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LUKASZ LECH STELINSKI

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INTEGRATED MANAGEMENT STRATEGIES FOR CONTROL OF APPLE MAGGOT AND BLUEBERRY MAGGOT FLIES

By

Lukasz Lech Stelinski

A THESIS

Submitted to
Michigan State University
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ABSTRACT

INTEGRATED MANAGEMENT STRATEGIES FOR CONTROL OF APPLE MAGGOT AND BLUEBERRY MAGGOT FLIES

By

Lukasz Lech Stelinski

The apple maggot, *Rhagoletis pomonella* (Walsh), and the blueberry maggot, *Rhagoletis mendax* Curran are the two most important late-season pests of apples and blueberries, respectively. In an attempt to develop integrated management strategies for control of these two key *Rhagoletis* species, insecticide-treated biodegradable spheres, sphere deployment tactics, and synthetic host-volatile compounds were evaluated over three field seasons in commercial apple orchards and blueberry plantings. Spheres treated with imidacloprid killed significantly more apple maggot and blueberry maggot flies compared to spheres treated with thiamethoxam, thiocloprid and untreated spheres. In sphere deployment experiments, there were no significant differences in fruit injury between the three tactics tested. Also, fruit injury in plots that received azinphos-methyl spray applications did not differ significantly from plots containing insecticide-treated spheres. In the host volatile studies, a mix blend of apple volatiles attracted significantly more apple maggot flies than other lures and baits evaluated. In blueberries, ammonium acetate was significantly more attractive to female blueberry maggot flies in both early- and mid-season cultivars compared with other compounds. In addition, green sphere traps baited with the synthetic fruit-volitiles, cis-3-hexen-1-ol and geraniol, captured statistically equal numbers of blueberry maggot flies compared with ammonium-baited traps when deployed within an early ripening blueberry cultivar (Earliblue).

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INTRODUCTION

Michigan apple and blueberry industry. Michigan ranks second or third, depending on annual yield and production, in the U.S. apple industry. Apples are grown commercially on more than 58,000 acres and more apples are produced by volume than all other Michigan fruits combined (Michigan Apple Committee). The state also maintains the lead in highbush blueberry (*Vaccinium corymbosum L.*) production, generating 45 percent of U. S. blueberries on more than 17,000 acres (Michigan Blueberry Growers Association).

In Michigan, apple orchards and blueberry plantings experience high infestations of apple maggot and blueberry maggot flies, respectively. Adults, responding to visual and olfactory cues, migrate into commercial orchards and plantings where mating and oviposition occurs on host fruit (Liburd and Stelinski 1999). Economically, the apple maggot fly, *Rhagoletis pomonella* (Walsh) and the blueberry maggot fly, *Rhagoletis mendax* Curran are the most important late season pests of commercially grown apples, *Malus domestica* Borkhausen and blueberries, *Vaccinium* spp. L., respectively in the northeastern and midwestern United States (Bush 1966). Each fly species restricts its attack to host plants within a few closely related genera and larval development within host fruit renders it commercially unmarketable.

Federal (USDA) regulations, phytosanitary restrictions, and consumer demands have resulted in a zero tolerance to maggot infested fruits such as apples and blueberries. In order to ensure the absence of maggot injury due to *Rhagoletis* species, growers generally apply three to five insecticide treatments regardless of whether flies are present in their orchards or plantings (Stanley et al. 1987, Liburd et al. 1998 a). Registered insecticides for fruit fly control include malathion, carbaryl (Sevin), phosmet (Imidan), dimethoate (Cygon) and azinphosmethyl (Guthion). These insecticides are highly toxic and kill both pest and beneficial insects indiscriminately. The presence of insecticide residues on food and their impact on human health has also created widespread apprehension among the general public regarding the use of insecticides. Future management practices for protecting apples and blueberries from apple maggot and blueberry maggot flies with insecticides, particularly organophosphates, are subject to restriction by the regulations being implemented under the Food Quality Protection Act (FQPA).

Michigan fruit fly integrated pest management (IPM) program. For almost three decades, integrated fruit fly management programs in Michigan have relied on the use of visual and olfactory traps to make control decisions. The strategies adopted by most growers involve the deployment of baited Pherocon AM yellow sticky boards and colored spheres in apple orchards and blueberry plantings (Reissig 1976, Prokopy and Hauschild 1979, Liburd et al.1998 b). Ground and aerial applications of insecticide sprays usually follow the detection of one fly per trap (Liburd et al. 2001).

The use of visual and olfactory monitoring traps can be a valuable IPM strategy. However, the monitoring traps currently used by most growers and scouts become inundated with a wide variety of non-target insects after only two weeks of deployment, which drastically reduces their effectiveness (Liburd et al. 1999). In addition, the odor of decomposing insects on the sticky surface of these traps overpowers the insect attractant (bait) used with them, which further reduces trap effectiveness. Under these circumstances, traps need to be cleaned and replaced frequently in the field to maintain accurate monitoring. Trap preparation, replacement, and maintenance is a time consuming and labor-intensive operation (Prokopy *et al.* 1990). Trap monitoring efficacy could be improved by increasing selectivity to apple maggot and blueberry maggot flies. This could be accomplished by identifying appropriate synthetic fruit volatiles that selectively attract each fruit fly species. The development of traps baited with selective lures may extend the longevity of trap effectiveness, possibly allowing growers to make more informed and precise management decisions.

In addition, the development of insecticide-treated sphere technology (attract-and-kill devices) as part of an IPM strategy could potentially reduce broad-spectrum insecticides for the control of key *Rhagoletis* species. Laboratory and field studies have shown that insecticide-treated spheres are effective in attracting and killing both apple maggot and blueberry maggot flies (Hu et al. 1998, Liburd et al. 1999). However, there have been no published studies on optimization of sphere deployment strategies in the field and little work has been done on determining performance, longevity, and compatibility of various novel insecticides for use with this technology.

Life-history of temperate *Rhagoletis* species. The majority of temperate *Rhagoletis* species are univoltine (Fletcher 1989). Puparial diapause is followed by emergence of adults beneath host plants that yielded fruit the previous year; emergence nearly coincides with appearance of suitable host fruit for egg laying in the current year (Lathrop and Nickels 1932). Sexual maturity is reached within 1-2 weeks of fly emergence and is followed by mating on fruiting host plants, which culminates in oviposition by the females into ripe or ripening host fruits (Smith and Prokopy 1981 and

1982, Liburd et al. 1998 b, Lathrop and Nickels 1932). Prior to sexual maturity, the females exhibit an attraction to ammonia (Liburd et al. 1998a), apparently seeking a protein source that may be important for egg maturation (Prokopy and Roitberg 1989, Prokopy 1993, and Prokopy et al. 1994). Odor emitted by ripening fruit, which is attractive to both sexes at distances of at least 20 m may serve as a long-range attractant to *Rhagoletis* flies (Prokopy et al. 1973). In addition, *R. pomonella* have been reported to detect the visual stimuli of host trees at distances of at least 3 m (Green et al. 1994). After locating an appropriate mating site (species-specific host fruit), males defend their territory by various displays including wing waving, foreleg kicking, and boxing (Messina and Subler 1995). Observations by Prokopy and Bush (1973) and Smith and Prokopy (1981,1982) suggest that *R. pomonella* and *R. mendax* males force copulation upon the females. The female fly usually visits several fruits prior to oviposition. After identifying an appropriate site, she inserts her ovipositor into the fruit and deposits a single egg just below the surface of the fruit. Immediately after the egg is deposited, the female walks around the fruit dragging her extended ovipositor on the fruit surface and laying down an epidictic pheromone, which deters other females from ovipositing into the same fruit (Prokopy 1972 b). Interestingly, studies have shown that flies from different species do not recognize each others host-marking pheromones, while different populations of the same species show marked recognition of each others' pheromones (Prokopy 1976, Prokopy et al. 1982).

Rhagoletis larvae feed and develop inside the fruit, which results in destruction of the internal fruit tissues (Liburd et al 1998 b). During the third or fourth instar, the larvae bore a hole in the skin of the decaying fruit and drop or crawl to the ground. Emergence from the fruit takes place in the early morning hours and is stimulated by the onset of light and an increase in surrounding temperature (Goeden and Ricker 1971, Boller and Porkopy 1976). Larvae usually burrow 3 to 5 cm below the surface of the soil where they pupariate and overwinter in diapause, with 20 % of the puparia remaining in the soil for more than two seasons (Lathrop and Nickels 1932).

Biogeographic range distribution. Species of the genus *Rhagoletis* are widely distributed over the Holarctic and Neotropical regions (Bush 1966). More than 60 species with their related host plants have been described (Smith and Bush 1996), and it is likely that speciation within the genus *Rhagoletis* is associated with the ecological separation of populations, resulting from flies shifting to new host plants (Bush 1992). Bush (1969) argued that the use of different hosts by these sympatrically speciating forms would generate immediate and complete reproductive isolation. A prominent characteristic of the genus is the frequent occurrence of morphologically indistinguishable sibling species that are ecologically independent.

The apple maggot fly is native to North America and its original host is believed to be hawthorn, *Crataegus* spp., which is a close botanical relative of apples (Dean and Chapman 1973). Although the apple maggot fly appears to be most common in the northeastern United States and southeastern Canada, it is widely distributed and present in all of the Atlantic States to northern Florida (Bush 1966). Its distribution also includes the central states as far south as Arkansas and states west to the Dakotas and southern Manitoba (Bush 1966). The apple maggot fly also occurs in the Pacific Northwest in Oregon, Washington and northern California (Larry J. Gut, personal communication).

The blueberry maggot fly is a sibling species of the apple maggot fly. The three primary ericaceous host plants used by *R. mendax* in the northeastern United States include blueberries, (*Vaccinium corymbosum* L. and *V. angustifolium* Aiton), and huckleberries, (*Gaylussacia* Humboldt, Bonpland, and Kunth spp.) (Bush 1966). In the south, *V. stamineum* L. (deerberry) is the primary host (Payne and Berlocher 1995 a, b). The blueberry maggot fly's distribution extends northward into Canada. The fly is known to occur in Nova Scotia, Prince Edward Island and New Brunswick (Wood 1965, Neilson and Wood 1985). In the northern United States, high populations occur in Maine, New Hampshire, Vermont, New Jersey, and Michigan. (Bush 1969). The blueberry maggot fly is also widely distributed within the southern United States into northern Florida and as far to the west as the Ozark Plateau (Bush 1969).

Morphology, ovipositional preference, and genetic isolation.

Considerable work has been carried out to determine the taxonomic placement of the apple maggot and blueberry maggot (Bush 1966, 1969, Feder 1998). Distinction based on morphological features has been difficult, while few structural characteristics can be discerned with which to separate the species. The blueberry maggot form is smaller than apple maggot, in every stage of the life cycle, yet the processes of oviposition, pupariation, as well as larval habits appear identical (Lathrop and Nickels 1932). In western Michigan, blueberry maggot flies of both sexes captured on sticky yellow pane traps and sticky sphere traps are consistently smaller than apple maggot flies captured on the same manner (personal observation). Bush (1966) concluded that *R. pomonella* and *R. mendax* are separate species based on the morphological differences of ovipositor length, femur coloration, and wing band ratios.

Divergent host-fruit preferences exist between the apple maggot and blueberry maggot. The literature documenting the apple maggot's attraction to its particular larval host-fruit volatiles is voluminous. Prokopy et al. (1973) and later Reisig (1974) provided evidence that apple maggot flies positively respond to apple odor in the field. Later, Fein et al. (1982) isolated apple volatiles from two apple varieties (Red Delicious and Red Astrachan) and found that a mix blend consisting of a series of short chain carbon esters, including butyl hexanoate was attractive to apple maggot flies. Also, Reissig et al. (1985) showed that sticky red spheres (8.5 cm diam) baited with synthetic apple volatiles were four times more effective than unbaited red spheres. Recently, Averill et al. (1998) determined that apple maggot flies exhibits a high degree of olfactory specificity, showing a minimum behavioral response to straight chain esters, 10-11 carbons in length and having an acid portion of six to eight carbons and an alcohol portion of three to five carbons. Finally, a recent study has shown that a lure consisting of a mix blend of synthetic apple volatiles combined in specific proportions and including butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate, and pentyl hexanoate was significantly more attractive to adult apple maggot flies than lures containing butyl hexanoate alone (Zhang et al. 1999).

Blueberry maggot flies are relatively more sensitive to the odor of blueberries, their specific host, than of apples and vice versa with apple maggot flies, indicating that antennal sensitivity may be adapted to the species-specific host (Frey and Bush 1990). Furthermore, hybrids of apple maggot and blueberry maggot flies exhibit significantly weaker peripheral responses to host odor compounds as measured by electroantennograms, (Frey and Bush 1996). In addition, the two species have evolved divergent egg-laying responses to chemical stimuli on the fruits of their respective hosts (Bierbaum and Bush 1990). Specifically, apple maggot flies lay more eggs compared with blueberry maggot flies when stimulated by extract from apples, while blueberry maggot flies lay more eggs when stimulated by blueberry extract.

Sibling species within the genus *Rhagoletis* also exhibit differential behavioral responses based on host-fruit size and color. Fruit size may be a particularly important influence on *Rhagoletis* fly orientation and response, while females of several species prefer a limited range of sizes of fruit hosts in which to oviposit (Weisman 1937, Reissig et al. 1990, Messina and Jones 1990). In studies using inanimate fruit-mimicking objects, it has been found that blueberry maggot flies (natural host fruit size ranges from 5 to 15 mm in diameter) deposited eggs in equal numbers in 10 and 20 mm models, with fewer eggs laid in 40 and 70 mm models (Prokopy and Bush 1973). The same study showed a contrasting result in the response of apple maggot flies (natural host fruit sizes range from 15 to 70 mm in diameter), which deposited eggs in equal numbers in of 20 and 40 mm models, with fewer eggs laid in 10 mm or 70 mm models. In other studies involving the closely related western cherry fruit fly, *Rhagoletis indefferens* Curran, and *R. pomonella*, females flies were found to preferentially bore into fruit models of similar or slightly larger size to that of native host fruit and they had a lesser inclination to oviposit into models smaller than or much larger than native host fruit (Prokopy and Boller 1971, Papaj and Prokopy 1986, Messina 1989). Given these observations, it was concluded that in *Rhagoletis* species whose native host fruits are small (5 to 15 mm in diameter) and which have not expanded their host range to host fruit of larger size (15 to 70 mm in diameter), oviposition is limited to a restricted range of host fruit sizes within close range of native host fruit diameter. On the other hand, in *Rhagoletis* species that have expanded their host range to fruit of a larger size, such as apples, the range of host fruit size acceptable for oviposition is more expansive and characterized by less selectivity.

Evidence for genetic divergence among *R. pomonella* and *R. mendax* has also been compiled. Feder et al. (1989) found species-specific alleles for 11 different allozymes. Further experiments by Bierbaum and Bush (1989) corroborate the genetic evidence for speciation, and provide support for the theory that sympatric speciation occurred as a result of a divergence in host preference and acceptance behaviors. To test the hypothesis that these two species have evolved viability differences on different hosts, Bierbaum and Bush (1989) measured the larval-to-adult viability of *R. mendax, R. pomonella*, and F_1 interspecific hybrid progeny reared on naturally growing highbush blueberries and apple (*Malus pumila* Miller). Fewer interspecific hybrids survived to adulthood than either *R. pomonella* reared in apples or *R. mendax* reared in blueberries. Thus, reduced hybrid viability compared with that of conspecific crosses provides a mechanism for restriction of gene flow among the two species.

Speciation between *R. pomonella* and *R. mendax.*

Allopatric speciation within various *Rhagoletis* sibling-species complexes may have occurred as a result of geographic separation among populations, resulting from changes in environmental conditions in particular localities (Bush 1966). Bush (1969) notes Mayr's (1963) proposal that populations may become adapted to a secondary host within an isolated locality when climatic or other changes lead to the extinction of the primary host. Such a host shift among temporally isolated peripheral populations could lead to rapid adaptation to the new host and explain the sympatric existence of sibling species within the genus *Rhagoletis*.

Bush (1969) proposed a mechanism for the evolution of *Rhagoletis* sibling-species complexes via sympatric speciation based on the correlation between mate and host selection within the genus, which involves courtship and mating on larval host-plants. Sympatric speciation may have occurred as follows: 1) Diapause and emergence times are under genetic control. 2). Orientation to and selection of hosts is in response to chemical cues. 3) Host selection has a genetic basis, ei. *AA* and *Aa* orient preferentially toward apples, while *aa* orient toward blueberries. 4) *A* mutates to *a* in a location where blueberries are available and a few homozygous individuals are eventually produced as a result of recombination. 5) Host plant and mate selections are positively correlated and thus individuals orient preferentially to their respective host plants (*AA*-apples or *aa*-blueberries), depending on genotype. Mating occurs on each sibling species' respective host plant. The result of this process would be the establishment of two ecologically separated sibling species.

Speciation within the genus *Rhagoletis* may have also taken place as a result of a temporal separation of host races, resulting in allochronic divergence. The emergence patterns of *Rhagoletis* species occur within a relatively short period and are tightly synchronized with the phenology of host plants (Lathrop and Nickels 1932). However, within naturally occurring populations of both *R. pomonella* and *R. mendax*, there are individuals that will emerge up to a month earlier or later than the peak emergence period (personal observation, Bush 1966). These early or late individuals are at a distinct selective disadvantage since host fruit are usually not readily available for oviposition during these time periods (Bush 1966). However, such genetic diversity within a population prevents extinction during years when peak emergence does not coincide with fruit maturation. In this case, the few early or late emerging flies could possibly propagate the population and save it from extinction.

Research objectives. As federal mandates on the use of organophosphates become more stringent, there is a growing need to develop effective and environmentally safe pest management tactics. The main objective of this study was to determine the effectiveness of biodegradable spheres treated with neonicotinoid insecticides for control apple maggot and blueberry maggot flies. The second research objective was to evaluate potential sphere deployment strategies within blueberry plantings and to determine their relationship to fruit injury. The final objective was to evaluate synthetic host-plant volatile compounds and determine appropriate load rates and blends to be used as attractants for effective monitoring and control of apple maggot and blueberry maggot flies. The overall goal of this project was to develop novel management tactics and improve existing monitoring and control techniques for apple maggot and blueberry maggot flies, thereby reducing fruit growers' reliance on organophosphate insecticides.

CHAPTER ONE

EVALUATION OF BIODEGRADABLE, INSECTICIDE-TREATED SPHERES FOR CONTROL OF *RHAGOLETIS* SIBLING SPECIES.

INTRODUCTION

In Michigan and the eastern regions of the United States, there is a zero tolerance policy on the part of the fruit industry to maggot infestation in fruit, including apples and blueberries. In order to avoid significant losses, growers must implement effective monitoring programs to detect the presence of fruit-parasitic *Rhagoletis* flies. Currently, most growers administer three to five applications of insecticides irrespective of whether or not *Rhagoletis* flies are present (Liburd et al. 1998 a). This type of management strategy increases the potential for environmental contamination, evolution of insecticide resistance, and poisoning of humans.

Yellow sticky boards and colored spheres (baited and unbaited) have been shown to be effective monitoring traps for *Rhagoletis* species (Johnson 1983, Liburd et al. 1998 a). However, after two weeks of deployment both sticky boards and spheres frequently become inundated with insects, which reduces their effectiveness. Replacing, coating, and hanging sticky boards and spheres is time consuming (Liburd et al. 2000) and labor intensive (Prokopy et al. 1990). Duan and Prokopy (1993) indicated that the reliance on sticky Tangle-Trap® for killing and monitoring *Rhagoletis* species in large-scale operations is a major impediment due to the previously mentioned labor intensity involved. The disadvantages associated with use of sticky boards and spheres necessitate seeking alternative management tactics for *Rhagoletis* species. One possible alternative is to develop a chemically and visually attractive biodegradable sphere that is coated with insecticide.

Although several researchers (Prokopy and Coli 1978, Prokopy and Hauschild 1979, Johnson 1983, Neilson et al. 1984) have reported on the use of sticky traps for the management of *Rhagoletis* species, only a few investigations have explored the potential of using combinations of traps, lures, and an insecticide (Duan and Prokopy 1993, Duan and Prokopy 1995 a, Duan and Prokopy 1995b, Hu et al. 1997). To date, mortality data on *Rhagoletis* species using insecticide-treated spheres have been based on visual observations and data collected from variation in fruit injuries. Currently, there are no field studies illustrating mortality of any *Rhagoletis* species using insecticide-treated spheres and tracking flies visually after they have visited a sphere has been a difficult operation (Duan and Prokopy 1995 b). Growers who may wish to adopt the use insecticide-treated spheres need information on mortality of *Rhagoletis* flies encountering these devices in their commercial orchards in order to evaluate the effectiveness of this tactic.

In laboratory studies using spheres treated with a combination of a pesticide, feeding stimulant, and a residue-extending agent, it was discovered that several insecticides (except dimethoate and malathion) reduced apple maggot fly visitation and feeding (Duan and Prokopy 1995 a). Spheres treated with combinations of an insecticide, corn syrup, and latex paint were effective in killing > 50 % of alighting flies. In a related laboratory study, Duan and Prokopy (1995 b) compared the effectiveness of insecticide-treated spheres with conventional sticky, baited spheres for control of apple maggot flies. They found that freshly baited insecticide-treated spheres were just as effective as freshly baited sticky spheres in killing released flies, as well as reducing oviposition. Similarly, under field conditions they sighted equal numbers of *R. pomonella* flies visiting apple trees containing insecticide-treated spheres and red sticky spheres.

The objective of this study was to document the mortality of *Rhagoletis* flies in the field using insecticide-treated spheres. The hypothesis was that insecticide-treated spheres baited with an appropriate lure and feeding stimulant would attract and kill *Rhagoletis* flies. Furthermore, we hypothesized that killed flies could be documented and counted on sticky coated plexiglas panes hung directly underneath the spheres.

MATERIALS AND METHODS

Research in 1998 was conducted at two sites near Fennville, Michigan: the Michigan State University Trevor Nichols Research Station and an organic grower's commercial blueberry farm. Additional experiments were conducted at Hood's Farm and Fox orchard located near Paw Paw, Michigan.

Biodegradable spheres (Apple and blueberry maggot). Biodegradable spheres (9-cm diameter) obtained from the United States Department of Agriculture (USDA) laboratory in Peoria, IL were used in the apple and blueberry experiments. These spheres were made from sugar (20%), water (15%), syrup (isosweet 100) (20%), and pregelatinized corn flour (45%) and produced mechanically by the process of extrusion. Spheres were hard and durable (resulting from extrusion process) which allowed them to withstand adverse weather conditions during the summer. Spheres were painted with red (Glidden Red Latex Gloss) and green (Shamrock green 197A111) enamel paint, respectively. The red spheres were used in both apple and blueberry experiments; green spheres (Liburd et. al 1998 a) were only used in blueberry experiments. Prior to field deployment, spheres treated with insecticide were brush-painted with a mixture containing 2% (AI) imidacloprid, 20% sucrose solution, 8% water, and 70% paint. Control spheres, lacking insecticide, were painted with the same mixture without imidacloprid. The decision to use of imidacloprid was based on the positive results from laboratory assays with *R. pomonella* (Hu and Prokopy 1998). All spheres were allowed to dry for 48 hours after brush painting prior to deployment in the field.

Apples. Two experiments were conducted to evaluate the effectiveness of 9-cm diameter, biodegradable, red spheres treated with insecticide for the control of apple maggot flies. Experiment 1 was located at the Trevor Nichols Research Station in Fennville and was designed to establish data on apple maggot mortality using insecticide-treated spheres. We also wanted to monitor the apple maggot population within a 2-5 m radius of the biodegradable spheres. Experiment 2 was established at Fox orchard to reevaluate the potential for controlling apple maggot flies in a different location.

The experimental designs were randomized complete blocks (blocked by apple variety) with 5 replications. Spheres were hung approximately 25 m apart and 30 m between one-acre blocks of Red Delicious in experiment 1, and Red and Golden Delicious in experiment 2. An apple maggot BioLure® dispenser (Consep. Inc. Bend, Oregon) containing 1.8 g (load rate) butyl hexanoate was affixed (using a staple gun) to the branch adjacent to each insecticide-treated sphere. A 60 x 45 cm plexiglas pane was placed approximately 30 cm below each sphere. Each plexiglas pane was lightly coated with insect Tangle-Trap® aerosol formula (Great Lakes IPM). In experiment 1, unbaited (9-cm diameter) red spheres (Great Lakes IPM) coated with 13 g of insect Tangle-Trap® (Great Lakes IPM) were placed within a 2 m radius of the biodegradable spheres to monitor the apple maggot population. Sphere positions were rotated every 5 days in experiment 1, and every 3 days in experiment 2.

<u>Blueberries.</u> Green and red spheres were used in blueberry experiments (Liburd et al. 1998 a and b) targeting the blueberry maggot fly. Experiment 3 was located in an area with a moderate blueberry maggot population (≈ 10 flies / trap over 2 weeks catch) based on trap data from previous years. Experiment 4 was located in a different area within the same plantation known to have a low population of *R. mendax* flies.

The experimental designs were randomized complete blocks arranged within Jersey and Earliblue cultivars and blocked by cultivar with 4 replications. Insecticide-treated and control spheres were used in both experiments. Spheres were hung within blueberry bushes approximately 15 m apart (20 m between blocks) in plantings of Jerseys, Rubel and Bluecrop. A 60 x 45 cm plexiglas pane was placed approximately 30 cm below each sphere. Each plexiglas pane was lightly coated with insect Tangle-Trap® (aerosol formula). A scintillation vial (National Diagnostics, Atlanta, GA) containing 1 g of ammonium acetate (Aldrich Chemical Co., Milwaukee, WI) dissolved in 5 ml of water was affixed (using a masking tape) to the branch adjacent to each insecticide-treated sphere. Each vial was plugged with cotton wool, which became damp whenever branch movement occurred. Sphere positions were rotated every 4 days.

Sampling. Both spheres and plexiglas panes were checked twice per week and the number of *R. pomonella* and *R. mendax* flies found on plexiglas panes in apples or blueberries were counted. Both *R. pomonella* and *R. mendax* flies captured were counted by sex on a weekly basis.

Statistical analysis. Data from biodegradable sphere experiments (except the red spheres used in the blueberry experiment) were analyzed by ANOVA followed by mean separation using the least significant difference (LSD) test (SAS Institute 1989). Data from red spheres in the blueberry experiment was square root transformed (x + 0.5) before subjecting to ANOVA. The means were separated by the LSD test.

RESULTS

Apple maggot (biodegradable spheres). Experiment 1 (Fennville The mean number of *R. pomonella* caught on plexiglas panes placed below insecticide-treated spheres (20.0 ± 3.0) was significantly greater ($F = 52.4$; df = 1,4; $P < 0.01$) than the number of flies that were caught on plexiglas panes placed under biodegradable spheres without insecticide (0.8 ± 0.6). Flies spent significantly ($F = 12.7$; df = 1,4; $P < 0.02$) more time alighting on spheres treated with imidacloprid (8.8 ± 1.9 min) than they did on untreated spheres (2.0 ± 0.6 min).

Experiment 2 (Fox Orchard). We observed more *R. pomonella* flies on sticky plexiglas panes under insecticide-treated spheres than on panes placed under spheres without insecticide. The mean number of *R. pomonella* caught on plexiglas panes under insecticide-treated spheres (17.4 ± 2.6) was significantly greater ($F = 34.2$; df = 1,4; $P < 0.01$) than the number of flies caught on panes placed under untreated spheres (1.0 ± 0.5). There was no significant difference in the number of females and males (*R. pomonella* flies) caught on plexiglas panes under insecticide-treated or untreated spheres. We caught an average of 10.8 ± 1.6 female and 6.8 ± 2.2 male *R. pomonella* flies on plexiglas panes placed below insecticide-treated spheres. However, we caught only an average of 0.8 ± 0.2 and 0.4 ± 2.2 females and males respectively, beneath untreated spheres.

The mean number of flies caught on unbaited sticky spheres placed within a 2 m radius of insecticide-treated spheres (33.4 ± 5.0) was significantly less ($F = 7.2$; df = 1,4; $P < 0.05$) than the number of flies caught on sticky spheres placed at the same distance from untreated spheres (103.2 ± 26.7).

Blueberry maggot (biodegradable spheres) Experiment 3 (Fennville). In a naturally infested area with a moderate population, the mean number of flies captured (10.8 ± 2.9) on sticky plexiglas panes placed under red insecticide-treated spheres was significantly ($F = 10.4$; df = 1,3; $P < 0.05$) more than the number of flies caught on panes placed under untreated spheres (3.0 ± 0.7). There were no significant differences in the ratio of male and female flies caught on plexiglas panes below insecticide-treated spheres. The mean numbers of female and male flies caught were 7.0 ± 1.6 and 5.8 ± 0.9 respectively, for plexiglas panes below insecticide-treated spheres. Plexiglas panes below untreated spheres captured an average of 1.5 ± 0.6 and 1.8 ± 0.3 female and male flies, respectively.

Experiment 4 (Fennville). In another area where the population of *R. mendax* flies was much lower, highly significant ($P < 0.001$) differences in mortality data occurred between sticky plexiglas panes placed under green, insecticide-treated spheres and sticky plexiglas panes positioned under untreated spheres. Sticky plexiglas panes placed under green, insecticide-treated spheres captured an average of 4.0 ± 0.4 flies versus 0.8 ± 0.5 for non insecticide-treated spheres. The amount of time flies spent alighting on spheres treated with insecticide (7.4 ± 1.9 min) was significantly ($F = 7.0$; df = 1,3; $P < 0.02$) less than the amount of time flies spent on untreated spheres (1.9 ± 0.6 min). More than 60 % of the flies caught on plexiglas panes were positioned directly under insecticide-treated spheres. The few flies caught on plexiglas panes under untreated spheres were randomly distributed throughout the panes.

DISCUSSION

Our findings showed that Plexiglas panes lightly coated with a sticky trapping material and hung 30 cm below baited, insecticide-treated spheres can be used to monitor populations and record the mortality of apple maggot and blueberry maggot flies in commercial orchards and plantings. These studies have also indicated that insecticide-treated spheres can be used effectively to suppress populations of *Rhagoletis* flies. Duan and Prokopy (1995 b) indicated that a nearly acceptable level of control (based on fruit injury) was achieved using insecticide-treated spheres in commercial orchards that contained the Liberty apple variety. In their study, repeated applications of sucrose were necessary after each rainfall to maintain sphere effectiveness, and spheres were retreated with an insecticide as the season progressed. Our spheres appeared to be more effective after each rainfall or heavy dew. This difference may have been due to the composition of our spheres. Biodegradable spheres used in this study were partially composed of sucrose, which progressively exuded from the spheres over the coarse of the season and continuously provided a fly feeding stimulus.

Insecticide-treated spheres killed 25 and 17 times as many flies respectively, as untreated spheres in our apple maggot studies (experiments 1 and 2). The observed mortality rate could have been significantly higher because in many situations flies were observed feeding on insecticide-treated spheres and then flying away. However, it was impossible to tell whether these flies were killed later. It is possible that flies feeding for an average of 8.8 ± 1.9 min ingest a lethal amount of insecticide. Moderate consumption of imidacloprid causes *R. pomonella* flies to regurgitate and cease feeding (Hu and Prokopy 1998). The small number of flies killed in the control was probably the result of accidental injuries and captures or the result of a lethal ingestion of the paint. Preliminary laboratory tests on enamel paints indicated that they are toxic to *R. pomonella* flies.

An equal ratio of captured male and female flies was observed. Both sexes may have been attracted to the apple maggot BioLure®, which releases butyl hexanoate at a constant rate (16 mg /d) (Edie Christensen, Consep, personal communication). Butyl hexanoate is a fruit odor that is attractive to *R. pomonella* flies seeking fruit resources for mating and ovipositing (Carle et al. 1987), which may have accounted for the observed nonsignificant differences in capture between males and females.

The results from our blueberry experiments were similar to those involving apples. A fairly equal number of both sexes were attracted to ammonia baits. Immature *R. mendax* females are attracted to ammonia (Liburd et al. 1998 a), which provides a protein source for reproductive development (Hendrichs et al. 1990). As flies mature, there is a change in the physiological state from feeding to ovipositing (Hendrichs et al. 1990). This results in a shift of female activities from leaves onto fruits (Smith and Prokopy 1981, 1982). Males usually respond by moving onto the fruit for mating purposes. Therefore, approximately equal numbers of both sexes are usually found on the fruit later in the season (Liburd 1997). This may account for the lack of a significant difference in capture between *R. mendax* males and females during our experiments.

Imidacloprid is a new systemic neonicotinoid insecticide with relatively low mammalian toxicity (Fleischer et al. 1998), that has been shown effective in controlling *R. pomonella* flies (Hu and Prokopy 1998). Flies appeared to be disoriented after feeding on insecticide-treated spheres for a short period of time. This was probably due to the imidacloprid since it has rapid lethal and sublethal effects when ingested orally (Hu and

Prokopy 1998). This may also account for the significantly longer feeding duration on insecticide-treated spheres compared to untreated spheres. The higher percentage (60% of total capture) of flies found on plexiglas panes directly under insecticide-treated spheres was probably due to the rapid effects of imidacloprid, which prevented the flies that had consumed it from flying away. The application rate for imidacloprid used in our experiment was higher than field application rates. However, since fruit was not sprayed, the potential risk due to insecticide residues was greatly reduced. In addition, due to its target specificity, the use of insecticide-treated spheres could also protect beneficial insects and prevent the potential for resistance development (Prokopy et al. 1990, Duan and Prokopy 1995 b).

Sticky spheres placed within a 2 m radius of insecticide-treated spheres captured significantly fewer *R. pomonella* flies than sticky spheres placed at the same distance from untreated spheres. This indicates that there is some level of suppression within the area where insecticide-treated spheres were deployed. However, population suppression could not be confirmed since the apple maggot BioLure® contained butyl hexanoate, which is known to draw flies from neighboring sites. Also, *R. pomonella* can move from infested areas to noninfested areas when the orchard perimeter is not protected to intercept immigrating flies (Prokopy et al. 1990).

This was the first study to document that biodegradable spheres treated with imidacloprid are effective in attracting and killing both *R. pomonella* and *R. mendax* in commercial orchards and plantings. Growers who employ the use of insecticide-treated spheres can monitor fly populations with sticky plexiglas panes placed directly beneath the spheres. This study documented that mortality data from these panes can also be used to evaluate the effectiveness of the insecticide on the treated spheres. Insecticide-treated spheres in combination with lures have potential for use in commercial orchards for the control of *Rhagoletis* sibling species. Their use could reduce the need for insecticide sprays that kill natural enemies, contaminate the environment, and pose a danger to humans.

CHAPTER TWO

COMPARISON OF NEONICOTINOID INSECTICIDES FOR USE WITH BIODEGRADABLE SPHERES FOR CONTROL OF KEY *RHAGOLETIS* SPECIES

INTRODUCTION

Recent studies have shown that non-sticky, imidacloprid-treated, biodegradable spheres are effective in killing apple maggot (Hu et al. 1998, Liburd et al. 1999) and blueberry maggot flies (Liburd et al. 1999). *Rhagoletis* flies foraging for suitable host plants are attracted to baited, insecticide-treated spheres for mating and oviposition (Liburd et al. 1999). Flies landing on these spheres die after consuming a lethal dose of insecticide. As discussed in Liburd et al. (1999), the use of biodegradable spheres offers several advantages over conventional trapping using sticky traps or insecticide spray applications, including the potential for season-long control of fruit flies by a single deployment of spheres at the onset of the season, a possible reduction of insecticide residues on the fruit, and a reduction in labor costs.

In field studies, Liburd et al. (1999) caught 25 times as many apple maggot and 4 times as many blueberry maggot flies on Plexiglas panes placed beneath imidacloprid-treated spheres compared with untreated (control) spheres. Further studies showed that the mean time that flies spent on imidacloprid-treated spheres was significantly longer than on untreated spheres. Most recently, Hu et al. (2000) achieved season long residual activity with 80 % fly kill in laboratory assays after weathering spheres treated with imidacloprid (Merit® 75 WP) at 1.5 % (AI) in an orchard for 3 months. However, there is no published research documenting the effectiveness of the insecticides thiamethoxam (Actara®) or thiocloprid (Calypso®) on apple maggot flies and only one recent paper (Ayyappath et al. 2000) has discussed the effects of thiomethoxam but not thiocloprid on blueberry maggot flies using insecticide-treated sphere technology.

As Food Quality Protection Act (FQPA) regulations lead to reduction in the use of organophosphate insecticides and public pressure against the use of broad-spectrum insecticides increases, it becomes necessary to identify effective non-organophosphate insecticides for inclusion into novel pest management tactics. The objective of this study was to investigate the duration of activity and performance of biodegradable spheres treated with the neonicotinoid insecticides imidacloprid (Provado® 1.6 F and Merit® 75 WP), thiamethoxam (Actara®), and thiocloprid (Calypso®) on apple maggot and blueberry maggot flies. A second objective was to evaluate whether the commonly used synergist, piperonyl butoxide (PBO), would increase the toxicity of biodegradable spheres treated with either imidacloprid (Provado®) or thiamethoxam, (Actara®) to alighting blueberry maggot flies.

MATERIALS AND METHODS

Field experiments to determine the effectiveness of biodegradable, neonicotinoid-treated spheres in killing apple maggot and blueberry maggot flies were conducted in commercial apple orchards and blueberry plantings in southwestern Michigan during the 1999 and 2000 field seasons. Biodegradable spheres (9-cm diameter) made from a combination of sugar, starch, and flour were obtained from the United States Department of Agriculture (USDA) laboratory in Peoria, IL. Specifications for sphere preparation were described in Liburd et al. (1999).

<u>Field evaluation of neonicotinoid-treated spheres.</u> The experimental designs were randomized complete blocks (blocked by apple varieties and blueberry cultivars) with 4 replications. In apples, spheres were spaced 20 m apart within blocks (25 m between blocks) and were hung 1.5 m above ground within the canopy of Red and Golden Delicious trees. Spheres were positioned 0.25-0.5 m from fruit and foliage according to the guidelines suggested by Drummond et al. (1984). All biodegradable spheres used in apple maggot experiments were baited with polyethylene vials (Isreal Andler and Sons, Evrett, MA) containing 4 ml of butyl hexanoate (Penta international Corporation, West Caldwell, NJ).

In blueberries, biodegradable spheres were hung within the canopy of 'Bluecrop' and 'Jersey' blueberries at a height 15 cm below the tops of blueberry bushes according to the recommendations of Liburd et al. (2000). Biodegradable spheres used in blueberry maggot experiments were baited with polycon dispensers (Great Lakes IPM, Vestaburg, MI) containing 5 g of ammonium acetate (Liburd et al.1998a).

Apple Maggot (1999). Three treatments were evaluated that included (1) spheres treated with imidacloprid (Provado® 1.6 F) [Bayer, Kansas City, MO], (2) spheres treated with thiamethoxam (Actara®) [Novartis, Greensboro, NC], and (3) untreated control spheres. A sample of apple maggot flies killed was collected using Plexiglas panes (60 x 45 cm) coated with sticky, aerosol-formula Tangle-Trap® (Tanglefoot, Grand Rapids, MI) and placed horizontally ~ 30 cm below each sphere (Liburd et al.1999). Killed apple maggot flies were counted by sex and removed from panes twice per week. Data on the number of apple maggot flies killed were separated into four monitoring periods to reflect the seasonal abundance of apple maggot flies in Michigan (Howitt, 1993) and to provide even comparisons with laboratory assays conducted at the University of Massachusetts, Amherst, MA. During the first monitoring period (8 July-19 July), flies were beginning to emerge. The second (22 July-2 August) and third (5 August-16 August) monitoring periods constituted peak fly activity, depending on the predominant apple varieties within the area. During the final monitoring period (19 August-9 September), fly populations were in decline.

(2000). During our 2000 field season, the three treatments from our 1999 apple maggot study were selected for further investigation. These treatments were (1) imidacloprid (Provado®), (2) thiamethoxam (Actara®), and (3) untreated spheres. Two additional neonicotinoid treatments, thiocloprid (Calypso®) [Bayer, Kansas City, MO] and another formulation of imidacloprid (Merit® WP 75) were included in our 2000 evaluation of spheres. Spheres were prepared according to the previously described protocol and all insecticide concentrations were prepared at 2% AI . The Merit® WP 75 formulation was prepared as a slurry by mixing the wettable powder with distilled water prior to mixing this insecticide with paint. The number of apple maggot flies killed was assessed in the same manner as described for 1999. Field data collected in 2000 were not compared with laboratory assays. Therefore, data in 2000 were not divided into separate monitoring periods. The numbers of apple maggot flies killed over the course of the entire season were compared to determine the most effective insecticide treatment.

Blueberry Maggot (1999). Field experiments to compare the effectiveness of imidacloprid and thiamethoxam in blueberry plantings paralleled our apple maggot studies. The three treatments evaluated included (1) spheres treated with imidacloprid (Provado® 1.6 F), (2) spheres treated with thiamethoxam (Actara®), and (3) untreated control spheres. The number of blueberry maggot flies killed was assessed twice per wk using the Plexiglas pane monitoring system described in our apple maggot experiments. Blueberry maggot fly data were separated into two distinct monitoring periods to coincide with the seasonal abundance of blueberry maggot flies in Michigan (Liburd and Stelinski 1999).

(2000). Our blueberry maggot fly experiment in 2000 likewise paralleled 2000 apple maggot study. Five treatments were evaluated, including (1) imidacloprid (Provado®), (2) imidacloprid (Merit® WP 75) (3) thiamethoxam (Actara®), (4) thiocloprid (Calypso®), and (5) untreated control spheres. All insecticide treatments were prepared at 2 % AI. The number of blueberry maggot flies killed was assessed using Plexiglas panes as described for the 1999 apple experiment. Treatments were compared by using total numbers of blueberry maggot flies found killed over the course of the entire season.

Laboratory bioassays. In 1999, we conducted a laboratory bioassay to determine the effectiveness of biodegradable spheres treated with imidacloprid (Provado® 1.6 F) and thiamethoxam (Actara®) in killing blueberry maggot flies. The second objective of this assay was to evaluate whether the commonly used synergist, piperonyl butoxide (PBO), would increase the toxicity of biodegradable spheres treated with either imidacloprid (Provado®) or thiamethoxam, (Actara®) to alighting blueberry maggot flies.

Bioassays were conducted with 5 flies per replicate and each treatment was replicated 6 times. Blueberry maggot flies were reared from larvae that were collected from blueberries of unsprayed bushes in Fennville, MI. Flies were maintained in aluminum screen cages (30 x 30 x 30 cm) and supplied with water and food strips consisting of 5 x 7 cm sheets of filter paper dipped in a solution of enzymatic yeast hydrolysate and sucrose [1:3] and dried 24 h prior to use. All flies used in bioassays were sexually mature (14-20 d of age) and deprived of food, but not water, 10 h before testing (Hu et al. 2000). Different sets of spheres were used for each replicate. Prior to laboratory bioassays, biodegradable spheres were brush-painted with a mixture containing 2% (AI) of the neoticotinoid insecticide tested with or without PBO, 20% sucrose solution, 8% water, and 70% latex paint. Control spheres were painted with the same mixture, lacking both insecticide and PBO. The sphere treatments were as follows: (1) Imidacloprid alone (Provado® 1.6 F), (2) Thiamethoxam alone (Actara®), (3) Imidacloprid plus PBO in 1:1 ratio, (4) Thiamethoxam plus PBO in 1:1 ratio, (5) Imidacloprid plus PBO in 1:5 ratio, (6) Tiomethoxam plus PBO in 1:5 ratio, (7) Control spheres lacking insecticide and PBO. Spheres were hung from the inner, top surface of cubical wire mesh cages in a central position. Spheres were lightly moistened 24 h prior to the start of bioassays to simulate natural dew accumulation in the field. Blueberry maggot flies were released into cages and allowed to feed on all sphere treatments. Feeding duration was defined as time blueberry maggot fly spent alighting on sphere while exhibiting proboscis extension in a continuous 'lapping' manner. After time of exposure to sphere treatments elapsed (10 minutes), flies that had not yet died were removed from cages and placed individually into plastic cups with food and water. Observations to determine mortality were made during the 10-minute feeding bout, as well as 1 and 24 hours after feeding.

Statistical Analysis. Data from all experiments were square - root transformed (x + 0.5) and then subjected to an analysis of variance (ANOVA). Means were separated by least significant difference (LSD) (P = 0.05) (SAS Institute 1989). The untransformed means and standard errors are presented in tables and figures.

RESULTS

Field-based evaluation of neonicotinoid-treated spheres. During our 1999 apple maggot study, significantly ($F = 35.8$; df $= 2,6$; $P < 0.01$) more apple maggot flies were found killed on Plexiglas panes hung beneath biodegradable spheres treated with imidacloprid (Provado® 1.6 F) compared with spheres treated with thiamethoxam (Actara®) for data embracing the entire growing season (Table 1). Both imidacloprid and thiamethoxam-treated spheres killed significantly more apple maggot flies compared with control (untreated) spheres (Table 1). The number of apple maggot flies killed by imidacloprid-treated and thiamethoxam-treated spheres averaged ~18 and ~8 times more, respectively, than the number killed by untreated spheres (Table 1).

As the growing season progressed, we noticed changes in the effectiveness of imidacloprid and thiamethoxam-treated spheres based on Plexiglas trap data. During the first monitoring period (8 July- 19 July), we recorded significantly ($F = 26.4$; df $= 2,6$; $P < 0.01$) more apple maggot flies on Plexiglas panes hung beneath imidacloprid-treated spheres compared with thiamethoxam-treated spheres (Table 1). During the second and third monitoring periods, there were no significant differences in the number of killed apple maggot fly beneath spheres treated with either insecticide (imidacloprid or thiamethoxam) (Table 1). During the fourth monitoring period, again significantly ($P < 0.01$) more apple maggot flies were killed by imidacloprid-treated spheres compared with thiamethoxam-treated spheres (Table 1).

Table 1. Effect of neonicotinoid-treated spheres on apple maggot fly, Michigan (1999).

Sphere Treatments	1st Monitoring period 7/8-7/19	2nd Monitoring Period 7/22-8/2	3rd Monitoring Period 8/5-8/16	4th Monitoring Period 8/19-9/9	Total Season 7/8-9/9
	Mean ± SEM no. flies found killed on Plexiglas				
Provado®-treated	98.8 ± 21.1 a	63.3 ± 16.1 a	48.0 ± 9.0 a	36.8 ± 4.8 a	280 ± 52.8 a
Actara® -treated	31.0 ± 6.1 b	33.3 ± 3.0 a	42.5 ± 9.6a	12.5 ± 0.9 b	119.3 ± 10.6b
Untreated	4.0 ± 3.0 c	8.3 ± 7.0 b	2.0 ± 1.4 b	1.0 ± 0.0 c	15.3 ±11.3 c

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

The results of our second year's apple maggot study showed no significant differences in the number of killed apple maggot flies beneath spheres treated with either formulation of imidacloprid (Provado® 1.6 F or Merit® 75 WP) (Table 2). However, biodegradable spheres treated with either formulation of imidacloprid (Provado® 1.6 F and Merit® 75 WP) killed significantly ($F = 54.9$; df $= 4,12$; $P < 0.001$) more apple maggot flies than any other insecticide treatment tested (Table 2). There was no significant difference in the number of dead apple maggot flies beneath spheres treated with thiocloprid (Calypso®) and our untreated (control) spheres (Table 2).

Table 2. Comparison of neonicotinoid insecticides at 2 % AI using biodegradable sphere for control of apple maggot, Michigan (2000).

Sphere treatments	Mean ± SEM no. killed apple maggot flies 6/26 – 8/11
Imidacloprid-treated (Provado® 1.6 F)	182.8 ± 13.6 a
Imidacloprid-treated (Merit® 75 WP)	190.0 ± 35.7 a
Thiomethoxam-treated (Actara®)	76.3 ± 8.6 b
Thiocloprid-treated (Calypso®)	10.8 ± 2.8 c
Untreated (control)	9.8 ± 1.5 c

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

The results from our 1999 blueberry field experiments were different from those observed for apples. There were no significant differences between the number of killed blueberry maggot flies found on Plexiglas panes hung beneath spheres treated with either imidacloprid or thiamethoxam over the course of the season (Table 3). Also, there was no significant difference in the number of flies killed between imidacloprid and thiamethoxam-treated spheres in either first or second monitoring periods. However, we recorded significantly ($F = 32.4$; df $= 2,6$; $P < 0.01$) more killed blueberry maggot flies on Plexiglas panes placed beneath treated spheres compared to untreated spheres (Table 3).

Table 3. Effect of neonicotinoid-treated spheres on blueberry maggot fly, Michigan (1999).

Sphere treatments	1st Monitoring period 7/16-7/22	2nd Monitoring Period 7/28-8/6	Total Season 7/8-9/9
	Mean ± SEM no. flies found killed on Plexiglas		
Provado®-treated	99.8 ± 17.2 a	52.3 ± 9.9 a	133.5 ± 30.4 a
Actara® -treated	71.3 ± 34.0 a	27.5 ± 11.9 a	127.3 ± 43.5 a
Untreated	12.3 ± 3.8 b	3.5 ± 1.8 b	15.8 ± 5.0 b

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

Our second year's blueberry maggot results were similar to those observed in our apple maggot 2000 field studies. There were no significant differences in the number of blueberry maggot flies killed by either Provado® 1.6 F or Merit® 75 WP (Table 4). However, both imidacloprid treatments killed significantly ($F = 53.2$; df $= 4,12$; $P < 0.001$) more blueberry maggot flies compared with spheres treated with thiamethoxam (Actara®) (Table 4). On average, imidacloprid-treated spheres killed 2.3 times as many flies compared with spheres treated with thiamethoxam (Table 4). Finally, spheres treated with thiocloprid (Calypso®) did not kill significantly more blueberry maggot flies compared with control spheres (Table 4).

Table 4. Comparison of neonicotinoid insecticides at 2 % AI using biodegradable sphere for control of blueberry maggot, Michigan (2000).

Sphere treatments	Mean ± SEM no. killed blueberry maggot flies 6/26 – 8/11
Imidacloprid-treated (Provado® 1.6 F)	512.0 ± 78.5 a
Imidacloprid-treated (Merit® 75 WP)	449.3 ± 72.0 a
Thiomethoxam-treated (Actara®)	216.3 ± 47.7 b
Thiocloprid-treated (Calypso®)	121.5 ± 8.6 c
Untreated (control)	93.8 ± 19.9 c

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

Laboratory bioassays. After the 10 minutes of feeding, significantly ($F = 13.4$; df $= 11,41$; $P < 0.001$) more blueberry maggot flies were killed by spheres treated with imidacloprid (Provado®), thiamethoxam (Actara®), imidacloprid + PBO (1 : 5 ratio), and thiamethoxam + PBO (1 : 5 ratio) compared with spheres treated with imidacloprid + PBO (1 : 1 ratio) (Table 5). Twenty-four hours after the allowed 10-minute feeding bout, 100 % mortality was achieved among all sphere treatments (Table 5). There was no mortality observed in the flies that alighted and fed upon the untreated control spheres 24 hours after exposure to spheres (Table 5).

Table 5. Percent mortality of blueberry maggot flies that fed upon neonicotinoid-treated spheres with and without piperonyl butoxide (PBO).

Sphere treatments	Percent of cumulative mortality after 10 minutes of feeding	Percent of cumulative mortality 1 hour after exposure	Percent of cumulative mortality 24 hours after exposure
	Mean ± SEM no. dead flies		
Provado®	73.4 ± 4.3 a	100 ± 0.0 a	100 ± 0.0 a
Actara®	63.4 ± 6.2 a	90 ± 6.4 ab	100 ± 0.0 a
Provado® : PBO (1:1)	36.6 ± 8.0 c	76.6 ± 12.0 bc	100 ± 0.0 a
Actara® : PBO (1:1)	40.0 ± 10.4 bc	60 ± 7.4 c	100 ± 0.0 a
Provado® : PBO (1:5)	56.6 ± 8.0 ab	80 ± 12.6 abc	100 ± 0.0 a
Actara® : PBO (1:5)	63.4 ± 8.0 a	73.4 ± 4.3 bc	100 ± 0.0 a
Untreated (control)	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 b

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

DISCUSSION

The study demonstrated that baited, biodegradable spheres treated with the insecticide imidacloprid, irrespective of formulation (Provado® 1.6 F or Merit® 75 WP), were more effective in killing apple maggot flies than identical spheres treated with thiamethoxam or thiocloprid. Upon initial field deployment, imidacloprid-treated spheres killed high numbers of apple maggot flies, but there was a gradual decrease in the number of flies killed as the season progressed. Alternatively, thiamethoxam-treated spheres killed fewer apple maggot flies initially and the numbers of flies killed also gradually decreased as the season progressed. The decreased numbers of dead flies over time may be due to a number of factors, including loss of insecticide from sphere coating, aging effects on spheres, decreasing fly populations in late season, or a combination of these factors.

During our second (2000) field season, spheres treated with either formulation of imidacloprid showed equal effectiveness in killing apple maggot flies. Thiamethoxam-treated spheres again killed fewer apple maggot flies than imidacloprid-treated spheres. Laboratory bioassays conducted in 1999 at the University of Massachusetts, Amherst, MA supported our field data by indicating that field-aged imidacloprid-treated spheres were more effective in killing apple maggot flies compared with similarly weathered thiamethoxam-treated spheres (Stelinski et al. in press). Spheres treated with thiocloprid (Calypso®), however, were no more effective than control spheres in the field. We believe that treating spheres with imidacloprid rather than thiamethoxam or thiocloprid may result in more effective control of apple maggot flies in commercial orchards.

The results from our blueberry studies were slightly different from those observed in apples. In 1999, imidacloprid- and thiamethoxam-treated spheres showed statistically equal effectiveness in killing blueberry maggot flies throughout the blueberry-growing season. By contrast, in 2000 imidacloprid-treated spheres performed better than thiamethoxam-treated spheres over the course of the season. In 2000, we captured our first blueberry maggot fly on monitoring traps two weeks earlier than in 1999. Therefore, all biodegradable sphere treatments were deployed in the field two weeks earlier in 2000 (26 June) compared with than the July 16 deployment date in 1999. However, during both years blueberry maggot fly mortality was monitored until the end of berry harvest (6 August in 1999 and 11 August in 2000). The longer period field exposure of thiamethoxam-treated spheres in 2000 may have resulted in the greater observed decline in efficacy near the end of that field season, while such a decline was not observed for imidacloprid-treated spheres. These results imply that thiamethoxam may have been lost from biodegradable spheres at a greater rate than imidacloprid. As observed for the apple maggot fly, spheres treated with thiocloprid were no more effective in killing blueberry maggot flies than control spheres in the field.

Laboratory bioassays conducted at the University of Massachusetts in Amherst using biodegradable and wooden spheres provided us with data similar to those obtained in the field and helped explain some of the differences and changes in effectiveness that were observed over the course of the growing season in Michigan (Stelinski et al. in press). The assays also showed that the effectiveness and period of activity of imidacloprid- and thiamethoxam-treated spheres could be lengthened by increasing the percentage of AI of insecticide in the spheres above 2 %. Such a change in formulation needs further research before it can be implemented in commercial apple orchards.

However, increasing the concentration of AI above 2 % may not be necessary for blueberry maggot fly management.

The Massachusetts bioassays also indicated that fungal infestation acquired by both biodegradable and wooden spheres over the course of the growing season does not significantly decrease the spheres' effectiveness in killing apple maggot flies (Stelinski et al. in press). Such fungal growth was observed in the field during the 4th monitoring period in apple orchards in Michigan. The problem of fungal growth was less apparent in our blueberry field experiments. The shorter sphere deployment period necessary for control of blueberry maggot flies may account for the observed differences in fungal growth on the spheres.

In addition to fungal growth, rodent feeding has been observed to be a problem with using biodegradable spheres. Assays showed that biodegradable spheres that were previously fed upon by rodents had a decreased effectiveness (Stelinski et al. in press). Also, the complete loss of spheres in the field due to animal feeding is a significant problem. In Michigan, rodent feeding appeared to be insignificant in 1999 when ~ 95 % of spheres deployed in field studies were retrieved at the end of the season. In 2000, however, ~ 20 % of spheres deployed in an abandoned apple orchard required replacement one month after initial deployment because of rodent feeding.

Our findings with respect to feeding stimulants were consistent with the earlier studies of Hu et al. (1998). The use of a feeding stimulant (sucrose) encourages prolonged feeding and increases the potential for *Rhagoletis* flies to ingest a greater dosage of insecticide. Although aged wooden spheres performed equally well or better than aged biodegradable spheres in the Massachusetts laboratory studies, previous field studies have shown that the use of wooden spheres requires the periodical reapplication of feeding stimulant, because the externally applied sucrose is washed off due to rain (Stelinski et al. in press). Therefore, we still support further development and future use of biodegradable spheres or the development of a constant release sucrose dispenser to be used with wooden spheres as a control tactic for key *Rhagoletis* species.

The laboratory bioassays conducted in Michigan showed that imidacloprid- and thiamethoxam-treated spheres that were not exposed to outdoor environmental conditions were equally effective in killing blueberry maggot flies. The fact that thiamethoxam-treated sphere are less effective than imidacloprid-treated spheres in the field provides further evidence that thiamethoxam is either broken down or washed off of spheres at a higher rate than imidacloprid. The addition of PBO in both 1 : 1 and 1 : 5 ratios to imidacloprid and thiamethoxam applied to biodegradable spheres did not increase their effectiveness in killing blueberry maggot flies. However, it was also observed that spheres containing PBO appeared to deter alighting blueberry maggot flies. Flies exposed to this treatment required periodic recapture and replacement, while flies placed on spheres treated only with the insecticide (imidacloprid or thiamethoxam) remained on spheres for the duration of the allowed 10 minute feeding interval.

Overall, both field and laboratory experiments confirmed several crucial requirements that must be met in order for insecticide-treated spheres to be effective throughout the growing season. First, they must deliver a lethal dose of insecticide over the course of the entire growing season in order to provide adequate fruit protection. Second, they must be able to withstand adverse weather conditions and present a feeding deterrent for rodents. Future prototypes of biodegradable spheres should include appropriate anti-fungal agents and rodent-feeding deterrents.

Recent studies have shown that insecticide-treated spheres can achieve comparable control of the apple maggot fly (Prokopy et al. in press) and the blueberry maggot fly (Stelinski and Liburd in press) to the use of organophosphate insecticides. This study demonstrated that field-deployed imidacloprid-treated spheres are more effective in killing apple maggot and blueberry maggot flies than similar spheres treated with thiamethoxam or thiocloprid. The study also provided initial evidence that the use of the neonicotinoid insecticide thiocloprid at 2 % AI with biodegradable spheres may be an ineffective tactic. Thus, we provide further evidence that imidacloprid is currently the most effective neonicotinoid insecticide tested for use with biodegradable spheres against both apple maggot and blueberry maggot flies.

CHAPTER THREE

EVALUATION OF VARIOUS DEPLOYMENT STRATEGIES OF IMIDACLOPRID-TREATED SPHERES IN HIGHBUSH BLUEBERRIES FOR CONTROL OF *RHAGOLETIS MENDAX* CURRAN

INTRODUCTION

Given their status as insect pests of economic significance, species within the genus *Rhagoletis* have been the subject of a vast number of studies focusing on the development of integrated management strategies (Boller and Prokopy 1976, AliNiazee 1978, Prokopy et al. 1990, Liburd et al. 1999). Many of these studies have concentrated on the development and optimization of monitoring techniques (Kring 1970, Prokopy and Hauschild 1979, Drummond et al. 1984, Liburd et al. 2000) for early season detection of adult flies. Effective monitoring techniques for fruit parasitic tephritids are of great importance given the strict tolerance levels imposed on maggot infested fruit (Liburd et al. 2000). Additionally, sensitive monitoring of adult flies in commercial settings can potentially reduce unnecessary, prophylactic pesticide applications.

In addition to the optimization of monitoring techniques for key *Rhagoletis* species, a considerable amount of research has dealt with the development of behavioral control methods designed to complement existing management techniques, such as pesticide spray applications (Prokopy and Mason 1996). Many of the behavioral control tactics involve the exploitation of fly response to visual and olfactory stimuli using fruit or foliage mimicking sticky traps baited with food or host-fruit mimicking synthetic attractants (Russ et al. 1973, Neilson et. al. 1981, Stanley et al. 1987, Duan and Prokopy 1992). In addition to their effectiveness as monitoring devices, Prokopy et al. (1990) showed that appropriately baited and visually attractive traps had the potential of reducing fruit injury when deployed within orchards to intercept immigrating adult apple maggot flies, *Rhagoletis pomonella* (Walsh). Furthermore, Reynolds et al. (1998) found that both perimeter and within-orchard deployment patterns of odor-baited red sticky spheres reduced oviposition by apple maggot flies compared with control blocks without such traps.

The use of odor-baited sticky traps (Neilson et al. 1984, Liburd et al. 1998b) and biodegradable attract-and-kill devices (Liburd et al. 1999, Ayyappath et al. 2000) have been evaluated against *Rhagoletis mendax* Curran and have potential for implementation as behavioral control tactics. Liburd et al. (1999) reported that biodegradable, fruit-mimicking spheres treated with the neonicotinoid insecticide imidacloprid effectively killed both blueberry maggot and apple maggot flies in field studies. In a later study, Ayyappath et al. (2000) demonstrated that biodegradable spheres treated with the neonicotinoid insecticide Actara® (thiomethoxam) were also effective in killing blueberry maggot flies in blueberry plantings. The results of this same study revealed that increasing the dosage of thiomethoxam used with biodegradable spheres prolonged their effectiveness when deployed in the field. Most recently, Prokopy et al. (in press) showed that both wooden and biodegradable insecticide-treated spheres were only slightly less effective than the use of organophosphate sprays or sticky red spheres in preventing fruit injury by *R. pomonella*.

The potential benefits of using insecticide-treated spheres instead of organophosphate applications or odor-baited sticky traps for control of *R. mendax* has been cited in recent studies (Liburd et al.1999, Ayyappath at al. 2000). However, there are no detailed studies demonstrating how insecticide-treated sphere deployment tactics within highbush blueberries (*Vaccinium corymbosum*, L.) affect the status of resident or immigrant populations of blueberry maggot flies. Also, no direct comparisons of conventional organophosphate sprays versus deployment of insecticide-treated spheres have been made with respect to controlling *R. mendax*. Consequently, there has been no documentation of preventing fruit injury caused by blueberry maggot fly oviposition with the use of insecticide-treated spheres.

The objective of this study was to evaluate three potential insecticide-treated sphere deployment patterns in highbush blueberry plantings to determine how they may impact fruit injury and infestation levels of *R. mendax*. Furthermore, the study aimed to compare insecticide-treated spheres, as a behavioral control tactic for blueberry maggot flies, with conventional spray applications of an organophosphate.

MATERIALS AND METHODS

Field experiments to determine the effectiveness of biodegradable spheres treated with the neonicotinoid insecticide Provado® (Imidacloprid) [Bayer, Kansas City, MO], for the control of blueberry maggot flies were conducted at an experimental blueberry farm in Douglas, Michigan in 1999 and 2000. Biodegradable spheres (9-cm diameter), made with the specifications outlined in Liburd et al. (1999), were obtained from the United States Department of Agriculture (USDA) laboratory in Peoria, IL. Spheres were brush-painted with two coats of a mixture containing DevFlex latex green paint (ICI Paints) (70 %), sucrose feeding stimulant (20 %), water (8 %) and Provado® (Imidacloprid) at 2 % AI (Liburd et al. 1999). Spheres were allowed to dry for 72 hours prior to field deployment. Biodegradable spheres were hung within the canopy of blueberry bushes within the cultivar Jersey at a height approximately 15 cm below the tops of the uppermost bush according to the recommendations of Liburd et al. (2000). Biodegradable spheres used in blueberry maggot experiments were baited with polycon dispensers (Great Lakes IPM, Vestaburg, MI). The dispensers were attached to the strings used for hanging spheres and contained 5 g of ammonium acetate (Liburd et al. 1998b). The experimental designs were completely randomized blocks with 4 replications.

1999. The experiment was designed to determine the most effective strategy for deploying (arrangement and interval distance) imidacloprid-treated spheres to control *R. mendax* and consequently reduce maggot injury and infestation. The experimental plots were 10 x 40 m rectangles, containing 3 rows of 12 highbush blueberries. Treatments were randomly assigned to the experimental plots contained within larger blueberry plantings. All treatment plots were spaced at least 20 m apart and treatments were assigned randomly with respect to field borders. Twelve biodegradable, imidacloprid-treated spheres were used in each treatment / replicate (Figure 1). The 4 treatments evaluated were: (1) perimeter deployment in which spheres were hung individually and spaced equally around the perimeter of experimental plots (Figure 1); (2) cluster deployment in which 4 groups of 3 spheres were hung in equally spaced perimeter locations of experimental plots (Figure 1); (3) uniform deployment in which spheres were placed 10 m apart (in a grid-like pattern) within experimental plots (Figure 1); (4) untreated experimental plots containing no spheres (Figure 1). All spheres were deployed on 30 June after the detection of the first adult blueberry maggot on Pherocon AM yellow sticky boards (Great Lakes IPM, Vestaburg, Michigan).

Fruit evaluation. At the end of the growing season (5 August), three hundred ripe blueberries (majority of berries turned blue around stem) were picked at random from each experimental plot and kept separate according to treatment. From each batch of 300 berries, 50 were randomly selected and examined under a magnifying lens for blueberry maggot fly oviposition scars. The numbers of berries with oviposition scars were used to calculate percent fruit injury. All 300 berries from each replicate were then placed over 0.5 cm mesh hardware cloth to allow larvae to exit the fruit and drop into containers filled with vermiculite (Liburd et al 1998 b). The vermiculite was sifted and blueberry maggot fly puparia were collected and counted in order to quantify fruit infestation.

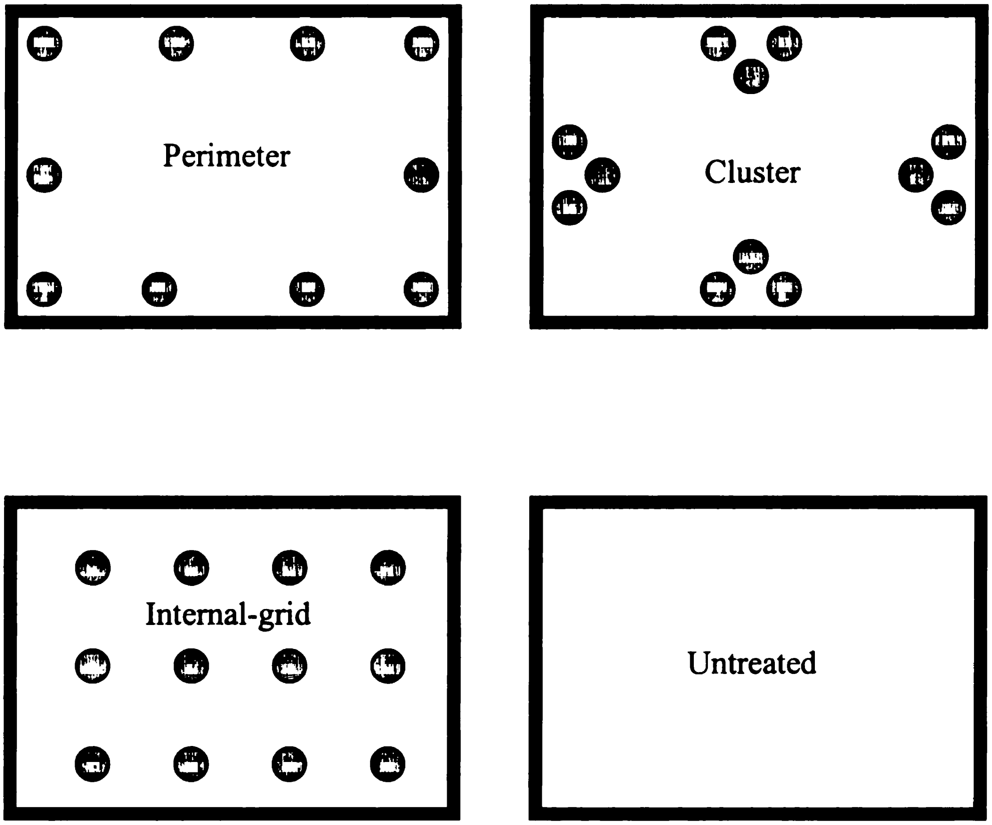

Figure 1. Imidacloprid-treated sphere deployment strategies within highbush blueberries.

- 9 cm diam, green imidacloprid-treated sphere

- 10 x 40 m Highbush blueberry plot

2000. During our second field season, we evaluated the same three imidacloprid-treated sphere deployment strategies as described for 1999 and compared deployment tactics with standard treatment of Guthion 50 W (azinphos-methyl) [Bayer, Kansas City, MO] sprays. Azinphos-methyl sprays were made on 15-June, 6- and 25-July with a tractor air-blast sprayer (Air-O-Fan, Reedley, CA). Plot sizes and experimental designs in 2000 were identical to those described for 1999; plots receiving azinphos-methyl sprays were spaced 50 m or further away from sphere-treated plots. The 5 treatments evaluated were: (1) perimeter sphere deployment; (2) cluster sphere deployment; (3) uniform sphere deployment; (4) Two applications of Guthion 50 W at a rate of 1.7 kg/hectare; (5) untreated experimental plots containing no spheres or azinphos-methyl sprays. All spheres were deployed on 20 June.

Fruit evaluation. Twelve hundred ripe blueberries were picked (August 8) at random from each experimental block and fruit was kept separate according to treatment. Similar to our procedure in 1999, we randomly selected 50 from each batch of 1200 and examined them under a magnifying lens for *R. mendax* oviposition scars. The numbers of berries with oviposition scars were again used to calculate percent fruit injury. All 1200 berries from each replicate were then placed over 0.5 cm mesh hardware cloth as described for 1999 and blueberry maggot fly puparia were later collected and counted in order to quantify fruit infestation.

Monitoring. During the second year of our study (2000), two unbaited Pherocon AM yellow sticky boards (Great Lakes IPM, Vestaburg, Michigan) were hung 20 m apart in the center of each treatment plot to monitor *R. mendax* activity within treatments.

Traps were positioned in the V shaped orientation (folded into a 45°- degree angle with apex downward and sticky surface outwards) (Prokopy and Coli 1978). Flies were counted and removed from traps two times per week and traps were replaced in the field every two weeks.

Statistical Analysis. Percentage data from fruit injury analysis were arcsine transformed and fly monitoring data were square - root transformed (x + 0.5) to stabilize variances and then subjected to an analysis of variance (ANOVA). Least significant difference (LSD) tests were used to show treatment mean differences (P = 0.05) (SAS Institute 1989). The untransformed means and standard errors are presented in tables.

RESULTS

(1999). Significantly ($F = 29.1$; df = 3,9; $P < 0.01$) more oviposition scars and puparia ($F = 71.1$; df = 3,9; $P < 0.01$) were recorded from blueberries that were collected from the untreated plots (not containing spheres) compared with berries from plots that contained imidacloprid-treated spheres, regardless of their deployment pattern (Table 6). The numbers of oviposition scars in untreated blocks were 3 times higher than sphere-treated blocks. Likewise, the numbers of puparia in untreated blocks were 11.6 times higher than in sphere-treated blocks. We recorded no significant differences in the numbers of *R. mendax* oviposition scars and puparia from blueberries collected from plots containing imidacloprid-treated spheres in the three deployment strategies tested. (Table 6). Finally, the percentages of fruit injury based on oviposition scars and puparia recorded were below 1 and 2 %, respectively, in all plots containing imidacloprid-treated spheres (Table 6).

Table 6. Percentage of fruit injury and infestation due to *R. mendax* oviposition, Michigan (1999).

	Mean ± SEM	
Control Strategy	Oviposition scars	Puparia
Perimeter deployment of spheres	0.3 ± 0.3 b	1.3 ± 0.6 b
Internal-grid deployment of spheres	0.3 ± 0.3 b	1.0 ± 1.0 b
Cluster deployment of spheres	0.0 ± 0.0 b	1.8 ± 0.5 b
Untreated plots (Control)	3.7 ± 0.2 a	21.0 ± 2.4 a

Means within columns followed by the same letter are not significantly different, (P = 0.05, LSD test).

(2000). The results of our second year's study were similar to those observed in 1999. Significantly ($F = 21.3$; df = 4,12; $P < 0.01$) more oviposition scars and puparia ($F = 10.1$; df = 4,12; $P < 0.01$) were found on berries picked from untreated control plots compared with plots containing imidacloprid-treated spheres or plots sprayed with Guthion (Table 7). Untreated (control) plots had more than 2.5 times as many oviposition scars and/or puparia compared with any of our plots treated with imidacloprid-treated spheres or Guthion sprays. We did not record any significant differences in the numbers of *R. mendax* oviposition scars or puparia from berries collected from plots containing imidacloprid-treated spheres in the three deployment strategies tested (Table 7). An important finding was that there were no significant differences in fruit injury, based on oviposition scar data, between plots containing imidacloprid-treated spheres and those sprayed with Guthion (Table 7). However, plots treated with Guthion had significantly ($F = 10.1$; df = 4,12; $P < 0.01$) fewer puparia compared with plots containing imidacloprid-treated spheres (Table 7).

In our monitoring program, significantly ($F = 9.3$; df = 4,12; $P < 0.01$) more *R. mendax* flies were captured on Pherocon AM boards placed within control plots compared with fly captures in plots containing imidacloprid-treated spheres in perimeter, uniform, and cluster orientations and plots sprayed with Guthion (Table 8). There were no significant differences in the numbers of blueberry maggot flies captured on unbaited Pherocon AM boards within plots containing imidacloprid-treated spheres, regardless of deployment pattern, and plots sprayed with Guthion (Table 8).

Table 7. Percentage of fruit injury and infestation due to *R. mendax* oviposition, Michigan (2000).

	Mean ± SEM	
Control Strategy	Oviposition scars	Puparia
Perimeter deployment of spheres	4.0 ± 1.8 b	1.3 ± 0.3 b
Internal-grid deployment of spheres	4.0 ± 0.8 b	0.8 ± 0.1 b
Cluster deployment of spheres	4.6 ±1.0 b	1.0 ± 0.2 b
Guthion (azin-phosmethyl) spray	2.0 ± 0.8 b	0.1 ± 0.1 c
Untreated plots (Control)	11.6 ± 1.0 a	3.9 ± 0.7 a

Means within columns followed by the same letter are not significantly different, (*P* = 0.05, LSD test).

Table 8. Mean number of *R. mendax* captured on unbaited Pherocon AM boards within treatment plots, Michigan, (2000).

	Mean ± SEM no. *R. mendax*
Control Strategy	25 June-8 August
Perimeter deployment of spheres	20.0 ± 4.4 b
Internal-grid deployment of spheres	19.3 ± 2.8 b
Cluster deployment of spheres	16.5 ± 2.1 b
Guthion (Azinphosmethyl) spray	14.5 ± 2.8 b
Untreated plots (Control)	47.0 ± 5.3 a

Means within each experiment followed by the same letter are not significantly different, (P = 0.05, LSD test).

DISCUSSION

Our results showed that deployment of imidacloprid-treated, biodegradable spheres decreased blueberry infestation levels to 1 % in 1999 and 0.8 % in 2000 (with internal-grid deployment patterns), while untreated (control) plots had maggot infestation levels > 20 % and > 3.2 % in 1999 and 2000, respectively. In both years, there were no differences in fruit injury levels obtained using the three different strategies for sphere deployment. Several factors may have contributed to the observed non-significant differences, including the fact that the blueberry planting used in our experiments had a residential population of *R. mendax* flies (emerging from within the planting), as well as immigrating populations invading from the surrounding areas and natural bogs. Perimeter trapping tactics could be effective in intercepting immigrants (Prokopy et al. 1990) into the planting, but the degree of fruit protection from fly oviposition is dependent on trap spacing, the pressure of immigrating flies, and field status with respect to the presence or absence of residential blueberry maggot fly populations. By contrast, the internal-grid pattern of sphere deployment may hold more potential in suppressing adult blueberry maggot flies and preventing fruit infestation in plantings harboring residential fly populations. Again, the effectiveness of the internal-grid pattern is dependent on the degree of sphere spacing and fly population densities within the planting. Also, factors such as fruit-load, degree of fruit maturity, and the physiological status of *R. mendax* (Liburd and Stelinski 1999), may influence the effectiveness of the internal-grid deployment pattern. Overall, our experiments have implied that specific sphere placement patterns (perimeter versus internal-grid) may be less important in blueberry plantings that are exposed to both residential and immigrating *R. mendax* populations.

The observed non-significant differences among the deployment strategies tested may have been also due to equal levels of visual and olfactory stimuli provided by the odor-baited, fruit-mimicking spheres in each treatment plot. Given the strong attraction of *R. mendax* to 9 cm diam green spheres baited with ammonium acetate (Liburd et al. 1998a, 2000), it is possible that flies foraging within each of our plots had approximately equal probabilities of encountering the bait-odor or visual stimulus provided by the imidacloprid-treated spheres, irrespective of sphere deployment patterns. This may have been due to our relatively small plot sizes and comparatively small differences between baited sphere spacing in the perimeter treatments versus the uniform or cluster treatments. Future studies comparing the effectiveness of imidacloprid-treated sphere deployment strategies should be conducted within plantings known to harbor resident populations of *R. mendax*, as well as in plantings that are only infested seasonally by immigrant blueberry maggot flies. By conducting a comparison of residentially infested plots versus those that receive immigrants only, it may be possible to gain further insight into the importance of sphere deployment patterns for effective fruit protection. Also, future studies must include larger scale treatment plots before this technology can be recommended to growers as a possible substitute to conventional pesticide applications.

During the second field season (2000), we found that field-deployed, imidacloprid-treated spheres were only slightly less effective than conventional applications of a sprayed organophosphate (Guthion 50 W at 1.7 kg/hectare) insecticide in providing fruit protection against *R. mendax* oviposition. We observed no difference in fruit injury (oviposition scars) and only a 0.9 % difference in fruit infestation between plots sprayed with Guthion and plots protected by imidacloprid-treated spheres. These results indicate that it is possible to achieve fruit protection against *R. mendax* oviposition equivalent to the level obtained with broad-spectrum, organophosphate sprays in highbush blueberries. However, the sphere density used in our experiments to compare the various deployment patterns was relatively high. At an estimated cost of a $1.00 per sphere, commercial use of this technology would necessitate a smaller number of deployed spheres in order for this technology to be commercially viable and comparable to insecticide treatments. Future research must also focus on optimizing sphere deployment densities and determining whether effective and economically viable densities can be achieved.

Our monitoring program confirmed that blueberry maggot fly activity in plots containing imidacloprid-treated spheres was suppressed to a level similar to that observed in plots that were treated with Guthion. Similar suppression of fly activity has been recorded with the apple maggot fly foraging in areas where imidacloprid-treated spheres were deployed (Liburd et al. 1999, Prokopy et al. in press). In a separate study, Stelinski et al. (in press) showed that imidacloprid-treated spheres at 2 % AI did not lose their effectiveness in killing *R. mendax* throughout the duration of the 9 wk period when sexually mature flies are ovipositing (Liburd and Stelinski 1999). We therefore believe that a single deployment of ammonium-baited, imidacloprid-treated spheres could potentially provide effective, season-long control of blueberry maggot flies in a commercial setting.

Despite their effectiveness, the current version of biodegradable spheres is susceptible to rodent feeding. We encountered this problem in 2000 and replaced ~ 10 % of our spheres two weeks after initial deployment. Future prototypes of insecticide-treated spheres must be more resistant to rodent feeding if this technology is to be effectively implemented as a control tactic for blueberry maggot flies.

To our knowledge, this is the first study that documents deployment of biodegradable, imidacloprid-treated spheres as a form of behavioral control reduces *R. mendax* infestation levels below 1 %. Although this is still above the currently mandated zero tolerance, we believe that even greater control can be achieved by making spheres more attractive with more effective and selective baiting systems, further optimizing sphere deployment and density strategies, and perhaps making fruit less attractive by coating it with visual or olfactory deterrents. Due to their target-specificity and reduced impact on the surrounding environment, imidacloprid-treated spheres have potential for integration into a second level IPM program (Prokopy 1990) by involving methods of cultural (Liburd et al. 1998b) and biological controls. Our study provides direct evidence for the potential of using biodegradable spheres treated with imidacloprid for control of blueberry maggot fly.

CHAPTER FOUR

ATTRACTION OF APPLE MAGGOT FLIES, *RHAGOLETIS POMONELLA* (WALSH) (DIPTERA: TEPHRITIDAE), TO SYNTHETIC FRUIT VOLATILE COMPOUNDS AND FOOD ATTRACTANTS IN MICHIGAN APPLE ORCHARDS.

INTRODUCTION

Michigan ranks second or third (depending on annual production) in crop value in the U.S. apple industry. Apples are grown commercially on more than 23,400 ha and more apples are produced by volume than all other Michigan fruits combined (Michigan Apple Committee, 2000). The apple maggot, *Rhagloletis pomonella* (Walsh), is an important fruit pest infesting commercially grown apples in Michigan and throughout the eastern United States. There is a zero tolerance for maggot infestation in apples bound for commercial sales. High numbers of apple maggot flies migrate into commercial orchards in Michigan, responding to visual and olfactory cues of host fruit, where mating and oviposition occurs (Liburd and Stelinski 1999). Sensitive and reliable monitoring techniques for the apple maggot fly are of extreme importance for making accurate and responsible control decisions.

Apple maggot flies are attracted to apple odors in the field (Prokopy et al.1973, Reisig 1974). Location of appropriate mating and oviposition sites by apple maggot flies is mediated in part by odors, which serve as primary cues for host site location and acceptance in fruit parasitic insects (Frey and Bush 1990). Among apple volatiles isolated from two apple varieties (Red Delicious and Red Astrachan), a blend consisting of a series of short chain carbon esters, including butyl hexanoate, is attractive to apple maggot flies (Fein et al. 1982). A comparison of visual stimuli showed that unbaited, 8 cm diam. red spheres captured significantly more apple maggot flies than unbaited, 10 cm diam. red spheres (Duan and Prokopy 1992). Furthermore, sticky spheres baited with vials of butyl hexanoate captured significantly more apple maggot flies than unbaited spheres, although there was no difference in catch with one, two, or four vials/sphere. A combination treatment containing one vial of ammonium carbonate and one vial of butyl hexanoate also significantly increased the capture of apple maggot flies compared to capture on unbaited spheres.

More recently, Reynolds and Prokopy (1997) evaluated synthetic odor lures (butyl hexanoate, ammonium carbonate, or butyl hexanoate plus ammonium carbonate on red sticky spheres) for their attractiveness to apple maggot flies. Butyl hexanoate plus ammonium carbonate was more attractive than either odor alone. Finally, a recent study has shown that a lure consisting of a mix blend of synthetic apple volatiles, including butyl butanoate, propyl hexanoate, butyl hexanoate, hexyl butanoate, and pentyl hexanoate combined in specific proportions, is significantly more attractive to adult apple maggot flies than lures containing butyl hexanoate alone.

In addition to synthetic fruit volatiles, other odor baits are used in tephritid monitoring programs. Attraction of the Queensland fruit fly, *Dacus tryoni* Froggatt, to several hydrolysed proteinaceous baits is mainly due to the ammonia they release (Bateman and Morton 1981). Ammonium acetate is also commonly used as an attractant in tephritid monitoring programs in Michigan (Liburd and Stelinski 1999). Ammonia is a by-product of the bacterial decay of several adult tephritid food sources (Prokopy and Roitberg 1984). Ammonia-producing baits may be attractive because flies are seeking a protein source important for egg maturation (Prokopy and Roitberg 1989, Prokopy 1993, and Prokopy et al. 1994).

The goal of this research was to establish a sensitive, reliable, and selective monitoring device for the apple maggot fly that can be implemented by apple growers in

Michigan and other apple producing regions. The objective of this study was to evaluate the attractiveness to of several synthetic fruit volatile lures, as well as ammonium-odor and protenacious baits, to the apple maggot fly using sticky-coated red sphere monitoring traps.

MATERIALS AND METHODS

Research on potential baits for apple maggot flies was conducted in apple orchards in Van Buren Co., Michigan during the 1998, 1999, and 2000 field seasons. Each treatment consisted of a 9 cm diam., red sphere coated with 13 g of Tangle Trap® (Great Lakes IPM Vestaburg, MI). Spheres were hung approximately 25 m apart and 30 m between one-acre blocks of Red Delicious and Golden Delicious. The experimental designs were randomized blocks (blocked by apple variety) with four replications. Traps were replaced every three weeks in the field and re-randomized weekly. Apple maggot flies caught in traps were counted and removed twice per week. They were counted by sex once per week. Visual estimates of beneficial and non-beneficial insects captured on spheres were made in the field in 1998 and 2000.

(1998). Experiment 1. The purpose of this experiment was to evaluate the importance of protein hydrolysate and ammonium baits for capture of apple maggot flies. Depending on the treatment, varying amounts of ammonium acetate, ammonium carbonate, and protein hydrolysate were mixed thoroughly into the 13 g of Tangle Trap® before it was applied to the spheres. Apple maggot BioLure® (contained in a plastic dispenser) was used in some treatments. The plastic dispenser with BioLure® contained 1.8 g (load rate) butyl hexanoate. The experiment evaluated nine treatments that included a red sphere coated with (1) 2 g of ammonium acetate (Aldrich Chemical Co., Milwaukee, WI) plus 0.5 g protein hydrolysate (Pfaltz & Bauer Chemicals Co., Waterbury, CT) and apple maggot BioLure®, (2) 2 g ammonium acetate plus 0.5 g protein hydrolysate, (3) 2 g ammonium acetate, (4) 2 g of ammonium carbonate plus 0.5 g protein hydrolysate and apple maggot BioLure®, (5) 2 g ammonium carbonate plus 0.5

g protein hydrolysate, (6) 2 g ammonium carbonate, (7) 2 g protein hydrolysate, (8) apple maggot BioLure®, (9) and untreated control.

(1998). Experiment 2. The second experiment examined the attractiveness of different types of volatile blends with and without ammonium acetate. In some treatments, polyethylene vials [5 ml] (Great Lakes IPM, Vestaburg, MI) containing 1.8 ml of butyl hexanoate or 1.8 ml of a mix volatile blend were used. The mix volatile blend contained butyl butanoate (10%), propyl hexanoate (4%), butyl hexanoate (37%), hexyl butanoate (44%), and pentyl hexanoate (5%). Six treatments were evaluated that included a red sphere baited with (1) apple maggot BioLure®, (2) apple maggot BioLure® plus 2 g of ammonium acetate, (3) mix blend of apple volatiles, (4) a mix blend of apple volatiles plus ammonium acetate, (5) butyl hexanoate, (6) butyl hexanoate plus 2 g of ammonium acetate

(1999). In 1999, we chose 4 of the treatments evaluated in 1998 and compared them with unbaited (control) spheres. The location of our study, experimental design, and sampling regime were the same as described for 1999. The five treatments evaluated were red spheres baited with (1) apple maggot BioLure®; (2) polyethylene vial containing 1.8 ml butyl hexanoate; (3) polyethylene vial containing 1.8 ml of the mix volatile blend; (4) 2 g of ammonium acetate impregnated into the Tangle-Trap® coating the spheres, (5) untreated spheres (control) covered in Tangle-Trap® alone.

(2000). During the 2000 field season, we carried out an exact replicate of our 1999 study, using the same treatments, experimental design, location, and sampling regime.

Statistical analysis. In order to stabilize the variances, the data from both experiments were square root transformed ($x + 0.5$) before subjecting it to ANOVA. This was followed by mean separation using the least significant difference (LSD) test (SAS Institute 1989).

RESULTS

(1998). Experiment 1. Spheres baited with protein hydrolysate captured significantly ($F = 4.1$; df = 8,24; $P < 0.05$) fewer *R. pomonella* flies in comparison to spheres baited with other lures (Table 9). With the exception of protein hydrolysate, there were no significant differences among the other baits and lures evaluated in this experiment. We noticed that spheres baited with BioLure® (n = 15) only were highly selective and captured an average of 70 ± 8.6 % of apple maggot flies in relation to other beneficial and non-beneficial insects. Alternatively, spheres baited with ammonium carbonate and ammonium acetate were non-selective capturing on average 38 ± 3.6 % apple maggot flies, as well as many beneficial and non-beneficial insects.

Table 9. Attraction of apple maggot flies to synthetic fruit volatiles with and without ammonium-odor and protenaceous baits, Michigan, (1998).

Treatment	Mean ± SEM flies per trap 3 July—19 August
Ammonium acetate + protein hydrolysate + Biolure® dispenser	175.3 ± 39.6 a
Ammonium acetate + protein hydrolysate	143.8 ± 29.3 a
Ammonium acetate	215.5 ± 54.2 a
Ammonium carbonate + protein hydrolysate + Biolure® dispenser	174.3 ± 43.8 a
Ammonium carbonate + protein hydrolysate	156.3 ± 23.3 a
Ammonium carbonate	171.8 ± 35.9 a
Protein hydrolysate	34.3 ± 9.6 b
Biolure® dispenser	200.5 ± 53.5 a
Unbaited (control)	133.0 ± 26.1 a

Means followed by the same letter are not significantly different ($P = 0.05$, LSD test).

(1998) Experiment 2. Significantly ($F = 4.8$; df = 5,15; $P < 0.01$) more *R. pomonella* flies were captured on spheres baited with the mix volatile blend compared with all other treatments tested (Table 10). Total counts throughout the season indicated that there were no significant differences between spheres baited with a combination of butyl hexanoate and ammonium acetate, or spheres baited with either compound alone (Table 10). However, with the exception of spheres baited with butyl hexanoate (in polyethylene vials), ammonium baits captured significantly more females than males early in the season (Figure 2).

Table 10. Attraction of apple maggot flies to synthetic fruit volatiles and baits, Michigan, (1998).

Treatment	Mean ± SEM flies per trap 25 June—9 August
Volatile mix blend polyethylene vial	175.0 ± 35.5 a
Volatile mix blend polyethylene vial + ammonium acetate	60.5 ± 25.4 b
Biolure® dispenser	48.0 ± 2.9 b
Biolure® dispenser + ammonium acetate	55.8 ± 16.8 b
Butyl hexanoate polyethylene vial	75.3 ± 10.7 b
Butyl hexanoate polyethylene vial + ammonium acetate	71.3 ± 22.1 b

Means followed by the same letter are not significantly different ($P = 0.05$, LSD test).

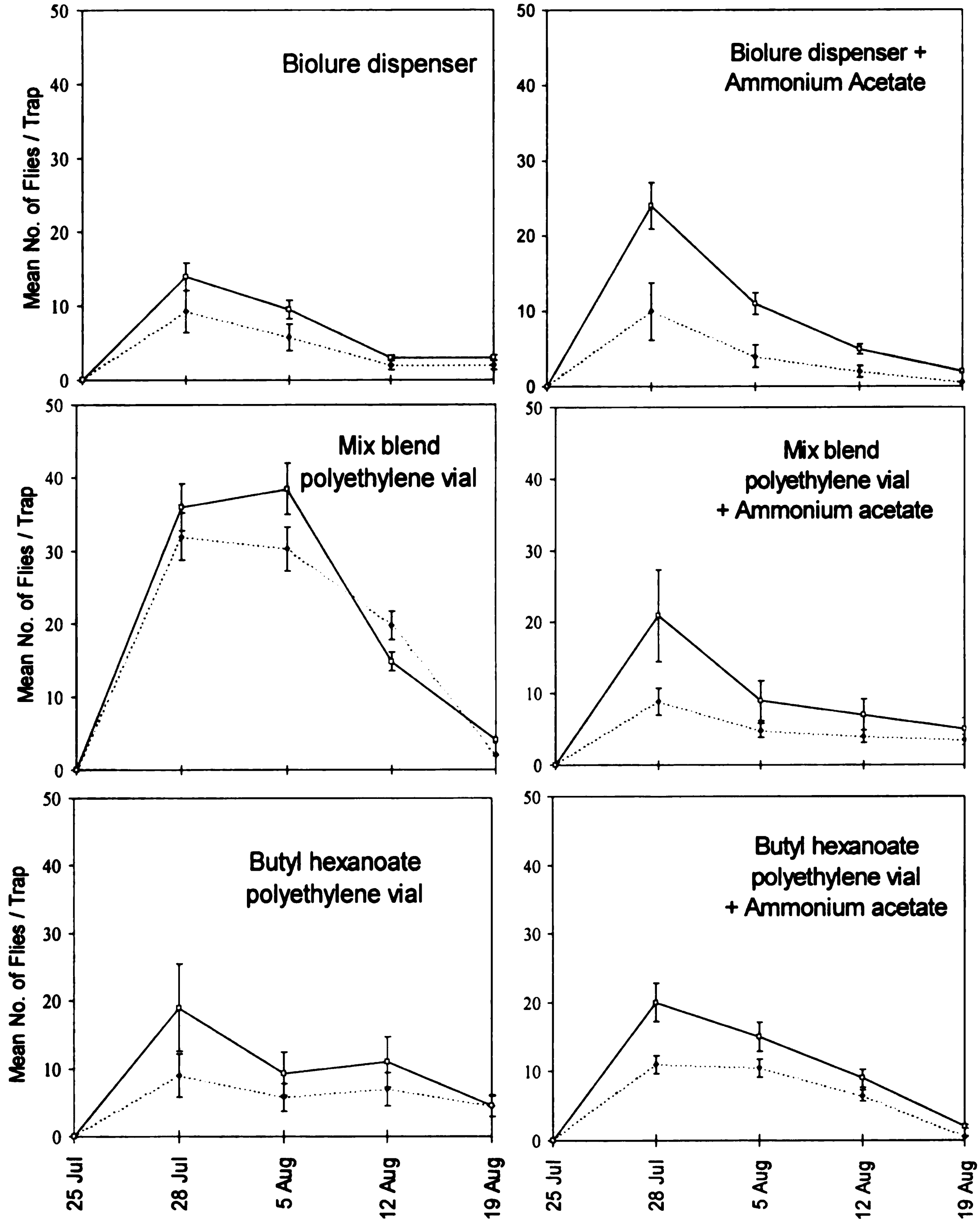

Figure 2. Attraction of adult *R. pomonella* to baited red spheres, 1998, [⋯⋯Male; —— Female]

(1999). As observed in 1998, significantly ($F = 3.2$; df = 7,12; $P < 0.05$) more *R. pomonella* flies were captured on spheres baited with the mix volatile blend compared with all other treatments evaluated (Table 11). In fact, spheres baited with the mix blend captured approximately twice as many apple maggot flies as spheres baited with the standard ammonium acetate bait and approximately three times as many apple maggot flies as unbaited (control) spheres (Table 11). There were no differences observed among the other treatments evaluated.

Table 11. Attraction of apple maggot flies to synthetic fruit volatiles and baits, Michigan, (1999).

Treatment	Mean ± SEM flies per trap
Volatile mix blend polyethylene dispenser	326.5 ± 67.2 a
Biolure® dispenser (Butyl hexanoate)	111.5 ± 13.8 b
Ammonium Acetate	164.3 ± 51.7 b
Butyl hexanoate polyethylene vial	150.3 ± 23.7 b
Unbaited (control)	105.5 ± 9.0 b

Means followed by the same letter are not significantly different ($P = 0.05$, LSD test).

(2000). During our third field trial, we once again observed that spheres baited with the mix blend captured significantly ($F = 13.5$; df = 7,12; $P < 0.01$) more apple maggot flies compared with spheres baited with any of the other lures evaluated (Table 12). Furthermore, in 2000, we recorded significantly ($F = 13.5$; df = 7,12; $P < 0.01$) more *R. pomonella* flies captured on spheres baited with butyl hexanoate placed in polyethylene vials compared with spheres baited with the Biolure® dispenser and unbaited (control) spheres (Table 12). We observed high selectivity to apple maggot capture on spheres baited with the mix blend (polyethylene vial), butyl hexanoate (polyethylene vial), and the Biolure® dispenser, which captured 74.7 ± 5.3 %, 84.2 ± 7.5 %, and 79.5 ± 7.1 %, respectively, of apple maggot flies in relation to other beneficial and non-beneficial insects. We observed a moderate degree of selectivity for the unbaited (control) spheres, which captured 60.3 ± 12.9 % of apple maggot flies relative to non-target captures. The lowest degree of selectivity was observed on spheres baited with ammonium acetate, which captured 19.8 ± 2.0 % apple maggot flies. Throughout the season, spheres baited with ammonium acetate captured significantly ($F = 17.5$; df = 3,4; $P < 0.05$) more *R. pomonella* females than males, while such a difference was not observed with the other treatments evaluated (Figure 3).

Table 12. Attraction of apple maggot flies to synthetic fruit volatiles and baits, Michigan, (2000).

Treatment	Mean ± SEM flies per trap
Volatile mix blend polyethylene dispenser	983.8 ± 203.5 a
Biolure® dispenser (Butyl hexanoate)	421.0 ± 32.1 cd
Ammonium Acetate	580.0 ± 60.8 bc
Butyl hexanoate polyethylene vial	610.5 ± 143.4 b
Unbaited (control)	336.3 ± 49.1 d

Means followed by the same letter are not significantly different ($P = 0.05$, LSD test).

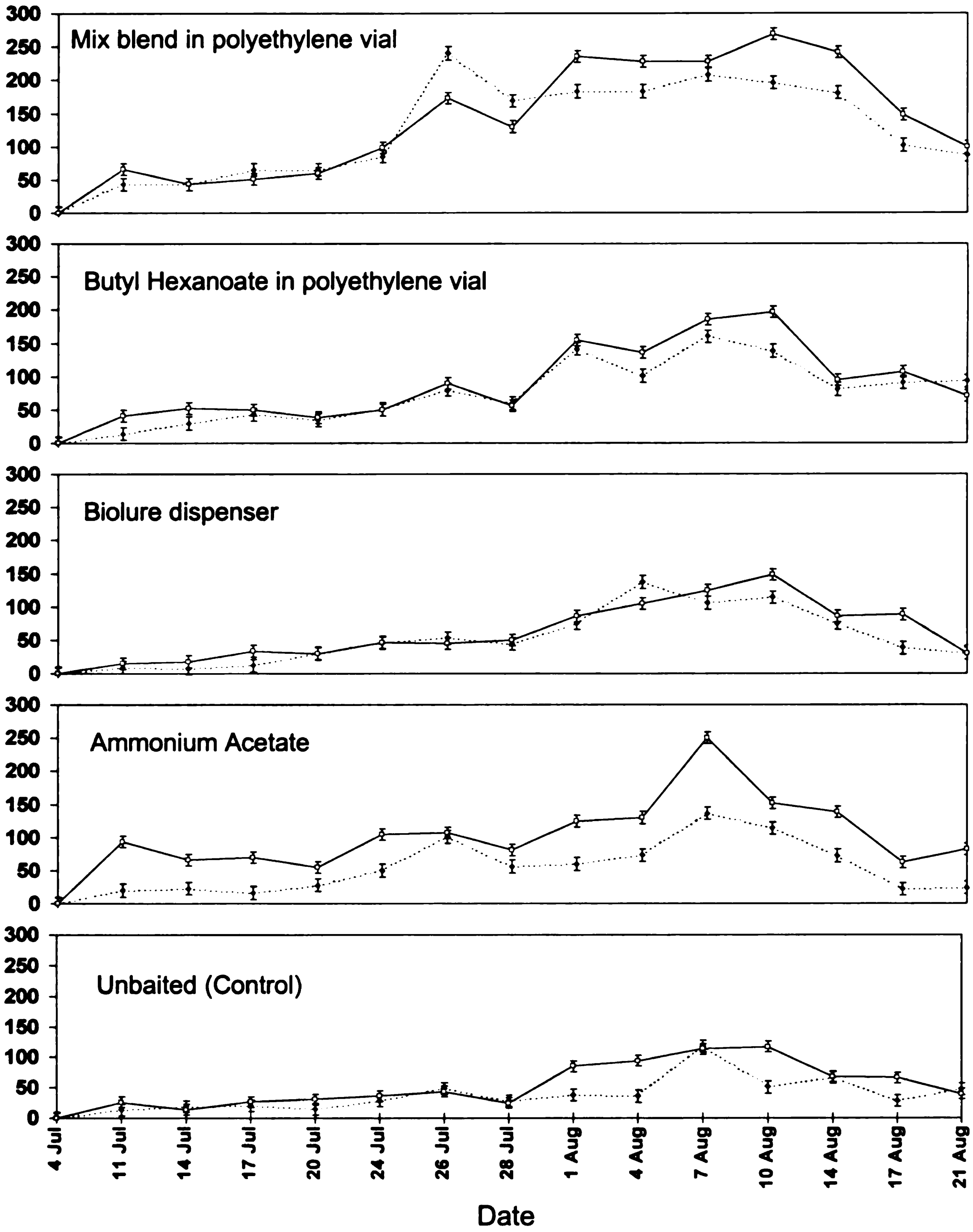

Figure 3. Attraction of adult *R. pomonella* to baited red spheres,

2000, [·······Male; _____ Female]

DISCUSSION

Red spheres (9 cm diam) baited with the mix volatile blend were the most effective traps tested for attracting the apple maggot fly. *R. pomonella* use chemical and visual stimuli in long range orientation to their host plants and use chemical cues alone to discriminate between host and non-host fruit (Prokopy and Roitberg 1984). When adult *R. pomonella* detect an appropriate blend of odors, such as a synthetic fruit volatile lure, they move upwind in a series of flights that eventually culminate in the arrival at the odor source (Aluja and Prokopy 1992). Over the course of three field seasons, we observed that the mix volatile blend was the most effective synthetic lure for attracting apple maggot flies to red sphere monitoring traps. In addition, the mix volatile blend, as a fruit odor source, was significantly more attractive to *R. pomonella* than butyl hexanoate alone. It is possible that the mix may produce a distinct, attractive odor due to a synergistic relationship existing between the individual compounds that elicits a greater response than either compound alone. More research is needed to investigate this hypothesis properly.

The results from our first experiment in 1998 indicate that spheres baited with protein hydrolysate alone are not an effective means of monitoring *R. pomonella* populations. Spheres baited in this fashion were less effective than spheres baited with various other treatments tested, as well as unbaited spheres. Gow (1954) also evaluated various protein hydrolysates and found that although they were initially very attractive to the oriental fruit fly, *D. dorsalis* Hendel, their attractiveness decreased when the protein hydrolysates experienced bacterial decay. We noticed that when protein hydrolysate was mixed into the Tangle-Trap and applied to a red sphere, the sphere became discolored, assuming a whitish appearance. Spheres baited in this fashion were possibly visually unattractive to *R. pomonella* flies. When spheres baited with protein hydrolysate were left in the field for 2 weeks, they began to lose their initial whitish color as the bait progressively dissipated (a possible reaction from bacterial decomposition). During the course of the season, we observed that spheres baited with protein hydrolysate alone gradually caught a greater number of flies compared to earlier catches. Based on this observation, we hypothesize that as the spheres began to regain their original red (dark) color, their effectiveness in catching *R. pomonella* increased.

The development of monitoring traps for *R. pomonella* has been based on the identification of synthetic visual and olfactory stimuli that are naturally attractive to *Rhagoletis* flies. *Rhagoletis* sibling species are strongly attracted to yellow color, which may be due to the visual stimulus exuded by yellow foliage, locations which may be associated with honeydew producing insects such as aphids (Kring 1970). Hodson (1943) was the first to show that ammonium baits are highly attractive to *R. pomonella*. Since that time, a plethora of published studies have described the effectiveness of ammonium baits for monitoring *Rhagoletis* species (Liburd et al. 1998, Liburd et al. 1999, Liburd et al. 2000). However, a study conducted by Drummond et al. (1984) found that spheres baited with ammonium compounds do not attract *R. pomonella* selectively. Instead, the traps used in their study attracted and captured a wide variety of Dipteran (beneficial) insects. This decreased the effectiveness of the traps for detecting *R. pomonella* due to the reduced trapping area caused by the inundation of non-target insects. We recorded similar results in our study; spheres baited with ammonium compounds became saturated with insects after 7-10 d in the field during all three field trials. We observed that the average life span of a sphere baited with ammonia was at most 3 wk, depending on fly pressure. After 3-4 wk, the decomposing insects caused a blunt odor that may have competed with the odor given off by the ammonia baits. This may have decreased the effectiveness of ammonia-baited spheres. Bateman and Morton (1981) studied the role of ammonium compounds and concluded that both ammonia and amino acids were essential to attract *Rhagoletis* species, the former for olfactory stimulation and the latter for feeding response. Additional studies revealed that although ammonia is the major attractant, an accompanying increase in the pH level of the bait compound further increases its attractiveness. They assumed that at this increased pH level, additional unidentified volatiles maybe produced, resulting in increased attractiveness to *Rhagoletis* flies. These variables may have effected the results of our study; however, we did not measure these factors.

The addition of protein hydrolysate or ammonium compounds to spheres baited with BioLure did not significantly increase the attractiveness of these spheres to *R. pomonella* flies. In fact, the only difference that was recorded by combining an ammonium bait with an apple volatile lure in comparison to using the fruit volatile alone was an increased capture of female *R. pomonella* relative to males in early season. However, unbaited spheres and spheres baited only with BioLure, butyl hexanoate, or the mix volatile blend, were far more selective in capturing *R. pomonella* flies than lures containing ammonium compounds. Such spheres, containing no ammonia or protein bait impregnated in the Tangle-Trap maintained the original deep red color of the plastic. Spheres that were treated with baits impregnated into the Tangle-Trap tended to become discolored (lighter in color, whitish appearance). The attractiveness of unbaited spheres observed in both 1998 and 2000 may be indicative of the visual stimulus, which closely mimics apples in both size and color (Prokopy 1968). The addition of apple volatiles to such a spheres increased their attractiveness to *R. pomonella* flies, while at the same time preserving the selectivity of the spheres. The principal component of BioLure is butyl hexanoate, a fruit odor that attracts sexually mature *R. pomonella* flies for mating and oviposition (Reynolds and Prokopy 1997). The possible reason why we did not observe an increased total trap capture by combining ammonium baits with BioLure may be the decreased selectivity associated with this type of trap baiting system in comparison to using the synthetic apple volatile alone. Also, apple maggot flies immigrating into commercial orchards are sexually mature and therefore exhibit a greater response to host-fruit volatiles than food-odor attractants (Rull and Prokopy 2000). Therefore, we feel that using synthetic apple volatiles without ammonium baits may produce more accurate and sensitive monitoring of *R. pomonella* flies in commercial apple orchards.

We found that by using synthetic apple volatiles (mix blend, butyl hexanoate, BioLure), the effectiveness of red-sticky sphere traps could be extended beyond the 3-week life span of spherical monitoring traps baited with ammonium attractants. This may be due in part by an extended available surface area on spheres baited with apple volatile lures, as well as a decrease in odors associated with insect decomposition found on spheres heavily inundated with non-target captures. The combination of attractiveness and selectivity associated with using the mix volatile blend may provide the highest degree of effectiveness for monitoring both sexes of *R. pomonella* populations in commercial apple orchards. Furthermore, the use of the mix volatile blend may produce the best possible results for controlling *R. pomonella* with mass trapping (Prokopy et al. 1990) or through the use of baited insecticide-treated spheres (Stelinski et al. in press).

CHAPTER FIVE

RESPONSE OF BLUEBERRY MAGGOT FLIES, *RHAGOLETIS MENDAX*, (DIPTERA: TEPHRITIDAE) TO SYNTHETIC VOLATILE COMPOUNDS IN BLUEBERRY PLANTINGS.

INTRODUCTION

The blueberry maggot fly, *Rhagoletis mendax* Curran, is a widely distributed pest of low- and highbush blueberries (Vaccinium *angustifolium* Aiton and *Vaccinium corymbosum* L., respectively) within the northeastern and midwestern United States, as well as Canada. High infestations have been found in several states including Maine, New Hampshire, Vermont, New Jersey, and Michigan (Bush 1968). Its range extends as far south as northern Florida, where its primary host is the deerberry, *Vaccinium stamineum* L. (Payne and Berlocher 1995). The distribution of the blueberry maggot fly also extends northward into Canada. *R. mendax* is known to occur in Nova Scotia, Prince Edward Island and New Brunswick (Wood 1965, Neilson and Wood 1985). Additionally, there have been sightings of blueberry maggot flies in areas previously thought to be uninfested in Canada as for example in Saint-Bruno, Quebec (Jobin 1965).

The behavior of *R. mendax* has been the studied extensively for the purpose of developing effective integrated control programs (Prokopy and Coli, 1978, Liburd et al. 1998 a,b, 2000). Several attempts have been made to develop and refine blueberry maggot fly monitoring programs in order to forecast the presence of *R. mendax* in commercial blueberry plantings (Liburd et al. 1998a, 2000). The current monitoring practices employed by commercial growers of low- and highbush blueberries involve the deployment of ammonium-baited Pherocon AM yellow sticky panels or green and red sticky spheres. (Prokopy and Coli 1978, Liburd et al. 1998 a). Such practices allow growers to detect fly emergence for more accurate timing of spray regimes. One of the primary problems with current monitoring techniques is that ammonium/protein hydrolysate baits attract non-target insects and other arthropods, including beneficials.

Inundation of monitoring traps with non-target captures significantly reduces their effectiveness (Liburd et al. 1999, 2000). Due to the blueberry maggot fly's status as a major economic pest of commercially grown blueberries, (Liburd et al. 1998b) and the fact that consumer demands have resulted in a zero tolerance for maggots in commercially sold berries, growers in the eastern and mid-western United States seek further improvements for monitoring and integrated control practices. Refined monitoring programs with a high degree of trap selectivity may alleviate some of the problems encountered with non-target insect captures discussed in Liburd et al. (2000) and improve growers' capabilities of accurately detecting the presence of adult *R. mendax*.

Host-plant volatiles are important behavior-modifying stimulants for *Rhagoletis* sibling species (Liburd et al. 2000). Odor emitted by ripening fruit, which is attractive to both sexes of *Rhagoletis* flies, (Prokopy et al. 1973), may serve as a long-range attractant since flies detect host fruit odor at a distance of at least 20 m. *R. pomonella* and *R. mendax* exhibit preferential ovipositional responses to their respective host fruit volatiles. Specifically, *R. pomonella* are more sensitive to the odor of ripe apples, their specific host, compared with blueberries and vice versa for *R. mendax*, indicating that antennal sensitivity may be adapted to the species specific host (Frey and Bush 1990). In addition, hybrids of *R. mendax* and *R. pomonella* display a significantly weaker peripheral responses to host odor compounds as measured by electroantennograms than either parental species (Frey and Bush 1996).

Parliament and Kolor (1975) identified twenty-one volatile compounds emitted by highbush blueberries and found that the compounds trans-2-hex-enal, trans2-hex--enol and linalool were associated with the human sensory characteristic flavor of fresh blueberries. Later, Horvat and Senter (1985) extracted fifty-one volatile compounds from blueberries and found that the compounds cis-3-hexen-1-ol and geraniol also had the characteristic fruity aromas of fresh blueberries.

Although *R. mendax* has been shown to be responsive to host-specific chemical cues as measured by electroantennograms (Frey and Bush 1990) and volatile compounds from blueberries have been collected and characterized, an effective synthetic volatile lure, load rate, and release rate have not been determined for effective monitoring of the blueberry maggot fly. In addition, there have been no published reports on the attraction of *R. mendax* to synthetic fruit volatiles in commercially grown blueberry plantings. Identifying synthetic volatiles attractive to the blueberry maggot fly could potentially make monitoring devices more selective to this species, allowing growers to make more informed and precise management decisions. The purpose of this study was to evaluate the behavioral responses of *R. mendax* to spherical monitoring traps baited with synthetic host-plant volatiles in early- and mid-season blueberry cultivars. We also wanted to document how the responses of *R. mendax* to synthetic host-plant volatile lures change over the course of the season.

MATERIALS AND METHODS

Two experiments to evaluate synthetic blueberry volatiles were carried out during the 1999 blueberry field season. Both experiments were located in southwestern Michigan. Experiments 1 and 3 were located at a large commercial organic blueberry planting within an early-season blueberry cultivar (Earliblue), where blueberry maggot fly emergence occurred in early June. Experiments 2 and 4 were located at a smaller organic blueberry farm within a mid-season blueberry cultivar (Bluecrop), where blueberry maggot fly infestation took place in late June and early July. Based on previous monitoring data, it was known that both sites contained large residential populations of *R. mendax*. In 1999, volatile treatments were selected based on preliminary experiments conducted by Liburd et al. 1997 (unpublished data). During 2000, volatile treatments that demonstrated potential for attraction in 1999 were included for further evaluation. In both years, experiments were conducted at the same Michigan sites. All treatments were arranged in a randomized complete block design. The treatments were blocked by location and replicated four times.

(1999). Experiment 1. (Earliblue). Our inaugural experiments in 1999 focused on evaluating previously identified synthetic blueberry volatile compounds and attractants at very high load-rates (Liburd et al. unpublished data). In treatments consisting of synthetic volatiles, 3 ml of each volatile was pipetted into 5 ml polyethylene vials (Great Lakes IPM, Vestaburg, MI). Treatments consisting of ammonium acetate had 2 g of solid ammonium acetate dissolved in 4 ml of water and placed into 5 ml polyethylene vials. Four pinholes were bored into the caps of each vial in the field to facilitate a high release rate of the volatile compounds and ammonium baits. Nine volatile compounds (treatments) were evaluated. Polyethylene vials containing the nine treatments were affixed to 9-cm diam green, spheres coated with 13 g of Tangle Trap® (Liburd et al. 1998a). All synthetic blueberry volatiles and ammonium acetate were obtained from Aldrich Chemical Co., Milwaukee, WI while protein hydrolysate was purchased from Pfaltz & Bauer Chemicals Co., Milwaukee, WI. The treatments included spheres baited with (1) 2.0 g of ammonium acetate, (2) cis-3-hexen-1-ol, (3) geraniol, (4) alpha-terpiniol, (5) trans-2-hexen-1-ol, (6) 6-methyl-5-hepten-2-one, (7) linalool, and (8) trans-2-hexen-al. Spheres were hung within blueberry bushes approximately 15 m apart (20 m between blocks) in plantings of Earliblue (cultivar) blueberries. Spheres were placed within the canopy of blueberries ~ 15 cm from the top of the highest bush (Liburd et al. 2000).

Experiment 2. (Bluecrop). Our second experiment was placed within a mid-season blueberry cultivar (Bluecrop). It was similar in design and served as a replicate for our first experiment and as a comparison among phenologically differing blueberry cultivars. The six treatments that were evaluated included spheres baited with (1) 2.0 g of ammonium acetate, (2) cis-3-hexen-1-ol, (3) geraniol, (4) alpha-terpiniol, (5) beta-caryophyllene and (6) unbaited spheres.

(2000). Our objective during the 2000 field trials was to evaluate different release-rates of blueberry volatile compounds. We also wanted to continue the evaluation of volatile compounds in blueberry cultivars with different phonologies by, once again, comparing volatile treatments within both early- and mid-season blueberry cultivars. Four of synthetic blueberry volatile compounds that were evaluated in 1999 were chosen for further study in 2000. These compounds were geraniol, cis-3-hexen-1-ol, trans-2-

hexen-1-ol, linalool. Volatile treatments were compared against the standard ammonium acetate bait used in current blueberry maggot fly monitoring programs as well as an untreated control.

All volatile-treatments were placed in BEEM® embedding (polyethylene) capsules (Ted Pella, Inc., Redding, CA). We loaded 0.3 ml of each volatile-treatment via pipette into the polyethylene capsules for field release. Release rates of all pure compounds from sealed BEEM® capsules were approximated by gas chromotography (HP-6980, Hewlett-Packard Co.) and time-of-flight mass spectrometry (Pegasus II, LECO Corp., St. Joseph, MI); the GC was fitted with a column of length 15 m and internal diam. 100 μ. The GC temperature program was 30°C/1 min, 10-250°C and held for 5 min; the carrier gas was He. BEEM® capsules containing volatile compounds were aerated in Teflon collection systems with an airflow ≈33 ml/min. Volatiles were sampled with solid-phase microextraction (SPME) using 1 cm long fibers coated with a 100 μm thick layer of poly(dimethylsiloxane) (Supelco Co., Bellefonte, PA); the absorption time was 5 min. Volatiles were sampled from aerating chambers 1, 3, 6, 9, and 12 d post loading at ~ 22° C. Each volatile released from BEEM® capsules and collected by SPME was compared with a previously prepared standard of known concentration. Steady emission from BEEM® capsules was observed for all compounds after 9 d; 12 d data were used to estimate release rates. All compounds were identified by comparison of collected mass spectra with the reference standards and spectra of the National Institute for Standards and Technology (NIST) mass spectra library, search version 1.5 (Mir and Beaudry 1999).

During the 2000 field season, BEEM® capsules containing treatments were affixed to 9 cm diam, green spheres coated with 13 g of Tangle Trap®. Volatile release rates were varied by placing treatments in one capsule (lowest release rate), five capsules (mid-range release rate), or single capsules with a single hole bored into its lid (highest release rate). Spheres containing treatments were hung within the canopies of blueberry bushes approximately 15 m apart within rows and 20 m between blocks in plantings of either Earliblue or Bluecrop blueberry cultivars.

Experiment 3. (Earliblue). In our first experiment of the 2000 field season conducted in the early-season blueberry cultivar (Earliblue), we evaluated treatments of geraniol, cis-3-hexen-1-ol, and ammonium acetate. Overall, a total of six treatments were tested. The compounds geraniol and cis-3-hexen-1-ol were deployed at two different release rates based on the number of BEEM® capsules attached. Each trap (sphere) had either one or five capsules containing each volatile, totaling 4 treatments for geraniol and cis-3-Hexen-1-ol. The fifth treatment of ammonium acetate was evaluated at a very high release rate. This was accomplished by boring a hole into the cover of the BEEM® capsule. The sixth treatment was a control that consisted of traps left unbaited (without volatile or bait compounds).

Experiment 4. (Bluecrop). In our second study carried out during the 2000 field season, we investigated the performance of synthetic volatiles within the mid-season blueberry cultivar (Bluecrop). A total of 8 treatments were evaluated including the two volatiles, trans-2-hexen-1-ol and linalool at the two load rates previously discussed (treatments 1-4). In addition, a new mixed blend consisting of 0.1 ml of geraniol, 0.1 ml of trans-2-hexen-1-ol, and 0.1 ml aqueous ammonium acetate was tested at the two load rates discussed (treatments 5,6). Our seventh treatment consisted of the standard ammonium acetate at a very high release rate (hole punctured in polyethylene capsule). Treatment 8 was an unbaited sphere.

Sampling. *R. mendax* flies caught on traps were counted by sex and removed from spheres twice per week. Traps were re-randomized on a weekly basis and replaced every three weeks.

Statistical Analysis. Data from all experiments were square - root transformed ($x + 0.5$) and then subjected to an analysis of variance (ANOVA). Means were separated by least significant difference (LSD) ($P = 0.05$) (SAS Institute 1989). The untransformed means and standard errors are presented in tables and figures.

RESULTS

(1999). Experiment 1. (Earliblue). Our 1999 study conducted within the early blueberry cultivars showed that during the first monitoring period (26 June-02 July) spheres baited with ammonium acetate captured significantly ($F = 3.0$; df = 8,24; $P = 0.02$) more blueberry maggot flies than spheres baited with Alpha-Terpiniol, Trans-2-Hexen-1-ol, 6-Methyl-5-hepten-2-one, Linalool, and Trans-2-Hexen-al (Table 13). The response of *R. mendax* to the conventional ammonium acetate bait during the first monitoring period was not statistically different from the response to Cis-3-Hexen-1-ol and Geraniol volatiles (Table 13). During our second monitoring period (06-13 July) spheres baited with the standard ammonium acetate attracted significantly ($F = 17.5$; df = 7,21; $P < 0.01$) more blueberry maggot flies than all of the synthetic fruit volatiles evaluated (Table 13). Spheres baited with ammonium acetate captured 2.4 times as many flies as all other treatments evaluated during this period. During the second period, Cis-3-Hexen-1-ol was the second most effective treatment, attracting significantly ($F = 17.5$; df = 7,21; $P < 0.01$) more *R. mendax* than all other synthetic volatile treatments, apart from Alpha-Terpineol. During the final monitoring period (July 16-23) spheres baited with ammonium acetate captured significantly ($F = 12.1$; df = 7,21; $P < 0.01$) more flies compared with all of the synthetic fruit volatiles evaluated (Table 13). The compound Cis-3-Hexen-1-ol, which performed well during our second monitoring period, attracted significantly ($F = 12.1$; df = 7,21; $P < 0.01$) more flies than Trans-2-Hexen-1-ol and Linalool volatile compounds during the final monitoring period (Table 13).

The mean number of females caught on spheres baited with ammonium acetate (255.5 ± 10.0) was significantly greater ($F = 11.8$; df = 4,3; $P < 0.05$) than the mean number of males (146.8 ± 18.3). There were no significant differences between male and female captures on any of the other treatments evaluated.

Table 13. Attraction of blueberry maggot flies to synthetic fruit volatiles and baits within an early-season blueberry cultivar (Earliblue), Michigan, (1999).

Sphere treatments	1st Monitoring period 6/26-7/2	2nd Monitoring Period 7/6-7/13	3rd Monitoring Period 7/16-7/23
	Mean ± SEM no. flies found killed on Plexiglas		
Ammonium Acetate	43.0 ± 14.8 a	172.3 ± 23.2 a	235.8 ± 37.6 a
Cis-3-Hexen-1-ol	24.8 ± 1.5 ab	71.8 ± 11.4 b	111.8 ± 12.2 b
Geraniol	21.5 ± 9.9 ab	42.0 ± 4.6 cd	94.3 ± 11.6 bc
Alpha-terpineol	16.5 ± 5.6 b	69.0 ± 18.2 bc	88.5 ± 13.7 bc
Trans-2-Hexen-1-ol	11.3 ± 4.0 b	38.5 ± 12.3 d	72.0 ± 6.2 c
6-Methyl-5-hepten-2-one	10.8 ± 1.9 b	34.3 ± 5.5 d	78.0 ± 11.7 bc
Linalool	13.8 ± 5.0 b	30.0 ± 5.4 d	74.5 ± 4.9 c
Trans-2-Hexen-al	13.5 ± 3.8 b	41.8 ± 11.2 d	86.8 ± 10.3 bc

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

Experiment 2. (Bluecrop). In 1999, the response of *R. mendax* to our synthetic fruit volatiles within mid-season blueberry cultivars was different from that observed in our trials conducted in the early-season varieties. During the first monitoring period (16-23 July), spheres baited with ammonium acetate captured significantly ($F = 7.35$; df = 5,15; $P < 0.01$) more flies than all of the fruit volatile treatments evaluated and unbaited spheres (Table 14). Ammonium baited spheres captured 2.8 times as many flies as any volatiles compound and 2.4 times as many *R. mendax* flies as the unbaited spheres. (Table 14). There were no differences observed among captures of flies on spheres baited with any of the synthetic blueberry volatiles. Also, there were no differences between captures on spheres baited with any volatile compound and the unbaited spheres. This same trend continued throughout the second (07 July-02 August) and third (05-12 August) monitoring periods (Tables 14).

Table 14. Attraction of blueberry maggot flies to synthetic fruit volatiles and baits within a mid-season blueberry cultivar (Bluecrop), Michigan, (1999).

Sphere treatments	1st Monitoring period 7/16-7/23	2nd Monitoring Period 7/26-8/2	3rd Monitoring Period 8/5-8/12
	Mean ± SEM no. flies found killed on Plexiglas		
Ammonium Acetate	230.3 ± 42.1 a	142.8 ± 44.2 a	65.3 ± 10.3 a
Cis-3-Hexen-1-ol	52.3 ± 15.9 b	44.8 ± 12.8 b	15.8 ± 4.6 b
Geraniol	81.5 ± 33.7 b	73.3 ± 30.7 b	28.8 ± 11.6 b
Alpha-terpineol	65.3 ± 16.9 b	46.0± 14.5 b	31.3 ± 11.2 b
Beta-Caryophyllene	48.0 ± 7.1 b	53.8 ± 13.3 b	27.5 ± 4.1 b
Control	96.5 ± 23.3 b	59.0 ± 17.5 b	18.8 ± 4.9 b

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

(2000). The approximate release rates for 0.3 ml of Geraniol, Cis-3-Hexen-1-ol, Trans-2-Hexen-1-ol, and Linalool from single, closed BEEM® capsules at 22° C were 0.034, 0.051, 0.041, and 0.0047 µl / h, respectively.

During the second field trial, we observed a slightly different response of *R. mendax* in our early-season blueberry cultivar trials, possibly due to the lowered volatile release rates. During the first monitoring period (22-29 June) when blueberry maggot flies were beginning to emerge, there were no differences in fly response to all of the treatments evaluated, including the control (Table 15). During the second monitoring period (3-10 July), we recorded our highest trap captures on spheres baited with Cis-3-Hexen-1-ol with a release rate of 5 capsules. Cis-3-Hexen-l-ol attracted significantly (F = 3.63; df = 5,15; P = 0.03) more flies than spheres baited with Geraniol (1 and 5 capsules) and unbaited spheres (Table 15). During the third monitoring period (13-21 July), during which peak blueberry maggot fly activity was observed, there was no significant difference in the performance of spheres baited with Cis-3-Hexen-1-ol at both release rates tested and the standard ammonium acetate bait (Table 15). During the final two weeks of monitoring (24 July-1 August and 3-10 August), as *R. mendax* populations were in decline, ammonium acetate attracted significantly (F = 6.9; df = 5,15; P < 0.01) and (F = 3.48; df = 5,15; P < 0.05) more blueberry maggot flies than all of the other treatments (Table 15).

There were no significant differences among male and female captures on spheres baited with any of the synthetic volatile compounds and unbaited (control) spheres. However, the mean number of females (123.8 ± 20.3) caught on spheres baited with ammonium acetate was significantly ($F = 15.8$; df $= 4,3$; $P < 0.05$) greater than the mean number (52.3 ± 5.6) of males.

Table 15. Attraction of blueberry maggot flies to synthetic fruit volatiles and baits within an early-season blueberry cultivar (Earliblue), Michigan, (2000).

Treatments	1st Monitoring period 6/22-6/29	2nd Monitoring Period 7/3-7/10	3rd Monitoring Period 7/13-7/21	4th Monitoring Period 7/24-8/1
	Mean ± SEM no. flies found killed on Plexiglas			
Geraniol (1 capsule)	2.5 ± 2.5 a	20.8 ± 9.0 bc	28.5 ± 7.0 b	11.5 ± 1.9 b
Geraniol (5 capsules)	1.3 ± 0.5 a	25.0 ± 7.0 bc	26.5 ± 9.3 b	12.0 ± 4.2 b
Cis-3-Hexen-1-ol (1 capsule)	1.5 ± 0.7 a	35.0 ± 8.0 abc	46.8 ± 9.4 ab	7.3 ± 1.7 b
Cis-3-Hexen-1-ol (5 capsules)	1.3 ± 0.5 a	57.0 ± 10.1 a	48.5 ± 15.3 ab	13.0 ± 3.5 b
Ammonium acetate	7.3 ± 3.1 a	37.5 ± 7.2 ab	88.3 ± 21.5 a	30.0 ± 7.5 a
Unbaited (Control)	2.3 ± 2.3 a	16.8 ± 5.2 c	42.8 ± 5.3 b	8.5 ± 0.9 b

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

In our second study of the 2000 field season, carried out in the mid-season blueberry cultivar, we observed no significant differences in trap captures among the volatile and bait treatments evaluated during all four monitoring periods (Table 16).

Table 16. Attraction of blueberry maggot flies to synthetic fruit volatiles and baits within a mid-season blueberry cultivar (Bluecrop), Michigan, (2000).

Treatments	1st Monitoring period 6/29-7/6	2nd Monitoring Period 7/10-7/17	3rd Monitoring Period 7/21-7/27	4th Monitoring Period 8/1-8/8
	Mean ± SEM no. flies found killed on Plexiglas			
Linalool (1 capsule)	2.5 ± 1.0 a	17.8 ± 1.9 a	7.3 ± 1.5 a	27.5 ± 6.0 ab
Linalool (5 capsules)	6.0 ± 3.8 a	18.8 ± 8.4 a	9.5 ± 2.4 a	14.5 ± 1.7 b
Trans-2-Hexen-1-ol (1 capsule)	2.8 ± 0.5 a	11.0 ± 3.5 a	5.8 ± 1.6 a	32.5 ± 15.7 ab
Trans-2-Hexen-1-ol (5 capsules)	2.8 ± 0.8 a	19.8 ± 7.9 a	11.3 ± 4.3 a	13.0 ± 3.2 b
Mixed blend (1 vial)	6.3 ± 5.3 a	18.3 ± 5.5 a	13.3 ± 5.9 a	39.8 ± 9.5 a
Mixed blend (5 vials)	10.5 ± 3.1 a	20.8 ± 6.7 a	12.5 ± 4.8 a	25.8 ± 13.1 ab
Ammonium acetate	12.0 ± 7.3 a	26.8 ± 8.0 a	10.5 ± 2.0 a	28.3 ± 5.2 ab
Unbaited (Control)	7.3± 2.8 a	22.5 ± 10.4 a	8.8 ± 3.3 a	24.8 ± 6.2 ab

Means within the same column followed by the same letter are not significantly different, ($P = 0.05$, LSD test).

DISCUSSION

This study confirmed that ammonium acetate can be highly attractive food-odor bait to blueberry maggot flies and that its use with sticky, green sphere monitoring traps produces high captures of adult flies in both early- and mid-season ripening blueberry cultivars. These results are in concordance with many previous studies, which have shown that synthetic ammonium baits are highly attractive to *Rhagoletis* species (Prokopy 1969, Reissig 1974, Liburd et al. 1998 a, Liburd et al. 2000). In addition, we found that the synthetic blueberry volatiles, cis-3-hexen-1-ol and geraniol, can be attractive to blueberry maggot flies during the first half of the season, when deployed within an early ripening blueberry cultivar.

Numerous studies have shown successful use of synthetic host-odor lures for attracting *Rhagoletis* flies, such as the apple maggot *Rhagoletis pomonella* (Walsh), in the field (Riessig 1985, Duan and Prokopy 1992, Zhang et al. 1999). Evidence for a similar attraction of blueberry maggot flies to synthetic fruit-odor lures in the field was observed during this study. However, the effectiveness of the synthetic volatiles evaluated here was dependent on several factors including host-plant phenology, fruit maturity, and volatile release rates.

During both field seasons, we observed a significant change in the response of blueberry maggot flies to synthetic host-fruit volatiles within the early ripening blueberry cultivar (Earliblue) over the course of season. In both years, traps baited with cis-3-hexen-1-ol and geraniol were equally attractive to blueberry maggot flies as traps baited with ammonium acetate in early season cultivars (26 June-10 July). However, the attractiveness of traps baited with these two synthetic host-fruit compounds decreased over the course of the season as host-fruit ripening occurred (after July 13). Such a decrease in fly captures was not observed on spheres baited with ammonium acetate, which remained highly effective in attracting blueberry maggot flies throughout the course of the blueberry growing season (26 June-1 August). We believe that early in the season, there is a lower concentration of blueberry volatiles being released from immature green fruit. Therefore, blueberry maggot flies immigrating into the early cultivar plantings may be responding to the point source traps that are emitting attractive synthetic blueberry volatiles, such as cis-3-hexen-1-ol and geraniol. Later in the season, as fruit begins to ripen, a greater overall concentration of blueberry volatiles may be emitted from the maturing fruit. The effect is a greater level of competition between with synthetic fruit-volatile lures and the volatiles being released from berries as fruit maturation progresses. This results in a decline in blueberry maggot captures on fruit-volatile baited traps later in the season.

During both seasons, both female and male blueberry maggot flies were equally responsive to synthetic blueberry volatiles because females were foraging for both mating and oviposition sites and males were searching for sites harboring females for mating (Prokopy et al. 1973). However, ammonium-baited traps captured approximately twice as many females as males. Recently, a study conducted by Liburd et al. (1998 a) showed that the olfactory stimulus provided by ammonium baits was more important than trap shape and color for attracting adult blueberry maggot flies. The greater captures of females over males on ammonium-baited traps occurred because female flies were seeking a protein source required for sexual maturation (Liburd et al. 1998 a).

In 2000, we reduced the release rates of our synthetic fruit-volatiles by decreasing the load rate from 3 ml to 0.3 ml and by using smaller 1 ml, sealed polyethylene vials compared with the 5 ml polyethylene vials containing holes in the lids used in 1999. Therefore, in 2000, the fruit volatiles were only allowed to escape through the matrix of the polyethylene and over a smaller surface area, which likely reduced the rate at which volatiles were released. The decrease in release rates caused a change in the response of blueberry maggot flies to cis-3-hexen-1-ol in the early-season blueberry cultivar. Specifically, the attractiveness of traps baited with this compound at the lower release rates remained high throughout the end of mid-season (until 21-July) in 2000, while it declined significantly at the high release rate before the onset of mid-season (2-July) in 1999. High volatile release rates may depict mature fruit that is no longer suitable for oviposition and generally not preferred by gravid females (Liburd et al. 1998 a). The lowered release rates in 2000 may have mimicked susceptible, ripening fruit, which are used preferentially by blueberry maggot flies for mating and oviposition (Liburd et al. 1998 a). Therefore, effectiveness of cis-3-hexen-1-ol may have been extended at the lower release rate because sexually mature females found it more attractive during mid-season, compared with fully mature (ripe) berries.

There was a substantial difference in the response of blueberry maggot flies to synthetic fruit-volatiles in the early-season blueberry cultivar (Earliblue) versus the mid-season cultivar (Bluecrop). Specifically, baiting traps with synthetic fruit-volatiles did not increase capture of blueberry maggot flies over unbaited spheres in the mid-season cultivar. Spheres baited with ammonium acetate, however, captured significantly more flies compared with the other treatments throughout the entire season. These results provide further evidence that later in the season (7-17 July), when fruit has ripened in early blueberry cultivars and is ripening in mid-season cultivars, there is a greater competition between fruit-volatile lures and the concentration of volatiles released from maturing berries. This may render traps baited with synthetic fruit-volatile lures less effective in capturing blueberry maggot flies.

In 2000, we recorded no differences in blueberry maggot fly captures between spheres baited with synthetic fruit-volatiles, ammonium acetate, or unbaited spheres within the mid-season cultivar. Furthermore, we observed decreased overall fly captures in comparison to 1999. However, in 2000, the planting of Bluecrop in which this study was conducted experienced unusually low pollination, as well as severe flooding with standing water accumulated from 25 June – 4 July (John Vanvoorheis personnal communication, personal observation). Factors such as decreased fruit load and flooding may have influenced our fly captures due to decreased emergence of resident flies and decreased immigration from surrounding populations.

Our study showed that ammonium acetate is an attractive lure for *R. mendax* throughout the season. In addition, the results provide evidence that blueberry volatiles may serve as important stimuli for host-plant location by foraging blueberry maggot flies infesting blueberry plantings early in the season. The effectiveness of traps baited with synthetic blueberry volatiles in capturing blueberry maggot flies decreases as blueberries mature and therefore such traps are less effective in monitoring flies during mid-season compared with early-season. Our results provide evidence that the synthetic blueberry-odor volatiles cis-3-hexen-1-ol and geraniol have potential as components of a lure attractant for blueberry maggot fly IPM monitoring programs, possibly replacing the non-selective ammonium acetate baits that are currently used. Future development of such a lure should focus on identifying other important blueberry volatiles, which may, in the correct proportions, act synergistically with cis-3-hexen-1-ol and geraniol to produce greater and extended attractiveness to blueberry maggot flies.

SUMMARY AND CONCLUSIONS

During our 2000 field season, spheres treated with either formulation of imidacloprid (Provado® 1.6 F or Merit® 75 WP) showed equal effectiveness in killing apple maggot and blueberry maggot flies. Thiamethoxam-treated spheres killed fewer apple maggot and blueberry maggot flies than imidacloprid-treated spheres. Spheres treated with thiocloprid (Calypso®) were ineffective, killing no more apple maggot or blueberry maggot flies than control spheres in the field. We therefore feel that treating spheres with imidacloprid rather than thiamethoxam or thiocloprid may produce better results for possible control of *Rhagoletis* sibling species.

The results we obtained from our 2000 deployment strategy experiment were similar to those that were obtained in 1999. The three deployment strategies that were tested in both years worked equally well in protecting fruit from *R. mendax* infestation. Based on the number of puparia reared from fruit collected from treated plots, we found that fruit infestation using insecticide-treated spheres was reduced from > 20 % in control plots to < 2 % in plots containing insecticide-treated spheres in 1999 and from 3.8 % to 0.8 % in 2000. The control offered by insecticide-treated spheres was comparable to organophosphate sprays, which produced fruit injury levels below 0.2 %, on average. Although our experimental replicate plots were only 10 x 40 m rectangles, we believe that similar fruit protection could be achieved on a larger scale, if adequate numbers of spheres were deployed in a commercial setting.

Our volatile work in 2000 with the apple maggot clearly showed that the mixed apple volatile blend is the most effective attractant when compared with the other treatments evaluated. We obtained similar data in both the 1998 and 1999 field seasons. We therefore recommend that the mixed apple volatile blend should be used in current monitoring and control programs for this species.

In blueberries, the most widely used attractant for the blueberry maggot fly has been ammonium acetate. This compound is very effective in attracting *R. mendax*, especially when maggots are seeking a protein source for ovarian development. Ammonium acetate, however, also attracts a wide range of Diptera and other beneficial insects, causing monitoring traps to become inundated with non-target arthropods, thus decreasing their effectiveness. In 1999 and 2000, we evaluated synthetic blueberry volatiles that can potentially replace ammonium acetate in future monitoring regimes. The blueberry volatile compounds cis-3-hexen-1-ol and geraniol were highly attractive to blueberry maggot flies early in the growing season. Early in the season, cis-3-hexen-1-ol and geraniol performed equally to ammonium acetate in attracting blueberry maggot flies to 9 cm diam. spherical monitoring traps. The use of synthetic blueberry volatiles that are equally attractive to the blueberry maggot flies as ammonium acetate may improve current IMP tactics by making traps more selective to blueberry maggot flies and reducing the capture rate of non-target insects on monitoring traps.

Overall, our research showed good potential for use of biodegradable, insecticide-treated spheres baited with a mix blend of apple volatiles for management of apple maggot flies. Other key *Rhagoletis* species, including *R. mendax* can also be suppressed using insecticide-treated spheres baited with ammonium acetate stored in plastic dispensers. In addition, growers who adopt this management tactic can use Plexiglas panes coated with sticky Tangle-Trap® to monitor *Rhagoletis* populations.

During the course of this study, we confirmed that imidacloprid is the best neonicotinoid available for use with insecticide-treated spheres. We were also able to demonstrate a significant reduction in fruit injury using insecticide-treated spheres in replicated blueberry plots. However, we encountered the problems of fungal growth on biodegradable spheres and the loss of spheres in the field due to rodent feeding. Future prototypes of these spheres should include more advanced anti-fungal agents and rodent deterrents before this technology can be recommended to growers for commercial application. Still, based on our data from 1998, 1999, and 2000, we feel that the use of biodegradable spheres treated with imidacloprid and baited with appropriate attractants has good potential as an alternative to conventional organophosphate sprays for control of *Rhagoletis* sibling species.

The use of imidacloprid-treated, biodegradable spheres may be implemented commercially in the near future. The USDA has currently submitted this technology to the Environmental Protection Agency (EPA) for registration. The final prototype will be black, green, or yellow in color and treated with imidacloprid at 4 % AI (Stelinski et al. in press). In addition, a company has been formed (Fruit Spheres Inc.) to produce these spheres and market them nationally. The USDA is also in the process of developing more effective rodent feeding deterrents to help prevent the occurrence of such damage in the field. Preliminary trials of this technology in commercial apple orchards in Michigan have produced satisfactory fruit protection as demanded by Gerber Corporation (Stelisnki and Liburd, unpublished data).

LITERATURE CITED

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AliNiazee, M. T. 1978. The western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae), developing a management program by utilizing attractant traps as monitoring devices. Can. Entomol. 110: 1133 – 1139.

Aluja, M. and R. J. Prokopy. 1992. Host search behavior of *Rhagoletis pomonella* flies: inter-tree movement patterns in response to wind-borne fruit volatiles under field conditions. Physiol. Entomol. 17: 1-8.

Ayyappath, R., S. Polavarapu, and M. McGuire. 2000. Effectiveness of thiamethoxam-coated spheres against blueberry maggot flies (Diptera: Tephritidae). J. Econ. Entomol. 93: 1473-1479.

Bateman, M. A. and T. C. Morton. 1981. The importance of ammonia in proteinaceous attractants for fruit flies (Family: Tephritidae). Aust. J. Agric. Res. 32: 883 - 903.

Bierbaum, T.J. and G.L. Bush. 1990. Host fruit chemical stimuli eliciting distinct ovipositional responses from sibling species of *Rhagoletis* fruit flies. Entomol. Exp. Appl. 56: 165-177.

Boller, E.F. and R.J. Prokopy. 1976. Bionimics and management of *Rhagoletis.* Annu. Rev. Entomol. 113: 113-119.

Bush, G. L. 1966. The taxonomy, cytology, and evolution of the genus *Rhagoletis* in North America (Diptera: Tephritidae). Bull. Mus. Comp. Zool. 134: 431--562.

Bush, G.L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). Evolution. 23: 237-251.

Bush, G. L. 1992. Host race formation and sympatric speciation in *Rhagoletis* fruit flies (Diptera: Tephritidae). Psyche. 99: 335-357.

Bush, G.L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). Evolution. 23: 237-251.

Carle, S. A., A. L. Averill, G. S. Rule, W. H. Reissig, and W. L. Roelofs. 1987. Variation in host fruit volatiles attractive to apple maggot fly, *Rhagoletis pomonella*. J. Chem. Ecol. 13: 795-805.

Dean, R. W., P. J. Chapman. 1973. Bionomics of the apple maggot in Eastern New. York. Search Agric. Entomol. Geneva 3: 62 pp.

Drummond, F., E. Groden, and R. J. Prokopy. 1984. Comparative efficacy and optimal positioning of traps for monitoring apple maggot flies (Diptera: Tephritidae) Envirn. Entomol. 13: 232-235.

Duan, J. J. and R. J. Prokopy. 1992. Visual and odor stimuli influencing effectiveness of sticky spheres for trapping apple maggot flies *Rhagoletis pomonella* (Walsh) (Dipt., Tephritidae). J. Appl. Entomol. 113: 271-279.

Duan, J. J. and R. J. Prokopy. 1993. Toward developing pesticide-treated spheres for controlling apple maggot flies, *Rhagoletis pomonella* (Walsh) (Dipt. Tephritidae). Carbohydrates and amino acids as feeding stimulants. J. Appl. Entomol. 115: 176-184.

Duan, J. J. and R. J. Prokopy. 1995a. Development of pesticide-treated spheres for controlling apple maggot flies (Diptera: Tephritidae): pesticides and residue extending agents. J. Econ. Entomol. 88: 117-126.

Duan, J. J. and R. J. Prokopy. 1995b. Control of apple maggot flies (Diptera: Tephritidae) with pesticide-treated spheres. J. Econ. Entomol. 88: 700-707.

Feder, J., C.A. Chilcote, and G.L. Bush. 1989. Are the apple maggot, *Rhagoletis pomonella* (Diptera: Tephritidae), and the blueberry maggot, *R. mendax*, distinct species? Implications for sympatric speciation. Entomol. Exp. Appl. 51: 113-123.

Feder, J.L. (1998) The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? Pp. 130-144 in: Endless Forms: Species and Speciation (D.J. Howard and S.H. Berlocher, eds.) Oxford University Press, Oxford, UK.

Fein, B. L., W. H. Reissig, and W. L. Roelofs. 1982. Identification of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella.* J. Chem. Ecol. 8: 1473-1487.

Fletcher, B. 1989. Life history strategies of tephritid fruit flies. In Fruit Flies: Their Biology, Natural Enemies and Control. (A.S. Robinson and G. Hooper, eds.), pp. 195-208. In World Crop Pests (W. Helle ed.), Vol. 3A. Elsevier Science Publishers, Amsterdam.

Fleischer, S. J., M. D. Orzolek, D. D. Mackiewicz and L. Otjen. 1998. Imidacloprid effects on *Acalymma vittata* (Coleoptera: Chrysomelidae) and bacterial wilt in cantaloupe. J. Econ. Entomol. 91: 940-949.

Frey, J. E. and G. L. Bush. 1990. *Rhagoletis* sibling species and host races differ in host odor recognition. Entomol. Exp. Appl. 80: 163-165.

Frick, K. E., H. G. Simkover, and H. S. Telford. 1954. Bionomics of the cherry fruit flies in eastern Washington. Wash. Agric. Exp. Stn. Tech. Bull. 13.

Goeden, R.D. and D.W. Ricker. 1971. Biology of *Zonosemata vittigera* relative to silverleaf nightshade. J. Econ. Entomol. 64: 417-421.

Green, T.A., R.J. Prokopy, and D.W. Hosmer. 1994. Distance of response to host tree models by female apple maggot flies, *Rhagoletis pomonella*: interaction of visual and olfactory stimuli. J. Chem Ecol. 20: 2393-2413.

Gow, P. L. 1954. Proteinaceous baits for the oriental fruit fly. J. Econ. Entomol., 47: 153-160.

Hendrichs, J., M. Hendrichs, J. Prokopy, and R. J. Prokopy. 1990a. How do apple maggot flies detect the presence of distant food? Fruit Notes 55: 3-5.

Hendrichs, J. and R. J. Prokopy. 1994. Food foraging behavior of frugivorous fruit flies. *In* C.A. Calkins, W. Klassen, and P. Liedo [eds.], Fruit flies and the sterile insect technique. CRC Press, Boca Raton.

Hodson, A. C. 1943. Lures attractive to the apple maggot. J. Econ. Entomol. 36: 545-548.

Hu, X. P., A. Kaknes and R. J. Prokopy. 1997. Improved pesticide-treated wooden spheres for controlling apple maggot flies. Mass. Fruit Notes. 62: 2.

Hu, X. P. and R. J. Prokopy. 1998. Lethal and sublethal effects of imidacloprid on apple maggot fly, *Rhagoletis pomonella* Walsh (Dipt. Tephritidae) J. Appl. Entomol. 122: 37-42.

Hu, X. P., B. S. Shasha, M. R. McGuire, and R. J. Prokopy. 1998. Controlled release of sugar and toxicant from a novel device for controlling pest insects. J. Control. Release 50: 257-265.

Hu , X. P., R.J. Prokopy, and J. M. Clark. 2000. Toxicity and residual effectiveness of insecticides on insecticide-treated spheres for controlling females of *Rhagoletis pomonella* (Diptera: Tephritidae). J. Econ Entomol. 93:403-411.

Jobin, L. 1965. Rapport d´enquetes su les insects nuisibles et les insects pollinisateurs du bleuet au Lac Saint-Jean, 1962-1963-1964, Services de recherches, Ministere de I´Agriculture du Quebec. Unpublished Mimeo Report. 74: 462-467.

Johnson, P. C. 1983. Response of adult apple maggot (Diptera: Tephritidae) to Pherocon AM traps and red spheres in a non-orchard habitat. J. Econ. Entomol. 76: 1279-1284.

Horvat, R. J. and Senter, S. D. 1985. Comparison of the volatile constituents from rabbiteye blueberries (*Vaccinium ashhei*) during ripening. J. Food Sci. 50: 429--436.

Kring, J. B. 1970. Red spheres and yellow panels combined to attract apple maggot flies. J. Econ. Entomol. 63: 466-469.

Lathrop, F. H. and C. B. Nickels. 1932. The biology and control of the blueberry maggot in Washington County, ME. USDA Tech. Bull. No. 275.

Liburd, O. E. 1997. Behavioral management techniques for broccoli and blueberry pests. A *Ph. D.* dissertation submitted to the Univ. of Rhode Island in partial requirements for the degree of Doctor of Philosophy. Published by the University of Rhode Island academic press. Kingston. Rhode Island.

Liburd, O. E., S. R. Alm. R. A. Casagrande and S. Polavarapu. 1998 a. Effect of trap color, bait, shape and orientation in attraction of blueberry maggot (Diptera: Tephritidae) flies. J. Econ. Entomol. 91: 243-249.

Liburd, O. E., S. R. Alm. and R. A. Casagrande. 1998 b. Susceptibility of highbush blueberry cultivars to larval infestation by *Rhagoletis mendax* (Diptera Tephritidae). Environ. Entomol. 27: 817-821.

Liburd, O. E., L. J. Gut, L. L. Stelinski, M. E. Whalon, M. R. McGuire, J. C. Wise, X. P. Hu, and R. J. Prokopy. 1999. Mortality of *Rhagoletis* species encountering pesticide-treated spheres (Diptera: Tephritidae). J. Econ. Entomol. 92: 1151-1156.

Liburd, O. E., S. Polavarapu, S. R. Alm, and R. A. Casagrande. 2000. Effects of trap size, placement and age on captures of blueberry maggot flies (Diptera: Tephritidae). J. Econ. Entomol. 93: 1452-1458.

Liburd, O. E., and L. L. Stelinski. 1999. Apple maggot fly and its sibling species: physiological and environmental status. MSU CAT Alert Ext. Bull. 14: 3-4.

Liburd, O. E., L. L. Stelinski, L. J. Gut, and G. Thornton. 2001. Performance of various trap types for monitoring populations of cherry fruit fly species (Diptera: Tephritidae). Environ. Entomol. 30: 82-88.

Mayr. E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge, Massachusetts, 797 p.

Messina, F.J. and J.K. Subler. 1995. Conspecific and heterospecific interactions of male *Rhagoletis* flies on a shared host. J. Kans. Entomol. Soc. 68: 206-213.

Mir, N. A., and R. Beaudry. 1999. Effect of superficial scald suppression by diphenylamine application on volatile evolution by stored cortland apple fruit. J. Agricul. and Food Chem. 47: 7-11.

Neilson, W. T. A., A. D. Knowlton, and R. J. Whitman. 1981. Capture of apple maggot adults on pherocon, rebell, and sticky sphere traps. J. Econ. Entomol. 74: 203-206.

Neilson W. T. A., A. D. Knowlton and M. Fuller. 1984. Capture of blueberry maggot adults, *Rhagoletis mendax* (Diptera: Tephritidae), on Pherocon AM traps and on tartar red dark sticky spheres in lowbush blueberry fields. Can. Ent. 116: 113-118.

Neilson, W. T. A. and J. W. McAllan. 1965. Artificial diets for the apple maggot. III. Improved, defined diets. J. Econ. Entomol. 58: 542-543.

Neilson, W.T.A. and G.A. Wood. 1985. The blueberry maggot, economic importance, and management practices. Acta Hortic. 165: 171-175.

Parliment, T. H. and M. G. Kolor. 1975. Identification of the major volatile components of blueberry. J. Food Science 40: 762--763.

Payne, J. A. and S. H. Berlocher. 1995 a. Distribution and host plants of the blueberry maggot fly, *Rhagoletis mendax* (Diptera: Tephritidae) in Southeastern North America. J. Kansas Entomol. Soc. 68: 133-142.

Payne, J. A. and S. H. Berlocher. 1995 b. Phenological and electrophoretic evidence for a new blueberry-infesting species in the *Rhagoletis pomonella* sibling species complex. Entomol. Exp. Appl. 75: 183-187.

Prokopy, R. J. 1968. Visual responses of apple maggot flies, *Rhagoletis pomonella* (Diptera: Tephritidae): orchard studies. Entomol. Exp. Appl. 11: 403-422.

Prokopy, R. J. 1972a. Response of apple maggot flies to rectangles of different colors and shades. Environ. Entomol. 1: 720-726.

Prokopy, R. J. 1972b. Evidence for a marking pheromone deterring repeated oviposition in apple maggot flies. Environ. Entomol. 1: 326-332.

Prokopy, R. J. 1976. Feeding, mating and oviposition activities of *Rhagoletis fausta* flies in nature. Ann. Entomol. Soc. Am. 69: 899-904.

Prokopy, R. J. 1975. Selective new trap for *Rhagoletis cingulata* and *R. pomonella* flies. Environ. Entomol. 4: 420-424.

Prokopy, R. J. 1993. Levels of quantitative investigation of tephritid fly foraging behavior, 165-171. *In* M. Aluja & P. Liedo [eds.], Fruit flies: biology and management. Springer, New York.

Prokopy, R. J. and B. D. Roitberg. 1989. Fruit fly foraging behavior, pp. 293-306. *In* A. S. Robinson & G. Hooper [eds.], Fruit flies: their biology, natural enemies and control. Elsevier, Amsterdam.

Prokopy, R. J. and B. D. Roitberg. 1984. Resource foraging behavior of true fruit flies. Am. Sci. 72: 41-49.

Prokopy, R.J. and G.L. Bush. 1973. Ovipositional responses to different sizes of artificial fruit by flies of *Rhagoletis pomonella* species group. Ann. Entomol. Soc. Amer. 66: 927-929.

Prokopy, R. J. and K. I. Hauschild. 1979. Comparative effectiveness of sticky red spheres and Pherocon AM standard traps for monitoring apple maggot flies in commercial orchards. Environ. Entomol. 8: 696-700.

Prokopy, R. J. and W. M. Coli. 1978. Selective traps for monitoring *Rhagoletis mendax* flies. Prot. Ecol. 1: 45-53.

Prokopy, R.J., and J. Mason. 1996. Behavioral control of apple maggot flies, pp. 555-559. *In* B.A. McPherson and G.J. Steck [eds.]. Fruit fly pests: a world assessment of their biology

Prokopy, R. J., S. S. Cooley, J. J. Prokopy, Q. Quan, and J. P. Buonaccorsi. 1994. Interactive effects of resource abundance and state of adults on residence of apple maggot (Diptera: Tephritidae) flies in host tree patches. Environ. Entomol. 23: 304-315.

Prokopy, R. J., V. Moericke, and G. L. Bush. 1973. Attraction of apple maggot flies to odor of apples. Environ. Entomol. 2: 743-749.

Prokopy, R. J., A.L. Averill, S.S. Cooley, C.A. Roitberg, and C. Kallet. 1982. Associative learning in egglaying site selection by apple maggot flies. Science. 218: 76-77.

Prokopy, R. J., S. A. Johnson, and M. T. O' Brien. 1990. Second-stage integrated management of apple arthropod pests. Entomol. Exp. Appl. 54: 9-19.

Prokopy, R. J., S. E. Wright, J. L. Black, X. P. Hu, and M. R. McGuire. Attracticidal spheres for controlling apple maggot flies: commercial-orchard trials. Entomol. Exp. Appl. *in press*.

Prokopy, R. J., S. S. Cooley, L. Galarza, C. Bergweiler, and C. R. Lauzon. 1993. Bird droppings compete with bait sprays for *Rhagoletis pomonella* flies. Can. Entomol. 125: 413-422.

Reissig W. H. 1976. Comparison of traps and lures for *Rhagoletis fausta* and *R. cingulata*. J. Econ. Entomol. 69: 639 - 643.

Reissig, W. H. 1974. Field tests of the response of *Rhagoletis pomonella* to apples. Environ. Entomol. 3: 733-736.

Reissig, W. H., B. H. Stanley, W. L. Roelofs, and M. R. Schwarz. 1985. Tests of synthetic apple volatiles in traps as attractants for apple maggot flies (Diptera: Tephritidae) in commercial apple orchards. Environ. Entomol. 14: 55-59.

Reynolds, A. H. and R. J. Prokopy. 1997. Evaluation of odor lures for use with red sticky spheres to trap apple maggot flies. J. Econ. Entomol. 1655-1660.

Reynolds, A. H., A. M. Kaknes, and R. J. Prokopy. 1998. Evaluation of trap deployment methods to manage the apple maggot fly (Dipt., Tephritidae). J. Appl. Ent. 122: 255-258.

Russ, K., E.F. Boller, V. Vallo, A. Haisch, and S. Sezer. 1973. Development and application of visual traps for monitoring and control of populations of *Rhagoletis cersari* L. Entomophaga 18:103-116.

SAS Institute, Inc. 1989. *SAS/STAT* User's Guide, version 6, Fourth Edition, Vol. 1, SAS Institute, Cary, NC. 943 pp.

Smith, J. J. and G. L. Bush. 1996. Phylogeny of the genus *Rhagoletis* (Diptera: Tephritidae) inferred from DNA sequences of mitochondrial cytochrome oxidase II. Mol. Phylogenet. Evol. 7: 33-43.

Smith, D. C., and R. J. Prokopy. 1981. Seasonal and diurnal activity of *Rhagoletis mendax* flies in nature. Ann. Entomol. Soc. Am. 74: 462-466.

Smith, D. C., and R. J. Prokopy. 1982. Mating behavior of *Rhagoletis mendax* (Diptera: Tephritidae) flies in nature. Ann. Entomol. Soc. Am. 75: 388-392.

Stanley, B. H., W. H. Reissig, W. L. Roelofs, M. R. Schwarz, and C. A. Shoemaker. 1987. Timing treatments for apple maggot (Diptera: Tephritidae) control using sticky sphere traps baited with synthetic apple volatiles. J. Econ. Entomol. 80: 1057-1063.

Stelinski, L. L., O. E. Liburd, S. E. Wright, R. J. Prokopy, R. Behle, and M. R. McGuire. Comparison of Neonicotinoid Insecticides for use with Biodegradable and Wooden Spheres for Control of Key *Rhagoletis* Species (Diptera: Tephritidae). J. Econ. Entomol *in press*.

Stelinski, L. L., and O. E. Liburd. Evaluation of various deployment strategies of imidacloprid-treated spheres in highbush blueberries for control of *Rhagoletis mendax* Curran (Diptera: Tephritidae). J. Econ. Ent. *in press*.

Vincent, C. and M.J. Lareau. 1989. Update on the distribution of the blueberry maggot, *Rhagoletis mendax* (Diptera: Tephritidae), in Canada. Acta Horticulturae 241, Vaccinium Culture, 333-337.

Wood, G. W. 1965. Can. (Agric.) Insect Pest Review. 43: 173.

Zhang, A., C. Linn Jr., S. Wright, R. Prokopy, W. Reissig, and W. Roelofs. 1999. Identification of a new blend of apple volatiles attractive to the apple maggot, *Rhagoletis pomonella*. J. Chemical Ecology. 25: 1221-1232.

APPENDIX

Appendix 1

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.: 2000-06

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

Integrated Management Strategies for Control of Apple Maggot and Blueberry Maggot Flies

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

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)
Lukasz Stelinski

Date 4/30/01

*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 1.1

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
Rhagoletis pomonella (Walsh)	Paw Paw, MI, Van Buren Co. 20, 30, 31 July 2000 Yellow stick trap					5	5		MSU
Rhagoletis mendax Curran	Fennville, MI, Allegan Co. 6, 14, 18, July 2000 Yellow stick trap					5	5		MSU

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Lukasz Stelinski

Date 04/30/2001

Voucher No 2000-06

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

[signature] 30 APR 2001

Curator Date