ROOT INITIATION AND DEVELOPMENT IN VACCINIUM CORYMBOSUM L. WITH REFERENCE TO THE INFLUENCE OF ASSOCIATED FUNGI

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John Peter Mahlstede

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ROOT INITIATION AND DEVELOPMENT IN *VACCINIUM CORYMBOSUM L.* WITH REFERENCE TO THE INFLUENCE OF ASSOCIATED FUNGI

By

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TABLE OF CONTENTS

INTRODUCTION

Many plants are known to exist in close association with fungi, an interrelation between the fungus and the roots of the higher plants that is commonly called a mycorrhiza. When the plant or any part thereof contains hyphal fundaments, the relationship is either symbiotic or parasitic. This relationship complicates the nutrition of these plants and introduces speculation concerning the role of the fungus element. Friesleben (1934) indicates that the influence of the mycorrhizal fungus on the growth of the associated plant species is the result of the inactivation, destruction, or absorption of repressive substances in the rooting medium. Stimulation of root activity may be induced by the release of natural auxins from the individual hypha to the surrounding medium (Rayner, 1938). More recently many investigations concerned with the vegetative propagation of plants have been based upon the stimulation of adventitious roots by plant auxins. The stage of maturity of most plant parts governs partially the quantity of auxin present, and consequently influences the ability to regenerate missing parts. To clearly interpret adventitious root initiation it becomes necessary to understand the anatomical phases of

development, whether one approaches this phenomenon from the point of view of the influence of the auxin or the influence of fungal activity.

The present problem is designed to determine the anatomical aspects of root initiation and development in Vaccinium corymbosum L. with the presence or absence of associated fungal forms.

DISCUSSION OF PERTINENT LITERATURE

Mycorrhizal: Association between fungi and higher plants is not a recent concept. Numerous references have been made (Osborn, 1909; Weiss, 1904; Halket, 1930) to the presence of mycorrhizae in plants belonging to the Palaeozoic era. It was not until the third century B.C. that any mention of the occurrence of fungi with the roots of higher plants did exist. Theophrastus in the third century B.C. made note of the occurrence of fungi with the roots of oak and other trees (Kelley, 1950). In 1934 Asai recorded the presence of mycorrhizae in one hundred and ten out of one hundred and thirty-four families of higher plants. He assumed that these fungi were parasitic deriving elaborated food from the plants. Frank described the morophological nature of ectotrophic mycorrhizae. He explained the association as one of symbiosis whereby the plant's supply of water and mineral salts is increased as a result of the presence of the fungus; the fungus in turn used carbohydrates that were manufactured and stored in the plant. Stahl (1900) supported Frank's observations and advanced the theory that the mycorrhizal fungi associated with the roots of higher plants growing in a soil rich in organic matter enable the plants to acquire food through the increased area afforded by the numerous hyphae.

He believed that this increase in surface area increases the supply of water and mineral salts instead of reducing them by competition from separate micro-organisms inherent in the medium.

In contrast to the opinion of Frank and Stahl, McDougall (1914) concluded that the fungus was a parasite. This parasitic relationship is regarded by Burges (1936) as an example of a controlled attack because the activity of the fungus is curbed by the reaction of the host. Melin demonstrated in 1925, by pure culture techniques, that there was no significant difference between absorption by an infected and non-infected root. To support the theory that endotrophic mycorrhizae liberate and make available soil bound elements, Ternetz (1907) isolated species of Phoma from Vaccinium and demonstrated that this genus possesses a limited capability for fixing nitrogen. Rayner (1929) using aseptic innoculation reported that Phoma radicis oxycocci and vaccinii are capable of fixing nitrogen and are stimulated by the presence of organic matter in the soil.

Knudsen (1929) using species of Calluna and Freisleben (1934) using species of Vaccinium have reported that seedlings could normally develop without the associated fungi. Since the fungus has been located in seeds by Rayner, the seedling may contain fungus soon after germination. Waksman (1916) maintains that in

soils high in organic matter the microflora are composed of many fungi in no way connected to the mycorrhizal complex. By attacking the humus of the soil they liberate critical nutrients required for the growth of higher plants. He states that these micro-organisms may synthesize fats, waxes, hemicelluloses or proteins which are absorbed to the humus in the soil. Jahn (1934) used the term "petotrophic mycorrhiza" to describe this concept and found Mucor and Penicillium to be the most common genera indigenous to this soil.

The general classification of the fungal component of the mycorrhizal complex consists of four general types; the ectotrophic, the endotrophic, the ectendotrophic, and the pseudomycorrhiza.

The ectotrophic mycorrhiza refers to a definite morphological organ which consists of a slow growing short root and fungi that are regularly arranged (Hatch and Doak, 1933).

The endotrophic mycorrhiza refers to the association of the plant part and the fungus that is located inside the plant. Fossil records indicate their presence in the pre-historic period of civilization (Butler, 1938). Rayner (1929) reported the presence of endotrophic mycorrhizae in the genus Vaccinium as an annual occurrence in the plant. She observed that the mycelium in the stem is extremely fine, is associated with the

cell wall, and is inter and intra-cellularly located. Addoms and Mounce (1931) working with the cranberry, Vaccinium macrocarpon reported that all parts of the stem contained equal quantities of the fungus. In the young stem the fungus was noted in all regions except the epidermis. McDougall (1914) has observed in Quercus and Acer that the mycorrhizae appear during the summer in small numbers, increase substantially during the fall and winter, and die during the spring. The endotrophic fungus of Vaccinium macrocarpon is found more easily near the tips of the rapidly growing stolons and is most conspicious in parenchymatous cells of the pith and cortex (Addoms and Mounce, 1931). They noted dark masses in the ray elements which they believed presented progressive phases in the digestion of the hyphal strands. Rayner (1929) suggests that mycorrhiza formation in the genus Vaccinium is an annual activity influenced by the existing soil conditions. This is in agreement with the findings of McDougall for Quercus and Acer. Dufrenoy (1917) and Doak (1928) have also reported the presence of endrotrophic forms in the various tissue systems of Vaccinium.

The ectendotrophic relationship, a combination of ectotrophic and endotrophic forms, is described by Hatch and Doak (1933) as being found consistently in a limited number of species.

Pseudomycorrhizae resemble the mycorrhizal complex in appearance. Fungal activity is not beneficial to the plant associate. Invasion of roots by pathogenic fungi result in a decreased absorptive ability which subsequently results in the death of the plant in severe cases of infection.

Physiological: The production of roots on a cutting is dependent upon the formation and maintenance of root initials from the tissues situated distally on the severed stem. Many external factors have been shown to influence the production of adventitious roots on stem cuttings. Hitchcock (1928) working with a variety of plant species observed death of tissue accompanied by browning on the lower end of cuttings that rooted poorly in an acid medium. Partial or complete neutralization of the media tended to permit the development of callus and subsequent rooting without any apparent injury to the stem base. Cuttings of some species produced roots in acid peat moss providing it was abnormally wet. It has been suggested that this may be attributed to the higher intercellular oxygen content in the cambium regions (Sledge, 1930). Although cuttings taken in April and September produced roots, the style of cutting had no consistent influence on the rooting response of Vaccinium corymbosum L. (Ware, 1930). He found that the number of roots produced was directly related to an increase in stem diameter. Chadwick (1932)

found that by collecting hardwood cuttings in late winter or early spring to reduce the storage period, he could avoid much bacterial and fungal infection as well as prevent dessication of tissues. O'Rourke (1944) observed that vegetative wood of Vaccinium corymbosum L. rooted more readily than flowering wood. He also obtained a higher percentage of rooting ability on basal than on terminal stem pieces, with intermediate rooting ability on the medial pieces. There is no evidence to show that the location of the basal cut influences the rapidity or quantity of adventitious roots. Chadwick (1930) working with deciduous softwood cuttings found more roots when the basal cut was one half inch below the node, while O'Rourke (1950) found with hardwood stem cuttings of the highbush blueberry, that the position of the basal cut made no apparent difference in the resultant number or type of adventitious roots.

The relationship between adventitious root production and age of the tissue has been known for many years. Gardner (1929) reported that in general, hardwood apple cuttings taken from the outer perifery of young trees produced the greatest number of adventitious roots. In 1932, Hitchcock concluded that the rooting response of various greenwood cuttings was dependent upon the age of the material used. In hardwood stem cuttings of Malus domestica Bock two distinct growth phases were observed

to be in effect (Stoutemyer, 1937). The young or juvenile condition was favored by repeated cell division, but was not anatomically different from older mature wood. Cuttings made from the juvenile wood produced a greater quantity of roots, and this was concluded to be caused by a physiological process rather than a direct anatomical manifestation. Van Overbeck (1945) attributed the lack of adventitious root formation in a white variety of Hibiscus to a deficiency of natural auxin produced in the leaves.

Anatomical: An attempt to learn the origin of adventitious roots in vascular plants has been made by many investigators. Van Tieghem and Douliot (1888) reported that young adventitious roots in stems arise endogenously from the pericycle. They observed that the semi-mature parts of stems may produce roots from the phloem parenchyma and in mature wood adventitious roots originate from the cambium. They noted that lateral roots of Hypericum pyramidatum arose in the pericyclic region of the root. The root initial was first observed by the radial elongation of several bilateral pericyclic cells, with subsequent tangential divisions that projected the primoridium radially. The endodermis, cortex, and epidermis was ruptured by rapid cell divisions and elongation of the primordium. The initiation and development of lateral roots on terminal roots was concluded to be

synonymous with initiation and development of adventitious roots on young stems, rhizomes, and stolons (Van Tieghem and Douliot). Van der Lek (1925) has reported that in older stems adventitious roots arise close to medullary rays and in association with secondary tissues. Apparently it is impossible to make generalizations concerning the exact tissue involved in the initiation of adventitious roots in stem cuttings. The origin varies with the stage of maturity of the shoot at the time of adventitious root development (Priestley and Swingle 1929). They have described the change of the arrangement of tissue with age. With the development of the vascular cambium the ray cells in the cambium region are compressed and adventitious roots arise in association with the cambium. They reported that as growth continues the root initials arise in a group of undifferentiated air-free cells adjacent to the cambium. Connard and Zimmerman (1931) observed that in young stem cuttings of Portulaca oleracea L. adventitious roots generally arose in rays adjacent to the primary bundles, in comparison to their appearance in smaller connecting bundles where in older stems they were independent of any previously formed vascular tissues. Adventitious roots in stem cuttings of Coleus blumei L. were produced between the fibrovascular bundles originating in one to several adjacent cells of the pericycle (Carlson, 1929).

Carlson (1933) found that in semi-woody canes of the Dorothy Perkins and American Pillar roses the origin of adventitious roots was centered in single or in small groups of parenchyma tissue of the vascular cylinder at the base of the shoots. Van der Lek (1925) reported that young branches of Salix sp. and Populus sp. produced adventitious roots in connection with the cambium at the end of a medullary ray. In young stem cuttings of Lonicera japonica adventitious preformed root initials were produced from a group of cells extending from the cambium to the pericycle in contrast to adventitious roots induced by vegetative propagation that developed from the cambium layer (Sandison, 1932). Wolfe (1934) reported that adventitious roots in Cotoneaster Dammeri were produced as a result of the activity of one of the two groups of parenchyma cells in the divided bud gap.

Priestley and Swingle (1929) have observed that the development of adventitious roots on hardwood cuttings is directly related to the activity of the buds. They attribute it to "conditions governing meristematic activity in the neighborhood of the proximal wound and that delicate internal balance which converts this activity from the production of ordinary new vascular tissue to the organization of root growing points." This relationship hinges on the cambium which is the medium between the activity of the bud and the initiation of new root

primordia. They further noted that the resumption in cambial activity was governed by many factors, one of which was the displacement of air by sap in the intercellular spaces in tissues near the region of root initiation.

The production of adventitious roots in relation to the production of callus tissue at the base of woody cuttings has been investigated with a variety of vegetatively propagated plants. Knight (1926) and Zimmerman (1925) concluded that callus formation and root production were two individual processes. Knight observed that, although abundant callus formation accompanied prolific adventitious root formation the two processes were "collateral manifestations of internal conditions" and that "the relation between them is very susceptible to external conditions." Although hardwood cuttings of Prunus domestica, P. cerasus, and Malus domestica produced abundant callus at the basal end of the cutting very few roots developed (Zimmerman, 1925). The formation of callus on stem cuttings of Hibiscus Rosa-sinensis L. and Hevia brasiliensis resulted from the activity of the medullary ray system and was not related to the activity of the cambium (Sharples and Gunnery, 1933). Adventitious roots may be produced from callus tissue under optimum environmental conditions (Swingle, 1929). However, Knight (1926) has reported that callus formation is favored

by high water content of the soil in contrast to root formation which is favored by a greater supply of oxygen. The development of root primordia is believed to be retarded in callus tissue and consequently root emergence is limited to older, suberized callus tissues (Zimmerman 1925). In 1926, Swarbrick reported that wounds made on growing Sycamore, Rhododendron, plum and apple plants exhibited a seasonal variation in "wound gum" formation. He noted that from wounds made between the months of May and October partial or complete blocking of the vessels occurred as contrasted to the November to April period when no blocking took place. The normal sequence of wound cork formation resulted from the alteration of a cellulose surface to cutin or suberin followed by the accumulation of sap along the cut surface and subsequent stimulation of activity of a phellogen in the parenchyma region (Priestly and Woffenden, 1922). Priestly and Swingle (1929) recognized the significance of callus and cork formation when they considered vegetative propagation by cuttings. The healing or blocking of these exposed surfaces prevented the influx of saprophytic and parasitic fungi and possible disintegration of contiguous stem tissue. The blocking of exposed tissues also reduced loss of moisture from the cut at the base of the cutting.

There has been considerable emphasis placed upon stem anatomy and its relation to the capability of a woody

cutting to produce adventitious roots. Van der Lek (1925) concluded that the production of adventitious roots (morphological roots) near the base of the stem cuttings is restricted to certain morphological locations which were determined by the arrangement and type of tissues of the cutting. In several species of willows he found preformed root primordia, to which he gave the name "root germs". In the course of his study, Van der Lek (1925) made the observation that, in general, root primordia arise from a definite group of cells at a definite location in the stem but are not discernible from surrounding tissue at the time the cutting is made. He did not name or locate these regions.

EXPERIMENTAL PROCEDURE

Materials: For the first experiment, one year old stem cuttings of Vaccinium corymbosum L., variety Jersey were collected in a dormant condition in March of 1949 from a four year old planting growing in a peat bog north of Lansing, Michigan. Some material used in the second experiment was obtained from an established planting at the experiment station, South Haven, Michigan. This material was collected in the greenwood stage of maturity during the month of August, 1950. Other material was of the same type used in the first experiment.

For the initial trial a modified solar frame, divided into four sections was constructed similarly to a conventional cold frame (Figs. 1 and 2). Two inch lumber was used for the frame; the overall dimensions being six feet in length, six feet in width and two feet eight inches in depth. The frame was divided into four separate compartments which were lined with four cutting trays. These inserts (two feet eleven inches square) were constructed of six inch boards, covered with one-quarter inch hardware cloth stapled to the underside, and placed on braces nailed to the inside of each compartment twelve inches above the floor of the frame.

The second experiment required a special propagating frame that would allow frequent sampling and complete aeration as well as insure aseptic conditions. For this

FIGURE 1.

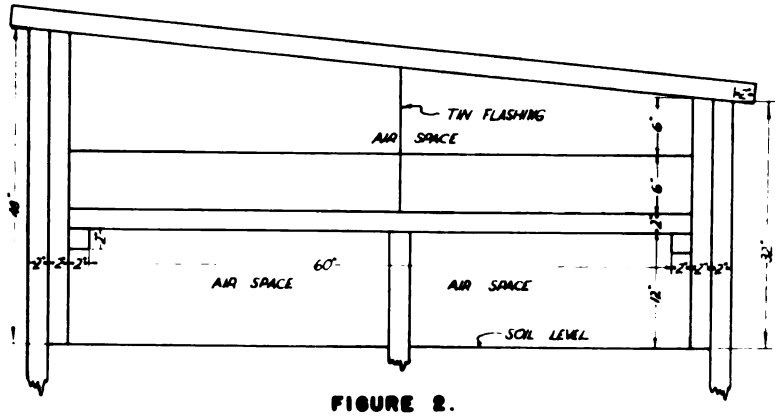

FIGURE 2.

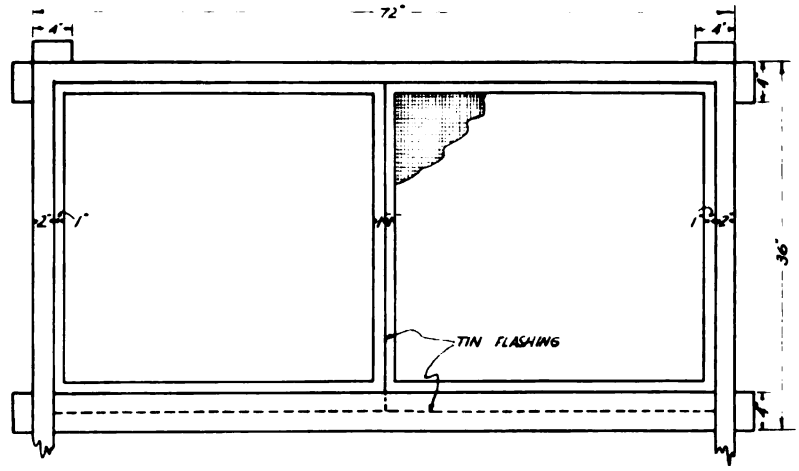

FIG.1. End elevation of propagation frame.

FIG.2. Plan view of propagation frame.

purpose a "Mahlstede" propagation chamber was designed and constructed. After a trial period in which Coleus blumei proved the success of the frame, ten additional boxes were constructed for the second experiment. Each box consisted of three parts: the outer shell, the insert, and the cover (Figs. 3 and 5). The outside measurements of the white pine shell were twenty inches in length, twelve and one-half inches in width, and twelve inches in depth. On one side of the box a board four inches in width was hinged to the shell to allow aeration of the structure. In the top of one end partition an isosceles triangle, (six by five by five inches) was cut out, grooved along the inner base and fitted to cover the removed section. The six inch deep insert was constructed of one-half inch white pine sheeting (eighteen and one-half by eleven and one-half inches). To the underside of this insert was stapled one-quarter inch hardware cloth, and it was covered with a one-half inch layer of rock wool held in place by cheese-cloth netting. To facilitate accessibility, a small triangular section was removed from one end partition to coincide with the opening in the shell. The cover (twenty-one and three-quarter inches by thirteen and one-half inches by three inches) was sealed with a single pane of glass. The entire structure was sealed with plastic wood and coated with aluminum oil paint.

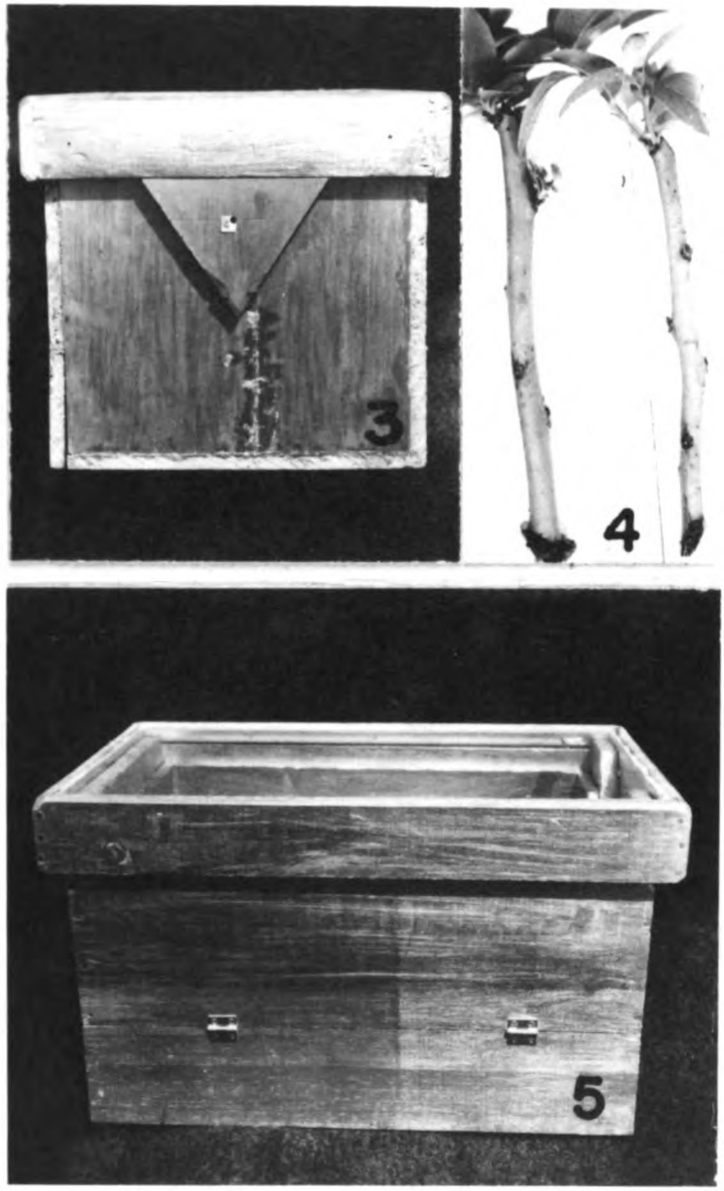

FIG.3. Propagation chamber, end view.

FIG.4. Hardwood cuttings of Vaccinium corymbosum L. variety "Jersey", showing callus (left) and beginning of roots (right) after 45 days.

FIG.5. Propagation chamber, front view.

Cultural techniques for the first experiment: To insure an accurate comparison throughout these investigations, cuttings were of uniform diameter, each shoot containing not less than four cuttings. The two distal parts were designated as terminal, and the two proximal parts as basal, cuttings. When a shoot could be divided into more cuttings, the medial section of the whip was discarded. Cuttings were four inches in length, their terminal horizontal cuts approximately one-eighth inch above, and the basal diagonal cuts immediately below, the bud.

All cuttings were placed in an air-tight container into which had been introduced 0.01 percent propylene oxide (Hansen and Snyder, 1947). The cuttings were allowed to remain for a period of two hours after which they were soaked in a solution of 7.2 percent slaked lime for a period of ten minutes. The cuttings were rinsed in two changes of sterile distilled water. Two hundred and forty terminal, and two hundred and forty basal, cuttings were placed in a complex of ten fungi, which had been isolated previously from similar stem tissue and root systems during the growing season. This group is later referred to as the treated material. An equal number of cuttings was soaked in a sterile chamber in sterilized distilled water for a period of ten minutes. This group is later referred to as the untreated material. Each insert in the modified solar frame which had been filled

previously with peat moss and sterilized in a steam chamber (180°F) for two hours was transferred under aseptic conditions to a transfer room. Each lot of cuttings was placed twenty per row with one hundred and twenty cuttings of each type confined to each insert. Cuttings were set on the ninth of July 1949 in the conventional manner in a vertical position with the apex of each cutting approximately one inch above the surface of the medium. This procedure was repeated for both treated and untreated plots, each insert subsequently being transferred to one of the four prepared culture chambers of the modified solar frame which had been sterilized with a ten percent solution of potassium permanganate. All plots were covered with standard sash. During the months of July and August, the sash was covered with an emulsion of lime, water, and oil to reduce the temperature within the frame. In addition, four inches of water was applied daily to the bottom of each section to maintain a high relative humidity and to eliminate opening for the purpose of adding water to the cuttings during the rooting sequence. The mean temperature maintained during the 88 day propagation period was 82.5°F above the cuttings and 80°F in the rooting medium. Peat moss used for the rooting medium gradually became less acid, changing throughout the period of rooting from a pH of 4.0 to 5.0, the untreated plots consistently having a slightly higher reading than the treated plots (plus 0.5).

The sash was allowed to remain on the frames for a period of one and one-half months after which all plots were watered with sterilized distilled water and covered with screen netting. Samples of five cuttings from each of the four lots were taken at ten day intervals after the first fifteen days had elapsed (Fig. 4).

Cultural techniques for the second experiment: The procedure adopted for the first experiment was repeated using instead of the mixture of ten fungi, a single fungus for each trial. Ten fungi were isolated from the mixture and one each of these was applied to individual lots of cuttings. Seven hundred cuttings made from the medial section of one year old whips (70 per glass dish) were soaked for ten hours in one of the following nine inocula: Ophiostoma pilifera, Echinobotrium laeve, Penicillium spinulosum, Epicoccum isolate 1, Epicoccum isolate 2, Fusarium isolate 1, Fusarium isolate 2, Streptomyces sp., and Chaetomium globosum.

Each insert was filled with peat moss and the entire structure was sterilized for a period of one and one-half hours at 180°F in a steam sterilizer. They were then transferred under aseptic conditions to the transfer room.

Each lot of cuttings was removed individually into an aseptic room where it was inserted in the previously prepared propagation chamber (Fig. 5). The cuttings were set ten rows per box, seven per row with the apex of each cutting approximately one inch above the surface of the

medium. This procedure was repeated for the eight remaining treated lots and for the untreated sample.

In order to control the temperature and light intensity in the propagation chambers, a walk-in refrigerator was designed to maintain sterile conditions throughout the experiment (Fig. 6). The floor of the refrigerator was re-covered with tar paper felt and the entire structure washed with a ten percent solution of potassium permanganate. Ten twin-tube banks of fluorescent lights were arranged to maintain a light intensity of 300 ft. candles above each individual growing chamber and the temperature was regulated to 65°F. In addition to the main storage room, a lead-in de-contamination closet was constructed of tar paper felt attached to a frame, and furnished with a small hinged door. This closet was coated with an oil base aluminum paint and fitted with a propolyne vaporizer system to de-contaminate the operator before entering the refrigerator. Two weeks after the experiment was initiated, the refrigeration failed to operate and as a result of the heat radiated by the light system, the temperature rose to 139°F. The entire procedure was then repeated using a second lot of hardwood cuttings. Three weeks after this trial had commenced, a second malfunction in equipment developed and the plant material was destroyed.

The following method was then adopted to substitute for the preceding procedure. Greenwood cuttings were the

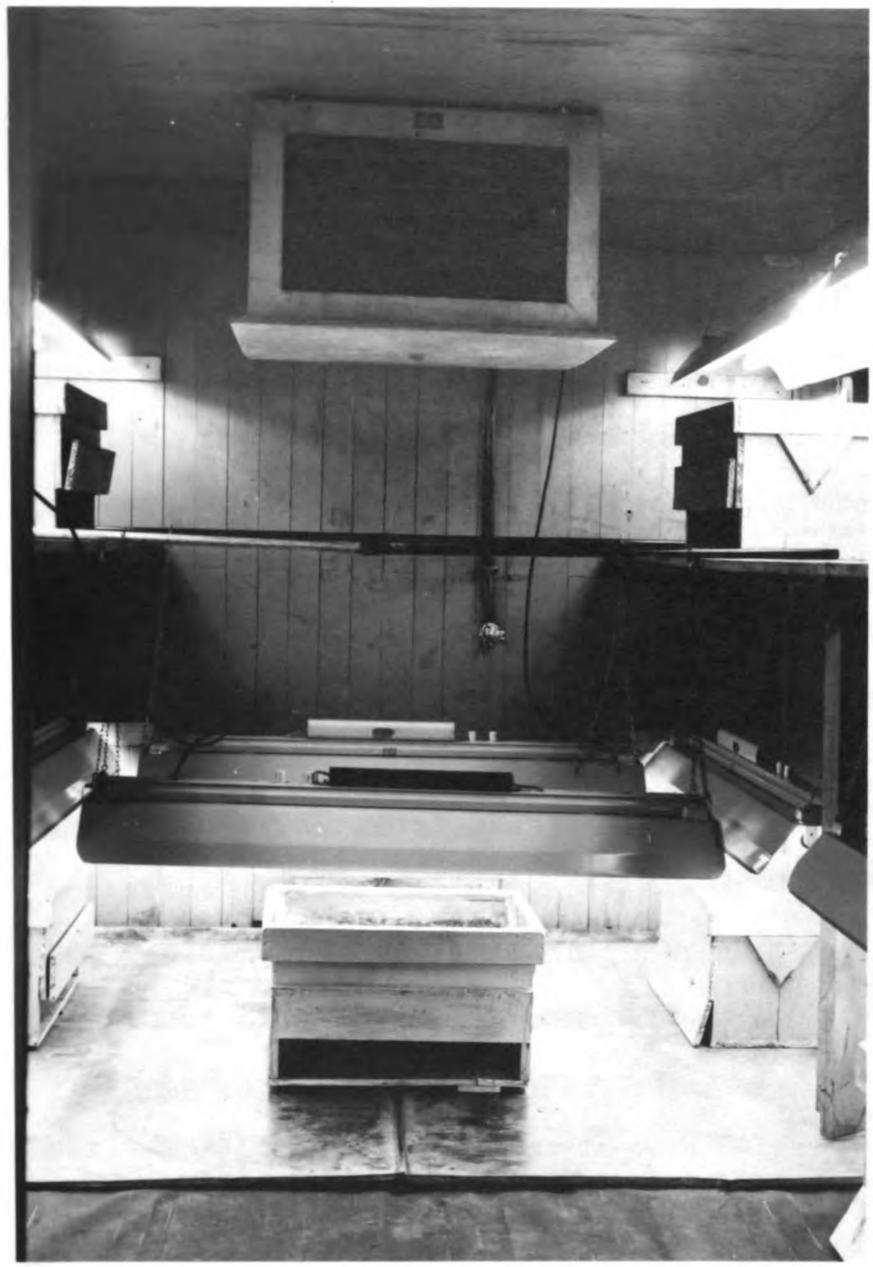

FIG.6. Propagation chamber under fluorescent lights in controlled temperature chamber.

only commercial stock available in August of 1950. To minimize the number of replications, each whip of the current seasons growth was divided into four sections, using only the two medial and discarding the terminal and basal parts. Cuttings were made four inches in length and leaves removed at all but the two terminal nodes. The terminal horizontal cut was approximately one-eighth inch above, and the basal diagonal cut immediately below, the bud. The cuttings were surface sterilized as before, soaked for a period of ten hours in separate fungal solution cultures covering the basal two inches of each cutting. On the 15th of August 1950, each lot was set seven per row in ten rows in its designated propagation chamber. The propagation chambers were then transferred under aseptic conditions to individual compartments of a divided cold frame. This frame was nine feet in length, six feet in width, and two feet eight inches in depth. The frame was further divided into ten compartments, each completely sealed from the other. The frames were covered with three glass hot bed sash. During the month of August, the sash was covered with one-quarter inch mesh camouflage netting to minimize the temperature within the frame. The entire structure was coated on the interior with aluminum paint and soaked with a twenty percent solution of potassium permanganate. After transferring all of

the chambers, the entire system was again surface sterilized with a ten percent solution of potassium permanganate and sealed. The frames remained sealed until the 13th of October 1950 (60 days), at which time they were opened and the individual propagation chambers transferred to a greenhouse bench illuminated by two banks of twin-tube fluorescent lamps. Samples of five cuttings from each of the ten lots were taken 60 and 105 days after the cuttings were set.

Mycological techniques: In order to secure fungi associated most commonly with Vaccinium corymbosum L. under Michigan conditions, weekly isolations from stem and root tissues were made during the period between August 1948 and March 1949 preceding the first experiment. Stem and root material from which isolations were to be made was collected from an established bog planting near Michigan State College. Buds were removed from the stems to eliminate all possibility of fungal contamination by spores and hyphae that might be enveloped within the overlapping bud scale system.

General laboratory procedures to avoid contamination were followed. Dissection and transfer instruments were sterilized in 70% alcohol, heated, and immersed in 70% alcohol when not in use. A 7.2% solution of slaked lime followed by two rinses of sterilized distilled water was used to sterilize the stem and root parts. This was followed by a dip of 70% alcohol and rapid flaming. One,

two, and three year old stems as well as primary, secondary, and teritiary roots were cut into thin slices approximately 500 microns in thickness to facilitate growth of the endotropic mycelia. The six different kinds of thin slices were re-sterilized before being placed on blueberry agar (infusion from 500 grams of blueberry stem tissue and 15 grams of agar). Five divisions were made on each agar plate to afford a place for one root or stem disc. After a two week period, single spores or hyphal tips were isolated from the various colonies that developed from each disc. These fungi were then cultured on potato-dextrose agar (infusion from potatoes 200 grams, Bacto-dextrose 20 grams, agar 15 grams) or Czapek's agar (water 1000 ml., $NaNO_3$ 3 grams, K_2HPO_4 1 gram, $MgSO_4.7\ H_2O$ .5 gram, $FeSO_4.7H_2O$ .01 gram, sucrose 30 grams, agar 15 grams), for identification. To identify non-sporulating types, it was necessary to culture some of the isolates on slide-culture (Riddel 1950).

The first experiment incorporated the use of a complex of nine fungi previously isolated and selected for use throughout these trials. This complex was cultured on 500 ml. of potato-dextrose agar in 1000 ml. Erlenmyer flasks. Five hundred ml. of sterilized distilled water was then added to the flasks after the colonies had developed fully. The fungus colonies and agar were thoroughly mixed with sterilized distilled water and

transferred to sterilized square glass refrigerator dishes to which the cuttings were subsequently added for a ten minute soaking period.

The techniques for the second experiment were modified to facilitate the addition of the fungi to the cuttings. Following isolation of the fungi, individual hyphal fragments from each culture were transferred to separate solutions of Fries' Media which was made up to the following specifications:

Ammonium Tartrate	10.0 grams
Ammonium Nitrate	2.0 grams
Potassium Dihydrogen Phosphate	2.0 grams
Sodium Chloride	0.2 gram
Calcium Chloride (Anhydrous)	0.2 gram
Magnesium Chloride	1.0 gram
Sucrose	30.0 grams

in 2000 ml. of sterilized distilled water. Seventy cuttings were soaked in sterilized glass refrigerator dishes containing 100 ml. of the culture solution of the specific fungus to be tested. Sufficient sterilized distilled water to submerge all cuttings was added and the entire system rotated to insure complete mixing.

Histological techniques: All cuttings were sectioned and prepared for microscopic observation in a similar manner. All cuttings were transferred from the individual propagation chambers to a moist atmosphere in square glass refrigerator dishes and stored for less than two days before sectioning. The basal slant cut

was divided into three regions: (1) the tip to the center of the pith (2) the center of the pith to the summit of the cut, and (3) remaining part of the stem. The distal one and one-quarter inch section of each fresh stem part was then removed and inserted in a vertical position in the clamp holder of a Bausch and Lomb sliding microtome. Transverse sections were cut approximately 35 microns in thickness. The remainder of the stem was then cut longitudinally at the same thickness. Four representative transverse sections from each of the regions one, two, and three in addition to eight longitudinal sections were selected and transferred for eight hours to a three percent solution of acetic acid containing orseillin BB (Strassburger, 1923). After rinsing in forty percent alcohol, the material was stained for ten minutes in a solution of analine blue in three percent acetic acid. Washing and dehydrating with alcohol was followed by clearing in clove oil and xylol. Permanent mounting in Canada balsam was done in such a manner as to orient sections for future identification. The most desirable staining was characterized by a light purple color. Lignin, suberin, nuclei, and cytoplasm stained red (orseillin BB); cellulose stained blue (analine blue); hypha stained shades of violet and blue (orseillin BB and analine blue). Water soaked and dead tissue appeared yellow or brown. It was necessary to

modify the portion of the stem selected for sectioning in those cuttings of the second experiment that portrayed basal browning in excess of one and one-quarter inches from the base. In this case a one and one-quarter inch part was cut in such a way as to include a three quarter inch region of each of the brown and normal regions. Resultant transverse sections were classified as to origin following the previous technique.

Serial sections of several stems were made in order to ascertain the region of root initiation. For this purpose distal three-quarter inch stem pieces were softened with sodium hydroxide and hydrofluoric acid (Hyland, 1941), and imbedded in the usual paraffin method. Transverse sections were cut on a rotary microtome and permanent slides prepared by dehydrating, staining with Haidenheins haemotoxylin and safranin and mounting in Canada balsam.

EXPERIMENTAL RESULTS AND EXPLANATIONS

Anatomy of the Stem of Vaccinium corymbosum L.:

(Fig. 7) The cuticular layer (a) on the surface of one year old stems of Vaccinium corymbosum L. covers a single epidermal layer (b) of cells. Many other plants from a moist habitat are frequently found to have a thin cuticle, however, on all plants examined, the cuticle is thick. It is approximately the same depth as the subtending epidermal cells. The epidermis consists of a single row of closely packed isodiametric cells, which toward the perifery of the stem have blunt, saw-toothed apices. The cuticle although entire is interrupted regularly by stomata (a) (Fig. 8) extending into the cortical region. The guard cells are thin walled, but the wall is thicker on the side close to the aperture. All of the cortical cells are isodiometric. They are smaller and more closely packed toward the outer edge of the cortical region. The elliptical to globose cortical cells subtending the stomatal mechanism are grouped more closely beneath the cavity and are somewhat smaller than those throughout most of the cortex. The cortical region varies from five to twelve cells in depth and deliniates a solid tissue system between the epidermis and air canals. Most cortical cells contain many chloroplasts. Centripetal to the cortical layer are large air ducts, usually two in a group, bilaterally located, and ranging from three to four

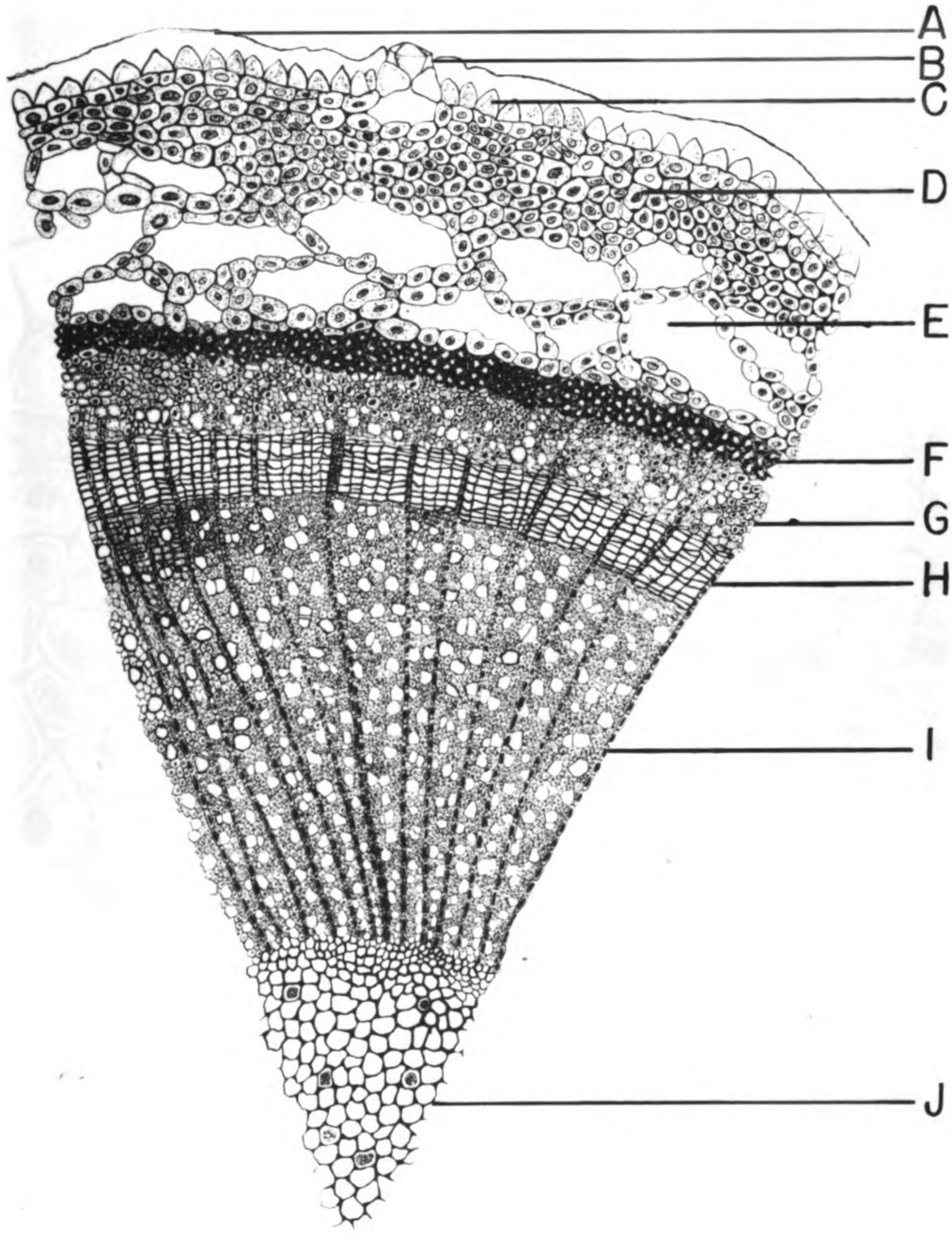

FIG.7. Segment of transverse section of hardwood stem of Vaccinium corymbosum L.:a.cuticle, b.stoma, c.epidermal cells, d.cortex, e.air canal, f.pericyclic fibers, g.phloem region, h.cambiom region, i.xylem region, j.pith, (x 200)

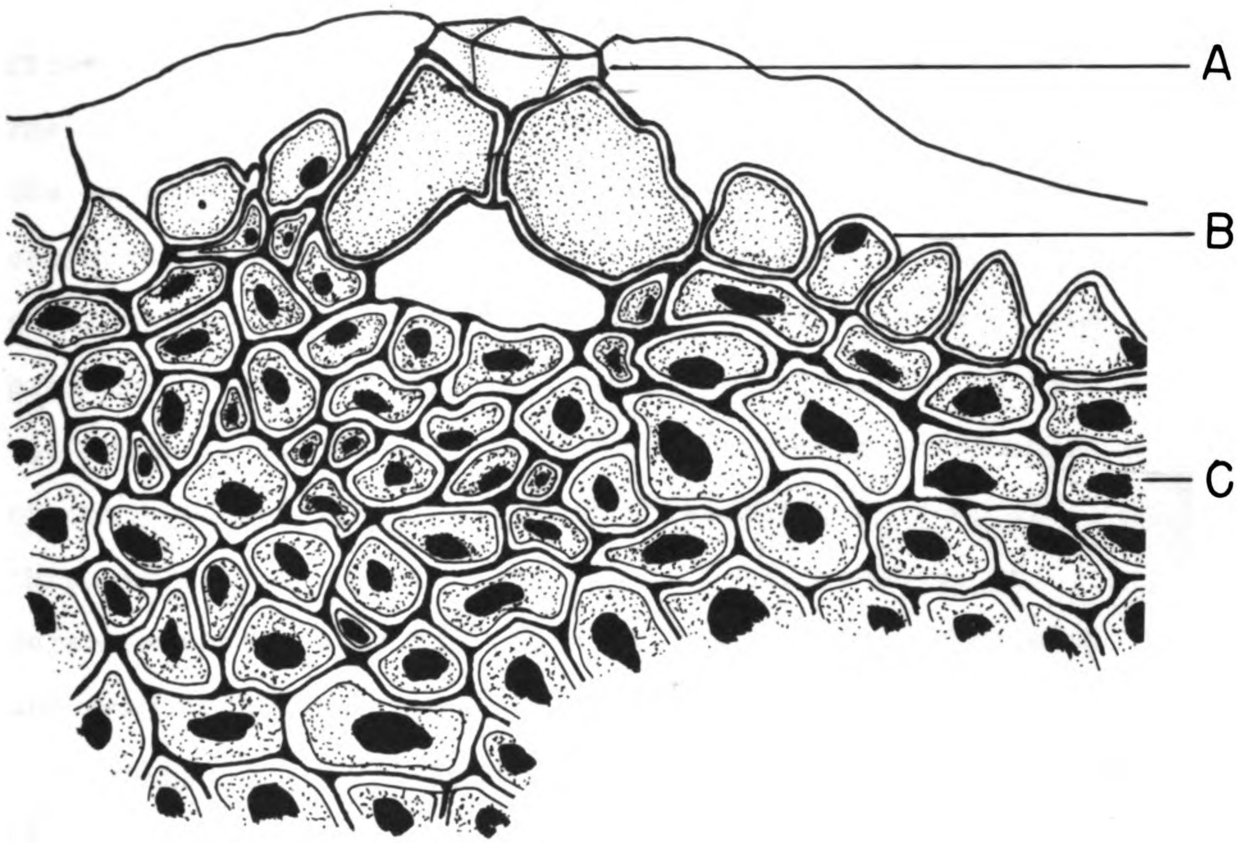

FIG.8. Transverse section through outer layer of hardwood stem of Vaccinium corymbosum L. showing: a.stoma with guard cells, b.epidermal layer, and c.part of cortical parenchyma.

cortical cells in thickness. These canals taper at either end and are bounded by one and two rows of globose cortical parenchyma. Immediately inside the ring of air canals is a band of long thick-walled pericyclic fibers, ranging from three to five cells in depth. The pericyclic fiber region is continuous. Each cell has a small lumen. The primary phloem region is no greater in thickness than the adjacent pericyclic fiber region, but in contrast is composed of more smaller cells. The cambium is often difficult to designate as a definite layer because of the presence of new and similar tissues on both sides of it. The xylem is composed of long angular vessels surrounded by thick-walled fibers bisected bilaterally by rows of thick-walled xylem parenchyma. The pith, composed of parenchyma with thin cellulose walls contains much starch and many crystals.

Origin and development of root primordia: The following discussion is concerned with those cells within the stem which give rise to the primary meristems of the adventitious root. The root primordium is intended to denote the root initial or the aggregation of associated, meristematic cells, as soon as they have become organized in a definite pattern.

Adventitious roots of hardwood and greenwood cuttings of Vaccinium corymbosum L. arise endogenously at the base of the cutting (Fig. 13). Microscopic examination of

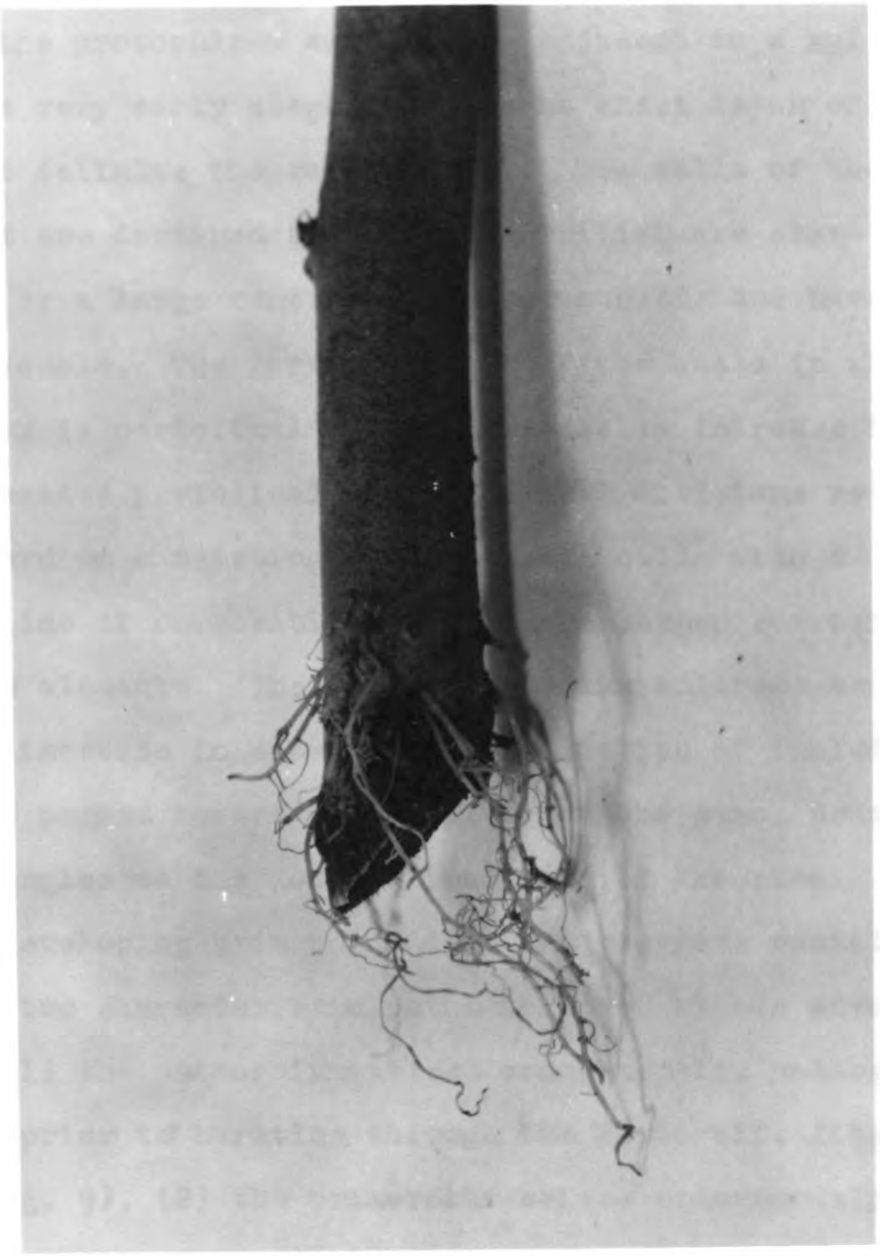

FIG.13. Base of cutting showing origin of roots and pustules where roots are pushing through the stem.

representative longitudinal and transverse sections from 650 stem cuttings showed that the root initials generally arise in the protophloem and usually adjacent to a xylem ray. At a very early stage there is no exact layer of cells that delimits the root initial. The cells of the group that are destined to become an initial are characterized by a large centrally located nucleus and have a small vacuole. The first division of the cells in the primoridium is periclinal without noticeable increase in size. Repeated periclinal and anticlinal divisions result in a primordium consisting of many small cells with a definite line of demarkation between the larger surrounding phloem elements. The young primordium enlarges as a result of increase in size and multiplication of included cells. It pushes toward the perifery of the stem, usually at right angles to the longitudinal axis of the stem. When the developing primordium does not progress radially there are two characteristic paths followed by the advancing tissues; (1) the primordium arises endogenously, enlarges and bends prior to bursting through the pericyclic fiber region (Fig. 9), (2) the primordium arises endogenously, enlarges and extends radially through the pericycle and the cortex, where it is forced to bend and discontinue development (Fig. 10). Differentiation within the root initial progresses slowly and is first apparent as a solid region of xylem elements after the root primordium has pushed through the primary phloem and pericyclic regions.

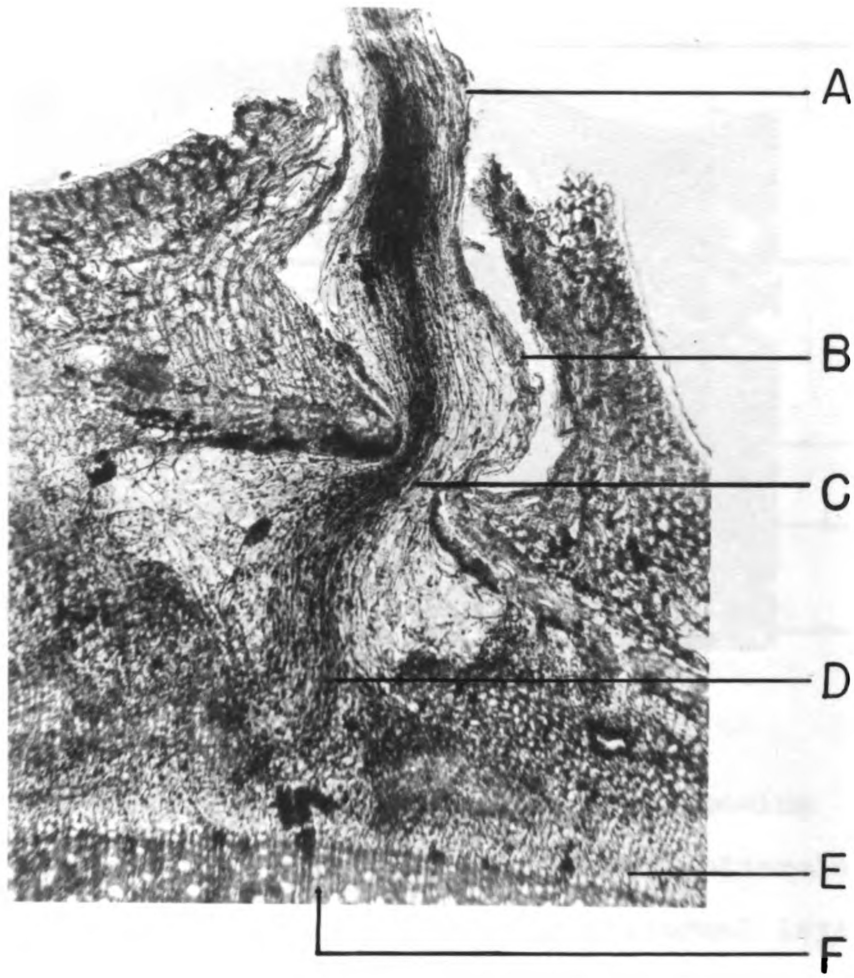

FIG.9. Transverse section through stem cutting showing bending of root beneath pericyclic fibers. a.developing root, b.bending in cortex, c.root passing through pericyclic fibers, d.root origin in protophloem region, e.cambium region, f.xylem. (x 91)

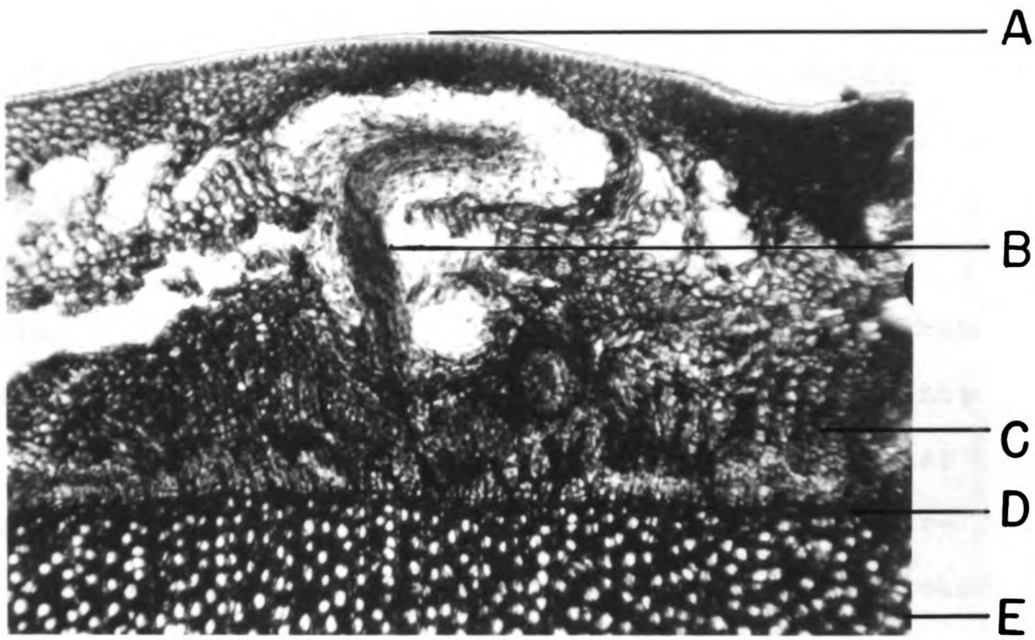

FIG. 10. Transverse section through stem showing developing root bending beneath epidermis. a. heavy cuticle cementing epidermal layer, b.root bending in cortex beneath cuticle, c. phloem region, d. cambium, e. xylem(X79).

As has been described for other plant species the location of the region of initiation is immediately adjacent to a medullary ray. There is no consistent evidence to show whether radial elongation of the developing primordium is one of dissolution or mechanical separation of adjacent tissue systems.

During the rooting sequence of hardwood cuttings, callus formation always preceded root formation in those cuttings that did not exhibit a basal browning of the proximal end. Microscopic examination of material exhibiting early stages of callus formation revealed that the callus tissue originated in the disorganized elements of the protophloem, immediately adjacent to the vascular cambium. This callus tissue is composed of large loose-walled parenchyma, which at the outset is undifferentiated. Later stages in callus differentiation are represented by the delimitation of xylem elements formed by multiplication, elongation and reorganization of parenchyma (Fig. 11). These are phloem precursors, cells closely related to the vascular cambium. Adventitious roots are produced from this callus tissue at the proximal end of the cutting, but only after considerable twining and grotesque weaving of the root primordium through the existing callus parenchyma. It would appear that if cuttings produced only this type of adventitious root, the vitality of the embryonic plant would be greatly reduced; the efficiency of the osmotic system would be

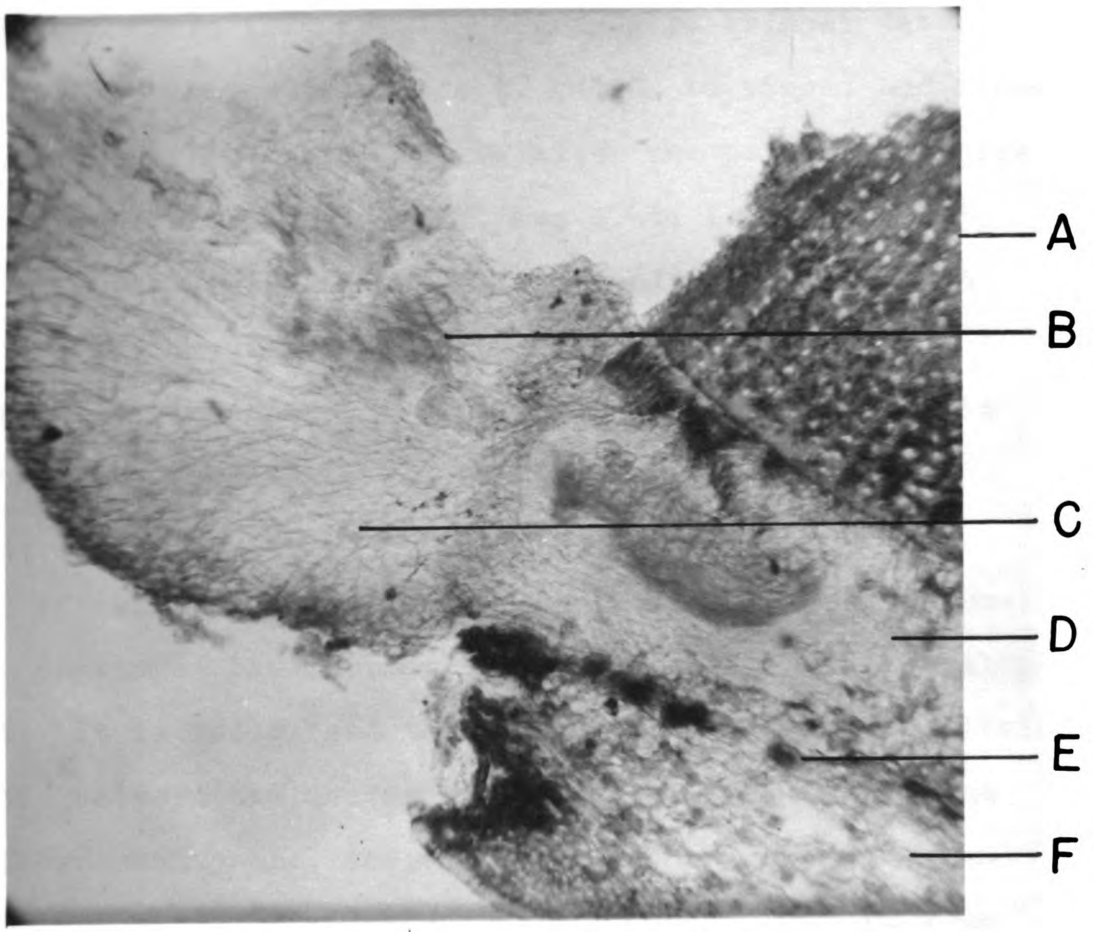

FIG.11. Transverse section through callus at base of stem showing callus growing out of cambium and phloem region; outer edge of root initial faintly visible. a.xylem, b.part of root, c. callus parenchyma, d.phloem region, e. pericyclic fibers, f. cortical parenchyma (X 91).

hampered by the curving and recurving of the xylem elements.

In blueberry cuttings, there are two types of roots produced; those that develop normally at or near the basal end of the cutting (Fig. 12, B. and C. to right) and those that emerge from callus tissue after the outer cells have become suberized (Fig. 12, B. and C. to left). Adventitious roots are produced most commonly in association with a bud gap, although they do infrequently occur anywhere at an internode, and at early stages were visible to the naked eye as slight swellings in the epidermis (Fig. 13).

If callus is produced at the proximal end of greenwood cuttings, its rooting ability is retarded in development. It is recognized that when wounds are made on living plants between the months of November and April, in the northern hemisphere, there is no blocking of vessels and medullary rays. Similarly, hardwood stems severed from the parent during this time produce no "wound gum" deposit at the cut surface. After the cuttings have been made, the activity of the cambium and protophloem elements is stimulated resulting in the formation of abundant callus along the outer stelar region at the basal cut surface. Wounds at the cut surface of greenwood cutting material, in August stimulates the blocking and suberization of vessels and medullary rays along the cut surface. This deposit of suberin and cutin, retards and often prevents

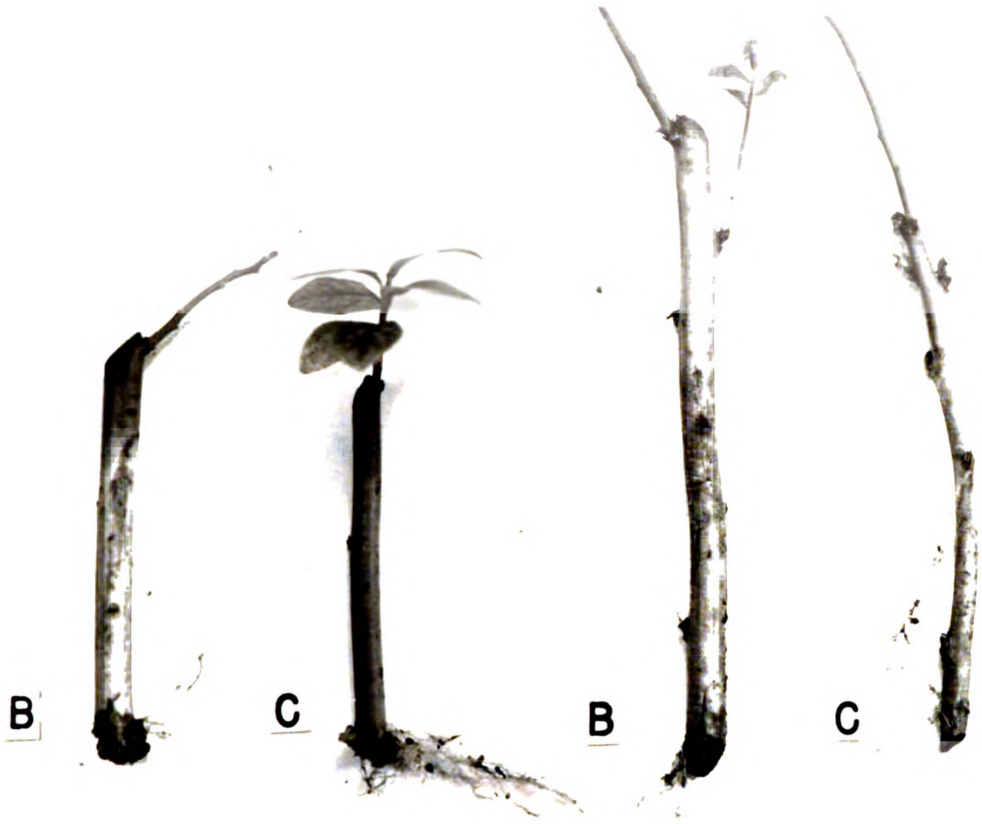

FIG. 12. Rooting of stem cuttings: b.and c. to left showing roots developing through callus; b.and c. to right showing roots developing from nodes and internodes without callus fromation.

the development of callus along the basal stelar region that is exposed to the rooting medium. Subsequent root production from this type of cutting material, under conditions of adequate aeration therefore is limited to nodal and internodal areas rather than to callus root primordia.

Description of associated fungi tested: The following descriptions are based on the development of the fungus mycelium on potato-dextrose agar. For the Penicillium species it was necessary to use the standard Czapek agar to insure positive identification.

1. Ophiostoma pilifera (Fries) Winter

Mycelium dense, light, greatly branched; Perithecia superficial to sunken in the substrate, globose, diameter at the base 105-120 microns; black with erect, rigid neck 320-492 X 20 microns; asci evanescent; ascospores hyaline, one celled, obovate, tapering toward tip, 2.7-3.9 X 2.0 microns.

2. Echinobotrium lavae Saccardo.

Conidia borne on short branched hyphae, septate, hyaline; Conidia 11-12 X 6 microns borne in clusters, ovate to spindle shaped with short hyaline stalks, glaborous; Conidiophores not differentiated from the vegetative hyphae.

3. Penicillium spinulosum Thom.

Colony with many aerial conidiophores and

sparsely floccose aerial hyphae; Conidiophores 200-300 X 3 microns with enlarged apex; Conidia globose 3.5 X 3.5-4 microns, thin walled, delicately warted.

4. Epicoccum isolate 1.

Sporodochia spherical, small, scattered, dark; Conidophores club-shaped, non-septate, brown; Conidia borne singly at tips of conidiophores, globose, spiny, one celled.

5. Epicoccum isolate 2.

Sporodocia spherical, small, scattered, dark brown; Conidiophores club-shaped, non septate, black, 12-14 X 5-6 microns; Conidia ellipsoid, finely warted, septate.

6. Fusarium isolate 1.

Mycelium white, becoming pink on the reverse; Macroconidia elongate, 1-6 septate, variable as to size; 5-6 X 8-16 microns; no sporodochia produced; Aerial mycelium cottony to fluffy and densely interwoven.

7. Fusarium isolate 2.

Mycelium white, floccose, rosy-white on the reverse; Microconidia 1-2 celled; variable as to size 4 X 8-10 Microns; numerous on the aerial mycelium; Macroconidia not produced; 1-septate 10-24 X 2.5-5 microns, 2-septate 15-25 X 5.5-7 microns; No sporodochia produced.

8. Streptomyces sp.

Straight or branched mycelium; long open spirals formed on Czapek's media. Colonies yellow and white when young, later yellow tan with brown reverse on potato-dextrose agar. Somatic hyphae up to 1.5 microns in width; conidia oval to rod shaped, mostly 1 X 3 microns, occasionally conidia up to 5 microns in length on Czapek's agar.

9. Chaetomium globosum Kunze.

Perithecia thin-membraneous, broadly ovate to ellipsoid, 25-300 X 250 microns, olivaceous with thin hairs; Ascospores simple 3-5 X 2.5-3 microns, greenish in color.

Relation between the isolated fungi and root growth: The percent of rooted cuttings after ninety-five days was conspicuously low for both the treated and untreated hardwood cuttings (Table 1). The average number of roots produced per rooted cutting, after ninety-five days was: 1.4 for the treated terminal, 4.4 for the treated basal, 2.6 for the untreated terminal, and 3.8 for the untreated basal lots. From these data it is apparent that the basal cuttings produced a higher average number of roots per cutting than did the terminal cuttings. The difference in the average number of roots produced (per rooted cutting) however, is not apparently influenced by the fungus treatment. Data from the hardwood cutting trial (Table 1) indicate that when the inherent quality of hyphae within

any specific type of cutting is supplemented by the addition of a mixed fungal complex, the quantity of adventitious roots produced is materially decreased.

Tables 1 and 2 indicate that in general for each treatment, the number of callused cuttings was in relation to the number that produced roots. The average number of roots per cutting (after 95 days) was not necessarily related to the percent of cuttings that was callused. There was more callus on cuttings that were not treated with the fungal extract than on those that were treated (Table 2). An apparent tendency for basal cuttings to produce more callus was observed, although the average percent of cuttings callused for the untreated basal lot was substantially higher than that for the treated basal cuttings.

Microscopic examination of longitudinal sections of hardwood cuttings showed that the concentration of endotrophic hyphae, in the outer xylem vessels (Fig. 14), adjacent to the vascular cambium was not consistent with the type of cutting (Table 3). This conclusion is based upon the compilation of observations from each treatment at five different times of sampling, twenty days apart. It is further substantiated by intermediate samplings not included in the table. The second experiment supported these observations (Table 5), although hyphal filaments were more widely distributed throughout the stem. The total number of hyphal strands were

FIG.14. Hyphal growth in xylem vessels (X480).

consistently high in treated and untreated basal cuttings, in contrast to the terminal cuttings (Table 3). Stems of the hardwood cutting trial showed no consistent increase in endotrophic hyphal activity with the elapse of time, but the greenwood cutting material in general, showed a decrease in the concentration of hyphal elements as time elapsed (Table 5).

The average number of perithecia per hardwood cutting noticeably increased from the date the cuttings were initially set (Table 4) until the observation on the ninety-fifth day. Data from the second experiment show that the average number of perithecia produced per greenwood cutting increased in cuttings that were treated with Ophiostoma pilifera and Chaetomium globosum, in addition to the check (Table 5). The average number of perithecia noticeably decreased as a result of the addition of any one of the other fungal supplements (Table 5). There was no apparent agreement between the amount of perithecial development at the proximal cut surface (Fig. 16) and the concentration of hyphal filaments contained in the cutting (Tables 3, 4, and 5).

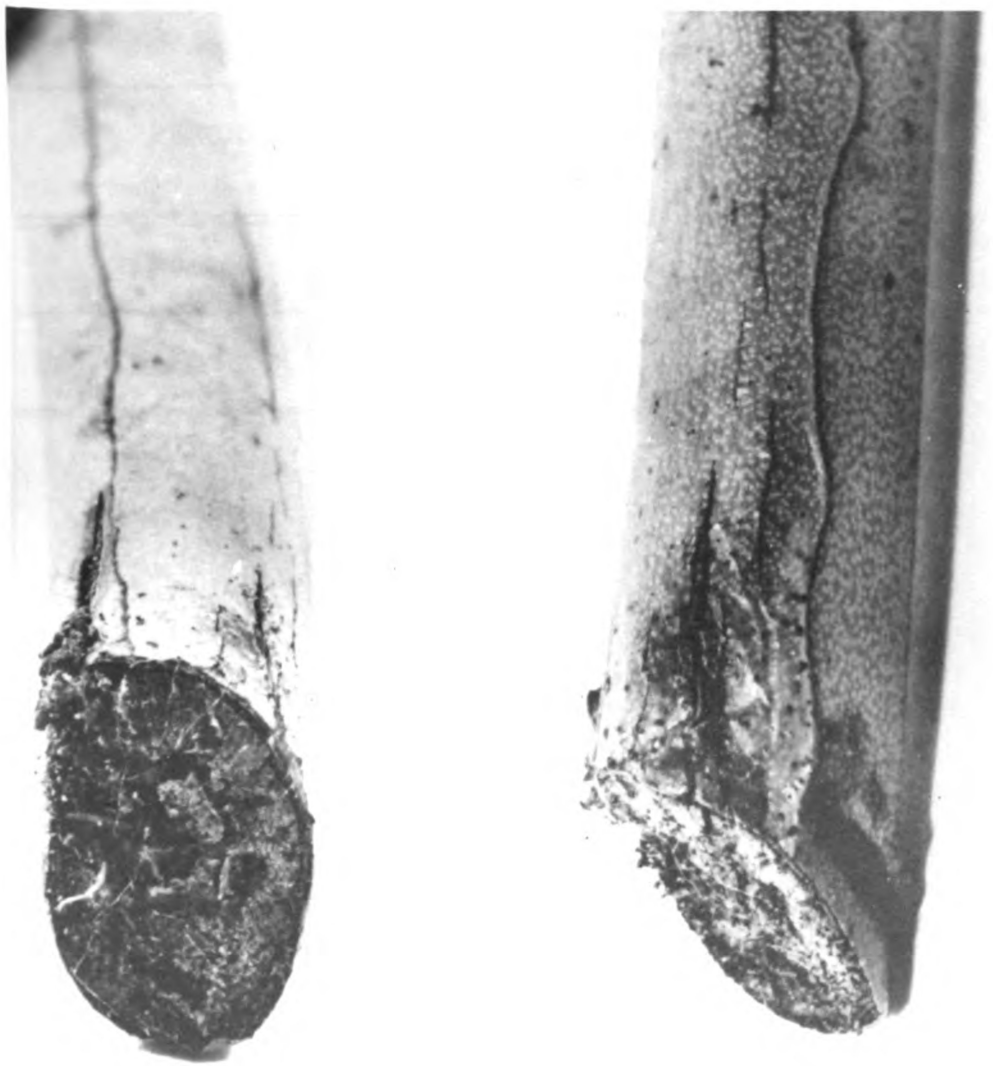

Fig.16. Perithecial development on the cut surface and crack in epidermis of stem.

TABLE 1.

PERCENT OF ROOTED HARDWOOD CUTTINGS (39 samples)				
Time	Treated*		Untreated**	
Days elapsed	Terminal	Basal	Terminal	Basal
15	0	0	0	0
35	7	0	7	0
55	4	8	12	8
75	9	20	23	26
95	13	23	36	33

* Cuttings soaked in fungal extract

** Cuttings soaked in sterilized distilled water

TABLE 2.

PERCENT OF HARDWOOD CUTTINGS CALLUSED
(39 samples)

Time	Treated*		Untreated**	
Days elapsed	Terminal	Basal	Terminal	Basal
15	0	0	0	80
35	0	27	40	70
55	0	42	48	68
75	3	26	51	66
95	3	23	54	69

* Cuttings soaked in fungal extract

** Cuttings soaked in sterilized distilled water

TABLE 3.

ENDOTROPHIC HYPHAL ACTIVITY IN HARDWOOD CUTTINGS

Time	Treated*		Untreated*	
Days elapsed	Terminal	Basal	Terminal	Basal
25	medium	low	low	medium
55	medium	medium	medium	medium
75	light	medium	medium	medium
95	light	heavy	medium	medium

TABLE 4.

AVERAGE NUMBER OF PERITHECIA PER HARDWOOD CUTTING

Time	Treated*		Untreated**	
Days elapsed	Terminal	Basal	Terminal	Basal
25	1.6	2.0	4.5	5.0
55	20.6	24.8	14.8	7.6
75	29.8	20.0	19.6	15.0
95	45.0	35.0	22.0	34.0

* Cuttings soaked in fungal extract

** Cuttings soaked in sterilized distilled water

TABLE 5.

ENDOTROPHIC HYPHAL ACTIVITY AND AVERAGE NUMBER OF PERITHECIA PER GREENWOOD CUTTING

Fungus	Hyphal activity		Perithecia	
	60 days	105 days	60 days	105 days
1. Ophiostoma pilifera (Fries) Winter.	medium	low	14	25
2. Echinobotrium lavae Saccardo.	medium	medium	28	18
3. Penicillium spinulosum Thom.	high	medium	17	4
4. Epicoccum isolate 1.	medium	low	30	15
5. Epicoccum isolate 2.	low	medium	18	6
6. Fusarium isolate 1.	high	low	29	18
7. Fusarium isolate 2.	medium	medium	28	18
8. Streptomyces species.	medium	medium	33	16
9. Chaetomium globosum Kunze.	medium	medium	28	33
10. Control	medium	low	13	24

DISCUSSION OF RESULTS

Negative relationship between the presence of fungi and the number of root initials: It is not proposed to suggest that all of the results are sufficiently comprehensive to suggest alterations in the practices of propagation of this plant. The number of roots produced was not sufficiently high to be considered typical for every season.

In general, the addition of fungi to the propagation medium did not increase the percentage of rooted cuttings. In all cases the untreated cuttings contained large numbers of endotrophic hyphal filaments. This would indicate that an appreciable amount of fungus was in the stem at the time the cuttings were removed from the plant. The mycelium was located adjacent to the vascular cambium in the outer xylem vessels in contrast to its location in all xylem, phloem, pith, and cortical regions of the greenwood stem. One probable explanation of the location of hyphae is the different amount of water available in various stem tissues at different stages of maturity. In the rapidly growing greenwood stem, the pith, xylem, phloem and cortical tissue contain large quantities of cellulose and bound water that would make a good medium for mycelial growth. As a result of the greater similarity of the chemical composition of all tissue at a young stage, the selectivity of a region suitable for fungal growth

would not be as great as in the older stem. As the stem matured, more lignified cells of the older xylem would not make as desirable a medium for the growth of fungi as would the young xylem vessels. The movement of large quantities of water in the older vessels might impede fungus growth. Less free water would be absorbed by the lignified, than by the cellulose, vessels and consequently the medium would not be as good for fungus growth. The quantity of carbohydrate in all tissues of the green stem might also improve growing conditions for the fungi. The results indicate that where there is a medium number of hyphal filaments in the stem tissue, the greatest number of roots develop. After ninety-five days all of the untreated hardwood cuttings display the best rooting ability and contain concentrations of hyphae in the medium range. Where hyphal activity is either heavy or light after ninety-five days for the treated lots, the number of rooted cuttings is considerably reduced. When the quantity of hyphae is light, the subsequent percent of rooted cuttings is not noticeably increased, and on the contrary, when the concentration of endotrophic hyphae is in excess there is no noticeable increase in the percent of rooted cuttings. This would appear to mean that a definite optimum quantity of endotrophic mycelia conducive to rooting might be established.

The greenwood cutting trial produced only a sparse number of rooted cuttings after 105 days in the rooting medium. This may have been caused by low temperatures in Michigan in September and October. The decrease in temperature would reduce the growth rate of both the cutting and the fungus. The various fungi apparently responded differently to this change in temperature. Epicoccum isolate 2. increased in concentration with the elapse of time reaching its optimum between 60 and 105 days. It may not have been given sufficient time to stimulate the root initials. Typical hyphal filaments observed in the longitudinal stem sections could not be identified or distinguished from any fungus that developed on agar culture media. The hyphae were much branched, septate, and brownish in color in regions where the death of the tissues occurred. In live tissue the fungus mycelium stained shades of violet. In comparison the longitudinal sections of the hardwood cuttings revealed a typically non-branched, single, non-septate hypha which stained a deep blue. These hyphae were observed passing through the pits in the cell walls of the young xylem vessels. It is suggested that the stimulation of root initiation in hardwood cuttings is brought about by the digestion of these hyphal strands and that these hyphae represent a source of available nutrient substances, which at a definite concentration stimulate the production of root

initials. This observation is in agreement with McDougall (1914) who reported that the mycorrhizae of Quercus and Acer appear during the summer in small numbers, increase during the fall and winter and die during the spring. However in the greenwood cuttings the hyphae are active and non fused in which state the substances assimilated by them is available in a small quantity.

Effect of associated fungi on root initiation and development: The cuttings that did not produce roots or callus at the end of 95 and 105 days exhibited a browning of the lower end of the stem in contact with the medium. It is thought that this is caused in part by the influx of saprophytic fungi into the non-living xylem elements. Adjacent ray and xylem parenchyma, as a result of the presence of the fungi form tyloses and subsequently secrete a wound gum deposit in the infected tissues. The formation of gum renders complete the functions of the secreting cell in the nutritional process of the cutting. The cells are dead but are physiologically important to the plant. They prevent the healthy wound tissue from absorbing water and air. This hindrance to the normal oxygen supply decreases the infection of the hyphal filaments, and these saprophytic forms generally require a certain amount of oxygen in order to carry on their life processes.

The breakdown of the cuticle, epidermis, and cortex is another probable function of associated fungi. The first action of some of these fungi is to dissolve the middle lamella, with subsequent direct penetration into the surrounding tissue. As the fungus progresses from the epidermis via the stomatal opening, through the cortex, its progress is frequently halted at the pericylic fiber region. Developing root initials are then aided in their lateral projection by the weaking of the pericyclic fiber region and the separation of the cells centripetal to it. Fig. 15 illustrates the quantity of adventitious roots emerging from this characteristic brown area at the proximal end of the cuttings.

Fungi associated with endotrophic hyphal activity: The identity of individual fungal supplements could not be determined in the longitudinal sections of material used in the second experiment. The lack of production of fruiting bodies within the "host" tissue coupled with the modification of the hyphal strands in the tissues of the stem further led to the complication of such a procedure.

In the first and second experiment, perithecia were produced very shortly after the cuttings were set in the sterilized rooting medium. They appeared at the surface of the basal slant cut, and where the epidermis was ruptured by the knife at the time the cutting was made. It would be reasonable to conclude that the

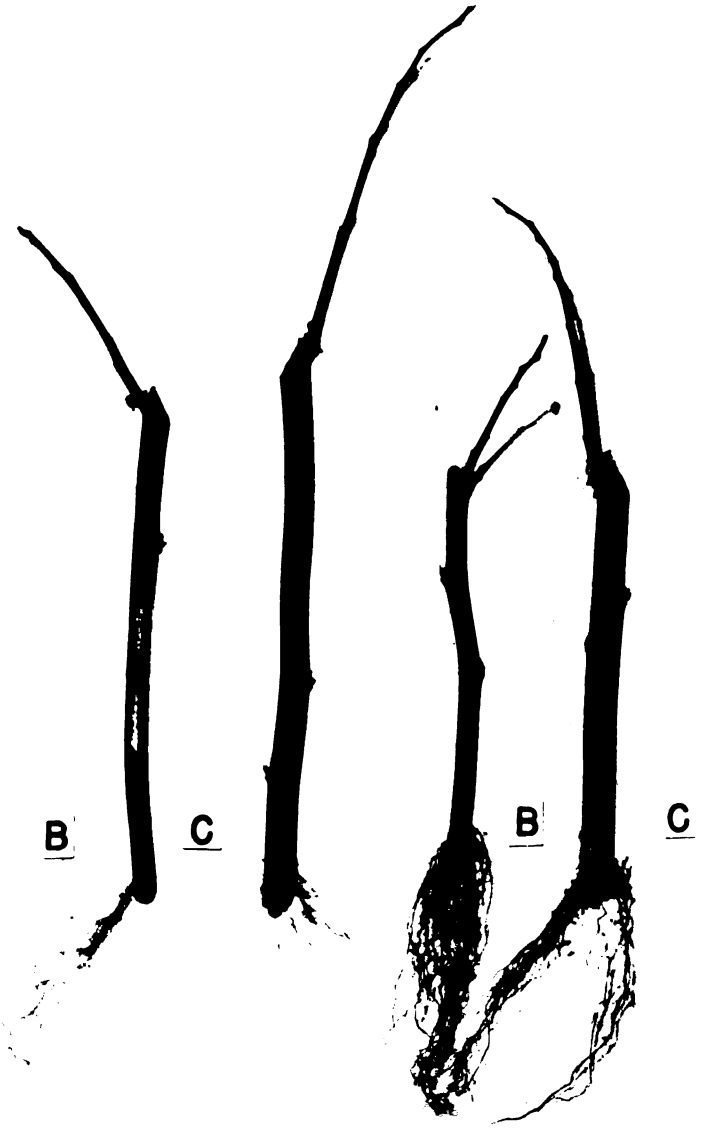

FIG.15.Roots developing through dead brown cortex and epidermis.

endotrophic hyphae present in the stem material at the time of collection produced these fruiting bodies at the exposed ends of the severed xylem elements. This fungus was identified to be Ophiostoma pilifera (Fries) Wint., which is commonly associated with the "bluing" or "red rot" of western yellow pine. The fungus could have been introduced from the surrounding plantations of Scotch pine, Pinus sylvestris, or from the lumber in the propagation frames in spite of the cuttings being maintained under what was thought to be sterile conditions. Intermediate trials in the laboratory have shown that perithecia were produced in all cases, no matter what particular method of surface sterilization was utilized. From these data it is suggested that hardwood and greenwood cutting material used in these trials contained Ophiostoma pilifera (Fries) Wint.....inherent in the stem.

This is further substantiated by the isolation and identification of Graphium from stem tissue during the initial isolation trials. Graphium sp. has been identified as the imperfect stage for several of the Ophiostoma species.

Mechanical inhibition of root projection: The ring of pericyclic fibers and heavy cuticle cementing the outer tips of the epidermal cells are suggested as two characteristic mechanical obstacles to the projection of the root. The photomicrograph of a transverse section through the developing root (Fig. 9) shows the bending of

the initial before it reaches the cortex. It is believed that the pericyclic bending is produced as a result of the inability of the developing initial to break through the continuous ring of thick-walled pericyclic fibers. There is no evidence to indicate that the initial at this point dissolves the surrounding tissue in its transverse movement toward the perifery of the stem. It is suggested therefore, that the pericyclic fiber region offers a mechanical barrier to the root initial, which is forced to change its course. There is evidence of contraction of the cell mass in the cortical region of the new root as it passes through the pericyclic fibers. The root attains a greater diameter both inside and outside of these fibers. A curvature of the developing root directly beneath the epidermis at as great an angle as at 90° is shown in Fig. 10. The radial projection of the developing root is halted as a result of the mechanical resistance offered by the thick cuticle and densely packed cortical parenchyma. Only in severe cases does the developing primordium make a right angle bend and discontinue further activity. In less severe cases specimens have been observed where a root bent slightly and subsequently forced its way through the cortex, epidermis and cuticle at some point far removed from the loci of a point directly radial to its origin. This would cause a root to be malformed. It might be injured mechanically.

It might not break the outer epidermal and cuticular layer. When the cuticle or epidermis does break, it may happen at a point of mechanical weakness or injury in the cuticle. Frequently when the epidermis is dead, degeneration of the tissue may precede root emergence. Either pericyclic fiber or cuticular restriction may be one cause of the low percentage of rooting on the stem cuttings of this species. If this is the primary cause of obstruction, rooting percentages could be raised by using a weak acid or solvent to destroy or dissolve the cuticle, the epidermal layer, and even the pericyclic fibers providing no damage was done to the phloem or cambium region where the initials originate.

Physiological inhibition of root projection: The bending and apparent retardation of elongation of the young roots (Figs. 9 and 10) may be attributed to an insufficient supply of nutrients, water, oxygen, or elaborated food at this stage in its development. It may be a result of a combination of mechanical and physiological causes. Because root development was less and less rapid in the greenwood cuttings, mechanical obstruction is a more likely cause of root inhibition. There would be a greater supply of food in the physiologically more active greenwood, than, in the less active hardwood stems. Anatomical differences as a result of age would further complicate this type of conclusion.

There was a great difference in the amount of lateral growth on each lot of hardwood cuttings. Treated basal cuttings for example averaged 42.1 cm. and untreated basal cuttings averaged 29.9 cm. in length after ninety-five days. It would be expected that these cuttings would produce roots in relation to the amount of leaf area but this was not true. It did not prove to be true for the terminal cuttings and it was concluded that there was no relation between the leaf area available for synthesizing food and the quantity of roots.

The lack of consistency between rooting ability and opportunity for additional supply of elaborated food tends to discount the theory of inhibition of root projection as a result of insufficient food. Better evidence might be obtained through tests of rooting in a media where sugar was supplied.

Superiority of root production by proximal contrasted to distil stem parts: The number of cuttings that produced roots after ninety-five days was conspicuously low for all hardwood lots of cuttings. This is in accord with the results obtained by Ware (1930) working with the southern blueberry. He reported eight percent rooting for cuttings made during the month of July. The low percentage of rooted cuttings is unfortunate since the experiment was designed to determine what influence associated fungal forms might have on the production of

roots. The experiment was terminated after a sufficient number of cuttings had produced roots, and it was possible to establish the influence of the fungus on rooting. The average number of rooted cuttings is therefore small because of the interruption at a time when all cuttings had not yet produced roots.

As has been previously noted (O'Rourke, 1944) hardwood cuttings taken from the basal portion of one-year old whips of Vaccinium corymbosum L. produced a greater quantity of adventitious roots in comparison to those cuttings made from terminal stem pieces. The untreated basal lot of cuttings of the first experiment did not substantiate these results. However the number of cuttings sampled is insufficient to indicate that there exists a significant difference. One explanation for the greater number of roots produced per rooted cutting may be the increased activity of the endotrophic fungi, which render mineral elements and elaborated food products available to the cutting. Ericaceous plants grow frequently in humus or acid peat soils, which are notably deficient in nitrates. It is recognized that certain mycorrhizal fungi can readily assimilate ammonium and organic nitrogen compounds, which are not available otherwise to the cutting. As has been previously suggested by Rayner (1927) for Calluna, the possible role of the mycorrhizal element in the cuttings of Vaccinium corymbosum L. is one of

availability and the absorption of organic food reserves, which are unavailable to the cutting in alkaline soils. The most important function of the endotrophic hyphal filaments is probably the increased absorptive surface afforded by the mycelium located in the xylem vessels of the stem and extending into the rooting medium. It must be pointed out however, that there is no adequate explanation for the means of absorption of substances by the hyphal filaments. Although the root-hair hypothesis for the absorption of substances exists, there is insufficient evidence to indicate that hyphal strands are able to act in this capacity. The possible formation of excretions by the endotrophic hyphae may also result in the higher percentage of roots per rooted cutting taken from the proximal end of one-year old whips. Subsequent diffusion of these substances along the medullary ray system to the region of root initiation might stimulate adventitious root formation.

SUMMARY

1. Greenwood and hardwood cuttings of Vaccinium corymbosum L. were rooted under aseptic conditions to study the influence on rooting of a fungal complex as well as nine separate associated fungi.
2. Detailed description is given of the methods used for these experiments including the techniques of innoculation and control of environmental influences.
3. Stem anatomy is discussed in relation to the plant part used for propagation purposes.
4. Roots were found to originate in the cambial and protophloem regions and project through the callus or through the outer stele, cortex and epidermis.
5. Descriptions are given of the mechanical inhibition of root projection by the lignified pericyclic fibers and closely packed epidermal cells cemented together by a thick cuticle on the outside of the stem.
6. Lack of elaborated food, water and oxygen at the time of early root development is suggested as a further possible reason for inhibition of root projection through the pericycle and the epidermal layer.
7. The nine fungi associated with the stems and roots of this species are identified and described. No relationship is established between their presence and the amount of root development.

8. Associated fungi were found to break down the outer tissues of the stem in preparation for root emergence. With a moderate amount of endotrophic fungal activity, there was more rooting than with a higher or lower amount of activity.

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