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CHEMICAL STIMULANTS AND DETERRENTS REGULATING ACCEPTANCE OR REJECTION OF CRUCIFERS BY CABBAGE BUTTERFLIES

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Abstract—Gravid *Pieris rapae* butterflies oviposit on many, but not all, crucifers. Rejection of *Erysimum cheiranthoides* and *Capsella bursa-pastoris* was initially explained by the presence of chemical deterrents in the plants. Analyses and bioassays of plant extracts indicated the absence of oviposition stimulants in *C. bursa-pastoris*, but similar chemical separation of *E. cheiranthoides* extracts revealed the presence of stimulants as well as deterrents. Choice tests illustrate how acceptance or rejection of a plant by an insect may depend on the balance of positive and negative chemical stimuli within the plant.

Key Words—Cabbage butterfly, *Pieris rapae*, Lepidoptera, Pieridae, oviposition, stimulants, deterrents, *Erysimum cheiranthoides*, *Capsella bursa-pastoris*.

INTRODUCTION

The host ranges of phytophagous insects are determined to a large extent by the presence or absence of specific chemicals in potential host plants (Thorsteinson, 1960; Städler, 1976). Discriminatory behavior has been linked to olfactory or contact chemoreception of attractants, repellents, stimulants, and deterrents (Dethier, 1947; Schoonhoven, 1968). The final response of an insect in accepting or rejecting a particular plant is thought to be mediated by a balance of sensory inputs from these positive and negative chemical stimuli in the plant (Dethier, 1982; Miller and Strickler, 1984). However, definitive proof of such a dynamic relationship has been difficult to obtain. One major problem is to determine whether a nonhost plant is avoided by a herbivore because of the presence of deterrents or a lack of stimulants.

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The cabbage butterfly, *Pieris rapae* L., specializes on members of the Cruciferae (= Brassicaceae) and a few related plant families that contain mustard oil glycosides (Verschaffelt, 1911; Ma and Schoonhoven, 1973). Yet several crucifers are unacceptable to this insect (Feeny, 1977), and the chemical basis of such discriminatory behavior is not clear. Previous studies on *P. rapae* have shown that recognition of host plants by ovipositing butterflies depends on the presence of water-soluble chemical stimulants that are detected by tarsal contact with the plant (Traynier, 1979; Renwick and Radke, 1983), but host plants may also contain lipid-soluble deterrents (Renwick and Radke, 1985). Under natural conditions, these compounds apparently do not interfere with recognition of host plants, probably because their concentration on the surface of undamaged leaves is negligible. However, in nonhost plants, additional water-soluble deterrents are present, and these could be responsible for rejection by gravid butterflies (Renwick and Radke, 1985).

The crucifers that are unacceptable to *P. rapae* include *Erysimum cheiranthoides* L. and *Capsella bursa-pastoris* L. Both of these species contain water-soluble deterrents to oviposition (Renwick and Radke, 1985). The study reported here was designed to determine whether these deterrents alone can explain avoidance of the plants, or if the stimulants necessary for host recognition by cabbage butterflies are lacking.

METHODS AND MATERIALS

Insects and Plants. Butterflies used for behavioral assays were from a colony started from field-collected insects each summer and maintained on cabbage plants. Seeds of *E. cheiranthoides* and *C. bursa-pastoris* were collected in Vermont and Connecticut, respectively, by Dr. Frances S. Chew.

Extraction of Plant Materials. Plants were grown from seed under uniform conditions in the greenhouse. After three to four weeks, leaves were harvested and immediately dropped into boiling ethanol to minimize enzyme degradation of constituents. After cooling, the tissue was homogenized in a Waring blender and the resulting macerate filtered through glass wool. The ethanol extract was evaporated to dryness and the residue was sequentially washed with hexane and water. The water extract was filtered, evaporated to 150 ml, and extracted three times with *n*-butanol. Standard cabbage extracts were prepared using the same sequence of boiling ethanol, homogenization, filtration, evaporation, lipid removal, and water extraction.

Stimulant Bioassays. The presence of stimulant was detected using artificial plants consisting of 77 × 64-mm green index cards supported on wooden stems (Renwick and Radke, 1983). The cards were painted with 0.5 ml extract at a concentration of 5 g original fresh wt/ml to approximate the concentration of material present in a plant. Control cards were painted with solvent alone. Five pairs of butterflies were presented with one test card surrounded by three

control cards in a 60 × 60 × 60-cm cage in a greenhouse with supplemental lighting. Eggs laid on each card were counted after 24 hr. The stimulatory effect was measured by comparison with that of standard cabbage extract offered to the same butterflies on preceding and following days. Control cards were included to ensure that no eggs were laid on substrates that lacked stimulant.

Deterrent Bioassays. Deterrent activity was assayed using identical cages in the greenhouse. Five pairs of butterflies were offered a choice of two cabbage plants. One was sprayed with 3 g equivalents (original fresh weight) of test plant extract in 100% or 70% methanol (depending on solubility). The control plant was sprayed with methanol alone. The plants were left in the cage for 4 hr during the peak daily oviposition period before counting the eggs.

RESULTS

Fractionation of polar extracts of the test plants by partitioning between water and *n*-butanol resulted in transfer of the deterrent into the butanol (Figure 1). The butanol extracts of both *E. cheiranthoides* and *C. bursa-pastoris* were just as deterrent as the original water extract, whereas the activity was almost completely removed from the postbutanol aqueous layer (Figure 1).

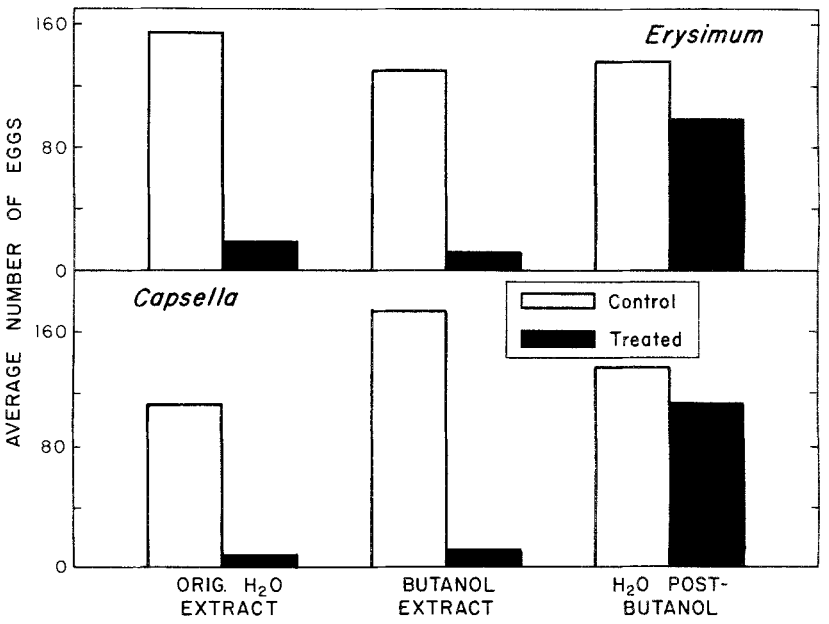

FIG. 1. Oviposition by *P. rapae* on cabbage plants treated with extracts of *Erysimum cheiranthoides* and *Capsella bursa-pastoris* or with solvent alone (controls) in choice assays.

TABLE 1. OVIPOSITION BY *P. rapae* ON CARDS TREATED WITH FRACTIONS OF EXTRACTS FROM *Erysimum cheiranthoides* AND *Capsella bursa-pastoris* COMPARED WITH STANDARD STIMULANT (H_2O EXTRACT OF CABBAGE)

Test material	No. of replications	Average No. eggs laid			Relative stimulation (%)[a]
		Day 1 (cabbage standard)	Day 2 (test extract)	Day 3 (cabbage standard)	
Erysimum cheiranthoides					
Orig. H_2O extract	7	92.7 (35.5)[b]	42.0 (51.8)	56.9 (27.7)	56
H_2O postbutanol	6	58.3 (28.3)	37.5 (40.2)	82.2 (28.8)	53
Butanol extract	9	76.5 (30.4)	27.0 (24.6)	68.1 (43.8)	37
Capsella bursa-pastoris					
Orig. H_2O extract	7	101.0 (38)	2.3 (3.5)	106 (23.5)	2.2
H_2O postbutanol	6	82.6 (17.1)	1.3 (2.8)	86.5 (27.6)	2.0
Butanol extract	5	137.6 (52.5)	0.6 (1.3)	154.2 (83)	0.4

[a]Relative stimulation (%) = No. eggs laid {day 2/[(day 1 + day 3)/2]} × 100, i.e., oviposition relative to mean oviposition on previous and following days in response to standard cabbage stimulant.
[b]Standard deviation is given in parentheses.

The same fractions from both plants were tested for stimulatory activity using artificial leaf bioassays. Activity was measured by comparison with standard cabbage extracts of known activity. The effect of possible changes in the butterflies' potential to lay eggs was eliminated by offering cabbage standard on days preceding and following the tests (Table 1). The results of these experiments clearly showed that the water-soluble fraction of *E. cheiranthoides* contains stimulant. Although less active than equivalent concentrations of cabbage extracts, this material was stimulatory before and after partitioning with butanol (Table 1). Some oviposition also occurred on cards treated with the butanol extract of *E. cheiranthoides* because of partial removal of the stimulant during the water–butanol partitioning.

Extracts of *C. bursa-pastoris* showed no significant stimulatory activity in any of the tests (Table 1).

DISCUSSION

The results indicate that the chemical explanation for avoidance of these two crucifers by cabbage butterflies is not the same. Since extracts of *C. bursa-pastoris* are not stimulatory, the presence of deterrent is not necessary for rejection of this plant. The lack of stimulant alone could account for unacceptability to landing butterflies. However, the presence of stimulant in *E. cheiran-*

thoides means that oviposition might be expected on this plant. Apparently the negative effect of the oviposition deterrent is sufficient to block the positive input from the stimulants. This result supports the suggestion of Jermy (1965) that the most potent stimuli inducing oviposition can be masked by inhibitory substances at an appropriate concentration.

Care is needed in interpretation of such laboratory results, especially when attempting to explain the factors that mediate oviposition in the field. Our green cards treated with standard cabbage extract are not as attractive as a host plant of comparable size. However, the concentrations of extracts used in these experiments were selected to represent levels of chemical stimuli comparable to those encountered by butterflies under natural conditions. Our efforts to mimic real plants are further complicated by the fact that little is known about the distribution of chemicals throughout the leaf. Since the ovipositional cues are contact stimuli, the active chemicals must be present at the leaf surface. Yet we have been unsuccessful in attempts to remove stimulant from cabbage leaves by solvent dipping (unpublished results). Despite these limitations in interpretation, we feel confident in concluding that we can readily determine whether stimulants are present in a plant. *P. rapae* does not lay eggs on blank green cards or cards that are treated with extracts of noncrucifer, nonhost plants (unpublished results).

The use of separate bioassay systems to detect oviposition stimulants and deterrents appears to be critical. The stimulant assay is particularly sensitive, and the use of three control cards in each cage emphasizes the ability of the butterflies to discriminate between low concentrations of active material or solvent alone (Renwick and Radke, 1983). Butanol extracts of *E. cheiranthoides*

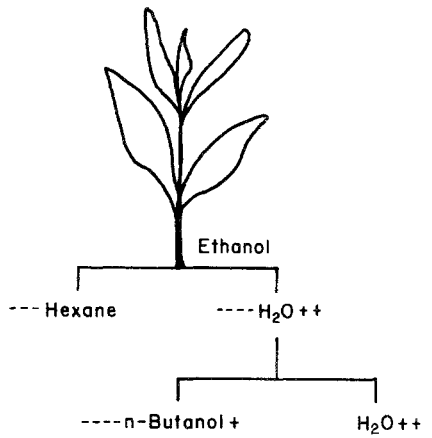

FIG. 2. Schematic representation of stimulant (+) and deterrent (−) activity of fractions from *Erysimum cheiranthoides* extracts affecting oviposition by *P. rapae*.

were slightly stimulatory when tested on green cards, even though most of the stimulant remained in the water fraction and despite the presence of deterrent (Table 1). When the same material was applied to cabbage plants, the deterrent effectively blocked the natural stimulant at the surface of the leaves (Figure 1). The reason for the lack of sensitivity to deterrent on the inert substrate provided by cards is not clear at this time. But the phenomenon does lend support to our conclusion that *Capsella* contains little or no stimulant since, even if other deterrents were present, they would probably have little effect.

The sequential extraction and the use of different bioassays for the study of *E. cheiranthoides* have effectively removed the mask from the stimulant which would not otherwise be detected (Figure 2). This may be the first demonstration that both oviposition stimulants and deterrents can actually occur in a plant that is rejected by an insect. The balance of such positive and negative signals within a plant is likely to play a major role in the acceptance or rejection of potential hosts by an insect.

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