Optimizing the Use of Semiochemical-Based Traps for Efficient Monitoring of *Popillia japonica* (Coleoptera: Scarabaeidae): Validation of a Volumetric Approach

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Abstract

Japanese beetle, *Popillia japonica* Newman, is an invasive insect, native to Japan. The species was detected in the United States in New Jersey in 1916, and then first confirmed in Minnesota in 1968. Since their arrival, *P. japonica* has become a major pest in turfgrass and several crop agroecosystems. As *P. japonica* continues to spread throughout the U.S., it’s important to discover more efficient ways to monitor adult populations. In 2018–2020, due to the high volume of *P. japonica* beetles collected in traps, a comparison of weight and volume calibration methods was conducted in Minnesota. Each method yielded a strong goodness of fit with counts of beetles captured. However, with a goal of cost-effective use of traps and in-field estimates, the volume-based approach was the preferred, most efficient method. In addition, a comparison of monitoring systems was conducted to observe differences in trap type, lure age, and check interval. Results from these studies indicate a standard green/yellow trap, and multi-component, semiochemical-based lure used for the duration of the *P. japonica* flight period, and a weekly check interval will minimize sampling time and resources, while providing accurate population estimates. In addition, results from these studies will benefit growers and researchers as they continue to explore integrated pest management (IPM) strategies for *P. japonica*. More importantly, by reducing the time required to quantify trap catches and rebait traps, these results may also facilitate area-wide tracking of *P. japonica* populations in newly invaded regions.

Key words: invasive species, pheromone, IPM, detection
propionate + eugenol + geraniol (3:7:3)) and the synthetic sex pheromone known as “Japonilure” (((R,Z)-5-1-decenyl)dihydro-2(3H)-furanone); both are highly attractive to both male and female *P. japonica* (Tumlinson et al. 1977, Ladd et al. 1981, Klostermeyer 1981). While these traps are most often used to monitor *P. japonica* for integrated pest management (IPM) purposes, other applications include early detection of the pest in new regions or countries (Althoff and Rice 2022). In addition, the traps, as well as other modified designs using the same lure, can be used for mass trapping, with a purpose of beetle suppression or eradication (Hungate et al. 2016). Mass trapping of other species has been seen as very effective where a proper trapping system has been established (Haniotakis et al. 1991, Hegazi et al. 2009). A proper system includes a trap design that allows ample space for the target insect to be captured, and a strong and specific lure to attract the target species (El-Sayed et al. 2006). Mass trapping of *P. japonica* was demonstrated with high beetle trap catches occurring in elderberry and blueberry over several years (Piñero and Dudenhoeffer, 2018). It is important to note that even with high beetle captures occurring, we are not aware of published studies that document a reduction in damage, or benefit to the crop when mass trapping is used. However, mass trapping efficiency depends heavily on proper trap design and the time necessary for growers or IPM managers to implement a program.

While there are several effective traps commercially available for *P. japonica*, there continue to be logistical questions related to how often traps should be checked or how often lures should be changed if used for monitoring or mass trapping. Moreover, due to the extremely high catch rates that are common in the Midwest U.S., often exceeding 1,000 beetles/day (personal observation), there is a need to efficiently estimate the numbers of beetles captured per trap, for both research and IPM applications. Streamlining the trapping process would aid growers in using traps more effectively, and researchers who use traps to study *P. japonica* population dynamics. It would also facilitate large-scale trapping programs for detection, surveillance, and regulatory control in areas where *P. japonica* has recently become established, such as northern Italy (Kisters-Thomas 2019).

We, therefore, conducted studies to develop and evaluate the usefulness of weight- and volumetric-based methods to estimate beetle numbers for rapid, cost-effective monitoring. Additional studies were conducted to determine the impact of trap type, lure age, and trap check frequency on mean trap catch for *P. japonica*’s flight period, to understand the most efficient way to utilize *P. japonica* traps whether it be for research, regulatory, or IPM applications. Our goal was to simplify the trapping and monitoring process for *P. japonica*.

**Materials and Methods**

**Weight-Based Estimation**

In 2018 and 2019, Trécé Pherocon *P. japonica* traps (Trécé, Adair, OK) consisting of yellow vanes, a green vented catch can, and the semiochemical-based dual lure (hereafter called standard traps) were deployed at the Rosemount Research and Outreach Center in Rosemount (RROC), MN (44° 43’ N, 93° 05’ W), and in vineyards near Stillwater (45° 02’ N, 92° 52’ W), Hastings (44° 41’ N, 92° 52’ W), and the Horticultural Research Center (HRC), University of Minnesota, in Excelsior (44°52’ N, 93°38’ W), Minnesota. Over the two years, 97 *P. japonica* samples were collected weekly, placed in 20 cm × 25 cm Minigrip Reclosable bags (Consolidated Plastics, Stow, OH), and brought back to the laboratory to be placed in a freezer overnight at –29°C. Once beetles were frozen, they were removed from the freezer to obtain the weight for each sample.

Weights (mg) were obtained using a bench scale (A&D Company, San Jose, CA, model# EJ-410). For the purposes of this paper, “fresh weight” will refer to beetles removed from the field without any unnatural manipulation of their moisture content. After the total fresh weight was acquired for each sample, individual beetle counts within a sample were recorded along with their corresponding fresh weight.

In 2018, after observations during trap collections indicated major differences in beetle weights, an additional study was conducted to measure percent water content in individual beetles. *Popillia japonica* standard trap contents were collected in Minigrip Reclosable bags from traps deployed at both HRC and RROC. The study consisted of two trap check intervals, 1 and 7 d. From the collected trap contents, 20 individual beetles were taken from both the 1 and 7 d traps, totaling 40 individual beetles, and both fresh and dry weights were recorded for each beetle. Again, fresh beetles were taken from the field, placed in the freezer at –29°C and weighed to obtain individual beetle weights after being frozen overnight. Dry weights of individual beetles were obtained by placing beetles in a brown paper bag, that was sealed, and dried in an oven set at 63°C; and samples remained in the oven for ~48 h.

**Volume-Based Estimation**

In 2019 and 2020, *P. japonica* standard traps with dual lures were deployed at RROC to obtain beetle samples. In 2019, a total of 32 samples were collected and processed for both fresh and dry weight, and in 2020, another 22 fresh weight samples were obtained. In 2020, no dry weight samples were added to the data set due to COVID regulations limiting access to the drying ovens. All samples were collected weekly from the field in 20 cm × 23 cm Minigrip Reclosable bags. All volumes (ml) were measured using an accu-pour measuring pitcher (Gempplers, Janesville, WI) with a capacity range of 100–2,000 ml and rounded to the nearest 100 ml measurement. For any beetle samples that were below 100 ml, a smaller accu-pour measuring pitcher (Gemplers, Janesville, WI) was used which could measure a range from 20 to 500 ml. To begin, fresh volumes were taken directly from field beetle samples. To minimize variability across samples, a standard practice was employed where the measuring pitchers were shaken and tapped several times to ensure beetles were settled in the pitcher and the top of the beetle mass appeared as flat as possible. After obtaining the sample volume, beetles were then hand-counted to record the associated beetle number for each volume. Next, beetle samples were placed in individual brown paper bags and then into drying ovens. Beetles were left in the drying ovens at a temperature of 63°C for 48 h. Dried samples were collected and brought back to the laboratory to obtain volume measurements of the dried beetles. After the volume measurement was obtained, beetles were counted again to ensure none were lost during the drying process.

**Trap Type**

Trap type studies were conducted using three different traps, standard trap, Tanglefoot Japanese beetle Xpando trap yellow/green (Tanglefoot, Grand Rapids, MI), and a modified trap with the same yellow top used on the Trécé Pherocon yellow/green trap but with a 3.8 L jug used as a base, (Trécé, Adair, OK) (Hereafter referred to as Xpando trap and jug trap, respectively). All traps received a *Trécé P. japonica* dual lure (Trécé, Adair, OK). Each trap type (i.e., treatment) had three replicates in 2019 and four replicates in 2020. Traps were deployed on 3 June 2019 and 9 June 2020 at the RROC. First trap catch was recorded for both years to determine if trap type would have an impact on first detection. Traps were placed about 3 m away from a raspberry crop (*Rubus idaeus*) and hung on a metal
stake about 1 meter off the ground, no insecticide applications were applied to these raspberries. Traps were placed 4.5 m apart in 2019 and 9 m apart in 2020. Following trial set up, trap contents were collected once or twice a week, and beetle counts recorded using accu-pour pitchers for fresh volumetric measurements, previously described. Trap type trials ended on 3 September in 2019 and 2020. Trap collections were eventually combined to reflect a week’s sample for a total of 9 and 10 weekly collection dates, in 2019 and 2020, respectively.

Lure Age
In 2019 and 2020, lure aging studies were conducted at the RROC. For these studies, standard traps with dual lures were used. In 2019, the study consisted of two treatments, and three replicates where lures remained in the trap for the entire flight period or were replaced every 2 wks. In 2020, treatments included an additional lure replacement of once a month, and replicates were increased to four, across the three treatments. Traps were deployed within 3m and 20m of a raspberry crop on 8 July in 2019 and 29 June in 2020, respectively. Traps were attached to a metal stake approximately 1 m high, 4.5 m apart in 2019, and then 9 m apart in 2020. Following trial establishment, trap contents were collected once or twice per week, and beetle counts were determined volumetrically, as previously described. After measurements were recorded, the trapped beetles were removed from the site and empty trap bases were re-attached. Studies ended on 3 September in 2019 and 2020.

Check Interval Between Emptying Traps
Trials with standard traps and lures were conducted at the RROC in 2019 and 2020 to determine if the time interval between checking and emptying traps affects the total number of beetles captured. The three treatments consisted of emptying trap contents and recording the associated beetle capture twice a week, once a week, or every other week. Captures were assessed volumetrically in the field; then trapped beetles were removed from the site and the empty trap base was re-attached. In 2019 there were three replicates, and in 2020 replicates were increased to four. Studies were deployed on 8 July in 2019 and 29 June in 2020. Traps were attached to a metal stake approximately 1 m high, 4.5 m apart in 2019, and then 9 m apart in 2020. Check interval study ended on 3 September in 2019 and 24 August in 2020 with a total of 4 bi-weekly collection dates for each year.

Data Analysis
Data collection consisted of both fresh- and dry-weights of beetles, volume measurements, and beetle counts of each sample. Depending on the year, each sample included either a weight or volume measurement with a corresponding beetle count. A linear regression was performed on these data to generate an equation that can be used for future trap catches of beetles. Linear regression included actual beetle trap count and was associated with a weight or volume measurement depending on the study.

Mean water content per beetle, mean dry weight per beetle, and percent water in individual beetles were calculated to compare fresh and dry weight of beetles that had been in traps for 1 d versus 7 d. For analysis purposes, percent water per beetle was converted to proportion water per beetle, and an arcsine transformation was conducted to normalize data. Water content and dry weight data met all analytical assumptions and transformation was not needed. All three data sets used an ANOVA to assess differences using R statistical software (R Core Team 2017). Untransformed means and standard errors are presented.

To assess differences in trap type, lure age, and check interval studies, an analysis of variance (ANOVA) with R statistical software (R Core Team 2017) was conducted comparing the mean trap catch for P. japonica’s flight period. For trap type and lure age studies, data were summed to represent weekly trap catch. Check interval studies were summed to represent a 2-week trap catch to equalize the means due to this longer interval. In cases where significant differences were observed (P < 0.05), a mean separation test was conducted using Tukey’s honest significant difference test [Agricolae, HSD.test, Mendiburu 2015]. Untransformed means and standard errors are presented.

Results
Weight-Based Estimation
Mean individual fresh weights were 74.1 and 51.5 mg at 1 and 7 d, respectively (Fig. 1A). The mean individual dry beetle weights were 29.2 and 27.9 mg at 1 and 7 d, respectively (Fig. 1B). Finally, mean percent water content for beetles was 60.4 and 43.8% at 1 and 7 d, respectively (Fig. 1C). Significant differences were observed between beetle collections after 1 and 7 d for fresh weight (F = 27.48, df = 1, 38; P < 0.001) and percent water content (F = 27.99, df = 1, 38; P < 0.001) but there was no significant difference in dry weight (F = 0.73, df = 1, 38; P = 0.39). These data confirm a significant amount of water loss, while beetles are held in traps between 1d and 7d trap collections. There was a positive linear relationship between

![Fig. 1. Mean fresh weight (mg) (A), mean dry weight (mg) (B), and mean percentage water content (C) per individual beetle for trap check intervals of 1 and 7 d. For each comparison, a total of 40 individual beetles were collected from traps. Different letters indicate significances (P < 0.05, Tukey’s HSD test; NS, not significant) between trap check intervals.](image-url)
beetle counts and beetle fresh weights (slope 0.015, \( P < 0.001, R^2 = 0.971 \)) (Fig. 2A).

**Volume-Based Estimation**

Fresh volume and beetle counts demonstrated a positive linear relationship (slope 3.529, \( P < 0.0001, R^2 = 0.994 \)) (Fig. 2B). We also found a positive linear relationship between beetle counts and dry volume (slope 3.62, \( P < 0.001, R^2 = 0.997 \)) (Fig. 2C).

**Trap Type**

During 2019, first trap sample with *P. japonica* captured was observed on 1 July for both the Standard and Jug trap where Xpando was on 8 July with peak beetle activity occurring on 29 July for all trap types (Fig. 3). Mean trap catch for the flight period was 2982 ± 443 (±SEM), 2,303 ± 316, and 1,722 ± 230 for the Standard, Xpando, and Jug traps, respectively (Fig. 4A). The Standard trap caught significantly more beetles than the Jug trap, but no other significant differences were observed (\( F = 3.65, df = 2, 77; P = 0.03 \)). In 2020 a similar trend was observed, despite lower beetle numbers compared to 2019. First trap catch was on 23 June and peak beetle activity occurred on 30 July for all trap types (Fig. 3). Mean trap catch for the flight period was 1,245 ± 171, 1,172 ± 94, and 806 ± 76 for the Standard, Xpando, and Jug traps, respectively (Fig. 4B). Like 2019, the Standard trap caught significantly more beetles than the Jug trap (\( F = 4.15, df = 2, 116; P = 0.02 \)), but no other differences were observed. All trap types in 2020 had a first trap catch date of 23 June.

**Lure Age**

Results for 2019 did not indicate significant differences in the mean number of adults per trap for flight period (\( F = 0.24, df = 1, 53; P = 0.63 \)). Mean trap catch for the flight period was 3,487 ± 434, and 3,700 ± 516 for flight period and 1-mo. lure ages, respectively (Fig. 4C). With the addition of a third treatment, lure age studies in 2020 indicated the mean trap catch for the flight period was 3,217 ± 347, 2,516 ± 262, 2,275 ± 202 for 2-wk, flight period, and 1-mo. lure age, respectively (Fig. 4D). Traps for which lures were changed every 2 wk caught significantly more beetles (\( F = 3.34, df = 2, 128; P = 0.04 \)) than did traps for which the lures were changed monthly or left in the trap for the entire flight period.

**Check Interval**

In 2019, mean trap catch for the flight period was 7,373 ± 1,189, 5,781 ± 780, and 3,491 ± 1,986 for twice per week, 1-wk, and 2-wk check intervals, respectively (Fig. 4E). Traps checked twice per week caught significantly more beetles than the 2-wk check interval (\( F = 9.77, df = 2, 32; P < 0.001 \)), but no other significant differences were observed. In 2020, mean trap catch for the flight period was 7,580 ± 652, 5,423 ± 481, and 4,109 ± 186 for twice per week, 1-wk and 2-wk check interval, respectively (Fig. 4F). Traps checked twice per week caught significantly more beetles than both the 1 and 2 wk check intervals (\( F = 16.79, df = 2, 44; P < 0.001 \)).

**Discussion**

The purpose of these studies was to further optimize the use of semiochemical-based traps by a) developing an accurate and efficient method to estimate *P. japonica* numbers caught in traps, and b) to utilize this method to compare the performance of different traps, lure ages, and trap-check intervals. Strong linear relationships were found between beetle counts, beetle weight, and volume (Fig. 2A–C). However, there are additional factors to consider when deciding on the most efficient method to quantify trap catch.

Considering the results of fresh vs. dry weights for individual beetles, there was a significant difference observed, with dry weights being less variable when compared to fresh weights. These results indicated that fresh beetle weights, used as a predictor of trap catch, lead to inaccuracies in beetle count. For example, in our water content (%) per beetle study, beetles that were “fresh”, collected after only 1 d in the trap, had a significantly higher moisture content when compared with beetles collected after 7 d in the trap (Fig. 1A). Because traps are often checked weekly for regulatory or IPM purposes (Ebbenga et al. 2020), this is a relevant time period comparison. However, the lower moisture content after 7 d is not surprising, and likely due to the extended exposure to ambient temperature fluctuation resulting in dehydration of beetles. This difference becomes a concern when using weight as a predictor for beetle counts, as both differences in trap check intervals as well as weekly weather conditions can interactively influence beetle weights and thus bias counts (Fig. 1A,C). One way to circumvent this concern is by drying field-collected beetles to minimize fresh weight influence in final count estimates, as observed in Fig. 1B. While this solution would produce more accurate results, it also required excessive weekly handling time, costs, and resources. The methods used for drying beetles requires constant access to drying ovens, and a minimum of 48h to allow the beetles to dry, creating a time lag between when trap contents are collected and when trap catch estimates are available for decision-making purposes. A final consideration regarding complications while using weights as a predictor is the seasonally changing male-female ratio in the traps. Females are larger than males (Fleming 1972) and if weighing a trap sample during a time when more females are present, this could bias the result of *P. japonica* captured (Ladd et al. 1981, Ladd and Klein 1986, Switzer et al. 2009).

Conversely, when using volume as a predictor for beetle counts, we observed much less variability between fresh and dry beetle samples (Fig. 2B,C) and are, therefore, less likely to experience biases based on sex ratios. This means that using fresh beetle samples will not substantially influence the associated beetle count and can drastically minimize the time and laboratory resources needed to obtain trap catch data. For example, in 2020 when access to laboratories and other resources was interrupted due to Covid-19 pandemic regulations, the volumetric approach facilitated rapid implementation in the field. This allowed us to avoid use of laboratory facilities, and thus estimate beetle counts in less than 2 min per trap.

While it is understood that trap catch results for any insect only provides relative population estimates, such monitoring continues to be useful for both IPM and research purposes (Southwood and Henderson 2000); for each application, the need for rapid and accurate estimates is critical. One example where this would be important is using trap catch results in tandem with degree-day models to forecast adult phenology (Hanson et al. 2015). If using weight as a predictor, depending on moisture content in the beetles, this could skew results for peak beetle activity if moisture content is artificially inflating trap catch. Conversely, when using volume as a predictor, our results indicate that moisture content of beetles does not substantially impact relative trap catch, with an \( R^2 \) of 0.99 explaining a high percentage of variance in the model. Previous studies by Gordon and Potter (1986) estimated beetle trap catches volumetrically using a scale of 325 ml = ca. 1,000 beetles. This calibration
Fig. 2. Linear relationship between observed number of *P. japonica* and mean fresh weights (A), mean fresh volume (B), mean dry volume (C). Solid lines indicate predicted regression line for estimating actual trap catch values; dashed line represents the 95% confidence interval.
is in close agreement with our results where approximately 325 ml = ca, 1,100 beetles. With our improved model proposed here, future trap estimates will now have a standardized calibration to estimate any beetle density up to 5,300 beetles. Although it would be most preferred to directly count beetles captured in each trap sample, this is very time consuming as some trap catches can easily exceed 4,000 beetles/day and take more than 20 min per trap, per person. Sub-sampling is another technique commonly used (Alm et al. 1994). However, this could be more time-consuming and labor intensive, whether this was done in the field or laboratory.

To address trap set up and maintenance, our trap studies aimed to generate greater efficiency and understanding of best use practices. Trap type studies suggested that when comparing a Standard trap, Xpando trap, and modified Jug trap, Standard trap consistently had a higher trap catch (Fig. 4A,B). One explanation for these differences could be explained by coloration of the traps. Both the Standard and Xpando traps have green bases with yellow tops, whereas the jug trap still uses a yellow top, but has a translucent base. Trap designs can increase or decrease overall beetle catch (Fleming et al. 1940, Fleming 1969, Hamilton et al. 1971, Alm and Dawson 2003). Specifically, Fleming et al. (1940) indicated that yellow seems to be most attractive to *P. japonica* adults, and secondarily green is important in optimizing trap effectiveness. The combination of both a yellow top and green base for the Standard and Xpando traps, compared with the Jug trap, likely contributed to the higher mean weekly and seasonal catch rates. Results from our trap type studies agree with past studies looking at comparisons between similar Jug traps and Xpando traps that when compared to a Standard trap, the other two variations yielded lower trap catch numbers (Klostermeyer 1985, Alm and Dawson 2003).

Lure age studies demonstrated that the current commercial recommendations of using one lure throughout the season or flight period (Trécé Incorporated 2016) may be as effective as changing lures at a frequency of every 2 wks or monthly (Fig. 4C,D). Use of just one lure throughout the season will also minimize maintenance and lure costs for growers and other stakeholders.

Finally, check interval studies indicated that when the trap is checked and emptied at a more frequent rate, there is an increase in overall trap catch (Fig. 4E,F). When observing treatments, traps that were emptied every 2 wks had their base filled with beetles and were overflowing. Conversely, traps checked once or twice/week did not demonstrate this. This observation shows that by emptying traps more frequently, the base is less limited by its volume and has more opportunity to trap beetles, which is similar to results from Alm et al. (1996). However, it is important to consider the intent of trapping. For example, if used in research for population monitoring, weekly check intervals would suffice since the differences in trap catch are not substantially different, and overflow was not observed on most sample dates. In some instances, traps have been studied for the use as a mass trapping tool for suppression of *P. japonica* populations (Piñero and Dudenhoef er 2018). While current recommendations do not warrant the use of traps to control *P. japonica* due to their dense populations (Gordon and Potter 1986),
emptying the trap more frequently for such purposes would be beneficial as it allows for higher total beetle catch.

Overall, utilizing the volumetric method, via fresh beetles, as a predictor of *P. japonica* beetle counts within a trap sample provides accuracy while also being time-efficient for various IPM and regulatory monitoring applications. For use in population monitoring, using the same lure for the duration of the flight period, and weekly check interval will yield a representative, relative estimate of population phenology and magnitude during the flight period. However, if traps are used with the intent of mass trapping *P. japonica*, increasing the frequency of trap checks and replacing lures every 2 wk would yield the highest trap catch.

Fig. 4. Mean adult trap catch for *P. japonica* flight period for trap type 2019 (A) and 2020 (B); lure age 2019 (C) and 2020 (D); and check interval 2019 (E) and 2020 (F). Traps were deployed at the Rosemount Research and Outreach center in Rosemount, MN. Different letters indicate significance exclusively for each study (*P* < 0.05, Tukey’s HSD test; NS, not significant).
Future research should seek an improved understanding of *P. japonica* behavior in relation to trap attractiveness. Some examples include determination of *P. japonica*’s range, or radius of attraction (Hamilton et al. 2007, Hungate et al. 2016), and the spillover effect in surrounding crops that occurs when traps exceed capacity (Gordon and Potter 1983, 1986, Switzer et al. 2009). Moreover, given the high volume of *P. japonica* adults captured weekly, per flight period, in this study, the commercial traps may have potential for mass trapping (Piñero and Dudenhoeffer 2018), or as an “attract and kill” tool, particularly for small berry crop growers. Improved knowledge of beetle behavior, and movement by adults toward traps, will greatly assist growers with new ways to implement the use of traps as part of a sustainable IPM strategy.

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