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Persistence of 2-Tridecanone on the Leaves of Seven Vegetables

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While significant research effort is currently directed toward biological and cultural control strategies against vegetable pests, the application of synthetic pesticides remains an essential activity in many production systems. Pesticide resistance is increasing and the development and registration rate of new pest control chemicals on vegetables "minor crops" is low compared to pesticides used for large-acreage crops. Because of the inherent toxicity of most existing synthetic pesticides to non-target organisms and because of their persistence in the environment (Antonious 2003; 2004a), there is increasing pressure on the agricultural industry to find more acceptable pest control alternatives. Concerns about pesticide safety usually involve two sides, the environment and the end-user. To protect the environment, the general trend is to use reduced levels of active ingredients. This trend creates a need for pesticide formulations with improved efficacy at low application rates. To protect the end-user, safe formulations that eliminate organic solvent-based formulations are needed. Some liquid formulations, such as emulsifiable concentrates (EC), are harmful not only because of the toxicity of their active ingredients but also because of the toxicity of their organic solvents. These formulations are also coming under more and more regulatory pressure due to their organic solvent content.

Developing efficient natural products with low mammalian toxicity and little or no impact on environmental quality for use against vegetable insects, that have gained resistance against many classes of insecticides, is needed. There appear to be no environmental studies conducted with specific reference to the use of 2-tridecanone (Figure 1, upper graph), a methylketone, on vegetables as an organic insecticide. Four methylketones (2-undecanone, 2-dodecanone, 2-tridecanone, and 2-pentadecanone) were detected in five *L. hirsutum f. glabratum* (Mull) accessions (PI 251304, PI 126449, PI 134417, PI 134418, and LA 407) (Antonious 2001). 2-tridecanone (the predominant methylketone in the five *glabratum* accessions analyzed) has shown insecticidal and acaricidal performance against several vegetable insects and spider mites (Antonious et al. 2003). Recent research on the wild tomato, *L. hirsutum* f. *glabratum,* has demonstrated that their glandular trichomes (plant hairs) and the exudates they produce contribute to insect resistance (Antonious 2001; Antonious et al. 2003; Antonious and Kochhar 2003). Such findings on the insecticidal performance of *L. hirsutum* extracts make wild tomato an attractive system for study against vegetable insects that have developed resistance to all major classes of modern synthetic insecticides. The objectives of the present study were 1) to prepare a new insecticide-acaricide formulation from the leaves of five wild tomato accessions (having insecticidal and acaricidal efficiency due to their high levels of 2-tridecanone), 2) to spray this new formulation on pepper, squash, radish, commercial tomato, broccoli, Swiss chard, and watermelon plants grown under greenhouse conditions, and 3) to determine the initial deposition, dissipation constant, and half-lives ($T_{1/2}$) of 2-tridecanone on the leaves of these vegetables.

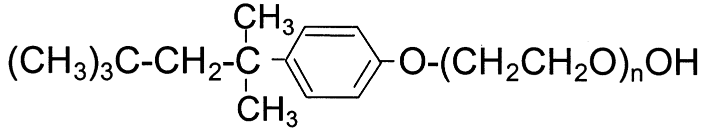
MATERIALS AND METHODS

For mass production of wild tomato leaves, seeds of wild relatives of tomato were obtained from the USDA/ARS, Plant Genetic Resources Unit, Cornell University Geneva, NY, USA. Wild tomato plants studied included five accessions of *Lycopersicon hirsutum f. glabratum* Mull: PI 126449, PI 134417, PI 134418, PI 251304, and LA 407. Seeds were germinated in the laboratory on moistened filter paper in Petri dishes kept in the dark. After germination, seedlings were maintained under fluorescent lighting in the laboratory. At the 6-leaf stage, plants from each accession were transported to the greenhouse, transplanted into plastic pots, 20 cm in diameter containing Pro Mix (Kentucky Garden Supply, Lexington, KY) and grown under natural day lighting conditions supplemented with sodium lamps providing additional photosynthetic photon flux of 110 $\mu mol\ s^{-1}\ m^{-1}$. Pots, spaced 30-cm apart, were distributed on the greenhouse benches and the plants were irrigated daily and fertilized twice a month with water containing 200 ppm of general purpose fertilizer of the elements N, P, and K (20:20:20). No insects were observed on the wild tomato foliage and no insecticides were applied.

A bulk crude extract of 2-tridecanone was prepared by soaking 5 kg wild tomato leaves collected from the greenhouse wild tomato plants in 10 L of water containing 15 mL of 2% Sur-Ten (sodium dioctyl sulfosuccinate), obtained from Aldrich Chemical Company, Milwaukee, IW, USA. After continuous manual shaking, the mixture was filtered through cheesecloth and each 1L of the filtrate was partitioned with 200 mL of n-hexane in a separatory funnel. The hexane layers were combined and the solvent was evaporated to dryness using a rotary vacuum evaporator (Buchi Rotavapor Model 461, Switzerland) at 35 ^{o}C followed by a gentle stream of nitrogen gas (N_2). To the concentrated wild tomato leaf extract, triton ×-100 (Figure 1, lower graph) was added at 0.1% (v/v) as an emulsifier and the contents were mixed and used for spraying the 45 day old pepper (*Capsicum annum* cv. Aristotle X3R), squash (*Cucurbita maxima* cv.Blue Hubbard), radish (*Raphanus sativus* cv. Brio), commercial tomato (*Lycopersicon esculentum* cv. Ponderasa), broccoli (*Brassica oleracea* cv. Italian Green), Swiss chard (*Beta vulgaris* cv. Lucullus), and watermelon (*Citrullus lanatus* cv. Stars & Stripes). To purify and determine the concentration of 2-tridecanone in the crude extract, one mL of the extract was re-dissolved in 10 mL of n-hexane and applied to the top of a glass chromatographic column (20 × 1.1 cm) containing 10 g alumina grade-II that had been pre-wetted and eluted with n-hexane (Fobes et al. 1985). The eluent was evaporated to dryness and

$CH_3-(CH_2)_{10}-CO-CH_3$

2- Tridecanone



t-octylphenoxypolyethoxy ethanol; triton x-100

Figure 1. Chemical structures of 2-tridecanone (upper graph) and triton ×-100 (lower graph). The value of "n" is an average of polymerization and not an exact figure.

reconstituted in n-hexane for GC/MSD injections. 2-tridecanone in the prepared insecticide formulation was used at the rate of 0.44 g AI L^{-1} of water in such a manner that the plants were drenched to the point of runoff. Spraying was applied with a microsprayer (Plant Care Sprayer, Delta Industries, PA) of 475 mL capacity capable of producing monosize droplets of 200 μm diameter. Spray drops were 20-30 drops cm^{-2} of treated leaf surface as determined by using water sensitive paper (Syngenta Crop Protection AG, CH-4002 Basle, Switzerland). Leaves were randomly collected at intervals of 1, 3, 5, 24, and 48 hr following spraying from both sprayed and unsprayed plants. Twenty g leaves were blended with 100 mL of chloroform for 2 min at high speed. After homogenization, the mixture was vacuum filtered through a Buchner funnel containing a glass microfibre filter containing 10 g Na_2SO_4 anhydrous and the extract was concentrated by rotary vacuum evaporator as described above. Clean-up of the concentrated extracts was achieved using the procedure described by Fobes et al. (1985) & Antonious and Snyder (1993). 2-tridecanone in the wild tomato crude extract and in the leaves of the seven sprayed vegetables was identified and quantified on a Hewlett-Packard (HP) gas chromatograph (GC), model 5890 equipped with mass selective detector (MSD) and a HP 7673 automatic injector. The instrument was auto-tuned with perfluorotributylamine (PFTBA) at m/s 69, 219, and 502. Electron impact (EI) mass spectra was carried out using an ionization potential of 70 eV. The operating parameters of the GC were as follows: injector and detector temperatures 210 and 275 ^{o}C, respectively. Oven temperature was programmed from 70 to 230 ^{o}C at a rate of 10 ^{o}C min^{-1} (2 min initial hold). Injections onto the GC column were made in splitless mode using a 4-mm ID single taper liner with deactivated wool. A 25 m × 0.2 mm ID capillary column containing 5% diphenyl and 95% dimethyl-polysiloxane (HP-5 column) with 0.33 um film thickness was used. Carrier gas (He) flow rate was 5.2 mL min^{-1}. Quantification was based on average peak areas from two consecutive injections. Retention time of 2-tridecanone under these conditions was 14.26 min. Peak areas were determined on a Hewlett Packard Model 3396 Series II integrator. Quantification was carried out using aliquots of 1-μL injections of diluted extracts. Area units were compared to an external standard solutions of 2-tridecanone of 99% purity (Aldrich, Milwaukee, IW). Linearity over the range of concentrations was

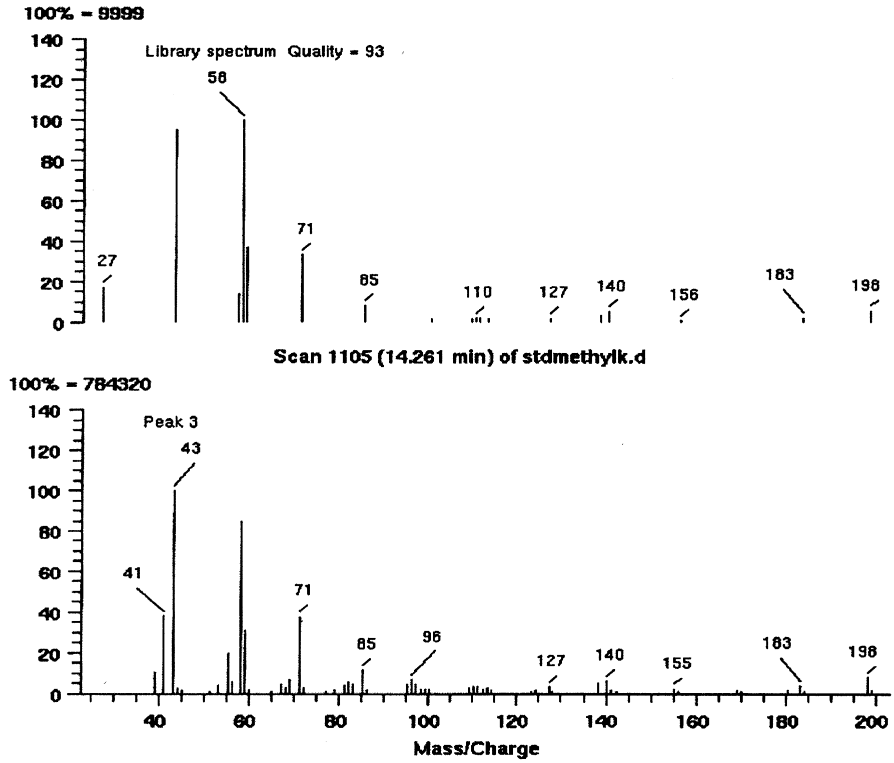


Figure 2. Electron impact mass fragment spectra of 2-tridecanone extracted from the leaves of *Lycopersicon hirsutum f. glabratum* accessions (PI-251304, PI-126449, PI-134417, PI-134418, and LA-407) indicating a molecular ion of m/z 198.

determined using regression analysis. Means were analyzed and separated using Duncan's LSD test (SAS Institute, 2001). The retention time and mass of 2-tridecanone isolated from *glabratum* leaf samples (PI-251304, PI-126449, PI-134417, PI-134418, and LA-407) matched those from Sigma standard. Recovery of 2-tridecanone was determined for each plant tissue. Twenty g samples of each untreated vegetable leaves were fortified with a known amount (5-10 $\mu g\ g^{-1}$) of analytical grade 2-tridecanone prepared in n-hexane. The fortified samples were then blended, extracted, and analyzed using the same procedure described above. Recoveries varied somewhat among crop species (93 ± 3.6 to 97 ± 1.8%). Residue data of all plant tissues were corrected according to their corresponding recoveries. Minimum detectable levels averaged 0.02 to 0.005 $\mu g\ g^{-1}$ leaves. Results indicated that 2-tridecanone spectral data, which showed a molecular ion peak (M^+) at m/z 198, along with other characteristic fragment ion peaks (Figure 2) are consistent with the assignment of the molecular formula of tridecanone ($C_{13}H_{26}O$). 2-tridecanone residue data were used to calculate half-lives on each type of leaf surface. Half-lives were calculated by regression lines using the methods described by Antonious (1982) and Anderson (1986). Residues and half-lives were statistically compared using analysis of variance (ANOVA) procedure (SAS, 2001).

RESULTS AND DISCUSSION

Surfactants are commonly used in agrochemical formulations to improve the performance of the active ingredient. Mechanisms of surfactants include stabilization of emulsions and/or suspensions, increased retention of the active ingredient on plant surfaces, and increased wetting of plant surfaces and subsequent penetration of the active ingredient into plant tissues (Shafer and Bukovac 1989). Surfactants also reduce droplet surface tension, increasing the contact area between the spray droplet and the leaf surface. These physical properties of surfactants increase droplet contact with the plant foliage and facilitate droplet penetration through the epicuticular wax layer. This is because plant cuticles are lipid membranes. They have high sorption capacity for lipophilic solutes like 2-tridecanone. Surfactants such as triton ×-100 (an inert nonionic surfactant) are amphipathic molecules (Shafer and Bukovac 1987). Many organic solvents which are insoluble in water but soluble in organic solvents may be solubilized in aqueous solution to a certain extent by the presence of surfactants. This characteristic is the primary basis for surfactants widespread use in agrochemical formulations. Dissipation curves for 2-tridecanone on the leaves of the seven greenhouse grown vegetables are presented in Figure 3. The initial deposits are highest on pepper leaves and lowest on broccoli leaves. These differences are due to the different physical and chemical properties of each plant surface that affect pesticide deposition and persistence on plants. The initial deposits, dissipation constants, and half-lives ($T_{1/2}$) of 2-tridecanone on the treated leaves are presented in Table 1. $T_{1/2}$-values on the seven types of leaves tested in this study are generally extremely low.

Decline of 2-tridecanone residues (Figure 3) on the leaves as a function of time indicated that $T_{1/2}$ values of 2-tridecanone were 1.3 hrs on squash leaves and 4.0 hrs on broccoli leaves (Table 1). The maximum residue limits (MRLs) of 2-tridecanone on vegetables is not known. The short persistence of 2-tridecanone on the leaves of the greenhouse vegetables tested in this study can be recognized as a desirable chemical characteristic. Ideally, safe pesticides remain in the target area long enough to control the specific pest then degrade into harmless compounds. This low persistence provides the safety needed for environmental quality, consumer safety, and for greenhouse and farm worker protection. The minimum intervals for worker reentry into treated areas are based upon pesticide deposits following spraying, repeated exposure, and residues remaining on the treated foliage and fruits (Antonious 1995). Foliage may accept and retain much greater pesticide deposits longer than fruits. This suggests that the plant foliage is important when considering worker reentry into pesticide-treated vegetables since they may be exposed to much greater surface areas of foliage than of fruits (Antonious 2002; Antonious 2004b). No phytotoxicity was observed on the leaves following spraying with 2-tridecanone formulation at 0.44 g L^{-1} of water. Many leaf surfaces represent the most unwettable of known surfaces. This is due to the hydrophobic nature of the leaf surface which is actually covered with crystalline wax of straight chain paraffin alcohols (Tadros 1987). An important factor which can affect the biological efficacy of foliar spray application of pesticides is the extent to which the liquid wets and covers the foliage surface. The optimum degree of coverage in any spray application depends on the

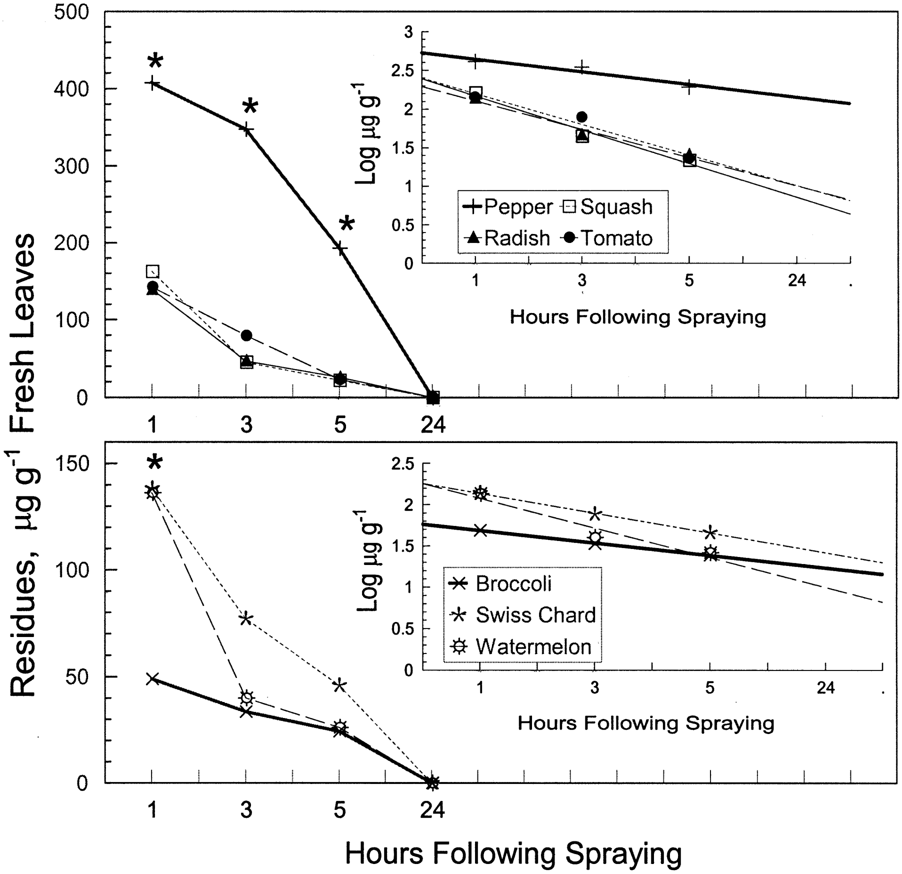


Figure 3. Dissipation of 2-tridecanone residues on pepper, squash, radish, and commercial tomato (upper graph) and broccoli, Swiss chard, and watermelon leaves (lower graph) following spraying of 2-tridecanone formulation under greenhouse conditions. Values accompanied by asterisks indicate a significant difference ($P < 0.05$; Duncan's multiple range test [SAS Institute 2001]) between residues detected on the leaves at a given time.

mode of action of the pesticide and the nature of the pest to be controlled. With non-systemic pesticides, the cover required depends on the mobility or location of the pest. The more static the pest, the greater is the need for complete coverage on the susceptible areas of the plant liable to attack. Under those conditions good spreading of the liquid spray with maximum coverage is required. The persistence of 2-tridecanone on vegetables and fruits can be tailor made by using appropriate surface active agents (Stevens 1993; Liu and Stansly 2000) and/or photo stabilizers for use under field conditions as described for the insecticide azadirachtin (Johnson et al. 2003). Most formulations of pesticides contain surfactants as emulsifiers and wetting agents to provide greater coverage and retention. In conjunction with other adjuvants they may function as spreaders, stickers, antifoamers, compatibility agents or as activators (Riederer and Schonherr 1990). Surfactants also play an important role in

Table 1. Average initial deposits, dissipation constant, and half-life ($T_{1/2}$) values of 2-tridecanone following spraying on the leaves of seven vegetables grown under greenhouse conditions.

Greenhouse Plants	2-Tridecanone		
	Initial Deposits ($\mu g\ g^{-1}$ Leaves)	Dissipation Constant (K)	Half-life ($T_{1/2}$) in hours
Squash	162.43	0.5204	1.33 c
Pepper	407.45	0.1871	3.70 a
Tomato	142.54	0.4567	1.52 c
Radish	139.16	0.4222	1.64 c
Broccoli	48.92	0.1744	3.97 a
Swiss Chard	138.27	0.2753	2.52 b
Watermelon	136.28	0.4133	1.68 c

Each value in the table is an average of three replicates. $T_{1/2}$ values in a column having different letter are significantly different from each other ($P > 0.05$; Duncan's multiple range test, SAS Institute, 2001).

the penetration and transport processes at the different barriers of the plant (the epicuticular wax layer, the cuticle, and the cell membranes). On the leaf surface, surfactants can modify the physical form of the pesticide deposits, increase the contact surface on the leaves, and the physical properties of the spray deposit such as sticking properties and persistence (Steurbaut et al. 1989). Therefore, the coverage of the leaf surface and the physical form of the initial deposit are important for good contact between the pesticide and the plant foliage. Liquid and jelly-like residues stick better to the leaf surface than solid or crystalline ones. In plant protection systems, the search for new efficient and safe compounds is very desirable. The medium chain length methylketones, in particular 2-tridecanone, have been shown to be potent agents against a variety of insects and spider mites. With more research on the persistence, environmental toxicology, and use of 2-tridecanone on vegetables and fruits, this compound could be a potential substitute for many synthetic pesticides used in plant protection across the country.

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