# Cucurbitacins: Specific Insect Attractants in Cucurbitaceae

Abstract. *In vivo studies of cucurbitacins, the bitter principles present in Cucurbitaceae, indicated a quantitative relation between the concentrations of cucurbitacin in the plant and the degree of insect feeding. In vitro studies demonstrated the attractiveness of these compounds when they were separated from influences of other components of the host plants.*

The cucurbitacins are a class of bitter toxic substances of almost universal occurrence in the angiosperm family Cucurbitaceae. They are classified chemically as tetracylic triterpenes, and their existence has long been known. Selective breeding has eliminated the cucurbitacins from the fruits of cultivated types, although they are still widespread in stem and leaf tissue. Reports of livestock poisoning in Africa, where some species of this family are indigenous, and a possible economic value of these compounds has led to extensive research on their occurrence, distribution, genetics, and biochemistry. Most of this work is reviewed by Rehm (*1*). Fourteen different compounds have been isolated from various species in this family; they have been given letter designations A through N. Although the cucurbitacins are important horticulturally and medically because of their extreme distastefulness, toxicity, and apparent carcinostatic activity (*2*), no specific function in the economy of the plant itself has been proposed for them.

Observations that sliced bitter fruits of a mutant watermelon strain (*Citrullus vulgaris* Schrad.), obtained from the commercial variety Hawkesbury, were fed upon voraciously by spotted cucumber beetles (*Diabrotica undecimpunctata howardi* Barb.), while fruits of the original strain were much less preferred, was the first clue that the cucurbitacins might be insect feeding attractants. The only apparent characters that distinguish the mutant from the original are the gene for fruit bitterness and a less intense fruit flesh color.

Our objective in this study was to determine the possible role that cucurbitacins play as insect attractants.

Various plant parts were exposed to natural and caged populations of insects for in vivo studies. Plant materials used were (i) samples of cut fruit from the Hawkesbury watermelon and its bitter mutant, (ii) seedlings of Hawkesbury (low in cucurbitacin E) and the watermelon line PI-274035 (high in cucurbitacin E), and (iii) seedlings of the squash (*Cucurbita maxima* Duch.), varieties Buttercup (low to intermediate in cucurbitacin B) and Hubbard (high in cucurbitacin B). Feeding preferences were determined by insect counts on pieces of fruit and by the damage to cotyledons. Seedlings were rated from 0 (undamaged) to 10 (killed). In vitro studies were made by exposing several cucurbitacins in inert materials to natural populations of insects. Extracts from fruits of the bitter mutant were incorporated into inert materials and exposed to insect feeding. The extraction procedure was similar to that used by Enslin (*3*). Water-, chloroform-, and pentane-soluble fractions were exposed to insects in cylindrical agar blocks wrapped in paper. Feeding punctures and defecations on the papers indicated relative attractiveness. Cucurbitacins B, D, E, and I, isolated from *Ecballium elaterium* L., and the bitter chloroform-soluble extract from the *C. vulgaris* mutant were also exposed to insects on filter paper, and evidence of feeding was observed.

Feeding response of the spotted cucumber beetle was observed in all experiments, and in one experiment the honey bee (*Apis mellifera* L.) and a species of yellow jacket wasp (*Vespula*) were also observed.

Table 1. Average number of insects present per piece of watermelon (*Citrullus vulgaris*), nonbitter and bitter, after exposure to natural insect populations of spotted cucumber beetle (*Diabrotica undecimpunctata howardi* Barb.), honey bee (*Apis mellifera* L.), and yellow jacket wasp (*Vespula* spp.). Average number on 24 pieces of fruit in the 15-minute experiment and 30 pieces in the 24-hour experiment.

| Insect | Insects per sample (No.) | |
|---|---|---|
| | Nonbitter | Bitter |
| *Exposure for 15 minutes* | | |
| Cucumber beetle | 2.2 | 8.1* |
| *Exposure for 24 hours* | | |
| Cucumber beetle | 7.5 | 78.4* |
| Honey bee | 3.5 | 0.6* |
| Yellow jacket | 6.0 | 0.0* |

* Significantly different from the number on the nonbitter sample at the 1-percent level.

Table 2. Average extent of damage by spotted cucumber beetles to seedlings of some watermelon (*Citrullus vulgaris*) and squash (*Cucurbita maxima*) varieties. Average of 50 seedlings rated for extent of damage, from 0 (undamaged) to 10 (killed).

| Variety of species used | Score of cotyledon damage per plant |
|---|---|
| *Citrullus vulgaris* | |
| Hawkesbury (low cucurbitacin E) | 0.7 |
| PI-274035 (high cucurbitacin E) | 6.6 |
| *Cucurbita maxima* | |
| Buttercup (intermediate cucurbitacin B) | 2.8 |
| Hubbard (high cucurbitacin B) | 6.5 |

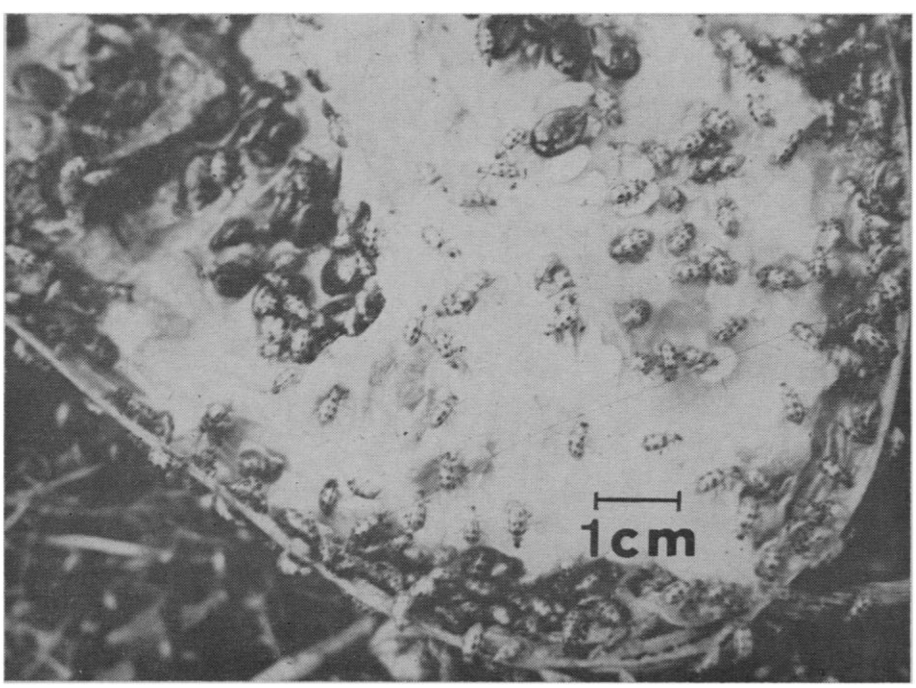


Fig. 1. Spotted cucumber beetles feeding on the cut surface of fruit of a bitter watermelon. Note collection of beetles immediately beneath the exocarp.

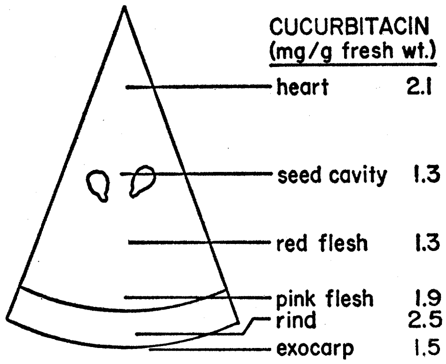


Fig. 2. Concentration (milligrams per gram, fresh weight) of cucurbitacin in different areas of fruit of a bitter watermelon.

Relative attractiveness of fruit samples is shown in Table 1. Bitter fruits had a much greater attractiveness for cucumber beetles than the nonbitter ones. After an exposure of only 15 minutes in one experiment the number of beetles on bitter samples was almost four times greater than the number on nonbitter samples. When exposure time was increased to 24 hours in a second experiment, the number of beetles on bitter samples was more than ten times the number on the nonbitter. Differences in the number of honey bees and yellow jacket wasps were also significant, but their response was opposite to that of cucumber beetles, that is, they exhibited greater preference for the nonbitter samples. The absence of yellow jackets from all 30 bitter samples, although not conclusive evidence for repellency, might suggest that the bitter samples are repellent to these insects. Cucumber beetles feeding on the cut surfaces of bitter fruit collected in the area adjacent to the exocarp (Fig. 1); this was the area of the fruit that contained the highest concentration of cucurbitacin (Fig. 2).

A quantitative relation was indicated in the feeding response of cucumber beetles to seedlings with different concentrations of cucurbitacin. Damage to cotyledons was also correlated with the concentration of cucurbitacin in the seedling (Table 2), as was the part of the seedling most damaged by the beetles. It was evident from the studies of damage to seedlings that the extent of the damage to cotyledons was greater than to hypocotyls. Our analysis, as well as that of others (*4*), of different plant parts from a number of commercial varieties showed that cotyledons and roots were high and hypocotyls low in cucurbitacin.

The average number of defecations and feeding punctures per sample on paper wrappings from water-, chloroform-, and pentane-soluble fractions of a bitter fruit extract in agar was 19.7, 45.4, and 3.3, respectively, which places the attractant primarily in the chloroform-soluble fraction. Chemical studies of these bitter compounds have shown them to be slightly soluble in water, highly soluble in chloroform, and highly insoluble in pentane (*1*). Moreover, cucumber beetles fed on dry filter paper in areas where chloroform solutions of purified cucurbitacins had been streaked. Cucurbitacin B was most heavily fed on, followed by cucurbitacins E and D. There was no evidence of feeding on cucurbitacin I. The positive response to several purified cucurbitacins definitely establishes them as specific feeding attractants for the spotted cucumber beetles, since induction of feeding on inert materials is considered conclusive evidence for a specific feeding attractant (*5*). Differences in chemical structure may explain the unattractiveness of cucurbitacin I.

Evidence that the toxic and repellent cucurbitacins are attractive to a limited group of insects has important implications in the evolution of the Cucurbitaceae. It may now be possible to study much more thoroughly the interactions of protective mechanisms of the plant against insect attack and the insect's adaptive responses. These results may also be important in the breeding of insect-resistant plants.

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**References and Notes**

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## High-Temperature Synthesis of Aromatic Hydrocarbons from Methane

Abstract. *Arenes of 1, 2, 3, 4 and more rings have been synthesized in a flow system by passing methane through silica gel at 1000°C. Yields of 4.8 to 7.2 percent have been obtained per single pass. As determined by combined gas chromatography–mass spectrometry, the major compound synthesized that are of less than 5 rings are benzene, naphthalene, acenaphthylene, phenanthrene, fluoranthene, and pyrene. Under certain conditions (presence of ammonia and water), small amounts of aliphatic hydrocarbons are also synthesized. Even-carbon-numbered hydrocarbons (aromatic as well as aliphatic) predominate, to the extent that about 97 percent of the total weight of the analyzed arenes have even numbers of carbon atoms.*

It is known that methane is one of the most abundant organic molecules in the solar system, being an important component of comets and the Jovian planets (*1*). It is also known that high temperatures are likely to be generated by the collision of either comets or large meteorites with planets (*2*) and that methane at high temperatures is converted to aromatic hydrocarbons (*3*). Because of the possible significance of these observations to organic cosmochemistry (*4*), we have studied in some detail the products formed from methane at temperatures of about 1000°C. The availability of a new gas chromatographic–mass spectrometric technique (*5*) has permitted us to carry out a qualitative and quantitative analysis of the reaction products with simplicity and accuracy not possible heretofore.

The experimental procedure can be summarized as follows: A Vycor tube (20 cm in length by 0.4 cm I.D., or 10 by 0.6 cm I.D.) filled with 100- to 200-mesh silica gel was heated at 1000°C in an electric furnace. Methane gas (Matheson, C.P.) was allowed to flow for 5 (or 10) hours at 0.2 $cm^3/sec$ (measured at about 25°C). A total of 3.6 (or 7.2) liters of methane gas