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INFLUENCE OF MONOTERPENE VAPORS ON SPRUCE SPIDER MITE, *Oligonychus ununguis*, ADULT FEMALES

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Abstract—Adult female spruce spider mite, *Oligonychus ununguis* (Jacobi), were exposed to various concentrations of four host conifer monoterpene vapors (limonene, β-pinene, α-pinene, and Δ^3-carene) for 24 hr to determine the lethal and sublethal effects. All four compounds were toxic to the mites. Further, at concentrations below the calculated LC_{50}s, all four compounds decreased oviposition by the mites and three of the compounds (limonene, β-pinene, and α-pinene) influenced movement. Whereas *O. ununguis* populations may not normally be exposed to high concentrations of host monoterpenes, trees continuously emit some monoterpene vapors, and when trees are damaged or under stress, oleoresin may accumulate at points on the external surface of tree tissues thereby exposing mites to the influence of monoterpene vapors.

Key Words—Arachnida, Acari, Tetranychidae, *Oligonychus*, conifer, monoterpene, toxicity, oviposition, dispersal

INTRODUCTION

The spruce spider mite, *Oligonychus unungis* (Jacobi) (Acari: Tetranychidae) is a serious pest of conifers throughout the world. It is capable of attacking most genera of conifers including spruce (*Picea*), pine (*Pinus*), fir (*Abies*), larch (*Larix*), and juniper (*Juniperus*) (Löyttyniemi, 1970; Jeppson et al., 1975; Johnson and Lyon, 1976; Drooz, 1985). Spruce spider mite is probably the most destructive conifer-feeding spider mite in the United States (Johnson and Lyon,

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1976) and has been suggested to be the only mite capable of causing economic injury in nurseries and plantations of coniferous trees (Kielczewski, 1966). Damage by spruce spider mite includes yellowing or browning of needles (Garman, 1923; Kielczewski, 1966), resulting in potential premature needle abscission (Peterson and Hildahl, 1969) and possible reductions in tree shoot growth (Löyttyniemi, 1971).

As with many herbivores, spider mite performance on a host plant may be influenced by the plant's chemical content. Researchers working with other spider mite species have demonstrated that nutritional components located in leaf tissue, especially nitrogen and amino acids directly impact spider mite biology (i.e., Cannon and Connell, 1965; Tulisalo, 1971; Dabrowski and Bielak, 1978; Hanna et al., 1982). Furthermore, there was a positive correlation reported between the essential amino acid content in spruce needles and the occurrence of spruce spider mite (Löyttyniemi and Tulisalo, 1972). However, along with nutritional compounds, plants have secondary metabolites within leaf tissues that may have negative impacts on the attacking mite population. Many terpenoid compounds may be placed within this category of potential plant protectants. For example, some components of the essential oils of spice plants are oxygenated monoterpenes, and when these essential oils are sprayed on the spider mite *Tetranychus cinnabarinus* (Boisd.), the result was an increase in mortality and repellancy (Mansour et al., 1986). Furthermore, sesquiterpenes present in tomato have been demonstrated to be both toxic and repellent to the spider mite *T. urticae* Koch (Patterson et al., 1975), and exposure of female *T. urticae* to the three principal monoterpenes in the essential oil of peppermint leaves (pulegone, menthone, and menthol) resulted in reduced oviposition and increased mortality (Larson and Berry, 1984) and spider mite injury increased the relative amounts of menthone and menthol in peppermint leaf essential oils (DeAngelis et al., 1983).

The potential influence of conifer needle monoterpenes on spruce spider mite has not been investigated. However, conifers usually have many terpenoid compounds present within their tissues, and these have been demonstrated to be toxic to several bark beetle species including *Dendroctonus frontalis* Zimmermann, *D. brevicomis* LeConte, *Ips calligraphus* (Germar), and *Scolytus ventralis* LeConte (Smith, 1965; Coyne and Lott, 1976; Raffa et al., 1985; Cook and Hain, 1988). Further, needle monoterpene content has been suggested to be important in tree resistance to the western spruce budworm, *Choristoneura occidentalis* Freeman (Cates et al., 1983). As part of an investigation of the interaction between spruce spider mite and its host conifers, this experiment was conducted to examine potential lethal and sublethal effects of four common conifer monoterpenes on adult female *O. ununguis*. Specifically, monoterpene influences on adult mortality, oviposition, and movement were examined.

METHODS AND MATERIALS

Experimental work was conducted from April 1990 through July 1991. Adult female *O. ununguis* were removed from an insectary-reared colony maintained on cuttings of Fraser fir, *Abies fraseri* (Pursh) Poir. Each adult female mite was placed on a single fir needle. The needles were individually placed either in a 1.0-ml glass vial that was sealed with a cotton plug (just enough to prevent mite escape) or on a circular (diameter = 16 mm) piece of black construction paper affixed with Tanglefoot, five per dish, to the bottom of an open-topped Petri dish (diameter = 9.0 cm). Vials and/or Petri dishes were placed in a 2-liter desiccator with an NaCl slurry to maintain relative humidity at ca. 75% (Winston and Bates, 1960). Desiccators were maintained for 24 hr at 26°C in a constant-light environment.

Monoterpene treatments were applied within each desiccator by suspending, from the top of the desiccator just prior to sealing, a piece of round no. 1 filter paper (diameter = 5.5 cm), which had the appropriate concentrations of the given monoterpene applied topically to it. In this manner, as the monoterpene volatilized, mites were exposed to various levels of monoterpene vapors. The monoterpenes tested were obtained from Aldrich Chemical Company and were (*S*)-(−)-limonene (92% purity), 1(*S*)-(−)-β-pinene (98% purity), 1(*S*)-(−)-α-pinene (98% purity), and Δ^3-carene (95% purity). The concentrations injected onto the filter paper were 0, 5, 10, 25, 50, 70, and 100 μl, which corresponds to ca. 0, 2.5, 5.0, 12.5, 25.0, 35.0, and 50.0 ppm within the sealed containers, respectively.

At 24 hr the mites were removed from the desiccators and those in vials were examined under a dissecting microscope for mortality at all monoterpene concentrations and oviposition at the 0-, 5-, 10-, 25-, and 50-μl injection levels. Mites on the round black arenas were examined to determine if they had moved beyond the 16 mm disk and were stuck in the surrounding Tanglefoot. This test was conducted at monoterpene injection concentrations of 0, 10, 50, 70, and 100 μl. Mites were exposed to the various treatments five per replicate with four replicates conducted at the 5-, 70-, and 100-μl injection levels, eight replicates at the 10-, 25-, and 50-μl injection levels, and 31 zero microliter (control) injections.

The influence of the monoterpenes on survival was determined using probit analyses to obtain LC_{50} estimates for each monoterpene. Regression analyses were conducted to determine the influence of the individual monoterpenes on oviposition (eggs per live female) and dispersal tendency (percentage of mites moved off the 16-mm disks). Correlation analyses were conducted between percent survival and oviposition and between percent survival and dispersal tendency for the mites exposed to each monoterpene. All statistical analyses were conducted using the SAS statistical package (Statistical Analyses System 1982).

RESULTS AND DISCUSSION

All four monoterpenes tested were toxic to adult female *O. ununguis* (Table 1). The order of toxicity based upon the LC_{50} calculations was limonene ≥ β-pinene ≥ α-pinene ≥ Δ^3-carene. Limonene is also one of the most toxic host monoterpenes to several conifer-feeding scolytid bark beetles (Smith, 1965; Coyne and Lott, 1976; Raffa et al., 1985; Cook and Hain, 1988). Host monoterpene vapors from peppermint have been demonstrated to be toxic to the mite *T. urticae* (Larson and Berry, 1984) as have topical applications of tomato sesquiterpenes (Patterson et al., 1975).

There was a significant decrease in oviposition (eggs per live female) as the concentration of each monoterpene increased {limonene, $F(4,26) = 6.16$, $[P > F] = 0.0013$; β-pinene, $F(4,26) = 4.49$, $[P > F] = 0.0068$; α-pinene, $F(4,26) = 4.43$, $[P > F] = 0.0080$; Δ^3-carene, $F(4,26) = 4.59$, $[P > F] = 0.0061$} (Figure 1). It is not surprising therefore to find a positive correlation between oviposition and survival of *O. ununguis* females for each of the monoterpenes {limonene, $r = 0.5687$, $[P > r] = 0.0008$; β-pinene, $r = 0.6631$, $[P > r] = 0.0001$; α-pinene, $r = 0.2403$, $[P > r] = 0.1930$; Δ^3-carene, $r = 0.4912$, $[P > r] = 0.0050$} . The decrease in oviposition began at monoterpene concentrations less than those resulting in significant mortality. Similarly sublethal affects of host monoterpene vapors have been reported for *T. urticae* exposed to peppermint monoterpenes (Larson and Berry, 1984).

There was also a significant relationship between monoterpene concentration and dispersal tendency (movement off of the circular disk) for *O. ununguis* females exposed to limonene {$F(4,27) = 4.73$, $[P > F] = 0.0051$}, β-pinene, {$F(4,27) = 6.22$, $[P > F] = 0.0011$}, and α-pinene {$F(4,27) = 37.46$, $[P > F] = 0.0001$} but not for those mites exposed to Δ^3-carene {$F(4,27) = 0.61$, $[P > F] = 0.6589$} (Figure 1). Again, given these results, it is not surprising to find a significant, positive correlation between dispersal tendency and survival of *O. ununguis* for three of the monoterpenes {limonene, $r = 0.4997$, $[P > r] = 0.0036$; β-pinene, $r = 0.5673$, $[P > r] = 0.0007$; α-pinene, $r = 0.7993$, $[P > r] = 0.0001$} but not for Δ^3-carene ($r = 0.0599$, $[P > r] = 0.7447$). The decreased movement at higher monoterpene concentrations for the three compounds may indicate a rapid effect of the toxic vapors with little movement by the mites being possible at these higher concentrations. The one monoterpene that did not demonstrate this result, Δ^3-carene, is the compound that had the highest LC_{50} concentration (Table 1). The increased movement in the presence of relatively low concentrations of host monoterpene

TABLE 1. PERCENT SURVIVAL ($\bar{X} \pm SD$) OF ADULT FEMALE SPRUCE SPIDER MITES EXPOSED TO VARIOUS CONCENTRATIONS OF FOUR MONOTERPENE VAPORS FOR 24 HOURS

	Concentration (μl)[a]								
Monoterpene	0[b]	5	10	25	50	70	100	LC_{50}[c]	95% CI[c]
Limonene	89.0 ± 14.4	86.7 ± 16.3	87.5 ± 14.9	77.5 ± 9.6	57.5 ± 24.9	30.0 ± 11.5	0.0 ± 0.0	49.0	41.7–59.0
β-Pinene	89.0 ± 14.4	81.3 ± 23.9	95.0 ± 14.1	74.2 ± 10.7	44.4 ± 19.5	60.0 ± 16.3	0.0 ± 0.0	51.7	36.1–86.3
α-Pinene	89.0 ± 14.4	85.0 ± 19.1	100 ± 0.0	92.5 ± 9.6	62.5 ± 25.9	55.0 ± 30.0	0.0 ± 0.0	61.3	41.1–121.1
Δ^3-Carene	89.0 ± 14.4	90.0 ± 11.5	87.5 ± 18.3	87.5 ± 5.0	68.1 ± 22.4	58.8 ± 14.4	0.0 ± 0.0	62.5	44.9–113.8

[a] Monoterpene concentration applied to filter paper suspended from ceiling of desiccators: equivalent ppm measurements are 0 μl = 0 ppm, 5 μl = 2.5 ppm, 10 μl = 5.0 ppm, 25 μl = 12.5 ppm, 50 μl = 25.0 ppm, 70 μl = 35.0 ppm, and 100 μl = 50.0 ppm.

[b] 0 μl concentration = control desiccators. One control desiccator was used during each replicate. Therefore, all control measurements were pooled for these analyses.

[c] Estimated concentration and 95% confidence interval for 50% mortality calculated using probit analysis (Statistical Analysis System, 1982).

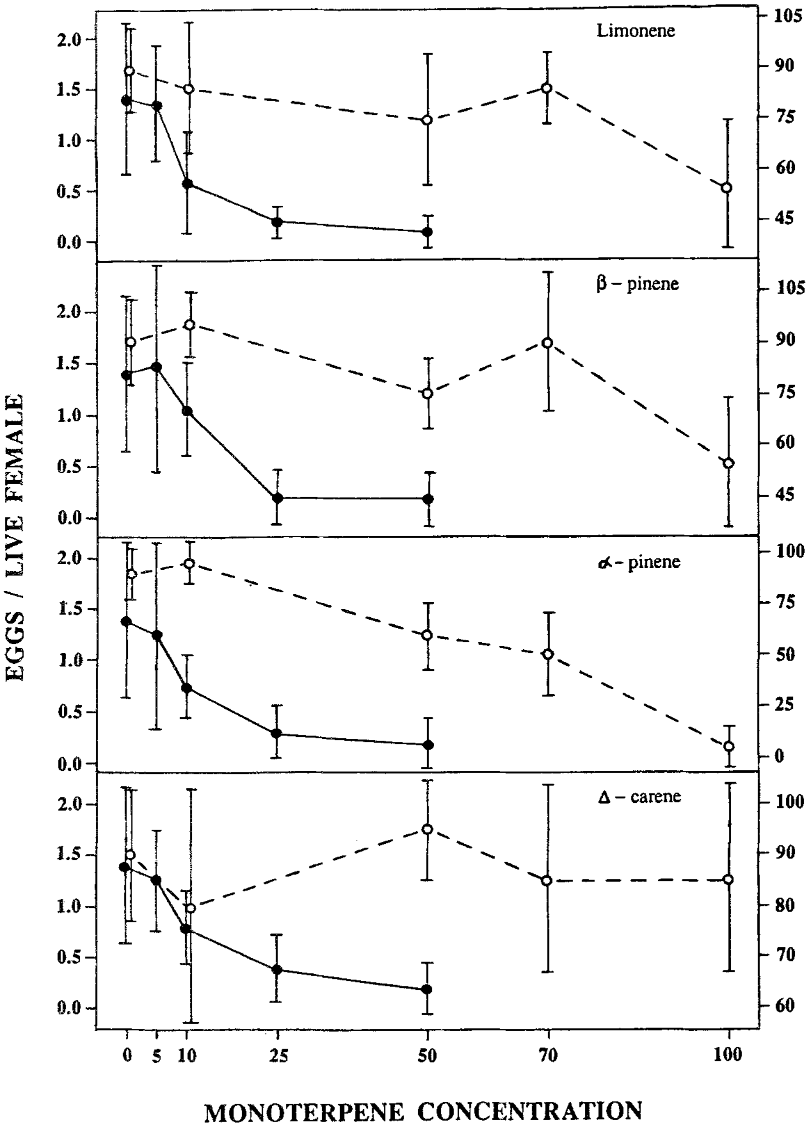

FIG. 1. Sublethal effects ($\overline{X} \pm$ SD) of monoterpene vapors on oviposition (●—●) and movement (○—○) of adult female spruce spider mites exposed to the vapors for 24 hr. 0 μl concentrations = control desiccators, one per replicate, which were pooled for these analyses.

vapors as was observed in the β-pinene and α-pinene exposures may be beneficial in the field by acting to disperse mites away from sites of needle or limb damage and the subsequent increased release of resin monoterpenes. However, a more accurate measure of dispersal tendencies in the presence of host monoterpene odors could possibly be obtained by placing mites on small twigs and monitoring movement in the presence of added host odors.

Although they feed by piercing leaf tissue and sucking out cell contents, *O. ununguis* may not normally be exposed to high concentrations of the monoterpenes contained within needle tissue. These compounds usually occur in specialized structures within the plant but they are continuously lost from tree tissue (Kramer and Kozlowski, 1960), which would constantly expose mites to a low dosage. Furthermore, under water stress, conifers may experience premature needle abscission with an accompanying deposition of resin droplets at the abscission point (Heikkenen et al., 1986). Since the leaf (or needle) is the basic microhabitat exploited by *O. ununguis* for population growth as opposed to the whole plant (Wanibuchi and Saitô, 1983), limited dispersal ability would expose the mites to higher monoterpene vapor concentrations released from these resin droplets and trapped within the canopy of the tree. Even if the concentration of these higher vapor concentrations was not great enough to cause mortality, populations of *O. ununguis* may experience some sublethal affects such as the decreased oviposition and movement observed during this experiment.

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