Bull. Environ. Contam. Toxicol. (2003) 71:400–407

DOI: 10.1007/s00128-003-0178-y

Bulletin of Environmental Contamination and Toxicology

Insecticidal and Acaricidal Performance of Methyl Ketones in Wild Tomato Leaves

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Received: 5 March 2003/Accepted: 18 April 2003

Health hazards created by synthetic pesticides have become a great public concern. Basic and applied research to reduce pest resistance and provide new and effective treatments that do not rely upon synthetic pesticides is needed. Many studies have indicated the potential ecological damage due to the widespread use of synthetic pesticides (Sances et al. 1992; Antonious and Snyder 1994; Antonious et al. 1998; Strang 1998). The US Food Quality Protection Act (FQPA) in 1996 initiated a systematic effort to identify and reduce potential risks posed by synthetic pesticides to safeguard public health. Among the provisions of the FQPA is a requirement for the EPA to reassess all synthetic pesticide tolerances (9,700+) within ten years of passage of the act. Among those that are significant to varying degrees to Kentucky growers are azinphos-methyl (Guthion), chlopyrifos (Lorsban), phosmet (Imidan), diazinon and malathion (Cythion) (Strang 1998; Anonymous 2002). Alternatives to synthetic insecticides are urgently needed to control vegetable insects.

The use of natural plant products for insect control (Xia and Johnson 1997; Pillmoor 1998; Rice et al. 1998; Antonious et al. 2001; Antonious 2001a; Antonious et al. 2003) may impart a selective advantage to plants by inhibiting, repulsing, and even killing non-adapted organisms that feed upon, or compete with, the plant. Production of toxic chemical compounds against insects is one method by which tomato trichomes (leaf-hairs) can impart resistance against insects. Recent review on the wild tomato, *L. hirsutum f. glabratum*, has demonstrated that glandular trichomes and the exudates they produce contribute to insect resistance (Eigenbrode et al. 1994, 1998; Xia and Johnson 1997; Guo 1992). Developing commercial insect resistant tomato varieties of *L. hirsutum* by genetic improvements has not been successful (Hartmann and StClair 1998). Such findings on resistance of *L. hirsutum* plants to herbivorous organisms make glandular secreting trichomes of wild tomato an attractive system for study against vegetable insects that have become resistant to all major classes of modern synthetic insecticides. The potential of using allelochemicals from the leaves of wild tomato accessions for controlling mites and herbivorous insects of vegetables is explored in this study. This investigation was designed to 1) assess the toxicity of four methyl ketones (2-undecanone, 2-dodecanone, 2-tridecanone, and 2-pentadecanone) against the two-spotted spider mite (*Tetranychus*

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urticae) and the green peach aphid (*Myzus persicae*) which represent two important worldwide vegetable pests, the tobacco hornworm (*Manduca sexta*) and tobacco budworm (*Heliothis virescens*); and 2) to screen and test the performance of ethanol and hexane extracts prepared from the leaves of eleven wild tomato accessions against the tobacco budworm and hornworm.

MATERIALS AND METHODS

Standard materials of 2-undecanone (Aldrich, Milwaukee, WI); 2-dodecanone (Pfoltz and Bauer Inc.,Waterbury, CT); 2-tridecanone (Aldrich, Milwaukee, WI) and 2-pentadecanone (Fluka Chemical Corp., Milwaukee, WI) each of 99% purity were dissolved in acetone. Dilutions were prepared for bioassays using filter paper test. Laboratory strains of tobacco hornworm (*Manduca sexta* Johannson) and tobacco budworm (*Heliothis virescens*) were obtained from a continuous colony maintained at the Department of Entomology, University of Kentucky (Lexington, KY). Tobacco hornworms were reared on artificial diet as described by Yamamoto (1969) and tobacco budworms were reared on artificial diet as described by Vanderzant et al. (1962). Laboratory strains of the two spotted spider mite (*Tetranychus urticae*) and the green peach aphid (*Myzus persicae*) were reared on bean leaves and mustard leaves, respectively. Ten replicates of ten neonate larvae (budworms or hornworms) or adults (aphid or spider mite) were used for each dose of each of the four pure methyl ketone tested. The test procedure was followed as described by Dimock et al. (1982). Acetone dilutions of methyl ketones were used to determine the minimum dosage needed to kill 50% of the test organisms. Concentrations were expressed as $\mu M.cm^{-2}$ of filter paper. One hundred µL of diluted solutions of each compound were placed onto a 4.2 cm Whatman No.1 filter paper, and the solvent was allowed to evaporate. Control filter papers were treated with 100 µL of solvent only. The dry filter paper was placed in a 4.5 cm diam Petri dish and moistened with 100 µL of distilled water. Ten newly hatched neonate larvae of the tobacco hornworm or tobacco budworm, ten adult females of the green peach aphid, and ten adult females of the two-spotted spider mite were each placed onto the treated filter paper and a tight fitting lid was placed onto the dish. The plates were then placed into a 27°C incubator in the dark for 6 h (Dimock et al. 1982; Kennedy 1984). Number of organisms alive at 6 hrs was recorded. Organisms were considered alive if they showed any movement after probing with a small soft brush. All organisms were then placed into another Petri dish containing prepared diet for hornworm and budworm or 2 cm diam disks of bean leaves for spider mite test or mustard leaves for aphid test on a moistened filter paper. The dishes were again placed into the incubator for an additional 18 hrs to allow for further death or recovery to occur.

Seeds of 11 wild tomato accessions were obtained from the USDA/ARS, Plant Genetic Resources Unit, Cornell University Geneva, NY, USA. Seeds of *L. esculentum cv.* Fabulous (included as control) were obtained from Holmes Seed Co. (Canton, OH). Wild tomato plants studied included five accessions of *Lycopersicon hirsutum f. glabratum* C.H. Mull: PI-126449, PI-134417, PI-134418, PI-251304, and

LA-407; three accessions of *L. hirsutum f. typicum* Humb & Bonpl.: PI-127826, PI-127827, and PI-308182; two accessions of *Lycopersicon pennellii* Corr. (D'Arcy): PI-246502, and PI-414773; and one accession of *Lycopersicon pimpinellifolium* (Jusl.) Mill: LA-1335. Seeds were all germinated in the laboratory on moistened filter paper in Petri dishes kept in the dark. At the six-leaf stage, plants from each accession were transported into the greenhouse and transplanted into 20 cm diam plastic pots containing Pro-Mix soil (Premier Horticultural Inc., Red Hill, PA) and grown under natural day lighting conditions with sodium lamps providing additional photosynthetic photon flux of 110 $\mu M.s^{-1}.m^{-1}$. Plants were irrigated daily and fertilized twice a month with water containing 200 ppm of general purpose fertilizer with the elements N, P, and K (20:20:20). Average greenhouse temperature and relative humidity were 30 ± 3.9°C and 49.5 ± 11.8% respectively. No insects were observed on the wild tomato foliage during the experimental period and no insecticides were applied. When the plants were 60 d old, leaves free of visible defects below the plant apex (designated as node number below the apical meristem) were sampled from the second, fourth, and sixth pairs of leaves. A regression line for each accession was established based on the relationship between leaflet surface area (cm^2) and leaflet weight (g). Leaflets surface area in cm^2 were measured using a Laser area-meter (CID Inc., Vancouver, WA).

Extracts of *L. esculentum* cv. Fabulous; *L. hirsutum f. glabratum*; *L. hirsutum f. typicum*, and *L. pennellii* leaflets were prepared by shaking 5 g of leaflets of each accession with ethanol for 10 min. Extracts from the same plants were also prepared in n-hexane. The solvent rinse was then decanted through a Whatman 934-AH glass microfibre filter of 55mm diam (Fisher Scientific, Pittsburgh, PA). The filtrate was evaporated under vacuum using a rotary vacuum evaporator (Buchi Rotovapor Model 461, Switzerland) at 35°C followed by a gentle stream of nitrogen gas (N_2). Insecticidal and acaricidal efficacy of the methyl ketones were expressed as per filter paper surface area. Insecticidal efficiency of the leaf extracts against tobacco budworm and hornworm were expressed as $\mu M.cm^{-2}$ filter paper surface area. LC_{50} values were calculated using PriProbit Analysis (Sakuma 1998) and considered significantly different when Fudicial limits did not overlap.

RESULTS AND DISCUSSION

Two methyl ketones, 2-tridecanone and 2-dodecanone, were more effective against the tobacco hornworm, *M. sexta* (LC_{50} of 0.015 and 0.028 $\mu M.cm^{-2}$, respectively) than 2-undecanone (LC_{50} of 0.096) and 2-pentadecanone. 2-pentadecanone at the highest concentration tested (5 $\mu Moles/cm^2$) killed only 27% of the tobacco budworms, 15% of the tobacco hornworms, 15% of the two-spotted spider mites, and none of the green peach aphids (data not shown). It was found that 2-tridecanone was the most effective methyl ketone against tobacco hornworm and budworm (LC_{50} of 0.015 $\mu M.cm^{-2}$ of filter paper surface area) (Table 1).

Based on LC_{50} values, the order of decreasing toxicity to neonate larvae of both Lepidoptera species, *Heliothis virescens* and *Manduca sexta* was 2-tridecanone, 2-

dodecanone, 2-undecanone and 2-pentadecanone. 2-Dodecanone and 2-tridecanone were about equal in toxicity against aphid adults, *Myzus persicae*, and required a significantly lower dose than 2-undecanone. Spider mite (*Tetranychus urticae*) was more sensitive to 2-undecanone and 2-dodecanone than 2-tridecanone and was also insensitive to 2-pentadecanone (15 % mortality at 5 $\mu M.cm^{-2}$).

Hexane and ethanol crude extracts prepared from the leaves of the 11 wild tomato accessions were tested against the tobacco hornworm and budworm using 6 h no-choice filter paper bioassay. When filter paper was treated with 100 µL of the leaf crude extracts results indicated that the ethanol extracts of *Lycopersicon hirsutum f. glabratum* accessions PI-134417, PI-134418, and PI-126449 were the most effective against the tobacco hornworm and tobacco budworm (Figure 1).

Table 1. LC_{50} values of three methyl ketones determined by a no-choice filter paper bioassay using four herbivors.

Compound	Test Organism	LC_{50} ($\mu Mole/cm^2$)	95% confidence interval
2-undecanone	*Heliothis virescens*	0.089 b	0.083, 0.095
	Manduca sexta	0.096 b	0.077, 0.21
	Myzus persicae	0.30 a	0.24, 0.35
	Tetranychus urticae	0.21 a b	0.11, 0.25
2-dodecanone	*Heliothis virescens*	0.031 c	0.026, 0.036
	Manduca sexta	0.028 c	0.024, 0.032
	Myzus persicae	0.059 b	0.053, 0.065
	Tetranychus urticae	0.15 a	0.00026, 0.24
2-tridecanone	*Heliothis virescens*	0.015 b	0.013, 0.017
	Manduca sexta	0.015 b	0.013, 0.017
	Myzus persicae	0.067 a b	$7.9272e^{006}$,$7.7818e^{-002}$
	Tetranychus urticae	2.03 a	1.69, 33.13

Each LC_{50} value in the table is an average of ten replicates. Statistical comparisons were done between test organisms for each compound. LC_{50} values for each compound accompanied by the same letter (s) are not significantly different from each other ($P< 0.05$; PriProbit, 1998).

We investigated differences in chemical composition of the leaves that may explain the observed differences in mortality between accessions. Major components of crude leaf extracts were identified and quantified using a gas chromatograph equipped with mass selective detector (GC/MSD, Hewlett-Packard model 5890) and a HP 7673

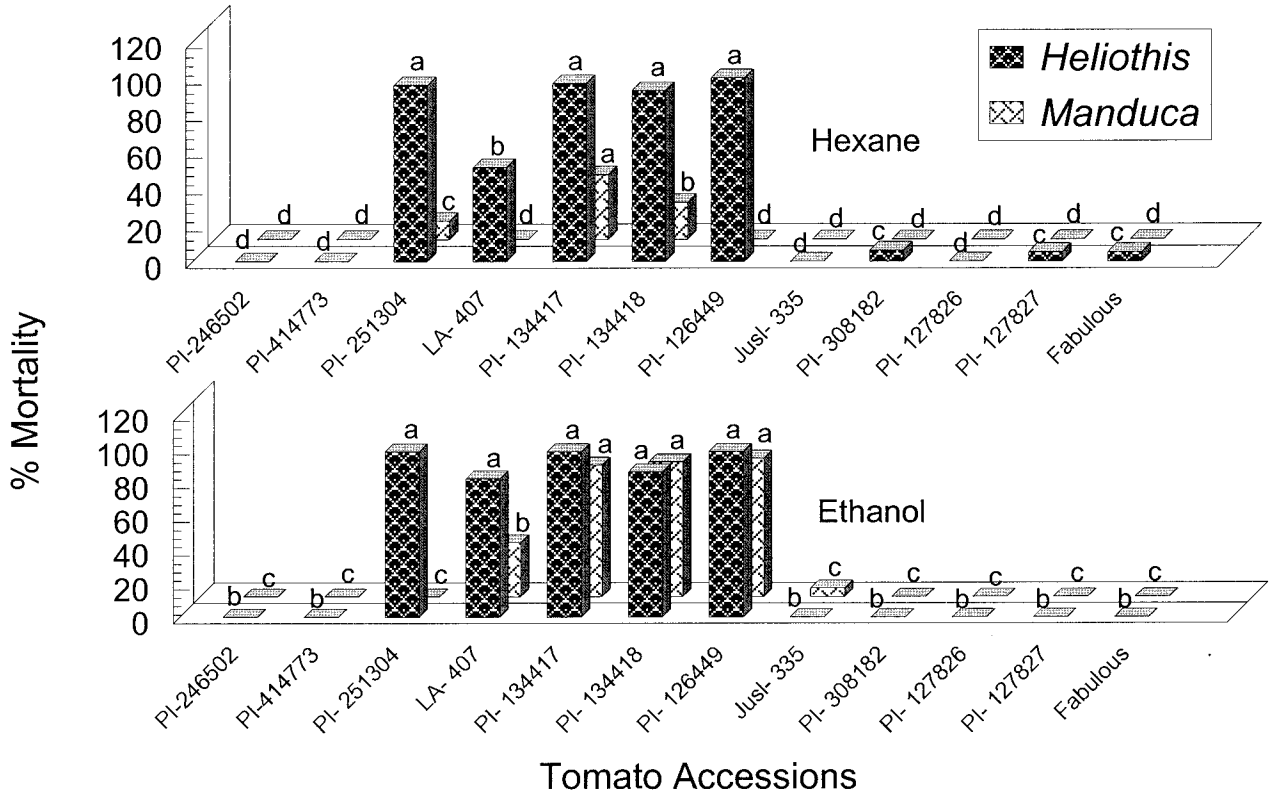

Figure 1. Mortality of *M. sexta* and *H. virescens* (average of ten replicates of ten neonate larvae) exposed to hexane (upper graph) and ethanol (lower graph) leaf extracts from eleven wild tomato accessions and one commercial cultivar (*L. esculentum* cv. Fabulous). Bars accompanied by different letter(s) for each insect indicated significant references ($P > 0.05$).

automatic injector model 5890. Concentrations of methyl ketones in the leaf extracts prepared using ethanol or hexane varied between accessions (Table 2). Concentration of 2-dodecanone was low compared to the other three methyl ketones. The hexane extract of accession PI-134417 contained substantially larger quantities of 2-undecanone and 2-tridecanone. The ethanol extracts of accessions PI-134417, PI-134418, and PI-126449 were more effective against the two lepidopteran insects compared to the hexane extracts prepared from the same accessions.

It could be concluded from the leaf extract tests that, in most cases the amount of 2-tridecanone present in the ethanol leaf extract was sufficient to explain the observed mortality of the two lepidopteran species tested. Some unresolved issues remain. It is unclear why *M. sexta* did not respond significantly to the hexane extracts which contained relatively sufficient amounts of the three methyl ketones (2-undecanone, 2-tridecanone, and 2-dodecanone). This probably suggests that other unidentified components in the ethanol crude extract are playing a role in *M. sexta* mortality, either directly as toxic materials or in some synergistic fashion. It suggests that some still to be identified compound in the leaf extracts that contributed to the toxicity effect.

Table 2. Concentrations of methyl ketones[†] in hexane and ethanol extracts[‡] prepared from the leaves of three *L. hirsutum f. glabratum* accessions grown under greenhouse conditions.

Accession	Undecanone (µg/g Leaves)	Dodecanone (µg/g Leaves)	Tridecanone (mg/g Leaves)	Pentadecanone (µg/g Leaves)
	Hexane Extract			
PI-134417	1049.1 a	11.6 a	3628.9 a	78.3 a
PI-134418	257.0 c	3.8 b	1018.5 c	22.5 c
PI-126449	981.1 b	9.6 a	2402.7 b	61.0 b
	Ethanol Extract			
PI-134417	446.6 ab	5.3 a	2034.7 a	117.5 a
PI-134418	350.9 b	6.1 a	1487.8 c	113.7 a
PI-126449	471.2 a	6.1 a	1873.1 b	124.3 a

[†] Each value in the table is an average concentration obtained from analysis of 9 wild tomato leaflets. [‡] Calculated using the methods described by Antonious, 2001b. Values within a column for each extract having different letter(s) are significantly different ($P< 0.05$) from each other, using Duncan's LSD test (SAS Institute, 1999).

Performance of methyl ketones, a potentially insecticidal and acaricidal natural product of Solanaceae can be explored for developing natural product for use as biodegradable alternative to synthetic pesticides. A formulation prepared from the

leaves of selected accessions may therefore create a mixture with the desired level of constituents. However, if methyl ketones and other constituents in wild tomato leaves are to be used within the organic production systems to control vegetable insects, further work is needed to investigate their performance under ultra-violet light and field conditions.

Acknowledgments. This investigation was supported by a grant from the USDA/CSREES to Kentucky State University under agreement No. KYX-10-99-31P.

REFERENCES

Anonymous (2002) Commercial Vegetable Crop Recommendations. Cooperative Extension Service, University of Kentucky, College of Agriculture, ID-36.

Antonious GF, Snyder JC (1994) Residues and half-lives of acephate, methamidophos, and pirimiphos-methyl in leaves and fruit of greenhouse-grown tomatoes. Bull Environ Contam Toxicol 52:141-148.

Antonious GF, Byers ME, Snyder JC (1998) Residues and fate of endosulfan on field-grown pepper and tomato. Pesticide Sci 54: 61-67.

Antonious GF, Snyder JC, Patel G (2001) Pyrethrins and piperonyl butoxide residues on potato and in soil under field conditions. J Environ Sci Health B36: 261-271.

Antonious GF (2001a) Insecticides from wild tomato: phase I- breeding, trichome counts, and selection of tomato accessions. J Kentucky Academy of Sci 62: 79, Published Abstract.

Antonious GF (2001b) Production and quantification of methyl ketones in wild tomato accessions. J Environ Sci Health B36: 835-848.

Antonious GF, Hawkins LM, Kochhar TS (2003) Foliar phenolic variation in wild tomato accessions. Bull Environ Contam Toxicol 70: 9-16.

Dimock M, Kennedy G, Williams W (1982) Toxicity studies of analogs of 2-tridecanone, a naturally occurring toxicant from a wild tomato. J Chem Ecol 8: 837-842.

Eigenbrode SD, Trumble JT, Millar JG, White K (1994) Topical toxicity of tomato sesquiterpenes to the beet armyworm and the role of these compounds in resistance derived from an accession of *Lycoperiscon hirsutum f. typicum*. J Agric Food Chem 42: 807-810.

Eigenbrode SD, Trumble JT, Millar JG, White K (1998). Topical toxicity of tomato sesquiterpenes to the beet armyworm and the role of these compounds in resistance derived from an accession of *Lycoperiscon hirsutum f. typicum*. J Agric Food Chem 46: 2062.

Guo Z (1992) Chemical deterrents to two-spotted spider mite, *Tetranychus urticae* Koch, associated with glandular trichomes of wild tomato, *Lycopersicon hirsutum* Humb. & Bonpl. MS.C Thesis, University of Kentucky, Department of Horticulture, Lexington, KY.

Hartman JB, StClair DA (1998) Variation for insect resistance and horticultural traits in tomato inbred backcross populations derived from *Lycopersicon pennellii*. Crop Sci 38: 1501-1508.

Kennedy G (1984) 2-tridecanone, tomatoes, and *Heliothis zea*: potential incompatibility of plant antibiosis with insecticidal control. Entomol Exp Appl 35: 305-311.

Pillmoor JB (1998) Carbocyclic coformycin: a case study of the opportunities and pitfalls in the industrial search for new agrochemicals from nature. Pest Sci 52: 75-80.
Rice MJ, Legg M, Powell KA (1998) Natural products in Agriculture- a view from the industry. Pest Sci 52: 184-188.
Sakuma M (1998) PriProbit and PriProbit NM. Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan.
Sances FV, Toscano NS, Gaston KL (1992) Minimization of pesticide residues on head lettuce. J Econ Entomol 85: 202-207.
SAS Institute (1999) SAS/STAT Guide, Release 0.03 Edition, SASA Inc., SAS Campus Drive, Cary, NC 27513, USA.
Strang J (1998) Kentucky Fruit Facts. Cooperative Extension Service, U.S. Department of Agriculture, University of Kentucky, College of Agriculture, Lexington, KY 40546.
Vanderzant ES, Richardson CD, Fort SW (1962) Rearing the bollworm on artificial diet. J Econ Entomol 55: 140.
Xia Y, Johnson AW (1997) Effects of leaf surface moisture and relative humidity on the efficacy of sugar esters from *Nicotiana gossei* against the tobacco aphid. J Econ Entomol 90: 1010-1014.
Yamamoto RT (1969) Mass rearing of the tobacco hornworm. J Econ Entomol 62: 1427-1431.