INTERACTION BETWEEN INSECTS AND

APPLE (*Malus ×domestica Borkh.*): INSECT BEHAVIOR,

GENOTYPIC PREFERENCE, AND PLANT PHENOLICS WITH

EMPHASIS ON JAPANESE BEETLE (*Popillia japonica* Newman)

By

Sirasak Teparkum

Dissertation submitted to the

Faculty of the Virginia Polytechnic Institute and State University

In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (Ph.D)

IN

HORTICULTURE

APPROVED

—————————————

Richard P. Marini, Chairman

—————————————   —————————————
John A. Barden   Richard E. Veilleux

—————————————   —————————————
Douglas G. Pfeiffer   L.T. Kok

May , 2000

Blacksburg, Virginia

Keywords: *Malus domestica*, Refined oil, Japanese beetle, Trichome, Y-tube

olfactometer

INTERACTIONS BETWEEN INSECTS AND APPLE (*Malus domestica Borkh.*): INSECT BEHAVIOR, GENOTYPIC PREFERENCE, AND PLANT PHENOLICS WITH EMPHASIS ON JAPANESE BEETLE (*Popillia japonica* Newman)

by

Sirasak Teparkum

Richard P. Marini, Chairman

Horticulture

(**ABSTRACT**)

Leaves and fruit of nine apple (*Malus x domestica* Borkh.) genotypes were evaluated for insect injury in 1998 and 1999. Foliar and fruit injury from 12 insect species was inconsistently affected by genotype. Spraying trees with oil affected neither fruit insect injury nor fruit phytotoxicity.

In choice feeding assays, incidence of Japanese beetle (JB) feeding and leaf area consumed was greater for 'Liberty' than for 'York.' Genotypes did not differ in no-choice feeding assays. Choice and no-choice feeding assays between apple and oak indicated that JB could distinguish host plants in an artificial environment. Trichome density appeared different among three genotypes. 'York', the non-preferred genotype, had highest specific leaf weight and concentration of phloridzin, a feeding repellent. 'Liberty' the preferred genotype, had the lowest specific leaf weight, and had the highest concentration of quercitrin, a feeding stimulant.

Olfactory stimuli of JB was evaluated with a Y-tube olfactometer. Beetles preferred the side of the Y-tube containing leaf tissue of apple or Virginia creeper over the side with no leaf. Beetles did not choose one plant species over the other. Bias test of beetle orientation in the Y-tube olfactometer indicated that in the morning, but not the

afternoon, beetles preferentially moved into the left side of the Y-tube. Humidity did not affect beetle orientation. In darkness JB preferred a leaf disc over a paper disc and beetles tended to remain on the leaf.

ACKNOWLEDGEMENTS

I would like to express my most honest gratitude to:

Dr. Richard P. Marini, who is my advisor and my guidance during the past three years. He has bestowed me the opportunity to work on my dissertation where its topic has been my curiosity for a long time. The completion of this dissertation will be impossible without his assistance;

My committee members, Dr. Richard E. Veilleux, Dr. John Barden, Dr. Douglas Pfeiffer, and Dr. L.T. Kok for their valuable and countless suggestions to improve the quality of this research;

Dr. Richard Helm and Lei Zhiengtien who provided equipments and technical support for analysis of phenolic compounds;

Donnie Sowers, a lab technician, for his help in fieldwork and whenever I had technical problems;

Finally, my family, particularly to my father and my aunt, Sermsakdi and Sompun Teparkum for their financial support, unconditional love, and encouragement that made my education possible.

Thank you very much.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 2

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Apple (*Malus x domestica* Borkh.) is possibly one of the most widely grown deciduous fruit trees in the world. The origin of apple is not exactly known, but it grows widely in the region of south Caucasus, from the Caspian Sea to the Black Sea.Superior apple cultivars have been vegetatively propagated in Europe for 2000 years. Apple seeds and grafted trees were brought to America by colonists (Smock and Neubert, 1950). Apple was commonly cultivated in the gardens, providing preserves, confections, and beverages in addition to being a food staple (Croft, 1983).

Nowadays, apple pests are extensively studied because of the tremendous effect on the tree and fruit, the diversity of ecology associated with the crop, and the environmental problem of heavy pesticide application to produce a marketable crop.

Apple has more than 400 direct and indirect insect pests worldwide (Slingerland and Crosby, 1930). In the United States, apple is heavily attacked by pests throughout all fruit growing regions. Over 760 species of insects and mites were found in Wisconsin apple orchards (Oatman et al., 1964). Some of them were incidental migrants or natural enemies. Approximately 100 species of those insects fed on the tree or fruit. More than 46 were economically important, but only 10 were considered serious pests (Oatman et al., 1964).

Since consumers place a high value on the cosmetic appeal of fresh market apples, the product must be almost blemish-free. Successful pest control involves applying pesticides repeatedly during a growing season. These fruits and the orchard environment receive heavy exposure to pesticides. Based on the total volume of nationally applied

pesticides in 1978, apple rated sixth among individual crops in the United States

(Pimentel et al. 1978). For the total apple acreage in the United States, more than 95%

was sprayed. The total amounts of 12 million kg of pesticide used on apple, about 28%

(3-4 million kg) are fungicides, 65% (7-8 million kg) are insecticides, and less than 5-6%

(1 million kg) are herbicides (Croft, 1983). On a per-hectare basis, apple probably

receives during a growing season the highest amounts of pesticide of any major crop

(Pimentel et al., 1978). In some areas it is possible for 20-30 different chemicals to be

applied annually in 10-15 applications.

Extensive pesticide use may adversely affect the environment. The ecosystem,

man, and other animals may be affected. The problems are not only the environmental

issues, but also the cost of pesticide application, energy requirements for pest control, and

pesticide resistance among insects. Therefore, the levels of pesticide use have been of

concern for more than 25 years. Pesticide accumulation in the soil also is a consideration

because apple is a perennial crop. Orchards become habitats of birds and other wildlife

which could be affected by pesticide residues. Many apple orchards are located near large

bodies of water such as the Great Lakes in Michigan and New York, and the Columbia

River basin of central Washington because of the favorable growing conditions.

Therefore, pesticides used in orchards can have a major impact on aquatic life forms. For

these reasons there is interest in finding ways to produce high quality fruit with less

dependence on pesticides.

Host selection by insects

Entomologists have observed the behavior and life history of insects for centuries.

Since the beginning of the twentieth century, several theories have been advanced about

how an insect locates a plant. The first to propose the theory of insect host selection was Brues (1920). He proposed a "botanical instinct" theory, suggesting that "insects select host plants that meet specific nutritional and ecological requirements for that insect not offered by other plant species" (Brues, 1920). Later, Fraenkel (1959) proposed "the token stimuli theory", suggesting that "insect host plant selection is determined by specific secondary plant substances or phytochemicals, i.e. glycosides, phenols, tannins, alkaloids, terpenoids, and saponins." He theorized that monophagous insects might have developed from polyphagous insects to overcome the adverse effects of secondary plant substances.

Kennedy (1965) and Thorsteinson (1960) proposed that host selection is based on insect responses to both non-nutrient and nutrient phytochemicals. Their theories stemmed from the fact that many insects can be stimulated to feed by nutrient chemicals such as amino acids, carbohydrates, and vitamins (House, 1969; Hsiao, 1969).

Japanese beetle **(<u>Popillia</u> <u>japonica</u> Newman)**

The Japanese beetle was brought to the United States around 1912. It was found in a commercial nursery in southern New Jersey and was first identified in 1916 (Dickerson and Weiss, 1918; Fleming 1972a). Now, it has spread widely because of favorable climatic conditions, a lack of natural enemies, and the beetle's ability to fly for as far as 8 km.

Adult Japanese beetle has a wide host range including more than 300 species of plants in eastern North America (Fleming, 1972a). Among 300 host plant species in 80 families, extensive adult feeding occurs on 47 species: 14 in the Rosaceae, 5 in the Malvaceae, 4 in the Vitaceae, and 24 species spread across another 19 plant families (Fleming, 1972a). The adult beetles are indirect pests feeding on non-harvested parts of

the plant. They feed almost exclusively on the foliage of the plant unless the fruits have been previously damaged (Fleming, 1972a; Hogmire, 1995).

Japanese beetle has one generation per year. The average life span of the adult beetle is 30-45 days, and the larva generally completes development in one year except in colder regions where two years may be required. After hatching in the summer, mating and oviposition occur. The larvae feed on roots of grass and weeds in the top 10 cm of the soil in late summer, then migrate to a depth of 10-20 cm where they enter diapause to pass the winter months. The larvae return to within 10 cm of the surface in the spring to continue feeding and to pupate (Smith, 1922).

Aggregation occurs during Japanese beetle feeding on host plants. Iwabuchi and Takahashi (1983) found that both male and female beetles would attract males to form clusters on the branches. Sex pheromone was found and might cause the aggregation of males around females on the ground (Ladd, 1970).

Environmental conditions and sex of beetles affect the rate of feeding. Japanese beetles tend to feed in direct sunlight beginning at the top of a plant when temperatures range from 29-35 °C and the relative humidity is above 60% (Fleming 1972b). When the relative humidity is lower than 60%, a flight response is induced. Even though female beetles spend less time on the host plant than males, they feed more often in a given time period. Fleming (1972b) showed that in a six-hour period, females spent approximately twice the time feeding on apple or grape foliage as did males.

Host preference and morphological characteristics within a plant species also affect feeding. There may also be considerable variation between cultivars of a species in degree of resistance to damage (Langford and Cory, 1948; Stevenson, 1970).

Chemoreception in Japanese beetles: the response to odor, color, and phenolics

The possible cause of insect preference could be explained by the selection of an appropriate host for feeding, survival, development, and reproduction, which might involve not only choosing the right species of plant, but also selecting an individual genotype within that species (Bernays and Chapman, 1994).

Plant chemical constituents that influence insect behavior may be classified as attractant, repellent, feeding or oviposition stimulant, or deterrent (Bjostad and Hibbard, 1992; Carle et al., 1987; Fein et al. 1982; Mitchell et al., 1991; Nielsen et al., 1989; Bernays and Chapman, 1976; Blaney et al., 1988).

To locate a host from a distance, insects may require olfaction (sense of smell), vision, or both. Even though plant odors can be taxon-specific, the insect olfactory system is often capable of distinguishing these odors from others (Bell, 1984; Carde′, 1984). Shape and color of plant, however, are usually less characteristic because of the variations even within the species. Vision is also essential in the final stages of host selection for a flying insect because it necessarily uses vision during landing (Prokopy, 1968; Prokopy et al., 1983). When an insect finds a plant, it has to make the 'decision' of whether or not to accept it. Olfaction may still be as important as contact chemoreception (Walters et al., 1989) or mechanoreception (Roessingh and Stadler, 1990).

Results from several studies indicate that adult Japanese beetles are attracted by a variety of volatile plant compounds (Fleming, 1969; Schwartz and Hamilton, 1969; Ladd et al., 1973; Ladd, 1980; Ladd and McGovern, 1980). Most of these studies were concerned with the identification of attractants that could be used for the detection, elimination, and mass trapping of beetle populations.

Trapping studies indicate that adult Japanese beetles are attracted by a variety of unrelated plant compounds (Fleming, 1969; Schwartz and Hamilton, 1969). Several monoterpenes (especially geraniol) and phenolics (e.g., eugenol) are potent attractants. These compounds occur in preferred hosts such as rose and sassafras, among other plants (Langford et al., 1943). Several phenethyl esters, most notably phenethyl acetate and phenethyl propionate, attract large numbers of beetles (Ladd et al., 1973). A number of other plant compounds (e.g., some acids, alcohols, and essential oils) also act as attractants (Fleming, 1969).

The combination of two or more attractant compounds often shows the synergistic effect for luring Japanese beetles (Fleming, 1969; Ladd, 1980). Many mixtures of plant volatiles have been tested in an attempt to optimize the efficiency of traps. The more attractive mixtures invariably contain eugenol and/or geraniol. In open areas, chemical baits may attract beetles from a distance of up to 400 m (Mehrhof and Van Leeuwen, 1930; Schwartz, 1968). However, studies of Japanese beetle attractants often have reported only the numbers of insects captured in traps and have not yielded much information on the nature of orientation mechanisms or on the relative attractiveness of trap baits compared to host plants. Although the attracting compounds such as geraniol and eugenol are found in preferred host plants, their role in attracting beetles to plants has not been established.

Recent evidence indicates that olfaction plays a major role in host location by adult Japanese beetles. Ahmad (1982) demonstrated in laboratory choice tests that intact beetles located highly preferred foliage much more frequently than less preferred foliage, but antennectomized beetles (beetle with antennae removed) appeared to locate different

types of foliage at random. Evidently, Japanese beetles are attracted by a wide range of chemicals and chemical blends, and many of them are found in a large number of plant species. The Colorado potato beetle *Leptinotarsa decemlineata* (Say) also responds to volatile chemicals that are common to a wide variety of plants. Unlike the Japanese beetle, the potato beetle does not appear to be attracted by specific compounds; rather, a blend of nonspecific chemicals in a definite ratio may be required for attraction (May and Ahmad, 1983).

For the Japanese beetle, variation in plant chemistry still would result in variation in attractiveness among plant species. Accordingly, the Japanese beetle should be attracted by a large number of plant species, but some host species will be more attractive than others. Currently, relationships among host attractiveness, host acceptability, and host suitability, are not well known. Attractant compounds affect acceptability as well as attractiveness. Major and Tietz (1962) found that beetles normally would not eat when they were offered leaves of *Gingko biloba*. However, when *Gingko* leaves were treated with cherry leaf extract, eugenol, or valeric acid, the beetles ate them readily without any harmful effect. Also, compared to intact beetles, antennectomized beetles fed for a shorter period of time and the consumption was reduced, indicating a possible integration of olfactory and gustatory cues (Ahmad, 1982). Relationships between host acceptability and tenure on the foliage have not been determined. Sugar content also affects the acceptability of foliage (Metzger et al., 1934).

Japanese beetles locate hosts using factors other than plant chemistry. Foliage developing in the sunlight is more susceptible to attack than is foliage developing in shaded areas. Foliage in the interior of dense woodlots is attacked only rarely (Fleming,

1972b). The beetles are somewhat gregarious, and plants upon which beetles are feeding

are more attractive to host-seeking beetles than are plants that are devoid of beetles

(Fleming, 1972a). Aggregation may result partially from the sex pheromone of the female

beetles (Tumlinson et al., 1977).

Resistance of Malus *taxa to Japanese beetle*

Goonewardene et al. (1976) showed that some apple cultivars, bred for disease

resistance, also have resistance to European red mite. Also, they found that European red

mites seem to prefer pubescent apple leaves which may provide greater protection for

mites from the environment as well as chemical sprays. Ranney and Walgenbach (1992)

and Spicer et al. (1995) found that defoliation by adult Japanese beetle varied from 0%-

83% among 33 taxa of crabapples (*Malus* spp.) and feeding damage varied among many

of the same cultivars.

Neriifolin, a cardiotonic glycoside found in yellow oleander (*Thevetia thevetioides*

(HBK) K. Schum.), was identified as an effective antifeedant (allomone) for Japanese

beetle (Reed et al., 1982). Also, prunasin, herniarin, and coumarin were found to be

potential antifeedants for Japanese beetle and important factors in host plant resistance of

Prunus L. taxa.

Phenolic compounds, i.e., phloridzin which is mainly found in high concentration

in the foliage of *Malus* taxa, may play an important role in insect resistance (Hunter et al.

1994; Pree, 1977; Fulcher et al., 1996). Phloridzin, and its hydrolysis product phloretin,

were highly effective at deterring Japanese beetle with an edible dose (amount of

consumed substance not harmful to 50% of population- ED_{50}) of 7.1 and 9.3 mM

respectively. Naringenin reduced feeding with an ED_{50} of 32.9 mM. Catechin only deterred feeding at high concentrations. Conversely, rutin and quercetin were phagostimulants, increasing feeding by 174% of the control at 100 mM. Chlorogenic acid was mildly stimulating at low concentrations but deterred feeding at higher concentration. Kaempferol had no effect on feeding. Under choice conditions, phloridzin was a more effective deterrent than under no-choice conditions (Fulcher et al., 1998).

Fulcher et al. (1998) also demonstrated that *M. baccata* ' Jackii', *M.* x 'Hargozam' Harvest Gold[TM], and *M. transitoria* 'Schmitcutleaf' Golden Raindrops[TM] were highly resistant to Japanese beetle in a 'no-choice feeding assay' (< 2 cm^2/day leaf area consumed). To the contrary, *M.* x 'Radiant' was the most susceptible (7.6 cm^2/day leaf area consumed).

Insect control using refined petroleum oil

Refined petroleum oils have been used as insecticides for controlling mosquito larvae and fruit-tree pests for decades. They also are used as carriers of other insecticides. Since petroleum oil is phytotoxic, it must be highly refined before it can be applied to plants. The lower the viscosity (resistance to flowing) and distillation (boiling point) range, the less phytotoxic the oil. Light oils probably are less toxic to plants because they volatize more quickly than heavier oils and do not remain on the plant surface as long. The amount of unsaturated hydrocarbons in an oil also affects its phytotoxicity. Unsaturated hydrocarbons are unstable and combine to form compounds toxic to plants (Pedigo, 1996).

Oils are usually emulsified with water for application. Summer oils are the most highly refined and can be applied to trees in full foliage. The summer oils are usually not

as toxic to insects as dormant oils but are effective against mites and scale insects (Homoptera: Coccoidea) on citrus (Calabretta et al., 1985; Vacante, 1985). Dormant oils are less refined and, to prevent phytotoxic effects, are applied to fruit trees and ornamentals during late winter before budswell occurs. They are applied mostly to control scale insects, aphid eggs, and mite eggs which are overwintering on branches and twigs.

Oil applications are advantageous because they are inexpensive, simple to mix and use, and safe to mammals. Also, only few insects have become resistant to refined oils. However, the disadvantage of oil applications includes phytotoxicity, instability in storage, and ineffectiveness against certain pests.

Rationale and Significance

Although Japanese beetle is not one of the more destructive pests of apple, it could become a greater problem if chemicals (i.e., carbaryl) used to control beetles become unavailable. Therefore, it is essential to find alternative methods to control Japanese beetle as well as other apple arthropod pests. Breeding cultivars with insect resistance may be one approach. However, apple breeders must first know how Japanese beetles locate and distinguish between preferred and non-preferred hosts.

A first step is to screen genotypes for preference and non-preference to insects, and then to identify plant chemical constituents and morphological characteristics of leaves that are associated with preference. Breeders may then be able to use molecular techniques to find DNA markers for repellent genes to develop Japanese beetle resistant apple cultivars.

Objectives

The objectives of this study were 1) to evaluate the potential of growing apples using refined petroleum oils to control insects, 2) to determine the relative preference of ten apple genotypes to Japanese beetle feeding, and 3) to determine if feeding preference is related to leaf morphological characters and concentrations of phenolics.

Literature Cited

Ahmad, S. 1982. Host location by the Japanese beetle: Evidence for a key role for

    olfaction in a highly polyphagous insect J. Exp. Zool. 220: 117-120.

Bell, W.J. 1984. Chemo-orientation in walking insects. *In* Bell, W.J. and Carde, R.T.

    (eds.) *Chemical Ecology of Insects*. Chapman & Hall, New York, pp. 93-109.

Bernays, E.A. and R.F. Chapman. 1976. Deterrent chemicals as a basis of oligophagy in

    Locusta migratoria (L.) Ecol. Entomol. 2: 1-18.

Bernays, E.A. and R.F. Chapman. 1994. Behavior: the process of host-plant selection. *In*

    Bernays, E.A. and Chapman, R.F. *Host-plant selection by phytophagous insects*

    (eds.) Chapman & Hall, New York. pp. 95-165.

Bjostad, L.B., and B.E. Hibbard. 1992. 6-methoxy-2-benzoxazolinone: a semiochemical

    for host location by western corn rootworm larvae. J. Chem. Ecol. 18: 931-944.

Blaney, W.M., M.S.J. Simmonds, S.V. Ley and P.S. Jones. 1988. Insect antifeedants: a

    behavioral and electrophysiological investigation of natural and synthetically

    derived clerodane diterpenoids. Entomologia Exp. Appl. 46: 267-274.

Brues, C.F. 1920. The selection of food plants by insects with specific reference to

    lepidopterous larvae. Am. Nat. 54: 313-22.

Calabretta, C., S. Inserra, G. Ferlito, F. Couti. 1985. The action of white mineral oil

    against the fig wax scale (*Ceroplastes resci* (L.)) on cv. Moro and Tarocco

    oranges and its influence on the organoleptic characteristics of the fruit. *In* R.

    Cavallors, E. Di Martino (Eds.) Integrated pest control in citrus-groves;

    proceedings of the experts meeting, Acireale, 26-29 March 1985. pp. 447-453.

Carde′, R.T. 1984. Chemo-orientation in flying insects. *In* Bell, W.J. and Carde′, R.T. (eds.) *Chemical Ecology of Insects.* Chapman & Hall, New York, pp. 111-124.

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

Croft, B.A. 1983. Introduction. *In* B.A. Croft and S.C. Hoyt. (Eds.) Integrated management of insect pests of pome and stone fruits. John Wiley & Sons, New York, pp. 1-18.

Dickerson, E.L. and H.B. Weiss. 1918. *Popillia japonica* Newman, A recently introduced Japanese pest. J. Econ. Entomol. 30: 217-221.

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

Fleming, W.E. 1969. Attractants for Japanese beetle. U.S. Dep. Agric. Tech. Bull., No. 1399.

Fleming, W.E. 1972a. Biology of the Japanese beetle. U.S. Dept. Agric. Tech. Bull. No. 1449. 129 pp.

Fleming, W.E. 1972b. Preventing Japanese beetle dispersion by farm products and nursery stocks. U.S. Dept. Agric. Tech. Bull. No. 1441. 258 pp.

Fraenkel, G. 1959. Evaluation of our thoughts on secondary plant substances. Science. 129: 1466-1470.

Fulcher, A.F., T.G. Ranney, J.D. Burton, J.F. Walgenbach, and E.P. Maness. 1996. Natural resistance to Japanese beetle among *Malus* taxa: Role of endogenous

foliar phenolics. Proc. Southern Nurserymen's Res. Conf., 41st Annu. Rpt. p. 75-60.

Fulcher, A.F., T.G. Ranney, and J.D. Burton. 1998. Role of foliar phenolics in host plant resistance of *Malus* taxa to adult Japanese beetles. HortScience 33: 862-865

Goonewardene, H.F., E.B. Williams, W.F. Kwolek, and L.D. McCabe. 1976. Resistance to European red mite, *Panonychus ulmi* (Koch), in apple. J. Amer. Soc. Hort. Sci. 101:532-537.

Hogmire, H.W.Jr., e.d. 1995. Mid-Atlantic orchard monitoring guide. Northeast Reg., Agric. Engin. Serv., Ithaca, New York. p 36.

House, H.L. 1969. Effects of different proportions of nutrients on insects. Entomol. Exp. Appl. 12: 651-669.

Hsiao, T.H. 1969. Chemical basis of host selection and plant resistance in oligophagous insects. Entomol. Exp. Appl. 12: 777-778.

Hunter, M.D., L.A. Hull, and J.C. Schultz. 1994. Evaluation of resistance to tufted apple budmoth (Lepidoptera: Tortricidae) within and among apple cultivars. Environ. Entomol. 23: 282-291.

Iwabuchi, K. and J. Takahashi. 1983. Aggregative distribution pattern of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and the role of former occupants in the formation of an aggregation. Appl. Entomol. Zool. 18: 324-329.

Kennedy, J.S. 1965. Mechanisms of host plant selection. Ann. Appl. Biol. 56:317-22.

Ladd, T.L. 1970. Sex attraction in the Japanese beetle. J. Econ. Entomol. 63: 905-908.

Ladd, T.L., Jr. 1980. Japanese beetle: Enhancement of lures by eugenol and caproic acid. J. Econ. Entomol. 73: 718-720.

Ladd, T.L., and T.P. McGovern. 1980. Japanese beetle: A superior attractant, phenethyl propionate + eugenol + geraniol 3:7:3. J. Econ. Entomol. 73: 689-691.

Ladd, T.L., T.P. Jr. McGovern, M. Beroza, B.G. Townshend, M.G. Klein, and K.O. Lawrence. 1973. Japanese beetles: Phenethyl esters as attractants. J. Econ. Entomol. 66: 369-370.

Langford, G.S., and E.N. Cory. 1948. Host preference in Japanese beetles with special reference to grape and apple. J. Econ. Entomol. 41: 823-824.

Langford, G.S., M.H. Muma, and E.N. Cory. 1943. The attractiveness of certain plant constituents to the Japanese beetle. J. Econ. Entomol. 36: 248-252.

Major, R.T. and H.J. Tietz. 1962. Modification of the resistance of *Gingko biloba* leaves to attack by Japanese beetles. J. Econ. Entomol. 55: 272.

May, M.L. and S. Ahmad. 1983. Host location in Colorado potato beetle: Searching mechanisms in relation to oligophagy. *In* S. Ahmad (ed.) *Herbivorous insects: Host-seeking behavior and mechanisms*. Academic Press, New York. p. 173-195.

Mehrhof, F.E. and E.R. Van Leeuwen. 1930. An electric trap for killing Japanese beetles. J. Econ. Entomol. 23: 275-278.

Metzger, F.W., P.A. Vander Meullen, and C.W. Mell. 1934. The relation of sugar content and odor of clarified extracts of plants to their susceptibility to attack by Japanese beetle. J. Agric. Res. 49: 1001-1008.

Mitchell, E.R., F.C. Tingle and R.R. Heath. 1991. Flight activity of *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae) with reference to host-plant volatiles. J. Chem. Ecol. 17: 259-266.

Nielsen, J.K., A.H. Kirkeby-Thomsen, and M.K. Petersen. 1989. Host plant recognition in monophagous weevils: specificity in feeding responses of *Ceutorhynchus constrictus* and the variable effect of sinigrin. Entomol. exp. appl. 53: 157-166.

Oatman, E.R., E.F. Legner, and R.F. Brooks. 1964. An ecological study of arthropod populations on apple in northeastern Wisconsin: insect species present. J. Econ. Entomol. 57: 978-983.

Pedigo, L.P. 1996. Entomology and pest management. Prentice Hall, Upper Saddle River, New Jersey. Pp. 399.

Pimentel, D., J. Krammel, D. Gallahan, J. Hough, A. Merrill, I. Schreiner, P. Vittum, F. Koziol, E. Black, D. Yen, and S. Fiance. 1978. Benefits and costs of pesticide use in US food production. Bio Science. 28: 772, 778-784.

Pree, D.J. 1977. Resistance to development of larvae of the apple maggot in crab apples. J. Econ. Entomol. 70: 611-614.

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

Prokopy, R.J., R.H. Collier, and S. Finch. 1983. Visual detection of host plants by cabbage root flies. Entomologia Exp. Appl. 34: 85-89.

Ranney, T.G. and J.F. Walgenbach. 1992. Feeding preferences of Japanese beetles for taxa of birch, cherry, and crabapple. J. Environ. Hort. 10: 177-180.

Reed, D.K., B. Freedman, and T.L. Ladd, Jr. 1982. Insecticidal and antifeedant activity of neriifolin against codling moth, striped cucumber beetle, and Japanese beetle. J. Econ. Entomol. 75: 1093-1096.

Roessingh, P. and E. Stadler. 1990. Foliar form, color and surface characteristics influence oviposition behavior in the cabbage root fly *Delia radicum*. Entomol. exp. appl. 57: 93-100.

Schwartz, P.H. 1968. Distribution of released Japanese beetles in a grid of traps. J. Econ. Entomol. 61: 423-426.

Schwartz, P.H. and D.W. Hamilton. 1969. Attractants for the Japanese beetle. J. Econ. Entomol. 62: 516-517.

Slingerland, M.V. and C.R. Crosby. 1930. Manual of fruit insects. MacMillan, New York.

Smith, L.B. 1922. Larval food habits of the Japanese beetle (*Popillia japonica* Newm.). J. Econ. Entomol. 15: 305-310.

Smock R.M. and A.M. Neubert. 1950. *Apples and Apple Products*. Interscience Publishers, New York.

Spicer, P.G., D.A. Potter, and R.E., McNeil. 1995. Resistance of crabapple (*Malus* spp.) cultivars to defoliation by the Japanese beetle (Coleoptera: Scarabaeidae) J. Econ. Entomol. 88: 979-985.

Stevenson, A.B. 1970. Strains of the grape phylloxera in Ontario with different effects on the foliage of certain grape cultivars. J. Econ. Entomol. 63: 135-138.

Thorsteinson, A.J. 1960. Host selection in phytophagous insects. Annu. Rev. Entomol. 5:193-218.

Tumlinson, J.H., M.G. Klein, R.E. Doolittle, T.L. Ladd, and A.T. Proveaux. 1977.

>   Identification of the female Japanese beetle sex pheromone: Inhibition of male

>   response by an enantiomer. Science 197: 789-792.

Vacante, V. 1985. Influence of white mineral oil on the population dynamics of some

>   mites in a lemon orchard in Eastern Sicily. *In* R. Cavallors, E. Di Martino (Eds.)

>   Integrated pest control in citrus-groves; proceedings of the experts meeting,

>   Acireale, 26-29 March 1985. Pp. 423-431.

Walters, D.S., R. Craig, and R.O. Mumma. 1989. Glandular trichome exudate is the

>   critical factor in geranium resistance to foxglove aphid. Entomologia Exp. Appl.

>   53: 105-109.

CHAPTER 2: INCIDENCE OF INSECT INJURY ON LEAVES AND FRUITS OF NINE APPLE GENOTYPES (*Malus x domestica* BORKH.) AS AFFECTED BY REFINED OIL

Apple is heavily attacked by arthropod pests throughout the United States. Oatman et al. (1964) found over 760 species of insects and mites in apple orchards in Wisconsin including incidental migrants, natural enemies, common pests, and serious pests. Even though apple has many pests, consumers place a high value on the cosmetic appeal of fresh market apples and expect a nearly blemish-free product. Therefore, pesticides are applied repeatedly during a growing season, exposing orchards and fruit to large amounts of pesticides. Based on the total volume of applied pesticide, apple was rated one of the most heavily sprayed crops in the United States (Pimentel et al. 1978). In some areas it is possible for 20-30 different chemicals to be applied annually in 10-15 applications.

A recent objective of the United State Department of Agriculture (USDA) is to reduce the use of pesticides and promote sustainable agriculture (Wallace Institute, 1993). Government regulatory agencies are enforcing the Food Quality Protection Act (FQPA), and the Environmental Protection Agency (EPA) is reevaluating the registrations of all pesticides. Currently, registrations for organophosphates and carbamates are being revised. These two classes of chemicals represent about 75% of all insecticides used in tree fruit (Warner, 1998). Pressure to reduce pesticide use is likely to continue for the foreseeable future, thus apple cultivars with genetic resistance to disease and insects may become more important as the options for chemical control of apple pests become more

limited. However, because development and evaluation of such cultivars is a time consuming process, alternative methods of insect control must be evaluated.

Refined petroleum oils have been used to control mosquito larvae and some fruit-tree pests for decades. Oils are usually emulsified in water for application. The most highly refined oils are summer oils which can be applied to trees in full foliage. The summer oils are usually not as toxic to insects as dormant oils, but can be useful against mites and scale (Homoptera: Coccoidea) on citrus (Calabretta et al., 1985; Vacante, 1985).

Oil applications are inexpensive, easy to use, and environmentally safe. However, the disadvantages of oil application are phytotoxicity in some plants, instability in storage, and ineffectiveness for certain pests.

The objectives of this study were to determine the incidence of insect injury on leaves and fruit of nine apple genotypes and to evaluate refined oil for insect control.

Materials and Methods

Field observations, 1998. In 1998, field observations were made to evaluate insect injury to trees and fruit in a plot where trees were sprayed with fungicides and insecticides until late May 1998, at the Virginia Tech College of Agriculture and Life Sciences Kentland farm. This plot was established in May 1993. Nine apple genotypes on M.9 rootstock were randomized within 30 blocks. They were 'Delicious', 'Freedom', 'Golden Delicious', 'Liberty', 'NY 74840-1', 'NY 74828-12', 'NY 7334-35', 'Redfree', and 'York'. Trees were supported to 2m with a wooden post and were trained as central leaders with minimal pruning.

Injury of indirect pests was evaluated on 15 July (79 days after full bloom). Five shoots were randomly selected per tree. There were nine replications of each genotype. The number of leaves per shoot and number of leaves injured by potato leafhopper (*Empoasca fabae* Harris), spotted tentiform leafminer (*Phyllonorycter blancardella* Fabr.), and leafrollers were counted. Each tree was rated for Japanese beetle (*Popillia japonica* Newman) injury by visually estimating the percentage of leaves on the whole tree that was damaged. The percentage of leaves injured by each insect species was calculated for each shoot, and percentage data were analyzed as a RCBD with subsampling (5 shoots/tree) using the GLM Procedure of SAS (SAS Institute, 1992).

All fruit on half of each tree in four blocks were harvested on 29 July, and the other half of each tree was harvested on 26 Aug. The number of the fruit injured by redbanded leafroller (*Argyrotaenia velutinana* Walker), rosy apple aphid (*Dysaphis plantaginea* Passerini), Japanese beetle, tufted apple bud moth (*Platynota idaeusalis* Walker), plum curculio (*Conotrachelus nenuphar* (Herbst)), and codling moth (*Cydia pomonella* Linnaeus) was recorded. The percentage of fruit injured by each pest was calculated and data were analyzed as a RCBD with the GLM Procedure of SAS (SAS Institute, 1992).

Insect control with refined oil, 1999. The experiment was conducted on the same trees in 1999, but trees were not sprayed with pesticides at all. The experiment was a 3x9 factorial in a split-plot design. The field was divided into five replicates (whole-plots) and each replicate was further divided into three subplots. Subplots, containing one tree per genotype (total of 9 genotypes), were assigned randomly to one of the three oil treatments. Refined oil (Sun Spray 6E, Sun Company, Inc., Philadelphia, PA) was

sprayed to runoff with a back-pack sprayer every 10 days at the rate of 0, 4, and 8% (v/v) during June to late Sept. Indirect pest injury on leaves was evaluated as described for 1998. The percentage of defoliation and phytotoxicity due to oil was also visually evaluated for each tree.

To evaluate the fruit injury caused by direct pests and oil, all fruit on half of each tree were harvested on 9 Aug., and the other half on 6 Sept. The number of fruit injured by red banded leafroller, tufted apple bud moth, tarnished plant bug, (*Lygus lineolaris* Palisot de Beauvois), Japanese beetles, plum curculio, and fruit with non-insect symptoms (sun burn, raised lenticels, and sun scald) were recorded. Sunburn symptoms were separated into two different levels (severe or slight) and referred to skin discoloration of the fruit. Lenticels were considered raised if they could be felt by rubbing the fruit surface with fingers. Sunscald was visually evaluated by halo-discoloration symptoms on the sun-side of the fruit (covered about 10%). All non-insect symptoms were recorded as a binomial (yes or no) response. A fruit might have more than one symptom. The percentage of fruit with each type of injury was calculated, and data were analyzed as a split-plot design with the GLM Procedure and contrasts were used to evaluate oil concentration and the interaction of genotype $\times$ oil concentration. Due to the missing trees, Least Square (LS) means were calculated and they were compared using probability of the differences (P-diff).

Results and Discussion

Field Observations, 1998. In 1998, foliar injury from potato leafhopper, spotted tentiform leafminer, Japanese beetle, and percentage of total insect injury differed among genotypes (Table 1). Injury from leaf rollers was not influenced by genotypes. The injury

from potato leafhopper was separated into two forms; mottled leaves (slight injury) and stunted curled leaves (severe injury). 'Delicious' had a higher incidence of mottled leaves than 'Liberty' and 'NY 73334-35'. 'NY 74840-1' had a higher incidence of stunted curled leaves than 'Freedom', 'Liberty', and 'NY 73334-35'. 'NY 73334-35' had a higher incidence of spotted tentiform leafminer than all genotypes except 'Delicious.' The percentage of the leaves on the whole tree with damage from Japanese beetle was greater for 'Liberty' than other genotypes except for 'NY 73334-35' and 'Redfree.' The percentage of leaves with insect injury symptoms was higher for 'York' than for 'Freedom,' 'Liberty' and 'NY 73334-35' (Table 1).

On the first harvest in 1998, the percentages of fruit injured by redbanded leafroller, tufted apple bud moth, rosy apple aphid, plum curculio, and codling moth were not significantly different (P=0.05) for the nine genotypes (Table 2). However, the percentage of fruit injured by Japanese beetle was greater for 'Freedom' than the other genotypes. Although the percentage of fruit with insect injury ranged from 24% for 'Redfree' and 'Delicious' to 44% for 'Freedom', genotype did not significantly affect incidence of fruit injury (P=0.68).

Results differed slightly for the second harvest (25 Aug.). All 'Redfree' fruit had abscised by the second harvest. There was no significant difference in percentage of fruit injured by redbanded leafroller, tufted apple bud moth, and codling moth among apple genotypes (Table 3). The percentage of fruit injured by rosy apple aphid and plum curculio differed significantly for genotype (P<0.005). 'Delicious' had more rosy apple aphid injury (>10%) than all genotypes except 'NY 74840-1.' 'NY 74840-1' had more plum curculio injury than all other genotypes except 'Liberty' and 'NY 73334-35.'

Although significant at only the 7% level, the percentage of fruit with insect injury varied from 13% for 'NY 74828-12' to 35% for 'NY 74840-1' (Table 3).

As expected, results from the first harvest differed from the second because insect populations vary during the season and the variation of fruit sampling. Fruit at the first harvest had more redbanded leafroller injury (5.6 to 28.1%) than that of the second harvest (1.2 to 5.6%) (Table 2 and 3). The percentage of fruit injured by rosy apple aphid and plum curculio was higher on the second harvest date and differences were significant among genotypes for the second harvest. Injury from both rosy aphid and plum curculio injury occurs during the early season and would not be expected to increase in August. Injury from rosy apple aphid usually occurs in specific areas of the tree. Therefore, the effect of harvest date on incidence of injury was probably due to sampling. I have no explanation for differences in incidence of plum curculio injury between dates. No fruit harvested on the second date was injured by Japanese beetle. Japanese beetle usually attacked fruit that were already injured by other insects such as redbanded leafroller. Therefore, before the second harvest, injured fruit rotted and dropped on the ground.

Insect control using refined oil, 1999. In 1999, the percentage of mottled leaves caused by potato leafhopper was not influenced by oil, but the percentage of curled leaves increased with increasing oil concentration (Table 4). The percentage of leaves injured by spotted tentiform leafminer, white apple leafhopper, and locust leafminer was not influenced by oil treatments. The percentage of leaves on sample shoots and the percentage of foliage on the tree injured by Japanese beetle declined with increasing oil concentration (Table 4). The incidence of leaf injury on sample shoots due to potato leafhopper (mottled), Japanese beetles, and percentage of Japanese beetle injury on the

24

whole tree differed among the nine apple genotypes. For potato leafhopper, 'NY 74828-12,' 'NY 73334-35,' and 'York' had more mottled leaves than 'Delicious,' 'Golden Delicious,' and 'NY 74840-1' (Table 4). The percentage of leaves on sampled shoots with Japanese beetle feeding was greater for 'Liberty' and 'Redfree' than for all other genotypes. Japanese beetle injury on the whole tree was greater for 'Redfree' than all other genotypes except 'Liberty'. White apple leafhopper injured less than 1% of the leaves for all genotypes. No genotype had more than 1.9% of the leaves injured by locust leafminer. 'Liberty' also had a higher incidence of total insect injury than all genotypes except 'Redfree'.

There was a genotype × oil concentration interaction for leaf phytotoxicity ratings (Table 5). 'Golden Delicious' and 'NY 74828-12' were the most sensitive to oil, but phytotoxicity on all genotypes increased with increasing oil concentration. Defoliation was greatest for 'Liberty', 'NY 74828-12' and 'Redfree.' Defoliation was not significantly related to oil concentration for 'Delicious', 'Liberty', and 'York' ($P>0.05$), but defoliation increased linearly with increasing oil concentration for 'Freedom,' 'Golden Delicious,' 'NY 74840-1,' 'NY 74828-12,' 'NY 73334-35,' and 'Redfree' (Table 5).

Due to alternate bearing (a characteristic where some cultivars crop every other year) only six genotypes cropped in 1999. About 30% of the fruit harvested on 9 Aug. were injured by insects, and heat (Table 6). Some fruit were injured by more than one insect species or were injured by insects and heat. Averaged over all oil treatments, about 7.5% of the fruit had insect injury and about 23% were injured by heat. The percentage of fruit injured by an individual insect species was not affected by oil treatment. The

percentage of fruit with symptoms resulting from insect injuries or sunburn was not influenced by oil treatment.

The total percentage of fruit with some type of symptom was affected by the interaction of genotype and oil concentration (Table 6). Fruit symptoms were not affected by oil for all genotypes (Table 7). The percentage of fruit damaged by redbanded leafroller, tufted apple bud moth, tarnished plant bug, Japanese beetle, and plum curculio was not influenced by genotypes (Table 6). The percentage of fruit with sunburn (slight and severe) injury was affected by genotypes. 'Golden Delicious' and 'Liberty' were the least sensitive genotypes for sunburn symptoms (severe and slight). 'NY 73334-35' had the highest incidence of severe sunburn, whereas slight sunburn was more common on 'Delicious,' 'Freedom,' 'NY 73334-35,' and 'Redfree' than 'Golden Delicious' and 'Liberty.'

By the second harvest (6 Sept.), all 'Redfree' fruit had abscised, so fruit from only five genotypes were harvested (Table 8). Averaged over all treatments, about 55% of the fruit had some type of injury. About 5.5% of the fruit were damaged by insects, and about 50% of the fruit were injured by factors other than insects. The percentage of total fruit injury and total non-insect injury increased with increasing oil concentration (Table 8). Overall fruit injury differed among genotypes (P<0.05). 'Golden Delicious' had the highest incidence of fruit damage, and 'Liberty' had the least damage. Oil treatments did not affect most of the insect injuries except for plum curculio. Tufted apple bud moth, Japanese beetles, and plum curculio injury were influenced by genotypes. 'NY 73334-35' had more tufted apple bud moth injury than all other genotypes except 'Liberty'. 'Freedom' had the greatest percentage of Japanese beetle injury, and 'NY-73334-35' had

highest injury of plum curculio. Increasing oil concentration promoted skin roughness ('raised lenticel'). 'Golden Delicious' had highest incidence of raised lenticels. Sunscald was significantly affected by oil. The percentage of fruit with sunscald was highest for 'Delicious' and 'Freedom' but lowest for 'Golden Delicious' and 'Liberty' (Table 8).

Plant chemical constituents and leaf morphological characteristics influence insect behavior; these may be classified as attractant, repellent, feeding or oviposition stimulant, or deterrent (Langford and Cory, 1948; Stevenson, 1970; Bernays and Chapman, 1976; Fein et al., 1982; Carle et al., 1987; Blaney et al., 1988; Nielsen et al., 1989; Mitchell et al., 1991; Bjostad and Hibbard, 1992). Therefore, cultivars varying in these characteristics, may also posses considerable variation in degree of resistance to insect damage. Differential susceptibility of cultivars to insects and mites has been carefully evaluated for only a few pest species. Alm et al. (1985) demonstrated that the apple blotch leafminer could survive on all apple cultivars tested, but that it favored some cultivars over others when given a choice. Goonewardene et al. (1975) compared 74 scab-resistant apple selections, and found that all apple selections had similar counts of plum curculio feeding scars, but 22.9% of the selections had fewer larval exit holes than did 'Jonathan.' Furthermore, some crabapple cultivars were found resistant to plum curculio, Japanese beetle and three common apple diseases (Crassweller et al., 1980; Alm and Hall, 1986; Fulcher et al., 1998). Langford and Cory (1948) divided 59 apple cultivars according to the host preference of Japanese beetles into four groups; 1) preferred cultivars; 2) attractive cultivars; 3) cultivars frequently attacked; 4) unattractive cultivars. They reported that 'Delicious' was one of the 'preferred cultivars' and 'York' was one of the 'unattractive cultivars'. From the results in 1998 and 1999, we were not able to

27

differentiate the feeding injury of Japanese beetle for these two cultivars possibly because of low insect pressure and replications. Nevertheless, they were among the unattractive cultivars.

Results for insect injury on leaves and fruit in 1999 were quite different from the previous year, possibly because of inconsistency of insect populations. Generally, insect injury in 1999 was less than in 1998, except for Japanese beetle that caused higher leaf injury in 1999 (Table 1 and Table 4). In 1998, mottled leaves caused by potato leafhopper ranged from 12.1%, on 'NY 73334-35' to 18.1% on 'Delicious', and curled leaf symptoms ranged from 12.8% on 'NY 73334-35' to 32.5% on 'NY 74840-1' (Table 1). However, in 1999, the leaf injury from potato leafhopper was much less severe ranging from 0.4% on 'Golden Delicious' to 4.9% on 'NY 73334-35' for mottled leaf and 0.1% on 'Redfree' to 1.3% on 'NY 74828-12' for curled leaf (Table 4).

The repellency and toxicity of a petroleum-based oil was tested against indirect and direct apple pests in 1999. Oil did not affect foliar and fruit injury for most insects. However, foliar feeding by Japanese beetle was reduced at higher oil concentrations (Table 4). Phytotoxicity varied among genotypes and increased with increasing oil concentration (Table 5). Larew and Locke (1990) reported that petroleum-based proprietary horticultural oil, Sunspray 6E plus, at 2% effectively repelled adult whitefly (*Trialeurodes vaporariorum* Westwood) on chrysanthemums (*Dendranthema* × *grandiflorum* Ramat.), grown in a greenhouse or an environmental chamber, without phytotoxicity on foliage or stems. The contradiction of these results may be because their experiment focused only on one insect and was conducted in the control conditions (light and temperature); therefore, insects were more exposed to oil and less phytotoxicity

28

developed. Differing results may also be due to the different plant and insect species that were studied or to the number of oil applications made.

In 1999, non-insect fruit injury symptoms were different between two harvest dates. At the first harvest (9 Aug.) non-insect injuries were severe and slight sunburn, but on the second harvest (6 Sept.) raised lenticel and sunscald were observed. Fruit with severe, but not slight, sunburn probably dropped before the second harvest because these fruit were so seriously damaged that ethylene was produced and caused fruit abscission. Slight sunburn injured fruit could further develop the injury and were observed as fruit with sunscald symptoms or abscised by the second harvest. At the first harvest raised lenticels were not observed, but became obvious by the second harvest for 'Golden Delicious.' Therefore, raised lenticels and sunscald were only observed at the second harvest.

I had speculated that there is natural insect resistance among the nine apple genotypes and refined oil might control some insect species. However, the level of insect injury was too inconsistent and too low to properly test our hypothesis. High insect pressure is needed to identify genotypes that possess insect resistance. It would also be advantageous to use more replication and more genotypes with greater genetic diversity, in the screen.

Table 1. Percentage of leaves with feeding symptoms for potato leafhopper (PLH) (mottled and curled leaves), spotted tentiform leafminer (STLM), leafroller (LR), and Japanese beetle (JB) on nine apple genotypes in 1998.

| Genotypes | leaves/shoot | Leaves with injury symptoms by the following insects (%) | | | | | Total insect injury (%) |
| | | PLH | | STLM | LR | JB | |
		mottled	curled				
Delicious	25^z	18.1a^y	25.1abc	2.2ab	0.0	0.3b	45.4abc
Freedom	25	17.1ab	19.4cd	0.5bc	0.3	0.8b	37.3bcd
Gold Del.	23	15.0abc	28.8ab	0.4bc	0.4	0.6b	44.6abc
Liberty	25	12.8bc	22.2bc	0.8bc	0.4	6.6a	36.2cd
NY 74840-1	24	13.6abc	32.5a	0.3c	0.3	0.1b	46.7ab
NY 74828-12	22	15.0abc	29.7ab	0.8bc	0.1	0.9b	45.6abc
NY 73334-35	20	12.1c	12.8d	2.7a	0.2	2.3ab	27.9d
Redfree	24	17.4ab	26.3abc	0.9bc	0.3	2.1ab	44.9abc
York	25	17.1ab	29.6ab	0.6bc	0.4	0.1b	47.6a
P-values	---	0.001	0.001	0.001	0.679	0.001	0.001

^{z} Values are means of 5 shoots on each of 9 trees per genotype

^{y} Means followed by the same letter do not differ at the 5% level of significance, Tukey's studentized range (HSD) test.

Table 2. Percentage of fruit injured by redbanded leafroller (RBLR), tufted apple bud moth (TABM), rosy apple aphid (RAA), Japanese beetle (JB), plum curculio (PC), and codling moth (CM) in 9 apple genotypes when harvested on 29 July, 1998.

Genotypes	Fruit harvested/tree	Fruit injured by the following insects (%)						Insect injured fruit (%)
		RBLR	TABM	RAA	JB	PC	CM	
Delicious	9[z]	14.6	4.2	0.0	0.0b[y]	2.8	2.1	23.5
Freedom	40	28.1	2.0	0.0	11.5a	2.0	0.0	43.8
Gold Del.	31	5.6	6.4	2.9	0.0b	12.4	0.7	28.0
Liberty	109	12.8	8.9	0.7	2.2b	11.4	0.0	36.0
NY 74840-1	65	13.7	4.5	3.7	0.3b	12.2	0.0	34.5
NY 74828-12	74	11.9	5.1	0.9	0.0b	12.6	0.3	30.8
NY 73334-35	96	7.6	3.3	0.0	0.0b	16.7	0.0	27.3
Redfree	40	9.6	6.4	0.0	1.8b	5.7	0.0	23.5
York	42	14.0	10.4	1.6	0.4b	0.8	0.8	28.3
P-values	---	0.083	0.531	0.207	0.001	0.096	0.571	0.677

[z] Values are means of 4 trees per genotype

[y] Means followed by the same letter do not differ at the 5% level of significance, Tukey's studentized range (HSD) test.

Table 3.   Percentage of fruit injured by redbanded leafroller (RBLR), tufted apple bud

moth (TABM), rosy apple aphid (RAA), plum curculio (PC), and codling moth (CM) on

8 apple genotypes when harvested on 25 August, 1998.

Genotypes	Fruit harvested/tree	Fruit injured by the following insects (%)					Insect injured fruit (%)
		RBLR	TABM	RAA	PC	CM	
Delicious	28^z	1.3	0.0	17.6a^y	3.5c	3.9	25.6
Freedom	26	5.6	2.0	0.0b	6.9bc	2.9	17.4
Gold Del.	43	1.2	2.8	3.1b	8.0bc	1.6	16.7
Liberty	68	4.3	2.9	0.0b	18.1ab	0.9	28.3
NY 74840-1	61	3.9	2.4	7.2ab	22.2a	3.2	35.1
NY 74828-12	84	3.1	2.1	0.0b	8.1bc	1.1	13.3
NY 73334-35	79	1.6	1.0	0.0b	14.8abc	3.2	20.3
York	54	5.2	6.6	0.0b	7.0bc	1.4	20.4
P-values	---	0.570	0.117	0.003	0.001	0.727	0.069

^{z} Values are means of 4 trees

^{y} Means followed by the same letter do not differ at the 5% level of significance,

Tukey's studentized range (HSD) test.

Table 4. Main effect least squares (LS) means for the percentage of leaf injury from

potato leafhopper (mottled and curled), spotted tentiform leafminer (STLM), Japanese

beetles (JB), white apple leafhopper (WALH), locust leafminer (LLM), and overall injury

of Japanese beetles (%JB) in 1999 as affected by three oil concentrations and nine apple

genotypes.

oil conc. (%)	Leaves with symptoms of injury by the following insects (%)							Total insect injury (%)
	PLH		STLM	JB	WALH	LLM	whole tree JB injury (%)	
	Mottled	Curled						
0	2.6^z	0.3	0.1	8.9	0.2	0.9	1.6	13.0
4	2.9	0.3	0.0	5.3	0.3	1.0	0.6	9.8
8	2.8	0.8	0.0	5.4	0.1	0.0	0.5	10.1
Genotypes								
Delicious	1.2bc^y	0.3	0.2	3.8cd	0.9	1.3	0.5c	7.6cd
Freedom	2.1abc	0.4	0.1	6.2bc	0.2	1.1	0.7bc	10.0c
Gold Del.	0.4c	0.5	0.0	3.3cd	0.3	0.9	0.2c	5.3cd
Liberty	3.7ab	0.4	0.0	15.7a	0.1	1.9	2.2ab	21.7a
NY 74840-1	1.8c	0.3	0.0	2.3cd	0.0	0.5	0.1c	4.8d
NY 74828-12	4.4a	1.3	0.0	0.6d	0.0	0.6	0.0c	6.8cd
NY 73334-35	4.9a	0.5	0.0	9.3b	0.2	0.8	1.3bc	15.7b
Redfree	2.0abc	0.1	0.2	14.6a	0.1	1.2	3.0a	18.3ab
York	4.5a	0.4	0.0	2.9cd	0.0	0.6	0.1c	8.4cd
P-value								
Oil	0.085	0.212	0.617	0.001	0.618	0.603	0.007	0.001
linear	0.680	0.020	0.389	0.006	0.681	0.799	0.013	0.048
Genotypes	0.002	0.156	0.445	0.001	0.180	0.336	0.002	0.001
G x oil	0.793	0.555	0.595	0.166	0.856	0.507	0.559	0.492

^{z} Values are least square means of 5 shoots on each of 45 trees.
^{y} Means followed by the same letter do not differ at the 5% level of significance by the
probability of the difference. Values are LS means of 5 shoots on each of 15 trees.

Table 5. Least squares means for leaf burn and defoliation caused by the interaction of genotype and oil concentration.

	Leaf burn rating[y]								
Oil conc (%).	Delicious	Freedom	Gold Del.	Liberty	NY 74840-1	NY 74828-12	NY 73334-35	Redfree	York
0	1.0	1.0	1.4	1.0	1.0	1.0	1.0	1.0	1.4
4	2.4	2.0	3.6	2.0	2.0	3.2	2.0	2.0	2.0
8	2.8	2.2	3.8	2.8	2.6	3.6	3.0	2.4	2.4
P-value									
Lin	0.001	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001
	Defoliation rating[z]								
Oil conc. (%)	Delicious	Freedom	Gold Del.	Liberty	NY 74840-1	NY 74828-12	NY 73334-35	Redfree	York
0	1.0	1.0	1.0	1.2	1.0	1.0	1.0	1.2	1.0
4	1.0	1.0	1.2	1.8	1.0	2.4	1.4	1.0	1.2
8	1.0	1.2	1.8	2.2	1.2	2.6	1.8	2.2	1.6
P-value									
Lin	1.000	0.004	0.009	0.506	0.009	0.048	0.001	0.001	0.506

[y] Values are LS means of 5 shoots on each of 5 trees. Leaf burn was rated on a scale of 1 = no injury to 5 = > 75% of the leaves on a tree with necrotic lesions.

[z] Values are LS means of 5 shoots on each of 5 trees. Leaf defoliation was rated on a scale 1 = no defoliation to 5 = > 75% leaf abscission on the whole tree.

Table 6. Least squares means for the percentage of fruit injured by insects: redbanded leafroller (RBLR), tufted apple bud moth (TABM), tarnished plant bug (TPB), Japanese beetle (JB), and plum curculio (PC); and oil phytotoxicity; necrotic burn, severe burn, and slight burn when harvested on 9 August, 1999.

Oil conc. (%)	Total fruit/tree	Total injury (%)	Insect injury (%)	Non-insect injury (%)	Fruits with symptoms of injury by the following insects (%)					Severe sun burn[y]	Slight sun burn[z]
					RBLR	TAB M	TPB	JB	PC		
0	58[v]	28.8	3.8	24.9	0.6	1.6	0.9	0.2	0.6	14.2	10.6
4	49	33.0	9.9	23.1	0.5	2.4	0.9	1.1	5.0	13.2	9.8
8	56	27.9	8.2	19.7	0.3	1.2	0.4	2.6	3.7	8.4	11.2
Genotypes											
Delicious	89[w]	33.5b[x]	6.1	27.4b	0.2	0.6	0.2	1.6	3.6	9.6bcd	17.2a
Freedom	105	30.2b	8.0	22.3b	0.6	2.7	0.0	1.1	3.6	8.3bcd	14.0a
Golden Del.	94	8.7c	7.2	1.5c	0.2	2.5	3.8	0.1	0.6	0.8d	0.8b
Liberty	74	12.3c	4.5	7.9c	0.8	1.8	0.4	0.2	1.3	3.7cd	4.1b
NY 73334-35	41	58.6a	13.7	44.9a	0.4	1.5	0.1	4.4	7.3	31.8a	13.1a
Redfree	61	35.9b	4.4	31.5b	0.5	1.3	0.0	0.8	2.1	17.5b	14.0a
P-value											
Oil	---	0.102	0.630	0.046	0.242	0.690	0.778	0.656	0.564	0.558	0.435
Lin	---	0.981	0.219	0.431	0.418	0.881	0.838	0.152	0.236	0.245	0.873
Genotypes	---	0.001	0.460	0.001	0.424	0.740	0.122	0.361	0.145	0.001	0.001
G × Oil	---	0.024	0.862	0.643	0.165	0.521	0.999	0.710	0.791	0.695	0.802

[v]Values are LS means of fruit on each of 45 trees/ oil concentration.
[w]Values are LS means of fruit on each of 15 trees/ genotype
[x]Values are LS mean percentage of total fruit injury from insect and non-insect on each of 15 trees/ genotype
[y]Severe sunburn defined as > 50 % burn damage on fruit skin
[z]Slight sunburn defined as < 50 % burn damage on fruit skin
LS means followed by the same letter do not differ at the 5% level of significance, by the probability of the difference.

Table 7. Interaction least squares means for the interaction of genotype and oil concentration on the percentage of fruit with injury symptoms caused to insects and non-insect factors for the first harvest in 1999.

Oil conc. (%)	Total fruit damage					
	Genotypes					
	Delicious	Freedom	Golden Del.	Liberty	NY 73334-35	Redfree
0	38.7^z	28.4	9.4	7.7	48.0	40.5
4	21.0	27.3	11.3	22.6	68.1	47.8
8	40.8	35.0	5.6	6.8	59.6	19.4
P-value						
Lin	0.863	0.923	0.339	0.087	0.583	0.541

[z]Values are LS means of fruit on each of 15 trees/genotype

Table 8.   Least squares means for the percentage of fruit injured by insects; redbanded leafroller (RBLR), tufted apple bud moth (TABM), tarnished plant bug (TPB), Japanese beetle (JB), and plum curculio (PC); and oil phytotoxicity; raised lenticel and sun scald when harvested on 6 September, 1999.

Oil conc. (%)	Total fruit/tree	Total fruit injury	insect injury	Non insect injury	Fruits with symptoms of injury by the following insects (%)					raised lenticel[y]	sun scald[z]
					RBLR	TABM	TPB	JB	PC		
0	43[w]	31.5	5.1	26.4	0.1	0.7	1.8	0.6	1.9	17.7	8.7
4	33	63.4	6.8	56.6	0.0	0.9	2.2	0.9	2.9	31.4	25.2
8	30	70.6	4.6	66.0	0.1	0.3	0.8	1.3	2.1	52.5	13.5
Genotypes											
Delicious	63[x]	49.6bc	1.0	48.5bc	0.0	0.2b	0.0	0.0b	0.9b	22.8c	25.8a
Freedom	63	65.6ab	4.5	61.0ab	0.3	0.4b	0.7	3.1a	0.1b	37.2bc	23.8a
Golden Del.	95	80.1a	8.5	71.6a	0.0	0.1b	6.9	0.3b	1.2b	65.7a	6.0b
Liberty	44	35.5c	4.1	31.4d	0.0	0.8ab	1.2	0.3b	1.9b	24.1bc	7.3b
NY 73334-35	18	45.2bc	9.3	35.8cd	0.0	1.7a	0.0	1.2b	7.3a	19.8c	16.1ab
P-value											
Oil	---	0.002	0.253	0.002	0.656	0.414	0.538	0.546	0.016	0.001	0.040
Lin	---	0.001	0.498	0.001	0.926	0.576	0.642	0.193	0.669	0.001	0.324
Genotypes	---	0.001	0.120	0.004	0.181	0.033	0.080	0.004	0.002	0.001	0.001
G × oil	---	0.878	0.831	0.839	0.912	0.437	0.909	0.277	0.314	0.988	0.755

[w]Values are LS means of fruit on each of 45 trees/oil concentration.
[x]Values are LS means of fruit on each of 15 trees/genotype
[y]Lenticels were considered raised when they could be felt with fingers.
[z]Sunscald defined as halo-discoloration on specific areas of skin.
LS means followed by the same letter do not differ at the 5% level of significance, by the probability of the difference.

Literature Cited

Alm, S.R. and F.R. Hall. 1986. Crabapple cultivar preferences of the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae). Fruit Var. J. 40:83-87.

Alm, S.R., R.W. Weires, R.C. Lamb, R.A. Nielsen, and J.R. VanKirk. 1985. Susceptibility of *Malus* spp. to the apple blotch leafminer (Lepidoptera: Gracillariidae). Environ. Entomol. 14: 228-230.

Bernays, E.A. and R.F. Chapman. 1976. Deterrent chemicals as a basis of oligophagy in *Locusta migratoria* (L.) Ecol. Entomol. 2: 1-18.

Bjostad, L.B., and B.E. Hibbard. 1992. 6-methoxy-2-benzoxazolinone: a semiochemical for host location by western corn rootworm larvae. J. Chem. Ecol. 18: 931-944.

Blaney, W.M., M.S.J. Simmonds, S.V. Ley, and P.S. Jones. 1988. Insect antifeedants: a behavioral and electrophysiological investigation of natural and synthetically derived clerodane diterpenoids. Entomol. Exp. Appl. 46: 267-274.

Calabretta, C., S. Inserra, G. Ferlito, F. Couti. 1985. The action of white mineral oil against the fig wax scale (*Ceroplastes rusci* L.) on cvv. Moro and Tarocco oranges and its influence on the organoleptic characteristic of the fruit. *In* R. Cavallors and E. Di Martino (Eds.) Integrated pest control in citrus-groves; proceedings of the experts meeting, Acireale, 26-29 March 1985. Pp. 447-453.

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

Crassweller, R.M., D.C. Ferree, and L.P. Nichols. 1980. Flowering crabapples as

> potential pollenizers for commercial apple cultivars. J. Amer. Soc. Hort. Sci. 105:
> 475-477.

Fein, B.L., W.H. Reissig, and W.L. Roeloffs. 1982. Identification of apple volatiles

> attractive to apple maggot, *Rhagoletis pomonella* J. Chem. Ecol. 8: 1473-1487.

Fulcher, A.F., T.G. Ranney, and J.D. Burton. 1998. Role of foliar phenolics in host plant

> resistance of *Malus* taxa to adult Japanese beetles. HortScience 33: 862-865.

Goonwardene, H.F., W.F. Kwolek, R.E. Dolphin, and E.B. Williams. 1975. Evaluating

> resistance of apple fruits to four insect pests. HortScience 10: 393-394.

Langford, G.S., and E.N. Cory. 1948. Host preference in Japanese beetles with special

> reference to grape and apple. J. Econ. Entomol. 41: 823-824.

Larew, H.G. and Locke, J.C. 1990. Repellency and toxicity of a horticultural oil against

> whiteflies on chrysanthemum. Hortscience 25: 1406-1407.

Mitchell, E.R., F.C. Tingle, and R.R. Heath. 1991. Flight activity of *Heliothis virescens*

> (F.) females (Lepidoptera: Noctuidae) with reference to host-plant volatiles. J.
> Chem. Ecol. 17: 259-266.

Nielsen, J.K., A.H. Kirkeby-Thomsen, and M.K. Petersen. 1989. Host plant recognition

> in monophagous weevils: specificity in feeding responses of *Ceutorhynchus*
> *constrictus* and the variable effect of sinigrin. Entomol. Exp. Appl. 53: 157-166.

Oatman, E.R., E.F. Legner, and R.F. Brooks 1964. An ecological study of arthropod

> populations on apple in northeastern Wisconsin: insect species present. J. Econ.
> Entomol. 57: 978-983.

Pimentel, D., J. Krammel, D. Gallahan, J. Hough, A. Merrill, I. Schreiner, P. Vittum, F. Koziol, E. Black, D. Yen, and S. Fiance. 1978. Benefits and costs of pesticide use in US food production. Bio. Science. 28: 772, 778-784.

SAS Institute. 1992. Technical Rep. P-229 SAS/STAT software: Changes and Enhancements Release 6.07.

Stevenson, A.B. 1970. Strains of the grape phylloxera in Ontario with different effects on the foliage of certain grape cultivars. J. Econ. Entomol. 63: 135-138.

Vacante, V. 1985. Influence of white mineral oil on the population dynamics of some mites in a lemon orchard in Eastern Sicily. *In* R. Cavallors and E. Di Martino (Eds.) Integrated pest control in citrus-groves; proceedings of the experts meeting, Acireale, 26-29 March 1985. Pp. 423-431.

Wallace Institute for Alternative Agriculture. 1993. Clinton administration confirms plan to reduce pesticide use. Alternative Agric. Resources Rept. 7(10):1.

Warner, G. 1998. Familiar pesticides may become a thing of the past. The Good Fruit Grower 49(6): 10-11.

CHAPTER 3: JAPANESE BEETLE (*Popillia japonica* Newman) PREFERENCE FOR APPLE GENOTYPES (*Malus domestica* Borkh.) RELATED TO LEAF MORPHOLOGY AND PHENOLIC CONTENT

Adult Japanese beetle, an indirect pest, has a wide host range including more than 300 species of plants in eastern North America (Langford and Cory, 1948). The beetles feed almost exclusively on apple foliage unless the fruits have been previously damaged (Fleming, 1972, Hogmire, 1995).

Morphological characteristics within a plant species may play an important role in host plant preference because it might affect insect feeding (Hoffman and McEvoy, 1985). There may also be considerable variation between cultivars of a species in degree of resistance to damage (Langford and Cory, 1948; Stevenson, 1970). Ranney and Walgenbach (1992) found that defoliation by adult Japanese beetles varied from 0% to 83% among 33 taxa of crabapples (*Malus* spp.). Spicer et al. (1995) found similar and consistent variation in feeding damage among many of the same cultivars.

Host plant resistance depends on many factors, including morphological characters; i.e. plant surface (Tingey and Laubengayer, 1982; Juniper and Jeffree, 1983), leaf thickness (Coley, 1983), and trichome density (Smith et al., 1975); and the presence of allelochemicals (Patton et al., 1997; Fulcher et al., 1998).

Plant surfaces are usually covered with a layer of wax (cuticle), and the toughness of cuticle was demonstrated to be a physical barrier of feeding insects (Tanton, 1962; Grime et al., 1968). 'Leaf thickness' may be associated with wax thickness on the leaf surface. Therefore, if cuticles affect insect feeding, the thickness of leaf might also affect

the feeding. Leaf thickness may be associated with hardness or toughness of leaf because penetrometer measurements indicated that leaf hardness was correlated with incidence of insect injury on grass from grasshopper (Williams, 1954), on Cruciferae from beetles (Tanton, 1962), and on oak from moth larvae (Feeny, 1970).

Trichomes may function as structural features, altering light reflectance of the leaf and the microclimate of its surface. They can act as barriers to herbivores, or may be glandular and secrete substances, such as resin, that can cover the surrounding surface (Upohf, 1962). Form and function of trichomes might vary within a plant species and serve as a mechanism of insect resistance in plants (Beck, 1965; Chapman, 1977). Where it has been investigated, the extent of pubescence is under the relatively simple genetic control of one or two genes (Knight, 1952; Nielsen et al., 1982).

Hairs on the leaf surface can influence the attachment of insects as well as their movement and feeding. The Mexican bean beetle (*Epilachna varivestis* Mulsant) fell off leaves of cultivars with long trichomes (Van Duyn et al., 1972), but for some insects on other plants the hairs seem to provide a 'foothold' and aid attachment to the plant (Ditman and Cory, 1933; Jonasson, 1980). For plant feeding insects, trichomes are a simple mechanical barrier to feeding; pubescence seems to be characteristic of species that are relatively poorly defended by secondary chemicals (Denno and Donnelly, 1981; Coley, 1983).

Japanese beetles are attracted to certain plant volatiles (kairomones), and some were commonly used as Japanese beetle lures (Ladd and McGovern, 1980; Ahmad, 1982; Loughrin et al. 1996a). Recently, Loughrin et al. (1995) demonstrated that Japanese beetles were able to locate host plants by feeding-induced odors. Moreover, Loughrin et

al. (1996b) stated that even though beetles appeared to be attracted to certain plant volatiles for locating potential host plants, nonvolatile factors such as sugars, nitrogen, and tannin concentration might play an essential role for host plant suitability.

Some plants contain strong repellent allomones (antifeedants). For example, neriifolin, a cardiotonic glycoside in yellow oleander (*Thevetia thevetioides* (HBK) K. Schum.) was identified as an effective antifeedant for Japanese beetle (Reed et al., 1982). Prunasin, herniarin, and coumarin were important factors in host plant resistance of *Prunus* L. taxa and could be antifeedants for Japanese beetle (Patton et al., 1997).

Phenolics are important defense chemical compounds. Apple leaves from different genotypes (*Malus* spp.) contain various levels of phenolic compounds such as chlorogenic acid, gallic acid, quercitrin, phloridzin, and phloretin (Garcia et al. 1995). Phloridzin, a major phenolic found in the foliage of *Malus* taxa, may play an important role in insect resistance (Pree, 1977; Hunter et al. 1994; Fulcher et al., 1996).

The objectives of this study were to determine the relative preference of ten apple genotypes to Japanese beetle feeding and to investigate the morphological characters and phenolic compounds in leaves of preferred and non-preferred genotypes.

Materials and Methods

Feeding assays. To screen host preference of Japanese beetles, no-choice and choice feeding assays were performed with 10 apple genotypes growing at the Virginia Tech College of Agriculture and Life Sciences Kentland Farm, near Blacksburg, VA. Genotypes were 'Delicious', 'NY 73334-35 (NY35)', 'Freedom', 'Fuji', 'York', 'NY 74840-1 (NY1)', 'Liberty', 'NY 74828-12 (NY12)', 'Redfree', and 'Golden Delicious'.

Choice and no-choice feeding assays were performed from June to August 1998 and 1999. Japanese beetles were collected from wild cherries, wild raspberries, wild roses, and peaches. One leaf and one beetle were placed in a petri dish (100×100×15 mm plate) at room temperature for the no-choice feeding assay. There were five petri dishes per genotype on each of 5 days. After 48 h, the percentage of leaf area consumed was visually estimated to the nearest 5%.

For the choice feeding assay, five Japanese beetles were placed in plastic containers (59.1×43.2×30.5 cm) containing moist paper towels and one leaf of each of the ten genotypes. Feeding injury was visually evaluated after 48 h at room temperature. There were five containers on each of 4 days. The experiment was repeated again in 1999. Both experiments were analyzed as randomized complete block designs, using date of the experiment as blocks and petri dishes or containers were subsamples. The GLM Procedure of SAS (SAS Institute, 1992) was used to perform ANOVAs and means were compared with Tukey's HSD at the 5% level. The block x genotype interaction was used as the error term to test equality of genotypes.

To determine if feeding activity was related to numbers of Japanese beetles, varying numbers of Japanese beetle (1, 2, 4, and 6) were placed in jars containing two leaves from one of the three genotypes ('York', 'Freedom', and 'Liberty'). Feeding injury was visually estimated after 24 h at room temperature. Genotypes were selected based on choice and no-choice trials where 'Liberty' was the most susceptible, 'NY1' was the least susceptible, and 'Freedom' was intermediate. The factorial experiment of three genotypes and four Japanese beetle levels had three replicates and was analyzed as a completely randomized design.

Additional choice and no-choice feeding assays between oak (*Quercus* spp.) and apple ('Freedom') were conducted in 1999 using the same choice and no-choice feeding assay methods previously described but observations were made after 24 h.

Morphological characteristics of leaves. Four fully expanded mid-shoot leaves from each of five trees of 'Liberty', 'Freedom', and 'York' were collected during June and July 1999. Four observations were made with a binocular at 20x on each leaf (two on each side of the mid-rib) to observe trichome density. Leaves were then weighed, leaf area was measured by portable area meter (LI-3000) (Lambda Instrument Coop., Lincoln, NE), equipped with transparent belt conveyer (LI-3050A). Leaves were dried at 70° C for four days. Specific leaf weight (SLW) was calculated as dry weight/ leaf area.

Phenolic Assay. Results from the feeding assays indicated that Japanese beetles preferred some genotypes to others. The most preferred genotype was 'Liberty', 'Freedom' was intermediate, and 'York' was least preferred. Endogenous levels of individual phenolic compounds were measured by using a reversed-phase high performance liquid chromatography (HPLC) method adapted from Picinelli, et al. (1995). Twenty leaves from each of five trees per genotype were collected during Sept. 1999, freeze-dried and ground in a mill. About 0.5 g of dry material was extracted with 10 ml of a methanol/water (80/20) mixture, plus 10 mg·g^{-1} of ascorbic acid to avoid oxidation, overnight at 4°C without stirring. After removing the methanol, the extract was washed with hexane three times to eliminate chlorophyll. The aqueous phase was freeze-dried and stored at –20°C until analysis. The residue was dissolved in 25 ml of the extractant mixture, filtered through a 0.22 μm PVDF filter.

Analyses of phenolic content were performed with gradient HPLC using a System Gold HPLC system (Beckman Instrument Inc.), equipped with 508 autosampler, 126 NM solvent module, 168 NM diode array detector monitoring at 280 nm, Gold Nouveau software, and a reversed phase LiChrospher 100 RP 18 column (3 x 125 mm; 5-μm, Hewlett Packard). The gradient used was based on a previously reported method (Picinelli et al., 1995) using solvents 2% acetic acid in water (solvent A) and methanol (solvent B). Flow rate was 0.6 mL/min, at 25° C.

Standard curves were prepared for phloridzin, phloretin, quercitrin, eugenol, chlorogenic acid, t-cinnamic acid, salicylic acid, gallic, and epicatechin from standards (Sigma, St. Louis, Mo.)

Phenolics analyzed in this study were selected because they may be involved in resistance mechanisms (Fulcher et al., 1998). They were identified by co-injection with the corresponding standards by retention time. Results are reported as $mg \cdot g^{-1}$ of dry weight. A typical chromatogram of a polyphenolic extract of apple leaves, at 280 nm is shown in Figure 7.

Results and Discussion

Feeding assays. In the 1998 choice feeding assays, genotypes differed significantly (P=0.045) in the incidence of feeding of Japanese beetle. 'Liberty' and 'NY35' had a higher incidence of feeding than 'York' and 'NY1' (Fig. 1). In 1998, 'Liberty' had the highest percentage of leaf area consumed, and 'NY1' had less damage than 'Liberty' and 'Freedom' (Fig. 2). However, in 1999, feeding incidence was not significantly affected by genotypes in choice feeding assays (P = 0.5).

46

In the 1998 no-choice feeding assay, there was no significant difference among genotypes, but the average leaf area consumed ranged from 4.4% to 12.5% (data not shown). The percentage of damaged leaf area increased linearly as number of beetles per jar increased. The pooled regression line of three genotypes ('NY1', 'Freedom', and 'Liberty') is presented in Fig. 3.

In 1999 the no-choice and choice feeding assays comparing oak and apple indicated that apple had a significantly higher percentage of leaf area consumed (Table 1). *Morphological studies of leaf characteristics.* The number of trichomes could not be quantified because individual trichomes could not be distinguished from each other. Based upon visual examination, trichome density appeared greater for 'York' and 'Liberty' than for 'Freedom' (Figures 4, 5, and 6).

Leaf fresh weight was not significantly affected by cultivar (Table 2). However, specific leaf weight (SLW) differed among the genotypes. 'York' had the highest specific leaf weight, 'Liberty' was intermediate, and 'Freedom' was the lowest (Table 2). *Phenolic assays.* Chlorogenic, epicatechin, eugenol, gallic acid, phloretin, phloridzin, quercitrin, salicylic acid, and t-cinnamic acid were tentatively identified and quantified by HPLC analysis from leaves of 'Liberty', 'Freedom', and 'York'. The concentrations of phenolics varied widely among genotypes (Table 3). Concentrations of chlorogenic acid, epicatechin, salicylic acid, and t-cinnamic acid were not influenced by cultivar. 'York' had the highest concentration of eugenol; 'Freedom' was intermediate, and 'Liberty' was the lowest. 'York' also had the highest concentration of gallic acid, and 'Freedom' had the lowest. For phloretin , 'Freedom' had the highest concentration, and 'Liberty' was the lowest. Phloridzin concentration was highest for 'York' and lowest for 'Liberty'.

'Liberty' had the greatest concentration of quercitrin, and 'York' had the lowest. Of all phenolics quantified in this study, phloridzin was present in the highest concentration.

Polyphenols have been associated with insect resistance mechanisms of plants. In a previous study of *Malus* taxa, Fulcher et al. (1998) demonstrated that in artificial diets phloridzin, and its hydrolysis product phloretin, were highly effective at deterring Japanese beetles with an edible dose (ED_{50}) of 7.1 and 9.3 mM, respectively. Quercetrin, a phagostimulant, increased feeding by 174% of the control at the 100 mM concentration. Chlorogenic acid stimulated feeding at low concentrations (<50 mM) and deterred feeding at higher concentrations (> 50 mM). However, in foliar analysis among 10 crabapple taxa (*Malus* spp.), only phloridzin was found to be significantly related to percent defoliation from choice feeding assays and to leaf area consumption from no choice assays by using stepwise multiple regression analysis. Also, under choice conditions, phloridzin was a more effective deterrent than under no-choice conditions.

Fulcher et al. (1998) showed that the concentration of phloridzin ranged from 34 to 177 mmol·kg^{-1} in 10 crabapple cultivars. *M.* × 'Schmitcutleaf' Golden Raindrops, had the least leaf area consumed (0.99 cm^{2}· d^{-1}), low percentage of defoliation (1%), and had high concentration of phloridzin (154 mmol·kg^{-1}). *M.* × 'Radiant', had the highest leaf area consumed (7.63 cm^{2}· d^{-1}), percentage of defoliation (73%), and had the lowest concentration of phloridzin (34 mmol·kg^{-1}).

After unit conversion (from mg·g^{-1} of dry wt to mmol·kg^{-1}), the concentration of phloridzin in this study ranged from 87.30 to 161.48 mmol·kg^{-1} in 'Liberty', preferred genotype, and 'York', non-preferred genotype, respectively. Therefore, my results agree with those of Fulcher et al. (1998) that phloridzin may play an important role in natural

resistance of apple genotypes to Japanese beetles. Furthermore, Langford et al. (1943) reported that eugenol was a feeding stimulant. Eugenol is currently used as a chemical bait to attract Japanese beetles (Ladd, 1980). Unexpectedly, 'York' (non-preferred genotype), had the highest concentration of eugenol, whereas 'Liberty' (preferred genotype), had the lowest eugenol concentration (Table 3).

This study showed that leaf morphological characteristics, such as trichome density and specific leaf weight, and plant phenolics might be associated with host preference for Japanese beetles. 'York', the non-preferred genotype, had the thickest leaves (highest SLW), and had the highest concentration of phloridzin, a feeding repellent. 'Liberty', the preferred genotype, had the lowest SLW, and highest concentration of quercitrin, a feeding stimulant. Chlorogenic acid, which is a feeding deterrent at high concentrations but becomes a feeding stimulant at low concentration, was variable and did not differ significantly among cultivars. Moreover, from visual observations, structural types of trichomes on 'York' might be different from 'Liberty' and 'Freedom'. However, this speculation needs the further investigation.

Plant defensive systems are very complex and natural insect resistance might not depend on only one basic chemical. A ratio of repellent and attractant might be important. For instance, although 'York' had the highest concentration of phloridzin, a strong repellent, it also had the highest concentration of eugenol, which is a strong attractant.

Using insect resistant plants is an important component of integrated pest management that can contribute to the development of more sustainable agriculture. Data from this study indicated that Japanese beetle prefers some apple genotypes over others. In addition to identifying less susceptible germplasm, information on the chemical nature

of pest resistance may help in further selection and improvement of apples for greater pest

resistance.

Figure. 1   The average percentage of leaves with Japanese beetle feeding injury for ten

apple genotypes in1998. Results are from a choice feeding assay, where beetles could

choose among the ten genotypes. Each value is the mean of 20 observations
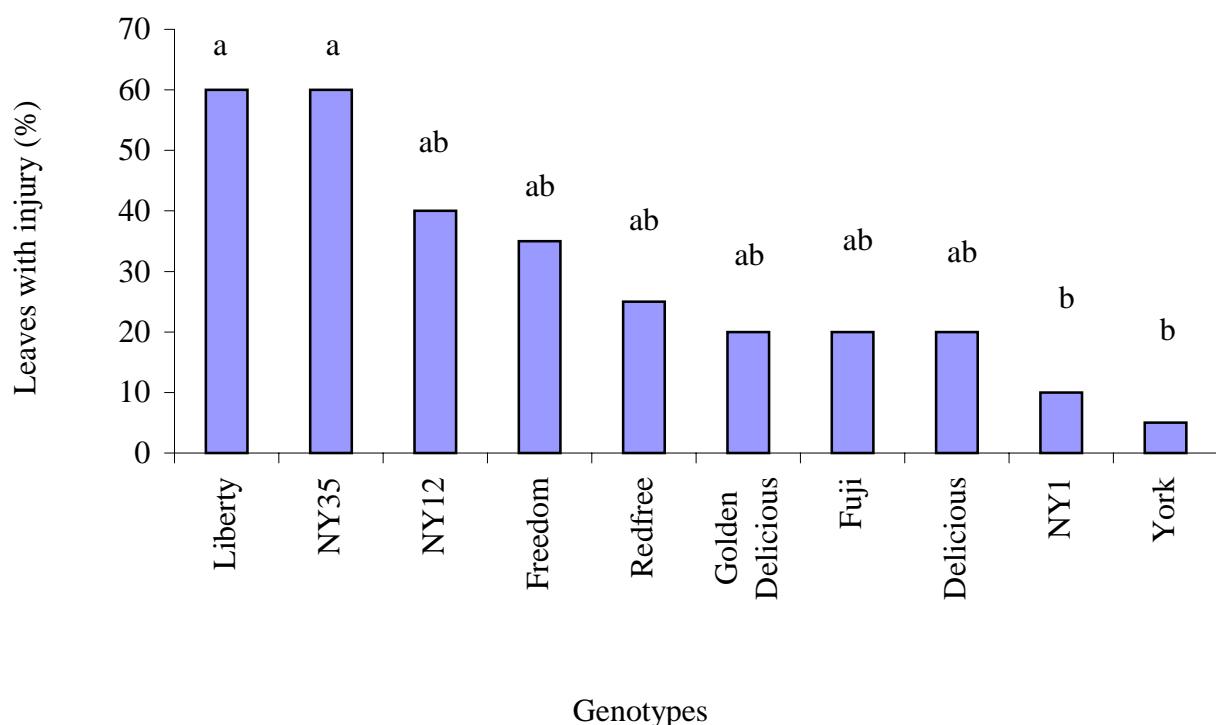
Mean separation by Tukey's HSD, 5% level

Figure 2.   The average leaf area consumed (%) for ten apple genotypes by the Japanese

beetle in 1998 and 1999 in choice feeding assays.
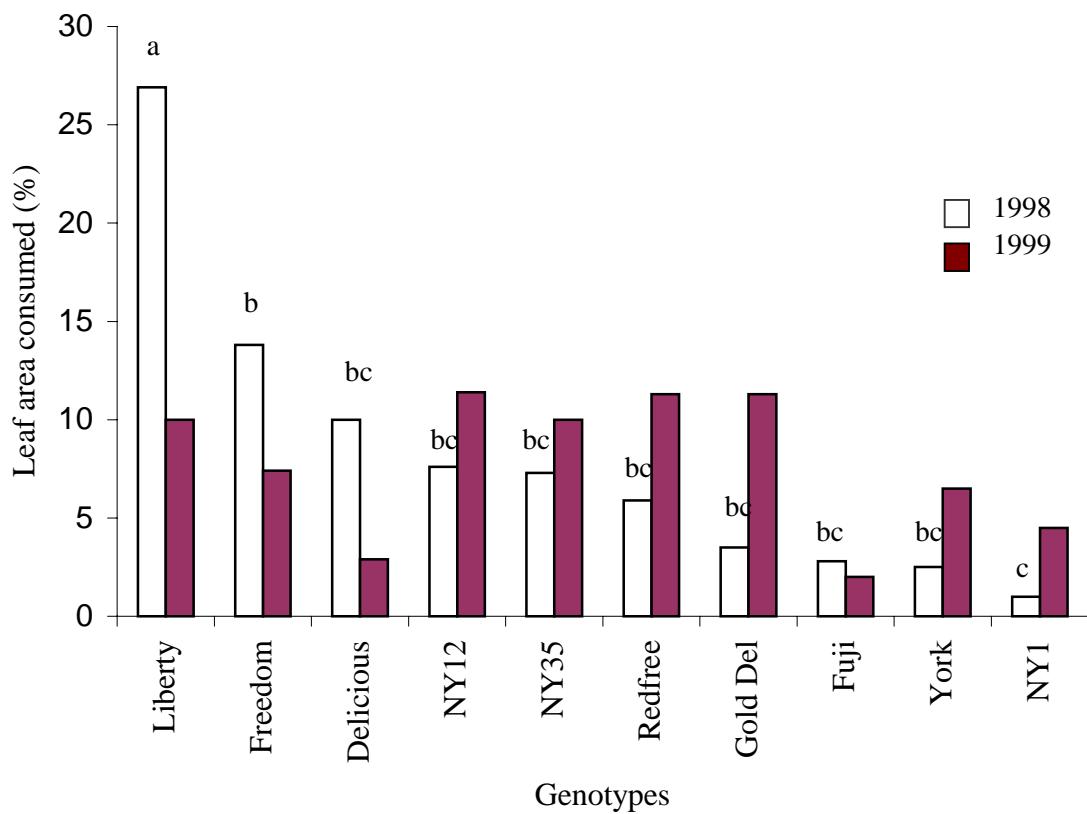
Mean separation by Tukey's HSD, 5% level.

Figure 3   The average leaf area consumed (%) by varying numbers of Japanese beetles on

three apple genotypes ('NY-1', 'Freedom', and 'Liberty'). Each point is the mean of 9

observations.

$P = 0.0002$

$y = 5.42x - 0.91$

$R^2 = 0.98$
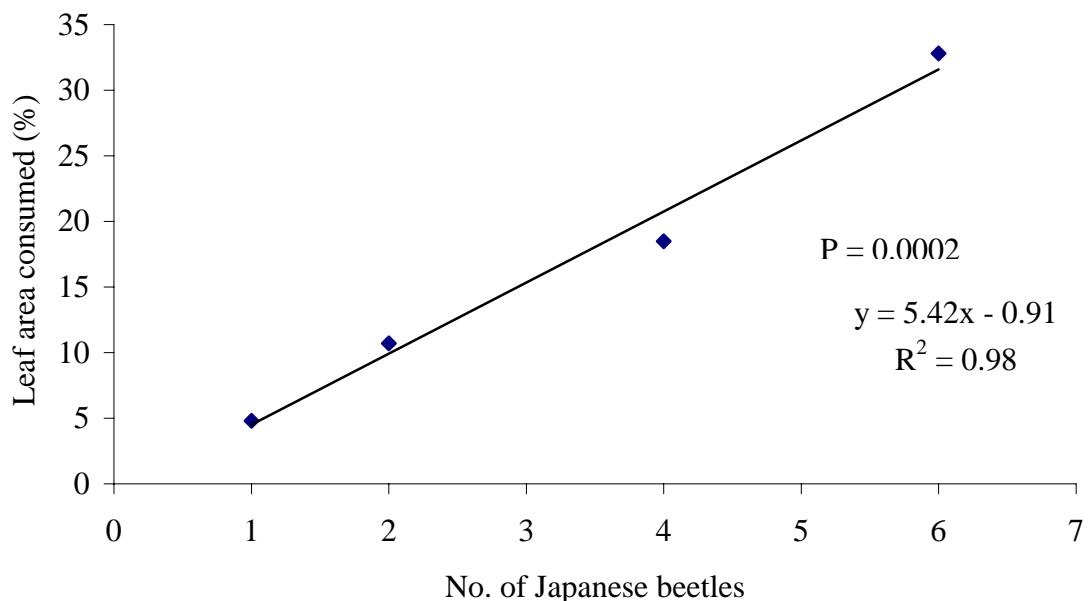
Table 1.   Percentage of apple and oak leaf area consumed by Japanese beetles for a no-

choice and choice feeding assay in 1999. Each value is the mean of 15 observations.

Species	No-choice feeding	Choice feeding
Apple	36.9	39.5
Oak	14.6	0.8
P-value	0.044	0.030

Table 2. Fresh weight (FW) and specific leaf weight (SLW) of three apple cultivars.

Each value is the mean of 20 observations

Genotypes	Fresh wt (g)	SLW (mg· cm^{-2})
Liberty	1.16	14.3ab[z]
York	1.02	15.8a
Freedom	0.86	11.9b
P-value	0.091	0.017

[z] Means separation by Tukey's HSD, 5% level.

Figure 4   Photograph (20x) of the lower leaf surface of 'Freedom' showing trichome
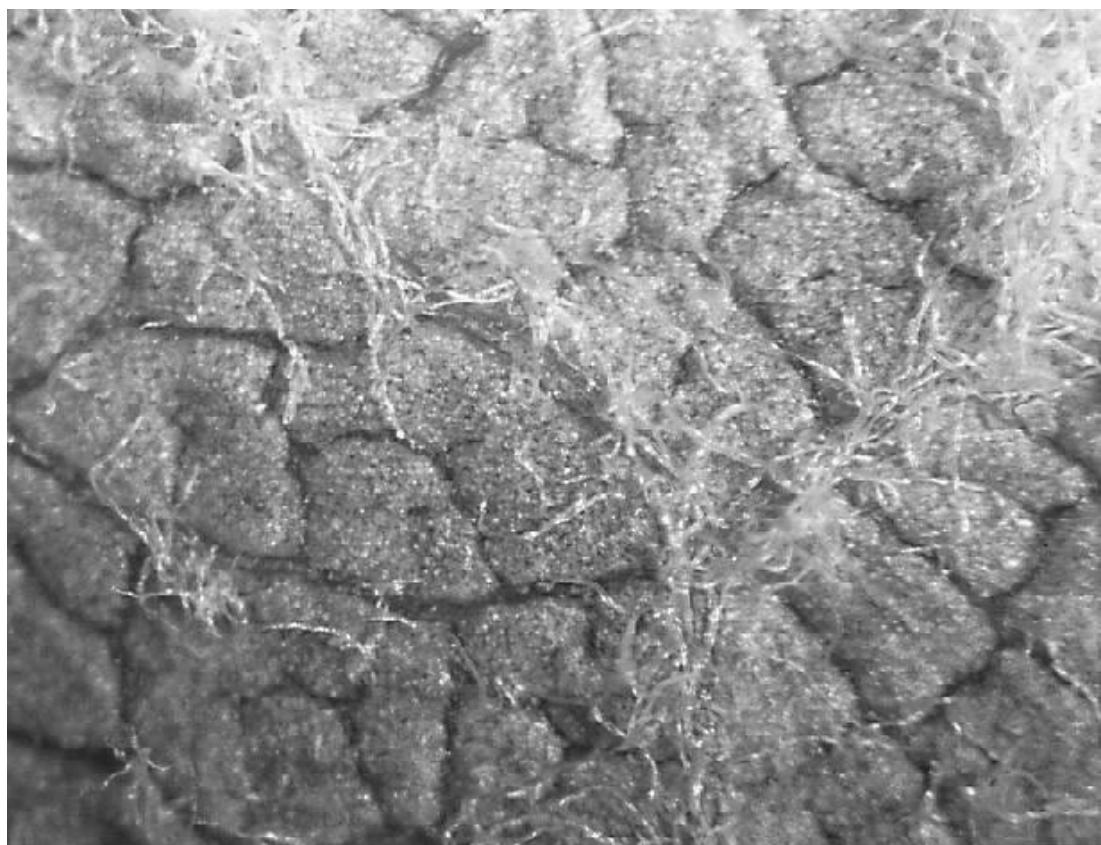
density.

Figure 5   Photograph (20x) of the lower leaf surface of 'Liberty' showing trichome
density.
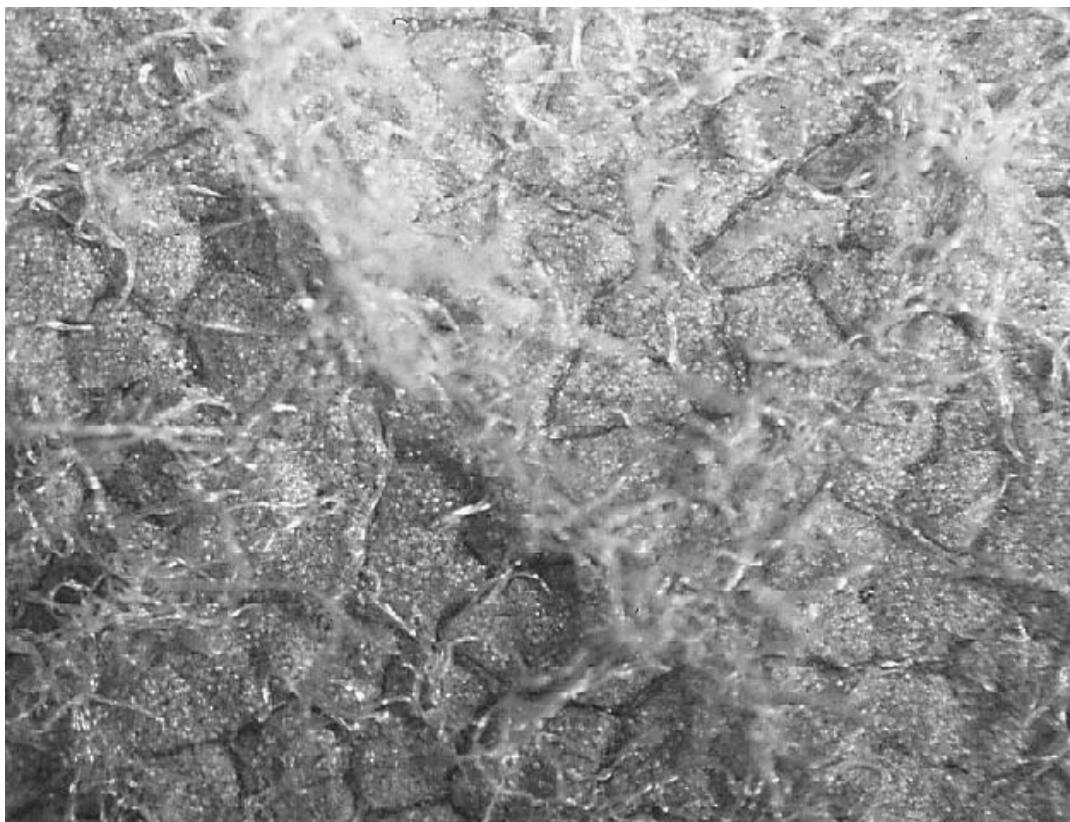
Figure 6   Photograph (20x) of the lower leaf surface of 'York' showing trichome density.
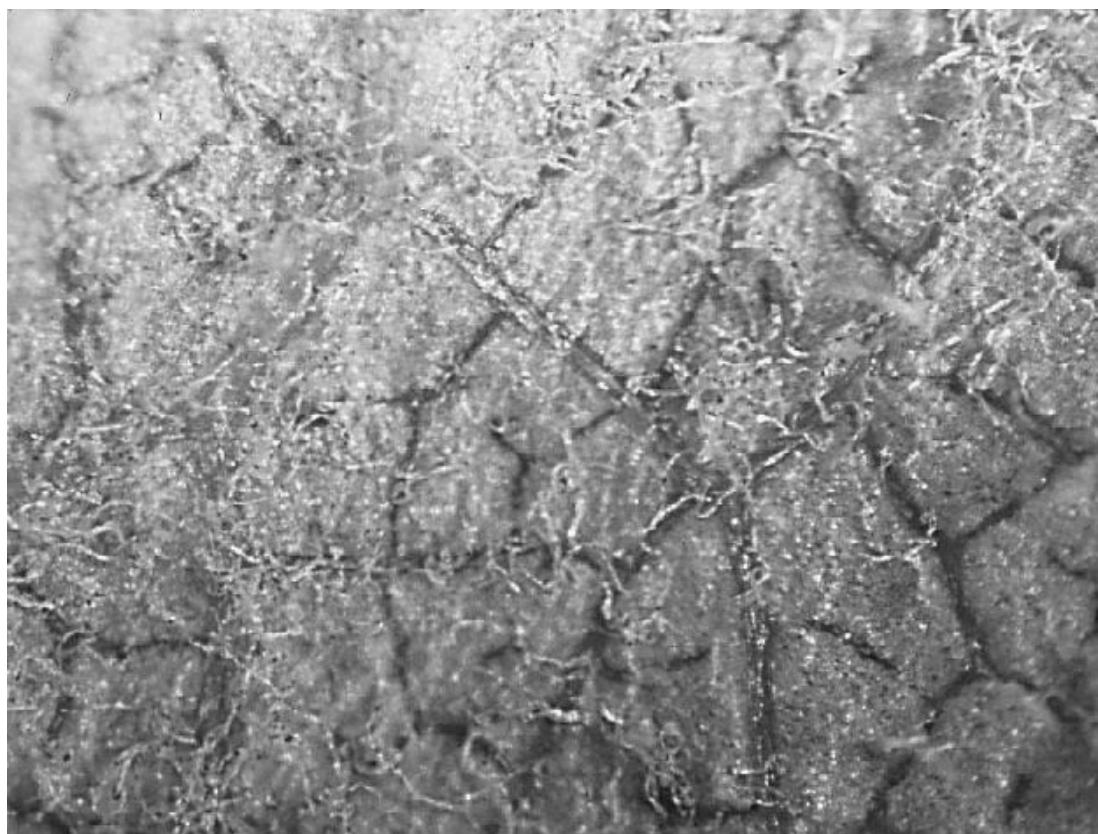
Figure 7.   Chromatogram at 280 nm of 'York' leaf extract. Conditions are described in

text. Peaks corresponded to; (1) gallic acid, (2) chlorogenic acid, (3) epicatechin, (4)

salicylic acid, (5) phloridzin, (6) t-cinnamic acid, (7) quercitrin, (8) phloretin, and (9)

eugenol.
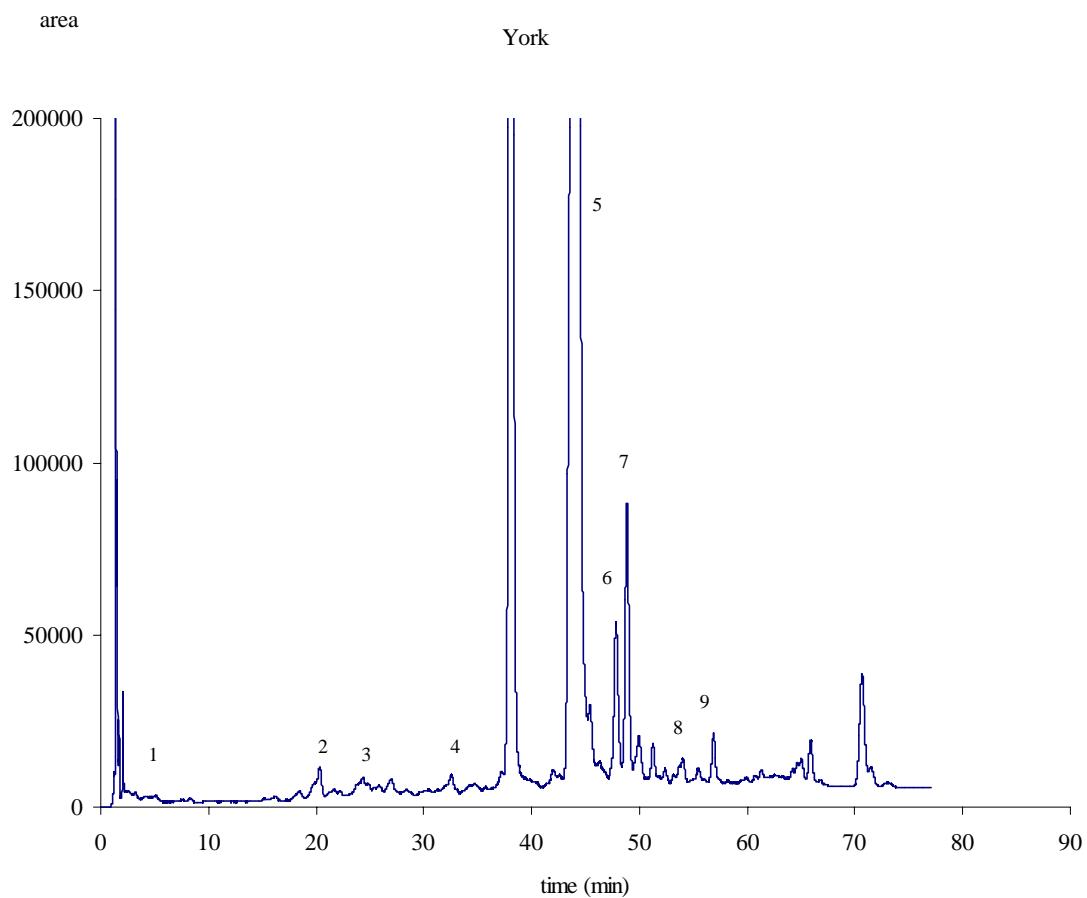
Table 3   Concentrations of nine phenolics in the leaves of three apple cultivars. Values

are means of five observations.

Cultivars	Phenolic concentration (mg·g^{-1} of dry wt.)								
	Chlorogenic	Epicatechin	Eugenol	Gallic	Phloretin	Phloridzin	Quercetrin	Salicylic	t-cinnamic
Liberty	0.60	0.05	0.41c^{z}	0.15ab	0.04b	38.10b	7.13a	0.15	0.04
Freedom	0.51	0.30	0.69b	0.14b	0.10a	47.48b	6.25a	0.13	0.06
York	1.43	0.34	0.95a	0.16a	0.07ab	70.47a	3.63b	0.19	0.10

[z] Mean separation by Tukey's HSD, 5% level.

Literature Cited

Ahmad, S. 1982. Host location by the Japanese beetle: Evidence for a key role for olfaction in a highly polyphagous insect J. Exp. Zool. 220: 117-120.

Beck, S.D. 1965. Resistance of plants to insects. Ann. Rev. Entomol. 10: 207-232.

Chapman, R.F. 1977. The role of the leaf surface in food selection by acridids and other insects. Colloquies Internationaux du Centre National de la Recherche Scientifique. 265: 133-149.

Coley, P.D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol. Monogr. 53: 209-233

Denno, R.F. and M.A. Donnelly. 1981. Patterns of hervibory on *Passiflora* leaf tissues and species by generalized and specialized feeding insects. Ecol. Entomol. 6: 11-16.

Ditman, L.P. and E.N. Cory. 1933. Corn earworm studies. Univ. Md. Agric. Exp. Stn. Bull. 348: 525-543.

Feeny, P. 1970. Seasonal changes in oak leaf tannins and nutrients as a cause f spring feeding by winter moth caterpillars. Ecology 51: 565-581.

Fleming, W.E. 1972. Biology of the Japanese beetle. U.S. Dept. Agric. Tech. Bull. No. 1449. 129 pp.

Fulcher, A.F., T.G. Ranney, and J.D. Burton. 1998. Role of foliar phenolics in host plant resistance of *Malus* taxa to adult Japanese beetles. HortScience 33: 862-865.

Fulcher, A.F., T.G. Ranney, J.D. Burton, J.F. Walgenbach, and E.P. Maness. 1996. Natural resistance to Japanese beetle among *Malus* taxa: Role of endogenous

foliar phenolics. Proc. Southern Nurserymen's Res. Conf., 41st Annu. Rpt. p. 57-60.

Garcia, M.E., C.R. Rom, J.B. Murphy, and G.W. Felton. 1995. Phenolic variation in *Malus* sp. HortScience 30: 830 (Abstr.)

Grime, J.P., S.F. MacPherson-Stewart, and R.S. Dearman. 1968. An investigation of leaf palatability using the snail *Cepaea nemoralis.* J. Ecol. 56: 405-420.

Hoffman G.D. and P.B. McEvoy. 1985. Mechanical limitations of feeding by meadow spittlebugs *Philaenus spumarius* (Homoptera: Cercopidae) on wild and cultivate host plants. Ecol. Entomol. 10: 415-426.

Hogmire, H.W.Jr., e.d. 1995. Mid-Atlantic orchard monitoring guide. Ithaca, New York. p 36.

Hunter, M.D., L.A. Hull, and J.C. Schultz. 1994. Evaluation of resistance to tufted apple budmoth (Lepidoptera: Tortricidae) within and among apple cultivars. Environ. Entomol. 23: 282-291.

Jonasson, T. 1980. Susceptibility of oat seedlings to oviposition by the frit fly *Oscinella frit* (Diptera: Chloropidae) Z. angew. Entomol. 89: 263-268.

Juniper, B.E. and C.E. Jeffree. 1983. Plant surfaces. Edward Arnold, London. P. 93.

Knight, R.L. 1952. The genetics of jassid resistance in cotton. J. Genet. 51: 47-66.

Ladd, T.L., Jr. 1980. Japanese beetle: Enhancement of lures by eugenol and caproic acid. J. Econ. Entomol. 73: 718-720.

Ladd, T.L., and T.P. McGovern. 1980. Japanese beetle: A superior attractant, phenethyl propionate + eugenol + geraniol 3:7:3. J. Econ. Entomol. 73: 689-691.

Langford, G.S., and E.N. Cory. 1948. Host preference in Japanese beetles with special

      reference to grape and apple. J. Econ. Entomol. 41: 823-824.

Langford, G.S., M.H. Muma, and E.N. Cory. 1943. Attractiveness of certain plant

      constituents to the Japanese beetle. J. Econ. Entomol. 36: 248-252.

Loughrin, J.H., D.A. Potter, and T.R. Hamilton-Kemp. 1995. Volatile compounds

      induced by herbivory act as aggregation kairomones for the Japanese beetle

      (*Popillia japonica* Newman). J. Chem. Ecol. 21: 1457-1467.

Loughrin, J., D. Potter, and T. Hamilton-Kemp. 1996a. Response of Japanese beetles

      (Coleoptera: Scarabaeidae) to some host plant volatiles in field trapping

      experiments. Univ. Kentucky Nursery and Landscape Program. 1996 Res. Rep.,

      SR-96.1: 28-29

Loughrin, J.H., D.A. Potter, T.R. Hamilton-Kemp, and M.E. Byers. 1996b. Volatile

      compounds from crabapple (*Malus* spp.) cultivars differing in susceptibility to the

      Japanese beetle (*Popillia japonica* Newman). J. Chem. Ecol. 22: 1295-1301.

Nielsen, M.T., G.A. Jones, and G.B. Collings. 1982. Inheritance pattern for secreting and

      nonsecreting glandular trichomes in tobacco *Nicotiana tabacum*. Crop Sci. 22:

      1051-1053.

Patton, C.A., T.G. Ranney, J.D. Burton, and J.F. Walgenbach. 1997. Natural pest

      resistance of *Prunus* taxa to feeding by adult Japanese beetles: role of endogenous

      allelochemicals in host plant resistance. J. Amer. Soc. Hort. Sci. 122: 668-672.

Picinelli, A., E. Dapena, and J.J. Mangas. 1995. Polyphenolic pattern in apple tree leaves

      in relation to scab resistance. J. Agric Food Chem. 43: 2273-2278.

Pree, D.J. 1977. Resistance to development of larvae of the apple maggot in crab apples. J. Econ. Entomol. 70: 611-614.

Ranney, T.G. and J.F. Walgenbach. 1992. Feeding preferences of Japanese beetles for taxa of birch, cherry, and crabapple. J. Environ. Hort. 10: 177-180.

Reed, D.K., B. Freedman, and T.L. Ladd, Jr. 1982. Insecticidal and antifeedant activity of neriifolin against codling moth, striped cucumber beetle, and Japanese beetle. J. Econ. Entomol. 75: 1093-1096.

SAS Institute. 1992. Technical Rep. P-229 SAS/STAT software: Changes and Enhancements Release 6.07.

Smith, R.L., R.L. Wilson, and F.D. Wilson. 1975. Resistance of cotton plant hairs to mobility of first instars of the Pink Bollworm. J Econ. Entomol. 68: 679-683.

Spicer, P.G., D.A. Potter, and R.E. McNeil. 1995. Resistance of crabapple (*Malus* spp.) cultivars to defoliation by the Japanese beetle (Coleoptera: Scarabaeidae) J. Econ. Entomol. 88: 979-985.

Stevenson, A.B. 1970. Strains of the grape phylloxera in Ontario with different effects on the foliage of certain grape cultivars. J. Econ. Entomol. 63: 135-138.

Tanton, M.T. 1962. The effect of leaf 'toughness' on the feeding of larvae of the mustard beetle *Phaedon cochleariae* Fab. Entomol. Exp. Appl. 5: 74-78.

Tingey, W.M. and J.E. Laubengayer. 1982. Defense against the green peach aphid *Myzus persicae* and potato leafhopper *Empoasca fabae* by glandular trichomes of *Solanum berthaultii.* J. Econ. Entomol. 74: 721-725.

Upohf, J.C.T. 1962. Plant hairs. Handbuch der Pflanzenanatonie, Berlin, 4(5): 1-206.

Van Duyn, J.W., S.G. Turnipseed, and H.D. Maxwel. 1972. Resistance in soybeans to the

    Mexican bean beetle. II. Reactions of the beetle to resistance plants. Crop Sci. 12:

    561-562.

Williams, L.H. 1954. The feeding habits and food preferences of Acrididae and the

    factors which determine them. Trans. Royal Entomol. Soc. London. 105: 423-454.

CHAPTER 4: THE ROLE OF OLFACTION IN HOST PLANT SELECTION BY JAPANESE BEETLES (*Popillia japonica* Newman)

Insect host selection has been studied for decades and several theories have been advanced about how an insect locates a plant. Brues (1920) proposed a "botanical instinct" theory, which implies that insects select host plants that meet specific nutritional and ecological requirements for that insect not offered by other plant species. Fraenkel (1959) proposed "the token stimuli theory," suggesting that insect host plant selection is determined by specific secondary plant substances or phytochemicals, i.e., glycosides, phenols, tannins, alkaloids, terpenoids, and saponins. He theorized that monophagous insects might have developed from polyphagous insects to overcome the adverse effects of secondary plant substances.

Kennedy (1965) and Thorsteinson (1960) proposed that host selection is based on insect responses to both non-nutrient and nutrient phytochemicals. Their theories stemmed from the fact that many insects can be stimulated to feed by nutrient chemicals such as amino acids, carbohydrates, and vitamins (House, 1969; Hsiao, 1969).

Finding hosts from a distance, insects may use olfactory system (sense of smell), vision system (sense of sight), or both. Plant odors can be taxon-specific and the insect's olfactory system is often capable of distinguishing these odors from others (Bell, 1984; Carde′, 1984). Plant morphology, however, is usually less characteristic because of the variation in a species. Vision is often critical in the final stages of host selection because a flying insect uses vision during landing (Prokopy, 1968; Prokopy et al., 1983). Once an insect arrives on a plant it is faced with the decision of whether or not to accept it

(Walters et al., 1989; Roessingh and Stadler, 1990). Host plant acceptance may depend on olfaction (long distance chemoreception), mechanoreceptionr (sense of touch), and gustation (contact chemoreception).

Japanese beetle is a polyphagous insect pest (Hawley and Metzger, 1940; Fleming, 1972), and is attracted by a variety of volatile plant compounds (Fleming, 1969; Schwartz and Hamilton, 1969; Ladd, 1980; Ladd and McGovern, 1980). Ahmad (1982) demonstrated that olfaction plays a very important role in host location by adult Japanese beetles. In laboratory choice tests, intact beetles located highly preferred foliage much more frequently than less preferred foliage, but antennectomized beetles could not locate the more preferred foliage as frequently as intact beetles (Ahmad, 1982). To test the role of olfaction of insects, Y-tube olfactometer has been widely used (McIndoo, 1926; Schanz, 1953; Kennedy, 1977)

The objective of this study was to investigate the role of olfaction in host plant selection by Japanese beetles.

Materials and Methods

Y-tube olfactometer. To confirm that olfaction plays a key role in Japanese beetle host location, a Y-tube olfactometer was used. The Y-tube olfactometer was a Y-shaped glass tube, 1 cm in diameter, each Y branch was 5 cm long, and the Y stem was 10 cm long. The Y-tube allows beetles to make one of two choices. The preference of plant species may be compared by placing leaf discs in each side of the Y. Three plant species were compared with a control (no leaf issue in one side of the Y) and with each other. There were six experiments: 1) Virginia creeper (*Parthenocissus quinquefolia*, L.) vs control (blank), 2) oak (*Quercus* spp.) vs control, 3) Virginia creeper vs oak, 4)

'Freedom'(*Malus × domestica*, Borkh) vs control, 5) Virginia creeper vs 'Freedom', and 6) oak vs 'Freedom'. Discs of leaves were placed in glass chambers (1×1×5 cm), attached to each side of the Y-tube. The chamber had a small hole with tygon tubing connected to a pump that allowed air to enter the chamber at approximately 0.25 l/min. Air flowed through a cotton/activated charcoal filter through the chamber, and into one side of the Y-tube olfactometer. It was assumed that plant volatiles were carried in the air stream from the chamber into a side of the Y-tube, to the beetle in the stem of the Y-tube where it had been placed (Fig.1). The beetle was able to walk inside the stem of the Y-tube and enter one of two chambers. The Y-tube and chambers were thoroughly washed after each observation to eliminate the effect of pheromone released by the previous beetle. Each side of the Y-tube was considered as a treatment. The response was binomial. A one was recorded for the treatment selected by the beetle and a zero was recorded for the side not selected. The observation was made within 30 min, and leaf tissues were changed after the beetle made a choice. There were 12 observations per experiment on each of five dates. Each experiment was analyzed as a randomized complete block design (RCBD), where dates were the blocks and there were 12 subsamples per block. Data were analyzed by analysis of variance (ANOVA) with SAS's GLM Procedure (SAS Institute, 1992).

Bias testing of Japanese beetle orientation in the Y-tube olfactometer. To confirm that there was no bias in the Y-tube olfactometer that might lead the beetle to one side of the Y more often than the other, orientation of Japanese beetles was tested. Also, humidity and time of the day were evaluated. Japanese beetles were divided into two groups and kept in moist or dry conditions. Beetles were held overnight in 500 ml beakers, covered with foil, with dry or moist paper towels, respectively. The experiment was 2×2 factorial

with two levels of humidity (humid vs dry) and two times of day (1000 to 1200 h vs 1300 to 1500 h). A beetle was placed in the stem of the Y-tube olfactometer as previously described, and nothing was placed in either side of the olfactometer. The movement of the beetle was observed and a one was recorded for the side that the beetle selected and a zero was recorded for the non-selected side. Y-tube and chambers were thoroughly washed after each observation to eliminate pheromone from the previous beetle. The experiment was a RCBD with 10 observations on each of three dates (blocks). Data were analyzed by ANOVA with the GLM Procedure of SAS (SAS Institute, 1992).

Mechanoreception and olfaction of Japanese beetles in host location. A 1.5 cm^2 disc of apple leaf tissue, (cv. Freedom) and a disc of green paper were placed in a plastic petri-dish (100×100×15 mm plate). One Japanese beetle was placed between the discs and the petri-dishes were placed in darkness. At 1, 3, 5, 7, 9, 11, 13, and 15 minutes after placement the beetles were observed and their positions were recorded. There were 15 blocks of 5 petri-dishes, for a total of 90 observations. At each observation time, there were three possible outcomes; the beetle could be on the leaf, paper, or neither. At each time, a one was entered for the location where the beetle was observed, and zeros were entered for the other two positions. Data were analyzed by ANOVA as a RCBD with the GLM Procedure of SAS (SAS Institute, 1992).

Results and Discussion

Y-tube olfactometer. Japanese beetles chose the side of the Y-tube containing apple or Virginia creeper, but not oak, rather than the side with no leaf. However, when given a choice of two plant species , the beetles did not consistently choose one species over the others (Fig. 2).

The role of olfaction in plant selection has possibly been studied more thoroughly with adult Colorado potato beetles than in any other insect (May and Ahmad, 1983). McIndoo (1926) demonstrated that Colorado potato beetles were able to locate unbruised potato foliage by using Y-tube olfactometer. Later, the result was confirmed by Schanz (1953). She reported that the attraction of beetles required the olfactory sensor at the terminal five antennal segments (Schanz, 1953).

Results from this study indicated that Japanese beetles used plant volatile compounds to locate leaf tissue, but they may require other mechanoreceptors to distinguish between plant species. Nevertheless, air flow through flow meter, activated charcoal filter, leaf chamber, and to Y-tube olfactometer might cause air turbulence that possibly confused beetles; so, they were not able to distinguish between plant species.

Bias testing of Japanese beetle orientation in the Y-tube olfactometer. Results from bias testing showed that beetles tended to move into the left side of the Y-tube more than to the right side in the morning but not in the afternoon (Table 1). Beetle movement was not affected by humidity. Beetles also tended to respond and move very slowly in the morning but became more active in the afternoon.

Bias should not affect the results from the Y-tube olfactometer because the experiments were conducted all day (morning and afternoon). Fresh leaf tissue was changed and placed in both left and right glass chambers after each observation. Glass chambers and olfactometers were thoroughly cleaned after each observation. The olfactometer was randomly placed on a lab bench in the middle of the lab with window facing southeast. The olfactometer was not exposed to direct sunlight. Beetle preference for the left side of the Y-tube in the morning, but not in the afternoon, cannot be

explained at this point. I speculate orientation to light might affect beetles' orientation. The orientation of insects may be explained by the cybernetic concept that has been developed by Mittelstaedt (1962). They demonstrated that there are two types of orientation; idiothetic control and allothetic control (Mittelstaedt-Burger, 1972; Mittelstaedt and Mittelstaedt, 1973; Mittelstaedt et al., 1979). Idiothetic control is the movement influenced by acquired information stored in the memory, and allothetic control is the movement influenced by external information, received by paired receptors (Visser, 1988). The equilibrium between idiothetic and allothetic control determines the output locomotion patterns. An increase in overall stimulus strength produces a more precise control of course direction, with less deviation from the target (Visser, 1988). Therefore, if beetles responded to the light, and they were facing southeast, they would tend to turn left in the morning more than in the afternoon. However, this speculation needs the further investigation.

Test of sense of touch and smell of Japanese beetles. When presented with a disc of apple leaf tissue or paper, 50% of the beetles were observed on the leaf after 1 min. Of the beetles that first chose the leaf, 92% stayed on the leaf, and 58% of the beetles were observed on the leaf after 15 minutes (P= 0.001) (Table 2).

When insects arrived on the plant in the previous studies, they were faced with the decision of whether or not to feed (Walters et al. 1989; Roessingh and Stadler, 1990). Results from the Y-tube olfactometer and the choice between apple and paper indicated that olfaction played an important role in host plant location, and gustation played the important role in host plant acceptance for Japanese beetles.

Figure 1. Y-tube olfactometer diagram showing the pairing between a plant leaf disc and control.

Air pump

Flow meter

chamber

Leaf disc
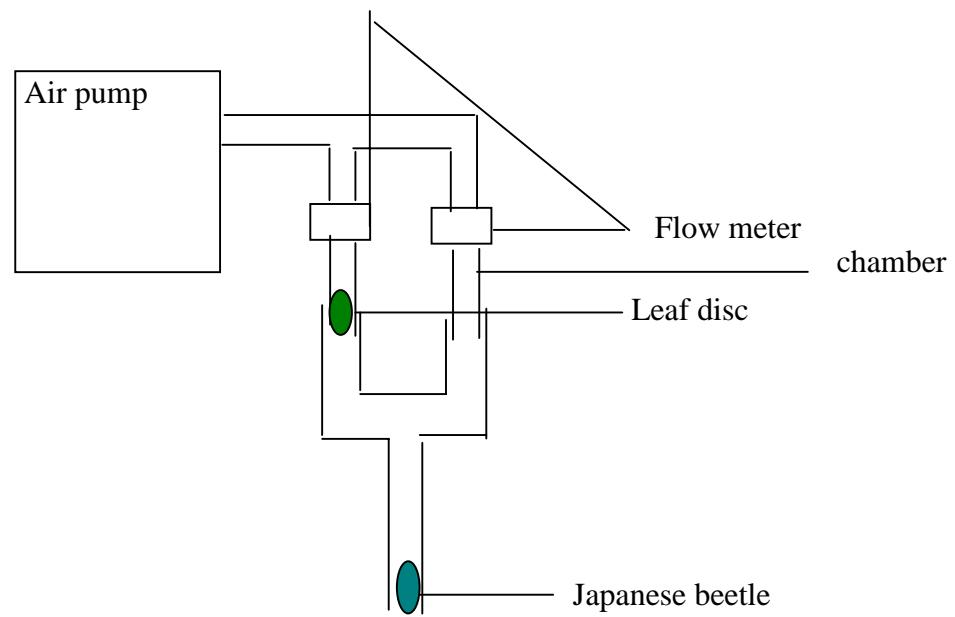
Japanese beetle

Figure 2. Percentage of Japanese beetles choosing plant species when given a choice between an empty side of the Y-tube or another plant species. Asterisks indicate that treatment means differ at the 5% level of significance by ANOVA. Each bar represents the mean of 60 observations.
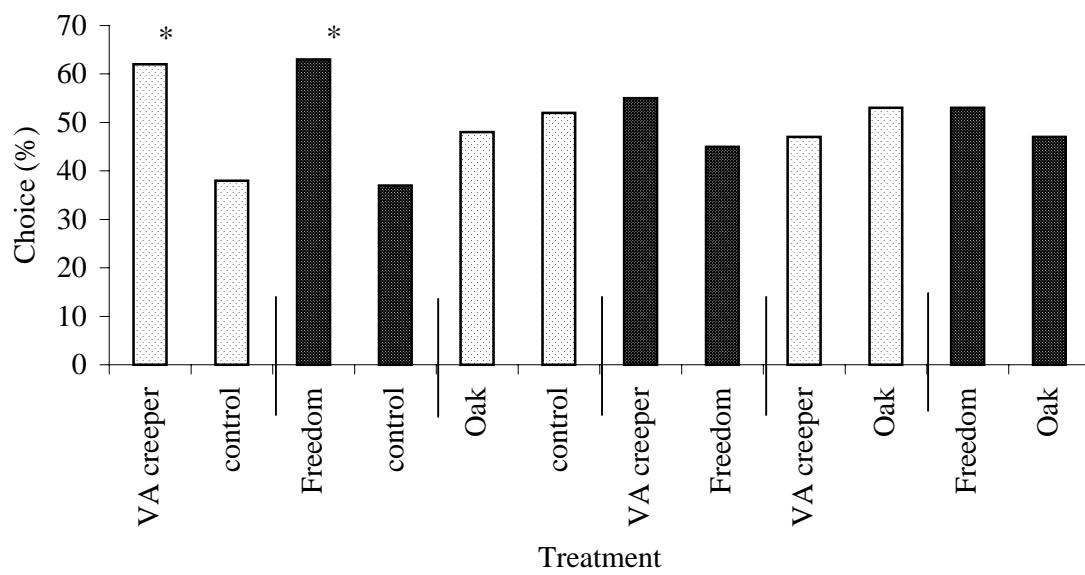
Table 1. Anova table and mean percentage of bias test for idiothetic orientation of Japanese beetle in Y-tube olfactometer as influenced by date, time of day, and humidity conditions when both sides of the olfactometer were empty. The response is 1 if beetle went to the left and 0 if it went to the right side of the Y-tube. Asterisks indicate means within a row differ at the 5% level of significance.

Source	df	Means square
Date	2	0.06
Time	1	1.63^{**}
Date $\times$ Time	2	0.21
Condition	1	0.13
Date $\times$ Condition	2	0.16
Time $\times$ Condition	1	0.13
Date $\times$ Time $\times$ Condition	2	0.01

Mean percentage of beetle orientation at different times and conditions		
Time and condition	Right	Left
Morning	27^z	73^{**}
Afternoon	50	50
Dry	35	65
Moist	42	58

[z] Values are mean percentage on each of 60 observations

Table 2. The percentage of Japanese beetles on the leaf, paper or neither when placed in

petri-dishes in the dark.

Observation	Position		
	Leaf	Paper	None
First choice (%)	49a^z	28b	23b
When leaf first chosen, beetles stayed (%)	92a^y	0b	8b
Position after 15 min (%)	59a^x	5c	36b

^{xyz} means followed by the same letter are not significant at the 5% level, by Tukey's HSD,

number of observations = 90/ treatment.

Literature Cited

Ahmad, S. 1982. Host location by the Japanese beetle: Evidence for a key role for olfaction in a highly polyphagous insect J. Exp. Zool., 220: 117-120.

Barton Browne, L. 1977. Host-related responses and their suppression: some behavioral considerations. p. 117-127. *In:* Shorey H.H. and McKelvey, J.J. Jr. (eds.). Chemical control of insect behavior: Theory and Application. Wiley, New York.

Bell, W.J. 1984. Chemo-orientation in walking insects. p. 93-109. *In:* Bell, W.J. and Carde′, R.T. (eds.). Chemical ecology of insects. Chapman & Hall, New York.

Brues, C.F. 1920. The selection of food plants by insects with specific reference to lepidopterous larvae. Amer.Nat. 54: 313-322.

Carde′, R.T. 1984. Chemo-orientation in flying insects. p. 111-124. *In:* Bell, W.J. and Carde′, R.T. (eds.). Chemical ecology of insects. Chapman & Hall, New York.

Fleming, W.E. 1969. Attractants for Japanese beetle. U.S. Dept. Agric. Tech. Bull., No. 1399.

Fleming, W.E. 1972. Biology of the Japanese beetles. U.S. Dept. Agric. Tech. Bull., No. 1449. 129 pp.

Fraenkel, G. 1959. Evaluation of our thoughts on secondary plant substances. Science 129: 1466-1470.

Hawley, I.M. and F.W. Metzger. 1940. Feeding habits of the adult Japanese beetle. U.S. Dept. Agric. Circ. 547.

House, H.L. 1969. Effects of different proportions of nutrients on insects. Entomol. exp. appl. 12: 651-669.

Hsiao, T.H. 1969. Chemical basis of host selection and plant resistance in oligophagous

    insects. Entomol. exp. appl. 12: 777-778.

Kennedy, J.S. 1965. Mechanisms of host plant selection. Ann. Appl. Biol. 56: 317-322.

Kennedy, J.S. 1977. Olfactory responses to distant plants and other odor sourses. *In* H.H.

    Shorey, and J.J. McKelvey (eds.) Chemical control of insect behavior. Pp. 215-

    229.

Ladd, T.L., Jr. 1980. Japanese beetle: enhancement of lures by eugenol and caproic acid J.

    Econ. Entomol. 73: 718-720.

Ladd, T.L., and T.P. McGovern. 1980. Japanese beetle: a superior attractant, phenethyl

    propionate + eugenol + geraniol 3:7:3. J. Econ. Entomol. 73: 689-691.

McIndoo, N.E. 1926. An insect olfactometer. J. Econ. Entomol. 19: 545-571.

Mittelstaedt, H. 1962. Control systems of orientation in insects. Annu. Rev. Entmol. 7:

    177-198.

Mittelstaedt, H. and M.L., Mittelstaedt. 1973. Mechanism der Orientierung ohne

    richtende Aussenreize. Fortschr. Zool. 21: 46-58.

Mittelstaedt-Burger M.L. 1972. Idiothetic course control and visual orientation. p. 275-

    279. *In:* Wehner R. (ed.). Information processing in the visual systems of

    arthropods. Springer, Berlin.

Mittelstaedt, M.L., H. Mittelstaedt, H. and W. Mohren. 1979. Interaction of gravity and

    idiothetic course control in millipedes. J. Comp. Physiol. 133: 267-281.

Prokopy, R.J. 1968. Visual responses of apple maggot flies, *Rhagoletis pomonella*

    (Diptera: Tephritidae); orchard studies. Entomol. exp. appl. 11: 403-422.

Prokopy, R.J., R.H. Collier, and S. Finch. 1983. Visual detection of host plants by
cabbage root flies. Entomol. exp. appl. 34: 85-89.

Roessingh, P. and E. Stadler. 1990. Foliar form, color and surface characteristics
influence oviposition behavior in the cabbage root fly *Delia radicum*. Entomol.
exp. appl. 57: 93-100.

SAS Institute. 1992. Technical Rep. P. 229 SAS/STAT software: Changes and
Enhancements Release 6.07.

Schanz, M. 1953. Der Geruchssinn des Kartoffelkafers (*Leptinotarsa decemlineata* Say).
Z. Vgl. Physiol. 35: 353-379.

Schwartz, P.H. and D.W. Hamilton. 1969. Attractants for the Japanese beetle. J. Econ.
Entomol. 62: 516-517.

Thorsteinson, A.J. 1960. Host selection in phytophagous insects. Annu. Rev. Entomol. 5:
193-218.

Visser, J.H. 1988. Host-plant finding by insects: Orientation, sensory input and search
patterns. J. Insect Physiol. 34: 259-268.

Walters, D.S., R. Craig, and R.O. Mumma. 1989. Glandular trichome exudate is the
critical factor in geranium resistance to foxglove aphid. Entomol. exp. appl. 53:
105-109.

SUMMARY

Since the government has passed the Food Quality Protection Act (FQPA), the registration of important pesticides, i.e., organophosphates and carbamates may be revised. Japanese beetle could become a more destructive pest of apple if the chemicals used to control beetles are unavailable. Thus, screening for natural insect resistant apple genotypes could be essential. Several experiments were conducted to evaluate the preference of apple genotypes to insect feeding

Insect injury of leaves and fruit of nine apple genotypes grown in a plot with low pesticide exposure were observed in 1998. The same trees were again evaluated for insect injury in 1999, but trees were sprayed with refined oil at the rate of 0, 4, and 8% (v/v). Foliar injury from potato leafhopper (PLH), spotted tentiform leafminer (STLM), Japanese beetles (JB), and total insect injury differed among genotypes. The results of fruit insect injury were different for two harvest dates, and the incidence of fruit insect injury did not significantly differ for genotypes. Refined oil decreased foliar insect injury for PLH and JB but did not affect fruit insect injury and fruit phytotoxicity. Leaf phytotoxicity symptoms and defoliation were affected by the interaction of oil and genotype. Total injury from insect and non-insect sources differed among genotypes. This study demonstrated that foliar insect injury might be influenced by genotypes. Fruit injury was inconsistent from year to year and within a year, so it is not possible to conclude if genotypes influenced fruit insect injury.

In the second experiment, JB were offered leaves of ten apple genotypes and oak in choice and no-choice assays. In 1998, JB preferred 'Liberty' to other genotypes; 'Freedom' and 'Redfree' were intermediate, and 'York' was the least preferred. However,

in 1999, the preference of genotypes was not significantly different for JB feeding. Also, genotypes did not differ for no-choice feeding assays. The percentage of leaf area damage increased linearly as number of beetles per leaf increased. Additional feeding assays between oak and apple ('Freedom') showed that JB preferred apple to oak; this indicated that JB can distinguish host plants in an artificial environment. Also, leaf morphological characteristics; i.e., leaf fresh weight, and specific leaf weight; and phenolic content were quantified for genotypes identified as preferred or non-preferred by JB. Trichome density was visually evaluated. 'York' and 'Liberty' appeared to have a higher trichome density than 'Freedom'. 'York' the non-preferred genotype, had the highest specific leaf weight, and a higher concentration of a feeding repellent (phloridzin). 'Liberty', the preferred genotype, had lower specific leaf weight, and the highest concentration of feeding stimulant (quercitrin), and yet the lowest concentration of eugenol, an attractant. This study demonstrated that feeding preference of JB may be associated with leaf morphological characteristics or phenolic compounds.

Host plant selection of JB was also investigated. Olfactory stimuli of JB was evaluated with Y-tube olfactometer. Virginia creeper, apple ('Freedom'), and oak were compared to a control (blank) and to each other. The results showed that beetles preferred the side of the Y-tube containing apple or Virginia creeper over the side with no leaf. Beetles did not choose one plant species over the other. Bias test of beetle orientation in the Y-tube odormeter due to the effect of time of day (morning and afternoon) and humidity (moist and dry) showed that beetles preferentially move into the left side of the Y-tube in the morning, but not the afternoon. Humidity did not affect beetle orientation. Host plant finding of JB was evaluated by offering the beetles discs of apple leaf or paper

in petri-dishes in the dark. The observations were made at 1, 3, 5, …and 15 min. Results indicated that 50% of the beetles first choose the leaf disc, and 92% of those beetles remained on leaf. After 15 min, 59% of the beetles were observed on the leaf. This study demonstrated that JB used sense of smell to locate a host plant.

Finally, the results of this investigation indicated that JB may prefer hosts with certain leaf morphology and phenolic content, and the beetles relied on smell and taste to select hosts. Therefore, if we are able to manipulate these factors such as breeding apple cultivars that contain non-preferred characteristics, we might be able to reduce beetle feeding without using pesticides. Furthermore, using refined oil or other environmentally safe chemicals that mask the smell or change the taste of plants could also be a simple technique to reduce JB feeding.

VITA

Sirasak Terparkum was born in July 15, 1968 at Bangkok, Thailand to Sermsakdi and Angkana Teparkum. He attended primary school and high school at St. Gabriel's College, Thailand and graduated in March of 1987. In March 1991, he received the degree of Bachelor of Science in Horticulture from Kasetsart University, Thailand.

In March 1992, he came to the United States of America and enrolled as an Undergraduate student at Concord College, West Virginia and received another degree of Bachelor of Science in Biology. In August 1994, he enrolled at Virginia Polytechnic Institute and State University and received the degree of Master of Science in Horticulture in December of 1997 under the direction of Dr. Richard E. Veilleux, who specialized in plant tissue culture and genetics. His master thesis was 'Embryogenic response of potato anther culture to colchicine'. Later, he decided to pursue the doctoral degree in integrated pest management program of apple orchard at the same department and university under the direction of Dr. Richard P. Marini. His Ph.D. dissertation was 'Interaction between insects and apple: insect behavior, genotypic preference, and plant phenolics with emphasis on Japanese beetle (*Popillia japonica* Newman).'