BUREAU OF SUGAR EXPERIMENT STATIONS

QUEENSLAND, AUSTRALIA

FINAL REPORT

SRDC PROJECT BS35S

ASSESSMENT OF VARIOUS FOOD CONSTITUENTS AS FEEDING ATTRACTANTS FOR CANEGRUBS IN A PEST CONTROL PROGRAM

by

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This Project was funded by the Sugar Research Council/Sugar Research and Development Corporation during the 1989/90 and 1990/91 financial years.

October 1991

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SUMMARY

Cellulose acetate filter disks were used to evaluate sugars, amino acids and ascorbic acid as feeding stimulants for canegrubs.

Fourteen sugars, 18 amino acids and ascorbic acid were tested with third instars of *Antitrogus parvulus* Britton and *Lepidiota negatoria* Blackburn. The sugars D-fructose, D-mannose, D-sucrose, D-trehalose, D-melezitose and D-raffinose stimulated feeding by *L. negatoria*; sucrose and raffinose stimulated feeding by *A. parvulus*. Fructose and sucrose were the most effective stimulants, at 1 M concentration for *A. parvulus* and at 2 M concentration for *L. negatoria*. The amino acids L-cysteine, L-glutamic acid and L-histidine monohydrochloride stimulated feeding by *L. negatoria*; only L-tyrosine stimulated feeding by *A. parvulus*. L-ascorbic acid did not stimulate feeding by either species.

The concentration responses for fructose and sucrose were also determined for *A. consanguineus* (Blackburn), *L. picticollis* Lea and *Dermolepida albohirtum* (Waterhouse). All three species responded to both sugars but peak responses were at different concentrations.

The addition of fructose or sucrose and perhaps some of the stimulatory amino acids to baits may make these more attractive to canegrubs, although the compounds may be degraded rapidly in the soil.

BACKGROUND

Sixteen species of melolonthine canegrubs are endemic to eastern Australia, where the larvae feed on the roots of grasses (Allsopp and Chandler, 1989). They are important pests of sugarcane, destroying roots and thus depriving the plant of moisture, nutrients and mechanical support (Allsopp and Hitchcock, 1987). Since 1982 canegrubs have been responsible for an average annual loss of cane valued at $2.4M and growers have treated an annual average of 31 600 ha with insecticides at a cost of $2.4M. The cost of insecticide treatment for canegrub control has increased significantly over the last 7 years with the replacement of low-cost organochlorines with more expensive controlled-release products. However, without insecticides, the level of losses would rise dramatically.

BSES is currently investigating the use of the fungus *Metarhizium anisopliae* and various insecticides as alternatives to controlled-release chlorpyrifos (suSCon Blue) for the control of canegrubs. The incorporation of feeding stimulants into these new materials could make them more effective against canegrubs. The development of resistant varieties of sugarcane also could be facilitated by an understanding of the phagostimulatory effects of plant compounds on canegrubs.

Larvae of the New Zealand grassgrub, *Costelytra zealandica* (White), and the African black beetle, *Heteronychus arator* (F.), are stimulated to feed by D-sucrose, D-glucose, D-maltose and D-fructose (Sutherland, 1971; Sutherland and Hillier, 1976). These sugars and D-trehalose also stimulate feeding by larvae of Japanese beetle, *Popilla japonica*

Newmann, (Ladd, 1988), and the first three are phagostimulants for larvae of the Australian melolonthine *Sericesthis geminata* Boisduval (Wensler and Dudzinski, 1972). Larvae of *C. zealandica* are also stimulated to feed by six amino acids, especially L-aspartic acid, L-glutamic acid and L-serine, and by L-ascorbic acid (Sutherland and Hillier, 1974) and larvae of *H. arator* are stimulated by L-alanine, L-aspartic acid and L-glutamic acid (Sutherland, 1983).

This study aimed to determine if various naturally-occurring sugars, amino acids, and ascorbic acid stimulate feeding by canegrubs, and to assess if such materials could be incorporated into bait materials.

OBJECTIVES

- To determine the attractiveness and stimulatory effects on feeding of a wide range of carbohydrates, amino acids and other compounds on canegrubs.

- To incorporate suitable compounds into baits in order to increase the attractiveness of such baits for delivery of insecticides or other biological agents.

RESEARCH METHODOLOGY

Preliminary experiment

Two techniques have been used to test for stimulatory effects of compounds on scarab larvae. Sutherland (1971) incorporated the test chemical into agar-based media and fed this to larvae. Stimulatory effects were assessed by counting the number of faecal pellets produced by the larvae. Ladd (1988) moistened cellulose acetate membrane filter disks with solutions of test compounds and placed these in soil with test larvae. Stimulatory effects were assessed by determining the proportion of the filter disk eaten by the larvae. The use of such disks in feeding assays was reviewed by Doss and Shanks (1986).

I evaluated the usefulness of these two techniques in a preliminary experiment with third instars of *Lepidiota negatoria* Blackburn. Sucrose was incorporated into water-agar media at 1 M concentration and compared with media with no added sucrose. Each larva was given 40 ml of media. Sucrose was also tested by moistening 25 mm diameter cellulose acetate membrane filter disks (Sartorius 11106, Sartorius, Göttingen, Germany) with 70 μl of water or of an 1.0 M aqueous solution of sucrose. Each test disk was placed in sand with one larva. Twenty larvae were tested with each of the four method-concentration combinations and feeding was assessed after 24 h.

Stimulatory effects of compounds on *Antitrogus parvulus* and *L. negatoria*

I used the filter-disk technique to test for feeding responses in larvae of *Antitrogus parvulus* Britton and *Lepidiota negatoria*. I tested 14 sugars separately: the pentoses D-

arabinose, D-ribose, and D-xylose; the hexoses D-fructose, D-galactose, D-glucose, D-mannose, and L-sorbose; the disaccharides D-maltose, D-melibiose, D-sucrose, and D-trehalose; and the trisaccharides D-melezitose and D-raffinose. I tested 18 amino acids separately: L-alanine, γ-aminobutyric acid, L-arginine monohydrochloride, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, glycine, L-histidine monohydrochloride, L-leucine, L-lysine monohydrochloride, L-methionine, DL-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-tryptophan. L-ascorbic acid was also tested.

Compounds were tested by moistening 25 mm diameter cellulose acetate membrane filter disks (Sartorius 11106) with 70 μl of an aqueous solution of the test compound.

Third instars of *A. parvulus* and *L. negatoria* were collected from infested sugarcane fields near Bundaberg, southeastern Queensland, and were allowed to feed on sections of sugarcane stalk at 25°C for at least 1 wk before the tests. Before testing, larvae were placed in moist sand (about 5% gravimetric soil moisture) without food for 24 h; sand had been sterilised at 105°C for 48 h. Tests were done in plastic cups (lower diameter 60 mm, upper diameter 70 mm, 60 mm high) containing moist sand. A cup was filled 40 mm deep with sand, a 20 mm deep depression was made in the centre, and a single larva placed in the depression. A treated filter disk was placed over each larva, and the cup was filled with sand and capped. After 24 h at 25°C, the portions of the disks not eaten by larvae were removed from the cups and washed free of sand. The disks were dried over silica gel for at least 2 wk and then weighed. As the amounts remaining in each treatment were usually not normally distributed (Shapiro-Francia statistic, $\underline{P} < 0.01$), the data were analysed by Kruskal-Wallis nonparametric one-way analysis of variance using STATISTIX 3.1 (Analytical Software, 1989). The mean ranks were separated at the 5% level using the method of Conover (1980).

Compounds were tested in a series of no-choice tests using completely-randomised designs. Tests 1-3 tested responses to a 0.1 M solution of each sugar. Tests 4-8 tested responses to 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 M solutions of either fructose, mannose, sucrose, or trehalose, and to 0.005, 0.01, 0.05, 0.1 and 0.5 M solutions of raffinose (Not enough raffinose would dissolve to give higher concentrations.) In these tests, areas eaten (A) were corrected for the mean area of water-treated disks eaten in the same test (W) by (A-W)/(1-W).

Tests 9-13 tested responses to a 0.01 M solution of each amino acid, and a 0.01 M solution of ascorbic acid was also evaluated in test 13. All tests included disks moistened with distilled water as controls. Twenty larvae of each species were used to evaluate the response to each compound or concentration and to the water controls.

Stimulatory effects of fructose and sucrose on other canegrubs

The filter-disk technique was used to test the stimulatory effects of fructose and sucrose on third instars of *Antitrogus consanguineus* (Blackburn), *Lepidiota picticollis* Lea and *Dermolepida albohirtum* (Waterhouse). Methodology was the same as in tests 4-8 above with the two sugars being tested in 0.005, 0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 M solutions.

RESULTS

Preliminary experiment

Larvae placed with agar-water media (with or without sucrose) appeared to work their way through the media without necessarily eating it. This movement mixed whatever faecal pellets were produced with the media, making assessment of the number of pellets impossible.

Larvae removed significantly more of the filter disks moistened with 0.1 M sucrose (mean of 37.2%, SEM 5.2) than of the filter discs moistened with water (mean of 15.3%, SEM 6.2) (Kruskal-Wallis statistic = 20.73, $\underline{P} < 0.0001$). Assessment of feeding response was relatively easy and the technique was considered suitable for use in further studies.

Stimulatory effects of compounds on *Antitrogus parvulus* and *L. negatoria*

Table 1 shows the mean proportion removed by *A. parvulus* and *L. negatoria* larval feeding from filter disks impregnated with 0.1 M solutions of sugars (tests 1-3); the larger *L. negatoria* consistently removed more of the disks. The hexoses fructose and mannose, the disaccharides sucrose and trehalose, and the trisaccharides melezitose and raffinose stimulated feeding by *L. negatoria*. Only sucrose and raffinose stimulated feeding by *A. parvulus*.

The feeding responses of *A. parvulus* and *L. negatoria* to various concentrations of fructose, mannose, sucrose, trehalose, and raffinose (tests 4-8) are shown in Fig. 1. Both species were most stimulated by fructose and sucrose. Increasing the concentration of fructose and sucrose up to the highest tested (2 M) increased feeding by *L. negatoria*. For *A. parvulus*, 1 M solutions of either of these sugars were the most effective in stimulating feeding. Concentrations of 0.05 M and above of mannose, trehalose or raffinose differed little in their stimulatory effect on *L. negatoria*; *A. parvulus* was little stimulated by any concentration of any of these three sugars.

Table 2 shows the mean proportion removed by larvae of both species feeding from filter disks impregnated with 0.01 M solutions of an amino acid or ascorbic acid (tests 9-13). Cysteine, glutamic acid and histidine monohydrochloride stimulated feeding by *L. negatoria*, and tyrosine stimulated feeding by *A. parvulus*. Neither species was stimulated to feed by ascorbic acid.

Stimulatory effects of fructose and sucrose on other canegrubs

The feeding responses of *A. consanguineus*, *L. picticollis* and *D. albohirtum* to various concentrations of fructose and sucrose are shown in Fig. 2. All species were stimulated to feed by both sugars. In *A. consanguineus*, increasing the concentration of each sugar generally gave increased feeding stimulation. In *L. picticollis*, the reaction to fructose peaked at 0.1 M concentration and to sucrose at 0.5 M concentration. In *D. albohirtum*,

the reaction to fructose peaked at 0.05 M concentration, whilst the reaction to sucrose showed little change in the range of 0.05-2 M.

DISCUSSION

The results of the preliminary experiment showed that the agar-water medium was not useful for determining feeding responses in canegrubs. Sutherland may have had more success with *C. zealandica* larvae because they are much smaller than canegrub larvae and apparently did not disturb the medium as much. The filter-disk technique was useful in demonstrating a response to sucrose and was used in the following experiments.

The results of the remaining tests show that fructose and sucrose are highly phagostimulatory for *A. parvulus* and *L. negatoria* and that concentrations of 1 M and 2 M are the most stimulatory for *A. parvulus* and *L. negatoria*, respectively. Both sugars also stimulated feeding in three other canegrubs, *A. consanguineus*, *L. picticollis* and *D. albohirtum*. However, the peak response was sometimes at lower concentrations.

Sucrose is the most common plant sugar (Irvine, 1977) and it stimulated feeding in other scarab larvae (Sutherland, 1971; Wensler and Dudzinski, 1972; Sutherland and Hillier, 1976; Ladd, 1988). In *S. geminata*, sucrose was most stimulatory at a concentration of 1 M, and fructose was most stimulatory at 0.01 M (Wensler and Dudzinski, 1972). However, in both *C. zealandica* and *P. japonica*, concentrations of 0.1 M were the most effective concentrations of sucrose in stimulating feeding (Sutherland, 1971; Ladd, 1988). Higher concentrations of sugars often reduce feeding by insects in general (Chapman, 1974).

Sucrose, glucose and fructose are the most abundant sugars in sugarcane (Irvine, 1977). In the cultivar CP65-357, sucrose occurs at a concentration of 0.026 M in the roots and 0.45 M in the below-ground stubble (Irvine, 1977). Fructose occurs at lower levels; 0.007 M in roots and 0.017 M in stubble. As sucrose can be hydrolysed to fructose and glucose, responses to fructose and sucrose may be confounded in these experiments. Glucose does not stimulate feeding by *A. parvulus* or *L. negatoria*. Larvae of all canegrubs feed not only on the roots of sugarcane, but also on the below-ground portions of the stem. The higher sucrose concentrations in the stubble may make this part more attractive to these larvae, but this attractiveness may be balanced by the toughness of the stem. When roots are severed by larvae, sugar concentrations may rise in the portion attached to the plant, because of the downward movement of sugars. This may make these portions more attractive to larvae.

Raffinose slightly stimulated feeding in both species, and mannose and trehalose slightly stimulate feeding in *L. negatoria*. The concentration response curves (Fig. 1) show some evidence of a maximum response to raffinose and trehalose at 0.1 M concentration. Mannose is available from gums of sugarcane by hydrolysis (Irvine, 1977), but it is generally rare in plants. It was not phagostimulatory to *P. japonica* (Ladd, 1988), but was weakly phagostimulatory to *C. zealandica* and *H. arator* (Sutherland, 1971;

Sutherland and Hillier, 1976). Raffinose occurs in cane final molasses (Irvine, 1977), but at very low levels. It did not stimulate feeding in *C. zealandica*, *H. arator* or *P. japonica* (Sutherland, 1971; Sutherland and Hillier, 1976; Ladd, 1988).

Trehalose did stimulate feeding strongly in *H. arator* and *P. japonica* larvae (Sutherland and Hillier, 1976; Ladd, 1988) and weakly in *C. zealandica* larvae (Sutherland, 1971). It has not been found in vascular plants (Gussin, 1972), but is the major oligosaccharide in insect haemolymph, occurring at concentrations up to 0.15 M (Bursell, 1970). Ladd (1988) speculated that the response to trehalose in *P. japonica* larvae may be related to confined larvae biting each other. Both *A. parvulus* and *L. negatoria* larvae show the same aggressive behaviour and their response to trehalose is similar to that of *P. japonica*.

Melezitose slightly stimulated feeding in *L. negatoria*; it also stimulated feeding in *C. zealandica* and *H. arator* (Sutherland, 1971; Sutherland and Hillier, 1976) but did not in *P. japonica* (Ladd, 1988).

Neither glucose nor maltose stimulate feeding in *A. parvulus* or *L. negatoria*, despite doing so in other scarab larvae (Sutherland, 1971; Wensler and Dudzinski, 1972; Sutherland and Hillier, 1976; Ladd, 1988). Glucose occurs in sugarcane (Irvine, 1977), and it is one of the hydrolysis products of many oligosaccharides and polysaccharides including sucrose. Maltose hydrolyses to two glucose molecules and is a breakdown product of starch, but starch is absent from sugarcane roots (Irvine, 1977). Maltose differs from trehalose only in the site of linkage of the two glucose molecules.

Of the other sugars I tested (arabinose, ribose, xylose, galactose, sorbose and melibiose), none stimulated feeding by either *A. parvulus* or *L. negatoria*. None stimulated feeding in *C. zealandica* or *P. japonica* larvae (Sutherland, 1971; Ladd, 1988). In *H. arator* larvae, xylose, galactose and sorbose stimulated slight feeding, while arabinose and melibiose were not stimulatory (Sutherland and Hillier, 1976). All except sorbose are found in vascular plants, and can be hydrolysed from gums, muscilages and hemicelluloses; ribose is also a component of nucleic acids (McIlroy, 1967).

Of the amino acids, tyrosine stimulated significant feeding in *A. parvulus* and cysteine, glutamic acid and histidine monohydrochloride stimulated significant feeding in *L. negatoria*. Glutamic acid (0.01 M) stimulated feeding in *C. zealandica* and *H. arator* larvae, but cysteine and histidine HCl at 0.01 and 0.1 M and tyrosine at 0.01 M were not stimulatory (Sutherland and Hillier, 1974; Sutherland, 1983). Glutamic acid, histidine HCl and tyrosine are all found in sugarcane juice (Irvine, 1977). Aspartic acid and alanine are the most common free amino acids in sugarcane juice, but neither stimulated feeding by *A. parvulus* or *L. negatoria*, despite doing so in *C. zealandica* and *H. arator* (Sutherland and Hillier, 1974; Sutherland, 1983).

Ascorbic acid did not stimulate feeding in *A. parvulus* or *L. negatoria*. It is a strong feeding stimulant for larvae of *C. zealandica* and, in combination with sucrose, evokes a strong response (Sutherland and Hillier, 1974). However, it deters feeding in

Trichoplusia ni (Hübner) (Gothilf and Beck, 1967) and *Sitona cylindricollis* Fåhraeus (Akeson *et al*, 1970). If ascorbate is synthesised *de novo* by *A. parvulus* and *L. negatoria*, then a response would not be expected. This remains to be determined.

The discrimination shown by *A. parvulus* and *L. negatoria* larvae between chemicals and by all canegrubs tested between different concentrations of the same chemical provides direct evidence of the sensory capabilities which allow these larvae to choose food within their soil habitat. The responses to fructose and sucrose, which are widely distributed in the plant kingdom, indicate that third instars prefer to feed on materials rich in these sugars. The addition of fructose and sucrose and perhaps some of the stimulatory amino acids to baits may make these more attractive to canegrubs, although the compounds may be degraded rapidly in the soil. None of the substances tested here deterred feeding, but a wide range of substances are known to inhibit feeding in insects (Chapman,1974). The technique used here may be useful in testing such compounds to determine their usefulness in conferring improved resistance to feeding by canegrubs on sugarcane.

ACKNOWLEDGMENTS

I thank Norm McGill and Tom Morgan for their assistance and Bryan Stickley for his comments on the hydrolysis of sugars.

PUBLICATION OF THIS RESEARCH

Allsopp, P G (1992). Sugars, amino acids and ascorbic acid as phagostimulants for larvae of *Antitrogus parvulus* and *Lepidiota negatoria* (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* in press.

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Table 1

Feeding by *A. parvulus* and *L. negatoria* larvae on filter disks moistened with 70 µl of 0.1 M concentrations of sugars

Sugar	*A. parvulus*	*L. negatoria*
	% of disk eaten ± SEM[a]	% of disk eaten ± SEM[a]
Test 1[b]		
D-Fructose	6.9 ± 2.5 a	58.3 ± 5.1 a
D-Mannose	4.5 ± 1.6 a	45.0 ± 4.6 ab
L-Sorbose	5.8 ± 4.1 a	33.4 ± 6.2 bc
D-Galactose	6.4 ± 2.2 a	32.3 ± 4.9 bc
D-Ribose	1.1 ± 0.7 a	28.9 ± 5.1 c
Water	5.0 ± 2.5 a	28.6 ± 5.2 c
Test 2[c]		
D-Glucose	5.5 ± 1.7 a	36.0 ± 3.8 a
D-Maltose	8.9 ± 2.9 a	36.3 ± 5.2 a
D-Arabinose	6.9 ± 3.3 a	33.9 ± 4.9 a
D-Melibiose	2.3 ± 1.7 a	26.3 ± 5.3 a
D-Xylose	4.9 ± 1.9 a	20.3 ± 5.9 a
Water	3.2 ± 1.7 a	31.9 ± 4.1 a
Test 3[d]		
D-Sucrose	4.2 ± 1.5 ab	44.1 ± 5.1 a
D-Raffinose	3.8 ± 1.4 a	35.6 ± 3.7 a
D-Trehalose	1.4 ± 1.1 bc	36.3 ± 3.8 a
D-Melezitose	4.3 ± 2.8 abc	32.7 ± 5.6 a
Water	0.4 ± 0.4 c	17.9 ± 6.8 b

[a] Means within a test followed by the same letter are not significantly different ($P = 0.05$). Twenty larvae were tested on each compound.

[b] Kruskal-Wallis Statistic for *A. parvulus* 6.82, $P = 0.23$; for *L. negatoria* 20.08, $P = 0.0012$.

[c] Kruskal-Wallis Statistic for *A. parvulus* 7.14, $P = 0.21$; for *L. negatoria* 9.20, $P = 0.10$.

[d] Kruskal-Wallis Statistic for *A. parvulus* 15.23, $P = 0.0094$; for *L. negatoria* 11.95, $P = 0.0355$.

Table 2

Feeding by *A. parvulus* and *L. negatoria* larvae on filter disks moistened with 70 µl of 0.01 M concentrations of amino acids or ascorbic acid

Compound	*A. parvulus*	*L. negatoria*
	% of disk eaten ± SEM[a]	% of disk eaten ± SEM[a]
Test 9[b]		
L-Alanine	2.8 ± 2.0 a	31.9 ± 5.8 a
γ-Aminobutyric acid	0.5 ± 0.4 a	23.4 ± 4.7 a
L-Arginine mono-hydrochloride	3.0 ± 1.3 a	41.1 ± 5.4 a
L-Asparagine	2.1 ± 1.3 a	24.9 ± 5.5 a
L-Aspartic acid	2.3 ± 0.8 a	37.4 ± 5.7 a
Water	0.7 ± 0.7 a	21.8 ± 4.9 a
Test 10[c]		
L-Cysteine	1.6 ± 1.4 a	51.2 ± 5.3 a
L-Glutamic acid	2.8 ± 1.2 a	47.6 ± 5.0 ab
L-Histidine mono-hydrochloride	4.5 ± 2.6 a	45.3 ± 4.3 ab
L-Leucine	0.1 ± 0.1 a	35.7 ± 4.7 bc
Glycine	5.9 ± 2.9 a	30.7 ± 5.1 c
Water	0.7 ± 0.7 a	29.0 ± 4.6 c
Test 11[d]		
L-Tyrosine	12.0 ± 4.5 a	42.7 ± 5.5 a
L-Methionine	4.8 ± 2.6 b	41.5 ± 5.2 a
DL-Phenylalanine	3.8 ± 2.0 b	37.8 ± 5.5 a
Water	0.6 ± 0.5 b	36.2 ± 4.7 a
Test 12[e]		
L-Lysine mono-hydrochloride	4.2 ± 1.9 a	37.4 ± 5.5 a
L-Threonine	7.9 ± 3.4 a	21.9 ± 5.4 a
L-Tryptophan	8.3 ± 3.3 a	33.5 ± 5.9 a
Water	2.4 ± 1.2 a	31.3 ± 5.4 a
Test 13[f]		
L-Proline	8.2 ± 2.6 a	16.4 ± 5.3 a
L-Serine	3.7 ± 1.3 a	20.1 ± 3.7 a
L-Ascorbic acid	1.7 ± 0.8 a	19.7 ± 4.7 a
Water	2.6 ± 1.8 a	9.7 ± 3.6 a

[a] Means within a test followed by the same letter are not significantly different ($P = 0.05$). Twenty larvae were tested on each compound.

[b] Kruskal-Wallis Statistic for *A. parvulus* 7.26, $P = 0.20$; for *L. negatoria* 10.36, $P = 0.07$.

[c] Kruskal-Wallis Statistic for *A. parvulus* 6.32, $P = 0.28$; for *L. negatoria* 15.99, $P = 0.0069$.

[d] Kruskal-Wallis Statistic for *A. parvulus* 9.30, $P = 0.026$; for *L. negatoria* 1.73, $P = 0.63$.

[e] Kruskal-Wallis Statistic for *A. parvulus* 1.03, $P = 0.79$; for *L. negatoria* 4.14, $P = 0.25$.

[f] Kruskal-Wallis Statistic for *A. parvulus* 7.26, $P = 0.06$; for *L. negatoria* 4.38, $P = 0.22$.

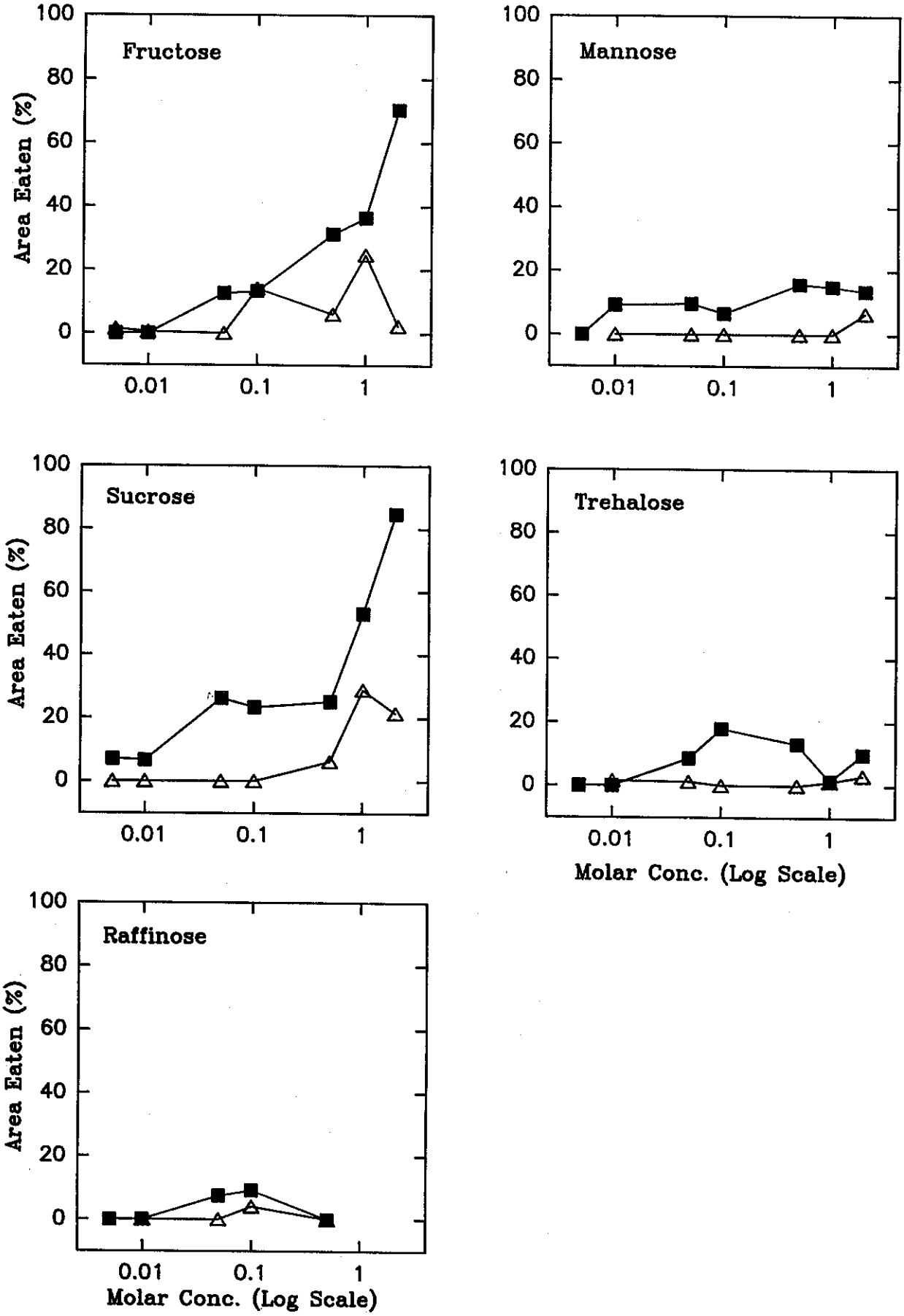

Fig. 1 Proportion of areas removed by feeding of larvae of *Antitrogus parvulus* (open triangles) and *Lepidiota negatoria* (closed squares) from 25-mm-diameter filter disks moistened with 70 µl of different concentrations of fructose, mannose, sucrose, trehalose and raffinose. Areas are corrected for feeding on water-treated disks.

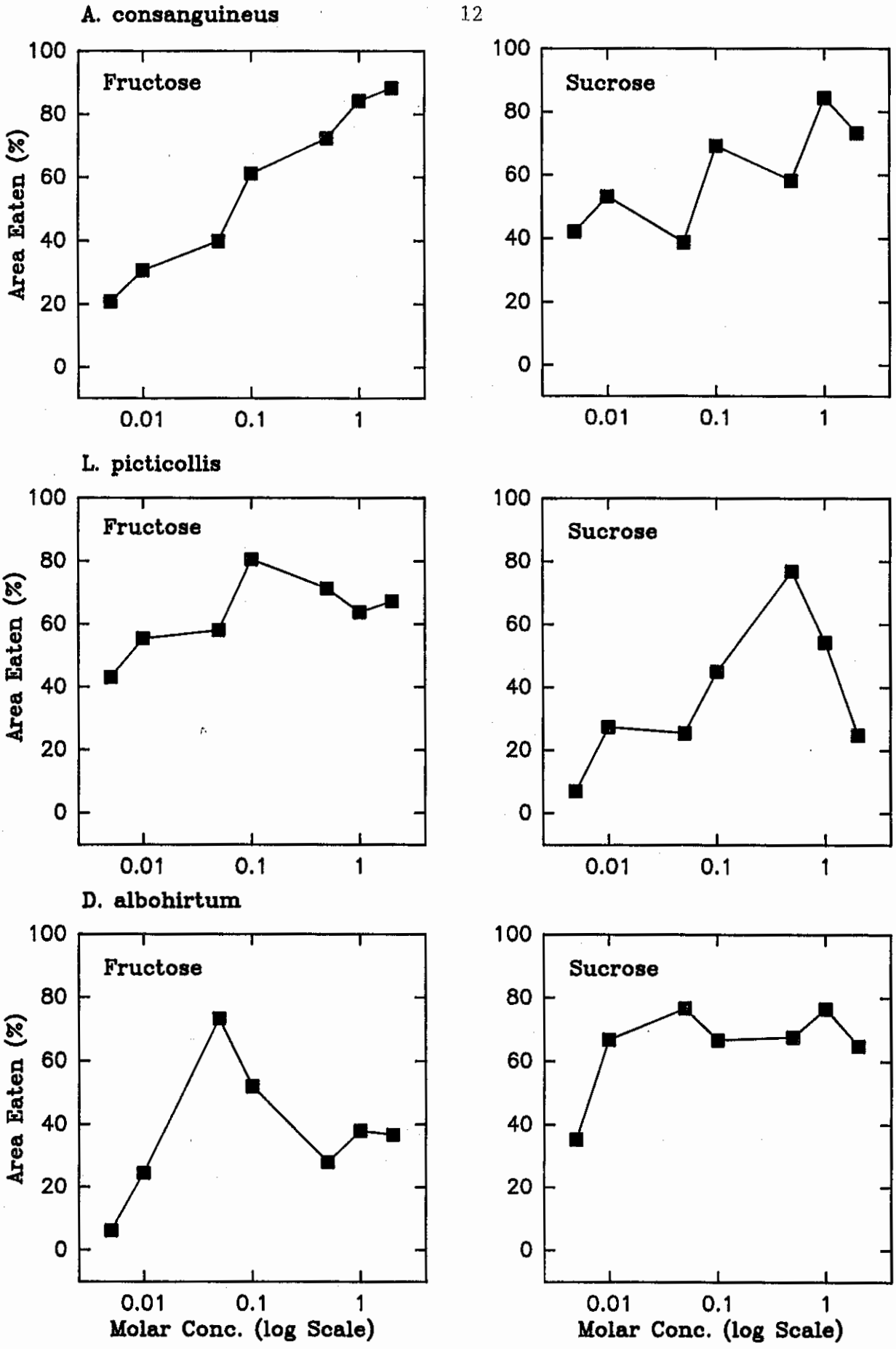

Fig. 2 Proportion of areas removed by feeding of larvae of *Antitrogus consanguineus*, *Lepidiota picticollis* and *Dermolepida albohirtum* from 25-mm-diameter filter disks moistened with 70 μl of different concentrations of fructose or sucrose. Areas are corrected for feeding on water-treated disks.