



## Biorational insecticides for the control of corn rootworm

**Abstract:** *Corn rootworm control accounts for the largest agricultural use of insecticides in Iowa. Most chemicals are applied in granular form, which makes them accessible to wildlife. Because these chemicals can be toxic, "softer" insecticides would be desirable from an environmental standpoint. Monoterpenoids, one type of softer insecticide, are natural products (constituents of essential oils from mints, cedar, pine, and citrus) that biodegrade fully and rapidly—which is one reason they are safer to nontarget organisms. This project tested several promising monoterpenoids on corn rootworm larvae in the laboratory, the greenhouse, and in field plots. Results showed that two monoterpenoids in particular showed promise for use as biological control agents, although formulations and delivery methods need to be developed to attain satisfactory suppression of corn rootworm in the field.*

### Background

Certain natural substances having a range of biological properties against insect pests, fungal, bacterial, and viral diseases, and weeds have been used for centuries to protect crops. These plant substances are more rapidly degraded in the environment, and some favor beneficial insects. Some plants can produce a diverse range of secondary metabolites such as terpenoids (mono-, sesqui-, and di-), alkaloids, polyacetylenes, flavonoids, and sugars. Terpenoids are some of the most successful examples of pesticides among these substances.

Monoterpenoids, ten-carbon compounds, are steam-volatile and odorous constituents that are widely distributed in the essential oils of plant materials. More than 1,000 monoterpenoids have been isolated from plants such as mint, pine, cedar, citrus, and eucalyptus. Originally used as food flavors, perfumes, decongestants, external analgesics, and antiseptics, some monoterpenoids have shown promise as biological control agents because they can aid plants in their chemical defense mechanisms against phytophagous (plant-feeding) insects and pathogens. Monoterpenoids are typically rather lipophilic (fat-soluble) compounds, and thus may interfere with basic metabolic biochemical, physiological, and behavioral functions of insects. Some exhibit acute toxicity, while others show repellent effects, antifeedant effects, or growth and development effects. Laboratory evaluations of

monoterpenoids on various insects have established their activity as ovicides, fumigants, and contact toxicants.

Other biological activities with insects, mites, nematodes, phytopathogenic bacteria and fungi, and growth-suppressing interactions in higher plants have also been well documented. The mechanisms of toxic action are not fully understood, but onset of symptoms is usually rapid, manifested as agitation, hyperactivity, and quick knockdown. Monoterpenoids also appear to have neurotoxic effects as indicated by adverse electrophysiological effects in earthworms. Some insects metabolize many terpenes and excrete them.

Several monoterpenoids are already in use as alternatives to conventional insecticides, *d*-Limonene is an active ingredient of commercially available flea shampoos; monoterpenoids such as pulegone and citronellal are used as mosquito repellents; and 1,8-cineole is the structural base of the herbicide cinmethylin. These compounds are favorable for development as environmentally safe insect control agents because of their insect toxicity and repellency, low mammalian toxicity, and biodegradability.

Better understanding of these complex relationships can provide a basis for using monoterpenoids for management of corn rootworm. The corn rootworm requires more

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insecticide than any other crop pest in Iowa, which made it a logical focus of this study. The goal of this project was to determine the feasibility of using natural monoterpene insecticides as alternatives to conventional insecticides, and to evaluate the insecticidal properties of monoterpenoids and their derivatives. Specific objectives included

- (1) evaluating candidate monoterpenoids against corn rootworm larvae in terms of insecticidal activity or corn root protection in the laboratory, the greenhouse, and field plots,
- (2) evaluating the insecticidal activity against a public health nuisance insect, a stored-grain insect, and a phytophagous mite,
- (3) evaluating the mode of action of monoterpenoids and side effects such as phytotoxicity, and
- (4) synthesizing simple derivatives of monoterpenoids to evaluate insecticidal activity relative to parent compounds.

### **Approach and methods**

Evaluations of corn rootworm mortality or corn protection were conducted in laboratory petri dish tests, greenhouse pot tests, and field plot applications. Two-spotted spider mites and house flies were also tested.

For topical application, monoterpene solutions were prepared with acetone and applied with an electric microapplicator that delivered measured amounts of acetone alone (as the control) or in serial dilution of each solution to the house flies and the third instar (growth stage) of the western corn rootworm. House flies were transferred to paper cups containing an aqueous 5% sugar solution with a cotton roll, and the western corn rootworm larvae were transferred to petri dishes with corn seedlings as food. Pyrethrins were used as a standard for comparison. At least 20 insects per dosage (three replications) were used at a minimum of five concentrations (based on results of tests to determine the appropriate range of concentrations). Toxicity was assessed after 24 hours.

A bioassay determined the larvicidal activity of monoterpenoids in soil against third-instar

corn rootworm larvae. Monoterpene stock solutions mixed with acetone were added to petri dishes containing untreated sandy clay loam soil. After processing, five germinated corn kernels were added to each petri dish, and ten larvae were placed in the center of the soil. At least seven concentrations per compound were replicated. Larvicidal activity and mortality were determined at 48 hours.

The greenhouse pot test offered the advantage of simulating field exposure to help determine the corn root protection effect of monoterpenoids against the western corn rootworm under greenhouse conditions as a longer-term soil bioassay. When one corn plant per pot reached the nine-leaf stage, 500 western corn rootworm eggs were placed in soil around the root zone in each pot and covered with the same soil. (Duration of the egg and larval stages were approximately two and four weeks, respectively.) Some pots were established to monitor larval growth in relation to the chemical application. The monoterpene solutions were applied to the soil surface when the majority of the corn rootworm eggs had hatched. The three concentrations used were calculated against the monoterpene concentration per gram of soil. Three weeks after treatment, roots were harvested and evaluated in terms of protection value, based on a rating system of zero for no damage and 100 for total damage. Roots were also weighed for comparison with a control treatment.

In a soil bioassay used for the phytotoxicity evaluation of monoterpenoids on corn plants and roots, two plants per cup were grown to the four-leaf stage under greenhouse conditions. Monoterpene solution was applied to the soil surface. Four concentrations were replicated six times in two groups. Phytotoxicity was determined via a rating system on both leaf and root parts: zero = no damage and 100 = total damage. Root dry weights were noted.

In 1994, protection of corn roots was studied in preliminary field plot trials at Chapin and Nashua. Numerous other corn rootworm field trials were under way at these locations. The majority of the rootworm insecticides were applied at planting time; others were applied at

cultivation. Because of the monoterpenoids' very short residual time, application was made at cultivation time. In these plots, formulations of two monoterpenoids were used. Citronellol was one of the more bioactive monoterpenoids in the petri dish lab assay, and it performed well in the greenhouse test as well. The other compound was  $\alpha$ -terpineol, which also worked well in the laboratory and is one of the less expensive, more readily available terpenoids. The toxicities of these two materials to corn rootworm larvae in laboratory assays suggested that 100 parts per million (ppm) of active ingredient in the soil in the root zone of the corn plant was optimal.

The application rates of the two monoterpenoid concentrates were 5 oz. and 10 oz. active ingredient per 1,000 row feet. Water and concentrate were mixed at a 9:1 ratio, and the spray volume rate was 20 gallons per acre. The mixture was sprayed as a 7-in. band at the base of the plants and then covered by the cultivator.

In 1995, field plots were conducted at Ames and Nashua. These trials were again conducted with two experimental monoterpenoid insecticides as part of larger field-plot trials on various commercial insecticides for corn rootworm control. As in 1994, the substances were applied at cultivation. Granular formulations were used in an attempt to achieve a slightly slower release of the active ingredient. Citronellol was used again, as well as perillaldehyde, which in later lab bio-assays was more toxic to rootworms than any other monoterpenoids.

## Findings

Of 34 monoterpenoids (see box) tested against house fly adults and western corn rootworm larvae, chlorothymol, citronellic acid,  $\beta$ -limonene, perillaldehyde, pulegone, and thymol were most effective on the house fly, while citronellic acid, citronellol, eugenol, perillyl alcohol, and thujone were most effective against the western corn rootworm.  $\beta$ -Carvone was more toxic than  $\alpha$ -carvone to the house fly; the reverse was true for the corn rootworm.  $\beta$ -Limonene was more effective

than  $\alpha$ -limonene to both insects, but the differences in house fly tests were insignificant.

Alcohol and phenol forms of monoterpenoids were more toxic than ketones, aldehydes, or acids in the corn rootworm test, while the most effective monoterpenoids in the house fly test were spread among the alcohol and phenol, ketone, and aldehyde groups. The structural characteristics of the monoterpenoids—shape, degree of saturation, type of functional groups—influence the insecticidal activity and species-specific insecticidal susceptibilities.

The acute topical toxicity of monoterpenoids was much less than that of commercial insecticides; compared to natural pyrethrins, the most effective monoterpenoid, thymol, was at least three times less toxic; other monoterpenoids were 4 to 30 times less toxic. The toxicity trend for the house fly differed significantly from that of the western corn rootworm. Insects responded to topical administration of some monoterpenoids with hyperactivity, then quiescence; surviving insects recovered after a few hours, suggesting immediately induced neurotoxicities after which the monoterpenoids are quickly metabolized or eliminated.

A wide range of monoterpenoids showed some degree of larvicidal activity against the western corn rootworm in the laboratory soil bioassay; some alcohol and phenol-type monoterpenoids such as carveol, citronellol, and thymol were significantly more toxic than others. Perillaldehyde was the most effective of the 34 tested, yet the soil insecticides carbofuran and chlorpyrifos used as comparison standards were three times more toxic. Limonene and 1,8-cineole were much less toxic than others.

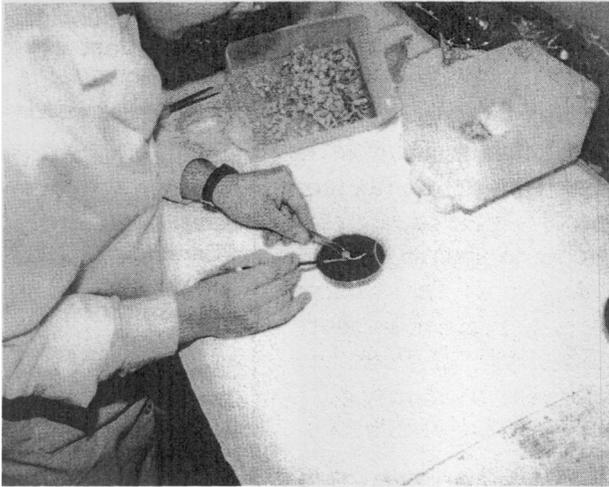
Carveol, citronellol, perillaldehyde, and  $\alpha$ -terpineol, which showed good larval activity, were evaluated under greenhouse conditions that resembled a field situation.  $\alpha$ -Terpineol, the most effective monoterpenoid, prevented any damage to corn roots. Carveol was the weakest rootworm insecticide of the four.

Some monoterpenoids showed phytotoxicity

## 34 monoterpenoids tested against corn rootworm larvae.

Borneol  
Carvacrol  
Carveol  
Carvomenthenol  
 $\alpha$ -Carvone  
 $\beta$ -Carvone  
Chlorothymol  
1,8-Cineole  
Citral  
Citronellal  
Citronellic acid  
Citronellol  
Eugenol  
 $\beta$ -Fenchone  
Geraniol  
Isopulegol  
Limonene (R)  
Limonene (S)  
Linalool  
 $\beta$ -Menthone  
Menthone  
Myrcene  
Perillaldehyde  
Perillyl alcohol  
 $\alpha$ -Pinene  
Pulegone  
 $\alpha$ -Terpinene  
 $\beta$ -Terpinene  
 $\alpha$ -Terpineol  
Terpinen-4-ol  
Thujone  
Thymol  
Verbenol  
Verbenone

*Pyrethrins also tested for comparison.*



**Petri dish test for corn rootworm bioassay.**

both on corn roots and leaves. *l*-Carvone was the most phytotoxic mono-terpenoid in comparison with control results. Thymyl ethyl ether, a synthetic derivative of thymol, also showed enhanced safety compared to

thymol, the parent compound. A longer exposure time showed more phytotoxic effects than a short one. *l*-Carvone, eugenol, and thymol were the most phytotoxic on both parts of the plant.  $\alpha$ -Pinene and pulegone did not show phytotoxicity. Derivatization of mono-terpenoid parent compounds might be able to reduce monoterpene phytotoxicity based on thymyl ethyl ether (one of the thymol derivatives), which showed 0% plant damage at 500 ppm while thymol showed 100% phytotoxicity.

In preliminary field trials, citronellol and  $\alpha$ -terpineol treatments failed to provide statistically significant control of corn rootworm as determined by root ratings (measured on Iowa State University's 1-6 scale). But in both locations, both rates of the monoterpene utilized yielded root ratings that were slightly better than the control plots. These trials showed the monoterpene to be extremely biodegradable.

In the 1995 field experiments, the use of granular formulations differed little from the 1994 results at the Ames location. At Nashua, all four monoterpene treatments gave *worse* control than the untreated plots, although the difference was statistically insignificant.

The primary drawback of monoterpene is their lack of persistence. The application method and placement (along with weather) likely exacerbated this tendency. A more sophisticated granular formulation may provide considerably better residual activity.

## Implications

Monoterpenoids vary considerably in potency, and their various chemical properties and structures can elicit different toxic effects. Some monoterpene demonstrated phytotoxic effects on corn.

This project showed that the degree of bioactivity was significantly less than conventional organic insecticides. Even so, monoterpene may be effective in high concentrations, and high concentrations may be acceptable because monoterpene are safe to wildlife and are so biodegradable. In these laboratory and greenhouse studies, good control of corn rootworm larvae was demonstrated. However, in field plots, there was no significant difference between monoterpene treatments and untreated plots. *Better formulations, different application modes, or multiple applications will be necessary to achieve satisfactory control in the field.*

This work has shown that in the greenhouse, these substances are sufficiently toxic to corn rootworms to protect corn roots, although at high concentrations compared to conventional insecticides. Field plot studies showed that formulations or delivery methods and timing must be improved to attain satisfactory suppression of corn rootworms. Insect control is usually marked by tradeoffs, however, and the extent of the trade-off in developing monoterpene for widespread use (i.e., balancing the advantage of biodegradability against the disadvantage of inadequate persistence) will depend on the degree of motivation for replacing commercial organophosphates, carbamates, and pyrethroids.

Some alternative formulations or delivery techniques may include slow-release granules, granules placed in the furrow or incorporated into the soil, larger volumes of liquid spray applied, or repeated applications over several weeks. The additional time and expense of special applications or formulations for monoterpene preclude their widespread use in field corn, but there still may be potential markets for these insecticides in organically grown sweet corn, popcorn, or seed corn.

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