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Development of a more effective behavioral approach to controlling Rhagoletis pomonella flies.

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DEVELOPMENT OF A MORE EFFECTIVE BEHAVIORAL APPROACH TO CONTROLLING *RHAGOLETIS POMONELLA* FLIES

A Dissertation Presented

by

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ABSTRACT

DEVELOPMENT OF A MORE EFFECTIVE BEHAVIORAL APPROACH TO CONTROLLING *RHAGOLETIS POMONELLA* FLIES

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The apple maggot fly, *Rhagoletis pomonella* (Walsh), is a key pest attacking apple fruit in eastern and midwestern North America. Sticky-coated 8-cm spheres baited with fruit odor (butyl hexanoate) have been the mainstay of a behavioral approach to direct maggot fly control. Improvements upon the red sphere trapping system are needed, however, if it is to be feasible and cost-effective for widespread commercial use.

Several aspects of visual and odor stimuli influencing apple maggot fly captures on sticky red spheres were investigated. Results indicated that the efficacy of spheres in capturing adults was not improved by increasing sphere size to a diameter greater than that of 8-cm or by using more synthetic fruit odor (butyl hexanoate). Significant improvement was attained by using synthetic food odor (ammonium carbonate) together with butyl hexanoate. Distance (15 - 60 cm) of a butyl hexanoate source from a red sphere had no significant effect on fly captures.

Semi-natural (field cage) conditions were used to examine response patterns of females to red spheres in relation to fly age and prior ovipositional experience. As fly age increased from a reproductively immature stage to a mature stage, the probability of a fly finding a sphere hung in a host tree increased. Simultaneously, the likelihood that a fly would deposit eggs in host fruit before encountering a sphere increased. Prior experience

with different species or cultivars of host fruit did not have significant effect on the ability of flies to find red spheres but reduced the likelihood of oviposition in unfamiliar fruit. Prior experience with the same species or cultivar of host fruit had no apparent effect on fly ability to find a red sphere trap or to oviposit in familiar fruit.

Various feeding stimulants, pesticides, and residue-extending agents were evaluated in laboratory and field cage experiments for suitability in developing a non-sticky lethal sphere. Spheres treated with a mixture containing 1.05% (a.i.) dimethoate (insecticide), 58.95% corn syrup (feeding stimulant) and 40% latex paint (residue extending agent) and not exposed to weather killed a great majority of alighting flies. However, these spheres became ineffective after exposure to weather (rainfall). Retreating weather-exposed spheres with feeding stimulant restored effectiveness.

Studies conducted in commercial orchards showed that pesticide-treated spheres, like the sticky spheres, had much potential for eliminating insecticide sprays against the flies. Current necessity of retreating pesticide-treated spheres with feeding stimulant after each rainfall compromises present utility for commercial use. Development of a polymer to protect residual effectiveness of feeding stimulant is key to further widespread commercial use of this simpler behavioral approach to controlling apple maggot flies.

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CHAPTER 1

INTRODUCTION

Apples are a crop of high economic value in the United States and elsewhere in the world. Since the colonization of this continent by Europeans, who included apples as part their agriculture, pest control has been a major component of apple production systems (Croft and Hoyt 1983). Until recently, control of apple pests has greatly, if not solely, relied on heavy use of chemical sprays on apple trees. Heavy pesticide use, however, has created a variety of environmental, ecological and toxicological problems. Concerns for these problems have called for an integrative approach to managing orchard pests (Prokopy and Croft 1994).

Apple trees and fruit are attacked by more than 500 arthropod species that feed upon them worldwide (Slingerland and Crosby, 1930). In the United States alone, apples are victimized by approximately 100 arthropod species in western, midwestern and eastern fruit-growing regions (Oatman et al. 1964). Among these 100 arthropod species, about 46 are of economic importance, 10 of which are considered serious pests. According to their economic importance and biological characteristics, Croft and Hoyt (1983) classified apple arthropod pests into three general categories: key pests, sporadic pests and induced pests. Key pests are the most destructive fruit pests and in most years must be controlled by chemical sprays (or other methods if feasible) to preserve fruit quality. Sporadic pests are generally less destructive to fruit but occasionally reach outbreak populations. The induced pests are usually pests of foliage and wood that frequently may be raised to pest status by insecticide treatments against key pests. Though each apple growing region of the world has its own peculiar set of orchard pests originating from endemic or introduced fauna, developing non- or low- insecticide approaches to controlling the key pests should be a main theme of any apple orchard integrated pest management (IPM) program. Success in developing the non- or low-

insecticide approaches to controlling key pests could greatly reduce environmental pollution from chemical sprays. Additionally, these approaches could reduce interference with natural enemies and thus facilitate the success of biological control of sporadic and induced pests (Prokopy et al. 1990b, 1990c, Christie et al. 1993).

The apple maggot, *Rhagolletis pomonella* (Walsh), is one of the most damaging key pests attacking apple fruit in eastern and midwestern North America. Native to eastern North America, it appears to have bred originally in the fruit of native large hawthorns (*Crataegus spp*), but it eventually invaded the fruit of cultivated apples about 150 years ago, after this plant was introduced to this continent during the colonial period (Bush 1966). In recent years, it has reportedly spread to the Pacific northwest region (Oregon, Washington and California) where it poses a threat to apple production. In the eastern and midwestern regions of North America, adults of apple maggot flies begin emerging from overwintering puparia beneath host apple and hawthorn trees about one month after petal fall and remain active until harvest (Dean and Chapman 1973). Eggs are deposited through the skin of the fruit into the flesh, where the larvae burrow and feed, giving rise to internal trails of bacterial decay. Even though there is only one generation per year, the two or three-month period of adult ovipositional activity demands constant attention by growers. Control of *R. pomonella* flies in commercial orchards, up to the very recent past, has been achieved mainly by 2 - 4 insecticide sprays per season against the flies.

Previous studies have shown that the apple maggot fly can build up to very large populations on wild host trees in the vicinity of commercial orchards and be capable of long distance dispersal (greater than one kilometer) when seeking host fruits (Dean 1941; Neilson 1971; Maxwell and Parsons 1968). The greatest threat of apple maggot infestations to most commercial apple orchards comes from migration of adults from adjacent unmanaged host trees, hedgerows, woods and home yards trees (Croft and Hoyt 1983, Prokopy et al. 1990b, 1990c).

Like many dipterans, apple maggot flies are adept at using both visual and/or odor cues to find resources such as food, mates, and oviposition sites that are essential for their reproductive success (Prokopy and Roitberg 1984). Over the past decade, studies on resource-finding behaviors of adult *R. pomonella* flies have led to the development of two types of traps that are attractive to both sexes. The first is a yellow rectangle, considered to represent a super-normal foliage-type stimulus to flies seeking feeding sites; the other is an 8-cm red sphere, considered to represent a super-normal fruit-type stimulus to flies seeking mates and egglaying sites (Prokopy 1968, Owens and Prokopy1986). Yellow rectangles are rendered more attractive by the addition of synthetic food odor in the form of compounds releasing ammonia (Jones 1988). Attractiveness of red spheres is enhanced by the addition of synthetic fruit odor in the form of butyl hexanoate and other fruit esters (Reissig et al. 1985). In commercial orchards in eastern North America, however, baited red spheres have proven far more effective than baited yellow rectangles in capturing *R. pomonella* flies throughout the growing season (Drummond et al. 1984).

Trapping methods using unbaited or baited sticky red spheres have proven successful as monitoring devices to time chemical sprays in chemically-based IPM programs (Prokopy and Hauschild 1979, Stanley et al. 1987, Agnello et al. 1990). Recently, they have also proven useful as a direct-control approach to trapping out adult flies in biologically-based IPM (Prokopy et al. 1990b, 1990c, Prokopy 1991b). Prokopy et al. (1990b, 1990c) showed that ringing perimeter trees of a commercial orchard with baited red spheres 5 m apart could provide control of *R. pomonella* flies. This approach eliminates all insecticidal sprays against *R. pomonella*, allowes natural enemies of foliar pests to build up during mid- and late-season in the absence of insecticide, and thereby facilitates biological control of mid and late season foliar pests. However, the success of this sphere trapping system in controlling *R. pomonella* flies relies on the effectiveness of red spheres to capture or kill the adult flies before they have initiated oviposition, which is under the influence of a number of environmental and fly factors. Understanding the

influence of various factors on trapping efficacy is critical to further improvement in effectiveness of traps in protecting apple fruit.

In addition, results of several years of pilot experiments by Prokopy et al. (1990b, 1990c) have shown that reliance on sticky as a mechanism to kill flies alighting on sphere traps has become an impediment to large-scale use of this system to control *R. pomonella*. This is because the sticky is too awkward to handle, and its deployment and mantainence is too labor-expensive to have appeal to growers. It is necessary, therefore, to develop a cost-effective alternative as a substitute for sticky to kill alighting flies.

The overall objective of the research reported in this dissertation was to seek improvements on the currently-used sticky-sphere system so that it could be feasible for large-scale use as a cost-effective control method against *R. pomonella* flies. The first research chapter, chapter 2, was designed to investigate various visual and odor factors influencing the effectiveness of red spheres to capture *R. pomonella* flies in commercial orchards. The purpose of this study was to identify the best visual and odor combination that could be used to enhance the attractiveness of *R. pomonella* to the spheres.

Chapter 3 concerns *R. pomonella* responses to red sphere traps in relation to fly age and prior-ovipositional experience. The purpose of this study was to investigate the influence of fly age and experiences on the effectiveness of red spheres in protecting host fruit . The results of this study provided base-line information on how these fly factors could influence the success and failure of the red sphere system to control *R. pomonella* flies in commercial orchards (which may reflect different fly experience and age structures).

Chapters 4 and 5 concern the development of pesticide-treated spheres as a substitute for sticky-coated spheres for controlling *R. pomonella* flies. Such development required in-depth assessment of the efficacy of different feeding stimulants, pesticides and residue-extending agents. Chapter 4 deals with fly-feeding stimulants, and chapter 5 deals with pesticides and residue-extending agents.

The final study, chapter 6, was designed to further evaluate the final product of Chapters 4 and 5 (pesticide-treated spheres) under semi-field and field (commercial orchard) conditions for controlling *R. pomonella*. This chapter required the integration of findings of all the above research chapters. In it, we discuss further improvements and provide a prospectus of using the sphere trapping system to control *R. pomonella* flies.

Although the studies presented in this dissertation do not involve any new or novel theories, the experiments conducted represent a blend of basic and applied research. Results from these studies offer a significant contribution to the success of an advanced (biologically-based) IPM program in apple growing regions in which *R. pomonella* flies are a key pest.

CHAPTER 2

VISUAL AND ODOR STIMULI INFLUENCING EFFECTIVENESS OF STICKY SPHERES FOR TRAPPING APPLE MAGGOT FLIES

2.1 Introduction

The apple maggot fly, *Rhagoletis pomonella* (Walsh), is a major pest of apples in eastern North America. Various traps to capture *R. pomonella* adults have been developed and used to monitor or control *R. pomonella* in commercial orchards. The most widely used trap in the eastern United States is a sticky-coated 8-cm diameter red sphere, considered to be a visual mimic of host fruit, the site of *R. pomonella* mating and oviposition (Prokopy 1968). Unbaited or baited sticky spheres have been used successfully to estimate abundance of *R. pomonella* and properly time insecticide applications against *R. pomonella* (Prokopy and Hauschild 1979, Reissig and Tette 1979, Prokopy et al. 1980, Stanley et al. 1987, Agnello et al. 1990). Baited sticky spheres have also been used successfully in ringing the perimeter of apple orchards to intercept *R. pomonella* immigrating into orchards from neighboring wild host trees (Prokopy et al. 1990b, 1990c). As pointed out by Prokopy et al. (1990b, 1990c), some improvements in the baited sticky sphere system are needed if it is to be feasible for widespread commercial use as a direct control measure.

One potential area of improvement lies in enhancing the attractiveness of baited sticky spheres to *R. pomonella* to ensure capture of a high proportion of immigrants on perimeter trees before they can penetrate into the orchard interior. Although much research has been carried out on trapping *R. pomonella*, there remain several gaps in our knowledge.

With respect to visual aspects of baited sticky spheres stimulating to fruit-seeking *R. pomonella*, spheres are more attractive than other shapes (Prokopy 1968), red is

equally or more attractive than other colors (Prokopy 1968, Owens and Prokopy 1986), and spheres of ca. 8 cm diameter are more attractive than spheres of ca. 4, 6, 15, 23, 30, or 45 cm diameter (Prokopy 1968, 1977). To date, however, *R. pomonella* responses to red spheres larger than 8-cm but smaller than 15-cm has not been studied.

With respect to olfactory aspects of baited sticky spheres, Fein et al. (1982) identified 7 volatile esters of Red Delicious and Red Astrachan apples attractive to *R. pomonella*: hexyl acetate, (E)-2-hexen-1-yl acetate, butyl 2-methyl butanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate, and hexyl butanoate. In field studies, Reissig et al. (1982, 1985) showed that sticky red spheres baited with this blend of volatiles [minus (E)-2-hexen-1-yl acetate] captured 2-4 times more *R. pomonella* than unbaited spheres. Subsequently, field studies revealed that a single component of this blend, butyl hexanoate, was just as attractive to *R. pomonella* as the combination of all components (Averill et al. 1988). Other investigations showed that attractiveness of butyl hexanoate and other volatile components varied substantially according to release rate (Reissig et al. 1982, 1985, Carle et al. 1987) and type of dispenser (Jones 1988). At very close range (within a few cm), butyl hexanoate released from a 2-dram polyethylene vial at a rate of ca. 700 apple equivalents per hour (the standard 500 ug/hour rate used for trapping) may even be repellent to approaching *R. pomonella* (Aluja 1989). To date, the influence on *R. pomonella* captures of varying amounts of butyl hexanoate at varying distances from a sticky red sphere has not been studied extensively.

Besides fruit odor, components of the odor of food also are attractive to *R. pomonella* (Hodson 1943, 1948, Neilson, 1960). The major food-type attractant, ammonia, has been used primarily in combination with sticky yellow rectangle traps to monitor presence of *R. pomonella* in and nearby orchards, particularly in western North American and eastern Canada (Prokopy 1968, 1975, Reissig 1974, 1975a, Aliniazee et al. 1987, Jones and Davis 1989, Warner and Smith 1989). Sticky yellow rectangles are considered to be visual mimics of foliage (Prokopy 1968). They are considerably less

effective than sticky red spheres in monitoring *R. pomonella* in eastern USA commercial orchards (Reissig 1975a, Prokopy and Hauschild 1979, Drummond et al. 1984). Until now, the combined value of butyl hexanoate and ammonia in attracting *R. pomonella* to red spheres has not been investigated.

A second potential area of improvement lies in substituting for sticky as the mechanism for controlling *R. pomonella* that alight on baited spheres when spheres are placed on perimeter apple trees to intercept immigrating *R. pomonella*. One potential substitute might be a mixture containing a fly feeding stimulant and a pesticide used in combination with some material to protect the residual effectiveness of feeding stimulant and pesticide against degradation by rainfall and sunlight (Prokopy et al. 1990b). *Rhagoletis pomonella* alighting on a sphere might feed, ingest pesticide and die before ovipositing. One potentially effective method of protecting residual effectiveness of feeding stimulant and pesticide might be to place a conical "roof" above a sphere as a shield. To date, the effect of such a roof on attractiveness of a sphere to *R. pomonella* has not been examined.

Here, we evaluated in a commercial orchard the effect on *R. pomonella* captures on sticky red spheres of (a) sphere size (8 vs. 10 cm), (b) different numbers of polyethylene vials (0, 1, 2, or 4) containing butyl hexanoate at 3 different distances (15, 30, or 60 cm) from a sphere, (c) presence or absence of vials of ammonia in combination with vials of butyl hexanoate as odor bait, and (d) presence or absence of a conical roof (yellow, green or clear) in combination with a vial of butyl hexanoate placed above, to the side, or below a sphere.

2.2 Materials and Methods

All trials were conducted in 1989 and 1990 in a mixed planting of Early McIntosh and Gravenstein apple trees in a commercial orchard in Deerfield, MA that received insecticide treatment through June but not thereafter. The orchard consisted of ca. 90 trees

each ca. 6 m in canopy diameter and supported a moderate population of *R. pomonella* during July and August. All spheres were hung ca. 0.5-1 m from the perimeter of the tree canopy and ca. 1.5 m above ground, with as much fruit and foliage as possible surrounding each sphere at a distance of 20 - 30 cm but little between 0 and 20 cm. We employed only 1 sphere per tree.

In experiment 1, we compared attractiveness of unbaited 8 cm versus unbaited 10 cm diameter spheres painted Tartar Red Dark and coated with Tangletrap®. Spheres were emplaced on July 23. Weekly, spheres were cleaned, captured *R. pomonella* were counted and sexed, and spheres were rotated to account for position effect. Spheres were removed on August 21. Fly captures on spheres were analyzed using the Student's t test.

In experiment 2, we evaluated *R. pomonella* responses to Tangletrap-coated 8 cm red spheres baited with different numbers of 2-dram (15 ml) polyethylene vials (0, 1, 2, or 4) containing butyl hexanoate (2.5 ml) fastened by wire at different distances (15, 30, or 60 cm) from the side of a sphere. We purchased vials from Andler Israel and Sons, Evrett, MA, USA and butyl hexanoate from Penta International Corporation, West Caldwell, NJ. Each vial was capped. The odor diffused through the polyethylene side wall. To minimize the effect of tree location, each of the 4 rows of 12 trees used in this experiment was divided into 3 units. Each tree in a unit was assigned the same distance (15, 30, or 60 cm) of vial from sphere. To minimize the effect of sphere location within a unit, treatments of different numbers of vials per sphere (0, 1, 2, or 4) were rotated every 4 days among trees within a unit during the 16 day period of the experiment so that each treatment appeared at each sphere location once. At each rotation, spheres were cleaned and captured *R. pomonella* were counted and sexed. The experiment was conducted initially from July 19 - August 3, 1989 and repeated from July 3 - July 19, 1990. Data from each year were analyzed separately using a two-way ANOVA subjected to strip-plot design (Milliken and Jonhson 1984), where columns consisted of numbers of vials and rows of distances

of vials from a sphere. A replicate was considered to be a sphere baited with same number of vials at the same distance over the 16-day test period

In experiment 3, we assessed *R. pomonella* responses to Tangletrap-coated 8-cm red spheres baited either with 2 capped 15-ml polyethylene vials (each with a 3-mm opening on the side wall just beneath the cap of ammonium carbonate (5 g), 2 capped vials of butyl hexanoate (2.5 ml), a vial of each of these 2 types, or no vial (unbaited control). Vials were positioned 30 cm from the side of a sphere. Each row of trees was divided into 2 units of 4 trees each. Each tree in a unit was randomly assigned one of the 4 treatments. To minimize effect of sphere location within a unit, treatments were rotated every 3 days among trees within a unit during the course of the 12-day experimental period (July 7-19). At each rotation, spheres were cleaned. *Rhagoletis pomonella* captured from July 13 - 19 were collected, soaked in paint thinner for 24 h to dissolve Tangletrap, placed in 70% alcohol and dissected to determine the proportion of females that contained ovaries with mature eggs. Data were analyzed by ANOVA (split-plot design). Mean numbers of *R. pomonella* captured per treatment over the 12-day period were separated by the LSD test criterion (0.05 level).

In experiment 4, we evaluated the effect on *R. pomonella* captures of placing a conical roof (green cardboard, yellow cardboard, or clear plastic) above Tangletrap-coated 8-cm red spheres. The rim of each cone was 16 cm diameter. The peak of each cone was 2 cm above the sphere, with the rim extending mid-way down the side of the sphere at a distance of 4 cm from the sphere surface. We reasoned that if a cone was any larger than the size we selected, it would be difficult to emplace among twigs and branchlets that normally surround a well-positioned sphere. If the cone was any smaller than the size we selected, it might not protect the sphere sufficiently from rainfall and sunlight. An uncovered sticky sphere was used as a control treatment. Each sphere was baited with a single vial of butyl hexanoate placed either 15 cm above, to the side or beneath the sphere. The experimental design and analysis were similar to that of

experiment 2. Spheres were emplaced on July 13. Treatments of the 4 different conical roof types were rotated every 5 days within a replicate to equalize position effect over the 20-day test period of the experiment. At each rotation, spheres were cleaned and captured *R. pomonella* were counted and sexed.

2.3 Results

In experiment 1 (Figure 2.1), over the entire 4-week test period, 53% more female *R. pomonella* and 80% more male *R. pomonella* were captured on 8 cm than 10 cm spheres (for females, $t=2.30$, $df = 38$, $p < 0.05$; for males, $t = 2.92$, $df = 38$, $p < 0.01$). The pattern of numerically greater captures on 8 cm than 10 cm spheres held true each of the 4 sampling periods.

In experiment 2 (Table 2.1), for the initial run in 1989, 20, 87, and 53% more females and 25, 54 and 46% more males were captured when 1, 2, or 4 vials of butyl hexanoate were placed around a sphere (all distances from a sphere combined) than when 0 vials were placed around a sphere. For the repeat run in 1990, respective values were 62, 57, and 90% more females and 63, 49, 47% more males captured when 1, 2, or 4 vials were used compared with 0 vials. For the initial run in 1989, 31 and 19% more females and 44 and 36% more males were captured when vials were placed 30 or 60 cm from a sphere (combined data for 1, 2 and 4 vials) than 15 cm from a sphere. For the repeat run in 1990, respective values were 30 and 32% more females and 49 and 25% more males captured at vial distances of 30 or 60 cm from a sphere than at 15 cm. ANOVA showed a significant effect of number of vials around a sphere on *R. pomonella* captures in both 1989 and 1990, no significant effect on captures of distance of vials from a sphere either in 1989 or 1990, and no significant effect of an interaction between number of vials around a sphere and distance of vials from a sphere either in 1989 or 1990 (Table 2.1). Separation by LSD of fly capture means among spheres treated with different numbers of vials over all distances showed that spheres baited with 2 or 4 polyethylene vials did not

capture significantly more female or male flies than those baited with 1 vial in either 1989 or 1990 (0.05 level). In nearly all cases, however, spheres baited with 1 or more vials captured significantly more flies than spheres with 0 vials.

In experiment 3 (Figure 2.2), 135, 74, and 68% more females were captured on spheres baited, respectively, with 1 vial each of ammonium carbonate and butyl hexanoate, 2 vials of ammonium carbonate, or 2 vials of butyl hexanoate than on unbaited spheres. For males, respective values were 208, 34, and 79% more captured than on unbaited spheres. Captures for each sex were significantly greater for 1 vial each of ammonium carbonate and butyl hexanoate than for 2 vials of ammonium carbonate or 2 vials of butyl hexanoate, between which there was no significant difference. Unbaited spheres captured significantly fewer flies than any of the baited sphere treatments. Of females captured on ammonium-carbonate-baited spheres, 48% were sexually mature compared with 63 and 62% mature females captured on spheres baited with butyl hexanoate or unbaited spheres ($X^2 = 5.563$, df = 2, $p = 0.06$). There was a significant interaction between sampling interval and comparative level of fly response among the 4 treatments (for both females and males $p < 0.05$). This indicated that for each sex, the level of response to one treatment compared with another was not consistent from one sampling interval to the next.

In experiment 4 (Table 2.2), only 9, 20 and 32% as many females were captured on spheres protected by a green, yellow or clear conical roof as on unprotected spheres (combined data for vials of butyl hexanoate positioned 15 cm above, to the side or below a sphere). For males, respective values were 13, 20, and 25% as many captured on protected as on unprotected spheres. ANOVA showed a significant effect of protective conical roofs on *R. pomonella* captures, no significant effect of position of vials on *R. pomonella* captures, and no significant effect of an interaction between type of conical roof protection and position of vial (Table 2.2). Separation by LSD of fly capture means among spheres covered with different types of conical roof showed that spheres covered

with clear roofs captured significantly more female flies than those covered with green roofs. For each sex, significantly more flies were captured on unprotected spheres than on protected spheres.

2.4 Discussion

Together, our findings indicate that significant improvement in use of baited sticky spheres for monitoring or controlling *R. pomonella* is unlikely to result from increasing sphere size to a diameter greater than that of the presently recommended 8 cm size or from baited spheres with 2 or 4 vials of butyl hexanoate rather than the presently recommended 1 vial. Our findings do indicate, however, significant improvement may be attained by using a vial of ammonium carbonate in combination with a vial of butyl hexanoate.

Prokopy (1968, 1977) hypothesized that red spheres of 8 cm diameter represented a super-normal fruit-type stimulus to *R. pomonella* because such spheres were larger than the size of fruit of the native host hawthorn (ca. 2 cm) or the recently acquired hosts of apple (ca. 5 - 6 cm) and cherry (ca. 2 cm). Apparently even a slight increase from 8 to 10 cm sphere size in our study was great enough to render a red sphere as representing to some *R. pomonella* an object different from a fruit, at least under the orchard conditions of our test where the apples on the trees were ca. 4 - 6 cm diameter. Evidence (Prokopy et al., 1993) suggests that *R. pomonella*, like *Ceratitis capitata* (Weidemann) and *Dacus dorsalis* (Hendel) flies (Prokopy et al, 1989, 1990a), are able to learn to find host fruit, with fruit size being the most important physical character learned. Thus, if a 10 cm red sphere were to be used in trees bearing exceptionally large apples (e.g. 8 cm), they might be just as attractive as 8 cm spheres.

Aluja (1989) found that the odor of fruit guides *R. pomonella* to a host tree or a portion of a host tree but that within a portion of a tree, individual fruit are found primarily on the basis of visual characteristics. Indeed, *R. pomonella* flies appear to use

odor as an aid in finding an individual fruit only when fruit are of rather unapparent color (light green or yellow) or masked by dense foliage (Aluja 1989). One might expect, therefore, that increasing the number of vials of butyl hexanoate in association with a red sphere would lure more *R. pomonella* to a portion of a tree but not necessarily to an individual fruit (or a red sphere fruit mimic). We found here that increasing the amount of butyl hexanoate released within 60 cm of a sphere from the standard 700 apple equivalents per hour (1 vial) to 1400 or 2800 apple equivalents per hour (2 or 4 vials) had no significant effect on *R. pomonella* captures. Nor was there any significant effect on *R. pomonella* captures of distance (15, 30, or 60 cm) of one or more vials of butyl hexanoate from a sphere. Reissig et al. (1982) showed that polyethylene caps loaded with 50 or 100 mg of the 6-ester blend of apple volatiles, when fastened near the top of a sphere, were more effective in attracting *R. pomonella* to spheres than caps containing 300 mg. Jones and Davis (1989) observed that despite an approximately 18-fold difference in release rate of apple volatiles in the field (from 120 to 2200 u_g/h), there was no significantly greater effect on captures of *R. pomonella* on spheres baited with volatile lures (blend or butyl hexanoate alone). Combined evidence to date therefore suggests that increasing the amount (or release rate) of apple volatiles above a certain high concentration (or release rate) would not increase effectiveness in attracting *R. pomonella* to red sphere traps.

Both ammonia and butyl hexanoate are olfactory stimuli to *R. pomonella,* but each is associated with a different type of response. Ammonia emanates from proteinaceous tephritid fly food (Bateman and Morton 1981, Morton and Bateman, 1981, Hendrichs et al, 1990). Butyl hexanoate is emitted by host fruit in a condition favorable to *R. pomonella* mating and oviposition (Carle et al, 1987). We were somewhat surprised to find that a combination of these 2 types of olfactory stimuli elicited a significantly greater number of alightings of both female and male *R. pomonella* on red spheres than did butyl hexanoate alone. Our surprise stems from the fact that sources of *R. pomonella* food in nature occur almost exclusively on foliage rather than on fruit (Hendrichs and Prokopy

1990). We found that compared with *R. pomonella* that alighted on spheres baited with butyl hexanoate alone, the proportion of *R. pomonella* that alighted on spheres baited with ammonium carbonate alone and that were sexually mature was somewhat less . This is consistent with data that indicate less mature *R. pomonella* feed more often than mature *R. pomonella* (Webster et al. 1979) and with data from other studies showing comparatively greater response of *R. pomonella* to food-type stimuli by immature than mature females (Hodson 1943, Hendrichs et al. 1990). Possibly many *R. pomonella* in the orchard in which our tests were conducted had originated within the orchard rather than immigrated from the outside of the orchard, and therefore they had more opportunity to search for food in the vicinity of vials of ammonium carbonate before becoming sexually mature. In some cases, by chance alone they may have alighted on an adjacent sphere. Whatever, further study is needed to verify the positive benefit on *R. pomonella* captures in commercial orchards of using a source of ammonia in conjunction with butyl hexanoate, especially in regard to variation in response among different weeks of the fly activity season.

It was disappointing to us that, irrespective of color, conical roofs used to protect spheres from rainfall and sunlight significantly reduced *R. pomonella* captures. Unlike *Rhagoletis fausta* (Osten Sacken) and *R. mendax* Curran flies, which approach host fruit and visual host fruit mimics primarily from below (Prokopy 1977, Prokopy and Coli 1978), *R. pomonella* flies approach fruit with almost equal probability from above and below (Prokopy 1977). Possibly this explains why comparatively few *R. pomonella* alighted on spheres where visual properties were partially obscured from the side and completely obscured from above by a conical roof. Interestingly, the obscuring effect was least (though still significant) in the case of clear acetate roofs compared with opaque roofs. These findings indicate that replacement of sticky by a coating of fly feeding stimulant and toxicant on a sphere will probably require using some sort of chemical

residue-extending agent as part of the coating rather than involve use of a roof as a protectant.

We conclude that a red sphere of 8 cm diameter baited with a single polyethylene vial of butyl hexanoate (500 ug / h release rate) hung 15 - 60 cm from the sphere is as effective in capturing female and male *R. pomonella* flies as a larger red sphere or a sphere baited with 2 or 4 vials of butyl hexanoate. Ammonia in combination with butyl hexanoate increased *R. pomonella* captures over unbaited spheres more than either odor alone and needs further study. Spheres partially obscured by a conical roof protectant are ineffective.

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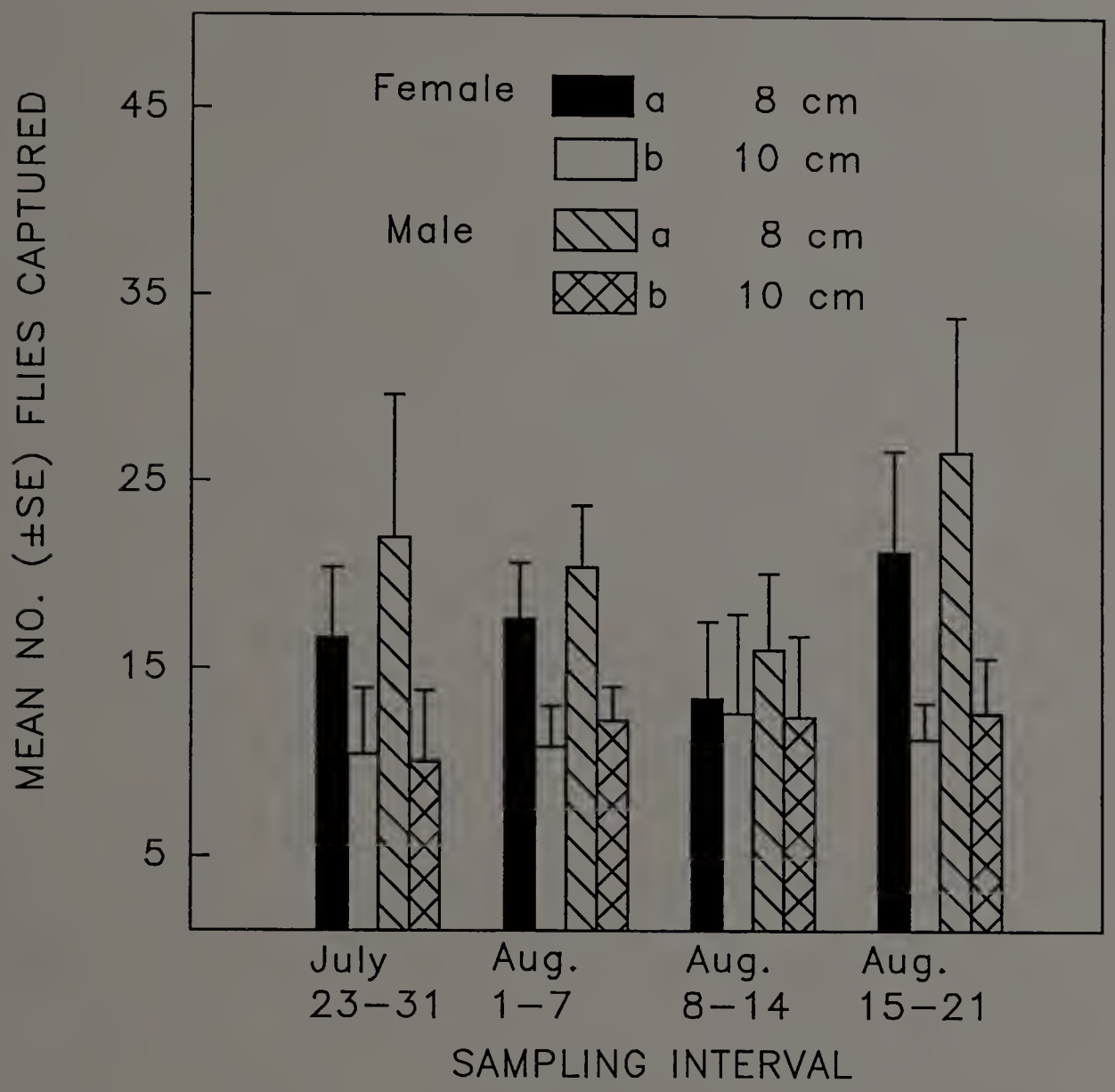

Figure 2.1. Mean number of *R. pomonella* captured on unbaited sticky 8 cm and 10 cm red spheres at each sampling interval (7 days). Within each sex, legend bars followed by the same letter are not significantly different in capture of flies throughout the trial period (28 days) according to the Student's t test ($p<0.05$).

Table 2.1. Mean number of *R. pomonella* flies captured on 8-cm sticky spheres baited with different numbers of 2-dram polyethylene vials containing synthetic apple odor (butyl hexanoate) at different distances from a sphere. Four replicates per treatment in 1989; 3 replicates per treatment in 1990.

No. of vials around each sphere	Distance of vials from a sphere[a]						Means (±SE) for each no. of vials around sphere over all distances[b]	
	15 cm		30 cm		60 cm			
	Female	Male	Female	Male	Female	Male	Female	Male
				In 1989 (July 19 - August 3)				
0	11.8	21.8	16.8	29.5	17.5	32.0	15.3 (1.5)b	27.8 (2.3)b
1	17.0	30.0	16.5	43.0	20.8	31.3	18.1 (1.9)ab	34.8 (3.1)a
2	22.3	30.1	37.3	45.0	24.0	52.3	27.8 (3.6)a	42.7 (4.4)a
4	19.5	32.5	22.8	46.8	25.3	43.5	22.5 (1.9)a	40.9 (2.9)a
				In 1990 (July 3 - July 19)				
0	16.0	23.0	20.7	41.7	24.0	31.3	20.2 (2.7)b	32.0 (7.1)b
1	26.3	48.7	38.0	60.0	33.0	48.3	32.4 (3.0)a	52.2 (9.3)a
2	31.3	43.7	30.0	52.3	34.0	54.0	31.8 (2.8)a	50.0 (7.8)a
4	27.7	27.3	42.7	66.7	45.3	47.3	38.6 (3.9)a	47.1(7.3)ab

[a]Means within each distance over the number of vials around each sphere are not compared since ANOVA (strip-plot design) showed no significant effect of distance: in 1989, for females, $F = 1.82$, $df = 2,6$, $p = 0.23$; for males, $F = 2.26$, $df = 2,6$, $p = 0.17$; in 1990, for females, $F = 1.05$, $df = 2,4$, $p = 0.50$; for males, $F = 0.55$, $df = 2,4$, $p = 0.87$. Nor was there any significant interaction between distance and number of vials: in 1989, for females, $F = 1.78$, $df = 6,18$, $p = 0.16$; for males, $F = 1.69$, $df = 6,18$, $p = 0.18$; in 1990, for females, $F = 1.10$, $df = 6,12$, $p = 0.42$; for males, $F = 0.86$, $df = 6,12$, $p = 0.55$. [b] ANOVA showed a significant effect of number of vials around a sphere ($p < 0.05$) in both 1989 and 1990 for females and males. Means followed by the same letter are not significantly different from each other at the 0.05 level according to LSD.

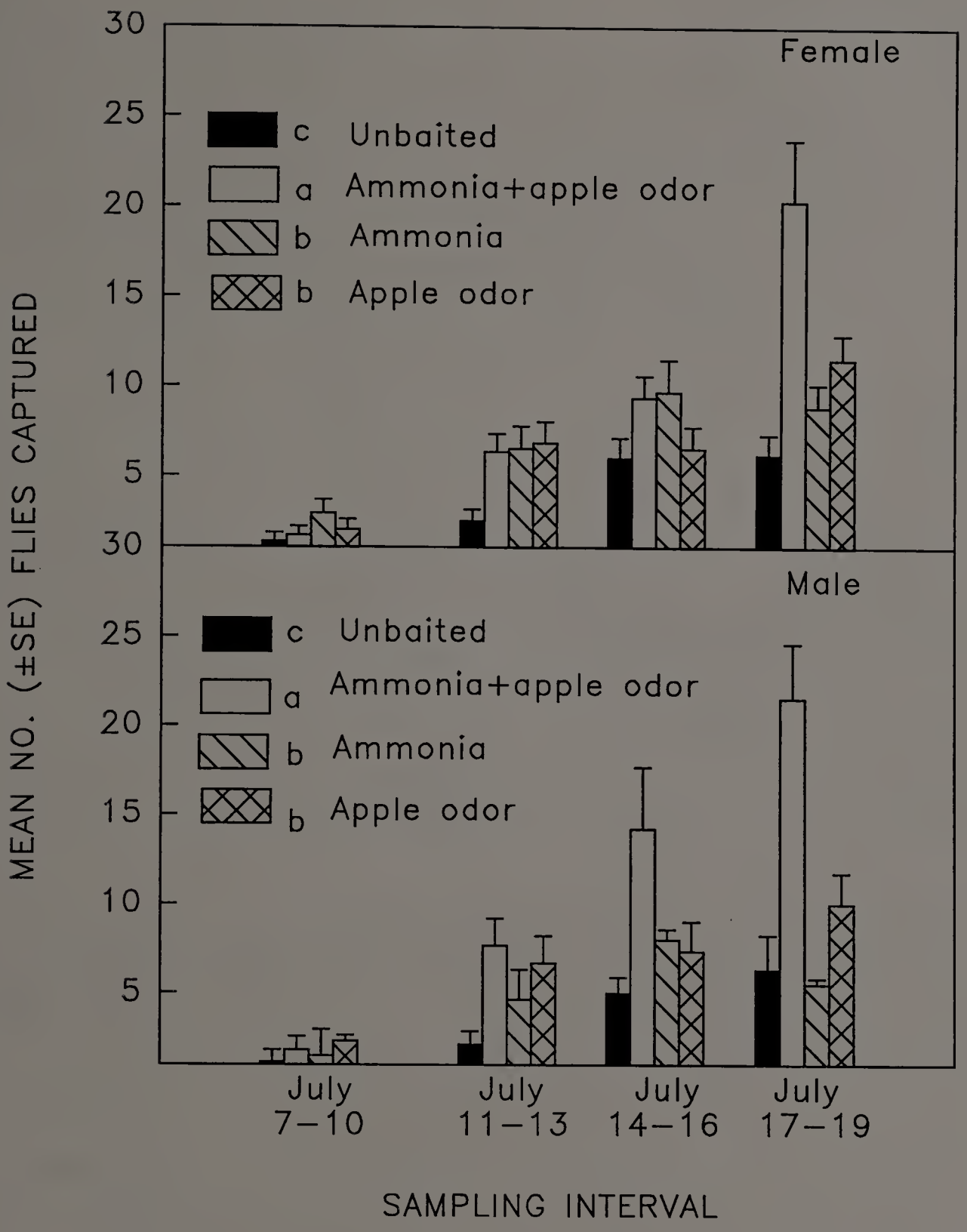

Figure 2.2. Mean number of *R. pomonella* captured on sticky 8 cm red spheres baited with either 2 vials of ammonium carbonate, 2 vials of butyl hexanoate , 1 vial of ammonium carbonate and 1 vial of butyl hexanoate or no vial at each sampling interval (3 days). Within each sex, the legend bars followed by the same letter indicate no significant difference throughout the trial period (12 days) according to LSD ($P<0.05$).

Table 2.2. Mean number of *R. pomonella* flies captured on 8-cm sticky spheres baited with a vial of butyl hexanoate without or with a green, yellow, or clear plastic conical roof above the sphere (July 13 - August 2, 1989). Four replicates per treatment.

Color of Conical roof	Position of vial relative to sphere						Means (±SE) for each type of conical roof over all positions[b]	
	15 cm above		15 cm to side		15 cm below			
	Female	Male	Female	Male	Female	Male	Female	Male
Green	2.0	3.7	1.0	6.5	1.5	4.3	1.5 (0.3)c	4.8(0.8)b
Yellow	5.0	13.0	3.0	5.0	1.8	5.0	3.3 (0.5)bc	7.6 (1.5)b
Clear	5.3	12.3	7.3	9.5	3.5	6.8	5.3 (0.9)b	9.5 (1.4)b
No roof	18.8	36.2	17.5	27.5	14.0	20.0	16.8 (2.1)a	27.9 (3.7)a

[a]Means for each position of vial over all types of conical roof were not compared with each other since ANOVA (strip-plot design) showed no significant effect of position: for females, $F = 1.74$, $df = 2,9$, $p = 0.18$; for males, $F = 1.97$, $df = 2,9$, $p = 0.18$. Nor was there any significant interaction between vial position and type of conical roof: for females, $F = 0.38$, $df = 6,18$, $p = 0.89$; for males, $F = 1.33$, $df = 6,18$, $p = 0.25$.
[b]ANOVA showed significant effect of type of conical roof. Means followed by the same letter are not significantly different from each other at the 0.05 level according to LSD.

CHAPTER 3

APPLE MAGGOT FLY RESPONSE TO RED SPHERE TRAPS IN RELATION TO FLY AGE AND EXPERIENCE

3.1 Introduction

An 8-cm red sphere represents an attractive super-normal fruit type stimulus to an apple maggot fly, *Rhagoletis pomonella* (Walsh), signalling a potential mating or oviposition site (Prokopy, 1968). Sticky red spheres, unbaited or baited with fruit odor (butyl hexanoate), have proven important to apple IPM programs in eastern North America through being an effective monitoring method for timing sprays against *R. pomonella* (Stanley et al., 1987; Agnello et al., 1990) as well as being a useful behavioral approach for directly controlling adults (Prokopy, 1975, 1985; Reissig et al., 1984). Recently, Prokopy et al. (1990b) demonstrated that ringing an orchard with fruit-odor-baited sticky red sphere traps placed 5 m apart on perimeter apple trees can provide control of *R. pomonella*. This approach eliminates all insecticidal sprays against *R. pomonella*, allows natural enemies of foliar pests to build up during mid and late season in the absence of insecticide, and has become an essential element in advanced (biologically-based) apple IPM programs in Massachusetts (Prokopy et al., 1990b; Christie et al., 1993).

As pointed out by Prokopy & Lewis (1992), however, the probable success of this approach is linked intimately with a variety of environmental and individual fly factors that influence the effectiveness of red sphere traps in capturing gravid females before they initiate oviposition into host fruit. While several researchers have investigated environmental factors such as tree size, foliar density, fruit cultivar, fruit density, fruit distribution and fruit maturity that influence fly captures on sphere traps in the field (e.g. Reissig, 1975b; Drummond, et al., 1984; Murphy, et al., 1991), no study has been

published on the influence of individual fly factors such as age and prior ovipositional experience (learning) on the efficiency of red sphere traps in capturing *R. pomonella* flies.

In many insects, adult age is one of the important physiological state factors that contributes to variation in behavior over time (Jaenike and Papaj, 1992; Browne, 1993). Like many other tephritids, *R. pomonella* flies do not commence reproductive behavior (mating, oviposition) until passage of a specific period of time after emergence (termed "pre-reproductive phase"), irrespective of the quantity and quality of food consumed (Fletcher and Prokopy, 1990). Information on how changes in fly age influence host fruit foraging behavior and/or responses to red sphere traps hung in host trees should be useful in further characterizing the value of sticky red spheres for controlling *R. pomonella.*

Learning, a process of behavioral change contingent upon individual experience, can markedly affect insects' response to particular resources (reviewed in Papaj & Prokopy, 1989; Szentesi & Jermy, 1990; Prokopy & Lewis, 1992). In three tephritids [*R. pomonella*; the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann); and the Queensland fruit fly, *Bactrocera tryoni* (Froggatt)], studies have revealed that females are capable of learning to find as well as to accept (bore into) or reject host fruit for oviposition (Prokopy et al., 1982; 1986; 1989; 1990a; 1991a; 1993; Prokopy & Fletcher, 1987; Papaj & Prokopy, 1988). To date, however, the effect of prior ovipositional experience on the probability of *R. pomonella* flies alighting on red sphere traps has not been studied. Information on this aspect would also be valuable in assessing the effectiveness of sticky red spheres in controlling *R. pomonella* flies originating from different habitats and having prior ovipositional experiences.

Here, we investigated the influence of fly age and prior ovipositional experience on the probability of a *R. pomonella* female finding a baited 8-cm red sphere hung in a field-caged potted apple tree containing green or red apples. We also examined other parameters related to fruit foraging behavior, such as frequency of fruit visitation and oviposition.

3.2 Materials and Methods

For all experiments, flies originated from puparia formed by larvae that infested apples collected from unsprayed trees in Amherst, Massachusetts. Females and males were maintained together from eclosion onward in 30x30x30 cm aluminum screen/plexiglass cages (at ca. 50 females and 20 males per cage). Each cage was supplied with water and food: a 5x7-cm strip of filter paper dipped in an aqueous slurry of enzymatic yeast hydrolysate and sucrose (1:4 ratio) and dried before use. All flies were kept at ca. 25^{o}C and 65% RH under an 18 h photoperiod regimen.

In experiment 1 (1991), we examined response patterns of females of five different ages (3, 7, 11, 15, and 19 days old) to a baited 8-cm red sticky sphere in a potted apple tree (ca. 1.5 m diam canopy) containing green (immature) or red (mature) Gravenstein apples. The tests were conducted outdoors within a 3x3x3 m field cage in which the potted tree was placed. The top of the cage was covered with a partly opaque green tarpaulin to exclude direct sunlight during tests. The green apples, picked June 17 from trees unsprayed since May 20 and stored at 3^{o}C, averaged 37 mm diam (range 35 - 41 mm) and were tested from June 20 - July 10. The red apples, picked August 1 from the same trees and stored in the same manner, averaged 49 mm diam (range 45 - 53 mm) and were tested from August 2 - 18. All fruit were rinsed thoroughly in tap water before use.

For each test, 50 apples (either green or red) were hung in the tree canopy by attaching the fruit stem to tree branchlets using copper wire. Fruit distribution was evenly spaced and was fixed throughout the entire experiment by marking initial fruit positions. Foliage was trimmed so that the ratio of leaves to fruit was kept at 30:1. An 8-cm red sticky sphere baited with one 2-dram polyethylene vial of synthetic fruit odor (butyl hexanoate) was hung in the upper 1/3 of the tree canopy. The sphere was surrounded by foliage and fruit except within a 15-cm radius cleared of foliage and fruit.

For each test, an individual fly selected randomly among flies of different ages was released onto a leaf located in the lower central portion of the tree canopy using a fruit (same variety as test fruit) coated with sucrose and attached to a probe. A fly spent ca. 10 sec on the fruit before release. Using a stop watch and datapad, we monitored the time at which the female left the release leaf, fruit visitation, ovipositional behavior, and incidence and time of landing on the sphere. Flies that did not leave the release leaf within 15 min were discarded. Oviposition was initially identified by ovipositor dragging after a fly had bored into a fruit (Prokopy, 1972), and was later confirmed by microscopic examination of the fruit flesh for eggs. A trial ended when a fly landed on the sphere, left the tree, or 1 h elapsed. Temperature in the tree canopy was measured at the end of each trial. It averaged 29.3 ($\pm$ 0.6) ^{o}C in tests with green fruit and 28.7 ($\pm$ 0.8) ^{o}C tests with red fruit. Each fly was collected after the trial and dissected to determine the number of mature eggs [completely elongated and fully yolked, as described by Dean (1935a)] remaining in the ovaries.

Because we tested green fruit earlier in the season than red fruit, data from tests with green and red fruit could not validly be compared statistically. One-way contingency table G-tests (SAS 1990) and non-parametric statistics (Krukal-Wallis one-way ANOVA and Mann-Witnny-U tests) (Statistix, 1992) were used in different analyses corresponding to the nature of parameters examined.

In experiments 2a and 2b (1992), we investigated response patterns of mature (17 day old) females, having different prior ovipositional experiences, to a baited red sphere hung in a field-caged potted host tree containing 50 green Gravenstein apples. In experiment 2a, flies received 2 days of prior exposure to green Gravenstein apples, green Red Delicious apples, or red *Crataegus mollis* hawthorn fruit. In experiment 2b, flies were exposed to green Gravenstein apples for 0, 1, 2, or 4 days before testing. Both the Gravenstein and Red Delicious apples were picked on July 13 (100% green) and averaged

42 mm diam (range 38 - 45 mm). Hawthorns were picked in September of 1991 when 100% red and stored at 3°C until use. They averaged 20 mm diam (range 18 - 22 mm).

On day 1 of experiment 2a, a cohort of 15-day-old females was separated individually into paper cups (473 ml) (James River Corporation, Dixie Products Group, Norwalk, CT). The entire bottom and part of the side of each cup was removed so that light could enter. The openings were covered with nylon screen. Each cup (containing one fly) was placed screen-bottom up on a tray and was supplied with water and a strip of food. Flies in cups were divided randomly into three groups. One group was provided with Gravenstein apples (one per cup). The second group with Delicious apples (one per cup) and the third group with hawthorns (two per cup). On day 3, exposure fruits were removed from the cups at 0800 h for flies tested in the morning . Flies selected randomly among different exposure treatments were tested from 0830 - 1200 h. Exposure fruits were removed from cups at 1200 h for flies tested in the afternoon. Tests occured from 1230 - 1600 h. Testing procedures were the same as described in experiment 1, except that each fly was introduced onto the release leaf using a small cup lined with moist filter paper.

For experiment 2b, all procedures were the same as in experiment 2a, except that on day 1, a cohort of 13-day-old females was separated into four groups, which were then provided with either no host fruit (naive flies) or with a Gravenstein apple on days 1, 3, or 4. Tests were conducted on day 5.

3.3 Results

In experiment 1 (Table 3.1) with immature (green) Gravenstein apples on the tree, 61 - 71% of flies 7 days or older landed on the sticky red sphere compared with significantly fewer (25%) of 3-day-old flies that landed. Time from leaving the release leaf to landing on the sphere was significantly longer for older (15 and 19 day) flies (12 - 15 min) than for younger (3 and 7 day) flies (ca. 6 min). No significant differences among

flies of different ages were found in number of fruit visited (Kruskal-Wallis ANOVA, Q=5.18, p=0.27). No 3-day-old flies were found to lay eggs; thus no fruit visited by flies of this age received eggs. Very few (6%) 7-day-old flies laid eggs; only 2% of visited fruit received eggs. In contrast, a significantly greater proportion (19 - 26%) of 11 day or older flies laid eggs; a significantly greater proportion (13 - 20%) of visited fruit received eggs from these flies.

In experiment 1 (Table 3.1) with mature (red) Gravenstein apples on the tree, 47 - 57% of flies 7 days or older landed on the sphere compared with significantly fewer (27%) of 3-day-old flies that landed. No significant differences were found among flies of different ages in time from leaving the release leaf to landing on the sphere (Q=1.28, P=0.87). Flies 7, 11, and 15 days old visited significantly more red fruit than those 3 days old. As in tests with green fruit, no flies 3 days old laid eggs. Very few (3%) 7-day-old flies laid eggs; few visited fruit (2%) received eggs from flies 7 days old. In contrast, a significantly greater proportion (17 - 30%) of 15- and 19-day-old flies laid eggs although the percentage of visited fruit receiving eggs was significantly greater (21%) only for flies 19 days old.

Data on the eggload of tested flies (flies pooled from tests with green and red apples) indicated that numbers of mature eggs in ovaries increased in a sigmoid manner as fly age increased (Figure 3.1). No eggs were found in ovaries of 3-day-old flies. As fly age increased from 7 to 11 days, however, the number of eggs in ovaries increased sharply, with increasing variation. Thereafter, the number of eggs in ovaries increased more slowly, reaching a mean of 18 eggs for 19-day-old flies.

In experiment 2a (Table 3.2), there were no significant differences among flies with 2 days of prior exposure to Gravenstein apples, Red Delicious apples or hawthorns in the proportion of flies landing on the sphere (G=0.07, p=0.97) or in the time elapsed between leaving the release leaf and landing on the sphere (Q=0.77, p=0.86). However, significant differences among groups of flies were detected in the number of fruit visited

(Q=7.8, p<=0.02), the proportion of flies laying eggs (G=5.75, p=<0.05), and the percentage of visited fruit receiving eggs (G=7.26, p<=0.03). Specifically, flies with 2 days of prior exposure to hawthorns visited significantly fewer green Gravenstein apples than flies with 2 days of prior exposure to Gravenstein apples. The proportion of hawthorn-experienced flies laying eggs (13%) was significantly less than that of Gravenstein-apple-experienced flies (34%). This pattern was also true for the percentage of visited fruit receiving eggs (12 vs. 30%). Response patterns of flies with 2 days of prior exposure to Red Delicious apples were intermediate.

In experiment 2a, results from examination of fruit held with flies during the 2 day pre-test exposure period indicated that significantly fewer eggs were deposited in Red Delicious apples (6.8±1.5 eggs/fly) than in Gravenstein apples (14.0±1.8 eggs/fly) or hawthorns (11.6±1.5 eggs/fly) (Figure 3.2). These results suggest that although pre-test fruit exposure time regimens were the same, the extent to which flies gained prior ovipositional experience with different host fruit might not have been the same. Results from ovary dissections indicated that after 2 days of pre-test exposure to fruit, flies exposed to Red Delicious apples contained slightly more mature eggs in ovaries (12.7.0±1.3 eggs/fly) than did flies exposed to Gravenstein apples (9.6±1.4 eggs/fly) or to hawthorns (9.0±1.1 eggs/fly); no significant differences were detected (Figure 3.2).

In experiment 2b (Table 3.2), no significant differences were detected for any of the parameters examined (same parameters as in experiment 2a) among flies with different durations of prior exposure to Gravenstein apples (same fruit as on the test tree). Results from examination of fruit held with flies during the 0, 1, 2 or 4 day pre-test exposure period (Figure 3.3) indicated that as the duration (days) of pre-test exposure to fruit increased, the number of eggs deposited in fruit increased progressively and the number of eggs contained in the ovaries (eggload) decreased.

3.4 Discussion

Our findings indicate that the probability of a *R. pomonella* female landing on a sticky red sphere hung in a tree containing 50 Gravenstein apples is affected significantly by fly age regardless of whether the fruit on the tree is immature (green) or mature (red). Fly age also affected significantly the time taken by a fly to reach the sphere after leaving the release leaf (with green fruit on the tree), number of fruit visited (with red fruit on the tree), probability that a fly would lay eggs, and probability that visited fruit would receive eggs. Neither two days of pre-test exposure to different types of fruit (red hawthorns, green Red Delicious apples or green Gravenstein apples) nor different durations (0, 1, 2 or 4 days) of pre-test exposure to green Gravenstein apples affected significantly the probability of a fly landing on a sphere hung in a tree containing green Gravenstein apples. Compared with two days of pre-test exposure to green Gravenstein apples, however, two days of pre-test exposure to red hawthorns reduced significantly the number of fly visits to green Gravenstein apples, the proportion of the flies ovipositing in such apples and the proportion of visited apples receiving eggs.

Although physiological mechanisms that control oviposition-site finding behavior are not understood in *R. pomonella* flies, our results (Table 3.1) suggest that readiness to commence ovipositon-site foraging behavior (which we equate with readiness to alight on red spheres) may be age-dependent and correlated (though not necessarily causally) with ovarian development. When supplied with ample protein and carbohydrate as food, females usually take 7 - 10 days (after eclosion) to become reproductively mature and to commence mating and ovipositional behavior (Dean, 1935b; Webster and Stoffolano, 1978; Webster et al., 1979). Previous laboratory studies by Prokopy et al. (1971), like our field-cage study here, suggest that sexually or reproductively immature *R. pomonella* flies show comparatively little tendency to visit or assemble at an ovipositon site when they have little ovipositional capability.

In practice, therefore, the effectiveness of red sphere traps in controlling *R. pomonella* flies in commercial apple orchards could vary according to the age structure of fly populations, which may change with fly season and orchard environmental conditions. In commercial orchards, *R. pomonella* populations usually consist of immigrants from unmanaged host trees. As the growing season proceeds, the proportion of mature flies increases and the ovipositional threat from mature flies becomes greater. Our findings here suggest that for greatest effectiveness in controlling *R. pomonella* flies in commercial orchards, red sphere traps should be employed early in the fly season before *R. pomonella* females have reached maturity.

Although the design of experiment 1 precludes legitimate statistical comparison of fly response patterns to fruit in two different stages of ripeness (immature green vs mature red), numerical differences in fly response pattern to these ripeness stages deserves comment (Table 3.1). In particular, 4 - 21% fewer mature flies (7 days or older) landed on the red sphere when the tree contained red fruit compared with green fruit, with red apples receiving 44 - 153% more visits than green apples. Even so, except for 19-day-old flies (which carried greatest eggload), the proportion of mature flies that oviposited in red fruit was only about half of that which oviposited in green fruit. In consequence, far fewer red apples (5 - 7%) than green apples (16 - 20%) received eggs from females 11 - 15 days old. Furthermore, significant differences in the time taken to reach the sphere (after leaving the release leaf) among different aged flies occurred with green apples on the tree but not with red apples, while significant differences in fruit visitation among different aged flies occurred with red apples on the tree but not with green apples. Because tests with these two different fruit ripeness stages were conducted at different times (6 weeks apart), these apparent differences in fly response could be partially or wholly the consequence of differences in weather conditions. The mean temperature in the test cages during the two test period was, however, almost identical (29.3 ± 0.6 and $28.7 \pm 0.8^{o}C$). It is possible that differences found here in fly response patterns to

immature green verse mature red apples stemmed at least in part from the effect of the different visual and/or chemical properties of green and red apples on fruit-foraging flies.

Upon arrival on host trees, *R. pomonella* flies locate host fruit and/or sticky red spheres in host trees via visual responses to physical properties (color, shape, and size) of fruit or fruit mimics, with 8-cm spheres of red or other dark color receiving the most alightings (Prokopy, 1968; Owens & Prokopy, 1986; Aluja & Prokopy, 1994). Recently, Prokopy et al. (1994) showed that the ability of *R. pomonella* flies to find apple or hawthorn fruit of green color is significantly less among flies having 3 days of prior experience with red fruit than green fruit. The ability of *R. pomonella* to find apple or hawthorn fruit of red color, however, is approximately equal irrespective of color of fruit with which flies have had 3 days of prior experience (Prokopy et al. 1994). Here, the probability of *R. pomonella* females landing on a red sphere (80mm) was not influenced by 2 days of prior exposure to red hawthorns (18 - 22mm) or green apples (38 - 45 mm) of different varieties (Red Delicious or Gravenstein) nor by different durations of prior exposure (up to 4 days) to green apples. However, *R. pomonella* females with 2 days of prior exposure to red hawthorns visited significantly fewer green Gravenstein apples than those with 2 days of prior exposure to green Gravenstein apples. Our results, together with those of Prokopy et al. (1994), are consistent with the suggestion of Wardle and Borden (1991) and Vet and Dicke (1992) that prior experience in insects is likely to have less impact on innate strong (genetically-controlled) responses (e.g., to red spherical objects by *R. pomonella*) than on initially weak responses (e.g., to less conspicuous green apples).

Prokopy et al. (1986) proposed two mechanisms by which *R. pomonella* learn to oviposit in host fruit: either by rejecting unfamiliar physical (fruit size) and/or surface chemical fruit stimuli or by accepting familiar physical (fruit size) and/or surface chemical fruit stimuli. Results of experiment 2a indicated that 2 days of prior exposure to red hawthorns had a significantly negative effect on the proportion of *R. pomonella*

laying eggs and the proportion of visited green Gravenstein apples receiving eggs before flies landed on the red sphere or flies left the tree patch. Although Prokopy and Papaj (1988) showed that *R. pomonella* females also learned to discriminate among different cultivars for oviposition, our results here did not show a significant negative effect of 2 days of prior exposure to Red Delicious apples on the proportion of flies laying eggs in Gravenstein apples or the proportion of visited Gravenstein apples receiving eggs. Perhaps physical and/or chemical properties of Gravenstein and Red Delicious apples are so similar at the stage when these apples were picked (early July) that no real difference in these two fruit types exists for flies. Furthermore, prior exposure to Gravenstein apples had no discernible effect on the proportion of flies laying eggs in Gravenstein apples or the proportion of visited Gravenstein apples receiving eggs. Together, these results are consistent with findings of Prokopy et al (1986) and Papaj & Prokopy (1988) that *R. pomonella* females mainly learn to reject unfamiliar fruit species for oviposition rather than increasingly accept familiar fruit species.

In summary, both fly age and prior egglaying experience appear to affect the success and/or failure of using 8-cm sticky red spheres to control *R. pomonella* flies in commercial orchards, but in different ways. As fly age increases from a reproductively immature stage to a mature stage, the probability of a fly landing on a red sphere hung in host trees increases. At the same time, however, the likelihood that a fly will deposit eggs in host fruit before encountering a sphere increases. Learning host fruit properties by *R. pomonella* recently has been suggested to have a potential effect on the success or failure of using red sphere traps to intercept immigrating flies (Prokopy & Lewis, 1992). Our results, together with those of Prokopy et al.(1994), suggest that the success of red sphere traps for intercepting immigrant flies could be facilitated by prior ovipositional experience of immigrant flies with different species or cultivars of host fruit. Such experience does not seem to affect the ability of flies to find red sphere traps but may reduce the likelihood that a fly will lay eggs in unfamiliar fruit before alighting on such a

trap. On the other hand, prior experience with the same species or cultivar of host fruit, regardless of duration (up to 4 days), has no apparent effect on the ability of a fly to find a red sphere trap nor on the likelihood of a fly laying eggs in familiar fruit.

Even for mature flies with a high egg load, the proportion of flies that oviposited in Gravenstein apples (a highly favored cultivar) did not exceed 34% in any of our experiments and the proportion of fruit visited that received eggs did not exceed 21%. These data indicate that apples, which have been a host of *R. pomonella* flies for only the past 150 years or so (Bush 1966), may receive frequent visits by oviposition-site foraging flies but receive relatively few eggs compared with hawthorns (Papaj & Prokopy, 1988). This supports the potential value of using red sphere traps for controlling apple maggot flies in commercial orchards.

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Table 3.1 Intra-tree fruit foraging behavior of different-age apple maggot females on an apple tree containing a baited red sticky sphere and 50 green or red Gravenstein apples (Exp. 1).

Fruit	Fly age (days after emergence)[a]	% of flies landing on sphere[b]	Mean time (min.) from leaving release leaf to landing on sphere (±S.E)[c]	Mean no. fruit visited per fly (±S.E.)[c]	% flies laying one or more eggs[b]	% of visited fruit receiving one or more eggs[b]
Green						
	3	25b	5.7 (1.2)b	1.5 (0.2)a	0b	0b
	7	68a	6.1 (1.1)b	2.7 (0.4)a	6b	2b
	11	61a	9.4 (1.2)ab	1.8 (0.3)a	25a	20a
	15	71a	15.2 (2.4)a	1.7 (0.3)a	26a	16a
	19	64a	12.3 (2.1)a	1.8 (0.3)a	19a	13a
Red						
	3	27b	8.7 (3.2)a	2.0 (0.5)b	0c	0b
	7	47a	10.3 (2.8)a	3.9 (0.8)a	3bc	2b
	11	57a	10.1 (2.7)a	3.8 (0.6)a	13ab	7b
	15	57a	11.9 (2.5)a	4.3 (0.8)a	17a	5b
	19	57a	10.8 (2.5)a	3.0 (0.4)ab	30a	21a

[a] No. flies tested: 35 and 30 per treatment for green and red fruit/respectively. [b] Values within each type of fruit in a column followed by the same letter are not significantly different at the 0.05 level according to pairwise G-tests with Bonferroni correction for the Type I error rate for each comparison. [c] Values within each type of fruit in a column followed by the same letter are not significantly different at the 0.05 level according to Mann-Whitney U-test criterion.

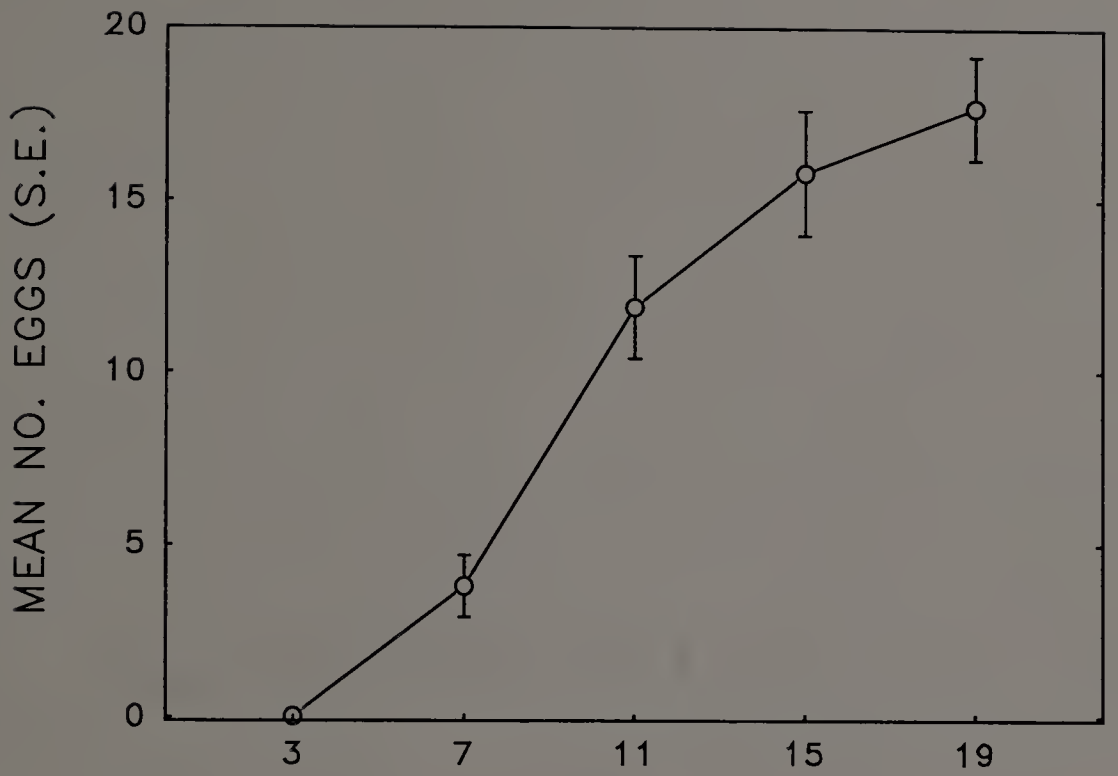

Figure 3.1. Mean number of mature eggs (numbers laid plus numbers found upon ovary dissection) in ovaries of *R. pomonella* females of different ages (days after emergence). Data were pooled across females released on trees with green and red Gravenstein apples.

Table 3.2 Intra-tree fruit foraging behavior of 17-day-old apple maggot females on an apple tree containing a baited red sticky sphere and 50 green Gravenstein apples. Females received 2 days of prior exposure to red hawthorns (RED HAW), green Red Delicious apples (GRN RD APL), or green Gravenstein apples (GRN GV APL) (Exp. 2a), or varying days of prior exposure to green Gravenstein apples (Exp 2b).

Exp.	Prior-experience with[a]	% flies landing on sphere[b]	Mean time (min.) from leaving release leaf to landing on sphere (±S.E)[c]	Mean no. fruit visited per fly (±S.E.)[c]	% flies laying one or more eggs[b]	% of visited fruit receiving one or more eggs[b]
2a	Red HAW	49a	11.5 (2.4)a	1.3 (0.2)b	13b	12b
	GRN RD APL	51a	17.1 (3.3)a	1.6 (0.2)ab	24ab	19ab
	GRN GV APL	49a	18.2 (3.2)a	2.0 (0.2)a	34a	30a
2b	GRN GV APL					
	0 days	56a	12.8 (2.3)a	1.5 (0.2)a	27a	27a
	1 days	49a	13.7 (2.4)a	1.8 (0.3)a	27a	23a
	2 days	66a	13.6 (2.3)a	1.8 (0.2)a	29a	31a
	4 days	51a	11.9 (2.7)a	2.1 (0.3)a	29a	28a

[a] No. flies tested: 41 per treatment in each experiment. [b] Values in each column in each experiment followed by the same letter are not significantly different at the 0.05 level according to pairwise G-tests with Bonferroni correction of the Type I error rate for comparison. [c] Values in each column in each experiment followed by the same letter are not significantly different at the 0.05 level according to Mann-Whitney U-test criterion.

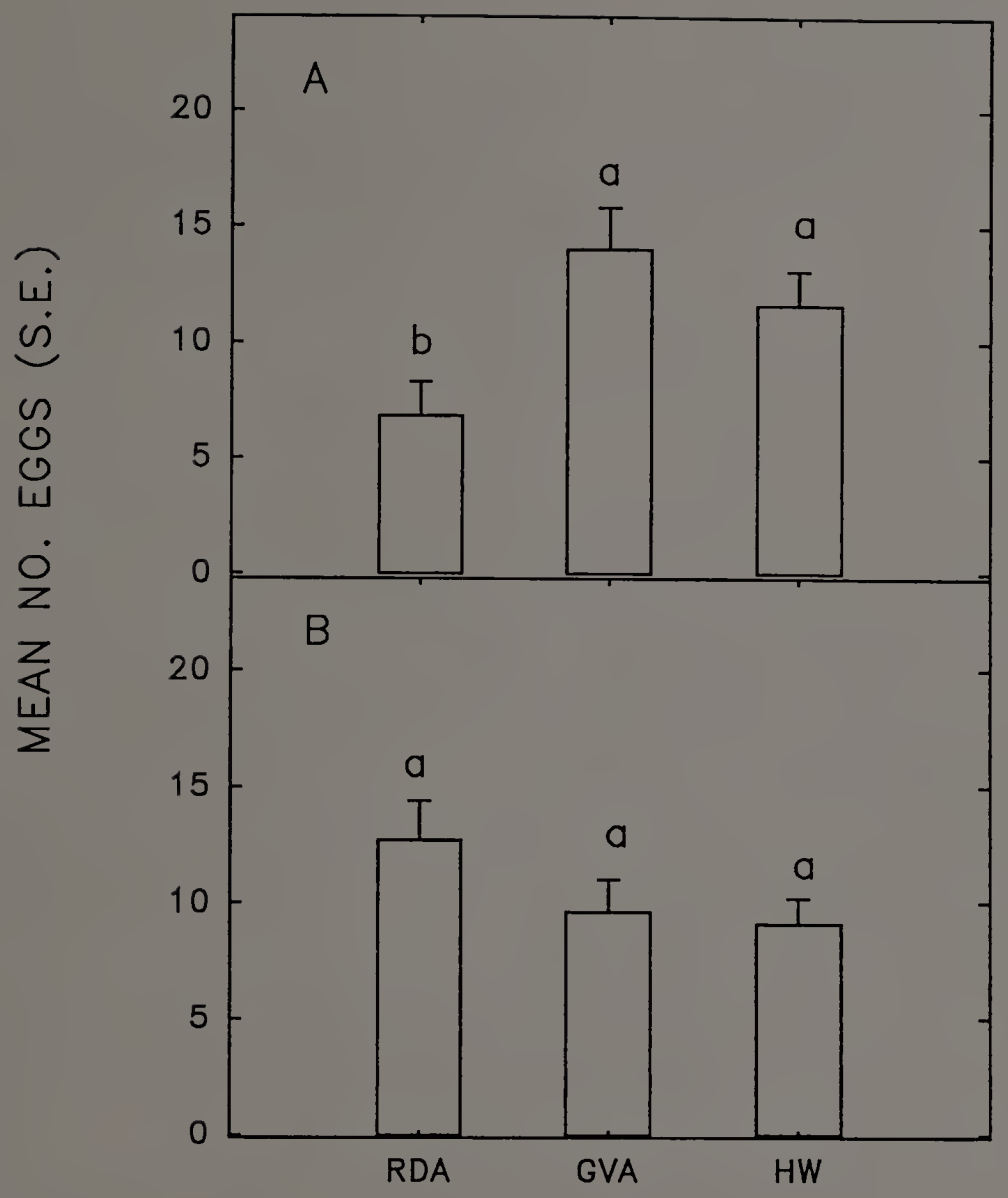

Figure 3.2. Mean number of eggs deposited during 2 days of pre-test exposure of *R. pomonella* females to different types of host fruit (A), and the number of eggs in fly ovaries after pre-test exposure (B). RDA = Red Delicious Apples. GVA = Gravenstein apples. HW = hawthorns. Bars in each graph followed by the same letter are not significantly different at 0.05 level according to Mann-Whitney U-test criterion.

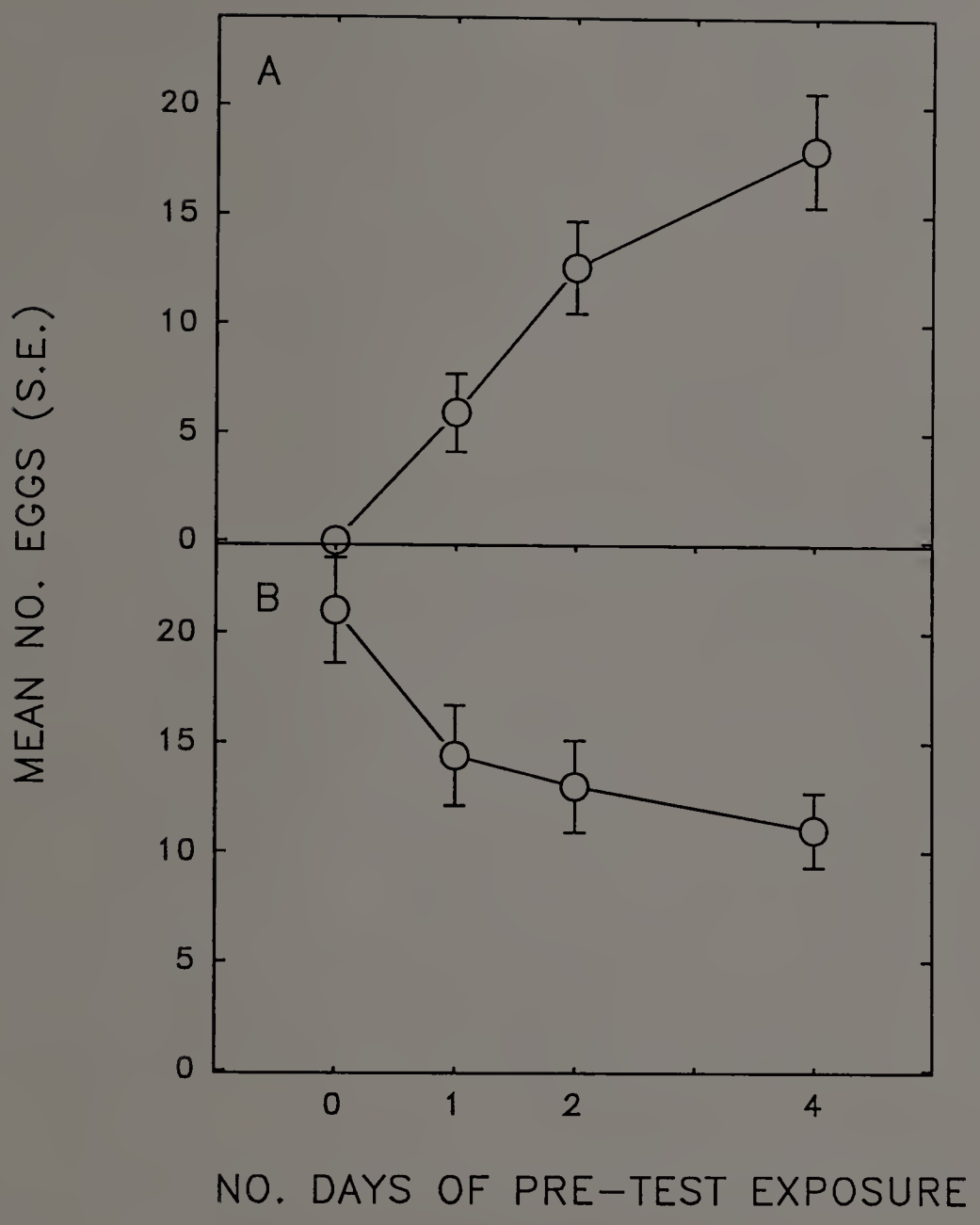

Figure 3.3. Mean number of eggs deposited by *R. pomonella* females in Gravenstein apples during pre-test exposure periods of different durations (A), and the number of eggs in fly ovaries after pre-test exposure (B).

CHAPTER 4

TOWARD DEVELOPING PESTICIDE-TREATED SPHERES FOR CONTROLLING APPLE MAGGOT FLIES: CARBOHYDRATES AND AMINO ACIDS AS FEEDING STIMULANTS

4.1 Introduction

A behavioral approach involving use of baited sticky red sphere traps to intercept immigrating apple maggot flies, *Rhagoletis pomonella* (Walsh), has become an essential element of current second-level IPM programs in Massachusetts apple orchards (Prokopy et al., 1991b). This approach is derived from knowledge that both sexes of *R. pomonella* exhibit a highly positive response to visual and odor stimuli of apple fruit and to fruit mimicking 8-cm red spheres baited with synthetic apple odor (Prokopy, 1968; Reissig et al., 1982). However, after several years of ringing small (1 ha) commercial apple orchard blocks with visual/odor interception traps 5 m apart on perimeter apple tree (Prokopy et al., 1990b, 1990c), it has become apparent that reliance on sticky (Tangletrap®) as the agent to kill *R. pomonella* that alight on the traps is an impediment to using traps in large numbers required for large orchard blocks. The sticky is too awkward and laborious to handle in large-scale trapping programs. As pointed out by Prokopy et al. (1990b, 1990c), a potential alternative of greater appeal would be to apply to spheres a mixture containing a contact pesticide, a fly feeding stimulant, and an agent that extends the residual effectiveness of the feeding stimulant and pesticide. We therefore initiated a project on developing pesticide-treated spheres for the control of *R. pomonella* in commercial orchards.

Pesticide-treated trap or lure systems have reportedly shown effectiveness in several pest control programs. Landolt et al. (1991) showed that a trapping system comprised of a floral lure, visual targets, a feeding stimulant, and a pesticide was

effective in attracting and killing cabbage looper moths, *Trichoplusia ni* (Hubner). Under this system, attracted *T. ni* moths were stimulated to feed at bait stations containing sucrose. Moths that fed were killed by 0.2% methomyl added to the sucrose (Landolt et al. 1991). Vale et al. (1988), working with the tsetse flies, *Glossina morsitans morsitans* Westwood and *G. pallidipes* Austen, found that odor-baited targets (consisting of a combination of black cloth 0.8x1 m and netting 0.7x1 m) sprayed with insecticide (deltamethrin) significantly reduced tsetse populations by 99.9% in the center of experimental blocks. This pesticide-treated target system, however, did not involve use of a feeding stimulant because *G. m. morsitans* and *G. pallidipes* were so susceptible to deltamethrin that apparently only a single brief visit of a few seconds at the target was required for high mortality (Vale, 1982; Torr, 1985). Recently, a study by Haniotakis et al. (1991) on the olive fly, *Dacus oleae* (Gmelin), showed that a method combining attractants (a food lure and a sex and aggregation pheromone) with a phagostimulant applied to insecticide-treated wooden rectangles eliminated 3-5 insecticide sprays required per season for the control of this pest, and was more economical and convenient to use than sticky traps. The phagostimulant (sucrose) was used presumably to cause *D. oleae* flies landing on rectangles to feed and remain for a longer period with consequent greater exposure to insecticide (Haniotakis et al., 1991).

For the control of *R. pomonella*, we hypothesized (and show in Chapter 5) that presence of a feeding stimulant in the pesticide mixture would induce *R. pomonella* flies that alight on treated spheres to ingest a lethal dose of pesticide and be killed in greater numbers than flies that alight on pesticide-treated spheres that do not possess a feeding stimulant. It has been well established that carbohydrate and protein are nutrients required by many species of Diptera, including Tephritidae, for survival and reproduction (Webster et al., 1979; Webster and Stoffolano, 1978; Tsiropoulos, 1980; Tsitsipis, 1989). Hasset et al. (1950), working with the blow fly, *Phormia reginia* (Meigen), studied the nutritive value of various carbohydrates and thresholds of carbohydrate acceptance by the

flies. *Phormia reginia* were particularly stimulated by sugars common in nectars, such as D-maltose, D-fructose, sucrose, glucose and melezitose, but were less sensitive or insensitive to other sugars. Gothilf et al. (1971) showed that consumption of a sugar solution by *Ceratitis capitata* (Wiedemann) generally correlated well with the degree to which the sugar stimulated the fly to initiate feeding. Stimulation to initiate and continue feeding can vary, however, according to species of fly and type and concentration of sugar (Gothilf et al., 1971; Dethier, 1976). Several amino acids have been shown to be phagostimulants for *C. capitata* (Galun et al., 1980), the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Sharp and Chambers, 1984), *D. oleae* (Tsiropoulos, 1984) and other Diptera (Robbins et al., 1965; Yamamoto and Jensen, 1967). The phagostimulatory power of a particular amino acid likewise appears to vary with the species of fly and the nature and concentration of the amino acid. To date, there has been no published study of phagostimulatory responses of *R. pomonella* to either carbohydrates or amino acids.

Here, for *R. pomonella* flies under laboratory conditions, we evaluated: (a) the degree to which different sugars stimulated feeding, as determined by median tarsal acceptance threshold; (b) the phagostimulatory power of several amino acids, as revealed by duration of feeding on saturated substrates; (c) duration of feeding on graduated concentrations of liquid or dry sucrose; and (d) the effect of texture of sucrose-treated surfaces (fibrous, polymeric, smooth-painted, smooth-plastic) on duration of fly feeding. Regarding the latter, we hypothesized that the texture of a red sphere surface could affect the feeding behavior of alighting flies and therefore the probability of dying as a consequence of ingesting pesticide.

4.2 Materials and Methods

Rhagoletis pomonella flies used in all experiments originated from puparia formed by larvae that infested mixed varieties of apple or hawthorn fruit collected from unsprayed trees in Amherst, Massachusetts. Flies of each sex were maintained together

from eclosion onward in 30x30x30-cm aluminum screen/Plexiglass cages at about 30 females and 30 males per cage. Each cage was supplied with water and food [a 5x7-cm strip of filter paper dipped in an aqueous slurry of enzymatic yeast hydrolysate and sucrose (1:4 ratio) and dried before use]. All flies were kept at ca. 25°C and 70% relative humidity under an 18-h photoperiod and tested at 13 - 17 days old (sexually mature).

We chose to test the 5 carbohydrates (sugars) that proved to be the most nutritive and stimulating to *P. reginia* in the study of Hasset et al. (1959) and some of which were also most nutritive and stimulating to *C. capitata* in the study of Gothilf et al. (1971): D-maltose, D-fructose, sucrose, D-glucose, and melezitose. We also tested 5 L-form amino acids (phenylalanine, arginine, methionine, glutamine, and leucine) which were shown to be phagostimulatory to several other species of fruit flies (Galun et al., 1980; Sharp and Chambers, 1984; Tsiropoulos, 1984). In addition, we chose one artificial sweetener for testing: saccharin. All of the tested compounds (except saccharin from Du Pont Corporation of Newark, DE USA) were purchased from Sigma Chemical Company, St. Louis, MO. USA. Red-painted wooden and red plastic spheres (8 cm) were obtained from Pest Management Supply, Amherst, MA. USA. The other types of red spheres were constructed by (a) enveloping a red-painted wooden sphere in a red cotton sock having ca. 60 strands of fiber per cm, and (b) coating a red-painted wooden sphere with transparent polymeric thickener supplied by Du Pont Corporation.

In experiment 1, we compared the stimulating power of the five sugars and saccharin by determining the median tarsal acceptance threshold (MTAT) of *R. pomonella* for these compounds. Flies were tested using a modification of Thomson's (1977) method of diet presentation as described by Stoffolano et al. (1990). Eleven aqueous solutions ranging in concentration from 2^{-10} to 1 M were prepared for each substance. All flies were deprived of food for 18 - 24 h before testing and were pretested for response to water. Because information on *P. reginia* (Dethier, 1969, 1976; Thomson, 1977) indicated that ingestion of water stimulates stretch receptors of the cibarial pump,

foregut, and ventral nerve cord and would be expected to have a subsequent effect on threshold response to sugars, those *R. pomonella* flies responding positively to water in the pretest were discarded. Immediately after the water pretest, each fly was captured in a small (50 ml) transparent plastic cup which was inverted so that the fly (positively phototactic) moved to the bottom of the cup. The cup was then placed on a Petri dish lid containing filter paper saturated with a test solution. The cup and Petri dish were then inverted and placed beneath a fluorescent light source, where the fly moved to contact the saturated filter paper. The one minute test period began at the moment of contact. Proboscis extension during this period constituted a positive response. The initial concentration of each substance tested was determined by randomly choosing one of the 11 concentrations to be tested. Thereafter, the order in which concentrations were tested was adopted from Thomson (1977). In all, 30 female and 30 male flies were tested on each of the experimental substances. The MTAT was determined according to Thomson (1977). MTAT values were compared with one another using the median test of Siegel (1956).

In experiment 2, we investigated the phagostimulatory capacity (PC) of the 5 amino acids, each at 0.1 M mixed either with water alone or with water and 4% sucrose (W/V). Unlike sugars, amino acids in many cases (those affecting water, sucrose or salt receptor cells located in labellum) provide only gustatory stimulation rather than contact tarsal stimulation to insects (Shiraishi and Kuwabara, 1970; Goldrich, 1973; Dethier, 1976). Thus, for determining phagostimulatory capacity we measured time of fly feeding on surfaces saturated with amino acids rather than MTAT values. Test procedures were essentially the same as described in experiment 1, except that total feeding time (equal to duration of proboscis contact with saturated filter paper) was recorded. A previous test indicated that flies that left the substrate or ceased feeding for more than 1 min often were not responsive again to the test solution in the 5 min observation period that followed (Duan, unpublished data). Thus, a trial ended when the fly left the filter paper for the wall

of the cup or ceased feeding for more than 1 min after contacting the substrate. Flies that did not extend the proboscis within 1 minute after contacting the filter paper were considered not to have fed and were given a 0 value for feeding time. The experiment was divided into 2 sets of tests: one set evaluated the PC of amino acids mixed with water alone, in which water was used as the control; the other set evaluated the PC of amino acids mixed with water plus 4% sucrose, in which a 4% sucrose solution was used as the control. We tested 25 females for each of the amino acids in each set of tests. The feeding time of *R. pomonella* on each amino acid substrate was compared only with that on the corresponding control (water or 4% sucrose solution) using a multivariate t-test (Milliken and Johnson, 1984).

In experiment 3, we evaluated behavioral responses of *R. pomonella* to wet and dry filter paper impregnated with 1, 2, 4, 8, 16, or 32% sucrose solutions. The wet treatment was filter paper saturated with a sucrose solution and not allowed to dry. The dry treatment was filter paper saturated with a sucrose solution and oven dried for 24 h at 35°C. The test procedure was the same as described in experiment 1 except that the fly was not pretested with water and that fly feeding time was recorded as described in experiment 2.

In experiment 4, we evaluated the influence of the nature of sphere surfaces on visiting and feeding times of *R. pomonella* on spheres. Flies were not deprived of food before testing. Each sphere was dipped in a solution of 8% sucrose, 46% water and 46% alcohol (which aided in an even spread of sucrose over the sphere surface). Spheres were dried at 25°C before testing. For testing, a sphere was hung at the top center of a cage (30 x 30 x 30 cm) in the laboratory. A single fly was released onto the sphere by a 0.5 X 1.5 cm triangular piece of filter paper (impregnated with 10% sucrose solution) affixed at the end of a probe. After release, time of feeding and duration of sphere visit during a 15 min test period were recorded. Data were analyzed by nonparametric statistical procedures

(Kruskal-Wallis one way ANOVA or Mann-Whitney U-tests). Means rather than medians are presented for the convenience of showing variation (± S.E.).

4.3 Results

In experiment 1 (Table 4.1), MTAT values for sugars for both female and male *R. pomonella* can be ranked in the following order: sucrose < fructose < melezitose < glucose <= maltose. Median-test paired comparisons for each sex indicated that values of MTAT were significantly greater for melezitose, glucose and maltose than for fructose and sucrose. No statistically significant differences in MTAT values were found between males and females for any of the sugars. Because few flies of either sex (less than 15%) responded to saccharin, MTAT values could not be calculated. Therefore, we considered that saccharin is not phagostimulatory to *R. pomonella*.

In experiment 2 (Table 4.2), *R. pomonella* did not appear to respond strongly to any of the amino acids mixed with water. Flies fed for only a short period of time (2 - 5 sec) on phenylalanine, glutamine, leucine, methionine, and arginine, with no significant difference from water as a control treatment. Feeding time was considerably longer (19 - 39 sec) when amino acids were mixed with 4% sucrose in water (Table 4.2). Under this condition, feeding time was significantly greater on phenylalanine, while shorter on methionine and arginine, than on the control treatment of water plus 4% sucrose. On the other treatments, the feeding time was not significantly different from that on the control treatment.

In experiment 3 (Figure 4.1), as the concentration of sucrose increased, the feeding time of *R. pomonella* on the sucrose substrate (filter paper) increased regardless of whether the filter paper was dried or wet. In the range of lower concentrations (1 - 8%), there was no significant difference in feeding time on dried versus wet substrate. When the concentration was greater than 8%, feeding time was significantly longer on the dried sucrose substrate than on the wet one.

In experiment 4 (Table 4.3), the nonparametric ANOVA showed significant effects of type of surface on visiting and feeding times of *R. pomonella* on spheres (Kruskal-Wallis statistic $Q = 52.69$, $p < 0.001$ for visiting; $Q = 58.97$, $p < 0.001$ for feeding). Visiting and feeding times of flies on spheres having a cotton-fibre surface were significantly shorter than on spheres having painted, plastic or polymeric surfaces, among which there were no significant differences for either visiting or feeding times.

4. 4 Discussion

The results presented here demonstrate that both female and male *R. pomonella* flies have greatest sensitivity to the sugars sucrose and fructose and less sensitivity to glucose, maltose and melezitose as feeding stimulants. Feeding duration of *R. pomonella* on dried and wet sucrose substrates increased as concentration of sucrose increased geometrically from 1% to 32%. Addition of the amino acid phenylalanine to sucrose significantly enhanced feeding duration above sucrose alone. The state of sucrose presented on filter paper (dried vs wet) and the surface characteristics (smooth vs fibrous) of red spheres treated with sucrose had a significant effect on the feeding duration of *R. pomonella*.

MTAT values provide an indication of the sensitivity of tarsal sensilla of *R. pomonella* to specific sugars. The lower the MTAT value, the more stimulating the sugar. Among the 5 carbohydrates tested, sucrose and fructose were the most stimulating, whereas melezitose, glucose and maltose were significantly less so. Evidence from electrophysiological and/or behavioral assays in studies by other researchers (e.g. Gothilf et al., 1971; Dethier, 1976; Mitchell and Gregory, 1979; Sharp and Chambers, 1984; Ramaswamy, 1988; Erhardt, 1991) indicates that among carbohydrates, sucrose appears nearly universally to elicit the strongest positive response by many species of insects (including Diptera). For some species (e.g. *Heliothis virescens*) (Blaney and Simmonds, 1990), sucrose is not discriminated from some other sugars such as fructose and glucose.

It should be pointed out that in our tests, MTAT values may not provide a true indication of preference for or discrimination of particular sugars by *R. pomonella* in that MTAT assays were carried out under no-choice conditions, which we favored for the purpose of developing pesticide-treated spheres. The most direct way to investigate the ability of insects to discriminate or select among sugars is to offer two or more types of sugars simultaneously, observe insect responses to each, and measure the quantity of each consumed (Dethier, 1976). Such an approach, however, was beyond the principal purpose of this study. Comparison of our findings with those of others who have studied insect discrimination among different carbohydrates should be done with caution because of the possible differences in methodology used.

Ross et al. (1977) reported that *R. pomonella* adults can not utilize maltose as nutrients. Our results indicate that *R. pomonella* flies nonetheless are quite sensitive to maltose (MTAT = 0.063 for both females and males), though less so than to sucrose and fructose. As indicated by Dethier (1976) and Hasset et al. (1950), the nutritive value of a food is not always a determinant of its acceptance threshold by a fly. Still, in that saccharin is a non-sugar sweetener, it is not surprising that *R. pomonella* was not responsive to it at any concentration offered. Previous work with *P. reginia* showed that the sugar receptors of this fly, although responsive to many different carbohydrates (Hasset et al., 1950), are not stimulated by a number of non-sugar compounds (including saccharin) which are sweet to humans (Hansen and Wieczorek, 1981; Schiff et al., 1990).

Regarding amino acids, Shiraishi and Kuwabara (1970) conducted an electrophysiological study of responses of the fleshfly, *Boettcherisca peregrina* (Ralineau-Desvoidy) and *P. reginia* flies to 19 L-amino acids. They concluded that on the basis of how amino acids react with labeller receptors, amino acids can be divided into 4 categories: (1) completely non-stimulating (glycine, alanine, serine, threonine, cystine, and tyrosine); (2) inhibiting sugar, salt and water receptor cells (aspartic acid, glutamic acid, histidine, histidine and arginine); (3) stimulating the salt cell (proline and

hydroxyproline); and (4) stimulating the sugar cell (valine, leucine, phenylalanine, and tryptophan). These findings were extended and generally confirmed by Goldrich (1973), who combined electrophysiological with behavioral studies. Our data show that the phagostimulatory capacity of the 5 amino acids tested, as measured by feeding time of *R. pomonella* females on saturated substrates, appears to depend on the presence of sucrose. Hungry *R. pomonella* females did not have strong or even moderate responses to any of the 5 amino acids when mixed with water alone at 0.1 M concentration. Flies fed only for a very short time (2 - 5 sec). Together with 4% sucrose in water, however, phenylalanine was significantly phagostimulatory to *R. pomonella*; leucine and glutamine had no significant effect; and methionine and arginine appeared to be inhibitory. Interestingly, the amino acid phenylalanine, when combined with sucrose, also has a phagostimulatory effect on other species of Diptera. Galun et al. (1980) and Sharp and Chambers (1984) showed, respectively, that phenylalanine is highly phagostimulatory to female *C. capitata* and *A. suspensa*. The other 4 amino acids tested here had different or opposite effects on *C. capitata* and *A. suspensa* (Galun et al.,1980; Sharp and Chambers, 1984). Differences in methodology and the insect species may have been responsible.

For sucrose, the greater the concentration, the longer *R. pomonella* fed on the substrate on which sucrose was presented. The manner in which sucrose was presented had a strong bearing on duration of feeding. When the concentration of sucrose solution on filter paper exceeded 8%, *R. pomonella* fed on dried filter paper significantly longer than on wet filter paper. The probable explanation of this is twofold: (1) when the filter paper was dried, evaporation of water increased the surface concentration of sucrose and thus increased its stimulating power to flies. (2) on dry filter paper, fly feeding time increased because uptake of dry food required that it be liquefied by salivary secretion, necessitating additional time for food handling and processing. On the other hand, at lower sucrose concentrations (1% - 8%) of sucrose, feeding time on dried filter paper was essentially the same as on wet filter paper. Perhaps at these lower concentrations,

potential gain in nutrients was too little to compensate for the extra amount of time spent in handling and processing.

Because of the potential ability of cotton fibre to retain impregnated insecticide under conditions usually leading to rather rapid insecticide depletion (e.g., direct sunlight and heavy rainfall), we were disappointed that the duration of *R. pomonella* visits to and feeding on cotton fibre-covered spheres was much less than that on smooth-surface spheres. This could have been due to an adverse effect of the cotton fibres on fly tarsal sensilla.

In summary, our findings suggest that either sucrose or fructose can be used as potent feeding stimulants to combine with pesticide applied to spheres for controlling *R. pomonella* in commercial orchards. Sucrose would be the stimulant of choice on account of its low cost and general availability. All of the 5 amino acids tested, however, would be precluded from use as feeding stimulants on spheres. Although phenylalanine appears to be phagostimulatory to *R. pomonella* when combined with sucrose, the effect is not pronounced enough to justify the expense of using it in a pesticide mixture applied to spheres. When sucrose is used in combination with a pesticide and a residue-extending agent on spheres, our findings suggest that the concentration should be more than 8% to stimulate alighting *R. pomonella* to remain and feed for a sufficiently long period to ingest pesticide. Our finding of a negative effect of cotton-fibre surface on the duration of fly feeding on red spheres [together with previous work in Chapter 2) that demonstrated that fly captures were significantly reduced by use of a conical roof above spheres to protect against the impact of rainfall and sunlight] indicates that replacement of sticky by a coating of fly feeding stimulant and toxicant on a sphere will probably require using some sort of residue-extending agent that forms a smooth sphere surface. As indicated by the study of Vale et al. (1988) and Haniotakis et al. (1991), absorption of an insecticide mixture by the trap itself (such as an unpainted plywood rectangle and a cloth-netting target) could contribute to the long residual effectiveness of an insecticide. Painted or

polymeric smooth sphere surfaces would limit the absorption of insecticide solution applied to spheres by dipping or spraying methods [as used in the studies of Vale et al. (1988) and Haniotakis et al. (1991)]. However, applying a mixture containing a residue-extending agent together with insecticide and a feeding stimulant to the sphere surface could overcome this disadvantage and possibly offer protection against weathering. A future paper (Chapter 5) will deal with the insecticide and residue-extending agent components of the mixture.

4.5 References

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Table 4.1. Median tarsal acceptance thresholds of five sugars and saccharin by *R. pomonella* flies.

	Median tarsal acceptance threshold[a]	
Compounds	Females	Males
Sucrose	0.008a	0.006a
Fructose	0.012a	0.008a
Melezitose	0.023b	0.031b
Glucose	0.063b	0.047b
Maltose	0.063b	0.063b
Saccharin[b]	-	-

[a]Threshold (molar concentration) values were determined using the technique of Thomson (1977). Within each sex, sample size was 30 flies per compound. Values in each column followed by the same letter are not significantly different at the 0.05 level according to Median Test of Siegel (1959).
[b] Because only 3 of 30 females and 4 of 30 males responded to concentrations of saccharin that ranged from 0.0005 to 1.0 M, it was not valid to determine the acceptance threshold.

Table 4.2. Feeding time of female *R. pomonella* flies on different amino acids presented on saturated filter paper.

	Mean (± S.E.) sec[a]	
Treatment	With water	With water + 4% sucrose
Phenylalanine	4.88 (1.11)a	38.88 (2.68)a
Glutamine	4.28 (0.76)a	22.80 (1.19)b
Leucine	3.68 (0.84)a	30.32 (2.49)b
Methionine	2.00 (0.81)a	18.88 (1.83)c
Arginine	1.80 (0.45)a	21.08 (1.93)c
Control (No amino acid)	2.88 (0.83)a	28.36 (2.41)b

[a] Sample size was 25 flies per treatment. Values in each column followed by the same letter are not significantly different when compared with control treatment at the 0.05 level according to multivariate t-test.

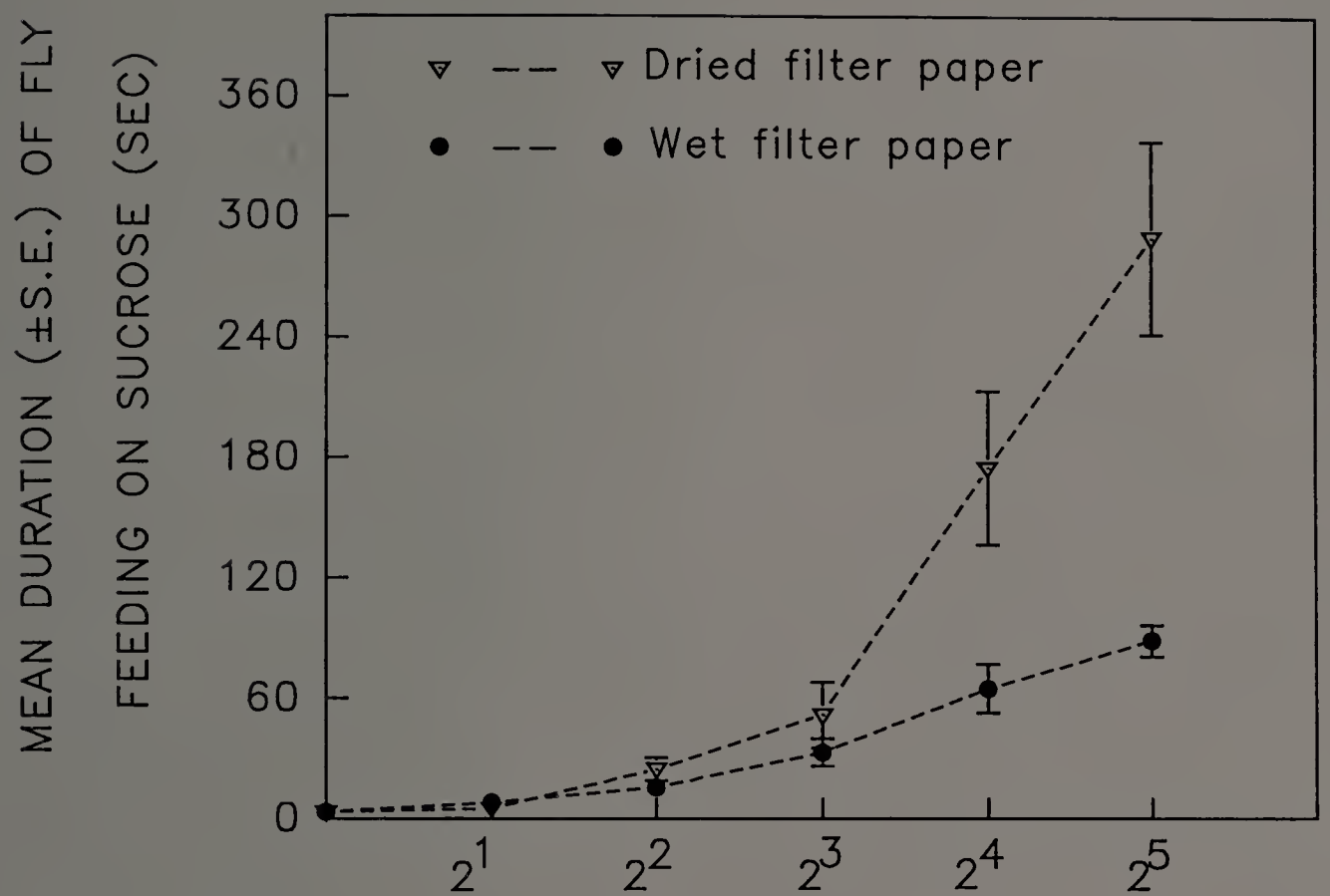

Figure. 4.1. Responses of *R. pomonella* to different concentrations of sucrose presented on dried or wet filter paper.

Table 4.3. Responses of *R. pomonella* flies to 8-cm red spheres of different surfaces treated with 8% sucrose solution (dried before testing).

	Mean time (± S.E.) on spheres (sec)[a]	
Surface	Visiting	Feeding
Cotton fibre	68.22 (22.10)b	16.98 (5.39)b
Painted-smooth	547.10 (59.27)a	464.30 (55.18)a
Plastic-smooth	592.00 (56.98)a	478.60 (50.85)a
Polymer-smooth	494.80 (59.39)a	370.50 (48.07)a

[a] Sample size was 40 flies per sphere type. Numbers in each column followed by the same letter are not significantly different from each other at the 0.05 level according to the Mann-Whitney U-test criterion.

CHAPTER 5

TOWARD DEVELOPING PESTICIDE-TREATED SPHERES FOR CONTROLLING APPLE MAGGOT FLIES: PESTICIDES AND RESIDUE-EXTENDING AGENTS

5.1 Introduction

The potential for using combinations of traps or lures and insecticide as a means of insect control has been suggested by a number of researchers [e.g. Vale et al., 1988 with tsetse flies, *Glossina spp*; Landolt et al., 1991 with the cabbage looper moth, *Trichoplusia ni* (Hubner); Haniotakis et al., 1991 with the olive fly, *Dacus oleae* (Gmelin)]. The purpose of this method is to attract insects to insecticide-treated lure stations or traps, where they are killed by insecticide residues either through tarsal contact or through feeding. This approach, if technologically feasible, could have much advantage over use of conventional chemical spray applications by reducing toxicological, ecological, and environmental problems associated with the latter (Haniotakis et al., 1991).

For developing a pesticide-treated red sphere trap system for the control of *R. pomonella*, an insecticide applied to spheres to kill alighting flies should: (a) not adversely affect the sphere's attractiveness to flies, (b) prove lethal to a fly visiting a sphere for only a brief period of time (e.g. a few minutes), (c) be resistant to weathering, especially rainfall, and (d) not be harmful to the applicator or various onlookers who might touch or handle treated spheres in an agricultural setting. In addition, Duan et al. (1990) hypothesized that combining a feeding stimulant with insecticide would cause *R. pomonella* alighting on spheres to ingest a lethal dose of insecticide before leaving and ovipositing in host fruit. Previous work in Chapter 4 showed that sucrose is a potent feeding stimulant for *R. pomonella*, and that a red sphere with a smooth surface treated

with sucrose induced *R. pomonella* released on it to remain for about 10 minutes, during which a fly spent most of the time feeding.

Several insecticides of different classes currently labeled for orchard use in USA (such as organophosphates, synthetic pyrethroids, and carbamates) have been tested extensively against *R. pomonella* flies in field spray programs and/or in the laboratory by topical application or exposure to treated surfaces (e.g., Dean, 1954, 1961; Maxwell, 1961; Dolphin et al., 1970; Bancroft et al., 1974; Pree et al., 1976; Reissig et al., 1980; Mohammad and Aliniazee, 1989; Stanley et al., 1989). Little information, however, can be drawn from existing literature on the suitability of such pesticides for developing pesticide-treated spheres, especially when combined with a feeding stimulant. The insecticide tralomethrin, which is not labeled for orchard use in USA, has been shown to be highly effective in controlling tsetse flies, *G. spp.*, when impregnated into treated targets (black cloth and netting) (Torr, 1985; Vale et al., 1988), and in controlling *D. oleae* when impregnated into unpainted plywood rectangle traps (Haniotakis et al., 1991). To the best of our knowledge, it has not been tested against *R. pomonella* flies.

In previous Chapters (2 and 4), we examined several ways to protect the residual effectiveness of fly feeding stimulant and insecticide on red spheres against degradation by weather. A conical roof placed above a sphere seemed to be effective in protecting surface residues of fly feeding stimulant and insecticide against rainfall and sunlight, but it strongly reduced numbers of alighting *R. pomonella* (Chapter 2). Spheres with a cotton fibre surface, which would absorb a liquid sucrose-insecticide mixture and possibly afford continued release over a long period, had a significantly adverse effect on duration of visitation and feeding by *R. pomonella* (Chapter 4). Until now, we had not examined the efficacy of materials such as paint or polymeric thickener as residue-extending agents in combination with fly feeding stimulant and insecticide.

Here, we conducted studies in the laboratory and under semi-natural conditions (field cages) aimed at selecting a suitable insecticide in combination with a residue-

extending agent and fly feeding stimulant for developing a pesticide-treated sphere system for the control of *R. pomonella*.

5.2 Materials and Methods

All *R. pomonella* flies used originated from puparia formed by larvae that infested mixed varieties of apple or hawthorn fruits collected from trees in Amherst, MA which had received no pesticide sprays. Flies were maintained by the same methods described in Chapter 4. All flies used for testing were 15 - 28 days old (sexually mature).

We originally chose to test 7 technical-grade insecticides: malathion (98%), fenvalerate (98%), azinphosmethyl (99%), and carbaryl (98%) (all purchased from Chem Service Inc., West Chester, PA), tralomethrin (95.9%) (provided by Roussel Bio Corp., Englewood Cliffs, NJ), dimethoate (98%) (provided by American Cyanamid Co., Wayne, NJ.), and methomyl (95%) (provided by Du Pont Corp. Newark, DE). Later we also chose to test commercial formulations of methomyl (Lannate^{TM} 1.8 SL) and dimethoate (Cygon® 4.0 EC). The above pesticides were chosen for testing in this study mainly because of registration for apple orchard spray application and/or toxicity to *R. pomonella* adults or other related species and/or low mammalian toxicity.

5.2.1 Laboratory Bioassays of the Toxicity of Insecticides Applied to Glass Jars.

To measure the toxicity of insecticides with or without the feeding stimulant sucrose, insecticide solutions were applied to the inside of glass Mason jars (ca. 500 ml) (Container Corp. of America, Dolton, IL). Jars were either pre-coated or uncoated with a film of sucrose by spreading 1 ml of a 32% granular sucrose/water solution evenly over the inside of each jar (equal to 1.31 mg sucrose/cm^2). Jars were then dried in an oven at 35^{o}C for 24 hours. Four to six concentrations of each insecticide (technical grade) were prepared in acetone from stock solutions (121.5 mg a.i./10 ml acetone). Each of the pre-coated or uncoated jars was treated with 1.2 ml insecticide solution. To ensure an even

distribution of insecticide deposit on the inside surface of each jar, treated jars were continuously rolled by hand until the inside surface was dry. Control jars were treated only with 1.2 ml acetone, with or without a pre-coating of sucrose. All treated jars were placed in a vented hood under a wind speed of 23 m per min for 8-12 hours before testing.

Five *R. pomonella* females were introduced into each Mason jar. There were 4-8 replicates (jars) for each concentration of insecticide. Flies were allowed to remain in a jar for 10 min and were then transferred into a clean Mason jar. Clean jars were covered with aluminum mesh screen secured with a screw-on ring top and were kept at 25±3°C, 60±5% RH and 16:8 LD photoperiod. Food and water were supplied by a cube of sucrose and a 8-ml water-filled plastic vial plugged with a cotton wick fastened to the inside of the screen top. Fly mortality was recorded 24 hours after initial exposure to insecticide-treated jars. Flies which were unable to walk or were moribund were considered dead. Dose and response data were used to calculate LC_{50} and LC_{90} values by the method of probit analysis using a POLO computer program (Russell et al. 1977). Insecticide concentrations were converted to ug (a.i.)/cm^2 by calculating the surface area of the inside of a Mason jar (244.2 cm^2). Because of the high heterogeneity associated with data, the confidence limits of the LC_{50} and LC_{90} values were calculated only at the 90% probability level.

5.2.2. Field Cage Bioassays of Toxicity of Insecticides Applied to Red Spheres.

All insecticides evaluated in the laboratory (except carbaryl, which showed little toxicity against *R. pomonella* females) were tested further in field cages for effectiveness against flies visiting treated spheres. Spheres painted red (Tartar Red Dark Enamel, Sherwin-Williams Corp., Cleveland) were coated with sucrose (in the same manner and at the same concentration as in the Mason jars) and dried before application of insecticide. Technical grade insecticides were dissolved in acetone and applied by brush

to sucrose-coated spheres at concentrations equal to 5 times the LC90 values determined from the laboratory tests. Amounts of insecticide applied to spheres are summarized in Table 5.1. Two spheres were treated with each insecticide. Treated spheres were placed in a hood for ca. 24 hours to dry before testing.

A single treated sphere was hung on a potted apple tree (ca. 1-m canopy diameter) in a 3x3x3-m Saran screen field cage for testing. For each trial, a single female was introduced onto the sphere by a 0.2x1.5 cm triangular piece of dry filter paper (impregnated with 10% sucrose solution). Following fly introduction, total time of fly visitation and total time of fly feeding on a sphere were recorded. Treatments were rotated after testing 2 - 3 flies. About 40 flies were tested per treatment. A trial was terminated when the fly departed the sphere or 10 min elapsed. At the end of each trial, the fly was captured and kept in a 35-ml cup covered with a screen top and provided with a small cotton wick dipped in a 10% sucrose solution. Cups were maintained in the laboratory at 25±3°C , 60±5% RH, and 16:8 LD photoperiod. Mortality was determined 24 hours later using the same criteria described for laboratory bioassays.

Data on the duration of visiting and feeding of *R. pomonella* on different insecticide-treated spheres were analyzed by non-parametric methods (Kruskal-Wallis one-way ANOVA). Paired comparisons of treatments were carried out by multiple comparison procedures using Mann-Whitney U statistics (Sokal and Rohlf, 1981). Means rather than medians are presented for convenience of showing variation (S.E.). However, we should point out that the 10 min test period used here was chosen on the basis of a laboratory study by Duan and Prokopy (1993). This inevitably underestimated the mean duration of fly visitation and feeding on spheres because there was always a small proportion (less than 30%) of flies tested which remained on spheres over 10 min, especially when spheres were freshly treated with feeding stimulant.

Unweighted logistic regression analysis was used to determine the relationship between death of *R. pomonella* flies and duration of visiting and feeding times on

pesticide-treated spheres. Maximum likelihood estimates of logistic regression model parameters were obtained by iterative procedures (reweighted least squares) using Statistix software (version 4.0, 1992). Data on percentage mortality or knockdown from different insecticides were compared by pairwise G-tests (Sokal and Rohlf, 1981).

5.2.3 Field Cage Bioassays of Residue-Extending Agents for Prolonging Effectiveness of Feeding Stimulant and Insecticide.

Glidden^{R} colonial red 100% acrylic latex paint (Glidden Company, Cleveland, OH) and a proprietary polymeric thickener were tested separately as residue-extending agents for prolonging effectiveness of insecticide and feeding stimulant on treated spheres. Light corn syrup (Karo®, containing 75% sucrose, fructose, and other carbohydrates) was used as the feeding stimulant in place of granular sucrose, as we found in a previous test (unpublished) that it was as effective as sucrose in stimulating fly feeding but easier to blend with latex paint. Azinphosmethyl, methomyl, and dimethoate were chosen as insecticides because of their high effectiveness found in previous laboratory and field cage bioassays.

The three components (residue-extending agent, feeding stimulant, and pesticide) were mixed together in paste form and applied to spheres using a small brush. The proportion of each component in different tests is given in Table 5.2. In tests of 1990, the proportion of azinphosmethyl or methomyl relative to residue-extending agent and feeding stimulant was equal to 5 times the LC90 value obtained in laboratory bioassays (0.3% and 1.05%, respectively). We later realized that this determination of insecticide toxicity based on laboratory data might not be appropriate because of change in solvent from acetone to a mixture of paint or polymer and corn syrup. In tests of 1991 and 1992, therefore, we chose to compare dimethoate and methomyl on the basis of equal active ingredient content (1.05%). Polymer was not tested in 1991 and 1992 because 1990 tests showed that it became whitish after treated red spheres were exposed to natural weather

conditions. Treated spheres were dried and hung in the canopy of apple trees in fields (unprotected) or placed in field cages covered with rainproof plastic tarpaulin (protected). They were evaluated for effectiveness after varying durations of exposure in trees or field cages. Rainfall on the unprotected apple trees was recorded with a rain gauge. Because we found that treated spheres in apple trees exposed to rainfall showed significantly reduced duration of fly feeding on spheres, we hypothesized that retreating with feeding stimulant would increase the effectiveness of unprotected pesticide-treated spheres in killing visiting *R. pomonella*. Therefore, we retreated all unprotected spheres with 16% sucrose/water solutions using a household sprayer. The spheres were sprayed until the liquid on the sphere surface started dripping off. The retreated spheres were dried for ca. 2 hours and were tested again for their effectiveness in killing visiting flies. Bioassay procedures and data analyses were the same as described in the previous field cage test.

5.2.4 Field Cage Bioassays of Responses of *R. pomonella* to Spheres Treated with Insecticide, Feeding Stimulant, and Residue-Extending Agent.

In the previous field cage bioassays, where flies were released directly onto treated spheres, adverse effects (if any) of pesticides or residue-extending agents on fly attraction to treated spheres could not be measured. The type of bioassay we had used reduced the amount of time required for conducting trials but did not assess fly propensity to alight on spheres. Therefore, we evaluated alighting responses of *R. pomonella* by releasing a test fly on the foliage of caged trees containing spheres treated with a mixture of 1.05% (a.i.) dimethoate (technical grade or Cygon 4.0 EC) or methomyl (technical grade or Lannate 1.8 SL) plus 58.95% corn syrup and 40% latex paint. Red spheres were treated in the same manner as described before. Two spheres of the same treatment were hung 50 cm apart near the center of the canopy of a potted apple tree. For each trial, a mature female was released on a leaf midway between and slightly beneath the spheres and followed until it visited a sphere, left the tree without visiting a sphere, or

10 min elapsed without the fly visiting a sphere. After the fly landed on a sphere, procedures for collecting and analyzing data were the same as described in the previous field cage bioassays.

5.3 Results

5.3.1 Laboratory Bioassays of the Toxicity of Insecticides Applied to Glass Jars.

Data on the toxicity to *R. pomonella* of insecticides applied with or without sucrose to the inner surface of glass jars are given in Table 5.3. The LC_{50} and LC_{90} values indicated that the effectiveness of each insecticide in killing exposed *R. pomonella* was increased by the addition of sucrose. Probit analysis revealed that the presence of sucrose did not alter slopes of regression lines significantly or affect toxicity relationships among insecticides significantly.

According to LC_{50} values obtained in combination with sucrose, dimethoate was the most toxic insecticide to *R. pomonella*. Azinphosmethyl, tralomethrin, and methomyl were more toxic than malathion, fenvalerate, and carbaryl. Carbaryl showed such low toxicity to *R. pomonella* in our test that an LC_{50} value could not be established validly.

Table 5.3 also shows a rather wide range in confidence limits, especially with LC_{90} values for insecticide toxicity. This result was not unexpected because of several possible sources of variation associated with this type of bioassay. One of these variations was probably a difference among individual flies in time since the last feeding bout and hence in propensity to feed on a treated surface. Variation in LC_{50} and LC_{90} values was not the same among all insecticides. Without sucrose, response of *R. pomonella* to tralomethrin, fenvalerate, and methomyl was more variable than to dimethoate, azinphosmethyl, or malathion. With sucrose, this pattern held true, but variation in response to all insecticides appeared to be reduced.

5.3.2 Field Cage Bioassays of the Toxicity of Insecticides on Treated Red Spheres.

Data in Table 5.4 show that in the 1990 test, azinphosmethyl, methomyl, and malathion applied together with sucrose to spheres at 5 times the LC_{90} value (obtained from laboratory tests in Mason jars having sucrose) killed significantly more visiting flies than tralomethrin and fenvalerate, between which there was no significant difference in mortality. All insecticides except malathion, however, significantly reduced the duration of fly visitation and feeding on sucrose-treated spheres compared with control spheres having sucrose only. In the 1991 test, dimethoate applied to sucrose-treated spheres at 5 times the LC_{90} value did not have a significant effect on visiting or feeding times and killed slightly but not significantly more visiting *R. pomonella* than methomyl (Table 5.4). Spheres treated with methomyl produced by far the fastest detectable effect within the 10-min test period, causing knockdown to the ground of 68% and 56% of visiting flies in 1990 and 1991, respectively; however, further examination of flies knocked down by methomyl (data not presented) indicated that about 25% recovered within 24 hours.

Results of logistic regression analysis of data pooled from all insecticide treatments in 1990 or 1991 (Table 5.5) indicated that for a sucrose-coated sphere treated with a lethal dose of insecticide (5 times the LC_{90} value), the probability of death of an individual fly visiting a sphere was significantly positively related to the duration of visiting (in 1991) and feeding on it (both years). Calculation of adjusted odds ratios based on the logistic regression coefficients indicated that feeding was an important cause of fly mortality. The odds ratio of a fly dying increased by a factor of 2.1 (1990) and 3.6 (1991) for each 60 sec increase in feeding time, and did so by a factor of 1.1 (1990) and 1.7 (1991) for each 60 sec increase in visiting time.

5.3.3 Field Cage Bioassays of Residue-Extending Agents Influencing Effectiveness of Feeding Stimulant and Insecticides.

In the 1990 tests, addition of latex paint or polymer thickener to 0.3% azinphosmethyl (= 5 times LC_{90} value) significantly reduced mortality of *R. pomonella* visiting treated spheres from 84% to 30% and 42%, respectively, when tested before exposure to weather (0 residual days) (Figure 5.1, A.). Mortality on 1.05%-methomyl-treated spheres (=5 times LC_{90} value) at 0 residual days was 74, 63, and 75%, respectively, in combination with latex paint, polymer thickener, or control (no residue-extending agent) (Figure 5.1, B), among which there were no significant differences. In 1991, we decided not to test polymer in combination with dimethoate because of its change to whitish in color after exposure to weather. Rather, we decided to test latex paint in combination with dimethoate, using the same amount of methomyl (1.05% a.i.) as a reference or control treatment. Addition of latex paint appeared to have little effect on mortality of *R. pomonella* visiting spheres treated with 1.05% dimethoate (Figure 5.2, A). Mortality for 1.05%-dimethoate- and 1.05%-methomyl-treated spheres was 83 and 62%, respectively, when tested before exposure to weather (0 residual days).

For both the 1990 and 1991 tests, results show that effectiveness of pesticide-treated spheres was sharply reduced regardless of treatment after the spheres were exposed to weather (especially rainfall). It appeared that exposure to rainfall had sharply reduced the duration of *R. pomonella* feeding on aged spheres though it had not greatly reduced the duration of visiting (Figure 5.1, C and D; and Figure 5.2, C). In 1991, when unprotected spheres aged 7, 21, or 35 days were retreated with 16% sucrose just before testing, the duration of *R. pomonella* visiting and feeding increased substantially (Figure 5.2, D) on both the dimethoate- and methomyl- treated spheres, and mortality of *R. pomonella* visiting 1.05%-dimethoate-treated spheres was completely or largely restored (Figure 5.2, B). Dimethoate-treated spheres retreated with sucrose killed significantly more visiting *R. pomonella* at 7, 21, and 35 days than methomyl-treated spheres retreated

with sucrose (Figure 5.2, B). Weathered 7- and 21-day dimethoate-treated spheres retreated with 16% sucrose killed almost as many visiting *R. pomonella* (75 - 70%) as freshly (0 residual day) treated spheres did (85%).

Similar results were obtained in 1992 tests (Figure 5.3.), where dimethoate and methomyl of different formulations were tested in combination with latex paint and corn syrup at 35 residual days under different weathering conditions (protected vs. unprotected against rainfall). Aged (35 residual day) spheres of all treatments, whether unprotected and retreated with sucrose or protected against rainfall (124.2 mm), killed considerably greater numbers of visiting *R. pomonella* than unprotected spheres not retreated with sucrose. Both formulations of dimethoate-treated spheres when unprotected and retreated with sucrose or protected against rainfall killed significantly more visiting flies than either formulation of methomyl-treated spheres when unprotected and retreated with sucrose or protected against rainfall (Figure 5.3., left). Retreating with sucrose or protecting against rainfall greatly enhanced the duration of *R. pomonella* feeding and visiting on the spheres (Figure 5.3., right), and therefore increased intake of insecticide by visiting flies.

5.3.4 Field Cage Bioassays of Responses of *R. pomonella* Flies to Spheres Treated with Dimethoate or Methomyl, plus Corn Syrup and Latex Paint.

The proportion of released *R. pomonella* landing on each type of pesticide-treated sphere (42 - 55%) was no different than the proportion that landed on control spheres treated only with corn syrup and latex paint (53%), indicating no negative effect on *R. pomonella* attraction to spheres due to the presence of pesticide (Table 5.6). After the flies landed, duration of visiting and feeding on methomyl-treated spheres, regardless of formulation, was significantly less than on control spheres. Duration of fly visits on dimethoate-treated spheres for both the technical grade and Cygon 4.0 EC formulations was not significantly different from that on control spheres. Duration of fly feeding on

spheres treated with technical grade dimethoate was not significantly different from that on control spheres. Spheres treated with commercially formulated dimethoate (Cygon 4.0 EC) appeared to have a reduced duration of fly feeding compared with control spheres. But there was no statistically significant difference in duration of fly feeding between the two formulations of dimethoate. Similar to results of previous experiments (section 5.3.3), mortality of *R. pomonella* that visited dimethoate-treated spheres was 76 and 83% for Cygon 4.0 EC and technical grade formulations, respectively. Both of these values were significantly greater than those for Lannate 1.8 SL and technical grade methomyl (50 and 61%, respectively). Both formulations of methomyl-treated spheres caused rapid knockdown (42 and 71%, respectively) of visiting flies. No knockdown on dimethoate-treated spheres was observed during the 10-min test period. About 30% of flies suffering knockdown from methomyl-treated spheres (pooled across both formulations) recovered after 24 h.

5.4 Discussion

The goal of our study was to develop an effective pesticide-treated-sphere system for controlling *R. pomonella* flies in commercial orchards. Together, our findings indicate that the effectiveness of insecticides in killing *R. pomonella* flies that contact residual deposits only briefly (10 min or less) can be increased by addition of a feeding stimulant. Among the insecticides tested, dimethoate showed the greatest toxicity to *R. pomonella* and the least adverse effect on visiting and feeding times on treated spheres at a deposit rate equal to 2.8 ug (a.i.)/cm^2 (5 times the LC_{90} value derived from laboratory bioassays). Thus, dimethoate would appear to be the most suitable insecticide for use in developing a pesticide-treated sphere system for controlling *R. pomonella*. Latex paint (but not polymer) as a residue extending agent protected the residual effectiveness of dimethoate (and to a lesser extent methomyl) but did not protect the residual effectiveness of feeding stimulant (corn syrup) to a degree sufficient to be of fly control value after

rainfall. Our results indicate, however, that retreating weather-exposed, aged, dimethoate-coated spheres with fly feeding stimulant restored effectiveness in killing alighting *R. pomonella* flies.

Steiner (1952) and Steiner and Hinman (1952), working with *Dacus dorsalis* (Hendel), showed that addition of the feeding stimulant sucrose to parathion and other organophosphate insecticides failed to improve control in field spray programs because too often the sugar reduced residual toxicity by adversely affecting the physical nature of deposits on foliage and fruit. Our experiments, however, indicate that the LC_{50} and LC_{90} values for each insecticide tested were decreased by the addition of sucrose. In our laboratory and field cage tests where a fly was exposed to a dried deposit of insecticide for a brief period of time (10 min or less), fly feeding on sucrose-insecticide surface residues may have increased greatly the amount of insecticide taken into the body compared with fly tarsal contact with insecticide in the absence of sucrose. This result could be expected, especially when an insecticide combined with sucrose has both contact and stomach toxicity. All of the insecticides that we tested here have these properties.

A control strategy using pesticide-treated spheres against *R. pomonella* involves alighting of attracted flies on insecticide applied to red spheres positioned optimally in orchards. Control is achieved when flies contact pesticide residue during the process of visiting a treated sphere. The extent to which visiting flies are poisoned through contact with pesticide-treated spheres will depend on the amount of toxicant picked up from a sphere, which in turn will depend in part on duration of visiting and/or feeding. Our results (Table 5.4) showed that all of the insecticides tested (except malathion and dimethoate) at 5 times the LC_{90} value for a 10 min exposure period reduced the duration of visiting and feeding on treated spheres. This reduction could have resulted from quick knockdown, contact repellency, sublethal poisoning, or behavior-modifying effects of insecticides (Hall, 1979; Reissig, 1983; Stanley et al., 1989). Reduced visitation and

feeding duration without acquisition of a lethal dose of insecticide could limit significantly the effectiveness of pesticide-treated spheres in killing *R. pomonella* visitors. For example, methomyl rapidly paralyses *R. pomonella*. It caused 42 - 70% knockdown of visiting flies within the 10-min test period. This action, however, reduced the duration of visiting and feeding on treated spheres and did not allow some visitors to pick up a lethal dose of toxicant before knockdown (about 25 - 30% of knocked down flies recovered after knockdown). This type of unwanted side effect on duration of visiting and feeding should be considered when selecting an insecticide suitable for use in a pesticide-treated-sphere control system.

Haniotakis et al. (1991), working with *D. oleae*, reported that unpainted plywood rectangles (20x20x0.4-cm) dipped for 30 min in a water solution containing deltamethrin and sucrose effectively reduced fly densities in test orchards after installation and required no replacement or other service throughout the fruiting period of olive trees (ca. 4 - 5 months). Under the prevailing conditions, absence of a residue-extending agent seemed to be unimportant, possibly because of absorption of the insecticide-sucrose mixture by the plywood and lack of any rainfall over the course of the trial, which occurred during Mediterranean summer months (Haniotakis et al., 1991).

For control of *R. pomonella* by pesticide-treated spheres, however, an effective residue-extending agent is necessary to reduce the need to retreat spheres frequently with pesticide and/or feeding stimulant during the growing season. This need exists because frequent and often heavy rainfall occurs during the fly activity period (July - September) under eastern North American conditions, and because there is very limited absorption capability of the surface of a red-painted smooth sphere (Duan et al. 1990, Chapter 4). A smooth, shiny dark surface is required to elicit a strong alighting and feeding response by *R. pomonella*. Our results show that latex paint or polymer thickener, as residue-extending agents, provided some benefit in protecting the residual effectiveness of insecticide applied to spheres but provided little protection for the feeding stimulant

(sucrose). Spheres treated with a mixture containing 1.05% a.i. dimethoate (technical grade or Cygon 4.0 EC) or methomyl (technical grade or Lannate 1.8 SL), 58.95% corn syrup, and 40% latex paint became nearly ineffective in killing *R. pomonella* visitors after exposure to natural weather conditions (rainfall of 6.6 mm or greater). Duration of *R. pomonella* fly visitation and especially feeding on aged (rain-washed) pesticide-treated spheres also was sharply reduced. Retreating unprotected dimethoate-treated spheres (aged 7 - 35 residual days) with sucrose, however, resulted in restoration of toxicity (as well as duration of visiting and feeding) to a level nearly equivalent to that of freshly (0 day) treated spheres. For unknown reasons, both latex paint and polymer thickener drastically reduced the toxicity of azinphosmethyl; however, such inactivation did not occur with latex paint or polymer thickener applied with methomyl.

One major concern regarding the use of a combination of insecticide, residue-extending agent and feeding stimulant in developing a pesticide-treated-sphere system is the influence on the attractiveness of red spheres to *R. pomonella*. Because we found that spheres treated with polymer thickener and azinphosmethyl or methomyl became whitish after exposure to weather (mainly rainfall), we did not test this thickener further, knowing that the dark color of a red sphere is essential to its attractiveness to *R. pomonella* (Owens and Prokopy, 1986). Our field-cage bioassay (Table 5.6) indicated, however, that latex paint (colonial red) in combination with corn syrup and insecticide [1.05% (a.i.) dimethoate (technical grade or Cygon 4.0 EC) or methomyl (technical grade or Lannate 1.8 SL)] had no negative effect on attraction of *R. pomonella* to red spheres. We did not evaluate the influence of latex paint alone on attractiveness of treated spheres to *R. pomonella* because the latex paint we used is similar in spectral reflectance pattern to the standard Tartar Red Dark Enamel (Owens and Prokopy, 1986; Duan, unpublished data). Although we did not investigate the influence of feeding stimulant (corn syrup or sucrose) on visual characters of treated red spheres, we have no reason to believe that either of these stimulants applied to red spheres would have a negative effect on

attractiveness to *R. pomonella* unless the amount of feeding stimulant were so high that the reflectance of spheres was altered.

In summary, we conclude that a paste mixture containing dimethoate, corn syrup, and latex paint shows the most promise as a substitute for the current sticky coating applied to red spheres for controlling *R. pomonella* in commercial orchards. Present data suggest, however, that spheres would require retreating with feeding stimulant (sucrose or corn syrup) to maintain high effectiveness whenever they are subject to substantial rainfall. Under field conditions, effectiveness of insecticide-treated spheres in killing alighting *R. pomonella* would be much more limited by loss of feeding stimulant than by loss of insecticide as a consequence of washing by rainfall. Further evaluation of the effectiveness of pesticide-treated spheres in protecting fruit under semi-field and field conditions needs to be accomplished before such spheres can be recommended for use in commercial orchards to control *R. pomonella*. In particular, refinement of the ratio of pesticide:feeding stimulant:residue-extending agent in the mixture should be made to achieve maximum benefit. In addition, we need to measure the potential danger of pesticide-treated spheres to various onlookers who might handle or taste the sphere surface.

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Table 5.1. Amount of insecticide applied to spheres in 1990 and 1991 at a concentration equal to 5 times the LC_{90} value. LC_{90} values were calculated from data obtained in previous laboratory bioassays conducted in glass jars with sucrose (see table 3). All spheres were coated with sucrose at a rate of 1.31 mg/cm^2 of sphere surface and dried before application of insecticide.

Treatments	Concentration (ug/cm^2)	Total amount/per sphere (mg)
	Summer 1990	
Azinphosmethyl	14.7	3.0
Methomyl	52.2	10.5
Tralomethrin	29.1	5.9
Malathion	51.6	10.4
Fenvalerate	159.2	32.0
Control	-	-
	Summer 1991	
Dimethoate	2.8	0.6
Methomyl	52.2	10.5
Control	-	-

Table 5.2. Composition of different mixtures of insecticide, feeding stimulant, and residue-extending agent tested in 1990, 1991, and 1992.

Insecticide (a.i.)	Residue-extending agent[a]	Feeding stimulant
	Summer 1990	
0.3% azinphosmethyl	40% latex paint	59.7% corn syrup
0.3% azinphosmethyl	40% polymer	59.7% corn syrup
0.3% azinphosmethyl	10% water + 30% methanol	59.7% corn syrup
1.05% methomyl	40% latex paint	58.95% corn syrup
1.05% methomyl	40% polymer	58.95% corn syrup
1.05% methomyl	10% water + 30% methanol	58.95% corn syrup
	Summer 1991 and 1992	
1.05% dimethoate	40% latex paint	58.95% corn syrup
1.05% methomyl	40% latex paint	58.95% corn syrup

[a] Includes a very small proportion of inactive ingredient contained in insecticidal formulations.

Table 5.3. Toxicity of dimethoate, azinphosmethyl, trallomethrin, methomyl, malathion, fenvalerate, and carbaryl to female *R. pomonella* flies 24 h after 10 min exposure in treated jars with or without a coating of sucrose.

Insecticides	Sucrose	N	LC_{50} (90% C.L.)[a]	LC_{90} (90% C.L.)[a]	Slope (±S.E.)
Dimethoate	Yes	182	0.1 (0.07-0.14)	0.6 (0.34-1.23)	2.9 (±0.4)
	No	198	0.2 (0.13-0.24)	0.7 (0.51-1.38)	3.5 (±0.5)
Azinphosmethyl	Yes	218	0.2 (0.11-0.29)	2.9 (1.56-7.42)	1.8 (±0.3)
	No	200	0.6 (0.38-0.83)	5.4 (3.08-11.95)	2.2 (±0.3)
Tralomethrin	Yes	218	0.2 (0.10-0.28)	5.8 (2.68-18.42)	1.4 (±0.2)
	No	167	0.6 (0.13-2.20)	16.5 (3.79-127.40)	1.5 (±0.2)
Methomyl	Yes	160	0.2 (0.11-0.42)	10.4 (4.53-40.23)	1.3 (±0.2)
	No	160	0.7 (0.14-2.34)	15.8 (3.80-112.91)	1.6 (±0.2)
Malathion	Yes	235	0.9 (0.61-1.25)	10.3 (6.237-20.59)	2.1 (±0.2)
	No	235	1.6 (0.59-2.75)	14.5 (7.23-73.82)	2.3 (±0.4)
Fenvalerate	Yes	200	0.7 (0.43-1.24)	31.8 (13.57-111.20)	1.3 (±0.2)
	No	160	2.2 (1.20-3.68)	40.3 (17.36-176.30)	1.7 (±0.3)
Carbaryl	Yes	160[b]			
	No	159[b]			

[a] ug/cm^2 of insecticide deposit on the inside surface of treated jars; [b]Tested up to 61 ug of toxicant / cm^2 of surface area. Mortality was too low for valid regression.

Table 5.4. Responses of *R. pomonella* flies to spheres coated with pesticide (at 5 times the LC_{90} value) and fly feeding stimulant (sucrose).

Treatment	N	Mean time spent on treated spheres (±S.E.) (sec)[a] Visiting	Feeding	Knockdown while on spheres (%)[b]	Mortality within 24 hours (%)[b]
Summer of 1990					
Azinphosmethyl	43	257 (±27)c	92 (±14)c	21.9b	81.4a
Methomyl	40	250 (±28)c	83 (±11)c	67.5a	80.0a
Malathion	42	386 (±31)ab	201 (±23)ab	12.2cb	78.6a
Tralomethrin	40	255 (±33)c	83 (±15)c	0.0c	57.5b
Fenvalerate	41	318 (±29)bc	132 (±14)b	7.3c	46.3b
Control	40	417 (±28)a	256 (±24)a	0.0c	0.0c
Summer of 1991					
Dimethoate	42	391 (±33)a	298 (±33)a	0.0b	68.9a
Methomyl	45	273 (±31)b	136 (±21)b	55.6a	62.2a
Control	42	365 (±31)a	300 (±33)a	0.0b	0.0b

[a] Numbers within year and in a column followed by same letter are not significantly different according to nonparametric multiple comparison procedures based on Mann-Whitney U statistics at 0.05 level. [b] Numbers within year and in a column followed by same letter are not significantly different according to pairwise G-tests, df = 1, at 0.05 level.

Table 5.5. Logistic regression analysis of *R. pomonella* fly mortality in relation to visiting and feeding times of flies on spheres coated with sucrose and pesticide (at 5 times the LC_{90} value). For each year, analysis is based on data for all pesticides combined.

Variable[a]	Coefficient	S.E.	P-value	Odds ratio (95% C.L.)[b]
		Summer of 1990		
Constant	-0.79	0.31	0.01	-
Visiting	0.11	0.06	0.07	1.1 (0.9 - 1.3)
Feeding	0.74	0.17	0.00	2.1 (1.5 - 2.9)
		Summer of 1991		
Constant	-3.52	0.83	0.00	-
Visiting	0.50	0.19	0.01	1.7 (1.1 -2.4)
Feeding	1.27	0.48	0.01	3.6 (1.4 - 9.2)

[a] Model deviance (G) = 205.38, df = 203, p = 0.440 for the test of 1990; G = 35.48, df = 87, and p = 0.999 for 1991. [b] Changes in the ratio of the likelihood of fly death for an increase of one unit of visiting and/or feeding time (min).

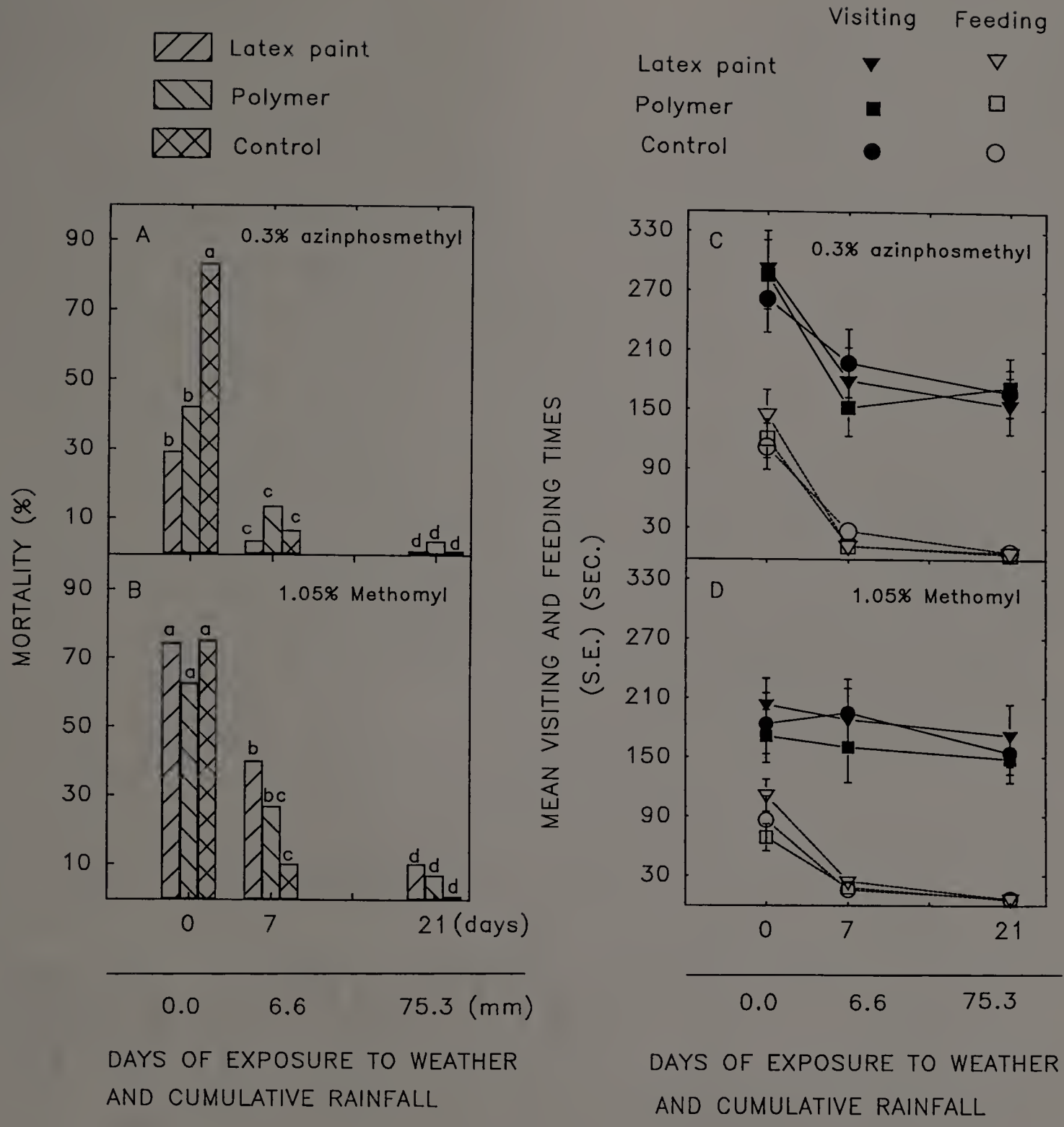

Figure 5.1. Responses of *R. pomonella* flies to spheres treated with azinphosmethyl or methomyl in combination with corn syrup (feeding stimulant) and latex paint or polymer (residue extending-agent) at different days after exposure to weather in 1990. Control treatment consisted of no residue-extending agent but insecticide and corn syrup. A and B: fly mortality 24 h after exposure to treated spheres. In each graph within each exposure period, bars having the same letter are not significantly different according to pairwise G-tests at the 0.05 level. C and D: duration of fly visiting and feeding on treated spheres.

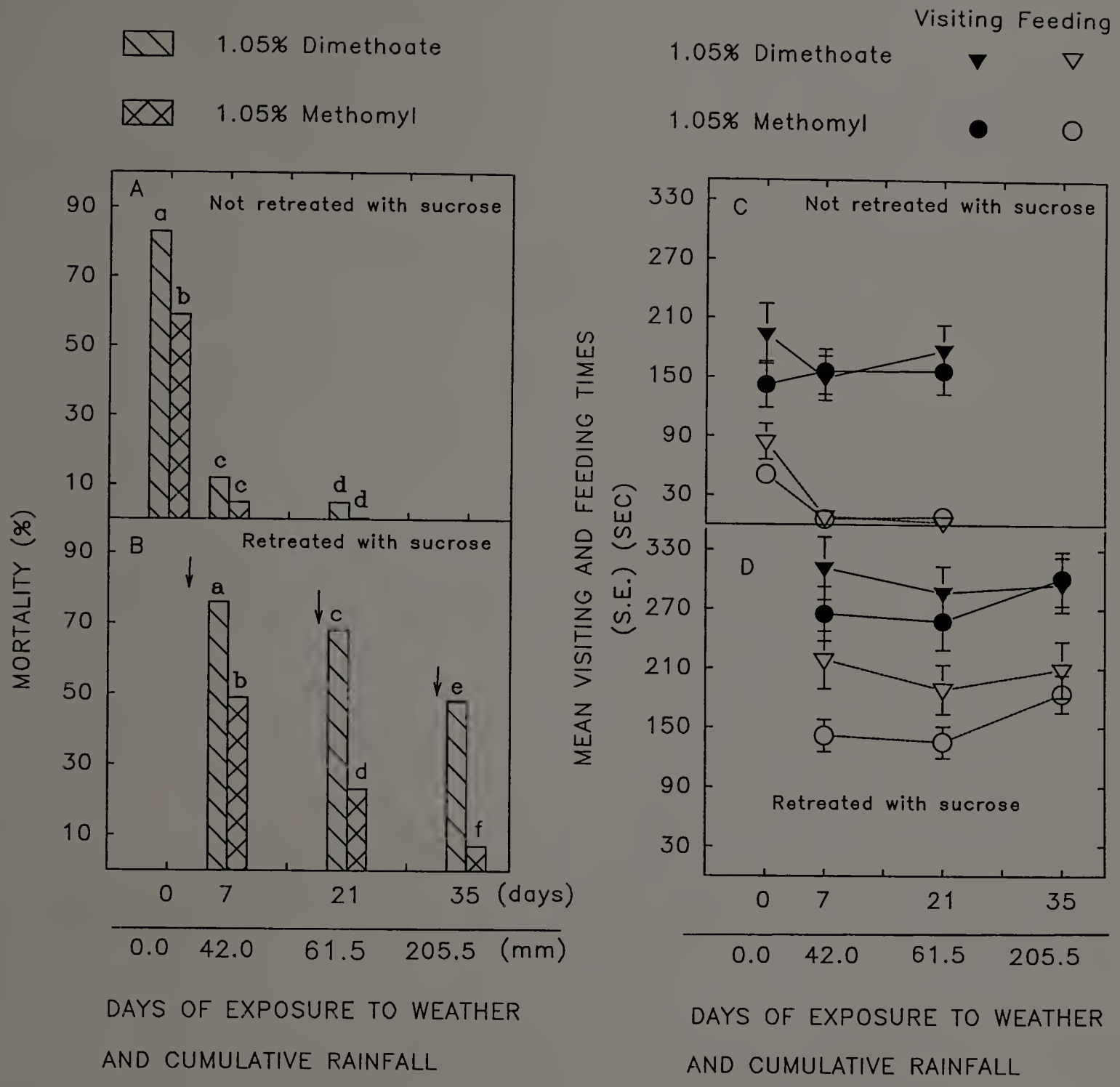

Figure 5.2. Responses of *R. pomonella* flies to spheres treated with dimethoate or methomyl in combination with corn syrup and latex paint at different residual days and to aged spheres after retreating with 16% sucrose just before testing. A and B: fly mortality 24 h after exposure to treated spheres. In each graph within each exposure period, bars having the same letter are not significantly different according to pairwise G-tests at the 0.05 level. C and D: duration of fly visiting and feeding on treated spheres.

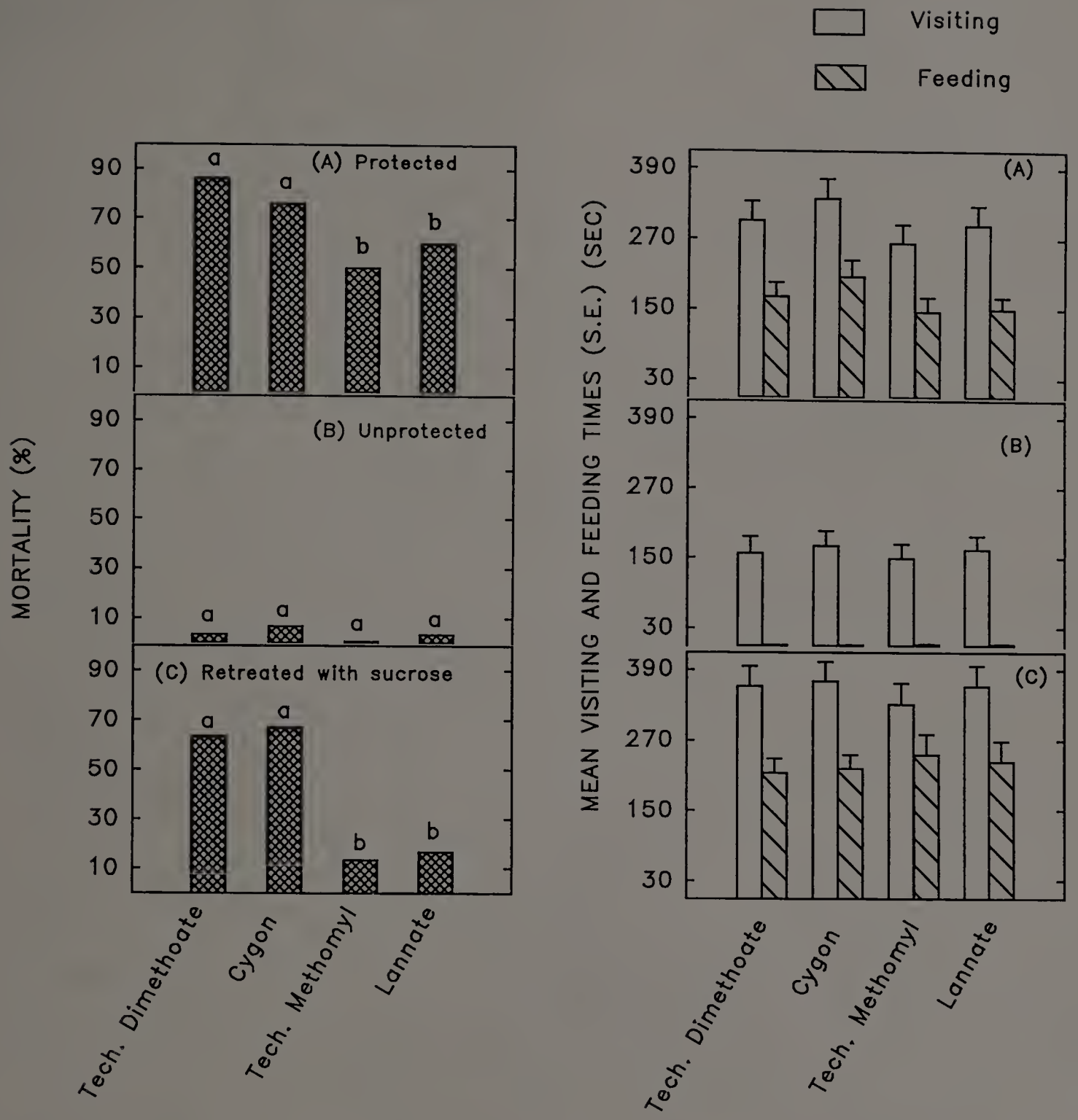

Figure 5.3. Responses of *R. pomonella* flies to aged (35 residual day) spheres treated with dimethoate or methomyl of different formulations in combination with corn syrup and latex paint under different weathering conditions (protected (A) vs unprotected (B) against rainfall, 124.2 mm) and after retreating (unprotected spheres) with 16% sucrose (C). Left - fly mortality 24 h after exposure to treated spheres. In each graph, bars having the same letter are not significantly different according to pairwise G-tests at the 0.05 level. Right - duration of fly visiting and feeding on treated spheres.

Table 5.6 Responses of *R. pomonella* flies to spheres treated with 1.05% dimethoate or 1.05% methomyl of different formulations in combination with corn syrup and residue-extending agent (latex paint) at 0 residual days.

Treatment	No. flies released	Landing on sphere (%)[a]	Mean time on sphere (±S.E.) (sec)		Knockdown while visiting sphere (%)[a]	Mortality within 24 h (%)[a]
			Visiting[b]	Feeding[b]		
Dimethoate						
Tech	53	41.5a	300 (±38)a	104 (±19)ab	0.0c	82.8a
Cygon	53	47.2a	269 (±39)ab	76 (±12)bc	0.0c	76.0a
Methomyl						
Tech	53	54.7a	192 (±28)bc	76 (±12)bc	71.4a	60.7bc
Lannate	53	45.3a	169 (±28)c	47 (±17)c	41.7b	50.0c
Control	53	52.8a	334 (±41)a	180 (±25)a	0.0c	0.0d

[a] Numbers in a column followed by same letter are not significantly different according to pairwise G-tests (df = 1) at 0.05 level. [b] Numbers in a column followed by same letter are not significantly different by nonparametric multiple comparison procedures based on Mann-Whitney U statistics at 0.05 level.

CHAPTER 6

CONTROL OF APPLE MAGGOT FLIES BY PESTICIDE-TREATED RED SPHERES

6.1 Introduction

The availability of effective visual traps (8-cm red spheres and yellow rectangles) and olfactory attractants (synthetic food and fruit odors) has facilitated development of behavioral approaches to controlling the apple maggot fly, *Rhagoletis pomonella* (Walsh). Prokopy (1975, 1991) showed that hanging unbaited sticky red spheres on each apple tree in a small orchard (1/7 ha) to capture alighting females was effective in protecting host fruit from apple maggot fly damage (fruit damage averaged less than 1%). Recently, ringing small (ca. 1 ha) apple orchard blocks with sticky red spheres 5 m apart on perimeter trees baited with butyl hexanoate has proven even more effective (fruit damage 0.4% or less) (Prokopy et al., 1990b). Also, MacCollom and Lauzon (1992) showed that placement of combined yellow rectangle and red sphere sticky traps baited with butyl hexanoate in a large proportion of host trees around and in orchards several hectares in size afforded effective apple maggot control (1% damage to fruit). In all cases, however, it has become apparent that reliance on sticky (Tangletrap®) as an agent to kill *R. pomonella* that alight on the traps renders this approach non-appealing for use in larger commercial orchards because coating and maintaining the sticky is awkward and labor-costly (Prokopy et al., 1990b). Development of a pesticide-treated (non-sticky) sphere system could be a potential alternative to currently used sticky-coated spheres for controlling *R. pomonella* (Duan et al. 1990).

Previously, we showed that spheres treated with a mixture containing a pesticide, feeding stimulant and residue-extending agent killed 76 - 90% of alighting *R. pomonella* before exposure to weather (Chapter 5). High effectiveness of these spheres in killing alighting *R. pomonella* lasted at least 35 days under natural weather conditions, provided

that the spheres were retreated with an aqueous solution of 16% sucrose after each rainfall. However, these mortality data were obtained in field cages, where the spheres were hung on single non-fruiting apple trees. Further study of the effectiveness of pesticide-treated spheres on fruiting apple trees under semi-field and field conditions is needed before recommendation of their use in commercial orchards to control *R. pomonella* can be made.

Here, we evaluated pesticide-treated spheres for controlling *R. pomonella* flies under semi-field and field conditions.

6.2 Materials and Methods

Standard 8-cm red wooden spheres and Tangletrap® were purchased from Pest Management Supply Co. (Amherst, MA). The insecticide dimethoate (technical or Cygon® 4.0 EC) was provided by American Cyanamid Company (Wayne, NJ). Light corn syrup or sucrose (feeding stimulants) and Glidden colonial red 100% acrylic latex paint (residue-extending agent) were purchased from local stores.

The pesticide-treated sphere design used here was the product of studies in Chapters 4 and 5, in which 8-cm red wooden spheres were brush-painted with a mixture containing 1.05% a.i. dimethoate, 58.95% corn syrup, and 40% latex paint. Here, we added 15% water to the mixture while correspondingly reducing the proportion of corn syrup to 43.95%. This was done because we found that addition of water greatly facilitated thorough mixing and application of components without reducing sphere effectiveness. All the components were mixed together into a paste form and applied to spheres using a small brush. Treated spheres were dried 48 h before use in tests.

6.2.1 Field cage tests.

All flies used in field cage tests originated from mixed cultivars of infested apples collected from unsprayed trees in Amherst, MA the previous summer. Flies were

maintained by methods described in Chaper 4. For testing, only reproductively mature flies (15 - 25 days old) were used.

In experiment 1 (1992), we evaluated the comparative effectiveness of spheres coated with technical dimethoate, Cygon 4.0 EC or Tangletrap in protecting host fruit from *R. pomonella* oviposition. The control treatment consisted of no traps of any type in the tree. Four test arenas were used, each consisting of a single potted apple tree (1.5 m diam canopy) placed in a 3 m diam x 3 m tall clear nylon screen cage. For tests, 35 green Gravenstein apples were hung in the canopy of each tree by attaching fruit stems to branchlets using copper wire. All fruit were picked on July 19 from a commercial orchard unsprayed with insecticide since May. Fruit were stored at 3 ^{o}C and checked under a microscope to insure lack of pre-existing *R. pomonella* egg punctures before use in tests. The fruit on each tree were evenly spaced. Positions were fixed throughout all assays. Foliage was trimmed so that the ratio of leaves to fruit was kept at 30:1 in each tree. On each tree, a single sphere was placed in the upper 1/3 of the canopy and was baited with one 2-dram polyethylene vial of butyl hexanoate and one packet (5 mg) of ammonium acetate (Consep Inc., Bend, OR), each 15 cm from the sphere.

For each trial, 25 females were released individually (using a small plastic cup lined with moist filter paper) onto 10 leaves located in the lower 1/3 of the tree canopy. Release occurred between 1000 and 1040. Following release, flies caught on sticky spheres were counted and removed every 30 min until the trial ended (after 5 h). Flies killed following alighting on pesticide-treated spheres fell to bottom of the cage and were counted there every 30 min. At the end of a trial, remaining flies were collected in a small (15x15x15-cm) cage and held in the laboratory for 18 h to assess post-trial mortality. For all trials, we found that the post-trial mortality was 5% or less for pesticide-treated sphere treatments and 0% for sticky sphere and control treatments. For data analysis, we included post-trial mortality in total mortality. To eliminate effects of differences between individual test arenas, treatments were systematically rotated every test day until each

treatment had one replicate in each arena (total of 4 replicates per treatment). Fruit from each treatment were examined under a microscopes for egg punctures and eggs.

Data on the proportion of flies killed by pesticide-treated spheres or caught on sticky spheres and data on the proportion of fruit receiving eggs were transformed logarithmically [ln (1+x)] and analyzed by ANOVA procedures (Statistix, 1992). Transformed mean percentages of flies killed or caught and fruit injuries were separated by Tukey's HSD (honest significant difference) tests at the 0.05 level. Means and standard errors are presented in table 6.1 for the untransformed data.

In experiment 2 (1993), we investigated the effect of exposure of pesticide-treated (Cygon 4.0 EC) and sticky spheres to climatic conditions in nature on trap efficiency in killing or capturing alighting flies. Beginning on June 28, we placed both pesticide-treated and sticky spheres in apple trees of a commercial orchard for 0, 7, 14, or 28 days. All spheres were baited as described in experiment 1 and positioned optimally for trapping *R. pomonella* flies as described by Drummond et al. (1984). Exposed spheres were stored in a dark room at 3 - 5 ^{o}C until they were tested during July 28 - August 15 in the same arenas used in experiment 1, except that no fruit were hung on the trees. Eight exposed spheres per treatment were tested. With pesticide-treated spheres, all except those aged 0 days were dipped into an aqueous solution of 16% sucrose for 2 sec and dried 24 h before testing.

For tests, 10 females per trial were released on 5 leaves in the lower 1/3 of the tree canopy using the same methods as in experiment 1. Following fly release, we observed continuously for 1 h the number of flies landing on a sphere and the number that escaped from a sticky sphere. After the 1-h observation, the trial continued for an additional 1.5 h. At the end of each trial (2.5 h), the number of flies killed by pesticide-treated spheres or caught by sticky spheres was recorded. We also determined post-trial mortality over 18 h (as in experiment 1) and found it to be 10% or less for flies that were tested with

pesticide-treated spheres. Again, post-trial mortality was included in total mortality for data analysis.

Data on the number of released flies sighted on a sphere, proportion of released flies killed or caught during a trial, and proportion that escaped from a sticky sphere were analyzed by linear regression procedures (Statistix, 1992). Natural logarithm transformation [ln (1+X)] was used when necessary to stabilize variance and normality of the data. In addition, we also quantified the accumulation of insects captured on sticky spheres during exposure in the orchard prior to testing. The spheres captured not only *R. pomonella* flies but also many other kinds of insects. Rather than counting the exact number captured, we decided to measure the proportion of sphere surface area occupied by captured insects. We quantified this by removing all insects, placing them immediately next to one another on a sheet of paper , and measuring the area occupied. The accumulation of captured insects in relation to duration of sphere exposure was described by a polynomial regression model (Dixon et al. 1990). A logistic regression model (Dixon et al. 1990) was fit to predict the probability of an alighting fly escaping from a sphere in relation to the area occupied by insects on the sphere surface.

6.2.2 Field observations.

Previous studies (Chapter 5) showed that duration of fly visit to a pesticide-treated sphere and, more importantly, duration of feeding are key variables that determine the probability of a fly dying. Thus, one of our major concerns with the strategy of using pesticide-treated spheres to control *R. pomonella* involves duration of visitation and feeding by a wild fly alighting on a sphere in the field. We are also concerned about the degree of attractiveness of pesticide-treated spheres to wild *R. pomonella* flies under field conditions. During July 23 - August 8, 1993, we observed responses of wild *R. pomonella* flies to a baited pesticide-treated (Cygon 4.0 EC) sphere placed in a fruiting tree under field conditions. A baited red sticky sphere was used as a control.

Observations were made in an unmanaged fruiting orchard harboring a high population of *R. pomonella* flies. Two potted apple trees, each with a 1.5 m diam canopy, were placed 3 m from an unmanaged orchard tree. On each observation day, we hung 60 Gravenstein apples (picked on July 19 from a commercial orchard and stored at 3°C until use) and one baited pesticide-treated or baited sticky sphere (unexposed to weather or insects) on each observation tree in the same manner described for field cage tests. Two observers were assigned to each tree. They assessed continuously for 1 h the number of flies arriving in the tree canopy, ovipositing in the fruit, and landing on a sphere. The duration of fly visitation and feeding on the pesticide-treated sphere was recorded. We were unable to quantify the efficiency of a pesticide-treated sphere in killing all alighting flies because 70% of flies that had visited such a sphere subsequently flew to nearby foliage, where they disappeared from view. Those flies that alighted on a sphere and were observed to have fallen to the ground were assumed dead. During our observations, we also found that some flies repeatedly visited a pesticide-treated sphere with only 1 - 2 sec between visits. We considered such a pattern to be a single continuous visit.

Observations usually commenced at 0900 h and ended at 1500 h. We interrupted observations when the ambient temperature fell below 22 °C or exceeded 32 °C or when there was strong wind and/or dense cloudy conditions. The two types of spheres were rotated between observation trees after each 1 h of observation. Data were analyzed according to Chi-squire ($\chi^{2)}$ and Wilk-Shapiro tests (Statistix 1992).

6.2.3 Field tests.

We selected four commercial apple orchards located in different parts of western Massachusetts for field evaluation of pesticide-treated (Cygon 4.0 EC) spheres in controlling *R. pomonella* flies.

In test 1, we used a single experimental orchard (ca. 0.4 ha) (Clarkdale Farm, Deerfield), which consisted of Gravenstein apple trees (each ca. 6 m in canopy diam)

spaced 12 m apart. This orchard was infested by a high population of *R. pomonella* that originated primarily from dropped fruit within the orchard. For tests, we divided this orchard into two equal-size blocks of 12 trees each. We deployed 3 baited pesticide-treated spheres on each tree in one of the blocks and the same number of baited sticky spheres in the other block (as a control treatment).

In test 2, we used 2 blocks (each ca. 0.4 ha) in each of three orchards (Rice Farm, Palmer; Horticultural Research Center, Belchertown; Apple Valley Farm, Ashfield). Five of the 6 blocks consisted of Liberty apple trees, the remaining block of McIntosh. All trees were ca 2 - 4 m in canopy diam and spaced 5 m apart. They were subjected to moderate populations of *R. pomonella* flies that originated primarily from wild host trees outside the orchards. For tests, a block of Liberty trees in each orchard received baited pesticide-treated spheres deployed 5 m apart on perimeter trees. The remaining block in each of the 3 orchards was sprayed twice (once in July and once in August) with azinphosmethyl (1.8 kg/ha/application) to protect against *R. pomonella.* All blocks received 2 applications of azinphosmethyl in May against other insect pests.

In both tests, spheres were deployed in late June or early July when adult *R. pomonella* flies began emerging. Each sphere was baited with one vial of butyl hexanoate and one packet of ammonium acetate. Spheres were placed 1.5 - 2 m above ground in an optimal position relative to surrounding foliage and fruit as described by Drummond et al. (1984). After deployment, pesticide-treated spheres were retreated with feeding stimulant (by dipping the sphere into a bucket containing an aqueous solution of 16% sucrose) within 1 - 2 days after rainfall of 5 mm or more. During the entire season, spheres were retreated on average 11 times with feeding stimulant. This procedure was important to maintaining presence of feeding stimulant and hence sphere effectiveness (see Chapter 5). Every two or three weeks, sticky spheres (in test 2) were cleaned and supplemented with new sticky and captured *R. pomonella* were counted.

To evaluate the effectiveness of pesticide-treated spheres, we sampled *R. pomonella* adult density and fruit infestation levels. To estimate adult density, in early July we placed unbaited sticky monitoring spheres in each block. In test 1, we used 6 monitoring spheres placed in 6 randomly selected trees in each block. In test 2, we used 8 monitoring spheres in each block (one per tree), four of which were placed in trees near the block perimeter and four near the center of the block. Every two or three weeks, monitoring spheres were checked and maintained as described for control sticky spheres. To estimate fruit injury levels, once every two or three weeks beginning in mid-July, we sampled 25 randomly selected on-tree fruit on each of 8 randomly selected trees within each block. All sampled fruit were examined under an optivisor for oviposition punctures and larval trails in the fruit flesh.

Data on the number of flies captured on monitoring traps were pooled over the season and were analyzed by ANOVA procedures (Statistix, 1992). To compare fruit infestation levels between treatments, we decided to focus on peak fruit injury rather than on average season-long injury. We did this because fruit injury generally increased as the fly season proceeded and because the peak fruit injury occurred at about the same time for the same cultivar in each orchard regardless of treatment. The Chi-squire (χ^2) test criterion (Statistix, 1992) was used for comparison of percent fruit injury. Standard errors (S.E.) for proportion of fruit injury were calculated by the formula of $\sqrt{p(1-p)/N}$ (where p=proportion of fruit injury, N=sample size). We wish to point out that for test 1, all statistical inference is based on pseudo-replication: number of flies captured per monitoring sphere and number of injured fruit per tree in the single block containing pesticide-treated spheres and the single block containing sticky spheres. For test 2, statistical inference is based on block replication: number of flies captured on monitoring traps per block and number of fruit injured per block.

6.3 Results

6.3.1 Field cage tests

In experiment 1 (table 6.1), 58, 54 and 61% of released flies were killed or caught by spheres treated with technical dimethoate, Cygon 4.0 EC and Tangletrap, respectively, when hung in field-caged potted apple trees. No significant differences were detected among the treatments in proportion of flies caught or killed ($F=0.17$, $df=2, 6$, $p=0.85$). Compared with potted trees without spheres (control), sticky or pesticide-treated spheres reduced significantly the amount of fruit injury (defined as % of fruit receiving eggs) by 58 - 68%. No significant differences were found in fruit injury on potted trees containing pesticide-treated or sticky spheres (Tukey's HSD test, $p>=0.05$).

In experiment 2 (Figure 6.1, A), as time of exposure in commercial orchard trees under natural weather conditions increased from 0 to 28 days, the proportion of released flies killed by pesticide-treated spheres (Y_1) decreased slightly (but not significantly) from 47.5 to 35% [$\ln(Y_1+1)=1.43 - 0.003*day$, $r^2=0.1$, $F=3.24$, $df= 1$ and 30, $P=0.08$]. The proportion of released flies caught on sticky spheres (y_0) decreased significantly from 49% to 13% [$\ln (Y_0+1) = 0.36 - 0.01*day$, $r^2=0.48$, $F=27.09$, $df=1$ and 30, $p=0.000$]. Further statistical analysis on the transformed data [$\ln (Y+1)$] indicated that the rate of decrease in proportion of released flies caught on sticky spheres was significantly greater than that for flies killed by pesticide treated spheres ($t=2.59$, $df=30$, $p<=0.005$).

Data from 1 h observations on released flies (Figure 6.1, B) indicated that days of exposure in orchard trees had no significant effect on number of flies alighting on either pesticide-treated spheres ($r^2=0.0004$, $F=0.01$, $df =1$ and 30, $p=0.92$) or sticky spheres ($r^2= 0.11$, $F=3.76$, $df=1$ and 30, $p=0.08$). During observations, numerically more flies were observed alighting on pesticide-treated spheres than on sticky spheres for each exposure treatment. This was probably because a fly could repeatedly visit a pesticide-treated sphere before it was poisoned. Few alighting flies (2.5%) escaped from sticky

spheres exposed in orchard trees for 0 days. However, 38, 43 and 73% of alighting flies escaped from sticky spheres exposed for 7, 14, and 28 days, respectively (Figure 6.1, C). As days of exposure in orchard trees increased, the percentage of sphere surface area (PSSA) occupied by captured insects increased significantly by a function of $PSSA=0.02*day - 0.0004*day^2$ ($r^2=$ 0.9680, F=453.05, df=2 and 29, p=0.000; model was forced through the origin of axes) (Figure 6.1, D). Accumulation of captured insects on sticky spheres was apparently a major reason accounting for alighting flies escaping from field-exposed spheres. The probability of a fly escaping from a field-exposed sphere (PE) can be predicted by a logistical model of PE=exp(-11.03) / [1+exp(-0.11.03 * PSSA)] (model deviance G=136.33, df=31, p=0.000).

6.3.2 Field observations

Over the 15 h of field observations, about an equal number of *R. pomonella* flies was sighted in trees containing a pesticide-treated sphere (55) as in trees containing a sticky sphere (52). Of those sighted, 56 and 49% were found visiting a pesticide-treated and sticky sphere, respectively (χ^2=0.74, df=1, p=0.39). Of flies sighted, 11% oviposited in host fruit on trees with a pesticide-treated sphere compared with 8% on trees with a sticky sphere (χ^2=0.33, df=1, p=0.57). All flies (100%) found alighting on a sticky sphere were caught immediately. Those found alighting on a pesticide-treated sphere stayed for a mean of 6.8 (±0.6) min, and fed for a mean of 5.2 (±0.6) min. Statistical analysis indicated that mean durations of visiting and feeding were normally distributed (Wilk-Shapiro test: W=0.9760, n=31, p>=0.5 for visiting and W=0.9839, n=31, p>=0.90 for feeding). Because of the difficulty of tracking flies that had visited and later left a pesticide-treated sphere, only 14 such visitors could be tracked. Of these, 93% were dead. These results indicated that a fresh pesticide-treated sphere was as effective as a fresh sticky sphere in attracting and killing *R. pomonella* flies.

6.3.3 Field tests.

In test 1 (Table 6.2), an unknown number of *R. pomonella* flies was killed by the 36 pesticide-treated spheres installed in the block. On the 36 control sticky spheres, a mean of 181 flies per sphere was caught. Such high capture of *R. pomonella* flies on control sticky spheres reflects a very high population of this insect in the experimental orchard. About 38% more flies were caught on monitoring traps in the block with sticky spheres than in the block with pesticide-treated spheres ($F=4.7$, $df=1, 5$, $p=0.06$). Fruit injury averaged 4.0 and 2.5%, respectively , indicating that a commercially desired level of control (fruit damage less than 0.5%) was not achieved by either sticky or pesticide-treated spheres ($\chi^2=0.72$, $df=1$, $p=0.4$).

In test 2, about 32% more *R. pomonella* flies were captured on monitoring traps in blocks surrounded by pesticide-treated spheres than in blocks treated with a mean of 2.0 insecticide sprays against *R. pomonella* ($F=3.9$, $df=1, 7$, $p=0.09$). Mean percent fruit injury was 1.0, and 0.8%, respectively, indicating a level of control approaching that desired by commercial growers ($\chi^2=0.4$, $df=2$, $p=0.8$). *R. pomonella* populations in all blocks in test 2 appeared considerably lower than in either block in test 1.

6.4 Discussion

Judged by effectiveness in protecting apple fruit from *R. pomonella* infestation (oviposition), the field cage and field studies reported here demonstrate that pesticide-treated spheres compete effectively with sticky spheres or insecticide sprays in controlling apple maggot flies. Pesticide-treated spheres have both advantages and disadvantages compared with sticky spheres and insecticide sprays. These must be considered by prospective users.

Compared with sticky spheres, deployment and handling of pesticide-treated sphere traps are much simpler and have more appeal to prospective users. For example, a single treatment of one pesticide-treated sphere with feeding stimulant (16% sucrose) for

seasonal maintenance takes approximately 20 sec; however cleaning and retreating one sticky sphere with sticky takes approximately 4 min. Currently, the requirement of treating pesticide-treated spheres with feeding stimulant immediately after each rainfall (see Chapter 5) may limit greatly potential use in commercial orchards because this operation will inevitably interfere with normal working schedules of commercial orchardist.

In reality, it is very difficult to maintain retreatment schedules immediately after each rainfall, as rainfalls occur in an unplanned fashion. The 32% more flies caught on monitoring traps and the 0.2% greater fruit injury in blocks managed with pesticide-treated spheres than in blocks receiving insecticide sprays (though not significantly different) may have been due in part to delayed retreatment of rain-washed pesticide-treated spheres. On several occasions, it was not possible for us to reach each experimental orchard within 24 h after rainfall.

Like baited sticky spheres, baited pesticide-treated spheres have potential for eliminating need for insecticide sprays against *R. pomonella* in commercial orchards and therefore facilitating the buildup of natural enemies in controlling mid- and late-season foliar pests (Prokopy et al. 1990b). Compared with pesticide sprays, pesticide-treated spheres eliminate deposition of pesticide and residues on the fruit and reduce drastically the amount of toxicant required for apple protection against *R. pomonella*. In commercial orchards, an average of 2.5 applications of azinphosmethyl (Guthion 50% WP at 1.8 kg formulated material per ha per application) is used in each growing season for controlling *R. pomonella* (Prokopy et al. 1990b). With pesticide-treated spheres (2 g of 1.05% a.i. dimethoate mixture per sphere), one retreatment of the sphere with the original pesticide mixture is enough to maintain high trap efficacy throughout the season because the efficacy of the sphere lasts more than one month (see results of experiment 2, and Chapter 5). For a 1 ha orchard, control of *R. pomonella* by pesticide sprays would require 2250 g (a.i.) (azinphosmethyl) per season. In contrast, ringing the perimeter of a 1 ha

orchard with baited pesticide-treated spheres placed 5 m apart would require 80 spheres (assuming it is approximately 100 m x 100 m) and only 1.7 g (a.i.) of dimethoate. This results in 1324 fold reduction in toxicant required per season.

Both fresh pesticide-treated spheres and clean sticky spheres are highly effective in killing or capturing alighting *R. pomonella.* But their effectiveness decreases as days of exposure to weather under orchard conditions increase. The decrease was only slight and insignificant in the case of pesticide-treated spheres exposed for 28 days, provided that the spheres were retreated with feeding stimulant following each rainfall. The decrease was substantial and significant in the case of sticky spheres. Accumulation of insects on the sticky sphere surface reduced efficacy in capturing alighting flies. Several researchers working with other dipterans, including tephritids (e.g. *Bactrocera spp*), have also reported a decrease in efficiency of different forms of sticky traps in capturing target insects as numbers captured increased. However, decline in efficiency has generally been attributed to a negative effect of captures on trap attractiveness through "blurring" of trap visual and/or odor cues (Hill and Hooper 1984; Vernon and Bartel 1985; Jenkins and Roques 1993). Results from our field cage study (experiment 2) indicated that numbers of insects captured on a sticky sphere up to a proportion of 37% of the sphere surface area occupied by captures had no significant effect on sphere attractiveness to *R. pomonella* (based on number of flies alighting). Rather, decreasing captures resulted from alighting flies escaping the sphere.

A control strategy using pesticide-treated spheres against *R. pomonella* depends upon alighting flies contacting pesticide residue after alighting. The extent to which flies are poisoned after alighting depends on the amount of toxicant absorbed from a sphere, which in turn depends in large part on the duration of visiting and/or feeding on the sphere surface (see Chapter 5). Results here indicated that in the field, *R. pomonella* flies alighting on a pesticide-treated sphere remained on average for about 7 min, during which the flies spent most of their time (ca. 5 min) feeding (provided feeding stimulant was

present). Whether such mean duration of visiting and feeding would be great enough for a fly to absorb a lethal dose of insecticide would depend on both the degree of toxicity and the amount of insecticide available on the sphere surface. Previous studies by Duan and Prokopy (1994) indicated that a mean time of 5 min visiting and 1 min feeding on a sphere treated with same type and amount of insecticide used here (1.05% a.i. dimethoate) resulted in 76% mortality of alighting flies. Repeated visits to a pesticide-treated sphere, as observed in our field cage and field observations, may play an important role in providing effective control of *R. pomonella*. Repeated visitation may occur both before development of poisoning symptoms and after recovery from initial poisoning. Importance of repeated visits to insecticide-treated sex pheromone traps or lure stations has also been suggested by De souza et al. (1992) in lure and kill studies of *Spodoptera littoralis* (Boisduval).

A control strategy against *R. pomonella* using pesticide-treated spheres is identical to that using sticky spheres except for use of a different lethal agent to kill attracted flies. The success of both pesticide-treated and sticky spheres in controlling *R. pomonella* depends on the efficiency of spheres in killing or capturing adults before they have initiated oviposition in host fruit, which is influenced by several environmental and fly factors such as fruit cultivar, tree size, site of fly origin, and fly population size. Differences in test orchard conditions probably accounted for the different results of field tests 1 and 2. In field test 1, a desired control effect (less than 0.5% fruit damage) was not achieved by either pesticide-treated or sticky spheres. The unusually high fly population and low cultivar resistance to fly oviposition might have been key factors accounting for lack of commercial-level control. Even so, it appeared that pesticide-treated spheres were more effective in reducing both fly density and fruit injury than were sticky spheres. Possibly, this was because high abundance of *R. pomonella* (as well as other insects) in the orchard caused a rapid accumulation of captures on the control sticky spheres, and the frequency of cleaning and retreating sticky spheres (every two to three weeks) was not

enough to maintain high effectiveness in capturing alighting flies. In field test 2, nearly acceptable commercial-level control was achieved by pesticide-treated spheres. Lower fly population density and high cultivar resistance to fly oviposition were probable contributing factors.

In summary, our results suggest that effectiveness of pesticide-treated spheres in controlling *R. pomonella* flies is subject to varying orchard conditions. The major operational obstacle in using currently-formulated pesticide-treated spheres for replacing sticky spheres for behavioral control of *R. pomonella* lies in the need for retreating the former with feeding stimulant immediately after rainfall. Improvement through developing a way to protect the residual effectiveness of feeding stimulant is key to future operational success of pesticide-treated spheres in replacing sticky spheres for *R. pomonella* control. An additional challenge of receiving government approval for using pesticide-treated spheres in commercial orchards also remains to be addressed.

6.5 References

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Table 6.1. Effectiveness in controlling apple maggot flies of a pesticide-treated sphere (technical dimethoate or Cygon 4.0 EC) or a sticky sphere when hung in apple trees containing 35 fruit each in field cages.

Treatments	No. trials (replicates)[a]	% flies caught or killed(± S.E.)[b]	% fruit receiving eggs (± S.E.)[b]
Technical dimethoate	4	58 (4.8)a	14 (5.1)b
Cygon 4.0 EC	4	54 (3.8)a	11 (1.0)b
Sticky	4	61 (8.8)a	12 (5.0)b
Control (no sphere)	4	-	34 (9.6)a

[a] 25 flies were released for tests in each trial (arena). [b] Values in each column followed by the same letter are not significantly different at the 0.05 level according to ANOVA and Tukey's HSD test.

Table 6.2. Comparative efficacy of baited pesticide-treated spheres (PTS), baited sticky spheres (SS) or grower sprays (GS) in controlling apple maggot flies (AMF).

Test	Treatment	Trap density	No. blocks	Mean no. insecticide sprays against AMF	Mean no. AMF adults captured per sphere over season (±S.E.)		% fruit infestation (±S.E.)[b]
					control trap	monitoring trap[a]	
1							
	PTS	3 spheres on each tree in block	1	0.0	-	126.8 (15.3)a	2.5 (1.1)a
	SS	3 spheres on each tree in block	1	0.0	181.0 (14.3)	175.0 (16.1)a	4.0 (1.3)a
2							
	PTS	1 sphere every 5 m on perimeter trees	3	0.0	-	34.3 (4.1)a	1.0 (0.7)a
	GS	-	3	2.0	-	26.1 (4.1)a	0.8 (0.6)a

[a] Values in each test within the same column followed by the same letter are not significantly different according to ANOVA and Tukey's HSD test criterion at the 0.05 level . Statistical analysis in test 1 was based on pseudo replication (i.e. no block replication). [b] Values in each test within the same column followed by the same letter in each test indicate no significant differences between treatments according to Chi-squire tests at the 0.05 level.

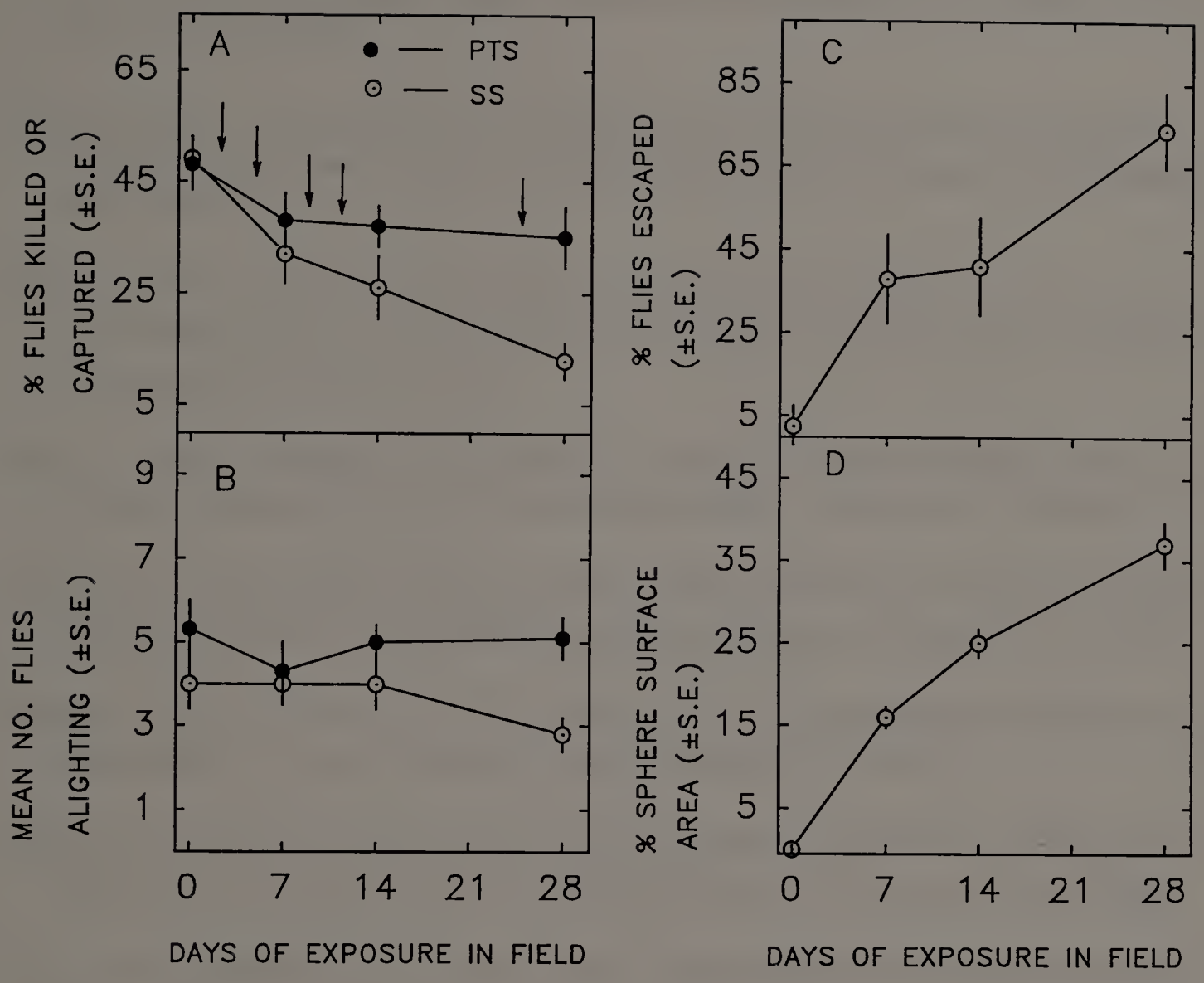

Figure 6.1. Effect of duration of exposure to weather in an orchard on residual effectiveness of pesticide-treated spheres (PTS) and sticky spheres (SS) in killing alighting flies:(A)proportion of released flies killed or captured, (B) mean number of flies observed alighting on spheres, (C) proportion of alighting flies that escaped from sticky spheres, and (D) mean % of surface area occupied by previously captured insects on sticky spheres. The arrow (↓) in graph represents the occurrence of a rainfall event (over 5 mm) and the retreatment of PTS with feeding stimulant.

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