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Olfactory and visual cues guiding plum curculios (Coleoptera: Curculionidae) to host plants.

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OLFACTORY AND VISUAL CUES GUIDING PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE) TO HOST PLANTS

A Dissertation Presented

by

TRACY CHRISTINE LESKEY

Submitted to the Graduate School of the
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Department of Entomology

OLFACTORY AND VISUAL CUES GUIDING PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE) TO HOST PLANTS

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DEDICATION

To my parents for believing in me

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ABSTRACT

OLFACTORY AND VISUAL CUES GUIDING PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE) TO HOST PLANTS

MAY 2000

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The plum curculio, *Conotrachelus nenuphar* (Herbst), is a major pest of stone and pome fruit in eastern North America. I determined generalized olfactory and visual cues guiding plum curculios to host plants as a first step in the creation of a reliable monitoring trap for this important tree fruit pest.

Olfactory attraction of overwintered adults to volatiles from extracts of McIntosh apple tissues was evaluated in the laboratory using a still-air dual choice bioassay system to determine the most attractive odor sources. Greatest numerical responses were recorded to volatiles from extracts made between bloom and 2 weeks after bloom.

This same laboratory bioassay system was used to evaluate the influence of barometric pressure on degree of olfactory discrimination by adult females; response indices used as a measure of discrimination were significantly and positively correlated with barometric pressure.

Olfactory attraction of overwintered adults to individual volatile components of unripe plum odor was evaluated in the laboratory and in the field. Two compounds, ethyl isovalerate and limonene, were significantly attractive.

Olfactory attraction of overwintered adults to volatiles released from same- and opposite-sex conspecifics alone, synthetic grandisoic acid alone, wild plums alone, or combinations of live single-sex conspecifics or grandisoic acid with plums was observed in the laboratory. Females may produce a sex pheromone attractive to males, but in general both male and females were strongly attracted to host plum volatiles and only mildly attracted to conspecific odors or synthetic grandisoic acid, with little evidence for synergy or enhancement of host plum odors when combined with conspecific odors or synthetic grandisoic acid.

Laboratory, semifield, and field experiments were conducted to determine the most visually-stimulating and acceptable version of a branch-mimicking trap (PVC cylinder topped with an inverted screen funnel) to capture adults in the canopy of host fruit trees. Tall cylinder traps (50 cm tall x 6 cm diam) coated with flat black latex paint were significantly more visually stimulating than cylinder traps of lesser height and/or different diameter. Lining inverted screen funnels with paper cones and joining cylinders to inverted screen funnels with wooden inserts increased surface acceptability of traps.

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CHAPTER 1

INTRODUCTION

The plum curculio, *Conotrachelus nenuphar* (Herbst), is native to North America. Originally believed to breed principally on wild plum, *Prunus* species (Chapman 1938), plum curculios have expanded their host range to include a number of exotic host plants belonging to the family Rosaceae (Maier 1990). The geographic range of plum curculios extends as far north as Winnipeg, Manitoba at 50°N latitude and south to Florida and Texas at 28°N latitude. The western limit is at 100th meridian from Texas to central Nebraska and 105th meridian northward. This geographic area coincides roughly with the geographic range of their original host plants; *P. americana* Marshall, *P. nigra* Aiton and *P. mexicana* Watson (Chapman 1938).

Plum curculios north of Virginia are generally univoltine, while those in and south of Virginia are multivoltine (Schoene 1936). Both sexes of northern strain plum curculio enter an obligatory diapause (Smith and Flessel 1968). However, there is some disagreement about diapause of southern strain adults. Smith and Salkeld (1964) reported that neither sex entered an obligatory diapause, but McGiffen et al. (1987) found that adult male southern strain plum curculios enter an obligatory diapause regardless of day length, while females can remain active for several more months if held under extended day length conditions.

There is evidence of post-mating reproductive isolation between univoltine and multivoltine plum curculios. Matings between the two strains resulted in reduced fertility and fecundity (Padula and Smith 1971, Stevenson and Smith 1961). Padula and Smith (1971) reported that sperm degeneration occurred within 1 week in univoltine male and multivoltine female crosses compared to 3-4 weeks for multivoltine male and female crosses. Furthermore, southern strain plum curculios could not survive overwintering conditions of Geneva, New York (Smith and Flessel 1968). The following descriptions of behavior and ecology will focus on northern strain plum curculios, the strain that inhabits Massachusetts.

Plum curculios overwinter as adults under leaf litter or grass roots, near or in orchards. A preferred overwintering site is under a thick layer of fallen sugar maple foliage (LaFleur et al. 1987). Female plum curculios enter diapause in a state of arrested ovarian reproductive development. By the time females emerge from overwintering, approximately 50% of females have begun oogenesis and all females have low fat reserves. Therefore, females must feed on suitable host plant material to complete ovarian development (Smith and Salkeld 1964). Males have a slightly higher overwintering mortality believed to be related to a greater sensitivity to desiccation. Males reportedly emerge from overwintering slightly before females (Smith and Flessel 1968).

Although emergence can occur over 3-4 weeks (LaFleur and Hill 1987), up to 60% of the population has been reported to emerge within a single day (Smith and Flessel 1968). After emergence, there is lag period before appearance in host trees. Smith and Flessel (1968) hypothesize that physiological conditions of plum curculios at the termination of diapause are not conducive to movement into host trees immediately because of their extreme sensitivity to desiccation, and that plum curculios may need to consume water before beginning to move toward orchards. Plum curculios are likely to move to host trees when humidity is high and air movement is low (Smith and Flessel 1968). The mean speed of spring dispersal was measured using radiolabelled plum curculios. From green tip to tight cluster, plum curculios remained within 1 m of their overwintering sites, either inactive or walking slowly. Plum curculios then began to move toward host trees though they remained mainly on the ground until petal fall. In general, there were peaks of activity coinciding with tree fruit phenology, with the greatest peaks between petal fall and fruit set. Further, plum curculios appear to prefer cultivars with dense foliage (LaFleur and Hill 1987).

Racette et al. (1991) found that between full bloom to petal fall, plum curculios spent most of the day on the ground, but that at fruit set, plum curculios spent most of their time in the trees. In studies of plum curculios on caged dwarf apple trees, the number of adults in trees peaked at petal fall with most plum curculios present on flowers, developing fruit, twigs, and spurs (Chouinard et al. 1994).

Plum curculios are mainly active at night (Smith and Flessel 1968, Owens et al. 1982, Racette et al. 1990, Racette et al. 1991, Chouinard et al. 1992, Chouinard et al. 1994). Greatest levels of activity have been recorded during fruit set. Nocturnal activity coincided with periods of low wind speed and high humidity (Racette et al. 1991). In studies of plum curculios on caged dwarf apple trees, diel periodicity of rates of movement and activity levels was recorded in 70% of the plum curculios (Chouinard et al. 1992). In the morning, plum curculios tend to move to protected areas including the ground, branches, crotches, or spurs regardless of weather conditions (Racette et al. 1991). The diurnal period was spent mainly resting with 45% of plum curculios observed on wild plum or apple branches engaged in this behavior. Feeding was observed in 23% of the plum curculios. Crawling was observed in 19% of plum curculios with percentages increasing into the evening. Approximately 12% of females were observed ovipositing with most observations of this behavior made in the afternoon and evening. Very few plum curculios were observed in flight (Owens et al. 1982). On small dwarf apple trees radiolabelled plum curculios engaged in mating from full bloom to fruit set with most matings occurring in the upper half of trees. At the onset of mating at full bloom, an increase in aggregation was also observed (Chouinard et al. 1993).

Oviposition is affected by a number of factors that include temperature, size, and rate of growth of apples and likely, physiological stage of plum curculios (Lanthrop 1949). Females lay eggs singly in apples and use their mouthparts to cut a small crescent shaped flap in the fruit skin and then turn around and deposit an egg (Chapman 1938, Quaintance and Jenne 1912). In a study in Quebec, females laid an average of 73 eggs (Paradis 1956). However, Quaintance and Jenne (1912) report nearly twice that number with over 144 eggs laid per female on average. Most oviposition occurs in tree rows next to wood lots (Quaintance and Jenne 1912, Chapman 1938, LeBlanc 1984). In studies by Chouinard et al. (1993), egg laying began at petal fall and peaked at fruit set.

The egg stage lasts from as little as 3 days to as much as 12 days (Paradis 1956), with average estimates between 6-7 days (Chapman 1938). Larvae hatch from eggs and feed within developing fruit, releasing pectic enzymes and cellulase (Levine and Hall 1978a, 1978b) resulting in premature fruit drop (Levine and Hall 1977). Mature 4th instar larvae then leave the fallen fruit and burrow into the soil to pupate (Quaintance and Jenne 1912). Pupation generally lasts between 4-5 weeks with little emergence occurring after week 6 (Quaintance and Jenne 1912).

New generation adult plum curculios emerge from the soil in July and August and have been observed feeding on fallen fruit or fruit still on trees (Racette et al. 1992). Generally adults will feed until they have enough fat stores to begin migration to overwintering sites (Smith and Salkeld 1964). In late summer and early fall, adults migrate to overwintering sites in wooded areas (Racette et al. 1992) by possibly orienting to the horizon where high tree silhouettes occur. Nevertheless, predominant directions recorded were not always toward the nearest woodlot (LaFleur et al. 1987).

As adults, male and female plum curculios are difficult to distinguish. However, there are three major characteristics that can be used for this purpose. First, an enlarged and modified tibial spur is present on the metathorax of males while the tibial spur of females is smaller and unmodified. Secondly, the first abdominal sternite of females is slightly convex, while the male is more or less flattened. Finally, the metathoracic sternite of the male plum curculio has a well-defined groove extending from the inner edge of the posterior coxa toward the mid coxa; this is not present on the female (Thomson 1932).

The plum curculio is a principal pest of stone and pome fruit in eastern North America (Racette et al. 1992) and the most destructive fruit pest of peaches and plums in the southeastern United States (Yonce et al. 1995). Plum curculios damage fruit in two ways: females oviposit in young fruit, leaving behind crescent-shaped scars, and both males and females puncture fruit when feeding, leaving behind round feeding scars.

Insecticide application is the only reliable control strategy for this insect. Furthermore, one of the major problems for growers who practice Integrated Pest Management in fruit orchards is the lack of a reliable monitoring system to detect the appearance as well as the abundance of plum curculios in orchards each spring. At the present time, growers examine fruit for the appearance of fresh egg-laying scars as a sign that plum curculios are active within an orchard and likely to produce economic damage, indicating a need for insecticide application (Prokopy and Croft 1994).

However, reliable monitoring traps have been created for many other species of weevils. Some of these include pea weevils, *Sitona lineatus* (L.), (Nielsen and Jensen 1993), pepper weevils, *Anthonomus eugenii* Cano, (Riley and Schuster 1994), pecan weevils, *Curculio caryae* (Horn), (Tedders and Wood 1994), West Indian sugar cane weevils, *Metamasius hemipterus sericeus* (Olivier), (Giblin-Davis et al. 1996), and cabbage seed weevils, *Ceutorhynchus assimilis* (Paykull), (Smart et al. 1997).

Only traps for pepper weevils, *A. eugenii* (Riley and Schuster 1994) and pecan weevils, *C. caryae*, (Tedders and Wood 1994) did not employ some sort of semiochemical to enhance trap attraction to weevils. Furthermore, since these trapping studies were published, male aggregation pheromones for the pepper weevil, *A. eugenii* (Eller et al. 1994) and for the pecan weevil, *C. caryae* (Hedin et al. 1997) have been identified and suggested for use as semiochemical baits in conjunction with traps.

Thus, attractive olfactory cues that can be used as semiochemical baits will probably be an important component for a successful monitoring trap for plum curculio as well. The plum curculio likely uses olfactory cues to locate attractive host fruit trees. In the laboratory, overwintered adults chose host plum fruit more than plum leaves, nonhost tomato fruit, maple leaves, or wax models of plum fruit indicating that they use olfactory cues to locate host fruit trees at close range (Butkewich and Prokopy 1993). Significantly more color-marked released plum curculio adults arrived on screen cages containing apple branches compared to maple branches; evidence from this study supports the idea that plum curculios locate host fruit trees over longer distances of at least 3 m by relying olfactory cues as well (Butkewich and Prokopy 1997). Furthermore, the major component of a male-produced pheromone, grandisoic acid, was identified and found to be attractive to both male and female plum curculios (Eller and Bartelt 1996). Therefore, evidence suggests that plum curculios are attracted to olfactory cues, both host plant volatiles and pheromones, although attractive host plant volatiles have not been identified, and the influence of combining host plant volatiles with grandisoic acid has not been tested.

A number of different unbaited and a few baited traps have been evaluated in terms of their ability to capture plum curculios. LeBlanc et al. (1981) evaluated inverted polyethylene funnels hung beneath tree trunks to capture falling adults. Yonce et al. (1995) tested unbaited sticky-coated green plastic spheres (3 and 8 cm diam) and sticky-coated green thinning apples (3 cm diam) hung from host trees, PVC pitfall traps placed beneath host trees, 5 cm band coated with a sticky substance encircling tree trunks, and unbaited and baited (baited will boll weevil pheromone, grandlure) boll weevil traps placed on vertical stakes between woods and commercial orchards. However, no plum curculios were captured on any sticky traps and very few were captured in pitfall and boll weevil traps (Yonce et al. 1995). Prokopy and Wright (1998) found that black pyramid traps, originally designed to be visual mimics of tree trunks to monitor populations of pecan weevil, *C. caryae* (Tedders and Wood 1994, Mulder et al. 1997), placed next to apple tree trunks captured significantly more plum curculios than those traps placed between apple trees, between apple trees and an adjacent wood lot and between apple trees and an adjacent field. However, Prokopy et al (1999) found that temporal occurrence of plum curculios in these pyramid traps placed next to trunks of small, medium-size, or large apple trees did not coincide with temporal occurrence of ovipositional injury by overwintered plum curculios nor did amount of adults captured coincide with amount of ovipositional injury. One reason for this disparity of results is likely due to abiotic factors. In experiments designed to learn how plum curculios move into host fruit trees, plum curculios dislodged from apple trees tended to walk off a small collecting frame when ambient temperatures where below 20°C, but fly to a host tree canopy or inter-tree space when temperatures were 20°C or above (Prokopy et al. 1999b). This indicates that plum curculios are likely to bypass any sort of trap designed to intercept crawling individuals such as black pyramid traps placed next to tree trunks (Mulder et al. 1997, Prokopy and Wright 1998, Prokopy et al. 1999a) when temperatures are above 20°C. Therefore, developing a trap that can be used to capture plum curculio adults after arrival in the host tree canopy is needed. One example of such a trap is the "circle trap," consisting of a wire screen cage, capped with an inverted screen funnel, and attached to a limb; this trap is designed to intercept foraging adults walking on tree limbs (Mulder et al. 1997), and not to attract them based on stimulating visual cues.

The overall objective of the research reported in this dissertation was to identify attractive olfactory and visual cues used by plum curculios to locate host plants in order to create a reliable monitoring trap for this important tree fruit pest.

Chapter 2 focuses on determining the most attractive odor sources of McIntosh apple tissues (twigs, leaves or fruit) at seven phenological stages of development (pink through 5 weeks after bloom); the purpose of this study was to determine the best McIntosh apple odor sources for volatile profile identification. Chapter 3 concerns the influence of barometric pressure on olfactory responses and oviposition behavior of female plum curculios. Chapter 4 describes experiments designed to identify attractive individual volatiles of unripe host plum odor to adult plum curculios using laboratory bioassays and field trials; the overall objective was to find attractive compounds that can be used in conjunction with traps. In Chapter 5, olfactory attraction of overwintered adult plum curculios to volatiles released from same- and opposite-sex conspecifics alone, synthetic grandisoic acid alone, wild plums alone, or combinations of live single-sex conspecifics or grandisoic acid with plums were evaluated to determine optimal semiochemical combinations to be used in conjunction with traps. Chapter 6 concerns the development of a visually stimulating version of a branch-mimicking trap for plum curculios; these traps were placed in the canopy to intercept adults that may bypass traps placed on the ground.

CHAPTER 2

ELUCIDATING SOURCES OF APPLE ODOR ATTRACTIVE TO ADULT PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE)

Abstract

I evaluated olfactory attraction of overwintered plum curculio adults, *Conotrachelus nenuphar* (Herbst), to volatiles from extracts of host and non-host plant tissues in the laboratory using a still-air dual choice bioassay system. In experiments evaluating four different solvents, hexane and water proved to be better than dichloromethane and methanol for extracting nonpolar and polar attractive compounds, respectively, from host McIntosh apple tissues. Significantly more plum curculios were attracted to volatiles from hexane extracts of host wild plum fruit at bloom, and host wild plum and McIntosh fruit at 2 weeks after bloom, than to volatiles from hexane extracts of non-host honeysuckle fruit at either phenological stage. In every case, for hexane-extracted or water-extracted volatiles from McIntosh apple tissues (twigs, leaves or fruit) at seven phenological stages of development (pink through 5 weeks after bloom), greatest numerical responses were recorded to volatiles from extracts made between bloom and 2 weeks after bloom. I conclude that source material used to identify volatile components of McIntosh apple odor attractive to plum curculios should be collected between bloom and 2 weeks after bloom.

Introduction

The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), attacks stone and pome fruit (Racette et al. 1992) and is considered to be a key pest of apples in eastern and central North America (Chouinard et al. 1994) and the most destructive pest of peaches and plums in the southeastern United States (Yonce et al. 1995). The native host of plum curculio is believed to be wild plum, *Prunus americana*, although within the past 150 years, plum curculio has expanded its host range to include commercial apple, pear, plum, peach and cherry trees (Maier 1990).

In spring, overwintered adult plum curculios move toward potential host trees, beginning when apple buds are at the tight cluster stage of development (LaFleur and Hill 1987). Upon arrival, adults begin to feed and mate. Plum curculios feed principally on developing fruit, although observations of nonfruit feeding on blossom anthers, foliage, and bark of twigs have been recorded (Chapman 1938, Owens et al. 1982). Matings have been observed in host trees (Smith and Salkeld 1964), in apple blossoms (LeBlanc 1982) and on the ground under host trees (Racette et al. 1992). Females lay eggs in developing fruit by excavating a small cavity in the fruit, depositing the egg in the bottom of the cavity, and finally making a crescent-shaped cut in the fruit skin (Owens et al. 1982). Apples with oviposition damage either drop to the ground prematurely [allowing mature larvae to crawl from the fruit and pupate in the soil] or become increasingly deformed as they mature (Racette et al. 1992). A problem faced by growers is lack of a reliable monitoring trap to detect plum curculio entry and abundance into orchards. Many growers presently rely on the appearance of fresh egg laying scars as a sign that plum curculios have moved into orchard trees (Prokopy and Croft 1994).

Reliable monitoring traps have been developed for many other species of weevils. These include pea weevils, *Sitona lineatus* (L.) (Nielsen and Jensen 1993), American palm weevils, *Rhynchophorus palmarum* (L.) (Oehlschlager et al. 1993), pepper weevils, *Anthonomus eugenii* Cano (Riley and Schuster 1994), pecan weevils, *Curculio caryae* (Horn) (Tedders and Wood 1994), West Indian sugar cane weevils, *Metamasius hemipterus sericeus* (Olivier) (Giblin-Davis et al. 1996), cotton boll weevils, *A. grandis* Boheman (Hardee et al. 1996), and cabbage seed weevils, *Ceutorhynchus assimilis* (Paykull) (Smart et al. 1997). Most successful traps developed for these species of weevils use semiochemicals as attractants, either host plant volatiles and/or pheromonal odors. Only traps for the pepper weevil and the pecan weevil did not employ some sort of semiochemical, although recently, male aggregation pheromones for the pepper weevil (Eller et al. 1994) and the pecan weevil (Hedin et al. 1997) have been identified and suggested for use in enhancing trap attractiveness.

Thus far, several types of traps have been evaluated for monitoring plum curculio activity in orchards (LeBlanc et al. 1981, LeBlanc 1982, Yonce et al. 1995, Eller and Bartelt 1996, Mulder et al. 1997, Prokopy and Wright 1998, Prokopy et al. 1999a), although none have been found to be very effective. All traps were unbaited except those deployed by Eller and Bartelt (1996) that employed a potentially attractive semiochemical, a synthetic version of a component of male-produced aggregation pheromone. Trials of a 1997 formulation of this pheromonal component tested alone at the University of Massachusetts and in Quebec showed no attraction in the laboratory (Leskey and Prokopy, unpublished data, Chouinard unpublished data) and showed little attraction in the field (Prokopy and Leskey, unpublished data, Chouinard unpublished data). Laboratory and field studies of plum curculios suggest that they use host tree odor cues in locating hosts (Butkewich and Prokopy 1993, Prokopy et al. 1995, Butkewich and Prokopy 1997). Identification of attractive host semiochemicals and incorporation of synthetic equivalents into monitoring traps could enhance the success of traps for plum curculios. However, to date, no systematic studies have been published of volatile compounds emitted by host plants that are potentially attractive to plum curculios.

Using a previously designed bioassay system found to be successful for evaluating plum curculio responses to host fruit odor (Prokopy et al. 1995), I conducted a series of experiments to define optimal sources of apple odor attractive to plum curculio adults. First, I tested the ability of four solvents (ranging from nonpolar to polar) to extract attractive volatile compounds from host plant material. I then wanted to determine if volatiles extracted from host plant tissues by such solvents were characteristic of those used by plum curculios to locate host plants by comparing levels of attraction to volatiles from extracts of host fruits (McIntosh apple, *Malus domestica* (Rosaceae), and wild plum, *Prunus americana* (Rosaceae) and a non-host fruit (honeysuckle, *Lonicera* spp. (Caprifoliaceae)). Finally, I conducted experiments to determine the optimal source of attractive nonpolar and polar volatiles present in McIntosh apple tissues by evaluating plum curculio attraction to volatiles from extracts of different structures of McIntosh trees (twigs, leaves and fruit) at seven different phenological stages of tissue development.

Materials and Methods

Plum curculios

All plum curculios used here were collected as overwintered adults in the field in late May or early June from populations present on unsprayed wild plum or apple trees in Hampshire County, MA and were assumed to be approximately the same age. Adults were held in wax-coated paper cups (473 ml) with clear plastic lids. No more than 60 individuals were held in any cup. Fresh food, either unsprayed wild plum or apple fruit, was provided along with a wetted cotton wick as a water source. Plum curculios were segregated according to sex using criteria described by Thomson (1932) and held separately under a photoregime of 16L:8D to mimic long-day conditions in nature from late May to late June.

Plant Tissue Extracts

In 1996, tissue collections were made from unsprayed McIntosh trees at the Horticultural Research Center of the University of Massachusetts in Belchertown, MA at the following dates/phenological stages: 8 May (pink), 14 May (bloom), 24 May (1 week), 31 May (2 weeks), 7 June (3 weeks), 14 June (4 weeks) and 24 June (5 weeks after bloom). In 1997, McIntosh tissue collections were again made at the Horticultural Research Center on 7 May (pink), 18 May (bloom), 26 May (1 week), 3 June (2 weeks), 14 June (3 weeks), 19 June (4 weeks) and 23 June (5 weeks after bloom). In 1998, McIntosh tissue collections were made at the Horticultural Research Center on 12 May (1 week after bloom).

Tissue collections from wild plum trees were made in Amherst, MA on 29 April (bloom) and 16 May (2 weeks after bloom) in 1997 and on 9 April (bloom) and 24 April (2 weeks after bloom) in 1998. Tissue collections from honeysuckle bushes, which bloom at approximately the same time each year as McIntosh apple, were made in Amherst, MA on 20 May (bloom) and 6 June (2 weeks after bloom) in 1997 and on 8 May (bloom) and 20 May (2 weeks after bloom) in 1998.

After collection, plant tissues were taken to the lab for immediate processing. Extracts of tissues were made from twigs, leaves or fruit soaked in hexane, dichloromethane, methanol, or water as solvents (in ratios of 1 ml solvent per 2 g of tissue) for 24 h at room temperature. Hexane and dichloromethane were evaluated as solvents for their ability to extract comparatively non-polar volatile components from plant tissue, whereas methanol and water were evaluated to extract comparatively polar volatile components. After 24 h, tissues were pressed gently to remove any solvent that may have been absorbed and the extract was filtered through glass wool. Any solvent that had evaporated was added back during this step. All extracts were stored in a freezer at -20 ° C until testing.

Bioassay Procedure

A dual choice still-air system of enclosed Petri dishes, described by Prokopy et al. (1995), was employed to test attractiveness of volatiles from all plant tissue extracts. Test were conducted at ~ 26 ° C, 65 - 80% RH at the beginning of scotophase of a 16L:8D cycle, and were carried out from late May to late June in 1996, 1997, and 1998. A 75 μl aliquot of extract was pipetted onto a 1 cm square of cotton wick placed next to one of the two pipette tips that served as ports into the Petri dish chamber. Either 75 μl of hexane, dichloromethane, methanol, or water was used as a solvent control and was pipetted onto a second cotton square placed next to the other pipette port. Hexane-, dichloromethane-, and methanol-based extracts and controls were allowed to evaporate for approximately 4 min. and then each port was covered with a transparent polystyrene cup. Water-based extracts and controls were covered immediately by cups. Plum curculios were starved for 24 h and chilled for 15-20 min. in a cold room at 8 - 9 °C just before use. Handling of plum curculios was kept to a minimum. A single plum curculio was placed gently in the center of each bioassay dish. One replicate of an extract consisted of 12 female or male plum curculios tested singly in individual bioassay dishes held together on a tray. Dishes were then moved immediately to the testing room. All bioassays lasted 2 h.

A positive response to either the treatment or control was considered to have occurred when a plum curculio crawled up the inner surface of one of the two tubes to enter a cup containing either the treatment or control (Prokopy et al. 1995). Once a plum curculio crawled from a tube leading to a cup containing either the treatment or control, the plum curculio nearly always remained under the cup and did not re-enter the tube. There was no statistical difference between responses of males and females to volatiles in any of the experiments. Therefore, male and female responses were combined in the following analyses. To measure the attractiveness of volatiles from a single extract, I used two methods. First, I used a Response Index (RI) developed by Phillips et al. (1993). The RI was calculated by subtracting the number of plum curculios responding to the control (C) from the number responding to the treatment (T), dividing the amount by the total number of plum curculios tested, and multiplying by 100. Thus, RI = (T - C / total) x 100; the greater the RI value, the more attractive the stimulus. Further, I considered a RI value of 9 as the minimum for suggesting attractiveness and a RI value > 40 as being highly attractive [according to the mean and standard deviation values (Mean RI = 25.0 ± 16 SD) obtained from responses to volatiles from a standard attractive source (a hexane-based extract made from McIntosh fruit at 1 week after bloom that was tested during each day bioassays were conducted in 1996, 1997 and 1998)]. Secondly, data were subjected to a Chi Square Test of Homogeneity ($P < 0.05$) where the total number of plum curculios that did or did not enter a treatment cup and the total number of plum curculios that did or did not enter a control cup was compared for each treatment.

To compare responses of plum curculios within each experimental group, data were analyzed by one-way analysis of variance (ANOVA). For each treatment, a minimum of 3 replicates was completed. Treatment means (calculated to be the average RI over all replicates) were compared using the least significant differences test criterion ($P < 0.05$). For purposes of these analyses, a negative RI was entered as a zero.

Comparisons were made among plum curculio responses to volatiles from extracts of McIntosh tissues (twigs, leaves or fruit) at 1 week after bloom and extracted with 4 different solvents (hexane, dichloromethane, methanol, and water). Responses to volatiles from water or hexane extracts of host and non-host fruit at bloom or 2 weeks after bloom were also compared. Finally, comparisons were made among responses to volatiles from water or hexane extracts of McIntosh apple tissues (twigs, leaves, or fruit) at seven phenological stages. Responses to volatiles across tissue types (twigs, leaves, and fruit) were not made because of the potential for each tissue to contain very different compounds and because this approach obscured many of the repeatable patterns observed using within tissue comparisons.

Results

Volatiles From Extracts of McIntosh Tissues at 1 Week After Bloom Made With Different Solvents

All responses recorded to volatiles from twigs or leaves extracted by each of the solvents were significantly greater than responses to the control. Although treatments were statistically equivalent, high RIs (> 40) were recorded for twig volatiles extracted with hexane (RI = 45.8) and with water (RI = 60.4), and for leaf volatiles extracted with hexane (RI = 41.6) and with dichloromethane (RI = 43.8) (Table 1). Responses to volatiles from fruit extracted with each of the solvents were significantly greater than responses to controls except for those extracted with methanol. Further, those extracted with hexane or water were significantly more attractive than those extracted with methanol and equally attractive as those extracted with dichloromethane (Table 1). A high RI (> 40) was recorded for responses to volatiles from fruit extracted with hexane (RI = 58.3) (Table 1).

Because plum curculios were attracted to volatile compounds extracted by nonpolar as well as polar solvents, I chose one solvent of each type for subsequent experiments. Hexane was chosen over dichloromethane as the nonpolar solvent for the following reasons: 1) for fruit and twig extracted with hexane, percentages of plum curculios responding and mean RIs using this solvent were greater than values using dichloromethane and 2) for leaves, there was no difference between percentage of plum curculios responding to the treatments and very little difference between mean RIs to leaves extracted with either solvent (Table 1). Water was chosen over methanol as the polar solvent because I observed a significantly higher mean RI and a higher percentage of plum curculios responding to fruit extracted with water, and the mean RI recorded for responses to twig volatiles extracted with water was double that obtained from twig volatiles extracted with methanol. Little difference was recorded for responses to volatiles from leaf extracts using these two solvents (Table 1).

Volatiles from Host and Non-host Fruit

Among volatiles of McIntosh, wild plum and honeysuckle fruit at bloom extracted with hexane, responses to each of the treatments were significantly greater than responses to controls, but responses to volatiles from wild plum were significantly greater than responses to volatiles from McIntosh apple or honeysuckle fruit, and also resulted in a high RI (53.5) (Table 2). Volatiles from wild plum again provided the highest RI (RI = 48.8) for fruit at bloom extracted with water, although no significant difference in plum curculio responses to volatiles from the three fruit species was detected; responses to each of the treatments were significantly greater than responses to controls (Table 2). Among fruit volatiles at 2 weeks after bloom extracted with hexane, responses to volatiles from McIntosh apple and from wild plum were significantly greater than responses to controls whereas responses to honeysuckle were not. Responses to volatiles from McIntosh and wild plum were significantly greater than responses to volatiles from honeysuckle (Table 2). There were no statistical differences among responses to water-extracted fruit volatiles at 2 weeks after bloom for these 3 species (Table 2). Responses to each of the treatments were significantly greater than responses to controls; McIntosh apple yielded a high RI (51.2) (Table 2).

Volatiles from McIntosh Tissues at Different Phenological Stages of Development

For volatiles from twigs extracted with hexane, responses to each of the treatments for each phenological stage were significantly greater than responses to controls except for 4 weeks after bloom. Further, responses to volatiles at bloom (RI = 36.5) were significantly greater than responses to volatiles at any other phenological stage except for 1 week after bloom (RI = 34.5) and pink (RI = 29.2), while volatiles from 4 weeks (RI = 10.4) and 5 weeks (RI = 13.5) after bloom were significantly less attractive than those at bloom and 1 week after bloom and equally as attractive as those at

2 weeks and 3 weeks after bloom (Table 3). Responses recorded to volatiles from hexane-extracted leaves at all phenological stages were statistically equal to each other, although responses at 1 week after bloom were significantly greater than responses to the control and yielded the highest RI (18.8) (Table 4). Similarly, no statistical differences were recorded among RIs for volatiles from fruit extracted with hexane, although all treatments, except 5 weeks after bloom, yielded significant responses compared to controls, and highest RIs were recorded at bloom (35.4) and 1 week after bloom (33.4) (Table 5).

For volatiles from twigs extracted with water, responses to each of the treatments were significantly greater than responses to controls for each phenological stage, and RIs were statistically equal to one another. High RIs (> 40) were recorded at every phenological stage, with the highest RIs recorded for responses to volatiles from twigs at 1 week (55.2) and 2 weeks (57.3) after bloom (Table 6). Similarly, for volatiles from leaves extracted with water, responses to each of the treatments were significantly greater than responses to controls, except at 4 weeks after bloom, and RIs for all phenological stages were statistically equal to one another. Highest RIs were recorded for pink (25.0), bloom (28.1), and 1 week after bloom (25.0) (Table 7). For volatiles from fruit extracted with water, responses at 1 week (RI = 61.7) and 2 weeks (RI = 67.7) after bloom were significantly greater than responses to volatiles at any other phenological stage except for pink and 5 weeks after bloom. Responses to volatiles from bloom extracts (RI = 11.5) were not significantly different from responses to the control, and volatiles from bloom extracts attracted significantly fewer plum curculios than volatiles from extracts at any other phenological stage except 4 weeks after bloom (Table 8).

Discussion

My findings demonstrated that overwintered, field-collected plum curculios were attracted to volatiles from McIntosh twigs, leaves and fruit extracted with nonpolar and polar solvents (Table 1), with hexane and water chosen as the best nonpolar and polar solvents, respectively, for use in future extracts. Volatiles from host fruit extracted with hexane, specifically from wild plum at bloom and McIntosh apple and wild plum at 2 weeks after bloom, were significantly more attractive to plum curculios than volatiles from honeysuckle fruit extracted with hexane at either phenological stage. Although responses to volatiles from host and non-host fruit extracted with water were not statistically different, volatiles from wild plum at bloom and McIntosh apple 2 weeks after bloom yielded the highest RIs (Table 2). When responses to volatiles from extracts of twigs, leaves, or fruit of McIntosh were compared, volatiles from twigs and fruit extracted with hexane or with water generally yielded higher RIs than volatiles from leaves (Tables 3-8). Volatiles from twigs and fruit extracted with hexane were most attractive at bloom and 1 week after bloom (Tables 3 and 5), whereas volatiles extracted with water were most attractive at 1 and 2 weeks after bloom (Tables 6 and 8). Volatiles from McIntosh apple tissues extracted with water generally yielded higher RIs than those extracted with hexane, although a notably low RI was recorded for fruit at bloom extracted with water (Table 8).

The experiment involving apple tissue extracted with four different solvents was important in establishing whether plum curculios are attracted to volatiles containing polar and/or nonpolar compounds. A study of the coffee borer, *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae), followed a similar approach, using extracts of mature coffee berries in 6 solvents of different polarities. Volatiles from extracts in dichloromethane attracted the greatest numbers of beetles, but this result was not significantly different from results using solvents of greater polarity, including ethyl acetate, acetone, ethyl ether, ethanol and water (Gutierrez-Martinez and Ondarza 1996). Similarly, plum curculios were attracted to volatiles from extracts of McIntosh tissues in both nonpolar and polar solvents. High responses (RI > 40) were recorded for volatiles from extracts of McIntosh tissues made with water (Table 1), indicating that volatiles containing polar molecules were attractive to plum curculios. Like the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Prokopy et al. 1997), the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Nigg et al. 1994) and the olive fly, *Bactrocera oleae* Gmelin (Scarpati et al. 1996), plum curculios were highly attracted to volatiles from water extracts of fruit tissue. However, unlike the Mediterranean fruit fly (Prokopy et al. 1997) and the Caribbean fruit fly (Nigg et al. 1994), plum curculios are also highly attracted to volatiles present in tissues extracted with nonpolar solvents (hexane and dichloromethane) (Table 1).

Plum curculios may be attracted to compounds such as green leaf volatiles that are found throughout many species of green plants, including fruit of non-host plants such as honeysuckle. In my experiments, volatiles from McIntosh and/or wild plum fruit (both hosts of plum curculios) extracted with hexane attracted significantly greater numbers of plum curculios than did volatiles from non-host fruit of honeysuckle. However, because positive responses were recorded to volatiles from honeysuckle fruit at bloom extracted with hexane, and from honeysuckle fruit at bloom and 2 weeks after bloom extracted with water, plum curculios may be attracted to compounds not specific to host plants, such as green leaf volatiles. Green leaf volatiles (alcohols, esters, and ketones) are found within many green plants (Metcalf and Metcalf 1992) including unripe fruit (Light and Jang 1996), and olfactory responses to these compounds may constitute a generalized mechanism of host plant recognition (Metcalf and Metcalf 1992). Further, other oligophagous insects are known to respond to green leaf volatiles produced not only in their hosts but also in non-host plants (Bernays and Chapman 1994). Odors from unripe fruit of nectarines and papayas, hosts which are acceptable to Mediterranean fruit flies when ripe, are dominated by green leaf volatiles, especially 6-carbon aliphatic aldehydes and esters, which inhibit and disrupt fly attraction to other fruit odors (Light and Jang 1996). Unlike Mediterranean fruit flies, female plum curculios deposit eggs in unripe, developing fruit (Racette et al. 1992). Also, male plum curculios are known to be attracted to a 5 mg dose of a known green leaf volatile (cis-3-hexen-1-ol) in an olfactometer in significantly greater numbers than to higher doses or to a control (Cormier et al. 1998). Therefore, plum curculios may be attracted to compounds such as green leaf volatiles. However, my data demonstrates that plum curculios respond in greater numbers to nonpolar and polar compounds emitted from host fruit volatiles than those found in non-host fruit, and host plant recognition may require either the presence of these specific volatile compounds in host tissues (such as those of McIntosh apple or wild plum) or alternatively, specific blends of compounds present in host tissues.

Several consistent patterns emerged in this study involving volatiles from extracts of McIntosh twigs, leaves, and fruit at seven phenological stages of development. Volatile nonpolar compounds from tissues extracted with hexane were most attractive at bloom and 1 week after bloom, with highest responses recorded for twigs and fruit. The most consistently attractive volatiles were polar compounds from McIntosh tissues extracted with water at 1 week and 2 weeks after bloom (during the earlier stages of fruit development), with highest levels of attraction again recorded for twigs and fruit. In contrast, the coffee borer, *H. hampei*, was more attracted to volatiles of various fruiting tissues than to volatiles of twig tissues of the coffee plant and to volatiles from mature fruit and epicarp of ripe or green fruit (later phenological stages of fruit development occurring at least 5 months after bloom) (Gutierrez-Martinez and Ondarza 1996). These results are likely related to oviposition and mating behavior of plum curculios and *H. hampei*. Female plum curculios deposit eggs in early-developing fruit (Racette et al. 1992), whereas coffee borer females deposit eggs in mature coffee fruit (Gutierrez-Martinez and Ondarza 1996). Further, male coffee borers are flightless and are not attracted to coffee volatiles (Gutierrez-Martinez and Ondarza 1996), whereas male plum curculios are attracted to host volatiles (Prokopy et al. 1995). Male plum curculios often mate on or near host fruit (Smith and Salkeld 1964, LeBlanc 1982, Racette et al. 1992), and male plum curculios may respond to the same host volatiles as females.

An interesting result occurred with plum curculio responses to volatiles from water and hexane extracts of fruit made at bloom. Although the highest RI for McIntosh fruit volatiles extracted with hexane was recorded at bloom (Table 5), a significant and consistent drop in response to McIntosh fruit volatiles extracted with water was recorded at bloom (Table 8). Perhaps, for some unknown physiological reason, McIntosh trees stop producing attractive polar compounds at this time. Alternatively, McIntosh trees may be producing extremely large amounts of attractive polar volatiles at this time, in concentrations so great that they repelled plum curculios. A similar response pattern was recorded for the boll weevil, *A. grandis*, to the major volatile component of cotton, ß-bisabol. At lower concentrations, boll weevils were attracted to this volatile, but at the highest concentration tested, they were repelled (Dickens 1986). Plum curculios are known to migrate to host trees in bloom (LaFleur and Hill 1987). This would argue in favor of host trees at bloom being attractive. The fact that plum curculios were attracted in highest numbers to volatiles from wild plum fruit at bloom (Table 2) also support my explanation that volatiles emitted from host trees at bloom are produced in large amounts and may be extremely attractive.

In conclusion, my findings suggest that identification of host-specific volatile compounds or specific blends of volatile compounds attractive to plum curculios may be facilitated by comparing volatile components and their relative amounts present in host tissues of McIntosh apple and wild plum with those present in the non-host tissues of honeysuckle. Further, my findings suggest that McIntosh twigs and/or fruit from bloom to 2 weeks after bloom can be used as source material in future experiments aimed at identifying volatile components of apple odor attractive to plum curculios.

Table 1. Response indices (RIs) of plum curculio adults to volatiles of McIntosh twigs, leaves, or fruit collected at 1 week after bloom and extracted by different solvents.

			Plum Curculio Responding (%)		Response Index
Tissue	Solvent	N[a]	Treatment	Control	(Mean ± SE)[c]
Twigs	Hexane	4	65[b]	19	45.8 ± 5.1 a
	Dichloromethane	4	40[b]	4	37.5 ± 6.3 a
	Methanol	4	44[b]	17	29.2 ± 5.1 a
	Water	4	73[b]	13	60.4 ± 5.1 a
Leaves	Hexane	4	58[b]	19	41.6 ± 6.7 a
	Dichloromethane	4	58[b]	13	43.8 ± 5.2 a
	Methanol	4	40[b]	15	25.0 + 4.2 a
	Water	4	46[b]	27	18.8 + 4.0 a
Fruit	Hexane	4	71[b]	13	58.3 ± 5.1 a
	Dichloromethane	3	42[b]	14	27.7 ± 4.2 ab
	Methanol	4	19	27	4.2 ± 2.9 b
	Water	4	54[b]	19	37.5 ± 5.3 a

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[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05. level according to least significant differences tests.

Table 2. Response indices (RIs) of plum curculio adults to volatiles of McIntosh, wild plum, or honeysuckle fruit collected at bloom and 2 weeks after bloom and extracted by hexane or water.

				Plum Curculio Responding (%)		Response Index
Stage	Solvent	Plant	N[a]	Treatment	Control	(Mean ± SE)[c]
Bloom	Hexane	McIntosh	7	38[b]	11	26.2 ± 3.9 b
		Wild Plum	7	60[b]	6	53.5 ± 5.0 a
		Honeysuckle	7	46[b]	20	27.2 ± 4.1 b
Bloom	Water	McIntosh	7	45[b]	30	21.4 ± 4.8 a
		Wild Plum	7	62[b]	14	48.8 ± 5.4 a
		Honeysuckle	7	57[b]	19	39.3 ± 5.0 a
2 Weeks	Hexane	McIntosh	7	39[b]	8	30.9 ± 4.1 a
		Wild Plum	6	35[b]	10	25.0 ± 3.4 a
		Honeysuckle	7	20	21	4.8 ± 2.6 b
2 Weeks	Water	McIntosh	7	61[b]	10	51.2 ± 4.2 a
		Wild Plum	7	54[b]	14	39.2 ± 5.3 a
		Honeysuckle	7	49[b]	14	34.3 ± 4.6 a

[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

Table 3. Response indices (RIs) of plum curculio adults to volatiles of McIntosh twigs collected at pink, bloom, 1 week , 2 weeks, 3 weeks, 4 weeks and 5 weeks after bloom and extracted by hexane.

		Plum Curculio Responding (%)		Response Index
Stage	N[a]	Treatment	Control	(Mean ± SE)[c]
Pink	8	40[b]	11	29.2 ± 5.0 abc
Bloom	8	40[b]	5	36.5 ± 4.6 a
1 Week	7	40[b]	6	34.5 ± 3.0 ab
2 Weeks	8	36[b]	19	16.7 ± 3.5 cd
3 Weeks	8	33[b]	16	17.7 ± 3.2 bcd
4 Weeks	8	25	18	10.4 ± 3.8 d
5 Weeks	8	29[b]	16	13.5 ± 4.0 cd

[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

Table 4. Response indices (RIs) of plum curculio adults to volatiles of McIntosh leaves collected at pink, bloom, 1 week , 2 weeks, 3 weeks, 4 weeks and 5 weeks after bloom and extracted by hexane.

		Plum Curculio Responding (%)		Response Index
Stage	N[a]	Treatment	Control	(Mean ± SE)[c]
Pink	8	26	17	13.5 ± 3.4 a
Bloom	8	30	26	13.5 ± 4.3 a
1 Week	5	33[b]	15	18.8 ± 3.8 a
2 Weeks	8	34	23	16.6 ± 4.6 a
3 Weeks	8	28	18	13.5 ± 3.6 a
4 Weeks	8	22	20	14.6 ± 3.6 a
5 Weeks	8	10	21	4.2 ± 3.0 a

[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

Table 5. Response indices (RIs) of plum curculio adults to volatiles of McIntosh fruit collected at pink, bloom, 1 week , 2 weeks, 3 weeks, 4 weeks and 5 weeks after bloom and extracted by hexane.

		Plum Curculio Responding (%)		Response Index
Stage	N[a]	Treatment	Control	(Mean ± SE)[c]
Pink	8	41[b]	20	20.8 ± 4.2 a
Bloom	8	45[b]	9	35.4 ± 4.1 a
1 Week	7	43[b]	10	33.4 ± 3.1 a
2 Weeks	8	46[b]	19	29.2 ± 4.5 a
3 Weeks	8	40[b]	13	30.2 ± 4.6 a
4 Weeks	8	34[b]	15	21.9 ± 5.2 a
5 Weeks	8	21	16	10.4 ± 3.8 a

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[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

Table 6. Response indices (RIs) of plum curculio adults to volatiles of McIntosh twigs collected at pink, bloom, 1 week , 2 weeks, 3 weeks, 4 weeks and 5 weeks after bloom and extracted by water.

		Plum Curculio Responding (%)		Response Index
Stage	N[a]	Treatment	Control	(Mean ± SE)[c]
Pink	8	60[b]	13	46.9 ± 4.9 a
Bloom	8	52[b]	15	40.6 ± 4.6 a
1 Week	8	66[b]	10	55.2 ± 4.6 a
2 Weeks	8	66[b]	8	57.3 ± 3.6 a
3 Weeks	8	53[b]	8	44.8 ± 4.5 a
4 Weeks	8	54[b]	10	43.8 ± 4.9 a
5 Weeks	8	52[b]	8	43.8 ± 4.2 a

[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

Table 7. Response indices (RIs) of plum curculio adults to volatiles of McIntosh leaves collected at pink, bloom, 1 week , 2 weeks, 3 weeks, 4 weeks and 5 weeks after bloom and extracted by water.

Stage	N[a]	Plum Curculio Responding (%)		Response Index
		Treatment	Control	(Mean ± SE)[c]
Pink	8	46[b]	21	25.0 ± 4.2 a
Bloom	8	43[b]	17	28.1 ± 5.5 a
1 Week	8	47[b]	20	25.0 ± 4.5 a
2 Weeks	8	38[b]	22	16.7 ± 3.7 a
3 Weeks	8	32[b]	14	15.7 ± 4.8 a
4 Weeks	8	27	18	16.7 ± 4.4 a
5 Weeks	8	32[b]	13	21.9 ± 5.0 a

[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

Table 8. Response indices (RIs) of plum curculio adults to volatiles of McIntosh fruit collected at pink, bloom, 1 week , 2 weeks, 3 weeks, 4 weeks and 5 weeks after bloom and extracted by water.

		Plum Curculio Responding (%)		Response Index
Stage	N [a]	Treatment	Control	(Mean ± SE)[c]
Pink	8	61 [b]	15	46.9 ± 5.5 ab
Bloom	8	32	26	11.5 ± 3.8 c
1 Week	8	71 [b]	9	61.7 ± 4.4 a
2 Weeks	8	73 [b]	5	67.7 ± 3.8 a
3 Weeks	8	45 [b]	7	37.5 ± 4.9 b
4 Weeks	7	33 [b]	6	30.9 ± 4.2 bc
5 Weeks	8	40 [b]	9	47.9 ± 4.6 ab

[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments are significantly different from control at $P < 0.05$.

[c] Values in each group followed by the same letter are not significantly different at 0.05 level according to least significant differences tests.

CHAPTER 3

INFLUENCE OF BAROMETRIC PRESSURE ON OLFACTORY DISCRIMINATION AND OVIPOSITION BEHAVIOR OF THE PLUM CURCULIO (COLEOPTERA: CURCULIONIDAE)

Abstract

We conducted laboratory experiments to elucidate the influence of barometric pressure on olfactory, oviposition and feeding behavior of adult female plum curculios, *Conotrachelus nenuphar* (Herbst). Adult female olfactory discrimination of hexane-based McIntosh fruit odor was high when barometric pressure was high; Response Index values used as a measure of olfactory discrimination were significantly and positively correlated with barometric pressure. Females oviposited more eggs during periods of low barometric pressure; a nearly significant negative correlation was observed between female oviposition activity and barometric pressure. Observed feeding activity of females was independent of barometric pressure.

Introduction

It is well documented that specific abiotic factors such as temperature, relative humidity, and day length exert a strong influence on insect behavior (Rosomer and Stoffolano 1998). Laboratory studies are often initiated to control abiotic as well as physiological factors to facilitate observation of a particular behavior under known conditions (Opp and Prokopy 1986). However, one factor that is generally not possible to control in a laboratory setting is barometric pressure, a factor that only a few studies of insect behavior has addressed.

An example of a laboratory study affected by barometric pressure is reported by Ankney (1984), who found that mating activity of *Drosophila pseudoobscura* Frolova was significantly reduced under reduced barometric pressure during passage of storm fronts. In our laboratory, we have extensively studied olfactory attraction of adult plum curculios, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), a principal pest of stone and pome fruit (Racette et al. 1992), to host fruit volatiles in small still-air dual choice olfactometers (Prokopy et al. 1995, Leskey and Prokopy 2000). During these studies, we observed that plum curculio behavior markedly changed during the passage of storm fronts; plum curculios quickly crawled to tops of, and often were observed trying to fly within olfactometers. These behaviors were not observed in the absence of storm fronts. Furthermore, Dixon et al. (1999) found that increased fruit damage by plum curculios was significantly and negatively correlated with barometric pressure.

Therefore, we initiated quantitative studies to determine the potential influence of barometric pressure on olfactory discrimination, feeding and oviposition behavior of plum curculios in the laboratory.

Materials and Methods

Plum Curculios

All plum curculios used here were collected as overwintered adults in the field in late May or early June from populations present on unsprayed wild plum or apple trees in Hampshire County, MA and were assumed to be approximately the same age. Plum curculios were identified according to sex within several hours of collection using criteria described by Thomson (1932) and were held separately in wax-coated paper cups (473 ml) with clear plastic lids at ~ 26 ° C, 65 - 80% RH under a photoregime of 16L:8D to mimic long-day conditions in nature from late May to late June. No more than 60 individuals were held in any cup. Fresh food, either unsprayed wild plum or apple fruit, was provided along with a wetted cotton wick as a water source.

Olfactory Discrimination

Plant Tissue Extracts

In 1996-1998, fruiting tissue collections were made 1 week after bloom from unsprayed McIntosh trees at the Horticultural Research Center of the University of Massachusetts in Belchertown on the following dates: 24 May (1996), 26 May (1997), and 12 May (1998). After collection, fruit tissues were taken to the laboratory for immediate processing. Extracts were made from fruiting tissues soaked in hexane or water as a solvent (in ratios of 1 ml solvent per 2 g of tissue) for 24 h at room temperature. After 24 h, tissues were pressed gently to remove any solvent that may have been absorbed and the extract was filtered through glass wool. Any solvent that had evaporated was added back during this step. All extracts were stored in a freezer at -20 ° C until testing.

Bioassay Procedure

A dual choice still-air system of enclosed Petri dishes, described by Prokopy et al. (1995), was employed to test attractiveness of volatiles from all plant tissue extracts. Test were conducted at ~ 26 ° C, 65 - 80% RH at the beginning of scotophase of a 16L:8D cycle, and were carried out from late May to late June in 1996, 1997, and 1998. A 75 µl aliquot of fruit extract was pipetted onto a 1 cm square of cotton wick placed next to one of the two pipette tips that served as ports into the Petri dish chamber, and 75 μl of corresponding solvent was pipetted onto a second cotton square placed next to the other pipette port. Extracts and controls were allowed to evaporate for approximately 4 min. and then each port was covered with a transparent polystyrene cup. Female plum curculios were starved for 24 h and chilled for 15-20 min. in a cold room at 8 - 9 °C just before use. Handling was kept to a minimum. A single female was placed gently in the center of each bioassay dish. One replicate of an extract consisted of 12 females tested singly in individual bioassay dishes held together on a tray. One replicate was evaluated per day. Dishes were then moved immediately to the testing room. All bioassays lasted 2 h. The number of replicates conducted in 1996, 1997 and 1998 was 22, 28, and 18, respectively.

A positive response to either the treatment or control was considered to have occurred when a plum curculio crawled up the inner surface of one of the two tubes to enter a cup containing either the treatment or the control (Prokopy et al. 1995). Once a plum curculio crawled from a tube leading to a cup containing either the treatment or control, it nearly always remained under the cup and did not re-enter the tube. To measure the level of olfactory discrimination and attractiveness of volatiles, we used a Response Index (RI) developed by Phillips et al. (1993). The RI was calculated by subtracting the number of plum curculios responding to the control (C) from the number responding to the treatment (T), dividing the amount by the total number of adults tested, and multiplying by 100. Thus, $RI = (T - C / \text{total}) \times 100$; the greater the RI value, the greater the discrimination between the treatment and control and the more attractive the stimulus. Daily pre- and post-test barometric pressure readings were taken with a holosteric barometer (Forestry Supply, Inc. Jackson, MS), and the daily averages of these were related to daily RI values using linear regression (Statistix 1992). Overall average barometric pressure levels experienced by plum curculios during test periods were 75.20, 76.01 and 75.64 cm in 1996, 1997 and 1998, respectively. Subsequent reference to low or high barometric pressure refers to barometric conditions above or below these average levels.

Oviposition and Feeding Behavior

All tests were conducted using ten female plum curculios that were held with ten males and freshly picked wild plums for two days to ensure they were in a physiological state conducive to oviposition. On the third day, females were separated from males and plums for 24 h. The following day, a single female was held with either a clean, freshly picked unripe wild plum or approximately 5 blueberries (of similar volume to that of the wild plum), covered by a wax-coated paper container and left for 4 h. Five females were tested daily with each fruit type. After 4h, each female was removed and the number of feeding and oviposition sites present on each fruit was counted. Tests were conducted over 15 separate days during an 18 day period (26 May to 11 June, 1999), the same period of time in which oviposition and feeding activities were occurring in the field (Prokopy, unpublished data). Thus, 15 replicates were completed. Barometric pressure readings were taken immediately before the start and after the conclusion of tests, and daily averages of these were related totals of number of eggs laid and of number of feeding punctures on wild plums and blueberries using linear regression (Statistix 1992).

Overall average barometric pressure level experienced by plum curculios during test periods was 76.2 cm. Subsequent reference to low or high barometric pressure refers to barometric conditions above or below this average level.

Results

Olfactory Discrimination

Significant positive correlations were observed between daily average barometric pressure readings and daily RI values for standard hexane fruit extracts in 1996 ($r^2 = 0.41$, F=13.65, P< 0.01) and 1998 ($r^2=0.21$, F=4.22, P=0.05) (Figure 1), but not in 1997 ($r^2 = 0.03$, F=1.01, P=0.32). No correlations were observed between daily average barometric pressure readings and daily RI values for standard water fruit extracts in 1997 ($r^2 =0.03$, F=0.78, P=0.38) and 1998 ($r^2 < 0.01$, F=0.03, P=0.83), respectively.

Oviposition Behavior

A nearly significant negative correlation was observed between daily average barometric pressure readings and daily totals of eggs laid by female plum curculios in wild plums ($r^2 = 0.23$, F=3.82, P=0.07) (Figure 2) but not in blueberries ($r^2=0.03$, F=0.28, P=0.61).

Feeding Behavior

No correlations were observed between daily average barometric pressure readings and daily totals of feeding scars made by female plum curculios on wild plums ($r^2 < 0.01$, F=0.01, P=0.93) or on blueberries ($r^2=0.09$, F=1.14, P=0.31).

Discussion

Low barometric pressure resulted in diminished female olfactory discrimination of attractive volatiles from hexane-based McIntosh fruit extracts in 1996 and 1998 (Figure 1), but not in 1997, and not from water-based McIntosh fruit extracts in 1997 and 1998. Greater oviposition in host wild plums occurred during test periods of low barometric pressure (Figure 2); this response was not observed with nonhost blueberry. Numbers of feeding punctures on host wild plum or nonhost blueberry fruit were not associated with barometric pressure.

Absolute level of barometric pressure influenced degree of female plum curculio olfactory discrimination in the laboratory. In both 1996 and 1998, significant positive correlations were observed between daily response indices for host McIntosh fruit volatiles extracted with hexane and daily average barometric pressure readings (Figure 1). This was not the case in 1997 when response indices and barometric pressure were not correlated. However, the overall average barometric pressure level experienced by plum curculios during test periods was higher in 1997 (76.01 cm) than in 1996 (75.20 cm) or 1998 (75.64 cm), perhaps accounting for differences in results among years.

Barometric pressure has been related to olfactory responses recorded for other insect species in laboratory studies. Olfactory discrimination by *Cotesia (=Apanteles) glomerata*, an endoparasitoid of *Pieris* sp., was also influenced by barometric pressure in both glasshouse and wind tunnel experiments; greater responses to volatiles emitted from host plant material and *Pieris* larvae held together were recorded during test periods of steadily increasing barometric pressure than during test periods of steadily decreasing or fluctuating barometric pressure (Steinberg et al. 1992). Furthermore, greater numbers of the parasitoid species *Uscana lariophaga* Steffan arrived at attractive odor sources in a simple diffusion olfactometer when absolute changes in barometric pressure were large (Ormel et al. 1995). Thus, olfactory discrimination exhibited by insects can be influenced by particular barometric pressure conditions. Olfactory discrimination by female plum curculios could have been influenced not only by absolute level of barometric pressure but also by changes in barometric pressure as was the case for *C. glomerata* (Steinberg et al. 1992) and *U. lariophaga* (Ormel et al. 1995), but we did not detect many of these changes within the 2 hour time frame of our experiments. Furthermore, although diminished female olfactory discrimination of hexane-extracted host McIntosh fruit volatiles occurred when barometric pressure was low, this did not occur with water-extracted McIntosh fruit volatiles. Perhaps more stimulating attractive olfactory cues can override low barometric pressure, as evidenced by volatiles from fruit extracted with water being more attractive to plum curculios than those extracted with hexane (Leskey and Prokopy 2000).

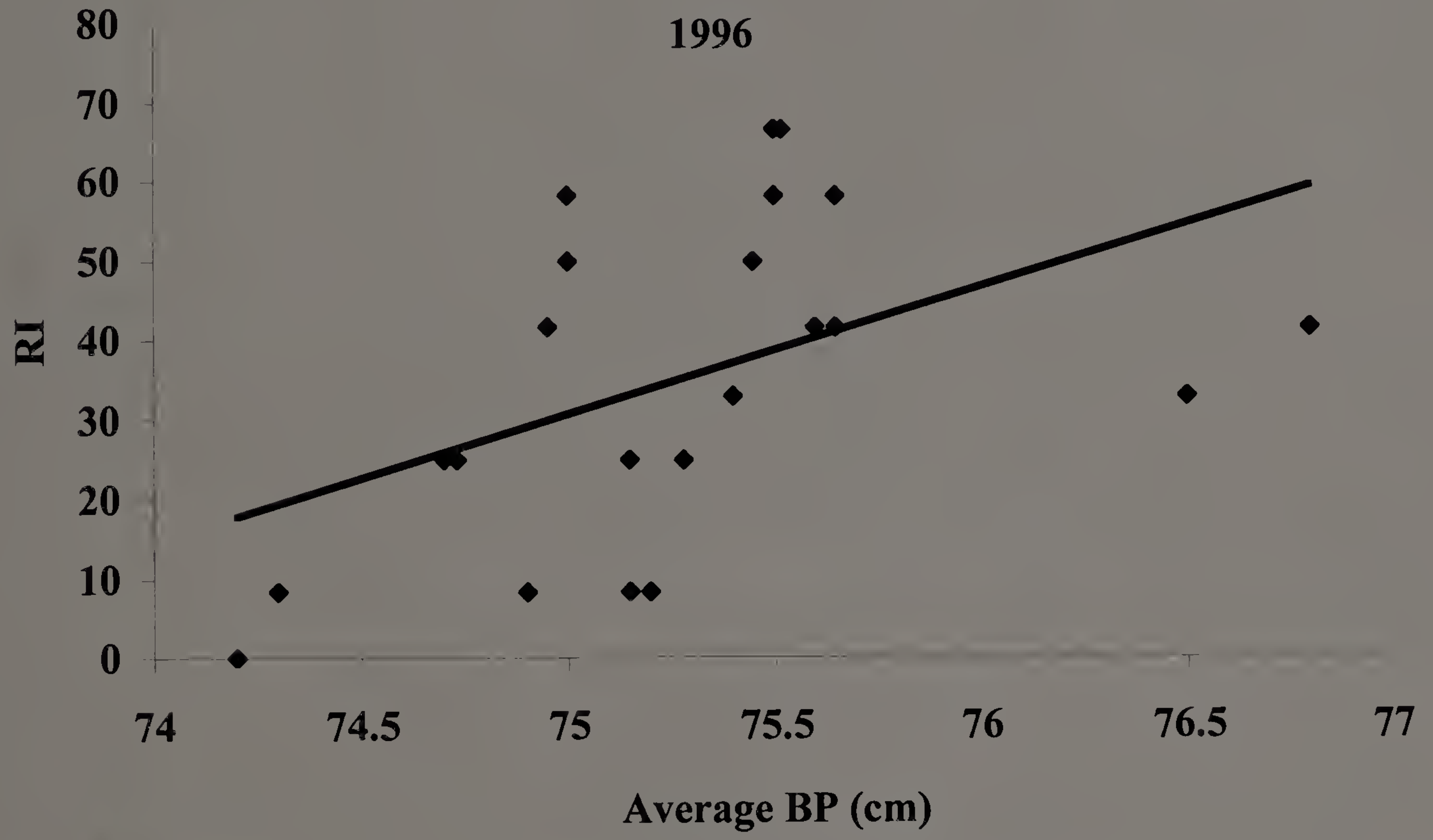
Diminished levels of olfactory discrimination during periods of low barometric pressure recorded for plum curculios may be due to increases in locomotory activity rather than decreases in olfactory capability. In the laboratory, we observed that plum curculios often would attempt to fly inside of Petri dish still-air olfactometers on days of low barometric pressure. Perhaps, low barometric pressure triggers specific locomotory responses in plum curculios. Levels of locomotory activity of other insect species have been found to be related to barometric pressure conditions. For example, increased locomotory activity relative to decreasing barometric pressure was noted for the potato leafhopper, *Empoasca fabae* (Harris); these insects initiated fall migratory movements when barometric pressure was declining due to approaching weather fronts (Shields and Testa 1999). Also, increased flight activity was recorded for the dragonfly *Libellula julia* Uhler under high barometric pressure conditions (Pilon et al. 1995). Similarly, foraging increased with increasing barometric pressure in female black flies, *Simulium luggeri* Nicholson & Mickel, (Fredeen and Mason 1991), and searching activity proceeded when barometric pressure was stable or increasing but declined when barometric pressure decreased for the parasitoid species *Encarsia formosa* Gahan (van Roermund and van Lenteren 1995). Furthermore, aerial dispersal of the two spotted spider mite, *Tetranychus urticae* Koch, was found by Li and Margolies (1994) to be associated with rising barometric pressure; these authors hypothesized that delayed aerial dispersal by these mites under conditions of reduced barometric pressure due to approaching storm fronts could increase chances of survival because mites could evade rainfall events associated with storm fronts. It seems possible that barometric pressure may be an important cue used by several kinds of in arthropods to assess prevailing or approaching weather conditions and to modify behavioral activity levels to maximize survivorship.

Barometric pressure also has been associated with changes in ovipositional activity of some insects. We found that increased oviposition by females in host plum fruit was correlated with low barometric pressure in the laboratory (Figure 2), and Dixon et al (1999) found that increased fruit injury by plum curculios was correlated with low barometric pressure in the field. Declining barometric pressure conditions were associated with increased oviposition in the parasitic wasp, *Leptopilina heterotoma* (Thompson) female wasps spent more time foraging for and ovipositing in both unparasitized and parasitized hosts under conditions of declining barometric pressure compared to steady barometric pressure (Roitberg et al. 1993). Roitberg et al. (1993) theorized that increased foraging and oviposition were a means to maximize lifetime fitness under conditions of decreased life expectancy. This result is in agreement with Wellington (1946), who demonstrated that storm fronts inflicted severe mortality on small, fragile insects. However, unlike *L. heterotoma,* which oviposited on a marginal host [a parasitized host], plum curculios did not oviposit on what could be considered a marginal host [blueberry], irrespective of barometric pressure. Perhaps because plum curculios are not soft-bodied insects, they are better able to survive inclement weather events and can be more discriminating concerning host choice. However, female plum curculios have a narrow time frame in which to locate host fruit trees and oviposit eggs in young fruit soon after fruit set (Chapman 1938). If females wait too long to deposit eggs, fruit will be too large and unsuitable for oviposition. Thus, waiting for better weather to oviposit eggs may not be a strategy that can be used by female plum curculios either.

Perhaps female plum curculios are similar to *L. heterotoma* (Roitberg et al. 1993) and increase ovipositional activity when barometric pressure declines to ensure successful reproduction in the event of weather-dependent mortality.

In conclusion, laboratory studies of plum curculio behavior and likely many other insect species should consider the influence of barometric pressure on behavior and it's relevance to behavior exhibited in nature.

Figure 1. Regression lines associated with average barometric pressure readings and subsequent Response Indices (RI) of female plum curculios to a hexane-based McIntosh fruit extract in 1996 and 1998.



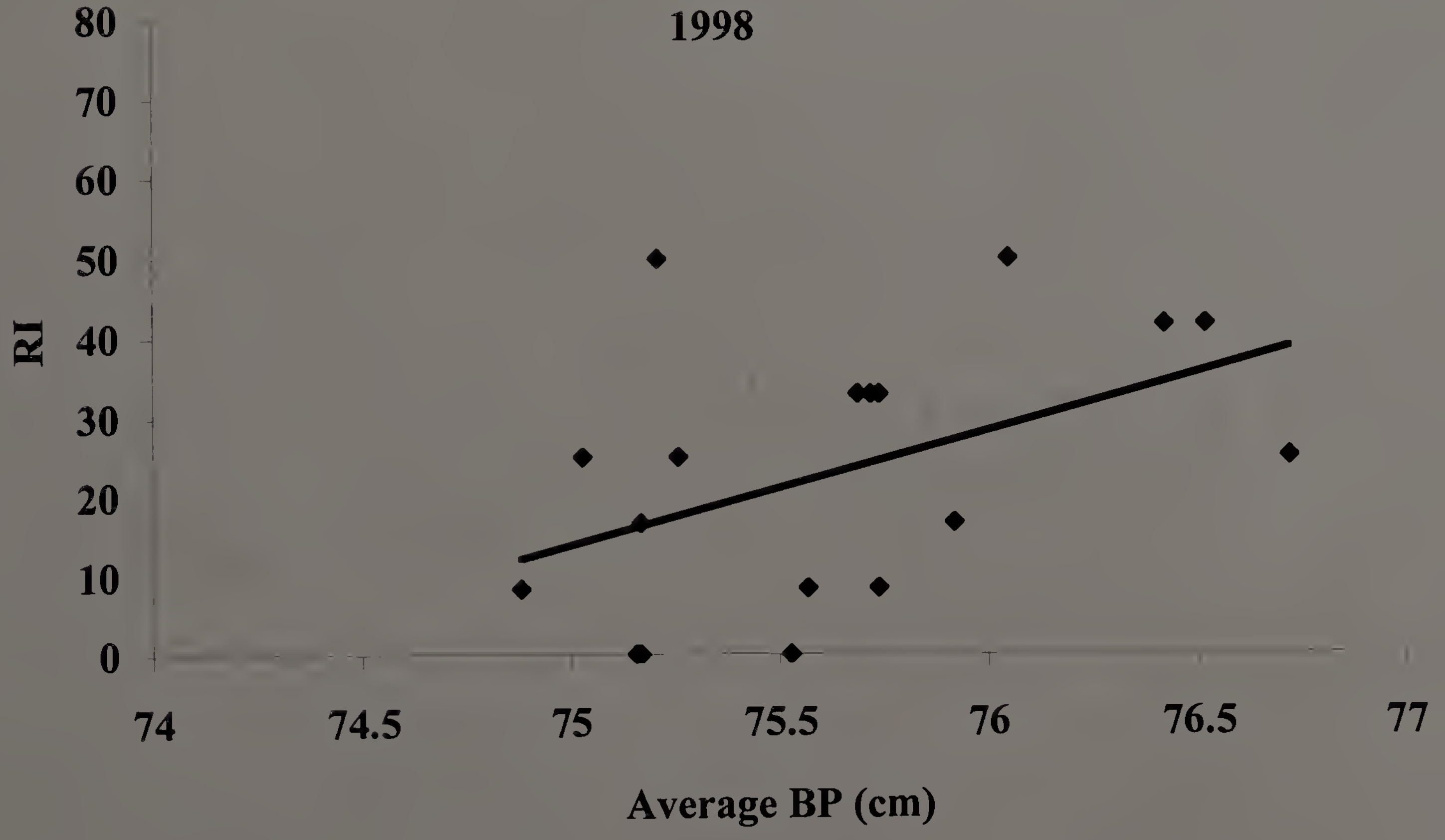
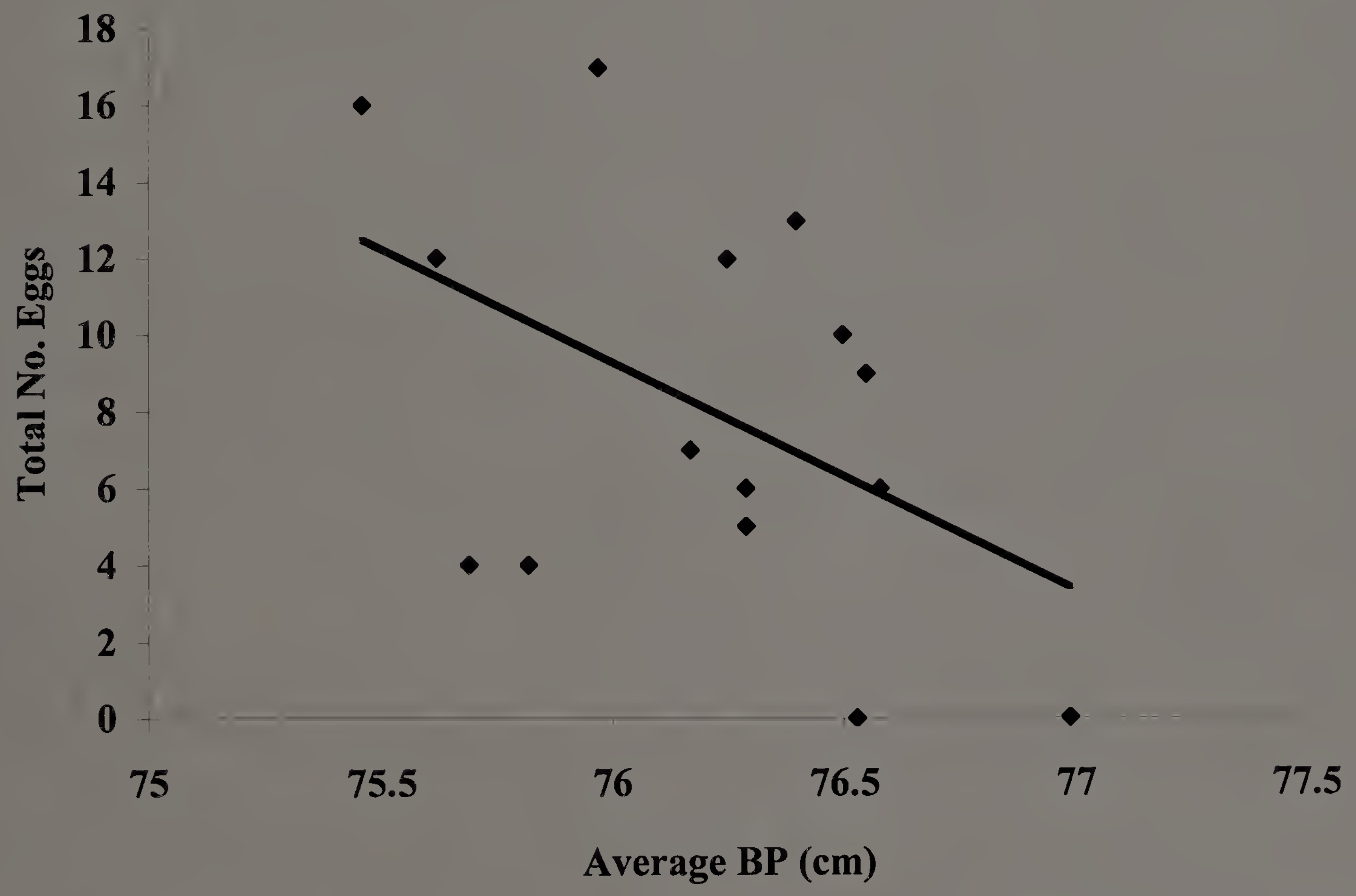


Figure 2. Regression line associated with average barometric pressure readings and total numbers of eggs deposited by five female plum curculios.



CHAPTER 4

EVALUATION OF INDIVIDUAL COMPONENTS OF PLUM ODOR AS POTENTIAL ATTRACTANTS FOR ADULT PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE)

Abstract

I evaluated olfactory attraction of overwintered plum curculio adults, *Conotrachelus nenuphar* (Herbst), in 1998 to individual volatile components of unripe plum odor in the laboratory using a still-air dual choice bioassay system and in the field using baited cotton dental wicks attached to boll weevil traps placed on the ground beneath the canopy of unsprayed apple trees. Two compounds, ethyl isovalerate and limonene, were significantly attractive in both laboratory bioassays and in field experiments. In laboratory bioassays, as concentration was decreased, a greater number of compounds elicited positive responses from plum curculios. Most of the green leaf volatile compounds tested in laboratory bioassays and field experiments appeared to be repellent, with 3-hexanone being repellent in both laboratory bioassays and in field experiments. Benzaldehyde, benzonitrile, 3-hydroxy-2-butanone, linalool and trans-2-hexenal were significantly less attractive than controls in laboratory bioassays at 1.00 % concentration, but yielded positive RIs in the field at 5.00 % concentration. I suggest that

the use of ethyl isovalerate and/or limonene as odor attractants offers potential to increase efficacy of current traps for monitoring plum curculios immigrating into fruit orchards during spring.

Introduction

Currently, no reliable monitoring device exists to detect initiation and amount of fruit injury by the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), a principal pest of stone and pome fruit in eastern and central North America (Racette et al. 1992) and one of the most destructive pests of peaches and plums in the southeastern United States (Yonce et al. 1995). Therefore, growers are forced to use the appearance of fresh egg-laying scars as a sign that plum curculios pose an imminent threat to fruit in orchards (Prokopy and Croft 1994).

Various trap designs have been evaluated for monitoring plum curculio adults. They include inverted polyethylene funnels hung beneath tree trunks to capture falling adults (LeBlanc et al. 1981), unbaited sticky-coated apples or plastic spheres hung from host tree branches (Yonce et al. 1995), pitfall traps placed beneath host trees (Yonce et al. 1995), unbaited cotton boll weevil traps placed on vertical stakes between woods and commercial orchards (Yonce et al. 1995), cotton boll weevil traps baited with a component of male plum curculio sex pheromone, grandisoic acid, and placed on cut ends of vertical host tree branches (Eller and Bartelt 1996), and tall dark-colored unbaited pyramid traps capped with detachable conical boll weevil trap tops, developed originally as super-normal visual mimics of tree trunks for attracting host-seeking pecan weevils, *Curculio caryae* (Horn) (Tedders and Wood 1994) and placed on the ground adjacent to orchard tree trunks for capturing plum curculios (Prokopy and Wright 1998, Prokopy et al. 1999a). Among these possibilities, only the cotton boll weevil traps baited with grandisoic acid (a component of a plum curculio male-produced aggregation pheromone) and placed over cut vertical host twigs (Eller and Bartelt 1996) and unbaited pyramid traps placed next to orchard tree trunks (Prokopy and Wright 1998, Prokopy et al. 1999a) captured even small numbers of plum curculios.

Numerous species of weevils are attracted to host plant volatiles or specific compounds present in host plant volatiles, including the banana weevil, *Cosmopolites sordidus* (Germar) (Budenberg et al. 1993), the cabbage seed weevil, *Ceutorhynchus assimilis* (Paykull) (Evans and Allen-Williams 1992, Evans and Allen-Williams 1993; Bartlet et al. 1997; Smart and Blight, 1997), the pecan weevil, *Curculio caryae* (Horn) (Collins et al. 1997), the pea weevil, *Sitona lineatus* (L.) (Landon et al. 1997), and the red weevil, *Rhynchophorus ferrugineus* F. (Gunawardena et al. 1998).

Plum curculios are attracted to host fruit odors (Butkewich and Prokopy 1993, Prokopy et al. 1995) and volatiles of host fruit odors (Leskey and Prokopy 2000) over short distances in the laboratory, and to host fruit odors in the field at distances up to 3 m (Butkewich and Prokopy 1997). A potential method for improving trap efficacy for plum curculios is to deploy specific attractive compounds present in host plant volatiles in conjunction with a trap, as has been done with the cabbage seed weevil for which water bowl traps painted yellow and filled with detergent solution were baited with specific odor compounds of oilseed rape, *Brassica napus* (Smart and Blight 1997). Although plum curculios are attracted to host fruit odors, the identity of particular compounds that could potentially be attractive to plum curculios and provide growers with a means of increasing trap efficacy over unbaited traps (LeBlanc et al. 1981; Yonce et al. 1995; Prokopy and Wright 1998, Prokopy et al. 1999a) or used in combination with grandisoic acid (Eller and Bartelt 1996) remains unknown.

Here, I evaluated responses of plum curculios in the laboratory in 1998 to 16 individual volatile components of odor of unripe host fruit of cultivated plum, *Prunus domestica*, to identify the most attractive compounds. Wild plum, *Prunus americana*, is the native host of plum curculios (Maier 1990), suggesting that volatiles from cultivated plum potentially could be attractive. I used a previously developed laboratory bioassay system designed to test plum curculio responses to host fruit odor (Prokopy et al. 1995). During the same time period and with the same 16 individual volatile components, I conducted experiments at a field site aimed at identifying volatiles attractive to plum curculios using baited boll weevil traps placed on the ground beneath the canopy of unsprayed apple trees.

Materials and Methods

Immature Plum Volatiles

Unripe cultivated plums (var. Fellenburg) were gathered approximately 2 weeks after bloom from an unmanaged orchard at the Ohio Agricultural Research and Development Center. Forty plums were placed in an 8-dram (32 ml) vial, held on ice, and transported to the laboratory for volatile collections. Volatile analysis commenced within 10 min of collecting the fruit. The cap of the vial was outfitted with two rubber septa, which allowed the flow of compressed air into the vial and flow of volatile-laden air out to a trap. Prior to entering the sample vial, the high-purity compressed air was filtered through activated charcoal and humidified by bubbling through distilled water. Fruit volatiles were collected using a trapping device previously described by Phelan and Lin (1991). The device consisted of a 60 cm x 0.1-cm-ID nickel capillary trap packed with Tenax GC (60/80 mesh) and made integral to a Hewlett-Packard 5890A gas chromatograph (GC) using a 2-position 6-port Valco valve (Houston, TX). This setup allowed volatiles to be collected from the sample and then thermally desorbed at 200°C directly to the capillary GC column. Plum volatiles were collected for 30 min. at 20 ml/min. The trap was purged with dry helium for 3 min., and then volatiles were desorbed for 2 min. to a DB-1 (0.32 x 30 m, µm film) capillary column. Components were eluted with a temperature program of 30-200°C at 10°C/min., followed by analysis using a HP 5970 Mass Selective Detector interfaced to the GC. Initial identifications were confirmed by comparison of retention times and mass spectra with authentic samples. Blank volatile collections using a clean empty vial were conducted each morning to isolate possible contaminants from the trapping system and GC. After the period of volatile collection, plums were placed immediately in hexane to permit extraction of possible additional compounds.

Ten compounds were identified from volatile emissions of field-collected plums (Figure 3). Six additional compounds not found in plum volatile profiles were identified from hexane extracts of plums: benzonitrile, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, and isopropyl acetate. All compounds identified from volatile emissions and from hexane extracts were evaluated as potential attractants for plum curculios in laboratory and field experiments. Compounds were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). For laboratory bioassays, each compound was diluted in hexane (with the exception of 3-hydroxy-2-butanone, which was diluted in water) to yield 3 concentrations: 1.00, 0.10, and 0.01%. For field experiments, all compounds were diluted in technical grade mineral oil to a 5.00% concentration; those compounds found to exhibit either high levels of attractancy or repellency were diluted again to a 0.50% concentration.

Laboratory Bioassay Procedures

All plum curculios used here were collected as overwintered adults in the field in late May or early June in 1998 from populations present on unsprayed wild plum or apple trees in Hampshire County, MA and were assumed to be approximately the same age but of unknown mating status. Adults were held for the duration of all experiments in wax-coated paper cups (473 ml) with clear plastic lids. No more than 60 individuals were held in any cup. Fresh food, either unsprayed wild plum or apple fruit, was provided along with a wetted cotton wick as a water source. Although some herbivorous insects respond differently to host plant odors according to type of diet provided prior to assay, in preliminary laboratory experiments with plum curculios, I saw no difference in plum curculio pattern of response to host fruit odors between individuals fed unsprayed apple or plum (Leskey and Prokopy, unpubl. data). Plum curculios were segregated according to sex using criteria described by Thomson (1932) within several hours after field collection. Plum curculios were held under a photoregime of 16L:8D to mimic long-day conditions in nature from late May to late June.

A dual choice still-air system of enclosed Petri dishes, described by Prokopy et al. (1995), was employed to test attractiveness of the 16 volatile components of plum odor. Tests were conducted at ~ 26°C, 65 - 80% RH, at the beginning of scotophase of the 16L:8D cycle, and were carried out from late May to late June in 1998. A 75 µl aliquot of a solution containing a single compound was pipetted onto a 1 cm square of cotton wick placed next to one of the two pipette tips that served as tubes into the Petri dish chamber. A 75 µl aliquot of hexane (or water, in the case of 3-hydroxy-2-butanone) was used as a solvent control and pipetted onto a second cotton square placed next to the other pipette tube. During bioassay preparation, volatiles and controls were pipetted beneath a fume exhaust system to prevent cross contamination between treatments and controls. Hexane-diluted compounds and controls were allowed to evaporate for approximately 4 min. and then each tube was covered with a transparent polystyrene cup. Water-diluted compounds and controls were covered immediately by cups. After cups were placed over both tubes, the bioassay became a closed system allowing volatiles from the treatment wick to diffuse through the opening of the adjacent pipette tip, into the treatment tube, and then into the Petri dish chamber, presumably creating a concentration gradient of treatment odor that did not, however, exclude treatment odor from entering the control tube. Plum curculios, chosen randomly from the available field-collected pool and held in groups of 40 males or 40 females, were starved for 24 h and chilled for 15-20 min. in a cold room at 8 - 9°C just before use. Handling of plum curculios was kept to a minimum.

A single plum curculio was placed gently in the center of each bioassay dish. Each replicate measuring response to a particular compound consisted of 12 plum curculios tested singly in individual bioassay dishes held together on a tray. Dishes were then moved immediately to the testing room. Plum curculios were fed immediately after the bioassay period had ended and were re-used in subsequent bioassays no less than 4 days later.

To assess attractiveness of a particular compound, I used a Response Index (RI) developed by Phillips et al. (1993). A positive response to either the treatment or control was considered to have occurred when a plum curculio crawled up the inner surface of one of the two tubes and entered a cup containing either the treatment or control within 2 h. Few plum curculios (< 3 %) leave a treatment or control cup after entering it. Plum curculios were allowed 2 h to respond because it takes longer than 1 h for the odor gradient and/or plum curculio response level to reach an asymptote; after 2 h, little change in plum curculio response level occurs (Prokopy et al. 1995). The RI was calculated by subtracting the number of plum curculios responding to the control (C) from the number responding to the treatment (T), dividing the amount by the total number of plum curculios tested, and multiplying by 100. Thus, RI = ((T - C) / total) x 100; the greater the RI value, the more attractive the stimulus. I considered a RI value of 9 as the minimum for suggesting attractiveness and a RI value > 40 as being highly attractive, according to the mean ± a single standard deviation unit (Mean RI = 25.0 ± 16.0 SD) obtained for responses to a standard attractive source: a hexane extract of McIntosh fruit collected 1 week after bloom, the most attractive phenological stage of apples (Leskey and Prokopy 2000). One replicate of the standard (involving assay of 12 plum curculios) was tested each day that bioassays were conducted to measure plum curculio responses to a standard olfactory cue. Under this system, a RI value of -13 or greater can be considered as indicating repellency (according to the subtraction of two standard deviation units from the mean RI for the hexane standard). In addition to assessment using RI values, data were subjected to a Chi Square Test of Homogeneity ($P < 0.05$), where the total number of plum curculios that did vs. did not enter a treatment cup was compared with the total number of plum curculios that did vs. did not enter a control cup for each treatment. Those plum curculios that remained in the dishes were excluded from the analysis because they did not respond.

Field Assay Procedures

For each compound tested, a 150.00 or 15.00 μl aliquot of compound in neat solution was diluted to a 5.00 or 0.50 % concentration, respectively, in mineral oil [3 ml total volume], or 3 ml of mineral oil alone served as a control. Each treatment or control substance was applied to a 7.6 cm piece of cotton dental wick (Absorbal, Wheatridge, CO), which was then wrapped with aluminum foil, sealed at one end, and suspended horizontally by a wire attached to the base of a 'fountain style' boll weevil trap top capping an accompanying solid green boll weevil plastic trap base (Gemplers, Inc., Belleville, WI).

Release rates were determined by exposing five wrapped wicks (identical to those deployed in field assays) containing each compound at each concentration tested to open-air indoor conditions at 26- 28^{0}C for 96 h and weighing each wick daily to determine mg compound lost per day over the 4 day period.

All compounds were evaluated simultaneously in a small unmanaged block of apple trees of mixed cultivars, each about 3 m in canopy diameter. Grass beneath tree canopies was maintained 5-10 cm throughout. One trap was placed at each cardinal point approximately 1 m away from the trunk beneath the canopy of an unsprayed apple tree, with the compound- or control-baited wick facing the trunk. For each tree, traps baited with the same compound were placed at north and south positions, and control-baited traps at east and west positions (or the reverse orientations determined randomly). After 48 h, plum curculios captured in the compound- and control-baited traps were counted, with totals from the 2 compound-baited traps combined into a single value and totals from the 2 control-baited traps combined into a single value, each value constituting one replicate. For the next replicate, trap location for each compound was re-randomized among trees, cardinal orientation of compound- and control-baited traps beneath trees was randomly assigned, and compound- and control-baited wicks were replaced.

In the first field experiment, each of the 16 compounds was evaluated in 12 replicates ran consecutively over a 24 day period at a 5.00% concentration of each compound. In the second field experiment, the most and least attractive compounds from the first field experiment were tested again, at a 5.00 % as well as a 0.50% concentration, with 10 replicates of each. Procedures were essentially the same as in the first field experiment except that each replicate lasted 24 rather than 48 h.

To measure the attractiveness of a particular compound, a Field Response Index was created by subtracting the number of plum curculios responding to the control (C) from the number responding to the treatment (T) [the particular compound], dividing by the total number of plum curculios captured in the treatment and control traps, and multiplying by 100. Thus, RI = (T - C)/(T + C) * 100; the greater the RI, the more attractive the compound. To analyze the extent of response to traps baited with a particular compound compared to corresponding control traps, data were subjected to a Wilcoxon Signed Rank Test ($P \leq 0.05$) where the total number of plum curculios that entered compound-baited traps and the total number of plum curculios that entered a control-baited traps were compared across all replicates for each compound (Statistix, 1992).

Results

Laboratory Results

There were no significant differences between males and females in response to compounds at any of the three concentrations tested. Therefore, results were combined across sex to increase statistical power. For compounds at 1.00% concentration, positive responses (RI > 9) were recorded to ethyl butyrate (RI = 14.6), while negative RIs were recorded for responses to benzaldehyde, benzonitrile, ethyl isovalerate, 2-hexanol, 3-hexanone, 3-hydroxy-2-butanone, isopropyl acetate, limonene, linalool, 2-pentanol, and trans-2-hexenal with repellent RIs (RI < -13) recorded for all of these except ethyl isovalerate, 2-hexanol, isopropyl acetate, limonene, and 2-pentanol (Table 9). There was a significantly greater response to control over treatment for benzaldehyde, benzonitrile, 3-hexanone, 3-hydroxy-2-butanone, isopropyl acetate, linalool, and trans-2-hexenal, indicating that plum curculios were likely repelled at 1.00 % concentration. At 0.10% concentration, positive responses were recorded to ethyl isovalerate (RI = 12.5), 2-pentanol (RI = 12.5) and 2-hexanone (RI = 10.4), with response to ethyl isovalerate being significantly greater than to control (Table 10). Negative RIs were recorded for benzaldehyde, benzonitrile, 3-hexanone, 3-hydroxy-2-butanone, isopropyl acetate, linalool, and trans-2-hexenal just as at the 1.00% concentration, with significantly greater response to control over treatment and RIs indicating repellency for benzaldehyde and trans-2-hexenal. Also, a negative RI was recorded at the 0.10% concentration for ethyl acetate (Table 10). At 0.01% concentration, positive responses were recorded to ethyl isovalerate (RI = 23.6), limonene (RI = 18.1), ethyl acetate (RI = 12.5), linalool (RI = 12.5) and benzonitrile (RI = 11.7), with responses to treatments of ethyl isovalerate, limonene and linalool being significantly greater than to controls (Table 11). Negative RIs were recorded for 3-hexanone, 3-hydroxy-2-butanone, and trans-2-hexenal, as was also the case at 1.00 and 0.10% concentrations, with a RI indicating repellency for 3-hydroxy-2-butanone at this concentration; responses to controls of 3-hexanone, 3-hydroxy-2-butanone, and trans-2-hexenal were significantly greater than to treatments. Other negative RIs at 0.01% concentration included responses to 3-hexanol, 2-pentanol, and 2-propanol.

Field Results

In the first field experiment, highest positive RIs were recorded for responses to traps baited with ethyl isovalerate (RI = 71.4), limonene (RI = 37.5), benzonitrile (RI = 33), and 3-hydroxy-2-butanone (RI = 26.3), with responses to ethyl isovalerate and limonene being significantly greater than to controls (Table 12). Negative RIs, indicative of more plum curculios caught in control-baited traps than compound-baited traps, were recorded for 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, isopropyl acetate, 2-pentanol and 2-propanol, with significantly greater responses to control-baited traps than to traps baited with 3-hexanol and 3-hexanone. In the second field experiment, highest positive RIs were recorded for responses to limonene (RI = 100) and ethyl isovalerate (RI=80) at 5.00% concentration, with response to each compound being significantly greater than to control. A lesser degree of positive response (none significant) occurred to both of these compounds at the 0.50% concentration, and to 3-hexanol at either concentration (Table 13).

Discussion

Two compounds, ethyl isovalerate and limonene, were significantly more attractive than controls in both laboratory bioassays and field experiments (Tables 10 - 13). One green leaf volatile, 3-hexanone, yielded significantly greater response to controls in both laboratory bioassays and field assays, and most of the other green leaf volatile compounds tested, including 2-hexanone, 2-hexanol, 3-hexanol, and trans-2-hexenal, elicited negative RIs in laboratory bioassays and/or field experiments (with trans-2-hexenal at all concentrations in laboratory bioassays and 3-hexanol at 5.00 % concentration in field assays being significantly less attractive than controls) (Tables 9 - 12). In laboratory bioassays, as the concentration of compound was decreased, a greater number of compounds elicited a positive response (RI > 9), including significantly greater response to treatment over control for linalool at 0.01% (Table 11). Benzaldehyde, benzonitrile, 3-hydroxy-2-butanone, linalool and trans-2-hexenal were significantly less attractive than controls in laboratory bioassays (Table 9) but yielded positive RIs in the field (Table 12).

Ethyl isovalerate and limonene were the only compounds tested here that yielded positive and significant responses from plum curculios in both laboratory bioassays (Tables 10 and 11) and field experiments (Tables 12 and 13). Volatiles from host plant odors can be used in conjunction with traps to markedly increase trap efficacy, as has been shown in the case of butyl hexanoate (a component of apple odor) that enhances attraction of apple maggot flies, *Rhagoletis pomonella* (Walsh) to fruit-mimicking red sphere traps (Reissig et al. 1982; Duan and Prokopy 1992; Reynolds and Prokopy, 1997). Addition of ethyl isovalerate and limonene to traps may provide growers with a means to increase trap efficacy over current unbaited plum curculio traps (LeBlanc et al. 1981, Yonce et al. 1995, Prokopy and Wright 1998, Prokopy et al. 1999a). In combination with grandisoic acid, an attractive component of the male-produced aggregation pheromone (Eller and Bartelt 1996), these fruit volatiles could be even more useful in enhancing trap efficacy, such as in the case of traps for the palmetto weevil, *Rhynchophorus cruentatus*

(F.) (Giblin-Davis et al. 1994) and the West Indian sugarcane weevil, *Metamasius hemipterus sericeus* (Olivier) (Giblin-Davis et al. 1996), where combinations of host plant tissue or host plant volatile compounds plus synthetic pheromones were employed.

Green leaf volatiles, including most importantly certain 6-carbon alcohols and aldehydes, result from metabolism of lipids and are produced by many green plants (Bernays and Chapman 1994). Chemotatic responses by herbivorous insects to these compounds are considered to be a generalized mechanism of host plant recognition (Metcalf and Metcalf 1992). In my laboratory bioassays, the green leaf volatiles trans-2-hexenal and 3-hexanone elicited negative RIs and significantly greater responses to controls by plum curculios at all 3 concentrations, except 3-hexanone at 0.10 % (Tables 9-11). In the field experiment, again a negative RI was recorded for plum curculio responses to 3-hexanone as well to the green leaf volatiles 2-hexanol, 3-hexanol, and 2-hexanone, with significantly greater responses to control-baited traps for 3-hexanol and 3-hexanone (Table 12). Perhaps at the concentrations tested, plum curculios perceived these volatiles to be from a non-host green plant, and thus avoided them. Similarly, traps baited with the green leaf volatiles 1-hexanol, (E)-2-hexen-1-ol, (Z)-2-hexen-1-ol and (Z)-3-hexen-1-ol alone or in combination resulted in reduced trap catches of the striped ambrosia beetle, *Trypodendron lineatum* (Olivier), in forests of Douglas fir trees in British Columbia, indicating that these beetles may use these compounds as cues to avoid non-host angiosperm logs in their search for host coniferous logs (Borden et al. 1997). On the other hand, unlike *T. lineatum*, plum curculios could be attracted to broad-spectrum green leaf volatile compounds, but at lower concentrations. For example, male plum curculios were attracted to a 5 mg dose of a known green leaf volatile, cis-3-hexen-1-ol, in an olfactometer in significantly greater numbers than to higher doses of this compound or to a control (Cormier et al. 1998).

The concentrations of 1.00 and 0.10% for most compounds tested in the laboratory bioassays were probably too high to elicit a positive response from plum curculios in the confined space and over the short distance between odor source and release point of a plum curculio in a Petri dish chamber. Linalool was significantly less attractive than control at 1.00% but was significantly more attractive than control at 0.01% (Tables 9 and 11). Similarly, the boll weevil, *Anthonomus grandis* Boheman, was repelled at the highest concentration tested and attracted at lower concentrations of β - bisabol, the major volatile of cotton evaluated in laboratory experiments (Dickens 1986). Therefore, plum curculios may in fact be attracted to some of the compounds that were significantly less attractive than controls at 0.01%, including 3-hexanone, 3-hydroxy-2-butanone and trans-2-hexenal (Table 11).

Concentration of a particular compound or amount released under respective conditions in which a compound is evaluated appears to have a strong effect on the nature of plum curculio responsiveness. Benzaldehyde, benzonitrile, 3-hydroxy-2-butanone, linalool and trans-2-hexenal were significantly less attractive than controls in laboratory bioassays at 1.00 % concentration (Table 9), but yielded positive RIs in the field at 5.00 % concentration (Table 12). Similarly, cabbage seed weevils were attracted to (Z)-3-hexen-1-ol in a linear track olfactometer in laboratory experiments (Bartlet et al. 1997), but in the field, this compound had no effect on trap captures during weevil colonization of crop fields or was slightly depressive to trap captures during dispersal from crop fields

(Smart and Blight 1997). Perhaps plum curculios and cabbage seed weevils express attraction to certain compounds under certain conditions, but do not do so under other conditions because of greater/lesser concentration of compound and/or the context in which a compound is offered.

This study provides the first evidence for plum curculio attraction to specific compounds present in host plant odor, namely ethyl isovalerate and limonene. However, both of these compounds as well as other compounds tested might be more attractive at concentrations or release rates different from these used here, or in combination. Although ethyl isovalerate and limonene significantly increased trap captures under the conditions tested here, further tests are needed to optimize formulation, release rate, type of trap used in association with compound deployment, and positioning of compounds on traps. However, the indication here of plum curculio attraction to these compounds increases the potential for creating a reliable monitoring device for this important tree fruit pest.

Table 9. Adult plum curculios (%) responding in the laboratory to individual volatile components of plum odor (at 1.00% in solvent) and corresponding response indices for each compound.

Compound	No. Plum curculios Asssayed[a]	Plum curculios Responding (%)[b]		
		Treatment	Control	Response Index
Benzaldehyde	72	1	29[b]	-27.8
Benzonitrile	48	2	31[b]	-29.2
Ethyl Acetate	48	31	25	6.3
Ethyl Butyrate	48	35	21	14.6
Ethyl Isovalerate	72	15	19	-4.2
2-Hexanol	48	15	19	-4.2
3-Hexanol	48	21	21	0.0
2-Hexanone	72	25	23	2.1
3-Hexanone	48	4	21[b]	-16.7
3-Hydroxy-2-Butanone	48	19	35[b]	-16.7
Isopropyl Acetate	48	13	25[b]	-12.5
Limonene	72	22	29	-6.9
Linalool	48	2	29[b]	-27.1
2-Pentanol	48	23	25	-2.1
2-Propanol	48	21	21	0.0
Trans-2-hexenal	48	4	25[b]	-20.8

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[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments and controls are significantly different from each other at $P \leq 0.05$ according to chi-square test of homogeneity.

Table 10. Adult plum curculios (%) responding in the laboratory to individual volatile components of plum odor (at 0.10% in solvent) and corresponding response indices for each compound.

Compound	No. Plum curculios Asssayed[a]	Plum curculios Responding (%)[b] Treatment	Control	Response Index
Benzaldehyde	84	13	26[b]	-13.1
Benzonitrile	60	15	25	-10.0
Ethyl Acetate	48	21	27	-6.3
Ethyl Butyrate	48	35	29	6.3
Ethyl Isovalerate	72	26[b]	14	12.5
2-Hexanol	48	23	15	8.3
3-Hexanol	48	23	23	0.0
2-Hexanone	48	31	21	10.4
3-Hexanone	48	10	21	-10.4
3-Hydroxy-2-Butanone	48	23	29	-6.3
Isopropyl Acetate	48	13	17	-4.2
Limonene	72	33	29	4.2
Linalool	48	13	19	-6.3
2-Pentanol	48	29	8	12.5
2-Propanol	48	19	19	0.0
Trans-2-hexenal	48	8	25[b]	-16.7

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[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments and controls are significantly different each other at $P \leq 0.05$ according to chi-square test of homogeneity.

Table 11. Adult plum curculios (%) responding in the laboratory to individual volatile components of plum odor (at 0.01% in solvent) and corresponding response indices for each compound.

	No. Plum curculios	Plum curculios Responding (%)[b]		
Compound	Asssayed[a]	Treatment	Control	Response Index
Benzaldehyde	84	24	24	0.0
Benzonitrile	60	33	22	11.7
Ethyl Acetate	48	38	21	12.5
Ethyl Butyrate	48	27	19	8.3
Ethyl Isovalerate	72	35[b]	11	23.6
2-Hexanol	48	21	13	8.3
3-Hexanol	48	19	21	-2.1
2-Hexanone	48	27	27	0.0
3-Hexanone	48	4	15[b]	-10.4
3-Hydroxy-2-Butanone	48	8	25[b]	-16.7
Isopropyl Acetate	48	23	19	4.2
Limonene	72	39[b]	25	18.1
Linalool	48	23[b]	10	12.5
2-Pentanol	48	17	23	-6.3
2-Propanol	48	17	21	-4.1
Trans-2-hexenal	48	12	25[b]	-12.5

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[a] Each replicate consisted of 12 plum curculios tested individually in bioassay dishes and held together on a single tray.

[b] Treatments and controls are significantly different each other at $P \leq 0.05$ according to chi-square test of homogeneity.

Table 12. Adult plum curculios responding in the field to individual volatile components of plum odor (at 5.00% in mineral oil) and corresponding response indices for each compound.

Compound	Replicates[a]	Release Rate mg/day	Total Captured	Plum curculios Responding (%)[b] Treatment	Control	Response Index
Benzaldehyde	12	14.64	16	56	44	12.5
Benzonitrile	12	11.33	15	67	34	33.0
Ethyl Acetate	12	30.54	25	56	44	9.1
Ethyl Butyrate	12	27.60	44	57	43	13.6
Ethyl Isovalerate	12	19.51	21	86[b]	25	71.4
2-Hexanol	12	18.59	16	44	56	-12.5
3-Hexanol	12	18.57	18	17	83[b]	-66.7
2-Hexanone	12	22.43	20	40	60	-18.2
3-Hexanone	12	26.37	26	31	69[b]	-38.5
3-Hydroxy-2-Butanone	12	27.29	19	63	37	26.3
Isopropyl Acetate	12	33.31	23	43	57	-13.0
Limonene	12	4.99	32	69[b]	31	37.5
Linalool	12	3.71	20	55	45	10.0
2-Pentanol	12	35.33	24	38	62	-25.0
2-Propanol	12	31.97	29	34	66	-31.0
Trans-2-hexenal	12	1.85	26	54	46	7.7

[a] Each replicate consisted of captures by 2 compound-baited and 2 control-baited traps placed on the ground beneath the canopy of an unsprayed apple tree for 48 h.

[b] Treatments and controls are significantly different from each other at $P \leq 0.05$ according to Wilcoxon signed rank test.

Table 13. Adult plum curculios responding in the field to individual volatile components of plum odor and corresponding response indices for each compound.

			Release Rate	Total	Plum curculios Responding (%)[c]		
Compound	%[a]	Reps[b]	mg/day	Captured	Treatment	Control	Response Index
Ethyl Isovalerate	5.00	10	19.51	10	90[c]	10	80.0
	0.50	10	2.82	4	75	25	50.0
3-Hexanol	5.00	10	18.57	8	63	37	25.0
	0.50	10	2.39	10	60	40	20.0
Limonene	5.00	10	4.99	10	100[c]	0	100.0
	0.50	10	1.03	19	63	37	26.0

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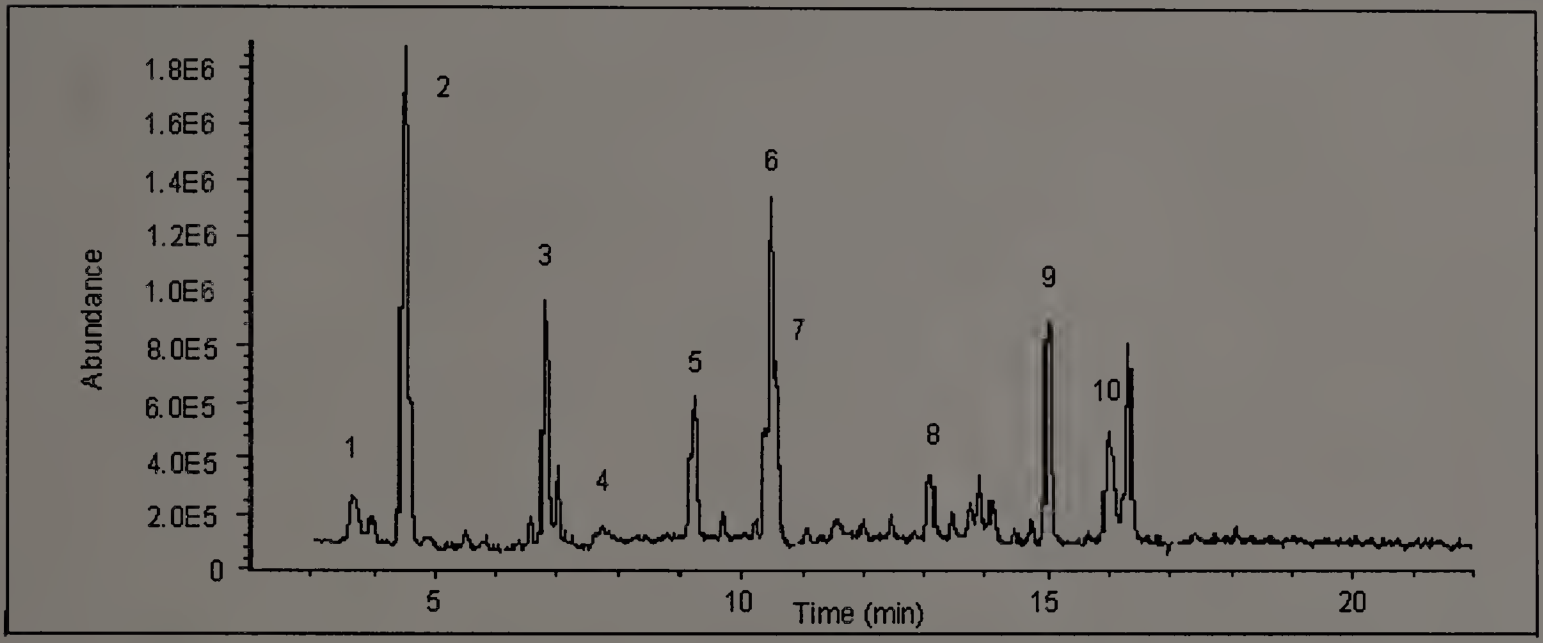
[a] % compound in mineral oil.

[b] Each replicated consists of captures by 2 compound-baited and 2 control-baited traps placed on the ground beneath the canopy of an unsprayed apple tree for 24 h.

[c] Treatments and controls are significantly different from each other at $P \leq 0.05$ according to Wilcoxon signed rank test.

Figure 3. Total ion chromatogram of natural plum volatiles collected on Tenax for 30 min. at 20 ml/min from 40 immature plums (10 g total, avg. = 7.3 mm) at 2 weeks after bloom. Compound identity: **1**, 2-propanol (8 ng); **2**, ethyl acetate (119 ng); **3**, 3-hydroxy-2-butanone (56 ng); **4**, 2-pentanol (4 ng); **5**, ethyl butanoate (40 ng); **6** + **7**, trans-2-hexenal and ethyl isovalerate, respectively (71 ng); **8**, benzaldehyde (11 ng); **9**, limonene (51 ng); **10**, linalool (29 ng). Unmarked peaks were incompletely identified.



CHAPTER 5

FRUIT ODORS ARE MORE ATTRACTIVE THAN CONSPECIFIC ODORS TO ADULT PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE)

Abstract

I evaluated olfactory attraction of overwintered male and female plum curculio adults, *Conotrachelus nenuphar* (Herbst), to volatiles released from same and opposite sex conspecifics alone, synthetic grandisoic acid alone, wild plums alone, or combinations of live single sex conspecifics or grandisoic acid with plums in large still-air arenas. Male plum curculios were most strongly attracted to volatiles released from females feeding on plums and to volatiles from mechanically punctured plums, which induced male copulations. Males were not attracted to volatiles of males alone or synthetic grandisoic acid alone, and were just as attracted to the odor of plums alone as to the odor to either of these other stimuli in combination with plums, indicating a lack of enhancement of male-produced volatiles by host plum odor. Females were attracted to the odor females alone, males alone, and synthetic grandisoic acid alone, but these attractive responses were quickly lost when plum odor was present (especially volatiles released from punctured plums), indicating the primary importance of host plum odor in attraction. I conclude that female plum curculios may produce a sex pheromone

attractive to male plum curculios, but in general both male and female plum curculios were strongly attracted to host plum volatiles and only mildly attracted to conspecific odors or synthetic grandisoic acid under the conditions of my tests, and that volatiles released from punctured plums are highly attractive to plum curculios and should be used as source material to identify potential attractants for plum curculios.

Introduction

For many phytophagous insects, host plant volatiles serve as attractants (Bernays and Chapman 1994) that aid in locating food, mates, and/or oviposition sites (Visser 1986). Further, pheromones produced by phytophagous insects also serve as attractants for other conspecifics of the same or opposite sex (Carde and Baker 1984). In combination, host plant volatiles often synergize or enhance insect responses to sex and/or aggregation pheromones (Landolt and Phillips 1997).

In the family Curculionidae, male-produced pheromones have been documented in at least 21 species, with 19 of these attracting both sexes and 18 enhanced by the presence of host plant volatiles. On the other hand, male-produced pheromones of only 2 species, the boll weevil, *Anthonomus grandis grandis* Boheman, and the pecan weevil, *Curculio caryae* (Horn), attract only females, with response enhanced by the presence of host plant volatiles (Landolt 1997). Female-produced odors also have been documented in the family Curculionidae, particularly in the sweet potato weevil, *Cylas formicarius elegantulus* (Summers) (Heath et al. 1986) which produces a pheromone attractive to males only (Mason et al. 1990) and in the cabbage seed weevil, *Ceutorhynchus assimilis*

Payk. which produces odor principally attractive to males (Evans and Bergeron 1994). The presence of host plant material in association with these female pheromonal odors has not been examined for evidence of synergy or enhancement in either species.

Attractive semiochemical combinations that include both host plant volatiles and pheromones have been deployed to create successful monitoring systems for a number of Curculionidae species, including American Palm weevil, *Rhynchophorus palmarum* (L.) (Oehlschlager et al. 1993), palmetto weevil, *R. cruentatus* (F.) (Giblin-Davis et al. 1994), and West Indian sugar cane weevils, *Metamasius hemipterus sericeus* (Olivier) (Giblin-Davis et al. 1996).

The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) is attracted to host plant odors in the field (Butkewich and Prokopy 1997) and in the laboratory (Butkewich et al. 1987, Butkewich and Prokopy 1993, Prokopy et al. 1995, Leskey and Prokopy, accepted) and to specific components of host plant odors in the laboratory and the field (Leskey et al. submitted). Further, plum curculio male-produced aggregation pheromone, grandisoic acid, was recently identified by Eller and Bartelt (1996). To date, no female-produced odor has been identified.

The plum curculio is a key pest of apples in eastern North America (Chouinard et al. 1994) and one of the most destructive pests of peaches and plums in the southeastern United States (Yonce et al. 1995). Lack of a reliable visual monitoring trap to detect plum curculio entry into orchards (Prokopy and Croft 1994) invites study of potential attraction of male and female plum curculios to male-produced pheromone, potential female-produced pheromone, and host plant volatiles to learn what may be the most attractive semiochemical combinations for use in conjunction with traps to create a reliable monitoring system.

Therefore, I conducted a series of experiments aimed at identifying the most attractive sources of semiochemicals to plum curculio adults. Tests took place in large still-air arenas throughout the active field season of plum curculio in Massachusetts. I tested male and female responses to odors of wild plum, *Prunus americana* Marshall., the native host of plum curculio (Maier 1990), male plum curculios, female plum curculios, synthetic grandisoic acid, and combinations of wild plums held with male plum curculios, female plum curculios, or grandisoic acid.

Materials and Methods

Plum Curculios

All plum curculios used here were collected as overwintered adults in the field in late May or early June from populations present on unsprayed wild plum or apple, *Malus* sp. trees in Hampshire County, MA and were assumed to be approximately the same age. Plum curculios were segregated according to sex within 1-3 h after collection using criteria described by Thomson (1932) and held as separate sexes in wax-coated cups (473 ml) with clear plastic lids under a photoregime of 16L:8D to mimic long-day conditions in nature from late May to late June. No more than 60 individuals were held in any cup. Fresh food, either unsprayed wild plum or apple fruit, was provided along with a wetted cotton wick as a water source.

Source Material

Odor sources evaluated in test arenas included male and female plum curculios starved for 24h, green fruit worms (*Orthosia hibisci* (Guenee), Lepidoptera: Noctuidae) collected from unsprayed wild plum and apple trees located in Amherst, MA, wild plums picked 1-2h before the start of the experiments from these same unsprayed wild plum trees, and synthetic grandisoic acid impregnated into rubber septa at two doses, a high dose of 3.00 mg per septum and a low dose of 0.03 mg per septum (IPM Technologies, Portland, Oregon). All source material comprising a particular treatment was placed in a small 6 x 9 cm cotton bag.

Treatments Including Females

Female plum curculio treatments included 5 females alone and 5 females + 5 plums held together within a bag and were designated as females and females + plums, respectively. To examine the influence of sounds made by movement of the plectra on the 6th abdominal segment against the stridulitra located on the ventral-medial surface of the left elytrum (Carlysle et al. 1975), 5 females with glued elytra to prevent stridulation [according to the procedures of Mampe and Neunzig (1966)] were held together within the same bag with 5 plums and designated as glued females + plums. Also tested were 5 females and 5 plums held separately within the same bag by a sewn barrier dividing the bag into 2 compartments and designated as (females)(plums). Additionally, a cotton bag containing 5 females feeding on plums for 24 h was divided into three treatments in an attempt to separate each of the potential odor sources: 5 females designated as fed females, 5 partially eaten plums designated as female-punctured plums, and the cotton bag in which females and plums were held for the preceding 24h designated as frass bag. To gauge degree of male responsiveness across the period of assay, a treatment of 5 females + 5 plums was tested for male responsiveness at three different periods throughout the active field season of plum curculios in Massachusetts in 1999: 23-26 May designated as females + plums (early), 7-10 June designated as females + plums (mid), and 21-24 June designated as females + plums (late).

Treatments Including Males

The following male plum curculio treatments were tested: 5 males, 5 males + 5 plums, 5 males and 5 plums held separately by means of a sewn barrier but within the same bag and designated as males, males + plums, and (males)(plums), respectively.

Treatments Including Plums Only

Plum treatments included 5 plums alone and 5 mechanically punctured plums [each with 2 punctures, 1 made 1h before, and 1 made immediately before the beginning of an experiment] designated as plums and mechanically punctured plums, respectively.

Treatments Including Non-Plum Curculio Insect with Plums

Green fruit worms, used to represent a non-plum curculio plum-feeding insect, were tested as one green fruit worm held with 5 plums and designated as GFW + plums. This treatment was compared with male or female plum curculios feeding on plums to look for evidence of male- or female- plum curculio produced odors that require either feeding on plums for their production or enhancement with host plum odor to be attractive.

Treatments Including Synthetic Grandisoic Acid

Synthetic grandisoic acid was tested alone at low and high doses designated as pheromone (l) and pheromone (h), respectively, and in combination with 5 plums at both doses, designated as plums + pheromone (l) or (h), respectively

Arena Set-up

Still-air arenas were constructed from clear Plexiglas with dimensions 61 x 61 x 30.5 cm and 61 x 61 cm Plexiglas lids. In the four upper corners of each arena, concentric curves were drawn on the two walls and the lid at a distance of 6 cm from the corner to create a concentric circle in each upper corner. Each treatment bag was securely tied and hung from the upper corner of an arena. Once the four treatment bags were hung in each arena, the lid was placed over the arena 1h before the experiment was to begin. Empty bags served as controls. Plum curculios that served as responders were starved for 24h and chilled for 15-20 min in a cold room at 8 - 9°C just before the start of the experiment to increase plum curculio responsiveness to odors by lessening their tendency to move instantly, perhaps in an escape response, upon release. Handling of plum curculios was kept to a minimum. Male or female plum curculios were held in cups in groups of 10 for release in the arenas. Chilled plum curculios were released onto the center of the arena floor, and the lid of the arena was replaced and repositioned to allow concentric curves on the walls and lid to meet. Tests were conducted at ~ 26°C, 65 - 80% RH at the beginning of scotophase of a 16L:8D cycle, and were carried out from late May to late June in 1997, 1998, and 1999. Responding curculios were considered to be those present on a cotton bag and those within the 6 cm circle and were counted every 10 min for 1 h using a red light.

In 1999, the amount of plum consumed by 5 plum curculios or 1 green fruit worm in all replicates of four treatments (females + plums, males + plums, glued females + plums, and GFW + plums) was estimated at the end of the experimental hour.

Statistical Analysis

The total number of plum curculio responders to a particular odor treatment in an arena was tallied over the six 10 min intervals for each of the four treatments to provide a total response score for each treatment for every replicate. Then for all replicates of each set of odor sources tested together in an arena, I carried out a one-way ANOVA with LSD pairwise comparison of means for total response scores. I also report for treatments with replicates completed in 1999, the mean percentage of injured and/or removed plum surface area by plum curculios and green fruit worms.

Male Plum Curculio Copulatory Behavior in the Presence or Absence of Plum Volatiles

In the presence of females feeding on plums, I observed that male plum curculios frequently attempted to mate with females as well as with other males or climbed and mounted males that were already copulating with females, often crawling upon and pushing one another. For this experiment, 2 male plum curculios, each marked with a small white dot on the right elytrum, were released into a Petri dish with 2 unmarked females in the presence of either 5 mechanically damaged plums (punctured as previously described), 5 uninjured plums, or nothing and observed continuously for 15 min during scotophase under red light conditions. The total number of male-attempted mountings of both male and female plum curculios was counted. One-way ANOVA with LSD pairwise comparison of means was calculated to compare differences in the number of attempted mounts made by males in the presence of volatiles from injured plums or uninjured plums or in the absence of plum volatiles.

Results

Male Responses to Females

Males did not respond to odor of females held alone compared to controls (Table 14, Experiment 14.1). However, when females were held with plums, males responded significantly to this treatment over controls throughout the activity period of overwintered plum curculios (Table 14, Experiments 14.2, 14.3, 14.4). Males also responded to treatment females + plums in significantly greater numbers than to males + plums (Table 16, Experiment 16.1) and GFW + plums (Table 16, Experiment 16.2), but females + plums and mechanically punctured plums were statistically equal in attractiveness to males (Table 16, Experiment 16.3). The greater response of males to odor of females feeding on plums than to odor of males feeding on plums can be accounted for by the nearly 3-fold greater amount of plum surface area punctured and removed by females compared to males (Table 16, Experiment 16.1). On the other hand, the treatment of GFW + plums showed a far greater amount of plum surface area damaged and removed, over a 16-fold difference, than the treatment of females + plums, even though males responded to females + plums in significantly greater numbers than to GFW + plums (Table 16, Experiment 16.2).

When males responses to females alone, plums alone, and females + plums were compared, males again responded in significantly greater numbers to females + plums (Table 18, Experiment 18.1). Similarly, when the elytra of females were glued to prevent stridulation, males responded in significantly greater numbers to glued females + plums than to glued females alone or plums alone (Table 19). When treatment females were separated from plums but held within the same bag, designated as (females)(plums), and were compared with treatments of plums alone and females alone, statistically equal numbers of males chose plums alone and (females)(plums), with response to each treatment significantly greater than to females alone or control (Table 18, Experiment 18.2). Statistically equal numbers of males responded the following treatments: females + plums, (females)(plums), and mechanically punctured plums, although the greatest numerical response was to females + plums (Table 18, Experiment 18.3). Because males tended to choose the treatment females + plums in greater numbers than any other treatment included in an experiment (with the exception of mechanically punctured plums in Table 16, Experiment 16.3), I compared male response to female-punctured plums alone, females alone that had fed on plums for 24 h, and bags alone containing frass left by females during feeding in an attempt to determine which of these odor sources was most attractive. Here, male plum curculios overwhelmingly chose female-punctured plums over the other treatments (Table 18, Experiment 18.4). Finally, in direct observations of male copulatory behavior in the presence of mechanically punctured plums, uninjured plums or nothing, significantly more males attempted to mount either female or male plum curculios in the presence of punctured plums than in other treatments (Table 21).

Male Responses to Males

Males showed no greater response to odor of males held alone than to controls (Table 15). Also, males responded in significantly fewer numbers to odor of males + plums than females + plums (Table 16, Experiment 16.1) and in statistically equal numbers to odor of males + plums and GFW + plums (Table 17). Males, however, did respond significantly more to odor of males + plums than to odor of males alone or plums alone (Table 20, Experiment 20.1). Males also responded significantly more to odor of (males)(plums) than to odor of males alone or plums alone (Table 20, Experiment 20.2). However, when the treatment (males)(plums) was compared to males + plums and mechanically punctured plums, male response to all treatments was statistically equal (Table 20, Experiment 20.3).

Female Responses to Males

Females responded significantly more to odor of males alone than to control (Table 22, Experiment 22.1). However, females did not discriminate statistically between odors of males + plums and odors of females + plums (Table 23, Experiment 23.1), of males + plums and GFW + plums (Table 23, Experiment 23.2) or of males +

plums and mechanically punctured plums (Table 23, Experiment 23.3). Similarly, females did not respond in significantly greater numbers to odor of males + plums compared to odor of plums alone, but did respond in significantly greater numbers to odors of these two treatments than to odor of males alone or controls (Table 25, Experiment 25.1). When female response to odor of males alone, plums alone, and (males)(plums) was compared, statistically equal numbers responded to plums and (males)(plums), with responses to both significantly greater than to males alone (Table 25, Experiment 25.2). Females responded equally to odor of males + plums, (males)(plums), and mechanically punctured plums (Table 25, Experiment 25.3).

Female Responses to Females

Females responded significantly more to odor of females alone than to controls (Table 22, Experiment 22.2). Female responses were equal to odors of females + plums and males + plums (Table 23, Experiment 23.1) and to the odor of females + plums and GFW + plums (Table 24, Experiment 24.1) with responses in all cases being significantly greater than to controls. When female responses to females alone, plums alone, and females + plums were compared, greatest responses were to plums alone and females + plums, which were statistically equal (Table 26, Experiment 26.1). Tests of female response to females alone, plums alone, and (females)(plums) yielded greater response to (females)(plums) over all other treatments (Table 26, Experiment 26.2). To evaluate the possibility of sound as a factor influencing results, female response to (females)(plums) was compared to (glued females)(plums), resulting in an identical level of response

(Table 24, Experiment 24.2). Finally, when comparing females + plums, (females)(plums) and mechanically punctured plums, numerically greatest response was recorded to females + plums, though it was not significantly greater than to mechanically punctured plums (Table 26, Experiment 26.3).

Male Response to Synthetic Grandisoic Acid

Males did not respond in significantly greater numbers to grandisoic acid tested alone at either low (Table 27, Experiment 27.1) or high doses (Table 27, Experiment 27.2) over controls. Males responded in significantly greater numbers to volatiles from plums alone and plums + pheromone (no difference between these treatments) at both a low (Table 28, Experiment 28.1) and high dose of pheromone (Table 28, Experiment 28.2) than to pheromone alone and controls.

Female Response to Synthetic Grandisoic Acid

Females responded in significantly greater numbers to pheromone tested alone at low dose than to controls (Table 29, Experiment 29.1), but did not do so to pheromone alone at high dose (Table 29, Experiment 29.2). In comparisons of response to low dose of pheromone alone, plums alone, and plums + pheromone, greatest response was to plums alone, although it was not significantly greater than to plums + low dose of pheromone (Table 30, Experiment 30.1). When the high dose of pheromone was tested, significantly greater response was recorded to plums alone and plums + pheromone than to pheromone alone or controls (Table 30, Experiment 30.2).

Discussion

Male plum curculios appeared to be attracted to a female-produced substance when females are feeding on plums (Table 16, Experiments 16.1 and 16.2, Table 18, Experiments 18.1 and Table 19), but were also strongly attracted to the odor the punctured plums alone (Table 16, Experiment 16.3 and Table 18, Experiments 18.3 and 18.4). Volatiles released from punctured plums stimulated males to attempt copulation (Table 21). Males were not attracted to odors of conspecific males held alone (Table 15) or synthetic grandisoic acid alone at either dose (Table 27, Experiment 27.1 and 27.2). However, males were attracted to odor of males feeding on plums over plums alone (Table 20, Experiment 20.1), again a possible attraction to odor of punctured plums because males were attracted in equal numbers to synthetic grandisoic acid held with plums and to plums alone (Table 28, Experiments 28.1 and 28.2) and males were more attracted to the odors of mechanically punctured plums (Table 20, Experiment 20.3) and green fruit worms feeding on plums (Table 17) than to odor of males feeding on plums. An interesting result in responses of both male and female plum curculios was that both sexes responded in significantly greater numbers to the same sex held separately within a bag with plums than to conspecifics alone or plums alone (Table 20, Experiment 20.2 and Table 26, Experiment 26.2), but not to the opposite sex held separately within a bag with plums compared to plums alone (Table 18, Experiment 18.2 and Table 25, Experiment 25.2). Females were attracted to odor of males held alone (Table 22, Experiment 22.1) and synthetic grandisoic acid at the low dose (Table 29, Experiment 29.1) in the absence of plum odor, but this response was overwhelmed when plums alone were included in experiments (Table 25, Experiments 25.2 and 25.3, Table 30, Experiment 30.1).

Female-produced pheromones in the family Curculionidae have not been shown to exist in as many species as male-produced pheromones (Landolt 1997), although one has been identified for the sweet potato weevil (Heath et al. 1986) and is apparently produced by the cabbage seed weevil (Evans and Bergeron 1994). Based on my observations, I believe that female plum curculios may produce a pheromone that is attractive to males because males were more highly attracted to females feeding on plums compared to males feeding on plums or a green fruit worm feeding on plums (Table 16, Experiments 16.1 and 16.2). Sex attraction responses of male phytophagous insects can be directly dependent on stimuli from host plants (Landolt and Phillips 1997). Male response to female-produced bark beetle sex pheromones is markedly enhanced by or highly dependent upon release of host plant odors (Wood 1982). Female plum curculio feeding on plums is necessary to elicit male plum curculio response to odor of females, and may bear similarity to the production of an aggregation pheromone by the flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae), which requires contact with its host plant for stimulation of attractive responses (Peng and Weiss 1992).

A physiological factor that bears consideration in terms of male response to a female-produced odor is mating status. All plum curculios were field-collected and thus were assumed to be mated, possibly influencing production of female-produced odor and/or male response to female-produced odor. Immediately after mating, female bruchid beetles, *Callososbruchus subinnotatus* (Pic), produce significantly less pheromone than virgin female bruchid beetles although this suppression only lasts 1 d (Shu et al. 1998). In an attempt to examine the influence of mating status on male plum curculio responses to female-produced odors, I reared larvae to adults, segregated them according to sex based on characteristics described by Thomson (1932), and housed them outdoors in single sex groups in overwintering containers based on methods used by Smith and Flessel (1968) to provide a source of known virgins for bioassays the following year. I repeated these procedures for 3 consecutive winters but obtained only minimal numbers of plum curculios surviving, thus prohibiting comparisons of mated and virgin plum curculios, although if better procedures were developed for overwintering adults and larger numbers could be obtained, comparisons of mating status could prove useful.

Although it is difficult to separate the response of male plum curculios to odor of a female-produced substance from that to odor of punctured plums, I would predict that if males were responding to the odor of punctured plums only, then more males should respond to the odor of green fruit worms feeding on plums (in which 19% of each plum was eaten) compared to odor released from females feeding on plums (in which approximately 1% of each plum was eaten). In fact, more males responded to odor of females feeding on plums than to odor of green fruit worms feeding on plums. Strong attraction of males to volatiles released from punctured plums may be related to the fact that sperm precedence occurs in plum curculios, with sperm from second matings replacing sperm from first matings by nearly 75% (Huettel et al. 1976). During the egg-laying sequence, a female plum curculio first excavates a tiny hole in the fruit with her mouthparts, turns around, deposits the egg and then cuts a tiny slit in the fruit (Quaintance and Jenne 1912, Owens et al. 1982). During this period, a male close enough to perceive volatiles released from punctured fruit could use this semiochemical cue not only to locate a potential female mate, but also to ensure his fathering of most of the progeny. In laboratory experiments, males in the presence of mechanically punctured plums were more likely to attempt copulations with both females and males than were males in the presence of uninjured plums or no plum odor (Table 21).

Most male-produced aggregation pheromones in Curculionidae are either synergized or enhanced by the presence of host plant volatiles (Landolt 1997). Therefore, it is not surprising that male plum curculios did not respond to synthetic grandisoic acid alone (Table 27, Experiments 27.1 and 27.2) or to conspecific males alone (Table 15). However, no increase in male responsiveness was ever observed when synthetic grandisoic acid [at either 3.00 or 0.03 mg doses] was held with uninjured plums compared to plums alone (Table 28, Experiments 28.1 and 28.2). Although boll weevil traps baited with a 5.00 mg dose of synthetic grandisoic acid captured more male plum curculios than unbaited traps in the field (Eller and Bartelt 1996), the influence of fruit odor in combination with grandisoic acid was not tested. I tested male plum curculio responses to grandisoic acid in combination with plum odor in the laboratory and found no enhancement of response to plum odor alone. Perhaps my pheromonal doses were too low to be enhanced and were overwhelmed by fruit odor, or too high and were repellent to male plum curculios. However, another possibility may be that the formulation procedure for grandisoic acid was inappropriate. For example, formulation of synthetic pheromone attractive to pepper weevil, *Anthonomus eugenii* Cano, required mixing the attractive synthetic compounds with BHT (an anti-oxidant) and then combining with Miraspers (a pregelatinized corn starch) followed by placing this mixture inside a glass tube that was subsequently sealed in a polypropylene bag (Eller et al. 1994). This procedure was used because it was observed in collections of synthetic pheromone volatiles released from impregnated rubber septa that little pheromone was released owing to chemical binding of synthetic compounds to septa(F.J. Eller, pers. comm.). This shortcoming may have existed for grandisoic acid impregnated into rubber septa and could explain the absence of enhancement of synthetic pheromone odor by host plum odor.

Live conspecific males in combination with host plant odors have also been used to examine male attraction to enhanced male-produced odors in other weevils. For example, male deodar weevils, *Pissodes nemorensis* Germar, were attracted in greater numbers to traps containing slash pine bolts with males than to traps containing slash pine bolts alone or females alone (Fontaine and Foltz 1982). In experiments conducted here, male plum curculios were equally attracted to odor of males feeding on plums and mechanically punctured plums (Table 20, Experiment 20.3), and to odor of males feeding on plums and green fruit worms feeding on plums (Table 17). Thus, there was no indication of enhancement or synergy of male-produced odor by volatiles from injured plums. Instead, evidence suggests a strong male attraction to volatiles released from punctured plums, and little or no response to male pheromone.

Male response to one treatment did appear to be enhanced by the presence of both males and plums. When both males and plums were held separately within the same bag, males responded in significantly greater numbers to this particular treatment than to plums alone and males alone (Table 20, Experiment 20.2). Similarly, females responded in significantly greater numbers to females held separately within the same bag with plums than to plums alone and females alone (Table 26, Experiment 26.2). In both cases, males and females responded in statistically equal numbers to plums alone and to the opposite sex held separately with plums (Table 18, Experiment 18.2, Table 25, Experiment 25.3). However, because plum curculios were able to stridulate, responses could have been initiated by sound. Plum curculios have stridulatory apparatus, the stridulitra, located on the ventral-medial surface of the left elytra. Movement of the plectra on the 6th abdominal segment against these file-like structures produces sound, with male and female stridulitra having different morphological configurations (Carlysle et al. 1975). In a study by Mampe and Neunzig (1966), single sex caged plum curculios with functioning stridulating apparatus attracted plum curculios of the opposite sex while those without did not. I tried to replicate the results of this experiment, but were unable to do so (Leskey and Prokopy, unpublished data).

My initial results here showed attraction to odor of the same sex in conjunction with plum odor. My subsequent experiments with female responders compared attraction to female plum curculios (with and without functioning stridulating apparatus) held separately with plums and revealed equal levels of responsiveness (Table 24, Experiment 24.2), thus eliminating the possibility of sound as the attractant and pointing to a possible semiochemical source of odor. This result is further supported by previous laboratory bioassays in which female plum curculio response to odor of hexane-based whole-body female or male extracts yielded highest responses to odor of female extracts (Leskey et al. 1996) and by experiments conducted here in which females did respond to female plum curculios held alone in the absence of plum odor (Table 22, Experiment 22.2). On the other hand, in experiments conducted here, females responded in significantly greater numbers to odor of females feeding on plums and mechanically punctured plums than to odor of (females)(plums) (Table 26, Experiment 26.3). Previously, female plum curculios were shown to be more strongly attracted to punctured plums than to uninjured plums, but responses of male plum curculios to these same odor sources were untested (Butkewich et al. 1987). For males, although the influence of sound was not ruled out in my experiments, males could have been attracted to male-produced odors that were not overwhelmed by the presence of plum odor [specifically, volatiles from punctured plums], and this response instead was enhanced by the presence of uninjured plums in close proximity to males under these particular experimental conditions. However, when the treatment of (males)(plums) was compared with treatments of male plum curculios feeding on plums and mechanically punctured plums, responses were statistically equal (Table 20, Experiment 20.3). Thus, results for both male and female plum curculios point to the primary importance of host plum odors and minor influence of odor of same sex conspecifics.

In the field in Illinois, more female plum curculios were captured by boll weevil traps baited with a 5.00 mg dose of synthetic grandisoic acid than by unbaited traps (Eller and Bartelt 1996), but traps were never baited with a combination of fruit odor and grandisoic acid or with fruit odor alone to assess comparative attractiveness of pheromone alone, fruit odor alone, or possible enhancement of response to grandisoic acid by host fruit odor. In experiments here, female plum curculios responded to odor of males alone (Table 22, Experiment 22.1) and synthetic grandisoic acid alone (Table 29, Experiment 29.1) in significant numbers compared to controls. However, when experimental conditions were changed to include plum odor, responses to live conspecifics and grandisoic acid were quickly lost. Therefore, as with male plum curculios, female plum curculios also seem to be more attracted to fruit odor and only secondarily to odor of male plum curculios. Similarly, females of the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* (L.), a major pest of sugar cane and vegetables in the West Indies, were more often attracted to odor of damaged food (broken beans) than to odor of males, and there was no enhancement of female responses when damaged food was combined with males or male frass in laboratory dual choice olfactometer experiments (Harari and Landolt 1997).

Although pheromonal odors appears to be attractive to plum curculios in the laboratory (results here) and in the field (Eller and Bartelt 1996), attraction to such odor seems to be minor in comparison to attraction to host plum volatiles. Current plum curculio trapping methods (Prokopy and Wright 1998, Prokopy et al. 1999a) have the potential to be greatly improved if the most attractive semiochemical combinations are deployed, as has been done for other weevil species. Specifically, the odor of punctured plums appears to be very attractive to both male and female plum curculios. Therefore, the identity of the particular volatiles released from punctured plums may have the potential to greatly improve effectiveness of traps.

Table 14. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which only one treatment per arena included odor source material and females represent one or all of the odor source components.

Exp	Treatment	F	df	P	N[a]	Mean No. Responders per Replicate ± SE[b]	% Plum consumed[c]
14.1		0.64	3, 28	0.59			
	Females				8	0.88 ± 0.44 a	
	Control 1				8	1.00 ± 0.71 a	
	Control 2				8	0.50 ± 0.38 a	
	Control 3				8	0.38 ± 0.26 a	
14.2		12.58	3, 12	<0.01			
	Females + Plums (early)				4	3.25 ± 3.55 a	1.0 %
	Control 1				4	0.50 ± 0.50 .b	
	Control 2				4	1.25 ± 0.95 b	
	Control 3				4	0.50 ± 0.29 b	
14.3		11.54	3, 12	<0.01	4		
	Females + Plums (mid)					10.75 ± 2.29 a	0.7 %
	Control 1				4	2.25 ± 1.03 b	
	Control 2				4	0.50 ± 0.50 b	
	Control 3				4	1.75 ± 1.03 b	
14.4		4.42	3, 12	0.03			
	Females + Plums (late)				4	9.25 ± 3.45a	0.6 %
	Control 1				4	0.75 ± 0.48b	
	Control 2				4	2.00 ± 1.00b	
	Control 3				4	1.75 ± 0.86	

[a] Each replicate consisted of 10 PCs released together in an arena.

[b] For each exp no. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate

Table 15. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which only one treatment included odor source material and males represented the only odor source component.

Treatment	F	df	P	N	Mean No. Responders per Replicate ± SE
	0.29	3, 28	0.82		
Males				8	0.88 ± 0.61 a
Control 1				8	0.38 ± 0.18 a
Control 2				8	0.75 ± 0.49 a
Control 3				8	0.50 ± 0.76 a

[a] Each replicate consisted of 10 PCs released together in an arena.

[b] For each exp no. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

Table 16. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which two treatments per arena included odor source material and females are included in at least one of the odor sources.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]	% Plum consumed[c]
16.1		18.56	3, 60	<0.01			
	Females + Plums				16	14.19 ± 2.45 a	1.4 %
	Control 1				16	0.38 ± 0.30 c	
	Males + Plums				16	6.44 ± 7.35 b	0.5 %
	Control 2				16	0.07 ± 0.06 c	
16.2		10.73	3, 44	<0.01			
	Females + Plums				12	14.75 ± 3.50 a	1.2 %
	Control 1				12	0.66 ± 0.33 c	
	GFW + Plums				12	8.00 ± 2.19 b	19.0 %
	Control 2				12	0.58 ± 0.19 c	
16.3		8.15	3, 44	<0.01			
	Females + Plums				12	9.50 ± 2.62 a	1.3 %
	Control 1				12	0.92 ± 0.56 b	
	Punctured Plums				12	11.67 ± 3.00 a	1.1 %
	Control 2				12	0.50 ± 0.34 b	

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[a] Each replicate consisted of 10 plum curculios released together in an arena

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 17.. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which two treatments included odor source material and males are included in one odor source.

Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]	% Plum consumed[c]
	10.02	3, 28	<0.01			
Males + Plums				8	11.13 ± 2.93 a	0.5 %
Control 1				8	0.13 ± 0.12 b	
GFW + Plums				8	8.13 ± 5.25 a	22.0 %
Control 2				8	0.50 ± 0.27 b	

[a] Each replicate consisted of 10 plum curculios released together in an arena

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 18. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which three treatments per arena included odor source material and females are included in at least one odor source.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]	% Plum consumed[c]
18.1		18.18	3, 44	<0.01			
	Females				12	0.17 ± 0.11 b	
	Plums				12	2.42 ± 0.70 b	
	Females + Plums				12	14.33 ± 3.07 a	
	Control				12	0.42 ± 0.23 b	
18.2		10.16	3, 60	<0.01			
	Females				16	0.94 ± 0.49 b	
	Plums				16	7.89 ± 1.75 a	
	(Females)(Plums)				16	7.50 ± 1.58 a	
	Control				16	1.00 ± 0.38 b	
18.3		4.88	3, 60	<0.01			
	Females + Plums				16	8.44 ± 1.82 a	1.2 %
	(Females)(Plums)				16	6.06 ± 1.42 a	
	Punctured Plums				16	5.93 ± 1.79 a	1.1 %
	Control				16	0.68 ± 1.96 b	
18.4		17.87	3, 28	<0.01			
	Fed Females				8	1.00 ± 0.38 b	
	Female-punctured Plums				8	13.25 ± 2.68 a	
	Frass Bag				8	1.88 ± 0.70 b	
	Control				8	1.00 ± 0.50 b	

[a] Each replicate consisted of 10 plum curculios released together in an arena

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 19. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which three treatments included odor source material and glued females are included in two odor sources.

Treatment	F	df	P	N[a]	Mean No. Responders per Replicate ± SE[b]	% Plum consumed[c]
	10.93	3, 60	<0.01			
Glued Females				16	1.32 ± 0.70 c	
Plums				16	6.50 ± 2.31 b	
Glued Females + Plums				16	12.63 ± 2.31 a	1.2 %
Control				16	0.69 ± 0.35 c	

[a] Each replicate consisted of 10 plum curculios released together in an arena

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 20. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles emitted from treatments in which three treatments per arena included odor source material and males are included in at least one odor source.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]	% Plum consumed[c]
20.1		13.31	3, 44	<0.01			
	Males				12	0.17 ± 0.11 c	
	Plums				12	5.17 ± 2.23 b	
	Males + Plums				12	11.33 ± 1.86 a	
	Control				12	0.17 ± 0.39 c	
20.2		8.77	3, 28	<0.01			
	Males				8	1.00 ± 0.46 bc	
	Plums				8	8.13 ± 3.55 b	
	(Males)(Plums)				8	15.88 ± 3.38 a	
	Control				8	0.25 ± 0.46 c	
20.3		3.56	3, 28	0.05			
	Males + Plums				8	7.88 ± 2.55 a	0.4 %
	(Males)(Plums)				8	6.38 ± 3.11 ab	
	Punctured Plums				8	8.75 ± 2.74 a	1.1 %
	Control				8	0.13 ± 0.13 b	

[a] Each replicate consisted of 10 plum curculios released together in an arena

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 21. One-way ANOVA comparing attempted mountings made by 2 males in the presence of mechanically punctured plums, uninjured plums, and nothing.

Treatment	F	df	P	N[a]	Mean No. Mountings attempted by Males per replicate ± SE[b]
	6.75	2, 12	0.01		
Uninjured Plums				5	2.00 ± 0.70 b
Punctured Plums				5	5.40 ± 1.60 a
Nothing				5	0.20 ± 0.20 b

[a] Each replicate consisted of 2 male and 2 female plum curculios released together in an arena

[b] Values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

Table 22. One-way ANOVA comparing laboratory responses of female plum curculios to volatiles in which only one treatment included odor source material.

Exp	Treatment	F	df	P	N[a]	Mean No. Responders per Replicate ± SE[b]
22.1		4.51	3, 28	0.01		
	Males				8	3.13 ± 1.29 a
	Control 1				8	0.25 ± 0.16 b
	Control 2				8	0.13 ± 0.12 b
	Control 3				8	0.38 ± 0.37 b
22.2		5.50	3, 28	<0.01		
	Females				8	3.25 ± 1.21 a
	Control 1				8	0.25 ± 0.16 b
	Control 2				8	0.00 ± 0.00 b
	Control 3				8	0.75 ± 0.36 b

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

Table 23. One-way ANOVA comparing laboratory responses of female plum curculios to volatiles in which two treatments per arena included odor source material and males are included in at least one of the odor sources.

Exp	Treatment	F	df	P	N[a]	Mean no. male responders per replicate ± SE[b]	% Plum consumed[c]
23.1		5.04	3, 44	<0.01			
	Males + Plums				12	6.58 ± 2.77 a	0.2 %
	Control 1				12	0.33 ± 0.19 b	
	Females + Plums				12	7.17 ± 1.63 a	0.6 %
	Control 2				12	0.92 ± 0.67 b	
23.2		5.32	3, 28	<0.01			
	Males + Plums				8	14.63 ± 5.33 a	0.6 %
	Control 1				8	1.13 ± 0.58 b	
	GFW + Plums				8	6.63 ± 2.19 ab	15.0 %
	Control 2				8	0.00 ± 0.00 b	
23.3		4.11	3, 28	0.01			
	Males + Plums				8	8.13 ± 4.05 ab	
	Control 1				8	0.13 ± 0.12 b	
	Punctured plums				8	13.38 ± 4.86 a	1.1 %
	Control 2				8	0.38 ± 0.26 b	

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 24. One-way ANOVA comparing laboratory responses of female plum curculios to volatiles in which two treatments per arena included odor source material and females are included in at least one of the odor sources.

Exp	Treatment	F	df	P	N[a]	Mean no. male responders per replicate ± SE[b]	% Plum consumed[c]
24.1		8.56	3, 28	<0.01			
	Females + plums				8	12.63 ± 2.82 a	1.1 %
	Control 1				8	0.38 ± 0.37 b	
	GFW + plums				8	12.25 ± 3.67 a	19.0 %
	Control 2				8	0.88 ± 0.39 b	
24.2		4.21	3, 28	0.01			
	(Females)(Plums)				8	10.38 ± 4.12 a	
	Control 1				8	0.63 ± 0.50 b	
	(Glued Females)(Plums)				8	10.38 ± 3.51 a	
	Control 2				8	0.75 ± 0.31 b	

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 25. One-way ANOVA comparing laboratory responses of female plum curculio adults to volatiles of treatments in which three treatments per arena included odor source material and males are included in at least one odor source.

Exp	Treatment	F	df	P	N[a]	Mean No. Responders per Replicate ± SE[b]	% Plum consumed[c]
25.1		4.72	3, 44	<0.01			
	Males				12	0.92 ± 0.52 b	
	Plums				12	7.00 ± 2.92 a	
	Males + Plums				12	7.58 ± 1.77 a	
	Control				12	0.66 ± 0.28 b	
25.2		8.89	3, 60	<0.01			
	Males				16	1.19 ± 0.48 b	
	Plums				16	7.94 ± 1.95 a	
	(Males)(Plums)				16	6.81 ± 1.55 a	
	Control				16	0.56 ± 0.23 b	
25.3		5.70	3, 60	<0.01			
	Males + Plums				16	4.06 ± 0.57 a	0.7 %
	(Males)(Plums)				16	5.94 ± 1.53 a	
	Punctured Plums				16	7.38 ± 1.82 a	1.1 %
	Control				16	0.19 ± 0.40 b	

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 26. One-way ANOVA comparing laboratory responses of female plum curculio adults to volatiles of treatments in which three treatments per arena included odor source material and females are included in at least one odor source.

Exp	Treatment	F	df	P	N[a]	Mean No. Responders per Replicate ± SE[b]	% Plum consumed[c]
26.1		3.24	3, 44	0.03			
	Females				12	0.58 ± 0.33 b	
	Plums				12	4.17 ± 1.64 ab	
	Females + Plums				12	6.66 ± 8.54 a	
	Control				12	1.25 ± 0.91 b	
26.2		9.39	3, 28	<0.01			
	Females				8	1.75 ± 0.67 b	
	Plums				8	4.75 ± 1.90 b	
	(Females)(Plums)				8	13.50 ± 3.26 a	
	Control				8	0.38 ± 018 b	
26.3		7.10	3, 28	<0.01			
	Females + Plums				8	13.38 ± 3.08 a	1.1 %
	(Females)(Plums)				8	5.00 ± 4.41 bc	
	Punctured Plums				8	9.63 ± 7.29 ab	1.1 %
	Control				8	0.13 ± 0.35 c	

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] Mean % plum consumed per replicate.

Table 27. One-way ANOVA comparing laboratory responses of male plum curculios to synthetic grandisoic acid emitted from treatments in which only one treatment per arena included odor source material.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]
27.1		0.25	3, 28	0.86		
	Pheromone (L)[c]				8	2.00 ± 0.98 a
	Control 1				8	1.75 ± 0.96 a
	Control 2				8	1.88 ± 0.74 a
	Control 3				8	2.88 ± 1.30 a
27.2		0.65	3, 28	0.59		
	Pheromone (H)[d]				8	1.25 ± 0.72 a
	Control 1				8	2.13 ± 0.58 a
	Control 2				8	2.25 ± 0.75 a
	Control 3				8	1.38 ± 0.42 a

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA ($P = 0.05$) and LSD pairwise comparison of means.

[c] One rubber septum impregnated with 0.03 mg dose of grandisoic acid.

[d] One rubber septum impregnated with 3.00 mg dose of grandisoic acid.

Table 28. One-way ANOVA comparing laboratory responses of male plum curculios to volatiles from synthetic grandisoic acid and/or plums emitted from treatments in which three treatments per arena included odor source material.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]
28.1		13.09	3, 44	<0.01		
	Pheromone (l)[c]				12	1.08 ± 0.36 b
	Plums				12	10.00 ± 1.75 a
	Plums + Pheromone (l)[c]				12	10.50 ± 2.42 a
	Control				12	0.50 ± 0.23 b
28.2		9.33	3, 28	<0.01		
	Pheromone (h)[d]				8	1.00± 0.50 b
	Plums				8	5.88 ± 1.06 a
	Plums + Pheromone (h)[d]				8	7.25 ± 1.40 a
	Control				8	1.50 ± 0.93 b

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] One rubber septum impregnated with 0.03 mg dose of grandisoic acid.

[d] One rubber septum impregnated with 3.00 mg dose of grandisoic acid.

Table 29. One-way ANOVA comparing laboratory responses of female plum curculios to volatiles from synthetic grandisoic acid emitted from treatments in which only one treatment per arena included odor source material.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]
29.1		4.20	3, 28	0.01		
	Pheromone (l)[c]				8	3.50 ± 0.21 a
	Control 1				8	0.88 ± 0.40 b
	Control 2				8	0.63 ± 0.38 b
	Control 3				8	0.63 ± 0.33 b
29.2		0.05	3, 28	0.01		
	Pheromone (h)[d]				8	2.00 ± 0.76 a
	Control 1				8	1.63 ± 0.50 a
	Control 2				8	1.75 ± 1.01 a
	Control 3				8	1.88 ± 0.58 a

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA (P = 0.05) and LSD pairwise comparison of means.

[c] One rubber septum impregnated with 0.03 mg dose of grandisoic acid.

[d] One rubber septum impregnated with 3.00 mg dose of grandisoic acid.

Table 30. One-way ANOVA comparing laboratory responses of female plum curculios to volatiles from synthetic grandisoic acid and/or plums emitted from treatments in which three treatments per arena included odor source material.

Exp	Treatment	F	df	P	N[a]	Mean no. Responders per Replicate ± SE[b]
30.1		4.72	3, 28	<0.01		
	Pheromone (l)[c]				8	1.88 ± 0.95 b
	Plums				8	11.00 ± 3.13 a
	Plums + Pheromone (l)[c]				8	5.63 ± 1.97 ab
	Control				8	2.25 ± 0.80 b
30.2		2.96	3, 28	<0.01		
	Pheromone (h)[d]				8	1.88 ± 0.88 b
	Plums				8	5.88 ± 2.26 a
	Plums + Pheromone (h)[d]				8	7.88 ± 2.27 a
	Control				8	2.13 ± 0.79 b

[a] Each replicate consisted of 10 plum curculios released together in an arena.

[b] For each experimental No. within the same column, values followed by the same letter are not significantly different from one another according to one-way ANOVA ($P = 0.05$) and LSD pairwise comparison of means.

[c] One rubber septum impregnated with 0.03 mg dose of grandisoic acid.

[d] One rubber septum impregnated with 3.00 mg dose of grandisoic acid.

CHAPTER 6

DEVELOPING A BRANCH-MIMICKING TRAP FOR ADULT PLUM CURCULIOS (COLEOPTERA: CURCULIONIDAE)

Abstract

I conducted laboratory, semifield, and field experiments to determine the most visually-stimulating and acceptable version of a branch-mimicking trap (PVC cylinder topped with an inverted screen funnel) to capture adult plum curculios, *Conotrachelus nenuphar* (Herbst) foraging within canopies of host trees. Cylinder traps (25 cm tall x 6 cm diam) coated with gloss or flat black latex paint captured slightly more adults than cylinder traps coated with other colors of latex paint in field tests. Tall cylinder traps (50 cm tall x 6 cm diam) coated with flat black latex paint were significantly more visually stimulating to released adults in semi-field tests than cylinder traps of lesser height (12.5 or 25 cm) and captured significantly more adults in field tests than cylinder traps of lesser height and/or different diameter (3 or 12 cm). Numerically more released adults arrived at a tall cylinder trap affixed to a horizontal limb at upright (standard position) and horizontal positions than at 45 ° or 135°, or upside down positions. A cylinder coated with gloss black latex paint and capped with an inverted screen funnel lined with a paper

cone provided a more acceptable surface than an unpainted cylinder or unlined funnel to crawling adults, with indication that addition of a wooden insert placed between the cylinder and screen funnel cap further increased trap capturing power in laboratory tests.

Introduction

Herbivorous insects can use visual stimuli to aid them in host plant finding (Jolivet 1998). Within a few meters of a host plant, host spectral quality and dimensional characteristics may be important cues used by insects (Prokopy and Owens 1983). In some insects, specific visual qualities of host plants have been exploited successfully to create traps. For example, cabbage seed weevils, *Ceutorhynchus assimilis* (Paykull), were captured in greater numbers in plastic bowl water traps coated with yellow paint compared to black, green, or white paint (Smart et al. 1997). A number of studies have shown that yellow represents a super-normal visual stimulus for many foliage seeking insects (Prokopy and Owens 1983). Also, apple maggot flies, *Rhagoletis pomonella* (Walsh) were captured in greater numbers on apple-mimicking red spheres coated with a sticky substance than on foliage-mimicking Pherocon AM yellow board traps (Prokopy and Hauschild 1979).

Little is known about the visual ecology of the plum curculio, *Conotrachelus nenuphar* (Herbst), a key pest of stone and pome fruit in eastern and central North America (Racette et al. 1992). Plum curculios overwinter as adults in hedgerows and woods outside orchards, but move back into orchards each spring (LaFleur and Hill 1987), where they arrive principally by flight and to a lesser extent by crawling

(Blanchett 1987, Dixon et al. 1999). Growers currently lack a reliable monitoring trap to detect plum curculio immigration into orchards and are forced to rely on fresh oviposition scars as a sign that adults have become active in fruit trees (Prokopy and Croft 1994).

In experiments designed to elucidate how plum curculios move into host fruit trees, plum curculios dislodged from apple trees tended to walk off a small collecting frame when ambient temperatures were below 20°C but fly to a host tree canopy or inter-tree space when temperatures were 20°C or above (Prokopy et al. 1999b). This suggests that when temperature is 20°C or above, plum curculios are likely to bypass traps designed to intercept crawling individuals, such as black pyramid traps placed next to tree trunks (Mulder et al. 1997, Prokopy and Wright 1998, Prokopy et al. 1999a).

There exists need for a trap that can be used to capture plum curculio adults that may have flown into host tree canopies. One example of such a trap is the "circle trap," consisting of a wire screen cage that is capped with an inverted screen funnel and attached to a limb. This trap is designed to intercept foraging adults walking on tree limbs (Mulder et al. 1997), but not to attract them based on stimulating visual cues. A number of herbivorous insects respond positively to visual cues provided by tall narrow dimensions of vertically growing plant structures (Prokopy and Owens 1983). Because plum curculios are highly prone to crawl rather than fly in a tree after arrival, traversing both the upper and lower sides of limbs (Prokopy and Leskey, unpubl. data), they too could orient to visual stimuli of vertically growing limbs or branches. An initial study of plum curculio visual orientation revealed that supernormal black branch-mimicking cylinders (20 cm tall x either 2.5 or 8 cm diam) positioned vertically on a horizontal branch were more visually stimulating than yellow cylinders of either diameter, or a clipped upright twig (approximately 20 cm tall x 1 cm diam) (Prokopy et al. 1998).

Therefore, I decided to further explore the possibility of creating a visually attractive trap for plum curculios based on supernormal branch-mimicking cylinders that could be used in host tree canopies for monitoring adults. In 1997-1999, I conducted laboratory, semifield, and field experiments to determine the most visually stimulating version of and most acceptable surface for a branch-mimicking trap.

Materials and methods

Laboratory Experiments

Plum Curculios

All plum curculios used for these experiments were taken from a multivoltine southern strain colony originating from Weslaco, TX. Adults were maintained on green thinning apples based on the methods of Amis and Snow (1985). Adults used in all experiments were 1-2 weeks post-eclosion.

Cylinder Surface Composition

I investigated the influence of differing surface compositions on propensity of plum curculios to crawl up and remain on varying structures. Two groups of cylindrical surfaces were tested. The first group included an unpainted black PVC cylinder (25 cm tall black x 6 cm diam), an identical PVC cylinder painted with flat black latex paint (Moor Guard Low Lustre Latex House Paint 10380), an identical PVC cylinder painted with gloss black latex paint (Royal Ace Hi Gloss Latex Enamel 102A105), a 29 cm tall x 6 cm diam cylindrical section of an apple tree limb, and a green plastic base (14 cm tall x 11.5 cm diam) of a cotton boll weevil trap (Gemplers, Mt. Horeb, WI). The second group was identical to the first except a non-host wood (spruce) banister railing (29 cm tall x 3 cm diam) was substituted for the apple limb to exclude potentially attractive olfactory cues (Leskey and Prokopy 2000). For each group, all structures were placed upright in a circle in a large Plexiglas arena (61 x 61 x 30.5 cm). Five adults starved for 24 h were released in the center of the arena and observed continuously for 15 min. The amount of time spent on a trap by each arriving adult was recorded. After each trial in each group, treatments were rotated clockwise until 20 trials were completed. One-way analysis of variance was used to determine if times spent by adults on different structures were significantly different.

Funnel tops

I investigated the influence of surface structure of funnel tops of cotton boll weevil traps (Gemplers, Mt. Horeb, WI) on the likelihood of adults ascending from tops of cylinders into attached inverted funnels, where they would be captured after crawling through the hole in the tip of the funnel. Funnels were lined with either black or white construction paper, were unlined and coated with black or white latex paint (Royal Ace Hi Gloss Latex Enamel 102A105 and 102A100, respectively) or were unmodified (aluminum screen control). These five funnel types were placed atop a PVC cylinder (25 cm tall x 6 cm diam) each with notches 2 cm deep and coated with flat black latex paint (Moor Guard Low Lustre Latex House Paint 10380) (Figure 1). Notches were created to facilitate adults crawling from a cylinder onto the detachable funnel top. Cylinder traps were placed upright in a circle in an arena as described above. In this case, 20 starved adults were released into the center of the arena and a Plexiglas lid capped the arena. After 24 h, the number of adults captured by each trap was recorded. Trials were repeated 5 times, with treatments (modified funnel tops and control) rotated clockwise by one position after each trial. One-way analysis of variance was used to determine if numbers of adults captured by different funnel types were significantly different, followed by LSD pairwise comparison of means.

Wooden Inserts and Cylinder Notch Size

To increase the likelihood that adults would crawl from cylinders into funnel tops, wooden pyramidal inserts coated with black paint (Tru-Test Weatherall Flat Black Acrylic Latex, Tru-Test, Cary, IL) were used to join cylinders (25 cm tall x 6 cm diam, coated with flat black latex paint) to inverted funnels (Figure 2). In addition, funnel tops were modified in one of the following ways: (a) lined with a black paper cone; or (b) lined with a 2.5 cm diam ring of black construction paper attached at the base of the screen funnel. One funnel was left unmodified. These three treatments were compared with a cylinder having standard sized notches 2 cm deep on the upper rim (control) and a cylinder with notches 1 cm deep to examine influence of notch height on propensity of plum curculios to crawl into screen funnel tops; neither of these cylinders was joined with a wooden insert but both were topped with a funnel lined with a black construction paper cone. Cylinder traps were placed upright in a circle in an arena as described above. Again, 20 starved adults were released into the center of the arena and a Plexiglas lid capped the arena. After 24 h, the number of adults captured by each trap was recorded. Trials were repeated 10 times, with treatments rotated clockwise by one position after each trial. One-way analysis of variance was used to determine if numbers of adults captured by different funnel types were significantly different, followed by LSD pairwise comparison of means.

Semifield Experiments

Cylinder height

All plum curculios used here were collected as overwintered adults in the field in late May or early June from populations on unsprayed wild plum or apple trees in Hampshire County, MA and assumed to be the same age. No more than 60 adults were held together in wax-coated paper cups (473 ml) with clear plastic lids. Freshly picked unsprayed apples as a food source plus a wetted cotton wick were introduced into each cup, held at 26 ° C, 65-80% RH and under a photoregime of 16L: 8D to mimic long-day conditions in nature from late May to late June.

For these experiments, a single notched cylinder (6 cm diam) of one of three heights (12.5, 25.0 or 50.0 cm) was coated with flat black latex paint and topped with a detachable inverted screen funnel top. The cylinder trap was attached to a horizontal limb of an unsprayed apple tree using a hose clamp fed through 2 slots cut near the base of the cylinder and tightened around the limb (Figure 3). Ten adults starved for 24 h were released 25 cm from the base of the cylinder from an 8-dram glass vial attached to the same horizontal limb, with the open end of the vial facing toward the cylinder trap and away from the tree trunk. I observed adult propensity to arrive at, touch, crawl up, and reach the top of each trap. Also, the number of adults captured in screen funnel tops was recorded. One-way analysis of variance was used to determine if numbers of adults arriving at different traps were significantly different, followed by LSD pairwise comparison of means.

Cylinder orientation

Using methods described above, I investigated adult propensity to arrive at, touch, crawl up, and reach the top of a cylinder trap (50 cm tall x 6 cm diam) affixed to a horizontal limb at one of five different cylinder orientations: upright (standard position), angled at 45°, horizontal, angled at 135°, and upside down. One-way analysis of variance was used to determine if numbers of adults responding to different trap orientations were significantly different.

Field Experiments

Cylinder color

To investigate the influence of cylinder color on visual attractiveness of cylinder traps to adults, in 1998 and 1999 white PVC cylinders (25 cm tall x 6 cm diam) were coated on the outside with either black, canary yellow, white, or deep green latex paint (Royal Ace Hi Glass Latex enamel 102A105, 102A108, 102A100, and 102A118, respectively) or on both the outside and inside with flat black latex paint (Moor Guard Low Lustre Latex House Paint 10380). Cylinders were notched as previously described and then capped with detachable screen funnel tops. Each cylinder trap was attached (as described above) to a horizontal limb of an apple tree in a small, unmanaged orchard in Deerfield, MA (Figure 3). In each tree, one cylinder trap of each color was positioned about two-thirds of the distance from the trunk to the dripline of the canopy (2 m from trunk), with traps equally distributed through the canopy. Traps were checked for captured adults every 4-5 days and were rotated by one position in the canopy throughout the active field season of plum curculios (from 16 May to 1 July, 1998 and from 15 May to 27, June 1999). One-way analysis of variance was used to determine if numbers of adults captured by traps with cylinders of different colors were significantly different.

Cylinder height and diameter

To investigate the influence of height and diameter of a cylinder on visual attractiveness of cylinder traps to plum curculios, in 1998 and 1999, black PVC cylinders of 3 heights (12.5, 25.0, and 50.0 cm), each of 3 different diameters (3.0, 6.0, or 12.0 cm), were coated on the outer surface with flat black paint (Moor Guard Low Lustre Latex House Paint 10380), notched, capped with detachable screen funnel tops, and attached to horizontal limbs of apple trees in a small, unmanaged orchard in Deerfield, MA. Each tree contained cylinder traps of a single height and three different diameters as well as a trap using a cylinder considered to be of standard size (25 cm tall x 6 cm diam). The four cylinder traps were distributed equally throughout the canopy as described above. Traps were checked for captured adults every 4-5 days and rotated by one position in the canopy throughout the active field season of plum curculios (from 16 May to 1 July, 1998 and from 15 May to 27 June, 1999). One-way analysis of variance was used to determine if numbers of adults captured by cylinders of different sizes were significantly different, followed by LSD pairwise comparison of means.

Results

Cylinder Color

In field experiments comparing cylinder traps (25 cm tall x 6 cm diam) painted flat black or gloss black, yellow, green or white latex paint, there was no significant difference in trap captures (F=0.13, P=0.9712) among colors although slightly more adults were captured by traps coated with flat black or gloss black than by traps of other colors (Table 31).

Cylinder Height and Diameter

In semifield experiments, significantly more released adults arrived at a 50 cm black cylinder trap than at 12.5 or 25 cm cylinder traps, all of 6 cm diam (F=9.28, P=0.005) (Table 32). Of those arriving at the 50 cm cylinder trap, only 23% touched the trap base, 8% ascended to the top and 3% crawled inside the funnel capping the cylinder (Table 32). In field experiments comparing black cylinder traps of three heights (either 12.5, 25 or 50 cm) each at three diameters (3, 6, or 12 cm), there was a significant effect of cylinder size (F=2.94, P=0.003) (Table 3); significantly more plum curculios (greater than two times more) were captured by traps 50 cm tall x 6 cm diam than by any other size of trap according to LSD pairwise comparisons of means. These results confirm findings of semifield experiments regarding influence of cylinder height.

Cylinder Orientation

In comparisons of cylinder traps (50 cm tall x 6 cm diam) at 5 orientations, numerically (although not significantly) more released adults arrived at traps oriented upright than traps of any other orientation (F=1.50, P=0.252) (Table 34). Numerically fewest arrived at traps oriented downward. In the upright position, 40% of the arrivers touched the cylinders and 12.5% ascended (Table 34).

Cylinder Surface Composition

When responses of released adults to five different surface compositions of cylinders were compared, including an apple limb, there were no significant differences among treatments in mean times spent on each (F=0.74, P=0.572) although numerically greatest time was spent on a PVC cylinder coated with gloss black latex paint, followed by an apple limb and a PVC cylinder coated with flat black latex paint (Table 35). The least time was spent on an unpainted PVC cylinder. When a piece of spruce banister railing was substituted for the apple limb to reduce possible response to host olfactory cues from apple wood, nearly significant differences were detected among surface types (F=2.25, P=0.079) (Table 36). Again, the greatest time was spent on a PVC cylinder coated with gloss black latex paint, followed by a PVC cylinder coated with flat black latex paint. The least time was spent on a boll weevil trap base (Table 36).

Screen Funnel and Cylinder Modifications

When screen funnels from boll weevil traps were modified by lining them with a cone of either black or white construction paper or by coating them with either black or white paint and comparing them with an unmodified screen funnel, significantly more released adults were captured in funnels lined with paper (either color) than in painted funnels (either color) (F=2.66, P=0.05) (Table 37). In a second experiment in which screen funnels were modified using a paper cone or a paper ring, and cylinders were modified using shallower notches or by attaching a wooden insert, numerically though not significantly (F=1.87, P=0.13) more plum curculios were captured by cylinder traps with an insert plus a paper cone or paper ring than by those without an insert (Table 38).

Discussion

Cylinder traps (25 cm tall x 6 cm diam) coated with gloss or flat black latex paint captured numerically more plum curculios than traps of other colors in field tests (Table 31). In semifield observations, tall cylinder traps (50 cm tall x 6 cm diam) coated with flat black latex paint were more visually stimulating, with significantly greater numbers of released adults arriving at them, than cylinder traps of equal diameter but lesser height, although few adults ascended any cylinder traps (Table 32). In field tests, cylinder traps with these same dimensions captured significantly more plum curculios than cylinder traps of lesser height and/or diameters of 3 cm or 12 cm (Table 33). Numerically more released adults arrived at a tall cylinder traps affixed to a horizontal limb at upright (standard position) and horizontal positions than at 45 ° or 135°, or upside down positions (Table 34). Cylinders coated with gloss black latex paint provided the most acceptable surface for adults to reside on in laboratory experiments (Tables 35 and 36). Lining inverted screen funnels with paper cones facilitated adult entry into trap tops (Table 37), with indication that a wooden insert joining the trap top with the cylinder further enhancing capturing effectiveness (Table 38).

The propensity of plum curculios to crawl up black-painted cylinders in laboratory experiments (Tables 35 and 36) and the fact that slightly more plum curculios were caught by black-painted cylinder traps in the field than by cylinder traps of other colors (Table 31) lends support to earlier studies by Prokopy et al. (1998) that black is likely the most visually stimulating color for a branch-mimicking object. However, at close range not only color but also dimensional qualities of host plants are important cues for herbivorous insects (Prokopy and Owens 1983), and the influence of both dimension and color can interact in terms of insect response to visual stimuli. For example, onion flies, *Delia antigua* (Meigen), alighted in equal numbers on white and green cylinders, but alighted more frequently on white spheres compared to green spheres (Judd and Borden 1991). Similarly, when my standard size black cylinder trap (25 cm tall x 6 cm diam) was compared with a taller black cylinder trap (50 cm tall x 6 cm diam), more than twice the number of plum curculios were captured on taller cylinder traps (Table 33). Released plum curculios also responded in greater numbers to these taller traps compared to those of lesser height (all with 6 cm diam) in semifield experiments (Table 34), indicating that at close range, the dimensions of taller black cylinders are more visually stimulating and may act as a stronger cue than shorter black cylinders for plum curculios seeking food and/or oviposition sites in host tree canopies. Thus, specific dimensions can be an important component of a visual cue. Foraging *Calosoma sycophanta* L., a predaceous beetle, responded in greater numbers to cylinders of larger than smaller diameters that more closely approximated dimensions of tree bases where they actively search for gypsy moth, *Lymantria dispar* L., larval prey (Weseloh 1997).

Surface properties can be important to the ability of an insect to crawl upon or accept a particular surface. For example, the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), can walk on clean plastic mulch but cannot walk on plastic mulch covered with fine soil particles because soil particles coat the adhesive tarsal pads (Boiteau et al. 1994). For plum curculios, PVC cylinders coated with gloss or flat black paint improved surface acceptability, with more time spent crawling and resting on these surfaces compared to uncoated PVC cylinders (Tables 35 and 36). Initially, unmodified boll weevil trap tops used atop cylinders were chosen because they are commercially available and have been used previously in conjunction with pyramidal traps (Tedders and Wood 1994, Prokopy and Wright 1998). However, these inverted screen funnels may not be the most acceptable surface for plum curculio adults to crawl upon. In the laboratory and field, I noted that plum curculios tended not to crawl onto screen or if they did, to crawl only a short distance before leaving (Leskey and Prokopy, unpubl. data). It was this observation that prompted us to modify the screen funnel. I found that paper cones lining the funnels significantly increased trap captures in laboratory experiments (Table 37) and when used in conjunction with wooden inserts joined to cylinders, captures increased even more in laboratory experiments (Table 38), suggesting that such modifications to boll weevil trap tops may increase their success in capturing plum curculios in the field.

Several unbaited trap types have been compared for ability to attract and capture plum curculios (Yonce et al. 1995, Prokopy and Wright 1998, Prokopy et al. 1999a) with moderate success at best. In field studies here, I found that significantly greater numbers of adults were captured by traps comprised of tall black cylinders (50 cm tall x 6 cm diam) compared to cylinders of lesser height and/or different diameter (Table 33). Although significantly greater numbers of plum curculios arrived at cylinder traps of these same dimensions compared to shorter cylinder traps in semifield experiments, rather few ascended (Tables 32 and 34), indicating that at close range, visual cues may become less important or stimulating than olfactory cues. Both laboratory and field studies suggest that plum curculios use host fruit tree odor cues to locate hosts (Butkewich and Prokopy 1993, Prokopy et al. 1995, Butkewich and Prokopy 1997; Leskey and Prokopy 2000). Furthermore, a number of attractive host fruit volatiles (Leskey et al. submitted, Prokopy at al. submitted) as well as an attractive component of a male-produced aggregation pheromone, grandisoic acid (Eller and Bartelt 1996), have been identified for plum curculios. Additive effects between visual (trap color, dimension and design) and semiochemical cues have been reported for other insect species (Schoonhoven et al. 1998). For example, corn rootworm beetles, *Diabrotica virgifera virgifera* LeConte, were captured in significantly greater numbers on yellow sticky traps baited with 4-methoxycinnamaldehyde than unbaited yellow sticky traps or traps of other colors with or without baits (Hesler and Sutter 1993).

Therefore, using known attractive semiochemicals as attractive olfactory stimuli in conjunction with a branch mimicking cylinder trap that most thoroughly exploits stimulating visual cues of cylinders (black cylinder, 50 cm tall x 6 cm diam) joined by a wooden insert and capped with a modified boll weevil trap top (inverted screen funnel lined with paper cone) might substantially improve the ability to capture adult plum curculios in host fruit tree canopies.

Table 31. Mean number of plum curculio adults captured per trap per tree over the entire season in 1998 and 1999 by cylinder traps (25 cm tall x 6 cm diam) coated with different colors of paint.

Trap Color	N	Mean ± SE[a]
Flat Black	12	3.08 ± 0.48
Gloss Black	12	2.92 ± 1.12
Yellow	12	2.58 ± 0.92
White	12	2.42 ± 0.57
Green	12	2.42 ± 0.95

[a] Values are not significantly different according to one-way ANOVA (F=0.13, P=0.9712).

Table 32. Mean number of released plum curculio adults arriving at cylinder traps (6 cm diam) coated with flat black latex paint from release point 25 cm away.

				Among Arrivers at Cylinder Traps		
Height cm	N	Mean ± SE[a]	% Arriving	% Touching Base	% Crawling To Top	% Crawling In Funnel
12.5	13	1.69 ± 0.34 b	17	18	0	0
25.0	14	1.21 ± 0.24 b	12	41	6	0
50.0	14	2.85 ± 0.30 a	29	23	8	3

[a] Values followed by a different letter are significantly different at $P < 0.05$ according to one-way ANOVA ($F=9.28$, $P=0.005$) and LSD pairwise comparison of means.

Table 33. Mean number of plum curculio adults captured per trap per tree over the entire season in 1998 and 1999 by cylinder traps of 3 heights and 3 diameters coated with flat black latex paint.

Height cm	Diameter cm	N	Mean ± SE[a]
12.5	3.0	8	1.75 ± 0.77 b
	6.0	8	2.25 ± 0.67 b
	12.0	8	1.63 ± 0.94 b
Standard[b]		8	1.50 ± 0.46 b
25.0	3.0	8	0.88 ± 0.35 b
	6.0	8	2.25 ± 0.78 b
	12.0	8	1.50 ± 0.65 b
Standard[b]		8	2.38 ± 0.77 b
50.0	3.0	8	2.25 ± 0.84 b
	6.0	8	6.25 ± 1.36 a
	12.0	8	2.62 ± 0.68 b
Standard[b]		8	2.75 ± 0.67 b

[a] Values followed by a different letter are significantly different at $P < 0.05$ according to one-way ANOVA ($F=2.94$, $P=0.003$) and LSD pairwise comparison of means.

[b] Standard size cylinder trap dimensions were 25 cm tall x 6 cm diam.

Table 34. Mean number of released plum curculio adults arriving on cylinder traps (50 cm tall x 6 cm diam) of different orientations from release point 25 cm away.

			Among Arrivers at Cylinder Traps	
Orientation	N	Mean ± SE[a]	% Touching	% Crawling Up
Upright	4	4.00 ± 0.82	40.0	12.5
45^0 Upright	4	2.50 ± 0.65	5.0	2.5
Horizontal	4	3.25 ± 0.63	38.0	7.5
45^0 Downward	4	1.75 ± 0.86	2.5	2.5
Upside Down	4	2.25 ± 0.63	2.5	2.5

[a] Values are not significantly different according to one-way ANOVA (F=1.50, P=0.252).

Table 35. Mean number of seconds spent by released plum curculio adults on five types of cylinders that included host wood in laboratory choice tests.

Cylinder Type	Total Responders	Mean Sec. ± SE[a]
PVC Gloss	8	269.25 ± 75.24
Apple Limb	5	207.80 ± 66.11
PVC Flat	10	198.20 ± 68.41
Boll Weevil Trap	9	156.66 ± 67.12
Unpainted PVC	8	40.00 ± 13.56

[a] Values are not significantly different according to one-way ANOVA (F=0.74, P=0.572).

Table 36. Mean number of seconds spent by released plum curculio adults on five types of cylinders that included non-host spruce wood in laboratory choice tests.

Cylinder Type	Total Responders	Mean Sec. ± SE
PVC Gloss	4	252.13 ± 53.06
PVC Flat	9	164.33 ± 60.52
Unpainted PVC	9	82.44 ± 48.55
Spruce rail	9	62.00 ± 15.22
Boll Weevil Trap	13	35.85 ± 20.18

[a] Values are not significantly different according to one-way ANOVA (F=2.25, P=0.079).

Table 37. Mean number of released plum curculio adults captured per 24 h trial by black cylinder traps (25 cm tall x 6 cm diam) with modified or unmodified inverted screen funnels in laboratory tests.

Modification	N	Mean ± SE[a]
Black Paper Cone	5	3.00 ± 0.63 a
White Paper Cone	5	3.00 ± 1.09 a
Unmodified (control)	5	2.00 ± 0.63 ab
Black Paint Coating	5	0.80 ± 0.37 b
White Paint Coating	5	0.80 ± 0.37 b

[a] Values followed by a different letter are significantly different at $P < 0.05$ according to one-way ANOVA ($F=2.66$, $P=0.05$) and LSD pairwise comparison of means.

Table 38. Mean number of released plum curculios captured per 24 h trial in black cylinder traps (25 cm tall x 6 cm diam) joined by wooden pyramidal inserts or modified with reduced cylinder notch height in laboratory tests .

Modification	N	Mean ± SE[b]
Insert + Black Paper Cone	10	3.10 ± 0.46
Insert + Black Paper Ring	10	2.80 ± 0.53
Insert	10	2.60 ± 0.68
Black Paper Cone (Control)	10	1.90 ± 0.43
Black Paper Cone[a]	10	1.40 ± 0.37

[a] Notch depth of cylinder top was decreased to 1 cm.

[b] Values are not significantly different according to one-way ANOVA (F=1.87, P=0.13).

Figure. 4. Branch mimicking trap: black cylinder (25 cm tall x 6 cm diam) with inverted screen funnel top.

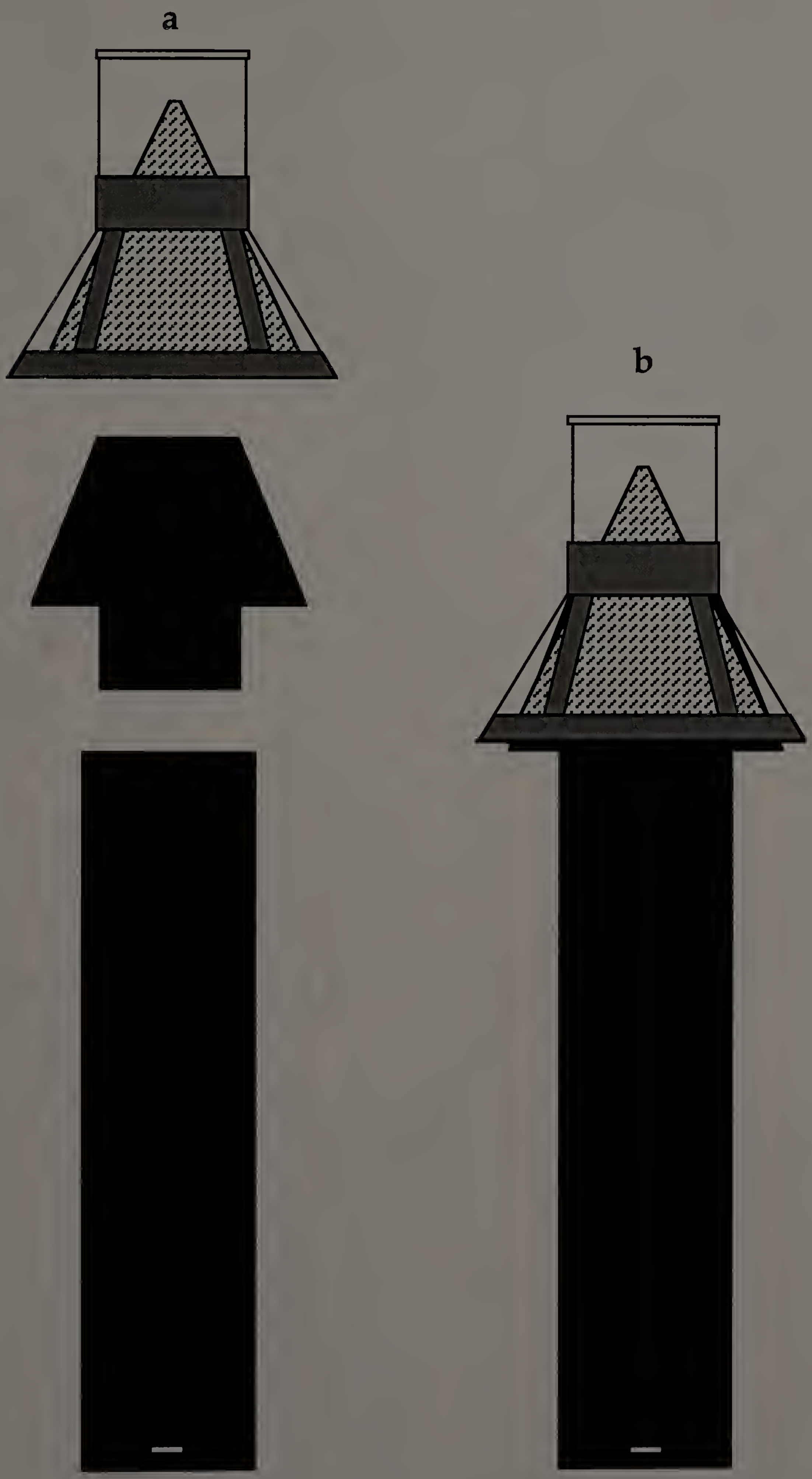
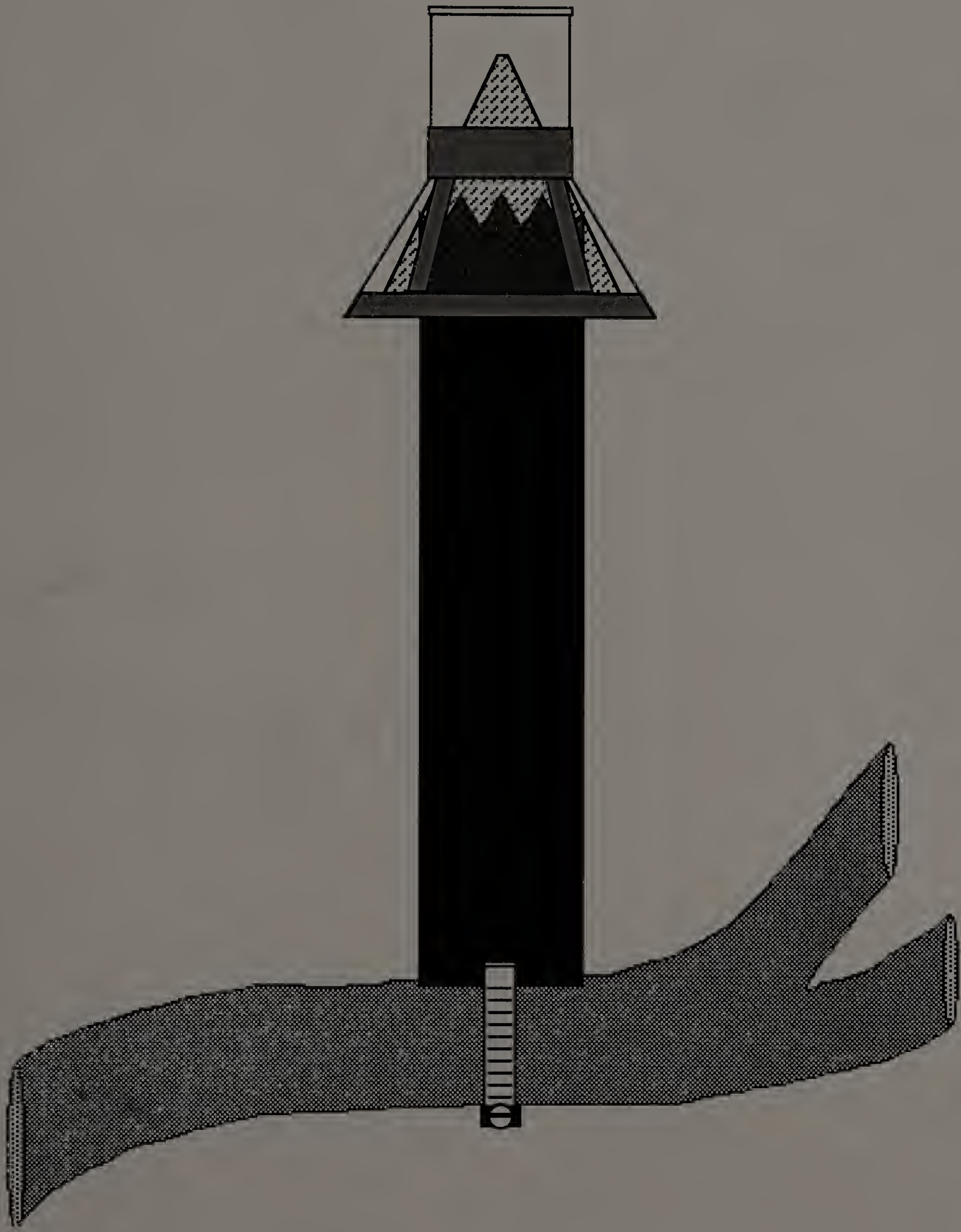


Figure 5. Branch mimicking trap: (a) black cylinder (25 cm tall x 6 cm diam), wooden insert and inverted screen funnel top, (b) trap components joined together.

Figure 6. Branch mimicking trap: black cylinder (25 cm tall x 6 cm diam) with inverted screen funnel top attached to a horizontal limb by a hose clamp.



CHAPTER 7

CONCLUSIONS

Plum curculio adults, *Conotrachelus nenuphar* (Herbst), were more attracted to volatiles from hexane extracts of host wild plum fruit at bloom, and host wild plum and McIntosh fruit at 2 weeks after bloom, than to volatiles from hexane extracts of non-host honeysuckle fruit at either phenological stage in the laboratory, indicating that host plant recognition likely requires the presence of specific volatile compounds present in host tissue. Source material used to identify volatile components of McIntosh apple odor attractive to plum curculios should be collected between bloom and 2 weeks after bloom based on plum curculio responses to hexane-extracted or water-extracted volatiles from McIntosh apple tissues (twigs, leaves or fruit) at seven phenological stages of development (pink through 5 weeks after bloom) using this same bioassay system.

In the laboratory, I found that adult female olfactory discrimination of hexane-based McIntosh fruit odor was significantly and positively correlated with barometric pressure. Furthermore, females oviposited more eggs in host plum fruit during periods of low barometric pressure, but feeding activity of females was independent of barometric pressure.

Two compounds, ethyl isovalerate and limonene, both individual volatile components of unripe plum odor, were found to be attractive to overwintered adult plum curculios in the laboratory using a still-air dual choice bioassay system, and in the field using baited cotton dental wicks attached to boll weevil traps placed on the ground beneath the canopy of unsprayed apple trees. Both of these compounds as well as other compounds tested could be more attractive at concentrations or release rates different from those tested here, or in combination. The use of ethyl isovalerate and limonene as odor attractants offers potential to increase efficacy of current traps for monitoring plum curculios immigrating into fruit orchards during spring.

Olfactory attraction of overwintered male and female plum curculios to volatiles released from same and opposite sex conspecifics alone, synthetic grandisoic acid alone, wild plums alone, or combinations of live single sex conspecifics or grandisoic acid with plums in large still-air arenas revealed that female plum curculios may produce a sex pheromone attractive to male plum curculios. In general, however, both male and female plum curculios were strongly attracted to host plum volatiles and only mildly attracted to conspecific odors or synthetic grandisoic acid. Volatiles released from punctured plums are highly attractive to both male and female plum curculios and should be used as source material to identify potential attractants for plum curculios.

The most visually-stimulating and acceptable version of a branch-mimicking trap (PVC cylinder topped with an inverted screen funnel) to capture adult plum curculios within canopies of host trees was determined to be a tall cylinder trap (50 cm tall x 6 cm diam) coated with black latex paint. This trap was significantly more visually stimulating to released adults in semi-field tests than were cylinder traps of lesser height (12.5 or 25 cm) and captured significantly more adults in field tests than cylinder traps of lesser height and/or different diameter (3 or 12 cm). Furthermore, an inverted screen funnel lined with a paper cone provided a more acceptable surface, with indication that addition of a wooden insert placed between the cylinder and screen funnel cap further increased trap capturing power in laboratory tests.

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