RESPONSE OF COLORADO POTATO BEETLES, *Leptinotarsa decemlineata* (Say), TO VOLATILE COMPONENTS OF TANSY, *Tanacetum vulgare*

OKSANA PANASIUK

Eastern Regional Research Center
Agricultural Research Service, U.S. Department of Agriculture
Philadelphia, Pennsylvania 19118

(Received July 21, 1983; revised January 19, 1984)

Abstract—The responses of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), to volatile components of tansy, *Tanacetum vulgare* L., were investigated in order to establish a chemical basis for observed reduction in beetle populations when potatoes, *Solanum tuberosum* L., were interplanted with tansy. Colorado potato beetles exhibited avoidance behavior to tansy oil, volatiles from intact tansy plants, a "hydrocarbon fraction" of tansy oil, obtained by fractionation on alumina, and five of the 13 known components of tansy oil that were tested. One constituent of tansy oil, α-pinene, attracted beetles.

Key Words—Colorado potato beetle, *Leptinotarsa decemlineata*, Coleoptera, Chrysomelidae, tansy, *Tanacetum vulgare*, potato, *Solanum tuberosum*, interplanting, volatile compounds, insect attractance, insect avoidance.

INTRODUCTION

Popular gardening literature is full of advice on how to prevent insect damage to horticultural plants, but much of this "gardening lore" is not supported by objective investigations. Interplanting of crop plants with other plants that are said to have insect-repelling properties is an often-recommended procedure. With the increased interest in integrated pest management, experiments in interplanting, as a means of insect control, have been reported recently in the literature (Latheef and Irwin, 1980; Theunissen and Denouden, 1980).

In a review of host-plant resistance to pests, Hedin et al. (1977) pointed out the paucity of work on naturally occurring insect repellents by finding only 12 such compounds reported in the literature. More recently, a number of insect-repelling or -attracting substances have been isolated from many plants, such as: bay leaves (Verma and Melvan, 1981), Javanese vetivar (Subhash et al., 1982), *Lasiantheae fructosa* (Mikami et al., 1981), alfalfa (Buttery and Kamm, 1980), and others. On searching the popular literature for plants that are alleged to have insect-repelling properties, one finds that most of them are herbs or other aromatic plants.

For three successive seasons, Rodale Organic Gardening and Farming Research Center (ROGFRC) Emmaus, Pennsylvania, with our cooperation, conducted interplanting experiments in the field. Various interplanting schemes were tried including: peppers (*Capsicum annuum* cv. Calif. Wonder), potatoes (*Solanum tuberosum*, cv. Katahdin), cucumbers (*Cucumis sativus* cv. Straight Eight), cabbages (*Brassica oleracea* cv. Danish Ballhead), with catnip (*Nepeta cataria*), coriander (*Coriandrum sativum*), eucalyptus (*Eucalyptus globulus*), marigold (*Tagetes patula*), nasturtium (*Tropaeolum majus*), onion (*Allium cepa*), wormwood (*Artemisia absinthium*), sage (*Salvia officinalis*), and tansy (*Tanacetum vulgare*).

Statistically significant reductions of insect populations were achieved at ROGFRC in some interplanting systems (Matthews et al., 1983). For example, Colorado potato beetle population on potato plants was reduced 60–100% when interplanted with tansy and 58–83% when interplanted with catnip, while green peach aphid (*Myzus persicae*) populations on peppers were reduced 82% when interplanted with catnip and 59% when interplanted with tansy as compared to monocultural plantings.

With the knowledge that statistically significant reductions of insect populations occur in crops interplanted with tansy, the purpose of the present study was to test the hypothesis that volatile compounds produced by tansy repel Colorado potato beetles in the vicinity of the plant. Von Rudloff (1963) separated 26 terpenes from the volatile oil of tansy and positively identified 22 of them. In a second investigation, Von Rudloff and Underhill (1965) studied seasonal variations in the volatile oil of tansy. Hethelyi et al. (1981) studied five genotypes of tansy and identified six more compounds in the oil. Thus, many of the volatile compounds that might be expected to occur in the vapor phase around the tansy plant are known. The Colorado potato beetle (*Leptinotarsa decemlineata*) was selected, since it has been widely studied for its response to volatile compounds produced by the potato plant, and it is known to be sensitive and specific in its responses to olfactory stimuli (Wei-Chun Ma and Visser, 1978).

METHODS AND MATERIALS

Tansy (*Tanacetum vulgare*) plants were harvested at ROGFRC, and the essential oils of the plants were isolated by steam distillation in our laboratory.

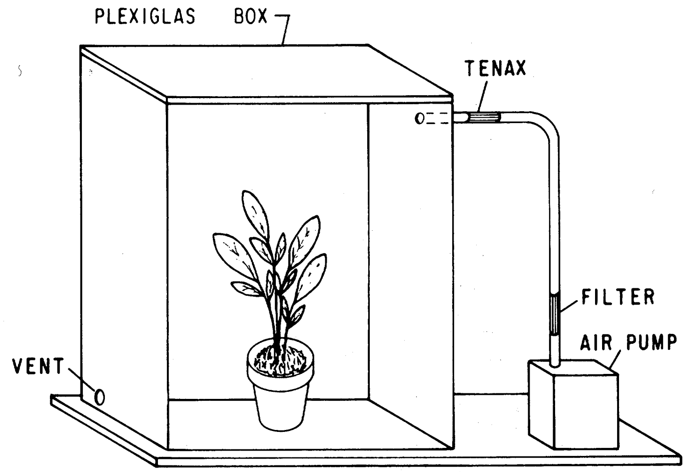

FIG. 1. Apparatus for collection of volatiles produced by intact tansy plants.

Commercial tansy oil from La Pine Scientific Company[1] of Norwood, New Jersey, was used for comparison with oil isolated in our laboratory. Individual authentic chemicals known to be present in tansy oil (Von Rudloff, 1963) were obtained from chemical supply houses.

Distillation. Tansy (620 g of stems, leaves, and some flowers) was steam distilled, and the oil was extracted from the aqueous distillate with methylene chloride. The extract was concentrated on a rotary evaporator to yield 9.3 g of tansy oil.

Headspace Vapor Collection. A method for collecting headspace volatiles from intact plants was developed to determine the volatile compounds given off by the plants. A box with a volume of 46.5 liters was constructed of clear Plexiglas (Figure 1). An air sampling pump was used to draw air through the box at a pumping rate of 1 liter/min. Intact tansy plants were placed in the box, and air circulated through the box was drawn by the pump into a glass tube filled with Tenax-GC (60/80 mesh). Tenax-GC was selected as the absorbent for volatiles of tansy, since once the volatiles are absorbed, they are stable on this polymer for up to 5 days at 0° C, and multiple collections can be made (Buckholz et al., 1980). Tenax-GC has a lower adsorption capacity than other polymers, but since volatiles given off by intact plants are produced in small quantities, this fact did not present a problem. After 24 hr of collection, material on the Tenax tube was eluted with acetone and analyzed by gas chromatography. Analysis of tansy oil obtained by steam distillation and the "headspace" vapor given off by intact tansy plants yielded similar chromato-

[1]Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

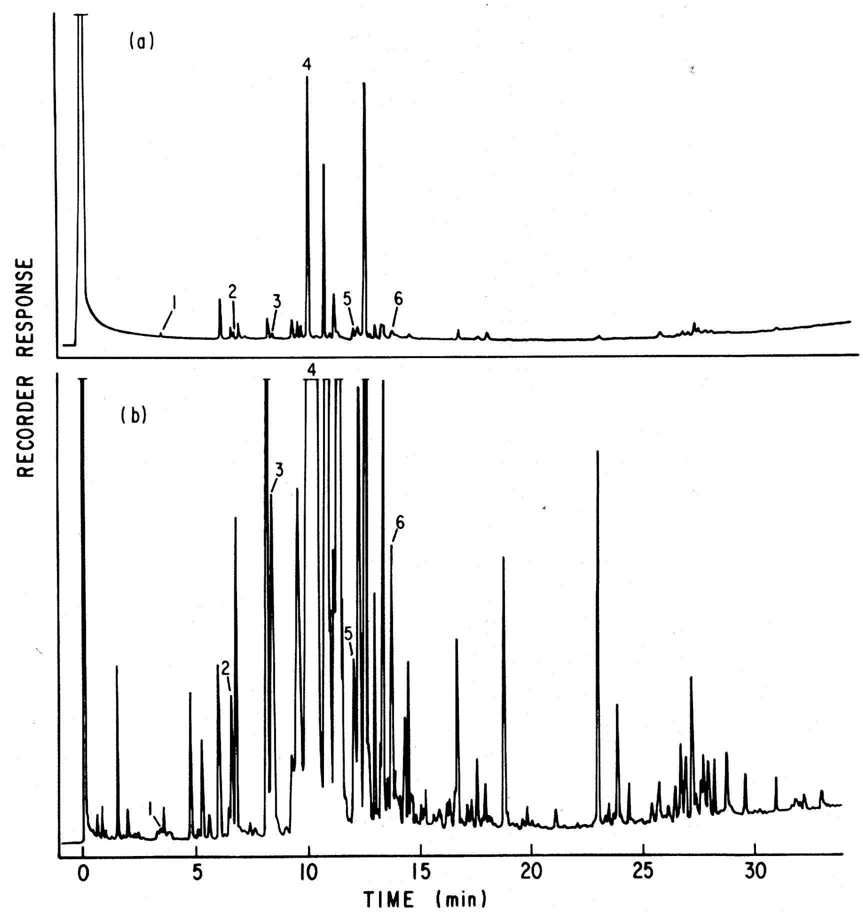

FIG. 2. Gas chromatograms of volatiles of tansy. (A) Volatiles collected from intact tansy plants. (B) Volatiles obtained by steam-distillation of tansy. Peaks: (1) α-pinene, (2) α-terpinene, (3) γ-terpinene, (4) thujone, (5) dihydrocarvone, (6) carvone.

grams (Figure 2), which indicated that the main components of the distilled oil also were given off in the vapor phase by intact plants.

Gas Chromatographic Method. A Varian gas chromatograph, model 3700 equipped with a 12-m, 0.25-mm-ID fused silica OV-101 capillary column was programed as follows: 5 min at 50°C then at 4°C/min to 170°C, and then held at 170°C for 10 min. The column inlet pressure was 15 psi helium.

Repellency Tests. Colorado potato beetle colonies were maintained on potato plants (grown in our laboratory) under long day conditions provided by timed, artificial light. Before each experiment, the beetles were starved for 24 hr (Visser and Ave, 1978). Five beetles were used per test.

The following preliminary experiments were conducted. In one case, a drop of full-strength tansy oil was placed on the rim of a large crystallizing dish. When a beetle that was placed on the rim approached the drop, it would back away, try to turn around, or fall from the rim; the latter is a typical reaction of the Colorado potato beetle when disturbed. A drop of water placed on the rim was used as a control. In another experiment, 20 mg of tansy oil was dissolved in 10 ml of acetone, and a strip of filter paper moistened with a 0.1-μl drop of this solution was introduced into the vicinity of beetles, and their reaction was observed. The beetles were repelled by strips of filter paper treated with tansy oil, but showed no reaction to acetone-treated strips. These experiments indicated that the beetles actually were repelled by tansy oil. In a subsequent experiment, two sprigs from a potato plant were placed in bottles filled with water and positioned at opposite ends of a cage containing 10 Colorado potato beetles. One sprig was moistened with tansy oil dispersed in water and the other with plain water. For the first 8 hr the beetles completely avoided the treated sprig. After 24 hr, six Colorado potato beetles had infested the untreated sprig, and it was almost completely eaten. The treated sprig was nearly intact except for one beetle that was feeding inside the top of the sprig where tansy oil solution had not reached.

Three additional repellency assays were conducted. First, to simulate the atmosphere produced by volatiles emanating from intact tansy plants, a cotton plug was saturated with tansy oil, or a component of tansy oil, and loosely inserted in a pipet. A stream of N_2 at a flow rate of 80 ml/min was directed through the pipet and exhausted in one side of a box. A second pipet was plugged with cotton, and the N_2 flow through this pipet was directed toward the other side of the box. Nitrogen was used instead of air to prevent possible oxidation of the substance tested. An overhead lamp was used to create even lighting. A freshly cut potato leaf was placed under each pipet, and five Colorado potato beetles were released in the middle of the box, and their activities were observed for 1 hr (Figure 3).

The second test involved comparison of feeding activity on two potato leaves, one plain and the other treated with the substance to be tested. One millimole of each individual compound to be tested was dissolved in 50 ml of acetone. For whole tansy oil, the concentration was 150 mg in 50 ml of acetone. Aliquots of acetone solutions were applied to potato leaves. A 100-μl aliquot was used in the 1-hr test and a 500-μl aliquot in the 2-hr test. As before, five beetles were placed in the box and observed for 1 hr. Substances that repelled or attracted beetles were repeated in a 2-hr test.

A third set of experiments involved placing beetles in a cheesecloth-covered dish (17 cm in diameter) containing one potato leaf moistened with a 500-μl solution of the substance to be tested and one untreated leaf for 24 hr. At the end of the test period both leaves were examined, and the repellency or the attractancy of the tested substance was determined by comparison of the extent of feeding on each leaf.

The three tests described above were conducted on the following sub-

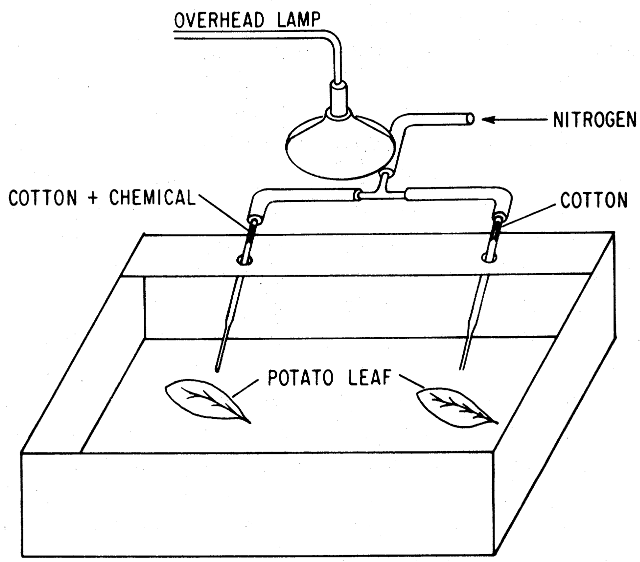

FIG. 3. Assembly for testing the response of Colorado potato beetles to volatiles produced by tansy.

stances: (1) tansy oil isolated in our laboratory; (2) tansy oil obtained from a commercial source; (3) tansy "headspace volatiles" collected for 24 hr, as already described, from intact plants, absorbed on a Tenax-filled tube, eluted with acetone, and concentrated under nitrogen (Table 1); (4) three fractions of distilled tansy oil: a "hydrocarbon fraction," an "aldehyde–ester fraction," and an "alcohol fraction" (Table 2), which were obtained by fractionating the oil on neutral Woelm's alumina with hexane, diethyl ether, and ether–methanol (90:10) eluents, respectively (Buttery and Kamm, 1980); and (5) thirteen individual compounds which are known to be present in tansy oil (Von Rudloff, 1963) in substantial amounts (Table 3).

RESULTS AND DISCUSSION

The phenomenon of repellency is complicated. These studies involved only one insect and one plant in very basic experiments. Even so, complications were encountered, because the composition of tansy oil varies considerably with season (Von Rudloff and Underhill, 1965) as well as from plant to plant, and because the compounds that vary are largely those that produced responses of Colorado potato beetles. Although all tansy plants were from the same source (ROGFRC), one batch of laboratory-distilled oil did not contain thujone (perhaps due to the fact that the seed company supplied seeds from

TABLE 1. COLORADO POTATO BEETLE RESPONSE TO VOLATILE OILS

	Potato leaf under atmosphere of tansy volatiles	Potato leaf treated with tansy oil or volatiles collected from tansy plants		
Oil	1 hr exposure	1 hr exposure	2 hr exposure	24 hr exposure
Tansy oil (commercial)	avoided[a]	avoided[b]	avoided[b]	avoided[b]
Tansy oil (laboratory preparation)	avoided[a]	avoided[b]	avoided[b]	sl. avoided[c]
Tansy headspace volatiles collected on Tenax	avoided[a]	avoided[b]	[d]	sl. avoided[c]

[a]Avoided leaf under atmosphere of tansy volatiles but fed upon leaf under nitrogen atmosphere.
[b]Avoided oil-treated leaf but fed upon untreated leaf.
[c]Slightly avoided—after 24 hr one beetle was found on the leaf but did not feed on it.
[d]Not observed at 2 hr.

different sources). The six components of the volatile oil of tansy which produced responses in the Colorado potato beetle were tentatively identified by GLC retention time. Presence of thujone was confirmed by mass spectral data, since this compound may be absent in some tansy plants.

The results of the experiments strongly suggest that there is a chemical basis for the repellent properties of tansy. First, tansy oil (both commercial and freshly distilled) produced avoidance behavior in Colorado potato beetles in all experiments (Table 1). Second, "headspace volatiles" from intact tansy plants had the same effect, demonstrating that the volatile components, in the

TABLE 2. COLORADO POTATO BEETLE RESPONSE TO FRACTIONS OF TANSY OIL

	Potato leaf under atmosphere of oil fraction	Potato leaf treated with fraction of oil		
Oil fraction	1 hr exposure	1 hr exposure	2 hr exposure	24 hr exposure
Hydrocarbon	avoided[a]	avoided[a]	avoided[a]	avoided[a]
Ester–aldehyde	accepted[b]	avoided[a]	sl. avoided[c]	no difference[d]
Alcohol	accepted[b]	avoided[a]	sl. avoided[c]	no difference[d]

[a]Avoided treated leaf, but fed upon untreated leaf.
[b]Fed upon treated leaf to some extent.
[c]Did not avoid the treated leaf completely, but did not feed upon it.
[d]Both leaves were fed upon.

TABLE 3. COLORADO BEETLE RESPONSE[a] TO INDIVIDUAL COMPOUNDS COMPRISING TANSY OIL

	Potato leaf under atmosphere of compound	Potato leaf treated with compound		
Compound	1 hr exposure	1 hr exposure	2 hr exposure	24 hr exposure
α-Pinene	attracted	attracted	attracted	attracted
dl-α-Pinene	no effect	no effect	[b]	no effect
α-Limonene	accepted	accepted	[b]	no effect
α-Terpinene	avoided	avoided	avoided	avoided
γ-Terpinene	sl. accepted	avoided	avoided	avoided
Cineole	no effect	avoided	avoided	no effect
ρ-cymene	sl. avoided	sl. avoided	accepted	avoided
α, β-thujone	avoided	avoided	avoided	avoided
L-Camphor	sl. accepted	avoided	sl. avoided	accepted
Terpinen-4-ol	no effect	sl. avoided	[b]	sl. avoided
Dihydrocarvone	avoided	avoided	avoided	avoided
Carvone	avoided	avoided	avoided	avoided
Borneol	accepted	avoided	sl. accepted	no effect

[a]Attracted = preferred treated leaf to untreated leaf; slightly avoided = did not avoid the leaf completely, but when on it, did not feed; slightly accepted = took a few bites before moving away; no effect = both treated and untreated leaf was fed upon; accepted = fed upon treated leaf to some extent; avoided = avoided treated leaf, but fed upon untreated leaf.
[b]Not observed at 2 hr.

aggregate, produce an avoidance response in Colorado potato beetle. As shown in Table 2, the strongest avoidance effect was produced by the "hydrocarbon fraction" obtained by fractionating tansy oil on alumina. Four components of tansy oil: α-terpinene, thujone, dihydrocarvone, and carvone, produced definite avoidance behavior in Colorado potato beetles (Table 3). To a slightly lesser degree, γ-terpinene had the same effect. One compound, α-pinene, attracted beetles.

While this study was limited in scope, the results confirm the validity of the concept of interplantings with tansy for the control of the Colorado potato beetle and identify at least some of the chemical substances responsible for avoidance behavior of Colorado potato beetles. Additional work under more rigid conditions is needed. Larger colonies of insects in more controlled environments should be used in experiments, quantitative composition of oils should be established, and different concentrations of compounds and their combinations should be examined in order to exploit more fully the use of tansy in interplantings, or the use of tansy oil or its constituents as insect repellents.

Acknowledgments—Rodale Organic Gardening and Farming Research Center is gratefully acknowledged for supplying tansy plants, and particular thanks is due to Diane Matthews of that organization for her constructive advice and support.

REFERENCES

BUCKHOLZ, L.L., JR., WITHECOMBE, D.A., and DAUM, H. 1980. Application and characterization of polymer adsorption method used to analyze flavor volatiles from peanuts. *J. Agric. Food Chem.* 28:760–765.

BUTTERY, R.G., and KAMM, J.A. 1980. Volatile components of alfalfa: Possible insect host plant attractants. *J. Agric. Food Chem.* 28:978–981.

HEDIN, P.A., JENKINS, J.N., and MAXWELL, F.G. 1977. Behavioral and developmental factors affecting host plant resistance of insects. Chemical compounds affecting insect behavior, feeding deterrents, repellents. *ACS Symp. Ser.* 62:231–275.

HETHELYI, E., TETENYI, P., KETTENES-VANDEN BOSCH, J.J., SALEMINK, C.A., HEERMA, W., VERSLUIS, C., KLOOSTERMAN, J., and SIPMA, G. 1981. Essential oils of five *Tanacetum vulgare* genotypes. *Phytochemistry* 20:1847–1850.

LATHEEF, M.A., and IRWIN, R.D. 1980. Effects of companionate planting on snap bean insects, *Epilachna varivestia* and *Heliothis zea*. *Environ. Entomol.* 9:195–198.

MATTHEWS, D.L., NICHALAK, P.S., and MACRAE, R.J. 1983. The effect of traditional insect-repellent plants on insect numbers in a mixed planting system. Proceedings of the 4th International Federation of Organic Agricultural Movements Conference, "Resource conserving, environmentally sound agricultural alternatives." Massachusetts Institute of Technology, Cambridge, Massachusetts, August 18–20, 1982. Praeger, Boston. pp. 117–127.

MIKAMI, K., TAYA, S., NIKAI, T., and FUJITA, Y. Lasidiol angelate: ant repellent sesquiterpenoid from *Lasiantheae fructosa*. *J. Org. Chem.* 46:5449–5450.

VON RUDLOFF, E. 1963. Gas–liquid chromatography of terpenes, Part VIII. The volatile oil of *Tanacetum vulgare* L. *Can. J. Chem.* 41:1737–1743.

VON RUDLOFF, E., and UNDERHILL, E.W. 1965. Gas–liquid chromatography of terpenes. XII. Seasonal variation in the volatile oil from *Tanacetum vulgare*. *Phytochemistry* 4:11–17.

SUBHASH, C.J., NOWICKI, S., EISNER, T., and MEINWALD, J. 1982. Insect repellents from vetivar oil: I. Zizanal and epizizanal. *Tetrahedron Lett.* 23:4639–4642.

THEUNISSEN, J., and DENOUDEN, M. 1980. Effects of intercropping with *Spergula arvensis* on pests of brussels sprouts. *Entomol. Exp. Appl.* 27:260–268.

VERMA, M., and MELOAN, C.E. 1981. A natural cockroach repellent in bay leaves. *Am. Lab.* 13:64–69.

VISSER, L.H., and AVE, D.A. 1978. General green leaf volatiles in the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata*. *Entomol. Exp. Appl.* 24:738.

WEI-CHUN MA, and VISSER, J.H. 1978. Single unit analysis of odor quality coding by the olfactory antennae receptor system of the Colorado beetle. *Entomol. Exp. Appl.* 24:520.