INTERACTIONS BETWEEN MOSQUITO LARVAE AND MUCILAGINOUS PLANT SEEDS

II. CHEMICAL ATTRACTION OF LARVAE TO SEEDS[1]

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ABSTRACT. Mucilaginous plant seeds show a positive chemo-attraction for *Culex pipiens quinquefasciatus* Say larvae. Seeds that produce mucilage and cause larval attachment are strongly attractive within 1 hour, while other mucilaginous seeds, to which larvae do not attach, are not attractive until after 2–4 hours. Half of the non-mucilaginous seed species examined also release a chemo-attractant for mosquito larvae.

INTRODUCTION. Mucilaginous plant seeds have been considered as possible agents for the control of mosquitoes. Reeves and Garcia (1969) reported that mosquito larvae, upon contact with certain mucilaginous seeds, become attached by their oral brushes and subsequently die because of lack of oxygen, exhaustion, or some other form of stress. The attachment of larvae to certain mucilaginous seeds (Barber et al. 1974) occurred at a much higher rate than could be accounted for by random location and suggested that certain seeds might be exerting a positive attraction for mosquito larvae. This paper represents the results of an investigation which pertains to the chemo-attraction of plant seeds for mosquito larvae.

MATERIALS AND METHODS. The seeds used in this study were as follows: Cruciferae-*Capsella bursa-pastoris* (L.) Medic (Shepherd's purse), *Descurainia sophia* (L.) Webb (Tansy-mustard), *Brassica sp.* (Mustard); Linaceae-*Linum usitatissimum* L. (Common flax); Plantaginaceae-*Plantago insularis* Eastw. (Plantain); Gramineae-*Sporobolus airoides* Torr. (Fine-top salt-grass); Leguminosae-*Pisum sativum* L. (Garden-pea); Magnoliaceae-*Magnolia grandiflora* L. (Evergreen magnolia). All of the Cruciferae are annual weedy species introduced accidentally from Europe.

Culex pipiens quinquefasciatus Say larvae were collected in the greater New Orleans area and used in all of the experiments. Larvae were maintained in covered rectangular pans (41 x 25 x 7 cm), in about 3 cm of spring water. The water was continuously aerated and the larvae were fed ground Purina Dog Chow.

The experimental chamber of Bonner and Etges (1967) was modified for study of chemical attraction of mosquito larvae to plant seeds (Figure 1). A plexiglass chamber, 43 x 6 x 5 cm was grooved for the insertion of a zone divider at the termination of each experiment. Permeable barriers of fine grade copper screen were located 2.5 cm from each end of the chamber; one of these two terminal areas was designated the seed area. The chamber was filled with 700 ml of charcoal-filtered deionized water. The following protocol was adhered to in studying chemical attraction. Approximately 100 3rd and/or 4th stage mosquito larvae were placed in a 2 cm diameter plastic tube, open at both ends, positioned in the center of the chamber to retain the larvae until the start of the experiment. The desired concentration of seeds was completely submerged in the seed area. After a 10-minute preincubation period for the seeds, the plastic tube restraining the larvae was removed and the entire chamber was placed in a light-tight box. A bubble level was used to keep the chamber horizontal. Temperature of the water varied from 23 to 25° C between individual experiments, however, no temperature differential was observed in the chamber, indicating that

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movement of larvae was not related to a thermal gradient.

All experiments were performed between 1000 and 1600 hours. At the termination of an experiment, either 1, 2, 4, or 6 hours, the zone divider release string was pulled from outside the box, allowing the zone divider, which had been suspended above the chamber, to fall into place and divide the chamber into five areas (7.5 cm apart) without disturbing the larval distribution. The chamber was removed from the dark and the numbers of larvae in each zone [+2 (closest zone to seed area), +1, 0, —1, —2] were recorded. Since the larvae were released in the middle of the chamber (zone 0), it was predicted that in experiments without seeds present random movement would result in equal numbers of larvae migrating in both directions from the release point. At the end of the experiment the number of larvae in zone 0 were divided equally between the positive and negative groups. Using this method for control and experimental data, the Chi-square test was performed to determine whether the larvae were significantly attracted to the seed area. At $P \leq 0.05$, seeds were considered to be significantly attractive.

Results. Preliminary trials indicated that mosquito larvae were attracted to *C. bursa-pastoris* seeds. To determine a working concentration of seeds to be used in later experiments, larval attraction was related to seed concentration for 1 hour (Figure 2). No significant attraction of mosquito larvae was found using 0.1 gram of seeds. Larval attraction first appeared using 0.5 gram of seeds, as evidenced by the increase of larvae in the +2 zone. The maximum larval attraction occurred using 5.0 grams of seeds and resulted in 85% of the larvae in the positive zone. A decrease of larvae in the positive zone occurred at 10.0 grams of seeds, indicating a possible saturation of the chamber with the chemical attractant. From the concentration curve, 2.0 grams were selected as the amount of seeds to be routinely used for testing larval attraction to various species of plant seeds.

The seeds used in this study were divided into three groups, depending on their ability to secrete mucilage and cause significant larval attachment. One group consisted of seeds that produce mucilage and cause attachment. The two seeds tested in this group, *C. bursa-pastoris* and *D. sophia,* both showed positive attraction after one hour (82% and 72%, respectively) (Table 1). *C. bursa-pastoris* was less attractive at 2 hours (73%) and 4 hours (67%) and not attractive at 6 hours. However, *D. sophia* was more attractive at 2 hours (80%), decreased at 4 hours (70%) and also was not attractive at 6 hours. Saturation of the chamber by the attractant probably results in even distribution of the larvae within the chamber following 6-hour incubations.

A second group consisted of seeds that produce mucilage but do not cause significant larval attachment. The three seeds in this group, *S. airoides, L. usitatissimum,* and *P. insularis,* did not attract mosquito larvae after 1 hour (Table 1). However, larvae were attracted to *S. airoides* after 2 hours (78%) and 4 hours (88%), and to *L. usitatissimum* after 2 hours (71%), 4 hours (84%) and 6 hours (70%). Both of these seeds, ultimately, had a greater attractive force for mosquito larvae than the seeds in the first group. The least attractive of all mucilaginous seeds examined was *P. insularis,* which drew only 66% of the larvae at 2 hours and 64% at 4 hours. Although the attraction by *P. insularis* was statistically significant, three-quarters of the larvae recorded were present in the +1 and 0 zones of the chamber, while all other seeds that attracted larvae had the largest number in the +2 zone. This low number of larvae in the +2 zone for *P. insularis* points out that the attractive nature of this seed is very weak.

A third group was composed of seeds that do not produce mucilage or contain an entrapment mechanism for larvae (Table 2). In this group, *Phaseolus* sp. and *M. grandiflora* did not show a positive attraction for mosquito larvae during the experimental time period of 6 hours. The

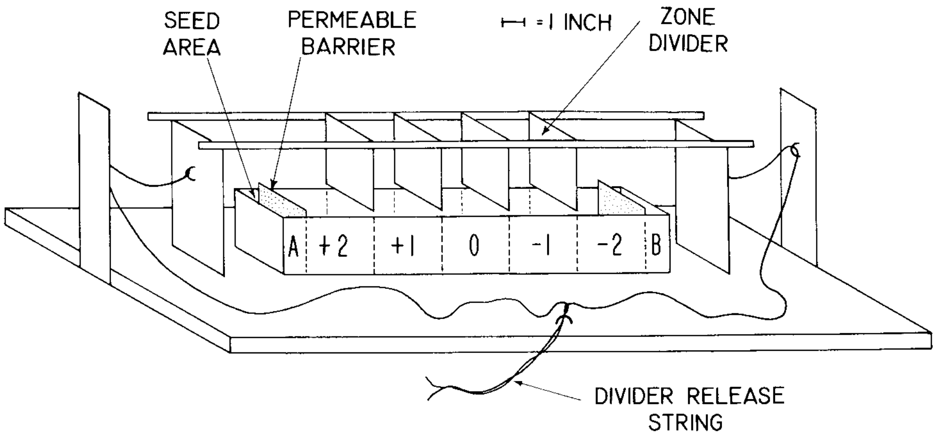

Fig. 1. Diagram of the plexiglass chamber used to test for chemical attraction of mosquito larvae to plant seeds.

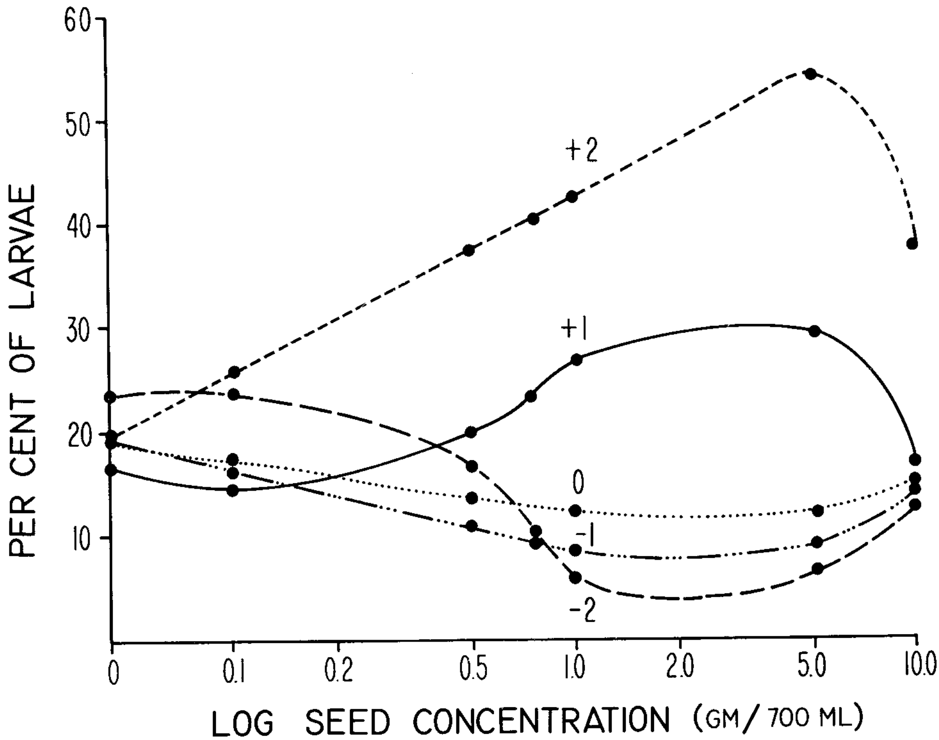

Fig. 2. Effect of seed concentration on the attraction of *Culex pipiens quinquefasciatus* larvae. Starting from the seed area, chamber zones are represented as +2 (●----●), +1 (●———●), O (●.....●), −1 (●—···—●), and −2 (●— —●). Each point is a mean of at least 3 experiments (100 larvae/experiment).

Table 1. Attraction of *Culex pipiens quinquefasciatus* larvae to mucilaginous seeds for 1 to 6 hour incubation.

Seeds tested	Time of seeds-larvae incubation[a]	No. of larvae (no. of trials)	Percent larvae per zone					χ^2 + = Attraction − = Repulsion	P
			+2	+1	0	−1	−2		
Capsella bursa-pastoris	1	995(10)	56	19	13	7	5	+39.69	<0.005
	2	202(2)	47	20	11	10	12	+20.25	<0.005
	4	205(2)	27	27	26	12	8	+11.56	<0.005
	6	202(2)	16	24	39	13	8	+ 3.61	>0.05
Descurania sophia	1	196(2)	47	18	13	8	14	+18.49	<0.005
	2	210(2)	46	26	16	7	5	+36.00	<0.005
	4	195(2)	35	24	22	7	13	+16.00	<0.005
	6	201(2)	24	21	20	15	20	+ 1.00	>0.25
Linum usitatissimum	1	212(2)	29	14	14	21	22	0.00	1.00
	2	198(2)	46	15	20	11	8	+17.64	<0.005
	4	206(2)	42	34	16	5	3	+46.24	<0.005
	6	202(2)	28	30	23	18	10	+14.44	<0.005
Plantago insularis	1	198(2)	26	16	22	15	21	+ 0.36	<0.75
	2	200(2)	18	22	52	5	3	+10.24	<0.005
	4	202(2)	7	40	34	10	9	+ 3.92	<0.05
	6	200(2)	27	16	11	19	27	− 0.09	>0.75
Sporobolus airoides	1	203(2)	19	17	27	15	22	− 0.01	<0.9
	2	200(2)	53	19	11	7	10	+30.25	<0.005
	4	188(2)	55	28	9	5	3	+56.25	<0.005
	6	206(2)	39	11	14	16	20	+ 1.96	<0.25
Seeds absent	1	327(3)	20	16	20	20	24	− 0.64	>0.50
	2	225(2)	20	19	19	21	21	− 0.09	>0.75
	4	208(2)	22	19	21	18	20	+ 0.09	>0.75
	6	281(2)	20	16	24	19	20	− 0.16	<0.75

[a] 2.0 grams of seeds in 700 mls of water and approximately 100 larvae/trial.

Table 2. Attraction of *Culex pipiens quinquefasciatus* larvae to non-mucilaginous seeds for 1 to 6 hour incubations.

Seeds tested	Time of seeds-larvae incubation[a] (hr)	No. of larvae (no. of trials)	Percent larvae per zone					χ^2 + = Attraction − = Repulsion	P
			+2	+1	0	−1	−2		
Phaseolus sp.	1	193(2)	15	22	27	23	13	+ 0.01	>0.90
	2	194(2)	27	21	23	19	10	+ 3.60	>0.05
	4	179(2)	25	14	15	16	30	− 0.48	>0.50
	6	198(2)	40	7	16	17	20	+ 1.00	>0.25
Magnolia grandiflora	1	201(2)	21	15	24	21	19	− 0.16	>0.50
	2	204(2)	25	24	16	21	14	+ 1.98	>0.10
	4	195(2)	25	12	22	18	23	− 0.16	>0.50
	6	181(2)	20	20	22	18	20	+ 0.04	>0.75
Brassica sp.	1	395(4)	24	17	20	20	19	+ 0.04	>0.90
	2	199(2)	47	18	10	7	18	+16.00	<0.005
	4	194(2)	42	21	13	11	13	+15.21	<0.005
	6	193(2)	60	13	17	5	5	+39.70	<0.005
Pisum sativum (Improved telephone)	1	195(2)	15	18	35	19	13	+ 0.01	>0.90
	2	202(2)	35	22	22	4	17	+12.96	<0.005
	4	191(2)	28	19	27	15	11	+ 4.41	>0.005
	6	148(2)	30	28	22	15	5	+14.44	<0.005
Pisum sativum (Mammoth melting sugar)	1	202(2)	18	23	33	14	14	+ 2.25	>0.10
	2	213(2)	11	11	35	28	15	− 4.41	<0.05
	4	188(2)	24	21	18	17	20	+ 0.64	<0.50
	6	165(2)	22	27	17	18	16	+ 2.25	>0.10
Pisum sativum (Early Alaska)	1	323(3)	44	17	11	14	14	+10.88	<0.005
	2	194(2)	39	25	13	8	15	+ 4.41	<0.05
	4	179(2)	25	9	19	21	26	− 1.68	<0.25
	6	198(2)	23	25	19	14	19	+ 2.25	>0.10
Seeds absent	1	327(3)	20	16	20	20	24	− 0.64	>0.50
	2	225(2)	20	19	19	21	21	− 0.09	>0.75
	4	208(2)	22	19	21	18	20	+ 0.09	>0.75
	6	281(2)	20	16	24	19	20	− 0.16	<0.75

[a] 2.0 grams of seeds in 700 ml of water and approximately 100 larvae/trial.

larvae exhibited a random movement, which resulted in equal numbers of larvae moving in both directions, plus and minus, from the starting point (zone 0). This random movement of the larvae was similar to that observed in the control group, where seeds were absent from the chamber. However, some of the non-mucilaginous seeds examined did affect the movement of mosquito larvae. *Brassica sp.* attracted larvae at 2 and 4 hours (70%) and increased its effect at 6 hours (82%). Also, two of the *P. sativum* varieties, Improved Telephone and Early Alaska peas, attracted larvae to a lesser degree. Improved Telephone peas attracted larvae at 2 hours (68%), 4 hours (61%) and 6 hours (69%) while the Early Alaska peas were attractive only after 1 hour (67%) and 2 hours (71%). A third pea variety, Mammoth Melting Sugar, did not attract mosquito larvae during the experimental time period but repelled the larvae at 2 hours (61%).

Discussion. Mosquito larvae are attracted to all of the mucilaginous seeds examined in the present study. There is an attraction demonstrable for mucilaginous seeds that entrap larvae (i.e., *C. bursa-pastoris* and *D. sophia*) and also those which do not entrap larvae (i.e., *S. airoides, L. usitatissimum,* and *P. insularis*). The soluble chemical attractant that diffuses from the seeds appears to be unrelated to the components that endow a mucilage with stickiness to entrap larvae. Barber et al. (1974) reported that a mucilage is "sticky" or "non-sticky" depending upon the presence or absence of a cellulose component. However, it should be remembered that cellulose is a relatively insoluble molecule and therefore would not be expected to diffuse throughout the chamber under the experimental conditions described. The results reported in this study on a limited number of seeds suggest that there is no correlation between mucilaginous seeds attracting mosquito larvae and larvae sticking to the mucilage.

Possibly the mechanism of mucilage release gives some clue as to the origin of the chemo-attractant. When mucilaginous seeds are placed in water they extrude mucilage from the seed coat; in some species the rapid imbibition of water causes the outer cell wall to burst (Youngken, 1934; Esau, 1960). Presumably not only mucilage will be released during this process but also other cell constituents. The mechanism of mucilage release appears to be similar in both group 1 and group 2 seeds. However, there appears to be a difference in the rate mosquito larvae are attracted to the mucilaginous seeds. Group 1 seeds, *C. bursa-pastoris* and *D. sophia,* show their highest attractive force (i.e., % positive) very soon after imbibition, while the second group of mucilaginous seeds, *S. airoides, L. usitatissimum,* and *P. insularis,* do not demonstrate any attraction at 1 hour and show a peak attractive force after 4 hours. The attractive force is similar among the mucilaginous seeds, with the exception of the somewhat lower attraction by *P. insularis.* This difference in attraction rate may be related to the differential release rate of the chemo-attractant from the seed following imbibition. The attractant may be released immediately on contact with water and the mosquito larvae would be attracted to the concentrated substance(s). Alternatively, it may be released slowly over a period of time, being too dilute to attract larvae, or, after a lag period following imbibition in which case it would take longer to build up to an attractive concentration. A second possibility is that several different chemo-attractants exist in plant-exuded substances and there is a range in the degree of attractive force exerted by these attractants upon the larvae. This could be related to the heterogeneity of chemical composition observed between cells of organisms from different genera, species, or even varieties.

An excellent example of variation within one species is seen in the three varieties of *P. sativum* used in our experiments. This non-mucilaginous seed has 1 variety that was attractive within 1 hour (Early Alaska), another after 4 hours (Improved Telephone), and a 3rd that showed no attraction throughout the experimental period (Mammoth Melting Sugar). Two of the other three non-mucilaginous seeds did not attract mosquito larvae. A chemical attractant specific for mosquito larvae may be lacking in some seeds (e.g., *Phaseolus, M. grandiflora* and *P. sativum*, Mammoth Melting Sugar), or possibly our experimental design has not permitted enough time for release of effective amounts of the attracting substance.

It is not uncommon for plants to chemically attract organisms from the environment. The roots of legumes are commonly associated with an extremely rich microflora (Conn, 1948). This is known to be due to the roots secreting numerous chemicals into the rhizosphere, such as amino acids, sugars, organic acids and even vitamins of the B complex, which attract microorganisms. This active secretion may start early in the life of the plant even prior to seed germination. It is possible that those chemicals which attract microorganisms could also attract mosquito larvae. Indeed, the attractant may possibly be a nutritional substance for the larvae, and their migration to mucilaginous seeds is of a survival nature.

A toxic substance for mosquito larvae has been shown to be released by mucilaginous seeds (Page and Barber, 1974; Supavarn, 1974). The seeds tested in the present study did not produce any mortality of larvae in the brief time periods examined. Significant percentages of mosquito larvae were never observed in negative zones, indicating that a repulsion influence was apparently not present, except for Mammoth Melting peas. However, it is possible that two orientation factors, a positive and a negative or repulsive one, are in simultaneous operation. If a positive chemo-response was stronger than a toxic or chemical repulsion, it is possible that the larvae would be directed toward the seeds by the attractant and simultaneously be drawn toward the toxic factor. Additional work dealing with a possible toxic factor or nutritional substance(s) released by the imbibed seeds is needed in this area of biological control of mosquito larvae by plant seeds.

Whatever the significance of the presence of a chemo-attractant in certain plants proves to be, it is of potential interest as the tool of a biological control mechanism. Work is now proceeding in our laboratory toward the isolation, characterization and identification of this attractant. Should this prove to be a relatively ubiquitous molecule, then its usefulness as a larval trap bait will be extremely limited, particularly in the nutritionally rich conditions under which mosquito larvae live. However, should it prove to be a unique substance with a limited distribution in nature, a chemical entrapment device appears to have a possible role in the control of mosquito larvae.

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