PLANT CHEMICALS AS ATTRACTANTS FOR *HELICOVERPA ZEA* (LEPIDOPTERA:NOCTUIDAE) AND OTHER INSECT SPECIES[1]

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ABSTRACT

Plant chemicals that have been identified and used for monitoring and controlling field populations of insects, including examples of attractants for noctuid and other species are reviewed. Methods for obtaining volatile chemicals from plants are discussed. A number of plant species have been identified as attractive to *H. zea* and other noctuids by direct observation of insects feeding on field plants or by identification of pollen found on body parts of captured insects. In several classes, the attractive chemicals in plants were determined to be extractable by solvents, especially methanol and methylene chloride. Methanol extracts of *Gaura spp.* flowers captured both sexes of corn earworm moths in a 1.3:1.0 ratio(♀ :♂).

INTRODUCTION

The concept of using natural plant-produced, insect-attracting chemicals as an integral part of the overall technology for managing insect pest species has been studied for many years. Many behavorial/physiological responses in insects are mediated through chemical cues produced by host plants or other plants which are beneficial to the insect. On the other hand, plants also may produce chemicals which can be detrimental to insects. An insect can be directed or attracted to a plant for shelter, food, or mating and oviposition. The plant often benefits from this interaction in the form of pollination when an insect is attracted to its flower to feed on nectar. In nature, there is a seemingly endless combination of volatile chemicals available for insect attraction. Hedin et al. (1974) listed some 54 examples of plant chemicals that were attractive to insects, but theorized that many other chemicals exist because more than 350,000 plant feeding insect species have been reported (Hedin 1976). Metcalf (1987) stated that there are more than 200,000 species of flowering plants and that about 500,000 species of insects interact with them.

Attractants for Chrysomelidae. Although the concept of using plant-produced chemicals for control has been considered for many years, the science has failed to progress to field application with noctuid moths. However, there is a considerable volume of literature dealing with other insect families. One example of an insect genus that has received considerable

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attention in this regard is *Diabrotica* (Chrysomelidae). Andersen and Metcalf (1986) identified the volatile attractant indole from blossoms of *Cucurbita maxim*. Other chemicals, such as 2-methoxy-4-propylphenol, veratrole, estragole, eugenol, beta-ionone, and phenylacetaldehyde are also attractive to several *Diabrotica* spp (Ladd et al. 1983, Lampman et al. 1987, Yaro et al. 1987) However, there have been several reports of species specificity for some chemicals as attractants to *Diabrotica*. For example, Yaro et al. (1987) reported that eugenol and 2-methoxy-4-propylphenol were highly attractive to *Diabrotica cristata* (Harris) and *Diabrotica barberi* (Smith and Lawrence), but not to *Diabrotica virgifera virgifera* (LeConte). Lampman and Metcalf (1988) demonstrated that *D. cristata* shares some chemosensory responses with both *D. barberi* and *D. virgifera virgifera. Diabrotica cristata* is attracted to estragole, beta-ionone, para-methoxybenzene, and a mixture of trimethoxybenzene, indole, and cinnamaldehyde, all of which are attractants for *D. virgifera virgifera. Diabrotica cristata* is also attracted to eugenol, isoeugenol, and cinnamaldehyde, which are all attractants for *D. barberi*. Also, Lampman and Metcalf (1987) reported that a mixture of veratrole, indole, and phenylacetaldehyde acted synergistically to attract *Diabrotica undecimpunctata howardi* Barber; the mixture caught eight times more beetles than was predicted by assuming there would be a simple additive response to the individual chemicals.

It has been demonstrated that chemoattraction of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Chrysomelidae), to potato plants depends to a large extent upon the composition of the so-called green leaf volatiles (Visser and Ave 1978). In general, the green leaf volatiles of plants consists of a group of six-carbon alcohols, aldehydes and acetates; in potato leaves, these include (Z)-3-hexenol, (Z)-3-hexenyl acetate, (E)-2-hexenal, (E)-2-hexenol, and hexenal. It is believed that the response of the insect towards the host depends not only on the presence of each of the components but also on the relative proportion of each component within the blend.

Attractants for Tephritidae. The genus *Dacus* (Tephritidae) includes many economically important species of fruit flies that have received considerable attention in relation to their response to plant attractants. Howlett (1915) reported that methyl eugenol attractive to males of three *Dacus* sp. in India, including the oriental fruit fly, *Dacus dorsalis* Hendel. Later, methyl eugenol isolated from the Australian plant *Zieria smithii* was reported to be attractive to *Dacus tyroni* (Fletcher et al. 1975). Steiner (1952) reported that oriental fruit flies were attracted to methyl eugenol from distances up to a half-mile. Other plant chemicals found to be attractive to *Dacus* spp. are beta-asarone and asarylaldehyde (*Dacus dorsalis* Hendel) and acoragermacrone (*Dacus cucurbitae* Coquillett, melon fly) (Jacobson et al. 1976). Feeding attractants from plants have been used to monitor and, in combination with insecticides, to control field populations of *Dacus* spp.

Attractants for Coleoptera. The boll weevil, *Anthonomus grandis grandis* Boheman (Curculionidae), has received extensive study in efforts to identify plant chemicals that might affect its behavior. In this work, conducted by USDA, ARS in State College, Mississippi, during the 1960's and 1970's, volatiles from cotton plants and alternate hosts were evaluated as *A. grandis* attractants. Various isolates were bioassayed and attractants were identified; these included limonene, alpha-D-pinene, beta-bisabolol, beta-caryophyllene, and beta-caryophyllene oxide (Minyard et al. 1969). Beta-bisabolene oxide (Hedin et al. 1972), and linalool oxide, geranyl acetate, neryl acetate, rose oxide, fenchone, menthone, isovaleraldehyde, and linalool were also attractive to *A. grandis* (Gueldner et al. 1970).

Attractants for Noctuids. Much of the literature pertaining to volatile plant chemicals attractive to noctuids centers around phenylacetaldehyde, which has been reported to be attractive to several important insect species including corn earworm, *Helicoverpa zea* (Boddie), and various looper and armyworm species. Smith et al. (1943) tested more than 500

chemicals as possible attractants for *Heliothis armigera* (Hübner) and *Autographa brassica* (Riley). While none of these were attractive to *H. armigera,* several chemicals strongly attracted *A. brassica* . The most attractive of these chemicals were phenylacetaldehyde, benzyl ether, benzyl acetate, palmitaldehyde, diphenyl ether and benzyl acetate; approximately half of the moths captured were females. Phenylacetaldehyde was reported by Cantelo and Jacobson (1978b) to be the attractive principle that attracted moths to bladder flower. Cantelo and Jacobson (1978a) also reported phenylacetaldehyde isolated from corn silk volatiles attracted *H. zea, Pseudoplusia includens* (Walker), *Caenurgina erechtea* (Cramer), *Ostrinia nubilalis* (Hübner) and *Lygus lineolaris* (Palisot de Beauvois) (Miridsel). These authors found that butanol and acetaldehyde, also identified from the corn silk volatiles, increased the attractiveness of phenylacetaldehyde to *P. includens* but not to *H. zea.* Pawar et al. (1983) reported that phenylacetaldehyde attracted *Heliothis armigera* (syn. *H. zea*), and that females represented ca. 39% of the total catch. Others have identified volatiles from corn kernels and husks (Buttery et al. 1978), corn tassels (Buttery et al. 1980), and corn silks (Flath et al. 1978) as possible attractants for *H. zea,* but no tests were performed to actually demonstrate attractancy. Haynes et al. (1991) identified phenylacetaldehyde, benzaldehyde, 2-phenylethanol, and benzyl alcohol from flowers of *Abelia grandiflora,* and a blend of these compounds was as effective as a cluster of flowers in stimulating upwind flight of male *Trichoplusia ni* (Hübner) in a wind tunnel test. Heath et al. (1992) identified benzaldehyde, benzyl acetate, and phenylacetaldehyde among volatile chemicals from flowers of jessamine, *Cestrum nocturnum* L. Female *T. ni* moths exhibited upwind flight and contact with dispensers containing the three chemicals, but response to phenylacetaldehyde alone was not significantly different from the blend.

Isolation of Volatile Chemicals from Plant Sources. Although there has not been enough work on plant attractants for noctuids to provide definitive answers concerning the chemistry of these attractants, some generalizations can be made about the methods that can be used to isolate and identify individual attractive compounds, or groups of attractive compounds, and to verify their attractiveness in laboratory and field tests. Primary factors in determining which chemical or group of chemicals in an attractive plant are responsible for the biological activity are isolation and analytical techniques and bioassays. Technological developments in the past few years have led to a tremendous increase in our ability to analyze and identify volatile plant chemicals. Of particular importance in the analysis and identification of chemicals among complex mixtures of volatiles emitted from plants is the availability of capillary gas chromatography coupled with mass spectroscopy. This instrumentation has sufficient sensitivity and mass range capability to identify many unknowns present in low concentrations in a mixture, and it is generally priced within the budget constraints of many laboratories. Several methods are available to obtain sufficient quantities of volatiles from plant material for analysis, including: (1) collection of volatiles from the headspace of a plant by trapping them on a sorbent such as Porapak, Tenax or charcoal; collections can be made from intact plants or from freshly harvested material of the plant part of interest, (2) extraction of the collected plant material with a suitable solvent such as hexane, diethyl ether, methylene chloride, or methanol, (3) steam distillation of the plant material and subsequent extraction of the volatiles with a solvent immiscible with water, or (4) pre-column evaporation of plant components through a special injector port on a gas chromatograph. The method of choice will depend largely on the plant and insect under study, but each method has inherent advantages and/or disadvantages. For example, solvent extraction or steam distillation of collected plant material extracts only the chemicals available at the time of extraction, whereas headspace collection techniques allow collection of the volatiles over a period of time, thus allowing enrichment and an increase in the range of chemicals produced over time. Damage to plant material such as maceration for some

extraction techniques can give rise to many volatile aliphatic aldehyde and alcohol compounds that are not present in the intact plant (Buttery et al. 1982). Also, certain enzyme systems can destroy some of the volatiles present in the intact plant. To fully understand the attraction of insect pests to specific plants, it is essential to identify the compounds emitted by the intact plant.

Identification of Plant Volatiles. A joint effort was made to identify feeding attractants for *H. zea* and other noctuid moths in certain attractive plant species, and to incorporate selected attractants into a control program for some of these insect pest species (Lingren et al. 1989, Lingren et al. 1994, Raulston et al., this supplement). A number of plant species were found to be attractive to *H. zea* and other noctuids by observing the insects feeding on field plants, or indirectly by analyzing pollen on body parts of captured insects (Lingren et al. 1993, Lingren et al. 1994, Lingren, et al., this supplement, Raulston et al., this supplement). In order to demonstrate that the attractive chemicals were extractable, flowers of three *Gaura* spp. were collected and extracted with hexane, acetone, methylene chloride, and methanol. These solvent extracts were then tested for activity by metering 50-300 bloom equivalents onto cotton dental rolls; impregnated cotton rolls were placed in inverted 75-50 cone traps (Hartstack et al. 1979) about sundown so they would be in place during the early evening feeding period of adult noctuids. In preliminary tests, traps baited with methanol or methylene chloride extracts of the *Gaura* blooms captured the greatest number of insects. In one test, 12 traps were placed in a 2 ac corn field for three days at tassel emergence (27-29 July, 1988), and three traps each were baited nightly with methanol extracts [300 bloom equivalents (BE)/trap in four cotton rolls] or fresh bouquets (300 blooms/trap) of *G. drummondii* or *G. longiflora*. Baits were replaced each day for three days, and captured moths were identified by species and counted. More corn earworm and true armyworm moths were captured in traps baited with bouquets than in traps baited with methanol extracts (Table 1). However, all baits captured some moths of all five insect species, except for the methanol extract of *G. longiflora* which did not capture sphynx moths. The sex ratio of captured corn earworm moths was 1.3:1.0 (♀:♂) in traps baited with methanol extract and bouquets of both *Gaura* spp.

TABLE 1. Captures of Noctuid Moths with Methanol Extracts and Flower Bouquets of *Gaura drummondii* and *Gaura longiflora*

	Capture/Trap/Night			
	Methanol Extract (300 BE)[a]		Bouquet (300 Blooms)[a]	
Insect	*G.d.*[b]	*Gl.*[c]	*G.d.*	*G.l.*
Corn earworm	10.8	2.0	21.0	8.8
Cabbage looper	7.5	0.8	7.8	5.8
True armyworm	3.0	1.3	12.0	14.3
Black cutworm	1.5	1.3	4.0	0.8
Sphynx moth	2.9	0.0	3.0	1.2

[a] Mean number of moths captured in three traps over three nights.
[b] *G.d.* = *Gaura drummondii*
[c] *G.l.* = *Gaura longiflora*

In another test, four traps were baited with a methanol extract of *G. longiflora* (150 BE/trap in 2 cotton rolls) each night for 3 nights (31 Aug-2 Sep 1988). Traps were monitored hourly to determine the number and sex of captured corn earworm moths. Also, two *G. longiflora* plants were observed for 30 min during each hourly period by observers wearing 6W head lamps equipped with a rheostat to obtain low light levels. Insects feeding on plants were captured for determinations of species and sex. Blooms on the test plants were counted at 0530-0600 h each morning ($\bar{x}$ = 575 blooms/plant/night). The hourly patterns of moth captures in traps baited with methanol extracts were similar to the patterns for moths observed feeding on flowering *G. longiflora* plants (Fig. 1). We found that 34 moths were captured in traps ($\bar{x}$ = 2.8/trap/night) and 47 moths were captured from plants (= 7.8 moths/plant/night). Sex ratios of moths captured in traps baited with the methanol extract or a flowering plant was 1.7:1.0 and 1.1:1.0, respectively.

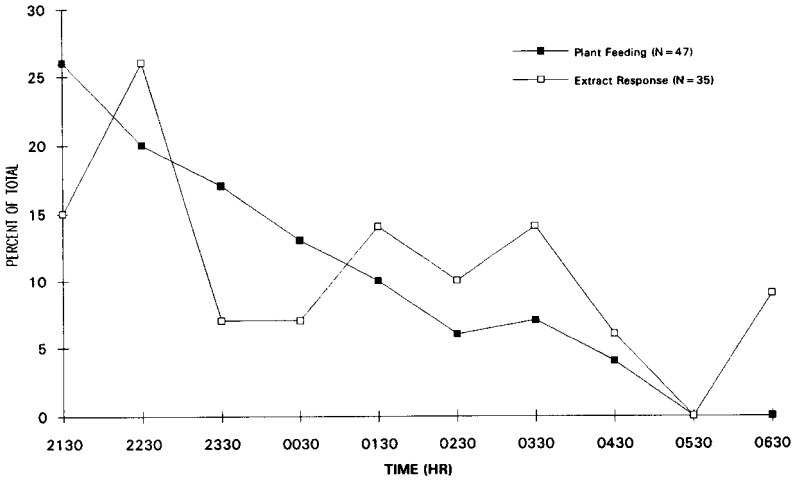

FIG.1. Response of corn earworm moths to *G. longiflora* plants and traps baited with a methanol extract of *G. longiflora*.

Most of our chemical identification work thus far has been concentrated on the hexane extracts of *G. drummondii, G. suffulta,* and *G. longiflora*, and a total of 12, 21, and 24 compounds respectively, have been identified from these species (Teranishi et al. 1991, Kint et al. 1993). Six compounds (phenylacetaldehyde, (E)-2-hexenal, (Z)-3-hexenol, 2-phenyl-ethanol, methyl salicylate, and 3-methyl-1H-benzopyran-1-one) were common to the three species. Additionally, two compounds (limonene and methyl 2-methoxybenzoate) were common to *G. drummondii* and *G. suffulta*, and two hexenyl acetate) were common to *G. suffulta* and *G. longiflora*.

Volatiles in the air stream were collected on Tenax sorbent tubes and the adsorbed chemicals were eluted with hexane. A small vacuum pump was used to pull air across 20-40 intact blooms from *Gaura* spp. contained in a bell jar. This hexane eluate was loaded onto cotton rolls which were placed in traps in the early evening. The Tenax-trapped volatiles volatilization of the attractants from the cotton roll. Several compounds previously identified attracted *H. zea* to the trap, but captures were limited to a period of 1-2 h because of rapid in hexane extracts were also found among volatiles collected on Tenax traps from *G.*

drummondii, including phenylacetaldehyde, (Z)-3-hexenol, 2-methylbutanal oxime (isomer A and B), 2-phenylethanol, and methyl salicylate (Shaver and Lingren, unpublished). The advantage of using the headspace collection technique rather than direct solvent extraction of collected plant material is that the volatiles are concentrated over time, and the profile of volatile chemicals is similar to that encountered by the insect responding to the plant. However, the range of chemicals collected on adsorbents is not identical to that of surrounding flowers in nature due to differences in adsorption efficiency of individual chemicals on the adsorbent. Also, the emission of volatiles from different plants varies widely and lengthy collection periods are sometimes required to collect sufficient quantities of the volatiles for quantitation or activity measurements. Because some chemicals are stored in the plant in forms different from those released as volatiles, and the vapor pressures of volatiles are different, large differences could occur in the composition of volatiles from a specific plant depending on the method of collection.

Bioassay of Potential Attractive Chemicals. Perhaps one of the most difficult and yet essential phases of isolating, identifying, and developing plant attractants for field use is the bioassay. Bioassays for measuring insect response to volatile chemicals are usually based either on electrophysiological or behavioral responses. The electrophysiological bioassay which is based on the assumption that the antennae of the insect contain the receptors used in receiving the olfactory messages, has been used successfully in many situations. Zhu et al. (1993) reported that flower volatiles from blooming plants observed to be attractive to *Agrotis ipsilon* (Hufnagel) adults in the field also elicited high electroantennogram responses. This bioassay is so sensitive that some workers have been able to use an effluent split from a gas chromatograph to simultaneously monitor detector signal and the insect's electroantennogram response. Bioassays based on behavioral responses commonly include of some form of olfactometer in which insects are placed in an arena, and then air is passed over one or more test chambers containing extracts, fractions, or chemicals of interest into the arena containing the insects. Relative response is then determined by observing the numbers of insects moving into chambers containing chemicals compared with movement into chambers containing only solvent or other suitable controls. Also commonly used are wind tunnels that have a directed flow of air from the test source to the insect. Some olfactometers include an electronic detection and recording device to automate much of the observation, recording, and analysis of insect activity (Wearing et al. 1973).

Test chemicals and extracts also can be evaluated directly in the field by comparing the numbers of wild insects caught in baited traps with numbers of insects caught in non-baited traps. One disadvantage of testing in the field is that an adequate population of the target insect is usually available for only a short time each year. However, field tests are advantageous because the materials are being tested against wild insects instead of colonized insects, and there is a possibility of detecting attractancy for other wild insect species. Bioassays also can be conducted in greenhouses or field cages by releasing known numbers of insects and monitoring numbers attracted to baited versus non-baited tra

Regardless of the method chosen as a bioassay tool for monitoring extracts and chemicals for attractiveness, the bioassay should be compatible with some phase of the observed interaction of the insect with the intact plant. When using bioassays to determine relationships between insect and plant, it is important to remember that, in its natural setting, the insect is often responding to the total profile of chemicals emitted from the plant and not merely to a series of concentrations of one specific chemical. Other factors such as additive effects, synergism, and even repellency by other chemicals in the total plant volatile complex affect the overall response of the insect. Environmental factors such as temperature, humidity, light, or wind speed could also affect insect response.

When considering an attractive plant of unknown composition, the attractive

chemicals can not be predicted. However, there are some generalizations that can be made. An attractive chemical must have a certain degree of volatility, and this limits the molecular weight and boiling point range of potential attractive chemicals. Metcalf (1987) stated that the various plant compounds that have been documented to function as volatile insect attractants range in molecular weight from about 99 to 222 and in boiling range from 20° to 340°C. Proven attractants include members of the various chemical classes such as alcohols, aldehydes, esters, acids and hydrocarbons, both as aliphatic and aromatic compounds. Although testing would be simplified if the plant attractants were exclusively single compounds, it is likely that optimum response is associated with a mixture of plant odorants.

Regardless of the structure of the chemicals responsible for attraction of the insect to a plant source, the final inclusion of the attractant in a pest management program will depend on a careful determination of insect behavior, the origin of the insect population, a time in the phenology of a target crop or cropping system when the insect will be most likely to respond in a desirable way to a plant attractant, and on the development of an effective controlled-release formulation.

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