Influence of Nectar Source Plants on the New Guinea Sugarcane Weevil Parasite, *Lixophaga sphenophori* (Villeneuve)[1, 2, 3]

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The New Guinea sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval), is at present the most serious insect pest of sugarcane in the Hawaiian Islands. Biological control work against this pest has been well documented (Muir and Swezey, 1916; Pemberton, 1948; Waggy and Beardsley, 1974). Following substantial increases in damage by *R. obscurus* on certain Hawaiian plantations, particularly in the windward areas of Kauai, ecological studies of its key natural enemy, *Lixophaga sphenophori*, were undertaken. This research sought to determine means by which the efficiency of this parasite could be enhanced.

Early in this work it was determined that nectar from floral and extra-floral nectaries of certain weed species of the family Euphorbiaceae was a major food source for adults of *L. sphenophori*. Leeper (1974) discussed certain aspects of the feeding behavior of this parasite. The present paper provides additional information concerning the influence of nectar source plants on the distribution, abundance and efficiency of *L. sphenophori*.

NECTAR SOURCE PLANTS UTILIZED BY LIXOPHAGA SPHENOPHORI

Leeper (1974) reported that the hairy, or garden, spurge (*Euphorbia hirta* L.) and the castor bean (*Ricinus communis* L.) were important nectar source plants for *L. sphenophori*. Graceful spurge, *Euphorbia glomerifera* (Millsp.), also was occasionally utilized. During 1971 the senior author found two additional species of *Euphorbia* which are preferentially utilized as nectar sources by *L. sphenophori*; *E. geniculata* Ortega (wild spurge) and *E. heterophylla* L. (various-leafed spurge or Mexican fire plant). Both species occur as weeds in and around cane fields on Oahu. *E. heterophylla* is more common in the drier, lowland areas, particularly on the Ewa Coral Plain, where it often forms thick,

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hedge-like stands on field margins. *E. geniculata* is a more widely distributed species, generally scattered throughout most of the cane-growing areas of the island and sometimes locally abundant on field margins.[6] Both are upright herbaceous annuals generally ranging between one and three feet in height (Pope, 1929; Neal, 1948). The upright form and dense foliage of these two *Euphorbia* species appear to favor their utilization as nectar sources as this type of growth habit provides some shade, which the flies require. Even at midday and during early afternoon flies often were seen feeding and resting within the foliage of these plants when they could not be found on the semiprostrate *E. hirta* plants.

We attempted to determine if the four *Euphorbia* species utilized by *L. sphenophori* on Oahu could be ranked according to their attractiveness to these flies. Ten potted plants of each species were randomly arranged within an enclosed greenhouse that was kept stocked with several hundred field caught *L. sphenophori* adults. For a period of one month, we counted the number of flies which visited and fed on each species during a five minute observation period each morning. Of a total of 812 feedings observed, 293 were on *E. geniculata,* 260 on *E. heterophylla,* 231 on *E. hirta* and 28 on *E. glomerifera.* A chi-square test showed that significantly fewer flies fed on *E. glomerifera,* confirming field observation that this species is much less attractive to *L. sphenophori* than any of the others. This may be due to the relatively small size of the nectar glands of that species. Differences between the other three *Euphorbia* species were not significant. The failure of the test to demonstrate a preference by *L. sphenophori* for *E. geniculata* and *E. heterophylla* over *E. hirta* may have been due to semishaded conditions which prevailed in the greenhouses. This could have nullified the positive effect of the shade provided the large leaves and upright growth habits of these species.

Distribution and Dispersal of Lixophaga sphenophori Adults in and around Cane Fields

Sex Ratios of Flies from Different Nectar Source Plants. Leeper (1974) suggested that behavioral differences between males and females probably accounted for unbalanced sex ratios of flies which he collected on two nectar source plants, *Euphorbia hirta* and *Ricinus communis.* Although the sex ratio of laboratory reared flies never differed significantly from 1:1, the flies collected from these plants were always predominantly males. During 1972 we recorded the sex of *L. sphenophori* adults collected on flowers of *Euphorbia geniculata* and *E. heterophylla.* Our results are compared with Leeper's data in Table 1. The sex ratios of flies from these plants were similar to that obtained by Leeper for *E. hirta.* However, Leeper pointed out that the ratio of ♂♂/♀♀ was significantly

[6] Stands of *E. geniculata* at Waialua Sugar Co. often were severely affected by a pathogenic fungus, *Alternaria* sp. (determined by Dr. A. Martinez, University of Hawaii, Dept. of Plant Pathology) which at times killed nearly all of the mature plants.

greater for flies caught on *R. communis* and suggested that this may have been due either to a greater attractiveness of *R. communis* for males, or because these plants generally were situated further away from field edges than *E. hirta* plants.

TABLE 1.—*Sex ratio of adult* L. sphenophori *collected on different nectar source plants on Oahu.*

Plant	Location	Period	♂♂	♀♀	Ratio
*Ricinus communis**	Ewa	Aug. 1970-Jan. 1971	981	273	3.6
*Euphorbia hirta**	Ewa	July 1969-Oct. 1970	573	224	2.6
E. geniculata	Waialua	Jan.-Sept. 1972	519	241	2.2
E. heterophylla	Waialua	Jan.-Sept. 1972	671	269	2.3

* Data from Leeper, 1974.

Comparison of Sex Ratios of Flies on Field Margins with Those Within Fields. A trapping experiment was conducted at Waialua Sugar Company plantation during 1973 to obtain additional data on the field distribution and behavior of *L. sphenophori*. Cylindrical traps 20 inches long and 4½ inches in diameter were fabricated from aluminum window screen. The bottom of each cylinder was closed with a circular piece of screen and the top was fitted with a lid from a wide-mouth gallon jar, held in place by rubber bands. A sheet of thin plastic, 8½ inches x 11 inches, was taped to the outside of the screen cylinder and coated with an adhesive mixture to serve as the trapping surface. Traps were baited by placing within each cylinder a split section of cane stalk, a two-week old *R. obscurus* grub inside an intact section of cane, and a rolled up sugarcane leaf which was sprayed with a mixture of honey and water. This combination bait, which provided both larviposition and feeding stimuli, was found to be superior to any of the three constituents alone. The baited traps were stood on end, the bottom of each placed within a partially buried half-gallon, plastic, ice cream carton. The outer rim of the carton above the soil level was sprayed with an adhesive to keep out ants.

Twenty such traps, distributed along two lines forming a perpendicular cross, were used in the test. One line extended along the edge of an open irrigation ditch, the banks of which supported an abundant growth of *Euphorbia hirta*. The second line extended directly into fields of eight-month old cane on either side of the ditch. Each arm of the cross contained 5 traps, distributed at 50 foot intervals away from the center point. Flies were trapped continuously for 26 days. Every second day fresh honey-water was applied and captured flies were removed.

Of 249 adult *L. sphenophori* which were trapped during the experiment, 207 were from traps along the irrigation ditch and 42 from infield traps. The sex ratio of the flies from the ditch bank traps was 2.0 ♂♂/♀; that of flies from infield traps was 3.2 ♀♀/♂. In both cases chi-square tests showed that the sex ratios of trapped flies differed highly significantly from a postulated 1:1 ratio. The sex ratio of flies from ditch bank traps was almost identical to that of flies which were collected by net from *Euphorbia hirta* plants in this area.

These data were interpreted as further evidence of behavioral differences between the sexes of *L. sphenophori* which affect the distribution of these flies in and around cane fields. The small number (10) of males captured on infield traps, and the fact that significantly more males than females were associated with nectar source plants along the irrigation ditch suggest that adult males rarely leave feeding sites to enter cane fields. The few males trapped within the fields may have been newly emerged individuals in search of food. That flies of both sexes were significantly more numerous along the ditch than infield, and the preponderance of males there, suggest further that concentrations of field margin nectar source plants may be important not only for food but also as mating sites. It seems likely that the male flies remain more or less continuously on field margins where nectar source plants are available, whereas females spend part of their time in such areas and part searching for larviposition sites within the fields.

Marking and Recapture Experiment. Large scale marking and recapture tests to study *L. sphenophori* population levels and dispersal were beyond the scope of this study due to limited facilities and personnel available. However, a marking technique was devised and a small-scale mark-recapture test was carried out using the traps and trap layout described in the previous section.

Leeper (1971) found that *L. sphenophori* adults were difficult to mark with fluorescent powders, dyes or paints because of the apparent toxicity of such materials and because of mortality resulting from anesthetization with CO_2 or low temperature. We found that these flies could be marked satisfactorily by feeding them radioactive glucose. D-glucose ^{14}C with a total activity of 250 m Ci was mixed with 70 ml tap water to provide a solution with a calculated activity of 7.92×10^6 DPM/ml. This solution was fed to newly emerged *L. sphenophori* adults either by mixing with equal parts of a 50% honey and water solution and spraying the resultant mixture on ti *(Cordyline)* leaves in fly cages, or by allowing the flies to feed on cotton saturated with the solution. All flies were adequately marked after a two day exposure period during which no other sources of food or water were provided. Enough activity was retained by the flies to give a significantly higher than background count thirty-three days after exposure, the maximum time that captive flies remained alive.

To test for radioactivity flies were placed individually in 20 ml glass scintillation vials, crushed with a glass rod, and digested for 24 hours in Protosol. After digestion, 15 ml of scintillation fluid (4.9 g PPO and 0.1 g POPOP per liter toluene) was added to each vial and samples were counted in a Nuclear Chicago Unilux II-A liquid scintillation counter with an external standard for quench correction.

In our small scale release-recapture trial 240 flies marked by the method described above were released in a field at Waialua Sugar Co. plantation. Four flies (3 ♂♂, 1 ♀) were recaptured, the last one eight days after release. Trapping was discontinued after 12 days because of poor weather. The three male flies were captured on traps along the irrigation ditch and the female fly on a trap 100 feet infield. The data, although meager, tend to support our hypothesis that male flies remain on field margins in association with nectar source plants.

Effect of Herbicidal Elimination of Nector Source Plants on Adult L. sphenophori Population Levels

To determine whether periodic herbicide applications to sugarcane field margins have an adverse effect upon population levels of *L. sphenophori,* we selected study areas along the edges of two fields in the Ewa section of Oahu Sugar Company plantation. These fields were about one-half mile apart but were similar in topography, age of cane and in the composition of their marginal weed flora. Both fields were about one year old at the beginning of the observation period, and in both the weed flora included the preferred species *Euphorbia hirta* and *E. heterophylla.* In one field the study area, designated Area A, was subjected to the regular plantation weed control program. Herbicide applications were made at somewhat irregular intervals five times during the study period. In the second field, marginal weeds in the study area, Area B, were not treated with herbicide during the entire seven months of the study. Weeds in this area occasionally were thinned out and cut back to maintain a stand density similar to that of Area A.

The number of adult *L. sphenophori* present in each of the two areas were counted at weekly intervals, between 8:00 a.m. and 10:00 a.m. when the flies were most active and easily seen. In Area A two counts were made each week; the number of flies observed on marginal weeds in two minutes, and the number which could be seen resting on cane leaves while slowly walking for a distance of 100 yards along the field margin away from the weed patch. The latter count was done to determine if the virtual absence of flies on the weeds during the weeks following herbicide applications was accompanied by low numbers on nearby cane. In Area B only the flies which could be seen on *Euphorbia* weeds during a two minute period were counted. The numbers of flies counted on *Euphorbia* in the two areas are compared in figure 1. In Area A, the peak numbers of flies counted while resting on cane leaves occurred almost exactly on the same days as peak numbers on flowers.

In Area A *Lixophaga sphenophori* populations declined virtually to zero, both on the treated weeds and on adjacent cane leaves, after each herbicide application, indicating a virtually complete absence of flies on the field margin. The time required for *L. sphenophori* adults to reappear on weeds in this area was directly correlated with the recovery rate of the

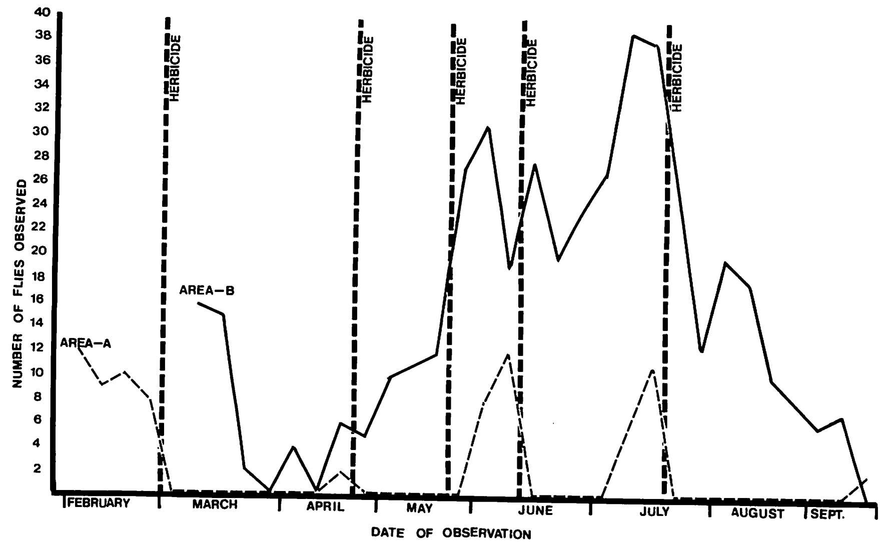

FIG. 1. Comparison of numbers of adult *Lixophaga sphenophori* observed on weeds in Area A (herbicide treated) and Area B (untreated), during 1972.

treated *Euphorbia* plants. The major factors affecting the recovery of *Euphorbia* were the thoroughness of herbicide applications and rainfall. With *E. geniculata* and *E. heterophylla* the smaller, younger plants and those which occurred as isolated individuals were most easily killed. Older plants, particularly where they occurred in dense stands, often were able to recover, after an initial dieback, by sending out new shoots from the bases of dead stalks, or at the nodes of lower branches. Incomplete coverage of individual plants by herbicides when applications were made to dense stands appeared to be the major reason for incomplete kill. Recovery of *Euphorbia* stands also occurred by seed germination when old plants were completely killed out. Although there was no apparent difference in the recovery rates of these two *Euphorbia* species under similar conditions, there was a pronounced seasonal effect on the recovery rates of both. Herbicide-treated stands died back more rapidly and recovered more slowly during the hot, dry summer months than during the cooler, wetter months of early spring (fig. 2). Both *E. geniculata* and *E. heterophylla* recovered sufficiently to begin flowering in about 22 days when herbicide was applied at the end of February. When application was made in mid-July, the same *Euphorbia* communities required about 36 days to attain this degree of recovery.

Euphorbia hirta is a relatively small species which grows only a few inches to a foot or so in height. These plants usually are killed out completely by herbicide applications and regeneration appears to be entirely from seed.

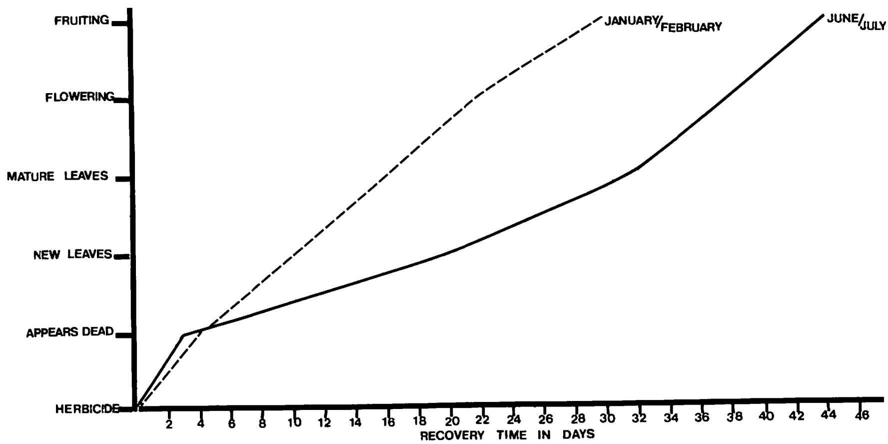

FIG. 2. Comparison of times required for dieback and recovery of a large stand of *Euphorbia geniculata* during January-February and during June-July, 1972.

From figure 1 it is apparent that the number of *L. sphenophori* observed on *Euphorbia* in Area B, where herbicides were not applied, often varied markedly from week to week. These variations were correlated directly with weather conditions at the time counts were made. On clear sunny days the flies were generally numerous, but they were relatively scarce on overcast or windy days. During periods of rain or unusually high winds flies were rarely seen. The marked decline in the numbers of flies observed in Area B during the last part of July, through September, probably was due, at least in part, to a reduction in nectar production by *Euphorbia* species. Many of the plants showed symptoms of water stress as irrigation of the cane field was curtailed in preparation for harvesting.

We assume that the major cause for the disappearance of adult *L. sphenophori* from cane field margins treated with herbicides was the migration of flies to other areas where nectar was available. However, there also may have been some direct mortality due to toxicity of the herbicides applied. In laboratory tests, Leeper (1974) showed that four herbicides which are commonly used in Hawaiian cane fields (Ametryne; 2,4-D; Diuron and M.S.M.A.) were toxic to *L. sphenophori* adults at concentrations of 0.1% when fed to flies in honey and water solution. In the field we observed that *L. sphenophori* adults continue to feed on nectar from *Euphorbia* plants that have been treated with herbicide mixtures containing Diuron and 2,4-D for a day or two after application, until the flowers dry up and cease nectar production. At times flies were observed to feed directly on droplets of freshly applied herbicides, possibly as a source of water. The possibility that herbicides may have contact toxicity for *L. sphenophori* has not been investigated. However, it has been shown that several of the commonly used herbicides have contact toxicity to honeybees (King, 1946).

Effect of Nectar Source Plant Elimination on Parasitization

After determining that herbicidal elimination of field-edge nectar source plants adversely affected population levels of adult *L. sphenophori*, we wished to determine if the elimination of these plants also resulted in a corresponding reduction in parasitization of *R. obscurus* grubs by this tachinid. Tests were conducted to develop and compare techniques for exposing *R. obscurus* grubs to parasites in the field. The results showed no significant differences in parasitization between grubs exposed in perforated plastic vials and those exposed by direct placement within uncut cane stalks or within cut sections of stalks. The plastic vial method was selected because of the relative ease with which these could be handled and recovered. Two week old grubs were exposed by placing them individually in perforated 5 dram snap-cap vials filled with a moist medium of mascerated green coconut husk. Grub vials were fastened to cane stalks with rubber bands. To minimize predation by ants and earwigs, tanglefoot was applied to cane stalks above and below grub vials. Further testing showed that significantly higher parasitization was obtained when vials were placed near the middle of cane stalks, 3 to 5 feet above the ground, than when they were placed near the tops of stalks, or at the bases of stalks under loose leaf trash. It was also determined that optimum parasitization and recovery of grub vials were obtained with field exposure periods of from five to seven days.

At Waialua Sugar Company plantation a corner of a large field of nine-month old cane with a heavy stand of *Euphorbia geniculata* growing along an irrigation ditch on one side was selected as a test site. Numerous adults of *L. sphenophori* were observed feeding on *Euphorbia* plants along the ditch. Nectar source plants along a road which formed the other side of the field corner were eliminated, and infield *Euphorbia* weeds were absent, having been shaded out by the closed cane canopy. Nectar source plants were therefore restricted to a stretch of about 200 feet along the irrigation ditch.

Grubs were exposed for parasitization along three parallel lines, each 50 feet apart, extending infield perpendicular to the irrigation ditch. Beginning at the field margin and at 50 foot intervals infields, up to a depth of 200 feet, groups of ten grub vials were attached to cane stalks (two to four vials per stalk on several adjacent stalks). After seven days, grub vials were recollected and returned to the laboratory for determination of parasitization. Three exposure tests were run. The first was made while the *Euphorbia* plants along the ditch were flowering profusely. Just prior to initiating the second test these plants were thoroughly sprayed with a mixture of Diuron, Dalapon and 2,4-D. The final test was begun 35 days after herbicide application. Concurrently with each test, a similar line of grub vials was exposed in another field of approximately the same age located about one-half mile away. Nectar source weeds on the margin of this field remained unsprayed during the entire experiment. Between 50% and 100% of the exposed grubs were recovered at each exposure site; the overall recovery rate was 67.8%. Losses were due to predation by ants and earwigs and to occasional loss of vials caused by breaking of the rubber band fasteners, and possibly rodents. The results of these tests are summarized in Table 2.

TABLE 2.—*Parasitization of* R. obscurus *grubs by* L. sphenophori *before and after application of herbicide to field margin.*

Time of Test	Distance infield from margin	Percent of recovered grubs parasitized	
		Herbicide treated field	Untreated control field
Before herbicide application	margin	95.0	100
	50 ft.	100	85.6
	100 ft.	100	87.5
	150 ft.	94.4	100
	200 ft.	26.4	100
	TOTAL	80.7	95.0
Immediately after herbicide application	margin	79.0	89.0
	50 ft.	86.5	71.4
	100 ft.	83.4	89.0
	150 ft.	92.8	70.0
	200 ft.	23.6	100
	TOTAL	76.5	83.6
35 days after herbicide application	margin	23.6	60.0
	50 ft.	5.3	71.4
	100 ft.	0.0	62.5
	150 ft.	16.7	50.0
	200 ft.	5.6	73.0
	TOTAL	10.0	64.9

Statistical analysis of data from the untreated field showed no significant differences in parasitization between any of the three exposure periods. A least significant difference test of data from the treated field showed no significant difference between parasitization before herbicide application and that during the period immediately following application. However, there were highly significant differences between parasitization during the first two exposure periods and the last one. These results, although they need further verification, suggest strongly that the complete elimination of nectar source plants from field margins can have a very detrimental effect on parasitization of *R. obscurus* by *L. sphenophori*. That a reduction in parasitization was not manifested during the seven day period immediately following herbicide application probably was due to the fact that *L. sphenophori* adults remained active in the test area until the *Euphorbia* plants had dried up almost completely, which required about three days.

During the first two tests parasitization of grubs placed 200 feet within the treated field was significantly lower than those placed at 150 feet or less. This seems to be further evidence of a correlation between parasitization and proximity of nectar source plants. It may be that the effective range of the parasite within cane fields is limited to around 150 to 200 feet from nectar sources. Further testing of this hypothesis is needed. The lack of a similar distance effect in the untreated field may have been due to the occurrence of some infield pockets of *E. geniculata* within 50 feet of the innermost exposure site along an unused irrigation flume, which we discovered after completion of the tests.

Conclusions

The results of the experiments and observations described in this paper corroborate Leeper's (1974) suggestion that continuous herbicidal elimination of field margin nectar source plants can have a detrimental effect on population levels of *Lixophaga sphenophori* and, therefore, lead to a decrease in the efficiency of this parasite as a biological control agent. Observations on Oahu sugar plantations suggest that completely abandoning all field edge weed control might not be beneficial. Where this was done at the Waialua plantation we found that other faster growing weeds often eventually crowded out the desirable *Euphorbia* species. *Euphorbia geniculata* and *E. heterophylla* appear to be somewhat resistant to herbicides, and *E. hirta* is a pioneer species which quickly reestablishes itself on denuded ditch banks, etc. For maximum utilization of the biological control potential of *L. sphenophori,* there appears to be a need for the development of an herbicide program utilizing materials and application intervals which will favor the continuous maintenance of field margin stands of nectar producing *Euphorbia* species.

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