12. Volatile Chemicals from Sweet Potato and Other *Ipomoea*: Effects on the Behavior of *Cylas formicarius*

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The most prominent component of chemical ecology has to do with the effects of plant chemicals on the behavior and development of phytophagous insects (Bell & Cardé 1984, Harborne 1988). Most of the chemicals involved are secondary metabolites -- presumed to have originated as by-products of plant metabolism -- of which there exist an estimated 50,000 to 400,000 (Metcalf 1987, Schoonhoven 1982). These include both volatile and non-volatile compounds which may mediate plant/insect interactions as attractants, repellents, stimulants or deterrents to feeding and/or oviposition. The subject of this chapter is the volatile compounds given off by one group of plants, the genus *Ipomoea*, and their influence on the behavior of one insect, the sweetpotato weevil, *Cylas formicarius* (Fabricius).

Volatility is an important property in the action of communicative compounds, or semiochemicals, both within and between species. Molecules that can be transported in air usually have fewer than 20 carbons and molecular weights of less than 300 (Wilson & Bossert 1963). Insects are known to respond to 64 plant volatiles that vary in molecular weight from 99 to 222 and in boiling point from 250° to 340°C (Finch 1980, Metcalf 1987, Visser 1986).

The genus *Ipomoea* (Convolvulaceae) comprises about 500 species worldwide (see Chapter 3), including the sweet potato, *I. batatas* (L.) Lam., water spinach, *I. aquatica* Forsk., and an array of species known

as "morning glories". A majority of species are climbing or creeping vines, but shrub-like plants and trees are also included in the genus.

Sweet potato is an important staple crop in much of the world, while water spinach is cultivated as a vegetable in some areas. Although the nutritional composition of these two edible species has been the subject of considerable study (e.g., Collazos 1967, Watt & Merrill 1975), the volatile chemistry of *Ipomoea* spp. has until recently received little attention. The practical value of such research is in understanding how some varieties and species attract insects which may become pests. Of particular interest are beetles of the genus *Cylas* (Brentidae: Apionidae; Kuschel, in press, also see Chapter 2), the most serious insect pests of sweet potato.

The best known member of the genus, *C. formicarius*, feeds and reproduces on a variety of *Ipomoea* species worldwide (Chalfant *et al.* 1990, also see Chapters 2 and 3) and is the most important pest of cultivated sweet potato. Adults feed on the leaves, stems, and roots of the plant and lay eggs in the stems and roots, where larvae develop to maturity. Sutherland (1986) and Chalfant *et al.* (1990) have reviewed the biology and management of *C. formicarius*.

We report here on responses of *C. formicarius* to plant volatiles and on recent advances in the volatile surface chemistry of *I. batatas*. The *Ipomoea/Cylas* relationship appears to be a promising model for studying interactions between oligophagous insects and their host-plants. Also, a better understanding of *Ipomoea*-derived volatile compounds that modulate the behavior of *C. formicarius* may help to improve management programs for this pest.

RESPONSES OF *Cylas formicarius* TO *Ipomoea* ODOR

No sweet potato cultivar is known to be immune to *C. formicarius*, but cultivars are not equally susceptible to damage (Barlow & Rolston 1981, Mullen *et al.* 1980, 1981, 1982, 1985, Rolston *et al.* 1979). In what follows, we use "susceptible" and "resistant" to refer to cultivars with relatively high and low susceptibility to sweetpotato weevil, respectively.

Based upon other plant/insect interactions, differences in susceptibility to the weevil may be largely due to chemical differences among cultivars, which could result in variation in initial attraction, host choice, the weevils' success in utilizing different plants, or a combination of these factors. This possibility was first explored by Barlow & Rolston (1981); however, they found no evident inhibition of

feeding or oviposition among various cultivars. They implied that resistance was probably due to lower levels of attractant and/c feeding-stimulant chemicals.

Nottingham *et al.* (1989a) used a dual-choice olfactometer (Figu: . 12.1) to compare responses of *C. formicarius* to leaf and storage-root volatiles from the highly susceptible sweet potato cultivar, Centennial, the moderately susceptable cultivar, Jewel, the moderately resistant cultivars, Regal and Resisto, and a non-host plant, *Plectranthur tuberosus* Poir Chev. et. Perrot. They tested female and male *C. formicarius* separately in both choice (*I. batatas* vs. *P. tuberosus*) and no-choice (*P. tuberosus* vs. a blank) tests, with the following results:

1. In a leaf-odor choice test of 'Jewel' vs. *P. tuberosus*, both adult female and male *C. formicarius* showed significantly greater attraction to 'Jewel'. Using an attraction index (AI) consisting of the percentage of weevils responding to the preferred treatment, 'Jewel', minus the percentage responding to the non-preferred treatment, *P. tuberosus*, they obtained AI values of 41.6 and 45.2 for females and males, respectively.

2. In a no-choice test of *P. tuberosus* leaf odor vs. a blank control, neither female nor male weevils showed a significant preference.

3. In leaf-odor choice tests of each of the four sweet potato cultivars vs. *P. tuberosus*, female weevils showed significantly greater attraction to sweet potato cultivars in each case (AI = 19.3 for 'Regal', 34.0 for 'Centennial', 40.7 for 'Resisto', and 46.7 for 'Jewel').

4. In no-choice tests of a methylene chloride extract from leaf surfaces of 'Jewel' vs. a solvent (methylene chloride) control, both female and male weevils showed a significantly greater attraction to a 1-leaf and a 0.2-leaf equivalent than to the control, but response to a 0.02-leaf equivalent extract did not differ from that to the control.

5. In no-choice tests of root odor of each of the four sweet potato cultivars vs. a blank control, female weevils showed significantly greater attraction to sweet potato root volatiles in each case (AI = 26.7 for 'Resisto', 30.0 for 'Centennial', 33.3 for 'Regal', and 52.0 for 'Jewel'). In similar tests of 'Centennial' and 'Jewel' root odor, male weevils showed no preference for either (AI = 7.2 and 6.8, respectively).
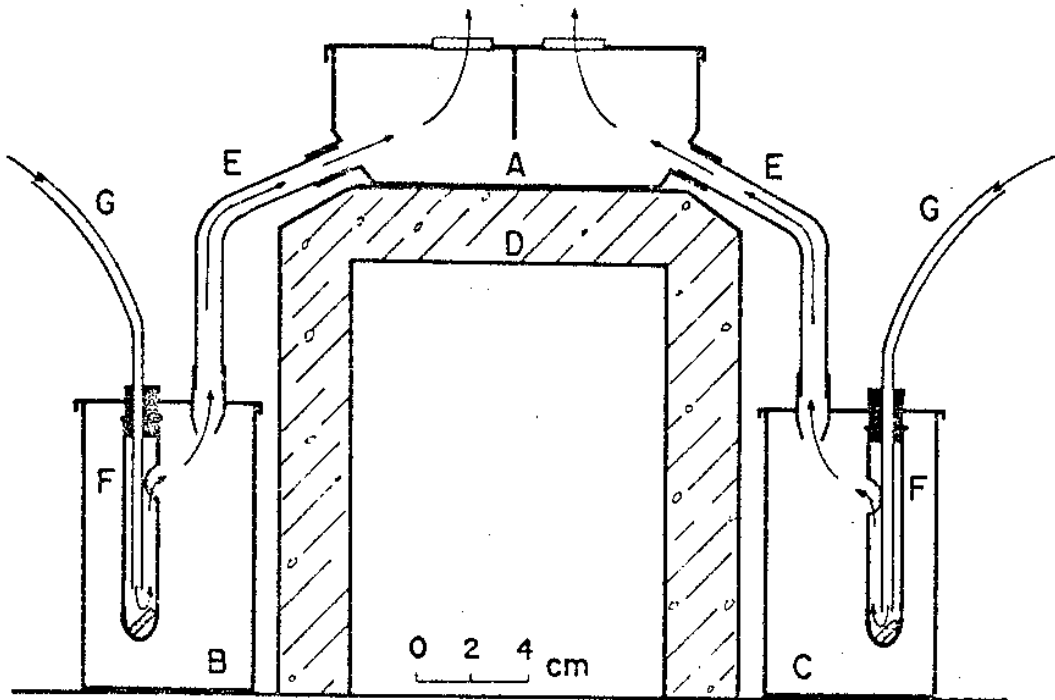
Fig. 12.1. Diagrammatic cross-section of dual-choice olfactometer used to compare attraction of *C. formicarius* to odor from various substrates. In each trial 25 female weevils were starved for 2 hours and then released in the main chamber. After an additional 2 hours, the numbers in the side chambers (B and C) were counted. Weevils found in the tubes that connected to the side chambers were regarded as having made a choice and were counted along with those in their respective side chambers. Arrows indicate the direction of air flow. The arrangement shown here was used for tests of extracts with treated filter paper placed in the test tubes in the side chambers. For tests of leaves, the test tubes were detached and placed on the floor of the side chambers, where they served as vases. For tests with roots, the test tubes were removed and the roots were placed on the floor of the side chamber. A. Main chamber. B. Treatment chamber. C. Control chamber (from Nottingham *et al.* 1989a; reprinted with permission from J. Chem. Ecol. [Plenum Publishing Corp., New York]).

6. In no-choice tests of a methylene chloride extract from 'Jewel' root surfaces vs. a solvent control, female weevils showed a significantly greater response to a 10-root and a 1-root equivalent than to the control, but their response to a 0.1-root equivalent extract did not differ from their response to the control. The attraction of males did not differ between 10-root or 1-root equivalents and the control.

These results suggest the following: 1) females respond to leaf and root volatiles from all tested sweet potato cultivars; 2) female response differs among cultivars; and 3) males respond to volatiles from leaves but apparently not those from roots.

In assays of feeding and oviposition preferences on storage roots and leaves, Nottingham et al. (1987, 1988, 1989b) found similar differences among cultivars. Thus, initial attraction and subsequent utilization may each contribute to variation in susceptibility. It remains to be seen whether C. formicarius shows comparable variation in its ability to reproduce in different sweet potato cultivars in the absence of a choice.

The range of host plants acceptable to C. formicarius has yet to be established, but present indications are that the weevil is restricted predominately to the family Convolvulaceae (see Chapter 3). It has been recorded feeding and/or breeding on at least 27 species of Ipomoea and 8 species from related genera (Austin et al. 1990, see Chapter 3).

In a modification of the olfactometer assay devised by Nottingham et al. (1989a), no-choice assays were conducted to determine the response of female weevils to volatiles from leaves ten Ipomoea spp. and a blank control. Of these species, nine have been recorded as host to the weevil: I. alba, I. aquatica, I. batatas, I. hederifolia, I. nil, I. pandurata, I. pes-caprae, I. purpurea, and I. setosa (Austin et al. 1990, Chalfant et al. 1990, Jansson et al. 1989, also see Chapter 3). The host status of I. carnea is unknown. Our experimental method differred from that of Nottingham et al. (1989a) in two ways: 1) we conducted assays under dim red light, rather than in total darkness; and 2) in tests with small-leaved plant species, we used two or three leaves together in each trial. These modifications were intended to simplify the experiment and it is unlikely that they affected the outcome. For I. batatas, we used the moderately susceptible cultivar, Jewel.

Figure 12.2 shows response data from two of the ten species, in which dosage was computed as [leaf area per side (cm^2)/rate of air flow $(ml. min.^{-1})$]. The data illustrate two general results. First, contrary
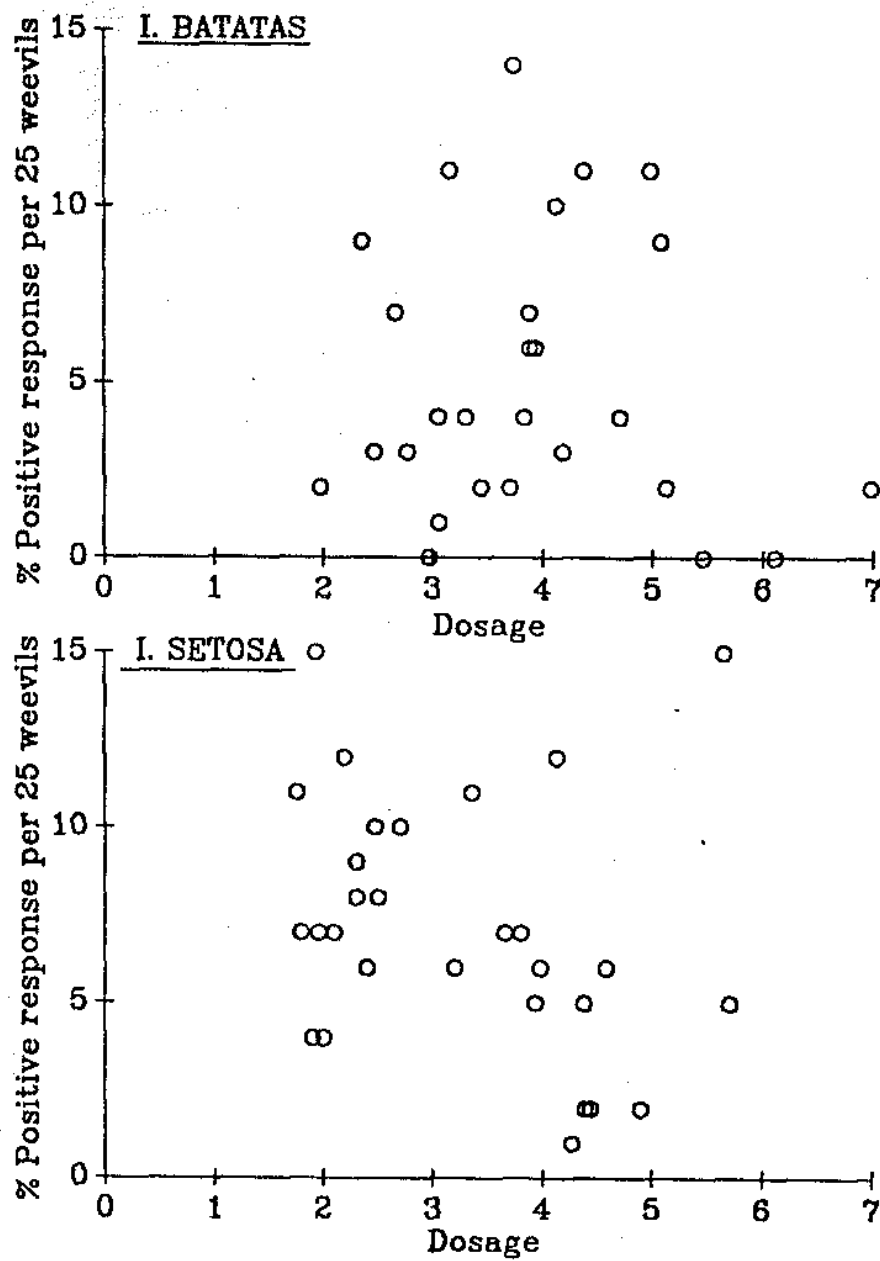
Fig. 12.2. Number of female *C. formicarius* (out of 25) responding to leaf odor from two *Ipomoea* species (*I. batatas* [top] and *I. setosa* [bottom]) in relationship to leaf volatile dosage. For simplicity, response to the blank control is omitted. Each data point corresponds to one trial.

to our expectation, we found no significant increase in weevil response with an increase in dosage of leaf volatiles (analysis of covariance, $P >$ 0.05) in any of the species. Variation in dosage could therefore be discounted as a factor within the range of dosages that were tested. Second, the response of weevils to a given species was not uniform among trials. The variance in response, which is illustrated in Figure 12.2, was typical of all ten species (Table 12.1).

As expected, the weevils did not respond equally to leaf volatiles from all plant species (Table 12.1; analysis of variance of ranked AI data; $F = 6.91$; $P < 0.05$). Four species, *I. batatas, I. carnea, I. nil*, and *I. pes-caprae*, elicited responses significantly greater than the control (Bonferroni *t*-test of mean AI ranks; $P < 0.05$). Furthermore, eight of 45 pairwise comparisons between species were significant (Bonferroni *t*-test of mean AI ranks, $P < 0.05$). These were: *I. batatas* > *I. aquatica*; *I. carnea* > *I. alba*; *I. carnea* > *I. aquatica*; *I. carnea* > *I. hederifolia*; *I. carnea* > *I. purpurea*; *I. carnea* > *I. setosa*; *I. nil* > *I. aquatica*; and *I. pes-caprae* > *I. aquatica*. It is our expectation that similar data from a broader range of species within the Convolvulaceae, coupled with analysis of surface volatiles, will help to understand what makes some plants more attractive to this particular insect.

Adult male and female *C. formicarius* were not equally attracted to root and leaf volatiles. Females were strongly attracted to both leaf and root volatiles, whereas males were attracted to leaf volatiles, but were not attracted to root volatiles. There are considerable morphological differences between the sexes of *C. formicarius* in their putative olfactory apparati (C.K. Starr *et al.*, unpubl. data) which may correlate with host finding behavior.

VOLATILES OF *Ipomoea batatas*

At present no class of chemicals is definitely known to be involved in the differential attraction of *C. formicarius* to *Ipomoea* spp. There are several lines of evidence, however, that point towards terpenoids as possible candidates. In contrast to the leaf surfaces of many plant species, *I. batatas* has copious amounts of triterpenes (S.J. Kays & R.F. Severson, unpubl. data). In addition, the ovipositional stimulant for *C. formicarius* found on the surface of the storage roots is a pentacyclic triterpene and weevil larvae induce the synthesis of a series of furanoterpenoids within the roots. It would appear, therefore, that terpene chemistry might be involved in the coevolution of the two species.

Table 12.1. Attractiveness of leaf volatiles from ten *Ipomoea* species and a blank control to *C. formicarius*.

Ipomoea species	Number of trials	Mean dosage ($\pm$ SE)	Response to plant, % ($\pm$ SE)	Response to control, % ($\pm$ SE)	AI ($\pm$ SE) [a]
I. alba	20	3.69 ± 0.26	21.6 ± 3.3	4.2 ± 1.2	17.4 ± 3.8
I. aquatica	32	3.92 ± 0.15	14.6 ± 2.8	7.0 ± 1.8	7.6 ± 3.5
I. batatas	30	3.23 ± 0.22	29.1 ± 2.6	2.9 ± 0.8	26.2 ± 2.4
I. carnea	29	3.11 ± 0.13	44.6 ± 3.4	3.0 ± 1.2	41.5 ± 3.9
I. hederifolia	30	3.30 ± 0.13	30.4 ± 3.2	11.6 ± 1.8	18.8 ± 4.2
I. pandurata	15	3.53 ± 0.27	27.7 ± 5.5	4.0 ± 1.6	23.7 ± 5.8
I. nil	30	4.53 ± 0.23	36.8 ± 3.3	14.4 ± 2.7	22.4 ± 5.4
I. pes-caprae	27	2.36 ± 0.12	34.4 ± 3.4	8.4 ± 1.8	25.9 ± 4.2
I. purpurea	30	3.52 ± 0.12	16.7 ± 3.0	5.3 ± 1.6	11.4 ± 3.6
I. setosa	32	3.78 ± 0.20	18.9 ± 2.8	3.1 ± 1.2	15.8 ± 3.0
Blank [b]	15	0.00 ± 0.00	5.6 ± 1.6	3.2 ± 1.4	2.4 ± 2.0

[a] Attraction index (see text for explanation).

[b] Both choices chambers were empty; one side was randomly designated as control and the other plant in each trial.

Terpenoids are a large, heterogeneous group of organic compounds based on a branched C_5 building block. Those of both lower [hemiterpenes (C_5), monoterpenes (C_{10}) and sesquiterpenes (C_{15})] and higher molecular weights [biterpenes (C_{20}) and triterpenoids (C_{30})] are widely distributed among plants and are frequently involved in modifying insect behavior (Harborne 1988, Metcalf 1987, Pant & Rastogi 1979). Several of these compounds are known to deter leaf-feeding insects (e.g., Howard *et al.* 1989). More importantly, several sesquiterpenes are known to act as kairomones, and help insect herbivores locate their host plants (Metcalf 1987).

The volatile fractions of storage roots and leaf surfaces of *I. batatas* contain several sesquiterpenes (Figure 12.3). Five of these have been identified as copaene, trans-caryophyllene, gamma-humulene, gamma-cadinene, and gamma-elemene (Nottingham *et al.* 1989a). There are distinct differences between roots and leaves in the concentrations of these compounds (Nottingham *et al.* 1989a). Nottingham *et al.* (1988) and Son (1989) also found large differences in the leaf-surface chemicals between cultivars with differing susceptibilities to *C.*
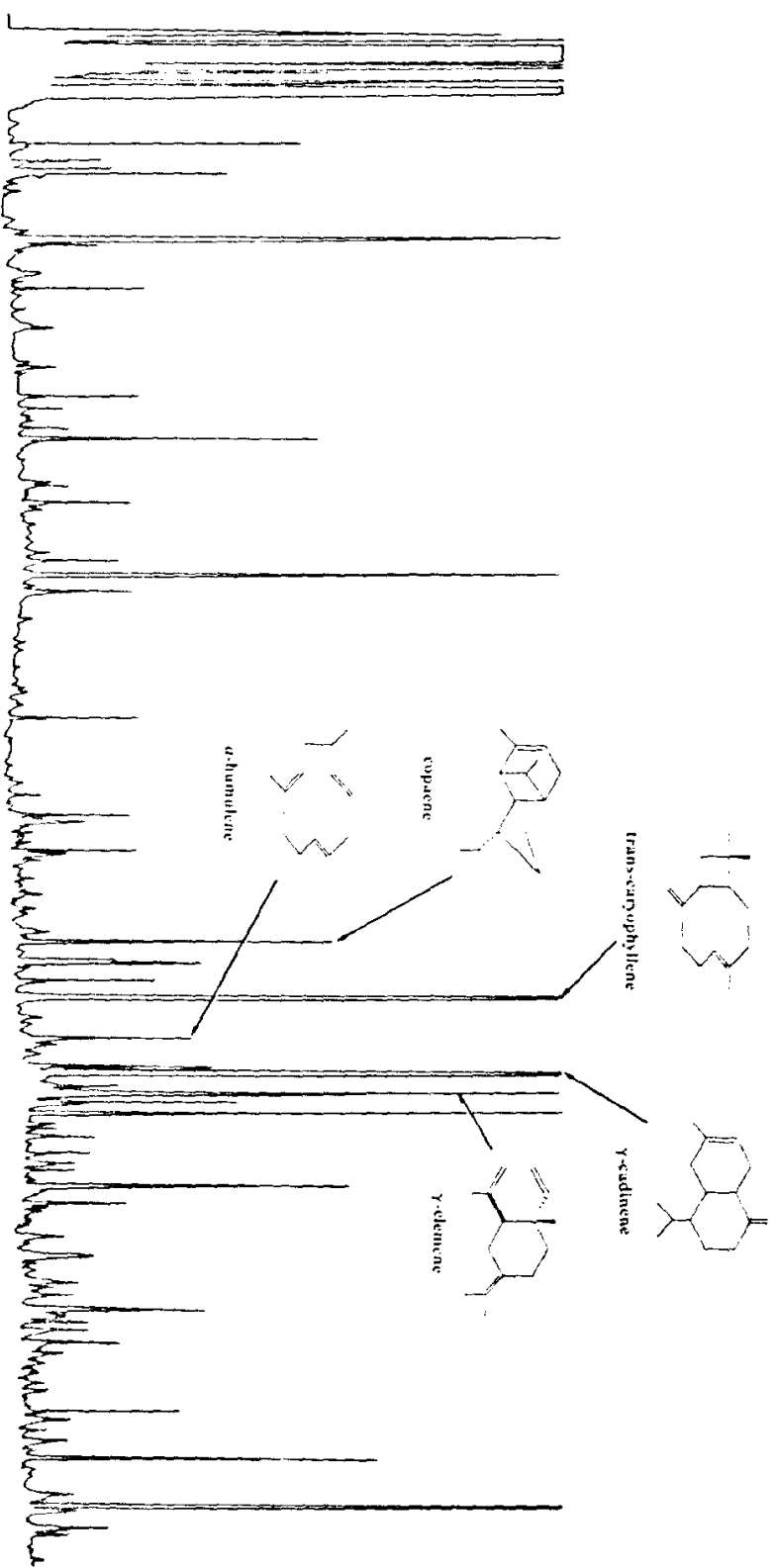
Fig. 12.3. Gas chromatogram of methylene-chloride extract from the surface of *I. batatas* (cv. Jewel) leaves. Sesquiterpenes identified in the volatile component are identified adjacent to their respective peaks.

formicarius. More work is needed, however, to determine the chemical(s) that attract the weevil to its host plants.

CONCLUSIONS

Investigations of the volatile chemistry of *Ipomoea* and its impact on insect behavior can be largely characterized as basic research. How an insect chooses among several potential host plants with differing apparency and unequal value as breeding sites is a compelling question. Comparative assays and chemistry in the *Ipomoea/C. formicarius* system can contribute to the elucidation of this more general problem. From a practical standpoint, volatile attractants from *Ipomoea* may be useful for trapping *C. formicarius* as part of an integrated pest management program. Current work with volatile attractants utilizes the female-produced sex pheromone of *C. formicarius* (see Chapters 5 and 6); however, this chemical only traps adult males. Since certain leaf volatiles from *I. batatas* attract both males and females, their identification and use may enhance the sex pheromone-based trapping systems currently in use world-wide.

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