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POTENTIAL FOR BEHAVIORAL AND CULTURAL MANIPULATION
OF JAPANESE BEETLE (*POPILLIA JAPONICA* NEWMAN,
COLEOPTERA: SCARABAEIDAE) POPULATIONS

presented by

Zsofia Szendrei

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POTENTIAL FOR BEHAVIORAL AND CULTURAL MANIPULATION OF JAPANESE BEETLE (*POPILLIA JAPONICA* NEWMAN, COLEOPTERA: SCARABAEIDAE) POPULATIONS

By

Zsofia Szendrei

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ABSTRACT

POTENTIAL FOR BEHAVIORAL AND CULTURAL MANIPULATION OF JAPANESE BEETLE (*POPILLIA JAPONICA* NEWMAN, COLEOPTERA: SCARABAEIDAE) POPULATIONS

By

Zsofia Szendrei

The Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is an invasive insect with potential for range expansion into many of the major agricultural production regions of the world. *Popillia japonica* is currently the most important insect pest for producers of highbush blueberry, *Vaccinium corymbosum* L., in the Midwestern United States. At fifteen commercial highbush blueberry farms in Michigan, fields with tilled row-middles had 72% fewer *P. japonica* larvae than fields with grassy row-middles. *Popillia japonica* larval populations were similar in the perimeters of tilled and untilled fields. Adult beetles on bushes were significantly less abundant in tilled fields compared to fields with grassy row-middles. The abundance of larvae inside fields during the spring was significantly correlated with early, but not late summer abundance of adult beetles on bushes. Grassy plots between rows of blueberry bushes tilled in spring and kept bare all year had fewer *P. japonica* larvae than plots retaining perennial ryegrass. Tillage in the spring and in the autumn caused 51% reduction in 2002 and 69% in 2003. Over three years, significantly fewer adult Japanese beetles were found on clover, ryegrass or bare ground than on buckwheat, whereas larval density from natural infestation in these plots in the fall was significantly lower under buckwheat and bare ground than under ryegrass and clover. Significantly fewer eggs that were artificially

placed into the soil in these plots developed into larvae under clover and bare ground, than under ryegrass and buckwheat. Female *P. japonica* observed in the field spend more time digging on ryegrass than buckwheat or clover. These field results indicate differential acceptance of females when choosing an oviposition site. In laboratory behavioral bioassays, females laid more eggs in soil with artificial grass stems compared to bare ground, and preferred for higher stem density and diameter, but did not respond to different stem colors. These findings suggest the possibility for reducing *P. japonica* populations by changing the vegetation characteristics of blueberry fields. In a survey, Michigan blueberry growers were asked to rate the extent to which *P. japonica* is a pest within this crop, and to reveal their responses to this pest management challenge. This insect was of concern to the majority of growers that returned the survey (84%), causing additional costs per acre of $72. Increased insecticide use was the major cause of the economic loss. Insecticides and clean cultivation were the main method for controlling Japanese beetle. Japanese beetle has driven changes in row-middle management, indicated by growers who have switched to clean cultivation recently. Many growers were willing to try new cover crops, if they are shown to be effective against Japanese beetle.

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CHAPTER 1:

BIOLOGY, ECOLOGY AND MANAGEMENT OF THE JAPANESE BEETLE

INTRODUCTION

History and distribution of the Japanese beetle

The Japanese beetle (*Popillia japonica* Newman, Coleoptera: Scarabaeidae) is endemic to the main islands of the Japanese Archipelago. Today, this insect is established in eastern North America and on Terceira Island (Azores, Portugal), where it escaped from a United States air base in the early 1970s (Lacey et al. 1994). The Japanese beetle is currently established in all states East of the Mississippi River, except for Florida, and parts of Wisconsin, Minnesota, Iowa, and Nebraska (Fig. 1.1). The beetle is capable of dispersing up to 12 km/year, and has the potential to spread further into southern Canada, and some of the western parts of the United States (Allsopp 1996). To prevent the further spread of Japanese beetle, the United States Department of Agriculture (USDA) has established the Japanese beetle Harmonization Plan that restricts interstate shipment of nursery stock with soil and movement of airplanes from regulated airports. The western states protected by the Harmonization Plan are: Arizona, California, Idaho, Nevada, Oregon, Utah, and Washington, with Montana in the process of being added to that list (Anon. 2003).

This exotic pest was brought from Japan into southern New Jersey in 1916 with a shipment of nursery materials (Fleming 1972). The beetle entered the United States without its natural enemies, and started spreading west as it found favorable climate, large areas of turf for the development of grubs and almost 300 species of plants for the adults to feed on (Fleming 1972).

Soon after it was identified in the US, its potential threat to agriculture was recognized and the USDA Japanese beetle Laboratory was established in 1917 to learn more about the biology of this insect. As soon as the first Japanese beetle appeared in Michigan in 1932 in Detroit, the USDA started eradication and control programs. Local eradication efforts brought success initially (Anon. 1959), but the lack of quarantine and the presence of the beetle in surrounding states made it clear that permanent eradication

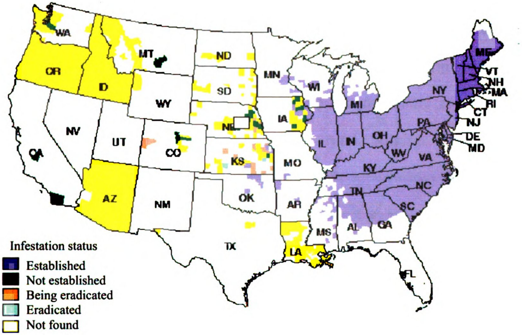


Figure 1.1. Distribution of the Japanese beetle in the US in 2004. (Anon. 2005)

was impossible. Beside the beetle's natural spread westwards, it was also accidentally transported by car, airplane and other means of human transport to new areas in the state. In the 1960s, area-wide eradication programs were organized to eliminate infestations, and treatments included soil insecticide against the larvae and aerial applications of insecticide against the adults (Anon. 1962, Anon. 1963). However, these efforts have failed to eliminate this pest. Recently, the Japanese beetle has become the most important production problem for numerous plant commodities in Michigan, including highbush blueberry.

Michigan leads the nation in the production of highbush blueberry (*Vaccinium corymbosum* L.); it produces over 40% of the US crop. However, the financial security of the Michigan blueberry industry has been threatened by the recent invasion of Japanese beetle into it's major production centers. The zero threshold for beetles (zero insects in fruit sampled by the buyer) by processors or the fresh market demands superlative pest control by the grower, and has led to increased insecticide applications close to harvest. It is clear from the recent contamination problems experienced by some blueberry growers that chemical control of adults is not wholly effective. This one-dimensional approach is also environmentally damaging, due to the toxicity of many broad-spectrum insecticides to non-target organisms and their persistence.

Biology of the Japanese beetle

Lifecycle and reproduction. The Japanese beetle is univoltine throughout most of its range in the United States, but in parts of its northern range it can take two years to develop (Vittum 1986). Adult emergence begins in early July in Michigan. Some researchers consider the Japanese beetle protandrous (Fleming 1972, Régnièr et al. 1981a, Vittum 1986), but in a recent study female emergence was skewed significantly earlier than that of males (Van Timmeren et al. 2000).

Emerging females carry an average of 20 eggs and begin to oviposit approximately two weeks after emerging and mating (Fig. 1.2). Females alternate between periods of feeding and egglaying; and they typically enter the soil 10 or more times and depositing 40-60 eggs over a 4-6 week adult life span (Fleming 1972). Eggs hatch in about 10-14 days, and development of the first and second instars requires about 2-3 weeks and 3-4 weeks respectively (Fleming 1972).

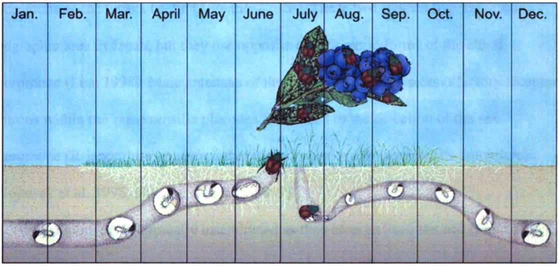


Figure 1.2. Lifecycle of the Japanese beetle in Michigan.

Most grubs are third instars by late September in Michigan. Young larvae are more sensitive, particularly to desiccation, whereas older larvae are less susceptible to environmental stress, partly because of their ability to move downward in the soil (Régnière et al. 1981b, Allsopp et al. 1992). The larvae overwinter below the frost line and start moving upwards in the spring once soil temperatures rise above 10°C (Fleming 1972). The larvae feed for another 4-8 weeks, and then move down in the soil to pupate. The prepupal stage lasts approximately ten days and the pupal stage lasts 7-17 days (Fleming 1972). Newly emerged adults remain in their earthen cells for 2-14 days before emerging (Fleming 1972).

Virgin females emit a sex pheromone ((Z)-5-(1-decenyl)dihydro-2(3H)-furanone), which is highly attractive to males (Tumlinson et al. 1977). The sex pheromone is produced in female-specific epithelial cells that line the inner surface of anal plates and two apical sternites. These cells are connected through many pores to the cuticule surface (Tada and Leal 1997). The sex pheromone has an identical chemical formula to the sex pheromone of the Osaka beetle (*Anomala osaka).* These two species live in the same geographic area in Japan, but they use opposite enantiomeric forms of the chiral pheromone (Leal 1998). Male antennae of the Japanese beetle possess olfactory receptor neurons within the same sensilla placodea, one tuned to the detection of the sex pheromone (R-japonilure), and the other to the behavioral antagonist, (S-japonilure) (Wojtasek et al. 1998, Nikonov and Leal 2002).

Virgin females are typically inseminated as they emerge from the soil (Ladd 1970). Japanese beetles are both polygynous and polyandrynous (Barrows and Gordh 1978), and sperm from the last mating is used to fertilize the eggs (Ladd 1966). Males guard females and experiments have confirmed sperm displacement (Ladd 1966), which is likely the driving cause of guarding behavior. Repeated mating is unnecessary for the sustained production of fertile eggs, therefore polyandry is likely to have evolved to increase fitness

(Ladd 1966, Ladd 1987). The food type and the age of females affect both their fecundity and their fertility (Goonewardene 1969, Ladd 1970).

Feeding habits. The adult Japanese beetle is highly polyphagous; it feeds on the leaves, flowers and fruits of over 300 plant species of wild and cultivated plants in 79 families (Fleming 1972). Host-finding is said to be mainly mediated by olfaction in adult *P. japonica* (Ahmad 1982), and both sexes are strongly attracted to blends of plant volatiles released from beetle-damaged leaves (Loughrin et al. 1995, Loughrin et al. 1996, Loughrin et al. 1997).

The stimulatory and inhibitory effects that plant chemicals, either primary or secondary, exert on the host-plant selection behavior of herbivorous insects counteract each other and their balance determines the possible outcome of the decision-making process: rejection or variable degrees of acceptance (Miller and Strickler 1984). In polyphagous species, several ubiquitous primary plant metabolites are sufficient to stimulate feeding on many plant species and rejection occurs only in those plants that produce deterrents of such quality or in such quantity that feeding stimulation is inhibited (Schoonhoven et al. 1998). Japanese beetles accept a range of host plants regardless of their suitability, thus it is likely that their decisions to accept a food source are based on non-specific plant cues like the ones that occur at the leaf surface, i.e. ubiquitous plant sugars (sucrose, maltose, fructose and glucose), that are phagostimulants for this species (Ladd 1986). Cucurbitacins (Tallamy et al. 1997), neriifolin (Reed et al. 1982), and phenolic glycosides (Orians et al. 1997) are examples of secondary metabolites produced by plants that deter feeding by Japanese beetle. The lactiferous secretion of milkweed has also been found to be a feeding deterrent for this species (Doussard and Eisner 1987).

Japanese beetle larvae are polyphagous, but because of their limited mobility they tend to be facultative monophages (Potter and Held 2002). The larvae feed on roots of grasses, weeds, garden and nursery crops (Fleming 1972, Potter et al. 1992, Crutchfield and Potter 1995, Smitley 1996).

Integrated pest management of the Japanese beetle

Integrated pest management programs emphasize ecosystem-based strategies that provide economical long-term solutions to pest problems (Flint and Gouveia 2001). Insecticide treatments alone may not always be economically optimal or ecologically acceptable. Integrated pest management practices may include managing water and fertilizer, adjusting cultivation techniques, the use of mating disruption, or disruption of the pest habitat (Flint and Gouveia 2001). Strategies that encourage natural enemies can also help limit pest numbers. With growing concern for the environment, methods of pest control have become more refined and require a precise understanding of the target insect's biology, physiology, behavior, distribution, etc. The shift in management practices from relying solely on insecticides towards integrated pest control necessitates the combination of different methods that complement each other in minimizing harmful effects on the environment and economic loss to growers (Matthews 1984).

Sampling populations. As an insect threatens a crop valued by humans, it becomes a target for control. Efficient control measures require an understanding of the presence and spatio-temporal distribution of the targeted pest population. Detection of the presence of Japanese beetle became a primary focus in early eradication programs (Fleming 1976), and the investigation for attractants began as early as 1919 (Fleming 1972). The female pheromone was identified in 1977 (Tumlinson et al. 1977), and effective attractants were developed in 1980 (Ladd and McGovern 1980). Male and female beetles can now be monitored by deploying traps baited with floral odor lures containing phenylethyl propionate: eugenol: geraniol (3:7:3) and sex pheromone lures ((R,Z)-5-(Dec-1-enyl)dihydro-2(3H)furanone) (Tumlinson et al. 1977).

Assessment of Japanese beetle grub populations requires soil sampling with a spade or a golfcup cutter. Larval populations are typically aggregated, so sequential sampling plans have been proposed in turf systems (Dalthorp et al. 1999).

Chemical control. Management of Japanese beetle in agricultural fields is complex because the adults are mobile and, in many cases, targeting one life stage may not provide sufficient control. There is a lack of information on the relationship between larval and adult abundance in a given area, making it hard to estimate the connections between controlling grubs and reduction in the number of subsequent adult beetles. Insecticides are widely used in turf and home lawns against Japanese beetle larvae, where toxicity of the residues in food products is not an issue, and where larval control is the primary concern. The soil-applied chloronicotinyl, imidacloprid, and the thianicotinyl, thiamethoxam, are effective insecticides against white grubs when applied to the soil as preventative treatments during or immediately after egg laying (Grewal et al. 2001). They have also been effective in preventing the downward movement of the overwintering grubs at the onset of winter, thus affecting their survival (Grewal et al. 2001). The recent registration of the soil-applied formulation of imidacloprid to control *P. japonica* larvae in blueberry provides a new alternative for growers to reduce pest pressure in and around their fields, especially in highly infested areas.

Insecticide applications against adults continue to be the foundation of management strategies for Japanese beetle in blueberries (Z. Szendrei, unpublished; Chapter 5) and many other fruit crops, as growers strive to meet the market's demand for contamination-free fruit. Broad-spectrum insecticides are effective against beetles that are treated directly (Isaacs et al. 2004), but many of the effective insecticides have long pre-harvest intervals, creating a potential for immigrating beetles to re-infest fields as residue activity declines. Use of a short-lived insecticide with rapid 'knock-down' activity, such as a pyrethrum (Casida and Quistad 1995), may be an effective approach for removing live beetles from bushes immediately before harvest. Extracted from chrysanthemum flowers, these botanical insecticides immediately paralyze treated insects and cause beetles to drop to the ground. However, the expense, environmental impact, and food safety concerns related to insecticide treated fruit mandate the search for non-chemical approaches.

Biological control. When the Japanese beetle was introduced into the United States, biological control agents of this species were limited to generalist predators such as birds, toads, mammals, and insects (Fleming 1968). These failed to reduce populations or limit the spread of the beetle. To enhance population control by natural enemies, predator and parasite species were imported from Japan starting as early as the 1920s, and reared for releasing into areas with known Japanese beetle infestation (Fleming 1968). Out of the 49 imported species, only a few became established. The most widely distributed were: *Tiphia vernalis*, a wasp that parasitizes overwintering grubs; *Tiphia popilliavora*, which attacks young grubs in late summer; and *Istocheta aldrichi*, a tachinid fly that parasitizes newly emerged adults (Fleming 1968). None of these species has been found in Michigan (Cappaert and Smitley 2002).

Japanese beetles with entomophagous nematodes were first found in New Jersey in 1929 (Fleming 1968). Some like *Steinernema glaseri* and *Heterorhabditis bacteriophora* can be effective biocontrol agents of Japanese beetle in turf and nursery stock (Potter and Held 2002). New species of nematodes, such as *Steinernema kushidai* (Koppenhöfer et al. 2000a) and *Steinernema scarabaei* (Koppenhöfer and Fuzy 2003), which are relatively specific to scarab larvae, may prove to be more effective. There is a synergistic interaction between certain species of nematodes (*Steinernema glaseri* and *Heterorhabditis bacteriophora*) and imidacloprid, where the action of the insecticide decreases the activity of the grubs, thus exposing them to increased nematode attack (Koppenhöfer et al. 2000b). Despite their potential, entomopathogenic nematodes were found to be uncommon in recent surveys of *P. japonica* larvae in Michigan (Cappaert and Smitley 2002).

Milky disease, a bacterial pathogen, was first discovered in central New Jersey in 1933, when a few abnormally white grubs were found in the field (Fleming 1968). Programs to disseminate *Paenibacillus* spores were conducted in the 1940s and although this effort suppressed Japanese beetle populations in treated areas, milky disease remained a factor that contributed to population suppression but was not by itself an effective microbial insecticide (Fleming 1968). Bacterial pathogens infected less than 1% of the Japanese beetle larvae in Michigan (Cappaert and Smitley 2002).

Pathogenicity tests with different species of fungi found attacking white grubs have been conducted since 1921 (Fleming 1968). *Metarhizium anisopliae* prevalence in natural Japanese beetle populations is low, and in Michigan and Connecticut this pathogen was detected in a very small percentage (<0.1%) of the samples (Cappaert and Smitley 2002).

Ovavesicula popilliae, a microsporidian, was shown not to affect larval weight or development of Japanese beetle in the laboratory. However, under field conditions, adult emergence was delayed and female fecundity was reduced at high infection rates (Hanula 1990). This pathogen was present in *P. japonica* grubs collected from a few locations in Michigan (Cappaert and Smitley 2002). Generally, gregarines have been considered weak pathogens having little affect on their hosts (in Cappaert and Smitley 2002). However, few gregarines have been studied carefully enough to determine the effect of infection on their hosts.

Behavioral and cultural pest control. Insect populations may be reduced by manipulation of the target insect's behavior. Stimuli that either inhibit or change the pestilential behavior are used, and for the protection of a crop it is necessary to understand the behavior (or aspect of behavior), the method by which it can be successfully manipulated, and the implementation of this method (Foster and Harris 1997). The basis by which the behavior for manipulation is chosen is usually driven by the available methods, and is often not linked to the type of damage caused.

Mechanism of cultural pest control. An important aspect of behavioral manipulation of plant pests is the process of resource acquisition by the insect in the context of the insect-plant interaction (Opp and Prokopy in Miller and Miller 1986). In the case of the Japanese beetle, plants provide essential resources for feeding, mating, egg-laying and refugia. As the insect through its environment, it meets a multitude of stimuli, mainly visual and chemical plant cues (Schoonhoven et al. 1998). Olfaction is a key factor in host finding by Japanese beetle (Ahmad 1982, Loughrin et al. 1995, Loughrin et al. 1997). Insects receive information from the environment through their peripheral sensory system, which is then processed to shape a response. Thus, there are two major phases that can be the target of behavioral manipulation: changing the inputs from the environment, or, altering the processing of these inputs (Foster and Harris 1997). Insect behavior is usually manipulated by changing the normal environmental stimuli leading to pest interaction with a crop. The stimulus chosen for behavioral manipulation has to be accessible to the insect, well defined for reproducibility, easily controlled, and practical to apply (Foster and Harris 1997). The more specific a stimulus is to a behavior, the more precisely the particular behavior can be controlled, and although precise definition of a stimulus allows for better control of a behavior, it is not essential.

Natural objects such as plants are frequently used to manipulate behavior to reduce pest pressure (Prokopy 1994, Bugg and Waddington 1994, Hartwig and Ammon 2002), and in this approach, the stimulus is generally not defined precisely. Behavioral manipulation of an insect population within an agricultural field can be achieved through increasing the species diversity and changing the composition of the plant community within the field by for example planting cover crops. Vegetation designs and other habitat manipulation practices such as cover crop management, crop row spacing, or trap-cropping have been extensively studied in annual crop systems (in Aluja et al. 1997), and less well studied in perennial crops, because in these systems it is more difficult to experimentally test the impact of orchard design (Bugg and Waddington 1994). Risch et al. (1983) reviewed 150 studies of insect populations in intercropped systems and found that of 198 insect species reported in these studies, 53% had lower populations in the intercrops, 18% had higher populations, 9% showed no significant difference, and 20% had a variable response. A meta-analysis by Tonhasca and Byrne (1994) found lower herbivore densities in polycultures in 60-70% of the studies. Andow (1991) indicated that insect pest densities were lower in polycultres in 52% of the examined cases when compared to monocultures.

The two most common mechanisms proposed to explain lower densities of insect populations in intercropped systems are the "Natural enemies" and the "Resource concentration hypothesis" (Root 1973). The "Natural enemy hypothesis" stipulates that, due to increased diversity of the ecosystem, there is enhanced natural enemy abundance and heightened rates of predation and parasitism, leading to pest suppression. Beneficial insects often require pollen and nectar as adult food sources, which are easier to find in a varied plant composition. According to the "Resource concentration hypothesis" it will become difficult for an insects to find host plants in a diverse system and therefore they will be more likely to leave. This is particularly important for controlling a specialist herbivore, where the cues from the plants influence colonization rate and behavior. Vandermeer (1989) has proposed the "Trap crop hypothesis" in addition to the "Resource concentration hypothesis" to explain how vegetation diversity can affect herbivore abundance. This supposes that pests will be attracted by certain plants, and thus will be more attracted to trap crops and will be less likely to move into the crop to be protected.

Approaches to cultural control. Cultural controls are the modification of normal crop or landscape management practices to decrease pest establishment, reproduction, dispersal, and survival (Flint and Gouveia 2001). Cultural practices include some of the oldest pest management tactics used, often exploiting weak links in the pest's life cycle or behavior. Habitat modification intentionally changes the managed ecosystem to limit availability of one or more of the requirements for survival and reproduction, making the environment less suitable for pest populations and improving survival and effectiveness of the pest's natural enemies (Flint and Gouveia 2001). Using cover crops can have long term economic benefits, by increasing soil fertility, suppressing weeds, preventing soil erosion, conserving soil moisture, and reducing the need for herbicides (Flint and Gouveia 2001).

Cultural controls and Japanese beetle. In the case of Japanese beetle the mechanisms of ovipositional decision-making are not clear. Japanese beetle adults are extremely polyphagous, and host selection may be influenced by relatively nonspecific stimuli common to many different hosts (Foster and Howard 1998). Compared to food-searching behavior, fewer critical cues may be involved in choosing an ideal egglaying site, since the larvae are facultative monophages. Because of limited larval mobility, the mother's decision is crucial for hatching and survival of the offspring. In the literature, oviposition preference and specificity is most often studied on species that lay eggs on above-ground plant parts (Wiklund 1981, Foster and Howard 1998, Bossart and Scriber 1999, Showler

2001). Since Japanese beetle lays eggs into the soil, qualities of the soil and the plants that grow in the soil may be correlated with cues that drive female ovipositional choice. In laboratory assays, females responded differentially to soil qualities such as texture, moisture, and organic matter before ovipositing (Allsopp et al. 1992). The incorporation of *Metarhizium anisopliae* (a fungal pathogen for the biological control of scarab grubs) into the soil increased oviposition (Villani et al. 1994), suggesting that female beetles could detect the pathogen. Data from these laboratory experiments reveal an important aspect of Japanese beetle searching behavior: namely that the female makes post-alighting decisions on where to lay eggs based on soil-born cues. Currently, no information is available on how the evaluation of the environment takes place by the insect and to what extent plant cues play a role in female ovipositional choice. Circumstantial evidence for female choice is available via the distribution of grubs. On this basis females prefer wet, sandy soils and low-mown grass, in sunny locations (Fleming 1972, Potter et al. 1996). They do not lay eggs into bare soil under natural conditions (Fleming 1972), but will readily do so in a confined arena (Allsopp et al. 1992, Van Timmeren et al. 2000). Based on these findings, it could be possible to use ground covers as natural barriers to avert ovipositing females from an area that provides ideal soil conditions for oviposition and larval development.

A suite of approaches is required by growers that can be integrated into their farming operations for reducing the likelihood of beetle infestation, fruit contamination, as well as to create a suppressive environment for Japanese beetle. Grubs potentially provide a better target for control than adults, because they stay in the soil for eight months. During most of this time growers are not actively involved in fruit production, thus grub control would be time saving, because there would be reduced need for Japanese beetle adult control during the harvest season. Grub control can also be preventive, because the beetles are eliminated as grubs, so they never emerge to cause further damage. In highbush blueberry production, on the other hand, the adult stage is responsible for the economic loss, since the grubs feed only on the roots of grasses and weeds, but not of blueberries. Thus the relationship between the density and location of grubs and adults needs to be established before effective grub control measures can be recommended or implemented.

Because of the zero-threshold for beetle contaminants in harvested blueberries, the goal for the growers should be to reduce the presence of both adults and larvae of this insect in their fields. The main reasons for Michigan blueberry growers' interest in planting cover crops in their fields are weed suppression, reduction of soil erosion, and to permit driving machinery through the field during wet conditions. Since Japanese beetle is currently the most important insect pest in blueberries in Michigan (Z. Szendrei, unpublished, Chapter 5), there is a need for testing cover crop species that satisfy grower's horticultural needs and do not attract Japanese beetle to their fields. In practice, the naturally occurring weed population is kept under control by mowing or different species of grasses are seeded in the row-middles. Blueberries are grown in low-lying areas with high soil moisture and fields are irrigated in the summer. The wet soil conditions and the vegetation in and around the blueberry fields create an ideal condition for Japanese beetle oviposition. The blueberry industry provides a model system for understanding how cultural and behavioral manipulation of an insect can be achieved for control in an integrated management program.

Objectives

The overall goal of this research was to evaluate behavioral control methods for reducing Japanese beetle adult and larval populations within blueberry fields. Ideally, these methods should be economically viable, practical, and environmentally harmless. In order to investigate this broad question, I undertook a set of investigations into the ecology and behavior of the Japanese beetle. The first objective was to determine the distribution of larvae within and around commercial blueberry fields, and the effect of soil tillage on Japanese beetle larval density. Since adult Japanese beetles are responsible for the economic loss in highbush blueberry production, the effectiveness of larval control for the protection of blueberries is dependent on whether there is a relationship between the adult and larval life stages within a field. If larval control translates into reduction of adult abundance within the field, then other options for larval control, such as rotovation and planting cover crops in row-middles that are not ideal for larval development and survival, could be considered for reducing adult Japanese beetle abundance. The second objective was to test if cover crops have the potential to reduce oviposition and larval and adult abundance within a blueberry field. If this approach were successful in reducing oviposition within fields, this would decrease the subsequent larval abundance, and the number of adults that emerge from the field. The third objective aimed to understand the underlying behavioral mechanisms that lead to the selection of grassy areas. This knowledge would be indispensable for developing a pest-control strategy that growers could use reliably and which would be based on this behavioral feature of the insect.

My fourth objective was to conduct a survey to quantify management tactics that growers have adopted to control Japanese beetle and to determine willingness of growers to incorporate alternative management strategies, such as the use of cover crops, in their future management programs if they are proved effective against Japanese beetle.

CHAPTER 2:

EFFECT OF TILLAGE ON ABUNDANCE OF JAPANESE BEETLE LARVAE AND ADULTS IN HIGHBUSH BLUEBERRY FIELDS

INTRODUCTION

The Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), is an invasive insect with potential for range expansion into many of the major agricultural production regions of the world (Allsopp 1996). *Popillia japonica* is currently the most important pest for producers of highbush blueberry, *Vaccinium corymbosum* L., in the Midwestern United States (Anon. 2001, Isaacs et al. 2004). It is a univoltine insect; adults are most abundant from mid-July to August (Fleming 1972), when beetles feed on leaf tissue and ripe blueberry fruits. If beetles are not controlled, fruit can be contaminated when harvesting machines knock beetles off the bushes. Over 70% of Michigan's 7,300 ha of blueberry production is harvested mechanically (Kleweno and Matthews 2002), and because the market demands fruit completely free of insect contamination, strategies are needed to minimize the risk of adult beetles being present during harvest. Foliar insecticide applications continue to be the foundation of *P. japonica* management in blueberries and many other fruit crops, as growers strive to meet exacting quality standards. Additional strategies targeting larvae, which develop in the soil, may help growers reduce populations of *P. japonica* within infested blueberry production regions and minimize the risk of beetles spreading into uninfested areas. Such strategies could also have the long-term benefit of reducing the number of foliar applications of insecticides.

Larval insect populations are influenced by initial adult distribution and density, oviposition preference and host plant acceptability, egg survival, and host plant suitability (Singer 1986, Renwick 1989). Conditions in and around crop fields can favor or inhibit development of *P. japonica* depending on whether requirements for population development are met (Vittum et al. 1999). Within many Michigan blueberry fields and along the perimeter of these fields, ground covers of seeded grass or the naturally-invading mix of grass and broadleaved weeds are commonly used to maintain soil structure, provide conditions where agricultural machinery can be driven during wet conditions, reduce soil erosion, and prevent pesticide and fertilizer runoff (Pritts and Hancock 1992). Fields are often irrigated to minimize crop stress and to maintain fruit quality and yield during periods of insufficient rainfall. Together, these factors provide ideal habitat for oviposition, egg hatch, growth, and survival of *P. japonica* larvae (Régnière et al. 1981, Allsopp et al. 1992, Potter et al. 1996).

Cultural practices such as tillage can have strong negative effects on arthropod pests, by modifying the soil habitat where many insects reside during at least a part of their life cycle (Funderburk et al. 1990). Immature stages of *P. japonica* feed on the roots of grasses, so mechanical manipulation of their habitat may alter their survival and development (Fleming 1976). In 1955 Cory and Langford (in Fleming 1976) reported that rototilling destroyed up to 90% of the *P. japonica* grubs. Fleming (1976 and references therein) reported that reduction of larval population in fallow and sodlands was greatest when the soil was rototilled in early autumn and late spring, when grubs are near the soil surface. Previous studies have also investigated the potential for control of white grubs through some cultural practices (mowing height, irrigation, application of organic fertilizer, aerification, heavy rolling) that are appropriate in turf systems (Potter et al. 1996). Tonhasca and Stinner (1991) explored the effect of tillage on adult *P. japonica* abundance in soybean fields, but there is a lack of information on cultural manipulation of *P. japonica* in perennial fruit crops and woody ornamentals.

To determine the response of *P. japonica* to tillage in the row-middles of highbush blueberry fields, we compared the relative abundance of larvae at fifteen commercial blueberry fields in row-middles that were either tilled or covered with mowed permanent sod comprised of seeded grass and local weed populations. The relationship between larval density and some soil parameters was examined to determine whether variation in grub abundance is explained by soil characteristics. Selected parameters describe conditions for nutrient uptake in the soil for the plants that serve as food sources for the grubs. Thus, these factors could have an indirect effect on herbivorous insect abundance. At six commercial blueberry fields (three tilled and three grassy), larval and adult densities were compared at the perimeter and interior of the fields. Spring larval populations were compared to subsequent summer adult populations, to determine whether there is a relationship between the two life stages within blueberry fields. In addition to these population studies, the effects of (1) the presence or absence of a grass ground cover and (2) different tillage timings on larval abundance of *P. japonica* were investigated experimentally over two growing seasons in a research planting of highbush blueberry.

MATERIALS AND METHODS

Larval density in commercial fields

The density of larval *P. japonica* populations was determined across the primary blueberry production region of Southwest Michigan (Ottawa, Allegan, Van Buren, and Berrien counties) in 2001 and 2002. Fifteen fields in 13 commercial blueberry farms with a history of *P. japonica* infestation were sampled. The sizes of the fields ranged from 1 to 5 ha.

Larval density was determined in April 2001, September 2001, May 2002, and September 2002 by taking 15 cm deep soil cores from the perimeter of each field and from the area between the rows of blueberry bushes (row-middles) of each field. This was done using a cylindrical golf cup cutter (area = 95 cm^2) (Parmenter & Andre Inc., Grand Rapids, MI). In each field, 80 samples were taken approximately 1 m from the edge of the blueberry field (in the drive lane around the field), by sampling in 20 positions spread out along each of the four sides of the field. Ten row-middles were selected that were spaced evenly across the fields, but more than 5 m away from the field border. In each selected row, six samples were taken equidistant between rows of bushes along the length of the field without sampling within 5 m from the row ends (60 interior samples / field). Soil cores were examined in the field, and all beetle larvae were placed in plastic bags with a small amount of soil. The bags were labelled with date, location, and number of larvae and transported back to the laboratory in a cooler containing an icepack. Larvae were identified to species using the diagnostic rastral patterns and other morphological features (Vittum et al. 1999).

For each field and sampling date, whether the fields were tilled or had grassy row-middles was recorded. Because row-middles were tilled based on growers' management decisions, the number of fields that had tilled or grassy row-middles varied by sampling date. Field perimeters were not tilled and had a grass-weed mix cover throughout the study, regardless of the type of row-middle management within the field. Foliar insecticides were applied to the bushes according to standard practices (Wise et al. 2003) at all of the farms.

The average numbers of *P. japonica* larvae in samples taken in the row-middles and in the perimeter of the fifteen fields were compared between tilled and non-tilled fields. Data were transformed to $\sqrt{(y+0.5)}$, before analysis to meet normality assumptions, and were analysed with a two-way ANOVA (tillage and date as factors) (SAS Institute 2001). *T*-tests were used at each sample date to compare samples from tilled and untilled fields. In cases where the population variance of the two compared groups were different, the Satterthwaite's approximation was used for calculating degrees of freedom for the *t*-tests (SAS Institute 2001).

Soil analysis

Soil cores (area 95 cm^2, 15 cm deep) were taken for soil quality analysis from all 15 fields in autumn 2002. One sample was taken from each of the four sides of each field, and four samples were taken from row-middles, distributed across the field. Each sample, was analysed for: pH, organic matter (%), and the concentration of P, K, Mg, and Ca (all in ppm). Samples were analyzed by A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN). Multiple regression was used to determine the relationship between the six measured soil parameters and average larval densities at the 15 fields in autumn 2002 (SAS Institute, 1999). Samples taken from the interiors and perimeters of the fields were analysed separately.

Relationship between larval and adult populations

In 2003, fields from the study described above that had consistent row-middle management history during 2001-2002 were selected. This group was comprised of three tilled fields and three with grass in their row-middles. Larval populations of *P. japonica* were sampled at all six fields in May using the methods described above. Eighty samples were taken from the interior and 80 from the perimeter of fields. Perimeter samples were taken approximately 1 m away from the bushes. Twenty samples were taken evenly on each of the four sides of the field. From the row-middles of fields, 8 samples were taken from 10 row-middles in a similar manner as described in the previous larval study.

The number of adult *P. japonica* per bush was sampled once in July, and once in August at each field by counting the number of adult beetles on 80 bushes on the perimeter (20 on each side of the field) and on 80 inside (10 bushes from 8 rows). All inside samples were taken at least 5 m from the edges of the field and the 10 sampling points were evenly spread out through the rows. Adult beetles were counted on sunny days with low wind velocity, and observers moved carefully through the field so as not to disturb beetles.

The abundance of adult *P. japonica* in July and in August was compared between grassy and tilled fields with the Mann-Whitney U test (SAS Institute 2001). Multiple regression analysis was used to determine relationships between larval and adult populations of *P. japonica* within fields (SAS Institute 2001). The regression model consisted of larval and adult numbers as variables and position as an indicator variable (perimeter = 0, interior = 1). Statistical analyses were performed on average numbers of adults per bush and average numbers of larvae per sample and were considered significant at α=0.05.

Effect of tillage on larval establishment

This experiment was conducted in a four-year-old 0.4 ha field of *V. corymbosum* cv. Bluecrop, at the Trevor Nichols Research Complex (TNRC) in Fennville, MI. The planting was established two years prior to the start of this experiment, on a 3.6 x 1.2 m spacing with 12 bushes in each row. The row-middles were kept clean of plants. *Popillia japonica* larvae were not found in soil samples taken before the experiment in April, 2002. Ten row-middles (14 x 2.5 m area between two rows of bushes) were tilled in May 10, 2002 and assigned to be grass or bare ground treatments. Treatments were assigned within the planting in a randomised complete block design with five replicates. Grass plots received perennial ryegrass seed at 27 kg/ha (*Lolium perenne* L., Michigan State Seed Solutions, Grand Ledge, MI) on May 23, 2002. Plots were rolled after planting to improve germination. This grass species was chosen because of it tolerates soil acidity. Bare-ground plots received further treatments of herbicide (Roundup Ultra 4WSL at 31.3ml/l) spot-applied with a 3.8 l hand-held sprayer when needed to keep the soil free of vegetation during the growing seasons. Fertilizers and pesticides were not used in the plots during this experiment. Samples were taken in September 2002, as well as May and

October 2003 to assess larval density of *P. japonica*. Five soil cores were taken with a golf cup cutter (area = 95 cm^2) from each plot, and collected larvae were identified to species as described above.

Larval densities in the two treatments were compared for each sample date with a Mann-Whitney U test, since normality assumptions were not met even after transformation (SAS Institute 2001; Ott and Longnecker 2001).

Effect of tillage timing in infested plots

This study was conducted in two 0.07 ha sections of a 1 ha field of *V. corymbosum* cv. Rubel, at TNRC. The blueberry plants were 10 years old on a 3.6 x 1.2 m plant spacing, with 12 bushes in each row. The 14 x 2.5 m row-middles between the rows of blueberry were comprised of naturally occurring weeds dominated by grass species, and were kept mowed. The experiment was conducted in 21 adjacent row-middles in 2002 and was repeated in 2003 in 21 adjacent row-middles located in a different section of the same blueberry field. These two sections of the field were selected based on prior sampling to verify the presence of *P. japonica* larvae across the field sections. The following three treatments were applied during both years to the different sections: tillage once in the spring, tillage once in the autumn, or tillage once in the spring and once in the autumn. Treatments were assigned to the row-middles in a completely randomised design with seven replicates. To apply tillage treatments, a tractor-powered BushHog rotovator (Model H72, Allied Products Corporation, Selma, Alabama) was driven between the bush rows twice, at 15 cm depth. Plots tilled in the spring were kept weed-free in the growing season by applying herbicide (as described above). Tillage treatments were applied on

May 10 and September 9 in 2002 and on May 2 and Oct 10 in 2003. All plots were sampled with a golf cup cutter as described above in the spring and the autumn, before and after tillage treatments. *Popillia japonica* larvae were sampled 2-4 d before and 10-14 d after tillage. Three samples were taken per row-middle in 2002 and six in 2003, and collected larvae were identified to species.

Larval count data were Poisson distributed, so pre- and post-tillage densities were compared using a log-linear model (PROC GENMOD, SAS Institute 2001). Comparisons of the percent reduction in *P. japonica* density between treatments were made after arcsine transformation with a one-way ANOVA procedure followed by Tukey's means separation (SAS Institute 2001).

RESULTS

Larval density in commercial fields

In spring 2001, the average density of *P. japonica* larvae across all commercial blueberry farms sampled was 0.28 ± 0.01 larvae/sample. This increased slightly in autumn 2001 and then decreased at each sampling time to 0.14 ± 0.01 larvae/sample by autumn 2002. Larval density was greater on the perimeter than in the row-middles of fields throughout this study, regardless of whether the row-middles were tilled. During the two sampling seasons in 2001, larval densities at the perimeter were approximately 4-fold greater than those in the interior, and by spring 2002 there was a 3-fold difference between these positions which decreased to 0.4-fold in autumn 2002. The highest average larval density in the perimeter of fields occurred in autumn 2001 (0.48 ± 0.02 larva/sample) and the lowest in autumn 2002 (0.17 ± 0.01 larva/sample). Eighty-seven percent of all the larvae were found in fields with grassy row-middles compared to those that were cultivated; the largest difference between the two treatments occurred in spring 2001 (0.14 grubs/sample) and the smallest difference occurred in autumn 2001 (0.09 grubs/sample) (Fig. 2.1). Larval density was significantly lower in tilled fields than in those with grassy row-middles in spring 2001 ($t = -3.41$, $P < 0.01$) and autumn 2001 ($t = -2.94$, $P < 0.01$) (Fig. 2.1a). At the other two sampling times, the differences between the two types of row-middle management were not statistically significant, even though on average there were twice as many larvae in grassy row-middles compared to tilled ones in autumn 2001 and 13 times more in spring 2002. Some growers changed row-middle management practices during the study, so between 6 and 13 of the 15 fields sampled had a grass ground cover, depending on the date of the sample (Fig. 2.1).

On average, larval density was 12 times greater in the perimeter than in the row-middles of tilled fields, whereas density was only 2.4 times greater at the perimeter of grassy fields (Fig. 2.1 a and b). Across the whole study, 78.5% of the larvae detected in soil samples were found in the perimeter of the fields; the highest value was 85% in autumn 2001, and the lowest value was 65% in autumn 2002. *Popillia japonica* larval density at field perimeters was not significantly different between fields with grassy or tilled interiors ($F_{1,3}$= 3.44, NS); but, in all of the samples, a greater density of larvae was found in the perimeter of grassy fields (Fig. 2.1 b).

In addition to *P. japonica*, larvae of European chafer (*Rhizotrogus majalis* Razoumowsky, Coleoptera: Scarabaeidae) and Asiatic garden beetle (*Maldera castanea* Arrow, Coleoptera: Scarabaeidae) were also found in the samples, but in very low numbers.

Soil analysis

Average organic matter content was similar in samples taken from the row-middles and from field perimeters, at 3.3 ± 0.1% and 3.5 ± 0.2%, respectively. The largest difference between positions was found for calcium, which was 668 ppm in the perimeter on average, compared to 558 ppm in the row-middles, but this difference was not statistically significant. The average concentrations of potassium and magnesium ions were not significantly different between the perimeter and the row-middles. There was no significant relationship between the measured soil parameters and larval densities, either for the soil samples taken in the row-middles ($F_{6,8}$= 0.57, R^2 = 0.3, NS) or in the perimeters ($F_{6,8}$= 2.85, R^2 = 0.68, NS).

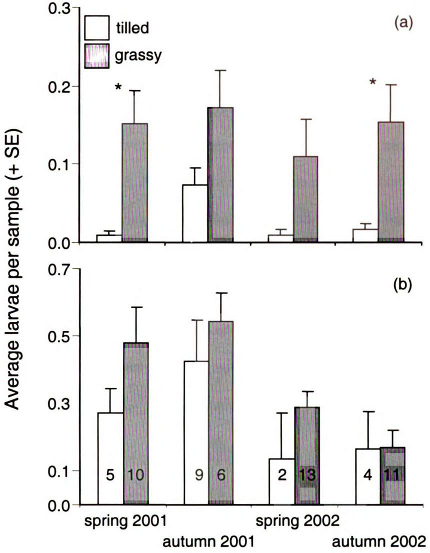


Figure 2.1. Density of *P. japonica* larvae (average + SE) in samples taken from (a) row-middles and (b) perimeters of commercial highbush blueberry fields with row-middles that were either tilled or grassy. An asterisk above the bars indicates a significant difference between tillage treatments (α=0.05). Numbers on the lower bars show the number of farms in each treatment during each date for (a) and (b).

Relationship between larval and adult populations

Adult beetles were less abundant in July (0.88 ± 0.41 beetles/bush) than in August (1.68 ± 0.59 beetles/bush). At both sampling times, greater numbers of beetles were detected on bushes in blueberry fields with permanent sod than in tilled fields (Fig. 2.2). This pattern was consistent across the positions of the sampled fields; but statistically significant differences in average adult beetle numbers were only detected in the perimeters ($Z = -1.96$, $n = 3$, $P = 0.049$ for both sample times). Fields that had greater larval abundance in the spring generally had more adult beetles on bushes in July, regardless of the position in the field (Fig. 2.3). Regression analyses showed that larval populations measured in the spring predicted 84% of the variability in July adult populations ($F_{2,9}$= 25.19, $P < 0.01$). In contrast to the July adult samples, beetle abundance in August was not correlated with earlier larval abundance ($F_{2,9} = 0.60$, NS) (Fig. 2.3).

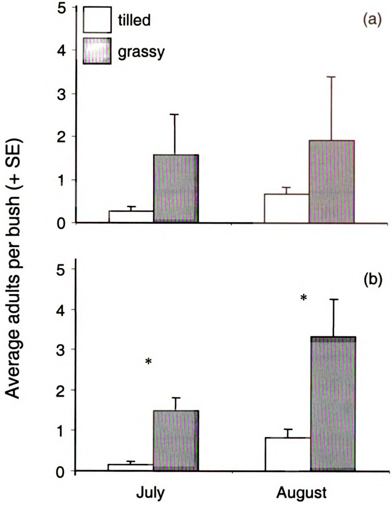


Figure 2.2. Average number (+ SE) of adult *P. japonica* per blueberry bush in July and August in fields with grass (N=3) or tillage in the row-middles (N=3). Values are presented for samples taken from (a) the field interior and (b) the field perimeter. Adjacent bars with an asterisk are significantly different (α=0.05).

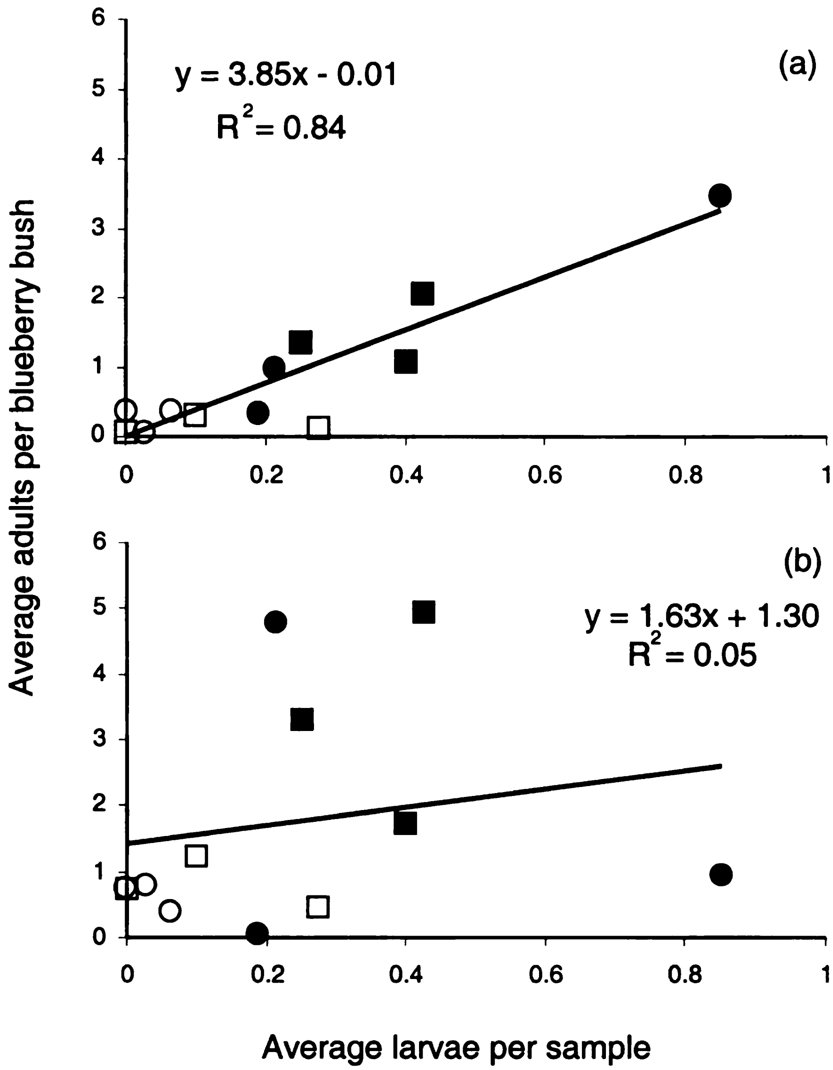


Figure 2.3. Relationships between *P. japonica* larval density measured in the spring and abundance of adults on blueberry bushes measured in July (a.) and August (b.) in the interior and perimeter of fields. White data points are from tilled fields and black data points are from fields with grassy row-middles. Circles indicate average values from interior and squares from perimeter samples.

Effect of tillage on larval establishment

Densities of *P. japonica* larvae increased during this experiment; the greatest increase occurred in plots that were planted with ryegrass (Fig. 2.4). Larvae in ryegrass plots were three times more abundant in autumn 2003 than in the previous autumn ($Z = -3.6$, $n = 10$, $P < 0.01$). During each of the three sampling dates, significantly fewer larvae were found in plots kept free of plants compared to those with ryegrass. The largest difference between the two treatments in the average number of larvae was in autumn 2003 ($Z = -3.8$, $n = 10$, $P < 0.01$).

Effect of tillage timing in infested plots

The change in *P. japonica* larval abundance due to tillage in the spring was not consistent over the two years; this treatment was associated with a significant decline in larval density in 2003, but not in 2002 (Table 2.1). Tillage in the autumn caused a decrease in *P. japonica* during both years when comparing pre- and post-tillage larval densities, but this trend was significant only in 2003 (Table 2.1). Tillage of the same plot twice, in the spring and in the autumn, significantly reduced larval density in both years, when comparing overall reduction from spring to autumn; by 51 ± 12 % in 2002 and 69 ± 15 % in 2003. The effect of tilling plots once was not consistent across the two years: although most of the treatments caused a similar reduction in *P. japonica* larval density in the row-middles, in each year one of the treatments did not provide any control. The percent control values (Table 2.1) were not significantly different in either year ($F_{3,20} = 0.65$, P = NS in 2002; $F_{3,20} = 0.13$, P = NS in 2003).

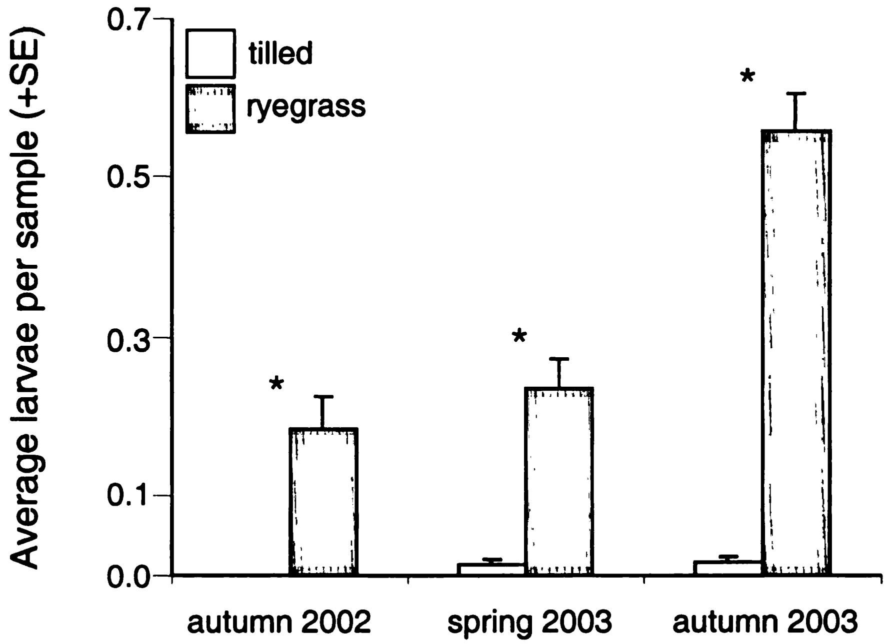


Figure 2.4. Density of *P. japonica* larvae (average + SE) in tilled (bare ground) or ryegrass row-middles. An asterisk above the bars demotes a significant difference between treatments (α=0.05).

Table 2.1. Effect of tillage timing on *P. japonica* larval density in plots within a blueberry planting. Comparisons of larval densities before and after tillage are represented by χ^2 values (NS = not significant).Percent control values show average reduction in larval density by treatment. Values in the last column are not significantly different from each other within years (α=0.05).

Tillage program	Sample timing	Average larvae per sample ± SE: Pre-tillage	Average larvae per sample ± SE: Post-tillage	χ^2	P	% control ± S.E.
2002						
spring	spring	0.9±0.3	0.9±0.3	0.0	NS	0±0
autumn	autumn	0.3±0.2	0.0±0.0	-	-	100±0
spring	spring	0.6±0.1	0.2±0.2	1.8	NS	58±27
-	autumn	0.3±0.1	0.1±0.1	3.4	NS	67±21
2003						
spring	spring	1.0±0.1	0.3±0.1	7.4	<0.01	66±14
autumn	autumn	1.0±0.5	0.1±0.1	10.1	<0.01	78±16
spring	spring	1.1±0.3	0.2±0.1	8.7	<0.01	72±12
-	autumn	0.2±0.1	0.3±0.1	0.6	NS	0±0

DISCUSSION

This study demonstrates that tillage can reduce larval *P. japonica* abundance in highbush blueberry fields. At commercial farms, tilled row-middles had lower larval and adult density of *P. japonica* than row-middles with grass cover. Smitley (1996) found lower densities of *P. japonica* larvae and adults in nurseries with clean fields compared to weedy ones, and this pattern is expected to be a general response of *P. japonica* to the lack of suitable hosts, leading to lower rates of oviposition and/or larval survival.

Tillage of the row-middles did not significantly reduce *P. japonica* larval populations in the uncultivated perimeters. Therefore additional control strategies, such as targeting early instars with soil-applied insecticides (Mannion et al. 2001) could be used to control *P. japonica* in regions of the farm that are not tilled. As the results depicted in Figure 2.4 suggest, the removal of grassy groundcover should reduce the number of beetles emerging in the field, providing fewer gravid females to move to the perimeter for oviposition. The higher larval density of *P. japonica* in the perimeters of fields, independent of the ground cover management regime, could be because gravid females arriving from outside the field are arrested at the field perimeters, and/or because females may leave insecticide-treated areas inside the field to lay eggs in the untreated soil around the fields.

The significant relationship between larval populations in the soil during the spring and adult populations on bushes in July (Fig. 2.3) suggests that spring larval sampling can be used to estimate local adult populations. This may allow growers to predict the risk of *P. japonica* adult presence across their farm and provide information to focus management inputs to fields with the greatest spring larval populations. Smitley

(1996) found a positive correlation between the number of *P. japonica* adults trapped and the number of larvae in nursery fields. Choo et al. (2002) found a similar positive relationship between adult and larval populations of oriental beetle (*Exomala orientalis* Waterhouse) in golf course fairways in Korea; adults were more abundant in fairways that had high larval densities. In their study of *P. japonica* spatial dispersion in golf courses, Dalthorp et al. (2000) suggested that grub dispersion patterns are dependent on location of adult feeding aggregations. *Popillia japonica* emerge from the soil in early July in Michigan, and the newly emerged adults may initially feed and aggregate close to their larval habitat if there is suitable food nearby. This may explain the greater predictability of adults in July compared to August. After 4-6 weeks of adult emergence and movement between fields, spring larval density no longer predicted the number of adult beetles on bushes.

The reduction of adult *P. japonica* densities by tillage may result fewer larvae emerging from these fields, but it could also be the case that fewer adult beetles may be attracted to blueberry fields with bare soil in the row-middles. In the small plot trials, tillage in row-middles was effective in preventing the establishment of *P. japonica* larval populations (Fig. 2.4). Tilling row-middles both in the spring and autumn reduced larval populations by 59%. The mechanism of larval population reduction through rotovation needs to be determined in the future.

The lack of relationship between soil parameters and larval density indicates that these factors do not affect the suitability of the environment in a blueberry field for *P. japonica*. Previous findings by Dalthorp et al. (2000) in turf found no relationship between mean *P. japonica* larval density and soil organic matter, but perennially low grub densities were associated with high organic matter content. Potter et al. (1996) have shown that the response of larval populations to spatial gradients in soil moisture may override the effects caused by soil pH, making soil micronutrient parameters of low value for predicting risk from *P. japonica*.

Targeting management tactics to the parts of the field or farm with the highest *P. japonica* larval population may be an effective and efficient way to help manage the adult pest populations. There are several advantages to controlling larvae in addition to adults. First, larval *P. japonica* are present for a longer period than adults, so there may be more opportunities for control. Second, larvae are not capable of moving large distances, so it would be easier to target controls against them, compared to the mobile adults. A recently available option for controlling *P. japonica* larvae in blueberry fields is the use of imidacloprid (Bayer CropSciences, Kansas City, MO) applied to the soil during the egg laying period (Mannion et al. 2001). Biological control methods are also being investigated, but parasites and pathogens of *P. japonica* are currently at low levels and cause only minor mortality in Michigan populations (Cappaert & Smitley 2002).

The presence of a groundcover, and the type of plant, are important in influencing larval *P. japonica* populations in turf (Potter et al. 1992, Crutchfield & Potter 1995), as well as in nursery (Smitley 1996) and Christmas tree plantations (Kard & Hain 1988). In sites where tillage is not appropriate, cover crops may be an alternative for management of *P. japonica*. Cover crops that are tolerant of the acidic soil conditions of blueberry fields are also being evaluated currently for their potential in reducing *P. japonica* populations.

CHAPTER 3:
COVER CROPS IN HIGHBUSH BLUEBERRY INFLUENCE JAPANESE BEETLE DENSITY AND OVIPOSITION BEHAVIOR

INTRODUCTION

Cultural control of insect populations in agricultural fields can be achieved by planting cover crops that affect pest dispersal, colonization, or reproduction (Bugg and Waddington 1994). A cover crop is any living groundcover that is planted in the main crop (Hartwig and Ammon 2002) for its positive horticultural effects, such as weed control, reduction of soil erosion, improvement of the biological, physical and chemical properties of the soil, or pest control. Increasing the diversity of plants in agricultural production can reduce pest abundance (Root 1973), but the mechanisms are dependent on the biology and behavior of the insect species. In many different agricultural systems, insect behavior is influenced by cover crops planted with a main crop, such that the pest is repelled or reduced in abundance (Risch 1981, Tonhasca and Stinner 1991, Bugg 1992, Bugg and Waddington 1994, Prokopy 1994, Hartwig and Ammon 2002). Despite its polyphagous feeding behavior as an adult, the more selective oviposition behavior of gravid females (Szendrei and Isaacs, in press, Chapter 4) may provide an opportunity for behavioral manipulation of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae). Following the steps provided by Foster and Harris (1997), development of such an approach will require an understanding of the behavior for manipulation, the method by which it can be successfully manipulated, and the implementation of this method for the protection of a commercial crop.

Selection of host plants for feeding by adult *P. japonica* may be influenced by relatively nonspecific stimuli common to many different hosts (Foster and Howard 1998). In contrast, the critical cues involved in choosing an ideal oviposition site are expected to be more specific since the female beetle is presumingly searching for specific habitat requirements. Insect pests are confronted with an array of potential hosts that vary in their suitability for larval development, and so most insects show some form of egg-laying preference (Jaenike 1978). The larvae of *P. japonica* are facultative monophages because of their limited mobility, thus the mother's decision is crucial in the hatching and survival of the offspring. Female preference for ovipositional sites, and larval performance on hosts chosen during egg laying by the adult are important in determining subsequent population densities in a field. Egg laying preference might be exploited for behavioral manipulation of *P. japonica*.

Popillia japonica is most likely to lay eggs into soil under monocotyledonous plants (Fleming 1972). Soil parameters such as moisture, texture, and organic matter content (Régnièr et al. 1981, Allsopp et al. 1992), short plant cover (Hawley 1944 in Fleming 1972) and light intensity (Dalthorp et al. 1999) can influence the number of eggs laid by *P. japonica*. In a comparison of larval abundance in nursery fields, *P. japonica* were 10-fold more abundant in weedy fields compared to clean fields (Smitley 1996), and clean cultivation of row-middles of highbush blueberry fields reduces the number of *P. japonica* larvae in the soil by 72% compared to field with permanent sod (Szendrei et al., in press, Chapter 2). These findings suggest that the presence of plants in the larval habitat is essential for development of *P. japonica*. The adult females of this species are also capable of evaluating plant cues on the soil surface to adjust oviposition investment

(Szendrei and Isaacs, in press, Chapter 4). Although clean cultivation between the rows of blueberry plants can reduce *P. japonica* larval abundance, the negative horticultural aspects of this management method such as wet fields and the potential for pesticide runoff and erosion require development of alternative strategies to reduce egg laying and/or larval survival by *P. japonica*. One such alternative is to plant ground covers in blueberry row-middles that reduce the egg-laying and/or larval development of this pest.

Foliar insecticide applications continue to be the foundation of Japanese beetle management in blueberries and many other fruit crops, as growers strive to meet the exacting quality standards and are looking for alternative solutions to reduce *P. japonica* pressure in their fields. Because of the zero-threshold for beetle contaminants in harvested blueberries, the growers' goal is to reduce the presence of both adults and larvae of this insect in their fields. The main reasons for Michigan blueberry growers' interest in planting cover crops in their fields are: weed suppression, reduction of soil erosion, and to drive machinery through the field during wet conditions. Since *P. japonica* is currently the most important pest in blueberries in Michigan, there is a need for testing cover crop species that satisfy grower's horticultural needs and do not attract *P. japonica* to their fields. Ideally, cover crops could be used as a natural barrier to avert ovipositing females from an area that otherwise provides ideal soil conditions for oviposition and larval development.

In this study, two acid-tolerant ground covers were evaluated for their potential to reduce the suitability of blueberry fields for *P. japonica*. Alsike clover (*Trifolium hybridum* L.) and buckwheat (*Fagopyrum esculentum* Moench) were chosen because their morphological and biological characteristics are different from the preferred perennial ryegrass (*Lolium perenne* L.), and these plants are recommended in other perennial fruit systems (Anon. 1992). These were compared to perennial ryegrass and bare ground within a blueberry planting to determine their ability to manipulate: 1) attraction to adult female beetles, 2) female beetle behavior after landing on the ground, 3) abundance of *P. japonica* larvae in the soil, and 4) survival of *P. japonica* from eggs to mature larvae.

MATERIALS AND METHODS

Experiments were conducted in a replicated field experiment at the Trevor Nichols Research Complex, Fennville, MI. The 4050 m^2 highbush blueberry planting (*Vaccinium corymbosum* var. Bluecrop) was established using two year old bushes in 2001, on a 3.7 m x 1.4 m plant spacing with 12 bushes in each row. Four row-middle treatments were established with either perennial ryegrass, alsike clover, buckwheat, or bare ground between the rows of bushes. Cover crops were seeded in the row-middles (53 m^2 / row-middle) in May and June 2002 in a completely randomized design. Buckwheat was reseeded in the third week of June in 2003 and 2004. One plot consisted of a set of two row-middles with the same treatment on either side of a row of blueberry bushes, and treatments were replicated five times. The seeding rates were 27 kg/ha for perennial ryegrass, 22 kg/ha for clover, and 67 kg/ha for buckwheat (Michigan State Seed Solutions, Grand Ledge, MI). The bare ground plots received herbicide (Roundup Ultra 4WSL at 31.3ml/l), spot-applied with a 3.8 l hand-held sprayer when needed, to keep the soil free of vegetation during the growing seasons. Overhead irrigation of all the row-middles was started from the time of seeding, and continued weekly or as needed throughout the growing season in each of the years of this study to create conditions typical of a commercial blueberry field. Ryegrass and clover plots were mowed throughout the growing season as needed. Measurements of larval density, adult abundance and individual adult beetle behavior were conducted in this site.

Effect of cover crops on adult **P. japonica**

One of the row-middles from adjacent pairs of the same cover treatment was selected at random, and the numbers of *P. japonica* adults that naturally occurred in these plots were counted weekly in July and August in 2002, 2003, and 2004. Observations were made from 12 - 2 PM on sunny days with low winds. Each visual survey of a row-middle lasted two minutes, and was measured with a timer. The observer walked down one side of the row-middle and back on the other side, focusing on the area in the row-middle and counting the number of beetles. The observer walked on the periphery of the area covered by the crops, wore neutrally colored clothes, and moved evenly and slowly so as to minimize disturbance of the beetles. At the end of the two minutes the total number of beetles per row-middle was recorded on a data sheet.

Larval density under different cover crops

The naturally-established population of *P. japonica* was surveyed by taking soil samples in May 2002, before the cover crop seeds were sown in the plots. Subsequently, larval density was sampled in October 2002, September 2003, and October 2004 in the same plots. Larvae were sampled by taking five 15 cm-deep soil cores from each row-middle using a cylindrical golf cup cutter (area = 95 cm^2) (Parmenter & Andre Inc., Grand

Rapids, MI). Soil cores were examined in the field, and all beetle larvae were placed in plastic bags with a small amount of soil. The bags were labeled with date, treatment, and number of larvae. Bags were transported to the laboratory in a cooler containing an icepack. Larvae were identified to species using the diagnostic rastral patterns and other morphological features of the labrum (Vittum et al. 1999).

Survival of* P. japonica *larvae under cover crops

Plots for this experiment were established in a different part of the same blueberry field as above, to create row-middles with bare ground, clover, buckwheat, or ryegrass in a completely randomized design with five replicates (same seeding rates as above). To obtain first instar *P. japonica*, adults were collected from a grassy field (East Lansing, MI) with Japanese beetle Expando Traps baited with floral and sex lures (Great Lakes IPM Inc., Vestaburg, MI). Traps were deployed on July 1, 2002, 2003, and 2004. Adults were transported to the laboratory daily and approximately 500 (~50:50 sex ratio) were put into each of ten 36 x 24 x 12 cm plastic containers. The lid of each container had a 10 x 15 cm hole for ventilation, which was covered with nylon mesh to stop beetles from escaping. A 5 cm deep layer of moist sand (Dixie Cut Stone & Marble Inc., Bridgeport, MI) was provided as an ovipositional medium in each container. Slices of apple were placed on top of the sand as *ad libitum* food and to provide moisture. Containers were kept at constant environmental conditions (16L:8D, 30% RH, 24-26 °C) and fresh slices of apples were added as needed. The sand was sifted for first instars by placing a handful of sand in a sieve (#18 mesh size) and washing the sand with running tap water. Larvae were transferred using a paintbrush (Loew-Cornell®, #12) to moist filter paper (90 mm diameter, Whatman International Ltd., Maidstone, UK) in Petri dishes (Becton Dickinson and Co., Franklin Lakes, NJ) (10 x 1.5 cm) kept at room temperature in the laboratory until further use in the field, but not longer than 12 h.

First instars were transported from the laboratory to the field plots in a cooler. Five PVC pipes (15 x 8 cm) were pounded into the soil in each row-middle, for a total of 25 pipes per treatment. A 10 cm-deep hole was made in the soil in the middle of the PVC pipe with the end of the paintbrush; five larvae were carefully placed in the hole with the brush. Then the hole was covered with soil and irrigated with 500 ml water to wash soil particles close to the larvae and to provide moisture. After placement of larvae in the soil, the PVC pipes were covered with mesh to prevent oviposition by naturally occurring *P. japonica*. First instars were placed in the field in the last week of July and PVC pipes were removed in the first week of September in 2002, 2003, and 2004. Soil was shaken from the pipes onto a tray and examined for *P. japonica* larvae. The number of *P. japonica* larvae per PVC pipe was recorded.

Effect of cover crops on* P. japonica *behavior

The behavior of individual adult female *P. japonica* was observed in row-middles planted with clover, buckwheat, or ryegrass. Beetles were very rarely observed on bare ground plots. Gender was distinguished by observing the shape of the front tibia (Fleming 1972) without disrupting the insect's natural behavior. Data were collected in July and August 2002 and 2003 weekly from 10 am – 4 pm on sunny days with low winds. A total of 35 observations were made in buckwheat and ryegrass and 39 in clover during the two years of this study, and observations were recorded by the same observer throughout. The order of observations on the three cover crop treatments was randomized. An observation began as soon as a beetle was located by the observer on one of the cover crops, and lasted for a maximum of one hour or until the beetle left the plot. The observer wore neutrally colored clothes, and moved evenly and slowly so as to minimize the disturbance of the beetles. Continuous focal sampling, in which all of the behaviors of the subject are recorded, was conducted using The Observer 3.0 (2002) and 4.0 (2003) (Noldus Information Technologies, The Netherlands) loaded onto a laptop computer that was carried by the observer. Behaviors that were observed and recorded are defined in Table 3.1.

Table 3.1. Description of behavioral elements of *P. japonica* observed on four row-middle treatments in highbush blueberry.

Behavior	
Stationary	Standing motionless
Walk	Moving on a surface by means of leg motions
Groom	Using legs or mouthparts to clean abdomen or antennae
Feed	Moving mouthparts for the purpose of ingesting food
Dig	Moving first pair of legs to loosen the soil surface, associated with oviposition behavior
Position	
Crop	At least two thirds of insect's body is in contact with plant
Soil	At least two thirds of insect's body is in contact with soil surface

Data analysis

The natural larval and adult densities of *P. japonica*, and the larval survival values were transformed to $\sqrt{y+0.1}$ to meet the assumptions of normality and homogenous variance. Sub-samples from each row-middle taken for the natural larval infestation experiment and larval survival experiment were averaged. Data for these three experiments were analyzed separately for each year with a one-way ANOVA using PROC GLM (SAS Institute 2001). Adult density data were analyzed with a two-way ANOVA (time and treatment) using PROC GLM (SAS Institute 2001). Tukey's test was used to determine significant differences between means at the 5% probability level. Average rate (frequency of behavior/minute), average duration (min) of each behavior, and duration as a percent of the total observed time (% observation) of behavioral elements on the three cover crops were compared with a multivariate analysis of variance (MANOVA, SAS Institute 2001) and contrasts of mean values were made to compare behavior of different cover crops. The proportion of time spent on plant or soil surfaces was compared with a two-way ANOVA (PROC GLM, SAS Institute 2001), with type of surface (plant vs. soil) and cover crop treatments as factors.

RESULTS

Effect of cover crops on adult **P. japonica**

The number of adult *P. japonica* observed was significantly different among the row-middle treatments throughout this study (2002: $F_{3,111} = 11.29$; 2003: $F_{3,153} = 47.09$; 2004: $F_{3,111} = 6.95$; $P < 0.01$ for all dates). The general trend in *P. japonica* density on the four treatments in this study was, in decreasing order: buckwheat, clover, ryegrass, bare ground. On average there were 7 times more beetles on buckwheat than on the other planted row-middle treatments (Table 3.2). Bare ground had consistently fewer beetles (0.06 beetles per sample) than the other row-middles with plants (2.24 beetles per sample) over the three years (Table 3.2). In 2002 and 2003 there were significantly fewer beetles on clover and ryegrass than on buckwheat (Table 3.2). In 2004, beetle abundance on clover was not significantly different from that on buckwheat, but ryegrass still had significantly fewer beetles than buckwheat. In 2002 and 2004, no adult beetles were observed on bare ground, but during the higher overall population levels of 2003, a few beetles were observed on bare ground at a density significantly lower than the number seen on all the other treatments (Table 3.2). There were significantly more beetles on clover than ryegrass in 2003 and 2004.

Larval density under different cover crops

The larval density of *P. japonica* varied significantly among the four row-middle treatments throughout the three years of this study (2002: $F_{3,39} = 5.33$; 2003: $F_{3,39} = 45.52$; 2004: $F_{3,39} = 6.32$; $P < 0.01$ for all dates). Larval density was highest on ryegrass and clover in the three years, and the average larval densities were not statistically different in any of the years between these two treatments (Table 3.3). Across the three years, larvae were 3 times more abundant on average in ryegrass and clover than in buckwheat. In 2002 and 2003 bare ground had significantly fewer larvae than the other treatments, while in 2004 the larval density in bare ground and buckwheat was significantly lower than in the two other row-middle treatments.

Survival of **P. japonica** *larvae under cover crops*

Popillia japonica larval survival was significantly different among the four row-middle treatments in 2002 ($F_{3,19} = 3.76$, $P = 0.03$), but not in the other two years (Table 3.4). There was high variability in larval survival within treatments, but survival was numerically the highest in plots with ryegrass throughout this experiment. In 2002, a similar number of larvae survived in clover and ryegrass, and buckwheat and bare ground had significantly fewer larvae per PVC pipe than ryegrass. This trend was not consistent throughout the study; in 2003 there was no significant response to row-middle treatments, but the lowest larval survival was in clover and the highest in ryegrass and bare ground. In 2004 ryegrass still had the highest numbers of surviving larvae, but in this year the lowest values were recorded in buckwheat. Variation between years was the lowest when larvae were placed in plots of ryegrass (Table 3.4).

Table 3.2. Comparison of *P. japonica* adult density in four different row-middle treatments in highbush blueberry. Samples were taken in the summer of 2002, 2003, and 2004. For each year, averages within a column followed by the same letter are not significantly different (α = 0.05).

treatment	Average (± SE) *P. japonica* adults/row-middle/2 min			
	2002	**2003**	**2004**	**grand mean**
Bare ground	0.00 ± 0.00 b	0.20 ± 0.12 c	0.00 ± 0.00 b	0.07 ± 0.12
Buckwheat	2.07 ± 0.39 a	11.45 ± 1.78 a	0.67 ± 0.27 a	4.70 ± 2.44
Clover	0.87 ± 0.47 b	2.50 ± 0.53 b	0.37 ± 0.18 b	1.30 ± 1.20
Ryegrass	0.60 ± 0.34 b	1.55 ± 0.38 b	0.03 ± 0.03 b	0.70 ± 0.80

Table 3.3. Comparison of *P. japonica* larval density in four different row-middle treatments in highbush blueberry. Samples were taken in the fall of 2002, 2003, and 2004. For each year, averages within a column followed by the same letter are not significantly different (α = 0.05).

treatment	Average (± SE) *P. japonica* larvae/sample			
	2002	**2003**	**2004**	**grand mean**
Bare ground	0.00 ± 0.00 c	0.10 ± 0.04 c	0.06 ± 0.03 b	0.05 ± 0.05
Buckwheat	0.30 ± 0.08 ab	0.64 ± 0.14 b	0.00 ± 0.00 b	0.31 ± 0.22
Clover	0.88 ± 0.31 ab	2.06 ± 0.19 a	0.22 ± 0.07 a	1.05 ± 0.57
Ryegrass	0.88 ± 0.19 a	2.58 ± 0.24 a	0.30 ± 0.08 a	1.25 ± 0.51

Table 3.4. *Popillia japonica* larval survival in four row-middle treatments in highbush blueberry. Numbers are averages of larvae survived out of five first instars, in the summer of 2002, 2003, and 2004. For each year, averages within a column followed by the same letter are not significantly different (α = 0.05).

treatment	Average (± SE) *P. japonica* larvae/PVC pipe			
	2002	**2003**	**2004**	**grand mean**
Bare ground	0.41 ± 0.24 c	1.21 ± 0.27 a	0.40 ± 0.17 a	0.67 ± 0.68
Buckwheat	0.40 ± 0.09 bc	1.11 ± 0.19 a	0.13 ± 0.06 a	0.55 ± 0.34
Clover	1.04 ± 0.19 ab	0.46 ± 0.13 a	0.20 ± 0.09 a	0.56 ± 0.40
Ryegrass	1.33 ± 0.36 a	1.21 ± 0.59 a	0.57 ± 0.15 a	1.04 ± 1.10

Effect of cover crops on **P. japonica** *behavior*

Beetle behavior was affected by the different cover crops tested in blueberry. Beetles were stationary at least twice as frequently on buckwheat than on the other two treatments ($F_{2,110} = 10.47$, $P < 0.01$) (Fig. 3.1a). Beetles walked almost 50% less frequently on clover than on the ryegrass or buckwheat ($F_{2,110} = 3.89$, $P = 0.02$) (Fig. 3.1a), and fed significantly more frequently on buckwheat and clover than on ryegrass ($F_{2,110} = 12.49$, $P < 0.01$). The rate of digging was affected by the ground cover ($F_{2,110} = 3.95$, $P = 0.02$), and was six times higher in ryegrass than in buckwheat. Ryegrass and clover treatments were not significantly different ($F_{1,110} = 2.56$, $P > 0.05$) (Fig. 3.1a).

The average durations of each bout of stationary, walking and grooming behaviors were not significantly different among the ground covers ($F_{2,110} = 0.07$; $F_{2,110} = 0.71$, $F_{2,110} = 0.01$ respectively, $P > 0.05$) (Fig. 3.1b). In contrast, the durations of feeding and digging behaviors were highly affected by ground covers, in opposite ways. Feeding bouts lasted 1.65 ± 0.73 and 1.87 ± 0.45 minutes on buckwheat and clover respectively, but *P. japonica* spent significantly less time feeding on ryegrass (0.006 ± 0.006 min) ($F_{2,110} = 11.60$, $P < 0.01$). The total number of feeding behaviors throughout all the observations was only 12 in the case of ryegrass, 63 for buckwheat and 112 for clover. Digging bouts lasted an average of 1.32 ± 0.44 minutes in ryegrass, significantly longer than in clover (0.52 ± 0.24 min) or in buckwheat (0.05 ± 0.05) ($F_{2,110} = 6.18$, $P < 0.01$).

The proportion of time allocated by *P. japonica* females to particular behaviors was also affected by ground cover ($F_{2,110} = 4.11$, $P = 0.02$) (Fig. 3.1c). A significantly higher proportion of their time was spent stationary on buckwheat (34.2 ± 6 %) than on clover (21.0 ± 7 %), but this proportion was not significantly different between ryegrass

(25.6 ± 5 %) and the other two treatments (Fig. 3.1c). A significantly greater proportion of time was spent walking on ryegrass (36.3 ± 6 %) than on either buckwheat or clover ($F_{2,110} = 3.97$, $P = 0.02$), but grooming was not affected by cover crop. The proportion of time spent feeding was significantly different among the three cover crops ($F_{2,110} = 20.19$, $P < 0.01$). The greatest proportion of time spent feeding occurred on clover (47.8 ± 9 %); this was 2.5 times higher than the proportion of time spent feeding on buckwheat, and 15 times higher compared to ryegrass. The proportion of time spent digging was significantly different among treatments ($F_{2,110} = 4.26$, $P = 0.02$); beetles spent most of their time digging in ryegrass (16.4 ± 7 %), and approximately 8% was spent digging in clover, but this was not significantly different from the other two treatments. Only one beetle was observed digging (< 1% of time spent) in buckwheat.

On average, *P. japonica* spent significantly more time on the plants than on the soil on all of the treatments ($F_{5,220} = 51.58$, $P < 0.01$). Beetles spent 99.4 ± 0.5 % of their time on buckwheat plants, 82.6 ± 7 % on clover plants and 73.9 ± 8 % on ryegrass. The amount of time spent on clover and buckwheat was not statistically different ($P = 0.21$), but less time was spent on ryegrass than on the other two treatments ($P < 0.05$).

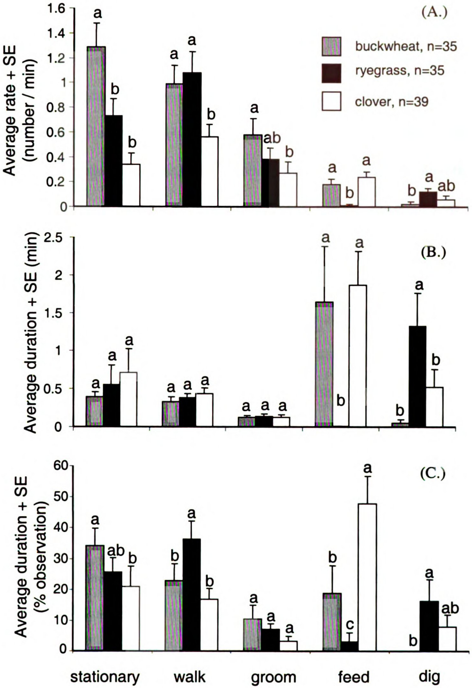


Figure 3.1. *Popillia japonica* behavior on different cover crops (n= number of observations within a treatment) in a blueberry field in 2002 and 2003. Within each behavioral element, bars with the same letter are not significantly different ($\alpha = 0.05$).

DISCUSSION

Cover crops can reduce pest damage in agricultural fields, by serving as a sink for pests, or confusing pests visually or olfactorally and thus reducing colonization, or changing microclimate within the field, thereby reducing pest success (Bugg 1992). In this study, the adult and larval density and behavior of *P. japonica* were influenced by different cover crops in row-middles of highbush blueberry fields. While *P. japonica* feeds on many hosts, its choice for an oviposition site is more restricted; the results presented here indicate that ryegrass is a preferred host for ovisposition and larval development, while the other tested plants are less suitable or are not utilized for oviposition. This knowledge can provide information when making management decisions about when and how to use cover crops to reduce pest populations.

Larval *P. japonica* density was highest in ryegrass and clover, whereas adult population density was lowest on ryegrass and highest on buckwheat throughout three years. In a choice test conducted on potted plants in the greenhouse, *P. japonica* larvae preferred to feed on roots of perennial ryegrass compared to five other turfgrass species (Crutchfield and Potter 1994). Previous studies have also found that survival varies with plant species: third instar *P. japonica* survived better in a greenhouse study on large crabgrass than on dandelion, white clover and Kentucky bluegrass (Crutchfield and Potter 1995). Thus, selection of cover crop species can influence pest abundance.

Bare ground row-middles harbored the fewest larvae, indicating that *P. japonica* density is increased by the presence of plants in the row-middles. This result is similar to that of Crutchfield and Potter (1995) who found in a greenhouse study that survival of third instar *P. japonica* larvae was higher in the presence of plants than in no-plant treatments. The inconsistency between years in *P. japonica* larval survival on the different treatments could be due to abiotic factors, such as temperature differences between the years. The results of this experiment are not consistent with the outcome of the larval density study, in that naturally occurring larvae were not found in bare ground plots, but first instars survived and developed in PVC pipes in bare ground treatments. This means that there is enough organic matter naturally in the soil of the experimental field to provide developing larvae with food. Inconsistencies between the results of the larval density study and the larval survival experiment could also be because the PVC pipes altered the microclimate, or that it prevented natural enemies from killing the larvae.

Fewer adult beetles were found in bare ground plots than in plots with cover crops, so it is likely that adult beetles avoid landing on brown (non-green) surfaces (Kostal and Finch 1994); alternatively, tenure time on bare ground could be brief. This would help to explain the lower abundance of larvae in bare ground plots. Among the three cover crops, the highest numbers of adult beetles were found on buckwheat throughout this study, indicating that this plant attracts beetles to land on them, making it unsuitable for reducing adult populations in blueberry fields. Observations of female beetle behavior under field conditions provide insight into the mechanisms driving the differences in beetle abundance and larval density in the three tested ground covers. These are likely to be due to a combination of plant suitability for feeding and oviposition. The observations revealed that although beetles spent more time and more frequently fed on buckwheat and clover than on ryegrass, they dug more frequently and for longer durations on ryegrass than on the other two treatments. Since *P. japonica* adults fed only minimally on ryegrass, but laid eggs in this treatment, it is clear that host plant evaluation for oviposition involves a different mechanism from food plant evaluation. Duration and frequency data revealed the dominant behaviors on the different plants: stationary on buckwheat, feeding on clover, walking and digging on ryegrass.

Popillia japonica utilize plants in different ways, and this ultimately translates into variation in larval density under the different ground covers. The high larval density on ryegrass is associated with a high proportion of digging behavior on this plant, and the low larval density in buckwheat is due to the lack of digging behavior. Interestingly, the rate and duration of digging behavior on clover was not statistically different from either of the other two treatments (Fig. 3.1), but larval densities were similar to those in ryegrass (Table 3.3). Clover is different from the other two plants in that it is a suitable host for feeding, oviposition, and for larval survival and these qualities together will likely make this plant unsuitable as a cover crop in blueberry fields. Buckwheat is not a host for oviposition and is not ideal for larval survival, but adults used it for basking and sometimes fed on the flowers (Z. Szendrei, personal observations). Ryegrass is not consumed by the adults, but is ideal for oviposition, and larval survival. These qualities should be considered when selecting plants that have a potential to supress *P. japonica* populations within agricultural fields.

The morphological differences among the tested plants may explain the behavior patterns observed. Buckwheat is 50-60 cm tall during the beetle flight period, whereas the other two plants are 10-30 cm tall; so the taller plants could serve as a barrier separating the beetles from the soil surface. Perennial ryegrass is a monocotyledonous plant with a thin leaf blade, while the other two plants have broad leaves; this feature could be significant in influencing *P. japonica* behavior if the leaf shape is used as an orientation or landing cue. Some of these morphological features could divert beetles from landing on the cover crops or after landing, could avert them from approaching the soil surface for digging. In a study with artificial grass blades, gravid females responded differently to stem density and diameter, suggesting that *P. japonica* uses visual cues to evaluate potential oviposition hosts (Szendrei and Isaacs, in press, Chapter 4). Visual cues have been shown to be important factors in host orientation, location, and choice for many species of Diptera, Lepidoptera, Hemiptera and Coleoptera (Prokopy and Owens 1983). In *P. japonica*, vision has already been proven important during the development of an effective monitoring trap (Fleming 1969). Olfactory and/or tactile cues may also be involved in oviposition, but the chemical basis of oviposition decisions needs further investigation.

Our small-plot trials were effective for measuring behavioral responses of *P. japonica* to candidate cover crops, but larval and adult density studies should be repeated at larger, commercial farm scales. Future studies should focus on adult density on blueberry bushes and *P. japonica* damage to berries in farms that have different row-middle treatments, to get a direct estimate of how cover crops affect *P. japonica* control and of the economic value of having a certain species of cover crop. These findings could be useful in other agricultural systems where cover crops are used for the control of an insect pest, because they provide valuable insight into the principles of insect-plant interactions that is critical in the selection of the appropriate plant species. For example, depending on the type of main crop and on growers' pest control goals, certain cover crop species are more suited to control adults while others reduce larval abundance.

CHAPTER 4:

DO PLANT CUES INFLUENCE THE OVIPOSITION BEHAVIOR OF JAPANESE BEETLES?

INTRODUCTION

Vegetation characteristics of agricultural landscapes can be manipulated to control insect pest populations (Tonhasca and 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 (Prokopy 1994, Bugg and Waddington 1994, Hartwig and Ammon 2002). This type of habitat modification changes the managed ecosystem to limit availability of one or more of the resources needed for insect development and reproduction, making the environment less suitable for pest populations (Flint and Gouveia 2001).

As an adult, the Japanese beetle (*Popillia japonica* Newman, Coleoptera: Scarabaeidae) has an extremely wide diet breadth; it feeds on the leaves, flowers and fruits of more than 300 plant species (Fleming 1972, Potter and Held 2002). This has made this species an important pest of horticultural crops in the eastern United States. Host selection by generalist species is likely to be influenced by relatively nonspecific stimuli common to many different hosts (Thorsteinson 1960, Renwick 1983, Renwick 1989, Foster and Howard 1998), which for *P. japonica* are likely to be green leaf odors (Ahmad 1982, Loughrin et al. 1995) and color (Held and Potter 2004). Compared to food-searching behaviors, fewer cues may be involved in choosing an ovipositional site in this species, since the larvae are facultative monophages (Potter and Held 2002). Because of the limited mobility of larvae, the mother's ovipositional 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 ovipositional sites have not been investigated.

Ovipositional preference and specificity are most often studied in species that lay eggs on the above-ground sections of plants (Wiklund 1981, Foster and Howard 1998, Bossart and Scriber 1999, Showler 2001), in part because of the challenge of measuring ovipositional preference of insects that lay eggs in the soil. Circumstantial evidence for ovipositional site selection can be gathered from the 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* ovipositional 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. 1981b, 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 ovipositional decisions, but the role of specific plant cues in ovipositional 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 ovipositional investment decisions. Oviposition was compared between grass and clover, which are both commonly used 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, I 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 ovipositional choice bioassays.

MATERIALS AND METHODS

Oviposition arena

Bioassays were conducted in a four-choice oviposition arena made of a Plexiglas box (internal dimensions 15 x 18 x 18 cm) (Fig. 4.1). 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 x 18 x 2.5 cm) was inserted into each arena. The Styrofoam square had four 4 x 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 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 to supply beetles with an inert surface for walking and provide access to the top of the soil blocks, and the potential ovipositional resources described below were inserted into each of the four openings. All arenas were washed between experiments with a weak detergent solution and thoroughly rinsed with water.

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 from July1 through 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 out into open, grassy fields and were emptied after one day of deployment. Beetles were transported to the laboratory as needed for the experiments. To ensure that all females were mated, males and females were placed together in a 1 m^3 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.

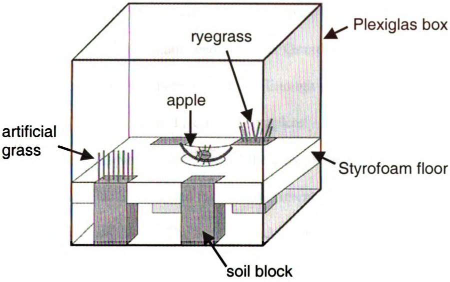


Figure 4.1. Bioassay arena for measurement of oviposition decisions by *P. japonica*.

Soil and plant material

The soil used in all experiments and for all of the treatments was a 1:1 (vol/vol) mixture of organic potting soil (Scotts garden soil, The Scotts Company, Marysville, OH, USA) and sand (Dixie Cut Stone & Marble Inc., Bridgeport, MI, USA). After thorough mixing, the soil was placed into 4 x 4 x 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, (Trifoilum hybridum L.) (Michigan State Seed Solution, Grand Ledge, MI, USA) were grown in a greenhouse 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; new plants were 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 with an even, flat surface, in the paper sleeves.

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 use 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 ovipositional investment in 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 was established for this experiment.

Experiment 2. Arenas were set up with bare ground, artificial grass, bare ground with holes and 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 above. To imitate grass roots loosening the soil, ten 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, both the above and below ground plant parts of 40 g fresh perennial ryegrass 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 ten holes were punctured in a similar manner as described above. Thirty replicates were set up for this experiment.

Experiment 3. In this experiment, the acceptance by *P. japonica* females of clover or grass as ovipositional 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 ovipositional response by *P. japonica* to the density of artificial grass stems, the number of wire stems per soil block was varied. Either zero, five, 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, green, blue or 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 fume 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 approx. 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 one 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 and Longnecker 2001). Ovipositional 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 that each contained soil blocks with perennial grass, artificial grass, grass roots and 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 9 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 hr and was terminated at 19.00 hr. 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), which recorded for 24 hr on an 8 hr videotape. Video tape recordings were then converted into digital format using Broadway Pro Capture Software (Data Translation, Inc., MA, USA) for quantification of beetle behavior.

Beetle behavior was scored using The Observer 5.0 (Noldus Information Technologies, 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 observational 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 determined at $\alpha = 0.05$ level.

RESULTS

Bioassays

Experiment 1. The average number of eggs per soil block was significantly different among all of the treatments (Fig. 4.2a); 60% of the eggs found in the arenas were under ryegrass. Bare ground had the fewest eggs on average; it received only 2.5% of the eggs laid. The maximum number of eggs in a soil block showed a similar trend; a maximum of 25 eggs were found 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 with eggs was significantly different from a random distribution in bare ground (10%), and in perennial ryegrass (71%) ($\chi^2 = 10.78$ for both tests, $P < 0.05$). Ovipositional preference was not significantly correlated between average numbers of eggs and proportion of soil blocks with eggs ($r^2 = 0.8$, $P = 0.2$) (Fig. 4.3a).

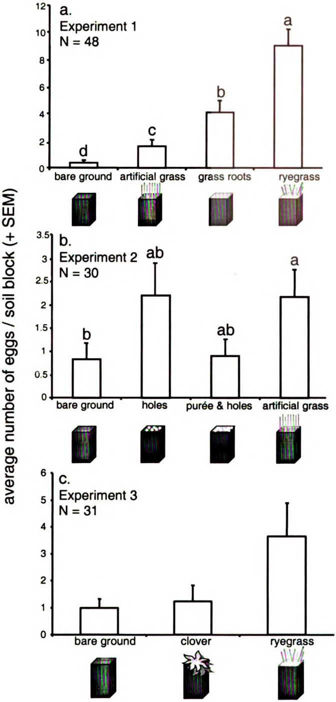


Figure 4.2. Average number (+ SE) of eggs per soil block testing the effect of grass cues on ovipositional behavior (Experiments 1-3). Drawings below graphs indicate respective treatments. Bars within a graph with the same letter above them are not significantly different (α= 0.05). 'N' represents the number of replicates in each experiment where one beetle was used per replicate.

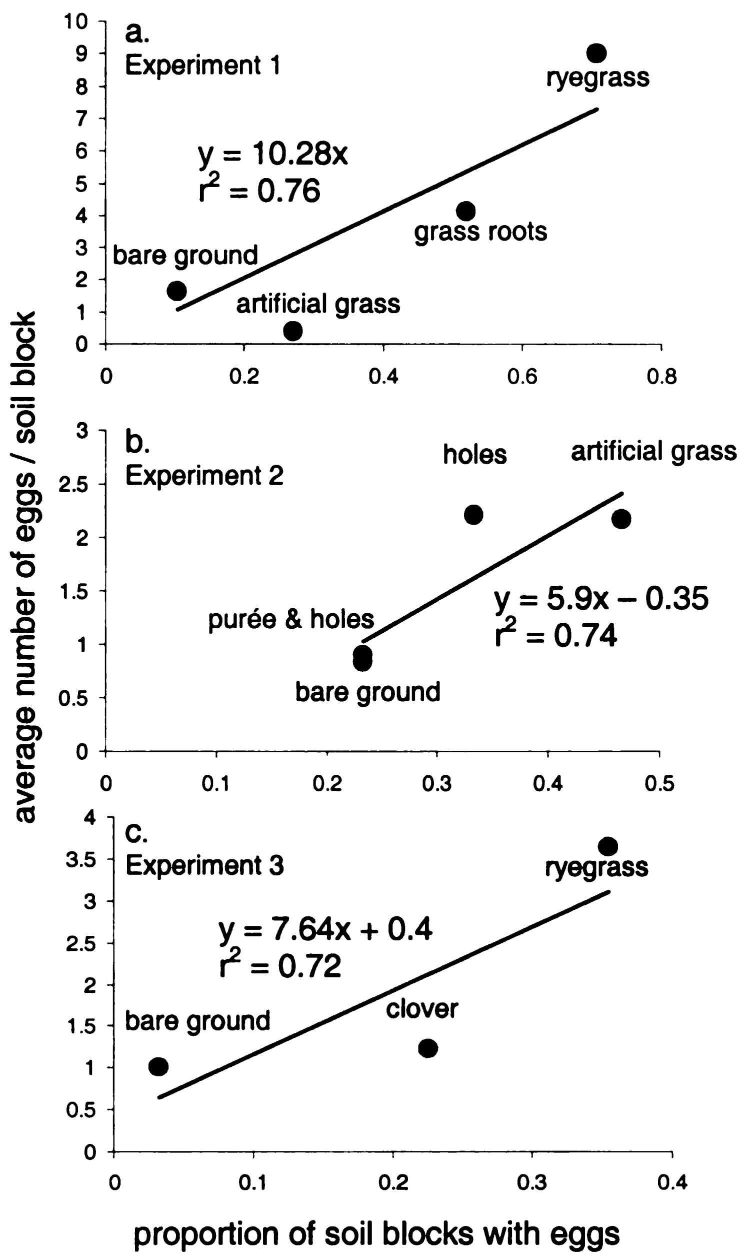


Figure 4.3. Relationships between the proportion of soil blocks receiving eggs and average number of eggs per soil block, for different soil block treatments in Experiments 1-3.

Experiment 2. Thirty-five percent of the eggs in this experiment were found in artificial grass, significantly greater than the investment expected by chance (χ^2= 14.72, P < 0.01). Artificial grass received significantly more eggs on average than bare ground (χ^2 = 4.47, P = 0.03) (Fig. 4.2b). 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 (χ^2 = 3.0, P = 0.08). Artificial grass was also not significantly different from soil with holes (χ^2 = 3.32, P = 0.97). 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. Ovipositional preference was not statistically significant (r^2 = 0.74, P = 0.26) (Fig. 4.3b).

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 among the treatments (Fig. 4.2c). 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. Ovipositional preference was not statistically significant (r^2 = 0.76, P = 0.051) (Fig. 4.3c).

Experiment 4. The number of eggs laid per soil block increased with increasing stem diameter (Fig. 4.4a), but there was no significant difference among the stem diameters. Medium diameter artificial stems received significantly more eggs on average than bare ground (χ^2 = 4.43, P = 0.03) (Fig. 4.4a). Maximum egg 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. Ovipositional preference was not significantly correlated between the two variables ($r^2 = 0.71$, $P = 0.2$) (Fig. 4.5a).

Experiment 5. Average number of eggs per soil block increased with increasing stem density, although there was no statistical difference between the stem-containing treatments (Fig. 4.4b). Treatments with 10 and 15 stems had significantly more eggs on average than bare ground ($\chi^2 > 4.62$, $P < 0.03$). Soil blocks with five stems had 6.5 times as many eggs on average compared to bare ground. Ovipositional preference increased nearly linearly with increasing stem density ($r^2 = 0.98$, $P < 0.01$) (Fig. 4.5b).

Experiment 6. Stem color had no effect on the average numbers of eggs per soil block (Fig. 4.4c) 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 (Fig. 4.4c). The correlation between proportion of soil blocks with eggs and average number of eggs per soil block was the weakest in this experiment ($r^2 = 0.27$, $P = 0.26$) (Fig. 4.5c).

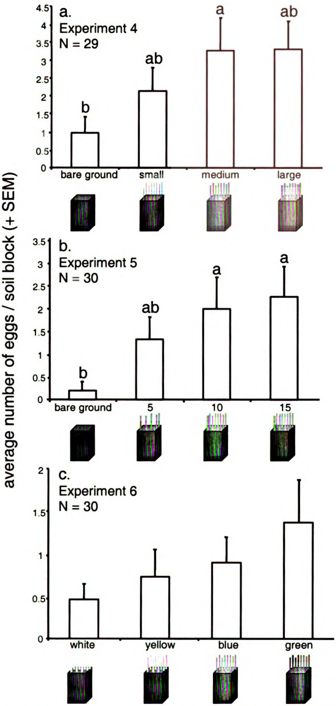


Figure 4.4. Average number (+ SE) of eggs per soil block testing artificial grass cues on oviposition behavior (Experiments 4-6). Drawings below graphs indicate respective treatments. Bars within a graph with the same letter above them are not significantly different (α= 0.05). 'N' represents number of replicates in the experiment.

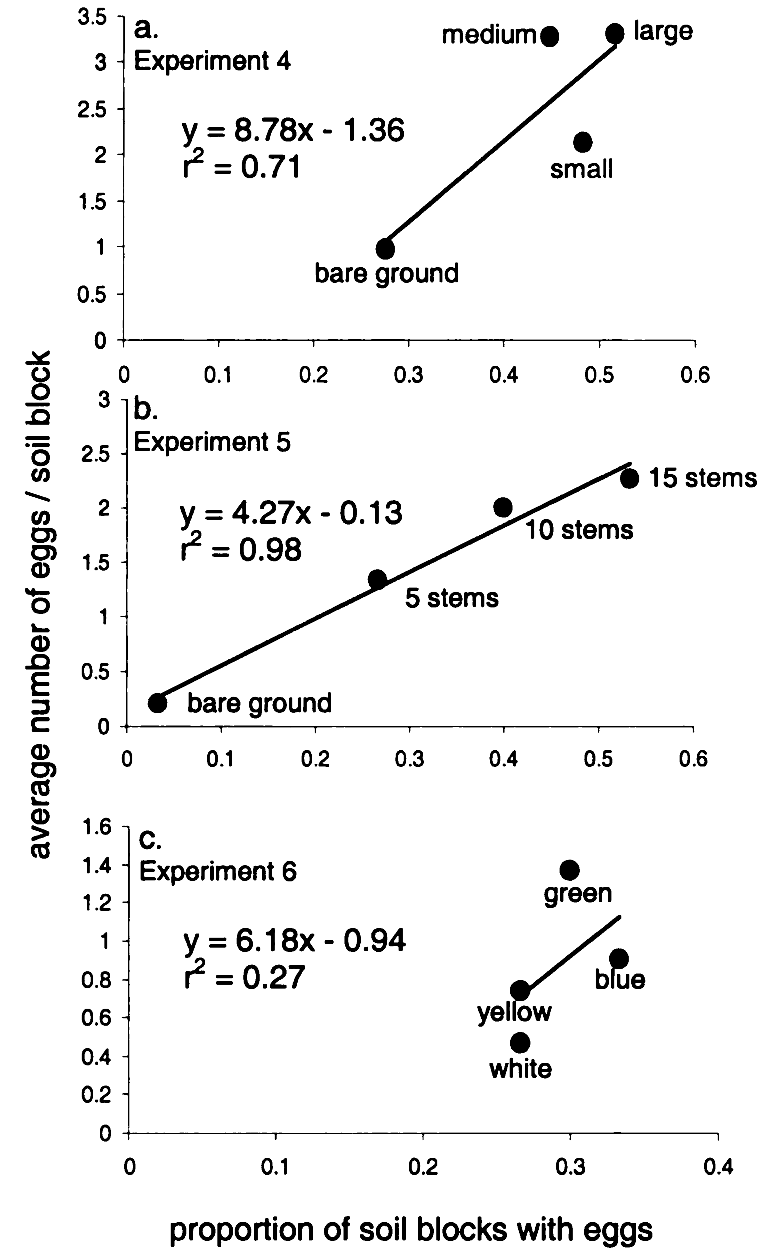


Figure 4.5. Relationship between the proportion of soil blocks receiving eggs and average number of eggs per soil block, for different soil block treatments in Experiments 4-6.

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 dig significantly more frequently on ryegrass than on bare ground (t = -3.01, d.f. = 16, $P = 0.02$) (Fig. 4.6a). Although no statistically significant differences were documented 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 and 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, $P = 0.01$) (Fig. 4.6b). 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. Despite these numerical differences, the proportions were not significantly different.

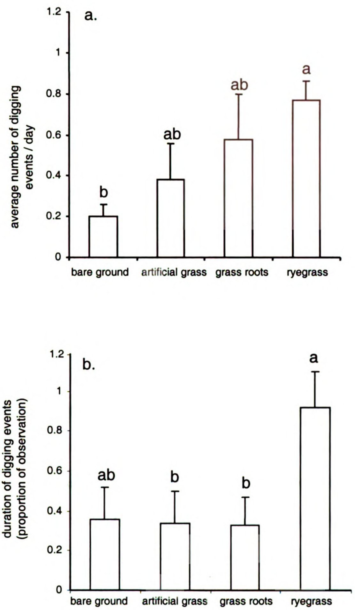


Figure 4.6. Frequency and duration of digging behavior by *P. japonica* in bioassay arenas, as a function of treatment. Average number (+SE) of digging events per day (a) and average duration of digging bouts (b) on the four treatments. Bars in a graph with the same letter are not significantly different (α= 0.05).

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 ovipositional 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, Rondon and Gray 2004), although these studies did not investigate the mechanisms involved in ovipositional site selection. The bioassay developed in this research could be useful for studying ovipositional behavior of generalist or specialist insect species that lay their eggs in the soil, as well as in determining the factors involved in oviposition decision-making.

The number of eggs per soil block increased as an increasing number of plant cues was 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, unpublished data), but, to date, it has not been measured directly. When female *P. japonica* were offered a choice between natural and artificial ovipositional cues at ovipositional sites, beetles preferred ryegrass more than artificial stimuli (Experiment 1), demonstrating either that the artificial grass was lacking some visual, tactile, or olfactory quality that enhances oviposition. Manipulation of the soil surface by punching holes (Experiment 2) increased the average number of eggs laid compared to that in bare ground, suggesting 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 egg deposition. This may have been because *P. japonica* do not use volatile or tactile chemical cues from grass to select an ovipositional 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 procedure are different from those 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 and 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 ovipositional 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 occurred because 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 ovipositional 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 accepted 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 ovipositional resource translates into increased larval survival and development, as has been found in other herbivorous insects (Jaenike 1978, Craig et al. 1989, Bernays and Chapman 1994, Showler 2001). *Popillia japonica* larval survival and growth are highest on perennial ryegrass (Crutchfield and Potter 1995) compared to other cool-season turfgrass species, but the degree of correlation between *P. japonica* female preference and larval performance is not known. The use of sub-optimal resources as ovipositional sites suggests that spreading offspring survival risks (Root and Kareiva 1984, Seger and Brockman 1987, Freese and Zwolfer 1996, 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 block with eggs and the numbers of eggs per soil block was the highest. The variation in ovipositional activity between experiments could be because females of varying ages were used in the different experiments, although Van Timmeren et al. (2001) found that in laboratory bioassays *P. japonica* ovipositional 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, therefore enabling quick decisions to lay eggs (Finch and 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 and Leather 2002), and based on the present results (Fig. 4.3, 4.5) 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 distinguishes them from turfgrass species.

While the bioassays revealed cues that stimulate *P. japonica* females to lay eggs in soil, the observations showed 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 ceased 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) (Fig. 4.6a), but beetles spent significantly more time in the soil under ryegrass than under any of the other treatments (Fig. 4.6b). *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 increase time spent in the soil, and the number of eggs laid. Alternatively, there may be ovipositional 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 *P. japonica* from ovipositing in areas that would otherwise provide suitable conditions for larval development.

CHAPTER 5:

SURVEY OF JAPANESE BEETLE MANAGEMENT PRACTICES IN MICHIGAN BLUEBERRY

INTRODUCTION

Michigan leads the nation in production of highbush blueberry (*Vaccinium corymbosum* L.), with 33% of the US crop produced in this state. In Michigan in 2003, there were 15,900 bearing acres of blueberries producing an average of 3,900 pounds of berries per acre (Kleweno and Matthews 2004). Of this, 24 million pounds were for the fresh market, receiving $1.30 per pound, and 38 million pounds were for the processed food market, receiving $0.84 per pound. In the past five years in Michigan, farm gate value of the whole industry has ranged from $49 (2001) to $63 (2003) million (Kleweno and Matthews 2004).

This industry has been challenged by the recent invasion of the Japanese beetle (*Popillia japonica* Newman, Coleoptera: Scarabaeidae) into it's major production centers. Although this pest was first detected in Michigan in 1932 (Anon. 1962), populations of Japanese beetle in west Michigan have increased in the past ten years, and some growers are applying increasing amounts of insecticides to maintain beetle-free fruit. Japanese beetle adults start emerging in early July and feed and mate on the bushes until mid-September (Fig. 1.2), which coincides with the period of blueberry harvest. The primary economic effect comes not from feeding damage, but from the risk of contamination of harvested fruit. About 75% of Michigan's blueberries are sold for processing, and for this purpose berries are harvested by machines that shake the fruits off the bushes. If beetles are present on the fruit or foliage as the harvester passes over the bushes, there is a risk of beetles being collected along with the berries. Buyers require the harvested fruit to be completely free of insect contamination, and although post-harvest techniques are available for removal of beetles and other items from the fruit, this situation demands superlative pest control by the growers facing this pest in their fields. In 2001, Japanese beetle was identified as the most important production problem for blueberry by commercial growers in Michigan (Anon. 2001), and has become a major focus for research and extension programs.

This survey was developed in 2003 to collect information from commercial blueberry growers in Michigan about the extent and magnitude of the problem Japanese beetles cause in blueberry production. The survey was also conducted to quantify management tactics that growers have adopted to control Japanese beetle and to determine willingness of growers to incorporate alternative management strategies, such as the use of cover crops, in their future management programs if they are proved effective against Japanese beetle.

MATERIALS AND METHODS

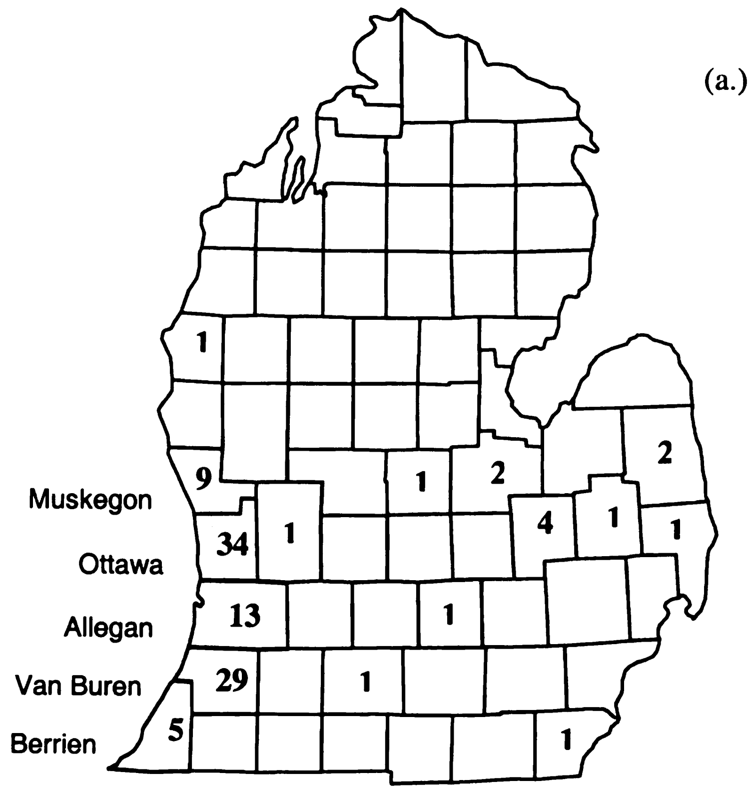
The survey (see Appendix 1) was developed to cover both sides of a letter sheet of paper and to be quick to complete (ca. 10-15 min). It was approved by the University Committee on Research Involving Human Subjects (UCRIHS) at Michigan State University (see Appendix 2) prior to being mailed to commercial highbush blueberry growers in South Michigan in 16 counties across the main blueberry production regions of the state. Surveys were mailed to 215 growers in the winter of 2003 by the Michigan

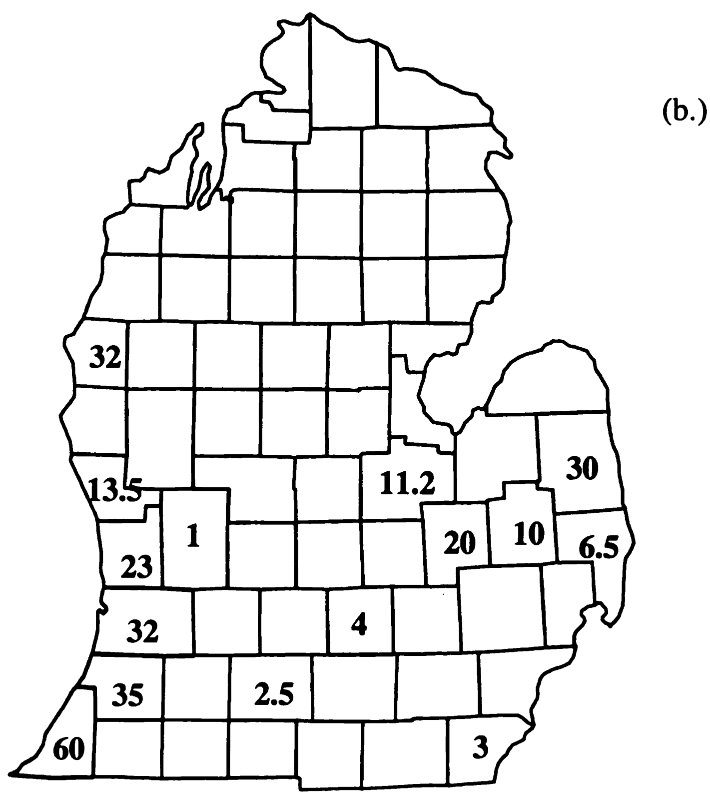
Blueberry Growers' Association, Grand Junction, MI and by Genesee County Extension, Flint, MI who used their address lists to mail the surveys to growers. Reply envelopes with postage were enclosed with the surveys, and reminders to return surveys were made at MSU Extension meetings in December 2003. Each survey that was returned was given a unique identification number used for the data organization and analysis. The name and address fields were optional to fill out, thus the answers could remain anonymous, based on the decision of the respondent. Data were entered into a Microsoft Excel spreadsheet, with the answers for each question entered on a separate spreadsheet. Some growers had farms in multiple counties, and these were treated as separate entries for the analysis of the first three questions. This was the case for 7 farms, and for these the total acreage was equally divided among the counties the farm was located in. Median and maximum values were calculated for values such as farm size and economic losses. Economic loss per acre was calculated by dividing the estimated loss per year by the total acreage from each farm. Percentage of respondents answering questions in a certain way were calculated based on the number of respondents for each question. Answers were analyzed with a χ^2 test, to determine significant departures from that expected by chance.

RESULTS AND DISCUSSION

Surveys were received from commercial blueberry growers in 16 counties of the southern tier of Michigan (Fig. 5.1a). A total of 99 surveys were received, and these were used in the analyses. The median farm size throughout this study was 21 acres (range: 1-375 acres) (Fig. 5.1b).

Figure 5.1. Map of Michigan's lower peninsula with the number of responses (a.) and median farm size (acres) (b.) per county, in 16 counties in the southern tier of Michigan. Names of Michigan counties in the main blueberry growing region are indicated on the left of the map (a.).


(a.)
1
9
1
2
2
Muskegon
34
1
4
1
1
Ottawa
13
1
Allegan
29
1
Van Buren
5
1
Berrien


(b.)
32
13.5
11.2
30
1
20
10
6.5
23
32
4
35
2.5
60
3

Eighty-four percent of respondents considered Japanese beetle a pest on their farms. On a 1-5 scale, (1-not important, 5-extremely important), 31% found Japanese beetle an extremely important production problem, and this value is significantly higher than expected (χ^2=11.52, $P < 0.05$). Only 6% of the respondents thought it was not an important insect pest. Responses ranged between growers considering this pest to be the most important production problem on their farm, to not having seen a Japanese beetle.

Among the major blueberry producing counties (in southwest Michigan), 53% of respondents considered Japanese beetle as an extremely important production problem in Allegan county, whereas only 22% of respondents in Muskegon county (Fig. 5.2). The reason for fewer growers considering Japanese beetle an extremely important pest in Muskegon county could be because of its geographic location: it is in the northern part of the main blueberry growing region, and Japanese beetles are not as abundant in the northern counties in Michigan as in the south part of the state. This could be because this beetle was first introduced into the state near Detroit, so it initially established in the southeast and/or because the climatic conditions in the northwestern part of the state are less suitable for sustaining Japanese beetle populations.

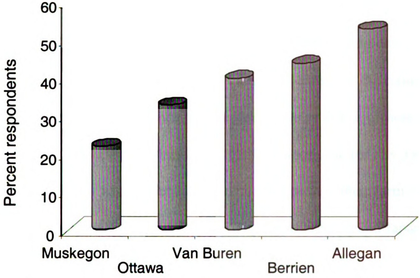


Figure 5.2. Percent of respondents that ranked Japanese beetle as the most important pest in blueberry production, by county.

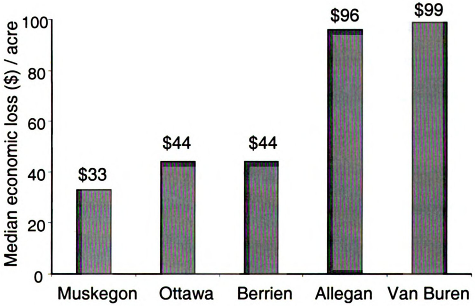


Figure 5.3. Estimated economic loss per acre from Japanese beetle at blueberry farms in SW Michigan counties.

The median economic loss per acre due to Japanese beetle as estimated by respondents was \$72 per year, and this value varied greatly among counties (overall range: \$10-1000) (Fig. 5.3). In order to understand the causes of the ratings respondents gave to the magnitude of importance of Japanese beetle as a pest, growers were asked about some of the production factors that might be responsible for this loss. More respondents were likely to consider Japanese beetle a pest on their farm if they had overhead irrigation, than if they didn't: 64% of growers who ranked Japanese beetle as an extremely or very important production problem have overhead irrigation (χ^2 =124.19, P < 0.05). More people rated Japanese beetle a very or extremely important production problem who didn't cultivate the soil around their fields (76%, χ^2 = 5.38, P < 0.05), than if they cultivated the soil around their fields (23%).

Seventy-nine responses were received to the question investigating the reason for the economic loss due to Japanese beetle. Significantly more than expected (63%, χ^2 = 67.12, P < 0.05) of the respondents indicated increased insecticide use as the major reason (Fig. 5.4). According to others, the damaged fruit reduced product value, damaged bushes yield less than undamaged bushes, there is an increased need for cultivation, harvest time is prolonged, or more labor and time are needed to remove beetles from harvested fruit. The risk of load rejection was considered to be a reason for increased cost by only 11% of growers, perhaps because if this occurs, it would be after the fruit have left the farm.

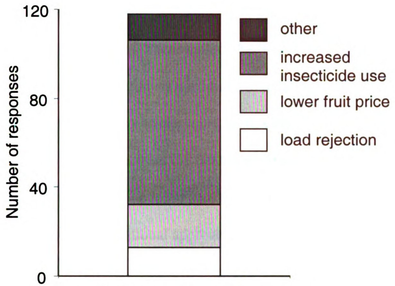


Figure 5.4. Causes of the economic impact due to Japanese beetle.

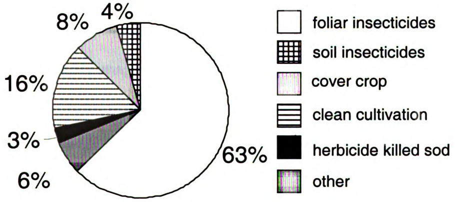


Figure 5.5. Growers' responses to the question "What is the most important Japanese beetle control method?"

The two most widely used methods of Japanese beetle control were foliar insecticides (63%) and clean cultivation (16%) (Fig. 5.5). The other methods listed were used by fewer than 21% of respondents. These include herbicide-killed sod, cover crops, and the use of soil insecticides. Four growers used traps to reduce the number of beetles (although this strategy could potentially attract more beetles into the crop), while others (6 responses) removed non-crop plants from the field that attract Japanese beetle. These results show that growers rely heavily on foliar insecticides for their Japanese beetle control, but that they are adopting additional approaches to help reduce the incidence of this pest.

Eighty-five of the respondents answered question 6 about the type of ground cover used at their farm. Thirty-one out of the 85 respondents (37%) had permanent sod throughout the row-middles across their farms. The second most commonly used row-middle treatment was clean cultivation, but only 24% of the growers who answered the question had this type of row-middle on their whole farm, suggesting that different fields have different row-middle management strategies. Almost twice as many acres were clean cultivated (2,067 acres) as were kept with permanent sod (1,254 acres) (Fig. 5.6). Only 15% of the 85 answers indicated the use of multiple ground covers at an individual farm.

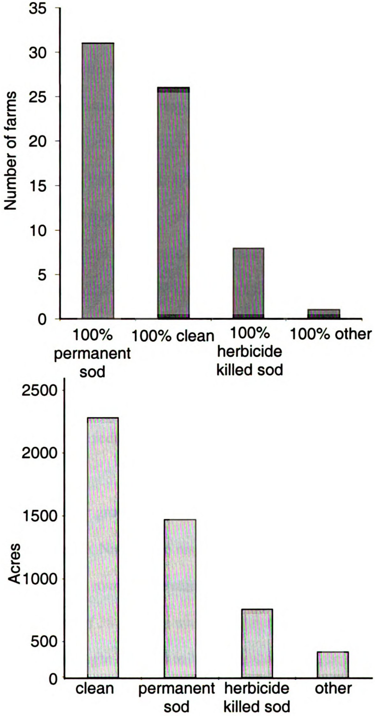


Figure 5.6. Number of farms with different types of row-middle management (top). Total acreage of farms with different row-middle management (bottom).

Of the growers that replied to the survey, there were approximately 544 acres of farms with herbicide-killed sod, and 207 acres of other types of ground covers. These included rye and herbicide-killed rye.

Conditions in and around crop fields can favor or inhibit development of Japanese beetle depending on whether fundamental requirements for the pest population development are met (Vittum et al. 1999). Within many Michigan blueberry fields and along the perimeter of these fields, ground covers of seeded grass or the naturally-invading mix of grass and broadleaved weeds are commonly used to maintain soil structure, provide conditions where agricultural machinery can be driven during wet conditions, reduce soil erosion, and prevent pesticide and fertilizer runoff.
These areas provide ideal conditions for Japanese beetle where both the egg-laying and larval developmental requirements are met. Changing these parameters, as some growers already are doing, may reduce pest pressure over the long-term by affecting the insect's biology.

I asked whether growers had changed row-middle management practices to control Japanese beetle. Ninety-two responses were received to the question and 42% of these growers changed row-middle management practices, with their changes starting on average in 2000. Out of 38 growers who changed management methods, 27 switched to clean cultivation and eight changed to herbicide-killed sod. In one case the grower started narrowing the sod strip in row-middles, and in another the grower switched to mowing more often. This clearly shows a tendency to move away from grassy, living row-middle ground covers. Fifty percent of growers who had made a change said that the changes reduced Japanese beetle pressure, and 45% were not sure. When asked whether they would be willing to incorporate new cover crops into their management strategies to control Japanese beetle if they were effective, 59% of the 90 respondents answered "yes" (Fig. 5.7), indicating a potential for integrating alternative ground covers into Michigan blueberry fields.

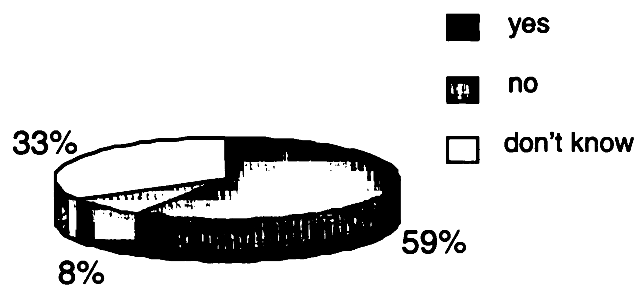


Figure 5.7. Responses to the question "Would you be interested in using new ground covers if they were effective against Japanese beetle?"

Eighty-nine responses were received to questions 9 and 10 about the perceived advantages and disadvantages of cover crops and clean cultivation in blueberry fields (Table 5.1). The two most important advantages of cover crops according to growers were prevention of soil erosion (65%, $\chi^2 = 34.5$, $P < 0.05$) and preservation of soil moisture (42%, $\chi^2 = 16.0$, $P < 0.05$). The most important disadvantage of having cover crops was that they compete with the blueberry bushes (44%, NS) and they might serve as an alternative host for pests (40%, NS). Seventy-six percent (68 answers out of 89) of the growers answered that having clean cultivation provides control of pests ($\chi^2 = 31.1$, $P < 0.05$), and half as many answers (34) indicated that clean cultivation is advantageous because it decreases frost damage in the fields. The main disadvantage of clean cultivation according to the survey was that it creates muddy and dusty conditions ($\chi^2 = 16.1$, $P < 0.05$), and the second disadvantage of clean cultivation was that it facilitates soil erosion. Interestingly increased cost, such as the expense of cover crop seeds or cultivation (as opposed to maintaining row-middles with cover crop) was never considered of high importance. Growers' answers indicated that the issues they consider when deciding what soil management method to apply in their fields are mostly horticultural, based on the effect on bushes (competition of cover crops for soil moisture) and insect pests (cover crops could provide alternate hosts for pest and clean cultivation is a method of pest control). Complaints about mud, dust and erosion in blueberry fields are common among growers who have clean cultivation (Z. Szendrei, personal communication with growers), and most of the blueberry fields in west Michigan have light, sandy soils that are prone to erosion and create dust when dry.

Table 5.1. Advantages and disadvantages of cover crops and clean cultivation for integration into blueberry production, and the percentage of survey respondents that consider these important (in order of importance). Eighty-nine responses were given.

ADVANTAGES	Response (%)	DISADVANTAGES	Response (%)
Cover crop			
1. Prevents soil erosion	65	1. Competition with bushes	44
2. Conserves soil moisture	42	2. Alternative host for pests	41
3. Controls pests	36	3. Too expensive	30
4. Cuts fertilizer costs	12	4. Difficult to seed	21
Clean cultivation			
1. Controls pests	76	1. Mud and dust in field	76
2. Decreases frost damage	38	2. Soil erosion	54
3. Easy to manage	30	3. Soil moisture loss	34
4. Low cost of maintenance	12	4. Need for herbicides	25

Seventy two percent of respondents did not cultivate the soil around their fields. Growers maintain this area to have permanent sod cover in the driveways and around the fields for the maneuverability of tractors and harvesters. These areas therefore cannot be switched to clean cultivation and there is a great need to develop effective approaches to control Japanese beetle grubs that are developing here in this area of the blueberry farms.

I asked growers if there were any changes to their current Japanese beetle management programs that they were planning for the future. Twenty-two out of 73 answers (30%) were "yes". The types of changes growers were planning included: switching to clean cultivation, planting cover crops, applying herbicide to row-middles, use more foliar insecticides, removing weeds, spraying the field perimeters, or using soil insecticides. One grower was planning on planting grapes around the field as a trap crop and spraying them with insecticide (although this may not be beneficial, because of the potential for attracting beetles to the area of the field). Thirteen out of the 30 respondents who said that Japanese beetle was an extremely important pest on their farm would not change their current management program. Twelve said they would change their management program, and the most common form of change was to spray more foliar insecticide.

Seventy-two answers were received to the question about whether there are other equally or more important pest management problems besides Japanese beetle. Blueberry maggot (*Rhagoletis mendax* Curran) was the most common other pest problem for growers (23%), but other common answers were cranberry fruitworm (*Acrobasis vaccinii* Riley) (17%), diseases (9%), weeds (7%), birds (6%), mammals (groundhog and deer, 6%). There were a few other sporadic problems with other insect pests mentioned, these included rose chafer (*Macrodactylus subspinosus* (Fabricius)), tussock moths (*Orgyia leucostigma* (J.E. Smith)), blueberry gall wasp (*Hemadas nubilipennis*), blueberry aphids (*Illinoia pepperi* (McGillivary)), and oblique banded leafrollers (*Choristoneura rosaceana* (Harris)). One grower said that government regulations were an equally or more important problem than Japanese beetle.

Among the growers who listed Japanese beetle as an extremely important pest on their farm, cranberry fruitworm and blueberry maggot were the most common other production problems, while weeds and cherry fruitworm (*Grapholita packardi*) were the second most common responses.

When comparing farm size and the importance of Japanese beetle in production we found that 67% of the owners of large farms (60-375 acres) said that Japanese beetle is an extremely important pest on their farm, 43% of the 13-20 acre and 53% of the less than 13-acre farm owners said the same (Fig. 5.8). Twice as many large farm owners (60-375 acres) gave rank 5 (extremely important pest) than rank 4 (very important pest), whereas this difference was only 1.3 –fold in the case of small farm owners (<13 acres). This could mean that growers who could potentially suffer a large economic loss due to Japanese beetle are more aware of the importance of this pest. It may also be that more Japanese beetles are attracted to a large patch of blueberries than a small one, or that the pest management program is easier to manage on a smaller farm than in a large one, particularly around harvest time.

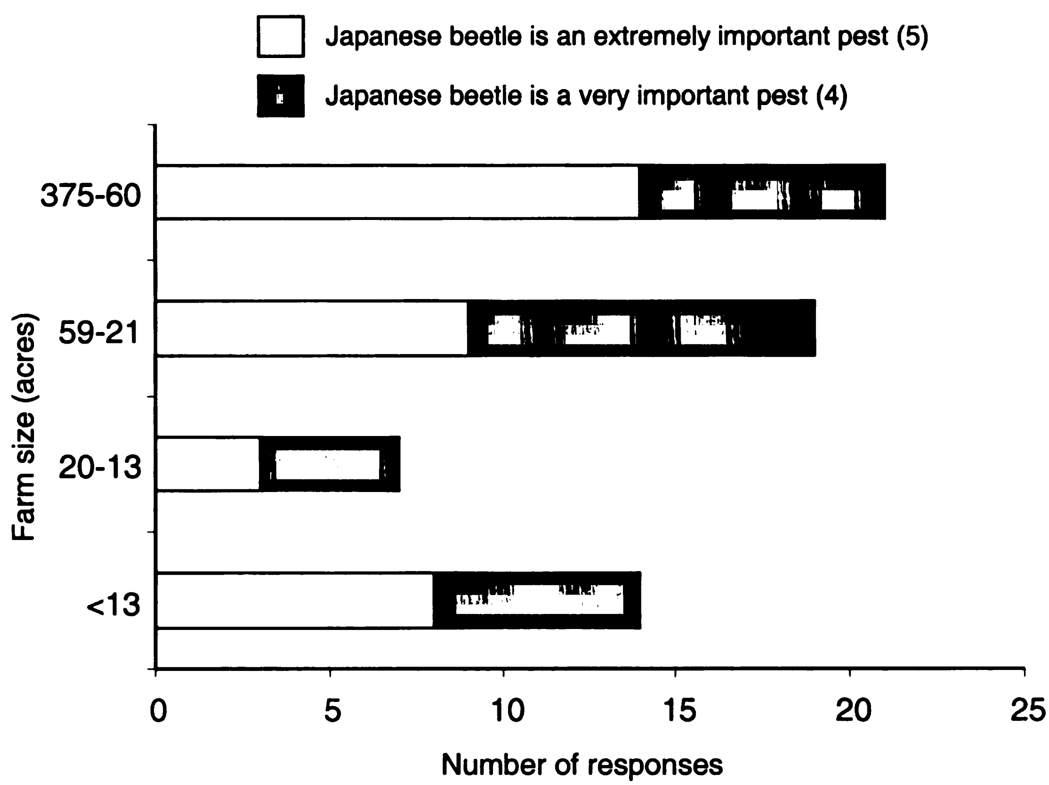


Figure 5.8. Relationship between farms size and the importance of Japanese beetles as a pest in blueberry production. Importance of the pest was on a scale from 1 (not important) to 5 (extremely important); results shown in the graph are ranks 4 and 5 only.

CONCLUSION

This grower survey shows that Japanese beetle is an important pest management challenge for blueberry producers in southern Michigan, with significant economic impact. Increased insecticide use was the major cause of the economic loss due to this pest for the producers. For the blueberry industry, the major causes of the economic loss included lost customers and sales due to grade reduction of berries that were chewed by beetles and secondarily infected with fungus (Dave Trinka, personal communication). It is estimated that at least half of the fruit loss is caused by this factor, which translates into an economic loss of $7-10 million per year (Dave Trinka, personal communication). Considering the $63 million dollar value of the industry in 2003, this translates into a ~14% value loss for growers.

The most widely used methods of controlling Japanese beetle in Michigan highbush blueberry fields are insecticides and clean cultivation. Szendrei et al. (in press) showed that clean cultivated fields had fewer Japanese beetle larvae than fields with permanent sod. In Michigan blueberry fields, more farms have permanent sod coverage than clean cultivation, but the acreage of clean cultivated farms is higher. This means that larger farms tend to be clean cultivated, and this could be correlated with the risk factor and size of economic loss a grower could suffer if a Japanese beetle was detected in the harvested fruit. It is likely that this trend is because growers who have larger farms and rely on blueberry farming for income are taking more precautions to minimize the chance of contamination of fruit with Japanese beetle. Currently, Japanese beetle is driving changes in row-middle management, as indicated by growers recently switching to clean cultivation. The changes implemented to control Japanese beetle are considered to be effective by growers responding to this survey, and most growers are not planning on making more changes to their pest management programs. Many growers would be willing to try new cover crops if they are shown to be effective against this pest.

This type of survey is important because the results point out a number of further opportunities for research and extension toward the adoption of new strategies to control Japanese beetle in highbush blueberry. The information gathered here could be used to develop control strategies that rely on realistic management needs and existing methods. Usefulness of grower surveys in developing future research directions has also been found in the case of strawberry growers in Ohio (Scheerens and Brenneman 1990) and New York (Stivers-Young and Tucker 1999). In the latter survey, the authors identified the types of cover crops that were currently being planted and the advantages and disadvantages that growers perceived when using them. In the future, participatory research that actively includes blueberry growers may speed up appropriate adoption of new cover crops or other strategies to help control Japanese beetle and other pests.

CHAPTER 6:

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

The research presented in this thesis has, in the broad sense, provided new information in the study of polyphagous insects and their interaction with plants, with relevance for pest management. Currently, there is an increased need for alternative methods of pest control to reduce the use of pesticides in agricultural production and this demands research into approaches that provide environmentally sustainable solutions to reduce insect pest damage. Behavioral manipulation of polyphagous pests, such as the Japanese beetle, through changing the resource composition within an agricultural field is challenging because of the lack of specificity in the insect's feeding behavior, and their ability to utilize multiple resources. The behavior chosen for manipulation is however not necessarily the one causing injury to the plant, but rather a behavior that can be targeted by management methods and is effective in reducing the subsequent population levels. Results of this thesis indicate that, in the case of the Japanese beetle, a possible target for behavioral manipulation is their oviposition, and its control thorough cultural practices.

Ultimately what matters for the success of pest control is the reduction of damage to the crop and economic loss related to this damage. Although I have been able to prove that spring larval populations are related to subsequent early-summer adult populations (Chapter 2) within a blueberry field, future research needs to investigate further the relationship of the two life stages on different spatio-temporal scales. This information is essential to understand the success of controlling adults through reducing the number of larvae within an agricultural field. If this is effectively done, then the benefits of disrupting oviposition could be two-fold: reducing the number of females that arrive to the field to oviposit and reduction of the following larval populations. This would also mean that soil applied insecticides targeting the larvae could be used to reduce adult density in the following summer. The larval density data in Chapter 2 provide useful information about where to target soil applied insecticides in the field. This information could be made more explicit if there was additional data on the spatial distribution of larvae and the change in distribution patterns over time. Currently we have no information about the year-to-year variation in the location of larvae within a field. Much of the success of pest management on a field scale is dependent upon the migration rates of the targeted insect from the surrounding habitats. In the future, detailed study of the immigration and emigration of Japanese beetle in and out of different types of habitats will aid growers' decisions on the use of different control strategies. This kind of information is also useful in predicting infestation rates depending on the type of habitat around the agricultural area.

In Chapter 3, I have investigated the interaction of cover crops and Japanese beetle. The economic damage of this pest in blueberry is due to the presence of adults on the blueberry bushes, so the next important step is to investigate how this interplay between cover crops and beetles will affect the number of beetles on the harvested crop. In this case the use of cover crops for reduction of crop damage is the goal, and this cannot be achieved reliably without understanding the particular aspects of insect plant interactions. Results in Chapter 3 give an indication of how some cover crop species might affect overall Japanese beetle abundance within a field, but the implications of these findings could be controversial. First, if a cover crop is more attractive for the pest than the main crop, such as was found for buckwheat, it could mean that the insects will be attracted out of the valued crop, thus reducing damage. On the other hand, it is possible that the higher overall abundance of the pest within the field will result in increased pest numbers on the bushes, and a higher level of damage and greater risk of contamination. In the future, more species of cover crops should be tested for their effectiveness to reduce Japanese beetle damage to blueberries. They also should be tested at larger scales. When selecting a cover crop for pest management, the horticultural aspects of adding a new plant into the agricultural field must be given high priority, thus the effect of the cover crops on the main crop has to be investigated. These effects can sometimes be deleterious, such as drawing water and nutrients away form the main crop, thus reducing yield, or it could change the microclimate within the field so that it creates ideal conditions for the development of diseases. In practice, weed control is one of the main reasons for using cover crops, and so it would also be important to measure the competitiveness of the chosen plant to weed species that are typically occurring in the main crop.

Field observations of Japanese beetle behavior in Chapter 3 have provided valuable information on how different plants change the behavior of Japanese beetle, or alternatively, how Japanese beetle utilize the different plant species. Further investigations are needed to examine what components of the plants act as cues for initiating particular behaviors that are characteristic on a plant species. Behavioral observations in the field provide valuable information about the behavior of the insect in its natural context, but to determine the effect of proximate factors influencing behavior, controlled studies are needed in a seminatural or laboratory setting. These types of experiments would allow the examination of the effect of the various components of the environment, and also allow the use of a rigorous experimental design.

For a successful application of behavioral pest management in this system, it is necessary to understand the mechanisms involved in ovipositional decision-making by Japanese beetle, which lives as larva in the soil and above ground as an adult. The larvae feed on plant material in the soil, whereas the adults feed on the above ground plant parts. Using bioassay arenas, I was able to prove that Japanese beetle females show selectivity in their oviposition decisions. I was interested to find out if this decision is made based on olfactory, tactile, or visual cues, or a combination of any of these (Chapter 4), because this information is essential for successful behavioral manipulation. In Chapter 4, I show the significance of visual and possibly tactile cues. The role of chemical cues from the soil and the plants is yet to be determined, but this could be done through similar bioassay experiments. The method developed in Chapter 4 for testing ovipositional choice has been found appropriate to answer some fundamental questions about Japanese beetle host selection, but other approaches to examine related research questions should be used in the future, such as no-choice assays, or olfactometers. It would also be of interest to investigate whether female preference is based on larval performance as has been found in the case of other herbivores (Jaenike 1978, Craig et al. 1989, Bernays and Chapman 1994, Showler 2001). This would be useful when evaluating characters of an agricultural field for predicting future infestation rates.

In my investigations described in this thesis, I kept in the forefront the practicality of my findings for growers. I often presented my results at growers' meetings and asked for feedback from them about the nature and magnitude of the problem Japanese beetles cause in highbush blueberry production and the control measures they use and would be willing to use in their pest management regimes (Chapter 5). My goal was to interact with growers to understand the needs and potential avenues in Japanese beetle control. I therefore had to combine horticultural aspects of blueberry production with our basic knowledge of Japanese beetle biology to make advancement in pest control through behavioral pest management in this system. Cover crops seemed like an ideal fit for this complex problem, since some growers have already applied them for alternative reasons. It is challenging to achieve a high level of control of Japanese beetle with non-chemical methods, like behavioral manipulation, because the rates of immigration and pest population levels are high in this species, but the incorporation of this new method into current management practices could contribute to achieving lower crop damage levels.

Some of the current findings could be used to build a theoretical model using the empirical data generated in this research. The integration of theoretical and empirical approaches offers the possibility to unravel the processes underlying habitat management strategies and generate hypotheses that can be tested in empirical studies. For example, by combining the information in Chapter 3 with the information from the literature on Japanese beetle biology, predictions can be made about how changing the plant composition will alter the density of the different lifestages of Japanese beetle in an agricultural field.

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APPENDICES

APPENDIX 1

Survey of Japanese Beetle Management Methods

This survey has been prepared to gather information about Japanese beetle and strategies for its control in blueberries. Your answers can be anonymous, but the survey results will be sent to respondents who provided their name and address. All information that is provided is treated as confidential. No information from individual replies will ever be used in a public way, and results will be shared with the industry at future MSU extension meetings.
Thanks for you time,

Dr. Rufus Isaacs and Zsofia Szendrei, MSU Small Fruit Entomology
You indicate your voluntary agreement to participate by completing and returning this questionnaire.
Completed surveys should be mailed to: Small Fruit Entomology, 202 CIPS, Michigan State University, East Lansing, MI 48824, using the enclosed envelope.

Name*: ______________________ *Name and address are optional

Address*: ______________________

Counties where your blueberry farms are located: ____________

Total area of commercial blueberry production: ________ acres

1. Is Japanese beetle a pest on your farm? ☐ Yes ☐ No

2. If Japanese beetle is a pest, how important is this insect in your blueberry production?
◯ extremely ◯ very ◯ somewhat ◯ slightly ◯ not

3. What do you estimate the economic cost of Japanese beetle to be per year on your farm?

______________________ $ per year

4. Is the economic impact of Japanese beetle due to:
☐ load rejections ☐ lower fruit price ☐ increased insecticide use
Other ______________________

5. What method(s) do you use to control Japanese beetle?

Check all circles that apply. Rank them in order of importance: 1= most important

- ◯ Insecticide, foliar application ____________
- ◯ Insecticide, soil application ____________
- ◯ Cover crops ____________
- ◯ Clean cultivation ____________
- ◯ Herbicide killed sod in row-middles ____________
- ◯ Other: ______________________ ____________

6. What row-middle ground cover do you use at your farm?

Check all circles that apply. % of acres

- ◯ Permanent sod ____________
- ◯ Clean cultivation ____________
- ◯ Herbicide killed sod ____________
- ◯ Other: ______________________ ____________

7. Have you changed row-middle management practices to help control Japanese beetl☐ ☐

Yes No

If YES, How many years ago did you change? __________

What changes did you make? ______________________________

____

Have the changes reduced Japanese beetle pressure? ☐ Yes ☐ No ☐ Don't know

8. Would you be interested in using new ground covers if they were effective against Japanese beet☐ Yes ☐ No ☐ Don't know

9. What do you think are the possible advantages or disadvantages of **cover crops**?

Advantages:

- ☐ Controls pests
- ☐ Prevents soil erosion
- ☐ Conserves soil moisture
- ☐ Cuts fertilizer costs

Disadvantages:

- ☐ Too expensive
- ☐ Difficult to seed
- ☐ Competition with bushes
- ☐ Alternative host for pests

10. What do you think are the possible advantages or disadvantages of **clean cultivation**?

Advantages:
- ☐ Controls pests
- ☐ Low cost of maintenance
- ☐ Easy to manage
- ☐ Decreases frost damage

Disadvantages:
- ☐ Soil erosion
- ☐ Soil moisture loss
- ☐ Need for herbicides
- ☐ Mud and dust in field

11. What kind of irrigation system do you have?

Check all circles that apply.

- ○ Overhead
- ○ Drip
- ○ None
- ○ Other: ____________

12. Do you cultivate the soil around your fields? ☐ Yes ☐ No

13. Are there any changes to your Japanese beetle management strategy that you are planning for the future? ____________________________

____

14. What other pest management problems do you have that are equally or more important than the Japanese beetle?

____

If you have any questions regarding this survey itself, please contact Rufus Isaacs at (517) 355-6619.

If you have any questions regarding your participation as a subject in this research, please contact:

Ashir Kumar, UCHRIS, 202 Olds Hall, Michigan State University, East Lansing, MI 48824-1046

APPENDIX 2

September 24, 2003

TO: Rufus ISAACS
202 CIPS

RE: **IRB# 03-706** CATEGORY: EXEMPT 1-2

APPROVAL DATE: September 22, 2003
EXPIRATION DATE:August 22, 2004

TITLE: SURVEY OF JAPANESE BEETLE MANAGEMENT METHODS

The University Committee on Research Involving Human Subjects' (UCRIHS) review of this project is complete and I am pleased to advise that the rights and welfare of the human subjects appear to be adequately protected and methods to obtain informed consent are appropriate. Therefore, the **UCRIHS approved this project.**

RENEWALS: UCRIHS approval is valid until the expiration date listed above. Projects continuing beyond this date must be renewed with the renewal form. A maximum of four such expedited renewals are possible. Investigators wishing to continue a project beyond that time need to submit a 5-year application for a complete review.
REVISIONS: UCRIHS must review any changes in procedures involving human subjects, prior to initiation of the change. If this is done at the time of renewal, please include a revision form with the renewal. To revise an approved protocol at any other time during the year, send your written request with an attached revision cover sheet to the UCRIHS Chair, requesting revised approval and referencing the project's IRB# and title. Include in your request a description of the change and any revised instruments, consent forms or advertisements that are applicable.
PROBLEMS/CHANGES: Should either of the following arise during the course of the work, notify UCRIHS promptly: 1) problems (unexpected side effects, complaints, etc.) involving human subjects or 2) changes in the research environment or new information indicating greater risk to the human subjects than existed when the protocol was previously reviewed and approved.

If we can be of further assistance, please contact us at (517) 355-2180 or via email: UCRIHS@msu.edu. Please note that all UCRIHS forms are located on the web: http://www.humanresearch.msu.edu

Sincerely,

Peter Vasilenko III, Ph.D.
UCRIHS Chair

PV: jm

cc: Zsofia Szendrei
202 CIPS

APPENDIX 3

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2005-02

Title of thesis or dissertation (or other research projects):

POTENTIAL FOR BEHAVIORAL AND CULTURAL MANIPULATION OF JAPANESE BEETLE (*POPILLIA JAPONICA* NEWMAN, COLEOPTERA: SCARABAEIDAE) POPULATIONS

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Zsofia Szendrei

Date April 19, 2005

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America.
Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.
Museum(s) files.
Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix 3.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Popillia japonica Newman	MI, Allegan Co. Fennville, TNRC in pheromone traps July 2001 Z. Szendrei					16	11		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Zsofia Szendrei

Date 19-Apr-05

Voucher No. 2005-02

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

31 MAY 2005

Curator Date