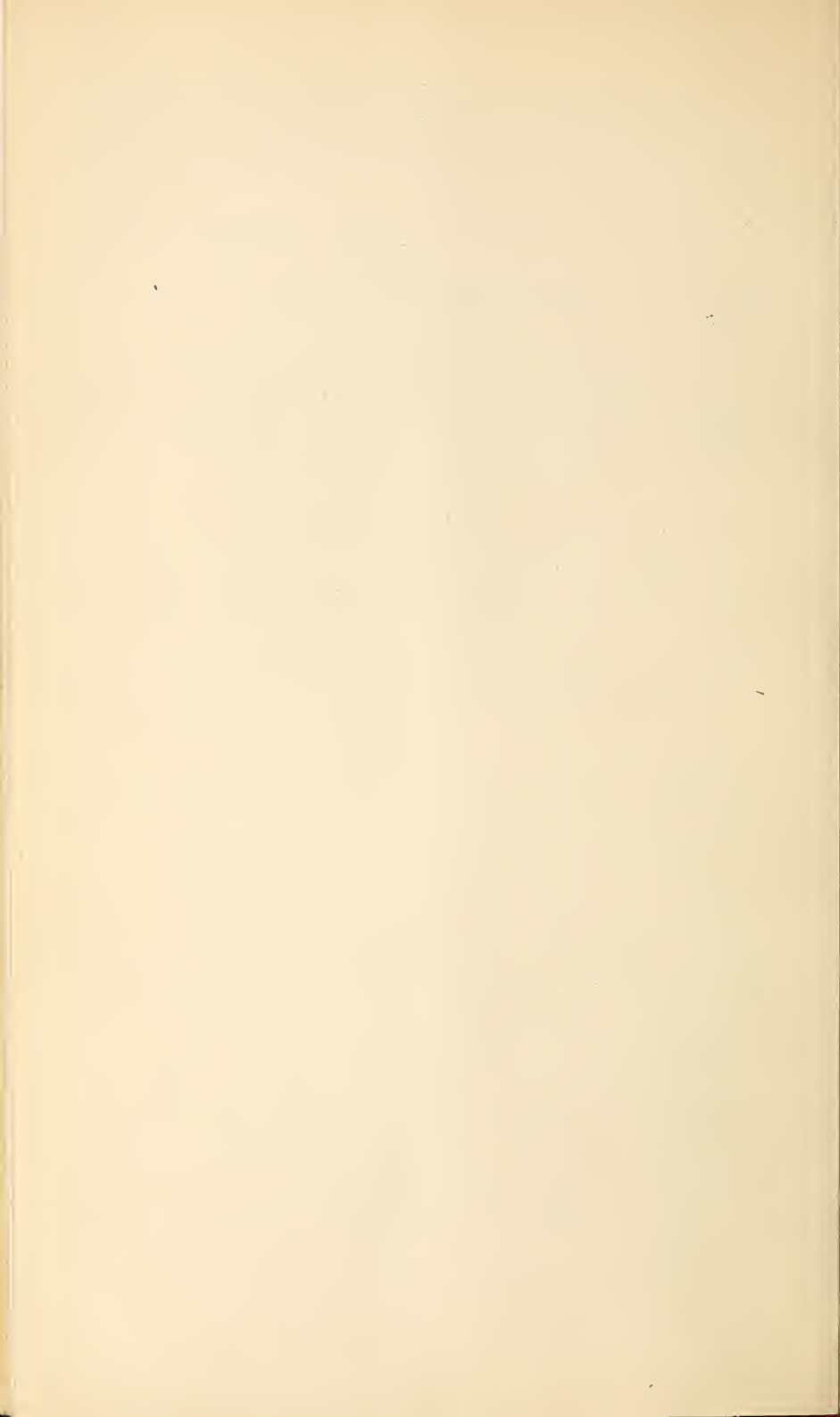


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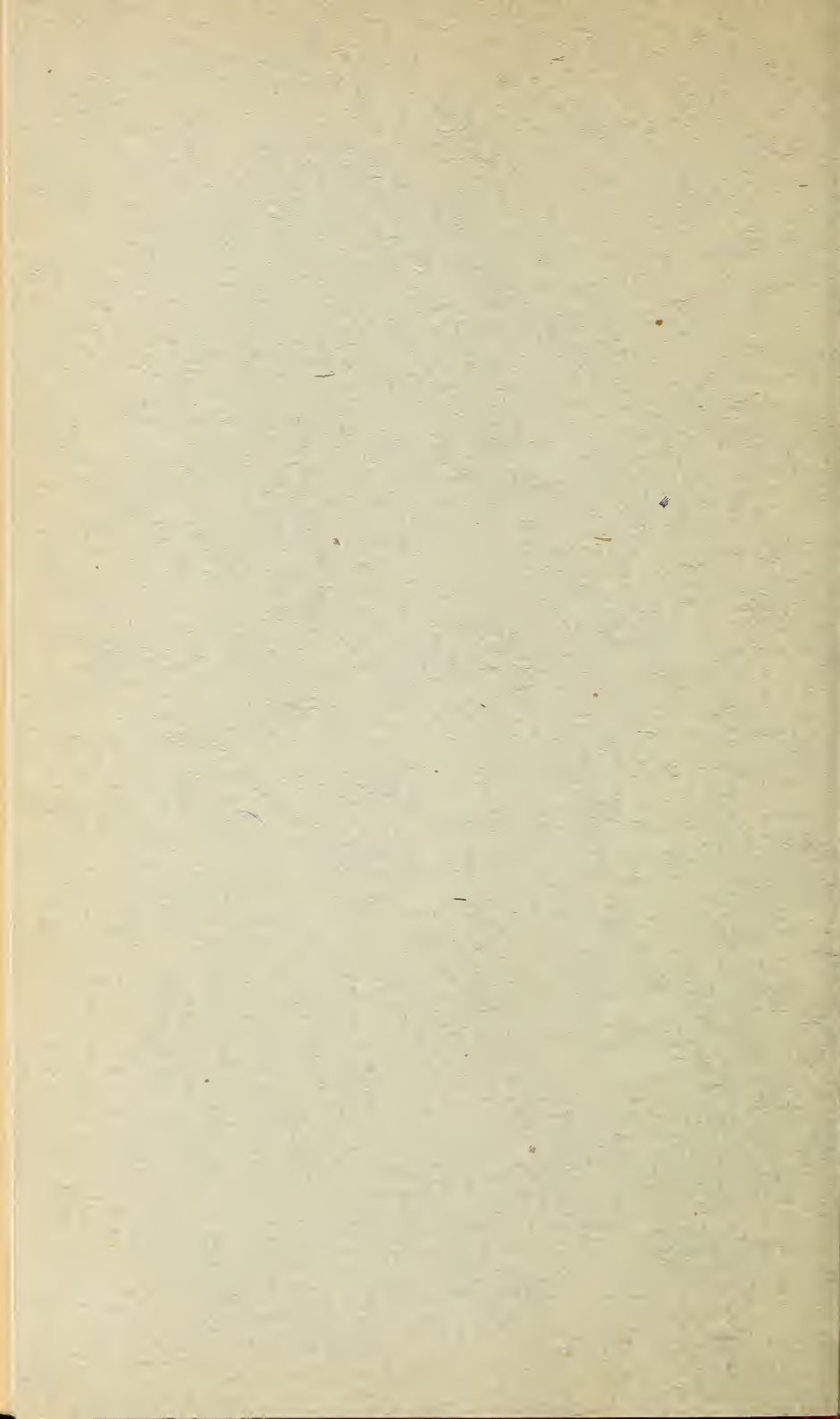
THE SEED COATS OF CERTAIN SPECIES  
OF THE GENUS BRASSICA.

BY

A. J. PIETERS AND VERA K. CHARLES.



WASHINGTON:  
GOVERNMENT PRINTING OFFICE.  
1901.



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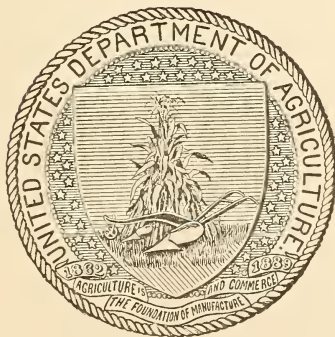
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## LETTER OF TRANSMITTAL.

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U. S. DEPARTMENT OF AGRICULTURE,

DIVISION OF BOTANY,

*Washington, D. C., June 1, 1901.*

SIR: I transmit herewith, and recommend for publication as Bulletin No. 29, of the Division of Botany, a manuscript by Mr. A. J. Pieters and Miss Vera K. Charles, entitled "The Seed Coats of Certain Species of the Genus Brassica." This genus of plants includes the cabbage, cauliflower, kale, brussels sprouts, turnip, ruta-baga, rape, kohlrabi, and various species of wild mustard. Heretofore it has been considered impossible to distinguish certain high-priced seeds of the group from some of the almost valueless ones, and this has sometimes been made the basis of frauds perpetrated either on the seedsmen themselves or on the public. The present investigation has resulted in the discovery of a method by which some of these seeds may be distinguished—for example, those of charlock and black mustard, which are common weeds, from those of the turnip, some varieties of which cost 50 cents to \$1 per pound, and turnip, the common sorts of which can be purchased at 10 to 20 cents per pound, from cauliflower, \$4 to \$30 per pound. The value of these results to the seed trade and to the public is evident.

Respectfully,

FREDERICK V. COVILLE,

*Botanist.*

Hon. JAMES WILSON,

*Secretary of Agriculture.*





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# THE SEED COATS OF CERTAIN SPECIES OF THE GENUS BRASSICA.

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By A. J. PIETERS and VERA K. CHARLES.<sup>1</sup>

---

## INTRODUCTION.

One of the troubles with which the seedsman has to contend is the complaint that he has sold turnip for cabbage or cauliflower seed, or the seed of a wild and weedy mustard for that of turnip or ruta-baga. The seedsman may have furnished the seeds in good faith, but he may have been deceived by the grower or by the wholesaler. It is, however, seldom possible to determine how far the trouble is due to carelessness on the part of the planter and in how far the seedsman is at fault. The seed bought has all been planted and the seedsman may insist that from other lots taken out of the same bag satisfactory results were obtained. Even when the purchaser has saved some seed, it is impossible without another season's test to determine what the seed really is. In external appearance the seeds of the different species and varieties of this genus are so similar that it is at all times a difficult matter to certainly identify a given lot, and it may be wholly impossible to do so.

The resemblance between the seeds of cheap and of high-priced Brassicas has always tempted certain seedsmen to mix more or less of the one with the other. This was at one time carried so far in England that factories were established where seeds of cheap Brassicas were heated to destroy their germination so that the fraud might escape detection. This practice was stopped by the adulteration of seeds bill which passed Parliament in 1869. While it is not difficult for an expert when examining a large sample of seed to pronounce it cabbage, rape, or mustard, it is impossible for him to pronounce upon mixtures, and even determinations of pure samples may be open to doubt. Fortunately we possess in the structure of the seed coat a means of identification by which most of the species studied may be readily distinguished. This method has been employed by various investigators, mainly for the purpose of determining the seeds used in the preparation of oil cakes, and the results have found their way into most pharmacopœias.

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<sup>1</sup>The laboratory work in histology was done by Miss Charles.

## PREVIOUS INVESTIGATIONS.

The first person, so far as we have been able to ascertain, to make practical use of the histology of the seed coats for the identification of the species of *Brassica* was Schröder, who in 1871 published the results of his work.<sup>1</sup> Previous to this the pharmacological works of Berg, Gaseke, and Fluckiger in 1865–1867 described the structure of *Brassica nigra* and of *Sinapis (Brassica) alba*, but the observations recorded were imperfect. Schröder's work was accepted by Nobbe and reproduced in the latter's *Handbuch der Samenkunde*. The drawings are in the main correct, but are imperfect in details and do not clearly bring out the essential differences between the species. Schröder studied the seeds of *B. napus* and *B. rapa*, *B. oleracea* and *B. nigra*, finding in them three types. He was unable to distinguish the seeds of *B. napus* from those of *B. rapa*.

In 1874 Sempolowski<sup>2</sup> published descriptions of some of the species included in Schröder's work and also of *Sinapis brassicata* and *S. alba*. He pointed out the error into which Schröder fell by interpreting the upper thin-walled portions of the supporting layer in *B. nigra* as being a separate cell layer irregularly distributed.

Hohnel's<sup>3</sup> work, which appeared in 1875, has not been seen by the writers of the present paper. It has been widely quoted in text books of pharmacy, and, judging from the quotations, contained not a few errors. Wittmack,<sup>4</sup> in 1877, and Kiaerskou,<sup>5</sup> in 1885, compared the structure of the testa of *Brassica glauca* with that of *B. rapa* and *B. napus*, and found no differences in any of these species. Kiaerskou's drawings of *B. ramosa* resemble ours of *B. juncea*. Harz, in *Landw. Samenkunde*, 1885, describes the seed coats of several species of *Brassica*. His drawings are very diagrammatic, and those of *B. napus* and *B. rapa* are nearly identical. He notes that in *B. napus* the lumen in the cells of the supporting layer is wider than the thickened common wall between two cells, while in *B. rapa* the opposite condition obtains. That this is incorrect we have shown below.

Many of the European investigators were led to this study from the necessity of determining what species of *Brassica* seeds entered into

<sup>1</sup>Schröder. Untersuchung der Samen der Brassica-Arten und Varietäten. Landw. Versuchs-Station, 1871, vol. 14.

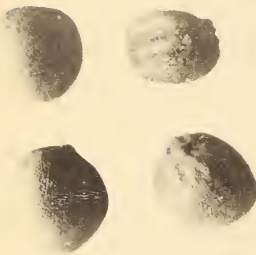
<sup>2</sup>Sempolowski, Anton. Über den Bau der Schale landwirthschaftlich Wichtiger Samen. Jahrb. wissenschaftliche Landw., Vol. III, p. 823 (1874).

<sup>3</sup>Hohnel, F. Bau der Samenschale der vier kultivierten Brassica-Arten. F. Haberlandt's wissenschaftl.-praktischen Untersuchungen auf dem Gebiete des Pflanzenbaues, Wien, 1875.

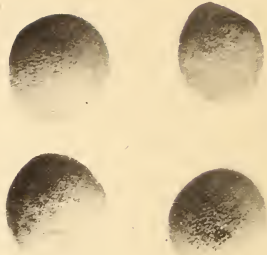
<sup>4</sup>Wittmack, L. Ueber die Unterschiede zwischen Raps-, Rüben-, Rüben- und Kohlsamen. Sitzungsberichte der Gesellschaft naturforschender Freunde in Berlin, 1887, No. 3, p. 83.

<sup>5</sup>Kiaerskou, H. Sur la structure du test de quelques sortes de Colza indien. Botanisk Tidsskrift, Copenhagen, 1884–85.

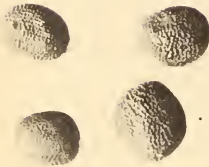




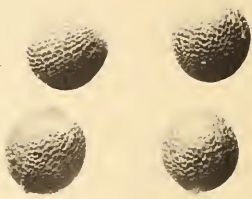
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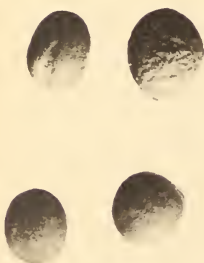
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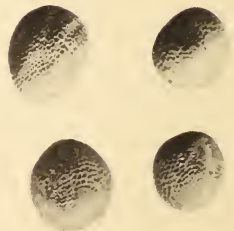
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5.



6.

SEEDS OF SIX SPECIES OF BRASSICA:

- 1, BRASSICA OLERACEA; 2, BRASSICA NAPUS; 3, BRASSICA NIGRA;  
4, BRASSICA JUNCEA; 5, BRASSICA RAPA; 6, BRASSICA ARVENSIS.

the composition of oil cakes. Among these were Steffek and Wittmack in 1887, Burchard in 1894 and 1896, and Kinzel in 1899. Burchard<sup>1</sup> found that the harmful effect that oil cakes sometimes exert on stock is due to the substitution of the seeds of other species of *Brassica* for those of *B. napus*. In this paper Burchard describes the structure of the seed coats of the seeds of thirteen species of *Brassica* and *Sinapis*. Burchard brings out the difference between the seed coats of seeds of *B. napus* and those of *B. rapa*, but his drawing of *B. juncea* does not clearly define the second cell layer. In a later paper<sup>2</sup> he gives descriptions of some additional eastern species and adds a key to such species as he had described.

Guignard<sup>3</sup> studied the development of the seed coat of *B. nigra* and found that the spaces just under the epidermis are really large cells. In the mature seeds these look like air spaces.

### EXTERNAL APPEARANCE AND GROSS ANATOMY OF SEEDS.

The seeds of the species of *Brassica* vary in shape from round in the wild species, as *B. arvensis*, to rounded-oblong in cabbage, while, with the exception of the white-seeded species, the color ranges from reddish brown to almost black. In general the seeds of the cultivated species are large and dark-colored and those of the wild species more nearly rounded, smaller, and reddish brown in color. This distinction has, however, little value as a means for detecting adulterations. When the conditions for seed production are unfavorable the seeds of cabbage, of cauliflower, or of turnips contain many small, poorly colored seeds that can not be separated from seeds of some wild species. It is even more difficult to detect the mixture of seeds of turnip or of rutabaga with those of varieties of cabbage and cauliflower.

The seed of *Brassica* is campylotropous, the hilum and the micropyle being near together. The large embryo fills the seed, the thick cotyledons being folded about the radicle in the manner known as conduplicate. The surface of the seed is more or less plainly reticulated. In some species as *B. nigra* and *B. arvensis*, the reticulations are large and prominent while in the cultivated species, especially in the varieties of *B. oleracea*, these markings become obscure so that they can not always be seen under an ordinary hand lens. The shape and marking of the seeds of six species is shown in Plate I, in which all the figures are magnified six times.

While characters drawn from external features, as size, form, color,

<sup>1</sup>Burchard, O. Über den Bau der Samenschale einiger Brassica- und Sinapis-Arten. Journ. für Landw., 42, p. 125.

<sup>2</sup>Burchard, O. Über den Bau der Samenschale einiger Brassica- und Sinapis-Arten. Journ. für Landw., 44, p. 337.

<sup>3</sup>Guignard, L. Recherches sur le développement de la graine et en particulier du tégument séminal. Jour. de Bot. 1893.

and markings, are useful for rough determinations or for giving direction to more careful research into the identity of the seeds in question, they can not be relied upon for exact determination. For critical work we must use the structure of the seed coat. In this bulletin the structure of the seed coat is described for seven species that are of economic importance in the United States. It will be seen that all the species are readily to be distinguished except that *B. campestris* is not to be separated from *B. rapa*.

In the Index Kewensis *B. rapa*, *B. napus*, and *B. campestris* are regarded as one species. We have not entered into the systematic position of these species, but call attention to the fact that *napus* and *rapa* are clearly distinguishable by the structure of the seed coat. Burchard has found the same differences as those we describe. A study of some of the varieties of *B. oleracea* was made, but no differences were found that could be regarded as satisfactory for a positive determination of the seed.

#### MATERIAL AND METHODS OF THE INVESTIGATION.

The material used in this study was the ordinary commercial seed, for the cultivated species; for the wild species, seeds of undoubted authenticity were selected. Several different methods were employed in order to determine the most satisfactory and expeditious way of obtaining clear sections. The paraffin and celloidin methods did not prove desirable because the firm tissue of the testa required a great deal of time for embedding. The tissue of seeds which were allowed to remain in celloidin for several weeks was not thoroughly penetrated by the latter. Free hand razor sections were the most satisfactory, as the seeds required no special preparation, but were best cut dry. This method of preparing sections could not be recommended for general use in studying the histology of seeds, but is valuable where a ready method is wanted for the identification of Brassica seeds. Seeds with extremely firm tissue, as those of *Trifolium* or *Medicago*, must either be placed between sheets of moist blotting paper or subjected to a preliminary soaking in a 2 per cent solution of formalin. In mucilaginous seeds, such as Brassica species, unnecessary dampness must be avoided. The razor sections were placed in a strong solution of potassium hydrate, in which they were allowed to remain about five minutes, when they were thoroughly washed, stained, dehydrated, and mounted in Canada balsam. Careful washing was found to be essential in order to produce a good stain and to prevent injury to the delicate tissues of sections destined for permanent mounts. Chloriodide of zinc was used to test for cellulose. Lignified walls were stained with fuchsin and washed in a solution of picric acid, and thus a permanent red stain was obtained. Permanent mounts were stained with hæmatoxylin, Bismark brown, fuchsin (aqueous solution), or aniline safranin. Hæma-



toxylin proved the most satisfactory permanent stain. Bismark brown did not differentiate tissues, but gave a clear stain and brought out folds, striations, and projections of the cell walls. The drawings and measurements were made before dehydration, as that process has a tendency to shrink tissues.

### LAYERS OF THE SEED COAT.

In the genus *Brassica* the seed coat consists of 5 or 6 layers. Of the species included in the present study, two—*B. juncea* and *B. oleracea*—have 6 layers; the others have 5. Where 6 layers occur the condition is due to the presence of a single layer of cells directly beneath the epidermis and between it and the supporting layer. The layers present in all species are, from without inward, epidermis, supporting layer, pigment layer, proteid layer, and endosperm. Of these the epidermis and the supporting layer furnish the best characters for identifying the seed, except in *B. oleracea*, in which the pigment layer is of importance. The other layers, except in *B. oleracea*, present few points of difference, and such as occur are not constant. The following descriptions apply to the mature seed, treated as above indicated. No attempt was made to interpret appearances in the light of the developmental history of the seed coat.

### HISTOLOGY OF SEED COATS BY SPECIES.

#### BRASSICA NIGRA.

(Black mustard.)

#### EPIDERMIS.

Seen in cross section the epidermis has a wavy outline, owing to the irregularity in height of the cells of the succeeding layer. The cells vary somewhat in shape and size. Viewed from above they are found to be mostly hexagonal, while in cross section they are rectangular in outline, 50 to 55 mic. mil. in length and from 35 to 40 mic. mil. in height. The cell wall is comparatively uniform in thickness and much thinner than the cell walls of the pigment or proteid layers. (Fig. 1. *a*.)

#### SUPPORTING LAYER.

Immediately below the epidermis is a layer known as the Stäbchenschicht or palisade tissue. This tissue has an extreme thickness of 60 to 66 mic. mil. The general form of the cells is cylindrical, and they vary in size from 35 to 66 mic. mil. The transition in height is gradual, the shortest cells being those midway between two groups of the tallest cells. At irregular intervals 3 to 7 cells attain a height of 60 to 63 mic. mil., and these longer cells support the epidermis.

According to Guignard's investigations there is a layer of large cells between the epidermis and the supporting layer. In the mature seeds these cells look like large air spaces (fig. 1) lying between the groups of longer cells of the supporting layer.

The walls of the cells of the supporting layer are thickened and lignified to a height varying according to the length of the cell. In

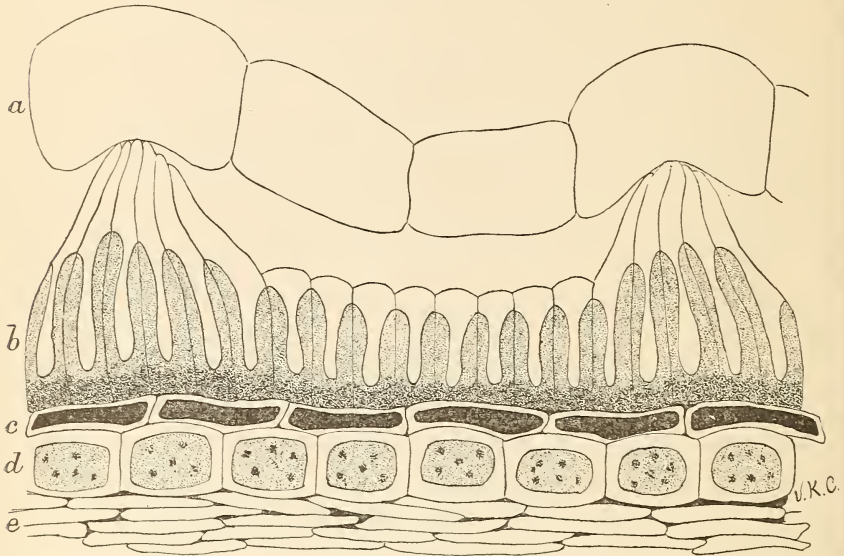


FIG. 1.—Section through the seed coat of *Brassica nigra*: a, epidermis; b, supporting layer; c, pigment layer; d, proteid layer; e, endosperm.  $\times 400$ .

no case does the thickening extend to the top of the cell, but from 5 to 10 mic. mil. of the upper portion of the wall remains thin. The lower cell wall is a solid mass of lignified matter 7 to 11 mic. mil. thick. (Fig. 1, b.)

#### PIGMENT LAYER.

The pigment layer consists of a single layer of cells, irregularly rectangular in cross section and filled with a dark pigment. This layer does not present any characteristic structures. (Fig. 1. c.)

#### PROTEID LAYER.

This layer consists of a single row of thick-walled, oblong cells containing proteid matter. In cross sections the lumen is oblong in outline and the granular contents are abundant; four or five of the grains generally exceed the others in size. (Fig. 1, d.)

#### ENDOSPERM.

This layer consists of 8 to 10 rows of colorless, thin-walled cells. In cross sections they are linear, with tapering apices, and measure

about 45 to 50 mic. mil. in length. Intercellular spaces are not prominent, owing to the crowded condition of the cells. (Fig. 1, *e*.)

*Brassica nigra* is one of the easiest species to identify. The epidermis responds quickly to the action of potassium hydrate, while the upper cellulose portion of the cells of the supporting layer to a great extent recovers its turgidity. The air spaces are most conspicuous in this species. According to Guignard<sup>1</sup> they represent the cells of the subepidermal layer.

### BRASSICA JUNCEA.

(Chinese mustard. Indian mustard.)

#### EPIDERMIS.

The cells of this layer are thin-walled, in cross sections appearing oblong, and are about 48 to 50 mic. mil. long, and 35 to 36 mic. mil. high. (Fig. 2, *a*.) Immediately beneath the epidermis is a layer of

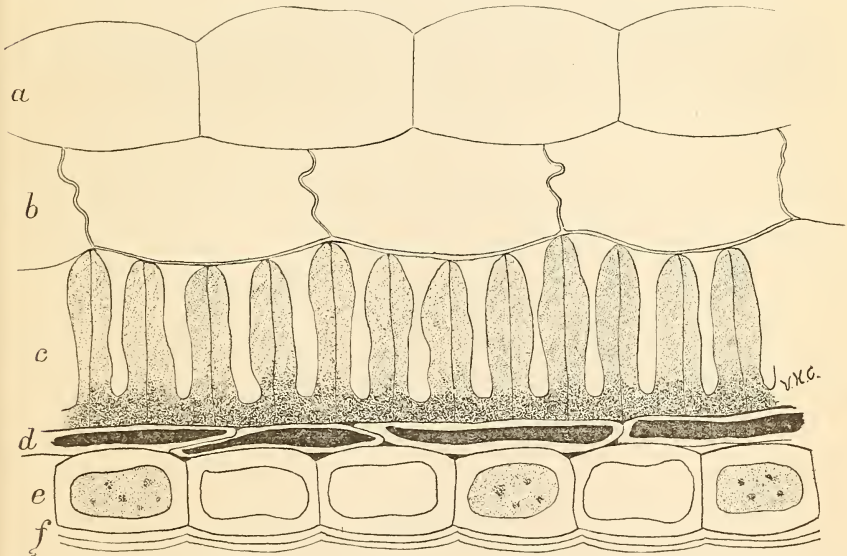


FIG. 2.—Section through the seed coat of *Brassica juncea*: *a*, epidermis; *b*, subepidermal layer; *c*, supporting layer; *d*, pigment layer; *e*, proteid layer; *f*, endosperm.  $\times 475$ .

cells that can not be seen until ten or fifteen minutes after the application of caustic potash, and even then the entire outline of the cells is not visible, only the radial and lower walls being distinguishable. The radial walls, which resemble a thin, twisted membrane, owing to the partially collapsed condition of the cells, start immediately above the summit of the intercellular walls of the tallest cells of the next

<sup>1</sup>Léon Guignard, *Récherches sur le développement de la graine et en particulier du tégument séminal*. Journ. Bot., 1893, p. 28.

layer. (Fig. 2, *b*.) In cross section the cells have a length of about 55 to 58 mic. mil. This second layer was very evident in seeds which had been preserved in alcohol, but in mature seeds the cells are easily confounded with air spaces.

#### SUPPORTING LAYER.

The extreme thickness of this layer is 40 mic. mil. The cells have the characteristic cylindrical form of the cells of the supporting layer in the genus *Brassica*. The thickening of the walls extends to the top of the cell, but varies in degree. Generally the wall is thinnest at the top and thickest at the center of the cell; however, this is not constant. The cells of this layer are closely applied to one another, and about two-thirds of the cell cavity is filled up with the thickening, which is a dark brown lignification. In cross sections the radial cell walls are almost invisible, and the thickening on the adjacent walls is linear, with a slightly wavy, entire marginal line. (Fig. 2, *c*.)

#### PIGMENT LAYER.

As a rule the cells of this layer are a little longer than, but not as broad as, the corresponding cells of *Brassica nigra*. (Fig. 2, *d*.)

#### PROTEID LAYER.

The mature cells of this layer are very apt to be empty and the lumen more or less constricted near the center. Though this is a help in distinguishing from *Brassica nigra*, it is not a constant condition. (Fig. 2, *e*.)

#### ENDOSPERM.

The individual cells of this layer can not be distinguished. The whole tissue appears as numerous wavy parallel lines. (Fig. 2, *f*.)

### BRASSICA ARVENSIS.

(Charlock.)

#### EPIDERMIS.

In cross section the epidermis appears to rest lightly upon the cells of the next layer. It consists of one row of thin-walled cells, oblong in shape, measuring from 38 to 40 mic. mil. in length, and from 22 to 25 mic. mil. in height. It was observed that the epidermal cells of seeds grown in different localities vary in size, some cells not exceeding 15 mic. mil. in height. (Fig. 3, *a*.)

#### SUPPORTING LAYER.

The cells of this layer are cylindrical in shape and of uniform height, usually about 36 to 40 mic. mil., and having a diameter of 14 mic. mil.

In this species the thickening of the wall extends to the apex of the cell, and the lower wall is thickened to a depth of about 6 to 8 mic. mil. In transverse sections the lumen appears to be inversely club-shaped. The radial cell walls can generally be distinguished. (Fig. 3, *b*.)

#### PIGMENT LAYER.

This consists of a single row of cells. In cross sections they appear elongated with narrow, pointed ends. (Fig. 3, *c*.)

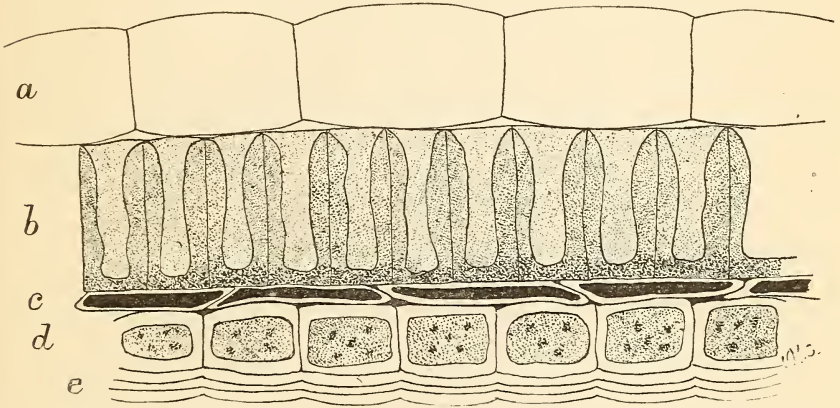


FIG. 3.—Section through the seed coat of *Brassica arvensis*: *a*, epidermis; *b*, supporting layer; *c*, pigment layer; *d*, proteid layer; *e*, epidermis.  $\times 575$ .

#### PROTEID LAYER.

Presents no distinctive features. (Fig. 3, *d*.)

#### ENDOSPERM.

The individual cells can not be distinguished. (Fig. 3, *e*.)

### BRASSICA NAPUS.

(Rape.)

#### EPIDERMIS.

The epidermis of *Brassica napus* is similar to that of *B. rapa* in resembling a compressed, yellowish membrane, but differs from it in the upper surface being entire and only faintly wavy. The differences in the epidermis of these two species are only apparent after the continued action of potassium hydrate. (Fig. 4, *a*.)

#### SUPPORTING LAYER.

The features presented by this layer are the principal means for distinguishing *B. napus* and *B. rapa*. The cells in *B. napus* are more regular in size than those of *B. rapa*, varying not more than 3 mic.

mil. in height, while those of *B. rapa* vary from 5 to 7 mic. mil. The thickening of the marginal line is quite apparent after the section has been allowed to remain in weak glycerin a few hours. The solution must be weak or severe dehydration will result. (Fig. 4, *b*.)

#### PIGMENT LAYER.

This layer is made up of one row of rather thick-walled cells. The pigment possesses the characteristic brownish red color. (Fig. 4, *c*.)

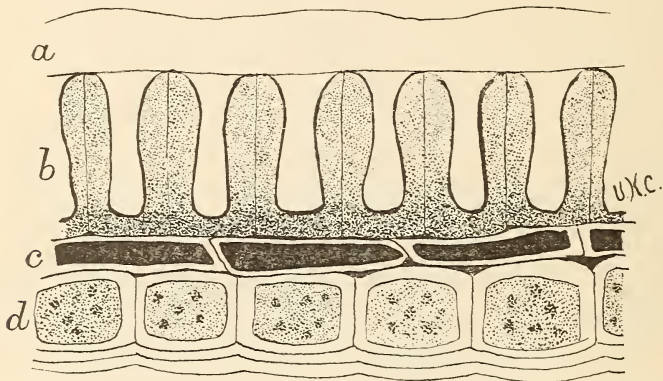


FIG. 4.—Section through the seed coat of *Brassica napus*: *a*, epidermis; *b*, supporting layer; *c*, pigment layer; *d*, proteid layer; *e*, endosperm.  $\times 450$ .

#### PROTEID LAYER.

This consists of a single row of comparatively regular, oval cells. (Fig. 4, *d*.)

#### ENDOSPERM.

As in *Brassica juncea*. (Fig. 4, *e*.)

### BRASSICA RAPA.

(Turnip.)

The structure of the seed coat of *Brassica rapa* and *B. napus* is very similar. There are, however, differences which afford a reliable means of identification. The seed coats of *B. rapa* can not be distinguished from those of *B. campestris* (Ruta-baga or Swedish turnip). This species is also regarded by Index Kewensis as identical with *B. napus* and *B. rapa*.

#### EPIDERMIS.

In mature and dry seeds of *Brassica rapa* the epidermis is so much compressed that all evidence of cells is obliterated. It appears, therefore, as a folded, yellowish membrane, closely adhering to the next layer. The upper surface is strongly wavy in outline and faintly

crenate. The lower line, representing the lower part of the epidermis, is slightly wavy, owing to the irregularity in height of the cells of the succeeding layer. (Fig. 5, *a*.)

#### SUPPORTING LAYER.

The cylindrical cells of this layer are not of equal height, but range from 43 to 50 mic. mil. In cross section the thickening on the walls of two adjacent cells presents a somewhat lanceolate outline, and shows a faintly sinuate marginal line. (Fig. 5, *b*.)

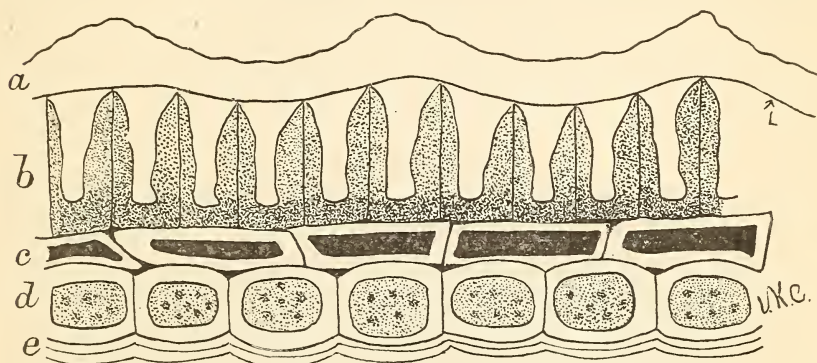


FIG. 5.—Section through the seed coat of *Brassica rapa*: *a*, epidermis; *b*, supporting layer; *c*, pigment layer; *d*, proteid layer; *e*, endosperm.  $\times 450$ .

#### PIGMENT AND PROTEID LAYERS.

These layers present no distinctive characters. (Fig. 5, *c* and *d*.)

#### ENDOSPERM.

As in *Brassica juncea*. (Fig. 5, *e*.)

#### BRASSICA CAMPESTRIS.

(Ruta-baga, Swede, Swedish turnip.)

The seeds of this species can not be distinguished from those of *B. rapa*.

#### BRASSICA OLERACEA.

(Cabbage, Cauliflower, Kale, Kohl-rabi, Broccoli, Brussels sprouts, Collards.)

#### EPIDERMIS.

At maturity the epidermis of *Brassica oleracea* consists of a single row of distinct, colorless, thin-walled cells (fig. 6, *a*) above a greatly collapsed, yellowish, membrane-like structure measuring about 5 to 6 mic. mil. in thickness (fig. 6, *b*). The latter is the remains of a row of cells lying directly beneath the epidermis. Sempolowski found these cells in the young seeds, and figures them as tangentially elongated thin-walled cells. In mature dry seeds these cells are obliterated. The

epidermal cells are easily separated from the underlying membrane, and in cross section appear somewhat inflated, averaging about 40 to 43 mic. mil. in length and from 20 to 25 mic. mil. in height. This species affords one of the best examples for the study of mucilage as it occurs in Cruciferae. Mucilage is contained only in the epidermal cells, and swells greatly on the addition of water. In cross sections of mature seeds there appears in the center of many of the cells a triangular structure which sometimes extends to the top of the cell, but generally terminates about one-third of the way from the apex of the cell. To this structure is due the mucilaginous character of the seed. It has the

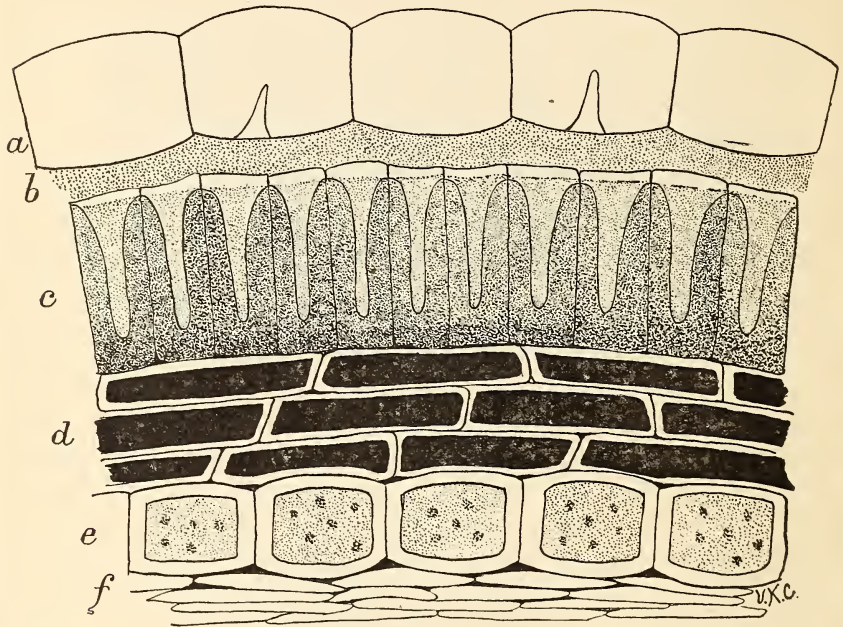


FIG. 6.—Section through the seed coat of *Brassica oleracea*: a, epidermis; b, subepidermal layer; c, supporting layer; d, pigment layer; e, proteid layer; f, endosperm.  $\times 550$ .

appearance of a conical thickening rising from the center of the lower cell wall (fig. 6, a). In surface view the epidermal cells are hexagonal, and the mucilaginous formation can be seen darker in color than the rest of the cell contents, circular in outline, with a diameter of about 20 mic. mil., and showing in the center a small refractive portion, according to Popovici the reduced cell lumen. Several concentric striæ are visible after the application of 95 per cent alcohol. So much can be seen by staining, varying the focus, and isolating a single cell and removing the thickened portion. This formation occurs in other species of *Brassica*, and it has been found that the larger the structure the greater the amount of mucilage in the seed.



## SUPPORTING LAYER.

This layer is uniform in height and in the thickening on the cell walls. About 5 mic. mil. of the upper part of the wall remains thin. In cross sections the thickening of the radial walls is broadly linear with entire, regular, marginal line. (Fig. 6, *c*.)

## PIGMENT LAYER.

This layer is of great value in distinguishing *Brassica oleracea*, and has an extreme thickness of 28 to 30 mic. mil. It differs from the pigment layers of other species in being composed of three or four rows of cells. These cells are apt to vary in size and shape, but in the ordinary commercial seed they generally appear rectangular in cross sections and are filled with a dark-red pigment. (Fig. 6, *d*.)

## PROTEID LAYER.

The cells of this layer present no constant distinctive differences. In cross sections the lumina generally appear rectangular, but this apparent difference is largely due to the age and quality of the seed, and is not a satisfactory means for identifying this species. (Fig. 6, *e*.)

## ENDOSPERM.

The cells composing this layer are colorless, and are abundant and crowded. In cross section they appear linear in form with slightly tapering apices, and in size measure from 30 to 40 mic. mil. in length. (Fig. 6, *f*.)

**KEY FOR DETERMINING THE SEEDS OF THE SPECIES STUDIED.**

- A. Seeds large, 2 mm. or more in diameter.
1. Seed coat of 5 layers. Proteid layer 1 cell row in thickness. *Brassica napus*.
  2. Seed coat of 6 layers. Proteid layer more than 1 cell row in thickness. *B. oleracea*.
- B. Seeds small, less than 2 mm. in diameter.
1. Reticulations on the surface conspicuous.
    - (a) Seed coat of 5 layers. Conspicuous air spaces between groups of long cells in the supporting layer. *B. nigra*.
    - (b) Seed coat of 6 layers. Cells of the supporting layer varying little in height. *B. juncea*.
  2. Reticulations usually inconspicuous.
    - (a) Epidermis wavy; cells obliterated. Cells of the supporting layer varying in height. *B. rapa*; *B. campestris*.
    - (b) Epidermis not wavy; cells evident. Cells of the supporting layer of uniform height. *B. arvensis*.

Williams