2-Tridecanone: A Naturally Occurring Insecticide from the Wild Tomato *Lycopersicon hirsutum* f. *glabratum*

Abstract. *A nonalkaloid insecticide was isolated from the wild tomato* Lycopersicon hirsutum *f.* glabratum *and identified as 2-tridecanone, a compound 72 times more abundant in the wild tomato than in the cultivated tomato* L. esculentum. *Lepidopterous larvae (*Manduca sexta *and* Heliothis zea*) and aphids (*Aphis gossypii*) died when confined on 2-tridecanone-treated filter paper.*

Wild varieties of cultivated crops or closely related species often contain potent chemical defenses. If these chemical defenses are to be exploited in the management of insect pests, it is important to know their chemical structures. Insects coming in contact with or feeding on the leaves of the wild tomato *Lycopersicon hirsutum* f. *glabratum* C. H. Mull soon die (*1–3*). We have isolated a toxin in this foliage and identified it as 2-tridecanone, a chemical that is also present in minute amounts in foliage of the cultivated tomato, *L. esculentum* Mill and in the defensive secretions of a termite and a caterpillar (*4, 5*). This report represents what appears to be the first positive identification of a nonalkaloid chemical defense against insects in plants of the genus *Lycopersicon* (*6*).

The foliage of the wild tomato *L. hirsutum* f. *glabratum* is covered with a dense vesture of glandular trichomes that physically entrap in their secretions tiny anthropods (*2*), but not larger insects such as *Manduca sexta* L. and *Heliothis zea* (Boddie). However, in at least one accession of this plant species (*7*), the trichome exudate is toxic to *M. sexta* and *H. zea* (*8*). When young *M. sexta* larvae are confined to resistant foliage, they die, often without feeding (*3*).

To isolate the toxin, resistant foliage was extracted with chloroform; the extract was evaporated to dryness in vacuo, and ethanol was added to make a 10 percent (weight to volume) solution. The ethanol-soluble fraction was decanted and dried, and the residue was dissolved in a minimum volume of chloroform. Heptane was added to make a chloroform and heptane (1:3) solution, which was chromatographed on a silicic acid column to remove various polar compounds and pigments (*9*). A second silicic acid column was used to fractionate the eluent from the first column (*10*). The active fractions, those that are acutely toxic to *M. sexta* larvae (*11*), were combined and fractionated by means of high-performance liquid chromatography (HPLC). A Whatman PAC column (9.4 by 250 mm) was eluted with a continuous gradient from 100 percent heptane to 100 percent methylene chloride, changing at 5 percent per minute. A single active peak eluted at 20.4 minutes and produced only one peak upon gas chromatographic (GC) analysis. This combination of HPLC and GC was strong evidence for the presence of a pure compound in the active HPLC peak.

Mass spectroscopy of the active fraction gave a molecular ion of 198. Infrared (IR) spectroscopy showed one ketonic function at 1705 cm^{-1}; ^{1}H and ^{13}C nuclear magnetic resonance (NMR) indicated a long-chain methyl ketone. The mass spectrum fragmentation pattern was also characteristic of a long-chain methyl ketone. The active compound was tentatively identified as 2-tridecanone (*12*). A commercially prepared sample (*13*) of 2-tridecanone yielded IR, ^{1}H NMR, and mass spectra that were identical to those of the isolated sample. Also, theoretical calculations of ^{13}C NMR shifts for 2-tridecanone matched the observed shifts. The commercial sample was also highly active (LD_{50}, 17.5 $\mu g/cm^2$ of treated surface compared to 44.2 $\mu g/cm^2$ for crude chloroform extract) when bioassayed for toxicity to *M. sexta* (*14*). Biological assay of 2-tridecanone against *H. zea* and the aphid *Aphis gossypii* Glover also indicated toxicity (*15*).

Crude chloroform extracts from the foliage of insect-resistant *L. hirsutum* f. *glabratum* and -susceptible *L. esculentum* plants (*16*) were analyzed for 2-tridecanone content by GC (*17*). 2-Tridecanone was about 72 times more abundant in the foliage of *L. hirsutum* f. *glabratum* than of *L. esculentum*, with an average (micrograms of 2-tridecanone per milligram of foliage fresh weight) of 9.40 (± 1.76) and 0.13 (± 0.019), respectively (*18*).

An analysis of trichome exudate collected (*19*) from the abaxial surface of *L. hirsutum* f. *glabratum* leaflets revealed an average 2-tridecanone content of 49.3 μg per abaxial leaflet surface equivalent of trichome exudate. This is just a fraction of the 672 μg of 2-tridecanone per leaflet observed when crude chloroform extracts from comparable leaflets were analyzed (*20*). Since the average area of a leaflet used in our bioassays and extractions was 2.6 cm^2, the amount of 2-tridecanone per leaflet was adequate to explain the resistance of *L. hirsutum* f. *glabratum* to *M. sexta*. This, however, does not preclude the involvement of other factors in the resistance to *M. sexta* and to other arthropod species.

The natural occurrence of insecticidally active quantities of 2-tridecanone in a wild tomato offers potential for its exploitation in the management of insect pests attacking the cultivated tomato. Since *L. hirsutum* f. *glabratum* and *L. esculentum* readily cross, it may be possible to breed commercially acceptable tomato cultivars having insecticidally active quantities of 2-tridecanone in their foliage, provided that acceptable fruit quality can be maintained (*21*). The availability of a relatively simple quantitative chemical analysis for 2-tridecanone should enhance the potential for success in such a breeding program. However, the effects of 2-tridecanone-based resistance on other insect pests of tomato and their natural enemies (*22*), as well as on humans, should be assessed prior to the widespread use of any tomato cultivars possessing high levels of 2-tridecanone in their foliage. The possibility also exists that 2-tridecanone or a structural analog may prove useful in managing insect pests on tomato and other crops when applied as a foliar spray to susceptible plants.

W. G. Williams
Department of Chemistry, North Carolina State University, Raleigh 27650
George G. Kennedy
Robert T. Yamamoto
Department of Entomology, North Carolina State University
J. D. Thacker
Jon Bordner
Department of Chemistry, North Carolina State University

References and Notes

1. R. L. Fery and F. P. Cuthbert, Jr., *J. Am. Soc. Hortic. Sci.* **100**, 276 (1975); A. G. Gentile and A. K. Stoner, *J. Econ. Entomol.* **61**, 1347 (1968); D. J. Schuster, *ibid.* **70**, 434 (1977); J. M. Schalk and A. K. Stoner, *J. Am. Soc. Hortic. Sci.* **101**, 74 (1976); R. E. Webb, A. K. Stoner, A. G. Gentile, *ibid.* **96**, 65 (1971); A. K. Stoner, *U.S. Dep. Agric. Prod. Res. Rep.* **102**, 9 (1968); D. J. Schuster, V. H. Waddill, J. J. Augustine, R. B. Volin, *J. Am. Soc. Hortic. Sci.* **104**, 170 (1979).
2. A. G. Gentile, R. E. Webb, A. K. Stoner, *J. Econ. Entomol.* **62**, 834 (1969).
3. G. G. Kennedy and W. R. Henderson, *J. Am. Soc. Hortic. Sci.* **103**, 334 (1978).
4. R. K. Soost, R. W. Scora, J. J. Sims, *Proc. Am. Soc. Hortic. Sci.* **92**, 568 (1968).
5. G. D. Presitvich, M. Kaib, W. F. Wood, J. Meinwald, *Tetrahedron Lett.* **52**, 4701 (1975); A. Quennedey, G. Brule, J. Rigaud, P. Dubois, P. Brossut, *Insect Biochem.* **3**, 67 (1973); T. Eisner, A. F. Kluge, J. C. Carrel, J. Meinwald, *Ann. Entomol. Soc. Am.* **65**, 765 (1972).
6. The steroidal alkaloid α-tomatine has been implicated in the antiherbivore defense of *Lycopersicon* spp. J. G. Roddick, *Phytochemistry* **13**, 9 (1974); D. A. Levine, *Annu. Rev. Ecol. Syst.* **7**, 121 (1976); S. L. Sinden, J. M. Schalk, A. K. Stoner, *J. Am. Soc. Hortic. Sci.* **103**, 596 (1978).
7. Plant 134417 was obtained from U.S. Department of Agriculture Plant Introduction Station, Ames, Iowa. Plants of this accession originally

0036-8075/80/0222-0888$00.50/0

obtained from this source varied in resistance to *M. sexta*. Resistant plant material used in our study consisted of a clone, propagated by cuttings, obtained from a single plant of *L. hirsutum* f. *glabratum* (plant 134417) selected as highly resistant to *M. sexta*. Potted plants were grown in a greenhouse under natural light between April and October and were fertilized as needed to maintain vigorous growth.

8. One hundred percent mortality of both species occurred when first instar larvae were confined for 2 hours on filter paper treated with crude trichome exudate (210 $\mu g/cm^2$) in a covered petri dish. As controls larvae were confined on filter paper treated with 1 ml of chloroform. No mortality was observed among controls.
9. Bio-Rad silicic acid (100 to 200 mesh) at a ratio of 100:1 (by weight) relative to the sample weight. The column (2.5 by 27 cm) was eluted with a step gradient from 100 percent heptane to 100 percent chloroform. Each collected fraction corresponded to a 77-ml addition of solvent. All fractions were combined for further purification.
10. The column (2 by 26 cm) was eluted with a step gradient from 100 percent heptane to 100 percent chloroform in 10 percent increments. Each fraction from the column corresponded to a 55-ml addition of solvent.
11. Only fractions 3 and 4, corresponding to 20 and 30 percent chloroform, showed activity. Bioassays were conducted by confining 20 newly hatched, first-instar *M. sexta* larvae on a filter paper disk (5.5 cm in diameter) treated with 5 mg of each fraction dissolved in 1 ml of chloroform. The chloroform was evaporated prior to addition of the larvae. Larval mortality was recorded after 6 hours. As controls, larvae were confined in an identical manner on filter paper previously treated with 1 ml of chloroform. No mortality was observed among controls.
12. 2-Tridecanone has been identified as one of many essential oils present in *L. hirsutum* f. *glabratum* (*4*), but no function was ascribed to it.
13. Pfaltz & Bauer, Inc., 375 Fairfield Avenue, Stamford, Conn.).
14. Bioassays were done as in (*11*), except that after 6 hours on treated filter paper larvae were transferred to *L. esculentum* foliage for 18 hours, after which mortality was recorded. The crude chlorofrom extract contained an average of 51 percent 2-tridecanone.
15. As bioassayed in (*11*), 63 μg per square centimeter of surface produced 100 percent mortality in both species.
16. The commercial *L. esculentum* cultivar 'Manapal.' Plants were grown in the Southeastern Plant Environment Laboratory at North Carolina State University, under 9 hours of photosynthetically active radiation from a combination of cool white fluorescent and incandescent lamps providing an illuminance of 451 hectolux at 95 cm from the lamps. Days were kept effectively long by interrupting the dark period from 11:00 p.m. to 2:00 a.m. with 12 W/m^2 of photomorphogenic radiation (41 hectolux) from incandescent lamps. Temperatures from day to night were 26° to 22°C, respectively. Extracts were prepared by steeping foliage in chloroform for 1 hour. The extract was then passed through Whatman phase separating filter paper (three chloroform rinses) onto Na_2SO_4 for drying and subsequently filtered to remove the Na_2SO_4 (Whatman No. 2).
17. Varian 3700 gas chromatograph with a flame ionization detector and a Varian CDS-111 processor.
18. Based upon eight foliage samples ($\bar{X}$, 360 mg fresh weight) *L. hirsutum* f. *glabratum* and ten samples ($\bar{X}$, 365 mg fresh weight) *L. esculentum*.
19. Trichome exudate was collected by brushing the abaxial surface of each of 100 *L. hirsutum* f. *glabratum* leaflets with a camel's hair brush and thoroughly rinsing the brush in chloroform. The resulting solution was analyzed for 2-tridecanone content by GC.
20. Based on analysis of extract from 100 leaflets collected at the same time as the trichome exudate and prepared as in (*16*).
21. Most of the insect pests that damage the fruit spend a portion of their life feeding on the foliage, for example, *H. zea*, *M. sexta*, and *Spodoptera exigua* (Hubner). *Lycopersicon hirsutum* appears in the pedigree of some newly released tomato cultivars [J. R. Baggett and W. A. Frazier, *HortScience* **13**, 598 (1978); *ibid.*, p. 599].
22. B. C. Campbell and S. S. Duffey, *Science* **205**, 700 (1979).
23. Paper number 6138 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh.

13 September 1979; revised 27 November 1979

Cellular Senescence in a Cloned Strain of Bovine Fetal Aortic Endothelial Cells

Abstract. *The life-span in vitro and other proliferative characteristics of a strain of endothelial cells cloned from the aorta of a fetal calf were examined. Cultures of these cells had a replicative life-span of approximately 80 cumulative population doublings. Growth rates in the logarithmic phase and plateau densities decreased as the cumulative population-doubling level increased. After approximately 65 percent of the life-span of a culture was completed, the percentage of cells that incorporated [^{3}H]thymidine during a 24-hour labeling period began to decrease rapidly. The cells expressed factor VIII antigen and their intercellular borders were stainable with silver nitrate throughout the life-span of each culture. Average cellular attachment size increased more than threefold between cumulative population-doubling levels 41 and 80. The facility with which cloned strains of endothelial cells can be isolated should encourage further exploitation of this important cell culture model.*

The vascular endothelium occupies a histologically unique and strategic location, is critical in normal physiological functioning, and figures importantly in such age-related diseases as atherosclerosis and neoplasia (*1*–*4*). Recent improvements in culture techniques have yielded endothelial cultures that retain many differentiated properties of the endothelium in vivo (*1*, *3*, *4*). The possibility that primary age-associated cellular changes take place in the endothelium in vivo makes the study of cellular senescence in cultured endothelial cells particularly relevant (*5*). Cellular senescence has been intensively investigated in human and chick fibroblast-like diploid cells that have a finite life-span in vitro

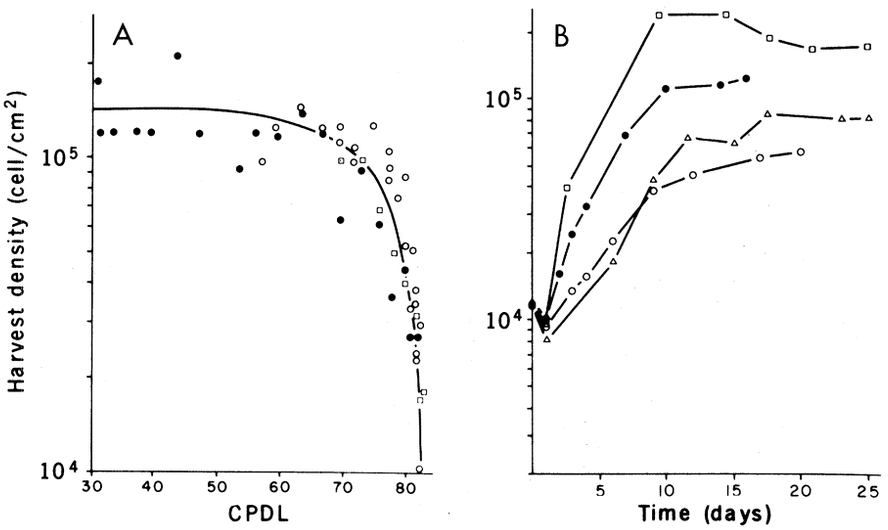

Fig. 1. (A) Cell density at subculture versus CPDL for endothelial cell clone BFA-1c. Data were obtained from (○) individual A (inoculation density, 2.5×10^4 cell/cm²); (●) individual B (1.0×10^4 cell/cm²); and (□) individual C (1.0×10^4 cell/cm²). The number of population doublings (PD's) that occurred in each subculture was calculated by using the formula: PD = $\log_2$ [cell density at subculture/(cell density at inoculation × attachment efficiency)], where attachment efficiency was the number of cells attached to the flask 24 hours after inoculation expressed as a fraction of the total number of cells inoculated (it varied from 0.70 to 0.95). The CPDL at any time, therefore, was the sum of all previously determined PD's. Weekly subculture cell density data could not be obtained until CPDL 30, because approximately 30 PD's occurred during the cloning procedure. Subcultivation was continued until inoculation cell density did not double after 2 weeks with weekly refeeding. The BFA-1c cells were frozen and stored in liquid nitrogen for various periods of time; when thawed, these cells proliferated similarly to cells that had not been frozen. All cultures remained free of detectable *Mycoplasma* infection, as demonstrated by agar plate cultivation, fluorescent staining for cytoplasmic DNA, and immunofluorescent staining for *M. hyorhinis* antigen (*28*). (B) Growth curves as a function of the CPDL for endothelial cell clone BFA-1c. Changes in proliferative rates and plateau densities associated with cellular senescence were monitored by comparing growth curves of cell density with time for cells taken from cultures at different CPDL's. Stock 75-cm² cultures at the desired CPDL were trypsinized and the cells were inoculated into numbered 25-cm² flasks at densities of 1.0×10^4 to 1.2×10^4 cell/cm² (8 ml of medium per flask). Flasks were incubated under standard conditions and given fresh culture medium every 6 or 7 days. At various times after inoculation, duplicate cultures were selected according to a random number table and counted with a Coulter counter to determine density. Replicate cell density determinations agreed to within 10 percent. The CPDL's studied were 55 (□), 59 (●), 70 (△), and 79 (○).

0036-8075/80/0222-0889$00.50/0