A HISTOLOGICAL ANALYSIS OF SHATTER AND SHATTER-RESISTANT TRAITS IN THE CASTOR BEAN (*RICINUS COMMUNIS L.*)

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PREFACE

During the spring of 1951, crossings were made between castor bean varieties with contrasting types of seed release to determine the method of inheritance of shattering. Segregation in the F_2 progenies was highly variable, and the relationship to genetic principles was indistinct. During the progress of this research, it was felt that a knowledge of the anatomy of the tissues responsible for seed release might aid in interpreting the expression of this phenomenon. With this in mind, the writer set out to determine the anatomy of structures causing seed loss before harvesting, and a possible method of inheritance of the structures. This thesis is the result of this study.

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CHAPTER I

INTRODUCTION

All fruits on individual castor bean plants do not mature at the same time. Further, in the common commercially grown varieties, the plants have a tendency to release their seed as soon as mature. This has frequently been observed to result in a loss to the grower of thirty percent of his crop. This fact plus the increased success of mechanical harvesting has stimulated greater interest in breeding for varieties which retain their fruits and seeds until the entire crop has matured.

This study of the castor bean plant was designed to trace by histological techniques the causes for seed loss by shattering. Seed loss resulted from the opening of capsules at maturity due to unlike structure and orientation of cells in the locules. Also the whole capsule may drop due to abscission. Both capsule opening and capsule dropping are influenced by moisture fluctuations.

CHAPTER II

REVIEW OF LITERATURE

The morphology and anatomy of the floral organs of the castor bean plant are described thoroughly by Ferry (1936). The tri-carpellate inflorescence or flower subtended by five bracts "is a highly reduced structure possibly derived from a primitive cyathium in which the gynoecium, except for the stigmatic tips, is buried within the walls of the cyathial receptacle." The distribution pattern of the major vascular elements in the capsule, such as the "central axis, the ovulary trace, the dorsal bundle, and the ventral bundles" are of importance in capsule growth and form a large part of the columella and its terminations identifiable in the mature, dehiscent capsule.

The amount of vascular tissue in castor bean plants varies directly with the concentrations of light intensity and soil moisture (Penfound, 1932).

The environmental factors of a sandy desert were noted to decrease the expression of seed loss by the castor bean (Zimmerman, 1951).

Capsule dehiscence and other traits in the castor bean were observed and investigated by means of genetic experiments by White (1918). The primary determiners of dehiscence were dominant factors segregating in the 9:7 ratio for two genes, thick leathery capsules being attributed to one gene, and capsule opening to the other gene. The genes for

capsule dehiscence were dominant in all of the F_1 progenies. In the F_2 progenies many indehiscent capsules would, "under favorable conditions," open slightly. These were thin-walled, brittle capsules showing that the characters for capsule tissues modify the dehiscent characteristics. The dehiscent characteristic is probably due to the presence of cells which rapidly lose their moisture content when the capsule matures and contracts, thus breaking apart the capsule and expelling the seed.

Zimmerman (1951) substantiated White's findings for the dominance of capsule dehiscence over indehiscence in the castor bean by observing segregation near the 9:7 ratio for two genes. Zimmerman attempted to further identify the genic composition of the plants by counting the number of capsules exhibiting dehiscence on each plant.

A dehiscence ratio near 3:1, suggesting the action of one gene pair, was obtained in the F_2 population by crossing a "mildly dehiscent" castor bean plant by an indehiscent plant (Zimmerman, 1952).

In contrast to previous results, Van Horn (1952) obtained a 9:7 ratio in the F_2 generation of a crossing between an indehiscent and a dehiscent capsule type of castor bean plant.

In further genetic crossings in the castor bean for the dehiscent character, it appeared dominant in F_2 populations with the following numerical ratios as reported by Parkey (1953):

3:1 in three crossings

9:7 in one crossing

15:1 in one crossing

92:0 in one crossing.

According to Zimmerman (1951) resistance to capsule dropping is distinct from resistance to capsule dehiscence. In a castor bean line

exhibiting the least amount of capsule droppage, he observed that the pedicel was attached flat against the capsule base. This construction is recessive to the formation of a swollen area on the pedicel about 1 mm. below the capsule.

Capsule droppage in the castor bean was dominant in the F_1 generation and segregated in a 9:7 ratio in the F_2 generation (Van Horn, 1952). The presence of an abscission zone at the capsule-pedicel attachment area was reported to segregate at a 3:1 ratio in the F_2 generation.

In two crossings of castor bean lines selected to exhibit capsule droppage the trait was governed by a single dominant factor (Parkey, 1953).

Capsule droppage by some castor bean plants was found to involve the phenomenon of abscission, which has been reinvestigated by Gawadi and Avery (1950). Three types of abscission behavior were observed as follows:

1. Secondary cell divisions at the base of the petiole formed a layer before the leaf dropped.

2. Dropping of the leaf occurred without any abscission layers having been formed.

3. In leaves that did not absciss, a layer of cells formed, but the leaf shriveled, remained attached, finally breaking off at or beyond the layer.

The three types of abscission described by Gawadi and Avery (1950) are essentially those presented by Esau (1953). The presence of surface grooves in the petiole does not necessarily coincide with the location of the abscission zone. The types of separation for fruits and seeds are very similar to those of leaf abscission whether separation results from differentiated cell types, cell collapse, cell shrinkage, or cell breakage.

In castor bean plants the arrangement of a separation tissue in the pedicel of flowers is completely established before the sexual organs are mature (Hilpert, 1939). The zone of separation can be distinguished chemically by means of starch and protein reagents. The flowers are later cast off by means of a turgor mechanism. The cellular junction is dissolved by the production of acid. The zone of separation differs for the male flowers and the female fruits; the former occurs in the stele and the latter in the cortex. After the male flowers are cast off, small groups of bast fibers are formed in the cortex, and concentric vascular bundles develop in the stele of the fruit-bearing pedicel.

A common characteristic of cells of the separation layer was that the cell walls were chemically changed during leaf abscission in such a manner that cell separation was easily accomplished. The cell walls or portions of the wall tended to increase in volume, swell, and assume a gelatinous appearance. Microchemical analyses revealed that changes involved conversions of calcium pectate into pectic acid and of the latter into water soluble pectin, with the cellulose remaining but assuming a gelatinous consistency. The cells may separate along the middle lamella without breakage, or the walls themselves may break (Esau, 1953).

There are extremes of conditions, both environmental and developmental, encountered during the growing season which lead to abscission (Gawadi and Avery, 1950).

All the developmental factors in plants determining the formation of the abscission zone and abscission itself are not known. However, the application of growth substances has been found to retard abscission (Esau, 1953).

The dropping of seed as a result of the formation of a callus layer accompanied by abscission just below the seed has been reported in American sorghum by Karper and Quinby (1947). Since two dominant genes caused callus formation, the breeding of a nonshattering plant was readily accomplished.

In grain sorghum, according to Karper and Stephens (1936), the effects of certain environmental influences resulted in the variation of heritable expression. This relationship was observed in six different tissue expressions for fruit formation.

CHAPTER III

MATERIAL AND METHODS

In studying seed loss or seed retention in the castor bean, capsules and pedicels from the following groups of plants were used:

10 Parental lines

3 Exotic lines

10 F_1 generations

7 F_2 generations.

Morphological characteristics of the parental and exotic lines are given in Table I. These thirteen lines include five local commercial varieties USDA 74, USDA 101, N-149-4, Cimarron, R-217-3, and eight other lines to show contrasting characters.

Immature fruiting material at various stages of development have been collected and preserved in formalin-acetic acid-alcohol fixative. Mature fruits have been retained in the dry condition. Tissues embedded in wax were cut into cross or longitudinal sections with a rotary microtome. Some sections were stained with safranin and fast green, others by Fosters' tannic acid - ferric chloride method (Johansen, 1940).

These histological sections were used in making morphological identifications, anatomical measurements and in the preparation of photomicrographs. From this information tables were prepared giving the structural composition of the capsule-pedicel attachment area and

TABLE I

GROWTH HABIT CHARACTERISTICS OF TEN PARENTAL AND THREE EXOTIC CASTOR BEAN LINES

Variety	Stem Color	Spines	Stem Bloom	Floral Node	Pedicel Bend	Pedicel Length	Pedicel Strength	Pedicel Abscission Zone	Capsule Droppage	Type of Dehiscence
D.P. 439	Red	-	+	5 - 9	+	Long	Weak	+	+	None
D.P. 443	Green	+	-	10 - 13	+	Long	Strong	+	-	Dehiscent
D.P. 438	Red	+	+	5 - 9	+	Long	Strong	+	-	Dehiscent
R-15	Red	-	-	6 - 9	+	Long	Weak	+	+	Semidehiscent
R-60	Green	+	+	7 - 9	+	Long	Weak	+	+	Semidehiscent
USDA 74	Green	+	-	6 - 8	+	Long	Weak	+	+	Semidehiscent
USDA 101	Red	-	-	7 - 9	+	Long	Weak	+	+	None
N-149-4	Green	+	-	6 - 8	+	Short	Strong	+	-	None
Cimarron	Green	+	+	10 - 12	+	Short	Strong	+	-	None
R-217-3	Red	+	-	5 - 6	+	Short	Strong	+	-	None
R-9/19	Red	-	+	7 - 9	+	Medium	Strong	-	-	None
R-13	Red	-	+	6 - 7	-	Long	Strong	-	-	None
R-12	Red	-	+	7 - 9	-	Long	Strong	-	-	None

of the capsules. The measurements of the capsule-pedicel attachment area forms a numerical basis for the comparison of the various structural types presented in the Results and Discussion.

CHAPTER IV

RESULTS AND DISCUSSION

Loss of seed by the castor bean plant were found to be the function of two different complexes of cellular interactions. Losses of seed accredited to capsule droppage are determined by the structure and orientation of the cells in the capsule-pedicel attachment area and are presented in Section A. A second aggregation of cellular interactions, which is located in the capsule, is responsible for capsule dehiscence and is presented in Section B.

Section A - Capsule Droppage

Capsule Droppage and Pedicel Structure in the Parental Lines

Capsule droppage is termed the separation in the apical region of the pedicel from the basal end of the capsule with the subsequent falling of the capsule.

The dropping of the fruit may occur soon after capsule maturity or may be delayed until after a killing frost or after several weeks of weathering in the field, depending on the genetic lines of the plants under consideration.

Capsule droppage in the castor bean plant depends upon the presence of a functional weakness in the pedicel, as shown by the ten parental varieties under study. The presence of a general weakness has been demonstrated on mature fruits by Emmett W. McCord* by a finger-flip test

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* verbal communication

in gross surveys. The test is performed by the lifting of the capsule apex to a position vertical to the inflorescence stalk and then releasing. Results from the testing of succulent capsules were compared with capsule droppage observed in mature, dry capsules. Many of the pedicels break just below the base of the floral bract in castor bean varieties, R-15, R-60, USDA 74, Cimarron, and N-149-4. Other pedicels break above the base of the floral bract in the base of the capsule. The pedicels of variety USDA 101 break easily; while, in the varieties, R-9/19, R-13, and R-12 the pedicels require repeated flippings for separation. Histological examinations indicated five causes for the localization of pedicel breakages, as follows:

1. Chemical dissolution occurred in the cell wall.
2. A sizable indented region existed in the pedicel.
3. Cork was formed in the pedicel.
4. A layer of small-celled parenchyma was present.
5. A thinness of the cell walls of the capsule columella was found above its basal extremity.

The five causes represented in the types of pedicel structure and breakage were as follows:

(a) Dissolution in the cell wall resulted in capsule droppage.

(b) Tissue indentation and cork formation gave capsule droppage.

(c) Tissue indentation, cork formation, and small-celled parenchyma produced capsule droppage.

(d) Tissue indentation, and small-celled parenchyma exhibited themselves by delayed capsule droppage.

(e) A thinness of the capsule columella above its basal extremity occurred with capsule non-droppage.

Spontaneous capsule droppage is exhibited by varieties, USDA 101, R-15, USDA 74, and R-60. In the USDA 101 variety chemical changes in

the cell wall produce a breakdown of the middle lamella and a softening of the cell walls resulting in their collapse (Figure 1).

In the varieties R-15, USDA 74, and R-60, there are special morphological structures which weaken the pedicels. The capsule-pedicel attachment area may be deeply indented in varieties R-15 (Figure 2) and USDA 74, moderately indented in variety R-60, or have no indentation as in variety USDA 101. The pedicel area adjacent to the indented region may be infiltrated with a corky layer of cells as in variety R-15 (Figure 3) or with corky cells and small-celled parenchyma in USDA 74 and R-60, or without a horizontal layer of specialized cells as in USDA 101.

Thus, one specific morphological arrangement of the capsule-pedicel attachment area does not necessarily determine capsule droppage; although, the presence and percentage of the components are related to it.

Six parental varieties R-15, USDA 74, R-60, Cimarron, N-149-4, and R-217-3 are classified as capsule dropping types. Varieties N-149-4, Cimarron, and R-217-3 had a delayed response to capsule droppage.

Variety N-149-4 exhibits capsule droppage when harvesting is delayed for a few weeks after frost. Cimarron variety shows capsule droppage when left standing in the field for several weeks after frost. Although capsule droppage has not been reported in variety R-217-3, its pedicel structure is similar to Cimarron.

In these three varieties, Cimarron, N-149-4, and R-217-3, the subcapsular pedicel area has a definite indented region. In variety N-149-4, an area of small parenchyma and corky cells was noted laid adjacently inward from the indentation; while, in the Cimarron variety

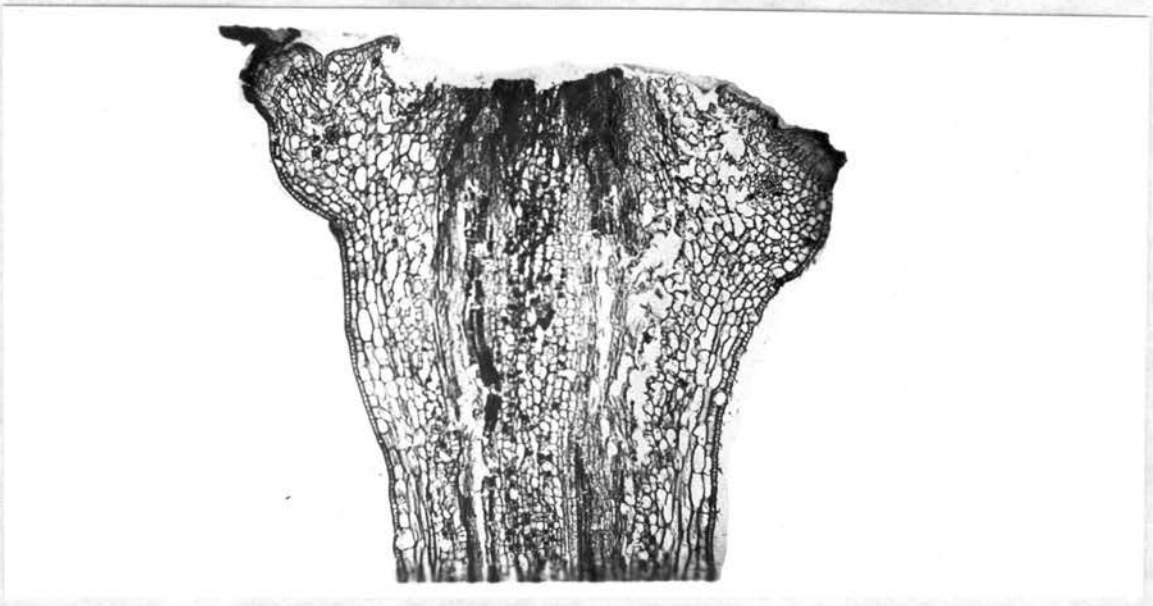

Figure 1. Longitudinal Section of the Apex of a Succulent Mature Pedicel Showing Dissolution and Collapse of Cells, Especially at the Capsule Separation Area in Castor Bean Variety USDA 101.

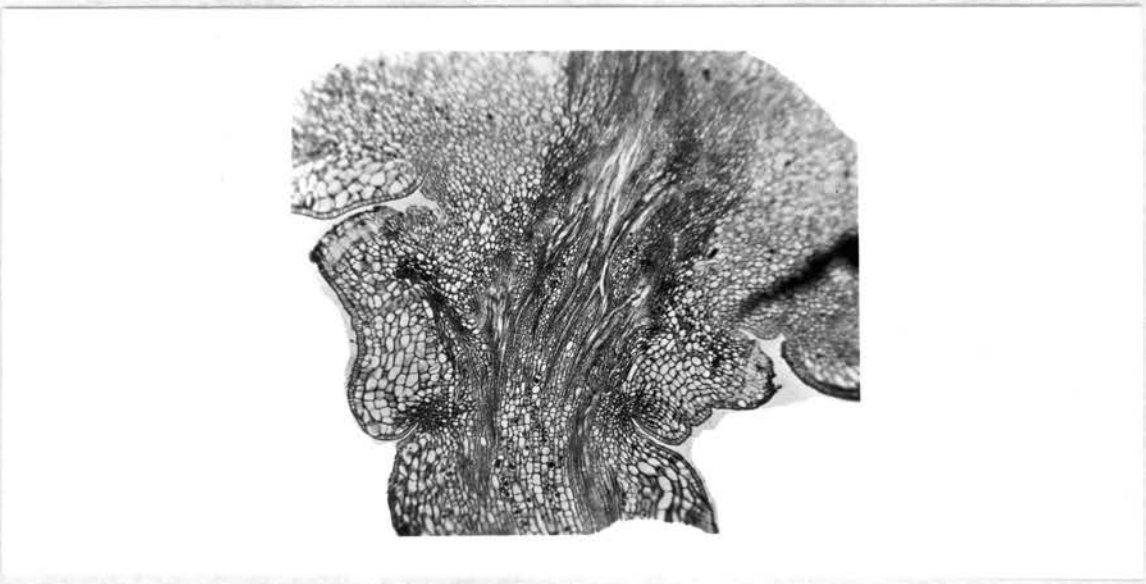

Figure 2. Longitudinal Section of the Succulent Mature Capsule-Pedicel Attachment Area Showing Indentation and Corky Cells of Castor Bean Variety R-15.

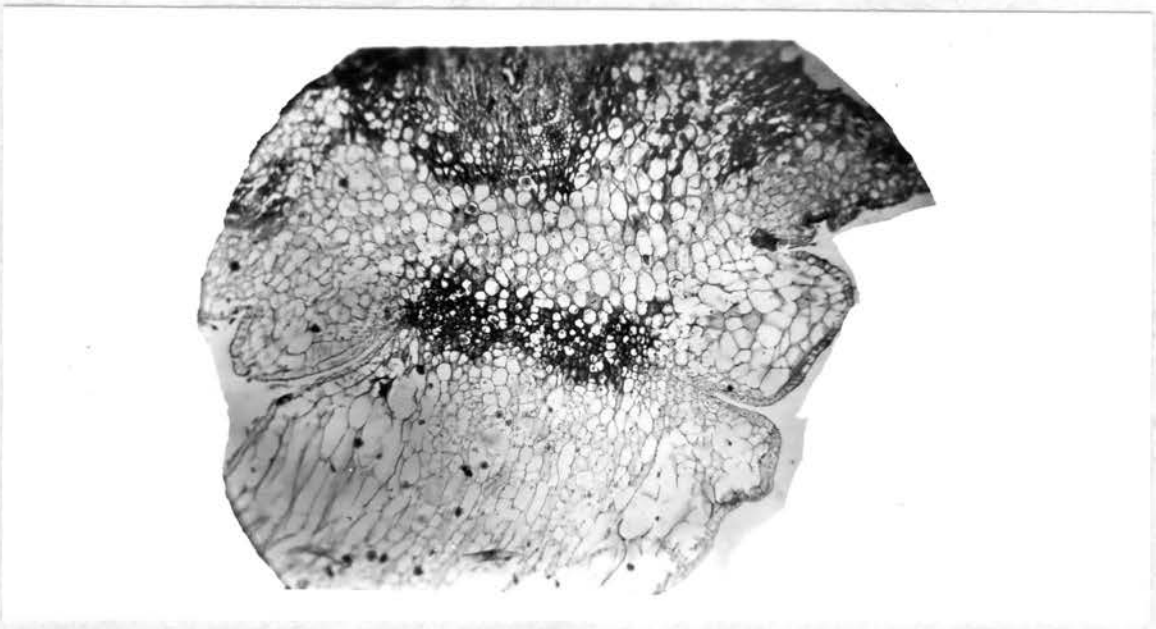

Figure 3. Darkly stained Cork-like Cells of the Pedicel Separation Area in Castor Bean Variety R-15.

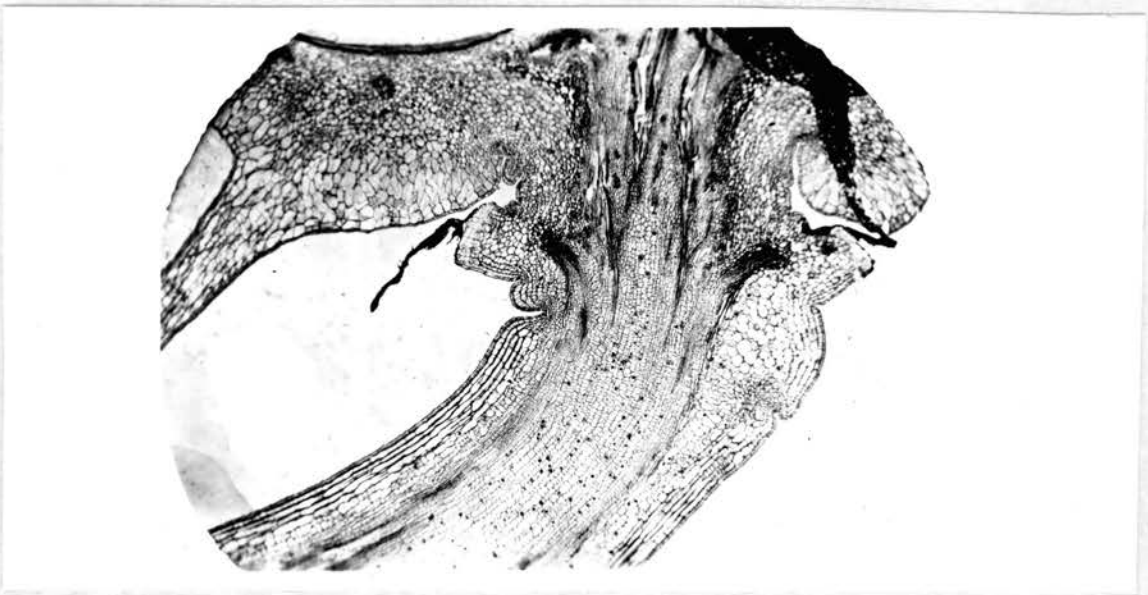

Figure 4. Longitudinal Section of the Succulent Mature Capsule-Pedicel Attachment Area of Cimarron Variety of Castor Bean Showing the Indented Region and Small Parenchyma Cells.

a large area of only small parenchyma cells occurred (Figure 4). These two traits, indentation and horizontal layering of small cells, lead to pedicel fracture from uneven stresses during the swelling and shrinking effects due to moisture fluctuations in weathering.

The three varieties, R-9/19, R-12, and R-13 (Figure 5) do not exhibit spontaneous capsule droppage. These varieties are devoid of any pedicel indentation below the floral bracts. They have neither cork-cell formation nor a horizontal area of small-celled parenchyma in the pedicel. The absence of any chemical dissolution, physical configuration, and tissue specialization for abscission at the subcapsular-pedicel area can be related to the lack of capsule droppage in these three varieties. Also, these varieties possess a capsule columella of thin-walled cells above its basal extremity.

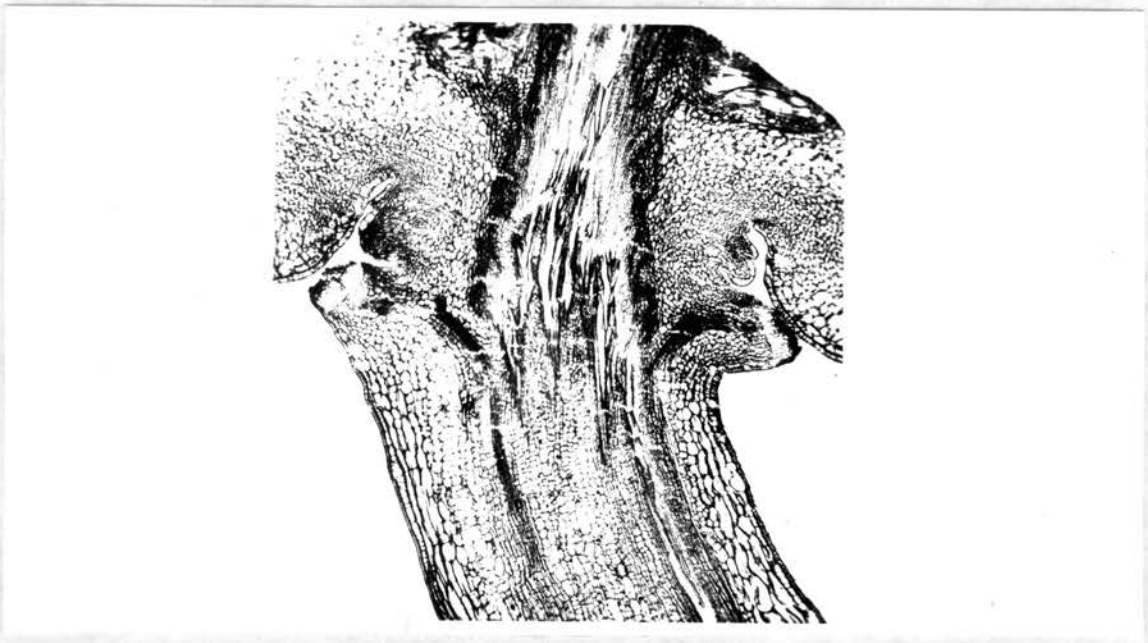

Figure 5. Longitudinal Section of the Succulent Mature Capsule-Pedicel Attachment Area Showing the Absence of Pedicel Weakening Structure in Castor Bean Variety R-13.

Thus, pedicel breakage with capsule droppage may be from one or a combination of causes. These include cell wall dissolution, presence, amount, combination of anatomical components, and width of actual supporting tissues at the point of breakage.

An Index For Capsule Droppage Based on the Tissue Composition of the Capsule-Pedicel Attachment Area

In making a quantitative analysis between capsule droppage and the composition of the capsule-pedicel attachment area, the tissue components of ten parental and two exotic castor bean lines were measured. The measurements were made across longitudinal sections showing the maximum content of tissue types and were converted into diameter percentages for use in varietal comparisons as shown in Table II.

Variety USDA 101, which shows drastic chemical changes in its mature cell walls of the pedicel apex, is omitted from the following considerations since the other capsule-dropping types are based on morphology.

A general index of capsule droppage is derived from a composite percentage of the pedicel indentation, corky cells, and small-celled parenchyma. These three components are the weakening factors replacing large-celled cortical parenchyma.

Capsule droppage occurs when the sum of the three weakening factors comprises from 40 to 60 percent of the pedicel diameter.

A value from 30 to 40 percent indicates probable capsule loss if harvest is delayed for a few weeks after frost.

Capsule non-dropping varieties have index values from 0 to 30 percent. However, as the factors and their combinations in each variety differ, the index values may vary 3 percent from the actual reading.

The most prominent feature of spontaneous capsule-dropping pedicels is the presence of the circling indentation averaging 27 percent of the diameter. Furthermore, the indentation is accompanied by

TABLE II

DIAMETER PERCENTAGE OF VARIOUS TISSUE TYPES IN THE CAPSULE-PEDICEL ATTACHMENT AREA FOR TEN PARENTAL AND TWO EXOTIC CASTOR BEAN LINES

Variety	Capsule Droppage*	PERCENT									
		Indentation	Cork	Small Celled Parenchyma		Index Total	Same Total Without Indentation	Large Celled Cortical Parenchyma	Vascular Bundles	Pith	Stele
				Horiz.	Vert.						
R-15	+ +	26	19	0	0	45	19	19	15	21	36
R-60	+ +	15	11	0	11	37	22	15	27	21	48
USDA 74	+ +	33	11	15	0	59	26	0	26	15	41
USDA 101	+ +	0	0	0	0	0	0	58	19	23	42
N-149-4	+	28	12	0	0	40	12	12	21	27	48
Cimarron	+	22	0	15	22	59	37	0	15	26	41
R-217-3	0	8	0	16	0	24	16	24	28	24	52
R-9/19	0	0	0	0	0	0	0	44	28	28	56
R-13	0	0	0	0	13	13	13	31	20	36	56
R-12	0	0	0	0	17	17	17	33	17	33	50
DP-439	+ +	7	32	0	0	39	32	22	21	18	39
DP-443	0	10.5	0	42	0	52.5	42	10.5	21	16	37

*Refers to the degree of capsule droppage: + +, +, and 0 represent distinct, intermediate, and negative degree respectively.

an inwardly adjacent, horizontal layer of small cells. These small cells may be of two types; one having thick, corky walls averaging 14 percent of the diameter and the other having thin, cellulose walls averaging 9 percent of the diameter. Some varieties, as R-60 and USDA 74, have both of these cellular types present. A layer of corky cells gives an easily fracturing pedicel, while a horizontal layer of small cellulose-walled cells withstands several weeks of weathering as exemplified by Cimarron variety and D.P. 443.

The two varieties which do not usually show capsule droppage are N-149-4 and Cimarron. These varieties have a pedicel indentation with corky cells or with small parenchyma cells. The amount of these three components present and their combination gives an indication of the stability of the pedicel. These account for 49 percent of the cross-sectional area in pedicels of the two varieties in which capsule droppage occurs only after standing in the field several weeks after frost.

In the three readily dropping varieties, R-15, R-60, and USDA 74, these three components account for an average of 48 percent of the pedicel diameter. In variety R-60 the percentage is lowest at 37 percent, but all three of the components are present with large-celled cortex adding to the diversity of the region. Further illustration to the fragility accompanying corky infiltration is shown by two foreign introductions selected for their extremes of function. D.P. 439, a South African spineless variety, has a small indented region with cork comprising 39 percent of the pedicel diameter and exhibiting 90 percent capsule droppage. D.P. 443, or Commercial Oil #2, has a small, indented region with small-celled parenchyma consisting of 53 percent of

the pedicel diameter and does not drop its capsules upon maturity. The other components of the pedicels of these two varieties are approximately equal. The comparison of varieties D.P. 439 and N-149-4 by indentation and cork content of the pedicel as favoring capsule droppage suggests the influence of another factor. Variety N-149-4, which reacts intermediately with respect to capsule droppage, develops approximately 9 percent more pith than D.P. 439, but some undisclosed type of middle lamella changes seems to be a more determining cause toward functional weakness in the D.P. 439 pedicel.

D.P. 443 is an exceptional variety having a strong pedicel with an index value of 53 percent which commonly indicates capsule droppage. The effects of a small indented region and a large layer of small parenchyma cells, the least fragile type of substituted cells, are further overwhelmed by occurring in a very broad pedicel.
By applying the general index for capsule droppage, a different morphological pattern is displayed by the capsule non-dropping varieties. This is exemplified by the lack of pedicel indentation, cork, of horizontal layer of small parenchyma cells in variety R-12, R-13, and R-9/19. Some small parenchyma cells occur but surround the xylem in a vertical layer.

The amount of stelar tissue in capsule non-dropping pedicels ranges from 14 to 20 percent higher than in the capsule dropping types. Also the amount of cortical tissues of capsule non-dropping pedicels ranges from 13 to 36 percent higher than in the capsule dropping pedicels when the indented region is omitted from the comparison.

Since the susceptibility to capsule droppage is correlated with the percentage of pedicel-weakening tissues in the capsule-pedicel

attachment area, capsule droppage can be predicted by the percentage of indentation, cork, and small-cell formation which are present in the zone of attachment.

The Developmental Anatomy of the Capsule-Pedical Attachment Area

Developing tissues of the capsule-pedicel attachment area were identified and measured in castor bean varieties R-12, R-13, R-60, USDA 74, and R-15; of these varieties R-12 and R-13 exhibit the capsule non-dropping trait, while R-60, USDA 74, and R-15 capsule-dropping varieties, show spontaneous capsule loss at fruit maturity. The development of the upper pedicel region shows an early separation of fundamental tissue into layers with varied subsequent differentiation.

In R-12 the anatomical composition of the pedicel remains constant from the time the young capsule is approximately two millimeters in diameter until they are matured. The stele, averaging 51 percent of the pedicel diameter, is composed of a central pith and six vascular bundles forming a dissected circle. The pith averages 24 percent of the pedicel diameter; while, the bundles average 26 percent. The xylem is surrounded by small parenchyma cells which are oriented vertically and overlayed by layers of large cortical parenchyma cells. Also, in this variety there is neither cork nor an indented region present. The small-celled parenchyma averages 18 percent of the pedicel diameter, and the large-celled parenchyma comprises approximately 31 percent.

Another capsule non-dropping variety, R-13, lacks the structural factors which cause a weakness in the capsule-pedicel attachment area. The vertically oriented, small parenchyma cells exterior to the xylem

vary in cell size and shape from the large parenchyma cells of the cortex lying adjacent. The proportion of tissue of the young pedicel is constant from the time the capsule attains 2 mm. of diameter until it matures at a 14 mm. diameter size. The constancy in appearance of the pedicel apex is due to the lack of development of specialized tissue.

The stele averages 49 percent of the pedicel diameter and consists of 22 percent pith and 28 percent vascular bundles. Small parenchyma cells are vertically oriented externally to the xylem and occupy 16 percent of the pedicel diameter, while large-celled cortical parenchyma accounts for 34 percent of the area.

In the capsule-dropping variety, R-60, the anatomy of the upper pedicel is complicated by the presence of pedicel-weakening factors in the cortex. At the 1 mm. diameter stage of capsule development, there is a definite indentation and large area of cork-forming cells which continue their presence in the pedicel through capsule maturity. When the capsule is approximately 1½ mm. in diameter, a vertically oriented layer of small-celled parenchyma surrounds the vascular tissue in the pedicel and persists through capsule maturity. Upon attainment of 3 mm. in diameter by the capsule, and remaining identifiable until capsule maturity, the cork-forming area of the pedicel is frequently surrounded by large-celled cortical parenchyma. After capsules have attained 5 mm. in diameter, horizontally oriented groups of small parenchyma cells make their appearance in the upper pedicel area. The horizontally oriented small cells do not develop thickened walls, and appear to be derived from the cork-forming cells. At the same stage of development, the pedicel contains the pedicel-weakening tissues and continues growth into maturity.

In USDA 74, another capsule dropping variety, cellular differentiation of the cortex proceeds in a different sequence. When the young capsule which is subtended on a pedicel devoid of cortical parenchyma is approximately 2 mm. in diameter, there is noted the presence of thicker-walled cork initials and a large indentation. The indented region occupies 25 percent, and the cork initials consist of 23 percent of the total pedicel diameter.

When capsules enlarge beyond 6½ mm. in diameter, a layer of horizontally oriented small-celled parenchyma is found in the pedicel and continues identifiable through maturation.

During later stages of development the tissue composition of the pedicel contains 15 percent of small-celled parenchyma, 17 percent of cork, and the indented region comprises approximately 23 percent of the pedicel diameter. Also, the stele averages 46 percent, the central pith occupies 20 percent of the total pedicel, and the vascular bundles comprise 25 percent of the total diameter.

In R-15, a capsule-dropping variety, the anatomical differentiation of the cortex is expressed in a different manner from R-12, R-13, R-60, and USDA 74. Some small parenchyma cells in the variety R-15 develop into large parenchyma cells.

In the upper pedicel region of young capsules of 2 mm. in diameter, small-celled cortical parenchyma is oriented horizontally and is composed of approximately 50 percent of the pedicel diameter. Also, in this area the indented region represents approximately 13 percent of the pedicel diameter. At this stage of development, there are no corky cells noted. After the capsule has attained 3½ mm. in diameter, the upper cortex is represented by 15 percent small parenchyma cells, 15

percent cork, and 38 percent indented region.

After the capsule has developed beyond 4 mm. in diameter the small-celled parenchyma comprises only 2 percent of the pedicel diameter, while the cork content increases to approximately 30 percent, and the indented region remains a constant 13 percent.

As the capsule attains approximately 12 mm. in diameter or nears maturity, the small-celled parenchyma of the pedicel is replaced by large-celled, cortical parenchyma which forms 10 percent of the pedicel diameter.

The anatomical sequence of the distal end of immature pedicels depends largely on the parental variety, and it was observed to develop by three different variations:

1. In variety R-12 and R-13 the developmental pattern remains relatively constant.

2. In variety R-60 and USDA 74 early pedicel development is characterized by common cell initials developing cork tissue, and as the pedicel matures small, thin-walled cells predominate.

3. In variety R-15 common cell initials of the immature pedicel predominately form cork and small-celled parenchyma and in the mature pedicel form large-celled parenchyma.

Capsule Droppage in the F_1 Hybrid Generations

Capsule droppage was expressed in the heterozygous condition in ten F_1 hybrid crosses between capsule-dropping and capsule non-dropping parental lines of castor beans.

The types of pedicel-weakening traits present in the parental lines vary greatly since the lines themselves were selected to show the inheritance of contrasting factors.

The percentage of weakening factors present in the capsule-pedicel

attachment area conforms to the index predicting capsule droppage. The variety USDA 101, which possesses a chemical cell dissolution, does not reveal its true function in this data based on morphology as shown in Table III.

The Expression of Capsule Droppage in F_2 Generations

Three general classes , one heterozygous class and two parental classes , can be discerned in the F_2 population from the seven crossings by external capsule and pedicel appearance. A primary separation is based upon the expressions of spines, pedicel angle, and capsule-pedicel attachment area , three characters showing dominance in the homozygous and heterozygous forms. Internal morphology usually substantiated the initial separation and displayed variation within the group. Then the fruits of the F_2 plants have been arranged according to one major character , the composition of the capsule-pedicel attachment area. Segregation has been at random and may include some plural gene traits giving the varying degrees of expression. These chance combinations can be depicted in series showing the intergradations of factors under consideration.

The F_2 plants from the crossing R-13 by R-60 were divided into ten classes by the anatomical content of the capsule-pedicel attachment area as shown in Table IV. Two of the classes, as indicated by the anatomical analysis of 13 individuals, were recessive parental types, while three classes, as indicated by eleven analyzed individuals, were dominant parental types. The intermediate types consist of eleven analyzed individuals falling into five different anatomical classes. These groupings do not indicate the inheritance ratio of capsule droppage but show the intergrading content of anatomical factors.

TABLE III

DIAMETER PERCENTAGE OF VARIOUS TISSUE TYPES IN THE CAPSULE-PEDICEL ATTACHMENT AREA OF TEN F_1 GENERATIONS OF CASTOR BEANS

F_1 Parents	PERCENT								
	Indentation	Cork	Small-Celled Parenchyma Horiz.	- Vert.	Index Total	Large-Celled Cortical Parenchyma	Vascular Bundles	Pith	Stele
USDA 74 x N-149-4	21	33	0	0	54	0	15	31	46
USDA 74 x Cimarron	23	31	8	0	62	0	19	19	38
USDA 74 x R-217-3	21.4	14	28.5	0	64	0	18	18	36
R-9/19 x R-15	15	8	11	4	38	16	23	23	46
USDA 74 x R-9/19	24	32	0	8	64	0	16	20	36
USDA 74 x R-12	15	4	11	4	34	15	23	28	51
R-12 x R-15	19	6	12.5	0	37.5	28	12.5	22	34.5
R-13 x R-15	26	17	9	0	52	0	26	22	48
R-13 x R-60	5	10	0	16	31	11	21	37	58
R-13 x USDA 101	0	0	0	10	10	40	17	33	50

TABLE IV

DIAMETER PERCENTAGE OF VARIOUS TISSUE TYPES IN THE CAPSULE-PEDICEL ATTACHMENT AREA FOR PARENTAL LINES, R-13 AND R-60, AND THE F_1 AND F_2 PROGENIES FROM THE CROSSING

Plant	Generation	PERCENT									
		Indentation	Cork	Small-Celled Parenchyma Hori. - Vert.		Index Total	Index Minus Vert. Par.	Large-Celled Cortical Parenchyma	Vascular Bundles	Pith	Stele
R-13	P_1	0	0	0	13	13	0	31	22	34	56
R-60	P_1	15	11	0	11	37	26	15	27	21	48
116	F_1	5	10	0	16	31	15	11	21	37	58
112	F_2	0	0	0	27	27	0	27	33	13	46
127	F_2	0	0	4	8	12	4	38	25	25	50
128	F_2	0	3	19	6	28	22	25	28	19	47
114	F_2	4	0	28	8	40	32	8	32	20	52
128-6	F_2	7	10	0	0	17	17	32	26	25	51
129	F_2	17	0	0	0	17	17	34	27	22	49
130	F_2	18	0	7	0	25	25	33	28	14	42
131	F_2	14	7	7	0	28	28	34	24	14	38
132	F_2	7	7	30	4	48	44	11	25	16	41
133	F_2	3	15	0	9	27	18	32	18	23	41

Capsule droppage in this cross depends upon the content of pedicel-weakening factors whose action can be indicated by the previously mentioned index number taken from their total percentage of the pedicel diameter.

The F_2 progeny of R-12 by R-15, arranged in a morphological sequence based on the percentage of weakening factors present in the capsule-pedicel attachment area, conform well to the three groups of the index predicting capsule dropping. Indentation and cork formation are the main weakening factors aided by small-celled parenchyma, especially in the heterozygous group. These factors are absent or negligible in the non-dropping recessive group (Table V). Another related series is the total percentage of cortex present varying from 33 to 78. Cortical content is higher in the dominant parent and dominant type of the F_2 segregates in contrast to the recessive type.

Among the F_2 progeny from the crossing R-12 by USDA 74 gross observations in the field reveal a separation of the pedicels into two types, the capsule-dropping type and the capsule non-dropping type. Microscopic determinations of the pedicel anatomy revealed a break in the pedicel index number series indicating capsule dropping. The presence of a varied intermediate group of individuals with pedicel composition unlike either parent was revealed (Table VI) but are not readily discerned by gross examination. The specific interaction of factors has not been determined which produces the obscure intermediate group.

The F_2 progeny from other parental lines possessing contrasting traits, R-9/19 by USDA 74, segregates into the two parental types and an intermediate type. The seven pedicels analyzed were selected as

TABLE V

DIAMETER PERCENTAGE OF VARIOUS TISSUE TYPES IN THE CAPSULE-PEDICEL ATTACHMENT AREA FOR PARENTAL LINES, R-12 AND R-15, AND THE F_1 AND F_2 PROGENIES FROM THE CROSSING

Plant	Generation	PERCENT								
		Indentation	Cork	Small-Celled Parenchyma Horiz. - Vert.		Index Total	Large-Celled Cortical Parenchyma	Vascular Bundles	Pith	Stele
R-12	P_1	0	0	0	17	17	33	17	33	50
R-15	P_1	26	19	0	0	45	21	15	19	34
301	F_1	19	6	12.5	0	37.5	28.5	12	22	34
302-5b	F_2	29	35	0	0	64	7	21	8	29
302-5c	F_2	24	28	0	0	52	14	27	7	34
302-5a	F_2	33	17	0	0	50	28	16	6	22
302-2	F_2	20	24	8	0	52	0	32	16	48
302-1	F_2	15	12	23	0	50	15	27	8	35
302-11	F_2	17	8	17	0	42	8	33	17	50
302-19	F_2	11	11	7	0	29	23	22	26	48
303-15	F_2	19	7	0	11	37	15	22	26	48
303-3b	F_2	0	0	5	5	10	32	32	26	58
303-3c	F_2	0	0	0	14	14	50	27	9	36
303-3a	F_2	0	0	0	8	8	25	33	34	67
303-13	F_2	0	0	0	0	0	48	30	22	52

TABLE VI

DIAMETER PERCENTAGE OF VARIOUS TISSUE TYPES IN THE CAPSULE-PEDICEL ATTACHMENT AREA FOR PARENTAL LINES, R-12 AND USDA 74, AND THE F_1 AND F_2 PROGENIES FROM THE CROSSING

Plant	Generation	PERCENT									
		Indentation	Cork	Small-Celled Parenchyma Hori. - Vert.		Index Total	Index Minus Vert. Par.	Large-Celled Cortical Parenchyma	Vascular Bundles	Pith	Stele
R-12	P_1	0	0	0	17	17	0	33	17	33	50
USDA 74	P_1	33	11	15	0	59	59	0	26	15	41
243	F_1	15	4	11	4	34	30	16	23	27	50
245-6	F_2	18	4	14	0	36	36	14	25	25	50
245-1	F_2	20	0	6	0	26	26	24	33	17	50
247-3	F_2	0	4	4	18	26	6	15	30	29	59
245-16	F_2	0	0	0	24	24	0	22	29	25	54
245-9a	F_2	0	0	13	0	13	13	32	29	26	55
248-13	F_2	0	0	0	8	8	0	42	25	25	50
245-3	F_2	0	0	0	12	12	0	30	33	25	58

probable intermediate types and did not show the recessive parental type (see Table VII). Horizontally oriented, small-celled parenchyma and indentation are the main weakening factors, while the expression of cork is less prominent.

Histological sections of pedicels from the F_2 progeny of crossings USDA 74 by N-149-4 and USDA 74 by Cimarron show a separation of traits into two parental types, and a broad, intermediate group. The variations present in the classes of the F_2 generations from these two crossings are not as extreme as the variations from the crossings R-12 by R-15 and R-13 by R-60. The difference in amount of variations in the F_2 progeny is related to the quantity of difference in the parental lines.

The inheritance of the anatomical traits of the upper pedicel have been found to follow basic Mendelian laws. The second generation produces the two parental classes and a broad, intermediate group.

The Occurrence of Altered Pedicel Anatomy

The occurrence of a horizontally oriented layer of small-celled parenchyma in the capsule-pedicel attachment area of crossings R-13 by R-60 and R-12 by R-15 has not been previously observed in the parental lines. Perhaps the new types of cells have originated from cork-cell initials or from the influence effecting horizontal layer orientation of the cork cells.

In the F_2 progenies from the crossing R-13 by R-60 horizontally oriented, small-celled parenchyma were found in six out of the ten anatomical classes which included members in both of the parent-like groups and in the intermediate groups. Clusters of small-celled parenchyma were present in the pedicels of the F_1 generation. Close

TABLE VII

DIAMETER PERCENTAGE OF VARIOUS TISSUE TYPES IN THE CAPSULE-PEDICEL ATTACHMENT AREA FOR PARENTAL LINES, R-9/19 AND USDA 74, AND THE F_1 AND F_2 PROGENIES FROM THE CROSSING

Plant	Generation	PERCENT									
		Indentation	Cork	Small-Celled Parenchyma Hori. - Vert.		Index Total	Index Minus Vert. Par.	Large-Celled Cortical Parenchyma	Vascular Bundles	Pith	Stele
R-9/19	P_1	0	0	0	0	0	0	44	28	28	56
USDA 74	P_1	33	11	15	0	59	59	0	26	15	41
256	F_1	24	32	0	8	64	56	0	16	20	36
257-4	F_2	15	0	33	4	52	48	7	22	19	41
260-7	F_2	23	10	20	3	56	53	0	26	18	44
257-8	F_2	22	6	15	12	55	43	0	22	23	45
259-3	F_2	20	12	8	4	44	40	8	28	20	48
259-13	F_2	17	5	0	13	35	22	17	30	18	48
260-8	F_2	22	6	0	3	31	28	25	25	19	44
262-29	F_2	0	0	7	7	14	7	34	30	22	52

investigation revealed the presence of this tissue layer at the five to ten percent level in many of the variety R-60 pedicels. This variation and low percentage of occurrence in the R-60 variety may represent a cytological response to ecological conditions. The appearance of a horizontally oriented layer of small-celled parenchyma which composes up to 27 percent of the pedicel diameter in three-fifths of the F_2 classes indicates there may be an inhibiting factor present in the R-60 variety which is not present in the R-13 variety.

This new cell type, horizontally oriented, small-celled parenchyma, is found in the F_1 and in the F_2 heterozygous classes of the crossing R-12 by R-15. The developmental anatomy of mature R-15 pedicels reveals such a tissue in the immature stages which enlarge upon maturity. The R-12 pedicel retains small-celled parenchyma as a vertical sheath around the stele. Possibly the traits in R-12 which retard the formation of a horizontal layer of cells prevent the final enlargement of the least differentiated, horizontal layer of cell type formed by the R-15 genes.

The presence of vertically oriented, small-celled parenchyma in the F_1 and F_2 progenies of the crossing R-9/19 by USDA 74 is of interest since neither parent possessed this tissue. The situation indicated three possibilities as follows:

1. The factor is recessive in a parental line.
2. A specific inhibitory trait was present in a parental line.
3. A tissue proliferation reaction accompanied the factors for indentation, cork, and horizontally oriented, small-celled parenchyma.

The anatomical composition of the parental lines, one dominant and the other recessive, for certain pedicel-weakening traits would suggest the possibility of an intermediate group being formed by genic inter-

action or combination. The presence of an inhibitory factor is neither substantiated nor disproven.

Although morphological analysis may reveal the presence of pedicel-weakening tissues, the application of mechanical force to the attached capsule may cause breakage above the indicated pedicel locus. Second generation individuals from crossings having a parent of variety R-12 R-13, or R-9/19 are subject to this type of capsule-pedicel separation. These individuals were found in the heterozygous class and in classes nearly like each of the parents. The lower extremity of the capsule columella is pulled from the capsule base, adhering to the pedicel. This reaction is noted especially in immature capsules undergoing rough handling. Pedicel measurements show the capsule-basal area as being morphologically strong. Microscopic anatomy shows thickenings of the vascular elements at the base of the capsular columella in the area originating the major traces to the outer capsule vascular system. Observations above this locus reveal vascular elements having much thinner walls where the breakage occurs (Figure 6). Upon re-examinations of an immature parental line, R-12, the lower capsule columella capsules displays a vascular thickening pattern like the F_2 generation.

Capsule-dropping line, USDA 74, reveals a similar-shaped capsule columella but with more-even vascular cell wall thickenings. The amount of large-celled cortex presents no differences in these varieties.

The presence of pedicel-weakening tissues does not eliminate the effects of weak capsule base structure. The F_2 heterozygous dominant class of the R-13 by R-60 crossings presents a clear example of this type (Figure 7) which shows a separation in the basal end of the capsule columella.

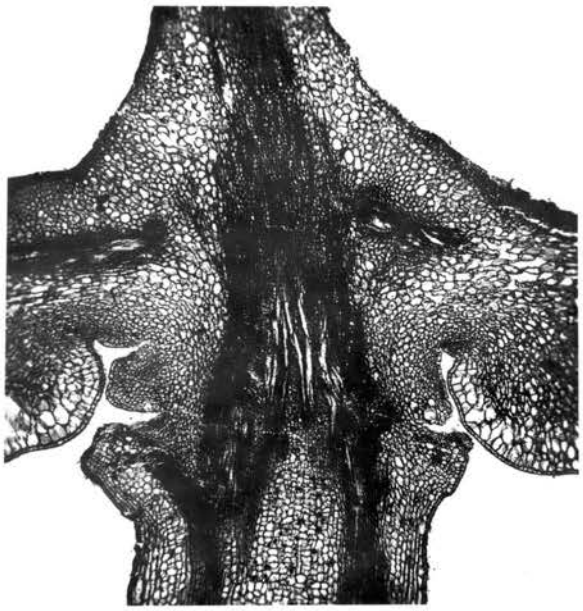

Figure 6. Longitudinal Section Through the Succulent Immature Capsule-Pedicel Attachment Area Showing the Capsule Separation Locus Above the Floral Bract Bases and Lack of Pedicel Weakening Tissues in an F_2 Plant of the Castor Bean Cross of Variety R-12 by R-15.

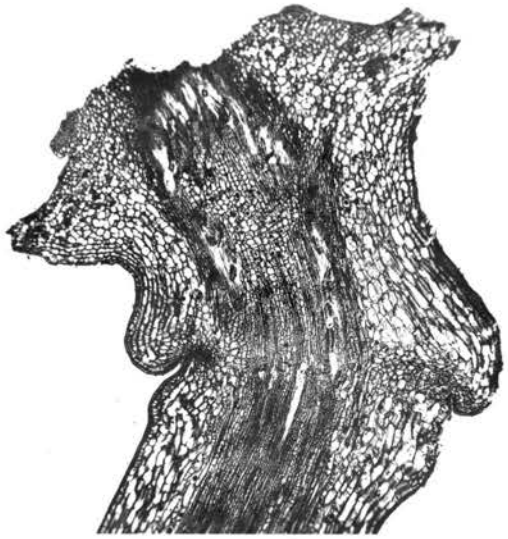

Figure 7. Longitudinal Section of the Apex of a Succulent Immature Pedicel Showing Capsule-Pedicel Attachment Area Containing Indented Region, Corky Cells, and Small Parenchyma Cells in an F_2 Plant of the Castor Bean Cross of Variety R-13 by R-60.

The heterozygous dominant nature of the pedicel-weakening factors was seen by their appearance in the F_1 generation (Table III).

Genetic Indications of Individual Pedicel-Weakening Factors

Pedicel indentation and cork formation occurred together in the F_1 generation of nine out of ten different contrasting crossings and in the F_2 progeny of the R-12 by R-15 crossing. In the progeny of the R-13 by R-60 crossing, there was a separate occurrence of cork or indentation in four out of the ten classes. Cork formation and pedicel indentation were frequently found together due to the dominance of both characters. Their joint presence was noted in eight out of the ten parental lines.

A similar type of association exists between capsule droppage and spiny capsules. These two dominant, unrelated characters are usually introduced by the same parent in a crossing.

When the F_2 progeny of the R-13 by R-60 crossing was separated by the three capsule types of spine characters, an anatomical series of pedicel composition types similar to the one shown by the pedicels from all the capsule types in Table I was shown by the pedicels of each of the three capsule types.

The pedicel composition series from all types of capsules in the R-13 by R-60 crossing were more complete than those from a single capsule type due to the limited number of individuals available in the latter and to the reduced number of recombinations in the smaller population.

In the F_2 generations these dominant pedicel-weakening traits make their appearance more frequently both singly and in combination than do the recessive alleles.

An indication of the general genetic nature of the pedicel-weaken

ing factors has been shown by an expression of dominance in first and second generation crossings from contrasting parental lines. The summations of pedicel-weakening factors present in three such crossings are shown in Table VIII.

TABLE VIII

FREQUENCY IN APPEARANCE OF PEDICEL-WEAKENING FACTORS IN THE F_2 PROGENY OF CROSSINGS FROM CONTRASTING PARENTS

Varietal Crossings	Number of Individuals Anatomically Analyzed	NUMBER OF INDIVIDUALS POSSESSING:			
		Indentation	Cork	Small-Celled Parenchyma - Horizontal	Vertical
R-12 x R-15	12	7	7	5	4
R-13 x R-60	35	21	13	20	22
R-9/19 x USDA 74	7	6	5	5	7
TOTAL	54	34	25	30	33

The total frequency of occurrence of these individual factors approximate a 1:1 ratio; however, the significance of the ratios in these small populations of selected individuals needs interpretation. There was a large proportion of individuals (13) belonging to the recessive parental type in the R-13 by R-60 crossing reducing the ratio of pedicel-weakening factors. The pedicels for sectioning from the R-9/19 by USDA 74 crossing were selected as probable intermediate types and did not include the parental type segregates. A primary reason for questioning the 1:1 ratio was the lack of such a known genetic ratio while a secondary reason was the clear dominance of capsule droppage found in genetic studies during 1952 (Van Horn) and 1953 (Parkey).

It has been previously noted in this report (Table II) that capsule droppage was not due to one cause but to a combination of pedicel-weakening causes. These causes, by the percentage of their presence, have been formulated into capsule dropping indexes. From the variations in amount of pedicel diameter occupied by each pedicel-weakening factor, it is probable that the 1:1 ratio represents the partial summation of another ratio: for example, the two factor ratio of 9:3:3:1 could separate into a 9:7 ratio based upon field counts of capsule-droppage or on regrouping of the intermediate F_2 types. This regrouping would be possible in the ten anatomical classes of pedicels of the crossing R-13 by R-60 and in the classes of the crossing R-9/19 by USDA 74 (Tables IV & VII). In the crossings R-12 by R-15 and R-12 by USDA 74, the regrouping is possible but the number of parental types included changes in the numerical ratio for these individuals, for they were selected to show the differences in pedicel anatomy.

For the crossing R-13 by R-60, Van Horn (1952) reported a 9:7 ratio for capsule-droppage versus capsule non-droppage using fruits from the first-formed rachis only. Anatomical composition indicates that 25 of these 35 pedicels will break if harvesting is delayed a few weeks. Since 13 of the 35 individuals were classed as the recessive parental type, the numerical dominance of capsule droppage becomes even more pronounced. The genetic ratio of Van Horn (1952), differs from that of Parkey (1953), and shows the predicted reaction of capsule droppage as indicated by the microscopic observations of the pedicel-weakening traits. However, the random segregation of the dominant, multiple pedicel-weakening traits may grossly appear to form a 3:1 ratio as observed in the field.

A cell type forming a low percentage of the total structural composition in the upper pedicel area of parental lines was found to form a high percentage of composition in the F_2 pedicels. The expression of these cell types was postulated as a result of genic interaction of altered substrate. A two-factor basis is indicated for the individual anatomical traits causing pedicel-weakness and capsule dropping.

Section B - Capsule Dehiscence

Dehiscence and Capsule Structure

Capsule dehiscence in the castor bean was divided into three classes:

1. Indehiscent or closed capsule ; generally the capsule does not open at maturity but will open after periods of prolonged, humid weather.
2. Semidehiscent or splitting capsule ; the capsule walls separate slightly at maturity and during periods of humid weather, locule separation is increased.
3. Dehiscent or flipping capsule ; the capsule walls separate violently at maturity and frequently flip the seed several feet.

A general pattern of structure is common in both the dehiscent and indehiscent types of capsules. Yet, by variation of common structural traits, the capsules of some varieties remain closed; some separate slightly; while others open violently. The capsule is composed of three carpels with their adjacent walls adhering around a central vascular core or columella to which the seeds are attached. The capsule wall or pericarp is composed of tissues representing three layers; the ectocarp, mesocarp, and endocarp. The ectocarp forms the epidermal layer. The mesocarp forms a thick, spongy layer which turns into a shrunken, paper-like mass at maturity. The endocarp is dividied into

three sublayers of cells which rapidly develop thickened walls by the formation of hard cellulose in the final stages of capsule growth. At capsule maturity, the endocarp is the most dense of the functional tissues of the pericarp. The composition and structural details of the endocarp are basically responsible for the forces which causes capsule dehiscence.

Dehiscence occurs visibly by the longitudinal opening of the dorsal suture of each carpel and is called the loculicidal type of opening. In castor beans the capsule apex most frequently remains intact, while the basal portion of the carpels separate into the two valves with a rupture of the ventral suture.

Generally the castor bean varieties have common structural areas in the woody endocarp tending toward locule openings, for example:

1. A horn terminates the apex of each locule.
2. The apical 1/3 of the locule has thick endocarp layers, and the basal 2/3 of the locule has thin endocarp layers.
3. The ventral surface of the locule is very thin and has a change of endocarp cell layers present.
4. The funiculus aperature, and caruncle tissue alter moisture relationships of the locule.
5. The basal ventral window is a very thin area.
6. The wide basal end of the dorsal suture matures as soft tissue.

The locule horn is a pointed protrusion located at the center of the apical end of each locule. The horn is primarily composed of large elongated woody cells from the central layer of the endocarp. These cells are orientated vertically to the seed axis and lie around a minute cavity. At capsule maturity in the Cimarron variety, the cavity and surrounding area are impregnated with a tenacious latex

which hardens with drying. The minute cavity and cells oriented around the cavity frequently act together as a hinge during dehiscence. The area bends at the cavity but remains attached to the apex of the locule valves. When the thin basal portions of the locule mature, they shrink and separate from the columella and adjacent locules. The thick apical horn tissue shrinks and twists less than the thin basal portion of the locule and in many varieties holds the three locules together at the apex.

The ventral surface of the locule is usually 3 to 7 times thinner than the dorsal surface of the locule. In the lateral extremes of the locule walls, the large middle layer of the endocarp usually is absent. Frequently, one or both of the endocarp layers of cells in the ventral surface of the locule show a horizontal orientation to the seed axis, while the dorsal and lateral layers are vertically orientated to the seed axis.

The funiculus aperture just below the ventral apex of each locule forms a large opening through the three thick endocarp layers. The large aperture is a point of structural weakness as the funiculus and surrounding succulent tissues shrink during the dessication which follows maturation.

The caruncle may influence capsule dehiscence by acting as a moisture reservoir during the humidity changes of weathering.

The basal ventral window is a narrow aperature or interruption in the endocarp layers covered with a thin, clear layer of tissue. This interruption of varying widths in different castor bean varieties does not add to locule strength.

Although containing these 6 recognizable weakening factors, the

capsules of indehiscent and semidehiscent varieties of castor beans remain intact during different periods of moist weathering conditions.

Some varieties of castor beans have further variations in the woody endocarp which tend toward dehiscence of the capsule, as follow:

1. A difference in size, shape, and orientation existed between cells of one endocarp layer and cells of the adjacent layer.
2. A difference in thickness of cell walls among the three endocarp layers was observed.
3. A difference in wall thickness between cells within each layer of the endocarp was found.
4. A grouping of specialized cells in one or more layers of the endocarp was present.

The combination or arrangement of the ten factors which have been listed may vary greatly depending on the variety under consideration.

The degree of capsule opening or type of dehiscence depends upon a delicate balance or interaction of the structural features in the endocarp layers.

Capsule Structure of One Exotic and Ten Parental Lines

A structural analysis of the capsules from the ten parental lines and one exotic castor bean varieties are presented in Tables IX and X. Uneven lines of stress in the maturing and drying endocarp layers form the forces for locule opening.

The Development of the Endocarp

The development of the woody endocarp occurs very slowly during the immature stages of capsule development but is rapidly accelerated prior to maturity. As early as ten or more days after syngamy, the seeds are well differentiated; however, at this time the immature

TABLE IX

STRUCTURAL ANALYSIS OF CAPSULES BY DEHISCENCE AND ENDOCARP CELL TYPES OF ONE EXOTIC AND TEN PARENTAL CASTOR BEAN LINES

Variety	Dehiscent			Dominance of Endocarp Layer			Orientation of Endorcarp Cell Layers and Cell Size			Extremes of Cell Shape and Cell Wall Thickness		
	N	S	VD	C	O	I	C	O	I	C	O	I
D.P. 443			X	+			V	H	V	slender v. ln. c.		v. thick w.c.
R-15		X		+ +		+	V l.c.	H s.c.	H s.c.	ln. c.		
USDA 74		X		+	+		V l.c.	H s.c.	H v.s.c.	ln. c.		
R-60		X			+ +	+	V s.c.	H s.c.	H s.c.	sq. c.		
Cimarron	X				+		V s.c.	V s.c.	H s.c.		sq.c.	ln.c.
N-149-4	X				+		V s.c.	H l.&s.c.	V s.c.			
R-9/19	X			+	+ +		V l.c.	H s.c.	H s.c.	ln.c. horiz. at locule sides		
R-217-3	X				+ +		V s.c.	H s.c.	H s.c.	short c.	thin cell	
R-13	X			+ +	+	+	V l.c.	H s.c.	H s.c.	ln.c.		
R-12	X			+ +	+		V s.c.	V s.c.	H s.c.		sq.c.	thick w. c.
USDA 101	X				+ +		V s.c.	H s.c.	H s.c.	sq.c.		

Refer to key of symbols on page 44.

TABLE X

VARIATION IN CELL ARRANGEMENTS OF THE ENDOCARP LAYERS OF ONE EXOTIC AND TEN PARENTAL CASTOR BEAN LINES

Variety	VARIATION IN CELL ARRANGEMENTS OF THE THREE ENDOCARP LAYERS		
	Center	Outer	Inner
D.P. 443	Not a constant cell orientation around the locule	Not a constant cell orientation around the locule Rods	Not a constant cell orientation around the locule
R-15	Arcs		Patches of round, thick-walled cells
USDA 74	Patches of small, thick-walled cells extending through all 3 layers	Patches of small, thick-walled cells extending through all 3 layers Rods	Patches of small, thick-walled cells extending through all 3 layers
R-60	Arcs	Rods	Rods
Cimarron	Arcs		
N-149-4	Arcs	Rods	
R-9/19	Patches of thick-walled cells Arcs	Patches of thick-walled cells	
R-217-3	Arcs		Rods of thick-walled cells
R-13	Arcs	Rods	Rods
R-12	Divided into three sub-layers	Horizontally oriented cells on ventral locule surface	
USDA 101	Thin layer of cells		

C - center layer
H - horizontal orientation
I - inner layer
N - nondehiscence

O - outer layer
S - semidehiscence
V - vertical orientation
VD - violent dehiscence

c - cells
l - large
ln - long
s - small

sq - square
v - very
w - walled
+ - relative amount present

arcs - cells lying at angles
rods - thick-walled cells

endocarp is represented only by a single-celled layer with cells of various sizes. When the capsule has attained one-half or more of its mature size, the endocarp layer is divided into three layers which are one cell in thickness. The inner and outer endocarp layers are composed of smaller cells than the central layer. These layers develop in the same relative proportion until after the capsule reaches its mature size. Subsequently and prior to capsule maturity, the cells of each layer divide rapidly to form layers of many cells in thickness depending on varietal characteristics. The cell walls of the endocarp form secondary thickenings of cellulose followed by a discoloration and drying of the epidermis. Upon dessication of the epidermis and spongy cortex, a papery mass surrounding the woody endocarp layers is formed. It is postulated that by the dehydration during maturation and weathering, the inner layers of the heterogenous woody tissue contract unevenly, and the locule breaks free of its adjacent locules with a violent flip depending upon the varietal characteristics.

Capsule Structure of Variety USDA 74

Cell irregularities in the capsule structure pattern are shown in the semidehiscent variety USDA 74 (Figures 8 and 9). The woody endocarp has a central layer which is composed principally of cells elongated vertically from the plane of the seed axis. The outer endocarp is a thickened layer composed of small cells, which are orientated horizontally and includes short rod-like associations of cells. The inner endocarp layer is composed of very small, horizontally oriented cells.

A special mechanism which tends to retard the dehiscence of three

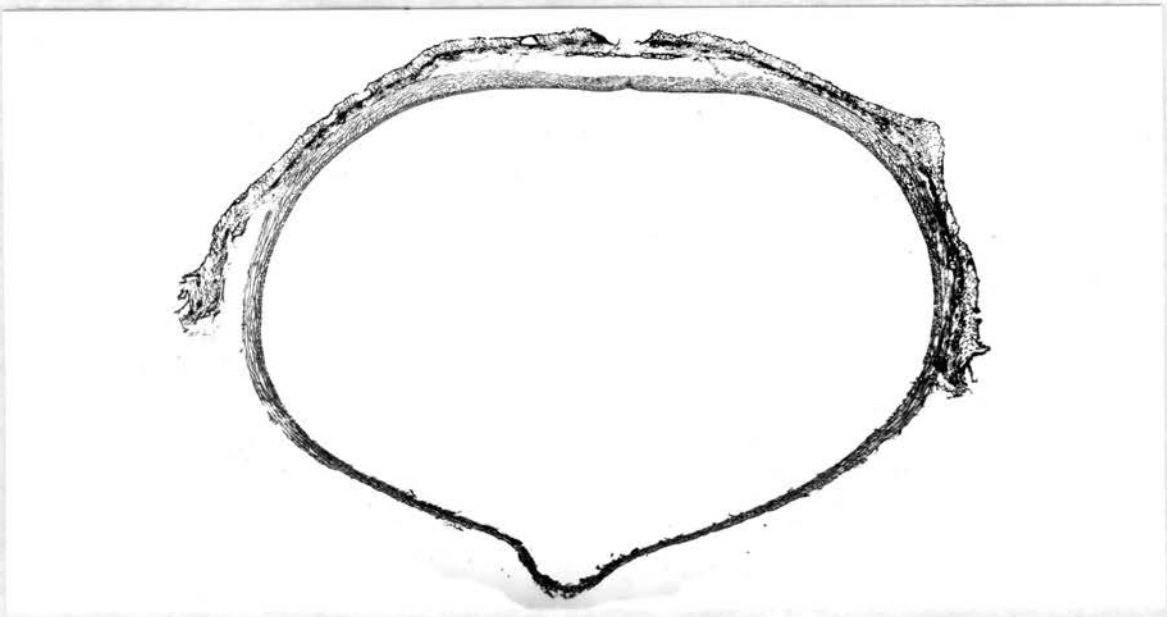

Figure 8. Cross Section of a Mature Drying Locule of a Capsule Showing the Outer, Spongy Mesocarp and the Inner Woody Endocarp in the Castor Bean Variety USDA 74.

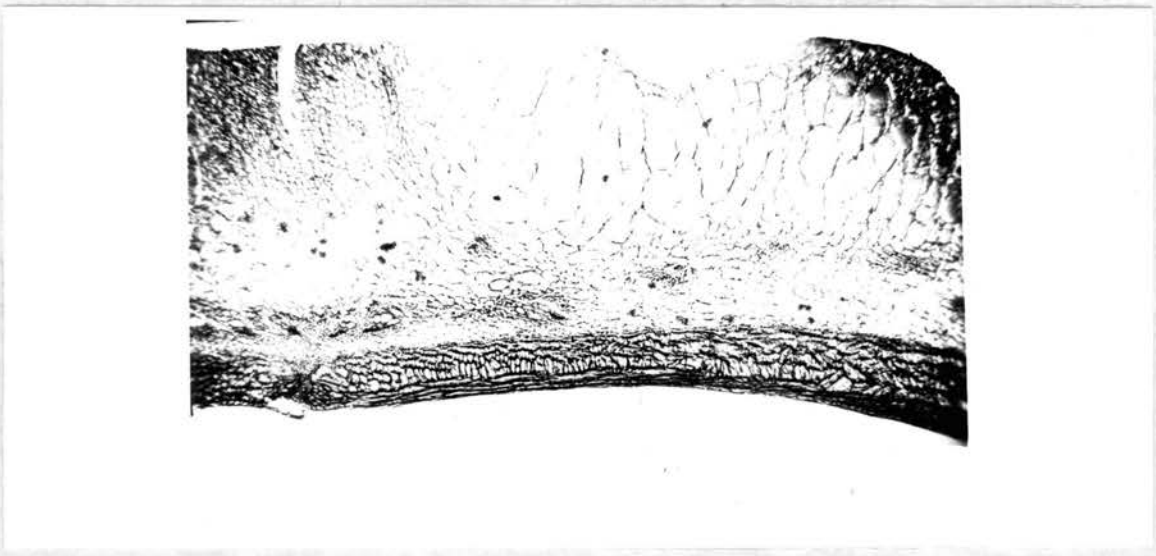

Figure 9. Cross Section of Portion of a Succulent Mature Locule Showing the Dorsal Suture at the Left Side and the Irregular Cellular Pattern of the Three Endocarp Layers in Castor Bean Variety USDA 74

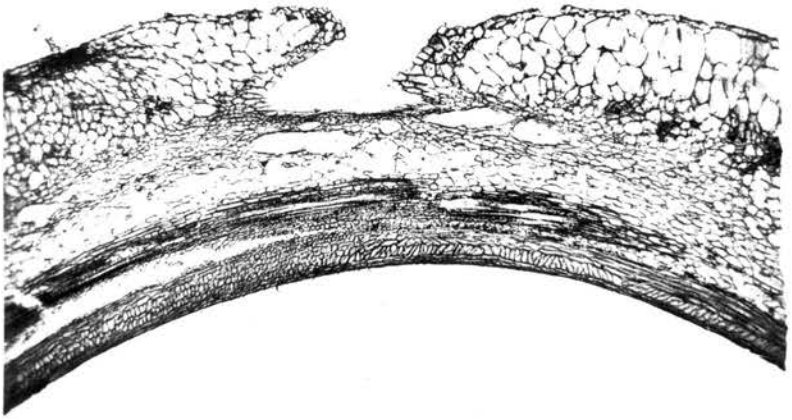

Figure 10. Band of Small Thick-Walled Cells Extending Through the Three Endocarp Layers in the Castor Bean Variety USDA 74.

Figure 11. Dorsal Suture of a Capsule From the Castor Bean Variety USDA 74 Showing Latent Opening at the Area of Small Thick-Walled Cells.

loci along the suture prior to dessication of the capsule is noted in this variety. The mechanism is in the form of local areas of small, thick-walled cells which extend through the three layers of the endocarp (Figure 10). The local areas of the endocarp are always adjacent to vascular tissue in the mesocarp. The small-celled areas are visible in longitudinal sections; one area lies near the capsule apex, a second lies near the capsule base, and a third area is frequently noted in the central portion of the suture. As the capsule matures there is observed the partial opening of two separate apertures along the suture (Figure 11). The location of these areas coincide with the portion of the suture which remain intact until after capsule dessication.

In the castor bean variety USDA 74 a variation of stress is formed in the three woody endocarp layers. This variation is probably accredited to the differences in cell size, shape, orientation, and wall thickness.

Capsule Structure of Variety USDA 101

In the capsule pattern of the indehiscent variety USDA 101 the central layer of the woody endocarp is uniformly constructed of mostly isodiametric cells. The occurrence of small cells at this location is exceptional, for in most varieties, the cells in this area are greatly elongated. The outer endocarp layer is thick and composed of numerous layers of small, isodiametric cells. The inner endocarp layers are thinner and composed of similar small cells. None of the woody endocarp cells have a notable increase in secondary wall thickening. No special varietal mechanism for dehiscence is present in this variety. The over-all composition of this capsule from the functional aspect is

symmetry of structure. The mature endocarp, composed of cells of similar types, may withstand, for several weeks, the effects of weathering which cause capsule dehiscence. By a similarity of cell morphology throughout the three endocarp layers, the effects of the six common structural areas tending toward dehiscence, although identifiable, are of little immediate effect. The capsule of variety USDA 101 displayed the best example in symmetry of structural pattern, but it was accompanied by an undesirable pedicel type.

Capsule Structure of Variety R-12

Capsule indehiscence in castor bean variety R-12 reflects the presence of cellular symmetry of capsule structure. The predominant layer of the endocarp is the central layer which is subdivided in this variety into three vertically adjacent sublayers. The three sublayers comprise the area occupied in most capsules by the central row of greatly elongated cells. The cells in each of the three sublayers are arranged singularly of varying lengths and lie in the vertical plane from the seed axis. The outer layer of the endocarp is composed of many small, isodiametric cells. Toward the ventral surface of the locule, these cells become elongated and horizontally oriented around the seed axis giving flexibility to its surface. The inner layer of the endocarp is composed of small, spherical cells with thick walls forming a sturdy inner shell. Although the six common structural areas tending toward dehiscence may be identified, however, no special varietal mechanism for dehiscence is present. The capsule in this variety maintains its over-all symmetrical pattern by possessing small cells which form three small sublayers in a central endocarp layer instead ofpossessing one row of long cells which occurs in most varieties.

Capsule Structure of Variety R-13

In the castor bean variety R-13 a reinforcement type of mechanism prevents capsule dehiscence. The small, spherical cells of the inner and outer layers of the endocarp contain horizontal rows of small, thick-walled cells which apparently function as strengthening areas in the capsule wall. The central layer of the endocarp is composed of long, vertically oriented cells which occasionally lie slightly at an angle. No special varietal mechanism for dehiscence is present in this variety, although the six common structural areas prone to dehiscence are identifiable. The small, thick-walled rod formation of cells appears to overcome the possible irregular stress, while the elongated, central layer of cells may encourage capsule dehiscence upon weathering.

Primitive Relationships of the Dehiscent Capsule

Dehiscence is an agressive trait and is frequently associated with other primitive traits observed in castor bean plants grown in tropical areas where the genus Ricinus is believed to have originated. Examples of these primitive traits are as follows:

1. A long growing period is generally required for flowering.
2. A large number of nodes to the first inflorescence is common.
3. The tall stature is characteristic of many wild forms.
4. The perennial growth of many tropical forms produces large diameter stems.
5. The dominance of stem cutin is noted.
6. An abscission-like zone in the upper pedicel appears characteristic.
7. The large seed character with variations of the brown seed coat color are common.

8. The genetic dominance of dehiscence is predominant.

The indehiscent capsule probably represents the recessive type for several capsule genetic factors based on the latent action of the dehiscent prone characters common to this species. The characteristics spineless and non-dropping capsule, traits of known recessive genetic reaction, are most frequently associated with indehiscence.

Genetic Segregation of Capsule Dehiscence

Capsule dehiscence tends to be a dominant trait in the F_1 generation. In the crossings of two contrasting parental lines as R-13 by R-60 and R-12 by USDA 74, capsule dehiscence segregated on a 9:7 ratio, therefore indicating the activity of two major genes.

In the crossing of line R-217-3 by USDA 74 a 15:1 segregation ratio was obtained for capsule dehiscence.

Crossings between lines Cimarron by USDA 74, and N-149-4 by USDA 74, indehiscent and semidehiscent lines, respectively, gave a segregation ratio of 3:1 indicating the dominance of one gene.

Histological sections were prepared from selected capsules of the F_2 populations from crossings for the dehiscence studies. Preliminary observations indicated that dehiscence is the externally visible result of the gross function of the locule. Microscopic examination of the woody endocarp reveals many varietal traits for dehiscence such as the predominance of rod-like associations of thick-walled cells, the variation in suture morphology, and other traits for dehiscence which tend to segregate independently (Tables IX and X).

Environmental Influence on Capsule Dehiscence

Examples illustrating the influence of environment upon capsule dehiscence were observed during the summer of 1955 at Stillwater, Oklahoma. Castor bean plants of the variety Ill. 48-36 exhibited different dehiscent reactions at two different locations during this season. The beans shattered severely when grown on a sandy clay loam soil with generally satisfactory moisture relationships; while those grown on a sandy soil with limited available moisture did not shatter excessively. The effects of environment on a dwarf castor bean line (R-1669) and a foreign introduction of Manchurian origin (R-1321) were observed. By mid-August there was no dehiscence observed except for the border or end plants in the row. These plants were obviously favored by a greater source of soil moisture and other favorable environmental conditions. During this season the rainfall was below normal and numerous fruits died with incompletely filled seeds. However, an occassional rachis continued to start seed formation.

Since rainfall occurred during the latter part of August, there was noted a renewal of plant growth. There was observed within two weeks dehiscence of both the filled and unfilled dried capsules. The immature fruits which were developed during this period later matured into dehiscent capsules.

Resolution of Capsule Dehiscence

Microscopic observations reveal that capsule dehiscence of the castor bean is based on the function of many separate morphological factors. This fact does not reduce the importance of genetic factors, although previously, only two genes have been considered responsible for

dehiscence. The many types and degrees of variations observed in the structure of the woody endocarp would indicate the action of many genes. The dominant genetic action of the many separate morphological factors for capsule dehiscence frequently reveals their functional presence together from random segregation. The factors appear together functionally in numbers approximating the genetic ratio for two genes.

The controlling agents for the formation of individual structural traits of the woody endocarp appear to be determined by two genes which tend to make dehiscence appear as a two-gene character. Variations in dehiscence occur in the different lines of castor beans by a separation of the endocarp factors.

The occurrence of a few dehiscent progeny from the crossing of two indehiscent parental lines indicates the segregation together of two or more dehiscent factors which express themselves readily in a capsule having a structure balanced weakly against dehiscence.

Soil and atmospheric moisture exert a strong influence in the expression of capsule dehiscence by castor bean plants. Dehiscence is not fully expressed during dry seasons or in plants grown on sandy soils without supplementary water. The opening of immature dried capsules within 14 days after a rain suggests the necessity of moisture for enzyme-like conversions of insoluble wall substances to soluble substances allowing locule separation. In the dwarf line(R-1668) locule enzyme-like substances were present together with a structural pattern which caused capsule opening. Moisture fluctuations in weathering furnish a stimulus for the contractions of the endocarp walls initiating capsule dehiscence. The difference between sufficient moisture for maturation of a few

capsules with seed and the quantity of moisture to increase the amount of vascular tissue in the castor bean plant does not noticeably effect capsule dehiscence. Variety USDA 101 has strong hydrolytic reactions in the capsule-pedicel attachment area but lacks their presence in the capsule, which indicates a specific focus for an individual physiological trait.

The anatomical and functional information obtained during this study has made possible a correlation of the various genetic results obtained for the fruit of the castor bean plant.

CHAPTER V

SUMMARY

Capsule Droppage

Three degrees of capsule droppage were observed in the 13 castor bean lines. The variation in capsule droppage was correlated with the structural composition of the capsule-pedicel attachment area. Capsule droppage can be predicted from the percent of the tissue components in the capsule-pedicel attachment area. The development of three different patterns of tissue sequence in the apical area of pedicels were noted in the various castor bean varieties studied.

From the parental lines of castor bean varieties and the crossings observed, the genetic segregation suggests the presence of two genes for each of three pedicel-weakening traits; indented region, cork, and small-celled parenchyma.

Pedicel-weakening traits exert an influence on other tissues as indicated by the appearance of an augmented anatomical feature.

Capsule Dehiscence

The basic cause of capsule dehiscence by castor bean plants is an asymmetry of structural pattern in the woody endocarp. The asymmetry is expressed as differences among the three endocarp layers, and by special structural configurations of cells found in individual endocarp layers. An additional cause influencing dehiscence is the

action of hydrolytic enzyme-like substances in the locule suture. Also, sufficient moisture is required to permit the functions of the structural traits and enzyme-like reactions.

Capsule dehiscence is locule opening caused by the over-all functioning of many genetically segregating dominant capsule factors.

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