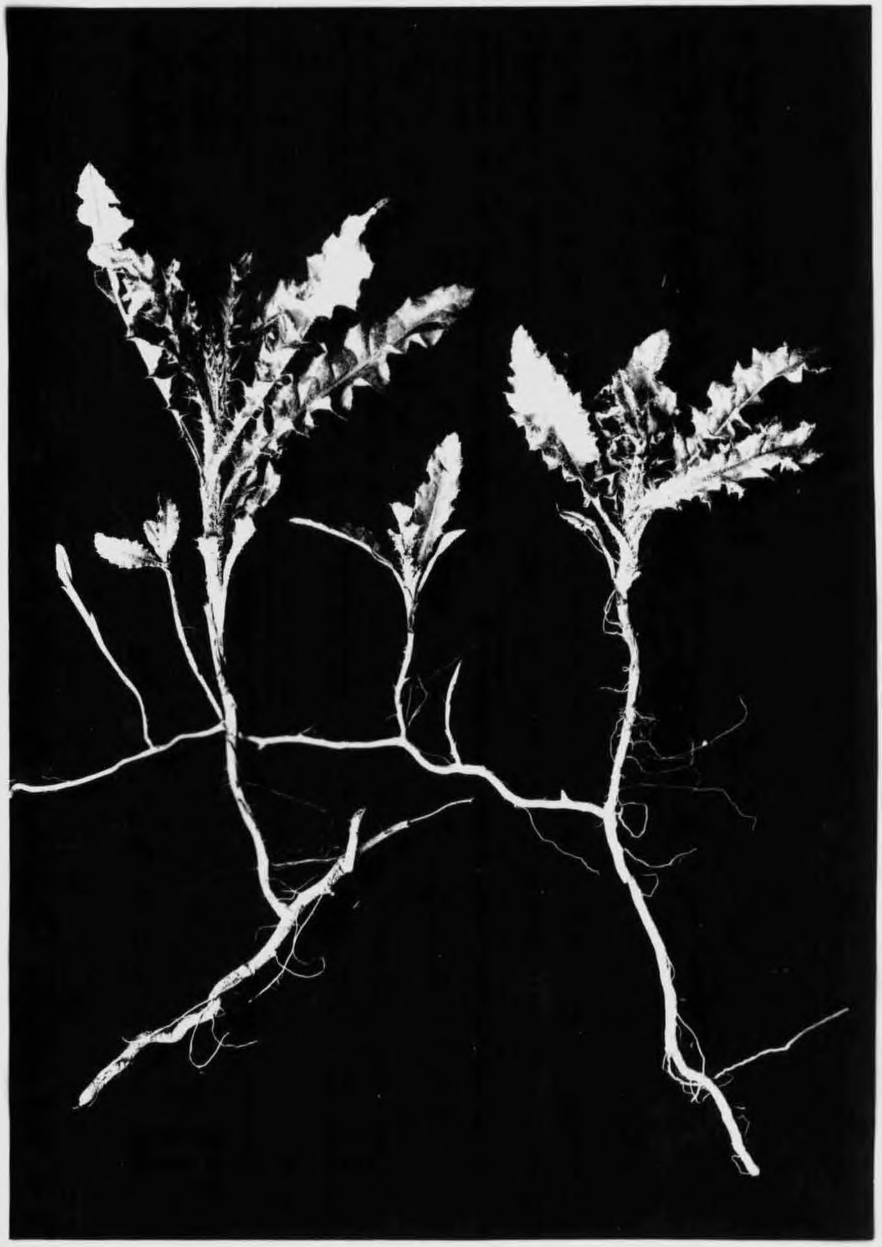
A morphological and histological study of the underground organs of the Canada thistle
by Chester W Griffin

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the Degree of Master of Science in Botany and Bacteriology
Montana State University


Abstract:
1. The underground creeping storage organs of the Canada thistle are true roots, because they exhibit typical root structures both externally and internally, 2. The young roots show the diarch arrangement of vascular tissues, 3, The branch roots originate in the pericycle of the mother root, 4, The origin of the adventitious shoot primordium is in the pericycle of the underground storage roots, 5, Adventitious roots originate in one to three cells of the inter-fasicular cambium in young, etiolated thistle shoots, 6. The typical root structures are formed in the primordium of an adventitious root before it breaks through the epidermis of the stem* 7. The vascular system of the root is a continuation of the tissues of the two fibrovascular bundles between which it arises, 8. The underground storage roots give rise to etiolated shoots early in the spring and these shoots develop adventitious roots in abundance, 9. The plant propagates itself by the horizontal root system, or segments of it, and by the shoots which may have become severed from the storage roots, 10, In nature, the shoot normally remains attached to the underground roots. The first adventitious roots, which arise on the young stems, are intemodal and just above the axils of the leaves, later, numerous roots develop along the internodes, 11, Numerous adventitious roots can be forced at the internodes by severing the shoot from the underground root and placing It in darkness and excessive moisture*



Cirsium arvense Tourn.
(Canada thistle)

The adventitious shoots of the "Canada thistle" develop upon the extensive creeping root system, and give rise to numerous adventitious roots.

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# A MORPHOLOGICAL AND HISTOLOGICAL STUDY OF THE UNDERGROUND ORGANS OF THE CANADA THISTLE

## INTRODUCTION

The creeping underground portions of *Cirsium arvense* (L.) Scop., which function as storage organs and as a means of propagation, have been referred to in the literature both as "underground roots" and "rootstocks." It is evident from the confusion of terminology pertaining to these underground portions, that no histological work has been reported which shows that they are "underground roots", or, that they are "rootstocks." The writer has found no records showing the anatomy of the Canada thistle with reference to the origin of the branch roots, the origin of the adventitious shoots, or the origin of the adventitious roots which develop upon the adventitious shoots. In the study reported in this paper, the writer has investigated: (1) the external and internal structure of the creeping underground storage organs of the Canada thistle; (2) the origin of typical branch roots; (3) the origin of adventitious shoots from the underground storage organs; and (4) the origin of the adventitious roots which arise from the adventitious shoots.

## NOMENCLATURE

In 1623, Caspar Bauhin (2) called the plant *Carduus in avena proviens*. Bauhin was the first man to use the generic name *Carduus* for this plant. In 1687, Theodorus Jacobus Tabernaemontanus (29) described and illustrated this thistle under the name *Carduus arvensis*. In 1700, Joseph Tournefort (30) named this thistle *Cirsium arvense*. According to Britton and Brown (3), Linnaeus, 1753, called the plant *Serratula arvensis* in his "Species Plantarum." In 1772, Johann Anton Scopoli (24) restored the name *Cirsium arvense* Tourn. In 1777, Steven Robson (22) referred to the thistle as *Carduus arvensis*, thus reviving the name previously used by Tabernaemontanus (29). In 1804, George Hoffman (14) published the name *Cnicus arvensis*.

*Cirsium arvense* Tourn., *Carduus arvensis* (L.) Robs., and *Cnicus arvensis* Hoffm., are used in the various manuals of flowering plants. Britton and Brown (3) separate the genus *Cnicus* from the genera *Cirsium* and *Carduus* by the manner in which the achenes are inserted upon the receptacle. In both *Cirsium* and *Carduus*, the involucral bracts are not hooked and the leaves are bristly. The separation of these two genera is based upon the characteristics of their pappus bristles. The pappus bristles of the genus *Cirsium* are plumose, while in the genus *Carduus*, they are not plumose.

Detmers (8) made a critical comparison of the characters of these named genera as given in the manuals, and she states that the Canada thistle belongs in the genus *Cirsium*. Jackson (16), in compiling the genera and species for the Index Kewensis, recognized the name *Cirsium arvense* Scop. Detmers (8) states that Scopoli (24) revived the name *Cirsium arvense* which had previously been used by Tournefort (30) in his description of the Canada thistle.

In this paper, the name *Cirsium arvense* (L.) Scop., which is in harmony with International Rules of Botanical Nomenclature, is adopted as the correct name for the plant which is commonly known as the Canada thistle.

## HISTORICAL

Tabernaemontanus (28) described the Canada thistle as "having a fusiform tap root with many smaller root branches." Knight (17) mentioned the occurrence on the stems of certain varieties of apples of "rough excrescences, formed by congeries of points which would have become roots under favorable circumstances." Lemaire (18) was concerned with the origin of naturally occurring endogenous adventitious roots in hypocotyls, stolons, and rhizomes of herbaceous dicotyledons. He made a study of the origin of adventitious roots in several families which included the following species: *Veronica beccabunga* L., *Veronica officinalis* L., *Mimulus luteus* L., *Valeriana dioica* L., *Hippuris vulgaris* L., *Primula elatior* Jacq., *Polemonium reptans* L., *Epilobium tetragonum* L., *Circaea lutetiana* L., *Ranunculus aquatilis* L., *Callitriche stagnalis* Scop., *Chrysosplenium oppositifolium* L., *Montia rivularis* Gm., *Mentha arvensis* L., *Alchimilla vulgaris* L., *Mercurialis perennis* L., *Hedera reticulata* DC., *Oxalis stricta* L., *Lotus uliginosus* Schk., *Viola palustris* L., *Vinca major* L., *Asperula odorata* L., and *Nasturtium officinale* R. Br. He grouped the plants studied into the following four classes, of which the first is the most common: (1) all tissues of the root originating in the pericycle of the stem; (2) the central cylinder of the root coming from the pericycle, and other regions from the endodermis and inner cortex of the stem; (3) all tissues of the root coming from the cambium; (4) the central cylinder of the root formed by the cambium, and the other tissues by the pericycle of the stem. Van Tieghem and Douliot (32) made a study of the origin and formation of endogenous and naturally occurring adventitious roots in stems. They concluded that most of the plants, which normally give rise to adventitious roots, have the origin of the adventitious root primordia in the pericycle of the stem. They found that the roots of different plants may develop in other tissues besides the pericycle, and that roots are usually formed endogenously. In _Alliaria officinalis_ Andrz., a crucifer, they report the endogenous development which is the most common type of root development, and that the origin of the root primordia is in the pericycle of the stem except in older stems where the pericycle has lost its "root forming character."

Goebel (11) discusses the development of normal and adventitious roots and shoots, and states that normal roots are usually endogenetic, beginning in the pericycle of the chief root and bursting through the peripherial tissue. The endogenetic type of root or shoot originates beneath the cortex and grows outward, penetrating the cortex and finally rupturing the epidermis. In the exogenetic type, the structures have a superficial origin and the tissue surrounding the developing organs persists for a short time and functions as the outer-most tissue of the newly formed organs. The exogenetic type of root formation is uncommon and has been observed in only a few cases. The epidermal and cortical cells in the axils of the leaves may divide to send out protuberances which are the beginnings of adventitious roots.

Gray (13) described the Canada thistle and called the underground creeping portion the "rootstock." Clark and Fletcher (6) state that the Canada thistle propagates itself by seeds and extensive "rootstocks." Coulter and Nelson (7) and Britton and Brown (3) have referred to the creeping underground portions of the Canada thistle as "rootstocks." Georgia (10) states "The jointed, horizontal rootstocks are the most obnoxious part of the plant; round, slender, like tough, white whipcords, lying so deep in the ground as to be always sure of moisture, they creep in every direction for rods, sending up new plants at short intervals; ......" Piper and Beattie (20) have referred to the creeping underground portions of the Canada thistle as "rootstocks." Swingle, Morris, and Jahnke (28) have referred to the underground portions of the Canada thistle as "rootstocks." Eames and MacDaniels (9) state "Adventitious roots develop by the formation of apical root meristems in the pericycle of stems and roots, or, in older axes, where the pericycle is no longer active, in the secondary phloem." The growing root then forces its way through the outer tissues. In some of the pteridophytes, and rarely in angiosperms, adventitious roots are said to arise from the cells of the outer cortex. It is possible that these roots are haustoria, or other emergences." Van der Lek (31) seems to have been the first to recognize the relation between the primary vascular system and the distribution of "root germs" on woody plants. Working with _Ribes nigrum_ L., _Salix_ sp., and _Populus_ sp., he found that most of the numerous "root germs" arise in definite relation to the primary vascular system. He described "root germs", present in the young branches, as being nodal and internodal roots. In some species of plants, the roots are largely nodal while in others, they are internodal. Swingle (27) has shown that our commercial varieties of apples can be grown from cuttings containing burrknot formations. He observed that in apple stems, the root germs may be initiated in the cambium ring at the branch gaps, the leaf gaps, the primary medullary rays, and the secondary medullary rays. Smith (26) reported that in cuttings of _Coleus blumei_ Benth., "the first recognizable sign of the development of an adventitious root is the appearance in the cambium of a nest of highly active meristematic cells." Detmers (8) studied the Canada thistle from the standpoint of gross morphology, propagation, and control. She states, "There is a complex root system, consisting of the fibrous absorbing organs and horizontally creeping branching thickened roots which are organs of storage and propagation." Carlson (4) reported that "adventitious roots arising between the fibrovascular bundles from the bases of young cuttings of _Coleus blumei_ Benth., originate in one to several adjacent cells of the pericycle." Wilson (33), working with _Roripa austriaca_ Spach., a crucifer, found that the origin of the adventitious roots was exogenous rather than endogenous. Rogers (23) described the general appearance of the Canada thistle and studied its methods of propagation and control.

In regard to the creeping underground portion of this plant, he states, "......growing in patches and by its horizontal branching roots." Aslander (1) made a study of the Canada thistle and its control with chlorates and other herbicides, in which he states, "The perennial part of Canada thistle is its root or root system, the principal part of which consists of the propagation roots which grow more or less horizontally 15-30 cm. below the surface of the ground. These roots form numerous shoots." Priestley and Swingle (21) have shown that in Crambe maritima L., the adventitious roots originate in the cambium. In Anemone baldensis L., and Convolvulus arvensis L., they have found that the adventitious shoots originate in the pericycle and develop endogenously. The branch roots of these species have the same origin and type of development as the adventitious shoots have. They state that the adventitious buds usually have their origin in the same tissue that the branch roots do, and that it is not their place of origin that determines whether the structure will be root, or shoot, but the organization of the growing structures. A root initial never forms a shoot bud, and a shoot initial never gives rise to a root. Mann (19) states, "Adventitious roots in the strawberry runner arise from small groups of meristematic tissue within the stele alternating with the primary vascular strands, and between the outer pericycle tissue and the medullary tissues of the centre of the stele." These groups of meristematic tissue are observed in greatest number in the region of departure of the leaf-trace bundles. Howe (15) found an exogenetic origin of the adventitious roots of Ceratopteris thalictroides L. The initial of the first adventitious root at the node developed from a hypodermal cell derived from the cells immediately below the leaf initials.

It is evident from a review of the literature, that very little work has been reported which pertains to the histology of the Canada thistle. Most of the investigators have been interested in its methods of propagation and control because it is such an undesirable weed which spreads rapidly and persists under adverse conditions in the agricultural centers. Since the Canada thistle exhibits such an abundance of storage roots, branch roots, adventitious shoots, and adventitious roots, it has been a very favorable species for histological study of these organs.

## MATERIALS AND METHODS

The Canada thistle, due to the mild winter of 1933-34, was available during the entire winter along the road-sides in the vicinity of Bozeman, Montana. The first thistle stems used were grown in the greenhouse from underground roots collected in February. The roots were dug beside a field west of the Montana State College campus. Portions of the roots were planted in a few inches of soil and covered with a foot of sawdust. After four or five days, the shoot buds began to appear upon the roots, and some of this material was prepared for sectioning for the study of the origin of the adventitious shoots. In three weeks, the shoots had developed to such an extent that they were emerging from the sawdust.

By the time that the stems emerged from the surface of the sawdust, the adventitious roots were just appearing through the ruptured epidermis.

The second collection of material was from the sandy banks of a ditch near Bozeman Creek. The shoots had nearly reached the surface, as it was in the middle of March, and it was not necessary to grow them in the greenhouse. Young stems, with adventitious roots just penetrating the epidermis, were collected for sectioning. During the first part of May, the underground portion of the Canada thistle, which gives rise to numerous etiolated shoots in the early spring, was collected and studied in order to determine whether it was an underground root, or a rootstock. Only young new growths of this material were collected near Bozeman Creek. The following portions were sectioned: (1) the main horizontal portion; (2) the branch roots from the horizontal portions; (3) the main vertical portion; and (4) the branch roots from the main vertical roots. This material was also studied for the origin of the normal branch roots. The same histological technique was used for all of the anatomical studies.

## HISTOLOGICAL TECHNIQUE

Fixing.- The paraffin method, as described by Chamberlain (5), was used throughout the anatomical studies of the Canada thistle. Three killing and fixing fluids, Bouin's, Chicago, and formal acetic-alcohol were used, all of which were satisfactory. The Chicago fluid was used for most of the material because of its advantage in revealing the origin of the branch roots, adventitious shoots, and adventitious roots before imbedding in paraffin. Sections of the material, one-quarter to one-half inch in length, showing roots and shoots just penetrating the surface of their mother parts, were killed in the above fluids for periods of time varying from twenty-four to forty-eight hours. The material was then washed in running tap water over night.

Hardening and Dehydrating.- The following series of alcohols was used: 5, 20, 35, 50, 65, 75, 85, 95, and 100%. The material was left in each grade of alcohol up to the 85% for two hours. At this point, the material became practically transparent and the primordia of the developing organs, which had been slightly darkened by the osmic acid of the Chicago killing fluid, could be easily detected with the aid of a disecting binocular microscope. The material was cut into smaller pieces so that selected short lengths contained one or more of the primordia of the developing structures. The material was left over night in 85% alcohol and after that, for one hour in each of three changes of 95% and 100% alcohols.

Clearing.- The following absolute alcohol-xylol series was used: 5, 10, 25, 50, 75, and 100% xylol. The material was left in each grade for one to three hours. Three changes into pure xylol were made to remove all of the absolute alcohol.

Xylol to Paraffin.- The material and five times its volume of xylol were transferred to a bottle having a stopper. A piece of paraffin (58° - 60° C.), the size of a pea, was added and allowed to dissolve in the xylol at room temperatures (20°-25° C.) Similar additions of paraffin were added as rapidly as the preceeding piece had dissolved, until the xylol became saturated with paraffin. The bottle was then unstoppered and set on top of the paraffin bath (30°-40° C.) for eight to twelve hours to let most of the xylol evaporate.

Paraffin Bath.- The material was passed through three changes of fresh paraffin (58°-60° C.) and left in the bath (60° C.) for one hour after each change.

Imbedding.- Melted paraffin (58°-60° C.) and three or four pieces of the material were put into paper trays and left in the bath for one half hour, after which the trays were taken from the bath, the material oriented into the desired positions, and the paraffin quickly cooled by submerging it in a dish of 95% alcohol.

Cutting.- Sections 10 microns in thickness were cut from the material on a rotary microtome.

Fixing Sections to the Slide.- Sections, in series, were mounted on 50 x 75 mm. slides. Approximately one hundred sections could be mounted at a time and this facilitated finding developing structures. Haupt's gelatin fixative, Chamberlain (5), was used to fix the ribbons to the slide. A 4% formalin solution in water was used to float the ribbons in the process of flattening them out. It was necessary to let the slides dry for at least twelve hours. After this time, the sections were thoroughly fixed to the slide and they never came loose in handling after the paraffin had been removed.

Removal of Paraffin.- The paraffin was removed with pure xylol. It was found that a minimum of one-half hour was necessary to completely remove all of the paraffin from the sections.

Removal of Xylol.- The xylol was removed with absolute alcohol. The alcohols, used in going from absolute alcohol to lower concentrations, were kept in dropping bottles and the sections were washed only two or three seconds in each. The series consisted of 35, 50, 65, 85, 95, and 100% alcohols.

Staining.- The sections were then run down through the alcohols and into tap water. An aqueous solution of basic fuchsin, Gourley (12), was used to stain the lignified tissues of the vascular system of the material and the nuclei of the meristematic tissue of the developing organs. Gourley used this stain to trace the vascular systems in living plants, but it gives excellent results when used for staining sections of the Canada thistle stem and root. It gives the same results as safranin, but it is much more rapid and it does not come out of the tissues in passing up through the alcohols in the dehydration process. It over-stains the sections in about five to ten minutes. De-staining was done with distilled water containing 0.5% of concentrated hydrochloric acid. The slides were de-stained until the tissues of the cortex showed only a faint tinge of red when held up to a white background. When they were observed with the microscope, the vascular bundles and the nuclei showed a bright red coloration as a result of the acid treatment. The acid was thoroughly washed from the tissue by leaving the slide in running tap water for a period of from fifteen to thirty minutes. The tissue was dehydrated through the dropping bottle series of alcohols and then washed several times with absolute alcohol. The sections were counter-stained and cleared at the same time by using Licht Grün dye dissolved in clove oil, Chamberlain (5). From three to five minutes was all that it was necessary to leave the slide in the mixture of light green and clove oil in order to stain the tissue. The Licht Grün, in clove oil, was removed with clear clove oil. At this point, the slides were observed under the microscope to see if any root or shoot primordia were present in any of the sections. If there were any, the slide was completed, otherwise, it was discarded. The clove oil was removed with xylol, and neutral balsam was used to mount the cover glasses on the slides. They were then allowed to stand until the balsam hardened.

## PROPAGATION

The Canada thistle propagates itself by seeds and by underground roots, and is a dioecious plant. In case plants of only one sex are present in an isolated district, the local propagation can be carried on by means of an underground root system. When a single seed of the thistle germinates, the first root system is normal and vertical. However, these vertical roots soon send out lateral roots which grow horizontally from one to two feet below the surface of the ground. Rogers (23) reported that these roots store food for use in the replacement of the above ground parts of the plant and for the extension of the root system. Shoots are sent up from buds which arise on the horizontal roots as often as the tops are killed until the roots starve. The growing shoots were observed in February while the surface of the ground was still frozen.

Roots grow horizontally through the ground as far as twenty feet in a single season. A single horizontal root grows in one direction for two to four feet and bends down to form a vertical root which may penetrate the soil to a depth of fifteen or twenty feet. At the point where the horizontal root bends to form the vertical root, a shoot is sent up and another horizontal root forms which continues in the course of the mother root and gives rise to other shoots. These shoots, which develop from the buds on the underground storage roots of the thistle, are adventitious structures.

The horizontal roots of the thistle were dug from the frozen ground in February, cut into pieces two to four inches long, and planted in root boxes in the greenhouse. In two to three weeks, etiolated shoots had developed upon the roots and had emerged from the covering of sawdust. All of the shoots bore a few adventitious roots at this time. These roots were internodal and more numerous toward the base of the stem. Severing the shoot from the underground root hastened the development of the adventitious roots which appeared more evenly distributed along the stem from the base to a short distance behind the growing point of the shoot.

Mann (19) states, "Some plants, such as the strawberry and various species of the genus Rubus, produce specialized shoots which develop adventitious roots readily, and may be severed from the parent plant to continue a vigorous separate existence." In the case of the thistle, all of the shoots develop adventitious roots which enable the plants to exist separately if they become severed from the underground root system. According to Mann (19), there are others, such as various members of the genus Ribes, that can be propagated by means of cuttings, and severed dormant or growing shoots, which readily produce adventitious roots under favorable conditions, and will continue to grow vigorously. On the other hand, many species rarely develop adventitious roots, and are propagated with difficulty, or not at all, from severed portions of a parent plant. Under favorable conditions, the fragments of either the stems, shoots, or roots of the Canada thistle will propagate themselves by the development of adventitious shoots and roots.

The adventitious roots of the Canada thistle occur normally on mature plants in great abundance, and form a part of the root system through which the above-ground parts receive nourishment from the soil. If the shoot is severed from the underground root system, it persists entirely because of the adventitious roots which have developed on the part of the stem which is below ground.

Recent workers have attempted to explain the formation of adventitious organs with theories pertaining to the factors which control or initiate growth activity in the meristematic tissues of plants. Smith (25) suggests, "that physiological changes, coupled with the modifications of mechanical tissues, and brought about by etiolation, are very important factors in creating the necessary internal conditions for the development of root meristems." It was observed by Smith that in Clematis sp., starch disappearance accompanied the reduced deposition of polysaccharide material in mechanical tissues in an etiolated shoot still attached to the parent plant. The growth of root meristems in etiolated regions is attributed to the fact that this diminuation of excess carbohydrate, without any interruption in the nigrogen supply occurring, produces the necessary chemical conditions for regeneration and development of meristematic tissue. Further development of the roots follows a period of carbohydrate accumulation as indicated by deposition of starch in the storage parenchyma of the plant as a whole and by marked increase in dry-matter content. Priestley and Swingle (21) state that the theory of polarity and numerous other theories, as advanced by other workers for the development of adventitious meristems, can only be regarded as an indication of the type of problem that has emerged and requires solution. They state "Adventitious roots, whether their formation was started before or after isolation from the plant, depend upon the formation and maintenance of a meristematic tissue which is organized as root meristem and it is not determined by its position in the tissue." They also state that adventitious structures are not determined in their nature and position by the movement of special formative food substances in the plant, or by the existence of preformed "anlagen." The external pH is likely to have a direct influence upon the activity of the cells of the meristematic tissue, because it does have an influence on the behavior of protein substances in water.

What initiates the development of an adventitious root from a cell or group of cells in a meristematic tissue is not definitely known, but it is probably a combination of genetical, chemical, and physiological factors, acting both internally and externally, and influencing the cells in the meristematic tissue.

The term "adventitious" has been widely used in the literature. Its general use signifies that it pertains to any plant structure which is not produced in the usual manner, with reference to its place or time of development. Roots produced upon leaves, roots upon stems, and shoots upon roots are classified as adventitious structures.

Eames and MacDaniels (9), and Priestley and Swingle (21) have mentioned adventitious roots upon roots. They have applied the term to secondary roots developing from older roots upon which normal development of roots has ceased. This type of root usually results from the bruising of the root tissue. Any meristematic tissue, which becomes activated and produces its corresponding organ, would then be considered adventitious regardless of the structure upon which it was born. In the latter case, the determining factor is abnormal time of development and can be confirmed only by actually observing the structure when it arises. This would include shoots upon shoots, and roots upon roots, in which the place of origin was normal, but the time of development of the primordia was abnormal.

In this paper, the writer restricts the meaning of the term "adventitious" to those organs produced abnormally with reference to the parts on which they normally occur, such as buds produced from the internodes of the stem instead of the axils of the leaves, or, roots which do not arise from the radicle or its subdivisions, but from other parts such as stems or leaves.

The first collection of Canada thistle roots were cut into short segments and planted in root boxes. The shoots which arose from the roots bore many adventitious roots by the time that the shoots had emerged from the sawdust. The etiolated shoots were very succulent and fragile as compared to those which were later collected near Bozeman Creek. The segments of the underground root, from which they were grown, were short and contained a limited amount of food materials. Consequently, the growing shoots developed adventitious roots abnormally and as a result, their own growth was stunted. Although the shoot was not completely severed from the root, it was limited to such a small food reserve that its formation of adventitious roots was very similar to the method of forcing adventitious roots from cuttings, as mentioned by Goebel (11). He states that some plants, which do not usually have adventitious roots, or have very few of them, can be forced to produce them by making cuttings from a parent plant and placing them in darkness and excessive moisture.

The roots, which developed from the thistle stems grown in the greenhouse, appeared at the internodes, there being a larger distribution of them at the base of the shoots, but in no case where there any directly at the nodes or in the axils of the leaves.

The shoots, which were collected in March, near Bozeman Creek, had remained in contact with the root system until they were a foot or more in length. They were about twice as large in diameter as the shoots grown in the greenhouse, and were very sturdy. The only visible roots were internodal and just above the axils of the leaves. The etiolated, sessile, leaves were wrapped tightly around the shoots. Invaribly, just beneath the leaf and just above its axil, an adventitious root could be found which was just penetrating the epidermis. In many cases, the tightly wrapped leaf hindered the outward growth of the root, and the root tip, instead of being normally pointed, was blunted as shown in Plate IV, fig. 15. In nature, the adventitious roots first appear just above the axils of the leaves and continue development as soon as the etiolated leaves die, blacken, and slough away. Other adventitious roots arise later at the internodes until a complete root system consisting entirely of adventitious roots, is developed. These roots function as normal roots and they are capable of supplying the plant with all of the necessary nutrients from the soil. Some of these stems were severed from the underground roots and placed in darkness and excessive moisture. In two days, the adventitious roots had developed very abundantly at the internodes, but only one appeared near the axil of the leaf. Because the adventitious roots could be forced so easily, it was easy to obtain plenty of material for sectioning.

In the Canada thistle, the origin of the adventitious root primordia is endogenous. Van Tieghem and Douliot (32) distinguish between endogenetic and exogenetic types of root formation. Endogenous refers to a method of growth by which a structure in the early stages of development is covered by a layer of tissue of the producing member which does not appear in the formation of the new structure. In exogenous development, the member arises superficially and the surrounding structure, from which it arises, serves as the outer-most tissue of the developing organ for a short time.

## EXTERNAL MORPHOLOGY

Underground storage roots.- The external appearance of the horizontal and vertical storage roots is identical. The horizontal root grows parallel to the surface for several feet and then turns downward to form a vertical root. The vertical root may penetrate downward for many feet into the sub-soil. The horizontal roots give rise to numerous adventitious shoots and small branch roots, but after it turns downward to form the vertical root, it very seldom gives rise to shoots, although it still produces branch roots in abundance. The main root, whether horizontal or vertical, varies from a light yellowish color, in the young growth, to a brownish color in the older portions upon which the numerous shoots appear. The roots are fleshy and they have a single central thread-like core which may be easily drawn from the fleshy part when the root is broken. All of the roots, whether horizontal, vertical, branch, or adventitious, showed this same characteristic which indicated that they all had the same internal structure. It is especially noticeable in the young roots in which the fleshy portion may be easily broken to form a chain of fleshy segments which are held together by the tough central thread-like core. The shoots and branch roots arise at irregular intervals along the storage roots. The horizontal and vertical roots, whether young or old, do not exhibit any nodes, and scale or rudimentary leaves are entirely absent. This external morphology of this underground portion indicates that the term "rootstock", as used by various investigators, has been erroneously applied to these underground storage organs of the Canada thistle. Further evidence will be presented on page 25 to show that this part in question is a true underground root and not a "rootstock."

Adventitious shoots.- The etiolated shoots which are produced on the underground storage roots of the Canada thistle, emerge from the soil and form the stem of the mature plant. The shoots first appear as buds on the horizontal roots and some of them are formed on the vertical roots. Their development begins early in the spring while the ground is still frozen. After the ground thaws, the shoots grow rapidly until they emerge. As the shoots grow upward, the sessile etiolated leaves, which are wrapped tightly around the stems, develop spirally. They arise at nodes which are at regular intervals from the base of the shoots to their growing tips. After the shoots emerge from the ground, this type of leaf becomes the functional leaf of the above-ground part, but those that are born on stem beneath the surface, die, blacken, and slough away. After the plant matures, the etiolated leaves, which were formed in the early development of the shoot, are not detectable, and the underground portion of the stem has the external appearance of a primary root, although it has the internal structure of a stem.

The growing shoot soon develops a loosely organized pith which develops into a hollow central shaft that continues to form in the above ground portion of the stem. The shoot has the typical internal structure of a dicotyledonous stem which is discussed on page 28 in connection with the origin of the root primordia of adventitious roots which arise on the young shoots.

## INTERNAL ANATOMY

Underground Storage Roots.- These organs exhibit typical root structures both externally and internally and, therefore, the term "rootstocks" has been erroneously applied to these true roots. The external structure of these storage roots has already been described on page 23. Plate V, figs. 17, 18, 19, show the internal arrangement of the root tissues. The cortex, which consists of parenchyma and intercellular spaces, occupies the larger portion of the root. Rogers (23) states that the nature of the cortex is an advantage from the standpoint of food storage and aeration. He showed that the vertical roots have typical root structure, but he did not consider the creeping horizontal roots and their branch roots. The young roots are diarch as shown in Plate V, fig. 17. The central portion of the stele in the young roots is composed of pith. This pith is bounded by two areas of xylem and two areas of the phloem, which are alternately arranged. Plate V, fig. 18, shows the stele after the xylem has grown inward and replaced the pith. The lateral arrangement of the xylem is definite and alternates with the phloem regions, and the cambium weaves between the alternating xylem and phloem. The pericycle is composed of a few layers of cells outside and near the stele (Plate V, fig. 19). Plate V, fig. 19, shows a transverse section of an older storage root near a