Insecticidal Activity of Selected Monoterpenoids and Rosemary Oil to *Agriotes obscurus* (Coleoptera: Elateridae)

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J. Econ. Entomol. 98(5): 1560–1565 (2005)

ABSTRACT Acute toxicities of three naturally occurring monoterpenoid essential oil constituents and the essential oil of rosemary were tested against late instars of *Agriotes obscurus* (L.) (Coleoptera: Elateridae). Both contact and volatile toxicities of thymol, citronellal, eugenol, and rosemary oil were determined. Also, phytotoxicity of these compounds was evaluated on corn germination and seedling development. Thymol had the greatest contact toxicity (LD_{50} = 196.0 μg/larva), whereas citronellal and eugenol were less toxic (LD_{50} = 404.9 and 516.5 μg/larva, respectively). Rosemary oil did not show any significant contact toxicity, even at 1,600 μg/larva. In terms of volatile toxicity, citronellal was the most toxic to wireworm larvae (LC_{50} = 6.3 $\mu g/cm^3$) followed by rosemary oil (LC_{50} = 15.9 $\mu g/cm^3$), thymol (LC_{50} = 17.1 $\mu g/cm^3$), and eugenol (LC_{50} = 20.9 $\mu g/cm^3$). Thymol, eugenol, and citronellal significantly inhibited corn seed germination and development, whereas rosemary oil had only minimal phytotoxic effects.

KEY WORDS *Agriotes obscurus*, monoterpenoids, insecticidal activity, contact toxicity, fumigant toxicity

WIREWORMS, *Agriotes* sp. L. (Coleoptera: Elateridae), the larval form of click beetles, consist of >800 species distributed worldwide. They are significant pests wherever they occur, infesting both cereal and forage crops, but they have recently become economically significant pests to corn and the single most damaging potato pest in western Canada (Vernon et al. 2001). These insects are most destructive during their larval stage by feeding on pregerminated seeds, the radical or young roots of juvenile plants, or directly on tubers, causing unmarketable product. Currently, the most effective control strategies rely on soil-incorporated synthetic pesticides. However, these chemicals often have adverse secondary effects in the environment, including long persistence and human health concerns. In addition, organic farmers, who cannot use synthetic chemicals, have no effective alternative for wireworm management besides field flooding, which first requires significant field modification. Therefore, alternatives to synthetic pesticides that are compatible with established control strategies and organic production guidelines need to be developed.

The use of natural, plant-based chemistry to control insect pests is gaining greater attention by the scientific community due both to increases in analytical capability and enhanced ability to dissect complex biochemical systems. Also, their general compatibility with organic farming practices makes them attractive topics for researchers interested in developing sustainable agricultural practices. In line with organic farming practices, identification of production system interactions have allowed for more targeted use of these compounds as crop protection agents. For example, when corn is protected from wireworm damage during the first 3 wk of growth, economic impact can be minimized (unpublished data). Therefore, acceptable qualities of these compounds include not only direct toxicity but also repellency effects on the pest. However, before this method can be field tested, potential phytotoxic effects of these compounds, on specific crops, need to be identified.

Phytochemicals have been used for many years to control insect pest damage on agricultural crops (Lee et al. 1997). Plants produce a wide range of secondary metabolites (e.g., terpenoids, alkaloids, and phenolics) that often possess insecticidal, fungicidal, bactericidal, antiviral, antifeedant, or insect growth retardant properties (Singh et al. 1989, Benner 1993, Wilson et al. 1997). Many of these chemicals are attractive alternatives to synthetic chemicals because none are likely to leach into groundwater or persist in soil or sediments (Isman 1999) as well as their reduced impact on beneficial insect populations, an important component in integrated pest management systems used by many organic farmers (Plimmer 1993).

Terpenoids, including the major constituents of most essential oils, are an important class of secondary metabolites possessing insecticidal activity. Essential oils refer to the steam-distillable fraction of plant tissues often responsible for their characteristic scent or odor (Isman 1999) and sometimes incorporated into

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natural pest control products. For example, citronellal, an acyclic monoterpene has become a popular natural alternative to *N,N*-dethyl-*m*-tolumide (DEET) for personal protection against mosquitoes and biting flies (Karr and Coats 1988, Isman 1999). In addition, rosemary oil, thyme oil, and eugenol, as pesticide active ingredients, are exempt from EPA regulation and are currently available in consumer pest control products, suggesting these materials have a relatively wide margin of safety to humans and the environment. Although considered safe for human use, essential oils often display significant biochemical effects on insects. Hough-Goldstein (1990) reported the antifeedent effects of essential oils against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), whereas Sharma and Saxena (1974) showed their effectiveness as growth inhibitors on house flies, *Musca domestica* L.

More specifically, many researchers have reported repellent, antifeedent, and toxic properties of selected essential oils against many agriculturally important pests. Thymol showed both repellent and toxic effects against the twospotted spider mite, *Tetranychus urticae* Koch (Gengaihi et al. 1996), whereas individually, thymol and citronellic acid were toxic to the common house fly; western corn rootworm, *Diabrotica vergifera vergifera* LeConte; and the twospotted spider mite (Lee et al. 1997). Thyme oil was toxic to cutworm *Spodoptera litura* F. (Isman et al. 2001), whereas rosemary oil showed repellent properties against the onion aphid, *Neotoxoptera formosana* (Takahashi) (Masatoshi and Hiroaki 1997) and the green peach aphid, *Myzus persicae* (Sulzer) (Masatoshi 1998). Finally, both contact and fumigant toxicities of eugenol and methyl eugenol were demonstrated on the American cockroach, *Periplaneta americana* (L.) (Ngoh et al. 1998). Unfortunately, despite these reports, there is no information on the toxic or repellent properties of these compounds against wireworms or their phytotoxic effects on specific plant species.

The mechanisms of toxicity of essential oils have not been fully identified (Enan 2001). However, regardless of the method of administration (e.g., oral, topical, or inhalation), insects acutely poisoned by certain essential oils display symptoms similar to toxins with a neurotoxic mode of action (Brattsten 1983, Grundy and Still 1985, Coats et al. 1991, Isman 1999), including agitation, hyperactivity, paralysis and quick knockdown.

In this study, we investigated the toxicity of three structurally different (i.e., aliphatic and aromatic) monoterpenoids (thymol, citronellal, and eugenol) and one complex essential oil (rosemary oil) on late instars of *A. obscurus* with the following specific objectives: 1) to quantify the contact and fumigant toxicities of selected compounds to *A. obscurus,* and 2) to evaluate the phytotoxic effects of selected compounds on corn seed germination and development.

Materials and Methods

Chemical Compounds. The selection of essential oils for inclusion in this study was based on reported efficacy on other insect pests, low mammalian toxicity, and benign effects on the environment. The following technical grade compounds were included citronellal (>95% purity; EcoSMART Technologies Inc., Franklin, TN), thymol (>98%; Sigma, St. Louis, MO), and eugenol (>95%; Arylessence. Inc., Marietta, GA). Rosemary oil (lot no. 0213142MB) was provided by EcoSMART Technologies Inc.

Insect Rearing and Preconditioning. Late instars (19–22 mm in length, 35–48 mg in weight) of *A. obscurus* were collected from fallow fields near the Agassiz Research Station, British Columbia, Canada, and stored at 5°C in soil-filled containers until use. Twenty-four hours before experimentation, the temperature was raised to 20°C, and slices of potato were placed 2 to 3 cm below the soil surface to identify actively feeding larvae. Every 6 h, wireworms of similar length (19–22 mm) and under the potato slices were collected for use in the bioassays.

For in vitro evaluation of chemical toxicity, it is essential to select wireworms of similar developmental stage and comparable feeding activities. Due to the difficulty in identifying instar stage through morphological observations, we classified wireworm developmental stage through length measurements. All wireworms were 3 to 4 yr old based on length criteria developed by Subklew (1934) for *A. obscurus.* We also selected only actively feeding wireworms for inclusion because these wireworms were found to be representative of wireworm behavior in the field.

Preparation of Bioassay Arena. Disposable 8-cm petri dishes were used for both topical and fumigation bioassays. Twenty grams of dry technical grade sand (prewashed with running water and autoclaved for 30 min at 120°C) and 3.5 ml of distilled water were added to each dish. The sand/water mixture was shown previously to allow for normal wireworm behavior (e.g., vigorous movements, molting, and pupation) over a 4-mo period and was deemed suitable for these bioassays. One untreated corn seed (Pioneer Brand Hybrid #39T68, Pioneer Hi-Bred Ltd., Chatham, Ontario, Canada), presoaked in water for 24 h, was added to the center of each dish as a food source.

Topical Bioassays. Five concentrations (100, 200, 400, 800, and 1,600 μg/10 μl) of each compound and rosemary oil were prepared in 100% methanol. Wireworms were held with insect handling forceps while applying the chemical with a micropipettor along the entire length of the dorsum. Each insect was treated with 10 μl of the treatment solution (i.e., 100, 200, 400, 800, or 1,600 μg/larva) with control insects receiving 10 μl of pure methanol. Immediately after treatment, individual wireworms were placed in petri dish arenas that were then sealed with Parafilm. Sealed arenas were placed in a dark growth chamber maintained at 15°C. Toxicity was assessed daily beginning 24 h after treatment. Larval death was confirmed by either probing the thoracic and head areas (if alive, larvae showed movement of body, legs, or mouthparts) or by noting changes in body color (once dead, body color changed from their natural color to dark brown to black). These

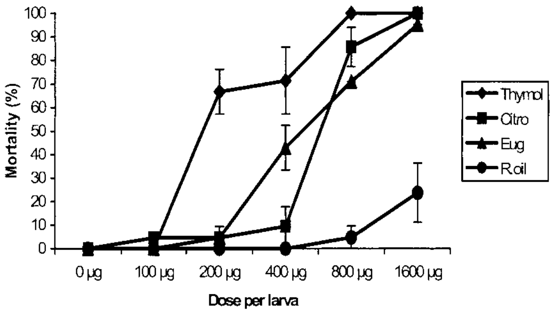

Fig. 1. Percentage of mortality of wireworms treated topically with either thymol (Thymol), citronellal (Citro), eugenol (Eug), or rosemary oil (R.oil). Error bars are standard errors of the mean.

procedures were found to be the most accurate ways to confirm death, because some wireworms seem initially moribund or dead but recovered after a few days. Observations were taken over 15 d to allow sublethally poisoned wireworms to recover. During this recovery period, corn seeds were replaced every 3 d. Each chemical–dose combination was tested on seven wireworms with three complete replications.

Fumigant Bioassays. Treatment concentrations were 5, 10, 20, 40, and 80 $\mu g/cm^3$, whereas the arena and wireworm cultures were as described above. Thirty microliters of treatment solution was added to a piece of paper towel (2 by 1 cm), attached to the lid of the petri dish with double-sided tape, and immediately sealed with Parafilm after placing a wireworm at the center of the petri dish. Sealed dishes were stored in the dark at 15°C. Observations were identical to those in the topical bioassays. Each chemical–concentration combination was tested on seven wireworms with three complete replications.

Phytotoxicity Test. Plastic pots (14 cm in diameter) with cleaned sand were used to test the phytotoxicity of the compounds and rosemary oil to corn. Corn seed (Pioneer Brand Hybrid #39T68, Pioneer Hi-Bred Ltd.) were presoaked in water for 24 h, and using a micropipettor, were coated with 20 μl of the test solution (i.e., 200, 400, 800, 1,600, and 3,200 μg/seed) described in the wireworm topical bioassay section. After treating seeds, they were planted 2.5 cm in depth in the sand. This experiment was conducted in the horticulture greenhouse, University of British Columbia, maintained at 22°C. To avoid drying, pots were immersed in trays with 1 cm of water every second day. Pure methanol was used as the control treatment. Germination percentage, cotyledon length, and radicle length were recorded 6 d posttreatment. Each chemical concentration was tested on 10 corn seeds with three complete replications.

Statistical Analysis. Data from both topical and fumigation bioassays were subjected to probit analysis to estimate LD_{50} or LC_{50} values (Finney 1971, SAS Institute 1991). Analysis of variance (ANOVA) procedures were performed by SuperANOVA software (Abacus Concepts, Berkeley, CA). Treatment means were compared by Fisher's protected least significant difference with an α value of 0.05 (SAS Institute 1991). For phytotoxicity assay, ANOVA procedures were performed by SPSS software (SPSS Inc., Chicago, IL).

Results and Discussion

Topical Bioassays. Differences in wireworm mortality were observed among the treatments ($F_{3,\ 30}$ = 6.79; P = 0.0001) and doses ($F_{5,\ 30}$ = 1.11; P = 0.0001). A compound × dose interaction also was noted ($F_{15,\ 30}$ = 1.28; P = 0.0001). Thymol, citronellal, and eugenol produced the highest topical mortality (Fig. 1), with LD_{50} values of 196, 405, and 517 μg/larva, respectively (Table 1). Rosemary oil elicited only slight (24%) mortality at the highest dose tested (Fig. 1), and no wireworm mortality or morbidity was observed in the methanol controls.

Previous studies using thymol and citronella indicate greater susceptibility in *S. litura*, LD_{50} = 25.4 and 111.2 μg/larva, respectively), *D. virgifera virgifera*, *T. urticae*, and *M. domestica* (Lee et al. 1997, Hummelbrunner and Isman 2001). We do not know why higher doses of these compounds were required to kill wireworms in our study compared with these previous reports. The wireworms we used may have weighed more than the other insects changing the effective dose required for mortality. However, a more plausible explanation is that wireworms possess a heavily sclerotized integument that could limit penetration of these compounds. The differences in toxicity to wireworms among the compounds tested herein might be due to differences in their absorption properties (Lee et al. 1997) or to differences in the ability of *A. obscurus* to detoxify or excrete these compounds, or a combination.

All compounds tested induced symptoms similar to those characteristic of neurotoxins. For example, thymol induced rapid paralysis at higher doses (800–

Table 1. Toxicities of the monoterpenoids thymol, citronellal, eugenol, and rosemary oil to *A. obscurus* in topical and fumigation bioassays

Chemical	Topical bioassay					Fumigation bioassay				
	n	Slope (± SE)	95% CL	LD_{50} (μg/larva)	χ^2 value	n	Slope (± SE)	95% CL	LC_{50} ($\mu g/cm^3$)	χ^2 value
Thymol	7	4.9 ± 1.6	127–291	195.5	2.39	7	5.4 ± 1.7	1.9–8.8	17.1	0.45
Citronellal	7	2.8 ± 0.8	243–675	404.9	3.36	7	5.6 ± 2.3	1.1–10.1	6.4	0.02
Eugenol	7	4.5 ± 1.3	344–777	516.5	1.14	7	3.9 ± 1.1	1.8–6.1	20.9	0.3
Rosemary oil	7	2.8 ± 1.6	Not determined	2378.7	0.34	7	3.5 ± 1	1.6–5.5	15.9	0.99

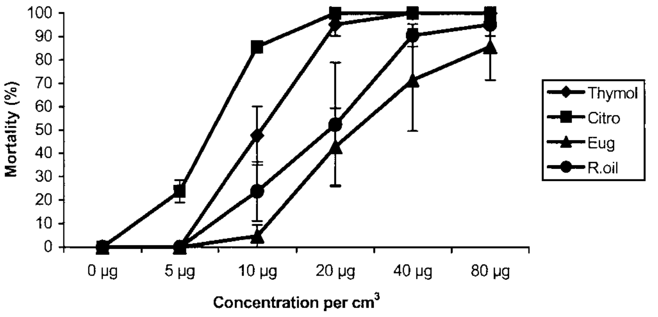

Fig. 2. Percentage of mortality of wireworms treated by fumigation with either thymol (Thymol), citronellal (Citro), eugenol (Eug), or rosemary oil (R.oil). Error bars are standard errors of the mean.

1,600 µg/larva), whereas eugenol and rosemary oil induced convulsion-like symptoms (e.g., stressful wiggling) or paralysis starting at 800 µg/larva. At lower doses of thymol and citronellal, some wireworms were able to recover from paralysis after a few days post-treatment. The recovery from paralysis observed at lower doses of thymol and citronellal suggests that these compounds may be internally metabolized and/or excreted; however, the exact means of recovery remain unknown (Harwood et al. 1990). Recovery did not occur with higher doses of any of the compounds tested, including rosemary oil.

Fumigation Bioassays. When presented to wireworms in fumigation bioassays, differences in wireworm mortality were observed among the four treatments ($F_{3, 48} = 15.5$; $P = 0.0001$) and concentrations ($F_{5, 48} = 80.3$; $P = 0.0001$). A treatment × concentration interaction was again noted ($F_{15, 48} = 2.51$; $P = 0.0081$). In contrast to the topical bioassays, all four compounds induced acute toxic effects (Fig. 2), with citronellal displaying the highest volatile toxicity (LC_{50} = 6.4 µg/cm^3), followed by rosemary oil (LC_{50} = 15.9 µg/cm^3), thymol (LC_{50} = 17.1 µg/cm^3), and eugenol (LC_{50} = 20.9 µg/cm^3) (Table 1).

Differences in acute toxicity via fumigation may be due to differences in vapor pressure and the ability of compounds to penetrate wireworms through the respiratory system and/or cuticle. Symptoms induced by the volatile forms of these natural compounds were similar to those observed in topical bioassays, suggesting a common mode of action despite different routes of exposure. The ability of wireworms to recover post-exposure varied by chemical and concentration but not by method of exposure. As observed in the topical bioassays, the ability of wireworms to recover decreased with increased concentrations of all compounds tested.

Phytotoxicity Bioassays. Differences in corn seed germination percentage, cotyledon length, and radicle length were observed among the four treatments ($P >$ 0.005) and concentrations ($P > 0.0001$). No phytotoxicity was observed with rosemary oil, whereas thymol, citronellal, and eugenol all inhibited germination and reduced cotyledon and radical growth as concentration increased (Table 2). The phytotoxicity observed with several of the compounds tested is not surprising in light of research that has shown many plant-produced secondary compounds are phytotoxic to some degree (Duke 1991). After applying thymol and eugenol around corn plants, Lee et al. (1997) observed damage to leaves and root death. In addition, these compounds often exhibit variable effects on different plant species and can include overall growth inhibition, prevention of seed germination, or plant death (Lee et al. 1997).

The phytotoxic effects of thymol, citronellal, and eugenol on corn germination and growth are of concern, because these effects may extend to other crops and limit their usefulness for wireworm control. However, our results suggest that some of the compounds tested might provide some level of wireworm control if applied to corn seed at doses below that causing phytotoxicity. Thymol, applied to corn seed at 400 µg/seed, caused no phytotoxicity, but when presented to wireworms either topically (400 µg/larva) or as a fumigant (20 µg /cm^3), induced 71.4 and 95.2% mortality, respectively. Under laboratory conditions, thymol (0.1 µg/µl in acetone) also was shown to repel *A. obscurus* when applied as a single 10-µl droplet placed on filter paper substrates (unpublished data). Therefore, thymol applied as a seed treatment to corn might kill, intoxicate, and/or repel attacking wireworms long enough for seed germination and establishment to take place. Other nonphytotoxic candidates that may provide some protection to corn seed from wireworm attack are citronellal at 800 µg/seed (topical and fumigant mortality of 85.7 and 100% mortality, respectively), eugenol at 1,600 µg/seed (topical and fumigant mortality of 95.2 and 85.7% mortality, respectively), and rosemary oil at 1600 µg/seed (topical and fumigant mortality of 23.8 and 95.2% mortality, respectively).

In envisioning how these results could best be applied to agricultural settings, two issues should be kept in mind: mode of exposure and the development of a broad-base cost–benefit analysis. The contact and fumigation mortality results clearly support continued development of some of these compounds for use in crop protection. However, in practical terms, not all modes of exposure are equal. For example, the high contact mortality observed with thymol would suggest some type of soil drench or spray application. Because

Table 2. Phytotoxic effects of monoterpenoids on corn seed germination and development

Compound	Dose (μg/seed)	Seed germination (%)	95% CL	Cotyledon length (mm)	95% CL	Radicle length (mm)	95% CL
Thymol	0	97	79–100	15.6	13.8–17.4	21.6	19.2–24
	200	97	79–100	15.3	14.5–16.7	21.9	19.1–24.9
	400	97	79–100	15.6	14.5–16.7	20.6	17.6–23.5
	800	70	53–87	14.7	13.6–15.9	19.5	16.6–22.4
	1600	77	59–94	13.9	12.7–14.9	17.7	14.8–20.6
	3200	20	3–37	6.8	5.7–7.9	8.1	5.1–10.9
Citronellal	0	97	79–100	15.6	13.7–17.4	21.6	19.2–24
	200	97	79–100	16.1	14.9–17.2	23.3	20.4–26.2
	400	93	76–100	15.6	14.5–16.7	21.8	18.9–24.7
	800	93	76–100	15	13.9–16.1	20.7	17.7–23.6
	1600	83	66–100	12.8	11.7–13.9	18.5	15.6–21.4
	3200	63	46–81	12.1	10.9–13.2	18.6	15.7–21.5
Eugenol	0	97	79–100	15.6	13.8–17.4	21.6	19.2–24
	200	100	83–100	14.7	13.5–15.8	20	17.1–22.9
	400	97	79–100	16.9	15.8–18	23.4	20.5–26.4
	800	90	73–100	16.9	15.8–18	21.4	18.5–24.3
	1600	90	73–100	13.6	12.5–14.7	19.2	16.3–22.1
	3200	57	39–74	11.9	10.8–13	16.1	13.2–19
Rosemary oil	0	97	79–100	15.6	13.8–17.4	21.6	19.2–24
	200	93	76–100	16.7	15.5–17.8	22.9	20.1–25.9
	400	87	69–100	16.6	15.5–17.8	21.9	18.9–24.8
	800	97	79–100	16.4	15.3–17.5	20.4	17.5–23.3
	1600	93	76–100	16.4	15.3–17.6	25.9	22.9–28.8
	3200	90	73–100	15.3	14.1–16.3	21.2	18.2–24.1

wireworms are soil-borne pests, spray applications are not a viable option (although they are for other pests), whereas drenching large production fields is inappropriate. The high fumigation toxicities observed with citronellal or rosemary oil suggest development of a soil fumigation treatment. Using citronellal as an example, we calculate ≈64 kg ha^{-1} would need be applied to achieve the desired concentration to 1-m depth. Although this may be technically feasible, it would not be economically viable.

The strong repellent properties of some of these compounds may indicate the most appropriate use of these compounds in agricultural settings. A seed pretreatment, which is able to prevent wireworm attack for an initial growth period, seems to be the most straightforward and simplest application. Based on our results, 400 μg/seed thymol, 800 μg/seed citronellal, or 1,600 μg/seed eugenol or rosemary oil would be required for adequate protection. These amounts are similar to quantities used for synthetic pesticides. Compared with the recommended rate of 125–1,250 μg/seed for thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Greensboro, NC), essential oils offer a viable alternative.

In addition to mode of exposure, a broad-based cost–benefit analysis should be completed for each essential oil. These calculations should include both economic factors (e.g., cost per hectare, anticipated yield increase) as well as less-quantifiable factors, including environmental impact (e.g., persistence and residual effects), human health (e.g., acute and chronic effects), and utility in organic farming systems (e.g., collateral mortality on nontarget organisms). Economic factors aside (due to significant site- and operation-specific variation), all of the essential oils tested are biodegradable and photodegradable, thereby greatly reducing their persistence in the environment.

Regarding human health issues, acute oral LD_{50} values are available for eugenol (500 mg/kg rat) and thymol (980 mg/kg rat) with both possessing relatively high values. However, all the compounds we have tested (i.e., thymol, citronellal, eugenol, and rosemary oil) are listed in the U.S. Food and Drug Administration's (FDA) Generally Recognized As Safe (GRAS) list (FDA 2005), indicating their safe use in food products. Unfortunately, inclusion on this list does not guarantee human safety due to different evaluation criteria between the various governmental agencies (Trumble 2002). However, based on the available information, the selected compounds do not display carcinogenic, hepatotoxic, or teratogenic activity in humans or any other animals tested. On the contrary, rosemary oil has been shown to have both hepatoprotective and antimutagenic effects on laboratory rats (Fahim et al. 2000), whereas eugenol has been evaluated as both an anti-inflammatory and a cancer-chemopreventive agent (Kim et al. 2003).

Several companies have already developed pest control products that contain thymol, citronellal, eugenol, or rosemary oil as active ingredients. For example, California Organic Fertilizers Inc. (Fresno, CA), is marketing Phyta-guard, a combined insecticide and fungicide containing 10% rosemary oil and 20% clove oil. EcoSMART Technologies Inc. has introduced several pest control products containing plant essential oils as active ingredients (Ecotrol, 10% rosemary oil as a broad-based insecticide; Sporan, 16% rosemary oil as a fungicide; and Home and Garden Spray, 0.45% thyme oil as a garden insecticide). Therefore, these compounds are already in commercial use.

We conclude that the insecticidal activities of thymol, citronellal, eugenol, and rosemary oil on the late instar larvae of *A. obscurus* observed in the laboratory are sufficient to warrant further investigation. Future research should focus on use of thymol, citronellal, eugenol, and rosemary oil in actual field studies with broader concentration series to evaluate their toxicities to *A. obscurus* and phytotoxicity to corn seeds and seedlings.

Acknowledgments

We thank Shannon Binns, Yasmin Akthar, and Alain Boucher for editorial assistance and Wim Van Herk for technical guidance. This research was supported by grants from NSERC and EcoSMART Technologies Inc. to M.B.I. We thank EcoSMART Technologies Inc. for providing the rosemary oil, citronellal, and eugenol used in this study.

References Cited

Benner, J. P. 1993. Pesticidal compounds from higher plants. Pestic. Sci. 39: 95–102.

Brattsten, L. B. 1983. Cytochrome P-450 involvement in the interactions between plant terpenes and insect herbivores, pp. 173–195. *In* P. A. Hedin [ed.], Plant resistance to insects. American Chemical Society, Symposium Series 208-198.

Coats, J. R., L. L. Karr, and C. D. Drews. 1991. Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. *In* P. A Hedin [ed.], Naturally occurring pest bioregulators, ACS Symp. Ser. 449: 305–316.

Duke, S. O. 1991. Plant terpenoids as pesticides, 269–296. *In* R. F. Keeler and A. T. Tu [eds.], Toxicology of plant and fungal compounds handbook of natural toxins, vol. 6. Marcel Dekker, New York.

Enan, E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comp. Biochem. Physiol. 130: 325–337.

Fahim, F. A., Y. A. Esmat., M. H. Fadel, and K.F.S. Hassan. 2000. Allied studies on the effect of Rosemarinus officinalis L. on experimental hepatotoxicity and mutagenesis. Int. J. Food Sci. Nutr. 50: 413–427.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, London, United Kingdom.

Gengaihi, E., S. E. Amer, and S.A.A. Mohamed. 1996. Biological activity of thyme oil and thymol against *Tetranychus urticae* Koch. Anzeiger fur Scchadlingskunde Pflanzenscutz Umweltsschutz. 69: 157–159.

Grundy, D. L., and C. C. Still. 1985. Inhibition of acetylcholinesterases by pulegone-1,2-epoxide. Pestic. Biochem. Physiol. 23: 383–388.

Harwood, S. H., A. F. Moldenke, and R. E. Berry. 1990. Toxicity of peppermint monoterpenes to the variegated cutworm (Lepidoptera: Noctuidae). J. Econ. Entomol. 83: 1761–1767.

Hough-Goldstein, J. A. 1990. Antifeedent effects of common herbs on the Colorado potato beetle (Coleoptera: Chrysomelidae). Environ. Entomol. 19: 234–238.

Hummelbrunner, L. A., and M. B. Isman. 2001. Acute, sublethal, antifeedent and synergistic effects of monoterpenoid essential oil compounds on the tobacco cutworm, *Spodoptera litura* (Lep., Noctuidae). J. Agric. Food Chem. 49: 715–720.

Isman, M. B. 1999. Pesticides based on plant essential oils. Pestic. Outlook 10: 68–72.

Isman, M. B., A. J. Wan, and C. M. Passreiter. 2001. Insecticidal activity of essential oils to the tobacco cutworm, *Spodoptera litura.* Fitoterapia 72: 65–68.

Karr, L. L., and J. R. Coats. 1988. Insecticidal properties of d-limonene. J. Pestic. Sci. 13: 287–290.

Kim, S. S., O. J. Oh, H. Y. Min, H. J. Park, Y. H. Nam, and S. K. Lee. 2003. Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RA W264.7 cells. J. Life. Sci. 73: 337–348.

Lee, S., R. Tsao, C. Peterson, and J. R. Coats. 1997. Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrysomelidae), two-spotted spider mite (Acari: Tetranychidae), and house fly (Diptera: Muscidae). J. Econ. Entomol. 90: 883–892.

Masatoshi, H. 1998. Repellency of rosemary oil against *Myzus persicae* in a laboratory and in a screenhouse. J. Chem. Ecol. 9: 1425–1432.

Masatoshi, H. and K. Hiroaki. 1997. Repellency of rosemary oil and its components against the onion aphid, *Neotoxoptera formosana* (Takahashi) (Homoptera, Aphididae). App. Entomol. Zool. 32: 303–310.

Ngoh, P. H., E.W.C. Lionel, P. Y. Fung, Y. Huang, M. R. Kini, and S. H. Ho. 1998. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana* (L.). Pestic. Sci. 54: 261–268.

Plimmer, J. R. 1993. Regulatory problems associated with natural products and biopesticides. Pestic. Sci. 39: 103–108.

SAS Institute. 1991. SAS language and producers: usage 2, version 6, 1st ed. SAS Institute, Cary, NC.

Sharma, R. N., and K. N. Saxena. 1974. Orientation and developmental inhibition in the housefly by certain terpenoids. J. Med. Entomol. 11: 617–621.

Singh, D., M. S. Siddiqui, and S. Sharma. 1989. Reproduction retardant and fumigant properties in essential oils against rice weevil (Coleoptera: Curculionidae) in stored wheat. J. Econ. Entomol. 82: 727–733.

Subklew, W. 1934. *Agriotes lineatus* L. and *Agriotes obscurus.* (Ein Beitrag Zu thus Morphologie und Biologie). Z. Angew. Entomol. 21:96–122.

Trumble, J. T. 2002. Caveat emptor: safety considerations for natural products used in arthropod control. Am. Entomol. 48: 7–13.

[FDA] Food and Drug Administration. 2005. EAFUS: a food additive database (http://www.cfsan.fda.gov/~dms/eafus.html).

Vernon, B., E. Lagasa, and H. Philip. 2001. Geographic and temporal distribution of *Agriotes obscurus* and *A. lineatus* (Coleoptera: Elateridae) in British Columbia and Washington as determined by pheromone trap surveys. J. Entomol. Soc. Br. Col. 98: 257–265.

Wilson, C. L., J. M. Solar, A. El Ghaouth, and M. E. Wisniewski. 1997. Rapid evaluation of plant extracts and essential oils for antifungl activity against *Botrytis cinerea.* Plant Dis. 81: 204–210.

Received 5 April 2005; accepted 10 June 2005.