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Preliminary Feeding Assessments for Asiatic Garden Beetle, Maladera formosae (Coleoptera: Scarabaeidae), Grubs and Adults

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Preliminary Feeding Assessments for Asiatic Garden Beetle, Maladera formosae (Coleoptera: Scarabaeidae), Grubs and Adults

Cover Page Footnote
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Abstract

The Asiatic garden beetle, *Maladera formosae* (Brenske) (syn. *M. castanea* [Arrow]) (Coleoptera: Scarabaeidae), is an annual white grub species that was unintentionally introduced from east Asia to North America in 1921 in New Jersey, and has since spread to at least 25 states and two Canadian provinces. Grub populations in the Great Lakes region have recently emerged as significant early-season pests of field crops, particularly field corn, grown in sandy soils. Asiatic garden beetle has also recently become established in other regions including Alabama. Prior research on this species was conducted mainly in the 1930s in horticultural and turfgrass systems of New York and New Jersey. In this study, we document Asiatic garden beetle preference and performance on previously un-investigated food resources, in populations from Ohio and Alabama. The objectives of these experiments were to a) understand if grubs show preference to potential diet choices present in a typical Ohio corn-soybean rotation, and gained mass when provided a single diet, and b) to conduct a preliminary assessment on the development, survival, and fecundity of field-collected beetles on different diets present in suburban Alabama. In general, grubs were more likely to be found at corn and marestail and they significantly increased in body mass when subjected to those diets. However, they were also able to survive and gain mass when provided soybean, crop residues or bare soil. Adults consumed more rose flower petals than floral tissue of white clover and Queen Anne’s lace. In no-choice trials, only females that were fed a diet of rose petals laid eggs, and diet consumption rates were similar among males and females. These findings provide insight into the feeding behaviors of Asiatic garden beetle grubs and adults collected from novel environments.

Keywords: Asiatic garden beetle, annual white grub, Scarab, feeding preference, feeding performance

The Asiatic garden beetle (AGB), *Maladera formosae* (Brenske 1898) (syn. *Maladera castanea* [Arrow 1913]) (Coleoptera:Scarabaeidae: Melolonthinae), an annual white grub endemic to east Asia (Fabrizi et al. 2021), was first reported in North America in 1921 in a nursery near Rutherford, New Jersey (Hallock 1929). Historically a pest of ornamentals, turfgrass (Hallock 1929), and vegetables (Hallock 1934), most previous AGB research focused on the adult stage in horticultural settings in New York (Long Island) and New Jersey in the 1930s because adult feeding was the primary cause of economic damage in those regions. AGB adults are active in the summer and feed on the foliage and flowers of over 100 plant species (Hallock 1936a; b; MacKellar 2011; MacKellar and DiFonzo 2011). Since its introduction, AGB has been reported in at least 25 states and two Canadian provinces and has become a pest in new habitats (Eckman 2015). For instance, the grubs have emerged as a significant pest of field crops in the past 15 years, particularly field corn, *Zea mays* L., in sandy soils of northern Ohio (Turner 2013), northern Indiana (Krupke et al. 2007) and southern Michigan (DiFonzo 2007). In the expanding North American range, little is known about the preferred host ranges of grubs and adults and how diet affects overall fitness (e.g., increases in food consumption, mass gain, fecundity, and survival). Adults are attracted to common agricultural weed...
species like marestail (*Erigeron canadensis* L.) and giant ragweed (*Ambrosia trifida* L.), and Queen Anne’s lace (*Daucus carota* L.) which is commonly found along field edges, as evidenced by extensive defoliation and the presence of adults in the soil under the plant (personal observation, A.P., A.R.). It is likely mated females are laying their eggs near these hosts (Heller 1995, Costa 2018) and that these grubs will feed on the crop in spring of the following year. Previous studies have shown that adult beetle preference for host plant species can vary from year to year (Hallock 1932, 1936a; Eckman 2015) and among plant cultivars (Eckman 2015). Furthermore, Hallock (1931) showed that males were initially more abundant on plants in the summer and accounted for approximately 70% of foliar damage, while females were more likely to be found in sod or under preferred plants for egg laying (Hallock 1932).

The goal of this study was to provide preliminary assessments of AGB grub and adult host preferences in the extended geographic range. The first objective was to understand if grubs a) showed preference for potential diet choices present in a corn-soybean rotation, a common agricultural system in northwest Ohio which experiences AGB infestation, and b) gained mass when provided a specific diet. The second objective was to conduct a preliminary assessment on the development, survival, and fecundity of field-collected beetles on different diets in Alabama.

**Materials and Methods**

**Grub Diet Choice and Performance**

Third instar grubs were hand collected from an infested corn field in Wauseon, OH, in late May 2019 by sieving through the soil with bare hands and placing grubs in a zip-lock bag filled with soil. Active third instars were chosen since they are present in the springtime when infestations in corn occur; prepupal grubs were readily distinguished by their lethargic behavior, bloated body, and solid cream color and were not chosen. Grubs were brought back to the laboratory and temporarily kept under refrigeration at 10 °C until the start of the experiment.

**Experiment 1: Grub Diet Choice Test.** We performed a choice test (Crutchfield and Potter 1994, Stevens et al. 2007) in early June 2019 to assess grub response to four different diet options present in a corn-soybean crop rotation—roots of corn, soybean (*Glycine max* [L.] Merr.) and marestail (a common weed in this system), and corn residue, in addition to a soil-only control. Each replicate, or arena (Fig.1A), consisted of a clear circular plastic container (12.7 × 30.5 cm)
We established diet treatments in individual soybean, marestail, crop residue, and soil. Options evaluated in Experiment 1—corn, could feed and develop on the same five diet treatments was also sifted for grubs and any present were considered not to have made a choice. The remaining soil present between each treatment was recorded as making a difference of each arena (Fig. 1A). For the corn and soybean treatments, two clean seeds (i.e., no pesticide seed treatments) of each were planted directly in the arena before the start of the experiment and thinned to one plant following germination. The marestail treatment consisted of a 6-week-old plant grown from seed in the greenhouse. The marestail plants were older than the corn or soybean plants to adjust for differences in root size because the root system for young marestail plants is extremely fine relative to corn and soybean. Approximately 25 ml of crop residue or field soil was placed into a 5.1 cm diameter hole at its assigned place in the arena. Though container soil was autoclaved, the soil and crop residue treatments were not, to preserve the biological integrity of the offering. Debris and residues were sieved from the soil control. Once corn plants had one fully developed true leaf (i.e., V1 growth stage), and soybean plants had their first set of unifoliate leaves (i.e., VC growth stage), 10 randomly chosen third instar grubs were placed in the center of each arena (Fig. 1A). Grubs that did not bury themselves within one hour were considered unfit and replaced.

Soil moisture was checked with a soil moisture meter (EXTECH, Model #MO750, Taiwan) and maintained between 7–14% as grub species like Japanese beetle (Popillia japonica Newman) are sensitive to soil moisture extremes in sand (Régnière et al. 1981). A total of 12 arenas were maintained in the greenhouse at a temperature of 25.0 ± 3.0 °C and 14:10D natural photoperiod supplemented with lights before sunrise and after sunset as needed. Grub response (i.e., distribution) was assessed after 48 hours by cutting out a 5.1 cm diameter of soil around each treatment with a plastic tube and sifting the contents for the presence of grubs. Any grub found within a 5.1 cm diameter of each treatment was weighed (mg) on a scale and their lengths (mouth to raster along the back) measured (mm) by eye with a ruler before being placed individually into separate containers. Containers were maintained in the greenhouse under the same conditions described in Experiment 1. After two weeks, the containers were emptied and grub mortality, body mass (including gut contents), and length were recorded.

Adult Survival, Fecundity, and Consumption

Adult AGB were captured in Lee County, Alabama from June to July 2012. Most beetles were captured using ultraviolet light traps and Japanese beetle traps baited with the three-part, food-type lure (phenylethyl propionate [PEP], geraniol, and eugenol) (Held and Ray 2009). All traps (two light traps, four Japanese beetle traps) were checked daily and placed near turfgrass to maximize chances of collecting newly-eclosed, virgin adults. The vast majority of beetles (94%) were captured at one light trap which was standard throughout the experimental period. Beetle capture-rate (data not shown) was highest in early to mid-June and declined in July, suggesting an early summer flight period. Trapped beetles were kept alive before the experiment on a diet of canna lily flowers (Canna generalis Tropicana) flowers in a growth chamber set at 26.7 °C with a 14L:10D photoperiod. Canna lily was chosen because it has historically been reported as a preferred host of AGB (Hallock 1936a).

Experiment 2: Grub Diet Performance. We performed a no-choice test in June 2019 to determine whether AGB grubs could feed and develop on the same five diet options evaluated in Experiment 1—corn, soybean, marestail, crop residue, and soil. We established diet treatments in individual 300 ml Corning® Snap-Seal (No. 1730) plastic containers (Corning, Inc., Corning, NY) with a 64 mm diameter, filled about 2.5 cm from the top with autoclaved Ottokie Tedrow fine sand. Each container was assigned one of the five diet options and each diet was replicated 10 times (Fig. 1B). For the corn and soybean treatments, two clean seeds (i.e., no pesticide seed treatments) of each were planted and thinned to one plant following germination and allowed to grow to two true leaves (i.e., V2 growth stage) before grubs were placed into the containers. The marestail treatment consisted of a 6-month-old plant grown from seed in the greenhouse to account for differences in root size. The crop residue and bare soil diets were collected from the same field as the grubs. Individual third instar grubs were weighed (mg) on a scale and their lengths (mouth to raster along the back) measured (mm) by eye with a ruler before being placed individually into separate containers. Containers were maintained in the greenhouse under the same conditions described in Experiment 1. After two weeks, the containers were emptied and grub mortality, body mass (including gut contents), and length were recorded.
1936a), easily obtainable in the region of study, and represent three different plant families (Rosaceae, Apiaceae, and Fabaceae). All plants were locally sourced from nearby gardens or grassy lots. Six adult beetles including three male and three female, were randomly chosen, and provided with one of the four flower diets. Each flower was placed in a separate 473 ml plastic cup containing dampened soil (50% autoclaved soil and 50% peat moss), and one flower of each respective diet placed into a water-filled water pick (a plastic water container typically used to hold a single rose) that was inserted into the side (Fig. 2A–B). The no-flower control had an empty water pick with no flower. Flowers were replaced every three days or whenever they wilted. Mortality and the number of eggs laid per cup were recorded every seven days by carefully sifting through the soil for live beetles and/or eggs until there were no living beetles recovered (eight weeks). After each assessment, eggs and dead beetles were removed and the soil was replaced. The cups were maintained in a growth chamber at a constant temperature of 26.7 °C with a 14:10 photoperiod and soil was kept damp with distilled water.

**Figure 2.** No-choice test arenas to assess adult beetle survival and fecundity on different diets (A and B), and the no-choice test arenas used to assess adult feeding performance on rose petals (C).

**Experiment 4: Adult Food Consumption.** Immediately following Experiment 3, we conducted a no-choice test to examine the feeding rates of male and female beetles on rose petals. Wild-sourced beetles were individually placed in cups with rose petals and consumption was monitored for 24 hours (Fig. 2C). There were three treatments, each replicated six times—a female beetle with a rose petal, a male beetle with a rose petal, and a petal with no beetle (used to calculate petal weight reduction from water loss in the absence of feeding). First, we prepared each V-16 polypropylene cup (9.2 × 3.7 × 7.5 cm) to hold the individual flower petals; we filled each cup to the one-quarter mark with GULF wax (Royal Oak Enterprises; Roswell, Georgia), placed a circular, 9 cm diameter filter paper in the center of the cup and moistened it with distilled water to prevent desiccation. We then weighed individual rose petals (approximately equal in size), sandwiched each between two 3 × 3 mm acetate sheet squares and secured it to the filter paper and wax in the middle of each container using an insect pin so that it was elevated about 1.5 cm above the wax. Cups were covered with fitted lids with holes to prevent excessive condensation and were maintained in a growth chamber under a constant temperature of 26.7 °C and a 14L:10D photoperiod. Each petal was weighed again after the experiment was conducted. The average change in mass due to water loss was then subtracted from the mass of petals consumed by beetles.

**Statistical Analyses**

**Grub Diet Preference and Performance.** For Experiment 1 which evaluated grub diet preferences in a choice test, the average number of grubs recovered from within a 5.1 cm diameter of each treatment were compared with a one-way ANOVA test using PROC MIXED in SAS 9.4 (SAS Institute Inc. 2013). Treatment was nested within replicate as a random effect and multiple comparison tests were conducted using Tukey’s post-hoc test with least-square means at $P \leq 0.05$. For Experiment 2 which assessed grub performance on different diets in a no-choice test, the average initial grub masses per treatment were compared to those recorded at two weeks with a repeated measures ANOVA using PROC MIXED in SAS 9.4 (SAS Institute Inc. 2013). Multiple comparison tests were conducted using Tukey’s post-hoc test with least-square means at $P \leq 0.05$. For Experiment 2 which assessed grub performance on different diets in a no-choice test, the average initial grub masses per treatment were compared to those recorded at two weeks with a repeated measures ANOVA using PROC MIXED in SAS 9.4 (SAS Institute Inc. 2013). Multiple comparison tests were conducted using Tukey’s post-hoc test with least-square means at $P \leq 0.05$. The average grub mass and length differences per treatment were compared in separate analyses with ANOVA using PROC MIXED in SAS 9.4 (SAS Institute Inc. 2013). Multiple comparison tests were conducted using Dunnett’s post-hoc test; soil diet was designated as the control for one-to-many comparisons, and least-square means were performed at $P \leq 0.05$. The mixed linear model was chosen to accommodate missing datapoints (Wolfgang and Change 1995) due to grubs either dying...
or making no choice in the preference experiment and being discarded prior to analysis.

Adult Survival, Fecundity, and Feeding Performance. For Experiment 3 which assessed beetle survival and fecundity on different flower diets, the number of surviving beetles per cup and number of eggs laid female were compared among treatments in separate analyses in R version 4.0.3 (R Core Team 2020) using a nonparametric Kruskal-Wallis test followed by a post-hoc nonparametric Dunn’s test using a Holm’s adjustment in the “FSA” package (Ogle et al. 2021). For Experiment 4 which evaluated male and female feeding rates on rose petals, a nonparametric Wilcoxon rank sum test using R version 4.0.3 (R Core Team 2020) was conducted to examine for differences \((P < 0.05)\) in feeding between the two sexes. We chose nonparametric tests because of the dataset had a nonnormal distribution and low sample sizes.

Results

Experiment 1: Grub Feeding Preference. In experimental arenas where grubs were offered a choice of corn, soybean, marestail, crop residue and bare soil (not autoclaved), approximately half of the grubs in each arena were found near living plants and the rest were found at residues, bare soil, or between treatments (i.e., no choice) (Fig. 3A). Nearly one-third of the grubs in this experiment failed to make a choice -- significantly more than were found at any individual treatment \((F = 11.49; df = 5, 78; P < 0.0001)\). For the grubs that did make a choice, majority were located near living plants (i.e., corn, soybean, marestail) relative to non-living diets (i.e., residues, soil). Significantly more grubs were at corn and marestail, than soil or residue, none of which varied from soybean.

Experiment 2: Grub Feeding Performance. Grubs of similar body mass \((82.0 \pm 1.7 \text{ mg})\) \((F = 0.83; df = 4, 36; P = 0.512)\) were subjected to a no-choice experiment and were provided with corn, soybean, marestail, crop residue, or soil. Average grub body mass (including gut contents) significantly increased after two weeks on the same diet for all grubs \((F = 64.55; df = 1, 36; P < 0.001)\) and the amount of mass gained varied by diet \((F = 2.95; df = 4, 36; P = 0.033)\). Grubs provided corn, marestail, and residue significantly increased in body mass after two weeks, but not grubs provided soybean or soil (Fig. 3B, asterisk). Grubs subjected to residue gained the most mass and were significantly heavier than grubs with soybean (Dunnett’s test: \(t = 2.51; df = 36; P = 0.017\) ); otherwise, final grub mass after two weeks was similar among diet treatments \((F = 2.04; df = 4, 36; P = 0.109)\) (Fig. 3B). Numerically, grubs subjected to crop residue gained nearly twice as much body mass as those provided marestail or corn, and three times as much as grubs with soybean or soil. The average initial and final grub body lengths \((F = 1.16; df = 4, 36; P = 0.345)\), and body length change over two weeks \((F = 0.77; df = 4, 36; P = 0.550)\) was similar regardless of treatment.

Experiment 3: Adult Survival and Fecundity. Adult survival varied significantly by flower diet \((F = 4.82; X^2 = 10.004; df = 3; P = 0.019)\). Adult beetles fed rose petals survived longer than to those which were fed clover \((Z = –2.59; P = 0.048)\) or starved \((Z = 2.76; P = 0.035)\). A total of 30 eggs were laid by two females fed rose petals during this experiment; 20 during the third week and 10 during the fourth week.

Experiment 4: Adult Food Consumption. In this experiment two males and one female were excluded from the reported

\[ \text{Figure 3. (A) Grub distribution among “diet” treatments after 48-hours during the diet choice test in Ohio, and (B) mean body mass change per grub after being subjected to one of the specific diets after two weeks in the no-choice test. Upper-case letter(s) above each bar indicates significant differences at the } P \leq 0.05 \text{ level from pairwise comparison analyses among (A) grub distribution, and (B) body mass after two weeks, using Tukey’s post-hoc test. For the no-choice test, diets with an asterisk (*) indicate that there was a significant increase in grub body mass after two weeks.} \]
corn seedlings (DiFonzo 2007, Krupke et al. 2007) and common agricultural weeds like marestail (MacKellar 2011, Costa 2018) in sandy soils of northern Ohio, northern Indiana, and southern Michigan. Weeds may deter grubs from feeding on the main crop as observed in other grub species (Norris and Kogan 2000); however, it is common practice for farmers to eliminate weeds early in the season to prevent competition with the crop (Hoef et al. 2000), and the non-availability of alternate hosts may encourage grub feeding on the crop. Numerically fewer grubs were found at soybean in the choice test, and grubs did not significantly increase in body mass after two weeks on a soybean diet. In the field, observable root feeding damage in soybean (i.e., discolored, stunted, and wilted plants, and stand losses) is rare relative to corn (personal observation, A.P., A.R., M.L., K.T.), though whether this is related to poor feeding performance, planting date (e.g., soybean is planted later than corn, often during grub pupation), higher density relative to corn, or some other environmental factor (i.e., shaded canopy or moister soils) that influence female oviposition preference, is unknown.

Half of all grubs in the preference study were found in either bare soil, at crop residues, or between treatments. Our finding that grubs survived and gained mass when provided soil with corn residue is consistent with Hallock (1936a), although we did not test whether grubs could survive and develop from first instar to adult solely on any given diet type, or what quantity of each diet is needed to complete development. Findings from the grub performance study more or less mirrored the preference study: grubs provided with live plants generally gained more mass than those with soil, with the exception of soybean which was less frequented by the grubs and did not contribute to significant body mass increase. Interestingly, grubs gained the most body mass on crop residue relative to the other diets.

Discussion

Since its introduction to North America 100 years ago, AGB has spread throughout the eastern half of the United States to new environments (Eckman 2015) where it continues to pose risks and introduce new challenges to plant health and production (DiFonzo 2007, Krupke et al. 2007, Turner 2013, Eckman 2015). Few studies (Eckman 2015) have been conducted on AGB since the extensive work of the 1930s, and data on grub and adult feeding behavior are needed as AGB spreads further across North America and emerges as a significant pest of economically important host plants. This study provides some of the first and only lab assessments of AGB grub and adult host preference in the extended geographic range.

Preliminary host range assessments of AGB grubs collected from an infested corn field in northwest Ohio suggest that they move towards, and gain mass when provided with live plant roots, particularly of marestail and corn. It is common to observe high grub densities at the base of exhumed

Figure 4. Comparison of beetle survival on different flower petal diets. Uppercase letter(s) above each bar indicates significant differences at the $P \leq 0.05$ level from pairwise comparison analyses using Dunn’s test with Holm’s adjustment.

averages because they died prior to feeding. The average rose petal consumption for beetles that fed (N = 9) was $27 \pm 5.5$ mg/day. Females (N = 5) consumed an average of $33 \pm 7.2$ mg/day of rose petals while males (N = 4) consumed $20 \pm 8.1$ mg/day (20.4%), but these differences were not statistically significant ($Z = 10.5; df = 11; P = 0.257$).

The adult studies provide the first preliminary diet assessments of AGB from Alabama. Here we presented novel approaches to assess adult feeding and survival in the lab, and of the few beetles evaluated, those provided a diet of rose petals consumed the most diet, survived the longest, and were the only to produce eggs compared to those fed flowers of Queen Anne’s lace or white clover. Beetles given the latter two diets, which were listed as preferred hosts by Hallock (1936a), fed very little. However, this study only evaluated feeding on flowers and not foliage. Interestingly, feeding by males and females in this study were similar despite the claims of Hallock (1936b) that males accounted for more defoliation. The reported defoliation
in the field likely reflects the male biased sex ratios on plants as females leave plants to oviposit. Being more abundant on host plants, males could account for more damage but do not necessarily feed more individually than females. With only flowers of one host and limited replicates in the adult trials, additional experiments will be required to better resolve meaningful conclusions about the adult feeding ecology.

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