Further Studies on Texas Plantago Seeds*

By MARTHA JANE JONES† and C. CLARENCE ALBERS‡

A comparative study was made of the seeds of the official *Plantago ovata* Forskal, the homegrown variety of *Plantago ovata,* and three Texas Plantago species: *Plantago Helleri* Small, *Plantago inflexa* Morris, and *Plantago rhodosperma* Decne. The investigation was carried out by making morphological and histological comparisons of the seeds and quantitative and qualitative comparisons of the mucilage content. It was found that the mucilage content of each of the Texas species is slightly less than that of *Plantago ovata* Forskal. However, the abundant occurrence and ease of cultivating and harvesting of the Texas species make them an excellent potential commercial crop. The homegrown variety of *P. ovata* was found to yield a percentage of mucilage equal to that of the imported variety, and commercial production of this species in the United States would seem to be warranted.

PSYLLIUM SEED MUCILAGE has attained considerable importance in the past few years, not only in the preparation of medicinal laxatives, but also in the arts and industries, such as the cosmetic and the textile industries. The mucilage for these purposes is obtained primarily from *Plantago ovata* Forskal (Blonde psyllium) and is imported from India chiefly as "Psyllium husks." The blond seeds are preferred as a source for this product, not only because they yield a practically colorless mucilage, but primarily because their mucilage layer cracks off under slight mechanical pressure applied to the seed and can readily be separated from the seed. One importer reports that his firm has not been wholly satisfied in its dealings with Indian sources, hence it has undertaken experimental cultivation of this species in the United States, especially in Arizona.

Greenberg (1) has directed attention to at least one Texas species of Plantago, namely *Plantago Wrightiana* Decne, as a possible source of mucilage and has made some quantitative and qualitative comparisons with the official seeds. The present work is a continuation of such studies applied to several additional native species of Plantago as well as to cultivated *P. ovata.* In addition to the study of the mucilage forming capacity of each species a morphological and histological study of the seeds was also made.

MATERIALS

The seeds designated in this study as *Plantago inflexa* Morris, *Plantago Helleri* Small, and *Plantago rhodosperma* Decne were obtained from plants authenticated by Dr. B. C. Tharp, Curator of the Herbarium, the University of Texas.

Plantago inflexa Morris.—The seeds of this species were collected in June, 1949, in Washington County, Texas. *P. inflexa* which is native to Texas was found in dry, deep sandy soil. The scapes of the plant were erect, very strong, and extended well beyond the leaves. The fruiting spikes, which varied in number per plant, were quite coarse and measured from 2 to 4 inches in length. The color of the seeds varied from light to dark brown.

Plantago Helleri Small.—The seeds of *P. Helleri* were obtained from plants collected in late June, 1950, north of Austin, Texas. This plant, smaller and less conspicuous than the other Texas species which were studied was usually only 5 inches high. This species is native to Texas and is found in dry, limestone soil. The spikes were oblong-cylindrical and measured up to $3^1/_2$ inches in length, usually extending only a short distance above the leaves. The seeds were medium brown in color.

Plantago rhodosperma Decne.—The seeds of this species were collected from plants grown in the Medicinal Plant Garden of the College of Pharmacy, the University of Texas, during 1948 and 1949. *P. rhodosperma* is found in sandy soil from Missouri and Oklahoma to Louisiana, Texas, and Arizona (2). In the Austin area it attains its maximum development in heavy limestone soils. The scapes were erect, usually extending above the leaves. The spikes measured from 2 to $7^3/_4$ inches in length and were densely flowered. The capsules dehisce less readily than those of the other species, making it more difficult to remove the dark red seeds. This would be an advantage in the harvesting of the crop since there would be less shattering and loss of the seeds.

Many of the plants collected near Austin were very large in comparison with the other Texas Plantagos. One plant was found to be 13 inches high and to have 63 fruiting spikes. The length of the spikes ranged from $3^1/_2$ to $7^3/_4$ inches, and the number of seeds from each spike varied from 190 to 218 and weighed from 108 to 208 mg. The total weight of the seeds from the entire plant was 9.978 Gm.

Plantago ovata (homegrown).—The cultivated *Plantago ovata* seeds were collected from plants grown in the Medicinal Plant Garden of the College of Pharmacy from seeds supplied by the Serutan Company. The seeds were planted in beds in sandy loam soil in the late fall of 1948 and the crop harvested in May, 1949. The scapes varied from one to several per plant, rarely surpassing the leaves.

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The spikes of these cultivated plants measured up to 1 inch in length and produced three types of seeds: silvery gray, partly gray and partly light brown, and entirely light brown.

Harvesting of all seeds was done by hand and the cleaning process was accomplished by winnowing with an electric fan.

EXPERIMENTAL

Swelling Factors.—In earlier studies Burlage (3) reported a wide variation in the swelling factor within the same lot of seeds of Plantago species. Because of such reports, methods were employed in order to determine if the official N. F. (4) method (I) and the procedure used by Greenberg (1) (II) had any effect on the swelling factor. Results obtained by each method are recorded in Table I.

Mucilage Content.—The seeds used in the swelling tests according to Method No. I were utilized as a source of the mucilage for the quantitative determinations. The mucilage was separated from the seeds by use of the hand press devised and described by Greenberg (1). The seeds were previously dried at 98° for ten hours before weighing. At the end of the twenty-four-hour period, as described in Method No. I, the contents of the cylinder were transferred to the hand press and the expressed mucilage collected in a tared evaporating dish. Both the cylinder and the hand press were washed two or three times, and the wash water added to the mucilage. The seeds were then carefully transferred to another tared evaporating dish. The evaporating dishes and their contents were thoroughly dried at 98° to a constant weight and the mucilage percentage was determined by (*a*) direct weight of the dried expressed mucilage, and (*b*) indirectly by loss of weight of the seed during the operation. The results are tabulated in Table I.

It will be noted that the figures check rather closely for all species except *P. rhodosperma* and show that very little mucilage is lost in the hand press. The discrepancy in the case of *P. rhodosperma* is apparently due to a greater tenacity of the mucilage of this species which interferes with its complete removal from the cylinder and hand press during the operation.

The mucilage separated from the seeds of commercial *P. ovata*, homegrown *P. ovata*, and *P. rhodosperma* was a clear jellylike mass varying in color with the different species. Mucilage expressed from *P. Hélleri* and *P. inflexa* seeds was a dirty-white, translucent mass. When examined closely, denser areas occur thickly scattered throughout the gelatinous mass. Greenberg (1) has referred to these dense areas as incompletely hydrated mucilage forcibly expressed from the seed.

The powdered mucilage from the different species possessed the following colors: pale cream color, *P. Helleri* and *P. inflexa;* brownish-tan, *P. rhodosperma;* grayish brown, *P. ovata* (homegrown); light tan, rose tint, *P. ovata* (commercial).

The solutions of the various mucilages varied in color and in viscosity. The latter property is tabulated in Table II.

Viscosity Determinations.—The dilutions used in determining the viscosities were prepared by adding 150 mg. of the powdered mucilage, dried at 98° to constant weight, to 20 cc. of distilled water. The determinations were made at 37.8° using the Ubbelohde viscosimeter and the results reported in Table II.

For the first trial the powdered mucilage was added to the water in a 125-cc. Erlenmeyer flask and allowed to stand, with occasional shaking, for eight hours. At the end of this period only the mucilage from the homegrown *P. ovata* yielded a clear, transparent liquid. The viscosity of this solution was found to be 19.02. The mucilage of the remaining species required a period of heating, ranging from fifteen to twenty-five hours, to effect a homogeneous solution for the determinations. The heating process was carried out by loosely corking the flasks and placing in an oven at 98°. After cooling to

TABLE I.—SWELLING FACTORS, MUCILAGE CONTENT, AND SIZE OF SEED

Species	Swelling Factors[a] (Av. Volume), cc. Method I	Method II	Av. Percentage of Mucilage[b] Direct	Indirect	Av. Wt. of 100 Seeds,[c] Mg.	Size of Seeds in mm.[d] Length	Width
P. inflexa	12.5	13.5	17.7	18.3	184.01	2.5–3.0	1.5–1.75
P. Helleri	16.5	16.0	17.4	17.7	229.93	3.25–3.5	1.5–1.75
P. rhodosperma	18.5	18.0	17.5	21.0	151.81	2.5–3.24	1.25–1.5
P. ovata (homegrown)	10.5	11.0	21.8	22.1	148.48	2.5–3.0	1.5–1.75
P. ovata (commercial)	11.5	10.0	21.5	21.7	189.71	3.0–3.25	1.5–1.75

[a] At least 5 determinations were made on each species.
[b] At least 5 determinations were made on each species.
[c] Six determinations were made on selected seeds of each species.
[d] Ten determinations were made on selected seeds of each species.

TABLE II.—KINEMATIC VISCOSITIES (CENTISTOKES)

Species	First Trial (Allowed to Stand for 8 Hours Then Heating from 0–25 Hours)	Second Trial (Heating for 34 Hours)	Third Trial (Allowed to Stand in Refrigerator for 12-Hours—Heating for 74 Hours) 5 Min.	12 Hrs.	18 Hrs.	32 Hrs.	74 Hrs.
P. inflexa	9.80	11.08	7.11	5.76	5.76	5.76	3.87
P. Helleri	6.63	4.96	17.39	7.94	6.84	5.53	3.54
P. rhodosperma	2.89	4.33	Too thick	Too thick	Too thick	1,445.00	2.93
P. ovata (homegrown)	19.02	3.85	177.38	45.01	26.83	17.83	3.31
P. ovata (commercial)	21.19	4.76	224.38	36.32	21.92	21.92	2.75

37.8° the mucilage was introduced into the viscosimeter and five determinations were made, in rapid succession, and the average was taken as the viscosity value.

Inasmuch as the solutions prepared by the above method were not uniformly treated, the values obtained were not truly comparable; therefore, a second trial was carried out with the solution of each mucilage subjected to similar treatment. The powdered mucilage was added to the water in a 50-cc. cylinder. The cylinder was then vigorously shaken and immediately placed in the oven and heated for thirty-four hours. During this treatment water lost by evaporation was replaced and the solutions were cooled to 37.8°.

Since the results obtained from trials 1 and 2 varied considerably and because it was observed that heating over a prolonged period of time obviously had some effects on the mucilages, a third trial was attempted to investigate further the causes for these vast differences in the viscosity values. Each mucilage was accordingly prepared by adding the powdered mucilage to the water in a test tube, placing it in the refrigerator, allowing it to stand for twelve hours, then transferring the test tube to a large beaker of boiling water and heating for five minutes. Each test tube was thereupon shaken vigorously to dissolve any small clumps of undissolved mucilage, after which the solutions were then placed in the oven and heated at 98° for a total of seventy-four hours, and the viscosity determinations were made at intervals of twelve-, eighteen-, thirty-two-, and seventy-four-hour periods of time, respectively. The results reported show a steady decrease in the kinematic viscosity of the solutions.

In analyzing the viscosities reported in Table II, second trial, it will be observed that after heating for thirty-four hours all the mucilages, except that from *P. inflexa*, show approximately the same values. Furthermore, it will be noted that the viscosities reported in the first trial, where the solutions were allowed to stand before heating, were higher than those obtained in the second trial, with the exception of those of *P. inflexa* and *P. rhodosperma* which were shown to be somewhat lower in the first trial. From these results it might be assumed that allowing the mucilage to stand for eight hours before heating tends to facilitate, in most instances, the hydration of the mucilages, causing an increase in the viscosities. If the mucilages are heated for thirty-four hours immediately after they are placed in water, heating either prevents hydration or else causes some other physical or chemical change in the colloidal solution, after hydration takes place, to cause a decrease in the viscosity. The mucilages from *P. inflexa* and *P. rhodosperma*, which have been the exceptions to the general trend in the previous trials, also show a difference in the third trial in that they change very little over a thirty-two-hour heating period, whereas the other mucilages steadily decrease after each period of heating. The behavior of *P. rhodosperma* mucilage indicates that it does not hydrate rapidly (requiring twelve hours to form a very thick mucilage); however, once it is hydrated it remains stable toward heat for a period of thirty-two hours before undergoing further change, either through hydrolysis or in the colloidal state of the solution. After thirty-two hours the solution is still extremely viscous, but measurable, but upon further heating the change in viscosity occurs quite rapidly to attain the value of 2.93 at the end of the seventy-four-hour period. Although the various mucilage solutions vary considerably under different treatment, it will be noted that after heating for seventy-four hours all the mucilage solutions tend to approach the same viscosity value.

The conclusions drawn from the viscosity values reported are that (*a*) heating has a definite effect on the mucilage solutions, (*b*) the period of time each solution is allowed to stand before heating is a significant factor, and (*c*) all the mucilages tend to approach the same viscosity value if heated for a long period of time.

A definite statement as to what changes occur in the mucilages to cause these variations in the viscosities cannot be made. A question arises as to the possibility of the change being brought about by enzymatic action, but since enzymes are thermolabile at 98° some other explanation must be sought. Instead, it appears to be a physical or chemical change brought about by hydrolysis. Hydrolysis might occur before, during, or after the process of hydration; or it is possible that a change in the micellar structure of the mucilage solution occurs. More information concerning the physical and chemical properties of the mucilages will have to be obtained before a satisfactory explanation can be given.

Since the viscosities vary so markedly under different conditions of time and temperature, it would be difficult to correlate this property with the medicinal and industrial use of the mucilages.

Microtechnique Methods.—In previous histological studies on certain Plantago seeds, Neva (5) used, with special adaptations, the double embedding procedure recommended by Johansen (6) and Sass (7) in getting suitable sections for study. It was found in the present study that if the fresh mature fruits were used, only the paraffin method was necessary to obtain satisfactory transverse sections of the seeds. Following Neva's (5) general procedure, with some modifications, complete cross sections of the seeds with the mucilage layer still intact were obtained.

The seeds were placed for five days in a formalin-aceto-alcohol solution in which the water concentration was approximately 16%. They were then washed with 95% alcohol and absolute alcohol, respectively. After washing, the seeds were then hardened and dehydrated by passing them, in succession, through 10, 30, and 60% chloroform-absolute alcohol mixtures and finally into 100% chloroform. After hardening with chloroform the paraffin method described by Sass (7) was employed. Sectioning was accomplished by (*a*) using a rotary microtome and (*b*) making freehand sections. The sections were stained by passing them, in succession, through 95% alcohol, the alcoholic staining solutions of 0.5% safranin and 0.5% fast green, absolute alcohol, and finally into xylol.

DESCRIPTION OF THE SEEDS

External Morphology.—A binocular dissection microscope (17×) was used in the examination of the external morphology of the seeds.

Plantago inflexa.—The seeds of *P. inflexa* measured from 2.5 to 3.0 mm. in length and from 1.5 to

1.75 mm. in width. They were ovate-oblong, typically boat-shaped, light brown over most of the top portion of the seed but darker brown along the seed margin. The light brown area visible on the convex dorsal surface coincided with the location of the straight embryo or possibly outlined the cavity on the concave ventral surface. This area extended almost the entire length of the seed and the division between the two cotyledons could easily be seen.

The dorsal surface was dull, obscurely papillose, and exhibited a distinct transverse groove near the mid-section of the seed.

The concave ventral surface showed a narrowed cavity bordered by a dark brown somewhat flattened ridge. The ventral hollow was completely covered with a white scaly tissue, leaving only the hilum exposed.

Plantago rhodosperma.—The seeds of *P. rhodosperma* varied in length from 2.5 to 3.24 mm. and in width from 1.25 to 1.5 mm. They were broadly ellipsoidal, pear-shaped, and light to dark red in color.

The convex dorsal surface exhibited a light-colored elongated central area, tapering at the broader end of the seed. The margin of the seed appeared as a white translucent band, especially conspicuous at the two extremities.

The ventral surface was slightly concave bordered by a clear, thin, low ridge. Near the center of the ventral surface lay the distinct white hilum. Both the dorsal and ventral surfaces were dull and slightly papillose.

Plantago Helleri.—The seeds of *P. Helleri* were larger than those of the other two Texas species, varying in length from 3.25 to 3.5 mm. and in width from 1.5 to 1.75 mm. They were ovate-ellipsoidal, larger at one end than the other, medium brown, but darker brown along the seed margin.

The convex dorsal surface was dull, slightly papillose, and showed a transverse groove nearer the broader end.

The concave ventral surface, showing a large cavity with the hilum located near the center of the base of this cavity, was bordered by a dark-brown flattened ridge. Most of the furrowed surface was covered with a white scaly tissue which was more dense around the hilum and around the base of the ridge.

Plantago ovata (homegrown).—The homegrown *P. ovata* produced seeds which varied considerably in appearance. There were three types: The first type was like the usual commercial form with the silvery-white layer covering all but a small central area on the dorsal side of the seed. They differed only in that they were slightly smaller. The second type was only partly covered with the silvery-white coating. In some instances one-fourth or one-fifth of the seed was covered with the white layer, the remaining portion being a light-brown color. The dorsal surface of some of the seeds showed only a narrow strip of the white coating formed across one end or along one side of the seed. The silvery-white coating seemed to be deposited in a very irregular fashion without any definite pattern. The third type possessed no silvery-white coating, but otherwise resembled the official variety. This last type of homegrown *P. ovata* seed was light brown in color. The dorsal surface showed a yellowish-brown center extending almost the full length of the seed, fading into a light brown (chestnut brown) border.

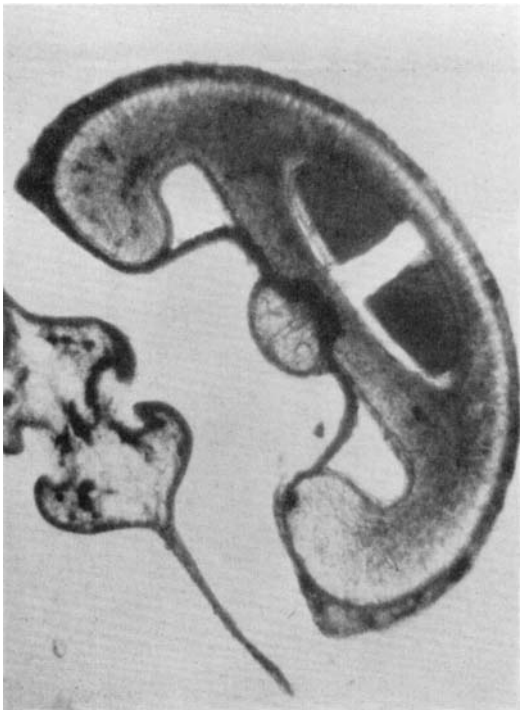

Fig. 1.—Transverse section of *Plantago inflexa* Morris seed showing cotyledons, endosperm, and outer mucilage layer. × 50.

The homegrown *P. ovata* seeds measured 2.5 mm. in length, rarely up to 3.0 mm., and from 1.5 to 1.75 mm. in width.

The silvery-white coating of these seeds appeared to be an extra thick layer of mucilage. Since the majority of the seeds obtained from the homegrown variety possessed no white coating, it was assumed that they would yield a smaller per cent of mucilage than the official variety. Experimental results, however, showed this assumption to be in error, as the quantitative yield of mucilage was approximately equal to that of the official variety, as shown in Table I.

Histology.—Transverse sections of the seeds of *P. inflexa* (Fig. 1), *P. rhodosperma* (Fig. 2), *P. Helleri*, and *P. ovata* (homegrown) were made and the three distinct regions, the embryo, endosperm, and seed coat were examined under low- and high-power magnifications.

Plantago inflexa.—*Embryo.*—The straight embryo was located in the center of the endosperm and consisted of two heel-shaped cotyledons adhering in the direction perpendicular to the ventral surface. Each cotyledon was composed of compact, circular, thin-walled parenchyma cells with the outermost layer being formed by slightly smaller, nearly square cells.

Endosperm.—The endosperm extended from both sides of the embryo in a curved ventral direction to form the two ridges which outlined the ventral cavity. These ridges conspicuously folded toward the center of the seed. The endosperm was composed of thick-walled, irregular shaped cells, with the

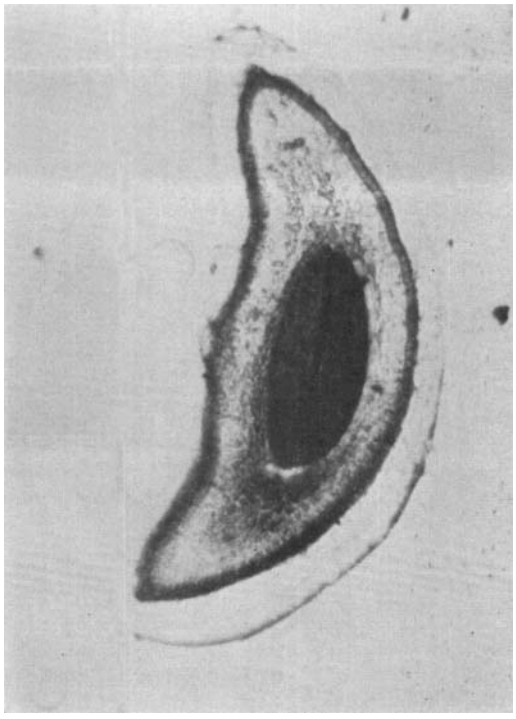

Fig. 2.—Transverse section of *Plantago rhodosperma* Decne seed showing cotyledons, endosperm, pigment layer, and outer mucilage layer. × 50.

outermost layer consisting of thick-walled palisade cells measuring from 20–28 μ in height.

Seed Coat.—The seed coat consisted of (*a*) the pigment layer, and (*b*) the outer epidermal layer. The pigment layer surrounded the endosperm and was formed by a single layer of flattened, wavy, somewhat collapsed, thin-walled cells measuring up to 8 μ in height. The single layer of outer epidermal cells was thin-walled, translucent, mucilaginous cells varying from a rectangular to a nearly square shape. On most of the dorsal surface, the cells measured in height from 30–40 μ. Along the incurved margins of the seed, the mucilaginous cells became somewhat elongated, measuring from 90–110 μ in height. At the top of the endosperm ridge, the region where the endosperm folded back toward the center of the seed, the outer epidermal layer became detached from the pigment layer and passed directly to the ventral surface near the hilum.

Plantago rhodosperma.—*Embryo.*—The two cotyledons of the embryo adhering in the direction parallel to the ventral surface, appeared as somewhat flattened, elongated semicircular structures. Each cotyledon consisted of several layers of compact, thick-walled, rectangular cells with ends being somewhat pointed, presenting a "storied cambial" effect.

Endosperm.—The endosperm did not form two ridges on the ventral surface as distinctly as those observed in other Plantago species. It formed a somewhat straightened half-moon shape, with the ends slightly rounded, the center region on the concave ventral surface being slightly raised. The cells, however, which made up the endosperm, were very similar in structure to those of *P. inflexa*, the palisade cells measuring from 8–15 μ in height.

Seed Coat.—The pigment layer differed considerably from that of *P. inflexa*. It was composed of rectangular and square shaped cells measuring from 12–28 μ in height and from 8–15 μ in width. The cells contained a dark red pigment. The single outer epidermal layer strongly adhered to the pigment layer around the entire seed, leaving only the hilum exposed. The clear, translucent cells were rectangular in shape measuring from 20–40 μ on most of the dorsal surface, 15–20 μ on the ventral surface, and from 70–90 μ near the ends of the half-moon shaped endosperm.

Plantago Helleri.—*Embryo.*—The two cotyledons of the embryo of *P. Helleri* were very similar to those of *P. inflexa*. They differed from *P. Helleri* in shape in that they were slightly larger and more rounded in appearance.

Endosperm.—The endosperm of *P. Helleri* differed from that of *P. inflexa* in that the endosperm ridges formed are thinner and fold toward the center of the seed to a lesser extent than those of *P. inflexa*. The cells of the palisade layer measured from 10–15 μ in height.

Seed Coat.—The cells making up the single pigment layer were structurally similar to those found in *P. inflexa*. They contained a dark brown pigment and measured from 5–8 μ in height. The cells which form the outer epidermis resembled those described under *P. inflexa*. They varied in height from 30–40 μ on most of the dorsal surface, to 90–110 μ along the incurved margins of the seed. The epidermal layer also became detached from the pigment layer near the top of the endosperm ridges; it did not extend directly to the ventral surface, but followed closely the cavity outline.

Plantago ovata (homegrown).—*Type I.*—The histology of this type seed produced by homegrown *P. ovata* was identical to that of *P. ovata* commercial, the description of which has been adequately covered in previous works. It was observed in this study, however, that the outer epidermal layer adhered to the pigment layer along the central area of the dorsal surface and along the top portion of the ventral endosperm ridges. It was detached from the pigment layer on the remaining portion of the seed. This could be the explanation for the particular coloration of the seed. The intercellular area between the pigment layer and the epidermal layer would tend to obscure the brown pigment layer, thus giving the seed a whitish appearance.

Type III.—The histology of the brown *P. ovata*, homegrown, also resembled that of the official variety. It differed from the latter in that the mucilage layer closely adhered to the pigment layer, which completely enclosed the seed.

SUMMARY

A study has been made of three Texas Plantago species and of the homegrown *P. ovata*. The morphological and histological characteristics of the seeds of each of these species have been described, the mucilage content of each has been quantitatively determined, and a study of the effects of time and temperature on the viscosity of the various mucilage solutions has been made. From these studies the following observations were made:

1. The mucilage content of each of the three Texas Plantagos, *P. inflexa*, *P. rhodosperma*, and *P. Helleri* was slightly less than that of *P. ovata* (commercial).

2. The homegrown *P. ovata* produces three types of seed, the majority of which differs in appearance from the imported seed of *P. ovata*. It was found, however, that the homegrown variety yielded a percentage of mucilage equal to that of the official variety.

3. The conclusions drawn from the viscosity values reported are that (*a*) heating has a definite effect on the mucilage solutions, (*b*) the period of time each solution is allowed to stand before heating is a significant factor, and (*c*) all the mucilages tend to approach the same viscosity value if heated for a long period of time.

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The Synthesis of C^{14} Carbonyl Labeled Dimethyl Phthalate*

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Special techniques and apparatus for the synthesis of C^{14} carbonyl labeled dimethyl phthalate, $C_6H_4(C_2CH_3)C^{14}{}_2CH_3$, are described.

DIMETHYL PHTHALATE is a compound showing considerable merit as a very effective insect repellent (1). In any study of the properties of this compound as an insect repellent, the use of a radioactive label would offer certain obvious advantages. Consideration of the structure of dimethyl phthalate, the ease of synthesis and the labeling isotopes available led to the conclusion that the C^{14} carbonyl labeled compound offered the most promise. Accordingly, the purpose of this study was to investigate the methods of synthesis of C^{14} carbonyl labeled dimethyl phthalate.

After careful consideration of various syntheses of dimethyl phthalate reported in the literature (2–5), C^{14}-labeled dimethyl phthalate was prepared by treatment of *o*-tolyl magnesium bromide with $C^{14}O_2$ (obtained from $BaC^{14}O_3$), oxidation of the resulting labeled *o*-toluic acid to labeled phthalic acid, and esterification via the acid chloride.

EXPERIMENTAL

***o*-Tolyl Magnesium Bromide.**—A twofold excess of this Grignard reagent was prepared using 3.42 Gm. of freshly distilled *o*-bromotoluene, 0.48 Gm. of magnesium turnings, one crystal of iodine and 30 ml. of anhydrous ether.

The magnesium turnings, previously crushed, were placed in a thoroughly dried 300-ml. three-necked round-bottomed flask, fitted with a mercury sealed stirrer, a 100-ml. dropping funnel and a Hopkin's condenser, fitted at its upper end with a drying tube filled with calcium chloride. Anhydrous ether, 6.5 ml., and a crystal of iodine were added to the flask. The *o*-bromotoluene was added to the dropping funnel and 20 drops of this undiluted halide were added to the flask. The mixture was refluxed without stirring, over a water bath at 45°. The remaining 23.5 ml. of anhydrous ether which had been used to rinse the apparatus in which the *o*-bromotoluene had been measured, was added to the dropping funnel. The *o*-bromotoluene-ether mixture was added with stirring to the flask over a thirty- to thirty-five-minute period. Stirring was continued as long as there was evidence of any reaction, after which the mixture was refluxed on the water bath for another thirty minutes.

***o*-Toluic Acid.**—The *o*-tolyl magnesium bromide was carbonated in the apparatus diagrammed in Fig. 1. Barium carbonate C^{14} (1.97 Gm.) with an activity of approximately 5 μc. was weighed into flask *G* and 15 ml. of concentrated sulfuric acid were added to funnel *F*. This carbon dioxide generator was attached and sealed to the manifold *L* through the drying tube *E*, filled with Drierite® and stopcock *D* was left open. The entire system was evacuated through stopcock *A* to less than 0.5 mm. pressure, as indicated by manometer *C*; stopcock *A* was closed and the system was tested for leaks. Stopcock *B* was opened and nitrogen admitted to bring the system to atmospheric pressure. Stopper *M* was opened and the *o*-tolyl magnesium bromide quickly pipetted into the reaction flask *K*. The stopper was quickly replaced, stopcock *B* was closed and the contents of the reaction flask frozen with liquid nitrogen and the system evacuated. Stop-

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The C^{14}-labeled barium carbonate was obtained on allocation from the Atomic Energy Commission. Other materials were standard chemical reagents.