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Abscission in Psoralea Argophylla

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ABSCISSION IN PSORALEA ARGOPHYLLA PURSH

by

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INTRODUCTION

Abscission is the natural process by which an organ or part of an organ is lost from the plant. Although of widespread occurrence in the plant kingdom, it is a more common and conspicuous phenomenon in the vascular plants. In these plants such structures as leaves, buds, bud scales, stipules, fruits, flowers and floral organs, and stems are abscised from the main plant body (Pfeiffer, 1928). The process typically involves the separation of cells by dissolution of the middle lamella as in the case of most angiosperm leaves; or, it may be caused by mechanical forces such as wind or tissue tensions acting on structurally weak areas in the plant. The latter type is exhibited by the various types of fruit dehiscence, by bark exfoliation, and by the shedding of mature leaves of investigated gymnosperms.

Most early studies of abscission have described the morphology of the abscission process. Extensive studies, primarily morphological, have been published by Tison (1900), Wiesner (1871, 1904a,b,c, 1905), Lee (1911), Hannig (1913), Lloyd (1914a,b, 1916a, 1920, 1927) and Sampson (1918). Much of this work was reviewed later by Pfeiffer (1928) in his "Pflanzlichen Trennunggewebe". More recent works on abscission, which for the most part combine morphological and

physiological studies, include those of Myers (1940), McCown (1943), Addicott (1945), Beal and Whiting (1945), Scott et al., (1948), Hoshaw and Guard (1949), Brown and Addicott (1950), Gawadi and Avery (1950), Facey (1950, 1956), Leinweber and Hall (1959), and Yager (1959).

Laibach (1933) first suggested the effect of hormones on the abscission of plant organs. Since then studies have been made primarily on the effect of chemical substances (mainly auxins) on the abscission process in general rather than on the chemical reactions involved. The work of Myers (1940), Gardner and Cooper (1943), Gawadi and Avery (1950), Addicott and Lynch (1951), Jacobs and co-workers (1953, 1955, 1958, 1962, 1964), Leopold and co-workers (1955, 1957, 1958, 1962, 1963, 1964) have led to various hypotheses regarding the regulation of abscission by auxin. These theories include the hormone-ethylene balance theory (Gawadi and Avery, 1950), the auxin-gradient theory (Addicott et al., 1955), and the two-phase auxin-action theory (Gaur and Leopold, 1955). The latter theory has since been modified into a two-stage theory of abscission, the first stage which is inhibited by auxin and a second stage which is promoted by auxin (Rubenstein and Leopold, 1963). In Coleus petioles Jacobs et al., (1964) has concluded that auxin indirectly inhibits abscission by promoting petiole growth, and therefore does not directly affect abscission. Osborne (1955) found that extracts from aging leaves affect abscission. Application of senescent leaf extracts to explants was found

to stimulate abscission even when inhibitory auxin concentrations were present. Osborne and Moss (1963) are of the opinion that cell separation in the abscission zone should be examined in regard to cellular senescence, and that the effect of various substances such as auxins and other growth regulators on the abscission process are mediated only through control of the aging process. More recently Rubenstein and Leopold (1964) have modified the two-stage theory of abscission to include factors other than auxins. They stated that "as the leaf ages, the inhibitory effect of auxin declines and various promotive substances, some of which are always present while others appear only as products of senescent metabolism, become important as possible natural inducers of the separation process".

Recent investigations by Osborne (1958) and Yager (1960) have dealt more directly with the chemical changes involved in abscission. They have suggested that pectic enzymes bring about changes in pectic materials. This possibility is likely since Deuel and Stutz (1958) reported that pectic enzymes are of common occurrence in higher plants. The softening of cells of ripe pears, avocados, and tomatoes has been found to be due to the activity of polygalacturonidases (Deuel and Stutz, 1958). Osborne (1958) suggested that the change of the pectic material is due to a decrease of pectin methylesterase activity and results in the accumulation of pectin in the middle lamella. Yager (1958) found that a methyl donor (methionine) and low concentrations of

indoleacetic acid decrease the activity of pectin methylesterase, while high concentrations of indoleacetic acid increase the activity of the enzyme. Yager (1960) suggested that other pectic enzymes are involved in the pectic changes. He brought about cell separation in the abscission zone with the use of other enzymes. This enzymatic separation of cells was accelerated also by applications of methionine alone, or by the simultaneous application of methionine and low concentrations of auxin. However, high concentrations of auxin alone or together with methionine either inhibited or prevented abscission. From these results it appears that varying concentrations of indoleacetic acid directly affect the action of pectic enzymes and methionine on abscission. Somewhat contradictory evidence appears from the work by Cleland (1963b). From the examination of four monocotyledonous and three dicotyledonous tissues, where cell elongation was promoted by auxin, it was concluded that auxin-induced methylation of pectin is restricted to monocotyledonous tissues. Since methylation is believed to be an important part in the formation of pectin in the abscission process, the failure of auxin to increase methylation in all growing tissues may mean that it has no direct effect on the pectic materials involved in abscission.

Although the abscission process has been investigated extensively, stem abscission of a heterogeneous group of plants known as the tumbleweeds has been neglected. The purpose of the present study was to investigate stem abscission in Psoralea argophylla, a native tumbleweed.

STATUS OF PSORALEA ARGOPHYLLA PURSH

Psoralea argophylla of the Fabaceae, together with the Compositae and the Gramineae form the dominant vegetation of the prairies and plains of North America. The Fabaceae is a large cosmopolitan group and according to Gleason (1952, p. 387) contains approximately 400 genera and 10,000 species. Along with the Compositae, Gramineae, and Cyperaceae it is included among the six most abundant families with worldwide distribution. Although abundant in both temperate and tropical regions, the Fabaceae are most abundant in the tropics of the Americas, Africa, Asia, and Australia (Good, 1947, p. 48-50).

The genus Psoralea, a widely distributed taxon, is found chiefly in North and South America, Africa, and Australia (Fernald, 1950, p. 587). Gleason (1952, p. 407) has reported about 150 species of Psoralea of which 35 have been reported as occurring in the United States (Britton and Brown, 1897, p. 280-281). The name Psoralea is derived from the Greek "psoraleos" which means scabby. This refers to the glands or dots found on the foliage of the plant. The genus has been characterized by Rydberg (1932, p. 463) as follows:

> Perennial herbs, with glandular-punctate foliage, leaves alternate and 3-5 foliolate with entire leaflets; calyx campanulate, lobes equal, or the lower longer; flowers

small, perfect, in racemes or interrupted spikes. Corolla white or purplish; banner usually broad and auricled at the base; wings clawed, scythe-shaped; keel shorter than the other petals, incurved. Stamens diadelphous or monodelphous. Pods broad, indehiscent, and 1-seeded.

According to Stevens (1950, p. 188) four species of Psoralea have been reported in North Dakota, and addition to P. argophylla, include P. esculenta, P. lanceolata, and P. tenuiflora.

Psoralea esculenta or the "Indian Breadroot" is widely distributed in North Dakota but does not appear to be very abundant. In a study of North American Prairie, Weaver (1954, p. 32) stated that P. esculenta ranked only 60th in importance on upland prairie and never occurred in lowland. Based on records cited in Bolley and Waldron (1900, p. 612), in Bergman (1912, p. 264), on specimens in the University of North Dakota herbarium, and personal observations, P. esculenta is known to occur in the following counties: Barnes, Billings, Cass, Grand Forks, Grant, Kidder, LaMoure, Logan, McKenzie, Ramsey, Ransom, and Sioux. P. lanceolata according to Stevens (1950, p. 188) is largely restricted to areas of North Dakota west of the Missouri River but there are records east of the river from Logan and Kidder counties. P. tenuiflora was reported by Bolley and Waldron (1900, p. 612) as being widely distributed in the state. If this statement was correct, however, it now appears that this species is rare, as Stevens (1950, p. 189) reported only two records, one from Bowman in 1918 and the other from the Badlands in 1892. P. argophylla is widely distributed in the state as

indicated by Bolley and Waldron (1900, p. 612), by Bergman (1912, p. 264), by specimens in the University of North Dakota herbarium, and from personal observations. It probably occurs abundantly in every county in the state but has only been reported or observed in the following counties: Barnes, Benson, Billings, Burke, Burleigh, Cass, Cavalier, Grand Forks, Grant, Griggs, LaMoure, McHenry, Morton, Pembina, Ransom, Richland, Stark, Steele, Stutsman, Walsh, and Ward.

The reported range of P. argophylla extends eastward to Minnesota and Wisconsin, southward to Missouri and Oklahoma, westward to Montana, Wyoming, Colorado, and New Mexico, and northward to Saskatchewan (Gleason, 1952, p. 407; Fernald, 1950, p. 898). Britton and Brown (1897, p. 283) reported that it was located as far north as Northwest Territories in Canada. Hitchcock et al., (1961, p. 348) stated that probably it is found exclusively east of the Rocky Mountains.

P. argophylla is not only abundant in the grasslands of North Dakota, but is one of the dominant herbs of the prairies and plains of central North America. Weaver (1954, p. 71-72) has reported that in 74% of the upland prairies studied P. argophylla was present and it formed societies of first rank in 37%. It was also found in 43% of the lowlands but was less abundant.

The plant consists of both aerial and underground stems, and of a taproot about 1/2 of an inch or less in diameter which tapers rapidly with depth and gives off fine branches. The roots may penetrate several feet

into the soil (Weaver, 1954, p. 113). The upper portion of the taproot is rhizomatous and produces buds which give rise to aerial shoots or to rhizomes extending laterally through the soil. The aerial stem is largely herbaceous while the underground portion of the stem is woody and farinaceous. The boundary between the herbaceous and woody portions lies in the area of the abscission zones at ground level. Following abscission the woody underground stem decays and eventually becomes detached from its base on the taproot (Pl. I, Fig. 1-2). The following characters for the aerial stem have been described by Rydberg (1932, p. 464):

> Stem erect, 3-6 dec. high, branched above, strigose-canescent; leaflets 1.5-4 cm. long, 6-20 mm. wide, obovate or oval, obtuse, densely white-silky on both sides or grayish strigose above; spike rather short, calyx silvery; tube about 2 mm. long; upper teeth 2-3 mm. lanceolate; the lower one narrower, 6 mm. long, in fruit over 1 cm. long (Pl. I, Fig. 3-4)

The following synonyms have been listed for P. argophylla (Britton and Brown, 1897, p. 283; Hitchcock et al., 1961, p. 348).

Psoralea argophylla (Pursh, Fl. Am. Sept. 475, 1814)

Psoralea incana (Name only 1813. Nutt, Fraser Cat.)
(Nutt, Gen. Pl. 2: 102, 1818)

Lotodes argophyllum (Kuntze, Rev. Gen. L: 194, 1891)

Psoralea argophylla robustior (Bates, Am. Bot. 20: 16, 1914)

Psoralidium argophyllum (Rydberg, N. Am. Fl. 24: 16, 1919)

TUMBLEWEEDS

Tumbleweeds are a diverse group of plants which have the adaptation for disseminule dispersal by tumbling or rolling over the ground. They are not only found among angiosperms, but also in the lower plants such as lichens, mosses, and lycopods (Ridley, 1930; Polunin, 1960). Ridley (1930, p. 33) defined tumbleweeds "as those in which the whole infructescence, or a part of the plant, or the whole of it carrying the seed, is torn off by the wind and drifted along, releasing and distributing the seed as it goes". According to Pound and Clements (1900, p. 156) they are characteristic of prairies, plains, deserts or level open stretches throughout the parts of the world where there are strong prevailing winds. Pound and Clements (1900, p. 156) also noted that in the ten species of tumbleweeds found in Nebraska, not a single one had fruits that were adapted for wind-dispersal, but rather the fruit was a small naked akene or caryopsis. Furthermore, they classified tumbleweeds into two types (1) typical tumbleweeds, characterized by a rounded, diffusely branched tip, which drys up in late summer and autumn, and breaks away from the root and goes tumbling over the ground and (2) tumble grasses in which the entire plant or only the large spreading panicle is transported.

The following species which have been reported as tumbleweeds

(Ridley, 1930; Polunin, 1960; Pound and Clements, 1900) are found in North Dakota (Stevens, 1950):

Typical Tumbleweeds

Amaranthaceae

Amaranthus albus
Amaranthus graecizans

Chenopodiaceae

Atriplex rosea
Axyris amaranthoides
Corispermum hyssopifolium
Cycloloma atriplicifolium
Kochia scoparia

Fabaceae

Psoralea argophylla
Psoralea esculenta
Psoralea tenuiflora

Cruciferae

Sisymbrium altissimum

Solanaceae

Solanum rostratum

Tumble Grasses

Gramineae

Agrostis scabra
Eragrostis pectinacea
Panicum capillare
Schedonnardus paniculatus

Although P. lanceolata has not been reported as a tumbleweed, it probably acts as one since observations of herbarium material indicate that stem abscission in this species also may occur. P. esculenta was first reported as a tumbleweed by Pound and Clements (1900, p. 157)

and later by Ridley (1930, p. 34). P. tenuiflora was first reported by Pound and Clements (1900, p. 157) and later by Weaver (1954, p. 72), who stated that in P. tenuiflora "an abscission layer forms in late summer weakening the stem near the ground". Todd (1883) first reported P. argophylla as a tumbleweed. He observed that a joint is formed in the stem very near the top of the ground as is done in a leaf when it is detached from a branch. Apparently referring to the joint he stated "it cuts through all the tissues so that when the top dries up and begins to sway in the wind, it is broken off very readily or evenly".

At first it appears that abscission in P. argophylla is just another adaptation so characteristic of tumbleweeds which enables the entire plant body to be detached and rolled so that disseminules may be dispersed. Stevens (1950, p. 188) reported that he had not seen a seed from P. argophylla until 1947. Cratty (1880) from northern Iowa stated that "after three seasons, I have not been able to find a single mature seed". Personal observations of the plants in the Barnes County study area during the summers of 1963 and 1964 revealed that seed production did occur. It was erratic, however, and if the plant produced seeds at all, the quantity was greatly reduced. Thus, it appears that vegetative propagation is currently the most important means of reproduction in P. argophylla, and that the tumbling habit may be of little value in seed dispersal and establishment of new colonies.

DESCRIPTION OF THE STUDY AREAS

The study areas are located in two of the three major physiographic regions of North Dakota, the Red River Valley, the Drift Prairie, and the Missouri Plateau (Wills, 1963, p. 31) (Fig. 1).

One of the study areas is in the Drift Prairie region on gently undulating topography which is pitted by numerous small and large kettles. This area is in northwestern Barnes County (Fig. 2) and is on the southwest quarter of Section 11, T. 142 N., R. 60 W. This site is occupied by a large kettle which lies 25-30 feet below the surrounding till plain. The bottom of the kettle is essentially level and usually is occupied by a shallow alkaline lake. The upper slopes along the edges of the kettle adjacent to the level upland are very short and steep, while the lower slopes are long and slope gently toward the lake bottom (Fig. 3). Thus, the area provides a variety of ecological situations, ranging from the level well-drained upland, through excessively drained steep slopes, to the moister more gentle slopes of the lowland.

The second study area is in Turtle River State Park in the Red River Valley (Fig. 1). This park comprises the southwest, southeast, and northeast quarters of Section 36, T. 152 N., R. 54 W., in Grand Forks County (Fig. 4). The valley of the Turtle River occupies most of the

park although smaller areas of till upland and isolated portions of three beaches of glacial Lake Agassiz are also present (Laird, 1944). Woodlands are most abundant on lower elevations in the Turtle River Valley on alluvial deposits, while prairie areas of grassland and low shrubs predominate on the till uplands and lake beaches (Fig. 4). Psoralea argophylla was collected in the prairie areas shown in Fig. 4 on sandy, well-drained soils on the lake beaches and till uplands. In these areas Symphoricarpos occidentalis and Poa pratensis were the dominant plants.

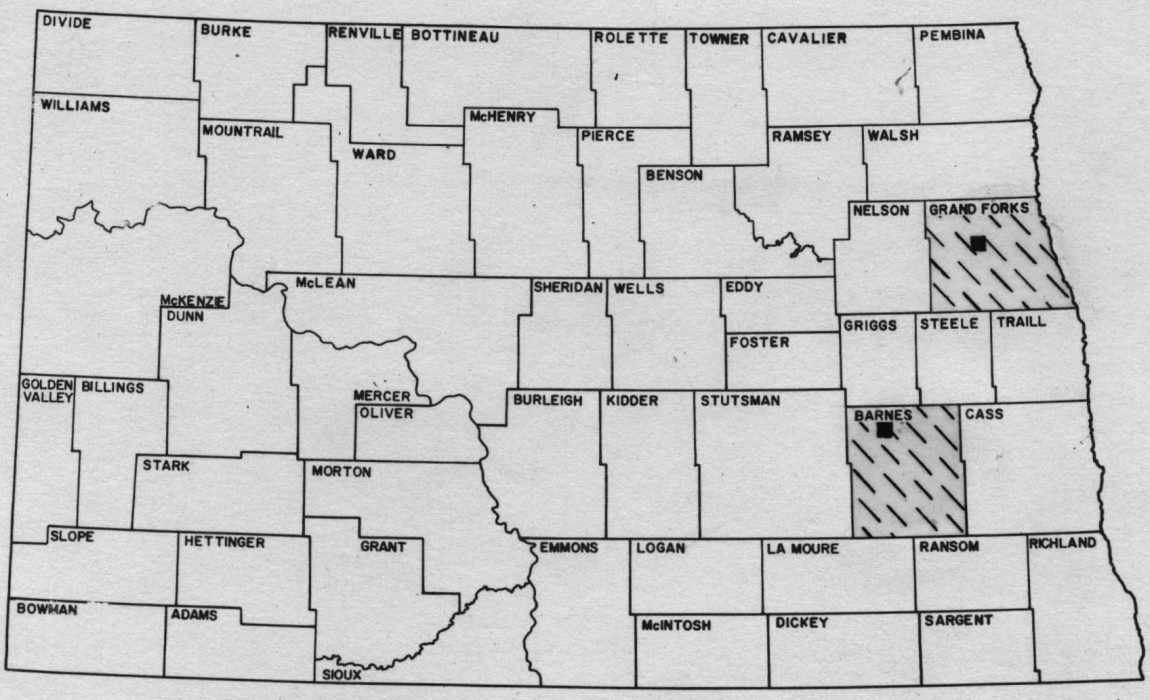

Fig. 2. County map of North Dakota and location of the study areas.

Fig. 3. Aerial photo of the S. W. 1/4 of Section 11, T. 142 N., R. 60 W., Barnes County. a, alkaline lake; b, study area. Courtesy of the Agricultural Stabilization and Conservation Service, Valley City, North Dakota.

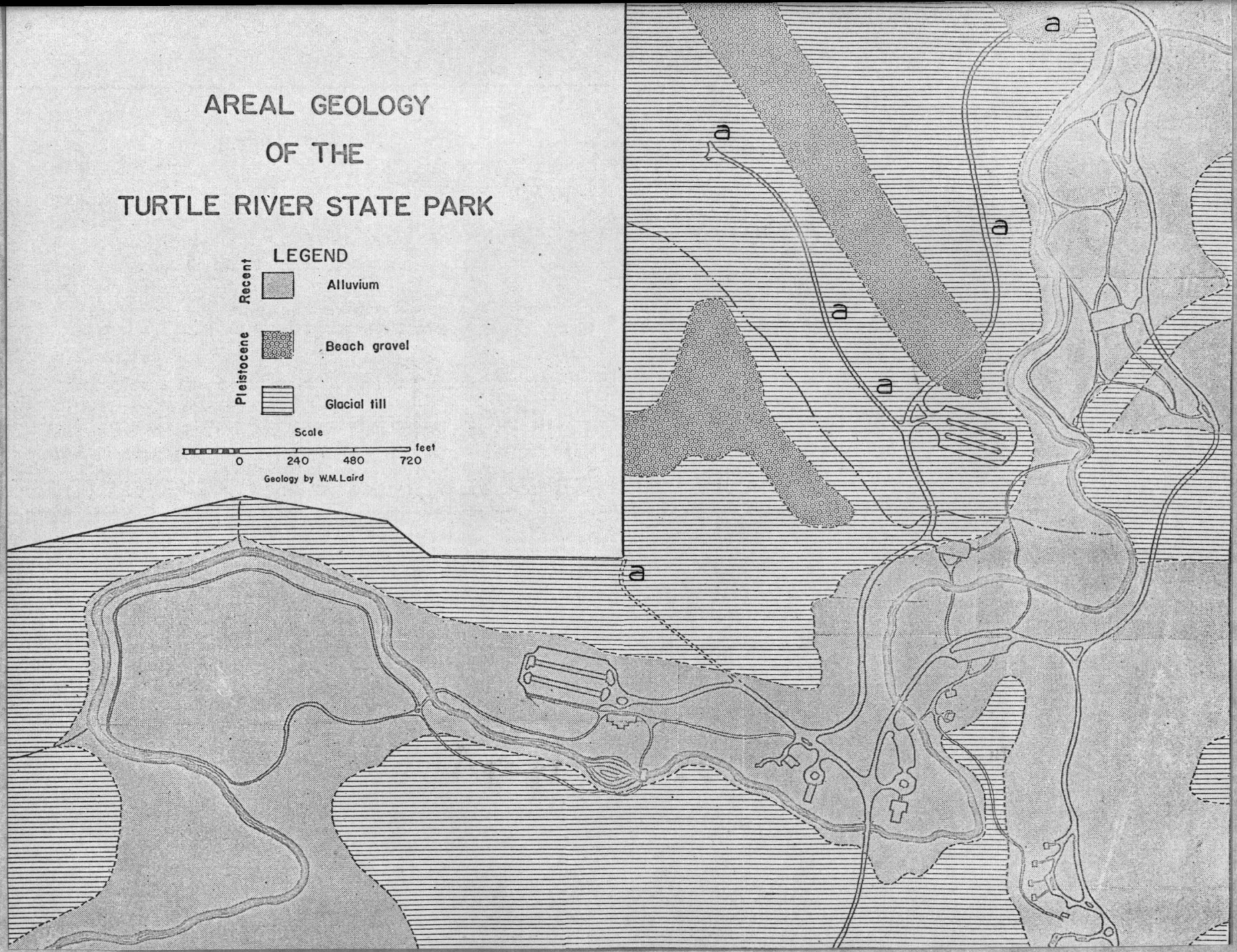

Fig. 4. Turtle River State Park study area. a, collecting areas (Courtesy of W. M. Laird, 1944).

METHODS

Psoralea argophylla was collected at weekly intervals during the summer months from early June to mid-September during 1963 and 1964 in the Barnes County study area. From each plant collected, a portion of the stem including the abscission zone was removed and preserved in 70% ethyl alcohol, while the remainder of the plant was pressed. Thus, a particular abscission zone could be associated with a particular plant, and the relationship between the phenological stage of the plant and the progression of abscission could be traced. In this area ecological observations were made also and the occurrence of abscission in the field was studied.

Other material was collected in Turtle River State Park, Grand Forks County in late abscission stages only, and was either examined immediately or allowed to dry naturally. The naturally dried material was placed in 70% alcohol for 1-2 weeks prior to examination in order to soften the tissue sufficiently for sectioning.

Alcohol-preserved material was generally hand-sectioned prior to examination, although some of this material was dehydrated, infiltrated, and finally embedded in paraffin. The n-butyl alcohol method of dehydration, modified after the tertiary butyl method of Johansen (1940), was

used as well as his methods of infiltration and embedding. Sections of thicknesses varying from 8-20 μ were cut on a rotary microtome. The paraffin was removed from these sections and they were then hydrated, stained with ruthenium red, and utilized for photography or morphological studies, while others were made into permanent mounts by staining with the safranin-fast green combination and mounting in canada balsam as outlined by Johansen (1940).

Microchemical tests were used to differentiate the substances directly or indirectly associated with the abscission process. Tests for cellulose, starch, suberin, and lignin followed those methods described by Johansen (1940). The pectic materials in the cell walls and middle lamella were differentiated by the use of ruthenium red, and by solubility tests and microscopic examination. This procedure was based upon a modification of the methods used by Facey (1950). The pectins were extracted with water, pectic acid with 2% ammonium hydroxide (80 C), and harder pectic materials with 1% hydrochloric acid (80 C) followed by treatment with 2% ammonium hydroxide (80 C).

Highly esterfied pectins were observed by the use of a more recent pectin test developed by Reeve (1959a). This method is based on the reaction of alkaline hydroxylamine hydrochloride with the methyl esters of pectins to produce pectin hydroxamic acid, which in turn produces insoluble red complexes with ferric ions. The amount of color produced with this reaction depends on the amount of the esterfied pectins present

and the degree of esterfication of the pectin present. The procedure outlined by Reeve (1959a) was used except that the reagents were made in 60% alcohol to prevent the loss of water soluble pectins, as suggested by Jensen (1962).

Most of the microphotographs were taken of relatively thick hand sections which were stained according to the qualities desired in the photographs. In all cases the tissue was photographed in water mounts shortly after the slide was prepared. The photographs were taken at magnifications of 100 X and 440 X and subsequently enlarged for printing.

ECOLOGY

Ecological Studies

The small societies or colonies formed by Psoralea argophylla appear to be most extensive on the moister areas of grasslands at the base of, or on the lower more gentle slopes of moraines, kames, kettles, ravines, and similar features, or on moist but well-drained level lowlands. Such areas generally occur where big bluestem (Andropogon gerardi) and little bluestem (Andropogon scoparius) form mixed stands (Weaver, 1954, p. 70). In the study area in northwestern Barnes County, the largest individual plants and the most extensive societies occurred in moister areas in the mixed bluestem stands on the gentle slopes near the bottom of the kettle. On adjacent drier level upland prairie, dominated by Poa pratensis, the societies and individuals are smaller. Still smaller societies and individuals were found on the sparsely covered, steep, short, west-southwesterly facing slopes of the kettle. In the latter area the mid and short grasses prevailed and consisted mostly of patches of Bouteloua gracilis, Stipa viridula, Stipa comata, Muhlenbergia cuspidata, Koeleria cristata, and Calamovilfa longifolia.

The colonies or societies of P. argophylla will maintain or

re-establish themselves in an area which has been disturbed by cultivation. Observations in an area of Section 14, T. 142 N., R. 60 W. in Barnes County, which had been cultivated 10-20 years, and then moderately grazed for 20 years, revealed the presence of well developed societies which appear to be as common as in other areas that have never been under cultivation. Examination of the roots of these societies revealed conspicuous scars formed at about the 6-8 inch level below the ground surface, which marks the depth at which plow lays penetrate the soil. That these roots have remained alive during the cultivation period is remarkable. One of the two following reasons may explain this phenomenon: (1) The roots were able to lie dormant below the plow sole layer during the many years of cultivation. Then, after the ground was left undisturbed and a grass cover was re-established, were able to renew their growth and form the present day colonies. (2) Growth was able to take place continuously during the period of cultivation. This is possible, since this area was cultivated before 1945 when cultivation was less intense than at present. The absence of the plants from cultivated prairies today can be explained by more intensive cultivation practices.

Abscission in the Field

Weekly observations of plants in the field and of pressed material reveal that approximately two or three weeks before the plant abscises there are changes in the appearance of the foliage of the plant. These

changes begin some time after flowering, or appear to be postponed if the plant produces seeds. The first visual signs of the approaching senescence leading to abscission are discoloration and withering of the lower leaflets of the main stem and branches. At about the same time, the green stem becomes mottled with brownish-black blotches near the ground level. The withering and subsequent loss of leaves progresses upward along the lower main branches of the stem. Simultaneously, the stem becomes progressively more mottled upward with blotches, and the green color of the stem gradually disappears. As these changes continue until shortly before abscission, the plant appears mostly withered and partly defoliated. All but perhaps 1-2 terminal branches are dried up and many of the leaves from the lower branches have been shed. The entire main stem, and also the lower portions of the main branches now have large brownish-black blotches over most of the surface. The long, white hairs, which were formerly inconspicuous against the green background of the stem, now stand out prominently against the dark background and produce an overall grayish hue to the stem. Not all abscised stems, however, have this coloration, for some are predominantly green with fewer blotches. Also, in some cases the entire plant is dried up before abscission. This later observation was also made by Christy (1887) who stated:

> By the time the fruit is ripe, flowers, stalks, and all become dry, brown, rigid, the plant separates just above the ground and is blown along; frequently dry

plants are found arrested and collected together in the wagon tracks of the settlers.

These differences in appearance of the abscised plants may depend upon external factors such as winds. By exerting force upon the widespread crown of the plant, wind can readily cause separation of cells in the softened abscission layer, and breakage of the vascular strands. Strong winds occurred frequently during late August and early September of 1964 when abscission was at its peak. Freshly abscised stems were more abundant on the prairie during and immediately following a strong wind storm than during periods of calm weather. Thus, this mechanical agency may hasten and promote the natural process of separation by providing a strong force to aid in severing the aerial plant from its underground connection.

Environmental Factors and Abscission

Observations of the abscission of Psoralea argophylla during the summers of 1963-1964 indicated differences in the time of abscission with respect to the topographical site and local climactic conditions. The time at which abscission occurred varied, depending on whether a society was located in the lowland near the bottom of the kettle, or on the steep slopes and upland areas at the edge of the kettle.

In the summer of 1963 abscission was first observed on upland areas on July 28. At this time flowering was still common in the lowland near the bottom of the kettle. Abscission in the upland areas was

at its peak by August 18, was nearly completed by August 25, but was not entirely completed until August 31. In the lowland areas, however, abscission had just begun in September when the majority of plants were green and healthy. Abscission was not completed in these areas until late September to early October.

In the summer of 1964 a midsummer drought adversely affected plants of P. argophylla growing on the steep slopes along the edges of the kettle. On July 15 many plants were in the process of drying up with or without previous flowering. By July 23 abscission was first noticed in those plants that were not affected by the drought. Plants in the moister lower areas did not show any apparent effects from the drought. In the upland areas abscission was completed between August 9-17. Abscission in the lowland areas did not occur until later, and was not completed here until September 26.

During both summers abscission began first in the upland areas in late July and was completed in lower areas by late September or early October. The differences in the time of abscission on the different topographical sites appears to be partially explained by differences in the moisture conditions of the soils. However, exposure and soil temperatures also probably cause differences in time of abscission. For example, on the upland areas the plant emerges from the ground in late May, but in the lower areas, where the temperature of the upper soil may be as much as 6 C below that of the upland areas, the plant

frequently does not emerge until mid-June. In the lowland areas flowering may persist until late August to early September, while it is completed by late July on the upland. On different topographical sites differences in exposure and soil temperatures are perhaps important factors in accounting for the differences in timing of such phenological events as shoot growth, flowering, and abscission in P. argophylla.

In an effort to explain some of the differences in the time of abscission of plants occupying similar topographical sites during the two summers, temperature and rainfall values for three summer months were compiled.

TABLE 1

MONTHLY RAINFALL AND AVERAGE TEMPERATURES
IN THE BARNES COUNTY STUDY AREA

1963			1964		
Month	Rainfall[a]	Temperature[b]	Month	Rainfall[a]	Temperature[b]
July	5.25	+0.4 F	July	1.70	-0.1 F
August	3.80	-0.3 F	August	5.35	-4.8 F
Sept.	--	+2.5 F (no frosts)	Sept.	--	-4.2 F (6 frosts)

a Total monthly rainfall in inches.

b Temperatures are above (+) or below (-) the average monthly temperatures recorded at the weather reporting station in Valley City.

The earlier occurrence of abscission in the summer of 1964 than in 1963 appears to be due largely to drought in July of 1964: from June 22 until July 21 no appreciable moisture fell, after which 1.70 inches fell during the remainder of the month. In 1963, however, 5.25 inches of rain were recorded during July which kept soil moisture levels adequate. The earlier completion of abscission on the upland areas during 1964 appears to be also due to moisture conditions, since no appreciable rain fell from August 1-22, after which there were 5.25 inches in heavy rains during the remainder of the month. In 1963, however, there were adequate moisture reserves on August 1 and the rain which fell during August (3.80 inches) was more evenly distributed. The more rapid abscission during September of 1964 than in 1963 appears to be better correlated with temperature than with moisture conditions. In September of both years moisture supplies were adequate during the month and the amount of rainfall was near normal. However, September of 1963 was unusually warm with above-normal temperatures and no frosts, while in 1964 temperatures were far below normal, since there were six nights in which the temperature was below 32 F, the coldest being in the low 20's. Since frost and extreme temperatures are known to affect abscission, these factors may have speeded abscission during September of 1964, while the warm, frost-free days of September of 1964 retarded abscission.

MORPHOLOGY OF THE ABSCISSION ZONE

External

Plants typically lose organs or parts of organs by the development of a single abscission zone. Psoralea argophylla, however, frequently develops two abscission zones. Less commonly, particularly on small stems, only one abscission zone is present, and occasionally three zones are formed, usually on large stems. These abscission zones are revealed by the presence of swellings or joints at the base of the aerial stem (Pl. II, Fig. 2). The presence of a joint at the base of the stem, however, does not guarantee that an abscission layer will eventually form, since not all joints function in abscission. These joints are actually nodes of the stem and are considered as such for several reasons: (1) There is typically a lateral bud that develops on one of the sides of the joint. This bud may be well developed and have branch or leaf traces early in the ontogeny of the aerial stem, but become arrested in growth, die, and finally abscise in later ontogenetic stages of the plant. In old plants the scars formed by the loss of these buds are clearly visible. (2) There is a intercalary meristem, particularly noticeable in the pith. (Pl. III, Fig. 1). (3) There are sheath- or scale-like leaves arising from the cortical region of the joint (Pl. III,

Fig 1). These scale-like projections are actually the old bud scales of the shoot left behind as the stem grows upward (Pl. II, Fig. 3-4). These scales are imbricate early in the development of the shoot and serve to protect its apex, but as the young shoot grows upward and internodal growth continues, the scales become located farther apart on the stem until they resemble leaves. At maturity of the plant these scales at the base of the aerial stem become brown, torn and shredded, resemble bark, and exist only as mere remnants of the former scale structure. As the leaf scales gradually become lost from the node with age, the constriction in the cortex marking the point of scale attachment at the node becomes noticeable. Externally, these constrictions appear as ring-like grooves in the middle of the joints, and mark externally the point at which the abscission layer develops internally across the stem (Pl. III, Fig. 1).

The abscised surfaces of the stem are related to each other as a ball-and-socket joint in much the same way as described by Eames and MacDaniels (1947, p. 272) for the abscission of branches in Populus grandidentata. The lower abscised surface of the stem of P. argophylla tends to be slightly concave with a peripheral ring of perforations. These perforations are the holes left by the fibers of the vascular bundles which protrude from the convex upper abscised surface (Pl. I, Fig. 2; Pl. II, Fig. 1).

The basal joints or nodes may be as close as 4 mm, or as far apart as 4 cm. If the joints are located close together, all develop abscission

layers and are usually at the same stage of abscission; if they are over 2 cm apart, only one will develop an abscission layer. Typically, if the joint is at or near ground level, a well-developed abscission zone and abscission layer will be present. In an effort to establish some relationship between field abscission and the number and position of the abscission zones involved in the separation process, 50 plants near or at the separation stage were selected on August 28, 1964. The plants selected were either those which had recently abscised, but were held in place above the exposed socket by adjacent vegetation, or those which could be separated by slight hand pressure. Of the 50 plants examined, 44 had two abscission zones as indicated by two separate breakages of the stem. Only six of the 50 plants had one abscission zone. In those plants which had two abscission zones, there was no preference for separation to occur at the lower or upper zone. Eighteen of the 44 plants separated at the upper zone; 21 separated at the lower zone; in five stems the two zones were at equal stages of development since both abscised with equal pressure.

In order to establish the relationship between the location of the different abscission zones and the ground level, 50 other intact plants were observed in five different societies. The lower abscission zone was found to be level with the ground surface in seven of 50 cases. In all other instances it ranged from 1/8-3/4 inches below the ground, typically being 1/4 inch below the ground level. The upper zone of

28 stems, however, was level with the ground. The upper zone of the other stems varied from 1/8 inch to 1-2 inches above the ground, the typical height above the ground being 1/8-1/4 inch. From these studies it appears that a joint at or near the ground level is more likely to develop an abscission layer than a joint located far below or far above the ground. In P. argophylla environmental factors then may play a more important role in determining the site of the abscission process than internal factors. The ecological approach to abscission needs further investigation since little is known about the ecological factors which may retard or prevent abscission from occurring in a particular area of a plant organ.

Internal

The shedding of leaves and certain other plant structures of angiosperms typically proceeds through the development of an abscission zone and abscission layer. Although P. argophylla possesses such an arrangement for stem abscission, the abscission zone and abscission layer are not as conspicuously defined as in the woody angiosperms which have a protective layer in the abscission zone formed before or after abscission. In this study the abscission zone was considered to be equivalent to the intercalary meristem and consequently extends as a broad region of 10-30 layers of cells across the pith, vascular tissue, and cortex. The abscission zone is more parenchymatous than other sections of the stem and

is responsible for the wider cortical, vascular, and pith regions in the abscission zone. While usually clearly delimited in the pith, the abscission zone is not as sharply defined in the other regions. In a freshly cut section of a green stem, the abscission zone appears light green to the naked eye due to the high concentration of chloroplasts. In older senescent stems this area appears brown due to the accumulation of dense protoplasm and starch in the abscission zone cells (Pl. II, Fig. 1).

The parenchyma cells of the cortex of the abscission zone are different in appearance from the parenchyma cells of the pith of the abscission zone. The former cells in longitudinal sections are generally isodiametric, are smaller, have many intercellular spaces, and have no definite arrangement or orientation (Pl. III, Fig. 2). In transverse sections they resemble collenchyma cells since the walls are thickened at the corners. The walls also possess a hydrophilic nature due to the high concentration of esterified pectic materials, and shrinkage results upon dehydration with alcohol.

In the vascular portion of the abscission zone the xylem and phloem are greatly reduced structurally and are also essentially non-lignified. In the phloem the most conspicuous reduction is in the phloem fibers. Outside the abscission zone, these phloem fibers are very conspicuous, since in these areas they are extremely thick-walled, heavily lignified cells aggregated in bundles that are located in the outer region of the

phloem adjacent to the pericycle. Although extending into the abscission zone from above and below, they are greatly reduced and non-lignified in the area where the abscission layer eventually forms, and may actually be discontinuous across the region of the abscission layer. In the xylem the vessels and fibers are reduced and all tissue except the vessels are non-lignified in the abscission zone. In this region they are composed of many short vessel segments that are approximately 1/2 of the length of the vessel segments found elsewhere. This increased segmentation of the vessels produces a knobby, jointed appearance, while the vessels in other areas are essentially straight and have smooth outer margins (Fig. 5) (Pl. III, Fig 1; Pl. IV, Fig. 1). Often these short vessel elements are not oriented in the plane of a longitudinal section, since they appear discontinuous across the abscission zone (Fig. 5). A vessel lying outside of this zone can be traced for some distance before it disappears from the field of the microscope. Transverse sections reveal that these vessels may be oriented for short distances in an oblique or horizontal plane before they return to the vertical plane. Transverse sections reveal further modifications of the vascular cylinder in the abscission zone, for: (1) The number of vessels in the vascular bundles are fewer per unit area than in adjacent tissue. (2) The diameter of the vessels is reduced in the abscission zone. (3) The arrangement of the vessels is different because in the vascular bundles in the abscission zone the vessels are scattered, while in vascular bundles out of the

abscission zone the vessels are typically arranged in a linear series radiating in the direction of elongation of the bundle. (4) There is a leaf gap in the vascular cylinder associated with the lateral bud, and a general increase of parenchymatous cells in the vascular bundles. (5) Pith rays are non-lignified in the abscission zone but lignified in other areas of the stem. Therefore, the combination of structural modifications and reduction of lignification makes the abscission zone a mechanically weak area in the stem. This can be demonstrated most simply by merely comparing the relative amount of force needed first to cut transverse sections through the abscission zone, and then through adjacent tissues. Sections can be readily cut through the abscission zone with a sharp razor blade, but in other areas greater force is required to section the tissue.

The intercalary meristem is most conspicuous in the pith. In newly emerging shoots it is not evident since the internode is uniformly meristematic. But as the stem elongates, part of the internode matures more rapidly than the remaining portion, and the younger cells are then left at the base of the internode or in the nodal region. In higher nodes of the stem, which do not develop abscission zones, the intercalary meristem has essentially lost the meristematic appearance. In the nodes near or at ground level, however, the meristematic appearance essentially persists throughout the ontogeny of the plant. The cells of the pith of the abscission zone or intercalary meristem are small, tabular-arranged parenchyma cells (Pl. III, Fig. 1; Pl. VI, Fig. 2).

They are non-lignified, unsuberized, they lack conspicuous pits, and have a greater abundance of calcium oxylate crystals than pith cells found elsewhere in the stem. The number of tiers or layers of cells in this part of the abscission zone varies according to the size of the stem, but typically ranges from 10-30; they are distinct from the mature parenchyma cells of the pith because they are smaller and are non-lignified. Thus, like the vascular tissue and cortex, the pith also is non-lignified in the abscission zone.

During senescence the abscission layer develops across the abscission zone (Fig. 5). It consists of 2-3 layers of cells, which in early stages of development, can be distinguished only on the basis of its cellular contents, since the cell size, shape, and orientation appears to be the same as adjacent cells in the abscission zone. In later abscission stages cell division occurs throughout much of this layer and 2-3 thin cross walls are formed. Cell division is most frequent in the pith, with less activity in the cortex, while no cell division occurred in the cells of the vascular tissue. The abscission layer cells consist of dense brown protoplasm and often are partially or completely filled with starch grains. When the starch-filled cells were stained with potassium iodide-iodine (IKI), they appeared as a black layer, the black contents of the cell contrasting sharply with the white-appearing cell wall (Pl. IV, Fig. 1). This layer with its easily removed cellular contents was found intact only in alcohol-preserved material. In naturally dried stems

much of the starch and dense protoplasm had already disappeared. In paraffin sections much of the starch was removed during the dehydration and embedding process and the abscission layer could be determined readily only if cell division had occurred previously. As a result, most of the work on the abscission layer was limited to hand-sectioned alcohol-preserved and naturally dried material.

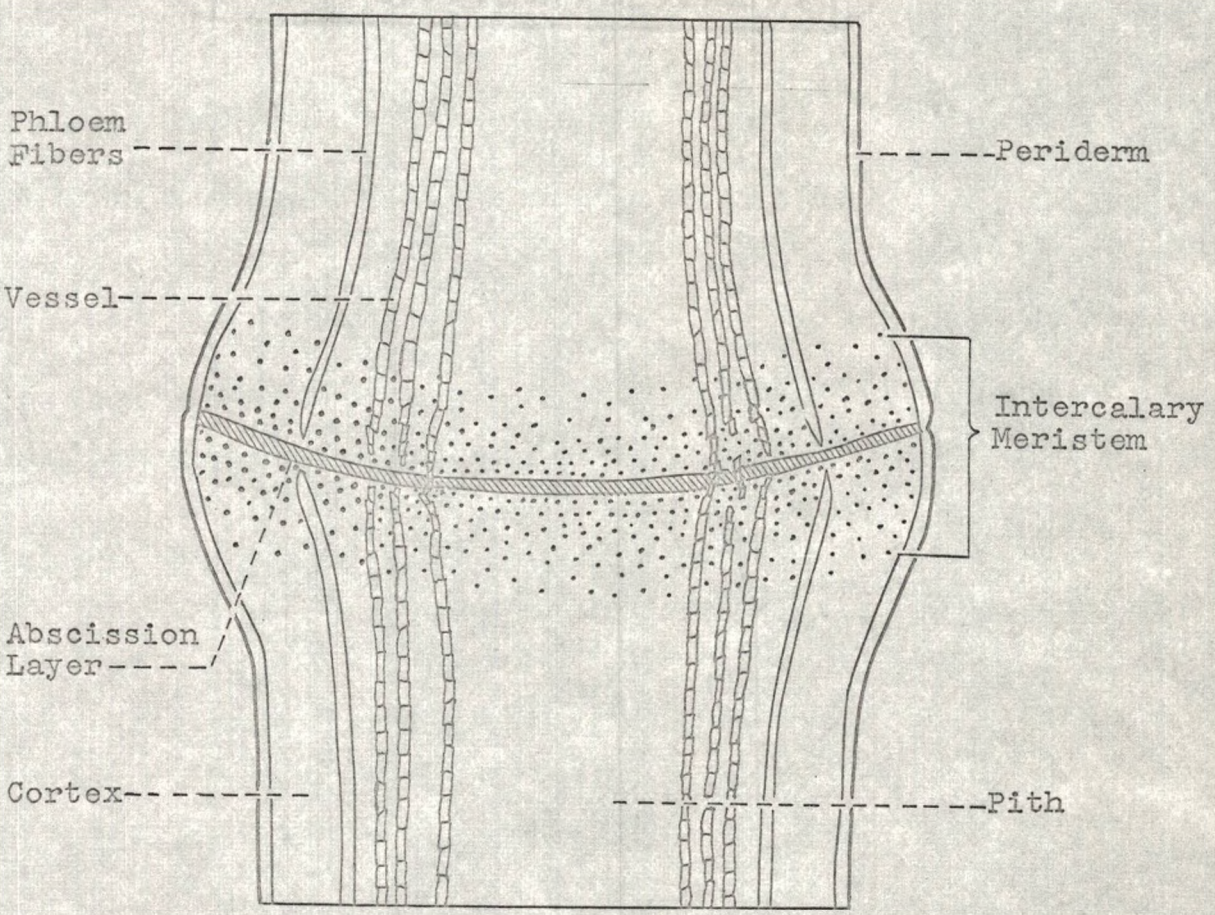

FIG. 5. DIAGRAM OF THE ABSCISSION ZONE (L.S.)

THE CELL WALL AND THE PECTIC SUBSTANCES

Since an understanding of the terminology of the cell wall and pectic substances is necessary for the understanding of the abscission process, a brief discussion of the cell wall and pectic substances will be included in this paper.

The term "middle lamella" is used interchangeably with "intercellular substance" as recommended by Bailey (1954, p. 69). This is the thin cementing layer, attaching contiguous cell walls; it is composed chiefly of pectic materials and is isotropic. The general term "wall" refers to the thicker, anistropic layer of cellulose, pectic materials, and other cell wall materials adjacent to the middle lamella. The cell wall of P. argophylla affected by the abscission process may be the primary wall of the cell because they are located in the intercalary meristem, contain much pectic material, and lack conspicuous pits. The term "compound middle lamella", used for convenience only, refers to the 3-layered structure visible under the light microscope. This structure in P. argophylla includes the intercellular substance and the two contiguous cell walls.

The pectic substances are a group of complex colloidal carbohydrates that occur in plants and are composed of long chains of linked

galacturonic acid residues. The carboxyl groups of these polygalacturonic acids may be partly esterified by methyl groups and partly or completely neutralized by one or more bases such as calcium or magnesium (Kertesz, 1951, p. 6). If the carboxyl groups of the polygalacturonic acid are free of methyl groups the substance is known as pectic acid. This substance is insoluble in water but soluble in hot dilute alkali. If the carboxyl groups of the polygalacturonic acid are partially esterified with methyl groups the substance is known as pectin. This substance is water soluble and the degree of water solubility depends on the degree of esterification and molecular weight. Deuel and Stutz (1958) mentioned that water solubility increases as the degree of esterification increases and size of the pectin molecule decreases. If the carboxyl groups of polygalacturonic acid are free of methyl groups and combine with magnesium or calcium, a pectate is formed which is insoluble in water and dilute acid. Such a material, however, becomes soluble if the dilute acid treatment is followed by a dilute alkali treatment. Another pectic substance, "protopectin", was defined by Kertesz (1951, p. 6) "as the water insoluble parent pectic substance which occurs in plants and which, upon restricted hydrolysis, yields pectinic acids". It is reportedly soluble in dilute acids but insoluble in dilute alkali. Protopectin is believed by most to be a separate pectic substance from those already described but there is no unanimity of opinion regarding its molecular structure (Joslyn, 1962). Roelofson (1959) believed it was "plausible to

regard protopectin as a network of chains of polygalacturonic acid held together by calcium ionically bonded between carboxyls of individual chains". According to Bonner (1950) protopectin is a macromolecule of pectin, which would explain its water insolubility. Henglen (1943) not only proposed calcium linkages between the polygalacturonic acid molecules, but also calcium linkages between the cellulose polygalacturonic acid molecules.

The state and distribution of the pectic materials in the cell wall of plant tissues is also somewhat confusing. Bonner and Galston (1952, p. 212) reported that in the living plant protopectin is found almost exclusively in the cell wall and that calcium or magnesium pectates largely make up the middle lamella. However, Frey-Wyssling (1959) stated "that the water-insoluble pectin in the middle lamella and primary cell wall occur in the young cell wall as protopectin". In view of these conflicting opinions it is probable that the type of insoluble pectic material and its distribution in the cell wall is quite variable and depends on the species of plant, the type of plant tissue, and the age of the tissue. In the present study, rather than attempt to distinguish between calcium pectate and protopectin, the original pectic material in the compound middle lamella will be designated as hard pectic material.

RESULTS

To facilitate the tracing of the changes in the cells of the abscission zone, stem sections of Psoralea argophylla were placed into three general classifications based on the phenological stage of the plant. Comparative studies were then made of sections from newly emerging and growing shoots, from plants in the bud and flowering stages, and from plants in senescent stages. Although progressive esterification and apparent increase of esterified pectic material was noted throughout the growing season, the pectic material remained insoluble until the abscission layer was finally formed in senescent stages. Other changes in the abscission zone cells, such as cell division and starch accumulation, are associated with the development of the abscission layer. Table 2 briefly summarizes the detailed results to follow.

TABLE 2

CHANGES IN THE ABSCISSION ZONE DURING ONTOGENY

Characteristic	Early Ontogeny	Flowering	Senescence
Dense protoplasm	+	-	+
Starch	-	-	+
Cell division	-	-	+
Hard pectic material	+	+	Trace
Pectic Acid	Trace	Trace	+
Pectin	-	-	+

+ present - absent

Abscission zone during early ontogeny

The cells of the abscission zone of Psoralea argophylla during the period of shoot growth and stem elongation are filled with a dense brown protoplasm. Calcium oxalate crystals are abundant but there is not starch accumulation in these cells. In this young tissue the ferric chloride-hydroxylamine test revealed the presence of significant amounts of esterified pectic material, insoluble in water, only in the vascular tissue. Comparable amounts of pectic material, however, were found not only in the vascular tissue of the abscission zone, but also distributed in the vascular tissue throughout the stem. Weaker esterification reactions in the abscission zone occurred in the cortex near the point of attachment of the bud scales, and in the pith. Staining these same tissues with ruthenium red prior to and following treatment with ammonium hydroxide at 80 C for two hours revealed the loss of small amounts of pectic material in the cell walls of the abscission zone, although the middle lamella was not noticeably affected. The same section treated with 1% hydrochloric acid for two hours at 80 C, followed by treatment with 2% ammonium hydroxide, resulted in the loss of the remaining pectic material in the compound middle lamella with concomitant maceration. Thus the pectic material of the compound middle lamella of the abscission zone cells at this stage, consists largely of insoluble pectic material.

Abscission zone during later ontogeny: budding and flowering

As the plant reaches its maximum size, and budding and flowering stages appear, the protoplasm of the abscission-zone cells becomes less dense, and the brown color so conspicuous in the earlier stages disappears. Meanwhile, the walls of the phloem fibers and cells of the cortex appear gelatinous in the abscission zone. The ferric chloride hydroxylamine test reveals that the area of gelatinous and swollen cell walls corresponds to areas of increased esterified pectic materials. Sections from plants in the flowering stage, when placed in water for 3-5 hours, did not lose any pectic material from these areas, thus revealing that the esterified pectic material is insoluble in water. At approximately the same stage the pectic material of the cells of the pith in the abscission zone also exhibit esterification reactions with the ferric chloride-hydroxylamine test, but again the pectic material is not affected by water treatment.

Abscission zone during senescent stages

As the plant begins to display signs of senescence, the abscission layer becomes visible microscopically and starch accumulates throughout the abscission zone. The abscission layer does not appear to develop simultaneously across the entire abscission zone, since in most cases it was found in varying stages of development. Large numbers of stems were examined before one was found with a recognizable abscission layer. In fact, of approximately 53 senescent-appearing stems, only 13

had abscission layers. Of these 13 stems examined, the abscission layer was present in both abscission zones in all but one, thus demonstrating the existence of two chemically active abscission zones, both of which may function in abscission. The abscission layers found in these sections were composed of mature-appearing cells filled with a dense, brown protoplasm and containing little or no starch, or of mature-appearing cells filled with starch, or of small starch-filled cells with 2-3 thin cross walls indicating cell division.

After the morphologically distinct abscission layers were found and recognized in intact stems, naturally abscised stems were examined to determine if remnants of the abscission layer could be distinguished. In such stems not only was the starch concentration greater in the 1-2 remnant layer of cells of the separated abscission layer, but also there were indications of cell division.

Ferric chloride-hydroxylamine tests

When intact sections with recognizable abscission layers were stained with ferric chloride-hydroxylamine, the abscission layers appeared as conspicuous light bands across the stem (Pl. IV, Fig. 2). The cells of the abscission layer produced no reaction, while the surrounding cells of the abscission zone gave an orange to very light red color reaction. These results indicate the absence of esterified pectins in the abscission layer and small amounts of esterified pectic materials

elsewhere in the abscission zone. With one exception, all of the 13 stems examined gave identical results. In the one exception the cells of the abscission layer exhibited the same staining reaction as the surrounding cells of the abscission zone.

The lack of staining of the abscission-layer cells, as well as the generally faint color of other cells of the abscission zone suggested the possibility that either highly esterified pectins were once present but had since been de-esterified, or that they were dissolved out in the 70% alcohol used to preserve the tissue during the period of storage. To determine if the former was the case, adjacent sections were methylated in .5N hydrochloric acid in dry methanol previous to staining as recommended by Reeve (1959a) and McCready et al., (1955). Little intensification of the reaction, however, was noted with this procedure. Next, the latter possibility was checked by processing the naturally dried material. With the use of the same methods, reaction times, and reagents, this material immediately produced a bright red color in the cells of the abscission zone, which in alcohol-preserved tissue formerly appeared orange to light red. At 1,000 X this color appeared to be concentrated in the middle lamella and wall of the cells. A large quantity of naturally dried material was then examined before a number of stems were found in which there were large concentrations of pectin across the abscission zone. Such abscission zones exhibited a bright red band extending across the abscission zone in the region of

the abscission layer (Pl. V, Fig. 1). This red layer is most conspicuous in the pith of the abscission zone, since in the cortex and vascular portion of the abscission zone the surrounding abscission-zone cells appear almost as bright as the cells of the abscission layer. In sections placed in water at room temperature for three-five hours, and then stained with ferric chloride-hydroxylamine, the red layer across the pith disappeared, and a light band, due to failure of staining and signifying absence of pectin, appeared across the vascular tissue and cortex in the region of the abscission layer (Pl. V, Fig. 2).

Ruthenium red and solubility tests

Staining with ruthenium red, together with solvent treatments and microscopic examination of alcohol-preserved tissue, confirmed and supplemented the results obtained with the ferric chloride-hydroxylamine test. Upon staining with ruthenium red, the cells of the abscission layer sometimes absorbed less stain, but usually stained uniformly with the remainder of the tissue of the abscission zone. Reduced staining of the cells of the abscission layer is compatible with that already discovered and again indicates that pectin has disappeared by dissolving in alcohol. Most sections with abscission layers, however, appeared to stain uniformly across the abscission zone. These same sections previously had not stained in the abscission layer with the ferric chloride-hydroxylamine test. This apparent contradiction may be explained if one

considers that only a portion of the hard pectic material had been converted to a highly esterified pectin, and hence dissolved out in alcohol. Still enough residual pectic material would then be left to absorb the ruthenium red stain and no perceptible loss of pectic material would result.

The pectic materials of stems containing dense protoplasm in the abscission layer, but little starch or cell division, responded less to solubility treatments than sections which had high concentrations of starch, or extensive cell divisions, or were partially separated in the abscission layer. These abscission layers with only dense protoplasm were not affected by hot water treatment, and when subjected to 2% ammonia for two hours at 80 C, the pectic material disappeared from only a few cell walls and middle lamellae, indicating the presence of only small amounts of pectic acid. However, treatment with 1% hydrochloric acid for two hours at 80 C, followed by treatment with 2% ammonium hydroxide, was necessary to remove the pectic material from the walls and middle lamellae. These sections tended to separate through the abscission layer only after a combination of the dilute acid and dilute alkali treatments. These results indicate that in early stages of abscission layer development the pectic material of the compound middle lamella is still largely insoluble.

Those abscission layers which had heavy starch concentrations, or extensive cell division, or were partially separated in the abscission

layer, or had various combinations of the above, were evidently in later abscission stages, since treatment of these sections removed larger amounts of pectic material. The pectic material of these abscission layers was also unaffected by water treatment at 80 C for two hours, but a widespread removal of pectic materials in the form of pectic acid occurred during a 2% ammonium hydroxide treatment at 80 C for two hours. In such sections the abscission layer stands out conspicuously as a light band across the abscission zone (Pl. VI, Fig. 1).

Separation of the abscission layer cells

In more advanced abscission stages the sections could either be separated with no prior treatment, or after a dilute hot ammonia treatment, by the application of slight pressure on a cover slip. Observations of these artifically separated sections, as well as of partially separated and abscised stem ends, indicate that separation proceeds through the middle lamella. This separation is not only made possible by the dissolution of the pectic materials of the walls and middle lamellae, but is also aided by preliminary cell division which forms a region of soft and delicate cross walls across much of the stem. Separation of cells appears to occur first in the cortex and pith and later through the vascular tissue. With the possible exception of a few of the vessels and the periderm, the separation of cells proceeds through the middle lamella across the entire abscission zone. Observations of separated vessel

ends revealed that while many have separated between the short vessel segments, others have separated across the vessel by rupture or breakage. Whether the separation tends to occur through the ends of the short vessel segments because these areas are structurally weak, or whether the middle lamella dissolves here cannot be answered. However, pectic changes in the walls of the vessels were not evident and associated features such as wall softening leading to the production of tyloses as reported by Facey (1950) were not observed either.

Concomitant with the change of the pectic materials in the compound middle lamella, the cell walls throughout most of the abscission layer become soft and easily ruptured or distorted by mechanical forces. In microtome sections the cell walls appear stretched and distorted in these advanced abscission layers (Pl. III, Fig. 2; Pl. VI, Fig. 2). The fine threads of wall material make it appear that cellulose is disappearing in this area. However, the potassium iodide-iodine-sulfuric acid (IKI-H_2SO_4) test was positive in walls affected by the dissolution of the pectic materials, and there was no evidence of disappearance of cellulose. Observations of numerous naturally abscised surfaces revealed not only the presence of intact cells with protoplasm and starch, but also the presence of unusually large cells, particularly in the pith of the lower concave abscission surface. Here, the cellulose walls appeared greatly stretched (Pl. VII, Fig. 1). Following abscission these large cells collapse and form a scar-like layer on the lower abscission surface.

The protective layer

The protective layer, unlike that of most angiosperms, does not form immediately below and adjacent to the abscission layer. Instead it forms at one or more nodes removed from the abscised surface, typically forming at the base of the shoot near its attachment on the root. Lignosuberization occurs at the base of the shoot shortly after abscission and is later followed by the development of a periderm. The persisting stump of the shoot then gradually decays to where the protective layer is formed. These stumps are woody and often persist for a year or more following abscission (Pl. I, Fig. 1-2).

DISCUSSION

Abscission in most plant organs typically involves chemical, physiological, and morphological changes of cells in an abscission zone. These include cell division, changes in the pectic materials in the compound middle lamella, and changes in the cellular contents. Since these changes also occur in P. argophylla, abscission is fundamentally the same as reported by earlier workers.

Cell division preceding abscission is a common occurrence in the angiosperms, particularly in leaf abscission. But cell separation during abscission without cell division occurs in many leaves (Lee, 1911; Gawadi and Avery, 1950), flowers (Dutt, 1928; Yager, 1959), and fruits (Pfeiffer, 1928; McCown, 1943). In P. argophylla cell division in the abscission layer was not found in the living cells of the vascular tissue and was frequently reduced in the cortex, indicating that separation in this species may be able to occur without cell division under certain conditions.

Chemical changes accompanying abscission were suggested by Sampson (1918) who thought cellulose was changed to pectose which in turn was converted into pectic acid and pectin. Facey (1950) first demonstrated the changes of the hard pectic material into pectic acid

and pectin in leaf abscission of Fraxinus americana L. The same change of pectic material appears to occur in P. argophylla. Initially, the compound middle lamella of cells throughout the abscission zone, as demonstrated by microchemical tests, is largely composed of insoluble pectic material. The presence of small amounts of soluble pectic material in young abscission zone tissue might be expected since Albersheim et al., (1960) reported the presence of soluble pectin in the middle lamella of onion root tip cells.

In naturally dried material the ferric chloride-hydroxylamine test revealed the presence of highly esterified pectic material across the abscission layer. This highly esterified material apparently is also highly water soluble since the pectins in naturally dried material were affected by water treatment, and the pectin in material preserved in alcohol was also affected during storage in the alcohol. This ferric chloride-hydroxylamine test is a highly regarded specific reaction for esterified pectins. McCready and Reeve (1955) following the testing of many different types of compounds found in plant tissues, concluded that in their studies only esterified pectic materials produced insoluble red complexes with the reagents at room temperature. Furthermore, this stain is confined to the compound middle lamella according to Albersheim and Killias (1959) and Albersheim et al., (1960).

The loss or alteration of the pectin in the 70% alcohol used to store the abscission zone tissues was not expected. There is other evidence,

however, that pectins may be unstable when preserved in alcohol. Gee et al., (1958) pointed out that fruit marcs prepared with ethanol as a solvent, should be analyzed as soon as possible, as progressive de-esterification in some marcs was detected within one week. McCready and McComb (1954) reported considerable quantities of pectin from ripe pears dissolved in the 63% alcohol used to extract sugars and inactivate enzymes. Joslyn (1962, p. 19) reported that appreciable quantities of pectins from apples could be lost by discarding alcohol extracts of fresh tissues.

The presence of large quantities of pectic acid in the compound middle lamella of alcohol preserved sections, where the pectin had previously been dissolved in the alcohol, was demonstrated by the use of ruthenium red and solubility tests. In such cases the removal of the pectic material enabled separation of the abscission layer cells through the middle lamella. Although pectin and pectic acid are both present in late abscission stages, pectin is evidently the final stage in dissolution of the pectic materials, since only pectins are water soluble (Kertesz, 1951).

Detecting pectic substances with the use of ruthenium red has been criticized by Jensen (1962). He reported that staining with ruthenium red is reliable only if high concentrations of pectic substances are present and various interferring substances are not present. Ruthenium red was also considered to be a partially non-specific stain. However, in the

present study no apparent interferring substances were recognized, dilute concentrations of ruthenium red solution were used, and rough quantitative estimates of pectic materials removed by various solvents are believed to be reliable. The results of the ferric chloride-hydroxylamine tests essentially agree with the results obtained using ruthenium red. For example, lack of staining of the abscission layer with the ferric chloride-hydroxylamine test was verified in many instances by reduced staining of abscission layer cells with ruthenium red.

Although Sampson (1918) thought that cellulose was altered during the abscission process, Facey (1950) found no evidence of a change in cellulose, as did Yager (1959). But the possibility of a change in cellulose should be considered, since recent studies by Kertesz et al., (1958), Sterling (1961), and Jermyn and Isherwood (1956) on pectic changes in ripening fruit presented considerable evidence that the amount of cellulose and hemicellulose decreases during the ripening process. Many believe that the thinning of cell walls during maturation may be due to the degradation of cellulose as well as pectic components (Sterling, 1961).

The large intact cells found on the abscised surface of P. argophylla (usually confined to the lower concave surface) are probably similar to those described by earlier workers (Tison, 1900; Lloyd, 1927; Brown and Addicott, 1950; and Scott, et al., 1948). Scott and the earlier workers have often interpreted these enlarged cells as evidence of turgor pressure or cell growth which causes abscission. However, Addicott and

Lynch (1955) concluded that these cells are "a consequence of abscission under conditions favorable to cell enlargement." In P. argophylla these conditions are believed to involve the following: (1) preliminary cell division, (2) softening of the old and new cell walls by removal of pectic materials, (3) stretching of the cells due to forces exerted by the upper convex abscission surface as it separates from the lower concave surface. Some residual pectic material could cause this adhesion.

The reduction of supporting tissues in the abscission zone in P. argophylla may be as important in the separation process as the chemical dissolution of the pectic materials. These morphological modifications are associated with the intercalary meristem and nodal position of the abscission zone and include the following: (1) general absence of lignification and small size of the abscission zone cells, (2) general reduction or absence of phloem and wood fibers in the vascular portion of the abscission zone, (3) modification of the vessel elements in diameter, position, length of the individual segments, and reduction in the number of vessels per unit area, (4) increased parenchyma cell production leading to wider cortical, vascular, and pith regions in the abscission zone, and (5) the frequent presence of a leaf gap in the vascular cylinder. Many of the above features have been associated with the abscission of plant organs, but as could be determined, all are not involved in the abscission of any single organ. In describing leaf abscission of the orange, Scott et al., (1948) stated that the abscission zones were areas

of structural weakness. Pericyclic fibers were unlignified, the vascular parenchyma increased, the length of the vessel segments were reduced, xylem fibers were reduced, and the entire vascular cylinder was reduced. Eames and MacDaniels (1947), in describing the abscission of branches of Populus grandidentata, mentioned that the vessels of the xylem in the abscission zone were reduced in number and were conspicuously modified with sclariform or reticulate pits. In this area the vessels, fibers, and other cells were also reported to be less strongly lignified, and parenchyma cells were more abundant than elsewhere in the xylem.

With the exception of the presence of multiple abscission layers in compound leaves, two or more abscission layers separating a single plant organ has not been reported. The development of one, two, or sometimes more abscission layers in P. argophylla appears to be related to the lack of internodal growth which maintains the intercalary meristem and increases the probability of the development of an abscission layer. Environmental factors affecting internodal growth likely play an important role in this phenomenon.

Although abscission in P. argophylla results in the development of a typical tumbleweed habit, disseminule production is erratic and often absent entirely. Thus, the adaptive value of the abscission process might be questioned, since it appears that vegetative propagation is the most important means of reproduction.

The position of the protective layer is unique. It typically forms

at the base of the shoot, usually one or more nodes removed from the abscised surface. Its position is probably determined by environmental factors, since in some instances it is not formed at the base of the shoot, but rather at one or more nodes upward toward the abscised surface. By this mechanism in a favorable environment net yearly growth increments will be added to the underground stem and the plant will colonize a new area.

In P. argophylla the cause of the abscission process can be only highly speculative, since adequate knowledge of the chemistry of the pectic materials, the action and occurrence of pectic enzymes, and the effect of auxins on pectic enzymes and pectic materials is lacking. However, it may be that as auxin production declines in the plant, signs of senescence begin to appear on the lower foliage. With the onset of aging, processes directly affecting the middle lamellae and cell walls would begin. Enzyme activity in the presence of favorable pH may be involved. Facey (1950) demonstrated that the change from calcium pectate to pectic acid could be brought about artificially only in a low pH range, and Yager (1960) noted that enzyme activity leading to cell separation is best in the pH range from 3.5-4.5. A combination of a decrease of pectin methylesterase activity and increased activity of a depolymerizing enzyme, like polygalacturonase, could produce the highly esterified, low molecular weight pectin. Whatever the processes may be, it appears that reactions involving methylation and molecular degradation

of the pectic materials cause the dissolution of the pectic substances in abscission.

SUMMARY

Abscission in Psoralea argophylla proceeds through the development of one or more abscission layers which develop internally across the nodes located at or near the surface of the ground. Within the abscission layer cell division occurs and hard pectic materials are changed into pectic acid and water-soluble pectin. Following dissolution of the pectic material in the compound middle lamella and the associated softening of the cell walls, separation of cells occurs in the abscission layer. A comparatively small amount of vascular and mechanical tissue (with corresponding increase of parenchymatous tissue) in the abscission zone reduces the amount of mechanical breakage and separation occurs through the middle lamella. Although the onset of abscission appears to be closely associated with senescence and internal factors, the site of the abscission process is probably affected more by external factors regulating internodal growth and the position of nodes relative to ground level.

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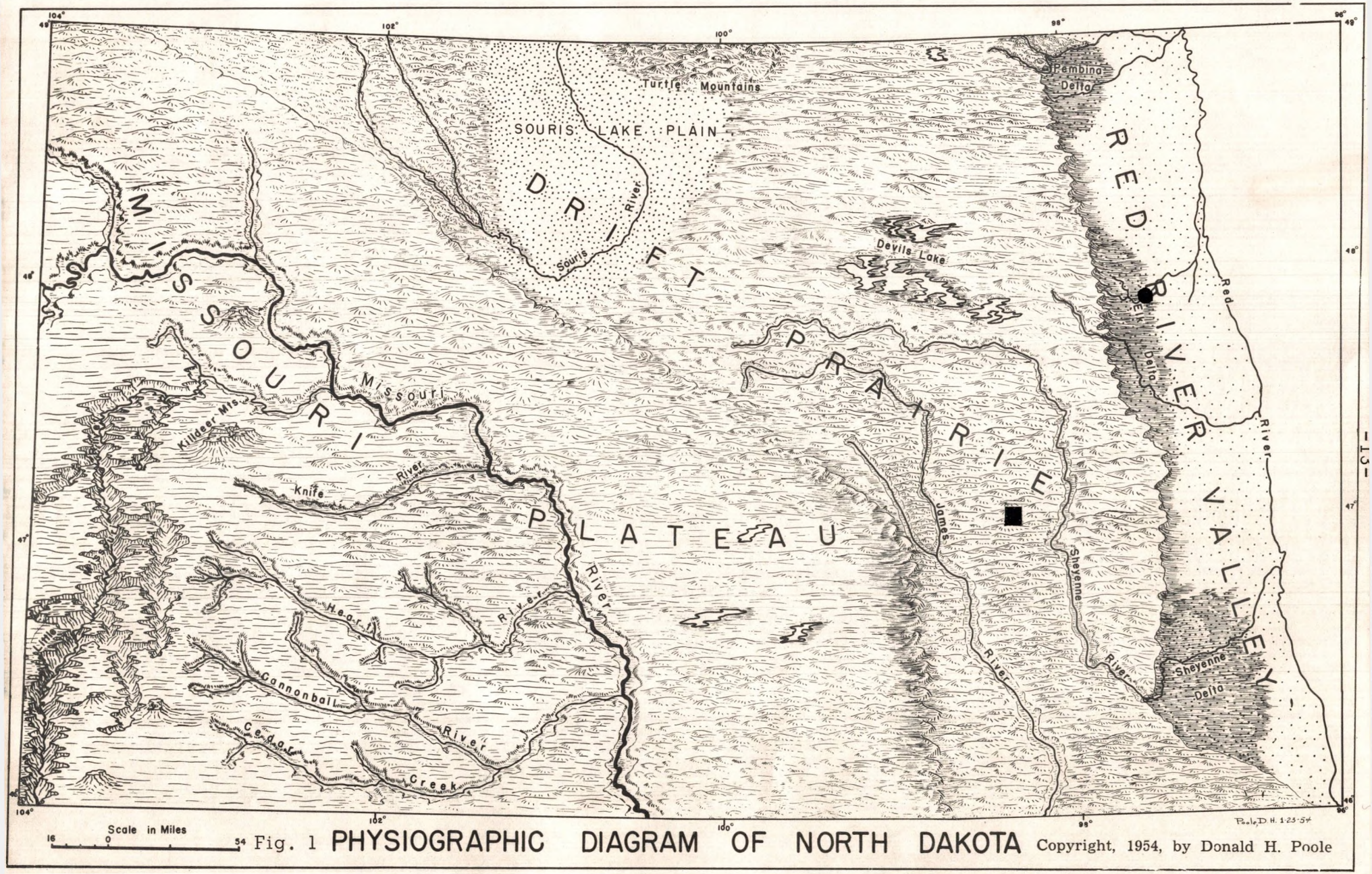


Fig. 1 PHYSIOGRAPHIC DIAGRAM OF NORTH DAKOTA

■ -Study area in Barnes County

● -Study area in Grand Forks County

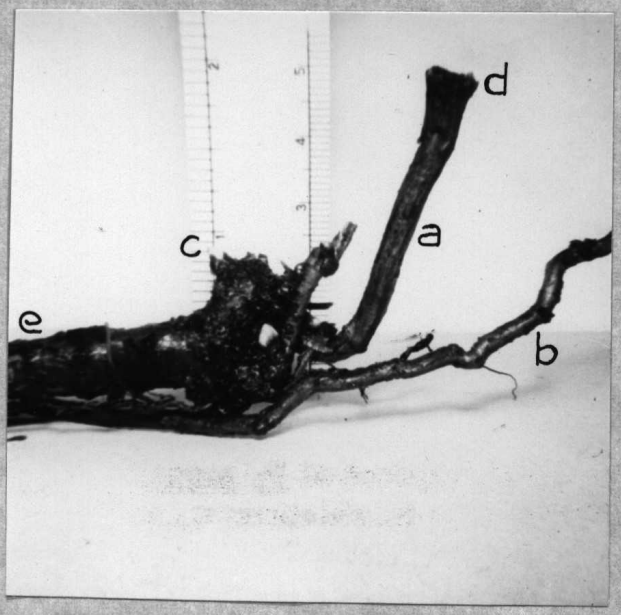
PLATE I

Fig. 1. Side view of the upper portion of the taproot of P. argophylla in fall condition. a, persistent stump; b, rhizome; c, young bud; d, socket of the joint; e. root.

Fig. 2. View from above of the upper portion of the taproot of P. argophylla in fall condition. a, persistent stump; b, rhizome; c, young bud; d, socket of the joint; e, root.

Fig. 3. A large plant of P. argophylla flowering and growing on a north-facing slope.

Fig. 4. A small but mature plant of P. argophylla growing on short grass prairie.



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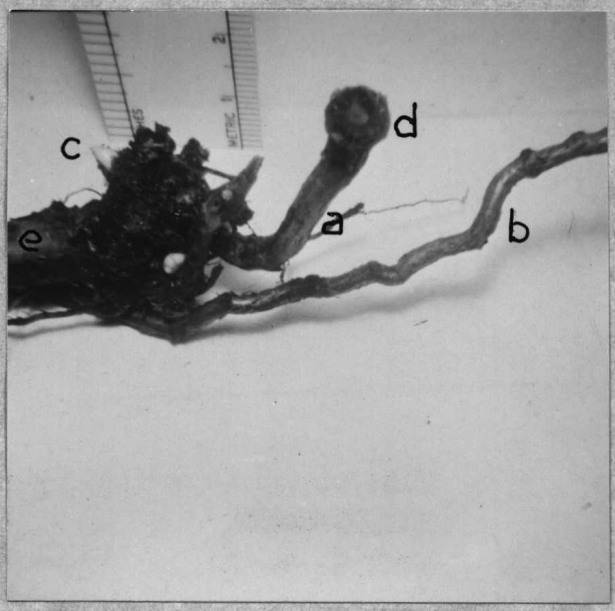

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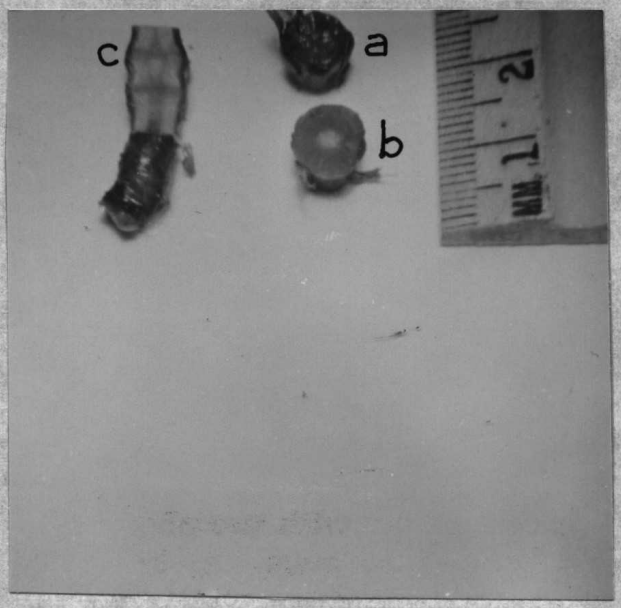
PLATE II

Fig. 1. Left, a median longitudinal view of a stem with two abscission zones; right, the separated surfaces of the stem. a, lower abscised surface; b, upper abscised surface; c, abscission zone.

Fig. 2. Three stems with (from left to right) 1, 3, and 2 joints.

Fig. 3. Bud of P. argophylla recently emerged from the ground. a, bud scale.

Fig. 4. Early stage of shoot development of P. argophylla. a, abscission zone; b, root; c, compound leaves.



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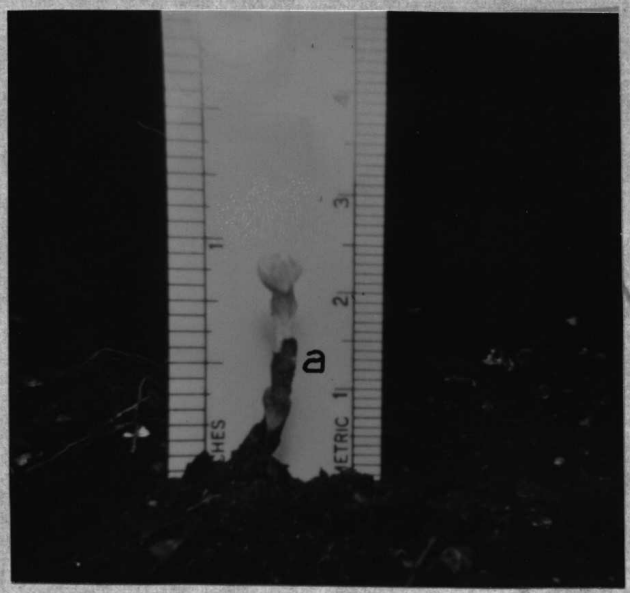

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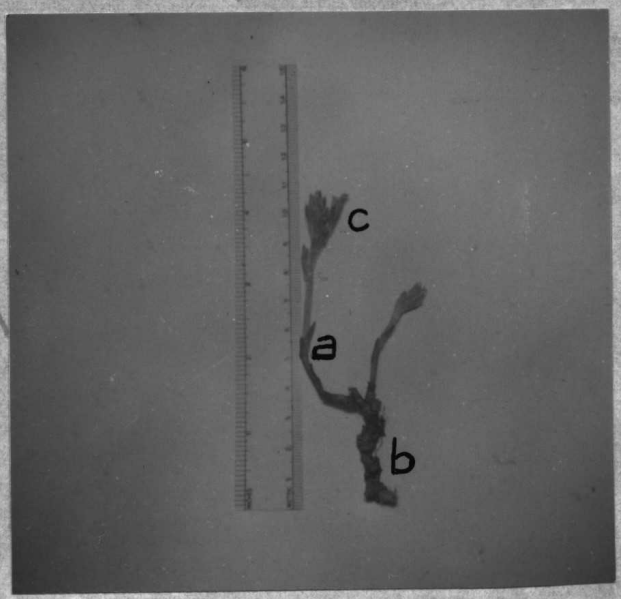

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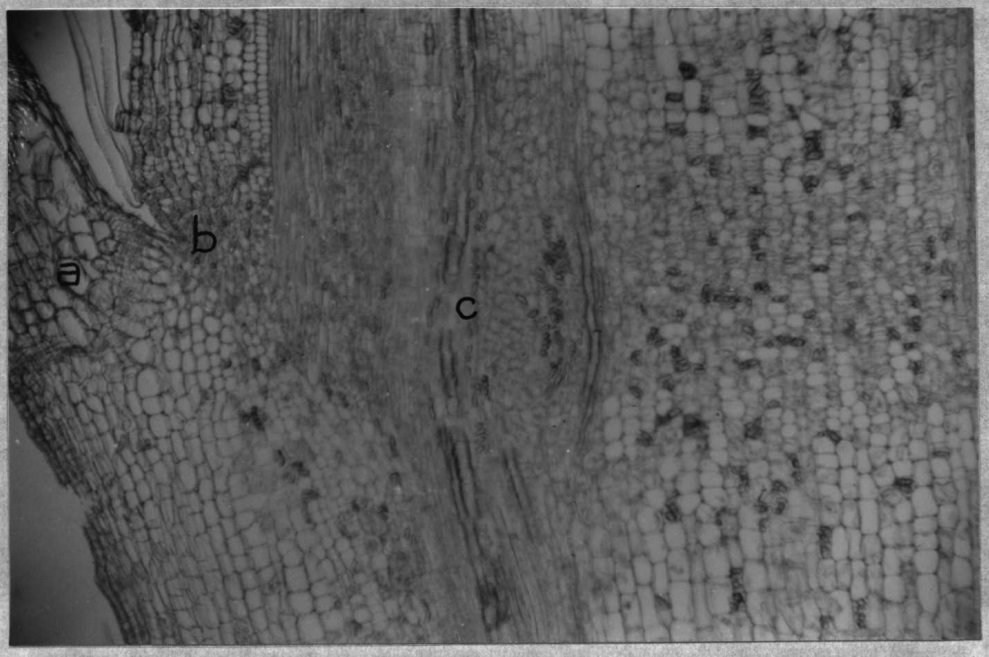
PLATE III

Fig. 1. Longitudinal section of the abscission zone of a young shoot (10μ). a, bud scale; b, groove; c. xylem vessels highly segmented and discontinuous, X 90.

Fig. 2. Longitudinal section of the cortex of the abscission zone. a, cortex; b, abscission layer with distorted and altered cell walls, X 400.



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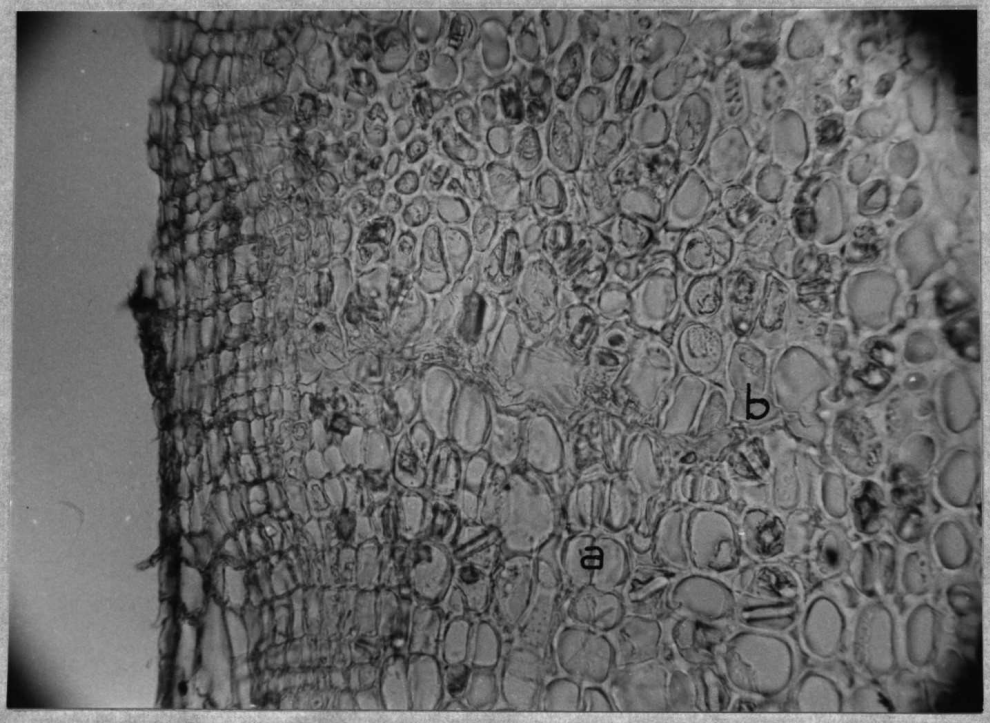

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PLATE IV

Fig. 1. Longitudinal section across the vascular tissue and pith of the abscission zone stained with IKI. a, increased segmentation of the vessels; b, the dark-stained abscission layer, X 90.

Fig. 2. Longitudinal section of the pith of the abscission zone stained with ferric chloride-hydroxylamine. The light band is the abscission layer, X 90.

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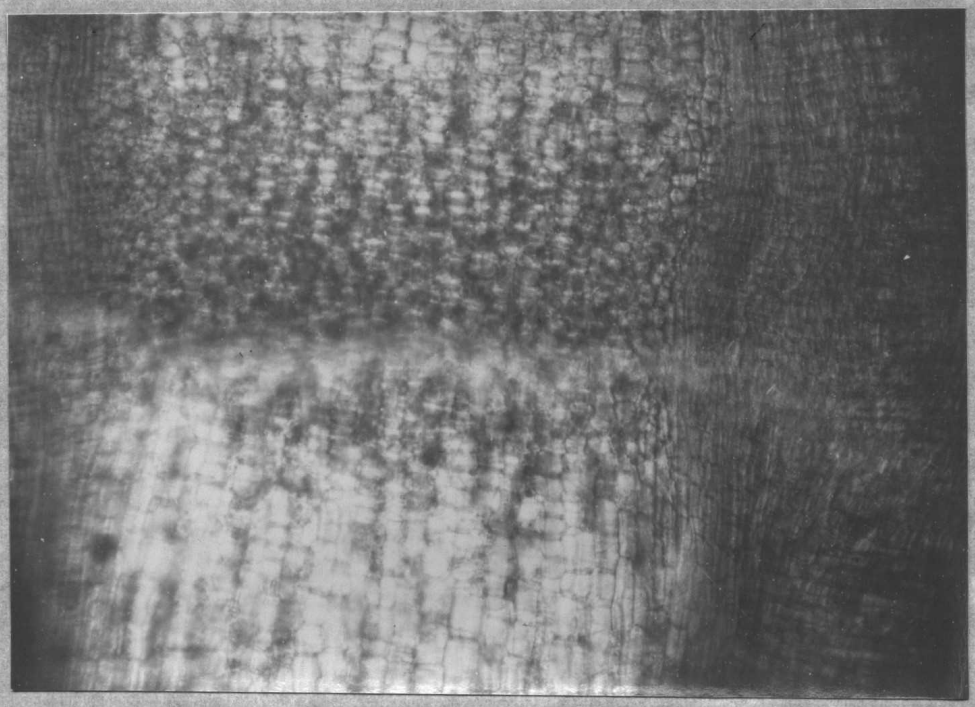

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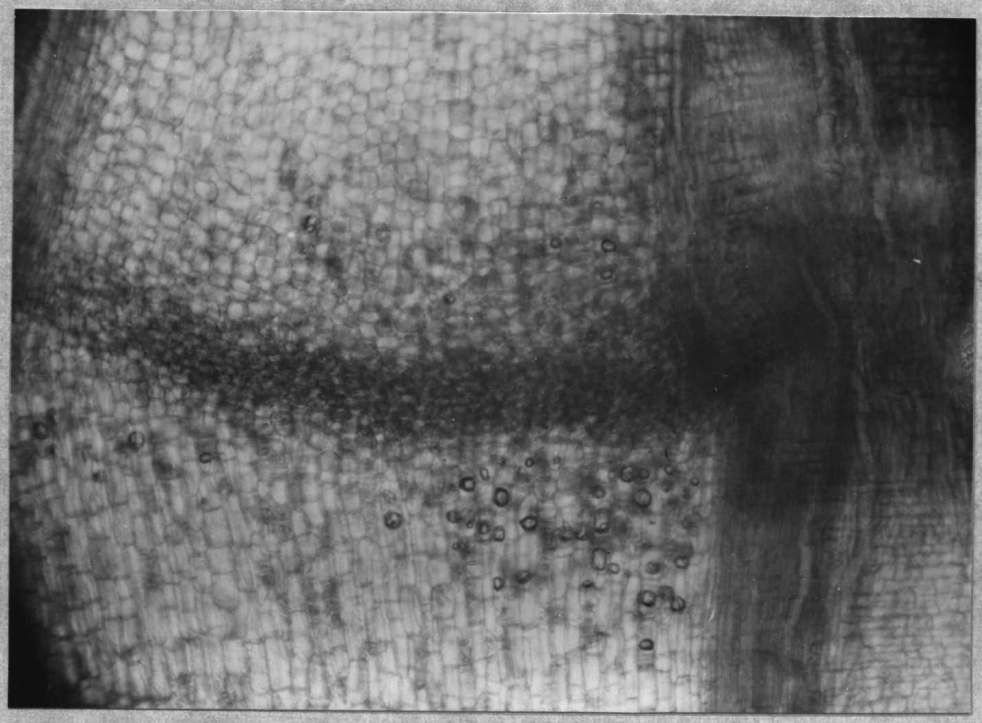
PLATE V

Fig. 1. Longitudinal section of the abscission zone stained with ferric chloride-hydroxylamine. The cells of the abscission layer produce an intense reaction, X 90.

Fig. 2. Longitudinal section from the same stem as above, but soaked in water for three hours. Although stained as above the red layer has disappeared in the pith and cells in the vascular tissue exhibit greatly reduced staining, X 90.



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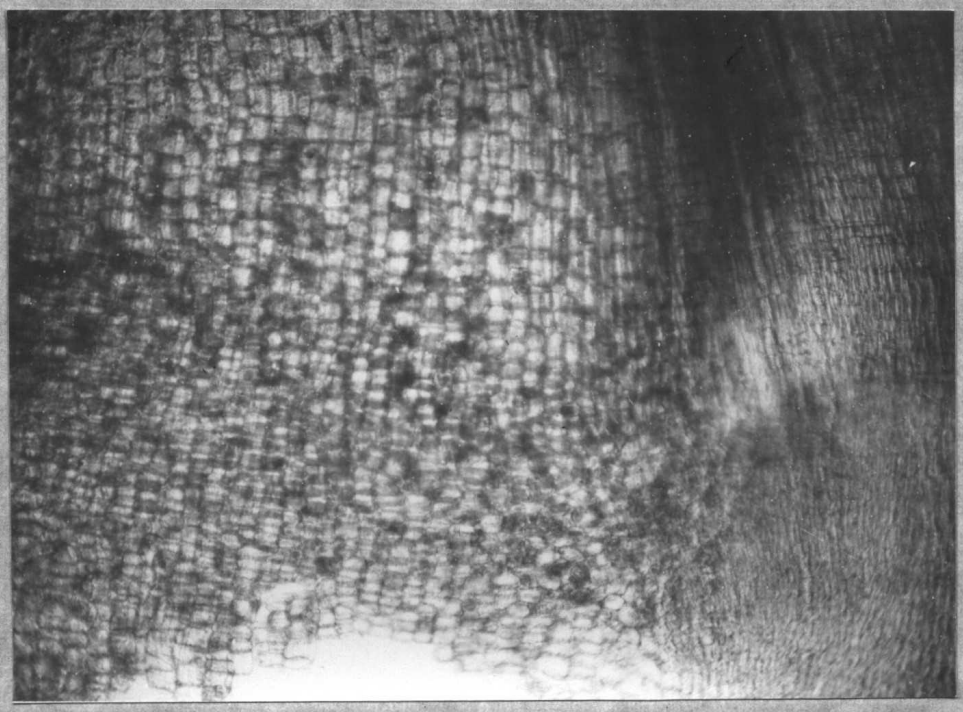

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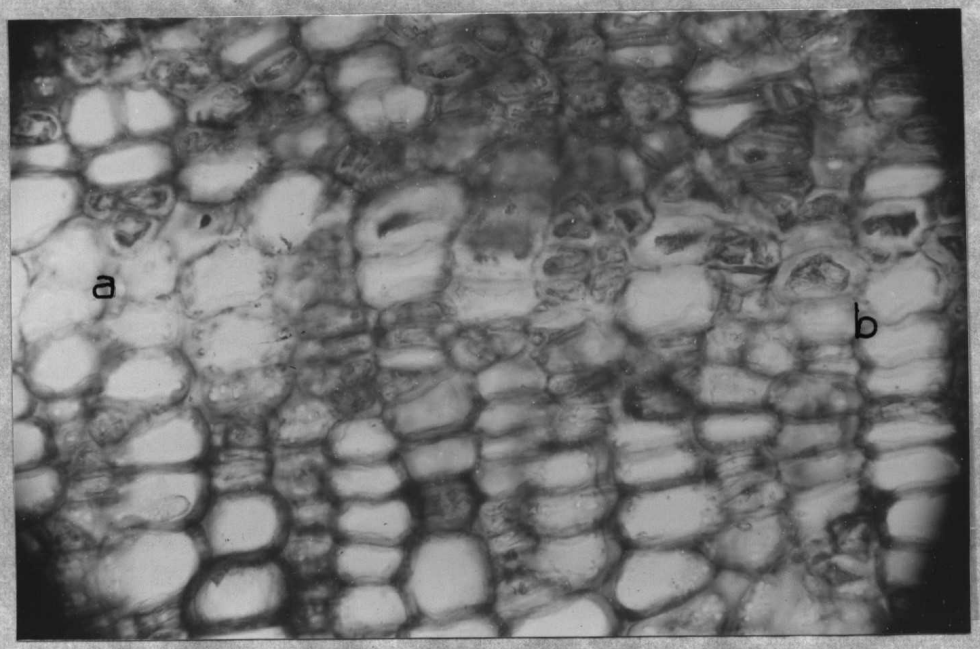
PLATE VI

Fig. 1. Longitudinal section of the abscission layer treated with 2% ammonium hydroxide and stained with ruthenium red. The abscission layer extends from left to right as a lighter staining area. a, cell which has lost the pectic material from both the wall and middle lamella; b, cell which has lost only the pectic material from the wall, X 400.

Fig. 2. Longitudinal section of the abscission zone in advanced abscission stage (15μ). The cell walls are soft and have been stretched, X 90.



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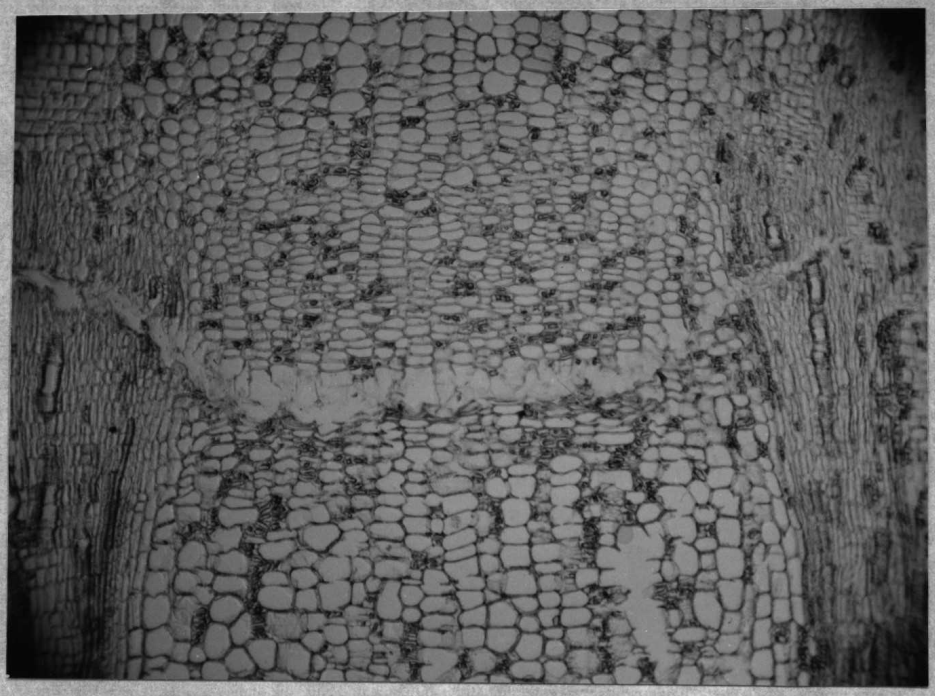

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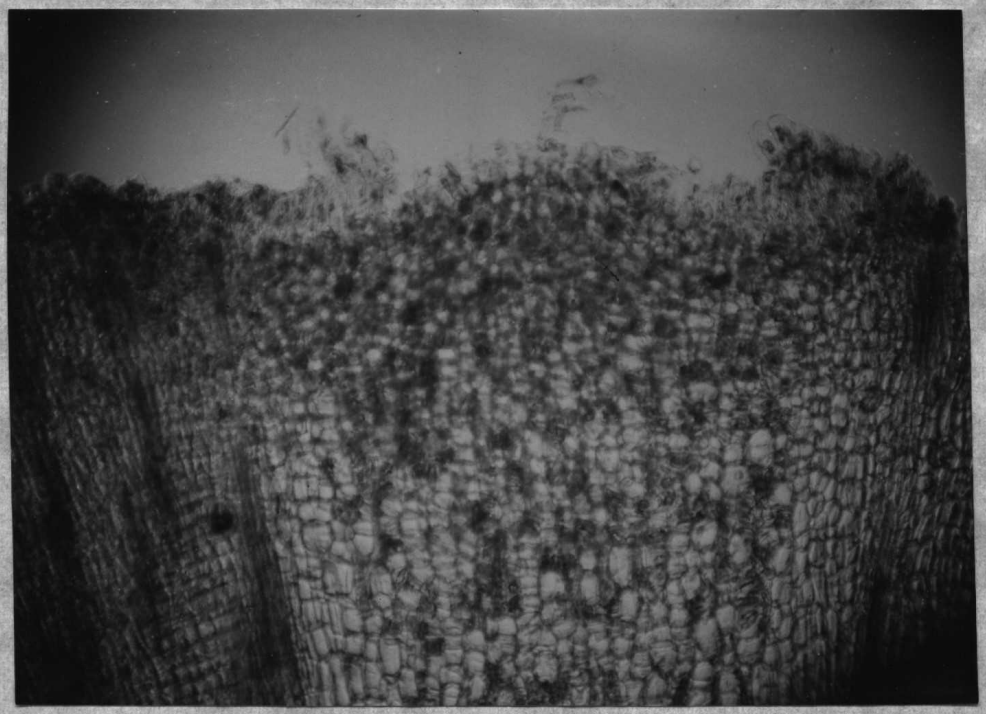
PLATE VII

Fig. 1. Longitudinal section of the lower abscised surface. The large cells are particularly noticeable at the upper right, X 90.



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