2,5-Dichlorophenol (from Ingested Herbicide?) in Defensive Secretion of Grasshopper

Abstract. *The defensive froth emitted by the grasshopper* Romalea microptera *contains several odorous compounds (phenols, terpenes, benzoquinone), including a chlorinated aromatic compound, 2,5-dichlorophenol. This compound, which is repellent to ants and therefore defensively useful to the grasshopper, probably stems from herbicide or herbicide derivative ingested by the insect with its diet. Although there is precedent for the defensive employ by one species of chemical agents produced by another, no instance was known involving secondary utilization of a pesticide dispensed by man.*

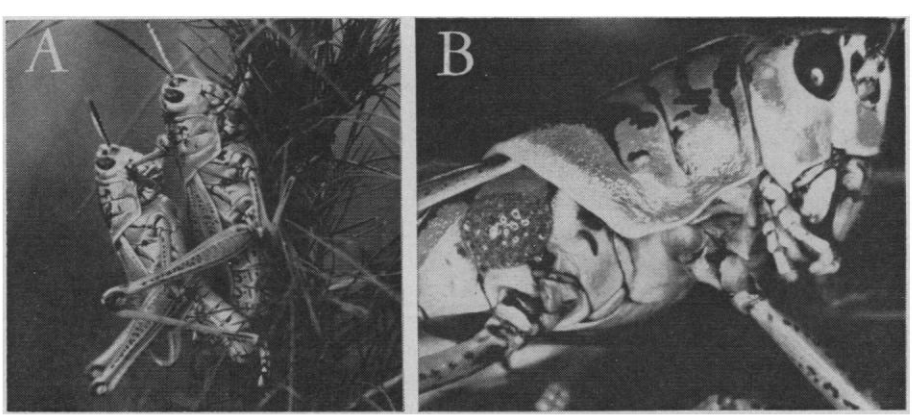
The large Southern flightless grasshopper *Romalea microptera* (Fig. 1A) responds to disturbance by emitting an odorous brownish froth from the anterior pair of respiratory openings (spiracles) on its thorax (Fig. 1B). The froth is generated by mixture of respiratory air with fluid produced by glandular tissue associated with the respiratory tubes (tracheae) that lead inward from the spiracles. Emission occurs with an audible hiss, and the fluid is repellent to predators, notably ants (*1*). The only compound previously isolated from the secretion is an odorless sesquiterpene (*2*) of no demonstrated repellent function. We have now identified several of the odorous constituents of the mixture, including phenols, terpenes, *p*-benzoquinone, and, most unexpectedly, 2,5-dichlorophenol.

Secretion was obtained from several hundred adult grasshoppers of both sexes by gently squeezing individuals between fingers and collecting the emitted froth in capillary tubes. Extraction with methylene chloride and analysis of the extract by gas chromatography and mass spectroscopy revealed the presence of nine major volatile components (Fig. 2) (*3*). Identification of each component was confirmed by comparison with an authentic sample. Concentrations were estimated by gas-chromatographic comparisons of the natural and authentic samples.

Quinones, phenols, and terpenes have been identified previously in the defensive secretion of many insects and other arthropods (*4, 5*), and the presence of such substances in the froth of *Romalea* comes as no surprise. Some of the compounds (I, II, VI, and IX in Fig. 2) have never been found as such in other species, but they are only minor variants of common repellents found elsewhere (*5*). The only truly anomalous constituent is 2,5-dichlorophenol. Chlorinated compounds are rare natural products, and among terrestrial organisms only certain fungi are known to produce them (*6*). They are, however, widely used by man as pesticides, and one compound, 2,4-D (2,4-dichlorophenoxyacetic acid), has been applied extensively as an herbicide in the Florida counties (Collier and Hendry) where the *Romalea* were taken (*7*). We suggest that the 2,5-dichlorophenol in the *Romalea* froth stems from herbicide or degraded herbicide ingested with plants (*8*). Froth from *Romalea* collected at a biological station (*9*), on wild acreage where no herbicide or other chemical control agents had been applied, lacked 2,5-dichlorophenol but contained the other identified components.

Although seemingly fortuitous in origin, the 2,5-dichlorophenol is not a mere superfluous additive to the froth. It has a strong phenolic odor, and in laboratory tests with ants (*Formica exsectoides*) it was an effective feeding deterrent. Droplets of aqueous glucose (0.1*M*, 5 μl) placed on the glass floor of a feeding arena with 12 to 15 ants were consumed by the group in 1.7 $\pm$ 0.2 minute. Addition of 2,5-dichlorophenol to the droplets at a concentration similar to that in the froth (14 ppm) caused a marked delay in the consumption time (5.2 $\pm$ 0.3 minute). A tenfold increase in concentration caused the droplets to be ignored altogether. The compound is thus capable of contributing significantly to the overall effectiveness of the froth, certainly as it is used against ants, which are important enemies of *Romalea*, and particularly of the ovipositing females, when they are relatively helpless with their abdomen stuck in the soil. Little can be said about the possible systemic effects of the 2,5-dichlorophenol to predators that might eat the grasshopper. The compound is relatively nontoxic to guinea pigs (*10*), but such insensitivity need not be the rule.

The appropriation by one species, for its own defensive purposes, of a chemical agent produced by another, is not without precedent and may even be commonplace among insects. For example, the swallowtail butterfly, *Pachlioptera aristolochiae*, contains aristolochic acid, which is apparently obtained by the caterpillar from plants of the family Aristolochiaceae on which



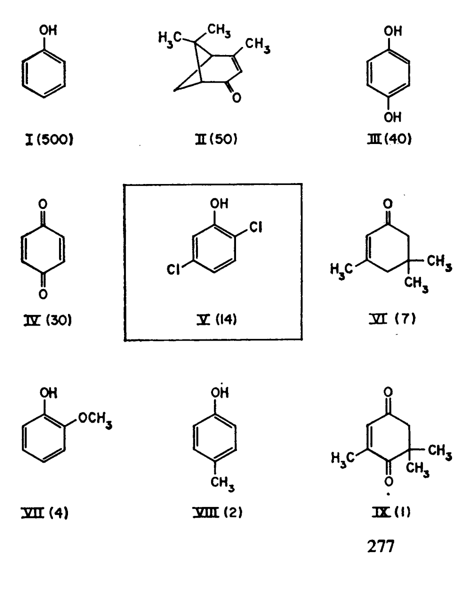


Fig. 1 (left). (A) *Romalea microptera*; mating pair. (B) Adult female discharging froth from anterior thoracic spiracle. Fig. 2 (right). Odorous compounds from *Romalea* froth, arranged in order of decreasing concentration (numbers in parentheses indicate parts per million in whole froth). I, Phenol; II, verbenone; III, hydroquinone; IV, quinone; V, 2,5-dichlorophenol; VI, isophorone; VII, *o*-methoxyphenol; VIII, *p*-cresol; and IX, 2,6,6-trimethylcyclohex-2-ene-1,4-dione.

it feeds and then is transferred through pupa to adult (*11*). Similarly, senecio alkaloids are incorporated by moths from food plants of the genus *Senecio* (*12*), and cardenolides are accumulated in grasshoppers and butterflies from milkweed plants (*13*). In *Romalea*, sequestration may account for more than just the 2,5-dichlorophenol in the froth. The other components all occur as such or in similar form in many species of plants (*14*), and the grasshopper may simply incorporate them with slight or no change from the diet. But 2,5-dichlorophenol would still be unique because of its apparent ultimate derivation from an exogenous source recently unleashed upon the ecosystem by man himself.

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References and Notes

1. Pieces of fresh integument (25 mm^2) of *Romalea*, treated with droplets (1 to 2 μl) of froth, were ignored by ants (*Paratrechina longicornis*) when placed in their foraging trail. Untreated controls were carried away. Tethered *Romalea* exposed on soil to foraging ants (*Pogonomyrmex badius*) discharged froth (and regurgitated crop fluid) when attacked, thereby repelling their assailants. After depletion of froth (and crop fluid) the grasshoppers were vulnerable.
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3. Over 30 additional components of the froth remain unidentified.
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5. J. Weatherston and J. E. Percy, in *Chemicals Controlling Insect Behavior*, M. Beroza, Ed. (Academic Press, New York, 1970), pp. 95–144.
6. J. Roche, M. Fontaine, J. Lepoup, in *Comparative Biochemistry*, M. Florkin and H. S. Mason, Eds. (Academic Press, New York, 1963), vol. 5, pp. 493–547. Alkaloids with chlorinated aliphatic side chains have been reported from some higher plants but are suspected of being artifacts rather than natural products [J. W. Hylin, R. E. Spenger, F. A. Gunther, *Residue Rev.* **26**, 127 (1969)].
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8. The rearrangement of chlorine from a 2,4 to a 2,5 substitution pattern presupposed by this assumption seems plausible in view of the demonstrated occurrence of such rearrangement in the degradation of 2,4-D by fungi and higher plants. [J. K. Faulkner and D. Woodcock, *Nature* **203**, 865 (1964); E. W. Thomas, B. C. Loughman, R. G. Powell, *ibid.* **204**, 765 (1964)].
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10. Lethal range: 0.5 to 2.8 g/kg (V. K. Rowe, Dow Chemical Co., personal communication).
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Aplysia californica: Analysis of Nuclear DNA in Individual Nuclei of Giant Neurons

Abstract. *The nuclei of the giant neurons of the marine mollusk* Aplysia californica *can contain more than 0.2 microgram of DNA. This is more than 200,000 times as much DNA as the haploid amount found in* Aplysia *sperm. On the basis of nuclear DNA content, the giant neurons R-2, P-1, and L-6 of adult animals can each be divided into at least two populations. The mean DNA content of these two populations (0.067 and 0.131 microgram of DNA) are approximately related by a factor of 2. This suggests that much and perhaps all of the genome replicates repeatedly (up to 16 times) during the growth and development of these neurons and that each replication is synchronous. The enormous amount of DNA in these cells opens up the possibility of characterizing the DNA and other constituents of chromatin from individual but phenotypically different neurons.*

The giant neurons of *Aplysia* represent one of the largest somatic cell types in the animal kingdom. These neurons, which range in size up to 1 mm in diameter, each contain a single nucleus which comprises approximately 30 percent of the volume of the cell body. The large size of the nucleus and the staining characteristics of the nucleoplasm with the Feulgen stain led Coggeshall (*1*) to suggest that the giant cell nuclei contain considerably more DNA than the diploid amount for *Aplysia*. This possibility was supported by an unpublished observation of Strumwasser (*2*) that the largest neurons contain 50,000 times as much DNA as mammalian cells. We have quantified the nuclear DNA from individual giant neurons and have found that the amount of DNA in *Aplysia* giant neurons is many thousands of times greater than the haploid value for these animals.

One of the remarkable features of the *Aplysia* nervous system is that individual neurons can be easily recognized in the abdominal ganglion; over 30 of these cells have been characterized electrophysiologically and morphologically (*3*). We chose to study cells R-2 and L-6 of the abdominal ganglion [nomenclature of Frazier *et al.* (*3*)] and the single giant cell of the left pleural ganglion, which we have abbreviated P-1. Cells R-2 and P-1 have been called the colossal cells and are the largest in the *Aplysia* nervous system; L-6 is somewhat smaller.

The giant cells are surrounded by thousands of glial satellite cells and contain a large complement of mitochondria. Therefore, in order to quantify the nuclear DNA of the giant neurons, it seemed necessary at the outset of these experiments to isolate the nuclei from the giant cells and rule out any contamination from glial or mitochondrial DNA. This precaution was justified in light of the finding that the glial cells and neuronal cytoplasm contribute 33 percent of the DNA to the intact giant cell (*4*).

An abbreviated description of the procedure for removing the giant cell nucleus from the cell body follows. A more complete description will be published separately (*4*). The abdominal and left pleural ganglia were removed from the animal and immersed in Millipore-filtered seawater (pH 7.4). The ganglia were incised under observation with the dissecting microscope, and selected cells were removed by severing the single axonal process. The neurons were transferred to a depression slide containing Millipore-filtered seawater. A small hole was made in the giant cell membrane, and the nucleus was extruded from the hole by gentle squeezing of the cell. The nuclei of the largest neurons are polymorphic and take on many odd shapes. However, when the nuclei are extruded into sea-