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# Morphology and anatomy of leafy spurge

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# I. Introduction

Most aggressive weeds in the prairie provinces of Canada and the great plains of north central United States are introduced perennials. One such weed is leafy spurge. The aggressiveness of the species can be related to the phenomenal ability of its roots to spread by producing horizontal roots, to propagate by producing buds profusely, and thus to establish long-living dense infestations. In view of the persistence and aggressiveness of the species, studies were undertaken several years ago in the laboratories of Professors R. T. Coupland and T. A. Steeves of the University of Saskatchewan, Saskatoon, to obtain a better understanding of its biology, especially the growth and development of the root system, with a view to developing effective control measures. Morphological and anatomical studies on leafy spurge are being continued in my laboratory, and the present report reviews a few aspects of these studies, including some unpublished information.

# II. Shoots

**Development of seedlings.** In natural populations of leafy spurge, different colored seeds, such as yellow, yellow-brown, brown, gray, and mottled are produced (1, 43). These colors are reported to be correlated with different developmental stages, that is, the yellow to gray seeds are immature and the mottled seeds are mature (4, 38). I have observed the yellow and yellow-brown seeds to be aborted, with no viable embryos in them. Each of the other three types represent mature seeds, although all seeds turn brown after complete imbibition with water.

Although the seeds show different degrees of dormancy (4, 38), a large number of seedlings appear very early in the growing season (about May) when environmental conditions are favorable. Seedlings appear in the field even when temperatures are about freezing. Sporadic seed germination and seedling development are observed throughout the growing season as long as soil moisture is adequate. The seedling shoots are identified by their pairs of opposite leaves in two successive nodes, including the cotyledonary node. As the season progresses, seedlings dry up and decrease in number (Figure 1). Although the shoots of seedlings dry up and appear dead, their underground parts persist and produce adventitious buds. In some instances the buds soon develop into aerial shoots, replacing the seedling shoot. A small number, about 11%, of seedlings do die and decay.

Young seedlings appear purplish-pink because of the anthocyanin pigments in the hypocotyl and eventually turn green. Ordinarily, the adventitious buds appear on the hypocotyl, especially in its more basal part (29). Similarly, the buds arise in the more proximal part of the primary root. The adventitious buds are initiated on seedlings in the field 2 to 3 days after their emergence aboveground. Root growth is more vigorous than shoot growth (38). In one observation in the greenhouse, the average length of the primary root of 58-day-old seedlings was about six times that of the shoot (n = 20,  $\times$  shoot length 36.05mm, sd = 3.46; root length 222.05mm, sd = 8.71). In the field, the first-year seedlings collected on the same day from open areas with little or no vegetation and from areas that were abundantly grassy showed significant difference in the length of the pri-

mary root (open area - n 62,  $\bar{\times}$  length 26.81cm; grassy area n = 92,  $\bar{\times}$  length = 17.60cm; t = 2.41). The epicotylary length of the seedlings in grassy areas was depressed more and reached its maximum length earlier than those in open areas (Figure 2).

Growth of the epicotyl, even under favorable conditions, is considerably depressed because of the development of adventitious buds/shoots on the hypocotyl and roots. In the field the seedling axis is replaced by adventitious shoots; consequently, it never matures into a flowering shoot.

Adventitious shoots. Seedling shoots may survive in the field until the end of the first growing season in sites where there is little or no competition by other plants. Most commonly they are soon replaced by adventitious shoots, which, with their seedling root system, persist in the field. In an established leafy spurge infestation, the shoots are all adventitious. The underground parts of stem (hypocotyl of seedlings) show considerable cambial activity and the production of adventitious buds is also increased (Figure 3). These buds show different degrees of development and most remain belowground. When conditions become favorable, a few buds emerge aboveground (Figures 3 and 4). The dormant buds are reddish-pink, and at ground level or above the buds turn purplishgreen to become shoots; the swollen tips of these shoots show different degrees of early floral differentiation. In other words, the floral primordia become evident after the emergence of shoots aboveground; the preformed floral buds in leafy spurge have not been observed belowground. Early in the season, the shoots increase in number until about the middle of June, and then the production



Figure 1. Changes in the number of seedling and adventitious shoots (per  $m^2$ ) in a leafy spurge infestation in a pasture (S.W. 34-35-6 W3) in Saskatchewan.



Figure 2. A comparison of seedlings growing in open (denuded) and grass areas in a pasture (S.W. 34-35-6 W3) in Saskatchewan. Measurements of the epicotylary length were made on emerged seedlings collected at known intervals from May to June. Each value is a mean of 28 seedlings.

of new shoots is considerably reduced (Figure 1). By about early July there is a decrease in the number of vegetative shoots and a concomitant increase in the number of flowering shoots, indicating the transformation of vegetative shoot apices to floral apices (Figure 1).

**Flowering shoots**. Flowering shoots in the field are predominantly of adventitious origin. In late April the early flowering shoots appear and their terminal umbellate inflorescences become obvious in June (Figures 5 and 6). Fruits mature by about the middle of July (Figures 5 and 7). Meanwhile, new shoots will have emerged in late June and early July and produced terminal umbellate inflorescences in August and fruits in September-October (Figure 5). Such shoots are termed late flowering shoots (Figures 5, 10, 11). Both the early flowering and late flowering shoots (with their inflorescences, called terminals) produce axillary shoots that terminate in umbellate inflorescences and they are called, respectively, the early flowering and late flowering axillary shoots (Figures 5, 6 and 10). If favorable growing conditions persist in the season, more axillary shoots are formed, some of which terminate in highly reduced umbellate inflorescences and others may remain vegetative (Figures 5 and 10 to 12). Thus, in an established infestation of leafy spurge, as Selleck *et al.* (38) have remarked, flowering occurs throughout the growing season (Figure 5).



Figure 3. Buds on the underground stem (collected at N.W. 9-17-17 W2) show different degrees of development early in the growing season. Apical dominance does not seem to have an effect at this stage of bud growth. (scale bar 5 cm.)

Figure 4. The leaves on shoots become obvious soon after the emergence of the latter aboveground. Notice the lack of apical dominance. (Scale bar 5 cm.)

**Histology of the shoot apex**. Observations on shoots have shown that adventitious shoots are more vigorous than seedling shoots; the apical meristem in the former has a greater stratification (28). The apical meristems of adventitious shows show distinct cytohistological zonations, which can be identified by histological, histochemical, and autoradiographic techniques. The meristem of the adventitious shoots apex shows a variable number of mantle layers at different stages of shoot development (14). The number of mantle layers tends to increase in the apices of older shoots, and this feature may be related to the transformation of the apices from vegetative phase to reproductive phase (14). The acropetal differentiation of procambium and early vascular differentiation are similar to those reported for other angiosperm species (8 9). In the early stages of shoot growth, when leaves are being vigorously produced, a conspicuous cytozonation is observed in the apical meristem (30). The peripheral parts of the meristem show a greater DNA distribution and synthesis than the central region. Similar patterns of zonation can be observed if the apex is stained for lysine-rich histones. However, the arginine-rich histones are abundant and their distribution is uniform, and consequently the cytozonation in the apical meristem is not obvious. At all stages of development, including active initiation of leaf primordia, the RNA distribution and synthesis are uniform throughout the meristem (30). Some anatomical details of the stem of leafy spurge have already been described (1, 12 and 23).

### III. Inflorescence

**Development**. Vegetative shoots of leafy spurge emerge in southern Saskatchewan between mid-April and May 1 (38). The development of the inflorescence is marked by a swelling at the tip of the shoot apex 7 to 10 days after emergence (38). The shoots attain a height of about 25 cm before the inflorescences begin to appear.

Shoots emerging in early spring are robust and eventually terminate in umbellate inflorescences (early-flowering terminals) (Figures 5 and 6). The terminal inflorescence is subtended by a whorl of bracts. The shape of these bracts may vary from linearlanceolate to oblong-ovate. but it is uniform in an inflorescence. The average length/width ratio of the subtending bracts is 4 (Table 1). In the axils of bracts, rays arise, which show different degrees of dichasial branching (Figures 6, 9 and 14). The number of cyathia (flowers) produced in each of the inflorescences is dependent on the extent of branching in it. The earliest formed (terminal or middle) cyathium of the umbellate inflorescence may persist, or it often drops off.

Immediately subjacent to the terminal inflorescence, rays appear in axils of leaves (Figure 14). Mature terminal inflorescences and their subterminal axillary rays appear in abundance in late May to early June, and together they mark the first flowering peak (Figure 5).

From mid-July to mid-August, the early-flowering shoots produce axillary shoots, which also terminate in umbellate inflorescences (early-flowering axillary) (Figures 6, 7 and 14). These axillary shoots may also produce subterminal rays, which morphologically resemble the rays of the terminal inflorescence. A second crop of shoots is developed from the persistent roots about mid-July. These are generally shorter and less

vigorous and have smaller and narrower leaves, possibly due to the drier conditions of midsummer. They may produce terminal inflorescences or remain vegetative. The inflorescences in such shoots are designated as late-flowering terminal inflorescences (Figures 5, 10 and 11). If favorable growth conditions persist late in the season, the late-flowering shoots may produce branches that may terminate in umbellate inflorescences (late-flowering axillary) (Figures 10 and 11). These inflorescences, together with the early-flowering axillary inflorescences, form the second crop or peak of flowering from late July to late August (Figure 5). Similar to shoots, four kinds of umbellate inflorescences can be recognized in the field: a) early-flowering terminal, b) early-flowering axillary, c) late-flowering terminal, and d) late-flowering axillary (Figure 5 and Table 2).



Figure 5. Phenological changes and the periods of peak flowering in leafy spurge infestations at a site near Jamieson (N.W. 9-17-17 W2) in 1975.

Table 1. Comparison of length/width ratios of bracts of terminal inflorescences of main andaxillary flowering shoots of leafy spurge collected at a site near Saskatoon (S.W. 34-35-6W3).

Inflorescence type	Total number of umbellate bracts	Mean length/width ratio
Early flowering terminal	1130	$4.00^{a}$
Late flowering terminal	1561	2.29
Early flowering axillary	1015	2.50
Late flowering axillary	1445	2.25

<sup>a</sup>Value significantly different (5% level from the other values according to t-test (Snedecor, G.W. 1953. Statistical methods. Iowa State College Press, Ames, U.S.A. Pages 75-88).



Figure 6. Early flowering shoot bears a terminal compound dichasium. Note the degree of branching in the rays and the shape (linear-lanceolate) of the bracts at base of rays. Subtending the compound dichasium are the axillary rays, which resemble the rays of the dichasium. Note also the determinate umbel at the axillary shoot tip. (Scale bar 5 cm.)

Figure 7. A late-flowering shoot with a compound dichasium bearing fruits. Note the shape of the bracts at the base of the inflorescence. (Scale bar 5 cm.)

Figure 8. Two flowering shoots, one with a cyme and the other with a determinate umbel, organically connected by the same root. Some shoots are vegetative. The bracts at the base of the umbellate rays are somewhat oblong-lanceolate. (Scale bar 5 cm.)

Figure 9. Early-flowering shoot with compound dichasium subtended by linear-lanceolate bracts and a determinate umbel at the tip of an axillary shoot. Oblong-ovate bracts subtend this latter inflorescence. (Scale in 5 cm.)

**Structure.** Like many other members of the Euphorbiaceae, leafy spurge has a cyathium consisting of staminate and pistillate flowers. The terminal inflorescence, consisting of several rays, has variously been described as umbellate, umbel-like, somewhat umbel-like, and so on (16, 20). According to the terminology of Rickett (36) and Lawrence (15), the determinate umbel of leafy spurge may be classified into three types depending on the degree of branching in the associated rays. The terminal inflorescence of early flowering shoots is a compound dichasium (Figures 14 and 15). Around the central cyathium of the inflorescence are the rays, which exhibit 2 to 3 sympodial or dichasial branching (Figures 6, 14, and 15 and Table 2). The second type of inflorescence is a determinate umbel in which the central cyathium is surrounded by unbranched rays (Figures 6, 11, and 15 and Table 2). The third, the intermediate type, is a cyme, where the central cyathium is surrounded by 1-branched rays (Figures 8, 10, and 15 and Table 2).



Figure 10. A late-flowering shoot with a terminal cyme. Lateral shoots terminate in determinate umbels. (Scale bar 10 cm.)

Figure 11. Unbranched and branched late flowering shoots with fewer rays in the determinate umbels. Note the shape of the bracts subtending the inflorescences and vegetative shoots in one. (Scale bar 10 cm.) Shoots bearing different types of inflorescence have been found to occur on the same root system (Figure 8). The early-flowering shoots with compound dichasia often produce axillary shoots with determinate umbels or cymes at their tips.

**Rays and bracts**. The number of rays in the terminal umbellate inflorescence of leafy spurge is variable. According to various authors (3, 13, 24, 26), the number ranges from 8 to 12, 7 to 13, 8 to 9, and 6 to 20. In the present study the ray number was also variable, and the mean value of 8.3 of early-flowering terminal inflorescence was significantly different from the means of other types of inflorescence (Table 2). The number of rays in the terminal inflorescences of lateral shoots of early- and late-flowering shoots is comparable. In addition, the inflorescence rays in the early-flowering shoots show a greater degree of branching than in the other types.

The bracts subtending the early-flowering terminal inflorescences are usually linearlanceolate and those of the other types are often oblong-ovate (Table 1 and Figure 14). The oppositely placed pairs of bracts on ray-nodes are morphologically similar in all types of the inflorescence (Figure 14).



Figure 12. A late-flowering shoot collected in September with many axillary shoots remaining vegetative. (Scale bar 10 cm.)

Figure 13. An early-flowering shoot whose tip has been damaged. Many lateral shoots have grown bearing cymes. (Scale bar 10 cm.)

### **IV. Roots**

**Establishment of the root system**. Although seeds are very important in the initial establishment of the species, the underground parts, especially the roots, are equally important in perennation and propagation. A thorough understanding of the morphology, anatomy, and behavior of roots is, therefore, fundamental.

Seeds buried at different depths may germinate, and consequently the length of the hypocotyl increases with an increase in depth at which the seeds germinate. Despite the variation in hypocotylary length, the early seedling root system shows a "heterorrhizic" pattern, which has been interpreted to contain the primary root of the "long-root" type and the laterals of the "short-root" type (32). The long roots persist for more than one growing season and are therefore potentially indeterminate. By contrast, the short roots are determinate, with limited growth, and they usually die at the end of the first growing season. The entire heterorrhizic primary root system appears white in the early stages of development. The cambium is soon initiated in the long root, followed by an increase in the diameter of the root. Due to the addition of secondary tissues, the primary cortex is soon obliterated and replaced by secondary phloem (Figures 16 and 17). In the more

peripheral regions of the "secondary cortex" the phellogen and cork appear. The primary root becomes yellowish-brown or brown and the lateral roots of the short-root type usually retain the same white color.

Spreading of the root system. Superimposed on the basic heterorrhizic pattern in the primary root system, additional roots appear, which include laterally spreading roots of the long-root type. Such roots arise on the primary root and also on the persistent subterranean part of the hypocotyl. The origin of these long roots apparently synchronizes with the appearance of cambium in the parent underground parts. These roots, especially in shallow depths and in sandy soils, are vigorous and grow horizontally for some distance; they eventually turn down to become vertical roots. It is not clear at present why and how such horizontally growing roots turn to become progeotropic. They also produce lateral short roots. More laterals of the longroot type may arise on the horizontal roots, although more of them are observed on vertical long roots. The horizontal roots, as with vertical roots, turn yellowish-brown and then brown after secondary tissues have been laid by secondary meristems.



Figure 14. A portion of the inflorescence of an early-flowering shoot (collected at site S.W. 34-35-6 W3) showing the terminal compound dichasium with branched rays and subtending whorl of bracts. The lateral shoot with a terminal inflorescence where the 2nd degree of branching is not as obvious as in the main compound dichasium. Notice the difference in shape of the bracts in the whorls of the two types of terminal inflorescences. x1/3

Table 2. Comparison of number of rays and degree of branching (0 to 3) in each of the rays in terminal inflorescences of early- and late-flowering shoots of leafy spurge collected at a site near Saskatoon (S.W. 34-35-6 W3).

Terminal inflorescence type (Number examined)	Number of rays $\_$ $\bar{\times} \pm$ s.d. (range)	Degree of branching of rays			
		Number of rays in each category			
		0	1	2	3
Early-flowering (145)	8.3 ± 1.8 (5-14)	14	55	916	224
Late-flowering (268)	$6.0 \pm 1.2$ (4-9)	194	858	513	36
Early-flowering axillary (179)	$5.8 \pm 1.4$ (3-9)	404	559	82	0
Late-flowering axillary (268)	6. 0 ± 1.3 (4-9)	625	762	98	0

The mature root system. The successive formation of permanent long roots and transitory short roots on the heterorrhizic roots initiated in the seedling results in a maze of roots.



Figure 15. Three types of dichasial inflorescences and their interrelationships: A) a compound dichasium, B) a cyme, and C) a determinate umbel. Only two rays in the inflorescence are shown in each. Interrupted lines indicate probable reduction of axes.

The framework of a mature root system then consists of abundant vertical and horizontal roots that ordinarily do not show continuous growth (Figure 18). New vertical roots arise on the old roots, resulting in successive sympodia; this mode of development allows the roots to penetrate vertically to greater depths (32). The horizontally spreading roots stop growing periodically, especially when the ground is dry, and resume growth when favorable conditions return. Consequently, the sympodial growth, which is seen in vertical roots, is less obvious in horizontal roots. The growth patterns in horizontal-spreading roots and vertical roots are doubtless related to the behavior of their apical meristems. The transitory short roots, although produced abundantly, do not add considerably to the permanent framework of the root system. The long roots, irrespective of the source of

their origin – root or underground stem – organize the perennial root system despite the fact that they vary greatly in length, thickness, and in their capacity to regenerate roots and shoots. The longevity of their survival in the field is, however, not known.



Figure 16. Transverse section of a long root showing the central stele surrounded by the obliterating cortex. Endodermal layer separates the two. x100

Figure 17. A mature vertical root section with central vascular tissue surrounded by secondary phloem (secondary cortex). The primary cortex has been completely removed. The periderm is present in the most peripheral region. Laticiferous cells are visible in the secondary phloem. x35

**Root anatomy**. Some details of the root anatomy of leafy spurge have already been published (1, 2, 23). In the seedling root anatomy a clear distinction between the lateral (short root) and the primary (long root) roots is observed, although both are protostelic. The short root has a diarch xylem in the stele and the cortex is preserved during the life of the root. Although storage materials, such as starch, are found in both cortex and stelar parenchyma of short roots, such materials are not so abundant as in comparable parts of long roots. The short roots do not show cambial activity.

The triarch primary root (long root) is thicker and more vigorous in growth than the short root. The primary cortex is transitory and soon is replaced by a secondary cortex. A large part of the secondary cortex consists of parenchyma filled with storage materials, and some functional sieve elements can be observed outside the secondary xylem. In addition, abundant laticiferous tissue is found in the secondary cortex of a mature root (Figure 17).



Figure 18. Reconstruction of a mature root system developed from a seedling. Note the disposition of horizontal and vertical roots and also the sympodial growth in roots.

The extent of secondary tissues in a mature long root is variable and is due to the behavior of cambium; the variation is also related to the neoformation of adventitious buds and roots. In the region where the adventitious primordia are initiated, the cambium produces abundant parenchyma and the vessel elements of different diameter are evident. Scattered in the secondary xylem are the xylem parenchyma and fibers of the gelatinous type. At certain levels of the root, wide ray parenchyma are present.

In a mature root system the anatomy of vertical roots is similar to the primary root. Secondary tissues are extensive and the primary cortex is soon replaced by secondary phloem (secondary cortex). In addition to obliterated sieve elements, secondary phloem contains abundant phloem parenchyma and a substantial amount of scattered laticiferous tissue (Figure 17). The horizontal long roots, on the other hand, are usually thinner, and the primary cortex is retained longer. They have the most common tetrarch type of xy-lem, in contrast to vertical roots in which the number of protoxylem poles ranges from 3 to 5. The mature root system, composed of vertical and horizontal roots, persists for a long time.

**Root apices**. Root apices determine the morphology and anatomy of mature roots. The structure of apical meristems of long and short roots is similar, but they differ in size; the long-root apical meristems are larger. The stratified promeristem in the apices of both root types consists of three layers: the inner or proximal layer produces the stele, the middle layer produces the cortex, and the outer or distal layer produces the epidermis root cap complex. Functionally, by <sup>3</sup>H-thymidine incorporation studies, the long- and short-root apices show differences. The long-root apex has a quiescent center, which is absent in the short-root apex (35).

It is not known how long the long-root apices of leafy spurge survive in the field. Some indirect evidences obtained from morphological and anatomical studies suggests that the same long-root apex may survive for 3 to 4 years in the field. Experiments and observations in the field show that the presence and extent of a quiescent center in longroot apices are variable. Early in the growing season, quiescent centers are absent in longroot apices (34). Later in the season, however, most roots show a quiescent center, with no DNA synthesis or mitotic activity. Other roots have quiescent centers, with DNA synthesis (34). Such sporadic activity in root apices suggests that roots periodically become inactive or stop growing in the field. Thus, some ecological factors, such as temperature, moisture and nutrition may have an effect on periodic inactivity of the long-root apices. Experimental studies by some authors (5, 42) on the control of the development of the quiescent center in the apices of roots and shoots do support this view.

### V. Buds

**Types of buds**. One of the most important traits of leafy spurge as a successful weed is its ability to produce a variety of ontogenetically different shoot buds. The buds can be classified into two major types, axillary and adventitious (9, 10). Although axillary buds contribute to branching of aerial shoots, they are not so significant as buds arising from underground stems (Figure 3). The latter buds persist longer and are important in the annual production of new shoots. In the field, most aerial flowering shoots are adventitiously developed from underground parts of older stems (Figures 4 and 8), and some shoots are developed from axillary buds present in the axils of scaly leaves on underground stems. It is, however, difficult to find aerial shoots developed directly from buds initiated on roots.

Adventitious shoot buds are produced abundantly and are important in the success of leafy spurge as a competitive weed (2, 23, 38). In leafy spurge, as in a few other perennial plants (25, 27), buds are produced on plant parts after an injury; these are the reparative buds (31). Buds also arise spontaneously on plant parts without any apparent injury and are classified as additional buds (31). Both types of adventitious buds are common on underground parts of leafy spurge. Buds also arise spontaneously (additional and (or) reparative) on the hypocotyl of seedlings. Axillary buds are not considered in the present review, since their origin and development have been investigated extensively (8, 9, 10).

**Buds on the hypocotyl**. In about 10 to 15 days after germination, or even earlier in the field, additional buds are formed. Most buds are initiated in the proximal part of the hypocotyl (29). The number of buds produced on the hypocotyl is limited, unlike in the roots, where bud production is abundant (Figure 19). Even in older seedlings, there is no increase in hypocotylary bud number. For the initiation of hypocotylary buds, cotyledons and leaves are essential (unpublished data). The development of buds inhibits seedling shoot growth, and this can be relieved by continuously removing the buds (Figure 20).

**Ontogeny of the hypocotylary buds**. Additional buds appear as pinkish or purplish protuberances, especially in the more proximal regions of the hypocotyl. They may also arise in the more distal regions when the cotyledonary node is removed and appear greenish. The bud primordia arise exogenously on the hypocotyl (23, 29). They are initiated in the hypodermal layers in the cortex and eventually differentiate into buds and shoots.

Young bud primordia at the time of their initiation lie opposite or alternate to the collateral bundles of the hypocotylary stele. During their development the procambia of buds gradually extend basipetally, connecting to and between the stelar bundles. In the more proximal regions of the hypocotyl, the bud primordia are initiated in the stelar region between the bundles (29). These primordia seem to be directly connected with stelar bundles because of their close proximity with the hypocotylary stele (29).



Figure 19. Formation of buds on the hypocotyl and the primary root of seedlings of leafy spurge in a greenhouse experiment. The examination of seedlings for buds was started 10 days after sowing.



Figure 20. The effect of removal of hypocotylary buds on the epicotylary growth (height) of leafy spurge seedlings in a greenhouse experiment.

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**Buds on the primary root**. Similar to buds on the hypocotyl, additional buds are developed on the primary root early in the development of seedlings. As the seedlings age, the number of buds on the primary root increases (Figure 19). In addition to root buds, many lateral long roots of the spreading type are produced and they behave as the primary root (32).

The development of buds on the hypocotyl and primary root, especially the latter, has a considerable depressive effect on seedling shoot growth. Growth of the epicotyl is very slow, presumably because of the increase in the number of buds developed on the primary root (Figure 20).

**Buds on mature underground parts**. In addition to roots, the underground parts of leafy spurge include the basal parts of adventitious shoots and in some instances the basal parts of hypocotyls. The underground parts show considerable cambia and phellogen meristematic activity (Figure 17). Because of the formation of cork tissue, the older underground parts are brownish. In some instances, especially in older roots, remains of obliterated cortex can be observed.

The adventitious buds are pinkish and there are more of them in shallower depths than in deeper levels; this trend also corresponds to the density of roots (6, 7). The number of additional buds on a vertical root decreases with increased depth, and roots at deeper levels do not contain any visible buds (33). When such roots are fragmented and brought to the surface, they do regenerate (33).

**Ontogeny of the root bud**. The root buds on the primary root, whether or not associated with lateral roots, are initiated endogenously in the pericyclic region opposite to the protoxylem pole of the parent root. The origin of such an initial is synchronized with the beginning of cambial activity. Many spreading long roots also arise in a similar way. Most root and bud primordia can be distinguished in the parent root before their emergence through the cortex to the exterior. (2).

In the old or mature roots, which are basically persistent long roots, the pattern of bud initiation is different. The primary cortex in these roots is obliterated and replaced by a secondary cortex, which is essentially secondary phloem (Figure 17) (33). In the parent root, the protostele is obvious and often the wide rays may be recognized opposite to protoxylem or in the secondary xylem. The secondary cortex and the xylem rays are filled with starch and other storage substances. Subjacent to the phellogen in the peripheral regions of the cortex, a few cells differentiate to become bud primordia (33). The bud procambia extend basipetally to establish connection with functional vascular tissue of the parent root. Such buds can develop further into shoots under favorable conditions.

### VI. Laticiferous tissue

Leafy spurge, like many other members of the Euphorbiaceae, contains laticiferous cells, which are branched and of the nonarticulated type. The initials are laid down in the embryo and they grow and ramify throughout the embryo cortex. Ramified laticiferous vessels are abundant in aerial and underground parts of leafy spurge and are similar to other investigated species of *Euphorbia* (17). The short roots and the primary cortex of all roots do not contain the laticiferous tissue. In old roots the secondary cortex, made up

of secondary phloem, contains proliferated masses of latex vessels, which do not extend into the woody tissue of the root. Observations on leafy spurge indicate that roots by themselves do not seem to produce laticiferous cells. It appears, therefore, that the buds on roots and the aerial shoots are the only organs responsible for the initiation and extensive development of nonarticulated laticifers in leafy spurge. The latex contains abundant starch, which is characteristic of *Euphorbia* (2, 12). It can also deter insect attacks (4).

# VII. Vegetative reproduction: An anatomical basis

One of the most important traits that has made leafy spurge an aggressive weed in cultivated and uncultivated areas is its ability to reproduce vegetatively (32, 33, 41). A wide variety of specialized organs exclusively for vegetative reproduction are developed on plant parts in unrelated taxa of vascular plants (11, 27, 37, 40). Morphologically specialized structures such as runners or rhizomes are not observed, but leafy spurge does have the ability to produce buds abundantly on underground parts, especially the roots. The production of vegetative buds on underground parts is greatly enhanced by injury, and consequently the distinction between the spontaneously produced buds and the ones formed after an injury in the field becomes obscure.

Whether the buds produced are additional or reparative, "It is certain that the behavior of the plant is closely correlated with its structure and mode of growth" (27). The root system in leafy spurge is important for perennation and propagation (41). The long root, which establishes the framework in a mature root system of leafy spurge, is anatomically dynamic and persistent. The lateral meristem (cambium) appears very early in root development and produces abundant phloem parenchyma, which is periodically replaced in the root. The phloem and the ray parenchyma, especially the latter, contain abundant storage materials and also become important sites of bud initiation (27). The ability to produce adventitious buds presumably is determined genetically. However, the extent of such regeneration may be controlled by a variety of internal and external factors. In leafy spurge, one anatomical feature correlated with bud regeneration, especially in roots, is the removal of primary cortex. Early in the development of the root, the primary cortex is obliterated or removed, and consequently the internal tissues, particularly phloem parenchyma, are exposed to the environment. Such an exposure of the internal tissues appears to have a stimulatory effect on cambium proliferation and bud initiation in leafy spurge.

# VIII. Conclusions

Many publications on leafy spurge suggest that the weed has spread westward very successfully since it was first recognized in eastern North America (21, 22, 38). The aggressive and colonizing ability of leafy spurge for the most part can be attributed to the vigorous and extensive growth of long roots, which are also capable of regeneration. The short roots do not regenerate and they do not contribute to the framework of the persistent root system. The vigorous growth and long retention of the ability to grow can be correlated with the organization of the long-root apices (34, 35).

Two types of roots, long and short, have been recognized in leafy spurge with a view to relate them meaningfully to their functional difference (32, 35). Esau (9, 10) has identified three types based on their ontogenies; the primary, the lateral, and the adventitious roots. The ontogenies of roots in leafy spurge (unpublished data) support Esau's classification. The primary root, which in function and structure is a long root, is unique because of its origin at a very early stage during embryo differentiation. The lateral roots of the short-root type arise in the pericycle of the parent roots. The third type is the adventitious roots, which have their origin anywhere but in the pericycle and whose origin is usually associated with the cambium in the parent organs. On this basis there are the embryonic primary root, the pericyclic lateral roots, and the adventitious roots in leafy spurge. Functionally, the primary and the adventitious roots are similar, and there is ample justification in recognizing two kinds of roots, long and short, in leafy spurge.

The long roots persist for several years and produce a large number of buds. Although numerous buds are present, only a few develop into shoots, exhibiting the phenomenon of apical dominance (18, 33). The early formed vigorous shoots establish dominance over the other buds. However, early in the season many buds on underground parts, especially on older stems (crowns), develop into shoots apparently showing no apical dominance (Figure 4). Observations in the field show that the developing shoots in an established root system do not seem to have control over buds on roots. Buds initiated on roots do not develop directly into aboveground shoots. Most adult shoots, especially the flowering shoots, are developed from buds initiated on underground older crowns.

The persistent long roots, especially in shallower depths, grow horizontally and invade new areas; they also produce buds and establish new growth centers. In some instances the lateral primordia produced on roots are morphologically indistinguishable. The nature and extent of vasculature and the base of the primordia connecting to the parent organ may have some influence on the determination of their potentiality to develop into roots or buds and this needs to be pursued.

Anatomical studies of leafy spurge indicate a strong correlation between extensive regeneration and structural behavior of roots. Early initiation of cambium and production of secondary tissues cause a breakdown of the primary cortex. Such obliterated cortex is soon replaced by secondary phloem consisting predominantly of parenchyma filled with reserve substances. This parenchyma forms the main site of initiation of lateral primordia. The vertical roots, which lose their cortex sooner than horizontal roots, have doubtless a greater ability to regenerate.

The obliteration of cortex, the periodic replacement of secondary phloem, and the lack of uniform cambial activity bring vascular discontinuity among roots; anatomical differences between thinner horizontal roots and thicker vertical roots are obvious. This is further complicated by the scattered development of shoots on the same root system. The lack of vascular continuity – especially of phloem – in the mature root system is of considerable importance in affecting chemical measures to control the spread of leafy spurge.

The analyses of the inflorescences in two populations of leafy spurge in Saskatchewan indicate some basic patterns of development. A cursory examination of inflorescences in other sites of leafy spurge infestation has revealed a comparable morphology of the inflorescence. The compound dichasia (15, 36) appear early in the growing season and are considered to represent the fundamental type, and the other types, such as cymes and determinate umbels, are derived from it (Figure 15). The terminal compound dichasia are borne on vigorous shoots, which arise on persistent thick underground stems. The determinate umbels appear on lateral shoots or on shoots arising on thin underground stems. The intermediate type, the cyme, appears on shoots arising on thin or thick underground stems. The degree of ray branching, which forms the basis in the recognition of inflorescence types in leafy spurge, apparently is controlled to a considerable extent by environmental factors, including nutrition. In some instances, careful excavations show that shoots bearing different types of inflorescence are connected by the same root (Figure 8). Therefore, the three types of inflorescence are interpreted to exhibit a developmental sequence by reduction from the basic type compound dichasium—the cyme—the determinate umbel. (The reverse sequence is perhaps also possible through amplification). Such an occurrence of variations in inflorescence morphology has considerable adaptive significance (39). Factors such as the accidental removal of apical dominance and the spraying of chemicals in the field may increase polymorphism in the inflorescence (Figure 13).

The development of an extensive root system and the ability of roots to produce buds enable leafy spurge to explore new habitats efficiently and to compete with other plants. The buds and their associated root parts may form independent units of propagation. Thus, in an extensive root system, where all roots are connected, despite their lack of vascular continuity, innumerable independent propagules are formed. Such a large reserve of propagules may last for several years. In addition, the roots are also capable of producing buds on injury (reparative buds) and thus augment bud production. The ability to produce adventitious buds no doubt is associated with the aggressiveness and persistence in both native and introduced prairie species (31, 41). Whereas introduced weeds with this ability, such as leafy spurge, have persisted under tillage, most native species having the same characteristics have not persisted. "Possibly the difference may lie in the general area of lack of biological control of introduced organisms" (41).

Leafy spurge shows considerable variation in vegetative and floral morphology, and this has caused some problems in the taxonomy of this species. Variations in the morphology of the inflorescence of leafy spurge reported in the present studies represent different developmental stages of the same fundamental pattern, the compound dichasium. Shoots bearing different inflorescence types and connected by the same root provide additional evidence that the same plant can have different, but closely related, morphological types of inflorescences (Figure 8). Different patterns of inflorescence do occur in the same plant showing various degrees of development (39). Similarly, leafy spurge plants grown in high nitrogen are more robust and vigorous in their vegetative growth than those grown in low nitrogen (18, 19). Therefore, only one highly polymorphic species of leafy spurge should possibly be recognized. More autecological studies are, however, needed to determine the factors that control polymorphism in this species.

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### Literature cited

- 1. Bakke, A. L. 1936. Leafy spurge, *Euphorbia esula* L. Iowa State Agric. Exp. Stn. Res. Bull. 198:209-246,
- 2. Bakshi, T. S. and R. T. Coupland. 1959. An anatomical study of the subterranean organs of *Euphorbia esula* in relation to its control. Can. J. Bot. 37:613-620.
- 3. Bentham, G. and J. D. Hooker. 1912. Handbook of the British Flora. Lovell Reeve and Company, London. 606 pp.
- 4. Best, K. F., G. G. Bowes, A. G. Thomas, and M. G. Maw. 1980. The biology of Canadian weeds. 39. *Euphorbia esula* L. Can. J. Plant Sci. 60:651-663.
- 5. Clowes, F.A.L. and H. E. Stewart. 1967. Recovery from dormancy in roots. New Phytol. 66:115-123.
- 6. Coupland, R. T. and J. F. Alex. 1954. Distribution of the underground parts of leafy spurge (*Euphorbia esula* L.) Can. J. Agric. Sci. 34:161-176.
- 7. Coupland, R. T. and J. F. Alex. 1955. Distribution of vegetative buds on the underground parts of leafy spurge (*Euphorbia esula* L.). Can. J. Agric. Sci. 35:76-82.
- 8. Esau, K. 1965. Vascular differentiation in plants. Holt, Rinehart, and Winston, New York. 160 pp.
- 9. Esau, K. 1965. Plant Anatomy. 2nd Edition. John Wiley and Sons, New York. 767 pp.
- 10. Esau, K. 1977. Anatomy of Seed Plants, 2nd Edition. John Wiley and Sons, New York. 376 pp.
- 11. Fernald, M. L. 1950. Gray's Manual of Botany. 8th Edition, American Book Company, New York. 1632 pp.
- Hanson, H. C. and E. Rudd. 1933. Leafy spurge life history and habits. North Dakota Agric. Coll., Agric. Exp. Stn. Bull. No. 266. 24 pp.
- 13. Hegi, G. 1925; Illustrierte Flora von Mittel-Europa. Band V. 1. Pages 170-172. München. 678 pp.
- 14. Ho, T.W.M. and M.V.S. Raju. 1972. Experimental studies on leafy spurge (*Euphorbia esula*). Histology of the adventitious shoot apex. Can. J. Bot. 50:635-641.
- 15. Lawrence, G.H.M. 1951. Taxonomy of Vascular Plants. Macmillan Company, New York. 823 pp.
- Looman, J. and K. F. Best. 1979. Budd's Flora of the Canadian Prairie Provinces. Res. Branch, Agric. Canada, Publication No. 1662. 863 pp.
- 17. Mahlberg, P. G. and P. S. Sabharwal. 1968. Origin and early development of nonarticulated laticifers in embryos of *Euphorbia marginata*. Am. J. Bot. 55:375-381.
- 18. McIntyre, G. I. 1972. Developmental studies on *Euphorbia esula*. The influence of the nitrogen supply on the correlative imbibition of root bud activity. Can. J. Bot. 50:949-956.
- 19. McIntyre, G. I. and M.V.S. Raju. 1967. Developmental studies on *Euphorbia esula* L. Some effects of the nitrogen supply on the growth and development of the seedling. Can. J. Bot. 45:975-984.
- 20. Moss, E. H. 1971. Flora of Alberta. University of Toronto Press, Toronto. 546 pp.
- 21. Muenscher, W. C. 1930. Leafy spurge and related weeds. Cornell Extension Bull. No. 192. 10 pp.

- 22. Muenscher, W. C. 1955. Weeds. 2nd Edition. Cornell University Press, Ithaca, New York. 586 pp.
- Myers, A., C. A. Beasley, and L. A. Derscheid. 1964. Anatomical studies of *Euphorbia esula* L. Weeds 12:291-295.
- Norton, J.B.S. 1900. A revision of the American species of *Euphorbia* of the section Tithymalus occurring north of Mexico. Missouri Bot. Gard. Report No. 11. 142 pp.
- 25. Peterson, R. L. 1975. The initiation and development of root buds. Pages 125-161, *in:* J.G. Torrey and D.T. Clarkson (eds.), The Development and Function of Roots. Academic Press, London. 618 pp.
- 26. Polunin, O. 1969. Flowers of Europe. Oxford University Press, London. 662 pp.
- Priestley, J. H. and C. F. Swingle. 1929. Vegetative propagation from the standpoint of plant anatomy. U.S. Dep. of Agric. Tech. Bull. No. 151. 98 pp.
- Raju, M.V.S. 1968. Developmental studies on leafy spurge (*Euphorbia esula*). Apices of seedling and adventitious shoots. Can. J. Bot. 46:1529-1532.
- Raju, M.V.S. 1975. Experimental studies on leafy spurge (*Euphorbia esula* L.). I. Ontogeny and distribution of buds and shoots on the hypocotyl. Bot. Gaz. 136:254-261.
- 30. Raju, M.V.S. and T.W.M. Ho. 1973. Developmental studies on leafy spurge (*Euphorbia esula*). Histochemical and autoradiographic studies of the adventitious shoot apices. Can. J. Bot. 51:211-219.
- Raju, M.V.S., R. T. Coupland, and T. A. Steeves. 1966. On the occurrence of root buds on perennial plants in Saskatchewan. Can. J. Bot. 44:33-37.
- Raju, M.V.S., T.A. Steeves, and R.T. Coupland. 1963. Developmental studies on *Euphorbia esula* L. Morphology of the root system. Can. J. Bot. 41:579-589.
- Raju, M.V.S., T. A. Steeves, and R. T. Coupland. 1964. On the regeneration of root fragments of leafy spurge (*Euphorbia esula* L.). Weed Res. 4:2-11.
- Raju, M.V.S., T.A. Steeves, and J. Maze. 1976. Developmental studies on *Euphorbia esula*: Seasonal variation in the apices of long roots. Can. J. Bot. 54:605-610.
- Raju, M.V.S., T. A. Steeves, and J. M. Naylor. 1964. Developmental studies on *Euphorbia esula* L.: Apices of long and short roots. Can. J. Bot. 42:1615-1628.
- 36. Rickett, H. W. 1944. The classification of inflorescences. Bot. Rev. 10:187-231.
- 37. Salisbury, E. J. 1942. The Reproductive Capacity of Plants. G. Bell and Sons, Ltd., London. 244 pp.
- Selleck, G. W., R. T. Coupland, and C. Frankton. 1962. Leafy spurge in Saskatchewan. Ecol. Monogr. 32:1-29.
- Stebbins, G. L. 1974. Flowering plants Evolution above the species level. The Belknap Press, Cambridge, Massachusetts. 399 pp.
- Steeves, T. A. and I. M. Sussex. 1972. Patterns in Plant Development. Prentice-Hall, Englewood Cliffs, New Jersey. 302 pp.
- Steeves, T. A., R. T. Coupland, and M.V.S. Raju. 1966. Vegetative propagation in relation to the aggressiveness of species. Pages 121-137, *In* R.L. Taylor and R.A. Ludwig (eds.), The Evolution of Canada's Flora. University of Toronto Press, Toronto. 137 pp.
- 42. Webster, P. L. and H. D. Langenauer. 1973. Experimental control of the activity of the quiescent center in excised root tips of Zea mays. Planta 112:91-100.
- 43. Wicks, G. A. and L. A. Derscheid. 1964. Leafy spurge seed maturation. Weeds 12:175-176.