MUCILAGINOUS SEEDS OF THE CRUCIFERAE FAMILY AS POTENTIAL BIOLOGICAL CONTROL AGENTS FOR MOSQUITO LARVAE

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INTRODUCTION. As man struggles to modify his environment in order to make it a more desirable place in which to live, he frequently has the option of either applying modern technology or making use of a naturalistic approach. Mosquito control agencies tend to select chemical control options because of their fast action, ease of application and availability, but in the process, the aquatic environment is forced to carry an additional burden of pollutants. Therefore, if alternative, non-chemical methods of suppressing pest and vector mosquitoes can be evolved, these may accomplish the desired modification of the environment without destroying it. The ideal solution to this problem is to obtain a pathogen, predator, or other means of controlling mosquitoes without injury to other animal or plant life. With this in mind, we recognized the potential of using seeds from certain wild plants to control mosquitoes.

The epidermal cells of the seed coats of a number of plant species belonging to several families contain a highly hygroscopic mucilage (Pieters and Charles, 1901; Winton and Winton, 1932; Bonner, 1950; Esau, 1953; Smith and Montgomery, 1959), notably the Cruciferae (mustard family), Linaceae (flax family) and Plantaginaceae (plantain family) (Murley, 1951; Robbins, Bellue and Ball, 1951; Munz and Keck, 1959; Robinson, 1963). When such seeds are placed in water, the mucilaginous material immediately exudes from the seed coat and, in some species, this rapid imbibition causes the outer cell walls to burst (Esau, 1960).

We have observed that when mosquito larvae come in contact with certain mucilaginous seeds, their oral brushes become irreversibly attached and the larvae subsequently die, due to drowning, exhaustion or other forms of stress. This paper presents data from preliminary tests on the use of mucilaginous seeds as agents for control of the mosquito larvae.

MATERIALS AND METHODS. Twenty-four kinds of seeds from three families, Cruciferae, Linaceae, and Plantaginaceae, were screened against mosquito larvae for desirable mucilage production and trapping efficiency. These seeds were obtained from the Colorado State University Seed Laboratory, the University of California, Riverside Horticulture Department and Herbarium, and other local sources (Table 1). After two preliminary tests, we selected five cruciferous species, *Descurainia sophia* (flixweed), *D. pinnata* (tansy mustard), *Lepidium flavum, Capsella bursa-pastoris* (shepherd's purse), and *Brassica geniculata* (short-podded mustard), for bioassay with 2nd and 4th instar *Culex tarsalis* and *C. peus* larvae. Five, 8, 10, 12, 15 and 20 seeds of each of the five species were placed in tap water-filled containers each preinfested with 60 second or fourth instar larvae from our laboratory cultures. The tests were conducted in 10 x 35 mm petri dishes with 2nd instar and 120 ml vials with 4th instar *C. tarsalis* and *C. peus* larvae. Each treatment was replicated three times. An untreated control of 60 larvae was run with each replicate. The tests were run at 23° C. for 24 hours.

An experiment was also conducted to determine if heat treatment of seeds could stop germination without inhibiting mu-

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TABLE 1.—Effect of screening seeds for detection of mucilage types suitable for mosquito larvae attachment and retention.

Seed tested	Production of suitable mucilage
Cultivated	
Cruciferae	
5018[a] Cauliflower	—
5038 Mustard	—
5072 Turnip	—
5102 Cress	—
5197 Cabbage	—
5211 Water cress	—
5234 Kohlrabi	—
5261 Mustard	—
5264 Mustard	—
5265 Mustard	—
6007 Broccoli	—
6035 Kale	—
6126 Rutabaga	—
6839 Rape	—
6851 Turnip	—
Linaceae	
Linum usitatissimum	—
Uncultivated	
Cruciferae	
Descurainia sophia	+
Descurainia pinnata	+
Lepidium flavum	+
Capsella bursa-pastoris	+
Brassica geniculata	+
Sisymbrium altissimum	—
Rorippa obtussa	—
Plantaginaceae	
Plantago insularis	—

[a] Cat. no., W. Atlee Burpee Co., Riverside, California.

cilage production. Seeds of each of the five species were heated at 121° C. for 20 minutes prior to introduction to larval containers. Germination, mucilage production and larval retention were compared with untreated seeds.

RESULTS. The criteria for the screening test were the production of mucilaginous material and the attachment and retention of mosquito larvae. The results are shown in Tables 1, 2, and 3. There was considerable variation in the quality and quantity of mucilage released from the seeds of different species (Table 1). None of the 15 varieties of cultured Cruciferae demonstrated satisfactory trapping qualities. Two uncultured Cruciferae, *Sisymbrium altissimum* and *Rorippa obtussa* also proved to be unsatisfactory. Seeds of *Linum usitatissimum* (common flax), produced mucilage in sufficient volume, but did not trap larvae. Seeds of *Plantago insularis* produced copious amounts of mucilage, but larvae did not become attached. The surface of this mucilage appeared to function as if it were a tough membrane which prevented attachment. Of the 24 kinds of seeds screened, five species of Cruciferae were found to possess the desired characteristics (Table 1). These five were evaluated against 2nd and 4th instar larvae of *C. tarsalis* and *C. peus,* and a summary of the data on their effectiveness is presented in Tables 2 and 3.

The average percent attachment data obtained from the various ratios of seeds to 2nd instar *C. peus* larvae are presented in Table 2. Data in Table 3 show that *D. sophia* demonstrated a high level of attachment and retention of 2nd instar larvae, holding 95 percent of *C. peus* and 89 percent of *C. tarsalis. D. pinnata* was nearly as high with 94 percent of *C. peus* and 83 percent of *C. tarsalis.* The retention of 4th instar larvae by the two *Descurainia* species was above 76 percent for both *C. peus* and *C. tarsalis. L. flavum* retained better than 91 percent of the 2nd instars of both mosquito species, but did very poorly against 4th instar larvae. The one species with mucilaginous seed that possessed the balance of physical characteristics required to attach and hold 2nd and 4th instar larvae of either species almost equally well was *C. bursa-pastoris.* It retained over 86 percent of the 2nd instar and over 81 percent of the 4th instar larvae. *B. geniculata* demonstrated the lowest adherance and retention rate of the five Cruciferae tested.

Data from the experiment on heat treatment of seeds show that viability could be completely destroyed by heating at 121° C. for 20 minutes. This treatment did not adversely affect the production of mucilage or larval entrapment at the temperature (23° C.) at which the experiments were conducted.

TABLE 2.—Percent attachment of second instar *Culex peus* larvae to mucilaginous seeds of five species of Cruciferae. Mean of three tests using 5, 8, 10, 12, 15 and 20 seeds per 60 larvae per container, 24 hour reading, 23° C.

	Percent larval attachment to indicated number of seeds					
Species	5	8	10	12	15	20
Descurainia sophia	88.5	95.1	95.1	95.8	94.6	96.8
Descurainia pinnata	78.0	90.8	94.1	96.3	92.5	94.1
Lepidium flavum	89.1	91.3	93.0	93.5	95.8	94.1
Capsella bursa-pastoris	74.7	76.9	86.8	90.8	93.5	96.8
Brassica geniculata	69.7	82.5	80.8	83.0	78.0	79.2
Control (percent mortality)	00	00	00	00	00	00

A photographic record of the seeds and larval attachment is presented in Figs. 1–4.

DISCUSSION. This new approach to the biological control of mosquito larvae has resulted from observations made while rearing laboratory cultures of mosquitoes. Mosquito larvae are commonly reared in the laboratory on a diet consisting of rabbit pellets or other enriched alfalfa meal animal feeds plus brewer's yeast added to the culture water at daily or tri-weekly intervals.

While following this feeding procedure, we observed that our culture pans occasionally contained one or two, or as many as eight larvae fastened together. The thrashing larvae were always attached by their mouthparts, as if they were fighting over a morsel of food. Once the larvae became attached in this manner, they invariably died. Microscopic examination of several of these masses of larvae revealed in each case the presence of a minute particle to which the larvae were attached. By detailed searching of the larval food, some particles were detected in the rabbit pellets that possessed the same microscopic characteristics as the particles securing the larval masses. These particles ranged from 0.2 to 0.5 mm in diameter. A halo of mucilage quickly formed around these particles when they were placed in water. Mosquito larvae introduced into containers with these mucilaginous particles soon became entrapped in the same manner as in the rearing pans.

At this point, we were concerned with two basic aspects: (1) These unknown particles, present in the rearing food, were interfering with the rearing of laboratory cultures of mosquitoes; and (2) If the nature of these particles could be determined, and the particles mass produced, they might be used to suppress mosquito populations under field conditions.

Samples of each of the 13 ingredients incorporated into the rabbit pellets were obtained from the manufacturer and each

TABLE 3.—Percent attachment of second and fourth instar *Culex tarsalis* and *C. peus* larvae to mucilaginous seeds of five species of Cruciferae. Mean of three tests each consisting of 10 seeds of each species per 60 larvae per container, 24 hour reading, 23° C.

	Culex peus Percent attachment		*Culex tarsalis* Percent attachment	
Seeds used	2nd instar	4th instar	2nd instar	4th instar
Descurainia sophia	95.1	77.5	89.1	80.2
Descurainia pinnata	94.1	78.5	83.0	76.4
Lepidium flavum	93.0	54.8	91.9	56.4
Capsella bursa-pastoris	86.8	81.3	89.6	86.3
Brassica geniculata	80.8	55.9	86.3	59.2
Control (percent mortality)	00	00	00	00

analyzed under the microscope. The particles were present in the alfalfa meal. It subsequently was determined that certain of the fields that supplied alfalfa contained cruciferous weeds that produced mucilage-containing seed.

The size of seeds varied in length from 1 mm in *Capsella bursa-pastoris* to 1.5 mm in *Lepidium flavum* (Fig. 1). The volume of mucilage produced by each of the five species was similar to that of *L. flavum* (Fig. 2). Each seed trapped considerably fewer large larvae than young larvae (Figs. 3 and 4).

If a larva is nearing the molting stage at the time it becomes attached, it is possible, under some conditions, for it to cast the larval skin and escape from the seed. The old exuvium remains attached to the mucilage, being held in place by the adhering oral brushes, while the newly molted insect swims away. If the newly molted larva returns to a seed, it then becomes permanently attached and succumbs.

In considering this approach to mosquito suppression, the question of source and supply of seeds arises. *D. sophia, D. pinnata, C. bursa-pastoris, B. geniculata* and several species of the genus *Lepidium* are classed as weeds in California (Robbins, Bellue, and Ball, 1951). We have observed extensive growth of some of these species along borders of alfalfa fields, in vacant lots and along roadsides. If sources of wild seed are not sufficient to meet the demands, seed probably could be produced on marginal land unsuitable

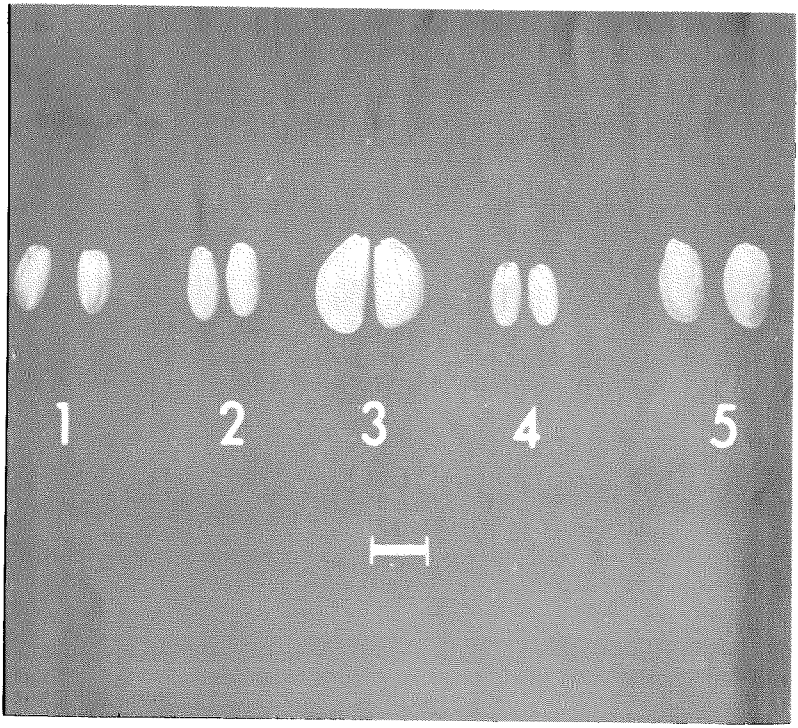

FIG. 1.—Five species of Cruciferae seeds: *Descurainia sophia* (1), *D. pinnata* (2), *Lepidium flavum* (3), *Capsella bursa-pastoris* (4), *Brassica geniculata* (5). Scale 1 mm.

for the production of conventional crops. To avoid any conflict of interest in distributing "weed seeds" to control mosquitoes, we have demonstrated the feasibility of heat treating these seeds to prevent germination. All tests reported in this paper were conducted with seeds heated to 121° C. for 20 minutes, thus reducing germination to zero percent. This heat treatment did not significantly reduce mucilage production. Alternative methods of eliminating germination would include irradiation of the seeds with cobalt 60 or cracking the seed in a feed mill.

The potential larval retention power possessed by a single kilogram of these seeds is considerable. The maximum number of 2nd instar *Culex* larvae observed attached to any single seed was 27 adhering to a *L. flavum* seed. There are over 4.4 million *L. flavum* seeds per kilogram (2 million seeds per pound). Therefore, the maximum projected catch would be 118 million larvae per kilogram (54 million larvae per pound) of *L. flavum.* A very conservative five larvae per seed would destroy 22 million larvae per kilogram (10 million larvae per pound). *C. bursa-pastoris,* our smallest seed, averages over 11 million seeds per kilogram (5 million seeds per pound). An equally conservative calculation of five larvae per seed could eliminate 55 million larvae per kilogram (25 million larvae per pound). Bailey, Bohart and Booher (1954) state that 1 acre of marsh land can produce more than 6 million mosquito larvae.

The practicality of this approach is yet

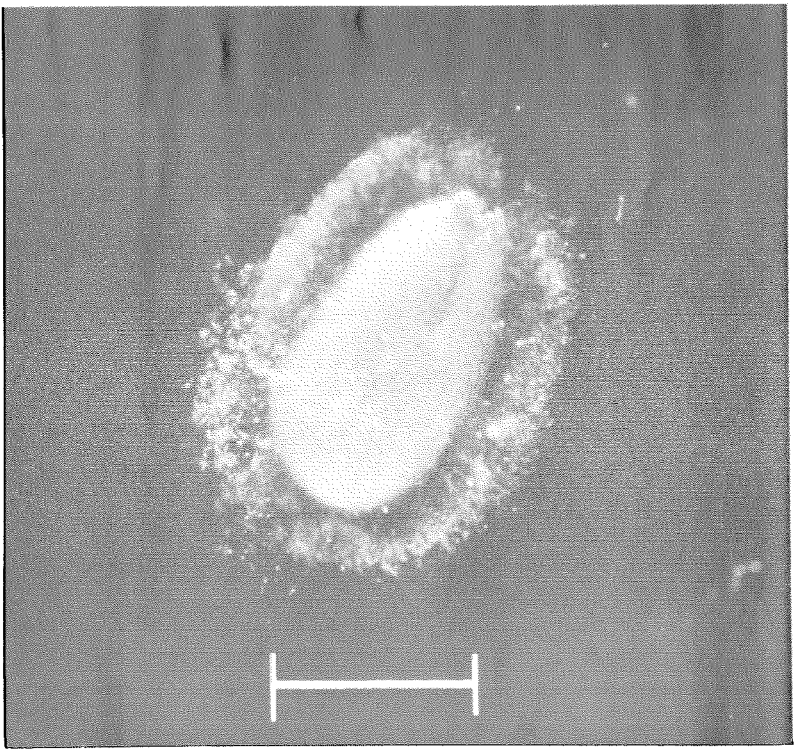

Fig. 2.—Seed of *Lepidium flavum* showing mucilaginous layer formed when placed in water (transparent halo of mucilage visualized by addition of chalk dust). Scale 1 mm.

to be determined. Its economic feasibility will depend to some extent upon the availability of a commercial source of suitable seeds. However, the growth habits of some of these plants should permit commercial production if there is adequate demand. Other factors yet to be evaluated under field conditions include the rate of mucilage breakdown and the effects of inactivating accumulations of sediments, unicellular algae, etc. on the mucilaginous coating. Preliminary field tests are planned for the summer of 1969. We envision this as a possible adjunct to a system of integrated control for mosquitoes. Mucilaginous seeds could complement established cultural, chemical, and biological methods of mosquito control. As new biological methods, i.e., pheromones, pathogens, and selective predators become operational, seeds should continue to be compatible and contribute as a population suppressant. The seeds could be broadcast by hand, ground applicator, or airplane.

The recognition of the potential biological control value of these and other mucilaginous seeds against mosquito larvae offers one more opportunity to suppress pestiferous and vector mosquitoes without increased chemical pollution of our aquatic environment.

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FIG. 3.—Second and third instar larvae of *Culex peus* attached to seeds of *Descurainia sophia* by mucilaginous layer.

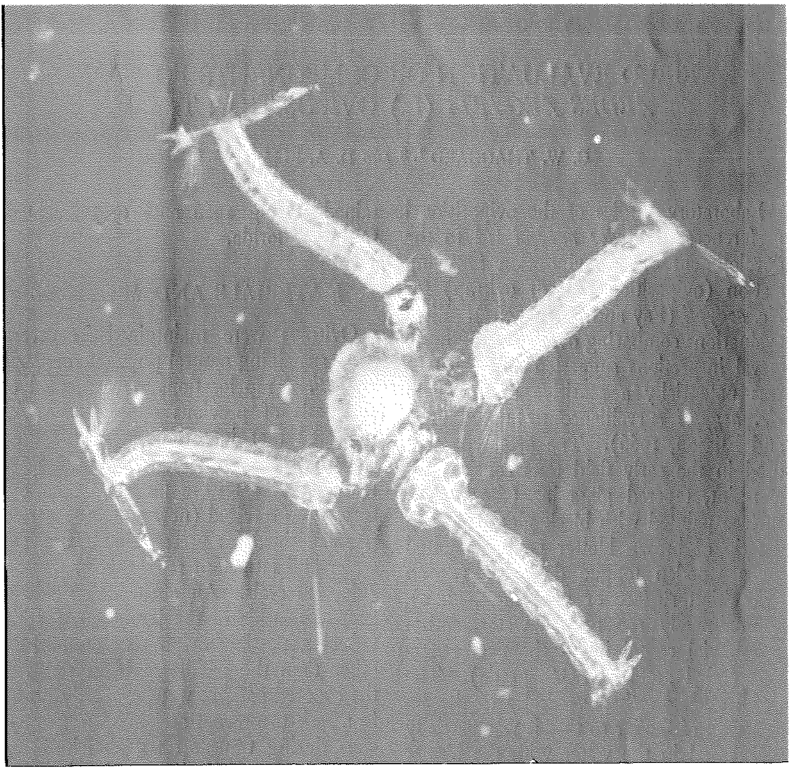

FIG. 4.—Fourth instar larvae of *Culex peus* attached to a seed of *Lepidium flavum* by mucilaginous layer.

of California, Riverside; and Mildred L. Thornton, Seed Analyst, Colorado State University are gratefully acknowledged. Photographs are by K. L. Middleham, University of California, Riverside.

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