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OVIPOSITIONAL RESPONSE OF THREE *Heliothis* SPECIES (LEPIDOPTERA: NOCTUIDAE) TO ALLELOCHEMICALS FROM CULTIVATED AND WILD HOST PLANTS[1]

E.R. MITCHELL, F.C. TINGLE, and R.R. HEATH

Insect Attractants, Behavior, and Basic Biology Research Laboratory
Agricultural Research Service, U.S. Department of Agriculture
Gainesville, Florida 32604

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Abstract—The role of plant allelochemicals on the oviposition behavior of *Heliothis virescens* (F.), *H. subflexa* (Guenee), and *H. zea* (Boddie) was investigated in the laboratory using a "choice" bioassay system. Fresh young leaves of tobacco, *Desmodium tortuosum* (Swartz) de Candolle, groundcherry (*Physalis angulata* L.), and cotton (*Gossypium hirsutum* L.) squares (flower buds) were washed in methylene chloride or methanol, concentrated to 1 g equivalent of washed material, and applied to a cloth oviposition substrate. Each of the extracts—including groundcherry, a nonhost—stimulated oviposition by *H. virescens*. *H. subflexa* were stimulated to oviposit by groundcherry extract, its normal host, and extract from cotton squares, a nonhost. None of the extracts stimulated oviposition by *H. zea*, although all except groundcherry were from reported hosts. The sensitivity of the bioassay was confirmed by giving *H. virescens* and *H. subflexa* an opportunity to choose between extracts that showed stimulant qualities when tested independently versus only solvent-treated controls. In these tests, tobacco showed the highest level of stimulant activity for *H. virescens*; groundcherry exhibited the highest level of stimulation for *H. subflexa*.

Key Words—Cotton, groundcherry, tobacco, *Gossypium*, *Desmodium*, *Physalis*, plant–insect interaction, host-plant resistance, *Heliothis* spp., Lepidoptera, Noctuidae, oviposition stimulant, oviposition deterrent.

[1]This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation of its use by USDA.

INTRODUCTION

Incorporation of factors into cultivated plant species that reduce or eliminate insect pest populations is an efficient and economical approach to the management of crop insect pests. Although there have been some dramatic demonstrations of the effects of plant resistance on the management of insect pests, progress has been extremely slow in some crops because of the empirical selection process that often requires many years of observation and experimentation before a cultivar can be released to growers. It now is generally believed that chemicals are the basis for many plants' defense against insect attack and that the chemical defense systems are composed of not one but a spectrum of chemical compounds (Alborn, 1988, and references therein). Although plant breeders have been successful in producing single-gene resistant material, little is known about plant chemicals imparting resistance in plants. To become more specific in breeding efforts, breeders must know how the behavior and biology of the insect pest is influenced by the plant's defense system, and how it can be modified to obtain an increased and more durable resistance.

Heliothis spp. rank high among the most destructive crop pests in the United States and around the world. Mated females of *Heliothis* spp. typically deposit their eggs on favored host plants (Johnson et al., 1975; Roome, 1975). Upon hatching, the feeding larvae often inflict extensive damage to flowers and fruit, resulting in millions of dollars in crop losses and control costs. The basis for the females of *Heliothis* spp. choosing one plant species or cultivar over another is poorly understood. Recently, Jackson et al. (1984a) showed that cuticular components from green leaves of a typical flue-cured tobacco (NC 2326), *Nicotiana tabacum* L., stimulates oviposition by *H. virescens* (F.) (Hv). Further, in comparative choice tests in oviposition cages, Hv females consistently oviposited fewer eggs on a primitive tobacco introduction, TI 1112, than on NC 2326 (Jackson et al., 1983). The primitive tobacco's "resistance" to oviposition by Hv is due, at least in part, to the absence or greatly reduced level of oviposition stimulant compounds present in the cuticular washes from the oviposition "susceptible" tobacco, NC 2326.

Johnson et al. (1975) reported on the ovipositional response of *H. zea* (Boddie) (Hz) to different phenological states of its major host plants in North Carolina. They showed that the flowering periods are the most preferred phenological states for corn, cotton, tobacco, and soybean, although Hz and other *Heliothis* spp. will oviposit on certain host plants in the absence of fruiting or flowering structures. Roome (1975) reached similar conclusions concerning the oviposition behavior of *H. armigera* (Hübner) (Ha) in corn and sorghum. He also suggested that susceptible crops attract adults and that, once within the crop, mated females are "trapped" by suitable physiological cues from the plants.

Identification of the chemical cues from plants that affect oviposition could assist in the breeding of crop cultivars resistant to *Heliothis* attack. The present study was conducted to determine the effect of cuticular washes from host and nonhost plants on the oviposition behavior of Hv, Hz, and *H. subflexa* (Guenee) (Hs). Unlike the polyphagous Hz, Ha, and Hv that attack a wide variety of cultivated crops, Hs is a monophagous species feeding exclusively on groundcherry, *Physalis* spp. (Brazzel et al., 1953). Groundcherry generally is regarded as a weed in the United States, but a commercial cultivar of "tomatillos" (*Physalis ixocarpa* Brot.) is widely grown in Mexico (Saray and Loya-Ramirez, 1978).

METHODS AND MATERIALS

Bioassay. The lack of suitable analytical chemical methods and appropriate bioassay techniques have heretofore hindered the study of insect–plant interactions relative to host finding and oviposition behavior. The methodology traditionally used in the study of insect–plant chemical relationships generally requires large quantities of plant material. For these bioassays, a recently developed olfactometer (Mitchell and Heath, 1987) was used that permits study of the effects of plant allelochemicals on insect oviposition behavior throughout the year in a controlled environment.

The bioassay chambers were located in an environmentally controlled room 3 m long, 2.6 m wide, and 2.1 m high. The room was equipped with an electric timer to turn overhead fluorescent lights (two banks of two bulbs, 40 W each) on and off; a separate timer was used to turn the red lights over the individual chambers on and off. A heat pump–air conditioner controlled room temperature at ca. 28.6°C. A room humidifier maintained the relative humidity at ca. 50%.

Test Insects. All test insects were reared in the laboratory on a modified pinto bean diet using methods described by Guy et al. (1985), King and Moore (1985), and Mitchell et al. (1988). The pupae of each of the three species—Hv, Hz, and Hs—were sexed and held separately in 3.8-liter paper cartons with screened tops until emergence. Upon emergence, females and males (21 : 14) were confined together for mating in 3.8-liter cartons for two days. Test insects were not routinely sacrificed to check mating status. However, periodic checks were made on the mating of sacrificed moths, and >90 were found to be mated as evidenced by the presence of a spermatophore in the bursa copulatrix. The pupal emergence and mating cages were held under a reverse 14 : 10-hr light–dark cycle in holding rooms maintained at ca. 28°C and 50–60% relative humidity.

Plant Material. One hundred grams of fresh cotton squares (i.e., flower buds), whole leaves of cotton (variety McNair 220), tobacco (susceptible, NC

2326; resistant, TI 1112), groundcherry (*Physalis angulata* L.), and *Desmodium tortuosum* (Swartz) de Candolle were washed in 500 ml solvent for 30 sec. *D. tortuosum* is an important late-season weed host for Hv and Hz in the Florida–Georgia tobacco belt (Jackson et al., 1984b; Jackson and Mitchell, 1984). The whole leaf wash was filtered through a white Viva paper towel into 1-liter containers, which were then capped and stored at 10°C until needed (1–6 months) (Mitchell and Heath, 1987).

The tobacco plants used were in the prebloom stage; the cotton, groundcherry, and *Desmodium* plants were in the flowering stage. All plant material was grown outdoors under natural light in small field plots. The leaves selected for washing were fully expanded yet tender and located on the upper half of the plants near flowering points. All plant materials except groundcherry were washed in methylene chloride; groundcherry was washed with methanol. Preliminary trials with whole-leaf washes showed that methanol was ineffective for extracting stimulant materials from cotton, tobacco, and *D. tortuosum*. Similarly, methylene chloride was ineffective for extracting oviposition stimulant compounds from groundcherry leaves (Mitchell and Heath, 1987).

Test Procedure. For testing, the plant extracts were concentrated in a rotary evaporator. A 1-g equivalent of whole leaves or squares in 1 ml of solvent was pipetted in a circular pattern onto the center of a piece of white broadcloth, the oviposition substrate (Mitchell and Heath, 1987). Broadcloth treated with 1 ml solvent only was used as the control. New cloths were used in every test. In preliminary tests, cloths treated with methylene chloride or methanol had no effect on egg deposition when compared to cloths without these solvents. After drying, the cloths were fitted over the open end of opposing collars and secured in position with a plastic O-ring. The other two openings in the chamber top were plugged with clear plastic cups. A cotton ball soaked with 10 ml water was then placed on the top of each cloth over the treated area and covered with a paper cup. Pretest measurements showed that the paper cup had no significant effect on air flow.

Each test chamber contained six Hv or Hs females or three Hz females. Fewer Hz females were used because of its more prolific egg-laying habit compared to Hv and Hs. The females were mated, as described, before being placed in the chambers. Periodic checks on the mating status of moths made throughout the course of the study showed that $>90\%$ of the females had mated after two days of confinement with males.

The females were preconditioned through one complete light–dark cycle in the test chambers before being exposed to the test materials; thus, the females were 3 days old when first subjected to treatment. Each test was usually repeated the following day (moths 4 days old) and sometimes on the third day (moths 5 days old), after which the females were removed from the test chambers. The position of the treatments in each compartment were alternated daily. The moths

were supplied daily with fresh food (10% honey–water solution on a saturated cotton ball in a paper cup placed in the chamber).

Choice tests also were conducted using either two plant extracts or three extracts and a control cloth in the same compartment. Dual-choice opportunities were presented to both Hv and Hs; the four-choice arrangement was presented only to Hv. Treatments in the dual-choice tests were alternated daily as described. Treatments involving four choices were randomly positioned in each compartment at the start of the test. The treatments then were rotated clockwise daily until each treatment (i.e., extract) was exposed once at each of the four ports in the test compartment. Freshly treated cloths were used daily in all tests.

The tests were carried out under a reverse photoperiod of 14 : 10 hr light–dark. Treatments were applied at 1200 hr. The test cloths were removed 4 hr later, and the eggs were counted. Treatment means were separated using the paired *t* test (Steel and Torrie, 1960) or Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Each extract tested—including groundcherry, a nonhost—stimulated oviposition by Hv (Figure 1). Hs adults were stimulated to oviposit by groundcherry extract and cotton squares (Figure 2). The positive oviposition response of Hv to washes from known host plants (cotton, tobacco, and *D. tortuosum*) is consistent with their polyphagous habit.

Laster et al. (1982) reported on the host acceptance and development of Hs, Hv, their hybrid, and backcross progeny on several plant species. In their study, none of the Hv larvae placed on leaves of groundcherry survived. Similarly, none of the Hs larvae placed on cotton leaves survived (Laster et al., 1982). These results suggest that although these species possibly may be stimulated to oviposit on nonhosts under forced conditions, e.g., Hv on groundcherry and Hs on cotton leaves, it is unlikely that any resultant larvae would survive in nature.

Cloths treated with extract from groundcherry and cotton squares had significantly fewer Hz eggs compared to solvent-treated control cloths. The chemical basis for this deterrence is unknown. However, among other moth species it is not unusual for chemicals extracted from nonhost and host plants to exhibit varying levels of deterrence by ovipositing females when sprayed onto otherwise acceptable host plants (Tingle and Mitchell, 1984, 1986; Mitchell and Heath, 1985; Williams et al., 1986).

The sensitivity of the bioassay was further demonstrated by giving Hv an opportunity to choose to oviposit between extracts of susceptible tobacco (NC 2326) and resistant tobacco (TI 1112) (Jackson et al., 1984a). In our test, Hv

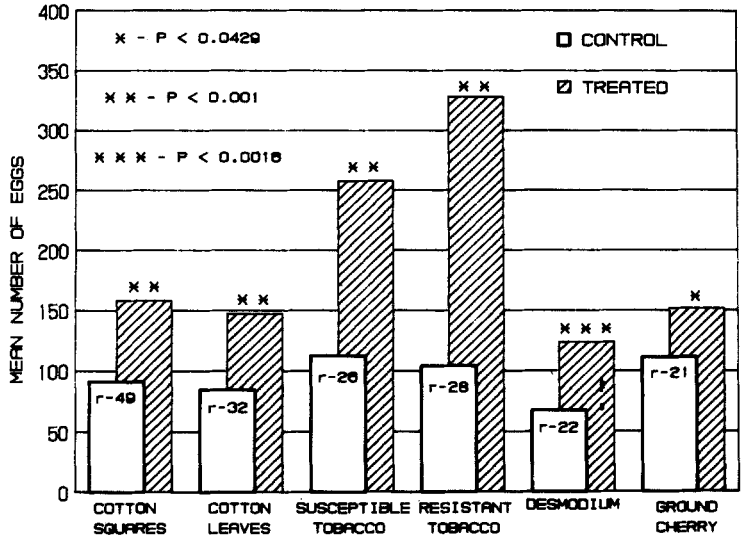

FIG. 1. Effect of plant extracts on the oviposition response of *Heliothis virescens* in laboratory bioassays. The *r* values represent the number of replications per test. Asterisks indicate the level of probability between means, paired *t* test, (Steel and Torrie 1960).

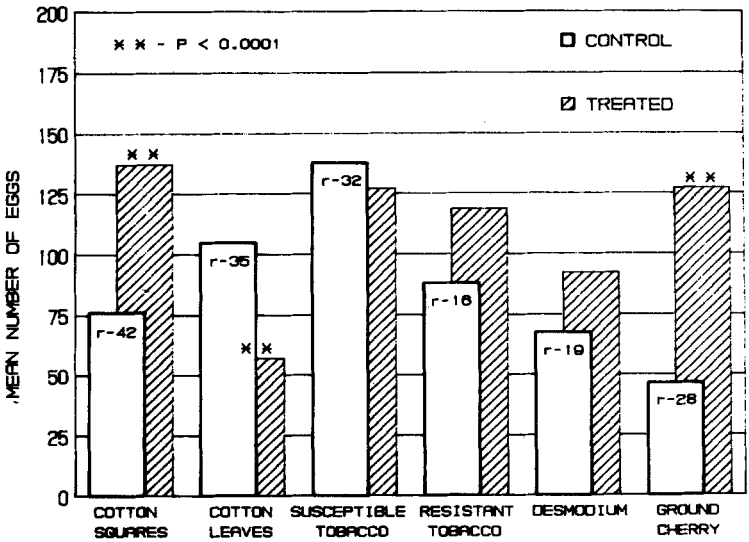

FIG. 2. Effect of washes from leaves and cotton squares (flower buds) on oviposition by *Heliothis subflexa*. The *r* values represent the number of replications per test. The asterisks indicate the level of probability between means; absence of asterisks indicates no significance between means, paired *t* test (Steel and Torrie, 1960).

laid more eggs on cloths treated with susceptible extract than on cloths treated with resistant tobacco extract (Figure 3). These results are consistent with those of Jackson et al. (1983), who conducted similar competitive tests outdoors in small field cages. It should be noted that the moths had free access to both treated and untreated cloths (this study) or treated and untreated plants (Jackson et al., 1983). Thus, the moths could either accept or reject each cloth or plant in a sequential manner. In oviposition tests such as these, it is the final egg numbers that are tallied and analyzed. These sorts of tests do not differentiate between olfactory and tactile stimuli. Moths may be attracted to a source over a distance—albeit small—but simply not oviposit on it. On the other hand, moths also may find a nonattractive substrate (cloth or plant) by accident but be highly stimulated to oviposit upon making contact.

In other dual-choice tests using susceptible tobacco (NC 2326) as the standard, there was no significant difference in the mean number of eggs deposited by Hv on cloths treated with tobacco extract or groundcherry extract (Figure 3). However, cloths treated with extract from cotton leaves received only ca. 50% of the number of eggs deposited on cloths treated with tobacco leaf extract. By contrast, cloths treated with *Desmodium* extract showed a slight but significant increase in the number of eggs deposited on them compared to cloths treated with tobacco extract (Figure 3).

Initial experiments with Hs suggested that cotton squares possessed ovi-

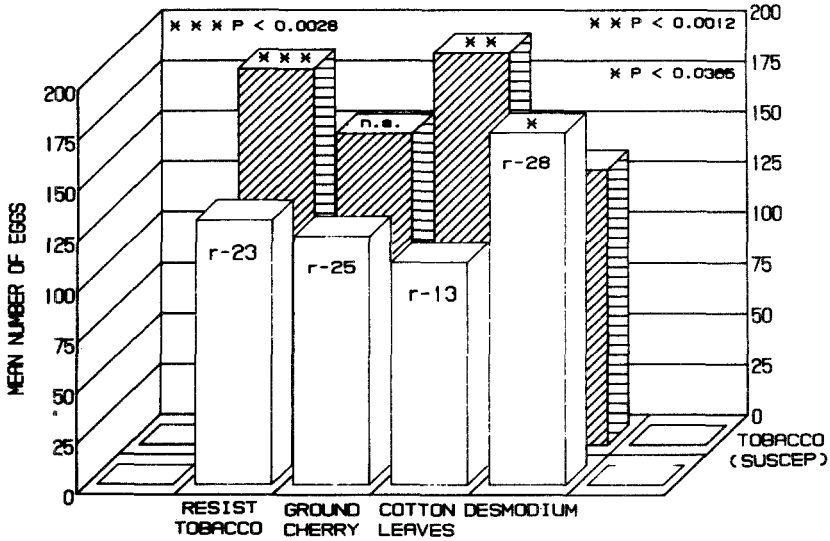

FIG. 3. Oviposition response of *Heliothis virescens* exposed simultaneously to two plant extracts. The *r* values represent the number of replications per test. Asterisks designate the level of significance between treatment means; n.s. indicates that there was no significant difference, paired *t* test (Steel and Torrie, 1960).

position stimulant qualities comparable to groundcherry, the only known host for this species (Figure 2). However, results of dual-choice tests with groundcherry and cotton squares or *Desmodium* clearly showed that this was not the case. In these two tests, cloths treated with groundcherry extract received ca. 70% of all the eggs deposited by Hs.

Results of the test in which Hv were exposed simultaneously to an untreated control and extracts from susceptible tobacco (NC 2326), *Desmodium*, and groundcherry are shown in Figure 4. When tested independently, each of the three extracts showed a significant increase in oviposition by Hv when compared to solvent-treated control cloths (Figure 1). The differences noted in egg deposition in the multiextract test were highly significant and consistent with the results of the tests involving a single treatment versus control cloths. The positive ovipositional response recorded from Hv from whole-leaf wash of susceptible tobacco, NC 2326, probably is due to the presence of duvane diterpenes secreted from leaf trichomes (Jackson et al., 1986). Conversely, the ovipositional nonpreference exhibited by Hv towards TI 1112 probably was due to the lack of or reduced level of duvane diterpene secretions.

It is possible that the stimulatory effects of the various extracts on egg-laying activity by Hv were caused by the same compounds or compounds similar to those found in tobacco, i.e., duvane diterpenes. A more realistic assump-

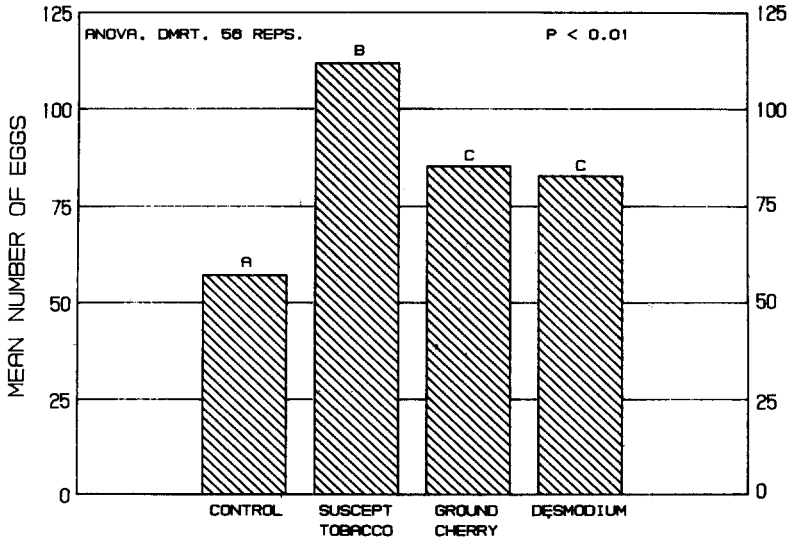

FIG. 4. Oviposition response of *Heliothis virescens* when exposed simultaneously to three plant extracts and a control. The *r* values represent the number of replications per test. Means with the same letter are not significantly different, Duncan's multiple-range test (Duncan, 1955).

tion, however, would appear to be that Hv are stimulated to oviposit by several different compounds. Such diversity in stimulant chemicals would have obvious advantages to an insect such as Hv, which is dependent for its survival upon many species of annual host plants.

The stimulatory oviposition response recorded for Hv from groundcherry extract (Figure 1) lends further support to the hypothesis that different species of plants may have different chemicals that elicit the same type of behavioral response, e.g., increased egg-laying. There is no evidence, however, that Hv oviposits on groundcherry plants in nature.

The lack of a stimulatory egg-laying response by Hz to any of the plant extracts was surprising, especially since all but groundcherry are known hosts. Johnson et al. (1975) reported on the ovipositional response of Hz to several crop hosts including tobacco and cotton. Comparing the flowering states of the crops, the maximum ovipositional response decreased in the following order: corn > tobacco > soybean > cotton. Their results clearly showed that Hz exhibited a strong ovipositional preference for flowering corn over tobacco, soybean and cotton.

Several researchers have cited chemical factors from corn silks as possible attractants or oviposition stimulants for Hz (Wiseman et al., 1988, and references therein). We did not test extracts of corn silk in our bioassay apparatus. Failure of our bioassay to demonstrate the presence of oviposition stimulating compounds for Hz in extracts from cotton and tobacco does not preclude their presence. Clearly, the work of Johnson et al. (1975) suggests that cotton and tobacco do have such compounds. All of the extracts used here were tested at only one dosage level—1 g equivalent of whole leaf or square wash. Thus, it is possible that the dosage used in these tests was below some as yet unknown critical threshold level for oviposition stimulant activity. It is also possible, of course, that Hz females simply did not respond "normally" in the confines of our bioassay apparatus.

Recent reports by Rembold and Tober (1985) showed that Ha females responded differentially in oviposition trials to odors emanating from two cultivars of pigeonpea, *Cajanus cajan* L. Millsp. Similarly, Tingle et al. (1989) found that Hs mated females exhibited positive flight responses to odors emanating from extracts of leaves of its host, groundcherry. Likewise, we have found that Hv females are attracted over a distance to volatile chemicals released from extracts from cotton, tobacco, and *Desmodium* (unpublished data).

Clearly oviposition per se is but one of a complex of behavioral events regulating the selection and successful colonization of host plants by insects. Nevertheless, identification of the chemicals stimulating oviposition in Hv presents opportunities for characterization of the behavioral and physiological factors regulating this essential process in the life cycle of this important pest. Further, it is conceivable that such chemicals may be greatly reduced or elim-

inated from otherwise desirable cultivars through genetic manipulations, thereby imparting a degree of "resistance" to attack by *H. virescens*.

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