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A Water-Soluble Factor in *Melilotus officinalis* Leaves Which Stimulates Feeding by the Adult Sweetclover Weevil

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A Water-Soluble Factor in *Melilotus officinalis* Leaves Which Stimulates Feeding by the Adult Sweetclover Weevil[1,2]

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Akeson et al. (1967) reported that the water-methanol phase of a water-methanol-chloroform extract, obtained from leaves of *Melilotus officinalis* L. Lam. (variety Goldtop), stimulated extensive feeding by the sweetclover weevil, *Sitona cylindricollis* (Fåhraeus), on bioassay disks prepared from sweetclover roots. Subsequent studies[5] have shown that a hot-water extract will elicit a similar response. When disks were treated only with the solvent used in extraction, an almost negligible amount of feeding was obtained. Thus, leaves of Goldtop sweetclover apparently contain 1 or more water-soluble feeding stimulants. Beck (1965) defined a feeding stimulant as a stimulus tending to promote continuous feeding. The present paper describes the feeding stimulant from a hot-water extract of Goldtop sweetclover leaves.

MATERIALS AND METHODS.—Fresh young leaves of greenhouse-grown Goldtop sweetclover plants were weighed, washed with distilled water, dropped into boiling water (10 ml/g of fresh tissue) in a glass beaker, and boiled for 5 min. The hot mixture was homogenized for 2 min in a blender and then boiled for an additional 5 min. The homogenate was cooled to room temperature and centrifuged at 2400 *g* for 30 min. The supernatant liquid was flash evaporated at 40°C to a volume of 5 ml/g of original dry plant material. The hot water extract was stored at −20°C until used. Occasionally a precipitate appeared in the extract after freezing and thawing. In such instances the extract was centrifuged as before and the precipitate was discarded.

Hot-water extracts were chromatographed on 18½ × 28½ cm sheets of Whatman no. 3 filter paper.[6] On each sheet, two 0.1-ml portions of extract were applied to an 18-cm base line which was 2.5 cm from the bottom of the chromatogram. The 7 solvents listed in Table 1 were used to develop the chromatograms. When the solvent had ascended 15 cm above the base line, the chromatograms were air dried at room temperature, and each sheet was cut into 6 strips of equal width (approximately 2.5 cm) parallel to the base line. In some later experiments strips approximately 1.5 cm wide were cut from the region containing stimulant activity to locate the active factor more precisely. The strips were eluted with distilled water in an apparatus similar to that used in descending chromatography. Elution was continued until 0.5 ml of eluate had been collected from each strip.

A bioassay essentially like that described by Akeson et al. (1967) was used to test the eluates for components that influenced feeding by the sweetclover weevil. Five root disks were treated with 0.15 ml of eluate. Little feeding occurred on disks treated with water or nonactive fractions; thus, the presence of a feeding stimulant was indicated in those disks that were appreciably consumed.

RESULTS AND CONCLUSIONS.—Bioassays for the detection of active factors were conducted with the 6 eluates obtained from each of 7 different chromatographic systems. Table 1 presents results expressed as the average percentage disk area consumed from 5 disks used for each treatment. With each of the solvent systems, feeding-stimulant activity appeared to be confined to a single area of the chromatogram. In several of the systems, activity was divided more or less evenly between 2 adjacent sections, but in no case was 1 section (or more) of low feeding-stimulant activity interposed between 2 sections of high activity. Thus, available evidence supports the conclusion that leaves of Goldtop sweetclover contain a single water-soluble feeding stimulant. This factor has been designated Stimulant A.

A more precise determination of the R_f value for Stimulant A has been accomplished by re-chromatographing the eluates and using narrower band widths cut from the general region of activity. With partially purified fractions, R_f values tend to be somewhat higher than

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[5] W. R. Akeson, unpublished data.

[6] Mention of a proprietary product does not necessarily imply its endorsement by the USDA.

Table 1.—Assay of chromatographic fractions for feeding-stimulant activity. Data are expressed as average percentage disk area consumed from 5 disks used in each treatment.

	Solvent systems[b]						
R_f range[a]	i-PrOH 8 NH_4OH 1 H_2O 1	i-PrOH 8 NH_4OH 1 H_2O 3	i-PrOH 20 HAc 4 H_2O 15	*n*-BuOH 5 HAc 1 H_2O 4 (upper phase)	*n*-BuOH 5 EtOH 1 H_2O 2	EtAc 6 HAc 2 H_2O 5 (upper phase)	MeOH
0.00–0.17	0.7	2.0	0.7	1.7	70.9	72.0	3.7
.17– .33	42.5	3.0	1.0	43.4	51.1	14.4	52.0
.33– .50	39.2	39.8	2.7	7.5	9.0	0.3	60.0
.50– .67	2.0	38.0	59.3	4.9	1.0	6.2	0.0
.67– .83	.7	2.7	31.5	0.7	13.3	8.3	.0
.83–1.00	2.7	6.0	.5	2.7	1.0	4.6	.0
Approximate R_f of stimulant	0.31	0.50	0.65	0.25	0.25	0.10	0.43

[a] 15-cm ascent on Whatman no. 3 filter paper.

[b] Abbreviations: i-PrOH, isopropyl alcohol; *n*-BuOH, *n*-butyl alcohol; EtOH, ethyl alcohol; MeOH, methyl alcohol; EtAc, ethyl acetate; HAc, acetic acid.

Table 2.—Refinement of R_f determination for the feeding stimulant. Solvent: isopropyl alcohol-acetic acid-water (20:4:15,v/v/v).

R_f range[a]	% avg. disk area consumed[b]
Water blank	0.0
0.53–0.62	2.3
.62–0.69	6.3
.69– .75	49.0
.75– .85	9.1

[a] 15-cm ascent on Whatman no. 3 filter paper.
[b] Average of 5 disks/treatment.

those observed with a crude preparation. Table 2 shows results obtained from 1 such series of strips cut from a chromatogram developed in the isopropyl alcohol-acetic acid-water (20:4:15 v/v/v) solvent. In this example, activity was confined primarily to the band extending from R_f 0.69 to 0.75; this fact indicated an R_f of 0.72 for Stimulant A in this solvent system. This value compares with a preliminary estimate of 0.65 (Table 1).

REFERENCES CITED

Akeson, W. R., G. R. Manglitz, H. J. Gorz, and F. A. Haskins. 1967. A bioassay for detecting compounds which stimulate or deter feeding by the sweetclover weevil. J. Econ. Entomol. 60: 1082–4.

Beck, S. D. 1965. Resistance of plants to insects. Annu. Rev. Entomol. 10: 207–32.