Trends in elm leaf chemistry and insect resistance

by

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Signatures have been redacted for privacy
# TABLE OF CONTENTS

## CHAPTER 1. GENERAL INTRODUCTION

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elms in the Urban Landscape</td>
<td>1</td>
</tr>
<tr>
<td>The Japanese Beetle</td>
<td>3</td>
</tr>
<tr>
<td>The Gypsy Moth</td>
<td>11</td>
</tr>
<tr>
<td>References</td>
<td>13</td>
</tr>
</tbody>
</table>

## CHAPTER 2. INFLUENCE OF ELM FOLIAR CHEMISTRY ON THE FEEDING PREFERENCE OF THE JAPANESE BEETLE, POPILLIA JAPONICA, AND THE GYPSY MOTH, LYMANTRIA DISPAR

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>22</td>
</tr>
<tr>
<td>Introduction</td>
<td>23</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>25</td>
</tr>
<tr>
<td>Results</td>
<td>30</td>
</tr>
<tr>
<td>Discussion</td>
<td>33</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>35</td>
</tr>
<tr>
<td>References</td>
<td>35</td>
</tr>
<tr>
<td>Tables</td>
<td>38</td>
</tr>
<tr>
<td>Figures</td>
<td>44</td>
</tr>
</tbody>
</table>

## CHAPTER 3. LEAF TOTAL PHENOLIC CONTENT OF ASIAN ELMS AND HOST PLANT SUITABILITY FOR GYPSY MOTH AND JAPANESE BEETLE

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>46</td>
</tr>
<tr>
<td>Introduction</td>
<td>47</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>50</td>
</tr>
<tr>
<td>Results</td>
<td>53</td>
</tr>
<tr>
<td>Discussion</td>
<td>55</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>56</td>
</tr>
<tr>
<td>References</td>
<td>56</td>
</tr>
<tr>
<td>Tables</td>
<td>60</td>
</tr>
<tr>
<td>Figures</td>
<td>61</td>
</tr>
</tbody>
</table>

## CHAPTER 4. GENERAL CONCLUSIONS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>65</td>
</tr>
</tbody>
</table>

## ACKNOWLEDGEMENTS
APPENDIX.

INTRODUCTION

Natural Products Research
References

STUDY 1. CATNIP, *NEPETA CATARIA* (LAMIALES: LAMIACEAE), A CLOSER LOOK: SEASONAL OCCURRENCE OF NEPETALACTONE ISOMERS AND COMPARATIVE REPELLENCY OF THREE TERPENOIDS TO INSECTS

Abstract
Introduction
Materials and Methods
Results
Discussion
Acknowledgements
References
Tables
Figures

STUDY 2. NATURAL INSECT REPELLENTS: ACTIVITY AGAINST MOSQUITOES AND COCKROACHES

Abstract
Introduction
Repellency Bioassay Method
Results
Discussion
Acknowledgements
References
Tables
Figures
CHAPTER 1. GENERAL INTRODUCTION

Elms in the Urban Landscape

Elm trees (Ulmaceae) are one of the most highly valued shade trees in North America. Even after the invasion of Dutch elm disease that first plagued North America in the 1930s, homeowners still take great pride and pleasure in the elm. A survey conducted in an elm leaf beetle-infested neighborhood in Sacramento, California found the public reporting major benefits, such as shade and visual aesthetics, to offset the negative aspects of the insect pests (Sommer and Summit 2000). Today, there are many new cultivars available from Europe and Asia offering a variety of natural defenses to disease. There have been immense efforts in exploring their natural resistance and plant breeders are developing new hybrids with low susceptibility to Dutch elm disease, elm yellows, verticillium wilt, and insect herbivory.

Some of the most common insect pests on elm include fall cankerworm, Alsophila pometaria (Harris), spring cankerworm, Paleacrita vernata (Peck), elm leaf beetle, Pyrrhalta luteola (Mueller), and elm leaf miner, Fenusa ulmi (Sundevall). More recently, invasive species including generalist feeders like the Japanese beetle, Popillia japonica (Newman), and the gypsy moth, Lymantria disbar L., have been targets for the study of host plant suitability and identifying mechanisms of insect resistance. Elm hybrids have been shown to have varying levels of resistance to numerous insect pests. Resistance can occur through insect-plant interactions such as: antibiosis, antixenosis, and tolerance (Painter 1951).

Past work has indicated that plant responses to these insect-herbivore interactions commonly involve plant secondary compounds. There are a wide variety of secondary
compounds isolated from plants and include large groups such as alkaloids, glycosides, terpenoids, iridoids, and phenolics.

Phenolic compounds have long been identified as a group of chemicals closely tied to insect-plant interactions (Feeny 1976, Rhoades and Cates 1976, Coley et al. 1985), but also function in U.V. protection, regulation of nutrient cycling within the ecosystem, pollinator attraction, seed dispersal, fungal defense, and allelopathy (Waterman 1992, Lois 1994). This large class of compounds can be identified by the presence of a hydroxy-substitued aromatic ring. Some of the more common plant phenolics are grouped as flavonoids, lignans, tannins, and hydroxy-quinones, all of which have been reported in numerous insect-plant relationships. Nishida et al. (1987) reported four flavonoids to stimulate oviposition by the citrus-feeding swallowtail, and Harborne (1988) noted several flavonoids as feeding stimulants. For *Bootetix argentatus* (Bruner), a specialist feeder, a surface lignan serves as a feeding stimulant, but deters other generalist herbivores (Chapman et al. 1988). *Betula resinifera* (Britton)'s tannins were found to possess high antiherbivore activity (Ayres et al. 1997).

There is a diversity of insect responses to phenols, which can depend upon the environment, specific concentrations that are present, or the physiological state of the receiver (Scriber and Slansky 1981). Past studies have most commonly exhibited a negative feeding response to higher phenolic content (Haukioja et al. 1985, Rossiter et al. 1988, Niemela et al. 1979, Wratten et al. 1984). However, this is not always the case. Insect attraction to phenolic compounds has also been described (Hartley and Lawton 1987).
The Japanese Beetle

Introduction and Impact

The Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae), is a successful invasive species in North America. This generalist pest started its progression by exploiting the diverse flora of the Eastern United States and has since continued to establish populations throughout the Midwest and southern parts of the U. S., in addition to inhabiting some areas of Canada. Since its arrival in the early 1900s, beetle populations have colonized areas in almost all states east of the Mississippi River and partial infestations frequently occur in the west. In 2004 more states were placed into quarantine status; this action included the states of Montana, Colorado, and Arkansas (APHIS 2004). The success of this insect pest in North America is in spite of our knowledge of its life history and various strategies for management programs. Efforts to control beetles go back to 1917 when a Japanese Beetle Laboratory was created by the former U. S. Bureau of Entomology. Much of the work on studying Japanese beetle biology and management was conducted by government employees with cooperation of participating universities. The prompt development of the Japanese Beetle Laboratory is an example of the initiatives for pest control at the time.

Japanese beetles were first discovered in the U. S. in August, 1916, at a nursery in southern New Jersey (Fleming 1976; Dickerson and Weiss 1918). Adult beetles were found feeding on *Crataegus*, but were believed to arrive as larvae on the roots of Japanese iris. Government guidelines are in place today that regulate interstate commerce in North America. Inspections of both nursery stock and soil are used to control the movement of the Japanese beetles. The Agriculture Plant Health Inspection Service (APHIS) has stressed the

**Nutritional Ecology**

Adult beetles have been observed feeding on a wide range of plant species. This means that adults are capable of consuming a vast array of plant primary and secondary metabolites. The physical properties of the different plant tissues consumed by the adult Japanese beetles will also differ in terms of external structures and internal tissues. In the plant kingdom, there exists a great diversity of physical structures and chemical components that are known to affect insect ecology (shelter, oviposition sites, food, etc.). In the case of the adult Japanese beetle, little information is available to help explain how different plant characteristics, if any, influence the biology and/or activity of this insect. Most of the work that is published consists of bioassays run on a select group of plant secondary chemicals, most commonly phenolics, which are usually implicated as the primary determinants of this insects host range.

The other common study that appears in the literature on Japanese beetles measures the release of volatile terpenes and green leaf compounds (C-6). However, none of these studies have been able to explain what specific chemicals adults use as cues for finding food sources or how these messages are carried and conserved through the environment to signal receptive adults. Several of the compounds that have been identified are released as volatiles in a large number of plant families, not all of which are preferred feeding plants. These studies conclude that adult Japanese beetles respond to a complex blend mixture of terpenes, aliphatics, and aromatics (Potter 2002). As the complexity and volume of volatiles emitted
by plants increases, so does the attractiveness of a plant to Japanese beetles (Loughrin et al. 1998).

Plant primary metabolites have also been investigated as determinates of Japanese beetle feeding preference. At least 16 sugars have been tested in laboratory bioassays with adult beetles. Sucrose, maltose, fructose and glucose were found to be strong phagostimulants, in addition to three other sugars that induced minor feeding responses (Ladd 1986). Several sugars were also found to be stimulatory to the insect in its’ larval stage (Ladd 1988). Data has also been collected to show that there are some effects of potassium and sodium on adult beetle survival and egg production (Stamp and Harmon 1991).

Host Plant Acceptance

Polyphagous or generalist insects represent only a small portion of insects. These individuals share a similar feeding strategy; the utilization of plants from many different families as a food source (Bernays and Chapman 1994). This feeding strategy is believed to be the more primitive condition as compared to specialists (Dethier 1954) and has since been studied at many different levels of specialization that may or may not change the definition above. This means species that feed on multiple tissues of a single plant can be considered as generalists (Futuyma 1976; Cates 1980; Fox and Morrow 1981).

Insects that can utilize multiple plants as food sources exhibit varied tactics for overcoming plant defenses. Individuals have been able to avoid detrimental effects from phytochemicals by increasing consumption rates, and/or modifying the nutrient quality of plant tissue. Some examples of different adaptations insects have evolved to alter food
source nutrients can include detoxification, sequestration, avoidance or other behavioral patterns, and even exploitation of the degradative properties in microbial systems through symbiotic relationships (Panda and Khush 1995).

As a polyphagous feeder, the Japanese beetle exhibits some of the generalists' adaptations for utilizing multiple plants as a food source. The digestive system contains a series of microsomal cytochrome P450 monooxygenases. These can increase in titers and complexity with multiple-plant feedings (Ahmad 1983). P450s, also referred to as mixed function oxidases, encompass a diverse group of enzymes that accompany the detoxification of allelochemicals, and are often components of insecticide resistance mechanisms. These enzyme systems may have influence on the adult beetles' ability to habituate to feeding on neem-treated leaf tissue (Held et al. 2001).

A behavioral adaptation often seen in generalist phytophagous feeders, food-aversion learning, was not observed with adult Japanese beetles feeding on two plant species: zonal geranium (Pelargonium x hortorum) and linden, Tilia cordata (Mill.). Beetles preferred the geranium petals over the linden foliage (preferred food source) and then become paralyzed for a 12-16 hour period. Following recovery, individuals continued to choose geranium tissue for feeding despite continued events of paralysis (Potter and Held 1999).

The current body of literature on Japanese beetle chemical ecology describes two semiochemical phenomena commonly seen in the Insecta: utilization of a sex pheromone, and exploitation of host plant volatiles as cues. Sex attraction was first characterized by Ladd in 1970, noting that male beetles were highly attracted to females in spring to early summer and that after mating commenced, females did not show signs of attraction to other males. Several years later, the sex pheromone was identified as (Z)-5-(1-decenyl)dihydro-2(3H)-
furanone (Tumlinson et al. 1977). This chemical is currently believed to be secreted by newly emerged adults in the spring by glands on both of the females’ anal plates and the two apical sternites (Tada and Leal 1997). After mating, release of the pheromone ceases even though females mate multiple times throughout the summer (Potter and Held 2002).

Additional experiments revealed that there was differential activity of the R and S­ enantiomers of 5-(1-decenyl)dihydro-2(3H)-furanone and that the specificity was related to the chemical ecology of another scarab beetle, the Osaka beetle, Anomala osaka (Sawada). The Osaka beetle occupies the same environment as the Japanese beetle in Japan (Leal 1998). Studies on the beetles’ neurophysiology show that male antennae contain two neurons, each with a specialized receptor for the two forms of the molecule. There is one sex pheromone transport protein present in both species (Wojtasek et al. 1998) and in P. japonica, the R orientation of the molecule acts as stimuli for the neuron while the S form acts as an antagonist. The opposite scenario occurs in the Osaka beetle where the S­ enantiomer stimulates the neuron, resulting in beetle activity.

The Japanese beetle sex pheromone, commonly referred to as japonilure and nuranone, was originally registered for use in the U.S. in 1979. It is now produced synthetically and sold with a mixture of eugenol, geraniol, and 2-phenylethyl propionate by several different companies as attractants and baits. Nuranone is currently registered for use by Spectrum Group, Springstar LLC, Suterra LLC, Trece Inc., and Woodstream Coporation for use in multiple applications: agricultural crops/soils, deciduous fruit trees, grapes, and several ornamentals (trees, shrubs, vines, herbaceous plants, etc.) (EPA 1995).

Originally, it was believed that adult beetle aggregation behavior was in response to an aggregation pheromone (Iwabuchi and Takahashi 1983). There has since been little
support for the existence of an adult aggregation pheromone and there are several laboratory
and field studies that show beetles respond to host plant volatiles (Klein 1981; Ladd 1970;

**Determinants of Host Range**

Japanese beetles are widely polyphagous plant-feeders, feeding on more than 300
species of plants (Fleming 1972). However, variation in Japanese beetle feeding
susceptibility has been reported in closely related taxa of crabapple (*Malus* spp.) (Ranney and
Walgenbach 1992; Spicer et al. 1995), linden (*Tilia* spp.) (Miller and Ware 1999), and elm
compounds that mediate differences in susceptibility are still poorly understood.

Several sugars have been indicated as phagostimulants to the Japanese beetle adult
and larval stages (Ladd 1986, 1988); however, no correlation with host susceptibility has
been determined. Cyanogenic glycosides found in Rosaceae (Patton et al. 1997), terpenes
produced in Cucurbitaceae (Tallamy et al. 1997), and neriifolin, a cardiotonic glycoside
present in yellow oleander, *Thevetia thevetiodes* (Kunth) K. Schum. (Reed et al. 1982), have
been identified in laboratory studies as Japanese beetle feeding deterrents. Foliar phenolic
compounds are often associated with feeding preference, and specific compounds in this
class have been indicated as phagostimulants and some as deterrents to the Japanese beetle
(Fulcher et al. 1998; Patton et al. 1997). Many of the recent studies on plant chemistry
emphasize leaf phenolics as the primary chemical determinates that limit Japanese beetle host
range (Keathley et al. 1999; Potter and Held 2002; Fulcher et al. 1996).
There are many studies that report adult beetle behavioral responses to plant volatiles. Multiple studies have helped to document the constitutive and induced plant chemistry of both preferred and non-preferred food sources.

Adult beetle attraction to chemical blends or lures, and leaf volatiles from both damaged and undamaged plant material is currently believed to be related to blend complexity (Loughrin et al. 1998) and is not consistent with cultivar susceptibility. Studies within *Malus* and *Acer* have failed to show relationships in leaf volatile composition as it relates to field data on adult beetle feeding behavior (Loughrin et al. 1996, 1997; Spicer et al. 1995). Conclusions based on pitfall bioassay data are that within the taxon tested, this generalist feeder does not distinguish potential food sources on the basis of plant volatiles and can be attracted to a wide variety of plant species.

Previously identified plant volatiles (both induced and constitutive) include chemicals classified as: terpenoids; aromatics; C6 green leaf volatiles; and aliphatics, consisting of alkenes, alkanes, ketones, aldehydes, ethers, esters, carboxylic acids and alcohols (Van Den Boom et al. 2004). Plants continuously emit volatiles and the composition can vary depending on a number of different factors such as: temperature, light, pollutants, water stress, and multiple interactions with the surrounding ecosystem. Several versions of mathematical models, most commonly incorporating physical, physiological, and physicochemical processes, have been developed to predict continuous plant volatile emissions (Ninnemets et al. 2004).

Plant volatiles that are released as plant tissue ruptures from insect activity can change in terms of quantity and quality of blend composition (Holopainen 2004). Plant defense studies compose a large area within the insect chemical ecology field and provide a
valuable source of information on plant volatiles. Defensive strategies against insects and pathogens can then be considered in terms of direct and indirect defenses (Van Den Boom et al. 2004). An example of indirect defense would be the induction of specific volatiles that act as synomones, attracting predators or parasitoids that prey upon the insect causing injury. Direct defenses include chemicals that have a direct negative effect on the insects’ physiology or behavior, such as the alkaloid nicotine present in tobacco, Nicotiana tabacum (L.). Species specific differences in the quality and quantity of volatile chemical profiles occur both before and after attack by herbivores. Studies on spider mite, Tetranychus urticae (Koch) feeding-induced volatiles produced from 11 different host plants nicely show how plants vary in defensive strategies relating to constitutive and induced phytochemistry (Van Den Boom et al. 2004).

There are many questions that still remain on the role of plant volatiles in Japanese beetle host-plant ecology. Adult beetles are attracted to feeding-induced plant volatile blends produced by multiple species that range in food source suitability. It is unknown if these volatile organic compounds function simply as an aggregation signal for the species, since numerous herbivores and their activities are capable of inducing plant volatiles. No specific chemicals or trends have been described except that Japanese beetles seem to be most attracted to compounds that are “floral like” (Potter 2002). More work is required to further explain the effect of host plant volatiles on Japanese beetle adults. Additionally, any information that relates to plant susceptibility or more specifically, identification of individual or groups of compounds that serve as attractants, deterrents, or toxins, would be valuable in for ecological pest management.
The Gypsy Moth

Introduction and Impact

The gypsy moth, *Lymantria dispar* L., has proven to be one of the most erratic invasive species in North America. Its ranges include areas of North Africa, Eurasia, and for at least 100 years, North America. Massachusetts suffered immense losses when this lymantriid was introduced from France in 1869, originally intended for silk production. Obviously, the gypsy moth did not prove adequate for silk production and has since plagued the Northeastern United States. It now causes $22 million a year in damage and management costs (USDA 2004a). Initially, the spread of the gypsy moth was by the movement of first instar larvae by wind, but later transportation of goods and recreational vehicles served to transport the gypsy moth greater distances. Monitoring efforts by the USDA, APHIS show that today, the gypsy moth occupies most of northeastern United States. The gypsy moth range has rapidly progressed west and south to include Virginia, Ohio, Michigan, and Wisconsin, with isolated reports in Washington and Oregon (USDA 1998).

In 1999 the USDA Forest Service, along with state cooperatives initiated the National Gypsy Moth Slow the Spread (STS) Project. Using an integrated management approach, this program prioritizes sampling for low population levels in order to first detect and then avoid movement and establishment of the gypsy moth from areas of North Carolina to Illinois, and then northwards into Michigan.

Determinants of Host Range

There is a large amount of literature available on preferred host plants of the gypsy moth and it is not surprising that most outbreaks occur in areas with highly preferred host
species. However, the scientific basis for host preference and survivorship has not been completely worked out. It has been shown that adequate amounts of defoliation can affect pupal weight, duration of development, fecundity, and survivorship (Wallner and Walton 1979, Hough and Pimentel 1978). It has also been hypothesized that induced responses are the cause of gypsy moth population fluctuations and outbreaks (Baldwin and Schultz 1983).

Further work on host foliage chemistry and more importantly, the quality from specific food sources has identified new factors that can considerably affect gypsy moth numbers. Preferred host species have long been identified but how the physical and chemical elements responsible for feeding preference and host suitability function are still unknown (Berryman 1996). This is probably due to a complexity of surface features as well as primary and secondary metabolites present in the leaf tissue. The influence of one class of secondary metabolites, tannins, have been determined to negatively influence transmission of the NPV pathogen by binding to virus particles in the midgut of gypsy moth larvae. The role of these tannin levels in viral transmission still remains a relevant area of study.

There is little doubt that certain host species can offer varying levels of viral transmission, with equal doses of virus present on leaf surfaces (Keating and Yendol 1978). Some experts speculate on the interaction of host plant chemistry with the environment. It is known that tannin levels are low during the early portion of the season in the northeastern U. S. (Hunter and Schultz 1995) and that the relevance of insect-induced tannin levels may not yield a significant impact on the population due to the forest system phenology. More specifically, in a 3 year field study by D’Amico et al. (1998), showed increased tannin levels resulting from gypsy moth defoliation of oaks that did not occur until late in the season until
after individuals had pupated. This leaves considerable questions about the role of induced-plant resistance in gypsy moth fluctuations.

An additional study by Hunter and Elkinton (2000) was centered on the synchrony of foliage development (incorporating the quality of larval food), larval feeding, larval dispersal, and parasitism. Results showed that the quality of foliage was greater closer to budburst and decreased over time, so fecundity was greater in individuals that acquired foliage closer to budburst. However, with the incorporation of natural enemies into this system, results showed that survival of late-feeding individuals was much higher than individuals emerging around budburst, the opposite effect of what was initially hypothesized. It is believed that the lower quality of food initiates larval dispersal, off-setting the impact of egg synchrony with budburst (Hunter and Lechowicz 1992). More importantly, this increase in dispersal had a direct effect on rates of parasitism, reducing the overall amount of mortality (Hunter and Elkinton 2000).

References


2. **(APHIS) Animal and Plant Health Inspection Service. 2004.** USDA Domestic Quarantine Notices; Japanese Beetle, Section 301. 48 [amended].

   [http://www.ceris.purdue.edu/napis/pests/jb/freg/04.07.06-jb.txt](http://www.ceris.purdue.edu/napis/pests/jb/freg/04.07.06-jb.txt)


polyphagous scarab, Popillia japonica, following intoxication by geranium, Pelargonium

Annu. Rev. Entomol. 47:175-205.


activity of nertifolin against codling moth, striped cucumber beetle, and Japanese beetle.
J. Econ. Entomol. 75:1093–96.


defoliation, red oak phenolics and gypsy moth growth and reproduction. Ecology.
69:267-77.

Annual Review of Entomology 26:183-211.

In: Dunn C. P. (eds.), The Elms: Breeding, Conservation, and Disease Management.

spp.) cultivars to defoliation by the Japanese beetle (Coleoptera: Scarabaeidae). J. Econ.
Entomol. 88:979–85.


CHAPTER 2. INFLUENCE OF ELM FOLIAR CHEMISTRY ON THE FEEDING PREFERENCE OF THE JAPANESE BEETLE, POPILLIA JAPONICA, AND THE GYPSY MOTH, LYMANTRIA DISPAR

A paper to be submitted to Journal of Chemical Ecology

Gretchen Schultz, Fredric Miller, and Joel Coats

Abstract

The Asian elm tree species and hybrids that are closely associated with the David complex (*Ulmus davidana* (Planch), *U. propinqua* (Koidz), *U. japonica* (Rehd.), and *U. wilsoniana* (Schneid)) show good levels of disease resistance and tolerance for urban forestry. Studies have shown that these species suffer moderate levels of injury from the adult Japanese beetle, *Popillia japonica* (Newman). The research presented here focuses on the influence of elm leaf chemistry on the feeding activity of the Japanese beetle and another invasive species, the gypsy moth, *Lymantria dispar* (L.). Leaf volatile complexity was surveyed for 26 different elm trees that vary in susceptibility to Japanese beetle feeding damage. Extracts were analyzed for trends relating lipid, phenolic, terpene, and alkaloid diversity with Japanese beetle and gypsy moth biological endpoints. Association of insect resistance and chemical data with elm parentage was also examined. Elm leaf surface wax extractions were assayed for effects on Japanese beetle feeding activity in the greenhouse. Trends in the data indicate elm tree susceptibility to gypsy moth and Japanese beetle injury is
linked to leaf chemistry; more specifically there is some support for elm leaf lipids playing a role in influencing Japanese beetle and gypsy moth preference for the Asian elms.

Introduction

The Japanese beetle, *Popillia japonica* (Newman), is a generalist herbivore that is known to feed on more than 300 plant species. In order to accommodate the diversity of food sources (var. plant species) generalist herbivores like the Japanese beetle often have complex metabolisms. The adult beetles' digestive system produces multiple microsomal cytochrome P450 (mixed function oxidases) monooxygenases that can increase in titers and complexity with multiple-plant feeding habits (Ahmad 1983). These P450 monooxygenases encompass a diverse group of enzymes that are active in the detoxification of allelochemicals, and are often components of insecticide resistance mechanisms.

Most of the research on chemicals that affect adult Japanese beetle feeding activity are bioassays with phenolic compounds and primary metabolites (Keathley et al. 1999; Potter and Held 1999; Fulcher et al. 1996). Sucrose, maltose, fructose, and glucose were found to be strong phagostimulants in laboratory bioassays, and three other sugars were capable of inducing minor feeding responses (Ladd 1986). In addition, several sugars were found to be stimulatory to the insect in the larval stage (Ladd 1988). Potassium and sodium affect adult beetle survival and egg production (Stamp and Harmon 1991).

Some studies have addressed the release of Japanese beetle feeding-induced volatile terpenes and green leaf compounds (C-6) from selected plant species (Loughrin et al. 1998). Some of these include field studies that measure volatiles released from crabapple and maple trees with various susceptibilities to adult beetles (Loughrin et al. 1996, 1997; Spicer et al.
The specific chemical cues adults use to find food sources and how these messages are carried and conserved through the environment to signal receptive adults are still poorly understood. Many volatiles sampled in previous studies are known to be produced by a number of plant families, not all of which are preferred feeding plants. The conclusions made from these studies are that adult Japanese beetles respond to a complex blend of terpenes, aliphatics, and aromatics (Potter 2002). As the complexity and volume of volatiles emitted by plants increase, so does the attractiveness of a plant to Japanese beetles (Loughrin et al. 1998).

Several studies have evaluated Japanese beetle injury to different elm species (*Ulmus* spp.) (Miller et al. 1999, 2001). These studies reported that elm species/hybrids with heavy leaf pubescence (*U. glaucescens* (Franch), *U. lamellosa* (C. Wang et S.L. Chang, ex L.K Fu et al.), and *U. macrocarpa* (Hance)) were less preferred by adult beetles. *U. davidiana* (Planch), *U. japonica* (Rehd.), *U. wilsoniana* (Schneid), and *U. propinqua* (Koidz), all elms belonging to the David complex, and the Szechuan elm, *U. szechuanica* (Fang) all show moderate to high levels of susceptibility to the Japanese beetles. This suggests that elm species/hybrid parentage might be related to host plant resistance to the Japanese beetle.

Our study presented here focuses on the influence of elm (*Ulmaceae*) leaf chemistry on the feeding activity of the adult Japanese beetle. Since there is little information in the literature on elm leaf chemistry, the first portion of this research covers qualitative data on the leaf volatile complexity of several elm trees that vary in susceptibility to Japanese beetle feeding damage. Specific chemical groupings and some individual chemicals of the extracts are discussed. Additional leaf extractions, focusing only on the surface waxes, were
performed and then used in a greenhouse bioassay to evaluate the effects of elm leaf waxes on the adult beetle feeding behavior.

**Materials and Methods**

**Analysis of Elm Leaf Volatile Complexity with Trends in Insect Preference**

**Chemical Analysis.**

Leaf material from 26 species of elm trees (Table 1) was collected at the Morton Arboretum, Lisle, Illinois on 17 June 2004 and sent to the Pesticide Toxicology Laboratory at Iowa State University for extraction.

Samples were weighed into 7g (wet weight) quantities per replicate and then placed into 500ml French square bottles for extraction. A solvent soak with hexane (300 ml) was used to pull out non-polar lower-weight leaf volatile components for analysis. Soaks were held at room temperature (21-23°C), in the dark, with no agitation, for a 72-hour period. Linalool and trans-caryophyllene (Sigma-Aldrich, St. Louis, Missouri) were used as internal standards for mono- and sesquiterpenes. Hexane extracts were then filtered using Whatman no. 1 qualitative filter paper to remove leaf tissue. Samples were concentrated under a gentle stream of nitrogen and brought to a final 1 ml volume. Due to large amounts of waxes in the extracts, a final filtration step was preformed with 0.45 micron, MAGNA, nylon filters (Osmonics, Inc., Minnetonka, Minnesota) before analysis with gas chromatography with flame-ionization detection (GC-FID) and with mass spectrometry (GC-MS). All sample extractions and GC preparation occurred over a four-day period.

Elm species’ profiles were determined first by analysis with a Hewlett Packard 5890 Series II gas chromatograph with a 30-meter x 0.25mm i.d. DB-5 column (J & W Scientific,
Folsom, CA) and flame ionization detection. Samples were injected at 250°C on to an initial column temperature of 75°C. This temperature was held for 3 min, then ramped at 10°C/min to final temperature of 295°C, holding for 15 minutes.

Samples were also analyzed on a Hewlett Packard 5890 Series II gas chromatography interfaced to a Hewlett Packard 5972 Mass Selective Detector (MSD). The gas chromatograph was equipped with either a DB-5 or a DB-wax column (30 m x 0.25 mm i.d., J & W Scientific). The injector temperature was 250°C, and the split valve was opened 1 min after injection. The oven initial temperature was set at 50°C for 3 min, and then increased to 250°C at a programmed rate of 15°C/min. Mass spectra were recorded from 30 to 550 a.m.u. with electronic impact ionization at 70 eV. The assignments of chemical identities to chemical compounds were confirmed by comparison of the retention indices and mass spectra with those of authenticate standards and reference spectra in a mass spectral library (Wiley 138K, John Wiley and Sons, Inc.). Some standards were available commercially and purchased including: N, N diethyl-m-toluamide (Sigma Aldrich, St. Louis, Missouri), and palmitic acid (Fisher Scientific, Hanover Park, Illinois).

Leaf toughness and thickness was recorded with the use of a Digital Force Meter leaf penetrometer (Chatillon, Greensboro, NC.). Data were collected on fresh leaves from trees at the Morton Arboretum.

The chemicals from the elm leaf volatile complexity analysis were classified into four chemical classes: phenolic, terpenoid, lipid, and alkaloid. The diversity of compounds in each class was analyzed with biological data from gypsy moth developmental studies (Miller unpublished) and Japanese beetle no-choice feeding trials and field defoliation observations (Miller et al. 1999). A principal component analysis with Proc PRINCOM (SAS Version
9.1) was used to analyze trends in the biological data (gypsy moth developmental studies and Japanese beetle no-choice assays), chemical data, and physical leaf measurement data relationships. A correlation matrix was used to equalize the variances between different units of measurement. Variables that showed high levels of correlation and/or were large contributors to the first two eigenvectors, were further analyzed for levels of significance in a multivariate analysis (as dependent variables) with PROC GLM (SAS Version 9.1). Elm tree parentage served as the independent variable. This included classifying elms according to whether or not they belonged to the David complex, including *U. davidiana*, *U. japonica*, *U. wilsoniana*, *U. propinqua*, and the hybrids Triumph (*U. japonica-wilsoniana x U. japonica-pumilia*) and Accolade (*U. japonica-wilsoniana*). *U. szechuanica* was grouped with the David complex in this analysis because it is a closely related species of elm (Fu 1980).

**Gypsy Moth Developmental Study.**

Gypsy moth laboratory no-choice suitability studies were conducted at the Morton Arboretum (Lisle, IL), in plastic vials with snap cap lids (2.5 x 6.5 cm) with one neonate larva female, given fresh leaf material every 2 days. Vials were checked daily for mortality, pupation, and emergence. 10 replicates of each elm species/variety were run until adult emergence or until pupae died. Gypsy moths were given no choice in these bioassays and therefore forced to complete development on the sample species. Data is reported as duration of larval longevity, pupal fresh weight, and percentage adult emergence. Natural log transformations were performed on pupal fresh weight and percent adult emergence for analysis.
Japanese Beetle No-Choice and Field Defoliation Study.

Japanese beetle feeding preference was determined in 1997. No-choice studies involved 1 adult female beetle in a clear petri dish (10 x 0.6 cm) with fresh foliage available every 2 days. Dishes were checked daily, and feeding was recorded by visual rating with the use of a defoliation template. Dry fecal pellet masses were also recorded throughout the study.

The field defoliation study was conducted on trees at the Morton Arboretum in 1997, assessing injury from Japanese beetle feeding activity. A scale from 0-4 was used with 0 representing the lowest level of activity (no feeding) and 4, the highest level of activity (>50% canopy damage). Details of both no-choice and field defoliation studies have been previously reported (Miller et al. 1999).

Japanese Beetle Wax-Disc Feeding Study

A greenhouse bioassay was used to assess the effect of elm leaf waxes on the Japanese beetle feeding behavior. Adult beetles were collected in mid-July from wild populations in Ames, Iowa with a Japanese beetle trap containing the pheromone lure: 2-phenethyl propionate, eugenol, geraniol, and (R,Z)-5-(1-decenyl)dihydro-2(3H)-furanone (Trécé, Inc., Adair, Oklahoma). Prior to testing, beetles were sustained in the greenhouse on a diet of Tiffany and Mirandy hybrid tea roses and peach fruit.

Extracts of elm leaf waxes from 23 different elm species/hybrids (Table 2) were prepared from leaf material collected at the Morton Arboretum. Multiple trees of each species/hybrid were sampled to account for variation within species. Leaves were weighed into 2.5g samples for wax extraction, approximating 6-7 large leaves. Leaf area was
measured using an LI-3100 Area Meter (LI-COR, Inc., Lincoln, Nebraska). Wax extractions were completed using a 10-second leaf dip in 40 ml of hexane. Extracts were then concentrated to a final 5ml volume under nitrogen.

Whatman no.1 qualitative filter paper discs (r=0.95 cm) were soaked in a 10% sucrose solution and then allowed to air dry before application of the leaf wax extract. 200 µl of the final wax extract was applied to individual paper discs. The amount of wax extract applied to the filter papers was determined by using average surface area from the leaf material extracted. The wax-disc feeding arena is pictured in Figures 1 and 2. The treated paper disc is suspended by a stainless steel pin in the middle of the paper cup arena at a height of 3 cm. A moist cotton wick was placed in each wax-disc feeding arena to serve as a water source. Beetles were maintained in the arena with a mesh screen covering the top.

Wax-disc feeding arenas were placed in a greenhouse conditioned at 27°C and beetles were allowed to feed for a maximum of 5 days. In most cases, feeding was observed within 24 hours. At the end of the 5-day test period, most beetles were found dead. Wax treated discs were placed into envelopes and consumption was measured by calculating the difference in area measurements from perfect circle estimates and the eaten discs. Adobe Photoshop CS2 (Version 9.0) and an HP Scan Jet 4670 digital flatbed scanner with a linear sensor (Hewlett Packard Company, Palo Alto, California) was used to quantify surface area of the wax-discs.

Data on surface-area consumption were analyzed with PROC MIX (SAS Version 9.1). A nested hierarchy was used to account for the variance component from within elm tree species. Pair-wise comparisons using least squares means are reported along with the overall adult beetle feeding frequency.
Results

Analysis of Elm Leaf Volatile Complexity with Trends in Insect Preference

Chemical Analysis.

Results from the survey of leaf volatile complexity yielded more than 100 chemicals. Major components are presented for specific elm species comparisons that were relevant in the gypsy moth developmental study and the Japanese beetle no-choice feeding preference studies (Tables 3 and 4). More components from the elm leaf volatile extracts are presented for specific species, based on relevance to the Japanese beetle wax-disc study (Table 5). The major components recovered included chemicals that fall into terpene, phenolic, lipid, and alkaloid groupings.

Gypsy Moth Developmental Study.

Principal component analysis incorporating all chemical groups surveyed and leaf physical properties (leaf toughness and thinness) for 16 of the elm species/hybrids yielded two eigenvectors that accounted for 67% of the variability in the data. The first eigenvector, accounting for 41% of the data’s variability, has the largest loading from the lipids (0.514). Terpenes (0.503), phenolics (0.441), and alkaloids (0.441) also showed large loadings on this vector. The second eigenvector has large loadings from leaf toughness (0.750) and thickness (0.594). Analyses, taking into account closely related species in the David complex, were preformed on all chemical and physical leaf data. The total number of lipids was the only variable that showed a significant effect relating to this parentage ($F=7.18$; df=1, 15; $P=0.0180$). The overall lipid diversity was greater in elm species closely associated with the David complex.
Separate principal component analysis with larval longevity, pupal mass, and percentage adult emergence from the gypsy moth developmental study yielded two eigenvectors that accounted for 87% of the variability in the data. When the data was analyzed with a multivariate analysis with elm parentage associating species/hybrids belonging to the David complex as the independent variable, there was a significant effect due to parentage ($F=3.48; \text{df}=3, 12; \text{P}=0.0502$). There was also a direct correlation found between adult emergence of gypsy moth and diversity of leaf lipids ($r=0.42916; \text{df}=15; \text{P}=0.0972$).

A graphic examination of the first and second eigenvectors from the data on chemical and physical leaf properties, and gypsy moth larval development reinforces that elms belonging to the David complex are better suited for gypsy moth larval development than other elms included in this survey. In general, the trees belonging to the David complex tended to have a larger diversity of leaf terpenes, phenolics, alkaloids, and lipids.

**Japanese Beetle No-Choice and Field Defoliation Study.**

All of the data on leaf physical and chemical properties was used for analysis with the Japanese beetle and was included in the first principal component analysis in this section. However, there were only 14 elm species/hybrids included in the analysis of Japanese beetle no-choice and field defoliation studies, so a separate principal component analysis was completed for comparison. The first two eigenvectors from the principal component analysis of this smaller data set now account for 68% of the variability in the data, and the eigenvectors show loadings similar to those previously described.
Principal component analysis with results from Japanese beetle tests (no-choice and field defoliation studies) on 14 different elm species/hybrids gave two eigenvectors that accounted for 94% of the variability. Elm species/hybrid parentage had a significant effect on percentage leaf defoliation \( (F=7.59; \text{df}=1, 12; P=0.0174) \) and fecal pellet masses \( (F=27.73; \text{df}=1, 12; P=0.0002) \) in the no-choice bioassay. Field defoliation rating was not significant when tested individually, but a multivariate test with all three Japanese beetle endpoints was significant \( (F=7.75; \text{df}=3, 10; P=0.0058) \) and the first eigenvector from the principal component showed large loadings from all three variables (leaf defoliation =0.650; fecal pellet mass=0.572; field defoliation rating=0.501). Overall, Japanese beetle adults showed higher preference and feeding activity on the elm trees that belonged to the David complex. No significant correlation was found between Japanese beetle feeding endpoints and elm leaf diversity of lipids.

**Japanese Beetle Wax-Disc Feeding Study**

Adult beetles consumed significant amounts of the sugar-only treated filter papers (positive control). There was no consumption of the untreated filter paper discs (negative control), however there was evidence of tasting and regurgitating. Considerable variation was found in surface area consumption of the paper discs treated with different elm species' hybrids' surface waxes (Table 6). Six of the species surveyed showed significant differences when compared to the negative control: Accolade; Vanguard; *U. japonica*; *U. chenmoui* (Cheng); *U. szechuanica*; *U. crassifolia* (Nutt.). There were only three species/hybrids that showed significantly lower rates of consumption than the positive control (Commendation; *U. propinqua var suberosa*; *U. wilsoniana*).
Discussion

Analyses presented here included physical leaf parameters that might limit Japanese beetle feeding activity. Leaf toughness and thickness were included with the principal component analysis of leaf chemical diversity. However, both leaf thickness and toughness had the largest loadings on the second eigenvector, which only accounted for 26% of the variability in the data. These two variables did not show a significant effect relating to the David elm parentage. In previous studies on elm tree susceptibility to Japanese beetle feeding activity, pubescence was identified as a major factor in determining beetle preference. The high density of trichomes present on the foliage of *U. glaucescens*, *U. lamellosa*, and *U. macrocarpa* is believed to explain the low levels of feeding seen in no-choice and multiple-choice laboratory studies (Miller et al. 1999).

Much of the significance from the leaf volatile extractions and elm species/hybrids belonging to the *U. davidana* complex (*U. davidiana*, *U. japonica*, *U. wilsoniana*, Triumph, Accolade, and *U. propinqua*) and the closely related species *U. szechuanica*, all show moderate to high levels of Japanese beetle feeding. Trends seen in the principal component and multivariate analysis with both Japanese beetle and gypsy moth suitability and preference showed that elm parentage is related to the diversity of multiple chemical groups present in the leaves (terpenes, phenolics, lipids, and blend complexity). The strongest connection to any one chemical group was the adult emergence of gypsy moth, which showed a positive correlation to elm leaf lipid diversity. There was no significant correlation found between elm leaf lipid diversity and Japanese beetle endpoints. This could be due to the lower number of observations, which resulted in a limited number of degrees of freedom in the analysis.
Significant effects with elm leaf waxes were seen in the Japanese beetle wax-disc feeding study. Extracts from three of the 23 elm species/hybrids surveyed negatively impacted Japanese feeding activity. Out of these three elms, *U. wilsoniana* was the only one that was included in the principal component analysis above and also had a lower lipid diversity compared to other elms in the David complex. It is possible that specific chemicals found in the epicuticular wax layer of these three species deter feeding, or there might be lack of a particular feeding stimulant.

None of the wax-treated discs showed an increase in consumption significantly differing from the positive control, which suggests that there is no feeding stimulant present in the wax extracts. It is possible that some of the leaf surface chemicals may interact with other compounds in the leaf to stimulate Japanese beetle feeding, but additional studies would need to be conducted to identify such interactions. Further studies will also need to be conducted on these wax extracts to trace the antifeedant activity to specific chemicals. The beetle behaviors might be related to leaf wax diversity, quantity, or specific compounds. Another interesting comparison for future studies would be evaluation of a hybrid with *U. wilsoniana x U. propinqua var suberosa* parentage.

To date much of the research on elms shows that species and hybrids closely associated with the David complex have good levels of disease resistance and tolerance for the urban environment. These trees are more tolerant of poor soil types, are less susceptible to elm leafminer injury (Miller 2000), and have excellent foliage and growth habit. Unfortunately, these trees may not be useful in areas with high Japanese beetle and gypsy moth populations based on suitability and preference testing (Miller et al. 1999 and Miller unpublished). Trends highlighted by this research show that susceptibility to gypsy moth and
Japanese beetle injury may be linked to leaf chemistry, perhaps in addition to leaf pubescence. Further work in characterizing elm leaf waxes is needed to trace activity to specific compounds and then understand the mechanisms of host plant resistance occurring in this system.

Acknowledgements

Support for this research was provided by the TREE fund, Champaign, Illinois and the Morton Arboretum, Lisle, Illinois. Thanks to Bryan Clark for assistance in the laboratory, Dr. Michael Collyer for statistical guidance, and the Iowa Department of Agriculture and Land Stewardship for use of the Japanese beetle pheromone traps.

References


Table 1. Elm (*Ulmus*) species/hybrids surveyed for leaf volatile complexity.

<table>
<thead>
<tr>
<th>Species/hybrid</th>
<th></th>
<th>Species/hybrid</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. castaneifolia</em></td>
<td>Danada Charm*</td>
<td><em>U. lamellosa</em></td>
</tr>
<tr>
<td><em>U. pumila</em></td>
<td></td>
<td><em>U. crassifolia</em></td>
</tr>
<tr>
<td><em>U. propinquu var suberosa</em></td>
<td></td>
<td><em>U. americana</em></td>
</tr>
<tr>
<td><em>U. davidiana</em></td>
<td></td>
<td>Commendation*</td>
</tr>
<tr>
<td>*U. glaucesens var. lastophylla</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. chenmou</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. pseudoprinqua</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triumph*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. wilsoniana</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accolade*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. thomasii</em></td>
<td></td>
<td>Vanguard</td>
</tr>
<tr>
<td><em>U. prunifolia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>U. japonica</em></td>
<td></td>
<td><em>U. macrocarpa</em></td>
</tr>
<tr>
<td><em>U. szechuanica</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Elm species/hybrids belonging to, or closely associated with the David complex.*
Table 2. Elm (*Ulmus*) species/hybrids evaluated for effects on Japanese beetle feeding in the wax-disc bioassay.

<table>
<thead>
<tr>
<th>Species/hybrid</th>
<th>Champion</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. castaneifolia</em></td>
<td>Danada Charm*</td>
</tr>
<tr>
<td><em>U. pumila</em></td>
<td><em>U. lamellosa</em></td>
</tr>
<tr>
<td><em>U. propinqua var suberosa</em></td>
<td><em>U. crassifolia</em></td>
</tr>
<tr>
<td><em>U. davidiana</em></td>
<td><em>U. americana</em></td>
</tr>
<tr>
<td>*U. glaucesens var. lasiophylla</td>
<td><em>U. propinqua</em></td>
</tr>
<tr>
<td><em>U. chenmoui</em></td>
<td>Commendation*</td>
</tr>
<tr>
<td><em>U. japonica</em></td>
<td><em>U. bergmanniana</em></td>
</tr>
<tr>
<td>Triumph*</td>
<td><em>U. szechuanica</em></td>
</tr>
<tr>
<td><em>U. wilsoniana</em></td>
<td><em>U. gausennii</em></td>
</tr>
<tr>
<td>Accolade*</td>
<td><em>U. parvifolia</em></td>
</tr>
<tr>
<td><em>U. thomasii</em></td>
<td>Vanguard</td>
</tr>
<tr>
<td><em>U. macrocarpa</em></td>
<td></td>
</tr>
</tbody>
</table>

*Elm species/hybrids belonging to, or closely associated with the David complex.
Table 3. Leaf volatile complexity of elm species/hybrids the gypsy moth was able to complete development on: Gaussen elm (*U. gaussenii*), Danada Charm (*U. japonica x wilsoniana*), Commendation (complex hybrid).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Match Quality</th>
<th>Percent of Fixed Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>U. gaussenii</em></td>
<td>Danada Charm</td>
</tr>
<tr>
<td>phenylethyl alcohol</td>
<td>93%</td>
<td>5.60</td>
</tr>
<tr>
<td>2,6-dimethyl-3,7-octadien-2,6-diol,</td>
<td>78%</td>
<td>5.04</td>
</tr>
<tr>
<td>methyl salicylate</td>
<td>95%</td>
<td>4.02</td>
</tr>
<tr>
<td>3-allyl-6-methoxyphenol</td>
<td>97%</td>
<td>5.76</td>
</tr>
<tr>
<td>2-me-,2-ethyl-3-hydroxyhexyl ester propanoic acid,</td>
<td>78%</td>
<td>6.12</td>
</tr>
<tr>
<td>diethyltoluamide</td>
<td>91%</td>
<td>19.35</td>
</tr>
<tr>
<td>(z)-9-octadecenamide</td>
<td>86%</td>
<td>9.39</td>
</tr>
<tr>
<td>n-hexadecanoic acid</td>
<td>90%</td>
<td>-</td>
</tr>
<tr>
<td>(z,z)-9,12-octadecadienoic acid</td>
<td>90%</td>
<td>-</td>
</tr>
<tr>
<td>octadecane</td>
<td>95%</td>
<td>-</td>
</tr>
<tr>
<td>(e)-3-tetradecen-5-yne</td>
<td>78%</td>
<td>-</td>
</tr>
<tr>
<td>9,12,15-octadecatrienal</td>
<td>91%</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4. Leaf volatile complexity of the least preferred species/hybrids elm species by the Japanese beetle in no-choice feeding preference studies: Gansu elm (*U. glaucescens*), golden-bark elm (*U. lamellosa*), and large-fruitied elm (*U. macrocarpa*).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Match Quality</th>
<th>Percent of Fixed Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U. glaucesens</td>
<td>U. macrocarpa</td>
</tr>
<tr>
<td>phenylethyl alcohol</td>
<td>93%</td>
<td>11.57</td>
</tr>
<tr>
<td>n-hexadecanoic acid</td>
<td>90%</td>
<td>27.21</td>
</tr>
<tr>
<td>(z,z)-9,12-octadecadienoic acid</td>
<td>90%</td>
<td>14.98</td>
</tr>
<tr>
<td>(z)-9-octadeNormamide</td>
<td>68%</td>
<td>6.93</td>
</tr>
<tr>
<td>diethyltoluamide</td>
<td>91%</td>
<td>-</td>
</tr>
<tr>
<td>n-hexadecanoic acid</td>
<td>90%</td>
<td>-</td>
</tr>
<tr>
<td>eicosane</td>
<td>92%</td>
<td>-</td>
</tr>
<tr>
<td>hexadecanal</td>
<td>91%</td>
<td>-</td>
</tr>
<tr>
<td>(e)-3-tetradecen-5-yne</td>
<td>78%</td>
<td>-</td>
</tr>
<tr>
<td>(z,z,z)-9,12,15-octadecatrien-1-ol</td>
<td>94%</td>
<td>-</td>
</tr>
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</table>
Table 5. Leaf volatile complexity of the least preferred species/hybrids evaluated in the wax-disc greenhouse study with the Japanese beetle: (*U. wilsoniana*), Commendation (complex hybrid), and (*U. propinqua var suberosa*).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Match Quality</th>
<th><em>U. wilsoniana</em></th>
<th>Commendation</th>
<th><em>U. propinqua var suberosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>phenylethyl alcohol</td>
<td>93%</td>
<td>7.24</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>9-octadecenamide</td>
<td>68%</td>
<td>10.40</td>
<td>-</td>
<td>3.21</td>
</tr>
<tr>
<td>eugenol</td>
<td>94%</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(z,z)9,12-octadecadienoic acid (z,z)</td>
<td>90%</td>
<td>24.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pentacosane</td>
<td>93%</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>heptadecane,9-octyl-n-hexadecanoic acid</td>
<td>95%</td>
<td>18.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(e)-3-tetradecen-5-yne</td>
<td>90%</td>
<td>-</td>
<td>14.37</td>
<td>8.1</td>
</tr>
<tr>
<td>9,12,15-octadecatrienal</td>
<td>78%</td>
<td>-</td>
<td>3.78</td>
<td>-</td>
</tr>
<tr>
<td>heneicosane</td>
<td>91%</td>
<td>-</td>
<td>50.66</td>
<td>26.3</td>
</tr>
<tr>
<td>diethyltoluamide</td>
<td>95%</td>
<td>-</td>
<td>-</td>
<td>13.8</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>96%</td>
<td>-</td>
<td>-</td>
<td>12.1</td>
</tr>
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</table>
Table 6. Japanese beetle feeding frequency and surface area consumption of wax-disc in the greenhouse feeding wax-disc bioassay.

<table>
<thead>
<tr>
<th>Species/Hybrid</th>
<th>Feeding Frequency</th>
<th>Disc Consumption (cm²)</th>
<th>Standard Deviation (Disc Consum.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U. glaucesens var lasiophylla</td>
<td>0.67</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>Accolade</td>
<td>0.89</td>
<td>0.17*</td>
<td>0.07</td>
</tr>
<tr>
<td>Vanguard</td>
<td>0.83</td>
<td>0.15*</td>
<td>0.10</td>
</tr>
<tr>
<td>U. davidiana</td>
<td>0.67</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Triumph</td>
<td>0.67</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>U. gausennii</td>
<td>0.44</td>
<td>0.07</td>
<td>0.10</td>
</tr>
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*Treatments that significantly differ from the negative control (no-sugar) by least-squares means analysis at α = 0.10.

*Treatments that significantly differ from the positive control (sugar treated) by least-squares means analysis at α = 0.10.
Figure 1. Japanese beetle wax-disc feeding arena.
Figure 2. Horizontal view of the Japanese beetle wax-disc feeding arena.
CHAPTER 3. LEAF TOTAL PHENOLIC CONTENT OF ASIAN ELMS AND HOST PLANT SUITABILITY FOR GYPSY MOTH AND JAPANESE BEETLE

A paper to be submitted to Environmental Entomology

Gretchen Schultz, Fredric Miller, and Joel Coats

Abstract

The total phenolic content of 13 species/varieties of elm tree foliage was measured and the relationship to Japanese beetle feeding preference studies and gypsy moth developmental studies was determined. The 13 elm species/hybrids, *Ulmus wilsoniana* (Schneid.), *U. japonica* (Rehd.), *U. glaucesens* (Franch), Accolade, Triumph, *U. davidiana* (Planch), *U. parvifolia* (Jacq.), *U. szechuanica* (Fang), *U. macrocarpa* (Hance), *U. chenmouyi* (Cheng), *U. lamellosa* (C. Wang et S.L. Chang, ex L.K. Fu et al.), *U. castaneifolia* (Hemsl.), *U. gausseennii* (Cheng), were grown at the Morton Arboretum, Lisle, Illinois and sent to the Pesticide Toxicology Laboratory at Iowa State University, Ames, IA for chemical analysis. There were significant differences found in the phenolic content of the 13 elm species/varieties surveyed. Results from Japanese beetle preference studies indicated that adult beetles moderately preferred feeding on Asian elms belonging to the “David” complex. Analysis presented here show that there is a positive correlation between gypsy moth adult emergence and total phenolic content, but this relationship does not follow trends in insect
susceptibility according to Asian elm parentage. In multiple-choice studies, Japanese beetles preferred to feed on elm species/hybrids with a lower level of total phenolic content. However, no significant trends were found between Japanese beetle no-choice and field defoliation studies and the variables tested here.

**Introduction**

Elm trees (Ulmaceae) are some of the most highly valued shade trees in North America. Even after the invasion of Dutch elm disease that first plagued North America in the 1930s, homeowners still take great pride and pleasure in the elm. A survey conducted in an elm leaf beetle-infested neighborhood in Sacramento, California found the public reporting major benefits, such as shade and visual aesthetics (Sommer and Summit 2000). Today, there are many new cultivars available from Europe and Asia offering a variety of natural defenses to disease. There have been immense efforts in exploring their natural resistance and plant breeders are developing new hybrids that are not susceptible to Dutch elm disease, elm yellows, verticillium wilt, and insect herbivory.

Some of the most common insect pests on elm include fall cankerworm, *Alsophila pometaria* (Harris), spring cankerworm, *Paleacrita vernata* (Peck), elm leaf beetle, *Pyrrhalta luteola* (Mueller), elm leaf miner, *Fenusa ulmi* (Sundevall). More recently, invasive species including generalist feeders like the Japanese beetle, *Popillia japonica* (Newman) and the gypsy moth, *Lymantria dispar* (L.) have raised concern for determining host plant suitability and identifying mechanisms of insect resistance. Elm hybrids have varying levels of resistance to numerous insect pests. Resistance can occur through insect-plant interactions such as: antibiosis, antixenosis, and tolerance (Painter 1951).
Past work has indicated that plant responses to these insect-herbivore interactions commonly involve plant secondary compounds. There are a wide variety of secondary compounds isolated from plants and include large groups such as alkaloids, glycosides, terpenoids, iridoids, and phenolics.

Phenolic compounds have long been identified as a group of chemicals closely tied to insect-plant interactions (Feeny 1976; Rhoades and Cates 1976; Coley et al. 1985), but also function in U.V. protection, regulation of nutrient cycling within the ecosystem, pollinator attraction, seed dispersal, fungal defense, and allelopathy (Waterman 1992; Lois 1994). This large class of compounds can be identified by the presence of a hydroxy-substituted aromatic ring. Some of the more common plant phenolics are grouped as flavonoids, lignans, tannins, and hydroxy-quinones, all of which have been reported in numerous insect-plant relationships. Nishida et al. (1987) reported four flavonoids to stimulate oviposition by the citrus-feeding swallowtail, and Harborne (1988) noted several flavonoids as feeding stimulants. For *Bootettix argentatus* (Bruner), a specialist feeder, a surface lignan serves as a feeding stimulant, but deters other generalist herbivores (Chapman et al. 1988). *Betula resinifera* (Britton)'s tannins were found to possess high antiherbivore activity (Ayres et al. 1997).

There is a diversity of insect responses to phenolics, and the responses can depend upon the environment, specific concentrations of phenolics that are present, or the physiological state of the receiving insect (Scriber and Slansky 1981). Past studies have most commonly exhibited a negative feeding response to higher phenolic content (Haukioja et al. 1985; Rossiter et al. 1988; Niemela et al. 1979; Wratten et al. 1984). However, this is
not always the case. Insect attraction to phenolic compounds has also been described (Hartley and Lawton 1987).

The purpose of this study was to evaluate the host plant suitability of Asian elms for the gypsy moth and compare foliar total phenolic content to growth and development of the gypsy moth. Thirteen different species/hybrids of elm were evaluated in this study (*Ulmus wilsoniana* (Schneid), *U. japonica* (Rehd), *U. glaucesens* (Franch), Accolade, Triumph, *U. davidiana* (Planch), *U. parvifolia* (Jacq.), *U. szechuanica* (Fang), *U. macrocarpa* (Hance), *U. chenmoui* (Cheng), *U. lamellosa* (C. Wang et S.L. Change, ex L.K. Fu et al.), *U. castaneifolia* (Hemsl.), *U. gaussennii* (Cheng)). Previous studies have cited that elms with heavy leaf pubescence (*U. glaucescens*, *U. lamellosa*, and *U. macrocarpa*) are less preferred by adult Japanese beetles (Miller et al. 1999; Miller et al. 2001). *U. davidiana*, *U. japonica*, *U. wilsoniana*, and (*U. propinqua*), all elms belonging to the David complex, and the Szechuan elm (*U. szechuanica*) all show moderate to high levels of susceptibility to the Japanese beetles. This suggests that elm species/hybrid parentage might be related to host plant resistance to the Japanese beetle. Knowledge of certain leaf properties that contribute to this relationship would be a valuable guide for elm breeding.

Total phenolic content is often times one of the preliminary steps in investigating chemical resistance among these elms (Hartley and Firn 1989; Suomela et al. 1995; Bergelson et al. 1986). This study points the direction for further research on the elm foliar chemistry and its effects on insect behavior. Eventually, species with more effective defense systems for deterring insect pests will serve a valuable function in breeding programs.
Materials and Methods

Measurement of Leaf Total Phenolic Content.

Elm leaves from 13 different species/hybrids were obtained from the Morton Arboretum, Lisle, Illinois, and shipped overnight to Iowa State University, Ames, Iowa where they were stored at -5°C. Before extraction, the leaves were ground to a powder in liquid nitrogen with a chilled mortar and pestle. A 100mg sample of each was stirred at room temperature for 2 hours in 20ml of 100% methanol (Fisher Scientific, Pittsburgh, PA). The methanol samples were then filtered and washed with 20 ml of hexane six times before final filtration through a 0.45µm syringe filter. Each remaining methanol solution was adjusted to a 10ml volume for total phenolic content measurements by a modified Folin-Ciocalteau procedure described by Torres et al. (1987). Samples were analyzed for total phenolic content by mixing 0.1ml of the methanol sample with 6 ml water. A 0.5ml volume of Folin-Ciocalteau Reagent (Sigma-Aldrich, St. Louis, MO), was added to the mixture and allowed to stand for at least 1 minute. Next, 1.5-ml of 20 % (wt/vol) sodium carbonate solution was added, and the final reaction mixture was brought to a 10-ml final volume with water. Each sample was maintained at 50°C for 2 hours in 25ml test tubes. The absorbance was measured on a spectrophotometer MV 21 at 765nm. For comparison, known concentrations of gallic acid (Aldrich) were used to develop a calibration curve.

Leaf toughness and thickness was recorded with the use of a model Digital Force Meter leaf penetrometer (Chatillon, Greensboro, NC.). Data were collected on fresh leaves from trees at the Morton Arboretum.

Significant differences among the 13 elm species/varieties in the current project were detected by a nested ANOVA using SAS (SAS Institute 1991). Total phenolic content was
analyzed with biological data from gypsy moth developmental studies (Miller unpublished) and Japanese beetle feeding trials (no-choice preference tests and field defoliation observations) (Miller et al. 1999). A principal component analysis with PROC PRINCOM (SAS Version 9.1) was used to compare biological data from gypsy moth developmental studies and Japanese beetle trials, chemical data, and leaf physical measurement data relationships. A correlation matrix was used to equalize the variances between different units of measurement. P-values for variables that showed high levels of correlation were obtained by using PROC CORR (SAS Version 9.1). Variables that were large contributors to the first two eigenvectors were further analyzed for levels of significance in a multivariate analysis with PROC GLM (SAS Version 9.1). Elm tree parentage served as the independent variable. This included classifying elms according to whether or not they belonged to the David complex, including *U. davidiana*, *U. japonica*, *U. wilsoniana*, *U. propinqua*, and the hybrids Triumph (*U. japonica-wilsoniana* x *U. japonica-pumilia*) and Accolade (*U. japonica-wilsoniana*). *U. szechuanica* was grouped with the David complex in this analysis because it is a closely related species of elm (Fu 1980).

**Gypsy Moth Developmental Study.**

Gypsy moth laboratory studies on host plant suitability were conducted at the Morton Arboretum (Lisle, IL), in plastic petri dishes (10 x 0.6cm) with one male and one female, given fresh leaf material every 2 days (Miller and Ware 1994). Dishes were checked daily for mortality, pupation, and emergence. Ten replicates of each elm species/hybrids were run for a maximum of 21 days. Gypsy moths were given no choice in these bioassays and therefore forced to complete development on the sample of one elm in the petri dish. Data are
reported as duration of larval longevity, pupal fresh weight, and percentage adult emergence. Natural log transformations were preformed on pupal fresh weights and percentage adult emergence.

**Japanese Beetle Study.**

Japanese beetle feeding preference was determined in 1997. No-choice studies involved 1 adult female beetle in a clear petri dish (10 x 0.6 cm) with fresh foliage available every two days. Dishes were checked daily, and feeding was recorded by visual rating with the use of a defoliation template. Dry fecal pellet masses were also recorded throughout the study.

The field defoliation study was conducted on trees at the Morton Arboretum in 1997 assessing injury from Japanese beetle feeding activity. A scale from 0-4 was used with 0 representing the lowest level of activity (no feeding) and 4, the highest level of activity (>50% canopy damage). Details of both no-choice and field defoliation studies have been previously reported (Miller et al. 1999).

Multiple-choice feeding studies were previously reported (Miller et al. 1999). Bioassays consisted of 3-7 elm species/hybrids individual leaf discs in a single petri dish (15 x 0.6 cm) with one adult beetle. Dishes were evaluated daily for 5 days, and visually evaluated with a defoliation template. Fresh leaf material was introduced each day into the petri dish. Placement of the leaf discs was randomized. Data from 4 individual multiple-choice feeding studies (including 3-7 different elm species/hybrids in each preference test) were analyzed for relationships with total phenolic content separately from the no-choice and field defoliation studies using PROC REG (SAS Version 9.1).
Results

Measurement of Leaf Total Phenolic Content.

Analysis of variance of the total phenolic content of the 13 elm species/hybrids showed significance in the overall model with $P<0.0001$ (Figure 1). *U. gaussennii*, had the highest phenolic content, and Accolade had the lowest.

Gypsy Moth Developmental Study.

Table 1 reports the summary of biological data from the gypsy moth developmental study. Of the 13 elm species/hybrids tested, *U. szechuanica* resulted in the longest duration of larval longevity, and *U. gaussennii* appears more suitable for development with a higher percentage survival, percentage pupation, and percentage adult emergence.

There was a significant positive correlation for elm leaf total phenolic content and gypsy moth adult emergence ($r=0.598; \text{df}=12; P=0.031$). Principal component analysis incorporating total phenolic content and physical leaf measurements (toughness and thickness) gave two eigenvectors that accounted for 71% of the variability in the data. The first eigenvector, accounting for 55% of the data’s variability, has large loadings from the leaf thickness and toughness (0.699; 0.697). Total phenolic content was a large loading on the second eigenvector (0.987).

A multivariate analysis with larval longevity, pupal mass, and percentage adult emergence dependent variables and elm parentage (associating species/hybrids belonging to the David complex) as the independent variable did not show a significant effect due to parentage ($F=32.19; \text{df}=3, 9; P=0.159$). However, the relationship between larval longevity and elm parentage was significant ($r=0.395207; \text{df}=12; P=0.021$).
Japanese Beetle Study.

Data from Japanese beetle no-choice and field defoliation studies was analyzed with elm leaf total phenolic content and physical leaf measurements (toughness and thickness) in a principal component analysis. The only significant correlation was found with adult beetle leaf defoliation and leaf thickness \( (r=-0.5154; \text{df}=12; P=0.071) \). The first two eigenvectors produced from the data set accounted for 58% of the variability. Leaf toughness (0.701) and thickness (0.707) were large loadings on the first eigenvector (PCA1). The second eigenvector (PCA2) was composed of a large loading from leaf total phenolic content (0.991).

Principal component analysis with results from Japanese beetle tests (no-choice and field defoliation studies) on 13 different elm species/hybrids gave two eigenvectors that accounted for 95% of the variability. Elm species/hybrid parentage had a significant effect on percentage leaf defoliation \( (F=6.91; \text{df}=1, 12; P=0.024) \) and fecal pellet masses \( (F=22.86; \text{df}=1, 11; P=0.0006) \) in the no-choice bioassay. Field defoliation rating was not significant when tested individually, but a multivariate test with all three Japanese beetle endpoints was significant \( (F=6.26; \text{df}=3, 9; P=0.014) \), and the first eigenvector from the principal component showed large loadings from all three variables (leaf defoliation =0.656; fecal pellet mass=0.572; field defoliation rating=0.493). Overall, Japanese beetle adults showed higher preference and feeding activity on the elm species/hybrids that belonged to the David complex. No significant trends were found between Japanese beetle feeding choice and field defoliation study endpoints and the variables tested here.
Simple linear regression tests with the individual data sets from four of the Japanese beetle multiple-choice tests showed significant negative relationships between total phenolic content percentage leaf defoliation (Figure 2).

**Discussion**

The elm trees surveyed vary significantly in total phenolic content. A correlation was found with gypsy moth adult emergence. However, this trend was not found to be significantly related to elm parentage (species belonging to the David complex). Results from the multivariate analysis showed a highly significant relationship between gypsy moth larval longevity and David complex parentage. When all of the gypsy moth developmental endpoints were incorporated into the model, the relationship was not significant. This could be due to the limited number of degrees of freedom. When more data points are included (as in the previous chapter), the model becomes significant. Neither of the remaining leaf variables tested (leaf toughness and thickness) were significantly related to elm host plant susceptibility to the gypsy moth.

Elm tree susceptibility to Japanese beetle injury has previously been tied to leaf pubescence. The high density of trichomes present on the foliage of *U. glaucescens*, *U. lamellosa*, and *U. macrocarpa* is believed to explain the low levels of feeding seen in no-choice and multiple-choice laboratory studies (Miller et al. 1999). Analyses presented here included leaf toughness, thickness, and total phenolic content as parameters that might limit Japanese beetle feeding activity. Total phenolic content was shown to mediate Japanese beetle feeding preferences in four multiple-choice tests. When given a choice to feed on a
variety of elm species/hybrids, Japanese beetle will prefer to feed on leaves with a lower level of total phenolic content.

Analysis of the Japanese beetle no-choice and field defoliation endpoints showed a high significance to David parentage, but this was not related to the variables tested here. Other factors must be responsible for determining Japanese beetle feeding behavior within the David complex.

Acknowledgements

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References


Table 1. Suitability of Asian elms for gypsy moth growth and development, May-July, 2001.

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<th>Mean Pupal Mass (mg)</th>
<th>Mean Percentage Adult Emergence</th>
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*Elm species/hybrids belonging to, or closely associated with the David complex.
Figure 1. Total phenolic content of the thirteen elm species/hybrids surveyed.
Figure 2. Japanese beetle percentage defoliation of elm tree species/hybrids in relation to total phenolic content of elm tree foliage in four separate multiple-choice studies (Miller et al. 1999).
CHAPTER 4. GENERAL CONCLUSIONS

There are currently many insects that injury elm trees, but there is significant concern for controlling emerging invasive species such as the Japanese beetle, *Popillia japonica* (Newman), and the gypsy moth, *Lymantria dispar* (L.). Research has shown that different elm species/hybrids exhibit various levels of resistance to numerous insect pests. A basic understanding of the general foliar chemistry and the effects of specific compounds on herbivore resistance will serve a valuable function in breeding programs.

To date much of the research on elms shows that species and hybrids closely associated with the David complex have good levels of disease resistance and tolerance for the urban environment. Previous studies have cited that elms with heavy leaf pubescence (*U. glaucescens* (Franch), *U. lamellosa* (C. Wang et S.L. Chang, ex L.K. Fu et al.), and *U. macrocarpa* (Hance) are less preferred by adult Japanese beetles (Miller et al. 1999; Miller et al. 2001). *U. davidiana* (Planch), *U. japonica* (Rehd.), *U. wilsoniana* (Schneid.), and *U. propinqua* (Koidz), all elms belonging to the David complex, and the Szechuan elm, *U. szechuanica* (Fang) all show moderate to high levels of susceptibility to the Japanese beetles. These trees are more tolerant of poor soil types, are less susceptible to elm leafminer injury (Miller 2000), and have excellent foliage and growth habit. Unfortunately, these trees may not be useful in areas with high Japanese beetle and gypsy moth populations based on suitability and preference testing (Miller et al. 1999 and Miller unpublished). Trends highlighted by this research show that susceptibility to gypsy moth and Japanese beetle injury is linked to leaf chemistry, in addition to leaf pubescence.
Trends seen in this analysis are that Japanese beetle preference and gypsy moth suitability is related to elm parentage and also connected to the diversity of multiple chemical groups present in the leaves (terpenes, phenolics, alkaloids, and lipids). The strongest connection to any one chemical group was the adult emergence of gypsy moth, which showed a correlation to elm leaf lipid diversity. Further work in characterizing elm leaf waxes is needed to trace activity to specific compounds and then understand the mechanisms of host plant resistance occurring in this system.

There were significant differences found in the phenolic content of the 13 elm species/hybrids surveyed. Results from Japanese beetle preference studies indicated that adult beetles moderately preferred feeding on Asian elms belonging to the “David” complex. Analysis presented here show that there is a positive correlation between gypsy moth adult emergence and total phenolic content, but this relationship does not follow trends in insect susceptibility according to Asian elm parentage. In multiple-choice studies, Japanese beetles preferred to feed on elm species/hybrids with a lower level of total phenolic content. However, no significant trends were found between Japanese beetle no-choice and field defoliation studies and the variables tested here.

More work is required to further explain the effect of host plant volatiles on Japanese beetle adults. Additionally, any information that relates to plant susceptibility, or more specifically, identification of individual or groups of compounds that serve as attractants, deterrents, or toxins, would be valuable for ecological pest management.
References


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APPENDIX. INTRODUCTION

Natural Products Research

Today the arena of natural product chemistries consists of multiple disciplines working on the discovery, and research and development of green chemicals, renewable plastics, natural fibers, and natural structural materials (USDE 2005). Botanical biopesticides and repellents are products derived from plant sources and constitute a category called green chemicals. These types of chemicals are currently the focus of much research. The economic worth of botanical insecticides has recently been estimated to be more than $150 million (Isman 2005), and $388 million (Hall and Menn 1999). Some of the major economic contributors include pyrethrum, neem, rotenone, and a few monoterpenes (i.e. limonene and citronellal). The activities of a few of these natural products have been known for some time, dating back to the nineteenth century in the case of pyrethrum.

Even with the most successful botanical insecticides, many challenges have held back wide commercialization, consumer acceptance, and usage. The history behind the discovery of pyrethrum and its development into one of the most widely used insecticide classes (pyrethroids) entails many of the limitations that still face natural products today. Pyrethrum, first utilized in ground up flower heads of Chysanthemum spp. for flea and lice control (Metcalf and Flint 1962) around the 1800s, contains a mixture of four active toxicant esters: pyrethrins I and II, and cinerins I and II. These compounds led to the development of synthetic analogs, the first of which was allethrin, made in 1949 by La Forge and associates (Metcalf and Flint 1962). Initially, there was a market for allethrin in the use of mosquito coils, but on a cost basis pyrethrins were still more efficient (Maciver 1963). Further work
on structural deviations from the naturally-occurring esters found in *Chrysanthemum* led to the replacement of pyrethrum formulations in the 1970s. Much of the work by Dr. Michael Elliott and Dr. Norman Janes through the National Research and Development Corporation in England on synthesizing new pyrethroids (resmethrin, bioresmethrin, permethrin, cypermethrin, and deltamethrin) caught the attention of agricultural marketing companies, leading to the increased use of pyrethroid-class insecticides (Gullickson 1995). Japanese researchers, including Dr. Junshi Miyamoto at Sumitomo, made structural improvements that increased insecticidal activity, photostability, and decreased mammalian toxicity. These improvements are highlighted by the classification of first, second, third, and fourth generations of pyrethroids.

In terms of research and development for product commercialization, most botanical insecticides and repellents are far behind today’s current market of synthetic products. There are many papers and reports on the insecticidal and repellent activity of botanical extracts. However, only few of these natural products are close to becoming commercially available. Even if more botanicals did make it to the market, it is still unclear whether they would replace synthetic chemicals in large-scale pest management operations. There are also other problems holding back the release of botanicals into the marketplace. Dr. Murray Isman proposed that some criteria for the development and commercialization of botanicals might include the availability of plant resources for production, standardization of the final product for quality control and performance, protection of technology, and regulatory approval on a global scale (Isman 2005).

In many ways, past and current uses of pesticides are shaping a new commercial environment for the introduction of new bioproducts. In the United States, registration of a
new biopesticide product with the Environmental Protection Agency often requires less data, allowing products to become registered in less than a year. This is a substantially less rigorous process than registering a new synthetic pesticide, which can average three years (Eisner 1964). There are also some biopesticides that qualify as a “minimum risk pesticide,” and are therefore exempt from Federal Insecticide Fungicide Rodenticide Act requirements, according to 40 CFR, section 152.25(g). Regulatory guidelines like these are making product registration of biopesticides a cheaper and quicker process.

Some consumer groups, in particular the organic groups, are also having an impact on shaping today’s market. Organic food production sales in 2001 were estimated at $9 to 9.5 billion by the World Trade Organization/United Nations International Trade Center (Greene and Kremen 2003). The practice of organic food production includes limitations on the use of certain chemicals for pest management. Only all natural (non-synthetic) pesticides are allowed on certified organic products, and there are currently very few effective control options available. Biopesticides and other alternative management practices, such as the use of natural products as insect repellents, can offer adequate protection and compatibility with organic production practices. These technologies are currently in demand. With organic sales on the rise, there should continue to be a need for them in the near future (USDA 2005).

In the chapters that follow, data are presented on the repellent properties of catnip, *Nepeta cataria*, and the Osage orange, *Maclura pomifera*. Previous literature has documented the repellency of Osage orange extracts to German cockroach, *Blattella germanica*, and the maize weevil, *Sitophilus zeamais* (Karr and Coats 1991; Peterson et al 2000; Peterson et al. 2002a). The catnip plant has also been a focus of multiple studies of insect repellency. One of the first was completed by Dr. Thomas Eisner in 1964, and showed that catnip had repellent
activity against 13 families of insects. Recent data shows German cockroach repellency to
catnip (Peterson et al 2002b) and biological activity of the active component, nepetalactone,
has been shown previously (Smith et al. 1979; Snook et al. 1993).

The purpose of this Appendix is to further advance research and development of the
active components and mixtures of catnip and Osage orange extracts into useful products for
insect management. Appendix Chapter 2 is an investigation into the seasonal ecology of
nepetalactone and continues to characterize the repellent activity of catnip extracts and
elemol, the major component of Osage orange essential oil. A comparison with current
commercial repellents is also included. In Appendix Chapter 3, extracts of catnip and Osage
orange, in addition to specific components of their essential oils, are examined for mosquito
repellency using the northern house mosquito, Culex pipiens.

Continued efforts are necessary in order to prepare and advance natural product
technologies toward commercial use. There are numerous botanical extracts that have been
identified as having insecticidal or repellent activity. In order for these to catch the attention
of large agricultural groups and successful niche markets, more research is needed to
recognize the specific chemical characteristics of active ingredients and mixtures (i.e.
structure-activity as it relates to potency, spectrum of activity, biodegradability, resistance,
and bioavailability). Background research is also required to analyze and outline the potential
for commercialization. Today’s society is enthusiastic about the adoption of natural products
for pest control, but it is important that basic studies are still conducted on natural product
chemistries. This will ensure that products developed today will be safe and an effective
means of insect management that can stand the test of time.
References


10. **Peterson, C. J., A. Fristad, R. Tsao, and J. R. Coats. 2000.** Examination of Osage orange fruits and two isoflavones, osajin and pomiferin, for repellency to the maize


STUDY 1. CATNIP, *NEPETA CATARIA* (LAMIALES: LAMIACEAE), A CLOSER LOOK: SEASONAL OCCURRENCE OF NEPETALACTONE ISOMERS AND COMPARATIVE REPELLENCY OF THREE TERPENOIDS TO INSECTS


Gretchen Schultz, Erica Simbro, Jason Belden, Junwei Zhu, and Joel Coats

Abstract

Evidence of repellent properties in catnip, *Nepeta cataria* (L.), to flies and cockroaches was observed in preliminary studies. This study compared catnip essential oil from steam distillation and elemol, a major constituent of osage orange essential oil, to current commercial repellents. These comparative studies found both the catnip steam distillate and elemol to be as good, and in some cases better, at repelling house flies, *Musca domestica* (L.), and American cockroaches, *Periplaneta americana* (L.), than N,N-diethyl-m-toluamide (DEET) or citronellal. Both short-term and long-term repellency bioassays were used to assess repellency. Catnip essential oil showed greater repellency than DEET and citronellal in the short-term. Extended repellency bioassays showed elemol to be more repellent than catnip steam distillate, citronellal, and DEET.

Nepetalactone, the major constituent of catnip essential oil is present as two isomers, and previous studies show the *E,Z*-nepetalactone (2-(2-hydroxy-1-methylethenyl)-5-methyl-
cyclopentanecarboxylic acid delta lactone) isomer to be even more repellent to cockroaches than the dominant isomer, Z,E-nepetalactone. This study examined the seasonal variation of the two isomers, Z,E and E,Z-nepetalactone, in catnip. Samples of fresh catnip mature leaves, immature leaves, and stems were steam-distilled separately, and isomer composition was analyzed using HPLC and GC. An ANOVA showed significant differences by week. The mature leaf essential oil samples were tested in a repellency bioassay and exhibited significant repellency to German cockroaches, *Blattella germanica* (L.). The catnip floral volatiles were sampled using SPME, and analysis with GC/MS showed the presence of Z,E-nepetalactone, E,Z-nepetalactone and β-caryophyllene as the major constituents.

Phytophagous insects and potential pollinators present on sampling dates were recorded.

**Introduction**

In today's society, there is a desire to reduce the use of synthetic pesticides. Several biological pesticides have high economic potential and currently make up a significant amount of the pesticide market (Hall and Menn 1998). These compounds are often specific to target species, and most have low toxicity to non-target species. Biological pesticides can have other advantages, such as rapid biodegradation, activity at low application rates, quick knockdown, and the appeal of producing safer foods, as well as a cleaner environment (Mandava 1985).

One specific area of study, the botanical pesticides, provides new perspectives in insect-plant interactions. Since these chemicals come from natural sources, they have the potential to offer novel modes of action for use in pest management, as well as unlocking the structure-function relationships of biologically active compounds (Hall and Menn 1998).
Several botanical insect repellents have also been commercially successful. Citronellal (3,7-dimethyl-6-octenal) is one of the most popular natural compounds and was first used for controlling fleas and lice. It is now found in candles, incense, aroma-therapy and many other commercial products. Citronella oil comes from Cymbopogon nardus (L.), a grass native to Southeast Asia. More recently, experiments have supported folklore about the repellent activity of the fruit of the osage orange tree or hedgeapple, Maclura pomifera (Raf.) Scheid (Moraceae) (Peterson, et al. 2002a) and catnip, Nepeta cataria (L.) (Peterson, et al. 2002b).

Catnip, has historically been known as an insect repellent and also as a folk remedy. In the 1960's it was reported to repel at least 13 families of insects (Eisner, 1964). Also, chemicals present in catnip have been found within defensive secretions of the coconut stick insect, Graeffea crouani (Le Guillou), (Smith, et al. 1979) and the lubber grasshopper, Romalea guttata (Houttuyn), (Snook, et al. 1993) and in the sex attractant pheromones of the damson-hop aphid, Phorodon humuli (Schrank), (Campbell, et.al. 1990) and black bean aphid, Aphis fabae (Scopoli), (Handie, et al. 1994). Specifically, much attention has focused on one of the active components of the essential oil, nepetalactone (5,6,7,7a-tetrahydro-4,7-dimethylcyclopenta[c]pyran-1-(4aH)-one) (McElvain et al. 1941).

Nepetalactone is an iridoid monoterpenoid that appears as two (or more) isomers in catnip, primarily as Z,E-nepetalactone and E,Z nepetalactone (Fig. 1) (Bates, et al. 1963). In a recent study, Peterson et al. (2002b) found that both of the isomers repelled German cockroaches (B. germanica). However, the E,Z-nepetalactone was found to be more repellent than the Z,E-nepetalactone, even at a lower concentration.
In the past, the ratio of \(Z,E\)-nepetalactone:\(E,Z\)-nepetalactone has been identified as 17:3 (Peterson, et al. 2002b). Research in the field of insect pheromones has indicated that many insects rely on specific ratios of compounds for attraction (Sorensen 1996). However, the field of insect repellency is still exploring various mechanisms and relationships to determine a physiological explanation for repellency.

In this study we evaluated changes in the ratio of the two isomers within three locations of the plant throughout the spring-summer season and their relationship to \(B.\) \textit{germanica} repellency. A short summary of the insects found on the collected samples is included, and an analysis of the floral volatiles was conducted, with observation of potential pollinators on collection day.

The other component of this study included short-term and extended repellency bioassays assessing differences between catnip essential oil from steam-distillation and elemol (a major component of osage orange essential oil) to some commercially available insect repellents.

\textbf{Materials and Methods}

\textbf{Collection and Isolation of \(Z,E\)-nepetalactone and \(E,Z\)-nepetalactone}

Aerial catnip biomass was sampled nine times, at seven-day intervals, from 22 May 2001 to 17 July 2001. Samples were from unsprayed, wild catnip stands found on the Iowa State University campus, Ames, Iowa. The majority was taken from established rootstocks bordering the north side of a railroad track on the north side of campus. The habitat was sunny and dry and surrounded by a variety of plant species, primarily mullein, \textit{Verbascum thapsus} (L.), common ragweed, \textit{Ambrosia artemisiifolia} (L.), bee balm, \textit{Monarda didyma}
(L.), crown vetch, *Coronilla varia* (L.), and common lambsquarters, *Chenopodium album* (L.). Each week’s sample consisted of a mass collection of catnip that was clipped at the base of the stem. After collecting, plants were immediately taken to the lab and separated according to three plant parts: mature leaves, immature leaves and stems. Leaf maturity was based on location of the plant and leaf size; leaves larger than 4 cm in length were considered mature. Each plant part was steam distilled according to the method in Pavia et al. (1988). The steam distillate was extracted twice in half volumes of hexane, and the water layer was discarded. Hexane was removed using a rotary evaporator with a 635-mm Hg vacuum at 36°C. The essential oil was stored in a 4°C refrigerator until used for HPLC analysis and repellency bioassays.

The concentration of each isomer within the extract was determined by diluting each essential oil in hexane, followed by analysis using high performance liquid chromatography (HPLC) coupled with UV detection. A Hewlett-Packard 1100 HPLC with a Pirkle Covalent phenylglycine Hi-Chrom preparative column (25 cm, 10 mm I.D., 5 µm S5NH Modified Stereosorb) made by Regis (Morton Grove, Illinois), was used with a mobile phase of 19:1, hexane: ethyl acetate, at a 2.5-ml/min flow rate under the detection of a Spectroflow 757 UV-detector at 254 nm. The ratio was calculated as Z,E concentration/E,Z concentration.

Samples were also analyzed by gas chromatography to confirm identification and ratio of the two nepetalactone isomers. A Varian 3700 model with a 15-meter DB5 column and helium carrier with an FID detector was used. The injection temperature was 250°C, with an initial column temperature of 40°C, held for two minutes, ramped at 10°C/min, finishing at 150°C for five minutes.
An ANOVA was performed on the resulting ratios of Z,E:E,Z-nepetalactone to determine significance of plant part, and week (date collected). Data was compared statistically by using SAS (PROC GLM, SAS Institute 1982).

Heat of formation for the two nepetalactone isomers was calculated by CAChe Work System (Oxford Molecular Ltd., 1999), using an AM1 basis set to optimize structures and energies of the molecules.

**Short-term Repellency Bioassays**

Test solutions consisted of 5%, 1%, 0.5% and 0.1% concentrations (volume/volume) of each compound or essential oil. Compounds tested included DEET, citronellal, and steam distilled catnip essential oil, all in acetone, as well as the samples of mature leaf catnip steam distillates (containing a known ratio of Z,E:E,Z-nepetalactone in hexane). DEET (N,N-diethyl-m-toluamide) and citronellal (3,7-dimethyl-6-octenal) were obtained from Aldrich Chemicals, St. Louis, Missouri.

Insect behavioral response to the repellent compounds was determined using adult male German cockroaches (*B. germanica*), adult male American cockroaches (*P. americana*), and house flies (*M. domestica*) of mixed sexes (Orlando regular strain). This experiment provides a measure of contact irritancy that is more applicable for use in the control of structure-invading pests. Insects were reared in colonies in the ISU Entomology/Toxicology Laboratory. One milliliter of test solution or solvent was applied to one-half of a 12.5-cm diameter round filter paper with an area of 61 cm² and then allowed to dry. A solvent-only half-piece of filter paper (control) was placed in the remaining half of the 15-cm plastic petri dish after solvent evaporation. At the time of initiation, a single insect
was introduced through a centered hole in the petri dish lid and then covered with masking tape. Time the insect spent on treated and control filter paper out of five minutes (300 seconds) was recorded by using two stopwatches. Ten replications for each concentration were performed. Percentage repellency was calculated with the following formula:

\[ \frac{(\text{Time on Untreated} - \text{Time on Treated})}{300} \times 100 \]

A random-number table determined the location of the treated filter paper in each replicate. Significant differences were determined by ANOVA, and the multiple comparison tests, including Dunnett and Tukey, were completed on SAS (PROC GLM, SAS Institute 1982). Details of this repellency bioassay have been reported earlier (Peterson, et al. 2002b).

**Collection of *Nepeta cataria* Floral Volatiles and Insect-Plant Ecological Survey**

Flowers were collected between 1 July 2001 and 10 July 2001 from established catnip bordering the site described above. Once clipped, the cut stems of the plants were put in water and allowed to stabilize for 30 minutes. The floral test was conducted in bottomless french square bottles, sealed off with Parafilm® (American National Can™, Menasha, Wisconsin). A single catnip raceme was sealed in at the mouth of the french square bottle, and a Supelco SPME portable field sampler with a polydimethylsiloxane fiber was suspended above, projecting through a parafilm seal. Three sampling collections and a control were conducted. Before each sampling, the SPME field sampler was pre-conditioned for 30 minutes at 250°C in a GC/MS. The SPME sampler was set up in the apparatus described and allowed to absorb for 30 minutes for three test replicates.
The samples were then run using GC/MS. A Varian 3200 model with a DB 5 ms nonpolar 30-m column (0.25-mm ID, 0.25-mm film thickness) and a Finnigan TSQ 700 triple quadrupole mass spectrometer with electron impact at 70 eV was used. β-Farnesene, β-caryophyllene were identified by comparing their GC retention times and mass spectra with those of synthetic standards from Sigma-Aldrich, St. Louis, Missouri. α-Caryophyllene was tentatively identified by the match of its mass spectrum with an available spectrum in the Wiley 138K mass spectral database. E,Z and Z,E nepetalactone were identified by comparison of proton NMR spectra from a Varian VXR-300 Spectrometer to NMR data reported in previous literature (Eisenbraun, et al. 1980).

The insect-plant ecological observations included the collection of phytophagous insects feeding on catnip at the time of plant tissue collection. Insect specimens were taken to the lab where they were placed in a growth chamber set at 12 hour light:12 hour dark at 30°C. Each day they were provided with fresh water and fresh catnip as food. At the end of the growing season the survival of each insect was recorded. Following week 5 of collection, the flowers had opened and potential pollinators were recorded.

**Extended Repellency Bioassay**

Test solutions were made consisting of 5%, 1%, and 0.5% concentrations by volume for catnip steam distillate, DEET, citronellal, and elemol, which was obtained from Augustus Oils Inc, Hampshire, England. One half of a 12.5-cm filter paper with an area of 61 cm² was treated with one milliliter of test solution and allowed to dry (30 seconds) before being placed in a 15-cm petri dish. The other half of the filter paper was treated only with solvent for control. One adult male American cockroach was placed in each petri dish and then
enclosed by a mesh, which eliminated any fumigation effects and allowed volatilization of the repellent under ambient laboratory conditions. Adult American cockroaches of mixed sexes were used for tests of the 1% and 0.5% concentrations of catnip essential oil. The location of the insect (presence on the treated or untreated filter paper) was recorded at seven time points after initiation: 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, and 24 hours. Ten replicates were done for each specific preparation.

Spearman Rank Correlation (Miller and Freund 1965) was used to examine the overall repellent response of cockroaches to the treated half of the test arena in this particular bioassay. Treatment and concentration differences were identified with a Cochran-Q test (Siegel 1956), assuming 100% repellency across all 7 time-points to be a successful response. Pair-wise comparisons among treatments were completed using Fisher Exact tests (PROC FREQ, SAS Institute 1982).

Results

Seasonal Variation of Nepetalactone Isomers and Repellency

The ANOVA showed that the overall model design was statistically significant ($F = 16.11; \text{df} = 10; 16; P < 0.0001$) with differences due to week ($P < 0.0001$); no significance due to plant part was found. The mean average values of the $Z,E:E,Z$ nepetalactone ratio, sorted by week, are represented in Fig. 2. Overall the mean ratio was 1.73.

With respect to percentage repellency, the individual catnip mature leaf distillates collected at different seasonal time points differed in an ANOVA ($F = 3.42; \text{df} = 27; 252; P < 0.0001$). Distilled catnip essential oil from weeks 3, 5, 6, 7, and 9 were found to significantly differ in percentage repellency compared to the control (Fig. 3). Direct
comparisons between weekly concentrations showed significant differences in percentage repellency for weeks 2 and 3 at the 1% concentration, and weeks 2 and 6 at the 0.5% concentration. The Tukey multiple comparison also showed significant differences in repellency between the 1%, 0.5%, and 0.1% concentrations of each mature leaf distillate, yielding a dose-response.

Overall, the catnip essential oil was significantly repellent in all 1% concentrations. No clear relationship was found between the Z,E:E,Z ratio and the percentage repellency between weeks.

Collection of Floral Volatiles and Insect-Plant Ecological Observations

The floral volatile analysis detected the presence of three major compounds (Table 1). Of the released volatiles, the Z,E-nepetalactone made up 54.6%, E,Z-nepetalactone made up 31.9%, and β-caryophyllene made up 11.6%. β-Farnesene and α-caryophyllene were minor constituents.

Generalist phytophagous insects found on catnip during the collection included: Aphididae, Noctuidae, Cicadellidae, and Tettigoniidae. Under laboratory conditions, only two loopers (Noctuidae) and one leafhopper (Cicadellidae) were able to complete development on a diet of only catnip. Insects families observed on the flowers included: Tachinidae, Apidae, Nymphalidae, as well as Pieridae.

Comparative Repellency

Percentage repellency of catnip essential oil, and citronellal to P. americana significantly differed from the control at all concentrations ($F = 4.02; \text{df} = 19; 180; P <$
0.0001). DEET only differed from the control at the 5% dose. All three concentrations of catnip essential oil, and citronellal were highly repellent (>80%). The 1% and 5% concentrations were more repellent than the 0.5% concentration, indicating a likely dose-response relationship (Fig. 4).

Similarly, percentage repellency of catnip essential oil, and citronellal to *M. domestica* significantly differed at 5% and 1% doses from the control. No DEET solutions differed from the control. The 1% concentration of catnip essential oil showed the highest percentage repellency value to *M. domestica* in short-term tests (Fig. 5). All concentrations of catnip essential oil and citronellal had greater than 50% repellency. In general, this data does not demonstrate a clear dose-response relationship.

**Catnip and Elemol Extended Repellency Bioassay**

The Spearman Rank Correlation coefficient, *r*<sub>s</sub>, equaled 0.321 with a critical *r*<sub>s</sub> value of 0.714 (*α* = 0.05), showing an overall repellent response to the compounds tested. The Cochran-Q test found significant differences among the four treatments (*Q* = 16.33; *Q*-critical = 16.227, *α* = 0.01) and three concentrations (*Q* = 17.87; *Q*-critical = 13.82, *α* = 0.01). The Fisher Exact test of each treatment against the control showed significance at the 5% concentration. Elemol was the only treatment significantly different from the control at the 1% (*P* = 0.0271) and 0.5% (*P* = 0.0027) concentrations (Table 2). Pair-wise comparisons of the four treatments indicated that elemol was significantly more repellent than the other treatments, with the highest amount of 100% repellency over the seven time points (*P* < 0.02). There were no significant differences found among the other essential oils.
Discussion

The seasonal variation of the two nepetalactone isomers was studied in the summer of 2001, and significant differences were found in the ratio of these two isomers over the 9 weeks of sampling (Fig. 2). Critical time points include: week 2 - generalist insects were first seen feeding on the plant; week 4 - floral buds were present but unopened; week 5 - the flowers first opened; and week 9 - flowers had senesced and ovaries were beginning to ripen.

Nepetalactone has previously been identified as a repellent to many insect species (Eisner, 1964). The data from this study shows the ratio of \( Z,E:E,Z \) at week 4 was 0.58, the lowest of the season, and it showed the lowest repellency value in the short-term bioassay with \( B. \) germanica (Fig. 3). This raises additional questions about the ecological role of the repellent nepetalactone isomers toward insect herbivores, indicating that more quantitative studies of the nepetalactone present within the plant are needed. Since the data presented did not show a significant relationship between the nepetalactone isomer ratio and time, it is difficult to make conclusions about how nepetalactone might be utilized by catnip for protection from injurious insects.

Following week four, the ratio increased again, reflecting a decrease in the amount of the more repellent isomer, \( E,Z \). The thermodynamics of the compounds show a 5.7 kcal/mol difference in the heat of formation of the two molecules, with \( E,Z \) being the higher and \( Z,E \) being the lower. It is possible that there is an adjustment at the flowering stage for the attraction of pollinators. However, the composition of the floral volatiles still contained a 1.7 \( Z,E:E,Z \) ratio. This is still a low ratio and poses many questions of possible species-specific olfactory response to the flowers.
The individual repellency comparisons of these weekly distillations yielded little information. In Fig. 3, the dose-response is shown from the Tukey analysis. 1%, 0.5%, and 0.1% concentrations significantly differed from each other. As apparent from Fig. 3, all 1% concentrations of mature-leaf essential oil were significantly repellent, which coincides with previous studies of catnip repellency (Peterson et al. 2002b).

The data provides insight into an optimal time-point for harvesting catnip material at maximum repellency to the German cockroach. This study indicates harvest should take place when the flower buds are first present till when they open. Additional studies are needed in order identify other factors that influence insect repellency and how they vary throughout the season before determining a specific time-point for catnip collection.

There are many interesting questions still to be answered regarding the insect-plant interactions and chemical responses of the catnip plant. Areas for further investigation are quantitative repellency bioassays of the two nepetalactone isomers with German cockroaches, as well as new bioassays for repellency or attraction to insects recorded at the flowers (Tachinidae, Apidae, Nymphalidae, and Pieridae). There may be specificity between species chemoreceptors or the specificity of the ratio of the two isomers, and additional chemicals present might be necessary to elicit repellency and attraction responses. The mechanisms of insect repellency are poorly understood. Improvements in our knowledge of possible receptors affected by repellent compounds and their correlation to neurobehaviors will add greatly to our understanding of insect repellency.

*P. americana* and *M. domestica* showed an avoidance response to all of the botanical solutions tested in the catnip comparative repellency bioassay in at least one concentration. In tests with *M. domestica* and *P. americana*, insects showed a significantly higher avoidance to
catnip essential oil at values lower than citronellal and DEET. These results indicate that catnip essential oil or components may have promise as commercially viable products.

In some cases the lower concentrations of the solutions were more effective in the closed petri dish arena than the higher concentrations. The dispersion of the compound molecules probably reaches equilibrium faster at higher concentrations. If the compounds rapidly reach equilibrium in the air, the insects may have more difficulty detecting which side is treated versus untreated. It is also possible that the insects may become desensitized to the high levels of chemical in the petri dish and not be able to choose an untreated area. Overall, these tests show the effectiveness of catnip steam distillate and osage orange constituents as repellents and indicate they may be effective at low concentrations.

The catnip and elemol extended repellency experiment showed that although all treatments tested were repellent, the current commercial repellents (DEET and citronellal) were not the most effective against the American cockroach. Elemol, a component of the fruit of the osage orange tree, provided the longest duration of repellency. This residual repellency may serve for improved protection of premises, materials, or animals from insect pests.

The results of these studies indicate that some compounds merit further investigation of botanicals as insect repellents, including catnip and osage orange components. Future studies should include various tests of repellency on additional insect species and further examination of elemol as a repellent. Distance and contact repellency on multiple mosquito species is currently in progress. More definitive testing on how effective nepetalactone and elemol are as mosquito repellents will need to be studied in a biting assay or field study.
Acknowledgements

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References


Table 1. GC-MS analyses of SPME collections of catnip flower, *Nepeta cataria*.

<table>
<thead>
<tr>
<th></th>
<th>Z,E</th>
<th>E,Z</th>
<th>β-Caryophyllene</th>
<th>β-Farnesene</th>
<th>α-Caryophyllene</th>
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</thead>
<tbody>
<tr>
<td>Mean</td>
<td>55%</td>
<td>32%</td>
<td>12%</td>
<td>0.9%</td>
<td>0.9%</td>
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<tr>
<td>SEM</td>
<td>3.5</td>
<td>3.5</td>
<td>1.2</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Table 2. Extended repellency of DEET, citronellal, elemol, catnip essential oil at various concentrations to the adult American cockroach, *Periplaneta americana*.

<table>
<thead>
<tr>
<th>Treatment Solution</th>
<th>Percentage of Cockroaches on Untreated Half of Arena</th>
<th>Fisher Exact Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>5% DEET</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>1% DEET</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>0.5% DEET</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>5% Citronellal</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1% Citronellal</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>0.5% Citronellal</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>5% Elemol</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1% Elemol</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>0.5% Elemol</td>
<td>80</td>
<td>90</td>
</tr>
<tr>
<td>5% Catnip essential oil</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>1% Catnip essential oil</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>0.5% Catnip essential oil</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>
Figure 1. Z,E and E,Z racemic nepetalactone isomers in catnip.
Figure 2. Seasonal variation of the ratio of Z,E:E,Z-nepetalactone isomers in catnip essential oil distilled from leaves and stems as analyzed by gas chromatography with a flame ionization detector (GC-FID).

Means that do not share the same letter are significantly different at $\alpha=0.05$. 
Figure 3. Repellency of catnip mature leaf essential oil, obtained by steam distillation, to the adult male German cockroach, *Blattella germanica*, in the five-minute arena bioassay.
Figure 4. Repellency of catnip essential oil, DEET, and citronellal to the adult American cockroach, *Periplaneta americana*, in the five-minute arena bioassay.

☆ Significantly different from acetone control at α=0.05.
Figure 5. Repellency of catnip essential oil, DEET, and citronellal to adult housefly, *Musca domestica*, in the five-minute arena bioassay.

☆ Significantly different from acetone control at $\alpha=0.05$. 
STUDY 2. NATURAL INSECT REPELLENTS: ACTIVITY AGAINST MOSQUITOES AND COCKROACHES

A paper in press in the American Chemical Society Symposium Series

Gretchen Schultz, Chris Peterson, and Joel Coats

Abstract

Recent research has focused on the repellent properties of extracts from the catnip plant (*Nepeta cataria*) and the Osage orange (*Maclura pomifera*) fruit. This chapter includes results on German cockroach (*Blattella germanica*), and house fly (*Musca domestica*) contact irritancy to catnip essential oil, and its major components, *Z,E*-nepetalactone and *E,Z*-nepetalactone, compared with the commercial standard, N,N-diethyl-m-toluamide (DEET). Both species showed high percentage repellency values when exposed to filter paper treated with catnip essential oil or the individual nepetalactone isomers. Of the two nepetalactone isomers evaluated, German cockroaches were most responsive to the *E,Z* isomer. House flies showed similar trends in contact irritancy, responding to surfaces treated with the predominant catnip isomer, *Z,E*-nepetalactone, more intensely than to the catnip essential oil. Catnip and Osage orange essential oils, and a sesquiterpene found in Osage orange, elemol, were evaluated for repellency to the northern house mosquito (*Culex pipiens*) and are presented here. Two mosquito bioassays were used to measure percentage and contact repellency. Mosquitoes responded initially with high percentage repellency to surfaces treated with catnip essential oil. From the residual repellency study, this trend in repellency by the
catnip oil significantly decreased over the 180-minute test period. Elemol, and DEET initially had lower percentage repellency values than catnip essential oil, but did not show the negative relationship between percentage repellency and time, retaining excellent repellency throughout the 3-hour bioassay. Solutions with elemol and DEET exhibited greater significance in contact repellency compared to catnip essential oil. These results show that catnip essential oil is a potent mosquito repellent, but does not provide the same residual effects as the commercial standard, DEET. Elemol, a sesquiterpene extracted from the fruit of the Osage orange, shows excellent promise as a mosquito repellent with comparable activity to DEET in contact and residual repellency.

**Introduction**

Over the last 20 years there has been intensive effort toward the development of natural products in pest control. Much of this initiative is due to increased regulations on the use of chemicals in insect pest management. Consumers have shown increased interest in and support for products that are safer to human health and more environmentally friendly than many of the traditional chemicals with high acute toxicity and long-lasting residues. Naturally derived biorepellents have been investigated as a group of chemicals that have biological activity and can cause repellent or insecticidal effects without negative impacts on human safety and the environment.

Some of the more common chemicals that have historically been used as mosquito repellents include dimethyl phthalate (DMP), 2-ethyl-1,3-hexanediol (Rutgers 612), dimethyl carbate, benzyl benzoate, butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate (Indalone), and N,N-diethyl-m-toluamide (DEET), which is currently the most widely used
and effective mosquito repellent available. Several reports on DEET toxicity, citing encephalopathy in children, anaphylaxis, urticaria syndrome, and hypotension (1, 2, 3, 4), have intensified the initiative for developing alternative insect repellents. In recent years, several botanical insect repellents have become available on the market and most commonly include components from at least one of the following extracts: citronella, cinnamon, cedar, eucalyptus, mints, lemongrass, geranium, and soybean (5). Neem oil, an extract of the Neem tree, *Azadirachta indica*, is another natural product that has shown repellency of *Anopheles* mosquitoes (6).

Many plant oils and extracts have been identified as insect deterrents, repellents or toxins. In addition to economic disadvantages holding back the commercialization of some natural products, one underlying limitation with these botanical materials is that many of them do not offer residual control equivalent to synthetic standards like DEET (5). Research in the Pesticide Toxicology Laboratory at Iowa State University has focused on the identification of compounds present in the extracts of two plants, the Osage orange (*Maclura pomifera*, Moraceae) and catnip (*Nepeta cataria*, Lamiaceae). Recent emphasis has been placed on understanding the mechanism of repellency and developing natural products that can offer increased potency and/or residual repellency.

### Catnip

*Catnip* (*Nepeta cataria*) is an herbaceous mint native to Eurasia and North Africa. Its present distribution includes most of North America, with great wild abundance around the Great Lakes, and commercial production in Alberta, British Columbia, Alaska, Washington, Oregon, and California. The first uses of catnip for insect control are referenced in folklore.
Over the past 50 years, experiments have validated its insect repellent activity (7, 8). Nepetalactone, the active ingredient present in catnip plant extracts, is known to occur as two isomers: Z,E and E,Z-nepetalactone (9). These two diastereomeric isomers are structurally very similar and differ only in the orientation of substituents across one bond. Past efforts from our lab analyzed the comparative repellency of these nepetalactone isomers. One particular study conducted previously on the German cockroach (Blattella germanica) is included in this report.

**Osage Orange**

The Osage orange is another source for natural products with insect repellent properties. *Maclura pomifera*, the osage orange or hedge apple tree, was used by early pioneers in the Midwest for dyes, the wood was used in bow making, trees were planted to create hedge rows (which served as living fences and windbreaks), and fruits were reportedly useful in repelling insects and spiders. Settlers placed whole fruits in their cupboards to ward off spiders, roaches, and other pests (10). Early studies on extracts of the Osage orange fruit focused on effects of two isoflavones, osajin and pomiferin (11), and five components of the essential oil obtained by steam distillation (12).Elemol, one of the major components of the essential oil, is a sesquiterpene alcohol. This compound has shown significant repellency to several species of insects in our laboratory studies, some of which are presented in this report.
Repellency Bioassay Method

German Cockroach and House Fly Bioassay

A choice-test arena was used to assess irritancy of test solutions to two common household insect pests, the German cockroach (*Blattella germanica*) and the house fly (*Musca domestica*). Catnip essential oil obtained by steam distillation, and the two major components of its essential oil, Z,E-nepetalactone and E,Z-nepetalactone (isolated from the essential oil by preparative TLC), were evaluated for behavioral effects of contact irritancy to the German cockroach (9); catnip essential oil and its major constituent Z,E-nepetalactone were tested against the house fly. N,N-diethyl-m-toluamide (DEET) (Aldrich, St. Louis, MO) served as a positive control for the choice-test arena assay and as a point of comparison for measuring insect behavioral effects that result from current commercial insect repellents. Test solutions ranging from 10% to 0.1% (vol/vol) active ingredient (a.i.) were made up in acetone and then delivered on to a filter paper for solvent evaporation. Resulting rates of a.i. were 1.63 mg/cm², 815µg/cm², 163µg/cm², 81.5µg/cm², and 16.3µg/cm². Choice-test arenas for German cockroaches and house flies were constructed from plastic Petri dishes. One-half of a 12.5-cm dia. filter paper was treated with 1 ml test solution, and the other was treated with 1 ml of only solvent (control). Both halves of the filter paper were placed in the choice-test arena. Position of the treated filter paper was randomized using a random-number table. Individual German cockroaches or house flies were placed in each choice-test arena through a centered hole in the lid of the Petri dish and evaluated for a 300-second period. The amount of time the insect spent on the treated and untreated filter papers were recorded and used to calculate a percentage repellency value:
Percentage Repellency = \((\text{Time on Untreated}-\text{Time on Treated})/300) \times 100\)

Ten replicates of each treatment solution were tested for both German cockroaches and house flies. Details of this assay design and some results have previously been described \((8, 13)\).

**Mosquito Repellency**

**Insects.**

A colony of *Culex pipiens*, 10 generations removed from wild mosquitoes collected in Ames, Iowa, was used for testing. The colony was blood-fed on the bobwhite quail, *Colinus virginianus*. Eggs from mosquitoes were dried and stored in an incubator until needed. Eggs were placed in deoxygenated water and two to three drops of a ground TetraMin™ fish food solution were added to the water to feed the larvae. Pupae were removed from the larval pans as they appeared and were placed into mesh-covered paper cups. Following emergence, adult females were tested over a six-day period. The mosquitoes were continually allowed to feed on a cotton ball soaked with 0.3 M sucrose solution. At 1-2 hours before testing began, the cotton balls were removed, and the mosquitoes were preconditioned in the bioassay environmental chamber, held at 26°C, for 1-2 hours.

**Percentage and Contact Repellency Bioassay.**

A static-air choice-test apparatus was used to determine the behavioral effects on the insects in this study. The apparatus consisted of a 9 x 60-cm section of glass tubing with a 2-cm hole drilled at the midpoint along the length for central introduction of the insects. All of
the testing was conducted in an environmental chamber at 26°C. Treatments included catnip essential oil, obtained by steam distillation previously described by Peterson et al. 2002 (9), Osage orange essential oil, obtained by steam distillation of whole fruits previously described by Peterson 2002 (13), elemol (Augustus Oils, New Hampshire, England), and DEET (Aldrich, St. Louis, MO) test solutions at 1%, 0.5% and 0.1% concentrations (wt/vol). The test solutions' solvent, hexane, served as a control treatment in this assay. One milliliter of the solution was applied to one half of a 9-cm diameter round filter paper with an area of 63.6 cm$^2$ and then allowed to dry before testing. This resulted in the following rates of exposure: 157, 78.6 and 15.7 µg/cm$^2$. Treated filter papers were placed inside the lids of 9-cm glass petri dishes, and placed over the ends of the glass tube. The position of the treated side, to the right or to the left, was selected by using a random-number table. Approximately fifteen unmated adult female mosquitoes were anaesthetized with CO$_2$ and then introduced to the 9 x 60-cm glass cylinder through the centered 2-cm hole. Timing began 2 minutes after mosquito introduction, and mosquito distribution inside the static-air choice-test apparatus was observed over a 180-minute period for each treatment. Mosquito distribution (number of individuals on treated and untreated side) was recorded at 15, 30, 60, 90, 120, and 180-minute timpoints. The data generated by this study was used to examine two measures of mosquito repellency, **percentage repellency** and **contact repellency**. Percentage repellency was calculated for with the following formula:

$$\text{Percentage Repellency} = \left(\frac{\text{Number of Individuals on Untreated Half} - \text{Number of Individuals on Treated Half}}{15}\right) \times 100$$
Contact repellency was defined in this assay as 100% avoidance of the treated filter paper (no contact). 15, 30, 60, 90, 120, and 180-minute time-points were used to assess contact repellency for individual observations.

The experimental design was a completely randomized design using three replications of each treatment. Analysis of variance was performed on SAS (PROC GLM; SAS Version 8) to identify significant differences of percentage repellency due to treatment, and concentration. Multiple comparisons were completed using Tukey’s procedure. Treatment pair-wise comparisons of contact repellency, which included data from the six time-points observed for each treatment, were completed using Fishers Exact (PROC FREQ; SAS Version 8).

**Mosquito Residual Repellency Bioassay.**

Aged applications of catnip essential oil, elemol, DEET, and hexane (control) were compared in the static-air choice-test apparatus under the same conditions as described above. The 0.5% and 0.1% (wt/vol) solutions of each test solution were made to yield the same rate of a.i. used in the above mosquito repellency bioassay. Individually treated filter papers were then placed in a fume hood and aged for 0, 30, 60, 120, or 180-minutes, allowing volatization to occur over a set period of time. After the specified ageing period, filter papers were placed on the inside of the 9-cm glass petri dish lids, and then placed over the ends of the glass tube. The position of the treated side was randomized. Approximately 18 unmated adult female mosquitoes were anaesthetized with CO₂ and then introduced to the 9 x 60-cm glass cylinder through the centered 2-cm hole. Timing began 2 minutes after mosquito introduction, and mosquito distribution (number of individuals on treated and untreated
sides) inside the static-air choice-test apparatus was recorded after 15 minutes for
determination of Percentage Repellency (calculations shown under Percentage and Contact
Repellency Bioassay). Experimental design was completely randomized with three
replications of each aged test solution. Analysis of variance was used to identify significant
differences related to a.i., concentration, and ageing period. Regression analysis was used to
examine percentage repellency relationship to filter paper ageing.

Results

German Cockroach and House Fly Repellency

The German cockroach and house fly both showed contact irritancy responses to at
least one concentration of each test solution evaluated (Table 1). German cockroaches gave
the highest percentage repellency value response when exposed to the 0.5% solution of E,Z-
nepetalactone. This percentage repellency response was more than four times the response
seen from testing the same concentration of Z,E-nepetalactone. In the cockroach experiment,
both Z,E and E,Z-nepetalactone isomers caused an overall higher percentage repellency
response at lower concentrations of the respective a.i., compared to treatments with DEET.
The house fly responded to the test solutions with a similar trend, although the E,Z isomer
was not tested. The higher percentage repellency values resulted from exposure to catnip
essential oil and to Z,E-nepetalactone, ranging from 70-96%, compared to DEET (39%)
(Table 2).
Mosquito Repellency

Percentage repellency of catnip and osage orange essential oil, elemol and DEET at 15 minutes is represented in Figures 3, 4, and 5. All compounds tested showed various levels of significance in percentage repellency and contact repellency. The overall concentration effect was not significant (P = 0.4569). Osage orange essential oil represented the lowest values in percentage repellency (<60%) and did not show any significant contact repellency (P = 0.1). Catnip essential oil showed high percentage repellency at the 15-minute time-point at all concentrations tested, including the highest value, 100% from the 0.1% concentration (Figure 5). This was also the most significant level of contact repellency (P <0.0001) resulting from the three concentrations of catnip essential oil (Table 3). The other concentrations of catnip essential oil varied in contact repellency (0.5% concentration, P = 0.5, and 1% concentration, P = 0.02). Elemol solutions yielded the second highest set of percentage repellency values of the test solutions, ranging from 81% to 63%. These treatments also resulted in highly significant contact repellency (Table 3). The commercially available standard for mosquito repellency, DEET, also showed high percentage repellency values, ranging from 63% to 44%, in addition to high significance for contact repellency.

Residual Repellency

Percentage repellency values were high for catnip essential oil, elemol, and DEET solutions immediately following application to the test surface (Table 4). The analysis of variance showed that there was a difference among the three different solutions and the control (P < 0.0001), and a significant interaction with treatment solution and time (P =
The only treatment solutions to show a significant decrease in percentage repellency over time were 0.5% catnip essential oil \( (P = 0.02) \) and 0.1% catnip essential oil \( (P = 0.003) \) in which 51% of the variability in the data was explained by this negative linear relationship.

Elemol, DEET, and control treatments did not show significant trends in the regression analysis, indicating maintenance of repellency with elemol and DEET over the 3-hour period.

**Discussion**

Bioassays in a choice-test arena were used to assess cockroach and house fly irritancy responses. The use of deterrents is a valuable tool for pest control, particularly when used with an integrated pest management program. In the studies we report, contact irritancy serves as a measure of deterrence and helps to identify compounds that may serve as effective protectants for premises. It should be noted that limitations of this method are that individuals are only exposed to the treated surface for a 5-minute period and can only characterize a short-term response.

German cockroaches and house flies responded negatively to all solutions evaluated. These results demonstrate the efficiency of the assay and add support for catnip essential oil as an insect repellent. Specifically, cockroaches showed greatest avoidance of filter papers treated with the purified nepetalactone isomers, \( Z,E \) and \( E,Z \), and house flies showed greatest avoidance of \( Z,E \)-nepetalactone. Both nepetalactone isomers were compared during trials on the German cockroach, and the result was a much higher percentage repellency from papers treated with \( E,Z \)-nepetalactone. These results raise the need for structure-activity relationship studies, since \( Z,E \)-nepetalactone and \( E,Z \)-nepetalactone are very similar compounds that only
differ in orientation of groups across one bond on the molecule. Additional studies on the mode of action of deterents are required before conclusions are drawn on how the minimal structural difference in \(Z,E\) and \(E,Z\)-nepetalactone cause significantly different responses from \(B. \) germanica.

Initial investigations of mosquito repellency with catnip and osage orange essential oil allowed us to directly compare with DEET, the current commercial standard, and further analysis helped identify differences in the activity of these compounds as insect repellents. At present, there is no one characteristic that fits all repellents or a single mechanism that explains how specific chemicals and blends act on insects. Studies have shown that an insect’s response to the chemicals in the environment is dependent on their physiological and developmental state (14). The studies presented in this report focus on adult female mosquitoes and their responsiveness to various rates of catnip and Osage orange essential oil, elemol, and DEET over time. Results from mosquito repellency assay show that after 15-minutes, the northern house mosquito was most significantly repelled from the filter paper surfaces treated with catnip essential oil (100%). The percentage repellency values from the DEET and elemol treatments resulted in a lower range (81%-44%) than catnip essential oil, but showed higher contact repellency. Observations during the assay showed that individuals exposed to catnip essential oil moved further away from the treated surface than in the DEET and elemol treatments. Over time, this effect started to decrease with catnip essential oil as mosquitoes redistributed through the tube, eventually reaching a distribution similar to the control.

Mosquitoes exposed to DEET and elemol settled far enough from the treated surface to achieve an adequate level of contact repellency. As time increased, individuals would
continually reject the treated surface up to the end of the 180-minute period, unlike the catnip essential oil, which exhibited an initially high repellency response that decreased over time. DEET and elemol showed a longer duration of repellency compared to catnip essential oil, as is evidenced with higher significance in contact repellency. Additional studies are needed to better understand how these differences occur, including studies on the chemical volatilization, and interference with behavioral stages of mosquito host-finding and acceptance.

The second mosquito assay focused on quantifying the residual repellency of the northern house mosquito to aged filter papers of catnip essential oil, elemol and DEET. All 0.5% and 0.1% test solutions showed significant percentage repellency following application (i.e., with no ageing period). This repellency effect slowly decreased over time for both concentrations of catnip essential oil (0.5%,\( P=0.02 \), 0.1%,\( P=0.003 \)). There was no significant loss in percentage repellency seen in the DEET and elemol treatment solutions, accounting for continual mosquito repellency over 3 hours from a treated surface. Olfactory repellency differs from contact repellency, and the method used here allows for some differentiation between the two types. The high initial repellency of catnip essential oil is not sustained over a 3-hour period, but elemol and DEET do show residual repellency to that time-point.

The series of experiments presented here give supporting evidence for catnip and Osage orange essential oils, elemol, and nepetalactone as effective insect repellents to common household pests and pests of human health. Investigations with mosquito behavioral responses in a static-air apparatus showed that catnip essential oil, and elemol can act as effective mosquito repellents from treated surfaces, but differ in residual efficacy.
Further studies are currently underway to evaluate residual repellency effects of other natural products in Osage orange essential oil and examine differences in the mechanism of repellency.

Acknowledgements

We appreciate the assistance from Dr. Wayne Rowley and Brad Tucker for providing the mosquitoes for the studies. We thank Dianna Wilkening, Theresa Redding, and Melinda Thede for their help with insect rearing and conducting bioassays. Contents of this paper were presented at the 226th National American Chemical Society Meeting, Anaheim, CA, March 28, to April 1, 2004.

References


Table 1. Percentage repellency of catnip essential oil, Z,E-nepetalactone, E,Z-nepetalactone, DEET and control to the German cockroach, *Blattella germanica*, in the choice-test arena bioassay. Treatments with the same letter are not significantly different by least-squares means analysis at $\alpha = 0.05$ (8).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Application Rate</th>
<th>Percentage Repellency ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Acetone</td>
<td>5.2 ± 7.5a</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>2.9 ± 3.7a</td>
</tr>
<tr>
<td>DEET</td>
<td>1.60 mg/cm²</td>
<td>58.3 ± 10.5b</td>
</tr>
<tr>
<td></td>
<td>800 µg/cm²</td>
<td>25.8 ± 9.5a</td>
</tr>
<tr>
<td></td>
<td>160 µg/cm²</td>
<td>20.4 ± 9.2a</td>
</tr>
<tr>
<td></td>
<td>80 µg/cm²</td>
<td>15.5 ± 5.4a</td>
</tr>
<tr>
<td>Catnip Essential Oil</td>
<td>800 µg/cm²</td>
<td>55.6 ± 9.8b</td>
</tr>
<tr>
<td></td>
<td>160 µg/cm²</td>
<td>27.7 ± 13.1ab</td>
</tr>
<tr>
<td></td>
<td>80 µg/cm²</td>
<td>33.7 ± 15.7ab</td>
</tr>
<tr>
<td>Z,E-Nepetalactone</td>
<td>800 µg/cm²</td>
<td>68.2 ± 5.7b</td>
</tr>
<tr>
<td></td>
<td>160 µg/cm²</td>
<td>56.8 ± 7.8b</td>
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<td>15.4 ± 6.9a</td>
</tr>
<tr>
<td></td>
<td>16 µg/cm²</td>
<td>16.1 ± 7.4a</td>
</tr>
<tr>
<td>E,Z-Nepetalactone</td>
<td>80 µg/cm²</td>
<td>79.4 ± 3.5c</td>
</tr>
<tr>
<td></td>
<td>16 µg/cm²</td>
<td>46.4 ± 11.0b</td>
</tr>
</tbody>
</table>
Table 2. Percentage repellency of DEET, catnip essential oil, Z,E-nepetalactone, and control to the house fly, *Musca domestica*, in the choice-test arena bioassay (ref. 13).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Application Rate</th>
<th>Percentage Repellency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-5.3</td>
</tr>
<tr>
<td>DEET</td>
<td>800 µg/cm²</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>160 µg/cm²</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>80 µg/cm²</td>
<td>38.7</td>
</tr>
<tr>
<td>Catnip Essential Oil</td>
<td>80 µg/cm²</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>160 µg/cm²</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td>80 µg/cm²</td>
<td>52.7</td>
</tr>
<tr>
<td>Z,E-Nepetalactone</td>
<td>800 µg/cm²</td>
<td>96.0</td>
</tr>
<tr>
<td></td>
<td>160 µg/cm²</td>
<td>69.3</td>
</tr>
<tr>
<td></td>
<td>80 µg/cm²</td>
<td>87.3</td>
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</table>
Table 3. Contact repellency of the northern house mosquito, *Culex pipiens*, measured at 15, 30, 60, 90, 120, 180-minutes in a static-air repellency chamber to catnip essential oil, Osage orange essential oil, elemol, DEET, and control. P-values in the table are from Fisher Exact test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Application Rate</th>
<th>Treatment vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catnip Essential Oil</td>
<td>157 µg/cm²</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>78.6 µg/cm²</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Osage Orange Essential Oil</td>
<td>157 µg/cm²</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>78.6 µg/cm²</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>0.5</td>
</tr>
<tr>
<td>Elemol</td>
<td>157 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>78.6 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DEET</td>
<td>157 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>78.6 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4. Residual percentage repellency of the northern house mosquito, *Culex pipiens*, to 0, 30, 60, 90, 120, 180-minute aged treatments of 0.5% and 0.1% solutions of catnip essential oil, elemol, DEET, and control in a static-air repellency chamber.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Application Rate</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
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<tbody>
<tr>
<td>Catnip Essential Oil</td>
<td>78.6 µg/cm²</td>
<td>71.5</td>
<td>88.6</td>
<td>59.8</td>
<td>24</td>
<td>31.9</td>
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<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>88.8</td>
<td>37</td>
<td>40.7</td>
<td>22.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Elemol</td>
<td>78.6 µg/cm²</td>
<td>84.7</td>
<td>76.5</td>
<td>96.5</td>
<td>80.8</td>
<td>76.5</td>
</tr>
<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>35.0</td>
<td>30.8</td>
<td>49</td>
<td>20.7</td>
<td>44.8</td>
</tr>
<tr>
<td>DEET</td>
<td>78.6 µg/cm²</td>
<td>74.0</td>
<td>37</td>
<td>59</td>
<td>77.7</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>15.7 µg/cm²</td>
<td>54.9</td>
<td>23.1</td>
<td>45.7</td>
<td>39</td>
<td>70.6</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-6.1</td>
<td>-9.3</td>
<td>1.3</td>
<td>25.5</td>
<td>-9.1</td>
</tr>
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</table>
Figure 1. Z,E and E,Z nepetalactone isomers in catnip.
Figure 2. Elemol, a sesquiterpene alcohol present in the essential oil of the Osage orange (*Maclura pomifera*).
Figure 3. 15-minute percentage repellency of the northern house mosquito, *Culex pipiens*, in a static-air repellency chamber to 157 µg/cm² application (1% concentration) of catnip essential oil, elemol, DEET, as well as osage orange essential oil, and a solvent control. Treatments with the same letter are not significantly different by Tukey analysis at α = 0.05.
Figure 4. 15-minute percentage repellency of the northern house mosquito, *Culex pipiens*, in a static-air repellency chamber to 78.6 µg/cm² application (0.5% concentration) of catnip essential oil, elemol, DEET, as well as osage orange essential oil, and a solvent control. Treatments with the same letter are not significantly different by Tukey analysis at $\alpha = 0.05$. 
Figure 5. 15-minute percentage repellency of the northern house mosquito, *Culex pipiens*, in a static-air repellency chamber to 15.7 µg/cm² application (0.1% concentration) of catnip essential oil, elemol, DEET, as well as osage orange essential oil, and a solvent control. Treatments with the same letter are not significantly different by Tukey analysis at $\alpha = 0.05$. 