Mass Trapping *Popillia quadriguttata* Using *Popillia japonica* (Coleoptera: Scarabaeidae) Pheromone and Floral Lures in Northeastern China

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ABSTRACT *Popillia quadriguttata* (F.) has caused extensive damage to ≈20 families and 25 species of plants in Asia, especially in China and Korea. Adult feeding causes serious damage to soybean leaves, and larvae develop on the roots of soybean, turf, and horticultural crops. As Japanese beetle (*Popillia japonica* Newman) lures have been used for trapping *P. quadriguttata* in a previous study, mass trapping this pest with various densities of the Japanese beetle pheromone, Japonilure, and floral lure, alone and in combination, were carried out during 2012–2013 in a northeastern China soybean field. Mass trapping in 2012 with Japonilure gave the best results with 72 and 75% adult and larval reduction, respectively. In 2013, mass trapping (30 traps per hectare) with Japonilure, floral lure, or the combination resulted in a 93, 70, and 74% reduction of adults trapped, and a 90, 77, and 93% reduction of overwintering larvae, respectively. In addition, field tests showed that almost twice as many beetles approached the lure combination compared with the floral lure alone, and the pheromone residual was ≈50% of the initial dosage after 30 d. Because reduction of overwintering larvae is the most critical parameter indicating treatment efficacy, the results here indicate that the lure or lure combinations can be recommended for use by Chinese soybean farmers.

KEY WORDS Japanese beetle lure, crop damage, adult and larval control

Soybeans are one of China’s most important crops, especially in Heilongjiang and Jilin provinces, and are grown on ≈1.0 × 107 ha, representing 10% of the total crop area. More than 20 insects cause serious damage to soybeans throughout the world (Hao and Ren 2003). *Popillia quadriguttata* (F.) is an important species within the complex of soybean insect pests, and is regarded as the dominant species in soybean fields and turfgrass in Liaoning province (Sang 1979). This scarab beetle is widely distributed throughout China, especially in the northeast region, which has been the primary Chinese soybean production region for the past 60 yr (Zhang et al. 1981, Wang et al. 1999, Hao and Ren 2003, Fang 2007).

In both China and Korea, *P. quadriguttata* was long misidentified as the Japanese beetle, *Popillia japonica* Newman, because of their extremely similar morphology. Consequently, 20th century references to the Japanese beetle in China and Korea (Fleming 1972) were in fact *P. quadriguttata* (Ping 1988, Ku et al. 1999). In addition, studies by Kim et al. (1990) and Reed et al. (1991) noted a common scarab species in Korea as *Popillia uchidai* Niijima and Kinoshita. That species has been reexamined (Ku et al. 1999) and determined to also be *P. quadriguttata*. *P. quadriguttata* has also been reported from Russian and Vietnam (Reed et al. 1991, Lee et al. 2007).

Known as a polyphagous pest, adults of *P. quadriguttata* have been shown to feed on ≈20 families and 25 species of plants in China and Korea (Sang 1979, Lee et al. 2002). Severe damage occurs to fruit trees, flowers, ornamental, and horticultural plants as well as other important agricultural crops (Zhang et al. 1981, Luo and Zhang 1981, Tan et al. 1998, Wang 2001, CAB International [CABI] 2002). Adult feeding causes damage to soybean flowers, buds, immature leaves, and shoots, whereas larvae feed on plant roots. This insect has resulted in ≈1% yield losses of Chinese soybeans per year (Wang and Zheng 2006, Fang 2007). In the recent past, feeding by *P. quadriguttata* has caused significant crop losses, especially on turfgrass, flower crops, soybeans, and tree fruit (Qu et al. 1993, Wang 2001, State General Administration of Quality Supervision, Inspection and Quarantine of the People’s Republic of China [AQSIQ] 2003a, b, Australian Government, Department of Agriculture, Fisheries and Forestry 2004, Fang 2007). In South Korea, this scarab is the dominant pest on golf courses, as adults feed on ornamental plants and larvae feed heavily on turfgrass roots (Reed et al. 1991, Lee et al. 2007). As with the
Japanese beetle, turf damage is often made worse by birds and mammals digging up the turf searching for larvae to eat. Therefore, P. quadriguttata will likely cause increased destruction of turf, as golf courses and green areas are developed in China. With increases in the Chinese economy, there will likely be a greater demand for ornamental plants, turfgrass, tree fruit, and agronomic production, all of which will be negatively impacted by P. quadriguttata larva or adult feeding.

Although the parasitoid Tiphia vernalis Rohwer has been reported to parasitize P. quadriguttata in Korea and China, and P. japonica in Japan and the United States (Clausen et al. 1927, 1932, 1933; Fleming 1968, 1972; Sang 1979; Ding and Fu 2000; Reding and Klein 2001), pest suppression from biological control is insufficient for either insect. In China, chemical insecticides, including diazinon (chlordane) remain the mainstay of P. quadriguttata control in field crops (Wang 2001, Fang 2007). However, chemical applications remain problematic because of environmental contamination, increasing pest resistance, and an overuse of chemicals. Because there is an increasing awareness and value of organic agricultural products in China, there is a corresponding increased emphasis on environmentally friendly pest control measures. Finally, some previous popular chemicals, such as benzene hexachloride and Carbofuran noted above, are now being prohibited in China. There is also an increasing realization that chemical sprays damage pollinating insects in crop flowers and may negatively impact crop yields.

Sex lures (pheromones) and floral lures (kairomones) can be used in pest management by providing early detection, mass trapping, lure and kill measures, and mating disruption of pest insects (Weinzierl et al. 2012). In addition, several scarabaeids have been attracted to the P. japonica sex attractant (Japonilure—[(R, Z)-5-(1-decenyl) dihydro-2(3H)-furane])—first described by Tumlinson et al. (1977), and many respond to the floral lures (Ladd et al. 1981: PEG—phenethyl propionate, eugenol, and geraniol [3:7:3]), or the combination (Chow 1986, Donaldson et al. 1986, Klein and Edwards 1989, Reed et al. 1991, Li et al. 1995, Cherry et al. 1996, Crocker et al. 1999, Chen et al. 2001, Toth et al. 2003, Lee et al. 2007). Attraction of P. quadriguttata to the P. japonica lures (Reed et al. 1991, Li et al. 1995, Lee et al. 2007, Chen et al. 2013b) provides an opportunity to exploit commercially available lures to protect Chinese crops from P. quadriguttata.

The objectives of this study were to evaluate the use of traps baited with Japonilure plus floral lures to evaluate the effect of trapping on P. quadriguttata by observing soybean damage and overwintering larval populations.

Materials and Methods

Experiments were conducted at the Jilin Agricultural University experimental farms and campus in 2012, and elsewhere in Shuangyang county in 2013, for field trial at Changchun, Jilin Province, northeastern China (GPS coordinates: 125° 26', 43° 23'), during July and August of both years. During this time, Trécé Japanese beetle traps and lures were used (Trécé, Inc., Adair, OK). The Trécé Japanese beetle trap is made of two perpendicular, yellow, high-impact styrene vanes and a plastic bag that forms a funnel and beetle receptacle (Chen et al. 2013b). The floral lure was contained in a felt pad in 2012 (Chen et al. 2013b) and a high void polyethylene disk in 2013 (Klein and Edwards 1989), either held in a solvent-resistant plastic pack. The sex pheromone (1 mg) was placed in a rubber septum held in its own compartment in the same pack, and attached to adjoining trap vanes (Chen et al. 2013b).

Control Efficiency, Trial and Trap Layout. In 2012, tests were conducted in a 3-ha soybean field bounded by corn on all sides. The tall corn reduced the movement of P. quadriguttata from other local soybean fields. The field was divided into 12 plots for trapping at 10 traps per hectare for each of three lure treatments (floral lure and Japonilure, floral lure or Japonilure alone, and an unbaited control). In 2013, tests were conducted in a 5.5-ha soybean field, divided into 30 plots (0.17–0.2 ha) for the three densities of mass trapping (10, 20, or 30 traps per hectare) baited with the same combinations as 2012. In both years, the trial field had never been previously trapped. Each lure treatment was replicated three times with lure-baited traps placed in a randomized complete block design. There were 25 m between plots as buffer to minimize the interaction between baited traps. However, because traps were placed inside the plot lines, the real distance from a trap in one replication to a trap in adjoining plots was <40 m. In addition, the corn in the buffer and surrounding fields reduced the movement of adults and attractant components between plots. Also, at several random points in the buffer, no lure components were detected on pieces of absorbent foam placed 48 h earlier to check for chemicals in the atmosphere. Traps were secured to wooden poles so the upper edge of the funnel was >75 cm above the ground. In both years, the investigations were conducted from 1 July to 30 August, and the lures were replaced on 1 August.

Evaluation of Treatment Efficacy. Lure-trap efficacy was compared with the untreated control plots for: 1) crop damage, 2) number of adults remaining on soybean leaves, and 3) overwintering larval populations. The first two indices can be a good indication that the trapping was effective, but the overwintering larval assessment provides the conclusive proof.

Crop Damage Assessment. Soybean leaf damage in the field from P. quadriguttata was assessed on August in both years by randomly selecting 10 soybean plants in each lure treatment plot. Leaves from each plant were returned to the laboratory, scanned (HP Scanjet 200, Hewlett-Packard Co., Palo Alto, CA), and damage accessed with Photoshop software (version 7.0, Adobe Inc., San Jose, CA). Damage values were: 0 (no damage), 1 (up to 1% leaf eaten), 2 (2–4% leaf eaten), and 3 (4–6% leaf eaten). Generally, when damage reaches >4%, soybean yield will be reduced by >3%.
Protection values were based on the damage value in the control minus the damage value in the treatments.

Adults on Foliage and Overwintering Larvae. Adults attracted to plants, but not into traps in each lure treatment, were counted and recorded from 10 random soybean plants every 5 d from 2 July to 12 August in both years. Because overwintering larvae are commonly found 30 cm deep under the ground in October, larvae were counted and recorded 10 October by digging 1.5-m² holes to a depth of 30 cm in 10 randomly selected areas in trapped and untrapped soybean plots.

Scarab Response to Lures. These observations were inspired by the research of Stelinski et al. (2013). Scarab beetle approaches to randomized dispensers during July–August 2013 were recorded in each treatment. Each treatment was simultaneously observed for 60 min by nine people. The number of *P. quadriguttata* orienting to a dispenser, duration of visit near the dispenser(s), and closest approach of adults relative to the dispenser(s) were recorded. Observations were conducted on three different sunny- and low-wind days. Wind speeds were estimated every 5 min by a wind velocity indicator (HHF2005HW, 1698 Yi Shan Road, Unit 102, Min Hang District, Shanghai 201103, China). It was considered a valid observation if wind speeds were <0.03 m/s during the 60 min. The sex of beetles was not determined because of the nature of observations.

Lure Release Rates. In 2013, the amount of the Trécé pheromone and Floral Lure components (phenethyl propionate, eugenol, and geraniol [3:7:3]) present after 5-d intervals in the field was monitored. This was accomplished by putting 18 dispensers of each type of lure and then removing 3 of them for residue assessment every 5 d from the fifth to thirtieth day after the exposure. The details on identification of components were similar to those by Chen et al. (2013a). The exceptions were that dispensers were stored at −22°C, and ethyl phenethyl was used as the internal standard for phenethyl propionate, acetone for (R, Z)-5-(1-decenyl) dihydro-2-(3H)-furanone, phenol for eugenol, and butyl acetate for geraniol.

Data Analysis. During the 2-mo investigations, all beetles were taken to the Agricultural Insects Research Laboratory at the Jilin Agricultural University, Changchun, where they were identified and counted. The number of adults on each soybean plant was recorded at eight different times and pooled. Adult captures (male or female), the number of adults in scarab response observation, overwintering larvae, and crop damage data were transformed by log(x + 1) to normalize the data. After transformation, data were analyzed by one-way analysis of variance, followed by a least significant difference (LSD) test to establish statistical differences.

Table 1. Control of *P. quadriguttata* in soybean fields treated either with 10 baited traps per hectare or in an untreated control in Changchun, Jilin Province, China in 2012

<table>
<thead>
<tr>
<th>Type of lure</th>
<th>Beetrots captured (male/female)</th>
<th>Adults on foliage (male/female)</th>
<th>Larval mean</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japonilure</td>
<td>185 ± 21a/13 ± 2c</td>
<td>83 ± 6c/353 ± 3a</td>
<td>401 ± 10c</td>
<td>71.9a/0.8b</td>
</tr>
<tr>
<td>Floral lure</td>
<td>56 ± 12b/65 ± 7a</td>
<td>198 ± 13b/255 ± 24b</td>
<td>830 ± 64b</td>
<td>33.1b/28.5a</td>
</tr>
<tr>
<td>Combination*</td>
<td>46 ± 9b/42 ± 5b</td>
<td>220 ± 21b/264 ± 17b</td>
<td>937 ± 26b</td>
<td>25.7b/26.3a</td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ± 1/2.3 ± 0.2</td>
<td>296 ± 25/358 ± 36</td>
<td>1630 ± 31a</td>
<td>42.5b</td>
</tr>
</tbody>
</table>

Numbers in a column followed by the same letter are not significantly different, LSD test (0.001).

* Combination of Japonilure and Floral lure.
Results

Control Efficacy. In 2012, significant differences ($F = 112.3, 92.3; df = 4, 10; P < 0.01$) were found in the numbers of adults on leaves and of overwintering larva in soybean fields with different Trécé lure treatments (Table 1). In addition, beetle trap captures and leaf damage also differed significantly between these treatments (Fig. 1). With all the lures, reductions of beetles on soybean leaves, and the decrease in overwintering larval populations compared with the unbaeted control were highly significant. The traps baited with Japonilure alone were superior with 71.9% reduction of adults on leaves and 75.3% reduction of overwintering larvae compared with the unbaited control (Table 1; Fig. 1). In comparison to the control, trapping with Japoniture alone, the combination of Japonilure and floral lure, and the floral lure alone also resulted in very low plant damage (0.62 + 0.46, 1.38 + 0.36, and 1.63 + 0.32; Chen and Klein, unpublished data) and provided 52.18, 47.34, and 39.16% damage reduction, respectively. The plant damage had a generally positive correlation (not statistically determined) with both beetles on leaves and overwintering larval populations (Tables 1 and 2).

In 2013, similar to 2012, significant differences ($F = 98.3, 56.4, 76.4; df = 9, 20; P < 0.01$) were found in the numbers of adults on leaves, overwinter larva in soybean fields, beetle trap captures, and leaf damage ($F = 167.7; df = 9, 90; P < 0.01$) with different lures treatment (Table 2; Fig. 1). Significantly more beetles were captured with Japonilure at 20 or 30 baited traps per hectare than with 10 baited traps per hectare, whereas the combination of floral lure and Japonilure captured significantly more female beetles at 30 baited traps per hectare (Table 2). Traps baited with Japonilure caught significantly more total adults, mainly males, followed by the combination of lures and the floral lure alone (Fig. 1; Table 2). This resulted in the largest reduction of adult damage and overwintering larvae by Japonilure (Fig. 1), especially male reductions of 92.6% with either 30 or 20 dispensers per hectare (Table 2).

Scab Response to Lures. During the highest period of *P. quadriguttata* flight activity (15 July to 5 August—Chen and Klein, unpublished data), the number of beetles captured per trap ranged from 8 to 22. Significantly more beetles ($F = 107.3; df = 3, 24; P < 0.01$) reached traps with the combination of lures than with Japonilure alone, which exceeded the floral lure alone. Almost twice as many beetles approached the lure combination compared with the floral lure alone (Fig. 2). The average duration of beetle visits to the traps ranged between 13 and 15 s, and there were no significant differences in duration of visits between treatments (Fig. 2). The average closest approach ranged between 13 and 15 cm from dispensers and the relative differences were significant ($F = 121.2; df = 3, 24; P < 0.01$) between treatments (Fig. 2). The sex of beetles was not obtained because of the inability to identify characteristic sex differences between adults flying in the field.

### Table 2. Control of *P. quadriguttata* in soybean fields, either treated with one of densities of baited traps per hectare or untreated control in Changchun, Jilin Province, China 2013

<table>
<thead>
<tr>
<th>Type of lure</th>
<th>Density</th>
<th>Traps per hectare</th>
<th>Beesoms captured (male/female)</th>
<th>Adults on foliage (male/female)</th>
<th>Larvae</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>65</td>
<td>0.4/0.4</td>
<td>1/1</td>
<td>764</td>
<td>764/0.01</td>
</tr>
<tr>
<td>Floral Lure</td>
<td>20</td>
<td>165</td>
<td>0.4/0.4</td>
<td>4/4</td>
<td>352</td>
<td>352/0.01</td>
</tr>
<tr>
<td>Japonilure</td>
<td>10</td>
<td>65</td>
<td>0.4/0.4</td>
<td>1/1</td>
<td>764</td>
<td>764/0.01</td>
</tr>
<tr>
<td>Japoniture</td>
<td>30</td>
<td>165</td>
<td>0.4/0.4</td>
<td>4/4</td>
<td>352</td>
<td>352/0.01</td>
</tr>
</tbody>
</table>

Numbers in a column followed by the same letter are not significantly different (0.01).

*Combination of Japonilure and floral lure. Numbers in each treated-control group are significantly different. LSD test (0.01).*
Lure Release Rates. The laboratory bioassay of the lures (Fig. 3) showed that the pheromone was released very slowly from the dispenser. After 15 d, >90% of Japonilure and >89% ßoral lure remained, and both lure residual was ≈80% of the initial dosage after 30 d. This is consistent with the label description of a 40-d field life.

Discussion

P. quadriguttata is a serious insect pest in Asia that has caused soybean yields decreases by ≈5–8% when flowers are seriously damaged by the beetle’s feeding (Qu et al. 1993). There are no reports about efficient ways to change planting and subsequent flower dates to avoid economic damage to flowers from this beetle. Furthermore, the recent increase in Chinese golf course turf, and ornamental grass, provides an ideal habitat where adults can lay eggs and the larvae can overwinter without disruption by natural enemies or agricultural practices such as tilling. In addition, the grass roots provide P. quadriguttata larvae a highly favorable and sustainable food resource each summer.

There are ≈20 insect species that damage soybean leaves, and several white grub species feed on the soybean roots (Hao and Ren 2003, Xu and Liu 2011). A reduction in soybean plant damage levels can be a good indication that a suppression technique is effective, but reductions of overwintering larvae provide the best proof of control. P. quadriguttata adult damage to leaves causes elliptical holes with the leaf veins, which looks very different from the damage caused by Lepidoptera larvae. Therefore, damage caused by these beetles can be easily assessed and evaluated.

Fig. 2. Mean total number, approach distance (cm), and visitation time duration (sec) of P. quadriguttata to dispensers in 2013 in Changchun, Jilin Province, China. Data of different density trap treatments of each type of lure were respectively pooled: J, Trécé sex attractant (SA); F, Trécé floral lure (FL); J+F, combination SA and FL.

Fig. 3. Percentage residual (a) Japonilure – (sex attractant): (R,Z)-5-(I-decenyl) dihydro-2(3H)-furanone and (b) ßoral lure; (PEG) in dispensers after a given number of days in the field in 2013 in Changchun, Jilin Province, China.
Soybean yield losses have been caused by several pest species including *P. quadriguttata*, and the soybean pod borer *Leguminivora glycinivorella* Matsumura (*Lepidoptera : Olethreutidae*) (Hao and Ren 2003). However, yield loss does not always correlate with pest density. However, the damage this year, and moreover for next year, will correlate with the overwintering larvae density. Therefore, overwintering larvae were used as the best indication for the effectiveness of mass trapping *P. quadriguttata*.

This is the first report of using attractants to mass trap the economically important *P. quadriguttata* in Asia. These beetles congregate diurnally and feed on flowers and young leaves, resulting in 5–10% soybean yield losses annually, and larval feeding causes mortality to newly planted trees by feeding on developing roots (Wang 2001, Wang and Zheng 2006, Yang and Wang 2008). Therefore, there is need to develop an effective means to reduce the density and damage from these beetles. Using the commercial Trécé Japanese beetle traps and lures to capture *P. quadriguttata* provides an adoptable control tactic that could be implemented by Asian growers. Interestingly, although *P. japonica* and *P. quadriguttata* feed on many of the same plants, the latter scarab is not a pest of rose (Sang 1979, Lee et al. 2002), which is one of the highly favored plants of the former (Fleming 1972). If the rose-derived attractant is not important for *P. quadriguttata*, elimination of the geraniol could make the floral lure cheaper. In any event, here in China, mass trapping provided ~80% reduction of overwintering larvae. This means the following year pest population will be significantly decreased, which may lead to lower crop damage without additional treatments. Overall, mass trapping with the Trécé system can provide a powerful means of early detection, forecasting, and control of *P. quadriguttata* in northeastern China fields.

Sex pheromones are an ideal component of integrated pest management programs (Liu and Zhang 2006, Chen and Li 2011, Chen and Klein 2012, Chen et al. 2013a), and are a suggested tactic for moth mating disruption when the proper number of dispensers are placed in fields (Stelinski et al. 2008, Witzgall et al. 2008). Information here correlates positively with previous research. In addition, studies have shown an increase in efficacy of pheromones by coreleasing pheromones with kairomones (Ladd et al. 1981, Knight et al. 2012, Stelinski et al. 2013). This is often based on food and floral lures being attractive to both sexes (Huler et al. 2011). Here, traps with Japonilure alone caught more beetles than the combination of sex attractant and floral lure, which is in agreement with our previous report (Chen et al. 2013b). The highest reductions of overwintering larvae in both years were found in the plots treated with Japonilure. This may indicate that reduction of males from mass trapping, or confusion of males due to the presence of the sex attractant, resulted in females in those plots not being mated. *P. japonica* females are normally highly mobile, and could be expected to move between plots and mated. These results indicate that *P. quadriguttata* females may not be as mobile at these population levels. Almost as many females were found on the foliage in the Japonilure plots as in the controls, but the larval population was greatly reduced in the Japonilure treatments. In any event, Japonilure at 30 dispensers per hectare provided greater larval reduction (93.2%) than any other treatments in 2013 (Table 2). This correlates positively with the number of beetles approaching the lures. The movement of female beetles, effects of lures without geraniol, effects of lures on high populations, and the necessity of pure Japonilure need to be investigated further.

Acknowledgments

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