INTERACTIONS BETWEEN MOSQUITO LARVAE AND MUCILAGINOUS PLANT SEEDS. I. CARBOHYDRATE COMPOSITION OF MUCILAGE IN RELATION TO ENTRAPMENT OF LARVAE [1]

J. T. BARBER, C. R PAGE III AND A. S. FELSOT [2]
Department of Biology, Tulane University, New Orleans, Louisiana 70118

ABSTRACT. Several plant seed mucilages have been prepared and analyzed with a view to determining a physical and/or chemical basis for their ability or inability to entrap mosquito larvae. A close correlation with the presence of a cellulose moiety in those mucilages which trapped larvae was demonstrated; mucilages which were ineffective in the entrapment of larvae lacked significant amounts of cellulose.

INTRODUCTION. Mucilaginous plant seeds have been the subjects of many and diverse studies. The chemistry of various mucilages have received much attention (e.g. Bailey and Norris, 1932; Smith and Montgomery, 1959; Tyler, 1965). Several publications have dealt with the production and structure of seed mucilages (e.g. Pammel, 1897; Hyde, 1970), and the development of the mucilaginous seed coat has even been used in a phylogenetic treatment of the Cruciferae (Janchen, 1942). Tookey and Jones (1965) conducted an extensive survey of 300 seed species with a view of finding new sources of galactomannans for use as paper or warp sizes, thickeners in drug preparations, or even as flocculants in sewage treatment.

However, while the explored uses of mucilaginous plant seeds may have been many and often original, it was not until the report of Reeves and Garcia (1969) that they were considered as potential agents for the biological control of mosquitoes. The observation of Reeves and Garcia (1969) that prompted this suggestion was that mosquito larvae could become attached by their oral brushes to the mucilaginous pellicle which surrounds certain immersed seeds and that these larvae subsequently died from unknown causes, presumably involving some form of stress. Such observations were sufficiently impressive to indicate that the "killing power" of certain seeds could approach 10 million larvae per pound.

Reeves and Garcia (1969) further noted that while many species of seeds produced a mucilaginous pellicle, not all were effective in trapping larvae. This raises the obvious question of what endows a particular mucilage with its physical and/or chemical basis for "stickiness" (i.e. the ability to entrap larvae). This paper presents the results of carbohydrate analyses of several plant mucilages from both "sticky" and "non-sticky" seeds; they are discussed in relation to the ability of those seeds to entrap mosquito larvae.

MATERIALS AND METHODS. The seeds used in this study were *Capsella bursa-pastoris, Descurainia pinnata* (from Mexico), *D. pinnata* var. *ochroleuca, D. sophia* (from Turkey), *D. sophia* (from Arizona–Mexico), *Lesquerella fendleri, Alyssum dasycarpum, Lepidium perfoliatum,* and *Sisymbrium altissimum* all of the Cruciferae, and *Plantago insularis* of the Plantaginaceae and *Sporobolus airoides* (Torr.) of the Gramineae. All species of seeds produced significant amounts of mucilage.

Larval entrapment trials were performed following the method of Reeves and Garcia (1969), except that *Culex quinquefasciatus* larvae (2nd–3rd instar) were used. Under these conditions, 60–75% of the larvae became attached, in 24 hours, to the various species of seed, with the

[1] Supported by a grant from the Louisiana Mosquito Control Association.

[2] Present address, Department of Entomology, University of Florida, Gainesville, Florida 32601.

exception of the *S. altissimum, P. insularis* and *S. airoides;* seeds of these species were unable to entrap larvae.

The mucilages from each seed were extracted and hydrolyzed by a modification of the methods of Bailey (1935). Figure 1 summarizes the protocol followed. The various fractions that resulted were: Fraction I (cellulose); Fraction II (aldobionic acids, i.e. acidic polysaccharides); Fraction III (monosaccharides from the neutral polysaccharides); Fraction IV (monosaccharides from the aldobionic acids of the acidic polysaccharides); Fraction V (uronic acids from the aldobionic acids of the acidic polysaccharides). Each fraction was dried and weighed.

The monosaccharides and uronic acids obtained as above were analyzed by ascending paper chromatography on Whatman No. 1 paper. Equivalent amounts of comparable samples were spotted on each chromatogram. Authentic samples of glucose, arabinose, galactose, rhamnose,

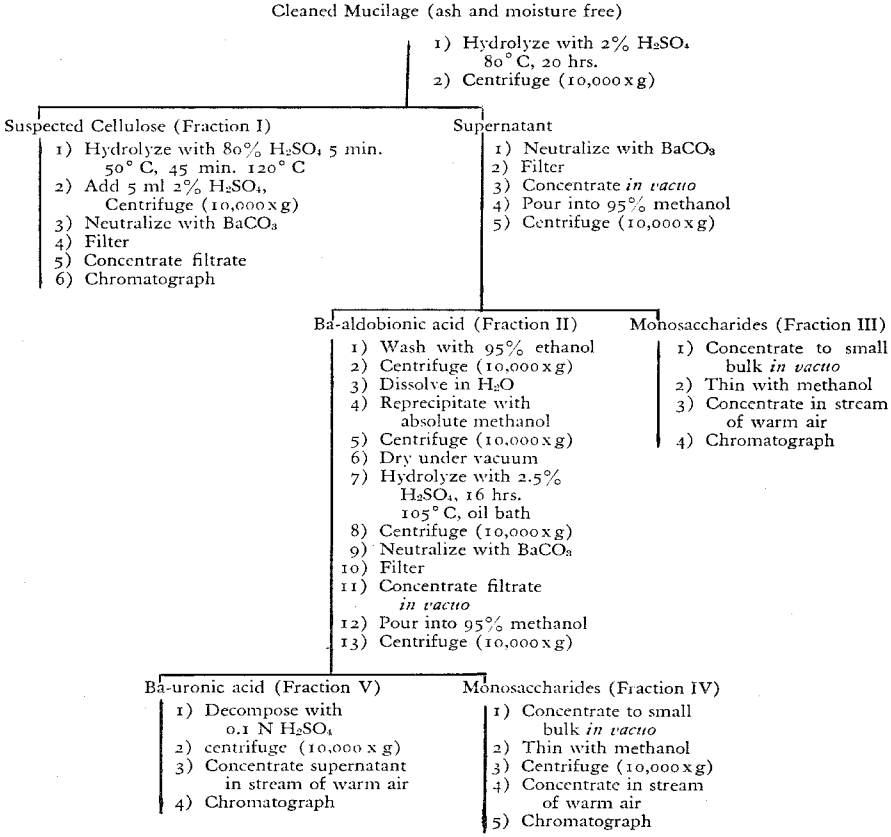

FIG. 1. Summary of mucilage hydrolysis.

TABLE 1. Carbohydrate composition of mucilages.

Seeds	Fraction I % cellulose	Fraction II % acidic polysaccharides	Fraction III % neutral polysaccharides
1. *C. bursa-pastoris*	40.9	15.1	44.9
2. *D. pinnata* (Mex.)	26.0	11.0	62.0
3. *D. pinnata* (var. ochro.)	41.2	20.8	38.0
4. *D. sophia* (Turkey)	27.0	15.2	57.8
5. *D. sophia* (Ariz-Mex.)	27.6	18.4	44.0
6. *L. fendleri*	27.3	8.0	64.7
7. *A. dasycarpum*	43.6	8.1	48.3
8. *L. perfoliatum*	52.2	26.1	21.8
9. *S. altissimum*	8.7	39.1	52.1
10. *P. insularis*	1.4	5.4	93.2
11. *S. airoides*	7.4	7.3	85.3

xylose, galacturonic acid and glucuronic acid were prepared at a concentration of 1% (w/v) in 10% (v/v) isopropanol and were co-chromatographed with unknown samples. The following solvent systems were used; isopropanol : water (240:60) for Fractions I, III, IV and V; ethyl acetate : pyridine : water (180:75:60) for Fractions I and III; isopropanol : pyridine : water (120:40:40) for Fractions IV and V. Chromatograms were run for 20–22 hrs., dried, and sprayed with one of the following detection reagents; 1% aniline+1% diphenylamine in acetone (Fractions I, IV and V) and *p*-anisidine HCL in butanol (Fractions I and III) (Smith 1958). Unknown sugars were identified by comparison of Rg's in at least two solvents.

RESULTS. Based upon the weights of each fraction recovered from the various mucilages, the percent composition of each is shown in Table 1. It is apparent that the various seed mucilages fall into two groups with regard to their quantitative compositions. The first 8 species listed have a relatively high cellulose content, while the last 3 species have a relatively low cellulose content; this result, of course, correlates exactly with the ability of these seeds to entrap larvae. The first 8 seeds are "sticky" insofar as larval entrapment is concerned, while seeds in the second group are not. No further correlation between "stickness" of mucilage and carbohydrate composition was evident (see Table 1).

Qualitative analysis of the various fractions substantiated the above conclusion. Fractions I revealed only glucose or a very high glucose content in the "sticky" mucilages, indicating pure, or almost pure, cellulose. Little or no glucose was found in the Fraction I of mucilages from "non-sticky" seeds, indicating not only the absence of cellulose but also, possibly, slight contamination of this fraction with sugars of Fraction III (giving rise to spurious weights in Fraction I for *S. altissimum, P. insularis* and *S. airoides*).

The same sugars were found in the neutral polysaccharide fraction (III) from each of the seed mucilages; these were arabinose, galactose, rhamnose and xylose. Quantitative differences were noted and were most pronounced between *S. airoides* (a monocot) and all the others (dicots).

Arabinose, galactose, rhamnose and xylose were detected in the monosaccharide fraction (IV) from the acidic polysaccharides in all seeds except *C. bursa-pastoris* and *D. pinnata* in which only rhamnose was demonstrable.

The uronic acids of Fraction V (from the acidic polysaccharides) of the various seed mucilages were either galacturonic acid and glucuronic acid or solely galacturonic acid. No correlation between the ability of the mucilages to entrap larvae and the uronic acids was evident.

DISCUSSION. There appears to be little connection between the compositions of Fractions III, IV and V from different mucilaginous seeds and the ability of those seeds to "catch" mosquito larvae. Thus, differences between the sugars of a particular fraction from two seeds were demonstrated but were not paralleled by differences in the "stickiness" of the seeds in question. Similarily, the same fraction from different seeds may have had the same sugar composition but different larval "sticking" abilities.

The only correlation between "sticky" and "non-sticky" mucilages seems to be in the presence or absence of a cellulose component (Fraction I). Very small amounts of cellulose were found in the "non-stickers" (*S. altissimum, P. insularis* and *S. airoides*) as compared with all other seeds ("stickers"). These small amounts may be attributed to contamination from unhydrolyzed mucilage (cf. Fig. 1) or organic debris that was incompletely removed prior to hydrolysis.

Supportive evidence for this thesis is provided by reports that mucilage from *Lepidium sativum* contains a cellulose moiety (Bailey, 1935; Tyler, 1965) as does *Brassica alba* (Bailey and Norris, 1932); while we have no evidence on the attachment of larvae to these particular species, Reeves and Garcia (1969) have demonstrated that both *L. flavum* and *B. geniculata* are effective in entrapping larvae as has been shown to be the case for *L. perfoliatum* in this study. Conversely, Reeves and Garcia (1969) reported that *Linum usitatissimum*, like *P. insularis*, produced large amounts of mucilage but could not "catch" larvae. Erskine and Jones (1957) and Hunt and Jones (1962) have shown that *L. usitatissimum* contains no cellulose in its mucilage while Laidlaw and Percival (1949, 1959) and Hirst *et al.* (1954) were unable to find cellulose in the mucilage of various *Plantago* species.

Thus, the results of this investigation, together with various reports in the literature, indicate a close correlation between the presence of cellulose in a particular seed mucilage and its ability to entrap mosquito larvae. The question remains, however, concerning what role the cellulose plays in determining the "stickiness" of an effective mucilage. It is attractive (but probably too simplistic) to envisage the oral brushes of the larvae becoming physically entangled in the cellulose units. Cellulose is usually considered to be a stable, insoluble substance. However, in mucilage its association with other polysaccharides allows it to become dispersed when placed in water (Bailey, 1935; Grant *et al.*, 1969). Therefore, while cellulose may itself endow a particular mucilage with "stickiness," it is also possible that it merely provides a matrix around which other branched (and "sticky"?) acidic and neutral polysaccharides may bind. The lack of a cellulose component in a "non-sticky" seed mucilage presumably allows these polysaccharides to disperse to the point where they are no longer "sticky."

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References Cited

Bailey, K. 1935. Cress seed mucilage. Biochem. J. 29:2476–2485.

Bailey, K. and F. W. Norris. 1932. The nature and composition of the mucilage of the seed of the white mustard (*Brassica alba*). Biochem. J. 26:1609–1623.

Erskine, A. J. and J. K. N. Jones. 1957. The structure of linseed mucilage Part I. Canad. J. Chem. 35:1174–1182.

Grant, G. T., C. McNab, D. A. Rees and R. J. Skerrett. 1969. Seed mucilages as examples of polysaccharide denaturation. J. Chem. Soc. D. 14:805–806.

Hirst, E. L., E. G. V. Percival and C. B. Wylam. 1954. Studies on seed mucilages. Part VI. J. Chem. Soc. 1954:189–198.

Hunt, K. and J. K. N. Jones. 1962. The struc-

ture of linseed mucilage. Part II. Canad. J. Chem. 40:1266–1279.
Hyde, B. B. 1970. Mucilage-producing cells in the seed coat of *Plantago ovata*: Developmental fine structure. Amer. J. Bot. 57:1197–1206.
Janchen, E. 1942. Das system der Cruciferen. Oestr. Bot. Zeit. 91:1–17.
Laidlaw, R. A. and E. G. V. Percival. 1949. Studies on seed mucilages. Part III. J. Chem. Soc. 1949:1600–1607.
Laidlaw, R. A. and E. G. V. Percival. 1950. Studies on seed mucilages. Part V. J. Chem. Soc. 1950:528–534.
Pammel, L. H. 1897. On the seeds and tests of some Cruciferae. Amer. Month. Micro. J. 18: 205–219, 269–274, 312–317.
Reeves, E. L. and C. Garcia. 1969. Mucilaginous seeds of the Cruciferae family as potential biological control agents for mosquito larvae. Mosq. News 29:601–607.
Smith, F. and R. Montgomery. 1959. The Chemistry of Plant Gums and Mucilages. Reinhold Publishing Corporation, New York. 627 pp.
Smith, I. 1958. Chromatographic and Electrophoretic Techniques. Vol. 1 Chromatography. Interscience Publishers, New York. 1080 pp.
Tookey, H. L. and Q. Jones. 1965. New sources of water-soluble seed gums. Econ. Bot. 19:165–174.
Tyler, J. M. 1965. The seed mucilage of *Lepidium sativum* (Cress). Part I. J. Chem. Soc. 1965:5288–5300.

BIOLOGICALLY ACTIVE PLANT EXTRACTS FOR CONTROL OF MOSQUITO LARVAE [1]

PIYARAT SUPAVARN, FRED W. KNAPP AND ROY SIGAFUS [2]
University of Kentucky, Lexington, Kentucky 40506

ABSTRACT. Thirty-six plant samples collected from Central Kentucky or grown in a greenhouse were extracted with methanol, dried, weighed and diluted with water to contain 100, 500, and 1000 ppm of the dry weight. These were then tested against larvae of *Aedes aegypti*.

Eleven plant samples resulted in 53% or greater larval mortality at 1000 ppm; 4 at 500 ppm and 1 at 100 ppm.

Recently new restrictions on the use of chemicals for insect control have stimulated investigations of the insecticidal properties of plant materials. One of the first reports of the toxic effects of plant alkaloids on mosquito larvae was that of Campbell and Sullivan (1933). They found that the plant alkaloids, nicotine, anabasine, methyl anabasine and lupinine, killed larvae of *Culex pipiens* L., *C. territans* Walk. and *C. quinquefasciatus* Say. Haller (1940) noted the extracts of Amur corktree fruit (*Phellodendron amurense* Ruprecht) were toxic to mosquito larvae. Wilcoxon *et al.* (1940) reported that extracts of male fern (*Aspidium filix-mas* (L.) SW.) killed larvae of *C. quinquefasciatus*. Hartzell and Wilcoxon (1941) found that several of the 150 species and varieties of plants which they tested gave 90 to 100% kill of *C. quinquefasciatus*. Hartzell (1944) reported 11 acetone extracts from various plant species to be toxic to larvae of *C. quinquefasciatus*, but no mortality was observed with water extracts of these same plants. Later, Hartzell (1948) found acetone extracts of "Pinaceae," Cucurbitaceae, Labiatae, Liliaceae, Compositae, Umbelliferae, Leguminosae and Euphorbiaceae to be toxic to *C. quinquefasciatus*.

Recently, Amonkar and Reeves (1970) extracted minced dehydrated garlic, *Allium sativum* L. and found it to be active

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[2] Graduate research assistant (now at Agricultural Research Center, Khon Kaen, Thailand) professor of Entomology and Agronomy, respectively.