

Host Preference and Plant Volatile Effects
On Black Vine Weevil (*Otiorhynchus sulcatus* F.) Behaviour

by

Hui Zhou

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Dedication

This thesis is dedicated to my lovely daughter, Wang, Wei (Juliette).

***Her birth made me become a mother
Her growth makes me getting younger***

Let's read a favourite poem together.

Youth by Samuel Erman

Youth is not a time of life; it is a state of mind
It is not a matter of rosy cheeks, red lips and supple knees
It is a matter of the will, a quality of the imagination, a vigour of the emotions
It is the freshness of the deep spring of life
.....

Nobody grows old merely by a number of years
We grow old by deserting our idea
Years may wrinkle the skin
But to give up enthusiasm wrinkles the soul
Worry, fear, self-distrust bows the heart and turns the spirit back to dust
.....

In the center of your heart and my heart there is a wireless station
So long as it receives messages of beauty, hope, cheer, courage and power from the
infinite
So long as you are young

When the aerials are down, and
Your spirits are covered with snows of cynicism and the ice of pessimism
Then you've grown old even at 20
But as long as your aerials are up to catch waves of optimism
There's hope you may die young at 80

Abstract

Host Preference and Plant Volatile Effects

On Black Vine Weevil (*Otiorhynchus sulcatus* F.) Behaviour

By Hui Zhou

Abstract: Black vine weevil (*Otiorhynchus sulcatus* F.) behaviour was examined based on host plant choice tests. Weevils fed significantly more on yew and strawberry than geranium and rhododendron in 4-choice tests, and laid the least eggs on rhododendron among the four plants. In 2-choice tests, weevils fed more on strawberry than on peppermint and yew. In no-choice feeding tests, there was no significant difference among the five plants tested. Weevils were attracted more to strawberry than to peppermint in olfactory assays by using the leaf disks and strongly attracted to volatile compound (Z)-3-hexen-1-yl acetate (from strawberry leaf) at concentrations 10^{-3} and 10^{-6} $\mu\text{l/ml}$. (Z)-3-hexen-1-ol (from strawberry leaf), (Z)-3-hexen-1-yl acetate and menthone (from peppermint) at the concentration 1 $\mu\text{l/ml}$ significantly repelled weevils. Weevils fed significantly less on strawberry leaf disks which were treated with menthone at 1 $\mu\text{l/ml}$ and 10^{-3} $\mu\text{l/ml}$ than untreated strawberry leaf disks.

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The education and influence will be here for long

Introduction

The black vine weevil

The black vine weevil (*Otiorhynchus sulcatus* F.), from Europe originally (Cowles, 1995; Shetlar, 2000), is one of many agricultural pests in the Order Coleoptera (Masiuk, 2003). It was recognized in Germany as early as 1834. In 1881, it was found that the black vine weevil (the term ‘weevil’ will be used throughout the thesis to refer to black vine weevil) injured strawberries and raspberries. In addition, this insect is not a new invader to Canada. In the early 20th Century, the weevil was recorded in Newfoundland, Nova Scotia, Quebec, Ontario and British Columbia (Smith, 1932). In recent years, as a pest of many plants in field and in greenhouse, it has caused serious economic losses in strawberries, particularly in eastern Canada and the United States (Fisher, 2004). Control challenges include the rapid development of insecticide resistance by weevils and the growing concern about environmental pollution (Cowles, 1995). So it is important to look for other options, especially to develop reduced-risk methods combined with the available techniques to manage the weevil (MacKenzie, pers. comm. Agriculture and Agri-Food Canada, Kentville NS).

Adult weevil cannot fly because the forewings are fused along the median line (Smith, 1932; Day and Lewis, 2003). However, it is a very active walker (Shetlar, 2000), and is carried or walks to uninfected areas (Baker, 2003). So far, only adult females have been observed (Weis, 1998; Masiuk, 2003; Smith, 1932). Evidence of the weevil’s parthenogenic reproduction can be traced back to the early 20th Century. In 1917, Feytaud examined over 3,000 adult individuals externally and dissected them without finding a male (Smith, 1932). In Feytaud’s study, six generations of the weevil adults were isolated

in the pre-pupal stage, and kept individually, as adults produced fertile eggs (Smith, 1932).

The weevil has a life span of several months (Shetlar, 2000), and can overcome poor living conditions. When 62 adults were confined as soon as they emerged, with soil, water, and dried cyclamen leaves, or no food, the individuals appeared normal and lived from 10 to 82 days (Smith, 1932). This would indicate that the adults may subsist in areas devoid of green crops until they can migrate to adjacent areas where food plants are growing (Smith, 1932).

Usually, a single generation occurs each year in North America (Shetlar, 2000; Smith, 1932). Adults emerge from soil in early summer (June). They are nocturnal, feeding on leaves mostly at night, and hide at day time (Kowalsick, 2003). They often drop from plants playing death when disturbed (Baker, 2003). Adults feed on leaf edges leaving notches from the outer margin of the leaf inward. The notches can be used as early indicators of the presence of adults, as well as of potential larvae in the soil. Weevil feeding often results in unsightly plants, but limited damage (Day and Lewis, 2003; Masiuk, 2003). Adults must feed on foliage for about one month (21-45 days) prior to laying eggs in July (Kowalsick, 2003; Masiuk, 2003; Shetlar, 2000). An effective management strategy would be to control the adults in fields early in July before the egg laying season, using multiple methods, such as insecticides (Baker, 2003), pitfall traps, and barrier pitfall traps. The later two are effective at trapping the flightless weevil (Fisher *et al.*, 2005). Thus, the one-month preoviposition period could be a golden opportunity to provide weevil control.

Adults lay eggs in July. One weevil was recorded laying 863 eggs, however, the average number of eggs deposited by each weevil is about 200 (Baker, 2003). The eggs hatch in 2-3 weeks (Baker, 2003). In the process of hatching, the larva's strong mandibles

open and close rhythmically until the shell is cut or torn about one-third of the way around the side. The mandibles, followed by the remainder of the head, are forced through the opening, and the larva is then drawn out of the shell (Smith, 1932). After emergence, the young larva feeds on rootlets, but after the third molt, the larva moves to the larger roots causing stunted and weakened plants, or even causing plant death (Baker, 2003). Therefore, more serious damage is done by the root feeding larva than by the leaf feeding adult (Masiuk, 2003). Immature and mature larvae spend the winter in the soil, and on occasion, adults survive over winter (Masiuk, 2003; Baker, 2003). In early spring, the larva matures and pupates inside an earthen chamber before emerging as an adult in late May through June (Masiuk, 2003).

Weevil host choice

Although adult weevils were reported to feed on up to 140 species, including evergreen, deciduous, and herbaceous plants (Masiuk, 2003; Shetlar, 2003; Smith 1932), they prefer to feed on certain plant species when given a choice (Tol *et al.*, 2004). Therefore, previous studies focused on a small group of host plants, such as strawberries, other small berries, yew (*Taxus* spp), rhododendrons, hemlock, *Euonymus* spp., *Humulus* spp., and pine trees (Tol *et al.*, 2004; Doss, 1983; Hanula, 1988; Tol and Visser, 1998; Masiuk, 2003; Shetlar, 2000). Some of the plants, such as yew, rhododendron and hemlock, are very toxic for most animals, but a few insect species, including the weevil, have adapted to these plant toxins (Tol and Visser, 2002). Although peppermint can be a host to the weevil, strawberry root weevil (*Otiorhynchus ovatus* L.) is more numerous in this crop (Anon. 1998) than the black vine weevil.

Tol *et al.* (2002, 2004) questioned the long list of weevil hosts and explained the

reason for the sudden drop from 140 plants to the small group of host plants that are typically used in research. Adult black vine weevils in Europe are limited to one gymnosperm genus (*Taxus* spp.), and some plants in two subclasses of Dicotyledonae (angiosperm plants). This indicates that the weevil has a more limited functional plant range than previously described. In the studies of Tol *et al.* (2002, 2004), *Taxus baccata* appeared to be the second-least preferred host plant because the weevils preferred this plant to rhododendron, but fed more on *Euonymus* spp., *Humulus* spp., and *Fragaria* spp. in two-choice tests. However, results from investigations of olfactory preference would argue that rhododendron is a non-host (Tol *et al.*, 2002). Also, in no-choice situations, weevils fed well on rhododendron, but fecundity was low and mortality high compared to those that ate *Taxus* spp. or *Euonymus* spp. (Tol *et al.*, 2004). Thus, the current list of host plants for the adult weevil may contain many non-hosts or poor hosts that would actually be avoided if the weevils were given a choice (Tol *et al.*, 2004). As mentioned, weevils are flightless, so they might often be isolated in small habitats and feed on any plant species that are growing in the area. Therefore, the ability of the weevil to feed and reproduce in low numbers on many non-host plants is an important factor in the successful survival of this flightless weevil species in different habitats (Tol, *et al.*, 2004).

In order to avoid an overestimation of weevil host range, a determination of host plants should be based on feeding preferences, oviposition or olfactory responses (Tol *et al.*, 2004). Rhododendron, for example, was avoided even though a rhododendron cultivar was used that stood out as a relatively more suitable host cultivar in terms of oviposition. Olfactory responses did not reveal attraction to this cultivar of rhododendron. Feeding preferences for equally suitable hosts, such as *Euonymus* spp., *Aronia* spp., *Fragaria* spp., *Taxus* spp. and one less suitable host, *Humulus* spp., correlated with oviposition. It

appeared that *Euonymus* spp., *Fragaria* spp. and *Humulus* spp. are preferred over *Taxus* spp. in the feeding preference tests, whereas oviposition on *Humulus* spp. is lower. These results indicate a mismatch between feeding preferences and olfactory responses (Tol, *et al.*, 2004). Other similar tests showed that weevils preferred ovipositing in containers with *Taxus* foliage over ten other hosts tested, regardless of which host they were reared upon or fed during their preoviposition period. Weevils preferred laying eggs in containers with strawberry foliage over those containing rhododendron when these hosts were paired. It was also suggested that *Taxus* spp. and possibly strawberry foliage contained an oviposition stimulant which the weevils perceived only by contact or feeding (Hanula, 1988).

It was also found that weevils had feeding or oviposition preference for different strawberry cultivars (Cowles, 2004). In many clones of *Fragaria* spp. (strawberries), resistance to weevil feeding was found (Shanks and Doss, 1986). In no-choice conditions, adult weevils fed less on several clones of the beach strawberry, *Fragaria chiloensis*, than on the cultivated strawberry (*F. fragaria*) 'Totem', and consequently had an extended preovipositional period, reduced survivorship and reduced oviposition (Shanks, 1980; Shanks and Doss, 1986). Cram (1980) found that there were significant differences among cultivars in the number of eggs laid during a ten-week period and in the number of larvae that hatched. The fewest eggs were laid and larvae hatched when weevils fed on the new strawberry cultivar 'Tyee'.

Host plant related volatiles

Plants release small quantities of chemicals called volatile compounds which can serve as airborne semio-chemicals, promoting or deterring interactions between plants

and insect herbivores (Pare and Tumlinson, 1999). Plant volatile compounds can indicate sources of appropriate food, oviposition sites and shelters (Pare and Tomulinson, 1999; Perveen *et al.*, 2001). Some of the volatiles are unique for a plant family, but many compounds can be found as blends in different and unrelated plants (Knudsen *et al.*, 1993). However, some relatively abundant compounds can elicit insect specific behavior (Roseland *et al.*, 1992). Insects interact with plants and detect plant volatiles through the antennae, or certain mouthparts (Hanula, 1988). Perveen *et al.*, (2001) reported the effects of cotton plant volatiles on insect behavior and found that volatiles in cotton leaves played some decisive role in insect attractions when assessed through various field and laboratory bioassays.

In addition, plant volatiles are involved in attracting natural enemies (predatory and parasitic insects) of pest insects, and in repelling the pest insects, especially when the plants were damaged by the pests (Dudareva *et al.*, 2004). The predators or parasites take an advantage of the signal to locate their prey (Vissotto, 1998). However, the black vine weevil has few predators or parasitoids in nature (MacKenzie, Pers. Comm.). The interaction between the weevil and its host plant volatiles seems more complicated (Tol, *et al.*, 2004). A bioassay showed these weevils strongly preferred mechanically and weevil-damaged foliage of *Euonymus* spp. This weevil-damaged *Euonymus* spp. was still attractive 24 hours after the insects were removed (Tol, *et al.*, 2004). Volatile components from wounded leaves may play an important role in attraction of the weevil (Tol, *et al.*, 2004). In other words, the odour of damaged plants might be the signal of suitable food for both adults and their offspring (Tol and Vissor, 2002).

As attractants, some plant volatiles have been used in agricultural pest control. For instance, a product called Magnet® (Scanlan, 2005) is a blend of five plant volatiles

combined with very small amounts of insecticides, along with a feeding stimulant and various other substances to control *Helicoverpa* spp. moths in cotton crops. Magnet® lures insects to it by odour, where they feed on insecticides and are killed. It gives a selective control by using low amounts of broad-spectrum insecticides, and it is less harm to beneficial insects (Scanlan, 2005).

Some plant volatile compounds evolved to repel insects (Pichersky, 2004), and this ability has not been effectively used in modern agriculture (Weis, 1998). Bruce, *et al.*, (2003) found that *cis*-jasmone, blackcurrant volatile, affected interactions between pest insects and crop plants. The volatile applied to wheat (*Triticum aestivum*) controlled the grain aphid, *Sitobion avenae*. In laboratory bioassays, plants sprayed with low levels of *cis*-jasmone as an aqueous emulsion were less attractive to aphids, but more attractive to their parasitoids. In the field, aphid infestations were significantly reduced in *cis*-jasmone treated plots compared to control plots (Bruce, *et al.*, 2003). Other plant-based repellent chemicals, such as peppermint oil and clove oil, have been used against ticks, mosquitoes and other blood-feeding arthropods present around porches, decks and picnic areas (Apperson and Waldvogel, 2002). Repellents against blood-feeding arthropods are much commonly used than against agricultural pests (Apperson and Waldvogel, 2002). Ansari *et al.* (2000) identified compounds in peppermint (*Mentha piperita*) oil. The yield of oil was 0.5% of fresh leaves, and two major components identified were menthol (81%) and menthone (5.8%). Peppermint oil showed a strong repellent action (84.5-100%) against adult mosquitoes *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* when applied on human skin. The larvicidal activity against the mosquito larvae (the third instar) ranged from 85% to 100% in 24-hour assays. Thorsell *et al.* (2005) extracted volatiles from birch, pine tar, citronella, cloves, eucalyptus, geranium, lavender, lily of the valley

and peppermint, and showed that the most pronounced effects on the tick, *Ixodes ricinus* L. were observed from the oils of citronella, cloves and lily of the valley. They possessed repellent activities of the same magnitude as the reference repellent DEET (N, N-diethyl-*m*-toluamide). These plant volatile repellent effects on other insects or arthropods suggest there may be uses for control of agricultural pests, including weevils.

The majority of volatiles collected from plants can be placed in the following four groups: terpenoids, fatty acid derivatives (green leaf volatiles (GLV)), benzenoids and nitrogen-containing compounds (Knudsen, *et al.*, 1993). The knowledge of occurrence and distribution of plant volatiles has been significantly increased in the last 15 years thanks to the adoption of simple, sensitive methods for headspace sampling, and to the availability of relatively inexpensive instruments for gas chromatography-mass spectrometry (GC-MS) (Dudareva, *et al.*, 2004).

From strawberry leaves, the essential oil composition of three strawberry genotypes, *Fragaria x ananassa* D. was examined by GC-MS (Khanizadeh and Belanger, 1993). Of the 37 compounds detected, the major components were linalool, nonanal, (Z)-3-hexen-1-ol, (Z)-3-hexen-1-yl acetate (throughout the text shorter names, hexenol and hexenyl acetate are used) and α -terpineol. Many of the other constituents were aliphatic in nature (Khanizadeh and Belanger, 1993). Hamilton-Kemp *et al.* (1988) found hexenyl acetate, hexenol, linalool, α -terpineol, hexanol, 2-phenylethanol and benzyl alcohol of 15 identified compounds from strawberry leaves. The amounts in strawberry fruits were 0.12-0.33 $\mu\text{g/g}$ hexenol, 0.20-0.46 $\mu\text{g/g}$ hexenyl acetate, 0.07-0.05 $\mu\text{g/g}$ benzyl alcohol, and 0.04-0.18 $\mu\text{g/g}$ linalool (Kafakas and Kafakas, 2005).

Volatile components of five Turkish *Rhododendron* species were analyzed, and 34 compounds were identified, with benzyl alcohol (16%), limonene (14.6%) and p-cymene

(8.4%) being the major compounds (Ruedi, 2003). Volatile compounds of *Taxus baccata* were reported by Erdemoglu *et al.* (2003), with hexadecanoic acid (19.6%) and decanoic acid (19%) as the predominant volatile compounds in this plant.

The electroantennogram (EAG) is a well known technique for detecting insect responses to volatiles (Jonsson, 2005). Tol and Visser (2002) used EAG to record the sensory response of black vine weevils (*Otiorhynchus sulcatus*) to a broad range (about 130 compounds) of volatiles known to occur in plant odors. The results revealed only a limited number of odors that evoked substantial responses on the weevils' antennae. Large EAG responses were recorded during exposure to the group of fatty acid derivatives which are generally called green leaf volatiles (GLV). In order of decreasing insect responses were (E)-2-hexen-1-ol, hexenol, hexenyl acetate, hexanal, heptanal, (E)-2-heptenal and (E)-2-hexenal. Tol and Visser's (2002) EAG response profile can give only limited indications about the possible attractiveness of the volatiles. Their study also stated that weevils prefer certain plant species in choice situations. GC combined with electroantennogram detection (EAD), (called GC-EAD) and bioassays with attractive plant extracts could further determine the compounds that attract the weevil, and provide better results than the EAG response to the single compounds only (Jonsson, 2005). These could then be combined with some more specific plant volatiles to select their host plants in the field (Tol and Visser, 2002).

Objectives

This study examined adult weevil feeding preferences, oviposition, and olfactory responses to strawberry and to other potential weevil host plants. The study also screened

the plants that produce attractant or repellent effects on weevil and identified abundant or unique plant volatile compounds in these plants, which may be responsible to the attractive or repellent effects.

Materials and methods

Newly emerged adult black vine weevils (*Otiorhynchus sulcatus* L.) were collected from strawberry fields in the Annapolis Valley of Nova Scotia, Canada, from June to July, 2004. The weevils were fed on strawberry foliage and either held at 4°C in a refrigerator or in a growth cabinet programmed for 16 hours light: 8 hours dark (16h: 8h, L: D), at 22°C and 75% relative humidity (RH). All weevils used in the experiments were held in the growth cabinet for at least one week prior to beginning the studies. Offspring of field-collected black vine weevils (from eggs to adults) reared on an artificial diet (Fisher and Bruck, 2004) were compared with the performance of field weevils.

Host plants including yew (*Taxus* sp.), strawberry (*Fragaria* sp. cultivar Annapolis), geranium (*Geranium* sp.), rhododendron (*Rhododendron* sp.), and peppermint (*Mentha piperita* L.) were used in a weevil preference study. Three strawberry cultivars (Annapolis, Cavendish, and Evangeline) were obtained (Keddy Nursery Inc., 982 North Bishop Road Kentville, Nova Scotia) as bare-root plants and grown in pasteurized soil for at least two months. The susceptibility of ‘Cavendish’ or ‘Evangeline’ to the weevil is not known, but ‘Annapolis’ is known to have some resistance. The yew, geranium, rhododendron, and peppermint were bought as plants from Blomidon Nurseries 10060 Hwy 1, Greenwich Corner, NS.

Weevil oviposition and egg viability on different strawberry cultivars

The adult weevils were held for 21 weeks individually in a 420-ml covered beer cup (Fig. 1) with some holes in the lid for air exchange. In total, 45 field-collected weevils (15 for each strawberry cultivar, 'Annapolis', 'Cavendish', or 'Evangeline') were randomly chosen from the colony and placed into individual cups for use in individual oviposition and egg viability studies.

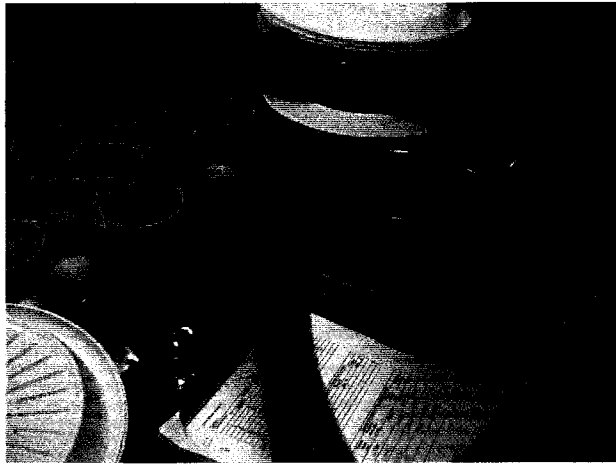


Figure 1. Beer cups used for individual weevil oviposition on different strawberry cultivars

Two strawberry leaflets of a cultivar were placed in each cup with one weevil. The cups were kept in a growth cabinet under 16h: 8h L: D at 22°C and 75% RH. Each week the eggs laid by individual weevils were collected and counted. Eggs for the viability assay were disinfected for 2-minutes in 1:10 Javex (sodium hypochlorite) solution, and rinsed with water five times in a Buchner funnel (Cowles, Pers. Comm.). Twenty eggs were placed on moist filter paper in a Petri dish which was sealed with Parafilm. Egg hatch was monitored weekly for a total of four weeks. Oviposition and egg viability for 10 weevils reared on artificial diet (Fisher and Bruck, 2004) were tested on strawberry 'Annapolis' using the same methods.

Fifteen (five for each cultivar) of the 45 weevils used for individual oviposition and egg viability were weighed to carry out the correlation between weight and oviposition over 37 weeks. Another forty-five field-collected weevils and 50 artificial diet-reared weevils were weighed individually in order to test for weight differences between the two colonies.

Weevil plant selection, oviposition and feeding preferences for plants

Weevil colony trials (10 weevils in each trial) (Fig. 2): Four-plant choice tests to assess feeding preference and oviposition were conducted in covered cake trays (bottom r: 15 cm, h: 8.5 cm, Fig. 2). Leaflets of each host plant yew, rhododendron, geranium, and strawberry (4-choice) were weighed (ranged 1.215 to 1.404 g) and placed at a regular spacing on the bottom along the edge of each cake tray. Adult weevils were starved for 24 hours before beginning trials.



Figure 2. Weevil plant selection, feeding and oviposition preferences for different plant leaves in a covered cake tray

Ten individual field-collected weevils were placed in the middle of each test tray (Fig. 2). Observations of weevil plant selection were made for intervals of 30 minutes, 2 hours and 24 hours by counting the number of weevils on different hosts after each time period. Forty-four replications were conducted for host selection observation.

At the same time, trials to measure 24-hour oviposition and leaf consumption of weevils, and controls to account for different leaf dehydration rates among plants tested, were run. The setup for the 24-hour trials were the same as the 30 min and 2 hour trials, except that the test trays were placed in a growth cabinet under 16h: 8h L: D at 22°C and 75% RH for 24 hours. Twenty-four-hour leaf consumption was measured by weight, and the number of eggs laid on different hosts was recorded. For the controls, similar amounts of leaf tissue as that used in the trials were placed in a tray and left for 24 hours in the growth cabinet. Then, the leaves were weighed. The percentage of leaf consumption (% LC) was calculated using the following equation:

$$\% \text{ Leaf Consumption} = [(T_0 - T_{24})/T_0 - (C_0 - C_{24})/C_0] \times 100$$

Where T_0 is the original trial leaf weight, T_{24} is the trial leaf weight after 24 hours of feeding, C_0 is the original control leaf weight and C_{24} is the control leaf weight after 24 hours.

Individual trials (1 weevil in each trial) (Fig. 3, A and B): Another set of experiments was designed to measure weevil feeding preferences in no-choice and two-choice conditions (Fig. 3, A and B).

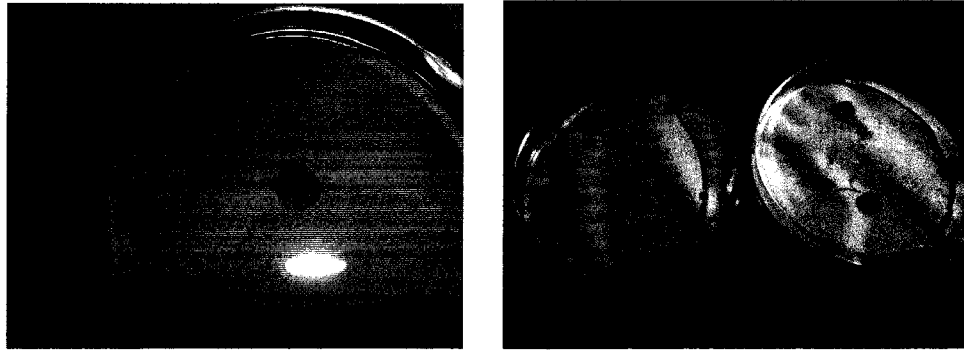


Figure 3. Petri dish feeding tests for weevils. A: no-choice feeding test showing one weevil and a cut yew leaf disk in a Petri dish, and B: No-choice and two-choice setups.

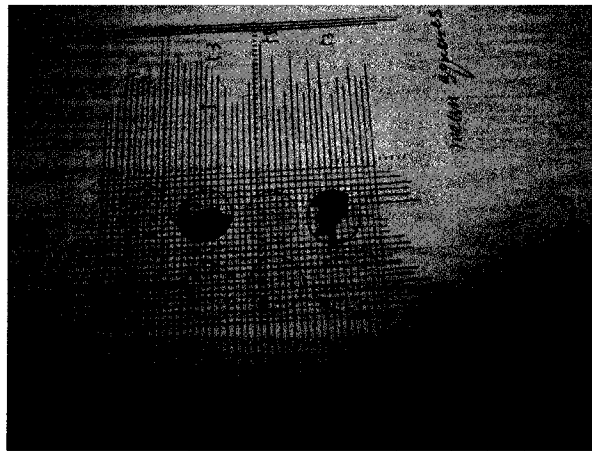


Figure 4. Method used to calculate the percentage of leaf disk consumption by weevils in 24 hours with 8-mm leaf disk outlines

A piece of 9-cm filter paper was placed inside each Petri dish, and 1 ml of distilled water was added (Cowles, 2004). An 8-mm diameter cork borer was used to punch leaf disks of yew, rhododendron, geranium, strawberry, and peppermint. Field-collected adult weevils were starved for 24 hours before being used in the trials. For no-choice trials, one leaf disk was placed in the middle of each Petri dish, and one adult weevil was introduced in it. Twenty replicates were tested for each plant species used. For two-choice trials, one leaf disk from each pair of plants (such as yew-rhododendron, or yew-geranium) was

placed on opposite sides of the Petri dish and one weevil was added in the middle of the Petri dish. A comparison of diet-reared and field-collected weevils were done for no-choice and 2-choice tests with strawberry and peppermint. Twenty replicates were done for each trial.

Trials were run in a box to keep weevils in the dark for 24 hours under room condition (about 22°C and 70% RH), which approximates their natural feeding conditions, and accounts for the influence of undetected environmental conditions. After the test period, leaf disk consumption was measured by tracing the 8-mm diameter leaf disk outline. There were 54 squares (1 mm²) in each leaf disk outline. The amount of leaf material eaten by weevils was estimated by counting the missing squares of the leaf disk (Fig. 4). Twenty replicates for each trial were tested.

Olfactory responses to plant leaf disks

Insect olfactory responses to yew, rhododendron, geranium, strawberry, and peppermint were tested in three-cell olfactometer (Fig. 5) with a single glass cover was used. The effects of plant leaf disks (diameter 8-mm) were tested by placing one leaf disk covered with a plastic screen in each cell (the left or the right cell). Weevils could walk on the screen, but could not touch the leaf disk. One 24-hour-starved adult weevil was placed on the covered glass and the glass was turned 180° so that the weevil was introduced into the centre cell of the olfactometer. The olfactometer was immediately covered with a box to keep the weevil in the dark. The position of the weevil (right, left, or centre) was checked after two minutes. The orientation of left cell and right cell was alternated for each weevil in order to account for possible influence of undetected atmospheric and environmental conditions. All the trials were conducted at about 22°C, 70% RH. Forty (40)

replicates for each trial and 10 replicates for each of five controls which were with same leaf disks were tested.

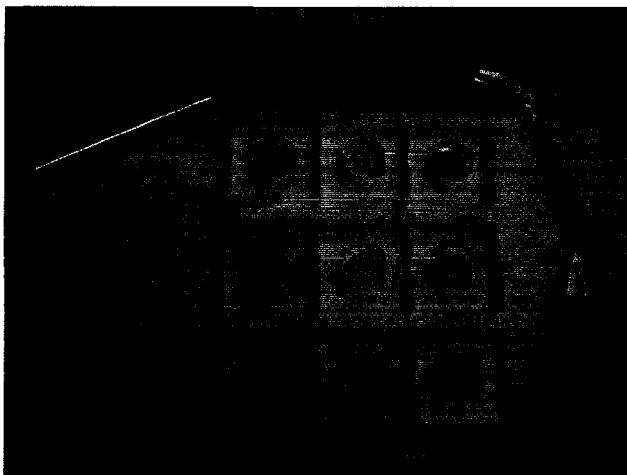


Figure 5. Three 3-cell olfactometers, an 8-mm OD cork borer and leaf disk preparation for weevil olfactory responses to plant leaf disks

Plant volatile compounds

Fresh strawberry leaves were cut from ‘Annapolis’ plants. About 20 g whole intact leaves or artificially damaged leaves (pieces cut from leaves with an 8-mm OD cork borer, and remaining damaged areas) were weighed, and placed into a 4-Liter (L) glass jar sealed with a Teflon lid. The headspace over the leaf sample was allowed to equilibrate for 1 hour. An 1 L sample of the headspace volatiles in the jar was pumped with an Alpha-1 air pump (6.2 V/DC power supply) and trapped on 120 mg of Tenax GR 20/35 (Alltech Associates, Inc., Deerfield, Ill.) and carbosieve III traps in a 100 x 6.4 mm (OD) glass tube (C. Forney and M. Jordon, pers. comm. Agriculture and Agri-Food Canada, Kentville NS).

Samples of headspace volatiles were analyzed on a Magnum gas chromatography-

mass spectrometer (GC-MS) system (Finnigan MAT, San Jose, Calif.) equipped with an LSC 2000 purge and a trap concentrator (Tekmar, Cincinnati). The valve and transfer line on the LSC 2000 were held at 170°C. Traps were placed in the Tekmar 2000 and desorbed at 250°C for 4 min directly onto a Supelcowax 10, 60m x 0.53 mm column with a film thickness of 1 µm (Supelco Inc., Bellefonte, Pa.). The column flow rate of helium carrier gas was maintained at 10mL·min⁻¹. The temperatures of the transfer line from the GC to the ion trap of the MS were 180°C and 220°C, respectively. The column temperature was held at 40°C for 2 min, increased to 120°C at a rate of 16°C ·min⁻¹, increased to 240°C at a rate of 15°C ·min⁻¹, and held at 240°C for 3 min. Quantifications were done using single ions of external standards. All peak areas were normalized using the peak area of a 4 ng dodecane standard that was run on the day of the analysis.

The effects of plant volatile compounds on adult weevils

Based on headspace GC-MS analysis in this study and from the results of previous studies, hexenol and hexenyl acetate, two of several abundant volatile compounds from strawberry leaves were used to assess their effects on weevils. Two volatiles of peppermint, menthone and menthol, were assessed as well. Hexenol (98%), hexenyl acetate (95%), menthone (90%), and menthol (99%) were purchased from Sigma.

For volatile assessment, hexenol, hexenyl acetate, and menthone were dissolved with distilled water to 1µl /ml, and then diluted to 10⁻³, 10⁻⁶, and 10⁻⁹µl /ml concentrations. Menthol (solid) was dissolved first in 40% ethanol at 1mg/ml concentration, and then diluted in water to 10⁻³, 10⁻⁶, and 10⁻⁹ mg/ml concentrations.

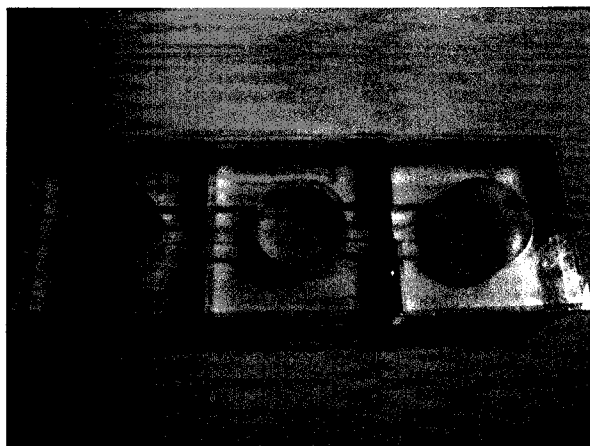


Figure 6. One weevil to be attracted by volatile compound-treated filter paper in the left cell of an olfactometer

Olfactory responses to these four compounds, hexenol, hexenyl acetate, menthone, and menthol, were assessed in olfactometers (Fig. 6). Five (5) μl of diluted compound (at 1, 10^{-3} , 10^{-6} , and 10^{-9} μl /ml, or for menthol at 1, 10^{-3} , 10^{-6} , and 10^{-9} mg/ml concentrations) was dropped on a small piece of filter paper, covered with a screen and placed in one cell of the olfactometer, same amount of solvent in the other cell. Weevil introduction into the olfactometer was conducted in the same method as described in Olfactory responses to plant leaf disks (page 15). Forty replicates for each compound concentration were tested. Controls were water for hexenol, hexenyl acetate and menthone trials, or 40% ethanol for menthol at concentration 1 mg/ml trial depending on the solvents used to dissolve or dilute the compounds.

Feeding preference for strawberry and rhododendron leaf disks treated with plant volatiles were tested in 9-cm Petri dishes (same method as leaf disk feeding preference with no-choice and two choices, page 14). The strawberry or rhododendron leaf disks were treated with 5 μL hexenol, hexenyl acetate, menthone, or menthol solutions at 1 or 10^{-3} μl /ml or mg/ml (menthol) concentrations respectively. Twenty-four-hour starved field-

collected adult weevils were introduced to the trials. For no-choice trials, either a compound-treated or untreated strawberry or rhododendron leaf disk was placed in the middle of each Petri dish, and one weevil adult was introduced (Fig. 3) into the middle of the dish. For two-choice trials, a pair of compound-treated and untreated strawberry or rhododendron disks was placed opposite one another in each Petri dish, and then one weevil adult was introduced into the middle of the dish. Assays were conducted in a box to keep weevils in the dark for 24 hours at about 22°C and 70% RH. After the test period, leaf disk consumption was measured by tracing leaf disk outline as described for feeding preference assays. Twenty replicates for each trial were run.

Statistics

Data were analyzed with the computer statistical program GraphPad Prism 4 (Miller, 2003). Firstly, D'Agostino and Pearson normality test was done to check the data for normality ($\alpha=0.05$) (Fig. 7).

If data were normally distributed, one way analysis of variance (ANOVA) and, if necessary, Tukey's multiple comparison test, or paired *t*-test was used for the significant differences (Fig. 7). For example, ANOVA and post test-Tukey's multiple comparison were for weevil oviposition on yew, rhododendron, geranium and strawberry, and *t*-test (paired or unpaired) for peppermint-geranium two-choice leaf disk feeding test, and some of compound-treated or untreated leaf feeding preference tests.

Nonparametric tests, such as Mann Whitney test for paired tests and Kruskal-Wallis tests, were used if the data were not normally distributed. Dunn's multiple comparison tests were used if Kruskal-Wallis test had a P value which was less than 0.05 (Fig. 7). In this study, most data were analyzed statistically by nonparametric tests.

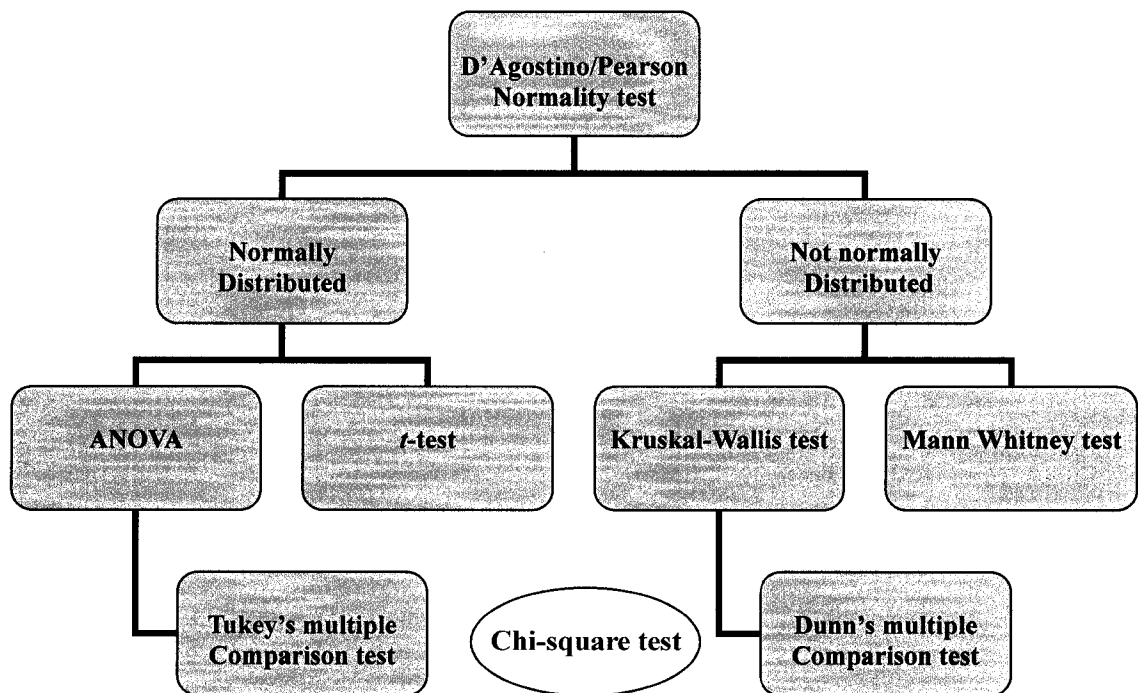


Figure 7. Statistical analysis

Chi-square tests were used for the contingency tables of olfactory response tests (Zar, 1999) (Fig. 7).

The correlation (Pearson r) between field-collected individual adult weight and the oviposition was analyzed (Miller, 2003).

All error bars shown in results are standard deviations.

Results

As mentioned, this study examined the effects of plant volatile compounds on black vine weevil behaviour which included weevil feeding and oviposition preferences, plant

selection, and olfactory responses to different host plants. Headspace analysis coupled with GC-MS was used to quantify volatiles from 'Annapolis' strawberry leaves. These results along with previous studies were used to select two volatiles, hexenol and hexenyl acetate from strawberry, and two, menthone and menthol, from peppermint for use in behavior bioassays.

Weevils were found to have the highest oviposition and egg viability when fed on strawberry cultivar 'Annapolis'. Weevils preferred strawberry to peppermint and to other plants when offered a choice. Weevils were significantly attracted to hexenyl acetate at concentrations of 10^{-3} and 10^{-6} $\mu\text{l/ml}$, while were repelled by hexenol, and hexenyl acetate at the concentration of $1 \mu\text{l/ml}$. Menthone at a concentration $1 \mu\text{l/ml}$ repelled weevils. Weevils significantly fed less on strawberry leaf disks treated with menthone at concentrations of 1 and 10^{-3} $\mu\text{l/ml}$.

The results are presented in following five sections respectively.

Weevil oviposition and egg viability on different strawberry cultivars

The average number of eggs laid over 21 weeks by 37 individual field collected weevils was 389 (range 86 - 909 eggs) on three strawberry cultivars: Annapolis, Cavendish, and Evangeline (Table 1). The number of eggs laid by individual weevils reared on artificial diet and then fed on cultivar Annapolis ranged from 126 to 1071 eggs ($n=10$) over a 21-week period (Table 1).

The oviposition of field weevils on 'Annapolis' was significantly higher than on 'Cavendish' and on 'Evangeline' (Kruskal-Wallis test, Dunn's multiple comparison tests: $p < 0.001$) (Table 1). There was also a difference between oviposition on 'Cavendish' and 'Evangeline' ($p < 0.05$). Weevils fed on 'Evangeline' laid the lowest average number of eggs

(264±147 eggs/weevil), those fed on 'Annapolis' the most (592±172 eggs/weevil), and 'Cavendish' (310±135 eggs/weevil) was intermediate. Over the 21 weeks, eight of the 45 weevils died, six that were fed on 'Cavendish', and one each for other two cultivars. Thus, higher mortality was seen in the 'Cavendish' group. There was no significant difference ($p>0.05$) in mean number of eggs laid per weevil between field weevils and the weevils reared on artificial diet when fed on the cultivar 'Annapolis'. However, diet weevil oviposition on 'Annapolis' was significantly higher than field weevils fed on 'Cavendish' or 'Evangeline' (Table 1), which was similar to field weevil oviposition on 'Annapolis' compared with 'Cavendish' and 'Evangeline'. Egg laying was highly variable among individuals (Table 1).

In addition, egg viability differed by cultivar. Only 31% of the eggs from 'Cavendish'-fed weevils hatched which was significantly less than the 62% egg hatch from 'Annapolis'-fed weevils (Kruskal-Wallis test, Dunn's multiple comparison tests: $p<0.01$), while 'Evangeline'-fed weevils had intermediate egg hatch (42%) similar to both other cultivar fed weevils (Table 1). No egg viability difference was found between diet weevils and field weevils on 'Annapolis', however, diet weevil egg viability was higher than field weevils fed on 'Cavendish' (Kruskal-Wallis test, Dunn's multiple comparison tests: $p<0.01$) (Table 1).

There was no correlation (Pearson $r = -0.2714$, $\alpha = 0.05$) between adult weight (range 54-84 mg), and oviposition (264-1382 eggs) observed in 37 weeks ($n = 10$, five of 15 weevils were died during the test period).

Field-collected weevil individual mean weight was significantly higher than the weight of artificial-diet-reared weevil's (Table 2). There was no significant weight difference among individual weevils in the same colony (Table 2).

Table 1. Field and diet weevil oviposition and egg viability on three strawberry cultivars

	Annapolis		Cavendish	Evangelina
	diet weevil n=10	field weevil n=14	field weevil n=9	field weevil n=14
total egg range	126-1071	391-909	120-500	86-524
Mean (eggs/weevil)	536±258 ^{a 1}	592±172 ^a	310±135 ^b	264±147 ^c
egg viability range %	35-80	0-100	0-95	0-95
Mean %	62±13 ^{a 1}	51±35 ^a	31±30 ^b	42±36 ^{ab}

¹ Kruskal-Wallis tests, Dunn's multiple comparison tests: means in the same row followed by the same letter is not significantly different at p=0.05

Table 2. A comparison of individual weevil weight between field-collected and diet-reared colonies

colony	Field-collected weevil	Artificial-diet-reared weevil
n	45	50
Range (mg)	51-83	32-82
Mean (mg)	70±7 ^{a 1}	63 ± 10 ^b
P value summary ²	ns	ns
P value	0.1496	0.1475

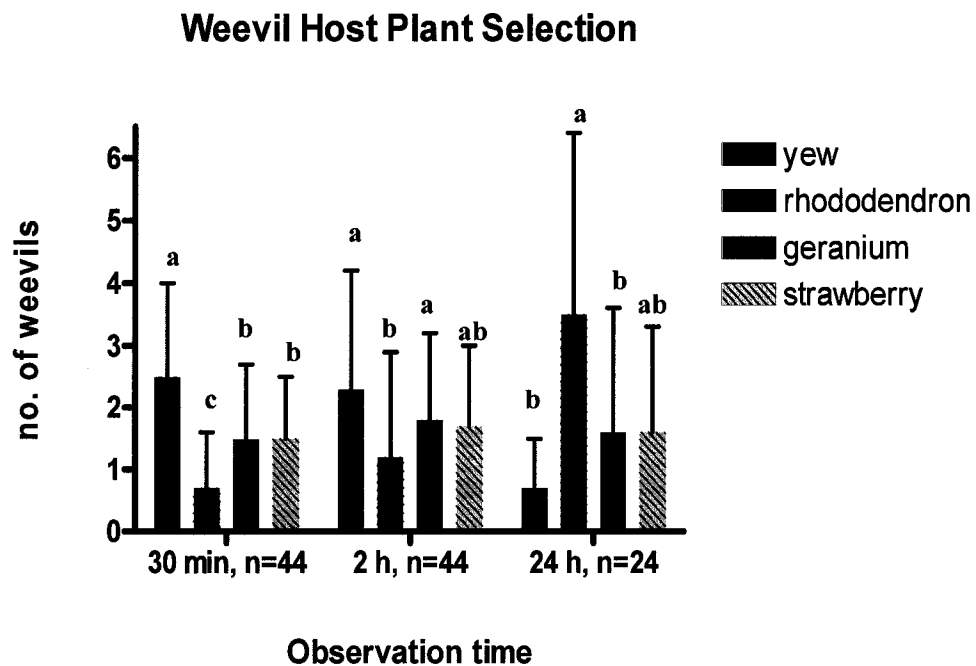
¹ unpaired t-test: means in the same row followed by different letter is significantly different at p = 0.05

² among the individual weevils in the same colony

Weevil plant selection, oviposition and feeding preferences for plants

Weevils were exposed to each of yew, rhododendron, geranium, and strawberry (4-choice) for intervals of 30 minutes, 2 hours, and 24 hours (Fig. 8). When ten 24-hour-starved weevils were placed in the test containers, they immediately moved around, searched and fed on leaf tissue. After 30 minutes, it was clear that weevils were found

more often on yew (2.5 ± 1.5 weevils/plant species) compared to rhododendron, geranium, and strawberry, and showed the least preference for rhododendron (0.7 ± 0.9 weevils/plant species) (Fig. 8). By the end of two hours, weevils were found to be more evenly distributed as the only statistically significant preference was for yew when compared to rhododendron (Fig. 8). After twenty four hours, the number of weevils hidden under rhododendron leaves was much greater than the number hidden under geranium leaves, and even greater than the number hidden under yew leaves. To sum up, statistically more weevils were found on yew than on the other three plants at 30 minutes; more on yew than on rhododendron at 2 hours; and more on rhododendron than on geranium and yew at 24 hours (Fig. 8).



Kruskal-Wallis test, Dunn's multiple comparison tests:
Means with the same letter are not significantly different at $p=0.05$

Figure 8. Number of weevils on four hosts at different observation times in four-choice tests

Regarding oviposition, there were no eggs laid during the two hour test. After twenty four hours, weevils laid significantly fewer eggs around rhododendron ($p<0.05$) than on yew and geranium, while on strawberry oviposition was similar to that on the other three hosts (Table 3). The 24-hour leaf consumption showed that yew and strawberry were chosen over geranium and rhododendron. However, there was no difference between strawberry and yew even though the adults were reared on strawberry prior to the test (Table 4).

Table 3. Weevil feeding and oviposition preferences in four-choice bioassays

N=24	Yew	Rhododendron	Geranium	Strawberry
24-h oviposition (n. of eggs)	13.1±7.5 ^{a 1}	6.3±3.8 ^b	14.3±12.5 ^a	8.0±5.3 ^{ab}
24-h leaf consumption (%)	12.5±7.0 ^{a 2}	4.6±3.7 ^b	4.8±5.0 ^b	11.6±5.3 ^a

¹ One way ANOVA, Tukey's multiple comparison tests

² Kruskal-Wallis tests, Dunn's multiple comparison tests

(Means in the same row followed by the same letter are not significantly different at $p=0.05$)

The weevil feeding bio-assay which was conducted in 9-cm Petri dishes showed that there was no significant difference in amount eaten by weevils among yew, rhododendron, geranium, strawberry and peppermint in no choice condition (Table 4). Although diet-reared weevils consumed less leaf amounts than field-collected weevils, there was no statistical difference between the two weevil colonies when fed on strawberry or on peppermint alone (no-choice) as well (Table 4).

Table 4. Proportion of leaf disk eaten by weevils on 24-hour no-choice tests

n=20 leaf feeding consumption %	Field collected weevils				
	yew	rhododendron	geranium	strawberry	peppermint
	8.8±12.2 ^{a1}	18.5±18.8 ^a	31.7±27.4 ^a	38.5±45.5 ^a	42.0±41.6 ^a
n=20 leaf feeding consumption %	artificial diet reared weevils				
				strawberry	peppermint
	/ ²	/	/	29.4±22.5 ^a	23.0±29.5 ^a

¹ Kruskal-Wallis tests: means with no-choice followed by the same letter are not significantly different at p=0.05² /: no data

Table 5. Proportion of leaf disk eaten by weevils on 24-hour two-choice tests

	Field weevil				Diet weevil
	Rhododendron %	Geranium %	Strawberry %	Peppermint %	Peppermint %
N=20					
Yew %	11.4±16.2 to 1.8±3.8 ¹	25.7±40.7 to 2.7±4.0	47.9±39.2 to 2.5±4.3 ¹	14.0±23.4 to 4.8±11.8	/ ³
Rhododendron %	/	24.8±30.7 to 1.5±3.7 ¹	9.5±13.41 to 1.1±15.1	0.3±0.9 to 14.1±16.8 ¹	/
Geranium %	/	/	33.2±31.0 to 21.1±30.4	19.3±23.5 to 37.3±33.7 ²	/
Strawberry %	/	/	/	3.9±12.4 to 66.4±38.7 ¹	9.9±24.9 to 35.4±23.6 ¹

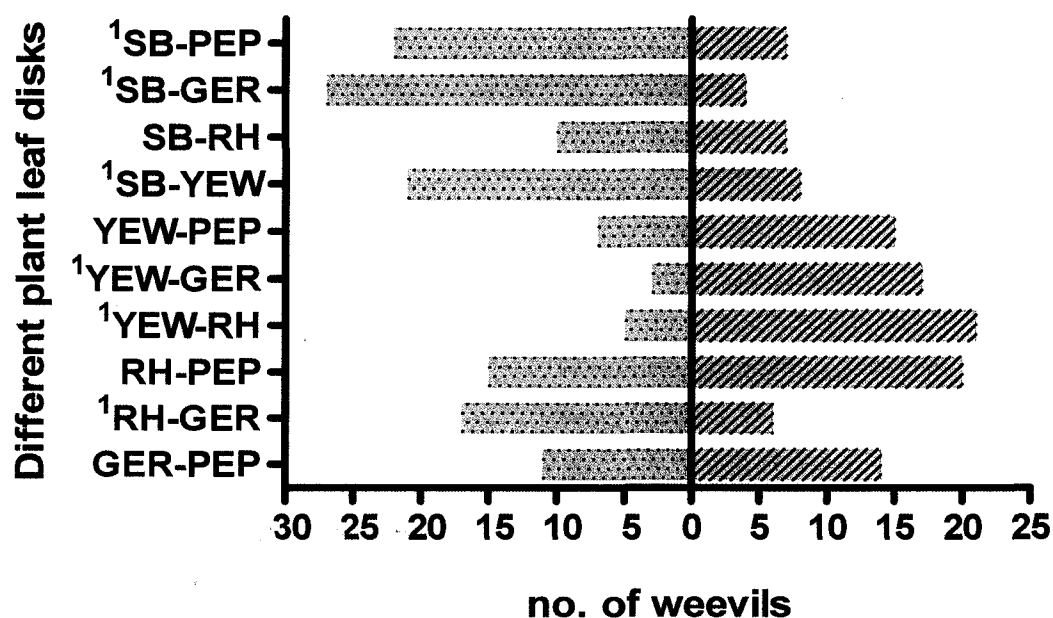
¹ Significant difference at p=0.05 by paired Mann Whitney test² Significant difference at p=0.05 by paired t-test³ /: no data

When the weevils had choice, the results were different. Field-collected weevils had a statistically significant feeding preference for rhododendron to yew, for strawberry to yew,

for geranium to rhododendron, for rhododendron to peppermint, and for strawberry to peppermint (Mann Whitney test: $p < 0.05$). Similarly, diet-reared weevils exhibited a significant preference for strawberry compared to peppermint. There was no difference between yew and geranium, yew and peppermint, rhododendron and strawberry, geranium and strawberry, geranium and peppermint respectively in pairs (Table 5). Weevils showed the least interest in yew. This was different from the 4-choice test results (back to Table 3).

Olfactory responses to plant leaf disks

Weevil responses to leaf disks with the same host (the controls) in each cell of the olfactometer were not statistically different (data were not presented). When weevils had two different kind of leaf disks, such as strawberry (SB)-peppermint (PEP), SB-geranium (GER), SB-rhododendron (RH), and SB-yew (YEW), they significantly preferred the cell with SB leaf disk to the cell with PEP, GER or YEW (Chi-square test, $p < 0.05$) (Fig. 9). There was no significant difference in preference between SB-RH (Fig. 9). Weevils chose GER or RH over YEW (Chi-square test, $p < 0.05$), while there was no difference in preference between YEW-PEP (Fig. 9). Weevils preferred RH to GER when given those two choices (Chi-square test, $p < 0.05$), but showed no preference for RH over PEP, GER and PEP, as well as YEW and PEP (Fig. 9).



¹ significant difference at $p=0.05$ by Chi-square tests

Figure 9. Paired comparison of weevil olfactory responses to five kinds of plant leaf disks

Plant volatile compounds

Fifteen compounds were detected by headspace-extracted strawberry ‘Annapolis’ leaf samples with gas chromatography-mass spectrometry (GC-MS), and six of them were identified. They were hexenyl acetate, hexenol, hexanol, nonen-1-ol, 2-ethylhexanol, 2-ethyl furan, and phenol (Table 6). In artificially damaged strawberry leaf samples, hexenyl acetate and hexenol were quantified and exhibited about a 4-fold increased abundance measured by GC-MS as those in whole strawberry leaves (Table 6).

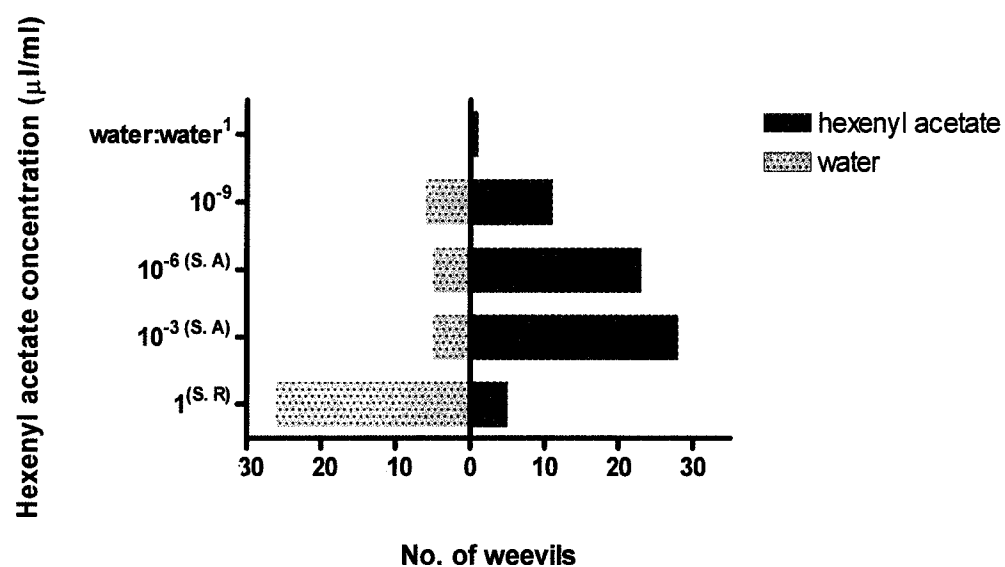
Table 6. Plant volatile compounds in strawberry “Annapolis” leaves

Compound	Compound GC peak area		%
	Damaged leaf sample	Whole leaf sample	
Z-3-Hexen-1-ol	2413156	66749	6.6
Z-3-Hexenyl acetate	7369946	212094	21.0
1-Hexanol	/ ¹	14797	1.5
Phenol	/	124614	2.7
Nonen-1-ol	/	31666	3.1
2-Ethylhexanol	/	98103	9.7
2-Ethyl furan	/	717969	7.9
Other compounds	/	/	47.5
Total	/	/	100.0

¹ /: no data

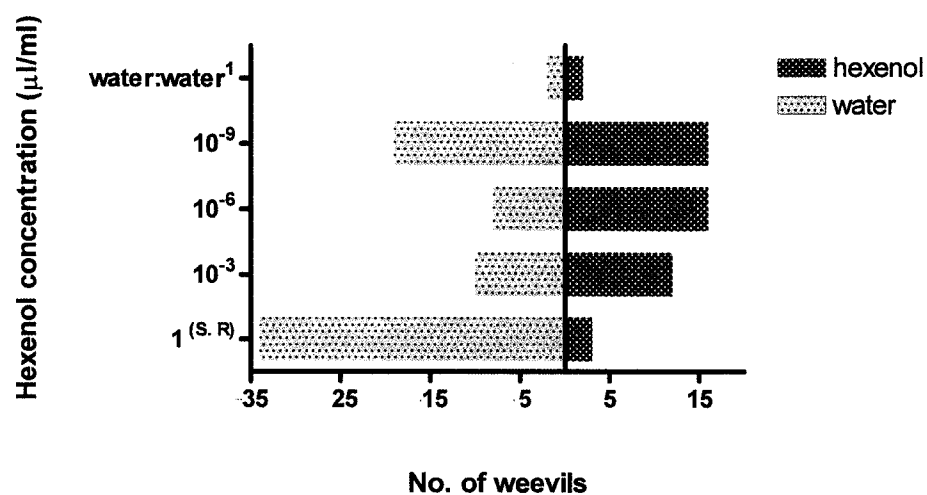
The effects of plant volatile compounds on adult weevils

The assay of volatile effects conducted in three-cell olfactometer showed that weevils were attracted to hexenyl acetate (from strawberry) at 10^{-6} and $10^{-3}\mu\text{l/ml}$ (Fig. 10), while hexenol did not show any attractive effect on the weevil. However, hexenol and hexenyl acetate at concentration $1\mu\text{l/ml}$ repelled weevils (Fig. 10-11). Menthone (from peppermint) at $1\mu\text{l/ml}$ had repellent effect on weevils (Fig. 12), while another peppermint volatile, menthol, had no effect on weevils at the concentrations tested (Fig. 13).



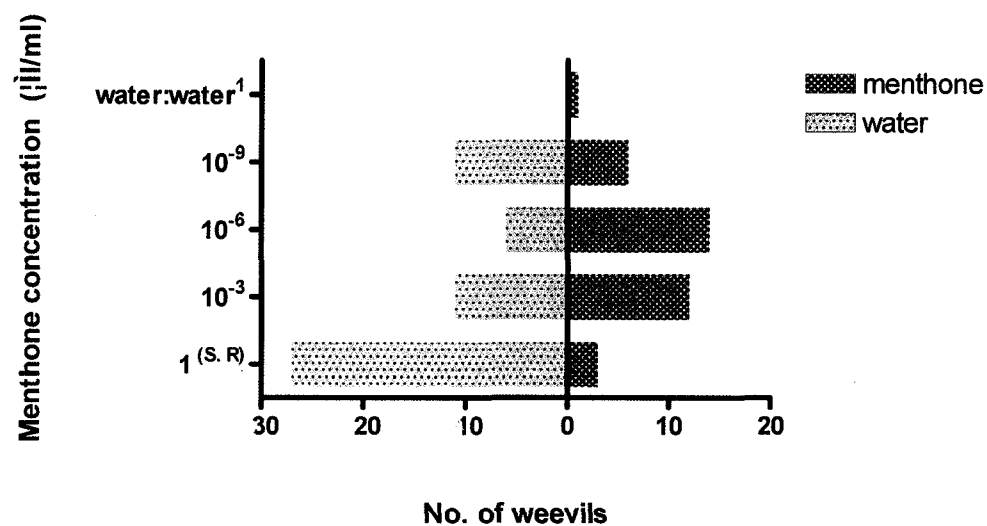
¹ Control
 S. A: significantly attracted at p=0.05 as judged by Chi-square tests
 S. R: significantly repelled at p=0.05 by Chi-square tests

Figure 10. Hexenyl acetate (from strawberry leaves) effects on weevils



¹ Control
 S. R: significantly repelled at p=0.05 as judged by Chi-square tests,

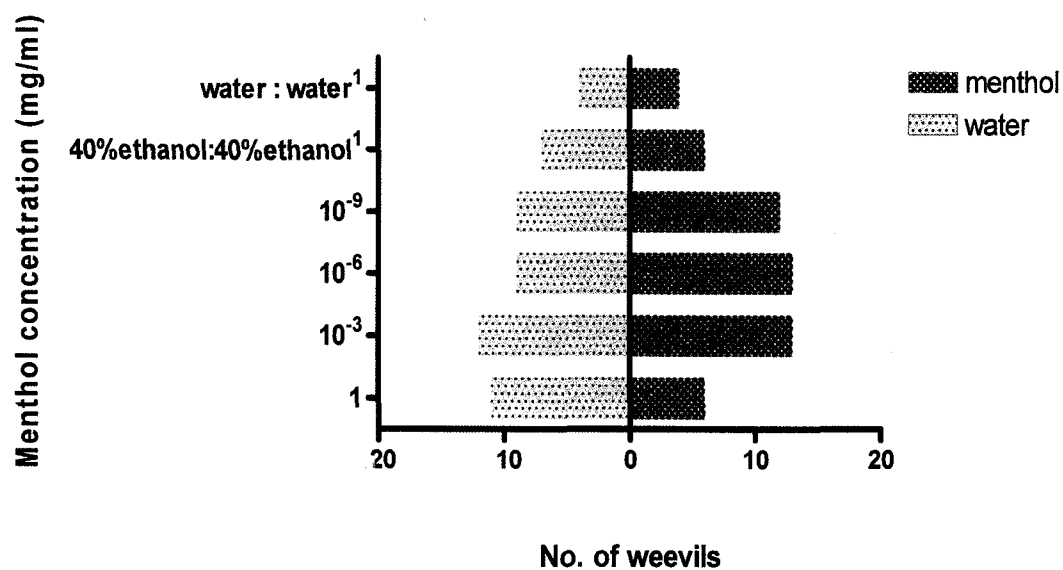
Figure 11. Hexenol (from strawberry leaves) effects on weevils



¹Control

S. R: significantly repelled at $p=0.05$ as judged by Chi-square tests

Figure 12. Menthone (from peppermint leaves) effects on weevils



¹Control

Figure 13. Menthol (from peppermint leaves) effects on weevils

In a separate set of experiments, weevil behaviour was observed when volatile compounds, hexenyl acetate and hexenol (from strawberry leaves); menthone and menthol (from peppermint leaves), were applied to strawberry or rhododendron leaf disks. Without choice (Fig. 3 B), there were no significant difference in feeding damage among compound-treated strawberry leaf disks at concentrations of 1 μ l/ml, 10⁻³ μ l/ml, and control (strawberry leaf disk only) (Table 7). However, weevils fed significantly less on rhododendron leaf disk treated with 10⁻³ μ l/ml hexenyl acetate (Table 8).

Table 7. Weevil feeding preference (%) for compound treated strawberry leaf disks (no-choice)

N=20	Menthone	Menthol	Hexenyl acetate	Hexenol
Strawberry +1 μ l /ml (mg/ml) ²	37.8 \pm 41.6 ^{a 1}	49.4 \pm 47.9 ^{a 1}	22.6 \pm 35.9 ^{a 1}	43.0 \pm 37.3 ^{a 1}
Strawberry +10 ⁻³ μ l /ml (mg/ml) ²	44.1 \pm 44.5 ^a	84.1 \pm 31.8 ^a	65.6 \pm 42.8 ^a	38.0 \pm 37.6 ^a
Strawberry (control)	50.4 \pm 46.3 ^a	50.4 \pm 46.3 ^a	49.6 \pm 41.9 ^a	49.6 \pm 41.9 ^a

¹ Kruskal-Wallis tests: means in the same column followed by the same letter are not significantly different at p=0.05

² mg/ml menthol

Table 8. Weevil feeding preference (%) for compound treated rhododendron leaf disks (no-choice)

N=20	Menthone	Menthol	Hexenyl acetate	Hexenol
Rhododendron +1 μ l/ml (menthol, mg/ml) ²	8.3 \pm 15.9 ^{a 1}	2.6 \pm 7.0 ^{a 1}	18.7 \pm 22.5 ^{a 1}	12.6 \pm 14.3 ^{a 1}
Rhododendron+10 ⁻³ μ l/ml (menthol, mg/ml) ²	6.5 \pm 16.2 ^a	6.7 \pm 14.6 ^a	2.2 \pm 4.8 ^b	8.3 \pm 14.2 ^a
Rhododendron (control)	2.4 \pm 4.2 ^a	2.4 \pm 4.2 ^a	18.5 \pm 19.6 ^a	18.5 \pm 19.6 ^a

¹ Kruskal-Wallis tests: means in the same column followed by the same letter are not significantly different at p=0.05

² mg/ml menthol

In two-choice bioassays, compound-treated and no-treated leaf disk comparison in Petri dishes, weevils significantly fed less on strawberry leaf disks treated with menthone at concentrations of 1 and 10^{-3} $\mu\text{l/ml}$ than fed on strawberry leaf disks (Table 9). There was no difference in weevil feeding on any of the volatile-treated rhododendron leaf pieces as compared to un-treated leaf pieces (Table 10).

In mean of the trials, the results were quite variable, and the standard deviations were large, even higher than their means. This resulted in no statistical differences though some means appeared very different from each other, or from others (Table 7-10).

Table 9. Weevil feeding preferences (%) for compound treated strawberry leaf disks (two-choice)

	Leaf disk treated with menthone		Leaf disk treated with menthol	
	1 $\mu\text{l/ml}$	10^{-3} $\mu\text{l/ml}$	1 mg/ml	10^{-3} mg/ml
Strawberry	28.2 \pm 31.5	10.7 \pm 15.1	32.2 \pm 26.8	43.2 \pm 35.8
	to 53.9 \pm 37.8 ¹	to 33.7 \pm 39.9 ²	to 38.7 \pm 33.4	to 35.4 \pm 31.5
	Leaf disk treated with hexenyl acetate		Leaf disk treated with hexenol	
	1 $\mu\text{l/ml}$	10^{-3} $\mu\text{l/ml}$	1 $\mu\text{l/ml}$	10^{-3} $\mu\text{l/ml}$
Strawberry	16.9 \pm 22.7	16.3 \pm 23.8	23.7 \pm 25.5	22.6 \pm 26.2
	to 27.0 \pm 35.7	to 21.1 \pm 32.6	to 22.4 \pm 22.3	to 15.6 \pm 17.9

¹ Significant difference at $p=0.05$ by t -test

² Significant difference at $p=0.05$ by Mann Whitney test

Table 10. Feeding preferences (%) for compound treated rhododendron leaf disks (two-choice)

	Leaf disk treated with menthone		Leaf disk treated with menthol	
	1 µl/ml	10 ⁻³ µl/ml	1 mg/ml	10 ⁻³ mg/ml
Rhododendron	3.5±11.1	0.2±0.6	1.9±5.9	7.2±12.6
	to 4.3±7.7	to 4.8±9.9	to 1.5±4.1	to 5.4±10.9
	Leaf disk treated with hexenyl acetate		Leaf disk treated with hexenol	
	1 µl/ml	10 ⁻³ µl/ml	1 µl/ml	10 ⁻³ µl/ml
Rhododendron	9.8±13.3	5.2±8.6	9.4±10.2	13.0±18.7
	to 20.4±15.4	to 3.3±6.7	to 5.4±9.0	to 9.6±9.8

Discussion

Weevil oviposition and egg viability on different strawberry cultivars

Fecundity varied greatly among individual weevils, which generated large standard deviations. For example, oviposition of field-collected weevils fed on the strawberry cultivar Annapolis was 592±172 eggs/weevil with a range from 391 to 909 (Table 1). This variation may have been influenced by weevils' egg laying history. Although all adult weevils were collected from one strawberry field, the sampling time lasted from June to July. Therefore, their egg-laying histories were unknown. Some of them might have laid eggs, and some might be in their preoviposition periods (Masiuk, 2003; Shetlar, 2000).

Another possible reason for the variation is due to the individual difference. Artificial-diet-reared weevils emerged over a couple of days, and their egg-laying

histories were known. They had not laid eggs prior to these bioassays, and the mean oviposition was 536 ± 258 (range from 126 to 1071) similar to field-collected weevils' (Table 1). This shows that the difference among individual weevils in oviposition is important. In this weevil study, variability occurred in other bioassays, too, which will be discussed later. Therefore, controls, efficient replications, and statistical analysis played important roles in dealing with variability, increasing data reliability, and ensuring proper conclusions in the weevil research.

Strawberry cultivars, in some cases, influenced weevil success, measured by oviposition and egg viability. When weevils fed on cultivar 'Cavendish', they had the highest mortality, and showed lower oviposition than on 'Annapolis'. These results are similar to those reported by Cram (1980). Cram found that there were significant differences among strawberry cultivars, such as 'BC 70-20R-15', 'Shuksan', and 'Tyee' in the number of eggs laid during a ten-week period and in the number of larvae that hatched. During the study, it was clear that strawberry cultivar Cavendish appeared to have some resistance to weevils, while 'Evangeline' and especially 'Annapolis' were more susceptible. This is somewhat surprising as 'Annapolis' was thought to be tolerant to weevils. Therefore, strawberry cultivars like Cavendish, that are more resistant to weevils are available and could be incorporated into management strategies for the weevil.

In this study, the highest record of oviposition was 909 eggs laid by one of the field-collected weevils fed on 'Annapolis', which is the same as Baker (2003) mentioned in that study (863 eggs). The average number of eggs laid by the 37 weevils in 21-week period was 389 which are higher than 200 eggs (the observation time period was not mentioned) recorded in Baker's study (2003).

One of variables influencing egg viability was mold even though eggs used were

disinfected by 1:10 bleach before being introduced into Petri dishes. Mold contamination could not be avoided completely during the four-week hatch period. According to previous research, weevil eggs hatch in 2-3 weeks (Baker, 2003), so it may be better to consider a shorter hatch period of two or three weeks in future studies rather than four weeks in this study.

Weevil plant selection, oviposition and feeding preferences for plants

In this study, weevil plant selection was tested by observing how many weevils were on yew, rhododendron, geranium or strawberry leaves after 30 minutes, 2 hours and 24 hours. In the results, the 24-hour starved weevils preferred yew to geranium, strawberry, and rhododendron 30 minutes after been introduced into test containers, while 24 hours later, many more were found under big oval-shaped rhododendron leaves than narrow needle-shaped yew. There was a significant difference in the location of weevils between yew and rhododendron when the data for 30 minutes were compared to that for 24 hours (Fig. 8). When the weevils were starving, searching for food and feeding on it was their first purpose even though the assays were conducted in day time. Weevils normally feed at night (Kowalsick, 2003), so this clearly shows their host preference. In contrary, 24 hours after (still in day time), they were fed, so hiding and resting became their main goals as if they hid near host plants in fields, which is less likely to show their feeding preference for host plants.

Weevil oviposition results were similar with a preference for yew, geranium, and strawberry over rhododendron (Table 3) no matter how many weevils hid under rhododendron at the 24 hour time period. Therefore, host selection, feeding and oviposition tests indicated that insects, like the weevil, depend upon attractants to guide

them to their food-host plants, and then deposit their eggs on the plants (Khanizadeh and Belanger, 1993). Rhododendron played a role more as weevil's shelter than the favorite food and oviposition site in this study.

Hanula (1988) observed that weevils preferred ovipositing in containers with *Taxus* (yew) foliage over ten other hosts tested (including rhododendron, strawberry and hemlock), regardless of which host they were reared on during their preoviposition period. This previous study also found that weevils preferred ovipositing in containers with strawberry foliage over those containing rhododendron when these hosts were paired and suggested that preference hosts may contain an oviposition stimulant (Hanula, 1988). The results in this thesis support Hanula's idea that yew and strawberry may contain an oviposition stimulant because weevils preferred yew when they were hungry, fed more on yew and strawberry, as well as laid more eggs on yew and geranium than on rhododendron which is similar to the number of eggs laid on strawberry.

Not surprisingly, weevils consumed similar amounts of leaf material of the five plants, yew, rhododendron, geranium, strawberry and peppermint when given no choice. However, once weevils had a choice, significant differences occurred among the species offered and the results showed that weevils are selective feeders. This observation was also made by Jonsson (2005) although it was reported that the weevil could feed on a wide variety of different kinds of plants (Smith, 1932). This again indicates that the ability of the weevil to feed on different plants, especially when they have little choice is an important factor in the successful survival of this flightless weevil species in different habitats (Tol *et al.*, 2004).

Olfactory responses to plant leaf disks

Yew produced the smallest weevil olfactory response compared with other plants, which was similar to the 2-choice feeding tests, but different from the results of the 4-choice feeding tests and oviposition studies. It is possible that there was a mismatch between weevil feeding preference and olfactory response, as found in the previous study of Tol, *et al.*, (2004), who reported that the weevil perceived suitability of a host only by contact or feeding.

Another possible explanation would be because of the quality of yew leaves used in the two-choice test. Yew plants were kept under cool conditions during the study period, so the quality might not be as good as those used in 4-choice feeding preference and oviposition tests. However, the results determined that the best host was strawberry and the poorest was peppermint. This provided a basis for further study of weevil host plants and the effects of their volatile compounds on adult weevils.

In olfactory assays, the method of introducing weevils into the olfactometer is critical. During the introduction, when weevils were handled, they either became very active or played dead, which might postpone the reaction to the stimuli offered to them, especially even over the short test period (2 minutes). Other unknown parameters, such as, how long weevils would respond to the stimulant, how many replicates should be set up, and if light or shadows in the laboratory would interrupt weevils' actions, must be assessed to ensure weevils have an equal opportunity to either go to the left cell or the right one, as well as to eliminate any unexpected variables.

Plant volatiles and the effects on adult weevils

In previous studies, the abundant volatile compounds in strawberry leaves included

hexenol, hexenyl acetate, linalool, nonanal, and 1-hexanol (Table 11). Most of these compounds caused a strong olfactory antennal response in the weevil (Tol *et al.*, 2002). Hexenol and hexenyl acetate (green leaf volatiles, GLV) were two of most abundant compounds identified from strawberry leaves by both Hamilton-Kemp *et al* (1998), and Khanizadeh and Belanger (1993), as well as identified from the leaves of strawberry ‘Annapolis’ in the study of the thesis. These GLVs also showed large electroantennogram (EAG) responses in the weevil according to Tol *et al.*, (2002) (Table 12). Therefore, these two volatiles, in addition to menthone and menthol, known insect repellents from peppermint (Ansari, *et al.*, 2000), were used in this study with effects of volatiles on the weevil.

Table 11. A comparison of volatile compounds in strawberry leaves

Compound	Hamilton-Kemp <i>et al</i> composition %	Khanizadeh <i>et al</i> composition %(3 cultivars)	This study composition %	Tol and Visser EAG ¹ peak response
Hexenol	60.3	2.0-5.1	6.6	92
Hexenyl acetate	24.5	3.0-5.0	21.0	<20
Linalool	8.4	16.1-18.8	/	54
1-Hexanol	1.9	/	1.5	77
2-Phenylethanol	1.7	/	/	/
Benzyl alcohol	1.6	/	/	62
trans-2-Hexen-1-ol	0.8	/	/	93
Methyl salicylate	0.3	/	/	/
11-Octen-3-ol	0.3	/	/	/
Hexyl acetate	0.2	/	/	/
nonanal	/ ²	5.9-16.6	/	/
α -terpineol	/	4.3-7.5	/	44
β -cyclocitral	/	3.5-5.8	/	/
C ₉ H ₁₀ O ₂	/	1.5-8.3	/	/
(E)-2-hexenal	/	1.5-2.4	/	58
Nonen-1-ol	/	/	3.1	/
2-Ethylhexanol	/	/	9.7	>40
Phenol	/	/	2.7	/
2-Ethyl furan	/	/	7.9	/

¹ EAG: electro-antennogram peak response to compounds

² /: no data

The composition of volatile mixtures in earlier studies is different because of the use of various GC analytical conditions, and the different strawberry cultivars analyzed. For example, in this study, volatile compounds were obtained from the headspace over the strawberry 'Annapolis' leaves, pumped into Tenax traps, and then desorbed to a GC column directly. There was no solvent involved, so the sample procedure is simple, and has low loss or damage to volatile compounds. In Hamilton-Kemp's (1988) report, headspace strawberry leaf samples in Tenax traps were washed with hexane and then the hexane-extracted and concentrated hexane extracts submitted to GC analysis. Khanizadeh and Belanger (1993) steamed strawberry cultivar 'Bounty', 'Honeoye', or 'Kent' leaves in distilled water, used hexane as the solvent and evaporated hexane to 2 ml before GC separation and identification.

The most significant factor found is that not only the compounds themselves, but their concentrations alter weevil behavior in this study, as weevils were more strongly attracted to hexenyl acetate at concentrations 10^{-3} and 10^{-6} $\mu\text{l/ml}$, but at concentration $1 \mu\text{l/ml}$, weevils were repelled. A similar repellent result was observed for hexenol at a concentration $1 \mu\text{l/ml}$. This phenomenon was obvious in the olfactory bio-assays. Most of the weevils avoided the cell with compound ($1 \mu\text{l/ml}$) and walked to the cell without compound quickly when they were introduced into olfactometers. Interestingly, the concentration $10^{-3} \mu\text{l/ml}$ is very close to the natural levels of hexenol and hexenyl acetate in strawberry. The amounts were 0.12-0.33 $\mu\text{g/g}$ hexenol, 0.20-0.46 $\mu\text{g/g}$ hexenyl acetate in strawberry fruits according to Kafakas and Kafakas (2005). Therefore, besides volatile compounds, the concentrations are another important consideration when doing further research in laboratory and applying volatile compounds to an effective and economical weevil control in fields.

Menthone showed obvious repellent effects on weevil olfactory responses and feeding preferences at relatively high concentrations 1 and 10^{-3} μ l/ml. This result showed that some volatile compounds in peppermint oil can repel agricultural pests, like the weevil, as Apperson and Waldvogel (2002), Thorsell (2005) and Ansari *et al.* (2000) reported the repellent effects on mosquitoes and ticks. However, menthol did not show significantly repellent effects on weevils although Apperson and Waldvogel (2002) reported 81% of the component was menthol and only (5.8%) was menthone in peppermint oil. It suggests that certain volatile compounds with abundant products in plants might not affect weevil behavior.

In the future, it is possible to consider not only single volatile compound effects, but the effects of blends of host related volatiles on the weevil. Jonsson (2005) and Tol *et al.* (2002) suggested that bioassays of attractive plant extracts, GC combined with electro antennogram detection (GC-EAD) (Table 12), could further determine the individual compounds and their mixtures that are attractive to the weevil. In this plant volatile study, the combinations to consider for further trial should include repellent plant extracts, as well as the attractive plant extracts that Jonsson (2005) mentioned. Another step would be to evaluate volatile compound residues. That is, how long do the effects of volatiles on adult weevils last and what are appropriate application techniques? It may also be possible to improve monitoring by including attractive plant volatiles (Tol *et al.*, 2002) in traps such as pitfalls (Fisher *et al.*, 2005), or to keep weevils away by applying repellent volatiles on strawberry plants in fields. This would be especially useful during the preoviposition period.

The interaction between the black vine weevil and its hosts is complicated because of the unique biological features of weevils such as the flightless adults, parthenogenic

reproduction and the ability to feed on many different plants. Therefore, obtaining a better understanding of the weevil and its hosts will be a very interesting hard job in the future.

Conclusion

In this research, differences in black vine weevil success and host choice were observed. Strawberry cultivar affected weevil reproductive ability, so it suggested that growers could choose the cultivars with resistance and/or tolerance to the weevil. Plant type also influenced weevil host choice. Yew and strawberry were weevil's favorite hosts, while rhododendron, geranium and peppermint were not suitable hosts. The plant volatile, hexenyl acetate, found in strawberry leaves attracted weevils at relatively low concentrations, while at higher concentrations repelled weevils as did menthone (from peppermint). These results are promising for the use of plant volatiles in the weevil management. Furthermore, the use of natural plant compounds, plant volatiles, could result in the elimination or minimal use of industrial insecticides, and thus reduce the actual or perceived human health risk of weevil management strategies when incorporated with other weevil population control techniques.

References

- Anonymous. 1998. Strawberry root weevil.
<http://mint.ippc.orst.edu/ipmpdemo.htm>.10/20/2005.
- Ansari, M. A., P. Vasudevan, M. Tandon and R. K. Razdan. 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Bioresource Technology. 71 (3): 267-271
- Apperson, C. S. and M. G. Waldvogel. 2002. Insect repellent products.
<http://ces.ncsu.edu/depts/ent/notes/Urban/repel.htm>.2/23/2005.

- Baker, J. R. 2003. Black vine weevil. <http://ces.ncsu.edu/depts/ent/notes/O&T/shrubs/note66/note66.html>.10/20/2005.
- Bruce, T., J. Pickett and L. Smart. 2003. Cis-jasmone switches on plant defense against insects. Royal Society of Chemistry 2003: 96-98.
- Cowles, R. S. 1995. Black vine weevil biology and management. J. Amer. Rhododendron Soc. Spring: 83-85, 94-97
- Cowles, R. S. 2004. Susceptibility of strawberry cultivars to the vine weevil *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Agricultural and Forest Entomology 6: 279-284.
- Cram, W. T. 1980. Fecundity of the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), fed foliage from some current cultivars and advanced selections of strawberry in British Columbia. J Entomol Soc BC 77:25-26.
- Day, E. R. and E. Lewis. 2003. Black vine weevil. <http://ext.vt.edu/pubs/entomology/444-210/444-210.html>.10/20/2005.
- Doss, R. P. 1983. Root weevil feeding on *Rhododendron*: A review. Journal of Environmental Horticulture 1: 67-71.
- Dudareva, N., E. Pichersky and J. Gershenzon. 2004. Biochemistry of Plant Volatiles. Plant Physiology 135:1893-1902
- Erdemoglu, N., Sener B., Demirci B. and Baser K.H.C. 2003. The glycosidically bound volatile compounds of *Taxus baccata*. Chemistry of Natural Compounds, 39:195-198.
- Fisher, J. R. and D. J. Bruck. 2004. A technique for continuous mass rearing of the black vine weevil, *Otiorhynchus sulcatus*. Entomol. Appl. 113: 71-75.
- Fisher, P. 2004. Root weevils in strawberries: have you seen them? <http://gov.on.ca/OMAFRA/english/crops/hort/news/hortmatt.05/17/2004>
- Fisher, P., H. Fraser, K. MacKenzie, J. Tolman, T. Sawinski and P. Bouchard. 2005. Root weevils in Ontario strawberry fields. <http://omafra.gov.on.ca/english/crops/hort/news/hortmatt/2005/06hrt05a9.htm>.10/20/2005.
- Hamilton-Kemp, T. R. R. A. Andersen, J. G. Rodriguez, J. H. Loughrin, and C. G. Patterson. 1988. Strawberry foliage headspace vapor components at periods of susceptibility and resistance to *Tetranychus urticae* KOCH. Journal of Chemical Ecology, 4(3): 789-796.
- Hanula J. L. 1988. Oviposition preference and host recognition by the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Environ Entomol 17: 694-698.
- Jonsson, M. 2005. Responses to oilseed rape and cotton volatiles in insect herbivores and

parasitoids. Ph. D. dissertation. Swedish University of Agricultural Sciences, SLU
P.O. Box 7070 SE-750 07 Uppsala SWEDEN. Page: 1-41

Kafakas, E. and S. Kafakas. 2005. Comparison of methodologies for aroma compounds in strawberry. Turk J. Agric. For. 29 (2005): 383-390.

Khanizadeh, S. and A. Belanger. 1993. Analysis of the essential oil of the leaves of *Fragaria x ananassa* Duch. J. Essent. Oil Res. 5: 109-111.

Knudsen, J. T., Tollsten, L. and Bergstrom, G. 1993. Floral scents-a checklist of volatile compounds isolated by headspace techniques. Phytochemistry. 33: 253-280.

Kowalsick, Tom. 2003. Black vine weevil.
<http://cce.cornell.edu/suffolk/grownet/tree-insect/blvnwvl.html>.10/20/2005

Masiuk, M.. 2003. Black vine weevil fact sheet-woody ornamental integrated pest management at Penn State.
http://woodypests.cas.psu.edu/FactSheets/InsectFactSheets/html/Black_Vine_Weevil.html.10/20/2005.

Miller, J. R. 2003. Step-by-step examples, GraphPad Prism 4. 999-2003 GraphPad Software, Inc., San Diego CA, pp 206

Pare, P. W. and Tumlinson, J. H. 1999. Plant volatiles as a defense against insect herbivores. Plant Physiology, 121: 325-331.

Perveen, S. Shahida, T.M. Qaisrani, F. Siddiqui, R. Perveen and S. H. M. Naqvi. 2001. Cotton plant volatiles and insects behavior. Pakistan Journal of Biological Sciences 4 (5): 554-558.

Pichersky, E. 2004. Plant biology: on plant volatiles. American Scientist. 2004 92:514

Roseland, C.R., Bates, M.B., Carlson, R.B. and Oseto, T.Y. 1992. Discrimination of sunflower volatiles by the red sunflower seed weevil. Entomologia Experimentalis et Applicata 62: 99-106.

Ruedi, P. 2003. Analysis of the volatile components of five Turkish *Rhododendron* species by headspace solid-phase microextraction and GC-MS (HS-SPME-GC-MS). Z. Naturforsch. 58c: 797-803.

Scanlan, J. 2005. Award for new approach to cotton pest control.
<http://une.edu.au/news/archives/000260.html>.11/24/2005.

Shanks, C. H., Jr. 1980. Strawberry and yew as hosts of adult black vine weevil and effects on oviposition and development of progeny. Environ. Entomol. 9: 530-532.

Shanks, C. H., JR., Doss, R. P. 1986. Black vine weevil (Coleoptera: Curculionidae)

feeding and oviposition on leaves of weevil-resistant and -susceptible strawberry clones presented in various quantities. *Environ. Entomol.* 15: 1074-1077.

Shetlar, D. J. 2000. Black vine weevil (and other root weevils).
<http://ohioline.osu.edu/hyg-fact/2000/2016.html.03/03/2005>

Smith, F. F. 1932. Biology and control of the black vine weevil. Technical bulletin 325, U.S. Dept. of Agriculture, pp 45.

Thorsell, W., A. Mikiver and H. Tunon. 2005. Repelling properties of some plant materials on the tick *Ixodes ricinus* L. *Phytomedicine* 13 (1-2): 132-134.

Tol, R. W. H. M. Van, N. van Dijk and M. W. Sabelis. 2004. Host plant preference and performance of the vine weevil *Otiorhynchus sulcatus*. *Agricultural and Forest Entomology*. 6: 267-278.

Tol, R. W. H. M. Van and J. H. Visser. 1998. Host-plant preference and antennal responses of the black vine weevil (*Otiorhynchus sulcatus*) to plant volatiles. *Proceedings of the Section Experimental and Applied Entomology*, N. E. V. Amsterdam. 9: 35-40.

Tol, R. W. H. M. Van and J. H. Visser. 2002. Olfactory antennal responses of the vine weevil *Otiorhynchus sulcatus* to plant volatiles. *Entomologia Experimentalis et Applicata*. 102: 49-64.

Tol, R. W. H. M. Van, J. H. Visser and M. W. Sabelis. 2002. Olfactory responses of the vine weevil, *Otiorhynchus sulcatus*, to tree odours. *Physiological Entomology* (2002). 27: 213-222.

Vissotto, S. 1998. How plants can help us to control insects in croplands.
http://colostate.edu/Depts/Entomology/courses/en570/papers_1998/vissott.htm.11/24/2005

Weis, P. H. 1998. Poison-free agriculture- Plants repel insect pests.
<http://truehealth.org/agnew04.html.11/22/2005>.

Zar, J. H. 1999. *Biostatistical Analysis*. Prentice-Hall. Inc., Upper Saddle River, New Jersey.