INVESTIGATIONS OF MUCILAGINOUS SEEDS AS POTENTIAL BIOLOGICAL CONTROL AGENTS AGAINST MOSQUITO LARVAE[1]

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ABSTRACT. Selected mucilaginous seeds were evaluated in the laboratory to determine their potential for controlling mosquito larvae. Larvae of *Aedes aegypti* were used as the test organisms. Seeds were placed in test tubes containing larvae and observed for larval attachment to the exuded mucilage. Seventy-three species in 22 genera and 10 families were evaluated with 8 species, *Lepidium medium, L. densiflorum, L. thurberi, L. austrinum, Lesquerella argyrea, L. mirandiana, L. fendleri* and *Alyssum minutum*, producing larval mortality ranging from 76 to 89%.

INTRODUCTION

Seeds of many plant species possess a mucilaginous substance in the seed coats or endosperm. Tookey and Jones (1965) reported that 300 species from 31 plant families produced water-soluble seed mucilages. Young and Evans (1973) found that 233 species of weeds from 119 genera in 39 families contained mucilages in seed coats. To plants, a mucilaginous seed coat is useful in drought resistance and seed germination. Those plants possessing this character exude a sticky material when the seeds are soaked in water. Reeves and Garcia (1969) found this to be a potential biological control agent for mosquito larvae. They found seed from species belonging to the family Cruciferae that produced mucilage to which larvae became attached. The attachment to the exuded mucilages was reported to be at the larval mouth brushes, thus, the larva cannot escape and finally succumbs. Page et al. (1975) reported seed mucilages containing chemo-attractants, but the details of the attractants were not reported. The factor that affects larval attachment was reported by Barber et al. (1974) to be the presence of the cellulose content in the mucilages. Those seeds possessing a greater amount of cellulose in the mucilage had a greater percentage of larval attachment. Whether this caused a greater retention value of the mucilage or was an attractant as suggested by Page et al. (1975) was not reported.

This study was designed to determine the potential of selected seeds as agents for mitigating mosquito populations.

MATERIALS AND METHODS

SEED MUCILAGE OBSERVATIONS. Seeds collected from plants of known identity, purchased through a local store or furnished by the USDA Northern Utilization Research and Development Division, Peoria, Illinois, were used. The seeds were observed for their mucilage-producing ability by placing 20 seeds of each species into 50 ml plastic cups each containing 40 ml of distilled water. After 24 hours the seeds were observed using a stereoscopic microscope for any exuded mucilage. Seeds producing visible mucilages were used for the larval retention tests. The quantities of mucilage produced by the seeds were compared with each other and then ranked as high, medium, low, or none. The floating ability of the seeds was also observed. Each of the above-mentioned tests was replicated 4 times.

LARVAL ATTACHMENT. All mucilage-producing seeds were tested against 2nd instars of *Aedes aegypti*. Twenty seeds

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[1] The investigation reported in this paper (No. 75-7-161) is published with approval of the Director of the Kentucky Agricultural Experiment Station.

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of each species were placed into separate 20 x 150 mm test tubes each containing 10 larvae in 20 ml of water. No seeds were placed in the tubes containing control larvae. The tubes were held at 76± 2° F. The larvae were not fed because of the possibility that the food might interfere with the tests. Tests were replicated 4 times, and the larval attachment to the seeds was determined at day 3.

LARVAL RETENTION QUALITY. Seeds of species found to entrap large numbers of larvae were again tested to obtain the larval retention quality. The technique was the same as previously described in the larval attachment study except the retention quality was determined by the percent larval mortality at day 7. A minute amount of ground dog chow was added as larval food to each tube at the beginning of the test and 3 days later.

LARVAL RETENTION QUALITY UNDER SIMULATED FIELD CONDITIONS. A simulated field condition was employed to determine the effect on retention properties of mucilaginous seeds. The procedure used was the same as described previously with the exception that 5 g of sterile soil were placed in each tube and were allowed to settle to the bottom to simulate field conditions. The seeds included in this test were those causing high mortality in earlier tests. Also some seeds that caused lower mortality were randomly selected to determine if simulated field conditions would change their effect against the test organisms.

Abbott's formula (Abbott 1925) was used to determine the corrected mortality among larvae exposed to mucilaginous seeds for 7 days. The Student's T-test was used to analyze the difference in the seed test under simulated field conditions (Duncan 1955).

RESULTS

THE QUANTITY OF MUCILAGE PRODUCTION. Seeds that were placed in water for 1 day were observed for exuded mucilages, and the results are presented in Table 1. Of the 73 species tested, 3 produced a large quantity of mucilages. One of these, *Gilia pinnata*, belongs to the family Polemoniaceae and the other two, *Salvia reflexa* and *Ocimum basilicum*, belong to the family Labiatae. Seeds of all the species of Cruciferae tested produced a medium amount of mucilage except for *Descurainia sophia* which produced a small amount. Only 4 other species, 2 from the family Leguminosae and 2 from the family Convolvulacae, produced small amounts of mucilages. Those seeds that produced small amounts of mucilages were not included in further testing.

The floating characteristics of the seeds are shown in Table 1. Those seeds that produced large and small amounts of mucilages sank to the bottom of the test chamber. Of the 55 species producing a medium amount of mucilage, 10 floated, 9 floated initially but sank after a few hours, and the remaining 36 sank almost immediately when placed in water.

LARVAL ATTACHMENT. Fifty-two of the 73 species tested had some larval attachment at day 3. These ranged from 1.25 to 92.5 percent attachment, with the majority of the attachment being on seeds from the family Cruciferae (Table 1). Only 1, *O. basilicum*, of 3 species that produced high amounts of mucilages, had a high rate of larval attachment (77.5 percent). All seeds that floated, except for *Alyssum* sp. (No. 47653), had a low rate of larval attachment (0 to 22.5 percent) at day 3 (Table 1). *Alyssum* sp. (No. 47653) had a larval attachment rate of 45 percent.

LARVAL RETENTION. The 52 species found to have some larval attachment properties at day 3 (Table 1) were further tested to determine the ability of the mucilage to produce larval mortality. All but 2 of these species, *Crotalaria usaramoensis* and *Ocimum basilicum*, belonged to the family Cruciferae. Eight species within 3 genera, *Lepidium*, *Lesquerella*, and *Alyssum*, gave a high larval mortality (ranging from 88.6 to 75.6 percent) at day 7. Nineteen other species produced a

Table 1. Plants, mucilage production of seeds, floating ability, propensity for larval attachment, and mortality of 2nd instar *A. aegypti* larvae.

Family	Scientific Name or Number	1-day mucilage production [a]	Seed floating ability [b]	3-day % larval attachment	Mean percent corrected motrality [c]
Boraginaceae	*Cynoglossum amabile*	–	F	0	–
Convolvulaceae	*Convolvulus tricolor*	*	S	0	–
	Ipomoea amnicola	–	F	0	–
	I. leptophylla	–	F	0	–
	I. pandurata	–	S	0	–
	I. purpurea	*	S	0	–
Cruciferae	*Alyssum argenteum*	**	F	2.5	13.3
	A. bertholonii spp. *scutonium*	**	F	5.0	30.0
	A. dasycarpum	**	S	56.2	29.7
	A. minimum	**	S	53.0	41.2
	A. minus	**	S	37.5	71.9
	A. minutum		S	67.5	85.2
	A. murale	**	F	0	0
	A. peltarioides	**	F	37.5	52.4
	A. sibiricum	**	S	72.5	34.8
	A. sp. (No. 43443) [d]	**	F	0	3.3
	A. sp. (No. 47653) [d]	**	F	45.0	40.9
	A. tortuosum	**	S	67.5	51.9
	Capsella bursa-pastoris	**	S	1.25	–
	Descurainia pinnata	**	S	40.0	51.2
	D. sophia	*	S	20.0	2.8
	Lepidium austrinum	**	S	67.5	78.6
	L. campestre	**	FS	25.0	59.0
	L. densiflorum	**	S	80.0	88.6
	L. graminifolium	**	S	10.0	40.9
	L. lasicarpum	**	S	22.5	33.3
	L. latifolium	**	F	15.0	6.6
	L. medium	**	S	92.5	88.6
	L. micranthum	**	FS	85.0	63.8
	L. montanum	**	S	54.0	65.4
	L. perfoliatum	**	S	50.0	56.1
	L. pinnatifidum	**	FS	72.5	65.7
	L. repens	**	FS	0	3.3
	L. ruderale	**	S	65.0	61.4
	L. sativum	**	S	52.5	70.5
	L. sp. (No. 43356) [d]	**	S	85.0	67.0
	L. sp. (No. 46940) [d]	**	S	65.0	52.7
	L. subulatum	**	S	35.0	55.2
	L. thurberi	**	S	85.0	83.3
	L. vesicarium	**	S	27.5	6.6
	L. virginicum	**	S	72.5	64.3
	Lesquerella argyrea	**	S	80.0	85.2
	L. auriculata	**	S	52.5	46.6
	L. engelmanii	**	S	56.0	57.7
	L. angustifolia	**	FS	22.5	59.0
	L. fendleri	**	S	79.0	75.6
	L. globosa	**	F	0	6.6
	L. gordoni	**	S	42.5	36.2
	L. gracilis	**	S	65.0	57.6
	L. grandiflora	**	S	35.0	47.6
	L. lasiocarpa	**	FS	60.0	33.3
	L. lescurii	**	F	45.0	36.2
	L. lyrata	**	F	25.0	32.9
	L. mirandiana	**	S	62.5	78.6
	L. palmeri	**	S	47.5	23.3
	L. perforata	**	FS	30.0	49.6
	L. stonensis	**	F	22.5	61.9

Table 1. (Continued)

Family	Scientific Name or Number	1-day mucilage production[a]	Seed ability[b] floating	3-day attachment % larval	Mean percent mortality[c] corrected
Labiatae	*Ocimum bascilicum*	***	S	77.5	28.4
	Prunella vulgaris	**	FS	2.5	–
	Salvia reflexa	***	S	2.5	–
Leguminosae	*Cassia bonariensis*	*	S	0	–
	Crotalaria spectabilis	–	S	0	–
	C. usaramoensis	–	S	0	23.3
	Cyamopsis tetragonaloba	–	S	0	–
	Parkinsonia aculeata	–	S	0	–
	Trigonella foenum-graecum	*	S	0	–
Linaceae	*Linum grandiflorum*	**	S	0	–
Plantaginaceae	*Papaver rhoeas*	–	F	0	–
Plantaginaseae	*Plantago ovata*	**	FS	0	–
	P. rhodosperma	**	S	0	–
	P. wrightiana	**	S	3.75	–
Polemoniacaeae	*Gilia pinnata*	***	S	2.5	–
Umbelliferae	*Eryngium giganteum*	–	S	0	–

[a] – none; * low; ** medium; *** high.
[b] F=float; FS=some would float for a while before sinking; S=sink.
[c] Only those seeds found to entrap large number of larvae were tested for larval retention qualities for 7 days.
[d] USDA seed identification number.

medium mortality of 51.2 to 71.9 percent. Fifteen species showed a low mortality of 28.4 to 49.6 percent, and 10 species were listed as very low, with 0 to 23.3 percent mortality (Table 2).

LARVAL RETENTION UNDER SIMULATED FIELD CONDITIONS. Sixteen species, 8 from the high, 5 from the medium, and 3 from the low retention group, from previous tests, were evaluated for their retention ability in a simulated field condition against 2nd instar *A. aegypti*. *Lesquerella argyrea* showed a significantly greater percent mortality than in the non-simulated test and *Alyssum minutum* showed a significantly lower mortality than in previous tests. The remaining 14 species were not significantly different at the 5 percent level (Table 3).

DISCUSSION

These tests showed that mucilage-producing seeds vary in the production of mucilage and in larval retention qualities. Mosquito larvae in contact with the mucilage usually became attached by means of the larval mouth brushes. Seeds producing large quantities of mucilage, such as species from the family Labiatae, had low larval retention qualities, but other seeds which produced less mucilage had greater larval retention qualities. The varied structure and composition of the mucilages as reported by Barber et al. (1974) may be the reason for this difference. Simply stated, they found a correlation between the stickiness of the mucilage, its cellulose component, and ability to trap mosquito larvae.

Of the 10 plant families tested, the seeds from the family Cruciferae were found to be the most effective in trapping larvae long enough for the larvae to be killed (Table 3). In some cases larvae were trapped but were not killed. This was possibly due to (1) the retention properties of the mucilage; (2) the retention time of the mucilage; (3) the size of the seeds in relation to volume of mucilage and its

Table 2. Effect of mucilaginous plant seeds on 2nd instar *A. aegypti* larvae in simulated field conditions at day-7.

Plant Name	Mean number larvae killed[a] Non-simulated	Simulated
Lepidium medium	8	10 NS[b]
Ocimum basilicum	7	9.7 NS
Lepidium densiflorum	9	9 NS
Lepidium thurberi	7.3	8.7 NS
Descuramia pinnata	5.7	7 NS
Lesquerella mirandiana	5.3	7 NS
Lepidium montanum	7	6.7 NS
Lesquerella argyrea	4.7	6.3*
Lesquerella fendleri	5.3	6 NS
Alyssum minimum	7.3	5.7 NS
Lepidium austrinum	7.7	5.7 NS
Lesquerella engelmanii	7.3	5.3 NS
Lepidium sativum	4.3	4.7 NS
Lepidium perfoliatum	4.3	3.3 NS
Alyssum minutum	7.3	3.3*
Alyssum dasycarpum	6	2 NS
Control	1	1.3

[a] Based on 10 larvae per treatment; 3 rep.
[b] *—significant difference at the 5% level by t-test.
NS—not significant at the 5% level by t-test.

floating ability, and (4) the stage of larval development. Seeds of many species showed viscous and sticky properties only in the early period. Thus, the larvae which came into contact with these seeds during the latter period did not become attached.

The length of time that mosquito larvae can survive when submerged in water varies with the age and size of the larvae (Sen 1914; Hagstrum and Mulla 1970). These workers found that when mosquito larvae are submerged in water the smaller ones can survive longer than the larger ones. Larvae reduce their oxygen consumption and in many cases induce cuticle respiration that enables them to survive (Fay 1964; Christophers 1960).

In the tests using 2nd instars, some larvae that were attached to the mucilage by the mouth brushes lived long enough to molt and escape entrapment. The late instars which became strongly attached were unable to escape. The mucilaginous seeds which were relatively small (mostly in the family Cruciferae) were not heavy enough to prevent larval movement once the larvae became attached. Consequently, relatively large larvae attached to small seeds could still move about and even surface for air. If attachment persisted for a long period of time the larvae became weakened and subsequently died.

Simulated field condition, i.e. test tubes with soil in the bottom, did not significantly reduce the trapping ability of seeds. However, one species, *Lesquerella argyrea,* did show a higher percentage of larval attachment and, thus, a higher larval mortality occurred. Why this one species exhibited this characteristic is unclear. However, it is important to recognize that water with a soil or mud bottom did not significantly affect the trapping ability of most seeds.

The practicality of using mucilaginous seeds for large area mosquito control is doubtful, owing to the number of the seeds needed. A more practical method would be to employ a synthetic mucilage, or an extracted seed mucilage which could be applied to some granular inert material and used as a granular larvicide. Sterile or cracked seeds, however, could be used in controlling mosquito larvae in small shallow pools or livestock watering areas where insecticide usage is prohibited. Also in certain areas of the world, various mucilage seed-producing plants could be grown around bodies of water and thus become a natural mosquito control method.

It is possible that some seed species that were not very effective against *A. aegypti* might produce a higher mortality in some other mosquito species having different larval grazing habits. Therefore further studies of mucilaginous seeds for control of mosquito larvae are indicated.

ACKNOWLEDGMENT

Sincere appreciation is given to Dr. W. H. Tallent of the USDA Northern Regional Research Center, Peoria, Illinois, for supplying many seeds used in this investigation.

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