure in SAS 9.2 (SAS Institute Inc., Cary, NC). In 2011, each petri dish was in its own sealed plastic bag to prevent moisture loss. In 2012, petri dishes were in shared plastic bags (Figure 11) for experimental efficiency. Petri dishes were placed in incubators for the 24 hour minimum experimental duration in 2011, or the 48 hour minimum duration in 2012. It was thought that the

beetles might act more naturally, and possibly feed more, if they were given more time to acclimate, as done in 2012. In 2011, the incubators were set to 15:9 h L:D, 26.7°C:18.3°C. In 2012, the incubators were set to a constant 22.2°C, with no light.

Beetles were removed from experimental petri dishes at the end of each trial period, and leaf circles were scanned to digital images once more (Figure 12). Leaf circles were then dried in an oven (Single-wall Transite Oven, Blue M Electric Company, Blue Island, IL) at 75°C in 2011, and 70°C in 2012 until they reached constant weight. Weights were recorded after drying. The computer program ImageJ (National Institutes of Health, Bethesda, MD) was used to calculate area of leaf circles from the scanned images, using the sequence of commands "Clear Outside", "Make Binary", "Fill Holes", and "Analyze Particles". This process effectively measures the surface area of a leaf circle by drawing an outline around its edge. The scale used was 8 pixels = 1 mm. Mass and area consumed were calculated. Paired control data were used to correct for changes in area and mass not caused by beetle presence. Corrections used were:

1. Mass consumed = Initial mass – (Final dry mass / Proportion dry mass in paired control)
   Proportion dry $\text{mass}_{\text{control}}$ = Final dry $\text{mass}_{\text{control}}$ / Initial $\text{mass}_{\text{control}}$

2. Area consumed = Initial area – (Final area / (1 + Proportion area change in paired control))
   Proportion $\text{area}_{\text{control}}$ change = (Final $\text{area}_{\text{control}}$ – Initial $\text{area}_{\text{control}}$) / Initial $\text{area}_{\text{control}}$

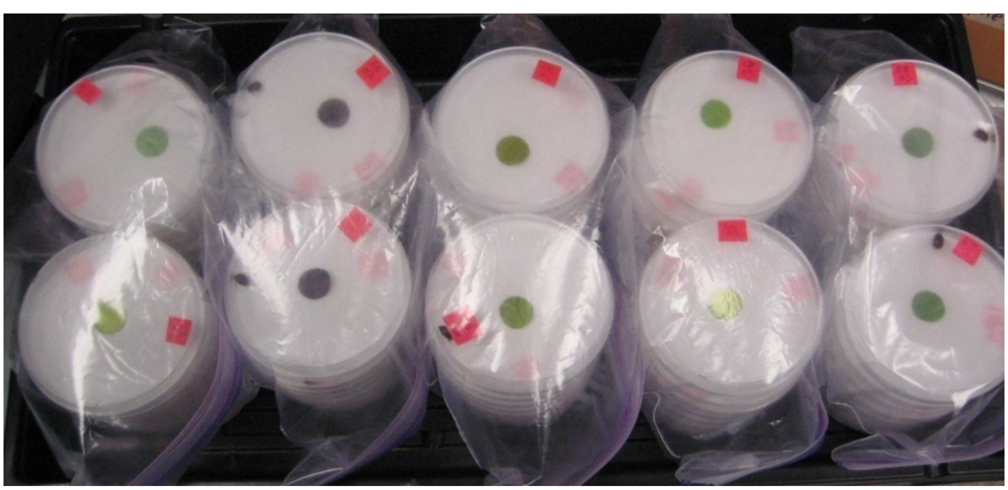

Figure 11. Experimental setup for no-choice laboratory feeding study in 2012.

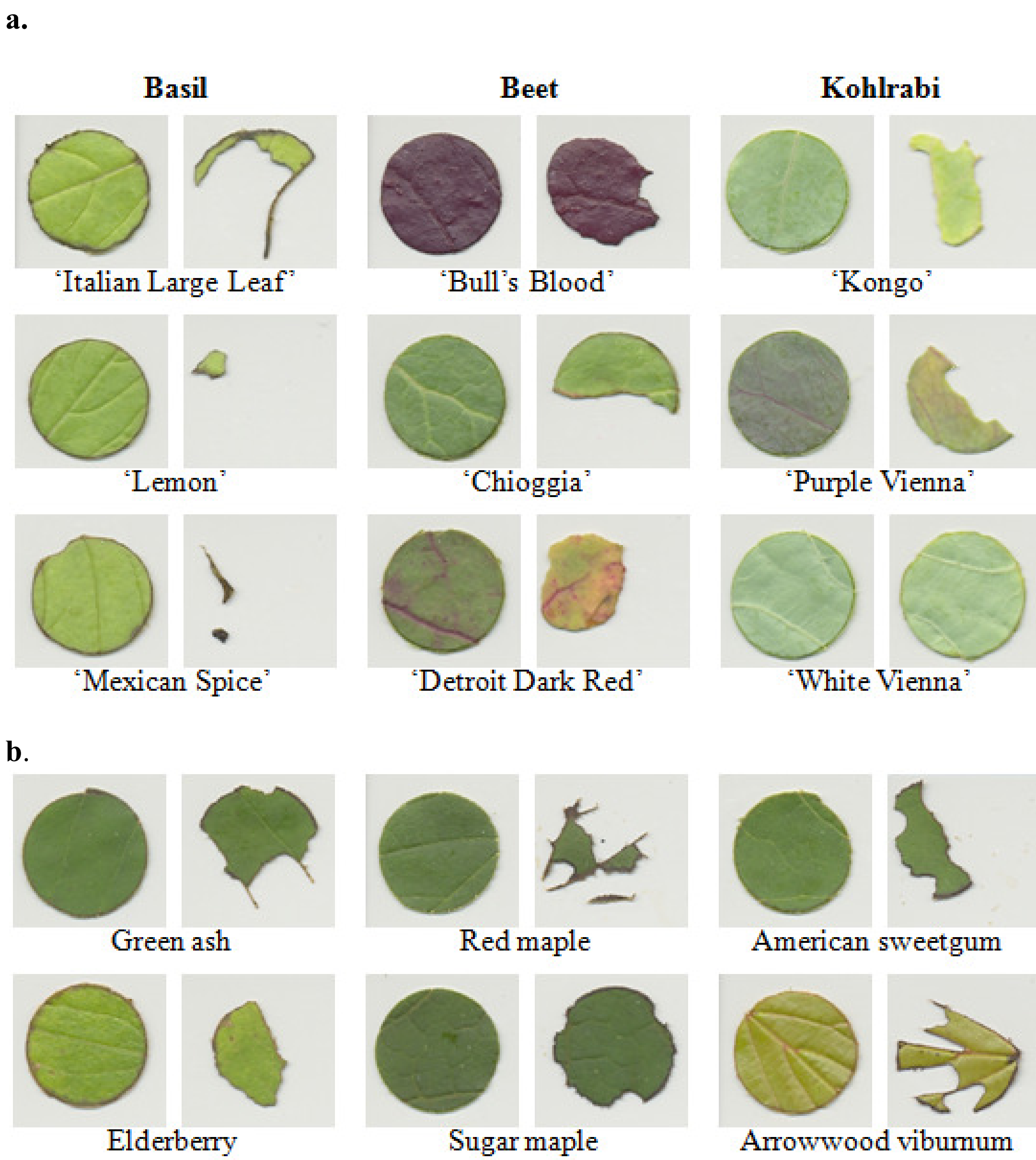

Figure 12. Scanned images of experimental leaf circles before and after trial period. Heavily-fed circles of each type from 2012 were chosen for this figure. **a**. Edible plants. **b**. Landscape plants.

Mass and area consumption were the only measures used to quantify feeding on leaf circles in this study. Some Japanese beetle feeding preference experiments have involved counting or weighing fecal pellets (Ladd, 1987, 1989; Miller & Ware, 1999; Spicer et al., 1995), but, in this AGB experiment, fecal pellets were too moist. Changes in leaf area have been used

by Spicer et al. (1995) and Miller & Ware (1999) to quantify Japanese beetle feeding. In this AGB experiment, both mass and area of leaf pieces were measured in an effort to compare the two measurement methods and obtain the most useful data.

Analyses of variance were performed separately on corrected mass and area consumption values for each year. Data from replicates in which beetles died during the experiment were removed from analyses, leaving a range of 11-15 replications per plant type in 2011 and 10-15 total replications per plant type in 2012, including both trials. Analyses were performed using the MIXED procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). To meet parametric analysis of variance assumptions, 2011 mass consumed was transformed by $(x + 20)^{0.25}$, 2011 area consumed was transformed by $\log(x^2 + 1)$, 2012 mass consumed was transformed by $1/(x^2 + 8)^{0.25}$, and 2012 area consumed was transformed by $1/\sqrt{(x + 20)}$. In 2012, a block design was used to account for the two trial dates.

Class contrasts were included in the main analysis of variance for each data set (mass 2011; area 2011; mass 2012; area 2012) to check for differences between cultivars within the basil, beet, and kohlrabi crop types, and also between the three crop types, combining all three cultivars of each. Within the main analysis, a Tukey-Kramer test was used for mean separation to determine which cultivars had significantly different amounts of feeding if a significant *P*-value (at $\alpha = 0.05$) was reported for differences between all cultivars. If *P*-values from contrasts were significant, a separate analysis of variance was run including a Tukey-Kramer test with the SLICE option in the MIXED procedure to separate means.

Results

Results from 2011 indicated no significant differences at the cultivar level for edible plants (at $\alpha = 0.05$) using mass as a measurement of Asiatic garden beetle feeding (Figure 13; $F = 1.96$, $df = 8, 107$; $P = 0.0582$). Class contrasts to test for cultivar differences within basil, beet, and kohlrabi crop types also showed no significant differences (basil $F = 0.17$; $df = 2, 107$; $P = 0.8426$; beet $F = 1.81$; $df = 2, 107$; $P = 0.1693$; kohlrabi $F = 2.09$; $df = 2, 107$; $P = 0.1289$). A class contrast to test for differences between basil, beet, and kohlrabi crops, combining all three cultivars of each, showed that beets were significantly more consumed than kohlrabi, while basil consumption was not significantly different from that of beet or kohlrabi (Figure 14; $F = 3.43$; $df = 2, 107$; $P = 0.0360$).

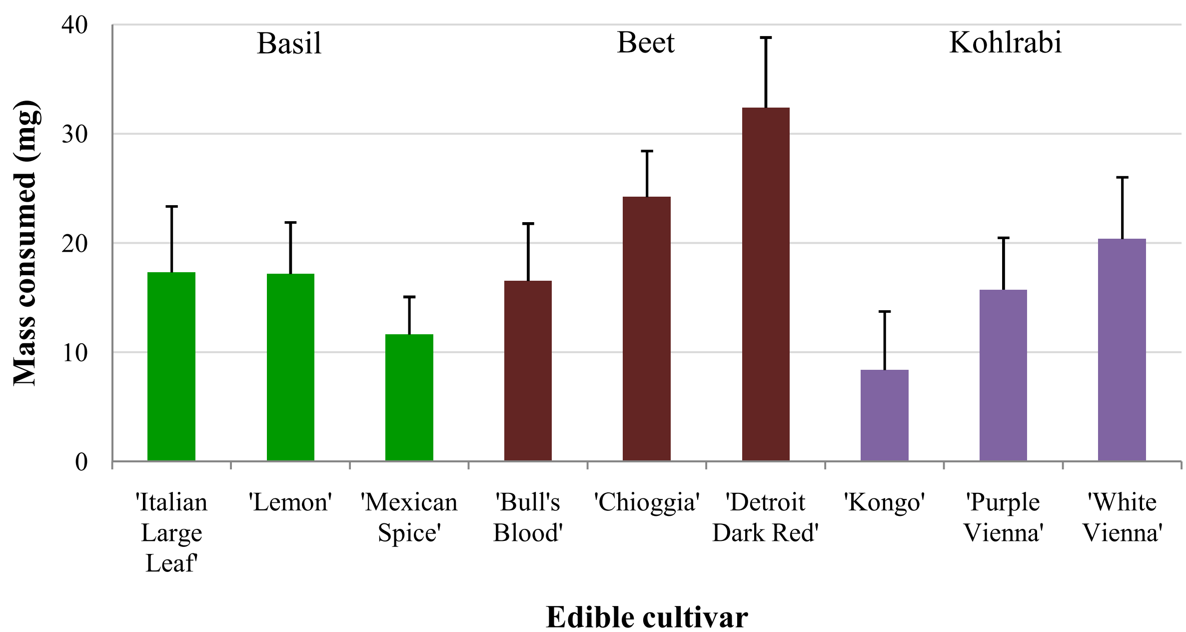

Figure 13. No-choice laboratory feeding test results for edible plants at the cultivar level showing mass (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2011, corrected using paired control leaf circle data (mean + SE). AOV on transformed values ($(x + 20)^{0.25}$); n = 12, 14, 14, 11, 11, 14, 13, 12, and 15, respectively; $P = 0.0582$.

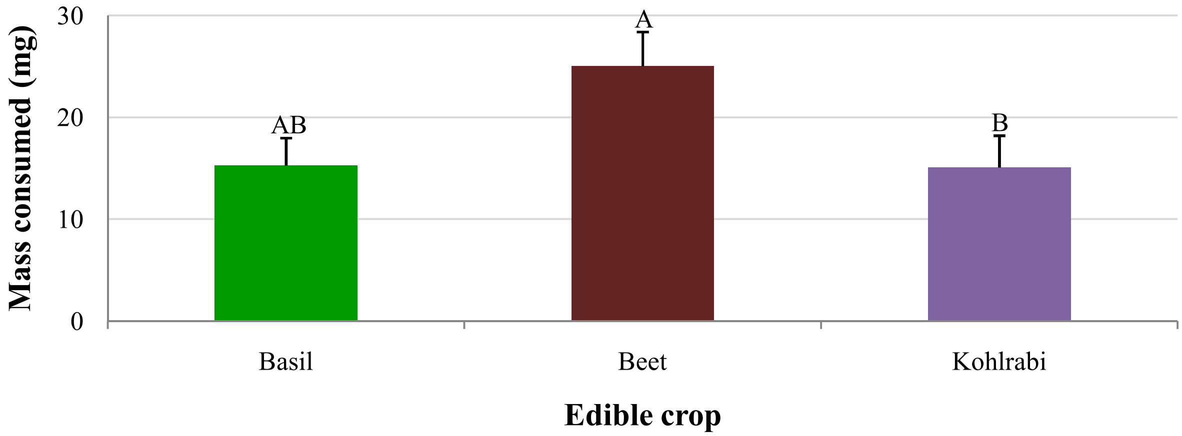

Figure 14. No-choice laboratory feeding test results for edible plants at the crop level showing mass (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2011, corrected using paired control leaf circle data (mean + SE). AOV contrast on transformed values ($(x + 20)^{0.25}$); n = 40, 36, and 40, respectively; $P = 0.0360$. Columns with the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey-Kramer test.

Analysis of variance using area consumption from 2011 indicated no significant differences at the cultivar level for edible plants (Figure 15; $F = 1.44$, $df = 8, 107$; $P = 0.1866$). Class contrasts to test for cultivar differences within basil, beet, and kohlrabi crop types also showed no significant differences (basil $F = 2.82$; $df = 2, 107$; $P = 0.0642$; beet $F = 0.94$; $df = 2, 107$; $P = 0.3936$; kohlrabi $F = 0.06$; $df = 2, 107$; $P = 0.9447$). A class contrast to test for differences between basil, beet, and kohlrabi crops also showed no significant differences (Figure 16; $F = 1.85$; $df = 2, 107$; $P = 0.1629$).

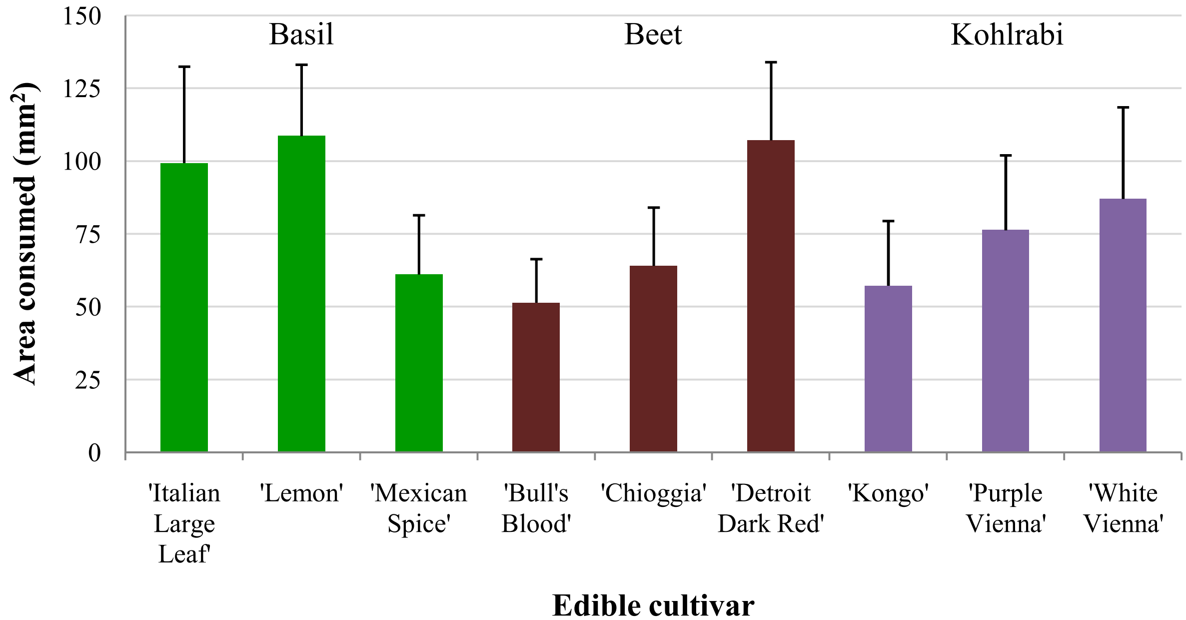

Figure 15. No-choice laboratory feeding test results for edible plants at the cultivar level showing area (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2011, corrected using paired control leaf circle data (mean + SE). AOV on transformed values ($\log(x^2 + 1)$); n = 12, 14, 14, 11, 11, 14, 13, 12, and 15, respectively; $P = 0.1866$.

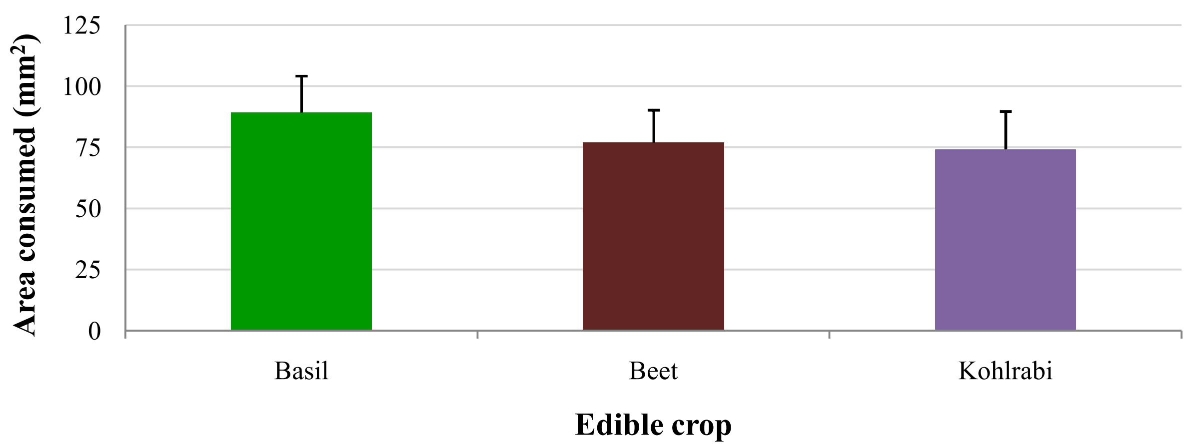

Figure 16. No-choice laboratory feeding test results for edible plants at the crop level showing area (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2011, corrected using paired control leaf circle data (mean + SE). AOV contrast on transformed values ($\log(x^2 + 1)$); n = 40, 36, and 40, respectively; $P = 0.1629$.

More significant differences in AGB feeding were found in 2012 than in 2011. As in 2011, 2012 mass and area data yielded similar, but not identical, results. Analysis of variance between all edible cultivars for mass consumption from 2012 showed that leaf circles from all three basil varieties were significantly more eaten than 'White Vienna' kohlrabi, and that 'Mexican Spice' basil was also significantly more fed upon than 'Chioggia' beet (Figure 17; $F = 5.06$; $df = 14, 163$; $P < 0.0001$).

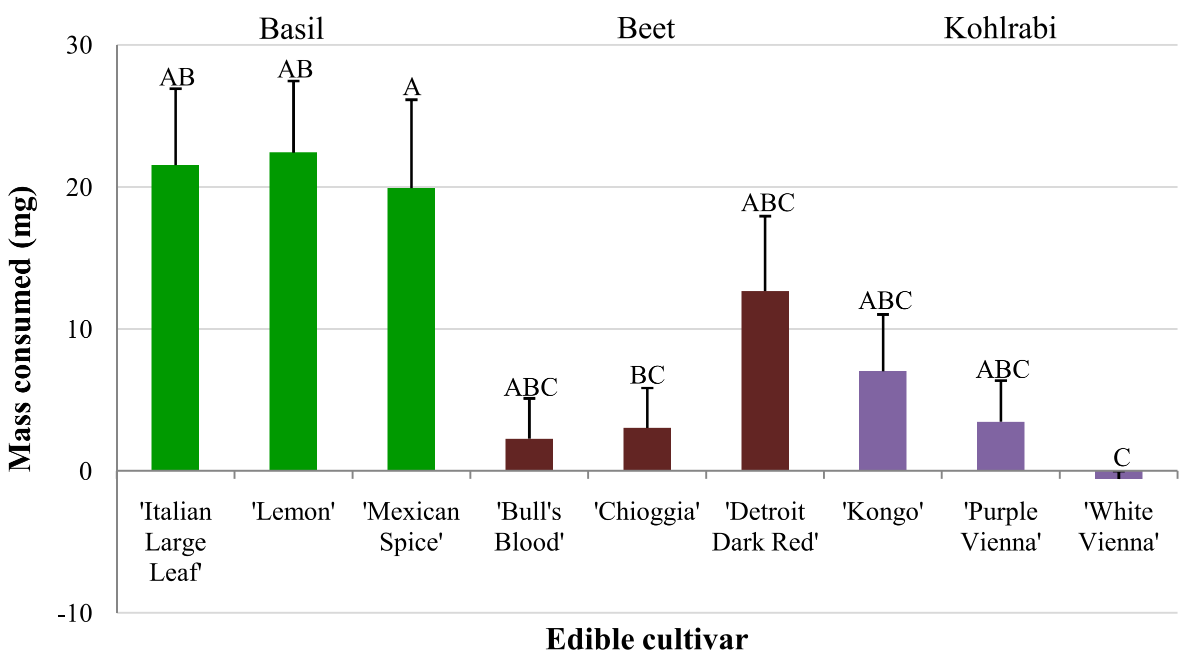

Figure 17. No-choice laboratory feeding test results for edible plants at the cultivar level showing mass (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2012, corrected using paired control leaf circle data (mean + SE). AOV on transformed values ($1/(x^2 + 8)^{0.25}$); n = 12, 11, 13, 12, 15, 10, 11, 13, and 11, respectively; $P < 0.0001$. Columns with the same letter are not significantly different at α = 0.05 according to the Tukey-Kramer test.

Class contrasts to test for cultivar differences within basil, beet, and kohlrabi crop types using mass 2012 data showed no significant differences (basil $F = 0.02$; $df = 2, 163$; $P = 0.9821$; beet $F = 1.34$; $df = 2, 163$; $P = 0.2654$; kohlrabi $F = 2.97$; $df = 2, 163$; $P = 0.0539$). A class contrast to test for differences between basil, beet, and kohlrabi crops, combining all three cultivars of each, showed that basil was significantly more consumed than beet or kohlrabi (Figure 18; $F = 16.97$; $df = 2, 163$; $P < 0.0001$).

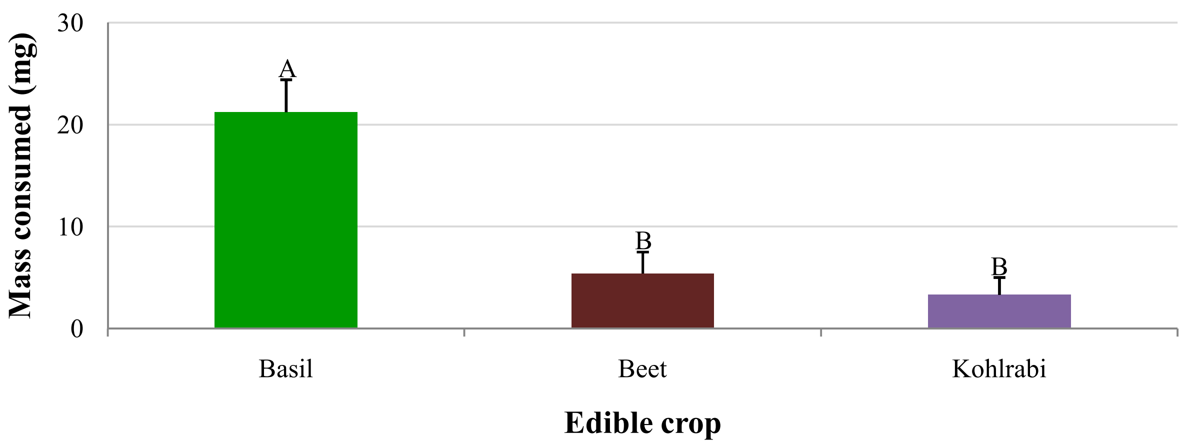

Figure 18. No-choice laboratory feeding test results for edible plants at the crop level showing mass (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2012, corrected using paired control leaf circle data (mean + SE). AOV contrast on transformed values ($1/(x^2 + 8)^{0.25}$); n = 36, 37, and 35, respectively; $P < 0.0001$. Columns with the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey-Kramer test.

Analysis of variance between all edible cultivars for area consumption from 2012 showed that all three basil varieties were significantly more fed on than 'Chioggia' beet, and that 'Italian Large Leaf' and 'Lemon' basils were also significantly more fed upon than 'Bull's Blood' beet, 'Purple Vienna' kohlrabi, and 'White Vienna' kohlrabi (Figure 19; $F = 4.71$; $df = 14, 163$; $P < 0.0001$). Class contrasts to test for cultivar differences within basil, beet, and kohlrabi crop types showed no significant differences (basil $F = 0.36$; $df = 2, 163$; $P = 0.6951$; beet $F = 1.36$;

df = 2, 163; *P* = 0.2598; kohlrabi *F* = 0.35; *df* = 2, 163; *P* = 0.7073). A class contrast to test for differences between basil, beet, and kohlrabi crops showed that basil was significantly more consumed than beet or kohlrabi (Figure 20; *F* = 24.30; *df* = 2, 163; *P* < 0.0001).

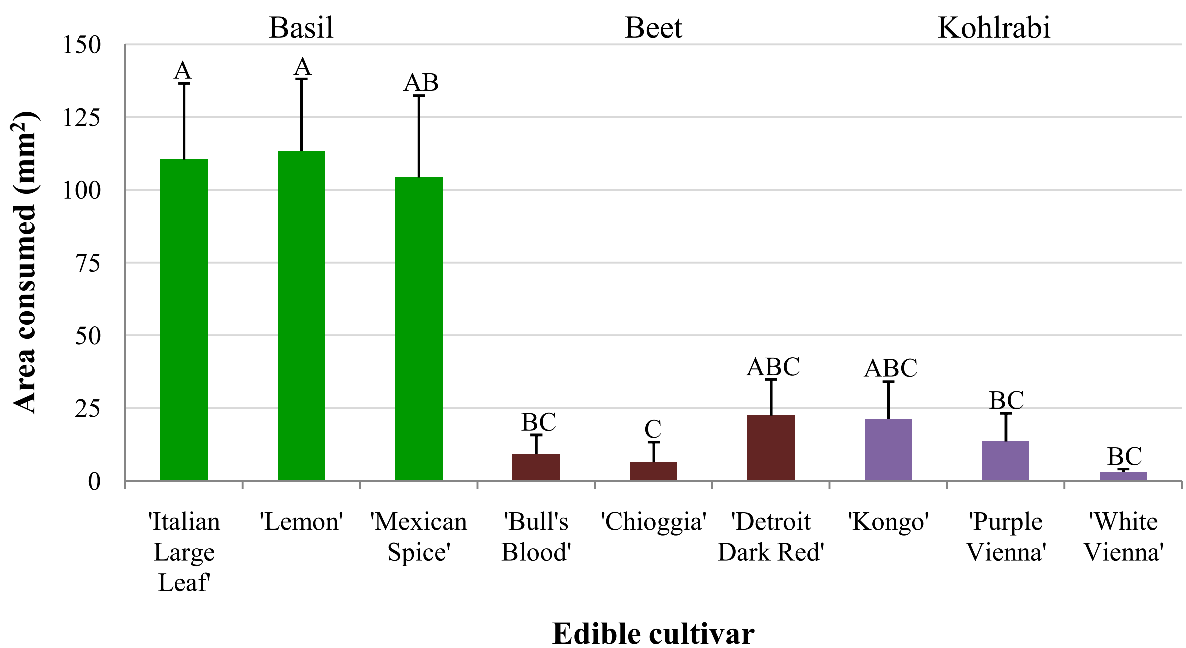

Figure 19. No-choice laboratory feeding test results for edible plants at the cultivar level showing area (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2012, corrected using paired control leaf circle data (mean + SE). AOV on transformed values ($1/\sqrt{(x + 20)}$); n = 12, 11, 13, 12, 15, 10, 11, 13, and 11, respectively; *P* < 0.0001. Columns with the same letter are not significantly different at α = 0.05 according to the Tukey-Kramer test.

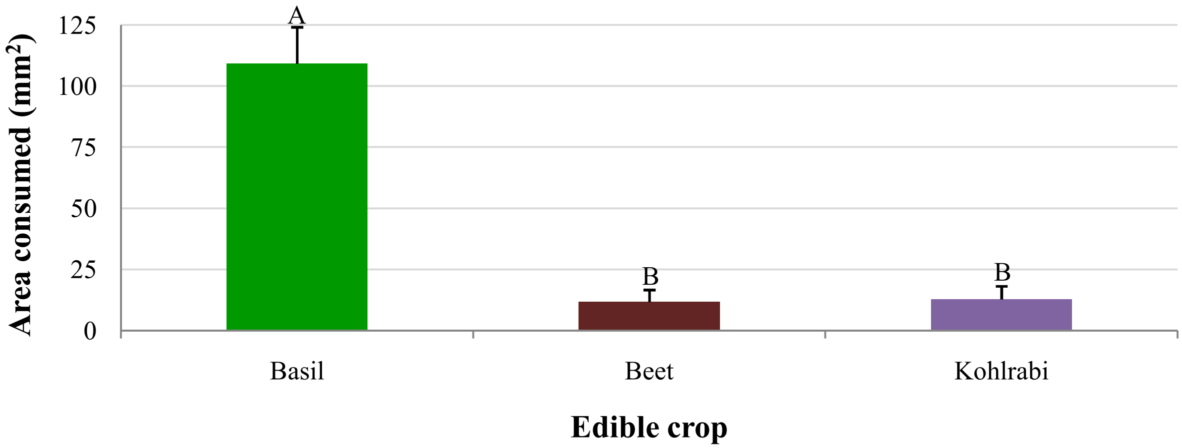

Figure 20. No-choice laboratory feeding test results for edible plants at the crop level showing area (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2012, corrected using paired control leaf circle data (mean + SE). AOV contrast on transformed values ($1/\sqrt{(x + 20)}$); n = 36, 37, and 35, respectively; $P < 0.0001$. Columns with the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey-Kramer test.

A class contrast to test for differences in beetle feeding on landscape plants using mass consumption from 2012 showed significant differences (Figure 21; $F = 2.85$; $df = 5, 163$; $P = 0.0170$). However, the Tukey-Kramer test for mean separation did not show significant differences between landscape plants. Similarly, a class contrast to test for differences in beetle feeding on landscape plants using area consumption from 2012 also showed significant differences (Figure 22; $F = 2.28$; $df = 5, 163$; $P = 0.0492$). However, as with mass consumption, the Tukey-Kramer test for mean separation did not show significant differences between landscape plants. A one degree of freedom contrast was also included in the main analysis for mass data, and the main analysis for area data to look for differences in beetle consumption of red maple and sugar maple. Contrasts between other landscape plants were not performed so that comparisons remained orthogonal, as contrasts for edible plants were also included in these analyses. Using both mass and area data, red maple was found to be significantly more consumed than sugar maple (mass $F = 4.76$; $df = 1, 163$; $P = 0.0305$; area $F = 4.82$; $df = 1, 163$;

P = 0.0296). It is likely that at least arrowwood viburnum, the landscape plant with the highest mean mass and area consumption, was also significantly more fed upon than sugar maple, the plant with the lowest mean mass and area consumption, but this has not been statistically verified. Elderberry, with the second highest mean mass consumption and similar area consumption to red maple, also seems especially likely to be significantly more fed upon than sugar maple, but, again, this has not been statistically verified.

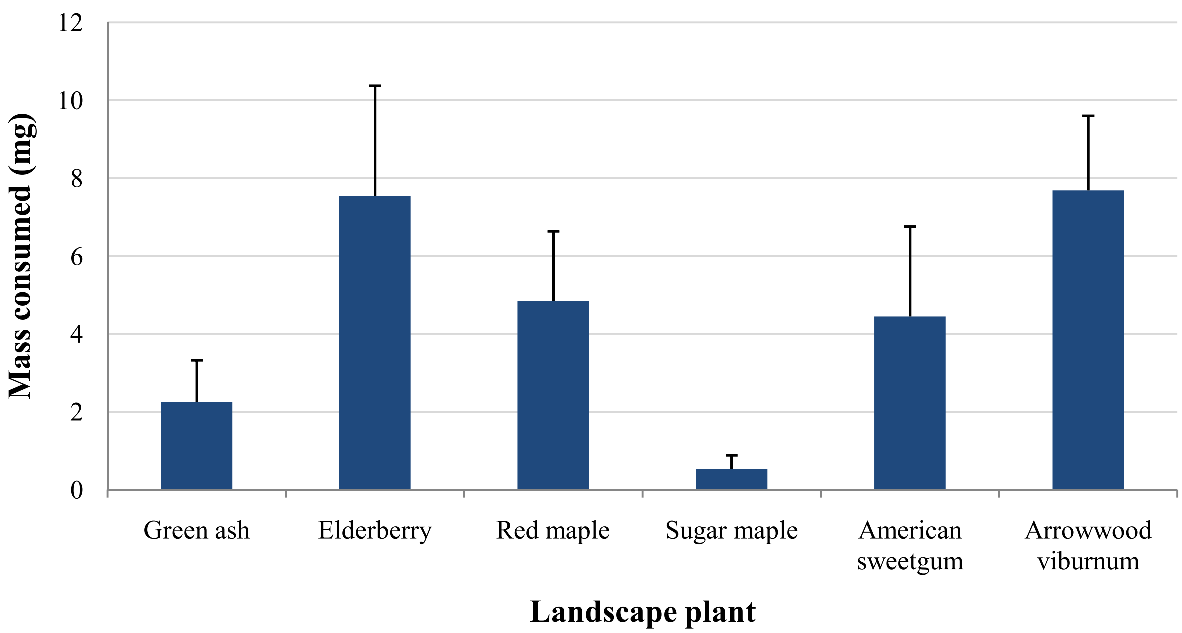

Figure 21. No-choice laboratory feeding test results for landscape plants showing mass (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2012, corrected using paired control leaf circle data (mean + SE). AOV contrast on transformed values ($1/(x^2 + 8)^{0.25}$); n = 13, 11, 13, 12, 12, and 10, respectively; P = 0.0170. Significant differences between individual plant types were not found when comparing all six types using the Tukey-Kramer test, however a contrast showed that red maple was significantly more consumed than sugar maple (P = 0.0305).

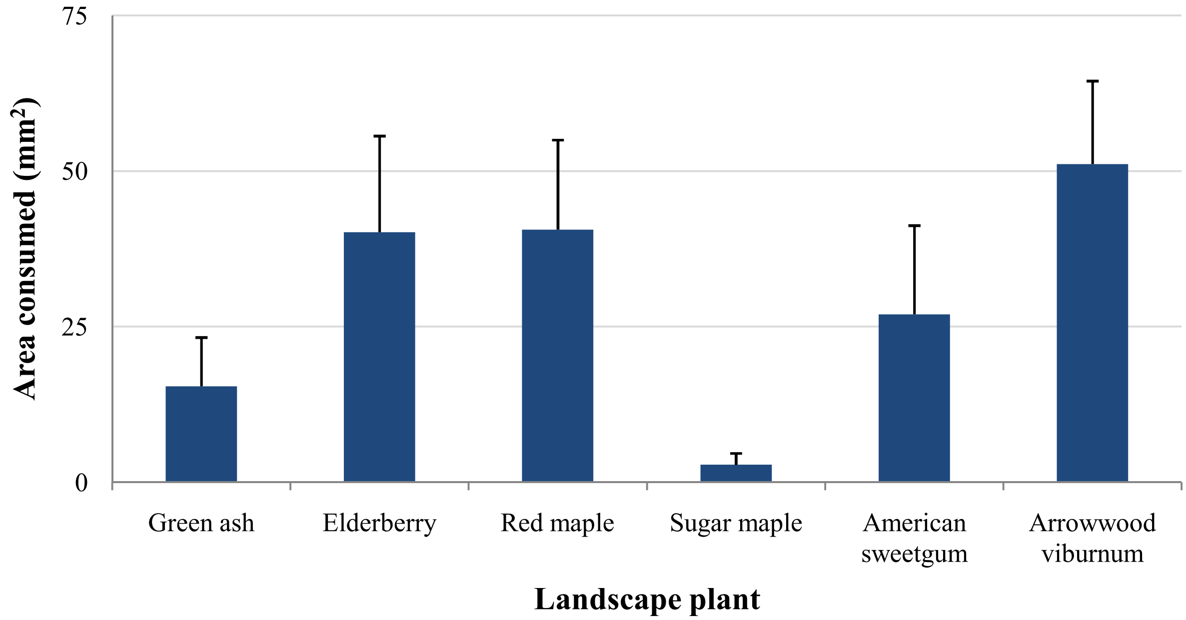

Figure 22. No-choice laboratory feeding test results for landscape plants showing area (non-transformed) of leaf circles consumed by Asiatic garden beetles in 2012, corrected using paired control leaf circle data (mean + SE). AOV contrast on transformed values ($1/\sqrt{(x + 20)}$); n = 13, 11, 13, 12, 12, and 10, respectively; $P = 0.0492$. Significant differences between individual plant types were not found when comparing all six types using the Tukey-Kramer test, however a contrast showed that red maple was significantly more consumed than sugar maple ($P = 0.0296$).

Due to the significant p-values at $\alpha = 0.05$ for differences between landscape plants measured using both mass and area consumption, it is possible that significant differences between landscape plants would be detectable using the Tukey-Kramer test for mean separation if more replications were included for each plant type. This inference is supported by the results of power tests done using the GLMPOWER procedure with contrasts in SAS 9.3. According to the power tests, there is a 70% chance of detecting any differences that existed between landscape plants using mass consumption, and a 60% chance of detecting possible differences using area consumption. To have at least a 90% chance of detecting the differences that might exist between landscape plants using either mass or area, the total number of replications in the experiment would need to be doubled, from 179 to 358 total replications.

To compare reliability of mass and area measurements, the average relative standard errors for mass and area data from each year were calculated as the average of the relative standard errors (SE/mean) for each edible cultivar and landscape variety used in that year's experiment. Spearman rank correlations were also calculated for each year to determine the extent of correlation between mass and area data. In looking at relative standard errors, it is important to consider that lower error indicates lower variance, and thus higher precision – not necessarily higher accuracy – of measurements. However, average relative standard error is the best measure of reliability of measurement methods in this situation. The average relative standard errors of the mass and area data from 2011 were both 31.50%, indicating that mass and area are similarly reliable measures to use. The Spearman rank correlation for 2011 mass and area data showed $r = 0.79$, $P < 0.0001$, indicating that the two measures are positively correlated. The average relative standard error of the mass consumption values from 2012 was 56.05%, while the average relative standard error of the area consumption values was 49.33%. These relative standard errors indicate that area might be the more reliable measure to use, but that the two methods of measurement produced similar levels of variability, as in the 2011 measurements. The Spearman rank correlation for 2012 mass and area data showed $r = 0.18$, $P = 0.0132$, indicating that the two measures are weakly positively correlated.

Discussion

In this no-choice laboratory experiment, statistical analyses using mass and area consumption measurements produced similar, yet different results. Using either mass or area consumption data from 2011, no significant differences were shown between feeding levels of Asiatic garden beetles on cultivars of edible plants either including all crop types or within basil,

beet, or kohlrabi crops. Analyses of mass consumption values from 2011 showed that beets were significantly more consumed than kohlrabi, while analysis of area consumption values from 2011 showed no significant differences between any of the three crop types. Despite these different outcomes, mass and area consumption were shown to be statistically correlated, and had the same average relative standard errors, suggesting that they were similarly reliable measures.

Analyses of mass and area consumption from 2012 also produced similar results to one another, with similar relative standard errors, and were statistically shown to be weakly correlated. Analyses of both mass and area measurements agreed that ‘Mexican Spice’ basil was significantly more fed upon than ‘Chioggia’ beet, and that ‘Italian Large Leaf’ and ‘Lemon’ basils were significantly more eaten than ‘White Vienna’ kohlrabi. Analysis of mass consumption also showed that ‘Mexican Spice’ basil was significantly more consumed than ‘White Vienna’ kohlrabi, while area data also showed that ‘Italian Large Leaf’ and ‘Lemon’ basil were more fed upon than ‘Chioggia’ beet, ‘Bull’s Blood’ beet, and ‘Purple Vienna’ kohlrabi. In the analyses of both mass and area consumption from 2012, basil was significantly more fed upon than beet or kohlrabi, and no differences were found between cultivars within any of the three crop types.

Some unexpected significant differences, indicated by different letters, appear in Figures 17 and 19, depicting 2012 mass and area data for every edible cultivar. The probable explanation is twofold. First, the two graphs in question consist of non-transformed data with lettering from analyses of transformed data. The transformed data has a slightly different rank order than the non-transformed data, explaining some of the oddities in lettering. Second, these experiments had varying sample sizes. Varieties with lower sample sizes were less likely to be found

significantly different from other varieties. One example of sample size possibly coming into play is the significant difference found between ‘Mexican Spice’ basil and ‘Chioggia’ beet. ‘Mexican Spice’ basil has a lower average value for mass consumed than the other two basil cultivars, yet it is shown to be significantly different from ‘Chioggia’ beet while the other two basils are not. This could be because ‘Mexican Spice’ basil had 13 replications, while ‘Italian Large Leaf’ and ‘Lemon’ basil had only 12 and 11 replications, respectively – not enough for the Tukey-Kramer test to find a significant difference between their mass values and those of ‘Chioggia’ beet. A similar situation occurs in which ‘Bull’s Blood’ beet has a smaller average value for mass consumed than ‘Chioggia’ beet, but is not significantly different from ‘Mexican Spice’ basil, while ‘Chioggia’ beet is. In this case, ‘Chioggia’ beet has 15 replications while ‘Bull’s Blood’ has only 12. With more replications, it may have been shown that all three basil cultivars had significantly different values of mass consumed from both ‘Bull’s Blood’ and ‘Chioggia’ beets. Similarly, for area 2012 data, ‘White Vienna’ kohlrabi, with only 11 replications, is not significantly different from ‘Mexican Spice’ basil, while ‘Chioggia’ beet is significantly different from ‘Mexican Spice’, despite having a higher average area consumed than ‘White Vienna’ kohlrabi. ‘Chioggia’ beet has 15 replications, suggesting that if ‘White Vienna’ had as many replications, it might also have been shown significantly different from ‘Mexican Spice’ basil.

P-values from contrasts in the analyses of variance using both mass and area measurements from 2012 show that at least one significant difference existed between consumption levels of landscape plants. However, the Tukey-Kramer test for mean separation did not show what the differences were; it detected no significant differences. Red maple was found to be significantly more eaten than sugar maple in the single one degree of freedom

contrast performed for both mass and area data, but more differences may have been picked up by the contrasts in the main analyses of variance, and narrowly missed by each Tukey-Kramer test. With more replications, particularly for viburnum and elderberry with only 10 and 11 replications, respectively, more differences may have been found by the Tukey-Kramer test.

Each of the landscape plants tested in this AGB experiment have been ranked by preference of another scarab pest, the Japanese beetle, *Popillia japonica*, by Fleming (1972) based on his and other authors' previous rankings, which were observationally-based (Held, 2004). Fleming (1972) ranked arrowwood viburnum as XXX, or moderately fed on by the Japanese beetle, followed by sugar maple, ranked as XX, or lightly fed on, followed by elderberry and American sweetgum, both ranked X, or occasionally lightly fed on, followed by green ash and red maple, ranked 0 for no record of feeding by Japanese beetles. Controlled, quantitative no-choice tests, using fecal pellet weight to measure Japanese beetle feeding, were performed by Ladd (1989) on three of the landscape plants included in this AGB experiment. Sweetgum was given a feeding index value higher than sugar maple, which was given a value higher than red maple. The feeding index values of these three landscape plants did not differ greatly. As each plant was included in a separate test, no statistical analyses were used to compare them to each other, so it is unknown if Japanese beetle preferences for each are significantly different from preferences for the others. All three plants produced a fecal pellet weight significantly higher than a control beetle presented with no leaf during the experiment, and significantly lower than a standard beetle confined with sassafras, a known favorite of the Japanese beetle. This indicates that the plants were fed on, but they are not as favored as sassafras. Arrowwood viburnum appears to be favored by both the Japanese beetle and the AGB, when compared to the other five landscape plants tested for AGB preference. Green ash appears

to be minimally eaten by both the Japanese beetle and AGB, compared to the other five plants. However, while the Japanese beetle appears to favor sugar maple over red maple, or possibly favors them equally, this AGB experiment showed that AGBs ate significantly more red maple than sugar maple leaf material, the latter of which was the least consumed of the six plants tested. Therefore, the Japanese beetle and AGB, while both scarab pests, do not always share food plant preferences. In choosing more resistant plants for preventative control, it is important to consider plant susceptibility and resistance to all potential pests, along with other factors (Held, 2004).

The use of paired corrections (detailed on page 44) was important for producing accurate estimates of feeding damage in this study. To estimate the mass of leaf material that was consumed during each experiment, the pre-experimental wet mass was measured, and the post-experimental dry mass was measured. Measuring post-experimental wet mass would not provide values of mass consumed as accurate as using post-experimental dry mass because the mass of the leaf disks changed for reasons other than AGB feeding during the experiment. Anticipated mass changes were either mass lost due to leaf desiccation, or mass gained due to increased moisture levels from the moistened filter paper petri dish base. In either case, the mass changes would be due to changes in moisture, which were negated by drying the leaf disks. Some post-experimental wet mass measurements were made, but not used to calculate mass consumed, and many leaf disks exited the experimental period with a higher mass than initially measured, presumably having gained water weight – this was even observed for some disks on which feeding damage was visually apparent, so weight would have been expected to have decreased. As wet mass was measured prior to experiments, and dry mass measured after, it was necessary to perform a correction in order to estimate the wet mass consumed by the AGB. The paired

control leaf disks, generally cut from the same leaf as the corresponding experimental disks, were measured when wet and then when dried to find the proportion of water weight in each leaf disk. This knowledge allowed the appropriate correction to be performed to estimate the wet mass consumed by the beetle. Water weight in control leaf disks varied from 47% to 95% of the total weight. Presumably, water weight varies between plants and between leaves even within one plant variety, so the use of paired controls was elected over the use of averaged control values to more accurately represent the experimental disks.

Area values were also corrected using paired controls in these experiments, in a manner similar to what was done for mass values. While leaf disk mass often increased during the experimental period when little or no feeding damage was present, so did leaf disk area. In control leaf disks, which were not exposed to beetles during the experimental period, area increased by a maximum of 26.630% of the pre-experimental area. However, area sometimes decreased in control leaf disks, losing up to 4.573% of the pre-experimental area. Changes in area not caused by AGB feeding are assumed to be due to leaf swelling or shrinking as a result of changes in moisture content. Expected non-feeding-related area changes were calculated using the paired control leaf disks, and these values were used to calculate the corrected values of area consumed. Without these corrections, the calculated values of area consumed would tend to be lower than the true area consumed, and many leaf disks with no beetle feeding would show negative amounts of area consumed (area gained). Again, it made sense to use paired controls instead of averaged controls for each plant variety because each plant and each leaf might have a different tendency to increase or decrease in area than the average for the plant variety.

The laboratory no-choice feeding study produced different results in each year experiments were performed, 2011 and 2012. Analyses of 2011 mass and area consumption

measurements showed no significant differences except for significantly more feeding, as measured using mass consumption, on beets than on kohlrabi. Analyses of both mass and area consumption measurements from 2012 showed significantly more feeding on basil than on beets and kohlrabi, and no significant difference between beet and kohlrabi feeding. Analyses using 2012 mass and area consumption also showed significant differences between some cultivars of basil and cultivars of the other two crops. Several experimental factors varied from 2011 to 2012, and any combination of these could have been the cause of the switch from beet preference over kohlrabi in 2011 to basil preference over beet and kohlrabi in 2012, assuming that amounts of feeding were representative of food plant preferences. Of particular interest are length of feeding tests, experimental light and temperature conditions, diameter of leaf circles, beetle diet prior to tests, and the number of petri dishes sharing a plastic bag during the feeding tests. Additionally, fertilizer and insecticidal soap spray were used on plants in 2011 only, plants were transplanted from smaller to larger pots in 2011 only, and plants were older in 2011, but these variables are not expected to have caused the differences in results.

One notable difference between the 2011 and 2012 no-choice feeding test results is that leaf consumption of edible cultivars appears to have been generally higher in 2011. In 2011, the lowest average mass consumed for a cultivar was 8.4 mg, and, in 2012, five out of nine cultivars had an average mass consumption below that value (Figures 13 & 17). The average mass of the most consumed crop in 2011, beet, was 25.0 mg, and the average mass of the most consumed crop in 2012, basil, was 21.2 mg (Figures 14 & 18). In 2011, the lowest average area consumed for a cultivar was 51.358 mm^2, and, in 2012, all cultivars except for the three basil cultivars had average area consumptions below that value (Figures 15 & 19). It appears that, in 2012

compared to 2011, basil cultivars were consumed somewhat more, and the other cultivars were consumed much less.

The length of feeding tests is one of the variables that changed most significantly between the 2011 and 2012 versions of this no-choice feeding study. In 2011, the minimum experimental duration was 24 hours, and, in 2012, the minimum duration was 48 hours. It seems unlikely that this factor was important in creating the difference in results between 2011 and 2012 due to the fact that beetles generally fed more in 2011. The expectation in lengthening the experimental duration was that beetles would feed more overall and would feed more naturally due to the additional time available for feeding and acclimating to the experimental setup. As 2012 measurements did not show feeding levels beyond what was seen in 2011, it does not seem that the expected results of lengthening the experimental duration occurred. Other possible effects of lengthened experimental duration on beetle feeding levels do not seem relevant.

The changes in light and temperature conditions between the 2011 and 2012 versions of the no-choice laboratory feeding study seem more likely than experimental length to have caused the differences in results. It is possible that the warmer, 26.7°C, daily high temperature (despite the corresponding 18.3°C daily low temperature) in 2011 encouraged beetles to be more active than the cooler, 22.2°C, constant temperature in 2012, which is closer to the 21°C minimum required for AGB flight, a time during which beetles also appear to be most active (Hallock, 1930, 1932), and the 16°C minimum temperature required for light AGB feeding (Hallock, 1936a). The feeding temperature at which AGB adults consume the largest quantities is unknown, however it has been shown that adults of the Japanese beetle (*Popillia japonica*), another scarab, showed a linear increase in feeding on soybean leaf disks at increasing temperatures beyond 16°C until around 37°C, after which feeding decreased, presumably due to

increased beetle mortality (Niziolek et al., 2013). This supports the hypothesis that AGBs fed more overall in 2011 than 2012 due to a higher maximum temperature in 2011. Interestingly, the difference in temperature between the two experimental years could account not only for the decreased overall feeding in 2012, but also for the shift in preference from beets over kohlrabi in 2011 to basil over beets and kohlrabi in 2012. Results from Lemoine et al. (2013) support results from Niziolek et al. (2013) in showing increased feeding of adult Japanese beetles at increasing temperatures. However, Lemoine et al. (2013) also show that Japanese beetle feeding preferences change at varying temperatures, presumably due to changes in nutritional requirements of the beetles and changes in attractive and repellent characteristics of leaf chemicals. In fact, while Japanese beetle adults consume more in general at higher temperatures, they also have high rates of feeding on fewer plant types than at lower temperatures, where they feed more vigorously on a larger number of plant types. Like Japanese beetles, AGBs could have different food preferences at different temperatures, explaining the preference for beets over kohlrabi in 2011 and basil over beets and kohlrabi in 2012. AGB diet breadth appeared to be more narrow in 2012 than 2011, but with the higher maximum temperature occurring in 2011, the Japanese beetle results suggesting more specific tastes at higher temperatures do not appear to explain the increased specificity of AGB feeding in 2012.

Photoperiod was another factor that varied between the 2011 and 2012 AGB experiments. It is possible that the 15:9 h L:D dynamic light cycle in 2011 encouraged beetles to be more active than the constant absence of light in 2012. This could perhaps be the case if the change from ambient light to darkness initiates AGB feeding behavior. *Oemona hirta*, a cerambycid beetle, has been found to feed the most from 0 to 3 hours after darkness in the lab, even when darkness occurred during different hours of the day (Wang et al., 1998). This could

indicate that the onset of darkness stimulates the onset of feeding, which would not occur if darkness were constant. If AGBs require a change from light to dark to initiate feeding, this could explain the lower feeding levels in 2012, when the beetles were subjected to constant darkness, compared to 2011, when there was a dynamic light cycle. However, the saw-toothed grain beetle, *Oryzaephilus surinamensis*, has a daily foraging activity cycle shown to be heavily influenced by an internal circadian rhythm in addition to external lighting cues. In the lab, Bell and Kerslake (1986) acclimated *O. surinamensis* to two photoperiods, and, for each photoperiod, the beetles showed peak foraging activity about 4 hours after the onset of darkness, indicating the importance of the environmental cue of lighting. However, beetles placed in total darkness for three days preserved the timing of their daily peaks of foraging activity for all three days. *O. surinamensis* therefore appears to have an activity cycle based on external darkness cues, and an internal circadian rhythm that appears stronger than the need for external cues, at least for three days. If the AGB has a strong internal circadian rhythm like *O. surinamensis*, then it is possible that constant darkness would not have affected levels of feeding activity, but it seems more likely that constant darkness would affect feeding because AGBs were kept in constant darkness prior to testing, and therefore, at least some of the beetles used were in darkness for many more than the three days during which *O. surinamensis* maintained its activity cycle.

Another explanation for higher feeding levels in 2011 than 2012 involving light is that the constant absence of light in 2012 could have caused the leaf circles to be less appetizing. Furutani and Arita (1990) showed that leaves from plants left in darkness for 24 hours had fewer carbohydrates than leaves from plants left in ambient lighting during that period. The dark-exposed leaves also were less fed on by Chinese rose beetles (*Adoretus sinicus*), which are also scarabs, and which feed in the early evening – in darkness, in this experiment. It is possible that a

similar change in leaf quality and preference occurred with AGBs and the experimental leaf circles, despite the circles being separated from the plants during the period of darkness. However, the darkness of the experiment seems less likely to have affected the leaf circles when considering that they were already kept refrigerated in the dark for up to three days prior to the experiment, although this period may have tended to be shorter in 2011 than 2012. While differences in photoperiod could have led to higher levels of feeding in 2011 than 2012 due to higher levels of beetle activity or higher leaf attractiveness, the switch from beet preference over kohlrabi in 2011 to basil preference over beet and kohlrabi in 2012 is less easily explained. Perhaps, if 2011 results are viewed as showing fewer differences between plant types instead of as showing beet preference over kohlrabi, which was only significant for mass consumption values, this could possibly be explained by greater beetle activity leading to higher, and more even, overall feeding, while decreased activity cues in 2012 could have led to higher activity, and therefore feeding, only on preferred plants which gave beetles an extra incentive to be active.

The use of different diameter leaf circles could also have caused the differences in results seen between 2011 and 2012. Similar to the possible activating effects of a warmer high temperature and a dynamic light cycle, a larger diameter food item might encourage an AGB to feed more than a smaller diameter item, without regard to the identity of the food item, leading to higher, and more even, feeding levels in 2011 on 2.2 cm circles, compared with feeding on 1.7 cm circles in 2012. This possible activation could be caused by the effect of size on a beetle's perception, or perhaps it could be caused by an effect that leaf circle size has on other properties of the leaf, such as wilting or chemical changes in response to damage. Jones and Coleman (1988) found that using both larger (1.7 cm) and smaller (1.0 cm) leaf disks in two otherwise identical experiments led to the same trend of willow leaf beetle (*Plagiodera versicolora*), a

chrysomelid, preference for cottonwood with higher prior exposure to ozone. However, there was only a statistically significant difference between feeding on ozone treatments using the larger leaf disk size. This shows that leaf circle size can affect feeding preference results, although in the case of the AGB experiment, more significant differences were found when the smaller of the two leaf circle sizes was used, in 2012. Jones and Coleman (1988) hypothesize that the effect of disk size is explained by the ratio of chemical signals given off by the cut leaf edge to signals given off by the undamaged center. Larger leaf disks have a lower ratio of damaged to undamaged material, so the signals given off by the edge do not overwhelm those given off by the undamaged leaf area, which is assumed to transmit the treatment signals to the insect. Jones and Coleman (1988) hypothesize that differences in leaf circle size would have different effects on different sized insects, and also on insects that feed in the center of leaves, like *P. versicolora*, compared to those that feed on edges, like the AGB. For an experiment like this AGB experiment, Jones and Coleman (1988) predict no difference between using small and large leaf disks.

Although starved for a minimum of 24 hours before feeding tests in both years, beetles may have been more hungry, or more attuned to feeding on leaf material, as a result of being fed a diet of leaves in 2011 compared to a diet of carrot pieces in 2012. Feeding preferences have been shown to be inducible for some insects, such as in larvae of the butterflies *Heliconius erato* and *H. ethilla* (Silva et al, 2014). It appears that there is less evidence for induction of food preference in adult insects, but Phillips (1977) showed that rearing adult *Haltica lythri* flea beetles, chrysomelids, on one plant, *Oenothera biennis*, led to the beetles having no preference for *Oenothera* or *Epilobium hirsutum* when, at emergence, they had shown preference for feeding on *Epilobium*. This indicates that adult beetle feeding history can affect adult feeding

preference. While these experiments tested preference between leaf types, it is possible that preference for carrot pieces over leaf pieces of any type could be induced by prior feeding of carrot pieces. If the AGBs were not very hungry even after being starved for 24 hours, those previously fed on carrot pieces might have preferred to eat carrot pieces instead of leaves, and therefore fed less overall on the experimental leaf circles in 2012 than beetles in 2011, which were accustomed to feeding on leaves. Increased hunger or interest in feeding on leaves due to prior feeding on leaves may have caused higher levels of more indiscriminate feeding in 2011 than in 2012, but this does not explain the switch from preference for beet over kohlrabi in 2011 to basil over beet and kohlrabi in 2012.

Finally, it is possible that putting multiple petri dishes into a single plastic bag in 2012 confounded the experiment by allowing beetles to detect plant volatiles emitted from leaf circles in petri dishes other than their own. In this situation, beetles may have reacted as in a multiple-choice test if they were not particularly hungry, even after starving for 24 hours: they may have spent time trying to get to their preferred food plant instead of eating from the leaf they were with, unless the leaf they were with was the preferred food plant. However, this explanation still would not account for the lower feeding levels on the preferred crop, basil, in 2012 compared to feeding levels on the 2011 preferred crop, beet, or the switch from beet preference over kohlrabi in 2011 to basil preference over beet and kohlrabi in 2012. The causes of the difference in results between 2011 and 2012 are unknown, but may include known differences in beetle and plant manipulation between the two years, particularly the difference in light and temperature settings during the feeding trials and the sharing of plastic bags by petri dishes representing separate experimental units in 2012.

This experiment could be improved by removing the variability in the experimental methods, perhaps after more preliminary tests to identify ideal conditions for the experiment. A 48 hour minimum experimental duration did not cause excessive mortality, and therefore can be recommended, although there was no evidence to support 48 hours leading to more feeding than a 24 hour minimum experimental duration. It is recommended that further tests be done to find the ideal temperature pattern and photoperiod for AGB laboratory experiments, based on natural conditions but allowing for moderately high activity levels. Providing a refuge of some sort to mimic the soil in which AGBs spend their daytime hours buried might be useful, however this would need to have little effect on leaf disk mass or area. Storing leaves for a shorter period prior to experimental trials might also be useful, primarily so that there is less time during which they are not exposed to light, potentially making them more appealing food items. Leaf disks 2.2 cm or larger are recommended, as this amount was almost entirely consumed by a small number of beetles during the experiment. Carrot-fed AGBs should be compared with leaf-fed AGBs in terms of subsequent feeding levels on different leaves, perhaps basil leaves, to make sure that prior feeding of carrots does not lower interest in leaf feeding. Finally, observations could be made of AGBs in petri dishes with less preferred food plants in the same plastic bag as petri dishes with basil leaf disks to ensure that they do not spend more time than beetles with basil obviously trying to escape their dishes, potentially to get to the basil leaves. Otherwise, it may be best to keep each petri dish in a separate, sealed plastic bag despite the extra time involved.

The overall conclusion of the laboratory no-choice feeding experiment is that basil is likely a preferred food of the AGB, compared to beet and kohlrabi, although it is possible that beet is a similarly, or more, preferred food, depending on conditions. When the results of the laboratory no-choice feeding experiment are considered alongside the results of the common

garden field count experiment, the preference of basil over other edible crop types is a more supported observation than the preference of beet over kohlrabi, which was seen in only one year of one experiment type, and only for mass, not for area, data. Differences between specific edible cultivars do not seem consistent throughout all experiments, and therefore seem less important than the prevalent data showing combined basil preference over other crop types. The observation that ‘Lemon’ basil seemed less preferred than the other two basil cultivars in the field count experiments was not supported in the no-choice experiments. However, this in itself may be important. Maybe AGBs are less attracted to ‘Lemon’ basil than the other cultivars, but, once on the plant, will feed in a similar fashion. A multiple-choice laboratory feeding test including all three cultivars of basil could be used to further test AGB preference for each cultivar. An olfactometer bioassay can be done to determine if plant volatiles play a role in basil cultivar attractiveness that may differ from the beetle’s plant utilization as food.

Regarding landscape plants, red maple was statistically shown to be preferred over sugar maple, and it seems that arrowwood viburnum and possibly elderberry are also preferred over sugar maple, but this is not statistically verified, nor are other differences that may exist between landscape plants. More replications would likely lead to an ability to distinguish more differences between the six landscape plants tested, and between more cultivars of crop plants. It appears that mass and area measurements produce similar results with similar levels of precision for no-choice laboratory feeding tests, and therefore the use of either measurement may be recommended in the absence of extreme differences of leaf characteristics from those used in this experiment.

Conclusion

This project's main objective has been to study the relationship between adult Asiatic garden beetles and commonly used or interesting cultivars of nine crop plants claimed to be heavily damaged by or preferred food plants of AGBs (Appendix A), in addition to six commonly used, native landscape plants, one of which (viburnum) is claimed to be a preferred food plant (Hallock, 1936a). These studies have approached the concept of adult AGB host plant feeding preference in a more controlled and quantitative manner than has previously been done, except for one study that included only one replication of each plant type (Hallock, 1936b), which limits the reliability of its results. This study is also the first to test for differences in AGB preference between cultivars of the same plant species.

The common garden field count experiment was partially based on Hallock's (1936b) one previous quantitative AGB plant preference study. This experiment provided beetles with a fairly natural situation and a choice of plants to use, and produced data indicating on which plants the beetles chose to locate themselves, which presumably may be correlated with feeding preference, as at least some of the beetles were observed feeding on the plants on which they were counted. The no-choice laboratory feeding experiment was based on similar feeding experiments performed on Japanese beetles (Ladd, 1987, 1989; Miller & Ware, 1999; Spicer et al., 1995). The data collected from this experiment were actual measurements of leaf matter consumed by AGB adults. These measurements indicate the potential of AGBs to feed on the experimental plant types, which is presumably related to plant preference, although beetles did not choose which plant to feed on, only how much of it to consume. The no-choice aspect of the laboratory feeding tests reduced the chances that the most favored food plant would draw attention away from slightly less favored plants, limiting our understanding of AGB preferences.

The results of both the field count study and the no-choice laboratory feeding study, when considered together, indicate that basil is likely to be favored as a food plant over beet and kohlrabi plants, although beet might be favored in some situations, and, with somewhat less certainty, basil appears favored over carrot, eggplant, parsnip, hot and sweet pepper, and turnip plants, at least when considering the three cultivars of each that were tested. To be more certain of this, it would be useful to perform a no-choice laboratory feeding experiment including all carrot, eggplant, parsnip, pepper, and turnip cultivars in addition to the basil, beet, and kohlrabi cultivars that have already been tested using this experiment type.

The results of these experiments also suggest that 'Lemon' basil may be less favored than 'Mexican Spice' basil (Figure 6), at least in terms of plant use in the field, if not in terms of feeding preference. The results of the no-choice laboratory feeding tests also suggest that more research should be done on AGB preference for beets, as they might be fed on as much as basil and more than kohlrabi in certain conditions (Figure 14). It is interesting and unexpected to have basil be the only clear favorite in these experiments when carrot, red pepper, and turnip were listed by Hallock (1936a) as preferred, as noted by visual observations, in addition to basil being listed as a favorite by Pundt and Smith (2005), presumably also as a result of visual observations. It is particularly interesting that, in the field count experiment, the other crop cultivars were hardly visited by AGBs despite being listed as heavily damaged by Hallock (1934, 1936b). Perhaps this was due to the number of AGBs on basil taking away from their presence on other plants. Regarding landscape plants, red maple is favored over sugar maple, and it appears that viburnum and possibly elderberry may also be favored over sugar maple; some other differences may exist between plant types tested, but Tukey-Kramer tests were unable to verify this, so a repeated no-choice laboratory feeding experiment with an increased number of replications is

recommended. Another useful follow-up to these experiments would be to repeat a common garden field count experiment, without including any basil cultivars, to see if more beetles are found on the other tested cultivars if they are not distracted by the basil. Differences between plant families and leaves of different color were not investigated due to the small number of significant differences found in these experiments.

If repeated or continued with a different choice of plants, the methods used in both experiment types in this study could be improved. The common garden field count experiment can be improved by more closely observing the hours during which the AGB is active, and potentially beginning beetle counts earlier in the night. Counting from earlier in the field season might also provide more useful information. Additionally, including a separate field count experiment in which AGBs and plants are enclosed in field cages would allow researchers to artificially create a higher population density, and would also allow estimates of feeding damage to be made in the field. The no-choice laboratory feeding experiment could be improved by finding the ideal temperature pattern and photoperiod that mimics the AGB's natural experience while allowing for moderately high activity levels, possibly adding a refuge to the experimental petri dishes, storing leaves for a shorter period, using slightly larger leaf disks, and keeping each petri dish in a separate, sealed plastic bag.

Using information gathered from the experiments detailed in this thesis, a recommendation can be made to monitor AGBs near basil plants in areas where the beetle is known to occur. More studies are required to determine the damage levels that AGBs can cause to basil plants, and the amount of AGB damage that affects the health of the basil plant or its value to humans, but, among eight other favored food plants of the AGB, basil appears to be the most in danger of severe attack. In cases of large AGB populations and severe AGB damage to

basil, it may be best to avoid growing the plant, although other measures, such as the use of row covers or pesticide sprays, may be used. Further research testing more cultivars of basil could provide insight into choices that are more resistant to AGB feeding. It is currently unknown whether the presence of basil in an area might attract more AGBs adults to the area and increase their feeding on other plants in the vicinity or the number of AGB eggs laid nearby, or if the presence of basil in an area might distract AGBs from damaging nearby plants, regardless of the possible increase in local population size encouraged by basil presence. The results of the common garden field count experiment suggest the latter occurrence, but, before recommending the use of basil as a trap or perimeter crop, or recommending the elimination of basil in vegetable gardens and fields containing other AGB-susceptible crops, more research should be done regarding the indirect effects of basil presence on damage to nearby plants.

More research is recommended on different plant types that may be attacked by AGBs, in addition to a deeper investigation into basil. As mentioned in Chapter 1, the AGB is nocturnal, and therefore may cause more damage than is understood. If plant leaves are being eaten from the outside edges inward by an unknown pest in an area where the AGB is known to exist (Figure 1), then it is recommended to dig in the soil near the base of the plant or to examine the plant by flashlight on a warm night 21°C (70°F) or higher to look for adult AGBs. Information from investigations of this type might help point to plants that would be relevant for inclusion in further AGB preference studies, including common garden field count and no-choice laboratory feeding experiments similar to those detailed in this thesis. Plants such as basil, that seem especially preferred, should be further studied to develop ways to incorporate that preference information into management strategies, such as using basil to monitor AGB populations, using it as a trap or perimeter crop, or eliminating it from areas with high AGB populations. Cultivars

of favored AGB food plants that are especially resistant could be recommended for use in areas with many AGBs, and could also be included in chemical ecology or plant breeding studies that could lead to the discovery of more AGB-resistant plants, their development, or even the creation of AGB repellents.

More research is needed before a new list of AGB preferences, and planting recommendations for areas of high AGB density, such as those for the Japanese beetle (USDA APHIS, 1998) can be published, but, with the existing information gained from these experiments, a recommendation can be made to further investigate the relationship between the Asiatic garden beetle and different cultivars of basil, and to be wary of AGB damage on basil plants. These experiments have re-opened the investigation into the adult Asiatic garden beetle, a topic that has been little studied since Hallock's research in the 1930s. With further study, it is possible that we will have a better understanding of this beetle and the ways in which it can be managed as an invasive pest.

Appendix A
Food plants of the adult Asiatic garden beetle

The foliage of all listed plants is eaten, except for gladiolus. ⚘Presence of flower symbol indicates that flowers are also eaten.

Preferred/always consumed

Plant	Category	Source
Ailanthus	ornamental shrub/tree	Hallock, 1936a
Aster⚘	flower	Hallock, 1936a
Basil	herb	Pundt and Smith, 2005
Carrot	vegetable	Hallock, 1936a
Chrysanthemum	flower	Hallock, 1936a
Dahlia⚘	flower	Hallock, 1936a
Devils-walkingstick	ornamental shrub/tree	Hallock, 1936a
Gerbera, flame-ray⚘	flower	Hallock, 1936a
Hemp	flower	Hallock, 1936a
Ragweed, common	weed	Hallock, 1936a
Ragweed, great	weed	Hallock, 1936a
Redpepper	vegetable	Hallock, 1936a
Rose⚘	ornamental shrub/tree	Hallock, 1936a
Strawflower⚘	flower	Hallock, 1936a
Sumac	ornamental shrub/tree	Hallock, 1936a
Sunflower⚘	flower	Hallock, 1936a
Turnip	vegetable	Hallock, 1936a
Viburnum	ornamental shrub/tree	Hallock, 1936a

Consumed when beetles are abundant/in absence of preferred plants

Plant	Category	Source
Ageratum	flower	Hallock, 1936a
Aquilegia	flower	Hallock, 1936a
Barberry, Japanese	ornamental shrub/tree	Hallock, 1936a
Bean	vegetable	Hallock, 1936a
Beet	vegetable	Hallock, 1936a
Beggarticks	weed	Hallock, 1936a
Begonia	flower	Hallock, 1936a
Blackberry	fruit	Hallock, 1936a; Hamilton, 1929
Blueberry	fruit	Hallock, 1936a
Boxelder	ornamental shrub/tree	Hallock, 1936a

Broccoli	vegetable	Hallock, 1936a
Burdock	weed	Hallock, 1936a
Butterflybush	ornamental shrub/tree	Hallock, 1936a
Cabbage	vegetable	Hallock, 1936a
Canna	flower	Hallock, 1936a
Castor-bean	flower	Hallock, 1936a
Catalpa	ornamental shrub/tree	Hallock, 1936a
Chard, Swiss	vegetable	Hallock, 1936a
Cherry	fruit	Hallock, 1936a
Cherry, Oriental	ornamental shrub/tree	Hallock, 1936a
Clover, white	forage	Hallock, 1936a
Cocklebur	weed	Hallock, 1936a
Currant	fruit	Hallock, 1936a
Eggplant	vegetable	Hallock, 1936a
Fleabane, daisy	weed	Hallock, 1936a
Forsythia	ornamental shrub/tree	Hallock, 1936a
Four-o'clock	flower	Hallock, 1936a
Foxglove	flower	Hallock, 1936a
Gaillardia	flower	Hallock, 1936a
Geranium	flower	Hallock, 1936a
Gladiolus (⚲ only)	flower	Hallock, 1936a
Goldenglow⚲	flower	Hallock, 1936a
Goldenrod	weed	Hallock, 1936a; Hamilton, 1929
Groundcherry, lantern	flower	Hallock, 1936a
Hollyhock	flower	Hamilton, 1929
Horsechestnut	ornamental shrub/tree	Hallock, 1936a
Hydrangea	ornamental shrub/tree	Hallock, 1936a
Kohlrabi	vegetable	Hallock, 1936a
Lambsquarters	weed	Hallock, 1936a
Larkspur⚲	flower	Hallock, 1936a
Lettuce	vegetable	Hallock, 1934
Locust	ornamental shrub/tree	Hallock, 1936a
Magnolia	ornamental shrub/tree	Hallock, 1936a
Mallow, velvetleaf	weed	Hallock, 1936a
Maple, silver	ornamental shrub/tree	Hallock, 1936a
Mockorange	ornamental shrub/tree	Hallock, 1936a
Morning-glory	flower	Hallock, 1936a
Mulberry	fruit	Hallock, 1936a
Orchid⚲	flower	Hallock, 1936a
Parsley	vegetable	Hallock, 1934

Parsnip	vegetable	Hallock, 1936a
Pea	vegetable	Hallock, 1934
Peach	fruit	Hallock, 1936a
Phlox⚘	flower	Hallock, 1936a
Pigweed	weed	Hallock, 1936a
Plantain	weed	Hallock, 1936a
Plum	fruit	Hallock, 1936a
Privet	ornamental shrub/tree	Hallock, 1936a
Pussywillow, *Salix* spp.*	ornamental shrub/tree	Hamilton, 1929
Radish	vegetable	Hallock, 1936a
Rhubarb	vegetable	Hallock, 1936a
Sage, scarlet	flower	Hallock, 1936a
Shrub-althea	ornamental shrub/tree	Hallock, 1936a
Smartweed	weed	Hallock, 1936a
Snapdragon	flower	Hallock, 1936a
Spinach	vegetable	Hallock, 1936a
Strawberry	fruit	Hallock, 1936a
Sweetpotato	vegetable	Hallock, 1936a
Willow	ornamental shrub/tree	Hallock, 1936a
Zinnia⚘	flower	Hallock, 1936a; Hamilton, 1929

Minimally/never consumed

Plant	Category	Source
Corn	vegetable	Hallock, 1936b
Hemlock seedling	nursery	Hallock, 1932
Orange hawkweed	weed	Hallock, 1936b
Peanut	x	Hallock, 1936b
Pine seedling	nursery	Hallock, 1932
Potato	vegetable	Hallock, 1936b
Sorrel	weed	Hallock, 1936b
Tomato	x	Hallock, 1936b
Yew seedling	nursery	Hallock, 1932

References

Abd El-Aziz, S.E., H.A. Salem, and S.F. Fahim. 2006. Effectiveness of certain nutrition, visual and olfaction cues in laboratory and field on the behaviour of *Epicometic (Tropinota) squalida* (Scop.) (Coleoptera: Scarabaeidae: Cetoniinae). *Annals of Agricultural Science (Cairo)*, 51(1): 283-293.

Ahrens, D. 2007. Taxonomic changes and an updated catalogue for the Palaearctic Sericini (Coleoptera: Scarabaeidae: Melolonthinae). *Zootaxa*, 1504: 1-51.

Arrow, G.J. 1913. Notes on the Lamellicorn Coleoptera of Japan and descriptions of a few new species. *The Annals and Magazine of Natural History*, 12: 394-408.

Australian Government, Department of Agriculture, Fisheries and Forestry. 2004. *Maladera castanea* (Arrow, 1913) [Coleoptera: Scarabaeidae]. *Longan and Lychee Fruit from the People's Republic of China and Thailand: Final Import Risk Analysis Report, Part B*. Commonwealth of Australia: 91-93.

Ballina-Gómez, H.S., S. Iriarte-Vivar, R. Orellana, and L.S. Santiago. 2008. Crecimiento, supervivencia y herbivoría de plántulas de *Brosimum alicastrum* (Moraceae), una especie del sotobosque neotropical (Growth, survival and herbivory of seedlings in *Brosimum alicastrum* [Moraceae], a species from the Neotropical undergrowth). *Revista de Biología Tropical*, 56(4): 2055-2067.

Bell, C.H. and P.R. Kerslake. 1986. A circadian rhythm influencing foraging behavior in the saw-toothed grain beetle *Oryzaephilus surinamensis*. *Physiological Entomology*, 11: 1-6.

Bixby, A., S.R. Alm, K. Power, P. Grewal, and S.R. Swier. 2007. Susceptibility of four species of turfgrass-infesting scarabs (Coleoptera: Scarabaeidae) to *Bacillus thuringiensis* serovar *japonensis* strain Buibui. *Journal of Economic Entomology*, 100(5): 1604-1610.

Brandenburg, R.L. and J.R. Baker. 1994. Major insect pests of turf in the U.S. *Handbook of Integrated Pest Management for Turf and Ornamentals*. Ed. A.R. Leslie. CRC Press, LLC, Boca Raton, FL: 219-236.

Brandenburg, R.L. and M.G. Villani, eds. 1995. Asiatic garden beetle. *Handbook of Turfgrass Insect Pests*. Entomological Society of America, Lanham, MD: 26-28.

Britton, W.E. 1930. The Asiatic garden beetle in Connecticut: *Aserica castanea* Arrow. *Twenty-Ninth Report: Connecticut State Entomologist 1929* (*Bulletin 315*). Connecticut Agricultural Experiment Station, New Haven, CT: 607-608.

Britton, W.E. 1934. Damage by the Asiatic or Japanese garden beetle. *Connecticut State Entomologist Thirty-third Report 1933* (*Bulletin 360*). Connecticut Agricultural Experiment Station, New Haven, CT: 466-468.

Capinera, J.L. 2001. Asiatic garden beetle. *Handbook of Vegetable Pests*. Academic Press, San Diego, California: 172-174.

Cappaert, D.L. and D.R. Smitley. 2002. Parasitoids and pathogens of Japanese beetle (Coleoptera: Scarabaeidae) in southern Michigan. *Environmental Entomology*, 31(3): 573-580.

Cavanagh, A.F., L.S. Adler, and R.V. Hazzard. 2010. Buttercup squash provides a marketable alternative to Blue Hubbard as a trap crop for control of striped cucumber beetles (Coleoptera: Chrysomelidae). *Environmental Entomology*, 39(6): 1953-1960.

Chandrasena, D., C. DiFonzo, and D. Wang. 2012. An assessment of Japanese beetle defoliation on aphid-resistant and aphid-susceptible soybean lines. *Crop Science*, 52: 2351-2357.

Chantal, C. 2003. Six nouvelles mentions québécoises de coléoptères. *Fabreries*, 28(1): 25-30.

Cutler, G.C. and R.E.L. Rogers. 2009. New record of the Asiatic garden beetle, *Maladera castanea* (Arrow), in Atlantic Canada. *Journal of the Entomological Society of Ontario*, 140: 40-45.

Eckman, L.E. 2011. Personal observation.

Estes, K. and S. McLaughlin. 2010. Asiatic garden beetle. *Home, Yard & Garden Pest Newsletter*. University of Illinois, Cooperative Extension Service, Issue 13. Accessed October 5, 2011, http://hyg.ipm.illinois.edu/pdf/hygpest201013.pdf.

Fleming, W.E. 1972. Biology of the Japanese beetle. *Technical Bulletin 1449*. United States Department of Agriculture, Agricultural Research Service: 1-129.

Furutani, S.C. and L.H. Arita. 1990. Effect of light exposure and carbohydrate content of snap bean leaves on Chinese rose beetle (Coleoptera: Scarabaeidae) feeding. *Journal of Economic Entomology*, 83(5): 2022-2025.

Grewal, P.S., S.K. Grewal, V.S. Malik, and M.G. Klein. 2002. Differences in susceptibility of introduced and native white grub species to entomopathogenic nematodes from various geographic localities. *Biological Control*, 24: 230-237.

Hallock, H.C. 1929. Known distribution and abundance of *Anomala orientalis* Waterhouse, *Aserica castanea* Arrow, and *Serica similis* Lewis in New York. *Journal of Economic Entomology*, 22: 293-299.

Hallock, H.C. 1930. Some observations upon the biology and control of *Aserica castanea* Arrow. *Journal of Economic Entomology*, 23: 281-286.

Hallock, H.C. 1931. Recent observations on distribution and abundance of *Anomala orientalis* Waterhouse and *Aserica castanea* Arrow in New York. *Journal of Economic Entomology*, 24: 204-212.

Hallock, H.C. 1932. Life history and control of the Asiatic garden beetle. *United States Department of Agriculture Circular 246*: 1-16.

Hallock, H.C. 1933. Present status of two Asiatic beetles (*Anomala orientalis* and *Autoserica castanea*) in the United States. *Journal of Economic Entomology*, 26: 80-85.

Hallock, H.C. 1934. The Asiatic garden beetle as a pest in vegetable gardens. *Journal of Economic Entomology*, 27: 476-481.

Hallock, H.C. 1936a. Life history and control of the Asiatic garden beetle. Ed. I.M. Hawley and H.C. Hallock. *United States Department of Agriculture Circular 246.* Rev. ed.: 1-20.

Hallock, H.C. 1936b. Notes on biology and control of the Asiatic garden beetle. *Journal of Economic Entomology*, 29(2): 348-356.

Hallock, H.C. 1936c. Recent developments in the use of electric light traps to catch the Asiatic garden beetle. *Journal of the New York Entomological Society*, 44(4): 261-279.

Hamilton, C.C. 1929. *Aserica castanea* Arrow, a recently recorded scarabaeid beetle injurious in New Jersey. *N.J. Agricultural Experiment Station Annual Report*: 203-207.

Hammond, R. not dated. Asiatic garden beetle could be cause for concern for northern Ohio corn. *About Us: News*. The Ohio State University, Department of Entomology. Accessed September 15, 2014, http://entomology.osu.edu/about-us/news/asiatic-garden-beetle-could-be-cause-concern-northern-ohio-corn.

Held, D.W. 2004. Relative susceptibility of woody landscape plants to Japanese beetle (Coleoptera: Scarabaeidae). *Journal of Arboriculture*, 30(6): 328-335.

Held, D.W. and C.H. Ray. 2009. Asiatic garden beetle *Maladera castanea* (Coleoptera: Scarabaeidae) grubs found in damaged turf in Alabama. *Florida Entomologist*, 92(4): 670-672.

Heller, P.R. 1995. Asiatic garden beetle. *Handbook of Turfgrass Insect Pests*. Eds. R.L. Brandenburg and M.G. Villani. Entomological Society of America, Lanham, MD: 26-28.

Invasive Species Advisory Committee (ISAC), Definitions Subcommittee. 2006. *Invasive species definition clarification and guidance white paper*. National Invasive Species Council (NISC). Accessed October 5, 2011, http://www.invasivespecies.gov/global/ISAC/ISAC_documents/ISAC%20Definititions%20White%20Paper%20%20-%20FINAL%20VERSION.pdf.

Jones, C.G. and J.S. Coleman. 1988. Leaf disc size and insect feeding preference: implications for assays and studies on induction of plant defense. *Entomologia Experimentalis et Applicata*, 47: 167-172.

Juliani, H.R. and J.E. Simon. 2002. Antioxidant activity of basil. *Trends in New Crops and New Uses*. Eds. J. Janick and A. Whipkey. ASHS Press, Alexandria, VA: 575-579.

Khan, A., W.M. Brooks, and H. Hirschmann. 1976. *Chromonema heliothidis* n. gen., n. sp. (Steinernematidae, Nematoda), a parasite of *Heliothis zea* (Noctuidae, Lepidoptera) and other insects. *Journal of Nematology*, 8(2): 159-168.

Koppenhöfer, A.M. 2010. An integrated approach to insect management in turfgrass: white grubs. *Rutgers New Jersey Agricultural Experiment Station Fact Sheet FS1009*.

Koppenhöfer, A.M., R.S. Cowles, E.A. Cowles, E.M. Fuzy, and L. Baumgartner. 2002. Comparison of neonicotinoid insecticides as synergists for entomopathogenic nematodes. *Biological Control*, 24: 90-97.

Koppenhöfer, A.M., R.S. Cowles, and E.M. Fuzy. 2003. Effects of turfgrass endophytes (Clavicipitaceae: Ascomycetes) on white grub (Coleoptera: Scarabaeidae) larval development and field populations. *Environmental Entomology*, 32(4): 895-906.

Koppenhöfer, A.M. and E.M. Fuzy. 2003a. Biological and chemical control of the Asiatic garden beetle, *Maladera castanea* (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, 96(4): 1076-1082.

Koppenhöfer, A.M. and E.M. Fuzy. 2003b. *Steinernema scarabaei* for the control of white grubs. *Biological Control*, 28: 47-59.

Koppenhöfer, A.M. and E.M. Fuzy. 2004. Effect of white grub developmental stage on susceptibility to entomopathogenic nematodes. *Journal of Economic Entomology*, 97(6): 1842-1849.

Koppenhöfer, A.M., E.M. Fuzy, R.L. Crocker, W.D. Gelernter, and S. Polavarapu. 2004. Pathogenicity of *Heterohabditis bacteriophora*, *Steinernema glaseri*, and *S. scarabaei* (Rhabditida: Heterorhabditidae, Steinernematidae) against 12 white grub species (Coleoptera: Scarabaeidae). *Biocontrol Science and Technology*, 14(1): 87-92.

Koppenhöfer, A.M., P.S. Grewal, and E.M. Fuzy. 2006. Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, and *Steinernema scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turfgrass in North America. *Biological Control*, 38: 397-404.

Lachowicz, K.J, G.P. Jones, D.R. Briggs, F.E. Bienvenu, M.V. Palmer, V. Mishra, and M.M. Hunter. 1997. Characteristics of plants and plant extracts from five varieties of basil (*Ocimum basilicum* L.) grown in Australia. *Journal of Agricultural and Food Chemistry*, 45: 2660-2665.

Ladd, T.L., Jr. 1987. Japanese beetle (Coleoptera: Scarabaeidae): influence of favored food plants on feeding response. *Journal of Economic Entomology*, 80(5): 1014-1017.

Ladd, T.L., Jr. 1989. Japanese beetle (Coleoptera: Scarabaeidae): feeding by adults on minor host and nonhost plants. *Journal of Economic Entomology*, 82(6): 1616-1619.

Legrand, A. 2010. Personal observation.

Lemoine, N.P., W.A. Drews, D.E. Burkepile, and J.D. Parker. 2013. Increased temperature alters feeding behavior of a generalist herbivore. *Oikos*, 122: 1669-1678.

MacKellar, B. 2012. Asiatic garden beetle larval feeding was more widespread in southwest Michigan in 2011: how to determine if your corn field is at risk of Asiatic garden beetles in 2012. Michigan State University Extension, Accessed September 15, 2014, http://msue.anr.msu.edu/news/asiatic_garden_beetle_larval_feeding_was_more_widespread_in_southwest_michi.

Marlatt, C.L. and H.A. Wallace. 1933. Japanese and Asiatic beetles. *Report to the Chief of the Bureau of Entomology*. United States Department of Agriculture, Bureau of Entomology: 12-14.

Metcalf, R.L. and R.A. Metcalf. 1993. Chapter 14: Insects injurious to vegetable gardens and truck crops. *Destructive and Useful Insects: Their Habits and Control*. McGraw-Hill, Inc., R.R. Donnelley & Sons Company, USA.

Meyer-Rochow, V.B. and N. Gokan. 1987. Fine structure of the compound eye of the Asiatic garden beetle *Maladera castanea* Arrow (Coleoptera: Scarabaeidae). *Applied Entomology and Zoology*, 22(3): 358-369.

Miller, F. and G. Ware. 1999. Feeding preference for selected *Tilia* spp. and cultivars by the adult Japanese beetle (Coleoptera: Scarabaeidae). *Journal of Arboriculture*, 25(3): 168-172.

Morales-Rodriguez, A., A. Ospina, and D.C. Peck. 2010. Variation in the laboratory susceptibility of turf-infesting white grubs (Coleoptera: Scarabaeidae) to biological, biorational and chemical control products. *Pest Management Science*, 66: 90-99.

Nielsen, G.R. 1997. Asiatic garden beetle. *EL 247*. University of Vermont Extension, Department of Plant and Soil Science. Accessed June 27, 2013, http://www.uvm.edu/pss/ppp/pubs/el247.htm.

Niziolek, O.K., M.R. Berenbaum, and E.H. DeLucia. 2013. Impact of elevated CO_2 and increased temperature on Japanese beetle herbivory. *Insect Science*, 20: 513-523.

Nurzyńska-Wierdak, R. 2013. Morphological and chemical variability of *Ocimum basilicum* L. (Lamiaceae). *Modern Phytomorphology*, 3: 115-118.

Parra, L., A. Mutis, R. Ceballos, M. Lizama, F. Pardo, F. Perich, and A. Quiroz. 2009. Volatiles released from *Vaccinium corymbosum* were attractive to *Aegorhinus superciliosus* (Coleoptera: Curculionidae) in an olfactometric bioassay. *Environmental Entomology*, 38(3): 781-789.

Phillips, W.M. 1977. Modification of feeding 'preference' in the flea-beetle, *Haltica lythri* (Coleoptera, [sic] Chrysomelidae). *Entomologia Experimentalis et Applicata*, 21: 71-80.

Pierce, C.M.F. Asiatic garden beetle. *Indiana's Most Unwanted Invasive Plant Pests*. Purdue University, Entomology Department, Indiana Cooperative Agricultural Pest Survey (CAPS) Program. Updated November 4, 2009. Accessed May 27, 2011, http://extension.entm.purdue.edu/caps/pestInfo/asiaticGardenbtl.htm.

Potter, D.A. 1991. Ecology and management of turfgrass insects. *Annual Review of Entomology*, 36: 383-406.

Pundt, L. and T. Smith. 2005. Beetles. *Pest Management for Herb Bedding Plants Grown in the Greenhouse*. University of Connecticut: 11.

Purdue University, Center for Environmental and Regulatory Information Systems (CERIS). 2014. Survey status of the Asiatic garden beetle – *Maladera castanea*. *Pest Tracker: Exotic Pest Reporting*. Accessed September 16, 2014, http://pest.ceris.purdue.edu/map.php?code=INBPAVA#.

Raupp, M.J. 2014. Midnight marauders: Asiatic garden beetles, *Maladera castanea*. *Bug of the Week*. University of Maryland Extension. Accessed September 15, 2014, http://bugoftheweek.com/blog/2014/7/21/midnight-marauders-asiatic-garden-beetles-imaladera-castaneai.

Risch, S.J. 1985. Effects of induced chemical changes on interpretation of feeding preference tests. *Entomologia Experimentalis et Applicata*, 39: 81-84.

Rowe, W.J., D.A. Potter, and R.E. McNiel. 2002. Susceptibility of purple- versus green-leaved cultivars of woody landscape plants to the Japanese beetle. *HortScience*, 37(2): 362-366.

Ruther, J. and C.J. Mayer. 2005. Response of garden chafer, *Phyllopertha horticola*, to plant volatiles: from screening to application. *Entomologia Experimentalis et Applicata*, 115: 51-59.

Shetlar, D. and H.D. Niemczyk. 1999. Asiatic garden beetle. The Ohio State University Extension Entomology. Accessed June 27, 2013, http://bugs.osu.edu/bugdoc/Shetlar/factsheet/turf/Asiaticgardenbeetle.htm.

Sideman, E. 2008. Maine Organic Farmers and Gardeners Association 2008 Pest Reports, June 11. Accessed June 27, 2013, http://www.mofga.org/Publications/PestReports/PestReports2008/tabid/926/Default.aspx?PageContentID=278.

Silva, A.K., G.L. Conçalves, G.R.P. Moreira. 2014. Larval feeding choices in heliconians: induced preferences are not constrained by performance and host plant phylogeny. *Animal Behaviour*, 89: 155-162.

Skelley, P.E. 2012. The Asiatic garden beetle, *Maladera castanea* (Arrow 1913) (Coleoptera: Scarabaeidae), a white grub pest new to Florida. *Entomology Circular Number 425* (*FDACS-P-01823*). Florida Department of Agriculture and Consumer Services, Division of Plant Industry.

Spicer, P.G., D.A. Potter, and R.E. McNiel. 1995. Resistance of flowering crabapple cultivars to defoliation by the Japanese beetle (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, 88(4): 979-985.

Tashiro, H. 1987. Scarabaeid pests: subfamily Melolonthinae. *Turfgrass Insects of the United States and Canada*. Cornell University Press, Ithaca, NY: 156-192.

United States Department of Agriculture, Animal and Plant Health Inspection Service (APHIS). 1998. *Managing the Japanese Beetle: A Homeowner's Handbook*.

United States Department of Agriculture, Bureau of Entomology. 1933. Asiatic garden beetle. *Insect Pest Survey Bulletin*, 13(7): 228.

Vittum, P.J. 2011. Current insecticide approaches for white grub control. UMass Extension Turf Program. Updated June, 2011. Accessed November 24, 2011, http://extension.umass.edu/turf/fact-sheets/current-insecticide-approaches-white-grub-control.

Wang, Q., G. Shi, and L.K. Davis. 1998. Reproductive potential and daily reproductive rhythms of *Oemona hirta* (Coleoptera: Cerambycidae). *Journal of Economic Entomology*, 91(6): 1360-1365.

Werner, R.A. 1972. Aggregation behavior of the beetle, *Ips grandicollis*, in response to combinations of host-produced attractants. *Journal of Insect Physiology*, 18: 423-437.

Werner, R.A. 1995. Toxicity and repellency of 4-allylanisole and monoterpenes from white spruce and tamarack to the spruce beetle and eastern larch beetle (Coleoptera: Scolytidae). *Environmental Entomology*, 24(2): 372-379.

Wolfe, D.W., L. Ziska, C. Petzoldt, A. Seaman, L. Chase, and K. Hayhoe. 2008. Projected change in climate thresholds in the Northeastern U.S.: implications for crops, pests, livestock, and farmers. *Mitigation and Adaptation Strategies for Global Change*, 13: 555-575.

50states.com. U.S. States (blank map). Accessed October 8, 2011, www.50states.com/maps/usamap.gif. Used to create Figure 1; AGB distribution map.