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Effectiveness of Hand Removal for Small-Scale Management of Japanese Beetles (Coleoptera: Scarabaeidae)

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ABSTRACT Hand removal is often recommended as a method for small-scale control of Japanese beetles (*Popillia japonica* Newman). In this study, we investigated the effectiveness of daily hand removal for controlling damage by Japanese beetles on grape plants. We also investigated whether the timing of the removal (at 0800, 1400, or 1900 hours, or at all 3 periods) influenced the effectiveness of the technique. We found that hand removal significantly lowered the number of beetles on, and consequently the damage to, grape plants relative to nonremoval controls. Of the single removal treatments, removal of beetles at 1900 hours was most effective, with results similar to removing beetles three times per day. The majority of beetles removed from plants during the experiment were female, a pattern that matches our understanding of aggregation formation behavior in the species, and which may serve to enhance the benefits of hand removal. Hand removal seems to work by decreasing the number of feeding beetles, which in turn reduces the release of aggregation kairomones from the plant, and subsequently decreases the attractiveness of the plant to future beetles.

KEY WORDS Japanese beetle, *Popillia japonica*, control, management, aggregation

As adults, Japanese beetles (*Popillia japonica* Newman) feed on >300 species of plants, including many horticultural and agricultural species (Fleming 1972). Depending on the plant, they may feed on foliage, flowers, or fruit (Fleming 1972, Potter and Held 2002, Held and Potter 2004). To control beetles on a relatively small scale (e.g., the scale of a home landscape), people may use pesticides or traps (Potter and Held 2002). Often, simple hand removal is frequently recommended; people are instructed to remove the beetles from the plant and place them in a jar of soapy water or something similar (e.g., Ladd 1976). Some recommendations suggest that hand removal should take place in the morning, because beetles are sluggish then and will drop off plants rather than fly (Ladd 1976, Kreuger and Potter 2001).

The mechanisms underlying Japanese beetle aggregation behavior indicate hand removal might be effective. Japanese beetles cause significant damage because of their tendency to aggregate on the host plants (Fleming 1972). These aggregations are formed as a result of attraction to plant volatiles that are released as a result of damage caused by Japanese beetles feeding on plant tissues and function as aggregation kairomones (e.g., Loughrin et al. 1995, 1996). Thus, aggregation formation seems to be the result of a positive feedback loop, where initial beetles cause damage that leads to more beetles arriving on the plant. Therefore, the idea behind removal is that removing existing beetles may limit damage not only because of fewer beetles at the time of removal, but also by limiting colonization of the plant in the future.

The effectiveness of hand removal might be influenced by multiple factors. First, the time of day at which the beetles are removed may impact the efficacy of removal. For example, Loughrin et al. (1996) found that the volatile release from Japanese beetle-damaged grape (*Vitis* sp.) vines peaked from 1200 to 1500 hours and that leaves damaged overnight were more effective at recruiting beetles than freshly damaged leaves. Furthermore, many aspects of beetle behavior seem to have a daily cycle (Fleming 1972; Kreuger and Potter 2001; Switzer et al. 2001, 2004; Tigreros and Switzer 2009). Of special importance is the fact that beetle flight behavior seems to be highest between 0900 and 1500 hours (Fleming 1972, Kowles and Switzer 2012). Thus, one would predict that recolonization would be greatest when the volatile release of the plant coincides with the peak of beetle flight.

Second, the part of the plant beetles feed on may also affect colonization, and thus the effectiveness of hand removal. Kairomone attraction in Japanese beetles was discovered from beetles foraging on leaves (Loughrin et al. 1995, 1996, 1997). Although it is likely that similar chemical and behavioral mechanisms are at work when Japanese beetles feed on flowers or fruits (e.g., as for the green June beetle, *Cotinus nitida* L.; Domek and Johnson 1988), studies have not been done to confirm this pattern for Japanese beetles. In

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addition, these plant parts may also have other stimuli that may attract beetles even in the absence of feeding damage (Loughrin et al. 1998, Held and Potter 2004).

To our knowledge, only Vitullo and Sadof (2007) have investigated the effectiveness of hand removal for Japanese beetles. In their study, they removed beetles three times per week from rose (*Rosa* sp.) leaves and flowers and found no significant difference in beetle damage between plants with hand removal and controls. Thus, it remains unknown whether removing Japanese beetles from plants with only foliage as the attractant or removing them at different times and frequencies is effective (Vitullo and Sadof 2007).

We designed the current study to assess the effectiveness of hand removal from grape plants, a preferred host plant that has been used in previous studies on Japanese beetles and on which the beetles consume the leaves (Fleming 1972, Gordon and Potter 1985, Loughrin et al. 1996). We also investigated whether the time of removal influences the effectiveness of the method. Finally, we examined both temporal patterns in colonization and the sex ratio of the beetle aggregations to see whether we could begin to explain the results of the removal experiments.

Materials and Methods

Experiments were conducted on the campus of Eastern Illinois University in Charleston, IL. The experiment was run for a total of 7 d, from 10 July 2008 until 16 July 2008. Our food plants were second year *Vitis labrusca* L. "Niagra" grapevines. Grapevines were potted in 11.3-liter pots with 1.5-m poles as support, and staked into the ground. Before the day of the experiment, the plants were kept at a separate location that experienced little beetle activity; during this time, plants were monitored regularly and any beetles that landed on the plants were removed. The day before the experiment, any damaged leaves were removed (irrespective of whether the damage was due to Japanese beetles, handling, etc.) and the plants were moved to the study site. Seventy plants were randomly assigned to a location within a 7 by 10 array with 3.5 m between plants in both the columns and the rows. This distance was chosen to maximize the distance between plants within our study site while still using a spatial scale that would be relevant for a homeowner. Total leaf area per plant did not differ significantly among treatments (see in Results for treatments and leaf area; $F_{4,65} = 1.0$, $P = 0.41$). Plants were watered as needed for the duration of the experiment.

Our experiment had five treatments that corresponded to when we removed beetles daily by hand for 7 d. Beetles were removed at 0800, 1400, or 1900 hours, all three times ("All Periods"), or never ("No Removal;" as a control) each day. Treatments were assigned to a location in the array in a quasi-random fashion, with the qualifications that 1) each treatment appeared exactly twice in each row of 10, with one appearance in the first five locations and one appearance in the second five locations, 2) no treatment appeared more than twice in each column of seven, and 3) no treatment was next to itself in either adjacent row or column. At each sample time, we collected beetles by hand from the designated plants and placed the beetles from each plant into a plastic bag for later sex determination.

On the morning of the eighth day, the experiment was terminated and we counted and collected all Japanese beetles from all plants across treatments and a cumulative leaf assessment was performed. The size of every leaf on every plant (over 5,500 leaves total) was estimated by placing each leaf into one of six different categories (5–25 cm^2, 25–60 cm^2, 60–90 cm^2, 90–150 cm^2, 150–200 cm^2, and 200–300 cm^2) using a standardized template. Percentage damage to each of those leaves was the recorded to the nearest 10% (Kreuger and Potter 2001). All beetles collected throughout the experiment were immediately frozen and were later sexed via foreleg morphology (Smith and Hadley 1926).

Analysis. We analyzed our data using analysis of variance (ANOVA) with Treatment as the main effect using the following methods for calculating variables. First, to examine differences among treatments in beetle number and sex ratio, we calculated the average number of beetles present in a day (i.e., over the three sampling periods, square-root transformed) or the sex ratio (i.e., proportion of individuals that were male) for each plant, and then calculated the average of all the days to obtain an overall average for each plant. We did not include the first sampling day in this comparison because beetles had insufficient time to settle before removal for some of the treatments (e.g., 0800 hours and All Periods). Second, for plant damage, we estimated the percentage of leaf area damaged (square-root transformed) for each plant by first multiplying the percentage damage for a leaf by the middle of range of the leaf's size category (e.g., 50% damage of the 60–90 cm^2 leaf category would be 37.5 cm^2 damaged) and then summing the leaf damage value for all leaves on the plant (mean = 80.4 ± 1.7 leaves per plant; $N = 70$). We then divided the total damage by the total leaf area for each plant to get the percentage of plant damaged. Thus, although each leaf size was categorized, the sum of the leaves on a plant resulted in more continuous estimate of damage per plant for analyses. Third, we looked for possible temporal differences in recolonization by using the All Periods treatment and calculated the proportion of occurrences that a plant had been recolonized by at least one beetle in the sampling period following the removal of at least one beetle in the designated removal period. We compared these proportions across all plants with ANOVA and within particular plants (e.g., 0800–1400 hours) using a sign test. Finally, to analyze sex ratio, we calculated the individual sex ratios (i.e., proportion of all beetles that were male) for each set of beetles removed from each plant.

Results

All of the grape plants in the study attracted beetles and sustained damage. However, the average number

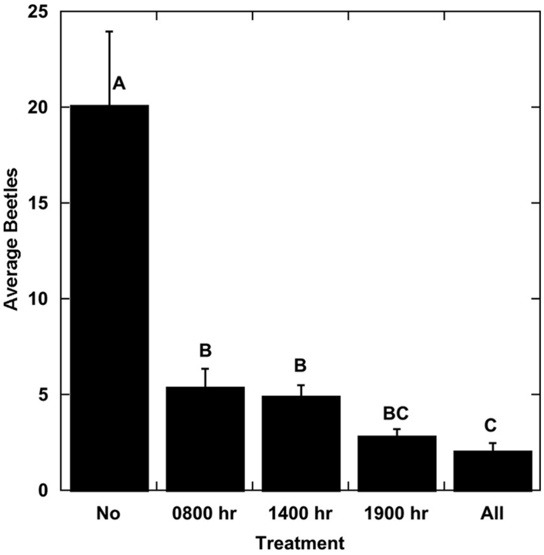

Fig. 1. Average (±SE) number of beetles found per sampling period for the five treatments (N = 14 for each treatment). Letters above bars represent Tukey post hoc comparisons among treatments. Treatments with the same letter are not significantly different at the 0.05 level.

of beetles on a plant significantly differed among treatments (Fig. 1; $F_{4,65}$ = 20.4, $P < 0.0001$). The No Removal treatment had the most beetles and the 1900-hour and All Periods treatments had the least. The total number of beetles removed also differed significantly between the removal treatments (Fig. 2; $F_{3,52}$ = 4.59, P = 0.006), with most beetles being collected for the 1400 treatment. Over the course of the experiment, the average number of beetles per plant increased sharply and nonlinearly for the No Removal treatment (R^2 = 0.89 for a second order polynomial function for these data), whereas the four different removal treatments maintained consistently lower levels (Fig. 3).

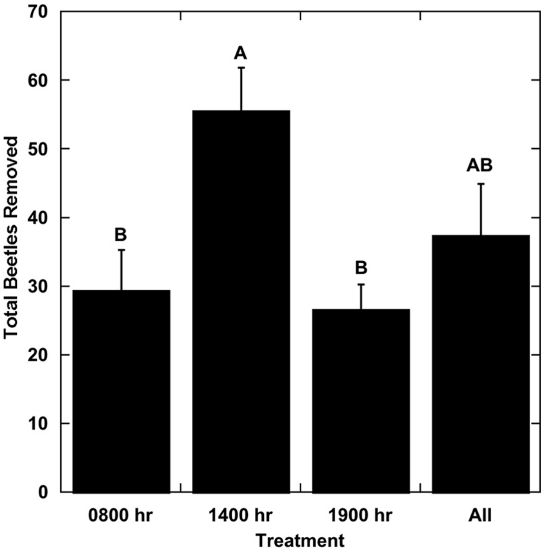

Fig. 2. Average (±SE) total number of Japanese beetles removed during the study for each of the four removal treatments (N = 14 for each treatment). Letters above bars represent Tukey post hoc comparisons. Treatments with the same letter are not significantly different at the 0.05 level.

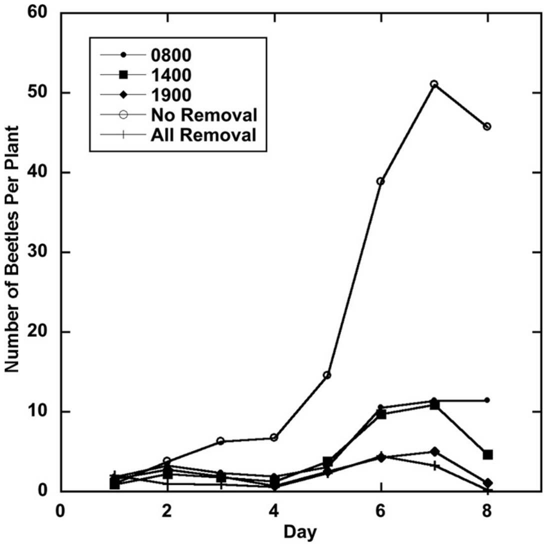

Fig. 3. Average number of beetles over the 8 d for the five treatments (N = 14 for each treatment each day). Error bars have been eliminated for clarity.

Not surprisingly, the average number of beetles on a plant explained a high proportion of the variance in leaf damage (R^2 = 0.90; regression equation: sqrt (% plant damage) = 0.089*sqrt (average beetle number) + 0.089; $F_{1,68}$ = 586.2, $P < 0.0001$). Consequently, plant damage differed significantly among treatments (Fig. 4; $F_{4,65}$ = 26.0, $P < 0.0001$). All of the removal treatments had significantly lower damage levels (15–20% less damage to the plants) than the No Removal treatment (Fig. 4). The All Periods treatment had significantly less damage than all other treatments with the exception of the 1900-hour removal. Thus, removal of any kind decreased damage, and removal at 1900 hours was almost as effective as removal three times per day.

Using the All Periods treatment, it was clear that the same plant was more likely to be recolonized after a 0800-hour removal and least likely after a 1900-hour removal (Fig. 5). Probabilities are significantly different when analyzed either with an ANOVA ($F_{2,35}$ = 29.5, $P < 0.0001$, all Tukey post hoc comparisons significant) or when a plant is compared with itself with a Sign test (For 8 out of 10 plants the recolonization probability at 0800 was >1400 [with one tie], P = 0.04; 9 out of 10 0800 >1900, P = 0.02; 13 out of 14 1400 >1900 [with one tie], P = 0.0002).

Overall, the sex ratio of recruited beetles was female-biased (Fig. 6). The sex ratio significantly differed among treatments, with the All Periods and

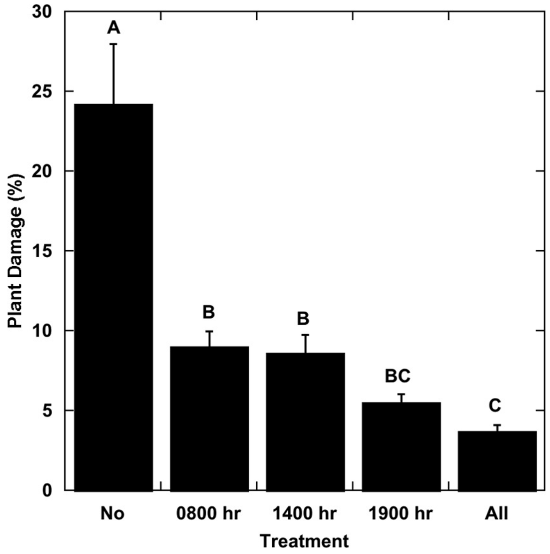

Fig. 4. Average percentage (±SE) of total leaf area of a plant eaten by Japanese beetles for the five treatments at the end of the experiment ($N = 14$ for each treatment). Letters above bars represent Tukey post hoc comparisons among treatments. Treatments with the same letter are not significantly different at the 0.05 level.

1900-hour treatments having the most female-biased and the No Removal treatment having the least female bias (≈50% male; Fig. 6; $F_{4,65} = 4.42$, $P = 0.003$).

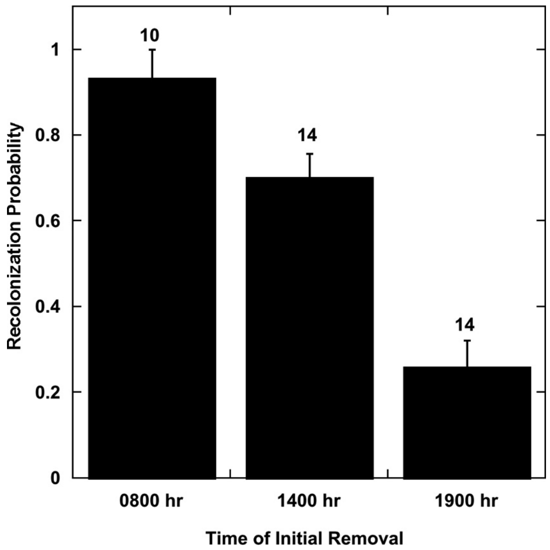

Fig. 5. Average (±SE) probability of the same plant being recolonized by at least one Japanese beetle in the next sampling period (i.e., by 1400 hours for the 0800-hour removal, 1900 hours for the 1400-hour removal, and 0800 hours the next morning for the 1900-hour removal) after the removal of at least one beetle in that sampling period. This analysis uses only plants from the All Periods treatment. Numbers above bars represent sample size. All times periods are significantly different from each other at the 0.05 level using Tukey post hoc comparisons.

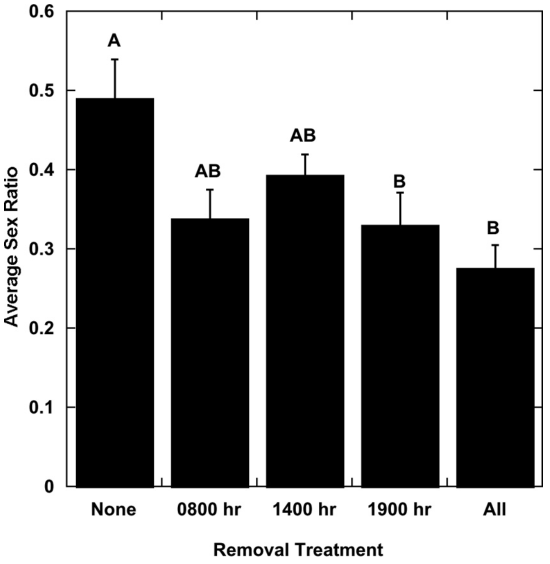

Fig. 6. Average (±SE) sex ratio (i.e., proportion of all collected beetles that were male) for beetles removed in the five treatments ($N = 14$ for each treatment). Tukey post hoc comparisons among treatments. Treatments with the same letter are not significantly different at the 0.05 level.

Discussion

Japanese beetles were attracted to and did damage to all plants, regardless of treatment. However, plants from which beetles were removed by hand had significantly fewer beetles, which led to considerably less damage than those plants without removal. The effectiveness of the hand removal seemed to be greatest if beetles were removed in the evening (1900 hours).

All of the removal treatments kept the numbers of beetles relatively low, a result that is probably due to the effect of removal on the positive feedback loop that exists between beetle presence and beetle attraction (Loughrin et al. 1996, Switzer et al. 2009). The plants in our No Removal treatment experienced a dramatic, nonlinear increase in beetle numbers over time. Although we cannot rule out the possibility of a general influx of beetles in the area on those particular days, such an influx would have occurred for all treatments. Thus, it seems more likely that the visual and chemical stimuli from beetles already present on the plants led to this increase (Loughrin et al. 1996, Kowles and Switzer 2012). We propose that our removal treatment kept the beetle density and kairomone release on these plants at low enough levels to minimize the positive feedback loop and maintain these plants at lower attractiveness levels for searching beetles. This proposal is also supported by the fact that the total number of beetles we collected for the All Periods treatment was not significantly higher than those removed only once per day.

The relatively high effectiveness of the evening removal treatment may be because of the timing of beetle activity. Beetles flight tends to peak during midday (Fleming 1972, Kowles and Switzer 2012), and therefore plants that are especially attractive to beetles during those times will be more likely to be colonized. We found that the likelihood of a plant being recolonized by the next time period varied with the time of the day, with the lowest and highest recolonization probabilities occurring for the 1900- and 0800-hour removals, respectively. Two related effects might explain this recolonization pattern. First, the 1900-hour treatment removed any settlers from the midday flight period, and fewer beetles would be searching and landing on plants between 1900 and 0800 hours. Fewer beetles searching would result in a lower chance that any particular plant is colonized. Second, Japanese beetles will feed overnight (Kreuger and Potter 2001). By having fewer beetles feeding overnight, the damage (and therefore damage-induced volatile release) will likely be lower for those plants, resulting in them becoming relatively less attractive for beetles when they resume searching for plants. Supporting this idea is the finding by Loughrin et al. (1996) that grape leaves that had been fed on overnight attracted more beetles than freshly damaged leaves and undamaged leaves. Therefore, the combination of fewer beetles and lower kairomone release makes it less likely for these plants to be recolonized in the next time period and throughout the next day, making 1900 hours an effective time for removal.

The sex ratio difference we found among treatments can likely be attributed differences in the stage of aggregation formation. Kowles and Switzer (2012) similarly found that more females overall were arriving on plants, and thus females tended to be the initial colonizers. However, established aggregations were more likely to be closer to a 1:1 sex ratio (Kowles and Switzer 2012). They suggested that females, especially those with relatively high egg loads, were more likely to initiate aggregations, while males and females with lower egg loads were more likely to join existing aggregations (Kowles and Switzer 2012). In our experiments, the plants in the four removal treatments were kept at initial stages of aggregation, and thus were female-biased. Therefore, regular hand removal of existing beetles from plants may have the dual benefit of removing the source of attraction for future beetles and removing predominantly females, especially females with the highest egg loads, from the population.

Our results differ from those of Vitullo and Sadof (2007), who found that hand removal did not significantly lessen damage on roses. Three, nonexclusive possibilities may explain this apparent contradiction. First, Vitullo and Sadof (2007) removed beetles three times per week, while in our study beetles were removed daily. More frequent removal of beetles may better prevent the positive feedback loop we describe above that exists between beetle presence and plant attractiveness. Second, Vitullo and Sadof (2007) removed beetles between 1200 and 1500 hours, the time we found was least effective in our study. Third, Japanese beetles feed on both the flowers and leaves of roses, while beetles only feed on the leaves of the grape plants if ripe fruit is not present (as in our study). Studies demonstrating plant aggregation kairomones for Japanese beetles have been conducted on leaf damage (e.g., Loughrin et al. 1995, 1996, 1997). The rose flowers likely emit other volatiles, as well as present other visual cues, beyond that which is present on host plant like grapes with leaves as food (Loughrin et al. 1998, Held and Potter 2004). Furthermore, if kairomone release does occur, the nature and pattern of the release may differ from that of grape plants.

Overall, our study has demonstrated that hand removal of Japanese beetles can be an effective means of reducing plant damage. This reduction is probably due to reducing the positive feedback that exists between existing beetles and the attraction of future beetles, and removal in the evening seemed to be the best practice. However, damage was not prevented completely by the removal schedules used in our study, and adherence to a daily collection routine may be important to achieve the best results. Future studies on different types of host plants, especially those in which beetles feed on different parts of the plant (e.g., flowers or fruits), would be particularly valuable in determining the generality of hand removal as an effective management tool for small-scale management of Japanese beetles.

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