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The Function of Seed Mucilage in Flax (*Linum usitatissimum*)

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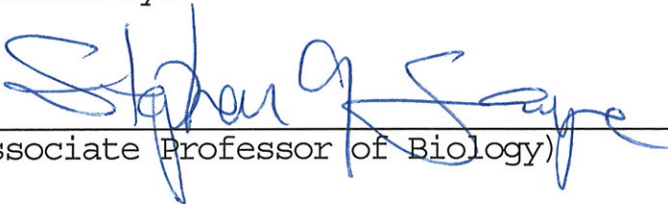
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by
Karla M. Gengler
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The Function of Seed Mucilage Flax (*Linum usitatissimum*)

Approved by:



(Associate Professor of Biology)



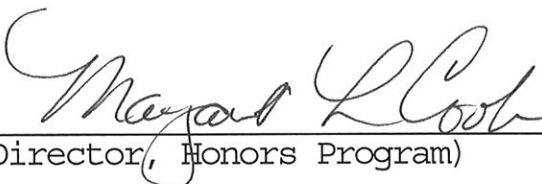
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INTRODUCTION

The seeds of flax (*Linum usitatissimum* L., Linaceae), when imbibed, produce a coating of mucilage, which has its origin in the testa. Economically, seed mucilages are important. The mucilage of psyllium (*Plantago ovata*, Plantaginaceae) is used as a laxative and to increase fiber and reduce cholesterol in humans (Bell et al., 1989). Seed mucilages are used in industry as thickening, stabilizing, or sizing agents (Tookey and Jones, 1965). The cosmetic and pharmaceutical industries also use flax seed mucilage (Vaughan, 1970).

Seed mucilage is a widely distributed in plants. Seeds of psyllium (*Plantago psyllium* L., Plantaginaceae) are noted for their copious mucilage production. Young and Evans (1973) found that about 8% of weed species tested in the rangelands of Nevada and California have mucilaginous seeds. The Cruciferae contributed to a large percentage of the total. Cruciferous species are also commonly found to produce mucilage in the plants of England; Swarbrick (1971) found 112 species distributed over 14 families that produce seed mucilage. Garwood (1985) found that the seeds of *Cavanillesia platanifolia* (H.&B.) H.B.K. (Bombacaceae), a tropical tree, produce copious amounts of mucilage. *Blepharis persica* (Burm.) Kuntze (Acanthaceae), a plant of

the Middle Eastern deserts, also exudes seed mucilage upon imbibition (Witztum et al., 1969).

Flax seed mucilage was classified by Mühlethaler (1950) as a "true slime" because it turns yellow on contact with iodosulfuric acid. He showed that the mucilage is an amorphous substance embedded with short, cellulose-like particles. Muralikrishna et al. (1987) reported that flax mucilage is a mixture composed of neutral and acidic polysaccharides. The neutral fraction contains an arabinoxylan with side chains of arabinose and galactose. The acidic fraction contains a rhamnogalacturonan with side chains of fucose and galactose residues.

Seed mucilages have been shown to serve a variety of functions. For example, Young and Evans (1973) reported that in scrubland plants mucilage helps seeds germinate at low water potentials and protects seeds from dessication. Harper and Benton (1966) also noted that the mucilaginous seeds of *Camelina sativa* (L.) Crantz (Cruciferae), *Lepidium sativum* L. (Cruciferae), *L. usitatissimum*, *Plantago major* L. (Plantaginaceae), and *Sinapis alba* L. (Cruciferae) germinate better than seeds without mucilage (such as *Clarkia elegans* Dougl. (Onagraceae), *Brassica napus* L. (Cruciferae), *Pisum sativum* L. (Fabaceae), and *Vicia faba* L. (Fabaceae)). Mucilage presumably increases the contact between the medium and the water, therefore increasing water uptake under low water potentials. Garwood (1985) reported

that the moisture-retentive property of mucilage helps *C. platanifolia* seeds to germinate earlier than competitors and to survive long periods of dry weather.

Mucilage may also be a nutrient source for the young plant. Ravenna and Zamorani (1911) reported that the ash of flax mucilage is composed of various minerals. When they deprived flax seeds of mucilage, the seeds germinated and grew poorly in comparison to intact seeds. When demucilaged seeds were provided with either a nutrient or sugar solution, they germinated with approximately the same frequency as intact seeds. They concluded that mucilage acts as a nutrient reserve for the germinating seed.

Mucilage may also inhibit germination by limiting the supply of oxygen to the growing embryo. Heydecker and Orphanos (1968), in their work with spinach (*Spinacia oleracea* L., Chenopodiaceae), found that the mucilage produced when the conditions were "wet" reduces germination by slowing the passage of oxygen, which diffuses through an aqueous solution approximately 10,000 times slower than through air, to the embryo. The desert plant *B. persica* (Witztum et al., 1969) also fails to germinate in wet conditions because it exudes enough mucilage to block oxygen uptake.

Mucilage may act as a mechanism of carnivory or defense against microorganisms. For example, the seeds of shepherd's purse (*Capsella bursa-pastoris* L. Medikus,

Cruciferae) attract, trap, and digest mosquito larvae (Page and Barber, 1975). Within one hour of placement in a tank with shepherd's purse seeds on one side, the mosquito larvae migrate to the seeds. The mucilage contains proteolytic enzymes which digest the larvae (Barber, 1978). Flax seeds, which they also tested, attracted larvae more slowly (after four hours), but they attracted more total larvae than shepherd's purse. Flax seed mucilage did not entrap mosquito larvae, however.

Finally, mucilage in flax seeds may aid seed dispersal. Young and Evans (1973) noted that mucilage, when dried, adheres to glass, clothing, and skin so tightly that the seeds must be individually removed. Swarbrick (1971) made similar observations pertaining to the transport of sticky seeds by animals and field workers. Seeds may be carried far from the source to extend the plant's range.

The purpose of this study was to establish protocols for the study of flax seed mucilage while examining the physiological role of the mucilage. I attempted to adapt the published methods for the study of mucilage in other seeds to flax seeds. A major effort was made to establish techniques for the isolation and quantification of mucilage as well. Focusing on the models suggested by the research discussed above, I tested mucilage as a nutrient source, as a means to protect the seed from dessication, as a mechanism of carnivory, and as barrier to oxygen passage. Since the

results of Harper and Benton (1966) and Ravenna and Zamorani (1911), who dealt with flax specifically, are contradictory, a special emphasis was placed upon attempting to confirm their results.

MATERIALS AND METHODS

Plant Materials. Flax (*Linum usitatissimum*) seeds were obtained from the Swany White Flour Mill in Freeport, Minnesota, courtesy of Mr. Walt Thelen.

Standard Equipment. Unless indicated otherwise, plastic disposable 100x15 mm petri dishes were half-filled (approximately 30 ml) with 1.5% water agar (agar dissolved in dH₂O) and 30 seeds placed on each plate. All dry weights were obtained after drying the seedling in a 102 C oven for at least 12 hr.

Mucilage Removal. I used three methods to remove mucilage from flax seeds: (1) the rinsing method (used by Ravenna and Zamorani (1911)) involved placing flax seeds in a large amount of water for at least an hr, then rinsing the seeds several times with dH₂O; (2) the wire mesh bag method involved running water over seeds placed in a wire mesh bag; and (3) the chemical method used cross-linked sodium pollyacrylate (trade name J-550 Water Absorber) obtained from Pampers brand disposable diapers to absorb excess water and mucilage from imbibed seeds upon which it was sprinkled.

Before the seeds were used in any treatment, they were rubbed lightly with paper toweling to remove remaining mucilage.

Mucilage Isolation. Seeds were imbibed in a large volume of water. I removed the mucilaginous liquid from the imbibed seeds using a Buchner funnel. Approximately one volume of this liquid was mixed with approximately nine volumes of 100% ethanol. I transferred the resulting white, slimy solid to centrifuge tubes and centrifuged the tubes for three min on high speed using the IEC Model HN-S Centrifuge. The supernatant was drawn off and the pellets either stored at 4 C or air-dried and ground with a mortar and pestle.

Mucilage Quantification. Because flax mucilage is composed largely of polysaccharides, I used the anthrone test described by Witham, Blaydes, and Devlin (1986) to measure the polysaccharide, and thus the mucilage, content of mucilage extract. To a mucilage sample (1 ml) I added 2 ml anthrone reagent (26 mM in concentrated sulfuric acid), then heated the samples in a boiling water bath for three min. After the samples cooled, I recorded the optical densities of the samples on a spectrophotometer at 600 nm. A standard curve was prepared, using concentrations of mucilage ranging from 10 to 100 $\mu\text{g/ml}$ derived from a 10

mg/ml stock solution prepared by dissolving dry, powdered mucilage in dH₂O.

Endpoint of Mucilage Production. To determine how long it takes for seeds to release their mucilage, I placed seeds (either 25, 50, or 100 seeds) into 100 ml water in flasks covered with parafilm. These seeds were left to imbibe overnight. 21 hr later, similar sets of seeds were prepared in the same manner and left to imbibe five hours. I quantified the mucilage contents of the mucilaginous liquids with the anthrone reagent and determined the amount of mucilage secreted by the seeds.

Rate and Quantity of Mucilage Release. Into a 250 ml flask I placed 35 seeds (0.154 g) and dH₂O (100 ml). I immediately withdrew three samples (1 ml) to serve as blanks and replaced their volumes with fresh dH₂O. Every 15 min for the first hour, I withdrew three samples, replacing each sample with dH₂O. After the first hour, aliquots were drawn every half hour for five hr. From Time 0 to 1.0 hr, 1.0 ml samples were withdrawn; at 1.5 hr and after, 0.5 ml samples were withdrawn instead of 1.0 ml samples. I added 0.5 ml dH₂O to these latter samples to give a total volume of 1.0 ml. Each sample was quantified with the anthrone reagent as previously described.

Germination of Unstressed Seeds. In order to determine the effect of mucilage upon seeds under normal conditions, I imbibed seeds for one hr in a petri dish lined with filter

paper soaked with 2.0 ml of water. Other seeds were demucilaged by the wire mesh bag method. The seeds were placed on water agar plates. Each demucilaged seed was treated with 0, 10, 15, or 25 μ l of a mucilage-water extract. In addition, I placed the intact seeds imbibed in the petri dish onto plates. The dishes were covered and transferred to a growth chamber set on a 12 hour day-night cycle at 29 C day and 24 C night temperatures. I recorded the number of seeds that germinated for three days. On the fourth day, the agar was dry, since the covers were either left off or pushed off by the seedlings. The seedlings were cut off at the junction of the hypocotyl and the root and the dry weight of the aerial portion recorded.

Seed Germination on Water Agar with or without Nutrient Solution. Thirty seeds, both intact and demucilaged, were placed on agar plates prepared with either full strength or half strength Hoagland's nutrient solution. The control agar plates were prepared with dH₂O only. The intact seeds were imbibed one hour in petri dishes, and the demucilaged seeds treated with the chemical method of mucilage removal. These plates were then transferred to the growth chamber set as described above. I noted the number of germinated seeds for five days, then took the dry weight of the entire seedlings. The experiment was performed twice.

Seed Germination in Perlite. Three flats (95 mm x 131 mm) of moist perlite were prepared. I planted

seeds demucilaged according to the rinsing method in two of the flats. An additional 11.1 ml of dH₂O were added to one of these trays. The third tray of moist perlite was planted with seeds which were soaked in a beaker of water. The mucilage left in the beaker was swirled with 11.1 ml of dH₂O, then poured over the seeds to supply the seeds with all their original mucilage. The trays were placed in the growth chamber set on a 12 hr day-night cycle at 29 C day and 22 C night temperatures. The second day after the planting, the trays were covered with plastic wrap to rapid dessication between waterings. After 13 d, I counted the number of germinated seeds. Seedlings were dried and weighed.

Effect of Mucilage on Embryo Development. In order to investigate the nutritive properties of mucilage, lima bean (*Phaseolus lunatus*, Fabaceae) embryos were treated with flax mucilage. I sterilized lima bean seeds in a 5% bleach solution, then rinsed them thoroughly with sterile water. After they imbibed sterile water overnight, the embryo was excised from the cotyledons. The length of the hypocotyls and epicotyls of 52 embryos were measured and the embryos transferred to plates of water agar. I divided the embryos into three treatments. The first set of embryos received a total of 466 μ g of mucilage in a 10 mg/ml solution. The second set of embryos received an equal volume of sterile water, and the third group, which served as a control, were

not watered so that their only source of water was from the agar. I sealed the plates with parafilm, then transferred them to a dark growth chamber set at 27 C. I recorded the lengths of the hypocotyls and epicotyls for three days.

Protease Activity in Mucilage. To determine whether flax seed mucilage could digest proteins such as those found in gelatin, I prepared six petri dishes of 5% gelatin and divided them into three treatments of two dishes each: intact seeds, demucilaged seeds, and dishes streaked with mucilage extract. The intact seeds were imbibed in petri dishes and the demucilaged seeds prepared by the wire mesh bag method. I pipetted two 0.5 ml streaks of mucilage onto the remaining two plates, using mucilage collected by the method described in the Germination of Unstressed Seeds experiment. These plates were incubated in a growth chamber under the same conditions as in the Seed Germination in Perlite experiment. I observed the plates for four days. Runny gelatin, a sinking of the seeds or mucilage into the gelatin, or the appearance of a hole around mucilage or seeds would have been interpreted as evidence of possible protease activity upon the protein in the gelatin.

Effects of Chemical Inducers of Water Stress. Flax seeds with and without mucilage were placed on water agar containing D-sorbitol to vary the water potentials from 0 (no D-sorbitol) to -0.5 megapascals (MPa). I imbibed the intact seeds in petri dishes and used the wire mesh bag

method to demucilage seeds. I used one plate per treatment. The plates were transferred to the growth chamber set as described in the Protease Activity in Mucilage experiment and the germinations tallied for five days.

In a second experiment, I placed demucilaged, intact, and unimbibed seeds on plates with various water potentials created by the addition of polyethylene glycol (MW 3,350). The plates containing 0.678 molal polyethylene glycol in water agar had a water potential of -0.5 MPa (at 24 C and assuming an ionic potential of 1). From a volume of about 180 ml ten plates were poured. The 14 plates with a -1.09 MPa water potential contained 1.48 molal polyethylene glycol. Because the agar failed to set sufficiently, I laid filter paper on the surfaces of these plates. I removed mucilage from the flax seeds by rinsing the seeds, then using the chemical method. The control dishes contained no polyethylene glycol. The plates were sealed with parafilm and incubated in a dark growth chamber at 24 C. I noted the germination for five days.

Germination of Flax Seeds in Different Volumes of Water. I placed seeds demucilaged by the chemical method and intact seeds in petri dishes lined with filter paper moistened with 1 to 5 ml of dH_2O . All dishes were sealed with parafilm and transferred to a growth chamber under the same conditions as in the Protease Activity in Mucilage experiment. I tallied germination for five days and

recorded the dry weights of the entire seedlings.

Data Analysis. Data collected were graphed using the Aseasyas graphing system. Minitab was used to statistically analyze the data, using Chi square and analysis of variance.

RESULTS

I measured three factors that I believe may be affected by mucilage: germination, dry seedling weight, and embryonic growth. All p values referring to germination were determined using a Chi square test. Those p values referring to seedling weights or embryo lengths were determined by analysis of variance statistical tests.

By measuring at intervals of 15 and 30 min the amount of mucilage secreted by 35 flax seeds, I established that the seeds had secreted approximately 86% (approximately 470 μg per seed) of their mucilage by 2.5 hr (Fig. 1). I also determined that 11 to 14% of the dry weight of the flax seed is composed of mucilage, since the average seed weighed 4.4 mg and the average amount of mucilage released per seed was between 500 and 600 μg . Measurements of mucilage content were not taken after 5 hr because a preliminary experiment indicated that there is no significant secretion of mucilage after 5 hr (Table 1).

In an initial experiment to determine the effect of mucilage on seeds under normal conditions (Germination of Unstressed Seeds), I plated intact and demucilaged seeds on water agar plates (Fig. 2). To some of the demucilaged seeds I added back different amounts of mucilage extract. Intact seeds did not germinate differently from seeds without mucilage ($0.5 < p < 0.7$). When mucilage extract was added back to the demucilaged seeds, significantly lower germination resulted for seeds with 10 μl ($p < 0.001$ and $0.001 < p < 0.01$ in comparison to demucilaged and intact seeds, respectively) and 25 μl ($p < 0.001$ in comparison to both intact and demucilaged seeds, respectively) added mucilage extract. Adding back 15 μl mucilage extract had no effect ($0.20 < p < 0.30$ and $0.30 < p < 0.50$ in comparison to demucilaged and intact seeds, respectively). In addition, seedlings with intact or added mucilage extract did not show any difference in dry weight over seedlings without mucilage ($p = 0.623$) (Fig. 3).

To test the hypothesis that mucilage was important in increasing the viability of seeds under water stress, I performed three experiments in which seeds with and without mucilage were germinated under various water potentials. In the first, D-sorbitol was used to vary the water potential from 0 MPa to -0.5 MPa. On day one of the D-sorbitol experiment, germination increased both for seeds with ($p < 0.001$) and without mucilage ($p < 0.001$) as water potentials

increased (Fig. 4). The difference in initial germination between seeds with and without mucilage was not statistically significant at any water potential. During the course of the experiment, I noted that dishes with higher concentrations of D-sorbitol supported the growth of more fungi and bacteria, especially in and near areas with mucilage. This contamination may have affected the germination of the flax seeds. When the experiment was repeated using polyethylene glycol in place of D-sorbitol to decrease the amount of contamination, no seeds germinated in plates containing the chemical, while the controls without polyethylene glycol germinated comparably to earlier experiments.

The final experiment, in which flax was germinated on filter papers moistened with various amounts of water (from 1 ml to 5 ml), showed that differences between germination of seeds with and without mucilage were not statistically significant at any added volume of water (Fig. 5). I found significant differences in germination for intact seeds between water volumes, however. Intact seeds tended to germinate better in 2 ml of water than in 1 ml of water ($0.05 < p < 0.10$). They also germinated significantly better in 3 ml of water than 4 ml of water ($0.001 < p < 0.01$). Mucilage had mixed effects upon the dry weights of the seedlings in this experiment (Fig. 6). The presence of mucilage tended, although not significantly, to depress

seedling dry weight at 1 ml of water ($p=0.097$) in comparison to seeds without mucilage in 1 ml of water. The dry weight of seedlings with mucilage tended to increase from 1 ml to 2 ml water ($p=0.070$), while the dry weight of seedlings without mucilage did not increase significantly ($p=0.294$). Finally, the dry weight of seedlings with mucilage increased in comparison to those without mucilage when the amount of water present was increased to 5 ml ($p=0.029$).

The effect of additional nutrients on flax seed germination and seedling growth was tested using Hoagland's solution in 1.5% water agar plates and by perlite. In the experiments involving the presence or absence of nutrients provided by Hoagland's solution, intact flax seeds did not germinate differently from demucilaged seeds (Figs. 7 & 8). Seeds with mucilage germinated better without nutrients, however ($0.02 < p < 0.05$ and $0.001 < p < 0.01$). Seeds without mucilage did not germinate differently when additional nutrients were provided ($0.10 < p < 0.20$ and $0.30 < p < 0.50$). In this case, either the presence of mucilage inhibited the germination of seeds in a nutrient-rich medium, or the presence of the water used to replace the nutrient solution increased the germination.

In the perlite experiment, germinated seeds planted in moist perlite with or without mucilage and water were counted on the tenth day after planting (Fig. 9). Seeds with mucilage extract ($p < 0.001$) and demucilaged seeds

treated with water ($p < 0.001$) both germinated better than demucilaged seeds without additional water or mucilage extract. Germination was better for demucilaged seeds with water added than for those with mucilage extract added ($0.01 < p < 0.02$). No seedling dry weights were obtained.

Lima bean embryos were used to measure the effects of mucilage on embryonic growth. On the first day, the increases in lengths of the epicotyls for each treatment were not significantly different (Fig. 10). After 48 hr, the lengths of epicotyls with added mucilage increased significantly more than those of the control, which had no mucilage and no water ($p = 0.016$). Increases in epicotyl length of embryos with only water, moreover, were not significantly different from the control ($p = 0.342$). Epicotyls with mucilage also did not increase significantly more than those with added water ($p = 0.113$).

Differences in hypocotyl growth were significant after 24 hr (Fig. 11). Embryonic hypocotyls treated with mucilage ($p = 0.017$) or with water ($p = 0.009$) were longer than the controls. The difference between the water and mucilage treatments was not significant ($p = 0.498$). After 48 hr no significant differences could be detected between treatments.

The experiment testing for protease activity, and thus carnivory, showed that mucilage probably has no digestive effect on gelatin, which is composed of proteins, and

therefore no proteases. I saw no depressions in the gelatin that could not be attributed to bacterial colonies or fungus, both of which were common on mucilagenous areas of the gelatin.

DISCUSSION

Data Analysis. There is a large investment of energy in the production of mucilage, which composes 11-14% of the seed's weight. This investment presumably serves an important function in the seed. One of the proposed functions for flax seed mucilage is carnivory, or the ability to digest proteins (Barber, 1978). My results, although not conclusive, do not suggest that flax seeds are carnivorous, as those of *C. bursa-pastoris* are believed to be. Proteases in flax mucilage capable of the hydrolysis of proteins such as those found in gelatin were not found.

Mucilage may also serve as a reservoir of nutrients, as it does in the model proposed by Ravenna and Zamorani (1911). They found that demucilaged flax seeds germinated poorly unless supplemented with a mineral or sugar solution. My data do not support this hypothesis. In the experiments in which I replaced water with Hoagland's solution, the intact seeds did not germinate differently from the demucilaged seeds. The data for my perlite experiment also suggest that the role of flax mucilage is not nutrition.

Demucilaged seeds with water germinated better than seeds with mucilage in the nutrient-poor perlite (Fig. 9), suggesting that water has a greater effect upon germination in this experiment than the absence or presence of any nutrients mucilage may contain.

Ravenna and Zamorani's results may have been skewed by their method of mucilage removal. I followed their method of mucilage removal in the perlite experiment, and found that much of the mucilage was not removed by merely rinsing the seeds. I had to rub the seeds in paper toweling to remove the rest of the mucilage for the experiment.

Harper and Benton (1966) observed that seed mucilages increase germination at low water potentials by increasing the contact between the seeds and water. I conducted several different experiments to attempt to repeat their results. The increases in length of the hypocotyls of the embryos in the lima bean experiment (Figs. 10 & 11) support, although not conclusively, Harper and Benton's conclusion that the prevention of water stress, rather than nutrient deficiency, is the function of mucilage in flax seeds. The hypocotyls treated with mucilage or water both lengthened faster than untreated hypocotyls. The difference between these treated groups was not significant. I expected that, if mucilage provides nutrients to the embryo, the embryos treated with mucilage would grow better than water-treated embryos. Just as in the perlite experiment, the

availability of water seemed to be more important than any nutrients that may exist in the mucilage. Mucilage did not significantly improve the ability of the embryo to absorb water. Epicotyls grew faster with mucilage, but this result is unreliable, since it was extremely difficult to measure the small increases in length I observed for the epicotyls.

In the experiment in which I used D-sorbitol to vary the water potential of agar, the presence or absence of mucilage did not affect germination. Since water flows from areas of high water potential to those of low water potential, seeds placed on media with a water potential lower than that of the seeds would not be able to imbibe water effectively. The results of this experiment are unreliable, however, due to fungal and bacterial contamination. When I used polyethylene glycol, which cannot support fungal and bacterial growth, as an osmotic agent, it proved to be toxic to flax seeds.

In the germination experiment in which I placed flax seeds on filter papers moistened with various volumes of water, germination was again unaffected by the presence or absence of mucilage. The germination rates of intact seeds, however, were affected by water volume. At low and high volumes of water, germination was poorer than at mid-range water volumes. I did not observe a similar trend in demucilaged seeds. Lower germination rates in dry or wet conditions may be an ecological asset. If germination is

delayed for even a day, the chance that the seed will be transported to a more favorable environment is increased. Since flax seeds, when imbibed, stick to fur or clothing tightly, the mucilage may act as both a germination inhibitor and a transport mechanism to ensure that the seed grows in favorable conditions.

The dry weights of the seedlings from intact seeds germinated in low volumes of water in the filter paper experiment were lower than the dry weights of demucilaged seeds. The seedlings from intact seeds germinated in large volumes of water were heavier than seedlings germinated from demucilaged seeds in the same volume of water. These data suggest that mucilage makes the environment more favorable for the seedling in wet conditions and less favorable in dry conditions. Each seed, whether it germinates or not, imbibes water and exudes mucilage, which holds water. The mucilage therefore dries the environment slightly, which is a disadvantage for seedlings in dry conditions and an advantage for those in wet conditions.

Although strong conclusions cannot be drawn from these experiments, my results do not tend to support the carnivory, nutrition, or water potential roles for mucilage. The work of Heydecker and Orphanos (1968) on spinach seeds and the work of Witztum et. al. (1969) on *B. persica* perhaps best explain my results. Both spinach and *B. persica*, when in excess water, produce a coating of mucilage which blocks

the passage of oxygen to the embryo. If flax seeds' mucilage production is linked to the amount of water present, greater amounts of mucilage would be exuded in excess water. This large amount of mucilage could prevent oxygen from reaching the embryo and thus inhibit germination. In dry environmental conditions, what little mucilage produced would have a greater chance of losing more moisture to the air than it could replace with water from the environment, causing the mucilage to thicken. Thicker mucilage may also prevent oxygen passage to the embryo.

The only model that I was unable to test in some way was Young and Evan's proposal that mucilage acts as a seed transport mechanism. I did observe, however, that imbibed flax seeds are indeed difficult to remove from clothing, especially from "fuzzy" cloth such as flannel.

Technique Analysis. Mucilage removal proved to be one of the most difficult aspects of the experiment. The technique Ravenna and Zamorani (1911) used, the rinsing method, did not remove mucilage effectively unless the seeds were rubbed in paper toweling. Rubbing the seeds may damage the seed coats, so this was not an ideal method. The wire mesh bag method and the chemical methods also failed to remove all of the mucilage, although the chemical method was somewhat more efficient. A combination of methods, I found, was the best method of mucilage removal. When I rinsed the seeds in water, as in the rinsing method, then sprinkled

sodium pollyacrylate on them, much of the mucilage was removed.

The experiments themselves often did not proceed as expected. In the experiment in which I used D-sorbitol to vary the water potential, contamination with fungi and bacteria was a problem. Polyethylene glycol, though not toxic to spinach seeds (Heydecker and Orphanos, 1968), was toxic to flax seeds. Therefore, some other, nontoxic osmotic agent that fungi and bacteria cannot utilize should be used. If the D-sorbitol is used again, the conditions must be sterile to avoid contamination to get reliable results. The experiment in which I tested for the presence of proteases using gelatin is reliable. If the experiment were to be repeated, a harder gelatin, perhaps 8%, rather than 5%, gelatin, should be used to prevent the seeds from sinking under their own weight.

Two experiments which gave surprising results, the experiment using Hoagland's solution to replace water and the experiment in which seeds were germinated on moistened filter papers, need to be replicated several times for greater statistical reliability. The experiments went well, and no changes in the protocols are necessary.

Germination in perlite also produced interesting results that I believe to be worth repeating. Since the results obtained seem to be due to the amount of water present, the experiment should be altered to reflect this

observation. If this experiment were run varying the amount of water given the intact and demucilaged seeds, then the results might be used to support or contradict those obtained in the petri dish water potential experiments. Further changes may include the use of vermiculite in place of perlite, which I found difficult to handle, and the sealing of the trays with plastic film at all times to prevent the water loss.

I also think that the experiment examining the influence of mucilage upon embryo growth should be repeated. The results of the experiment do not suggest to me that measurements of the increase in length of the epicotyls is reliable; the epicotyls grew so little that my ability to measure any changes was questionable. The measurements of the increases in hypocotyl length, on the other hand, were not difficult.

The experiments which provided the most reliable data were those in which I quantified the amount of mucilage secreted per seed. Not only was the anthrone test itself relatively simple, but the data were consistent.

In retrospect, the experimental process for this thesis was enjoyable, even if often frustrating. If I were to run these experiments again, I would alter most of the protocols and use the combination of the rinsing and chemical methods of mucilage removal. Conclusive results, because of the problems inherent in most of the experiments, were not

obtained, except in the mucilage quantification experiment; 11 to 14% of the flax seed's dry weight is mucilage. The other results only suggest that flax mucilage may be involved in oxygen transport to the embryo, water uptake, or seed transport, but not in carnivory or nutrition.

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Figure 1. Rate of the production of flax seed mucilage as measured by the anthrone reagent.

Figure 1

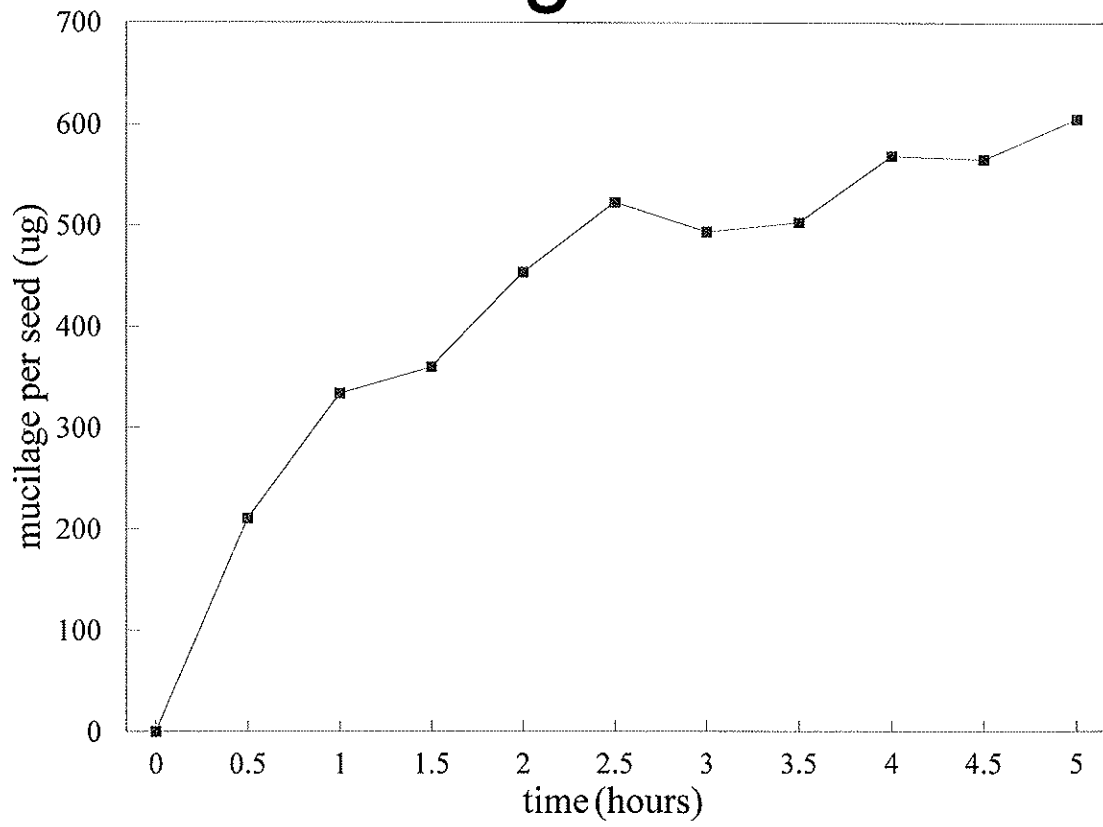


Figure 2. Percent germination after day 1 of flax seeds left intact, demucilaged, or treated with various amounts of mucilage extract.

Figure 2

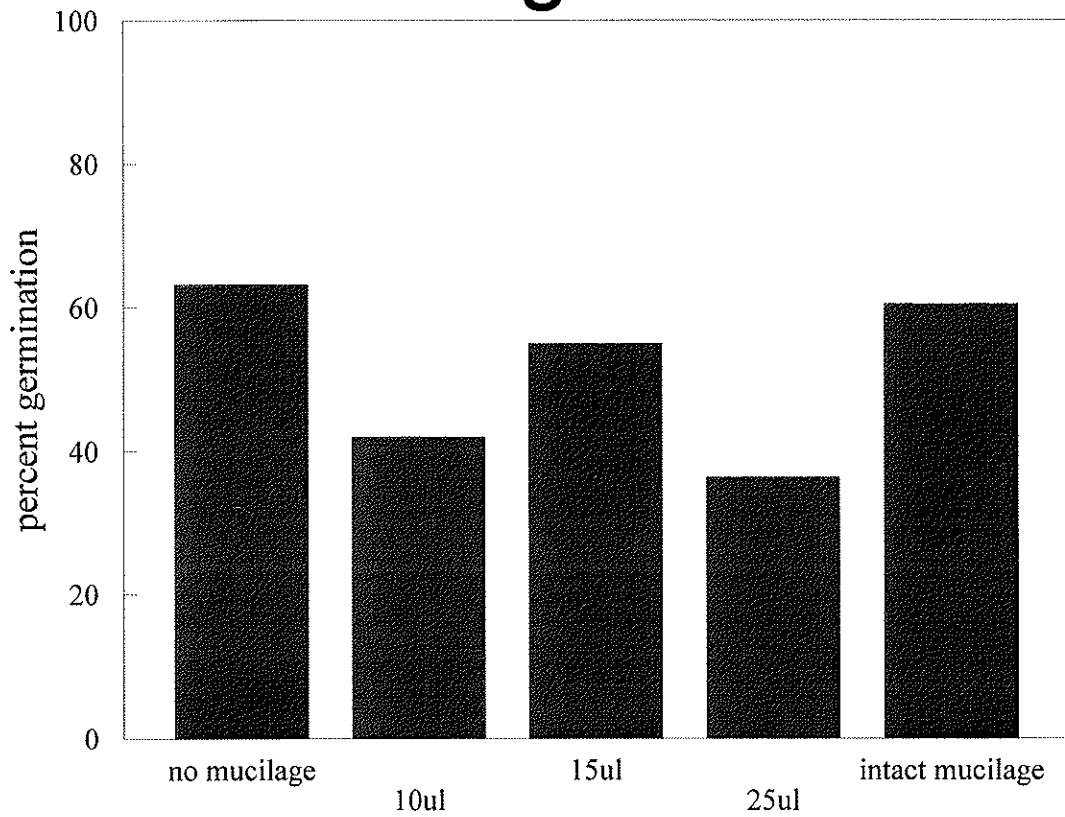


Figure 3. Dry weight per seedling of plants germinated from intact, demucilaged, and mucilage extract-treated flax seeds.

Figure 3

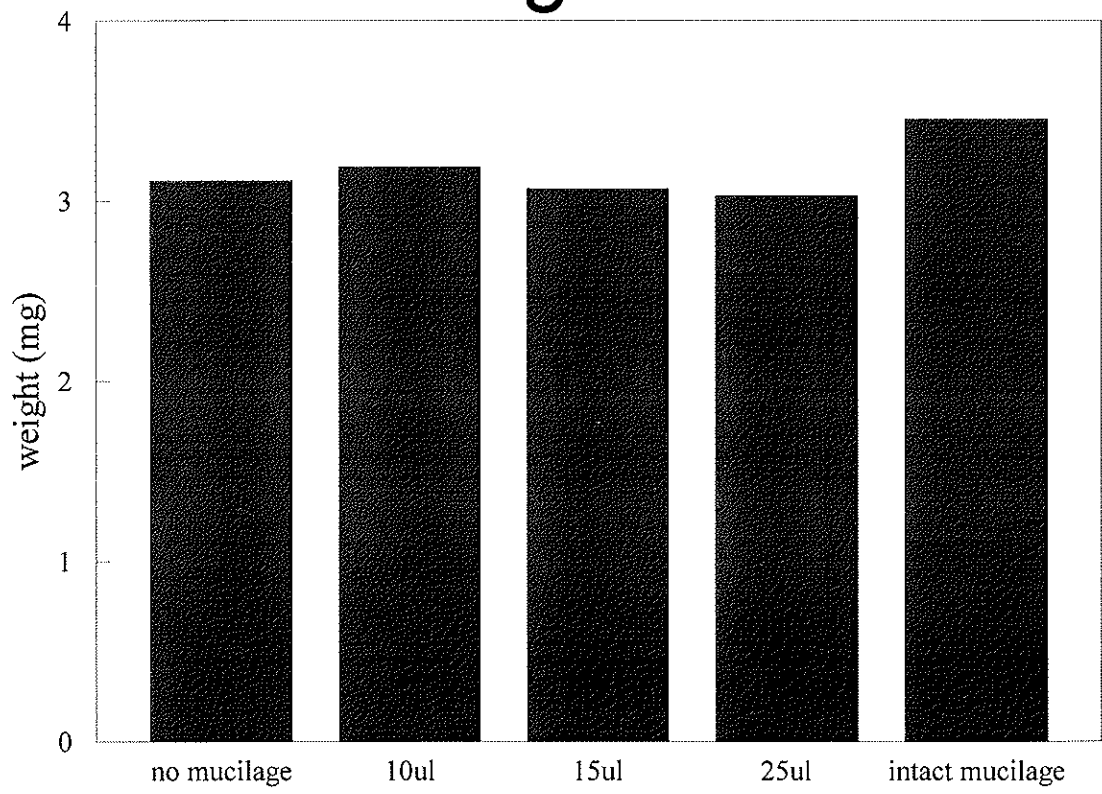


Figure 4. Percent germination after day 1 of intact and demucilaged flax seeds germinated under D-sorbitol-induced water stress.

Figure 4

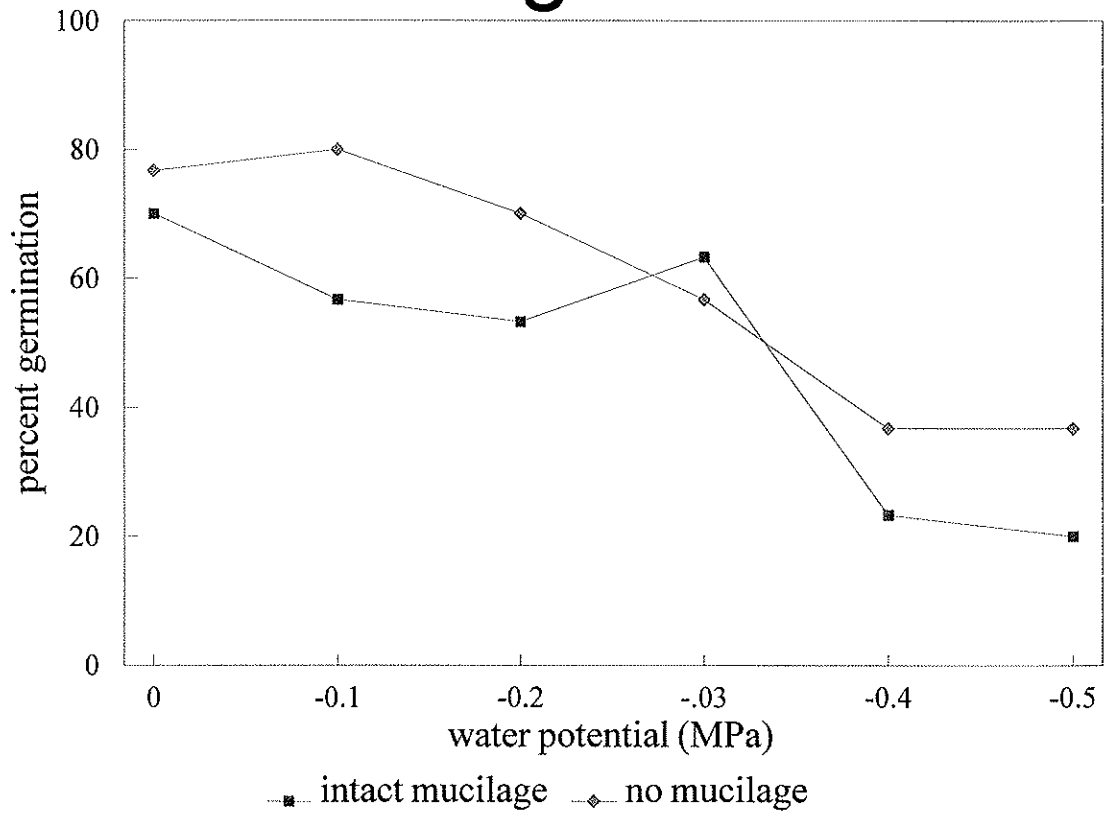


Figure 5. Percent germination after day 1 of intact and demucilaged flax seeds germinated on filter papers soaked with various volumes of water.

Figure 5

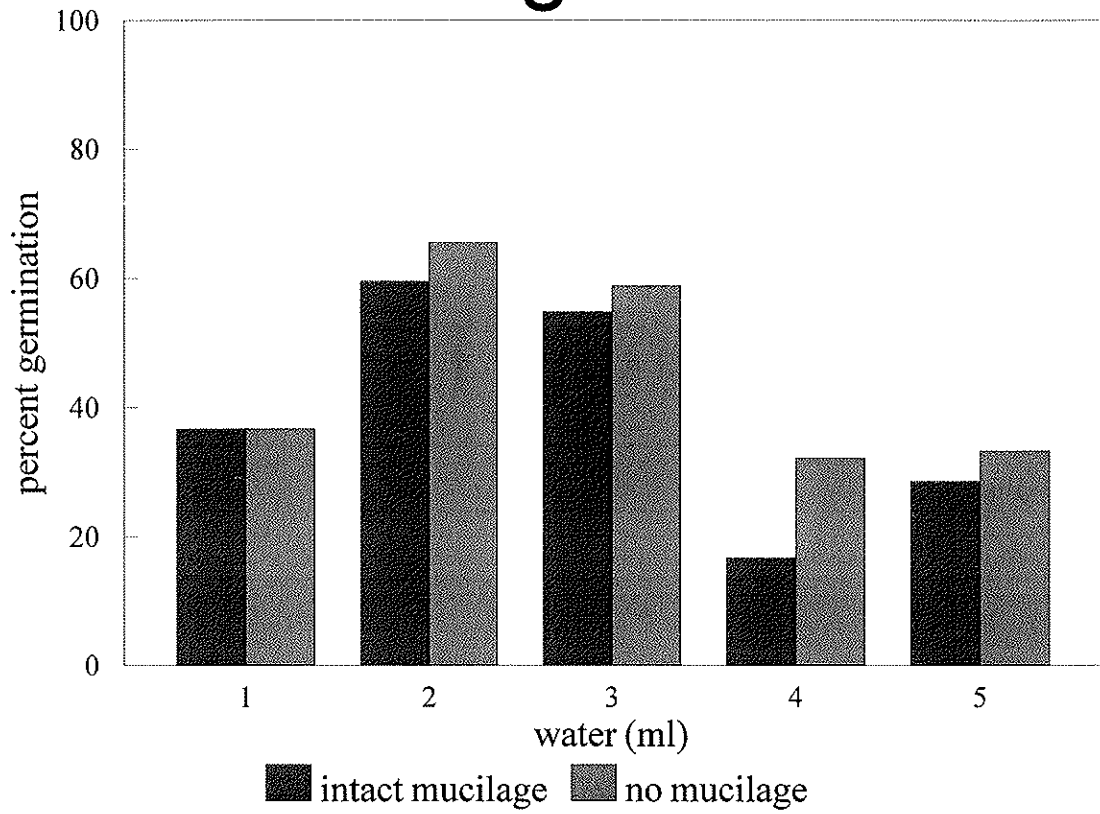


Figure 6. Dry weight per seedling of plants germinated from intact and demucilaged seeds in various volumes of water.

Figure 6

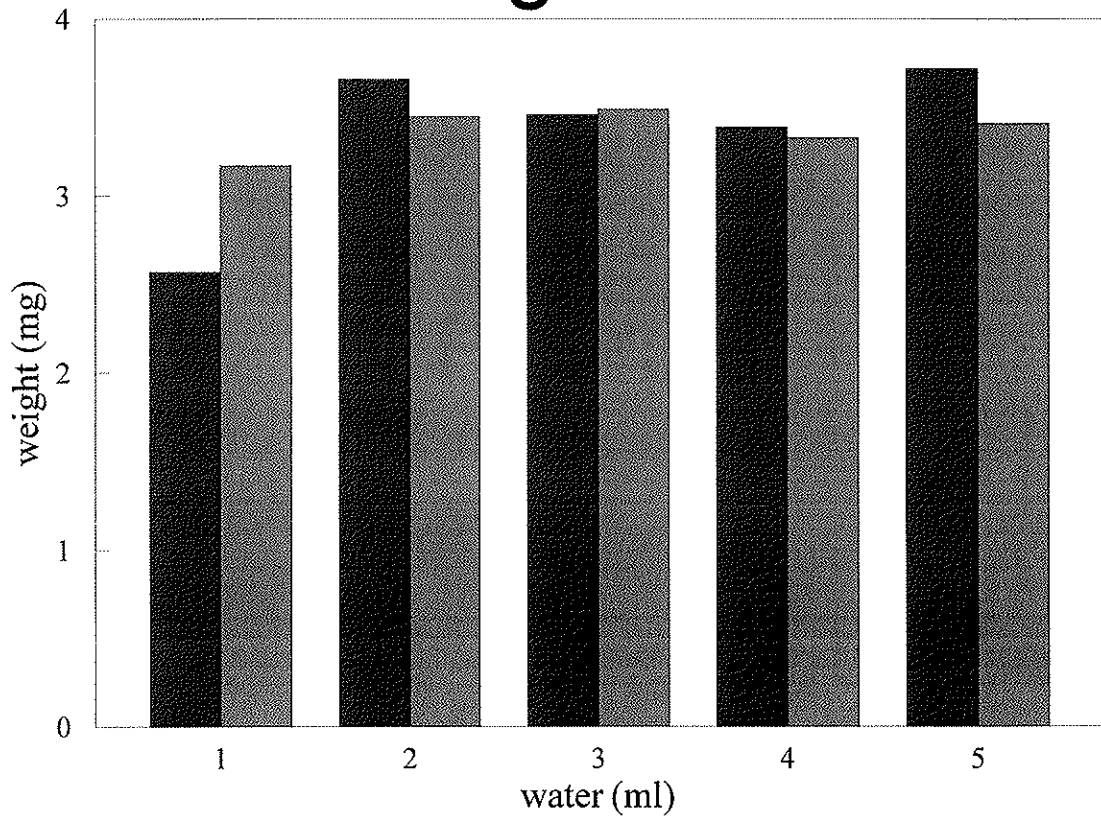


Figure 7. Percent germination after day 1 of flax seeds with and without nutrients and mucilage.

Figure 7

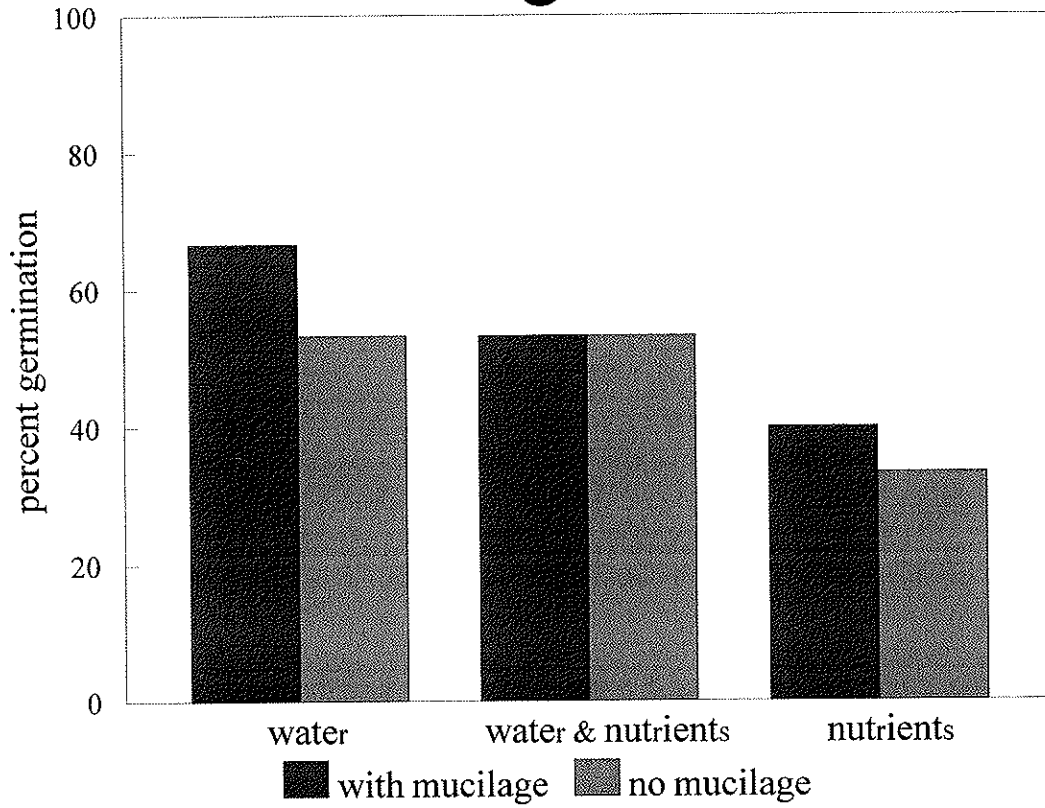


Figure 8. Percent germination after day 1 of flax seeds with and without nutrients and mucilage.

Figure 8

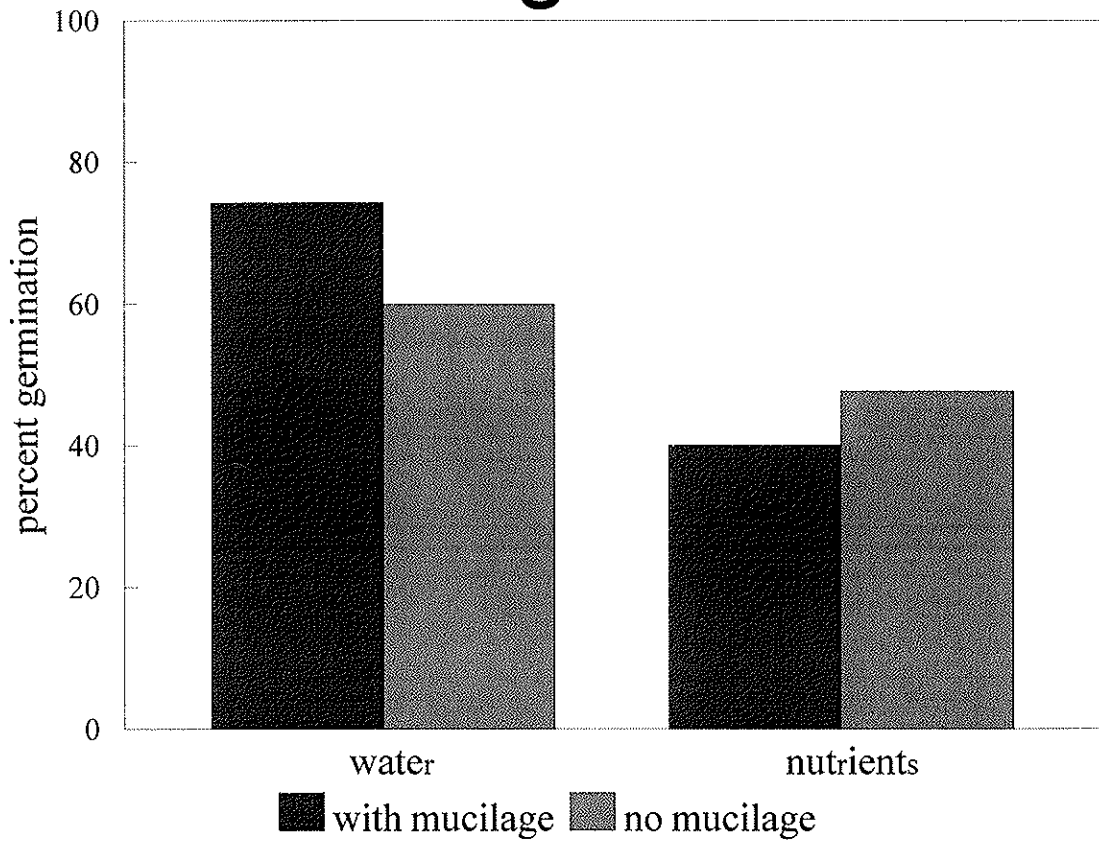


Figure 9. Percent germination after day 10 of flax seeds germinated in perlite with and without water and/or mucilage.

Figure 9

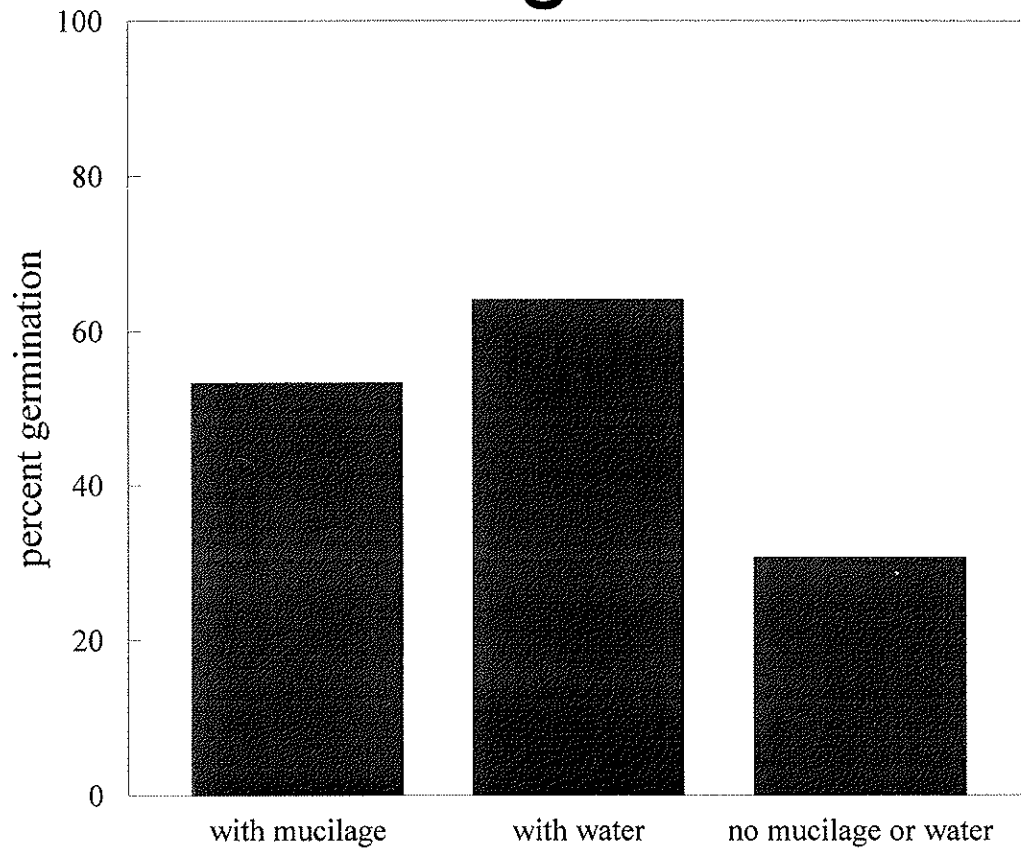


Figure 10. Average increase in epicotyl length of lima bean embryos treated with water and mucilage, mucilage, or nothing.

Figure 10

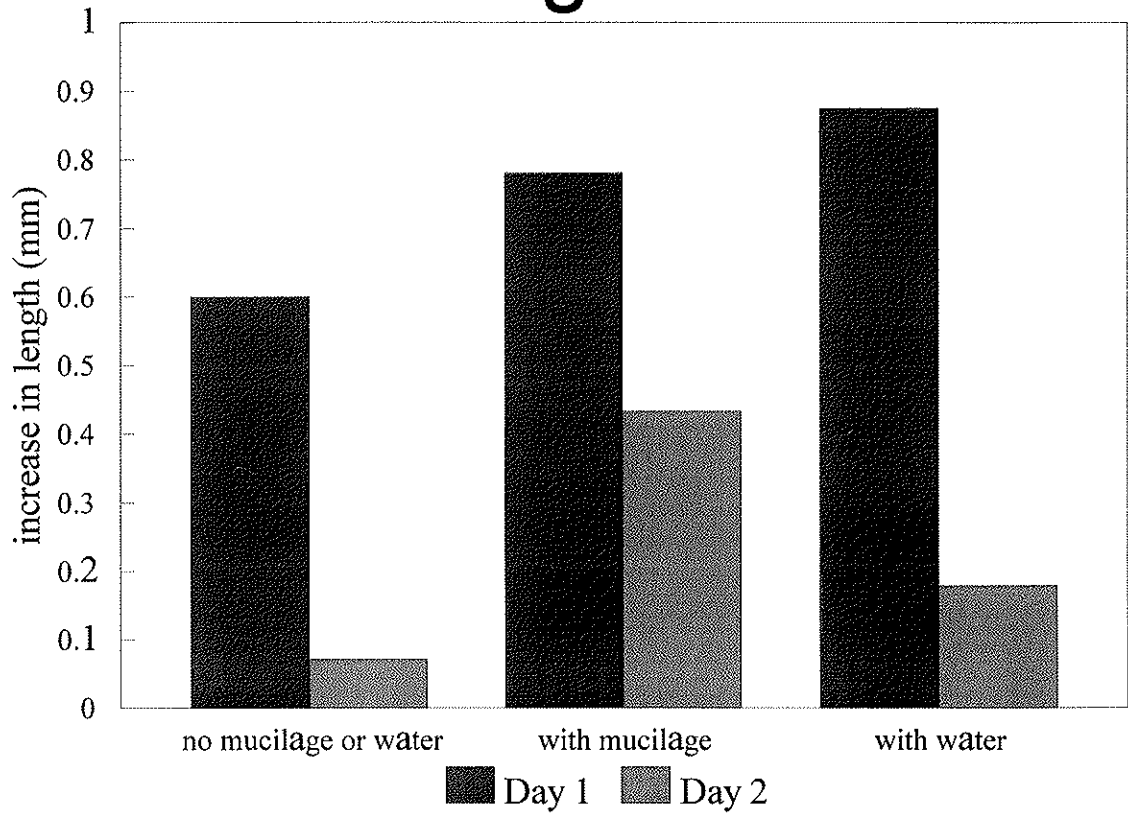


Figure 11. Average increase in hypocotyl length of lima bean embryos treated with water and mucilage, mucilage, or nothing.

Figure 11

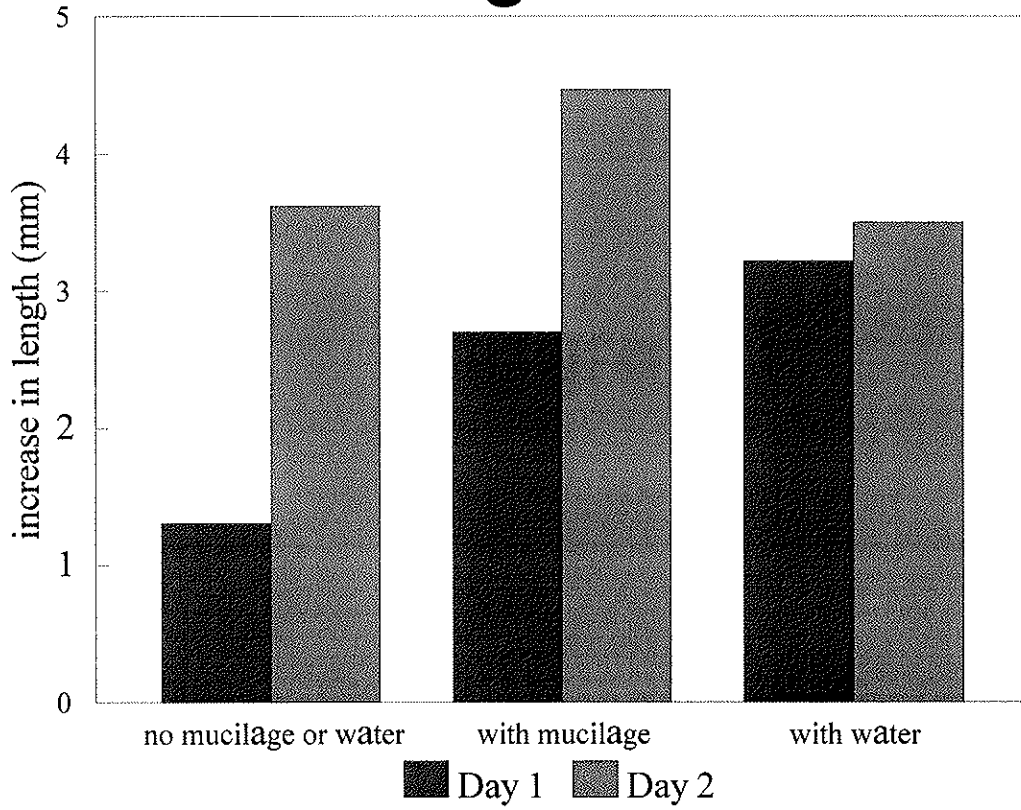


Table 1. Amount of mucilage released per seed after 5 and 26 hours. 25, 50, and 100 seeds were imbibed in 100 ml aliquots of water.

Table 1

Time Incubated in 100ml Water	Flask 1 25 Seeds	Flask 2 50 Seeds	Flask 3 100 Seeds
5 hours	566 ug/seed	514 ug/seed	351 ug/seed
26 hours	411 ug/seed	405 ug/seed	430 ug/seed