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Indican as a Feeding Stimulant to the
 Strawberry Leaf Beetle,
Galerucella vittaticollis BALY Attacking
 Polygonaceous Plants¹

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In previous papers, the role of plant chemicals in the host selection of Polygonaceae-feeding leaf beetles has been reported. The strawberry leaf beetle, *Galerucella vittaticollis* BALY, which feeds on plants of the family Polygonaceae and strawberry, *Fragaria chiloensis*, was stimulated to feed by some nutrient chemicals such as sugars and amino acids as well as flavonoids and esters of higher alcohols (MATSUDA and MATSUMOTO, 1974; MATSUDA, 1976; MATSUDA and SUGAWARA, 1978). The feeding of another beetle, *Gastrophysa atrocyanea* MOTSCHULSKY with a host range restricted to polygonaceous plants was stimulated by nutrient chemicals, flavonoids and organic acids (MATSUDA, 1981, 1976; MATSUDA and MATSUMOTO, 1975). The other two beetles, *Gallerucida nigromaculata* BALY and *G. bifasciata* MOTSCHULSKY, both feeding only on

Polygonaceae were stimulated to feed by flavonoids, organic acids and esters of higher alcohols (MATSUDA, 1976; MATSUDA and MATSUMOTO, 1975; MATSUDA and SUGAWARA, 1978). In the present study, the feeding stimulations of indican present in *Polygonum tinctorium* and related compounds were examined as the next step in understanding the host plant selection in Polygonaceae-feeding leaf beetles. The distribution of indican in Polygonaceae and *F. chiloensis* was also investigated.

MATERIALS AND METHOD

Insects. *Gal. vittaticollis* and *Galler. nigromaculata* used in the experiments were selected from laboratory colonies maintained on *Rumex obtusifolius*. Larvae of *Gastr. atrocyanea* were collected from *R. obtusifolius* and reared on the same plant to adults. Adults *Galler. bifasciata* were gathered from *Polygonum sachalinense* and reared on the same plant.

Chemicals. Indican, 3-indoxylsulfuric acid potassium salt and indoxyl acetate (Tokyo Kasei Kogyô Co., Ltd.) and 3-indole acetic acid, indigo, indole and isatin (Wako Pure Chemical Ind. Co., Ltd.) were used for the experiments. The first compound was recrystallized from methanol prior to use to remove contaminants.

Bioassay. The effects of indican and related compounds on the feeding of adult leaf beetles were examined in the choice trial test. Adult beetles were

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Table 1. Effects of indican and related compounds on the feeding of Polygonaceae-feeding leaf beetles^a

Compound	Concentration	Feeding response of			
		<i>Galerucella vittaticollis</i>	<i>Gastrophysa atrocyanea</i>	<i>Gallerucida bifasciata</i>	<i>G. nigromaculata</i>
Indican	0.1	SSS	N	N	N
	0.01	SSS	N	N	N
	0.001	SSS	N	N	N
3-Indoxylsulfuric acid potassium salt	0.1	SSS	N	N	N
	0.01	SSS	N	N	N
	0.001	SS	N	N	N

^a See the text for explanation of symbols "S" and "N."

released into a plastic petri dish (9 cm in diameter; 1.5 cm in depth) in which four pieces of filter paper (2 × 2 cm, 2: treated, 2: control) were placed. The degree of nibbling by the insects was compared after 24 hr between the pieces moistened with test chemical solutions and those with control solution. Details of the test have been described in a previous paper (MATSUDA, 1976). Since insects used for assays differed in physique and food consumption, test conditions (starvation period prior to the test, the number of released insects) varied with each species as follows: *Gal. vittaticollis* and *Galler. nigromaculata* (24 hr starvation; 20 insects were released); *Gastr. atrocyanea* and *Galler. bifasciata* (8 hr starvation; 15 insects were released). Comparisons were made at 3 concentration levels of 0.1, 0.01 and 0.001 M. Tests were replicated 5 times.

The feeding stimulation was judged from the differences in appearance of nibbled filter papers by insects between test and control pieces, and expressed by the number of Symbol "S." Symbol "N" was used to indicate no stimulative effect.

Detection of indican. The plants used in the chemical analysis were as follows: smartweeds, *Polygonum tinctorium*, *P. aviculare*, *P. blumei*, *P. nodosum*, *P. flaccidum*, *P. nepalense*, *P. thunbergii*, *P. sieboldi*, *P. perfoliatum*, *P. orientale*, *P. filiforme*, *P. cuspidatum*, *P. sachalinense*, buckwheat, *Fagopyrum esculentum*, docks, *Rumex acetosella*, *R. japonicus*, *R. obtusifolius*, *Rheum raphaniticum* and strawberry, *Fragaria chiloensis*.

Mature leaf samples were collected from the field. Fifty grams of finely chopped leaves was extracted with 500 ml of methanol for 24 hr, then extracts were filtered. The extraction was repeated two more times. After evaporation of the combined extracts under vacuum, the obtained syrup was dissolved in 100 ml of water and filtered. The

filtrate was washed with chloroform twice and extracted with 200 ml of ethyl acetate several times. The combined extract was evaporated under vacuum and residue was dissolved in a minimum amount of methanol. The crude ethyl acetate fraction and methanol extract were examined by silica gel 60 thin layer chromatography with indican as the control. The solvent was composed of chloroform: methanol (35:65) or chloroform: acetone (35:65). The indican was detected as a gray spot by spraying perchloric acid-ferric chloride reagent (BENNET-CLARK et al., 1952).

RESULTS

Effects of indican and related compounds

Effects of indican and related compounds on the feeding of Polygonaceae-feeding leaf beetles are summarized in Table 1. The feeding of the strawberry leaf beetle was strongly stimulated by indican at concentrations of 0.1, 0.01 and 0.001 M. Among the related compounds, only 3-indoxylsulfuric acid potassium salt elicited feeding at 0.1, 0.01 and 0.001 M. Indoxyl acetate, 3-indole acetic acid, indigo, indole and isatin did not show any stimulative effect. The feeding of *Gastr. atrocyanea*, *Galler. nigromaculata* and *G. bifasciata* was neither stimulated by indican nor its relatives.

Analysis of indican in Polygonaceae and F. chiloensis

Indican was detected in *P. tinctorium*, but in no other polygonaceous plants or *F. chiloensis*.

DISCUSSION

In the host selection of phytophagous insects, characteristic plant chemicals in the host plant sometimes play a significant role. Indican is obviously one of these chemicals in the strawberry

leaf beetles when they select smartweed, *Polygonum tinctorium* as a host. However, the feeding of *Gastr. atrocyanea*, *Galler. nigromaculata* and *G. bifasciata* was not stimulated by indican and related compounds, though they feed on *P. tinctorium*. As pointed out in previous papers (MATSUDA and MATSUMOTO, 1975; MATSUDA, 1978), organic acids such as oxalic, malic, tartaric and citric acid which are characteristic components of polygonaceous plants stimulated the feeding of the latter species, but not the former. To the contrary, the flavonoids which act as feeding stimulants for the strawberry leaf beetle were less effective on the feeding of the three other species. Taking these things into account, indican has the role of flavonoids in feeding stimulation in the strawberry leaf beetle, and the differences in host ranges between strawberry leaf beetle and the three other species may reflect the difference in their sensitivities to the three plant compounds.

Among the related compounds, only 3-indoxyl-sulfuric acid potassium salt showed an appreciable

effect on the strawberry leaf beetle. This compound occurs in the urine and blood plasma of mammals, but it has not been found in the plant kingdom. The feeding stimulative activity of 3-indoxylsulfuric acid potassium salt, therefore, seems to be due to its chemical structural resemblance to indican.

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Effect of Chlordimeform and Its Analogues on Wandering Behavior of the Cabbage Armyworm, *Mamestra brassicae* L. (Lepidoptera: Noctuidae)^{1,2}

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Chlordimeform has an antifeeding effect on some insect species, but the mechanism of this action is not well understood (DOANE and DUNBAR, 1973; HIRATA and SOGAWA, 1976; WATANABE and FUKAMI, 1977; NAKAMUTA and SAITO, 1977; LUND et al., 1979). LUND et al. (1979) reported that the *Manduca* larvae were not deterred from feeding, but rapidly showed fine tremors and dropped from the plants at chlordimeform levels much below acute

lethal doses. Recently, we have suggested that continuous repetitive bursts of mandibular movements induced by topical application of chlordimeform to the mouthparts disrupt feeding (SHIMIZU et al., 1981 a). Of the 11 compounds tested only 3 with 2-methyl substitution on the phenyl group evoked the continuous bursts (SHIMIZU et al., 1981 b). The present study has been undertaken to survey the structural requirements of chlordimeform analogues for wandering behavior of the cabbage armyworm, *Mamestra brassicae*.

Insect: The larvae of the cabbage armyworm, *Mamestra brassicae*, were reared at 24°C on an artificial diet under a 16L-8D photoperiod. The artificial diet consisted per 100 g of 50 g of powdered balanced mouse food, 20 g of soybean flour, 11 g of cellulose powder, 10 g of brewer's yeast and 9 g of agar. Wandering stage larvae were used in the present experiments.

Apparatus for monitoring wandering behavior: Apparatus as shown in Fig. 1. was devised to monitor wandering behavior. Wandering stage larvae were

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² This main points of this paper were presented at the annual meeting of the Japanese Society of Applied Entomology and Zoology (April 4, 1983, Tokyo).