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Effect of Coumarin and Related Compounds on Blister Beetle Feeding in Sweetclover

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Effect of Coumarin and Related Compounds on Blister Beetle[1] Feeding in Sweetclover[2]

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ABSTRACT

The ashgray blister beetle, *Epicauta fabricii* (LeConte); the striped blister beetle, *E. vittata* (F.); and the margined blister beetle, *E. pestifera* Werner, collected in the field from plants of sweetclover low in coumarin content, fed preferentially in the laboratory on low-coumarin leaves when offered a choice between leaves from high- and low-coumarin plants. The black blister beetle, *E. pennsylvanica* (De Geer), not known to feed on sweetclover foliage, also fed preferentially in the laboratory on leaves from low-coumarin plants, although the rate of feeding was much slower than for the other species.

By using appropriate genotypes of sweetclover and controlling the type of light to which the plants were exposed, it was possible to offer the beetles choices among leaves differing greatly in their content of specific coumarin-related compounds. Both *cis-o*-hydroxycinnamic acid (*cis-o*-HCA) glucoside and coumarin (the lactone form of *cis-o*-HCA) were strong feeding deterrents; *trans-o*-HCA glucoside had no significant deterring effect.

Sweetclover (*Melilotus* spp.) is one of a wide range of plants fed upon by blister beetles, *Epicauta* spp. No serious damage to sweetclover was observed until low-coumarin strains became available following the interspecific transfer of the gene conditioning low-coumarin content from a wild form to the cultivated white-flowered species, *M. alba* Desr. (Smith 1943). Preferential feeding by blister beetles on low-coumarin sweetclover plants was observed in the Great Plains region (Howe and Gorz 1960), but quantitative data on feeding differences were not obtained, nor were the species of *Epicauta* identified.

Although the term "coumarin" is used in the preceding paragraph, in reality, there is little if any coumarin in the intact sweetclover plant (Haskins and Gorz 1961). However, the term "coumarin" will be retained in some parts of this paper for convenience. The plant constituents previously assayed as coumarin are the glucosides of *cis-* and *trans-o*-hydroxycinnamic acid (*o*-HCA). Available evidence indicates that *trans-o*-HCA glucoside is synthesized in very young leaves and is then partially converted to the corresponding *cis* isomer by the action of UV light (Haskins et al. 1964). In plants grown in growth chambers equipped with cool-white fluorescent lamps (ca. 10,000 lux), most of the total *o*-HCA is present as the *trans*-glucoside, whereas similar chamber-grown plants exposed to sunlight for a few hours contain a preponderance of *cis-o*-HCA glu-

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coside. In those plants having a high content of *cis-o*-HCA glucoside as well as high activity of β-glucosidase (the enzyme which effects hydrolysis of *cis-o*-HCA glucoside), tissue disruption (such as that caused by blister beetle feeding) results in rapid hydrolysis of the *cis-o*-HCA glucoside to yield coumarinic acid. which lactonizes spontaneously to form coumarin (Kosuge and Conn 1961). However, no free coumarin formation would be expected during feeding on leaves containing only *trans-o*-HCA glucoside because the β-glucosidase in sweetclover does not hydrolyze the *trans* glucoside. Thus, by selecting proper genotypes and lighting conditions, leaves were obtained for use in feeding trials in which the effect of each glucoside could be assessed and the added effect of coumarin could be measured.

The objectives of this study were to (1) measure the relative amount of feeding by various *Epicauta* species among lines of sweetclover differing in content of *o*-HCA glucosides and the presence or absence of β-glucosidase activity, and (2) determine the deterring action of coumarin and the glucosides of *cis*- and *trans-o*-HCA on beetle feeding.

MATERIALS AND METHODS.—In 1970 and 1971, collections were made of the ashgray blister beetle, *E. fabricii* (LeConte); margined blister beetle, *E. pestifera* Werner; striped blister beetle, *E. vittata* (F.); and black blister beetle, *E. pennsylvanica* (De Geer)[6] The 1st 3 species were collected from low-coumarin sweetclover plants (variety 'Denta') and the black blister beetles were found on pigweed, *Amaranthus* spp.

Near-isogenic lines of biennial white-blossomed sweetclover (*M. alba*) of the *CuCuBB*, *CuCubb*, *cucuBB*, and *cucubb* genotypes were used. The derivation of these genotypes was described by Gilchrist et al. (1970). In commenting on the review of Whittaker and Feeny (1971), Jones (1971) stressed the importance of within-species comparisons to establish the role of secondary metabolites in the resistance of plants to insects and diseases. We submit that such intraspecific comparisons can best be made through use of near-isogenic lines of the type used in the present study. Sweetclover plants of the *CuCu* genotype are high in content of the glucosides of *cis*- and *trans-o*-HCA; *cucu* plants are low in content (Akeson et al. 1963). Similarly, preparations of *BB* plants are high in β-glucosidase activity; *bb* preparations appear to be inactive (Schaeffer et al. 1960).

A single feeding test with intact plants was conducted with *E. fabricii*. The test consisted of 8 replications of 4 genotypes of sweetclover offered to a population of 2 beetles/plant in a screened cage, 56×56×38 cm. A separate feeding cage was used for each replication. In each cage, each genotype was represented by 5 plants growing in soil in a 1-pint plastic-coated milk carton. Plants averaged ca. 15 cm high having been uniformly cut back 2 weeks prior to the feeding test. The extent of feeding was measured by visual estimation of the percentage consumption of each leaf.

All other tests utilized cut sweetclover shoots which were trimmed to a uniform number of leaves and placed in vials of water or various solutions to be tested. Shoots in which a high content of *cis-o*-HCA glucoside was desired were exposed to sunlight prior to the feeding tests. Feeding chambers consisted of 1-gal ice-cream cartons containing a 5-cm layer of silica sand and covered with a plastic screen. Vials containing the shoots were pushed into the sand to prevent tipping, and shoots of the various treatments were closely intertwined. One replication consisted of a single shoot of each treatment. A population of 2–3 beetles/shoot was sufficient in most cases to provide an adequate level of feeding. Feeding damage was recorded as described for the intact plants.

[6] We thank R. B. Selander, Department of Entomology, University of Illinois, Urbana, for identifying the species of *Epicauta*.

Table 1.—Feeding by blister beetles on *Melilotus* leaves of 4 genotypes differing in content of *o*-hydroxycinnamic acid and β-glucosidase activity.

Sweet-clover geno-type[a]	% of leaves consumed[b]			
	E. fabricii	*E. pestifera*	*E. vittata*	*E. pennsylvanica*
cucubb	97 a	100 a	100 a	88 a
cucuBB	95 a	100 a	100 a	81 a
CuCubb	38 b	52 b	3 b	26 b
CuCuBB	12 c	23 c	0 c	19 b

[a] Plants were grown in growth chambers and exposed to sunlight prior to initiation of the feeding tests.
[b] Values shown are means of 4 replications of cut shoots held in water except for the ashgray blister beetles, in which 8 replications of intact plants were used. Within each species, means not followed by the same letter are significantly different at the 5% level of probability.

RESULTS AND DISCUSSION.—Beetles of each of the 4 species fed preferentially on leaves of the *cucu* genotype, which contain a very low level of both the *trans*- and *cis-o*-HCA glucosides (Table 1). Thus, it appears that in *CuCu* leaves, one or both of the isomers of *o*-HCA glucoside have a strong deterring effect on feeding by blister beetles. Furthermore, the apparent preference of the beetles for *CuCubb* leaves over leaves of the *CuCuBB* genotype suggests that free coumarin also serves as a feeding deterrent. Both genotypes contain high levels of the *o*-HCA glucosides, but only *CuCuBB* plants contain the β-glucosidase necessary to hydrolyze *cis-o*-HCA glucoside, thereby permitting the formation of free coumarin during beetle feeding. In tests designed to determine whether blister beetles contain a β-glucosidase which hydrolyzes *cis-o*-HCA glucoside, assays were positive only when the beetles had fed previously on plants of the *BB* genotype. Prior feeding on *cucubb* plants for 2 days resulted in uniformly negative assays of various insect parts. Thus, the observed effects of β-glucosidase activity in the feeding tests were due entirely to plant-derived enzyme.

Table 2 shows the combined effects of *trans*- and *cis-o*-HCA glucoside content and β-glucosidase presence on blister beetle feeding. Shoots of the *CuCubb* and *CuCuBB* genotypes, grown in a growth chamber, were exposed to sunlight for 2 hr to effect conversion of most of the *trans-o*-HCA glucoside to the *cis* isomer (Haskins et al. 1964), while similar shoots were held in vials of water in the dark. Assays for content of *cis*- and *trans-o*-HCA glucosides (Haskins and Gorz 1970) in similarly treated shoots revealed that 90–94% of the total *o*-HCA glucoside content of the leaves was present as the *trans* isomer in shoots held in the dark, whereas only 18–20% was present as the *trans* isomer in leaves exposed to sunlight. In each of the 3 tests, there was a highly significant

Table 2.—Influence of content of *cis*- and *trans*-*o*-hydroxycinnamic acid glucosides and the presence of β-glucosidase activity in beetles and in sweetclover leaves on feeding preferences of margined blister beetles.

Test no.	Type of leaves fed[a] *o*-HCA content	Type of leaves fed[a] β-glucosidase presence	β-glucosidase presence in beetles[b]	% of leaves consumed when predominant *o*-HCA glucoside isomer is[c] *trans*[d]	*cis*[d]
1	High	—	—	68	30
2	High	—	+	74	11
3	High	+	+	81	3

[a] Genotypes of leaves fed were *CuCubb* in Tests 1 and 2 and *CuCuBB* in Test 3.
[b] Accomplished by previous feeding of beetles on *cucubb*(—) and *cucuBB* (+) leaves.
[c] Values shown are means of 5 replications. Differences within each of the 3 tests are highly significant.
[d] *trans*— leaves not exposed to sunlight: *cis*— leaves exposed to sunlight for 2 hr. See text for relative contents of these 2 isomers in the leaves.

reduction in feeding on shoots that contained a predominance of the *cis* isomer.

In Test 1, the beetles were purged of β-glucosidase activity by prior feeding on plants of the *cucubb* genotype. Leaves used in the feeding test also were devoid of this enzyme. More than a 2-fold difference in feeding was observed, demonstrating that *cis-o*-HCA glucoside acts as a strong feeding deterrent.

The leaves in Test 2 were essentially identical to those used in Test 1, but the beetles in the 2nd test had fed on *cucuBB* leaves immediately prior to this test and thus contained some plant β-glucosidase in various mouth parts and other portions of the digestive tract. This enzyme would be expected to hydrolyze *cis-o*-HCA glucoside, liberating coumarin. Feeding differences in Test 2 were somewhat greater than those observed in Test 1, probably because a coumarin effect was added to the *cis-o*-HCA glucoside effect in those leaves in which the *cis* isomer predominated.

The greatest feeding difference occurred in Test 3 in which the leaves had a high level of β-glucosidase activity. More extensive hydrolysis of *cis-o*-HCA glucoside would be expected in this test than in Test 2. Feeding was virtually eliminated on the cis-containing leaves. However, extensive feeding was observed on leaves of the same genotype in which the *trans* isomer predominated.

In other tests, the relative amount of feeding on leaves of the *CuCubb* genotype, having a predominance of *trans-o*-HCA glucoside (ca. 90%), was compared with that on leaves of the *cucubb* genotype. The beetles were purged of β-glucosidase activity prior to initiation of the test. In these tests, leaves of both genotypes were consumed extensively, and no significant difference in feeding was observed; thus, *trans-o*-HCA glucoside had no significant deterring effect on blister beetle feeding.

To determine the influence of coumarin on blister beetle feeding in the absence of the *o*-HCA glucosides, cut shoots of the *cucuBB* genotype were held 15 hr, immediately prior to the start of beetle feeding, in solutions containing 0, 1, 5, and 10 μmoles of coumarin/ml. The 10-μmole/ml solution caused a significant reduction in feeding compared with the lower concentrations of coumarin or the water control. Assays of leaves from the treated shoots gave no indication of large increases of coumarin. However, in the 10 μmoles/ml treatment, there was a small but consistent increase in coumarin, particularly in the lower leaves.

Coumarin administered to cut shoots of sweetclover is rapidly converted to melilotic acid (*o*-hydroxyhydrocinnamic acid) (Kosuge and Conn 1959). In 2 separate tests, consumption of cut shoots of the *cucubb* and *cucuBB* genotypes to which melilotic acid had been administered was not significantly different from consumption of similar shoots held in water.

The black blister beetles, which were collected from pigweed, did not feed readily on sweetclover in the laboratory. When compared with *E. pestifera*, twice as many black blister beetles required 3 times as many hours to eat an equivalent amount of low-coumarin leaves. However, these beetles did discriminate among the plant genotypes in much the same manner as the other 3 species, which fact is of interest because Horsfall (1941) stated of *E. pennsylvanica*—"The adults are strictly pollen feeders."—when he observed no foliage feeding on several legumes as well as other hosts. Horsefall's observations were made, of course, prior to the availability of low-coumarin sweetclover.

We conclude that blister beetles are able to discriminate between plants of sweetclover differing in *o*-HCA content because (1) *cis-o*-HCA glucoside acts as a strong feeding deterrent, and (2) in those plants containing β-glucosidase and *cis-o*-HCA glucoside, feeding is accompanied by hydrolysis of the glucoside to produce coumarin, which also serves as a deterrent. The content of *trans-o*-HCA glucoside has little influence on feeding preference, because the glucoside itself has no significant deterrent activity, and it is not hydrolyzed by sweetclover β-glucosidase during the feeding process.

While our results show that coumarin and *cis-o*-HCA glucoside have a pronounced deterring effect on feeding by 4 species of blister beetles on sweetclover leaves, there also is evidence that coumarin may have an attractive effect (Hans and Thorsteinson 1961) or no influence on some other species of insects which feed upon sweetclover (Howe and Gorz 1960, Manglitz and Gorz 1964).

REFERENCES CITED

Akeson, W. R., H. J. Gorz, and F. A. Haskins. 1963. Effect of genotype and growth stage on distribution of melilotic acid, *o*-coumaric acid, and coumarinic acid in *Melilotus alba*. Desr. Crop. Sci. 3: 167–71.

Gilchrist, D. G., F. A. Haskins, and H. J. Gorz. 1970. Comparison of protein constituents relating to β-glucosidase activity in *BB* and *bb* inbred lines of *Melilotus alba*. Genetics 66: 339–47.

Hans, H., and A. J. Thorsteinson. 1961. The influence of physical factors and host plant odour on the induction and termination of dispersal flights in *Sitona cylindricollis* Fahr. Entomol. Exp. Appl. 4: 165–77.

Haskins, F. A., and H. J. Gorz. 1961. A reappraisal of the relationship between free and bound coumarin in *Melilotus*. Crop Sci. 1: 320–3.

1970. Fluorometric assay of free and bound, *cis*- and *trans*-*o*-hydroxycinnamic acid in a single plant extract. Ibid. 10: 608–9.

Haskins, F. A., L. G. Williams, and H. J. Gorz. 1964. Light-induced *trans* to *cis* conversion of β-D-glucosyl *o*-hydroxycinnamic acid in *Melilotus alba* leaves. Plant Physiol. 39: 777–81.

Horsfall, W. R. 1941. Biology of the black blister beetle. Ann. Entomol. Soc. Am. 34: 114–26.

Howe, W. L., and H. J. Gorz. 1960. Feeding preferences of the cowpea aphid among species of *Melilotus*. Ibid. 53: 696–7.

Jones, D. A. 1971. Chemical defense mechanisms and genetic polymorphism. Science (Wash., D. C.) 173: 945.

Kosuge, T., and E. E. Conn. 1959. The metabolism of aromatic compounds in higher plants. I. Coumarin and *o*-coumaric acid. J. Biol. Chem. 234: 2133–7.

1961. The metabolism of aromatic compounds in higher plants. III. The β-glucosides of *o*-coumaric, coumarinic, and melilotic acids. Ibid. 236: 1617–21.

Manglitz, G. R., and H. J. Gorz. 1964. Host-range studies with the sweetclover weevil and the sweetclover aphid. J. Econ. Entomol. 57: 683–7.

Schaeffer, G. W., F. A. Haskins, and H. J. Gorz. 1960. Genetic control of coumarin biosynthesis and β-glucosidase activity in *Melilotus alba*. Biochem. Biophys. Res. Comm. 3: 268–71.

Smith, W. K. 1943. Propagation of chlorophyll-deficient sweetclover hybrids as grafts. J. Hered. 34: 135–40.

Whittaker, R. H., and P. P. Feeny. 1971. Allelochemics: Chemical interactions between species. Science (Wash., D. C.) 171: 757–70.