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USE OF CONIFER VOLATILES TO REDUCE INJURY CAUSED BY CARROT PSYLLID, *Trioza apicalis*, FÖRSTER (HOMOPTERA, PSYLLOIDEA)

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Abstract—The feeding and oviposition of the carrot psyllid, *Trioza apicalis*, were reduced by the application of fresh spruce and pine sawdust along the seedling rows in carrot fields. Turpentine and separate monoterpene hydrocarbons, mixed into old sawdust and/or placed in polyethylene tubes, were also effective. At a dose of 0.5 liter/m, fresh sawdust reduced the damage to 18% of the plants, compared to 100% damage in untreated plants. The sawdust materials were spread on the soil surface at four- or seven-day intervals during the oviposition period. The tubes were placed along the carrot rows before the oviposition started. Turpentine and separate monoterpene hydrocarbons afforded a protective effect of the same order of magnitude as that obtained from fresh sawdust. The volatile profiles of the spruce and pine sawdust as well as of the turpentine used were determined.

Key Words—Homoptera, Psylloidea, Triozidae, *Trioza apicalis*, *Daucus carota*, repellent, dispenser, sawdust, monoterpene, enantiomeric composition, TMP-turpentine.

INTRODUCTION

The carrot psyllid, *Trioza apicalis* Förster, can cause serious damage in carrot crops. Young carrot seedlings are attacked shortly after emerging. While feed-

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ing, the insects inject a substance that causes the leaves to curl and reduces growth, especially of the roots. Adult female psyllids are more injurious than adult males and immature individuals (Markkula et al., 1976). The adults pass the winter in diapause on coniferous trees, mostly Norway spruce, *Picea abies* (L.) Karst., (Rygg, 1977). After leaving their hibernation sites in spring, they seek their summer hosts, carrots, where mating and egg-laying take place. The eggs are laid one by one on the edges of the carrot leaves and one female can lay up to 900 eggs (Láska, 1964). The oviposition period, during which the plants are injured, lasts for about three to six weeks. There is one generation per year, and the adults die shortly after egg-laying. The eggs hatch after 9–14 days, and the nymphs remain sedentary on the underside of the leaves, passing through five instars before they become adults. The new adults emerge during August (with geographical variation) and leave the carrots two to three days after eclosion (Láska, 1976). They seek their winter hosts during autumn, and after hibernation they migrate back to the carrot seedings in spring.

This pest has been reported from all of Europe as well as from temperate Asia including Japan (Burckhardt, 1986). It is of great economic importance in carrot-producing areas located near conifer forests, where up to 100% of the carrots may be damaged.

In Sweden, carrot psyllids are commonly controlled by spraying at 10-day intervals with pesticides such as dimethoate, deltametrin, acephate, or fenitrothion (Kemiska bekämpningsmedel, 1991). Up to eight sprayings may be needed. A desirable alternative control strategy would be to manipulate the behavior of the insect.

An old gardener's trick, mentioned in the amateur-gardener literature, is to use sawdust to reduce injury by the carrot psyllid (Arman, 1977). Apsits (1931), who used sawdust as a soil mulch to control weeds in carrot fields, noted that the treatment also reduced the level of damage by the psyllid. This effect was verified by Rämert and Nehlin (1989).

Various products containing volatile conifer constituents have been used as deterrents against other insects. For example, pine oil, a by-product from certain pulp mills, has been shown to deter attacks by bark beetles, *Dendroctonus* spp. (Nijholt et al., 1981, Richmond, 1985), pine weevils, *Pissodes strobi* (Alfaro et al., 1984), and the onion maggot, *Delia antiqua* (Javer et al., 1987). Another product from the pulp industry, a terpene distillation residue with the commercial name XPQ, has also been used as a repellent against several forest insects (Eidmann and Nordenhem, 1984).

Our aim was to explore further the possibility of using sawdust and its volatile constituents, separate monoterpene enantiomers, as protectants against *T. apicalis*.

METHODS AND MATERIALS

Experimental Field Procedures; Evaluation Methods

Four randomized-block design experiments were performed during 1990–1992 in commercial carrot fields (in Karlstad and Sala, Sweden). Each experiment comprised four blocks of experimental plots. Each plot consisted of four rows of carrots. The lengths of the plots differed, depending on the space available in the field and on the type of experiment to be carried out. The carrots, cv. Duke, were sown in mid-May.

The protectants to be tested were either sawdust—with or without added chemicals—or chemical products enclosed in polyethylene tubes. The first application of the protectants was made along the carrot rows when the first seedlings were emerging. The sawdust was applied over the seedlings and additional applications were made at fixed intervals. The tubes were nailed with metal wires to the soil surface along the rows.

For estimation of the protective effect, two middle stripes of seedlings with the length of 1 m each were randomly chosen in each plot and the numbers of damaged and undamaged plants were counted. In total about 400 plants per treatment were examined. The psyllid injury was defined as the proportion of plants with curled, injured leaves. The effect of the treatment was evaluated using the method of analysis of variance after arc-sine transformation. The mean values found were compared by means of the least significant range (LSR) method (Sokal and Rohlf, 1969).

Experiments

Experiment 1 was a dose–response study using an undefined mixture of fresh sawdust from Norway spruce, *Picea abies* and Scot's pine, *Pinus sylvestris* L. The conifer logs were sawn at local sawmills about one to two months after being cut. The sawdust, which contained a small proportion of bark material, was collected immediately and then stored at $-18°C$ until its application to the carrot field. Six applications of sawdust in doses of 0.10, 0.25, and 0.50 liter/m were made at one-week intervals throughout the oviposition period. Each experimental plot was 4 m long in this experiment.

In experiment 2, the protective effects of spruce and pine sawdust were tested separately. The activity of an old sawdust preparation (see below) was tested for comparison. This old sawdust, used in experiments 2 and 3, was of the same origin as the fresh sawdust used in experiment 1 (a mixture of spruce and pine). After production, it had been stored outdoors, exposed to the weather during January–May. The volatiles present in the three sawdust preparations were analyzed using capillary GC (see below).

The application dose of the sawdust was 0.2 liter/m and the application started when about half of the seedlings had emerged. It was repeated every four days until the egg-laying of the carrot psyllids had ceased. Four applications were made before the oviposition period, which in 1991 was very short, lasting for just about two weeks. Four applications were made during this period (total of eight applications). The damage was recorded four days after the last application. Each of the experimental plots was 3 m.

In experiment 3, individual monoterpene hydrocarbons were assessed as protectants. α-Pinene, β-pinene, and limonene, major components of the volatile fractions of the sawdust and turpentine, were soaked up in old sawdust. The chemicals were mixed with the sawdust in plastic bags (Pingvin polyester oven bags) and kept at −18°C before use. The concentration of the monoterpenes on the sawdust was 1:500 (4 g of a compound in 200 ml of pentane was mixed with 2 kg of old sawdust). We tested both the (+) and the (−) enantiomer of α-pinene and limonene but only the (−) form of β-pinene. [The (+) form was not commercially available.] The origins and purities of the chemicals used were as follows: (+)-α-pinene [Koch-Light pract., 75% (+) enantiomer], (−)-α-pinene [Firmenich 95%, 91% (−) enantiomer], (−)-β-pinene [Firmenich 95%, 97% (−) enantiomer], (+)-limonene [Aldrich 97%, 97% (+) enantiomer], (−)-limonene [Fluka 97%, 97% (−) enantiomer].

A sawdust dose of 0.2 liter/m was applied every four days during the oviposition period in 1991 (total of three applications and damage recorded four days after the last application). The plots used in this experiment were 2 m in length.

In Experiment 4, separate monoterpene hydrocarbons and turpentine, containing a mixture of monoterpenes, were used as protectants. In this experiment polyethylene tubes were used as dispensers instead of sawdust. The volatile fraction of the turpentine was analyzed using capillary GC, as was done with the sawdust volatiles in experiment 2.

Preliminary studies of the release rate of a monoterpene from the tubes had been made (see below).

The chemicals used were: (+)-α-pinene, (−)-α-pinene, (−)-β-pinene, (+)-limonene, (−)-limonene, (+)-3-carene, and turpentine. The origin and purity of the chemicals were as described in experiment 3, (+)-3-carene [Fluka 95%, 99% (+) enantiomer]. The turpentine originated from thermomechanical pulping (TMP) of spruce wood (SCA, Sundsvall, Sweden).

The polyethylene tubes (Kaltoplast AB), with an outer diameter of 5 mm and an inner diameter of 4 mm, were cut in 2-m lengths. The chemicals were filled into separate sets of tubes (four pieces of 2-m tubes per plot). The ends of the tubes were sealed by first heating them and then pressing them with a pair of pliers.

The carrot seedlings were covered by insect nets (Agronest) until oviposition was observed on carrots nearby and the test started. The net was removed and the tubes were put close to the stem base of the carrot seedlings along the carrot rows. The plots were 2 m long. The damage was recorded two and seven days after the tubes had been placed in the carrot field.

Collection and Analyses of Sawdust Volatiles

Trapping of Volatiles from Sawdust. The volatiles emitted by samples of fresh sawdust from spruce and pine and of the old sawdust used in experiments 2 and 3 were analyzed separately. The sawdust (3 g) was placed on a glass Petri dish enclosed in a glass flask with two openings. During 4 hr, filtered air was passed at room temperature at the rate of 100 ml/min first through the flask and then through a plug filled with Porapak Q (100 mg, 80–120 mesh). The volatile compounds adsorbed in the plug were then eluted with pentane (0.5 ml), and the pentane solution was submitted to GC and GC-MS analyses, using the same instruments and techniques as were used for gaseous samples (see below).

Headspace Analysis Technique. The analyses of the volatiles thus collected from fresh spruce and pine sawdust and from old sawdust (see experiment 2) were made in order to estimate the proportions of the volatiles emitted by these three sawdust materials. A method of external calibration of the procedure was employed, using calibration samples of the old sawdust, containing adsorbed turpentine in different concentrations.

Preparation of a Calibration Sample. Old sawdust, 7 g, was placed in a 50-ml Erlenmeyer flask with a septum-equipped cap, and a 2-ml sample of a pentane solution of turpentine was added. The flask was shaken for 30 min and then placed in a freezer. The concentrations of turpentine in the four calibration samples prepared were 1 : 100 (70 mg), 1 : 1000 (7 mg), 1 : 10,000 (0.7 mg) and 1 : 100,000 (0.07 mg).

After 24 hr, a flask with one of the calibration samples was taken from the freezer. The flask had been sealed with a septum cap, which was now removed, and a piece of aluminum foil was placed over the opening. The septum cap was then replaced, and the flask was kept at room temperature for 20 min before the cap was removed again. The needle of a gas-tight syringe was inserted through the aluminum foil, and 20 μl of the air above the sawdust was taken and injected into a fused silica capillary DB-WAX column (30 m $\times$ 0.25 mm). A Varian 3400 gas chromatograph equipped with a splitless injector and a flame-ionization detector was used. The temperature program was: 40°C for 1 min, followed by 50°C/min to 65°C and then 8°C/min to 130°C.

Two-dimensional gas chromatography (Borg-Karlson et al., 1993) was used for the chiral analyses. Two Varian 3400 gas chromatographs were coupled in series. The first one was equipped with a DB-WAX column and the second one with two chiral columns. a Cyclodex B column (30 m $\times$ 0.25 mm) was used to separate the enantiometers of α- and β-pinene, camphene, limonene, and β-phellandrene. The sabinene and 3-carene enantiomers were separated on a Lipodex E column (30 m × 0.25 mm). The temperature during the chiral separations was kept at 30°C for 30 min and was then increased to 75°C at the rate of 3°C/min.

Studies of Release through Polyethylene Tubing Walls. Preliminary studies of the rate of release of a monoterpene from the polyethylene tubes (4 mm ID, 1 mm wall thickness) were made under laboratory conditions (25°C). A piece of tube (length 30 cm) was filled with α-pinene and closed at both ends. The tube was weighed at 24-hr intervals during a period of three weeks.

RESULTS

Experiment 1. Two weeks after the start of the oviposition period, 91% of the plants in the untreated plots were heavily attacked by the psyllids (Figure 1, June 14). One week later, all of the plants were injured. The lowest dose of sawdust, 0.1 liter/m, protected the carrot seedlings to some degree, but at the

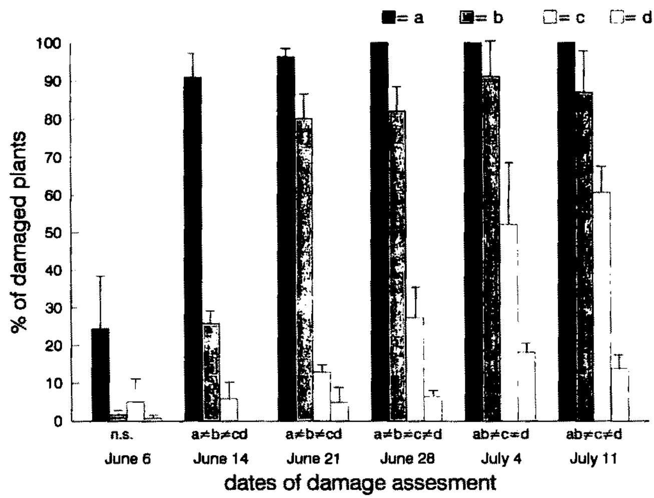

FIG. 1. Experiment 1: temporal variation in the frequency of psyllid-injured carrot plants during the oviposition period (mean values). a = untreated plants; b = sawdust 0.10 liter/m; c = sawdust 0.25 liter/m; d = sawdust 0.50 liter/m. An analysis of variance was made for each date, using arc-sine transformed values, and the results of the treatments were compared using the LSR method. (Significant differences at each date are marked below the bars in the diagram). LSD 5% for untransformed values were: 17.3, 10.1, 9.2, 10.5, 18.7, and 17.9, respectively.

end of the third week, the injury levels were high, 80%. By the end of the oviposition period, there was no significant difference in injury levels between the low-dose and control plots (Figure 1, July 4). In the 0.25 liter/m plots, the injury levels remained low for two weeks and then rose steadily to 61% at the end of the oviposition period. At the highest dose of sawdust (0.5 liter/m) the injury levels never rose above 18%. The final injury levels found in the medium- and high-dose treatments were significantly different ($P > 0.05$) from each other and from those found in the lowest dose treatment and the control plots.

The sawdust was effective only at very close range. Plants growing further than 10 cm away from the edge of the fresh sawdust were damaged. At the end of July, a slight yellowing of the carrot foliage could be seen in the sawdust-treated plots, indicating a deficit of nitrogen.

Experiment 2. by the end of the psyllid oviposition period, 85% of the untreated plants were injured (Table 1). The injury levels in the plots treated with old sawdust were somewhat lower, while fresh pine and spruce sawdust afforded the best protection (10–17% damaged plants). There was no statistically significant difference between the fresh sawdust of spruce and pine ($P < 0.05$), despite the quantitative differences in their head-space monoterpene composition (Table 4 below).

Experiment 3. Of the carrot plants in the control plots, 98% were injured (Table 2). The plants in the monoterpene-treated plots were significantly less damaged, with attack rates ranging between 12 and 38%. (−)-Limonene, with 12% damaged plants, showed the best protection and (−)-β-pinene the worst, 38% damage. There was no significant difference between the enantiomers.

Experiment 4. The weather in 1992 was warm and sunny during the pre-oviposition period. This led to a rapid invasion of a high field population of carrot psyllids. The psyllid damage was recorded two and seven days after the tubes had been applied to the field. Differences in effect between the protectants were seen only at the first recordings, two days after application (Table 3).

TABLE 1. EXPERIMENT 2: MEAN FREQUENCIES OF CARROT PLANTS INJURED BY CARROT PSYLLID (*Trioza apicalis*) IN PLOTS SUBJECTED TO VARIOUS TREATMENTS[a]

Treatment	Mean value (%)	SD
Untreated	84.6 a	8.3
Old sawdust	48.1 b	19.9
Spruce sawdust	17.3 c	5.3
Pine sawdust	9.7 c	3.8

[a] SD = standard deviation. LSD 5%: 18.9. All figures represent values before arc-sine transformation. Mean values in a column followed by different letters are significantly different (analysis of variance, LSR test, arc-sine transformation).

TABLE 2. EXPERIMENT 3: MEAN FREQUENCIES OF CARROT PLANTS INJURED BY CARROT PSYLLID (*Trioza apicalis*) IN PLOTS TREATED WITH OLD SAWDUST PLUS DIFFERENT SEPARATE MONOTERPENE HYDROCARBONS[a]

Treatment	Mean value (%)	SD
Untreated	97.8 a	0.4
(+)-α-Pinene	25.1 bc	9.5
(−)-α-Pinene	18.1 bc	10.6
(−)-β-Pinene	37.7 b	16.2
(+)-Limonene	21.4 bc	13.0
(−)-Limonene	11.6 c	4.5

[a]Figures represent values before arc-sine transformation. SD = standard deviation. LSD 5%: 15.8. Mean values in a column followed by different letters are significantly different (analysis of variance, LSR test, arc-sine transformation).

TABLE 3. EXPERIMENT 4: MEAN FREQUENCIES OF CARROT PLANTS INJURED WITHIN 2 DAYS BY CARROT PSYLLID (*Trioza apicalis*) IN PLOTS WHERE SEPARATE MONOTERPENE HYDROCARBONS WERE APPLIED IN POLYETHYLENE TUBES IN A CARROT FIELD[a]

Treatment	Mean value (%)	SD
Untreated	76.5 a	16.8
(+)-α-Pinene	64.3 a	12.5
(−)-α-Pinene	62.8 a	25.9
(−)-β-Pinene	68.5 a	18.0
(+)-Limonene	26.8 bc	10.2
(−)-Limonene	35.0 b	20.7
(+)-3-Carene	35.3 b	18.1
Turpentine	16.8 c	6.8

[a]Figures represent values before arc-sine transformation. SD = standard deviation. LSD 5%: 18.5. Mean values in a column followed by different letters are significantly different (analysis of variance, LSR test, arc-sine transformation).

When the damage was recorded after seven days, 100% of the plants in all treatments were damaged. Two days after the application of the tubes, 77% of the untreated plants were injured, whereas (+)- and (−)-limonene, 3-carene, and turpentine have reduced the injury to 17–35% damaged plants. Among these protectants, turpentine and (+)-limonene were the best according to the statistical analysis. In contrast to the low and stable release rates in the laboratory, the evaporation of the tested compounds in the field was quite fast due to the hot weather during the testing period.

Chemical Compositions. The volatile-constituent profile of the turpentine used was similar to the profile of the volatile fraction emitted by the spruce sawdust: α-pinene, β-pinene, 3-carene, and limonene were the main components (Table 4). In pine sawdust, 3-carene, α-pinene, limonene, and terpinolene were the main components. The enantiomeric composition of the monoterpenes, with the exception of 3-carene, varied between the samples. Pure (+)-3-carene was found in all three samples. As regards β-phellandrene, the (−) enantiomer was strongly prevalent in all samples. The spruce and pine sawdust used in our experiments differed most notably in their emissions of α-pinene, β-pinene, and 3-carene.

The old sawdust emitted less than 1 g of α-pinene per 100 kg of sawdust (according to GC analysis). The total monoterpene concentration in fresh sawdust was estimated at 1 : 1000–1 : 100 according to the calibration curve of the standard turpentine–sawdust samples.

Rates of Release. The polyethylene dispenser showed a stable release rate under laboratory conditions. During the first three days, the release was very low due to slow diffusion through the tubing walls. After three days, the release became stable, reaching about 2.4%/24 hr period; 0.02% of the compound had been released after the first 24 hr, 1.1% after 3 days, 22% after 12 days, and 43% after 21 days.

DISCUSSION

Our results show that the application of fresh sawdust from coniferous trees on the soil in carrot fields can reduce injury caused by the carrot psyllid. The good effect shown also by single monoterpenes as well as by turpentine indicated that the protective effect of fresh sawdust was due to the volatile monoterpenes emitted by it. The old sawdust showed a much lower capacity of reducing psyllid attack.

The most likely mode of action of the substances applied in our experiments is that they act as close-range repellents (Dethier et al., 1960), i.e., "a chemical which causes an insect to make oriented movements away from its source." Repellents can interfere at different stages of the host selection. Our repellents seem to work directly on the insects at very close range, yet before the insects settle on the carrot plants for sucking and egg-laying.

An explanation of repellency might be that the volatiles from sawdust interact with attractive chemical stimuli from the carrot plants. The reception of the stimuli by the insect neurons might then be blocked, and the insects might lose their orientation towards the hosts (Davis, 1985).

Although the psyllids hibernate in conifers and are probably attracted by conifer odors during autumn, they might be repelled by these odors in nature during their spring migration into the carrot fields. The concentration of the

TABLE 4. RELATIVE AMOUNTS AND ENANTIOMERIC COMPOSITIONS OF MONOTERPENE HYDROCARBONS IN VOLATILE FRACTIONS FROM SPRUCE (*Picea abies*) AND PINE (*Pinus sylvestris*) SAWDUST AND IN TMP TURPENTINE

	% monoterpene fraction (+/− enantiomer)		
Compound	Spruce	Pine	TMP turpentine
α-Pinene	44.7 (44/56)	33.2 (70/30)	57.4 (63/37)
Camphene	0.7 (22/78)	0.4 (50/50)	1.1 (40/60)
β-Pinene	23.6 (4/96)	1.2 (25/75)	23.7 (2/98)
Sabinene	0.5 (72/28)	1.3 (2/98)	0.3 (76/24)
3-Carene	13.5 (>99.5/<0.5)[a]	48.2 (>99.5/<0.5)[a]	6.8 (>99.5/<0.5)[a]
Myrcene	1.7 nonchiral	2.1 nonchiral	1.0 nonchiral
α-Terpinene	0.1 nonchiral	0.1 nonchiral	0.0
Limonene	4.7 (26/74)	5.0 (9/91)	6.5 (17/83)
β-Phellandrene	5.7 (1/99)	2.4 (0.1/99.9)	2.0 (7/93)
τ-Terpinene	0.3 nonchiral	0.4 nonchiral	0.0
p-Cymene	0.6 nonchiral	0.4 nonchiral	0.0
Terpinolene	1.9 nonchiral	4.7 nonchiral	0.4 nonchiral

[a]Corresponds to the detection limit of the Lipodex E column.

conifer volatiles from the protectants might be too high. It is known from other insects that substances normally attracting an insect may, if present in higher concentration, instead repel the same insect (Davis, 1985).

The similarity of the protective effects of spruce sawdust and turpentine was probably due to their similar contents of volatiles. The similar effects of different kinds of sawdust, turpentine, and monoterpene dispensers show that the presence of an individual active compound may not be crucial for the repelling effect. However, limonene showed a high protective effect in experiments 3 and 4 and will, together with turpentine, be of special interest in further investigations. (+)-Limonene has already been tested as a protectant against several insects (Karr and Coats, 1988). In their experiments, (+)-limonene was found to be both slightly toxic and repellent to some insects. Both α- and β-pinene showed different effects in our two tests, being active on sawdust (Table 2), but having little or no repelling effect when applied with the tubes (Table 3).

Except for limonene in the tube experiment, the enantiomers did not show any significant difference in effect, although this would have been expected due to several literature reports. Thus, (+)-α-pinene elicited an oviposition response from the female spruce budworm, *Choristoneura fumiferana*, but the (−) enantiomer did not (Städler, 1974). In EAG testing, *Ips typographus*, a bark beetle feeding an spruce, was more sensitive to (−)-α-pinene than to the (+) enantiomer (Dickens, 1978). This was found for both males and females. Thus, their choice of a breeding place will be the most suitable tree, since only (−)-α-pinene is transformed into the aggregation pheromone, (*S*)-*cis*-verbenol during feeding (Lindström et al., 1989). Monoterpenes from termite soldiers, acting as defense substances, showed different toxicities of their two enantiomers for other insects. (−)-α-Pinene was more toxic than the (+) enantiomer for *Formica rufa* ants, *Dysdercus congulatus* bugs, and *Tenebrio molitor* beetles (Everaerts et al., 1988). Samples of (+)-limonene showed a higher toxicity than the (−) enantiomer for *Monomorium pharaonis* ants (Valterová et al., 1988). Further examples of the activities of enantiomers of monoterpene derivatives were given by Silverstein (1979).

The continuing decrease observed in the protective effects of all treatments might have been due to loss of active material because of rapid evaporation at high field temperatures. There might also have been an overriding effect of psyllid population pressure, forcing psyllids to oviposit on plants that they had earlier considered unacceptable (Miller and Strickler, 1984).

Old sawdust, which contained only trace amounts of monoterpenes, still afforded some degree of protection. This indicated that the sawdust might have affected the visual perception of the host plant by the insect.

The effective high dose of fresh sawdust in experiment 1 (6 times 0.5 liter/m) is equivalent to a total dose of 15 liter/m^2 or 150 m^3/ha. On a commercial scale it would not be feasible to use such high application rates. The spreading is time-consuming when it must be repeated every four to seven days. Moreover, high doses of sawdust might have adverse effects on the crop; thus, being essentially organic, the sawdust might contribute to the immobilization of nitrogen during the mineralization, or it might release some phytotoxic substances. Therefore, applications of fresh sawdust cannot be recommended for large-scale use.

Our future research will focus on developing a dispenser with a constant terpene release rate. The carrier material should preferably be biodegradable and easy for the grower to handle and apply in practice. The repellent used could be turpentine or limonene. (+)-Limonene is a natural product of low toxicity. Turpentine is a complex by-product of the pulping industry and could be used as a cheap insect repellent if it were possible to spread it satisfactorily. However, before use, its possible plant toxicity must be investigated.

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