

Do plant cues influence the oviposition behavior of Japanese beetles?

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Accepted: 7 July 2005

Key words: Coleoptera, Scarabaeidae, pest, generalist, visual stimuli, bioassay, cover crop, *Popillia japonica*

Abstract

A bioassay arena was developed to test the response of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), to various plant-derived factors that may influence the selection of oviposition sites. In choice experiments with natural and artificial plant cues, females preferred to lay eggs in ryegrass rather than in artificial resources. Artificial grass stems received more eggs on average than bare ground, indicating the role of vertical objects on the soil surface as oviposition cues. *Popillia japonica* females were able to discriminate between the quality and quantity of the artificial stems, as more eggs were laid in soil blocks that had larger diameter stems or a higher number of stems per soil block. Beetles were capable of evaluating plant characteristics on the soil surface and adjusted their investment accordingly; more eggs were laid in resources that were chosen more frequently. Observations of females in choice arenas revealed how surface cues influence digging behavior and the time spent under the soil surface. The number of digging events was not significantly different among the three treatments with surface cues (ryegrass, grass roots, and artificial grass), but beetles spent significantly more time in the soil under ryegrass than any of the other treatments. These findings suggest that oviposition by *P. japonica* is selective and is influenced by plant-derived cues evaluated before and after digging into the soil.

Introduction

Vegetation characteristics of agricultural landscapes can be manipulated to control insect pest populations (Tonhasca & Byrne, 1994; Gurr et al., 2004), but this requires a thorough understanding of the mechanisms underlying the insect–plant interaction targeted for management. Plants are frequently used to manipulate the behavior of herbivores to reduce pest pressure, and this is often achieved by changing the plant composition (Bugg & Waddington, 1994; Prokopy, 1994; Hartwig & Ammon, 2002). This type of habitat modification changes the managed ecosystem to limit the availability of one or more of the resources needed for insect development and reproduction, making the environment less suitable for pest populations (Flint & Gouveia, 2001).

As an adult, the Japanese beetle (*Popillia japonica* Newman) (Coleoptera: Scarabaeidae) has a wide diet breadth, feeding on the leaves, flowers, and fruits of more than 300 plant species (Fleming, 1972; Potter & Held, 2002). This

has made the species an important pest of horticultural crops in the eastern United States. Host selection by generalist species is likely to be influenced by relatively non-specific stimuli common to many different hosts (Thorsteinson, 1960; Renwick, 1983), which for *P. japonica* are likely to be green leaf odors (Ahmad, 1982; Loughrin et al., 1995) and color (Held & Potter, 2004). Compared to food-searching behaviors, fewer cues may be involved in choosing an oviposition site in this species, as the larvae are facultative monophages (Potter & Held, 2002). Because of the limited mobility of larvae, the mother's oviposition site-selection decisions are crucial for hatching and survival of the offspring. However, despite the economic importance of *P. japonica* to agricultural systems and its potential for invasion into uninfested regions, biotic cues that are involved in the selection of oviposition sites have not been investigated.

Oviposition preference and specificity are most often studied in species that lay eggs on the above-ground sections of plants (Wiklund, 1981; Foster & Howard, 1998; Bossart & Scriber, 1999), in part because of the challenge of measuring oviposition preference of insects that lay eggs in

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the soil. Circumstantial evidence for oviposition site selection can be gathered from the location and distribution of larvae in the soil; *P. japonica* larval density is greatest in moist, sandy soils with grass cover in sunny locations (Fleming, 1972; Potter et al., 1996). Evidence for *P. japonica* oviposition response to specific abiotic cues is also available from laboratory experiments; females were found to be selective for soil qualities such as texture, moisture, and organic matter (Régnière et al., 1981; Allsopp et al., 1992). Laboratory bioassays have demonstrated that incorporation of *Metarhizium anisopliae* (a fungal pathogen for the biological control of scarab grubs) into soil influences oviposition by *P. japonica* (Villani et al., 1994). Taken together, these studies suggest that female *P. japonica* use soil-borne cues in post-alighting oviposition decisions, but the role of specific plant cues in oviposition decision-making remains unclear.

Female *P. japonica* have a strong preference to oviposit in grass, so cues from vegetation are expected to play a key role in female choice. We developed a bioassay method to allow quantification of *P. japonica* oviposition and to determine the influence of visual, tactile, and olfactory plant characteristics on oviposition investment decisions. Oviposition was compared between grass and clover, which are both commonly used as cover crops, to determine beetle response to plants. These plants were chosen because *P. japonica* larvae are more abundant in ryegrass compared to clover (Fleming, 1972), suggesting female preference for grass. However, we wanted to determine whether preference is caused by differential suitability for oviposition. To investigate mechanisms underlying *P. japonica* oviposition, observations of digging behavior were made in oviposition choice bioassays.

Materials and methods

Oviposition arena

Bioassays were conducted in a four-choice oviposition arena made of a Plexiglas box (internal dimensions 15 × 18 × 18 cm) (Figure 1). The arena had a removable Plexiglas top, while the other sides were permanently glued together. To supply beetles with an inert floor for walking and provide access to the top of the soil blocks, a rectangle of Styrofoam (15 × 18 × 2.5 cm) was inserted into each arena. The Styrofoam had four 4 × 4 cm openings cut into it, with two along each of the longer sides, so adjacent openings were 5 cm apart. The openings were cut with a Styrofoam-cutter (Flora Craft Co., Ludington, MI, USA) using a cardboard template to guide the cutter. A 2-cm-diameter hole was cut in the middle of the Styrofoam insert to facilitate handling and once in position, a 7-cm-diameter piece of plastic sheet was placed over this hole with a fresh piece of apple on

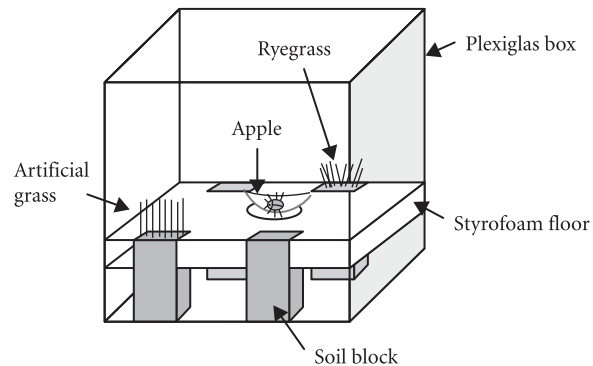


Figure 1 Bioassay arena for measurement of oviposition decisions by *Popillia japonica*.

top for the beetles to feed on. To assemble the bioassay chamber, the Styrofoam insert was wedged 8 cm above the bottom of the box, and the potential oviposition resources described in succeeding paragraphs were inserted into each of the four openings. All arenas were washed between experiments with a weak detergent solution.

Insects

To extend the availability of adult *P. japonica*, beetles were collected once in June 2004 (before *P. japonica* emergence in Michigan), from Auburn University, Piedmont Substation, Camp Hill, AL, USA, and this group of beetles was used in experiment 1. For experiments 2–6 and the behavioral observations, adult *P. japonica* were collected from Michigan State University campus Entomology Research Farm in East Lansing, MI, USA, in July, August, and September 2004. Japanese beetle Expando traps were baited with a floral lure (Great Lakes IPM Inc., Vestaburg, MI, USA) that attracts both sexes. Traps were placed in open, grassy fields and were emptied after 1 day of deployment. Beetles were brought back to the laboratory as needed for the experiments. To ensure that all females were mated, males and females were placed together in a 1 m³ screen cage in the laboratory at room temperature without an oviposition medium and were provided with cut apple slices ad libitum for 2–3 days prior to use in the experiments. Females for the bioassays were taken out of the screen cage just before use in the experiments. Beetles were sexed based on morphological differences on the foreleg tibia (Fleming, 1972), and a single female beetle was placed on the slice of apple in the center of each oviposition arena at the start of each assay. Beetles were used only once in the bioassays.

Soil and plant material

The soil used in all experiments and for all of the treatments was a 1:1 (v/v) mixture of organic potting soil (Scotts

garden soil, The Scotts Company, Marysville, OH, USA) and sand (Dixie Cut Stone and Marble Inc., Bridgeport, MI, USA). After thorough mixing, the soil was placed into 4 × 4 × 8 cm light material paper bands (Monarch Manufacturing Inc., Salina, CO, USA), and seeds were sown on top as needed for the experiments. Perennial ryegrass, *Lolium perenne* L. (The Scotts Company), and alsike clover, *Trifolium hybridum* L. (Michigan State Seed Solution, Grand Ledge, MI, USA), were grown in a glasshouse at 22–30 °C and ambient light. Enough water was added daily to the soil blocks from the top to moisten the column completely. New plants were seeded each week and ca. 3-week-old plants were used for the experiments, with new plants being used for each experiment. Soil blocks used for the experiments had uniformly healthy, vigorous, and densely growing plants. The bare ground treatment consisted of soil alone in the paper sleeves with an even, flat surface.

Artificial grass stems were arranged in the soil after the blocks had been placed into position in the oviposition arenas. Green floral wire stems (Panacea, Columbus, OH, USA) were washed with 70% ethyl alcohol and air-dried prior to using them in the experiments. Thereafter, all artificial stems were handled using gloves.

Bioassays

To start each assay, the soil blocks were installed into a cage in randomized positions, and a beetle was placed in the center of the arena on a slice of apple. Once all arenas had a beetle, they were closed with the lid and transferred to an environmental chamber at 25 °C, 60% r.h., and L16:D8 for 7 days. The orientation of each arena was randomized in the environmental chamber to compensate for any directional bias. Oviposition arenas in an experiment were set up at the same time.

Experiment 1. To determine the relative oviposition investment to artificial resources in the presence of a natural resource, beetles were assayed with bare ground, artificial grass, grass roots, and perennial ryegrass trimmed to 5 cm. For the artificial grass, 0.7-mm-diameter green floral stem wire was cut into 5.5-cm-long pieces with a wire cutter. Ten wire pieces were then inserted vertically ca. 0.5 cm into the soil, in a random arrangement. Grass roots were obtained by cutting perennial ryegrass at soil level with scissors. A total of 48 arenas were established for this experiment.

Experiment 2. Arenas were set up with soil blocks containing bare ground, artificial grass, bare ground with holes, or bare ground with holes and grass purée to determine whether beetles respond to plant cues from the soil surface. The artificial grass treatment was prepared as described previously. To imitate grass roots loosening the soil, 10

holes were punctured 0.5 cm deep into the bare ground with a floral stem wire. Holes were randomly distributed on the surface of the soil blocks. For the treatment with holes and grass purée, plant parts of 40 g fresh perennial ryegrass, both above and below the ground, were homogenized with 200 ml deionized water using a kitchen blender, and 5 ml of this was poured on the surface of soil blocks. Then, 10 holes were punctured in a similar manner as described previously. Thirty replicates were set up for this experiment.

Experiment 3. In this experiment, the acceptance by *P. japonica* females of clover or grass as oviposition resources was compared. A soil block containing clover was placed diagonally across from another soil block containing ryegrass in each of the arenas, and the two other positions were filled with soil blocks containing bare ground. Thirty-one arenas were set up for this experiment.

Experiment 4. The aim of this experiment was to determine whether oviposition by female *P. japonica* is affected by artificial grass stem diameter. Ten wires made of either 0.4-, 0.9-, or 1.5-mm-diameter green floral stem wire (Panacea, Columbus, OH, USA) were positioned vertically and were randomly distributed across the soil blocks. A bare-ground soil block served as the control. Twenty-nine arenas were established for this experiment.

Experiment 5. To determine oviposition response by *P. japonica* to the density of artificial grass stems, the number of wire stems per soil block was varied. Either 0, 5, 10, or 15 artificial stems made from 5.5-cm lengths of 0.7-mm-diameter green wire were randomly spread out on the surface of the soil blocks. Thirty oviposition arenas were set up for this experiment.

Experiment 6. The response by *P. japonica* to the color of artificial plants at an oviposition resource was assayed by providing beetles with a choice between 0.9-mm-diameter steel wire (Anchor Wire, Goodlettsville, TN, USA) stems painted with either white (2898 gloss white), green (205284 green), blue (205283 blue), or yellow (1945 sun yellow) spray paints (Aco Hardware, Farmington Hills, MI, USA). Steel wire was used instead of the green floral stem wire to provide an inert surface for the paint. After painting, wires were left to dry in a hood for 24 h. The wire was then cut into 5.5-cm-long pieces and 10 wire pieces of the same color were vertically inserted approximately 0.5 cm deep into a soil block, arranged randomly on the surface. Thirty arenas were set up for this experiment, with the four color treatments arranged randomly in the four positions.

Assessment of oviposition

After 1 week in the environmental chambers, soil blocks were removed individually from the arenas and visually inspected for eggs by gently breaking apart the soil. Eggs were picked from the soil with a moist paintbrush to verify their identity. The number of eggs found in each soil block was recorded.

Statistical analysis of bioassay data

The probability that the mean number of eggs laid in a treatment was different from that expected by chance (25% in each soil block) was evaluated using a logistic model (PROC GENMOD, SAS Institute, 2001), with cage as the repeated subject. The mean number of eggs per soil block was compared among treatments using a χ^2 test. Differences among treatments in the proportion of females choosing a resource, indicated by the presence of eggs in a soil block, were analyzed using a χ^2 test (Ott & Longnecker, 2001). Oviposition preference was determined by correlation analysis between the proportion of soil blocks with eggs and the average number of eggs per soil block (Spearman's correlation, PROC CORR, SAS Institute, 2001). The critical value for significance for all these tests was determined at $\alpha = 0.05$ level.

Behavioral observation

Detailed observation of oviposition behavior by *P. japonica* was performed using simultaneous video recordings of individual females placed in each of four bioassay arenas, each containing soil blocks with perennial grass, artificial grass, grass roots, or bare ground. Arenas were set up as described in experiment 1. Experiments were conducted between July 20 and September 2, 2004, producing a total of nine recordings from four arenas. Each recording lasted 2–4 days based on the condition of the insect, plant, and food material. On each day of the experiment, the video recording began at 10:00 hours and was terminated at 19:00 hours. Four closed-circuit security cameras (Shebar, Burton, MI, USA) simultaneously filmed the bioassay chambers from above and were connected to a time-lapse video recorder (Shebar, Burton, MI, USA), which recorded for 24 h on 8-h videotapes. Videotape recordings were then converted into digital format using Broadway Pro Capture Software (Data Translation, Inc., Marlborough, MA, USA) for quantification of beetle behavior.

Beetle behavior was scored using The Observer 5.0 (Noldus Information Technology, Wageningen, The Netherlands). The number of digging events per day and the proportion of time spent digging (digging on the surface plus time spent under the soil surface) were recorded. The total number of digging events was adjusted for the length of each observation by dividing the total number of events

by the number of days of the respective observation. Seventy-eight percent of observations lasted for more than 2 days. The effect of the four treatments on digging behavior was analyzed with a general linear-mixed model (GLIMMIX macro, SAS Institute, 2001) suitable for analyzing non-normally distributed data, with camera as a random factor and observation date as the block in the model. Treatment means were compared using the Tukey–Kramer adjusted LSMEANS t-test (SAS Institute, 2001). The critical value for significance for these tests was $\alpha = 0.05$.

Results

Bioassays

Experiment 1. The average number of eggs per soil block was significantly different among all of the treatments (Figure 2A); 60% of the eggs found in the arenas were under ryegrass. Bare ground had the lowest number of eggs on average, receiving only 2.5% of the eggs laid. The maximum number of eggs in a soil block showed a similar trend, with a maximum of 25 eggs in ryegrass soil blocks and only seven in the bare-ground treatment. Grass roots received significantly more eggs on average than artificial grass ($\chi^2 = 6.72$, $P < 0.01$), and the maximum number of eggs per soil block was also higher for grass roots (20) than for artificial grass (15). The proportion of soil blocks receiving any eggs was significantly different from a random distribution in bare ground (10%), and in perennial ryegrass (71%) ($\chi^2 = 10.78$ for both tests, $P < 0.05$). Oviposition preference was not significantly correlated between average numbers of eggs and proportion of soil blocks with eggs ($r^2 = 0.76$, $P > 0.05$) (Figure 3A).

Experiment 2. Thirty-five percent of the eggs in this experiment were found in artificial grass, significantly greater than the investment expected by chance ($\chi^2 = 14.72$, $P < 0.01$). Artificial grass received significantly more eggs on average than bare ground ($\chi^2 = 4.47$, $P = 0.03$) (Figure 2B). The addition of grass purée to soil with holes caused no significant change in the average number of eggs per soil block compared to soil with holes ($\chi^2 = 3.0$, $P > 0.05$). Artificial grass was also not significantly different from soil with holes ($\chi^2 = 3.32$, $P > 0.05$). Artificial grass and soil with holes received a maximum of 13 eggs, purée with holes in the soil received nine, and bare ground received eight. Oviposition preference was not statistically significant ($r^2 = 0.74$, $P > 0.05$) (Figure 3B).

Experiment 3. Although 3.6 times as many eggs were laid in ryegrass than in bare ground, and 3.0 times as many in ryegrass than in clover, there was no significant difference

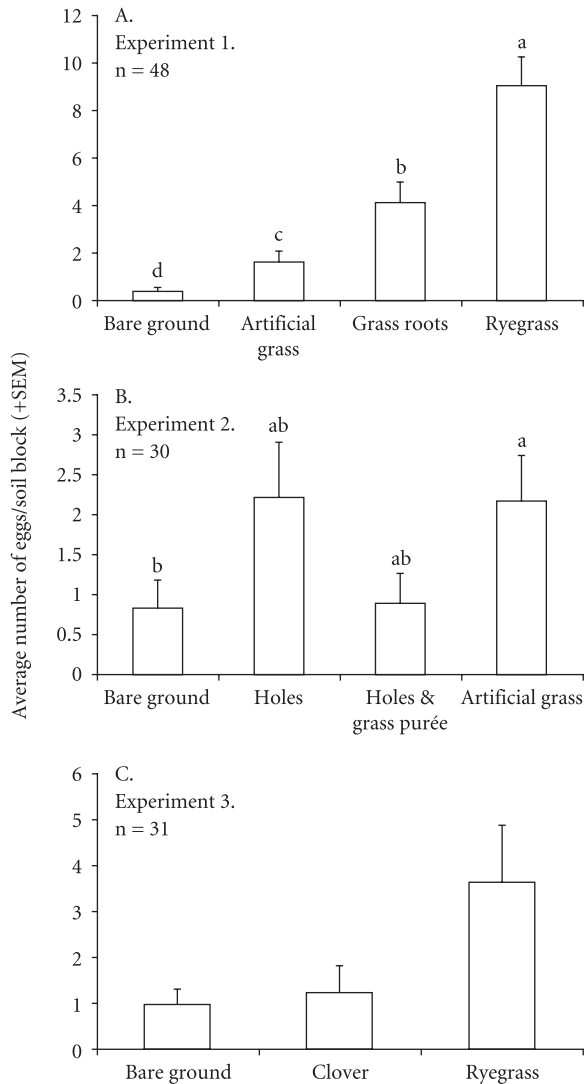


Figure 2 Average number (+SEM) of eggs per soil block in assays to test the effect of grass cues on oviposition behavior (experiments 1–3). Bars within a graph with the same letter above them are not significantly different ($\alpha = 0.05$). ‘n’ represents the number of replicates in each experiment.

among the treatments (Figure 2C). The maximum number of eggs per soil block was highest in ryegrass (25), and was lowest in bare ground (11). More than twice as many soil blocks with ryegrass had eggs in them than bare-ground ones, but there was no significant difference between the proportions of soil blocks with eggs. Oviposition preference was not statistically significant ($r^2 = 0.72, P > 0.05$) (Figure 3C).

Experiment 4. The number of eggs laid per soil block increased with increasing stem diameter (Figure 4A), but there was no significant difference among the stem diameters. Medium-diameter artificial stems received

significantly more eggs on average than bare ground ($\chi^2 = 4.43, P = 0.03$) (Figure 4A). The maximum number of eggs per soil block did not differ markedly among treatments, 16 being the highest in medium-stem diameter, and 10 the lowest in bare ground. Fifty-two percent of beetles laid eggs in soil with the largest stem diameter. Oviposition preference was not significantly correlated between the two variables ($r^2 = 0.71, P > 0.05$) (Figure 5A).

Experiment 5. The average number of eggs per soil block increased with increasing stem density, although there was no statistical difference between the treatments (Figure 4B).

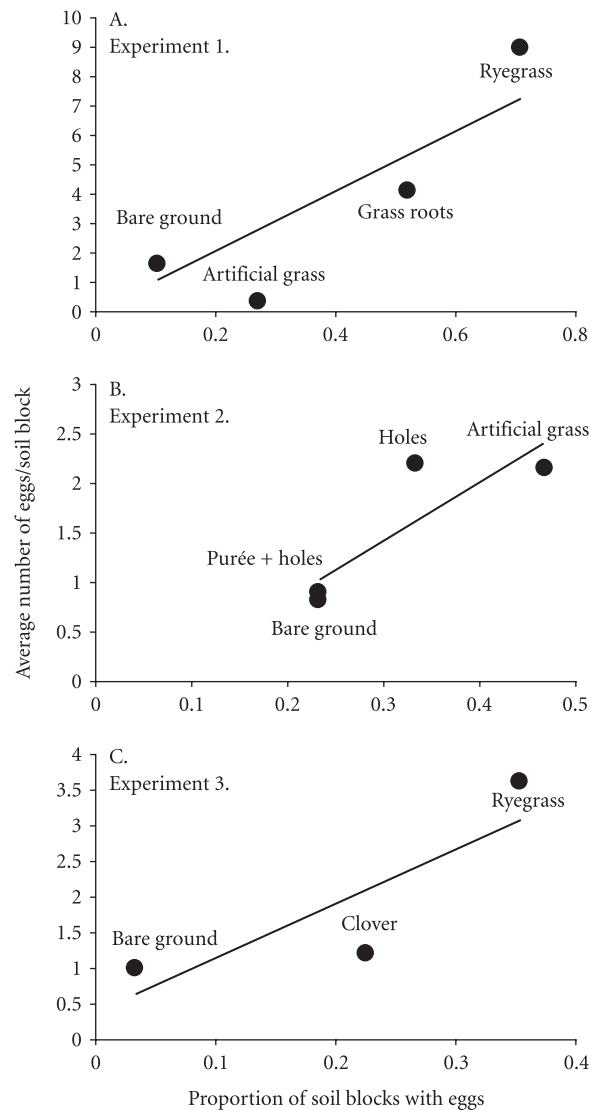


Figure 3 Relationships between the proportion of soil blocks receiving eggs and average number of eggs per soil block (i.e., oviposition preference), for different soil block treatments in experiments 1–3.

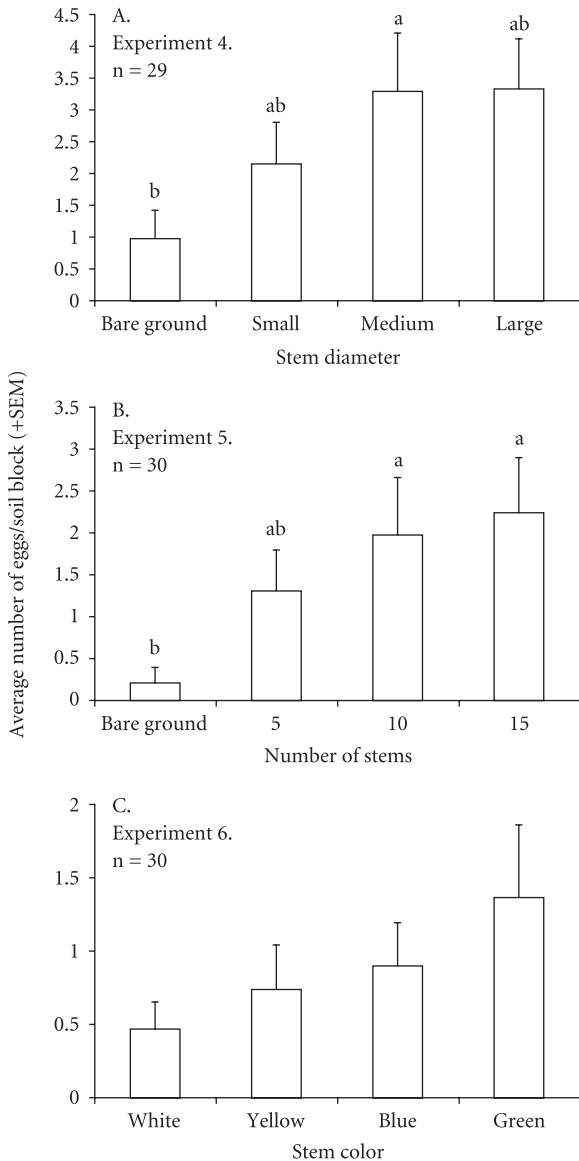


Figure 4 Average number (+SEM) of eggs per soil block in assays to test the effects of artificial grass cues on oviposition behavior (experiments 4–6). Bars within a graph with the same letter above them are not significantly different ($\alpha = 0.05$). ‘n’ represents number of replicates in the experiment.

Treatments with 10 and 15 stems had significantly more eggs on average than bare ground ($\chi^2 > 4.62$, $P < 0.03$). The soil blocks with five stems had 6.5 times as many eggs on average compared to bare ground. Oviposition preference increased nearly linearly with increasing stem density ($r^2 = 0.98$, $P < 0.01$) (Figure 5B).

Experiment 6. Stem color had no effect on the average numbers of eggs per soil block (Figure 4C), and the

proportion of soil blocks with eggs was also similar across the different treatments. Twenty-seven percent of the blocks with blue and green stems received eggs, whereas 30% of the yellow and 33% of the white had eggs in them (Figure 4C). The correlation between the proportion of soil blocks with eggs and the average number of eggs per soil block was the weakest in this experiment ($r^2 = 0.27$, $P > 0.05$) (Figure 5C).

Behavioral observation

Digging behavior of *P. japonica* was significantly different among the four treatments, for both the number of digging bouts ($F_{3,8} = 4.17$, $P < 0.01$) and the duration of the digging bouts ($F_{3,8} = 7.86$, $P < 0.01$). Beetles engaged in digging

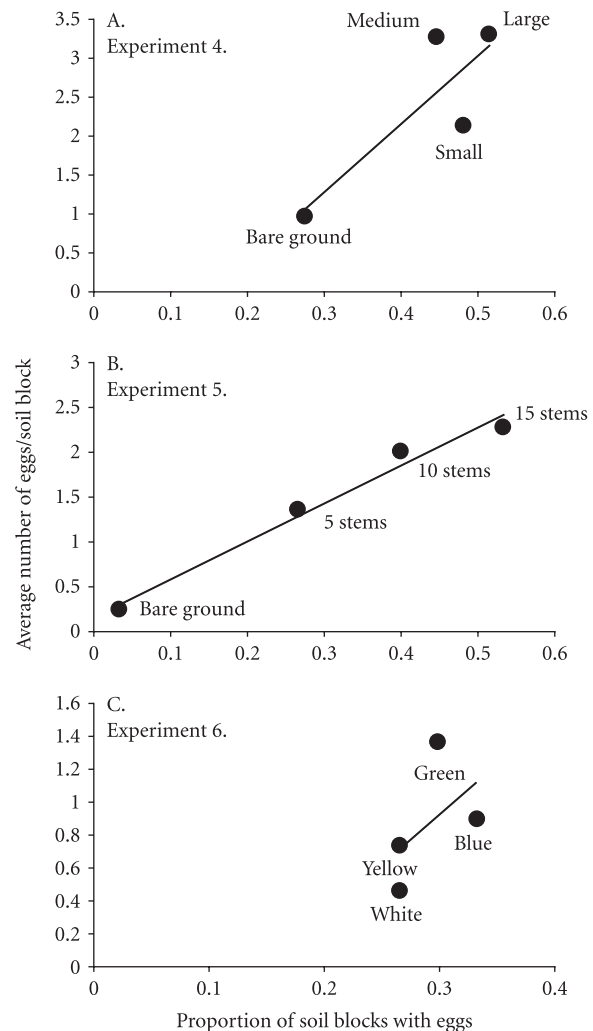


Figure 5 Relationship between the proportion of soil blocks receiving eggs and average number of eggs per soil block (i.e., oviposition preference), for different soil block treatments in experiments 4–6.

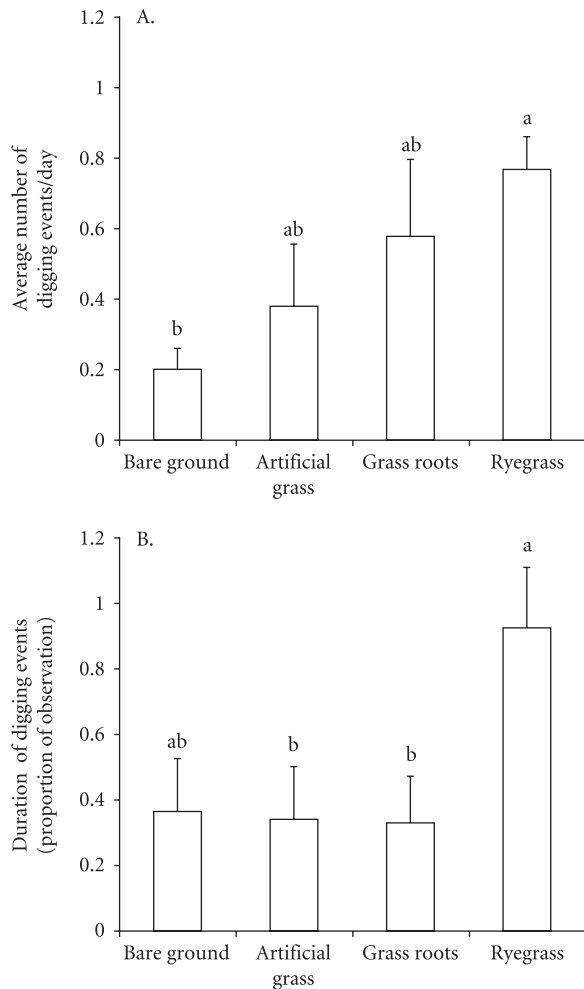


Figure 6 Frequency and duration of digging behavior by *Popillia japonica*, in bioassay arenas, as a function of the type of oviposition medium treatment. Data presented are the average number of digging events per day (A) and average duration of digging bouts (B) on the four treatments. Bars in a graph with the same letter are not significantly different ($\alpha = 0.05$).

significantly more times on ryegrass than on bare ground ($t = 3.01$, d.f. = 16, $P = 0.02$) (Figure 6A). Although no statistically significant differences were observed in any of the other mean comparisons, the number of digging events on grass roots was almost three times higher than on bare ground. Beetles started digging twice as many times on ryegrass (0.77/day) than on artificial grass (0.38/day).

Female *P. japonica* spent significantly more time digging under the soil surface in ryegrass than in artificial grass ($t = 3.94$, d.f. = 16, $P < 0.01$) or grass roots ($t = 3.16$, d.f. = 16, $P = 0.01$) (Figure 6B). Comparisons of proportion of time spent on or in a particular oviposition resource showed that the time spent digging in bare ground (36%) was

similar to that in grass roots (33%) and artificial grass (34%), whereas beetles spent 92% of their time digging on, or under the soil surface of ryegrass treatment. Despite these numerical differences, the proportions were not significantly different.

Discussion

Bioassays conducted in the oviposition arena developed for this study revealed that female *P. japonica* are selective in their oviposition investment decisions. Natural grass elicited the strongest response, and isolated structural and chemical cues also stimulated females to lay eggs in the soil. Plant-related oviposition preference has been examined previously in field studies with insects that lay eggs in the soil, including scarabaeid (King et al., 1981 and references therein) and chrysomelid beetles (Boetel et al., 1992), although these studies did not investigate the mechanisms involved in oviposition site selection. The bioassay developed in this research could be useful for studying oviposition behavior of generalist or specialist insect species that lay their eggs in the soil, and determining the factors involved in oviposition decision-making.

The number of eggs per soil block increased as an increasing number of plant cues were present on the soil surface. This trend in *P. japonica* oviposition has been inferred from grub abundance in soil samples in different agricultural systems (Fleming, 1972; Smitley, 1996; Szendrei et al., 2005), but to date it has not been measured directly. When female *P. japonica* were offered a choice between natural and artificial oviposition cues at oviposition sites, beetles preferred ryegrass more than artificial stimuli (experiment 1), suggesting that the artificial grass was lacking some visual, tactile, or olfactory quality that serves as an important cue for oviposition. Manipulation of the soil surface by punching holes (experiment 2) increased the average number of eggs laid compared to that in bare ground, indicating that female *P. japonica* use soil texture in the evaluation process of the oviposition medium. The presence of grass purée on soil blocks with holes did not increase the number of deposited eggs. This may have been because *P. japonica* do not use volatile or tactile chemical cues from grass to select an oviposition site, or because of the higher moisture content of these soil blocks with the addition of the grass purée. Alternatively, the odors released after our grass-extraction method may be different from that of real grass. Future investigations with different grass parts and plant-extraction methods are needed to examine the role of volatile and non-volatile chemical cues in oviposition decisions by *P. japonica*.

In experiments 1, 2, 4, 5, and 6, soil blocks with artificial grass received consistently more eggs than bare ground,

revealing that vertical objects on the soil surface serve as post-alighting pre-ovipositional cues. This kind of response to vertical plant cues on the soil surface was also observed to be one of the important cues stimulating onion fly oviposition behavior (Harris & Miller, 1984). In the presence of artificial stems (experiments 4–6), *P. japonica* were able to discriminate between the quality and quantity of the stimuli on the soil surface; more eggs were laid in soil blocks that had large stem densities or a higher number of stems. It is possible that females initially oriented towards a resource because there were more or larger diameter stems in the soil, which was perceived as a larger soil surface area covered by potential resources for the soil-dwelling offspring. After encountering the resource, the positive response in oviposition investment to stem diameter (experiment 4) and density (experiment 5) could be a result of the greater probability of encounter with the cues that are proportional to the diameter or density of the stems (Visser, 1988). It could also be that elevated number of eggs in artificial grass treatments compared to bare ground is due to the fact that the wire stems impaired the beetles' movement, causing them to stay below ground longer, although this is unlikely, since the stems reached 0.5 cm into the soil and the sandy soil allowed the stems to fall if they were pushed by the beetles. In experiment 3, more eggs were found in ryegrass than in clover, suggesting perception of visual, tactile, or olfactory differences between the two plants. However, in the context of our present findings, morphological (i.e., leaf shape) differences between the two plants may play a significant role in female oviposition choices.

Popillia japonica are not only capable of evaluating the plant characteristics on the soil surface, but our results also suggest that they adjust their investment decisions according to the strength of the resource cues, i.e., more eggs were laid in resources that were chosen more frequently. Correlations between the proportion of soil blocks with eggs and the average number of eggs per soil block were not statistically significant in most cases, but in experiments 1–5, the regression coefficients were all positive and above 0.7. This finding is further supported by the maximum egg numbers, which were typically the highest in those resources that were more often chosen by the females throughout all the bioassays. Additional investigation is needed to verify that choice of an oviposition resource translates into increased larval survival and development, as has been found in other herbivorous insects (Jaenike, 1978; Bernays & Chapman, 1994; Showler, 2001). *Popillia japonica* larval survival and growth are highest on perennial ryegrass (Crutchfield & Potter, 1995) compared to other cold-season turfgrass species, but the degree of correlation between *P. japonica* female preference and larval performance is not

known. Oviposition investment in suboptimal resources suggests that spreading offspring survival risks (Seger & Brockman, 1987; Roitberg et al., 1999) could be an important strategy where larvae have little mobility, as in the case of *P. japonica*. Although this might be an artifact of the amount of time the beetles were enclosed in the arenas, in 39% of all the bioassay arenas across the six bioassay experiments, females laid eggs in more than one treatment. Interestingly, the highest propensity to lay eggs in more than one resource was found in experiments 1 and 4, where the correlations between the proportion of soil blocks with eggs and the numbers of eggs per soil block was the highest. The variation in oviposition activity between experiments could be because females of varying ages were used in the different experiments, although Van Timmeren et al. (2000) found that in laboratory bioassays *P. japonica* oviposition rate was not affected by the age of females.

On a highly stimulating resource, *P. japonica* may accumulate sufficient stimulus for oviposition in less time than on a lower-quality resource, thereby enabling quick decisions to lay eggs (Finch & Collier, 2000). This may allow additional time for other behaviors, such as feeding and mating. Some insects can adjust their oviposition behavior based on resource quality, for example by reducing the number of eggs laid on a poor-quality resource (Awmack & Leather, 2002), and based on the present results (Figures 3 and 5), this might be the case for *P. japonica*. An implication of these findings is that most grass species used in lawns may be equally suitable for oviposition by *P. japonica*. Future investigations into potential ground covers for control of *P. japonica* through reducing oviposition should focus on plants that have broad leaves or other morphological or chemical features that markedly distinguish them from turfgrass species.

While these bioassays have revealed cues that stimulate *P. japonica* females to lay eggs in soil, the behavioral observations show how these surface cues might play a role in influencing digging behavior and the time spent under the surface. Beetles that engaged in digging either disappeared under the surface in a short time or gave up digging, so the longer digging bouts correspond to longer time spent in the soil. The number of digging events was not significantly different among the three treatments with surface cues (ryegrass, grassroots, and artificial grass) (Figure 6A), but beetles spent significantly more time in the soil under ryegrass than under any of the other treatments (Figure 6B). *Popillia japonica* may perceive contact cues for oviposition from ryegrass leaf blades on the surface that not only determine whether they oviposit in a resource, but also the amount of time spent in the soil, and the number of eggs laid. Alternatively, there may be oviposition cues detected after entering the soil under ryegrass that provide further

indication of resource suitability, modulating the number of eggs laid. These cues may be physical or chemical, and may involve interactions between soil particles and grass roots.

Further investigation of *P. japonica* oviposition behavior is expected to provide insight into potential avenues for disruption of this insect that is polyphagous as an adult, but more specific in its oviposition behavior. In annual and perennial agricultural systems, ground covers may be successful as natural barriers to avert fecund *P. japonica* from oviposition within areas that would otherwise provide suitable conditions for larval development.

Acknowledgements

Thanks to Anna Wright, Lydia Langdon, and Rozlyn Fulgoni for help in setting up and checking the bioassays, and to Elly Maxwell for help in collecting insects in Alabama. We are grateful for Dr M. Whalon for lending the video system. Members of the Small Fruit Entomology Laboratory provided helpful comments on earlier versions of the manuscript. Financial support was provided by Project GREEN. This research was completed in partial fulfillment of the requirements for a doctoral degree in entomology at Michigan State University.

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