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Infection of Juvenile Lodgepole Pine by *Endocronartium harknessii*

by

Eric Andrew Allen

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Infection of Juvenile Lodgepole Pine by Endocronartium harknessii submitted by Eric Andrew Allen in partial fulfilment of the requirements for the degree of Master of Science.

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Supervisor

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Date...28. March 1984.....

Abstract

Juvenile seedlings of lodgepole pine (Pinus contorta var. latifolia Engelm.) were artificially inoculated with the aeciospores of Endocronartium harknessii (J.P. Moore) Y. Hiratsuka , the causal agent of western gall rust. The study objectives were to identify how and where the fungus enters the host, how the fungus develops in the host, and how the host responds to the fungal infection. Pine seedlings from 11 to 76 days old were infected, and easily recognizable external symptoms of tissue discoloration were observed as early as 7 days after inoculation.

Immature epicotyl tissue was observed to be the only site of infections which led to successful gall formation. In this tissue, E. harknessii entered by direct intercellular penetration of the epidermis. Occasional successful infections were also observed on hypocotyls, where the fungus entered through stomata, as well as by direct penetration, and on needles, where it also enters through stomata. Infections on needles and hypocotyls did not result in gall development.

Red tissue pigmentation was observed to be a reliable indicator of infection on epicotyls. Increased tannin production was observed in host cells near the fungus. Most infections resulted in needle death near the infection site, necrosis and resinosis on the stem, and significant growth reduction of the entire seedling. The host response to epicotyl infection varied among seedlings, ranging from

rapid seedling mortality, to recovery from infection. This variation could have been due to causes such as spore density during inoculation, fungal virulence, and host susceptibility.

Acknowledgments

The author thanks Dr. Y. Hiratsuka, Dr. A.K. Hellum, Dr. J.P. Tewari, Dr. K.O. Higginbotham, and Dr. P. Blenis for their guidance during the course of this study. Thanks are also due to Mr. G. Braybrook for his assistance and advise with the scanning electron microscope.

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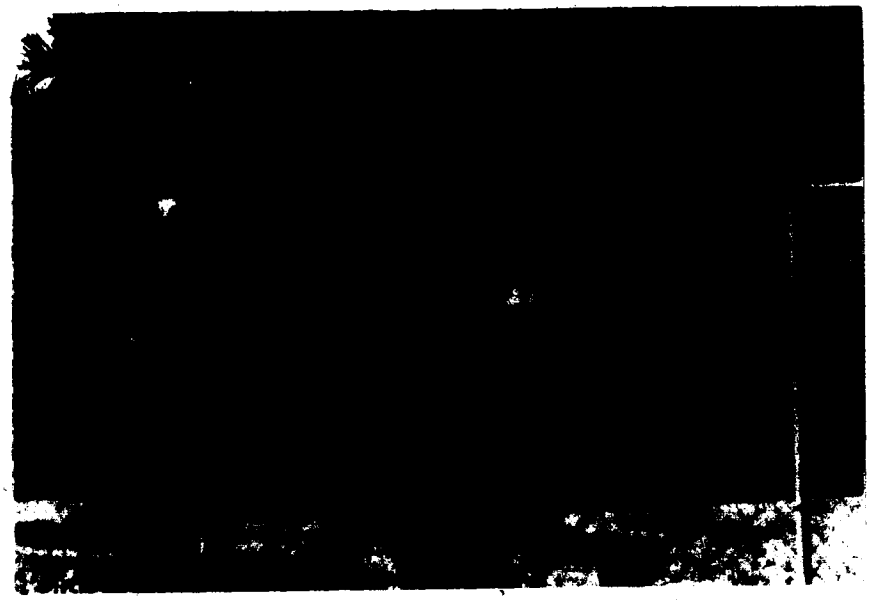
I. Introduction

Endocronartium harknessii (J.P. Moore) Y. Hiratsuka. is the causal agent of western gall rust, a fungal disease of hard pines (Figure 1). It is widely distributed throughout North America and can cause severe damage in infested stands (Hiratsuka and Powell 1976). Major outbreaks are rare and the impact of the disease is not high in natural forests. However, as intensive management techniques play a more prominent role in forest practice, the importance of western gall rust will increase. The use of potentially infected nursery grown tree seedlings in forest regeneration creates a new hazard from the disease. Whereas western gall rust infections on older trees can reduce growth rate and affect form (Figure 2)(Peterson 1960), infections on nursery seedlings often result in the death of trees before they reach a harvestable age (Hiratsuka pers. comm. 1982).

Current efforts to reduce the impact of the disease include studies on chemical and biological control methods (Merril and Kistler 1976, Kistler and Merril 1978, Huber 1980, Tsuneda and Hiratsuka 1981), and on the biology and genetics of the host-parasite relationship (Hildreth 1969, Nelson 1970, van der Kamp pers. comm. 1983). A lodgepole pine genetic improvement program now being initiated in western North America will include the selection of resistance to E. harknessii (van der Kamp pers. comm. 1983).

Figure 1 - Typical branch gall of western gall rust on lodgepole pine (magnification .66X).

Figure 2 - Lodgepole pine with sporulating western gall rust galls in St. Albert, Alberta.



Range and Hosts

The occurrence of western gall rust is as yet restricted to North America (Ziller 1974). Although found mainly in western Canada and the U.S., the fungus has also been reported in eastern Canada (Hiratsuka and Maruyama 1968), the Lake States (Anderson 1965), and New York State(York 1926). The disease as found in the northeastern U.S., earlier named the "Woodgate rust" by York (1926), is now thought to be synonymous with western gall rust (Boyce 1957, Krebill 1970). Western gall rust is found on "hard" pines including Pinus banksiana Lamb., P. contorta Dougl., P. mugo Turra., P. muricata D. Don, P. nigra Arnold, P. pinaster Ait., P. ponderosa Laws., P. radiata D. Don, and P. sylvestris L. (Ziller 1974). Many other pine hosts have been described, occurring naturally or identified by artificial inoculation (Nelson 1970).

Although P. radiata is a susceptible host for E. harknessii , there have been no reports of its incidence in areas such as Australia and New Zealand where this species is planted as an exotic (Parmeter and Newhook 1967). The disease poses a major threat in these areas where genetically similar strains of P. radiata have been planted over extensive areas. Canadian provenances of P. contorta that are being planted in Scandinavia are subject to the same threat (Martinsson 1980).

Taxonomic History

Efforts to describe the life history of E. harknessii have been complicated by confusing nomenclature with overlapping common names and questionable synonymy.

The fungus causing western gall rust was originally named Peridermium harknessii Moore by H.W. Harkness in 1876 (Peterson 1967). He found the fungus growing on Pinus ponderosa near Colfax, California. As more hosts were listed, and further observations of the fungus were made, there was question as to whether Harkness's description represented a single species (Boyce 1957). Meinecke (1916, 1920, 1929) identified two forms of P. harknessii, suggesting that the coastal form on Pinus radiata was identical to Peridermium cerebrum Peck (later changed to Peridermium cerebroides (?)). The other form, found in mountainous regions, was named as Cronartium harknessii Meinecke. Artificial inoculation experiments were conducted using pine and known alternate hosts of Cronartium species in an attempt to identify the life cycle of the fungus. He was able to infect both pine and Castilleja sp. with the aeciospores of P. harknessii. From these results he concluded that P. harknessii was

> "...a heteroecious rust possessing, in addition to the usual mode of spreading through basidiospores, faculty of directly infecting pines through aeciospores..." (Meinecke 1916).

He termed this phenomenon "facultative heteroecism" and used

the name Cronartium harknessii to describe the fungus as it existed on both the pine and Castilleja hosts (Meinecke 1920). Further reports of Castilleja infection by western gall rust aeciospores were made by Rhoades et al. (1918), Hedgcock and Hunt (1920) and Anderson and French (1965). Attempts by other researchers to duplicate these results have been negative (Wagener 1958, Zalasky and Riley 1963, Nelson 1970, and Ziller 1970). Moreover, in an analysis of Meinecke's early studies, Wagener (1964) suggests that the observed uredia formation was the result of contamination from other rust sources. The inclusion of Peridermium harknessii in the Cronartium coleosporioides Arth. complex (Arthur 1922, Anderson and French 1965, Peterson 1967) could have resulted in a misrepresentation of the rust's capacity to infect an alternate host. That is, the inoculation experiments demonstrating alternate host infection may not have been conducted with what we now recognize as Endocronartium harknessii .

A possible explanation of the conflicting evidence is that western gall rust is usually autoecious but heteroecious races may occur rarely in some locations. (Nelson 1970). Peterson (1967) suggested that the fungus is an extremely variable species and that physiological adaptations could account for differences in life cycle, host preference, and growth form.

Hiratsuka et al. (1966) compared the nuclear behavior and germ tube morphology between autoecious (Peridermium

pini Pers. and Peridermium harknessii) and heteroecious (Cronartium coleosporioides) rust species. They found that in the autoecious forms, the aeciospores function as teliospores which he termed "peridermoid teliospores" (Hiratsuka et al. 1966). Young aeciospores were usually binucleate, but later became uninucleate at maturity suggesting nuclear fusion. Upon germination of the diploid aeciospores, reduction division and septation occured producing a monokaryotic germ tube. The heteroecious rust had binucleate aeciospores and the dikaryotic condition was maintained through germ tube production, nuclear migration and appressoria formation. Similar observations were made regarding the nuclear characteristics of Peridermium pini and Cronartium flaccidum (Alb. & Schw.) Wint. (Hiratsuka 1968). As a result of this work, Hiratsuka placed Peridermium harknessii and P. pini into a new genus, Endocronartium , recognizing its endocyclic life cycle (Hiratsuka 1969).

Disease Development

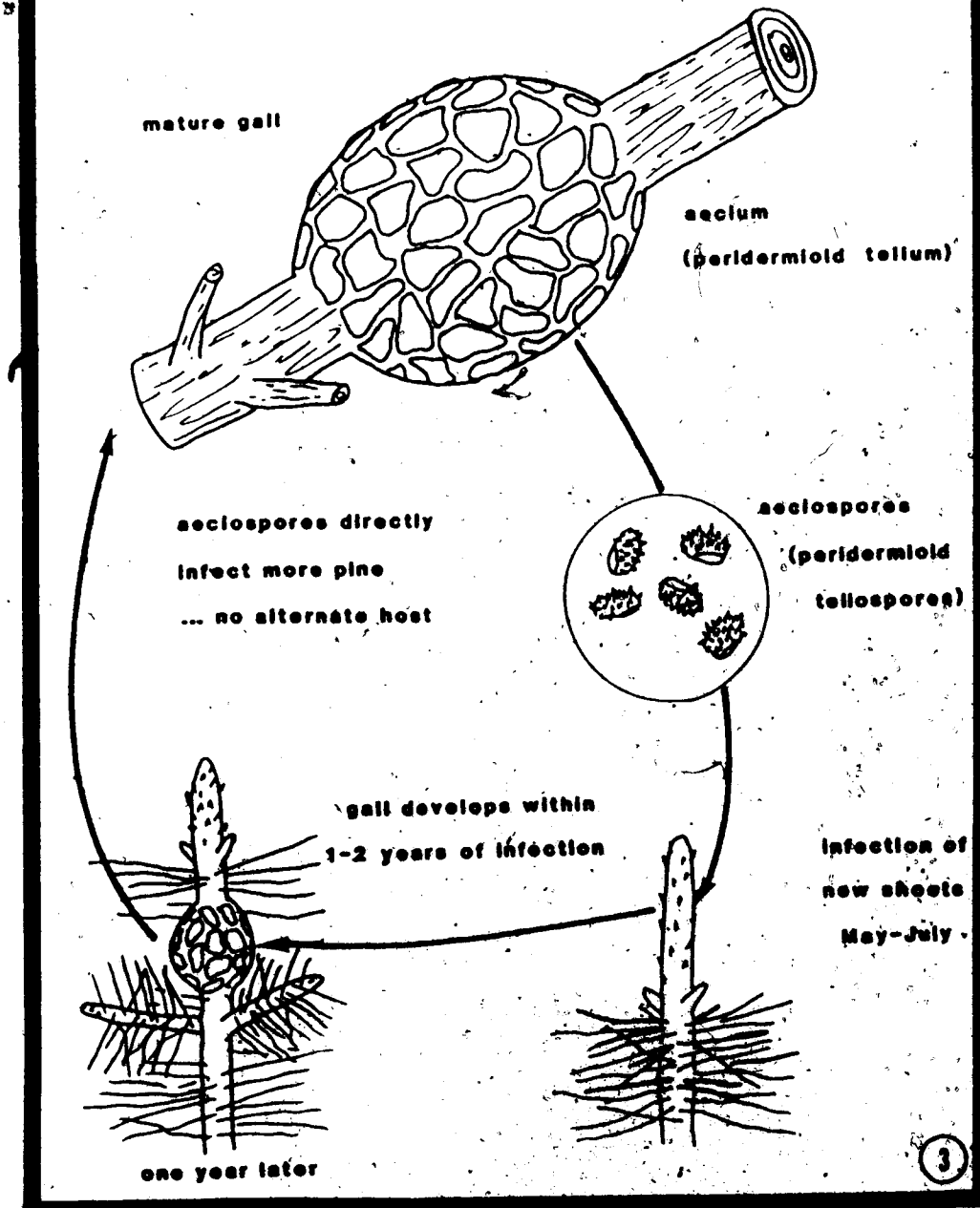
Many researchers have studied E. harknessii , attempting to describe its life history in detail. Presented here is a review of the life history of western gall rust as it has been observed on mature lodgepole pine.

Because E. harknessii is autoecious,its life cycle (Figure 3) is less complex than that of most forest tree rusts. Aeciospores are produced by mature galls on branches

Figure 3 - Life cycle of western gall rust.

LIFE CYCLE OF

Endocronartium harknessii



and/or stems of the pine host from mid-May until early July. Site elevation, aspect, and prevailing climatic conditions determine the time of sporulation for a given year. Warm dry weather seems required to complete aeciospore maturation and to dry and break the peridium encasing the spores. Peterson (1973) showed that on rainless days, E. harknessii spore release began in the morning, as the relative humidity decreased. Maximum daily release occurred at about 11:00 am. Aeciospores land on susceptible pine hosts and germinate, given proper environmental conditions of temperature and moisture. York (1929) observed that aeciospores infected the pines "directly through the epidermis of the current season's stem growths". True (1938) reported on the infection process of the Woodgate rust on Pinus sylvestris . All of the penetrations that he observed were through the epidermis, either inter- or intra-cellularly. No appressoria were noted. Some reports have been made of successful inoculations through wounds or bark slits (Boyce 1957, Anderson and French 1965), but the current season's new growth is generally recognized as the primary site of infection (Meinecke 1916, York 1929, Hutchinson 1935, True 1938, Boyce 1957, Nelson 1970, Hiratsuka and Powell 1976). No galls observed in field conditions are thought to have been infected through wounds of non-current year stems (Hiratsuka pers. comm. 1982).

The position of an infection on a stem seems to be determined by the maturity of the stem tissue. Most of the

successful infections observed by True (1938) were on tissues that had not yet developed a periderm. He felt that the presence of a normal periderm played a passive role in host resistance. Nelson (1970), studying Pinus radiata and Pinus ponderosa found that the elongating stem became nonsusceptible at the base first, and that the nonsusceptible zone "moved up" the stem as it matured. He felt that

> ...the nature of nonsusceptibility with stem maturation. ..appeared to be correlated with periderm formation as observed by True (1938) for the Woodgate rust on Scotch pine...".

Other factors contribute to the success of infection. Adequate inoculum levels and suitable temperature and moisture conditions must coincide with the presence of susceptible tissue for infection to occur. In some years, termed "wave-years" by Peterson and Jewell (1968), disease levels are very high in localized areas suggesting that conditions were optimal for infection. Attempts have been made to describe the environmental conditions prevalent in "wave-years" to aid in the prediction and possible control of the disease (Meyer et al. 1982).

Once the fungus has entered the host it grows intercellularly through the cortex, penetrating the cells with haustoria. The fungal mycelium proliferates in the cortex, and appears to grow toward starch containing cells (True 1938). When the fungus reaches the cambium, abnormal

growth of the xylem, and to a much lesser extent phloem, occurs. The most significant abnormality is hyperplasia of the xylem cells. An 80 to 100 per cent increase in the number of these cells has been reported (True 1938). Ring width is therefore affected; Peterson (1960) observed that rings in infected wood were up to 2-3 times as wide as those in uninfected wood. Another major change that results from infection is an increase in the proportion and number of parenchyma cells. Peterson's studies showed that infected gall tissue consisted of 32% parenchyma and 68% tracheids, whereas normal wood was 6% parenchyma and 94% tracheids. Xylem ray structure is also changed, most rays in infected wood are bi-seriate or multi-seriate whereas very few rays are more than one cell wide in uninfected wood (True 1938, Peterson 1960). Zalasky (1974) described changes in xylem cell structure due to infection by E. harknessii . He found that trachieds in galls "...follow a radial order, but with changes in polarity, curvations, intricate branching, and nodulation...".

Such changes begin to occur during the same season as infection, and a swelling of the stem is often noticeable within four months (True 1938, Boyce 1957). Gall growth continues and aeciospore production begins one to three years after infection (York 1929, True 1938, Boyce 1957). The galls grow each year, adding growth rings similar to, but wider than in a normal stem. Aeciospores are produced each spring until the branch or stem that the gall occupies

is killed or the gall is attacked and inactivated by hyperparasitic fungi (Tsuneda et al. 1980, Tsuneda and Hiratsuka 1981). Rodents and insects can also damage or kill western gall rust galls by feeding on the spores or the bark of the galls (Powell 1971, 1982, Powell et al. 1972).

Host Resistance

Within a population of pine, a range of reactions to infection by E. harknessii can be observed. Some trees are able to resist infection completely and remain gall-free in stands that are otherwise heavily infected. Other trees show no apparent resistance to gall rust infection. Such responses are visible, both externally and internally on the stem, shortly after infection takes place. Hutchinson (1935) studied the resistance of Pinus sylvestris to the Woodgate rust. He descibed three major reaction types. Type A, occuring in what he classed as susceptible trees, showed little external evidence of infection. Minimal host tissue reponse was observed, although some tannin[1] formation by the cortical cells was evident. Reaction type B showed definite stem discoloration following infection, with bark splitting and resinosis occurring during the following growing season. In these plants, tannin production by the cortex cells was much faster than in type A plants and the initial cells

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[1]Tannins or tannin-like substances are often produced by plants in response to fungal infection. This reaction has been studied with the fusiform rust disease caused by Cronartium quercuum (Berk.) ex Shirai f.sp. fusiforme (Walkinshaw 1978)

attacked by the fungus were killed. A barrier of cork cells produced by the host delineated these dead cells, excluding the fungus from the healthy part of the stem. The fungus sometimes broke through this layer, resulting in the development of a second cork barrier. This continued until the fungus was either successfully excluded or able to reach the cambium and initiate gall development. The third reaction type (type C) developed numerous small discolored spots on the stem a few weeks after inoculation, This was sometimes followed by swellings which did not increase in size, but were gradually sloughed off. In these trees, the infected host cells were usually killed immediately after invasion. A periderm formed around these dead cells and the necrotic area was sloughed off. Hutchinson felt that the production of tannin by the affected cells represented a local immunity and that the production of cork cells was a normal reaction to wounding and played a less important role in resistance.

These types of responses have been observed in resistant trees by other authors (True 1938, McKenzie 1942, Hiratsuka and Maruyama 1983). The rapid reaction to infection seen in the "resistant" trees has been described as a "hypersensitive response" (Hare 1966). The concept of hypersensitivity suggests that fungal infection invokes an immediate response in the host whereby the affected cells are killed. Obligate parasites are therefore unable to secure nutrients and thus die. In the case of western gall

rust, this reaction may be activated by the production of tannin compounds.

Damage and Importance

Western gall rust is a widespread disease on the hard pines of western North America. Mortality is not normally high in the natural forest (Gross 1983), but localized epidemics do occur. These have resulted in high mortality in young stands in the northwestern U.S. (Peterson 1960). Generally, infections that occur in the natural forest result in the development of branch galls that affect form, growth rate, and volume. Main stem galls occur less frequently and usually result in the loss of the tree to breakage.

The greatest threat from western gall rust lies in intensively managed stands, plantations, and nurseries. Christmas tree plantations in New Brunswick have been abandoned because of damage by western gall rust to tops and branches (Forbes et al. 1972). In Alberta, 63% of the 6 to 12 year old lodgepole pine stock on a Christmas tree plantation were infected (Powell and Hiratsuka 1973). Mortality in this case was not high but most of the trees were deformed and had no commercial value.

Western gall rust poses a major threat to plantations of Pinus radiata where it has been planted as an exotic species in Africa, South America, Spain, Australia and New Zealand (Scott 1960). The pathogen has not yet reached these

areas but seedlings from these plantations have been shown to be susceptible (Parmeter and Newhook 1967). The relatively small genetic base from which these trees originated further increases the potential danger.

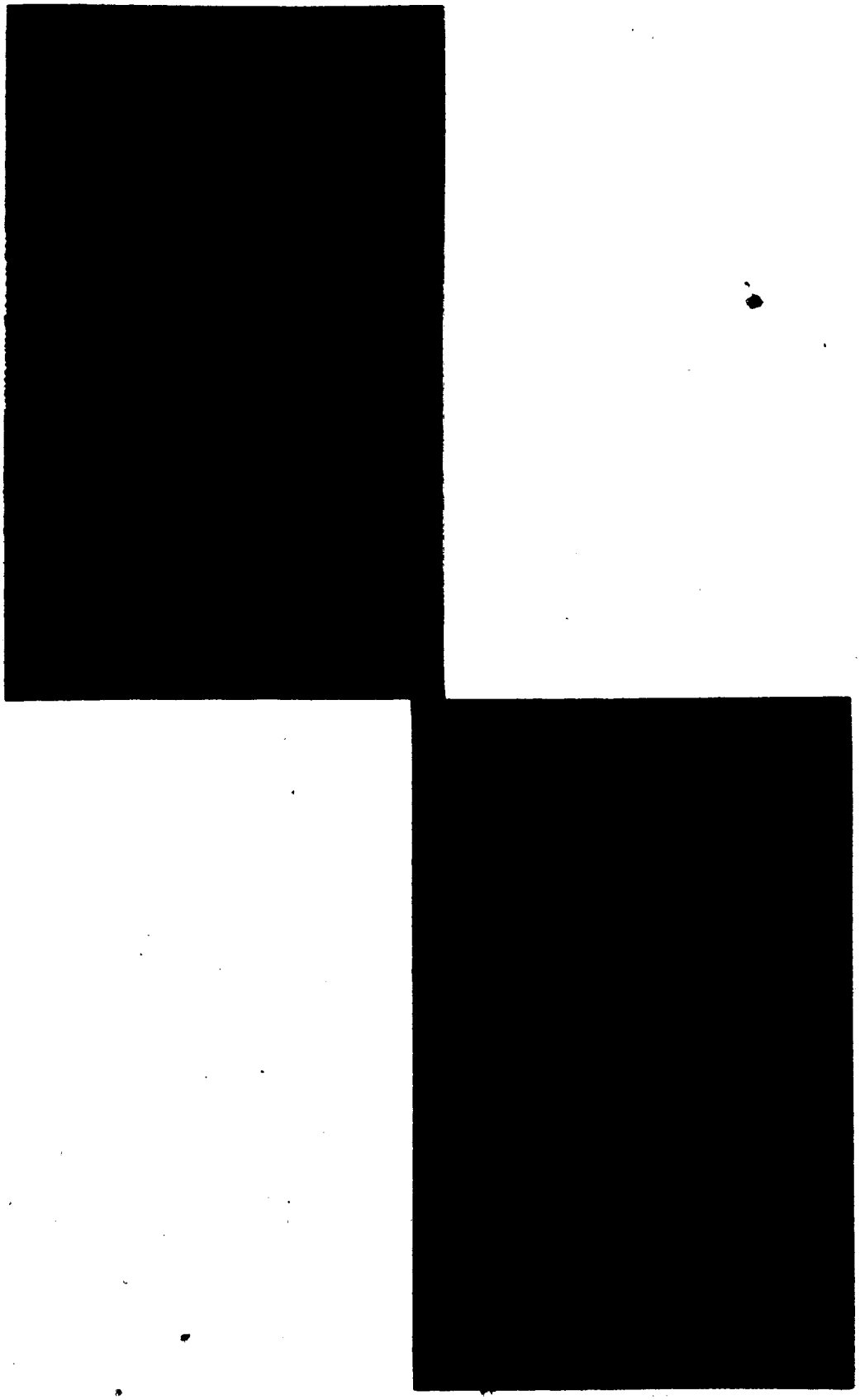
The worst danger from western gall rust is in forest nurseries that produce seedlings for regeneration of logged areas. Infections in the nursery almost always result in basal stem galls (Figure 4). These often go unnoticed in the nursery and the seedlings are planted out in the forest. In the plantation the infected seedlings occupy valuable space and may receive special silvicultural attention such as pruning, fertilization, etc. Furthermore, the diseased seedlings are counted as survivors in regeneration surveys. Mature galls produce aeciospores that can cause further infection of natural or planted stock. Growth reduction is common (Figure 5) and the trees usually succumb to breakage. Whereas older trees can sustain branch infections with little detrimental effect, infection of nursery seedlings is ultimately lethal. Controls, therefore, should be focused on preventing infection of young seedlings.

Control

Various control measures to reduce the incidence of western gall rust have been considered including silvicultural methods, chemical and biological control, and genetic improvement of seedling stock. The choice of control methods and their effectiveness will depend on the

Figure 4 - Basal stem galls on 2-0 lodgepole pine nursery stock (magnification .4X).

Figure 5 - Growth reduction caused by western gall rust on a 15-year-old lodgepole pine (magnification .3X).



particular nature of the disease problem.

The primary means of silvicultural control involve the mechanical removal of galls from infected trees (Hiratsuka pers. comm. 1982). This removes the source of inoculum preventing further spread of the pathogen. This type of control is obviously not feasible in mature natural stands, but can effectively reduce the incidence of the disease in a localized area. Infected trees in and around the Pine Ridge Forest Nursery in Smoky Lake, Alberta have been treated in this way. Further reduction or elimination of western gall rust problems in nurseries can be obtained if new operations are located in areas that are free of the disease or where pine does not occur. Pruning galls or removing whole trees during thinning and spacing operations can help to reduce inoculum levels in intensively managed stands.

Preliminary tests of chemical control of western gall rust have been conducted, (Merril and Kistler 1976, Huber 1980, Hiratsuka and Allen 1980) but as yet, field trials have shown limited success. Such controls, if possible, could be used in tree nurseries to prevent infection at times of the year when the risk of infection is high.

A number of organisms have been observed to hyperparasitize western gall rust galls (Byler 1970, Byler and Cobb 1969, Byler et al. 1972, Hiratsuka et al. 1979, Tsuneda and Hiratsuka 1979, 1980, 1981a, 1981b, Tsuneda et al. 1980). Some of these parasites are abundant in certain localities and have been reported to cause a significant

reduction in the activity and number of sporulating galls. Byler and Cobb (1969) reported that 44% of 2 year old galls, and 97% of the 3 to 6 year old galls on selected pine stands in California were inactivated by hyperparasitic fungi. Byler (1970) found that " Nectria fuckeliana Booth was the most common and efficient gall killer in the plots where it was present." In western Canada, Scytalidium uredinicola Kuhlman et al. and Cladosporium gallicola Sutton were both found to be strongly parasitic to E. harknessii (Tsuneda and Hiratsuka 1981a). A third mycoparasite, Monocillium nordinii (Bourchier) W. Gams. was reported by these authors to be a less effective biocontrol agent in the field. However, its ability to produce the antibiotics monorden and monocillin I (Ayer et al 1980) raise the possibility of its use in the production of antifungal compounds.

These and other mycoparasites are thought to be contributing factors to the natural control of western gall rust (Byler et al. 1972). Their use as biological control agents has been suggested (Byler 1970, Tsuneda and Hiratsuka 1981a) but they remain untested under field conditions.

Genetic tree improvement programs are well established for breeding white pine resistance to Cronartium ribicola and resistant stock has been produced (Bingham 1983). Similar efforts to develop hard pines that show resistance to western gall rust are now being initiated as part of a lodgepole pine improvement program that is being started in western Canada and the U.S.. Included in this program are

studies to determine the genetics of the host-parasite relationship, to examine the details of the infection process, and to further describe host-parasite interactions.

Study Objectives

The life history and epidemiological characteristics of western gall rust have been well described, but many features of the disease are unknown. In order to implement an effective control program, many aspects of the disease must be fully understood. These include the infection process, how the fungus develops in the host, and how the host responds to infection. The identification of susceptible and resistant host reactions may guide the selection of characteristics to emphasize in a breeding program.

Previous research on western gall rust has dealt with the disease as it affects older trees. In the course of study of the infection process of E. harknessii on various ages and types of pine tissue, juvenile seedlings were found to produce an easily recognizable response to infection. In light of the increasing importance of nursery seedlings in forest regeneration, and the potential dangers that E. harknessii presents to young seedlings, further study of this type of infection was warranted.

The remainder of this thesis presents an examination of the events surrounding the infection of lodgepole pine seedlings, that are less than one year old, by

E. harknessii . More specifically, the objectives of this study were to :

1) Describe the anatomy and development of Pinus contorta seedlings from germination to budset. Emphasis was placed on the anatomical changes that are important in relation to infection by E. harknessii .

2) Illustrate and describe the locations and methods of fungal entry into juvenile seedlings of Pinus contorta .

3) Outline where and how E. harknessii develops in the host following infection.

4) Characterize the host response to infection, describing the range of reactions exhibited by the host.

II. Materials and Methods

Test Materials

Lodgepole pine seeds used in this study were obtained from the seed extraction plant at the Pine Ridge Forest Nursery at Smoky Lake, Alberta. The original seeds were collected in the Grand Prairie forest.

Aeciospores of E. harknessii were collected from a number of Alberta locations including Maligne Lake road and Mt. Edith Cavell in Jasper National Park, the Robb road near Hinton, and from trees growing in St. Albert. The galls were removed from the trees and returned to the laboratory for extraction of spores. The extraction procedure involved sieving the spores through a fine mesh wire screen directly into vials for storage (Figure 6). The spores were stored frozen (-20 C) in capped glass vials in lots of appoximately 0.3 g (Nelson 1970). Efforts were made to minimize the time between gall collection and storage and to keep the spores as dry as possible. This helps to maintain the spores in a viable condition and reduces losses to hyperparasites.

Growth of Pine Seedlings

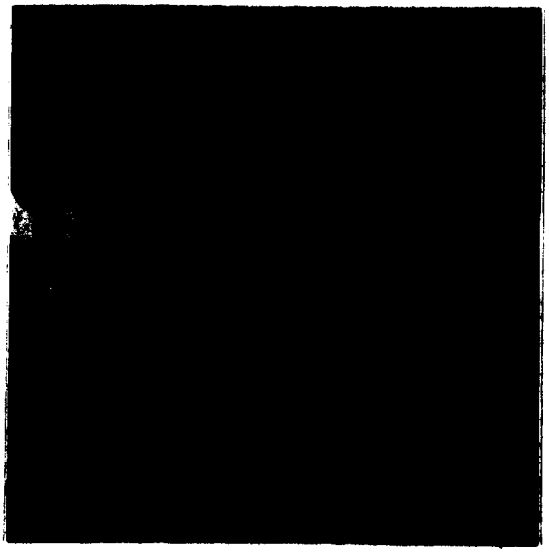
The pine seeds were planted in limed peat (2.6 g $CaCO_3$/l peat) in Spencer-Lemaire Rootrainers. Seedlings were grown in the greenhouse under an 18hr photoperiod. Weekly NPK fertilizer (229-29-154) (Carlson 1979) was applied starting approximately sixteen days after germination.

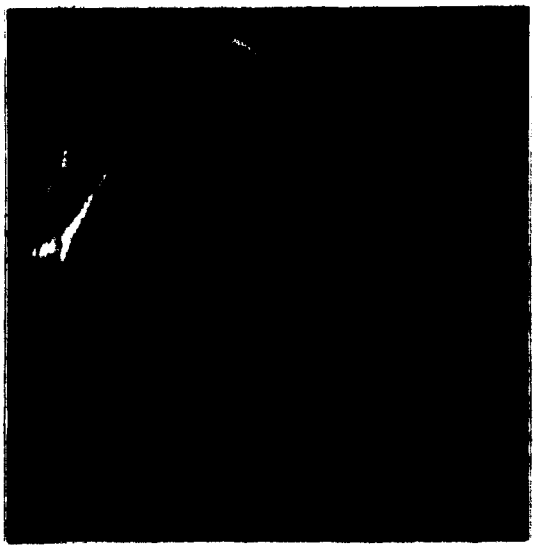
Figure 6 - Spore extraction from a western gall rust gall.

Figure 7 - Inoculation procedure, misting seedlings with distilled water.

Figure 8 - Inoculation procedure, blowing *E. harknessii* aeciospores onto seedlings.

Figure 9 - Inoculation procedure, moist chamber created with moist paper towels and plastic.





Inoculation procedure

Seedlings were removed from the greenhouse for inoculation at various ages, ranging from 11 to 76 days after the date of planting. The seedlings were first misted with distilled water (Figure 7). Spores were then dusted onto the surface of the plants. An effort was made to achieve complete coverage. Two methods of applying spores were used. Spores were "puffed" on to the plants using a "Devilbiss" aspirator. A second method involved scooping dry spores onto a spatula and blowing them onto the plant with a disposable glass pipette connected to an air line (Figure 8). The latter proved to be a more precise and less wasteful method of applying the spores. No attempt was made to control the number of spores applied to each plant. The seedlings were then loosely wrapped with moist paper towels and tightly covered with plastic, creating a moist chamber (Figure 9). The plants were then incubated at 15 C for 24 hours; then they were uncovered, and returned to the greenhouse. Normal growing conditions were resumed until the plants were needed for observation.

Methods of Observation

Seedlings were examined by light microscope using a variety of preparations including hand-sectioning live material, paraffin-sectioning fixed specimens, or by using scanning electron microscopy (SEM) methods.

a) Hand Sectioning

For immediate obervation of live material, hand-sectioning was done. Transverse sections of stems and needles were made under a dissecting microscope using single or double-edged razor blades. Epidermal peels were made by isolating an area of tissue with razor blade cuts and peeling off the tissue with the aid of forceps and micro-dissection needles. In both cases tissue sections were mounted in 70% Ethyl alcohol (EtOH) and/or lactophenol and stained with cotton blue-lactophenol.

b) Paraffin Sectioning

Permanent sections of tissue were prepared by conventional paraffin sectioning methods. This provided a permanent record and allowed for serial sectioning of tissue.

Specimens to be embedded in paraffin were first fixed in a 70% formalin-acetic acid-alcohol (FAA) solution for 24 hours or more. The dehydration, infiltration, and embedding procedures were all done under vacuum following the tertiary-butyl alcohol method outlined in Sass (1958). Sections 10 micromters thick were cut with a rotary microtome and affixed to glass slides using a solution of Haupt's adhesive and formalin (Bissing 1974).

A number of stains were tried in order to obtain clear views of fungal structures in the pine tissue. These included Orseillin BB - Aniline blue (Hutchinson 1935, True

1938, McKenzie 1942), Pianeze III-B (Waterman 1955), Safranin - picro-Aniline blue (Jewell 1958, Peterson and Shurtlef 1965), and Safranin - fast-green (Gurr 1965). The modified Safranin - fast-green method outlined below was found to be the most satisfactory.

1) Remove paraffin with 2 changes of xylene, 10 min. each.
2) Rehydrate the sections to 50% EtOH through a graded series (100, 95, 70, 50) of alcohols, 5 min. each.
3) Stain in 1% Safranin O in 50% EtOH for 8 min.
4) Dehydrate to 100% EtOH through 70, 95% EtOH, 2 min each.
5) Counterstain in 4% fast-green in 100% EtOH for 20 sec.
6) Rinse in 3 changes of 100% EtOH, 1 min. each.
7) Rinse in 50/50 xylene/100% EtOH for 5 min.
8) Clear in 50/50 xylene/clove oil for 5 min.
9) Rinse in 2 changes of xylene, 5 min. each.
10) Mount in Clearmount.

c) Scanning Electron Microscopy (SEM)

The SEM was used to identify potential germ tube penetrations, and to verify infections by viewing the tissued from below the entry points. Various methods, as outlined below, were used in the preparation of materials for scanning.

i) Osmium vapour fixation - freeze drying

Fresh tissue was cut into segments approximately 1 cm long and placed on a glass plate which was raised above the

base of a 6 cm glass petri dish. 1 to 2 ml of a 2% osmium tetroxide solution was pipetted into the space below the glass plate. The petri dish was covered with its lid sealed with Parafilm, and the tissue was fixed for 8 to 12 hours. By this method, osmium vapour filled the sealed petri dish, fixing the tissue without disturbing fungal spores resting on the surface. Fixed tissue segments were then plunged directly into liquid nitrogen and freeze dried prior to mounting for SEM observation. Surface tissue distortion was encountered, limiting the use of this method.

ii) Osmium liquid fixation - critical point drying

Fresh tissue was cut into segments approximately 1 cm long and placed directly into a vial containing a 3% aqueous solution of osmium tetroxide. The tissue was fixed for 8 hours, washed in several changes of distilled water, and dehydrated to 100% EtOH through a graded alcohol series. The specimens were then critical point dried and mounted for SEM observation. Although some of the spores washed off the surface of the host plant during this preparation, the tissue preservation was superior to the vapour fixation method. As well, cuticular wax which was present on the outer surface of the host tissue and which reduced the visibility of some fungal structures was removed by this process.

Specimens dried by both methods were affixed to observation stubs with double-coated sticky tape, carbon glue, or silver glue. Immediately prior to observation,

tissue specimens were sputter-coated with gold. Coated specimens were observed with a Cambridge 250 scanning electron microscope.

iii) Microdissection

The scanning electron microscope restricts observation to the surface of a specimen. Therefore, in order to show that a germinated spore had entered the host tissue, and was proliferating in the tissues beneath, a microdissection procedure was devised to allow observation of the internal plant tissues. The procedure as used in the preparation of Figures 19 and 20 is as follows. After the suspect infection (Figure 21) was located with SEM, the specimen was removed from the vacuum chamber and observed with a stereo dissection microscope. Using 50X maginification, the spore and stoma were relocated. A double edged razor blade was then used to make a transectional cut as close to the stoma as possible. This exposed the sub-stomatal chamber and underlying tissues. The dissected stem was then re-coated with gold and examined again with the SEM.

III. Results

A. Host Anatomy

Lodgepole pine seedlings were grown and observed at various stages of development (Figure 10).

Within a few days of planting, the radicle broke through the seed coat and began to elongate. At the same time, the hypocotyl was elongating, primarily by intercalary growth (Smith 1958). Within one or two days of germination the seedling root was able to penetrate the soil, and from that point on, the seedling stood erect. Cotyledons reached full development after the hypocotyl was fully extended at which time these were often held together at the tips by the remanins of the seedcoat. The hypocotyl and cotyledons generally reached their mature length in one week under optimal conditions. Primary needles began to appear in the axils of the cotyledons within about two weeks of germination (Figure 11). At about the fourth or fifth week, epicotyl elongation began, secondary needles appeared, and the color of the hypocotyl changed from green to red (Figure 12). The epidermis of the hypocotyl became drier and eventually began to crack and split logitudinally. By the time the seedling was 7 to 8 weeks old, epicotyl elongation was well under way, the hypocotyl epidermis was dry and dead, and the underlying tissues (periderm) began to mature and become lignified (Figure 13). Lateral buds developed and

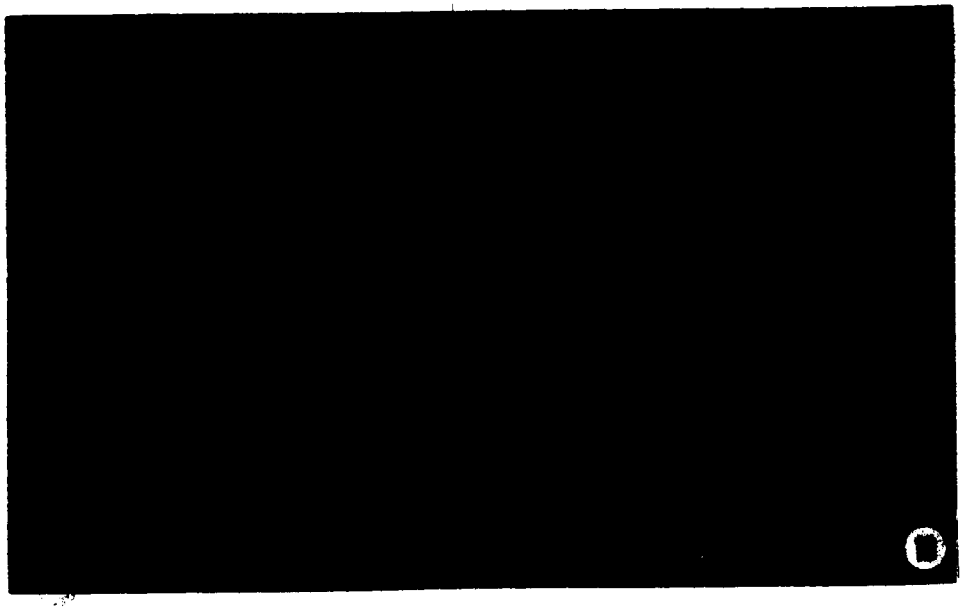
Figure 10 - 19, 33, 54, and 85-day-old lodgepole pine seedlings, showing differences in height growth and seedling maturity (magnification .5X).

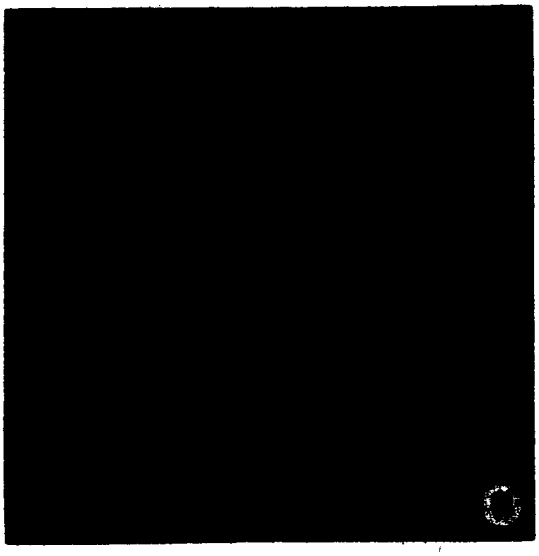
Figure 11 - 19-day-old lodgepole pine seedling showing incomplete development of hypocotyl and primary needles (magnification 10X).

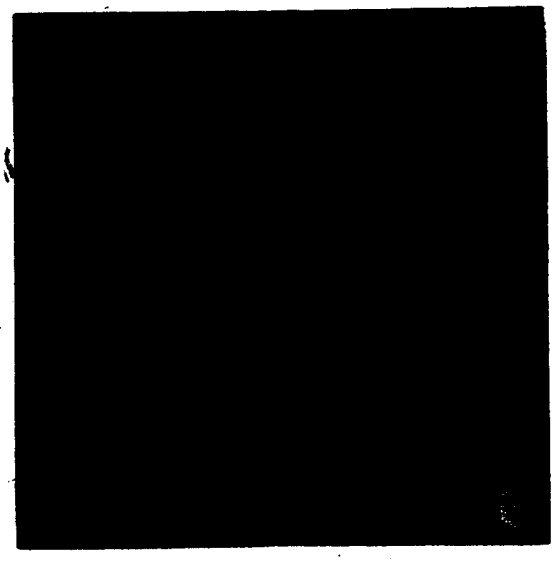
Figure 12 - 33-day-old lodgepole pine seedling showing extended primary and secondary needles and hypocotyl maturation (magnification 10X).

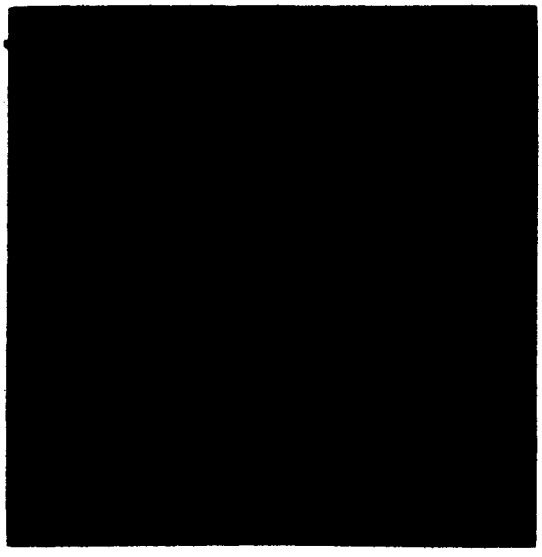
Figure 13 - 54-day-old lodgepole pine seedling showing elongation of ths stem and splitting of the hypocotyl epidermis (magnification 6X).

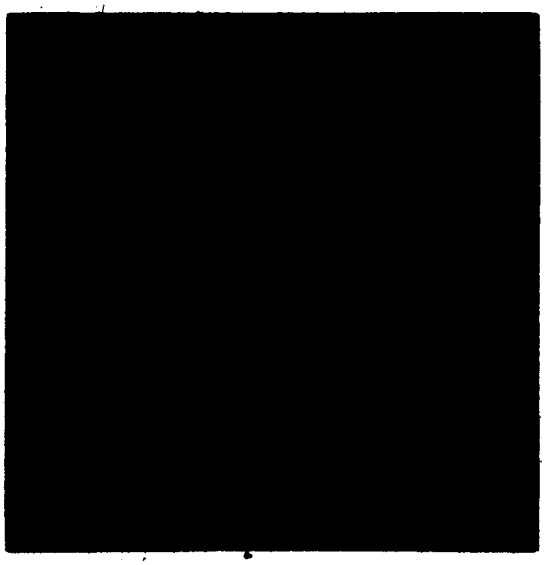
Figure 14 - 85-day-old lodgepole pine seedling showing development of phellem and rhytidome on the hypocotyl and stem, and lateral shoot initiation from the axil of a primary needle (magnification 6X).











elongated and growth continued until budset which started anywhere from 10 to 16 weeks after germination. The maturation of the elongating epicotyl was acropetal and was characterized externally by the drying and hardening of the epidermis (Figure 14). By the end of the growing season, when the terminal bud was set, this tissue maturation was evident on the entire stem. In contrast to the hypocotyl which appeared red, and then brown with inreasing development, the epicotyl remained green throughout most of the growing season (Smith 1958).

The internal development of the seedling reflected the events that were occurring externally. The hypocotyl, as it began to elongate was comprised of a thin epidermis which had stomata, underlain by the cortex, a wide zone of large thin-walled parenchyma cells, which surrounded the rudimentary vasculature and pith. Within two weeks of germination, the xylem and phloem cells began to develop and an endodermis formed, identifiable by Casparian thickenings, in the cortex external to the vascular tissues. By about the third or fourth week, cambial activity began and secondary xylem and phloem were produced. At the same time, the layer of cells immediately underlying the endodermis divided periclinally, initiating the development of the periderm, or cork cambium. The periderm appeared first near the base of the hypocotyl and developed acropetally (Borger and Kozlowski 1972). Within two weeks the periderm was well formed and the cortical cells external to it had died and

collapsed. As the hypocotyl continued to grow in diameter, these layers of dead cells, including the epidermis, dried and split longitudinally. In the subsequent development of the epicotyl, the periderm was formed in the subepidermal cells of the cortex. This produced phellem cells outwardly, resulting in the death of the epidermis and the initiation of the rhytidome.

Stomata were found on both surfaces of the needles and on the epidermis of the young hypocotyl, although in the latter case these were sloughed off as the hypocotyl matured. Stomata were not produced on the epicotyl; similarly none were produced on the elongating candles of older plants.

The developmental sequence of most young conifer seedlings is similar to that described here. Emphasis was placed on the formation of the periderm and the collapse of the cortex for this marked the end of the highly susceptible succulent period of and provided the seedlings with some mechanical protection against fungal attack (Baker 1950).

B. Penetration

Spore Germination

Aeciospores landed on all surfaces of the seedlings and began to germinate under the proper conditions of temperature and moisture. Germination involved the production of a single septate germ tube, one to four cells

long (Figure 15). Germ tubes were often branched, with branches originating from the proximal cells of the germ tube. Germ tube growth often followed the longitudinal "crevice" between the epidermal cells. Clumping of spores frequently occured, and was often accompanied by growth of the germ tubes away from the surface of the host. Such aerial growth could have been a result of the high humidity conditions established in the inoculation procedure.

Host Penetration

The fungus appeared to enter the host by two methods, direct penetration and stomatal entry. Direct penetration was observed primarily on elongating stem tissue, although some suspected direct penetrations were observed on young hypocotyls (Figure 23). Typically, germ tubes penetrated the host at some point in the crevice between the epidermal cells (Figures 16, 17, 18). Similar observations were made of spores germinating on elongating stem tissue of older seedlings (2-0 and 3-0 trees). Figures 17 and 18 show a germ tube emerging from a cluster of four spores, and terminating in an appressorium-like swelling. The epidermal cell immediately adjacent to this penetration shows red discoloration. This was the only discolored cell observed on the stem of this seedling. Red discoloration of affected cells is thought to be a typical host response to infection.

The fungus was also observed to enter through stomata on cotyledons and needles. Figure 19 shows a germinating

Figure 15 - Germinating E. harknessii spore on the stem of a lodgepole pine seedling. Arrow indicates septate germ tube (magnification 1570X).

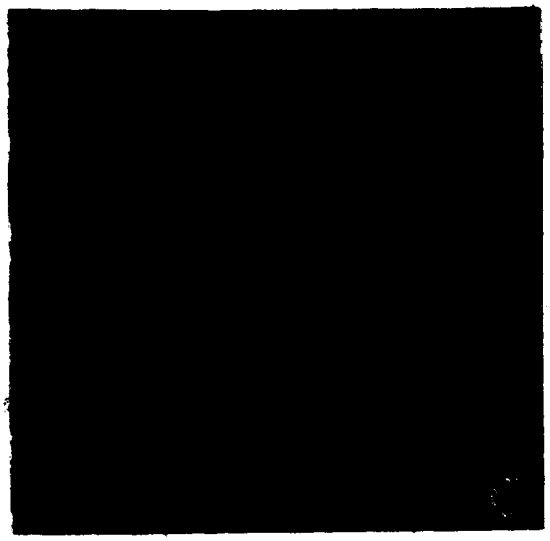
Figure 16 - Germinating E. harknessii spore on the stem of a lodgepole pine seedling. Arrow indicated possible point of direct penetration (magnification 1050X).

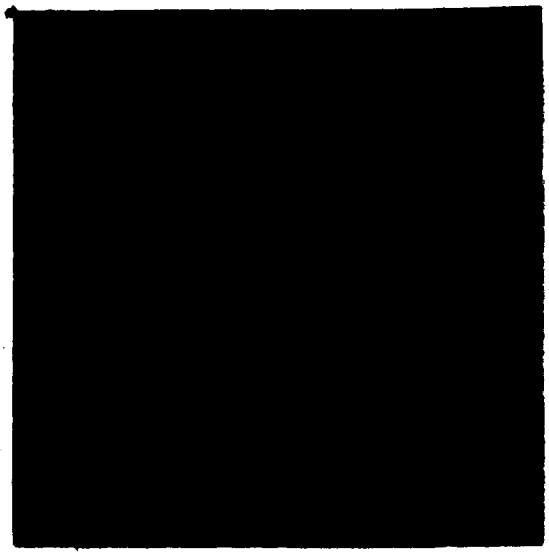
Figure 17 - Direct penetration of elongating stem tissue of a 57-day-old lodgepole pine seedling by E. harknessii . Arrow indicates germ tube. Note red epidermal cell at the point of penetration (magnification 400X).

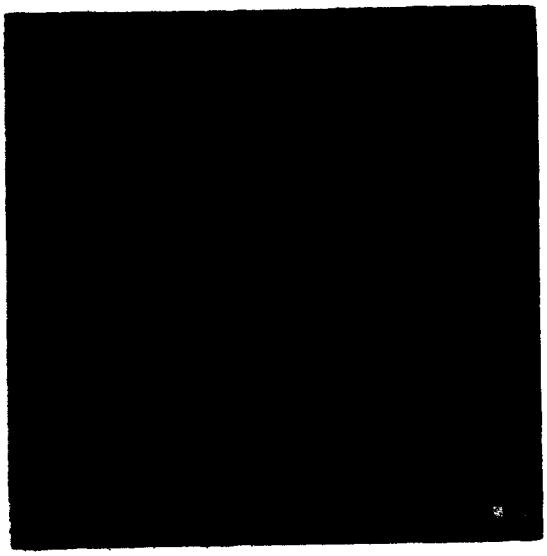
Figure 18 - Same as Figure 17. Arrow indicates appressorium (magnification 640X).

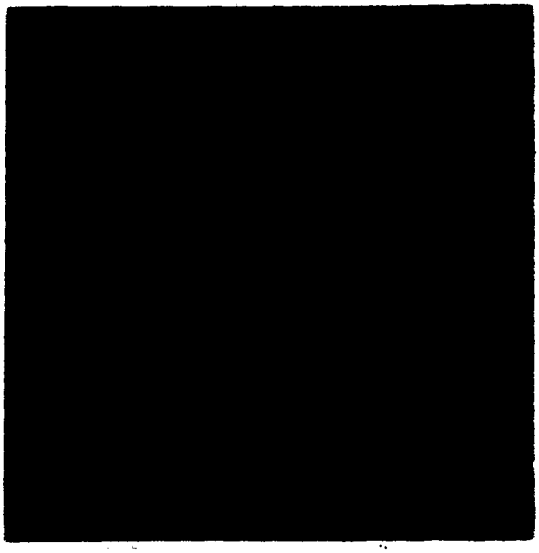
Figure 19 - Germinating E. harknessii spore with a branched germ tube entering a stomate on the cotyledon of a 40-day-old lodgepole pine seedling, inoculated when 11 days old (magnification 300X).

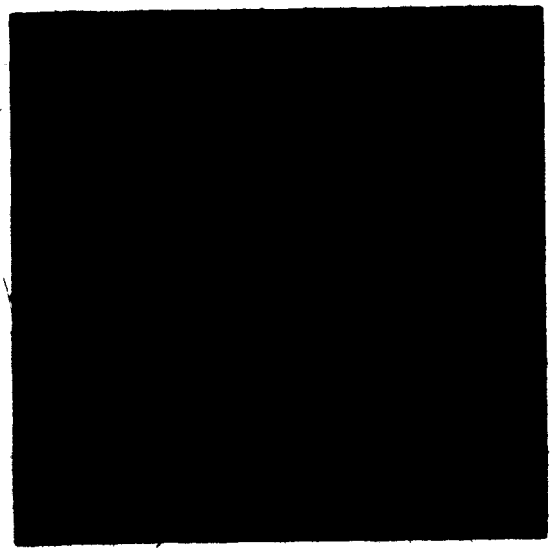
Figure 20 - Same as Figure 19, but viewed from inside the substomatal chamber, showing mycelial development inside the needle (magnification 3750X).

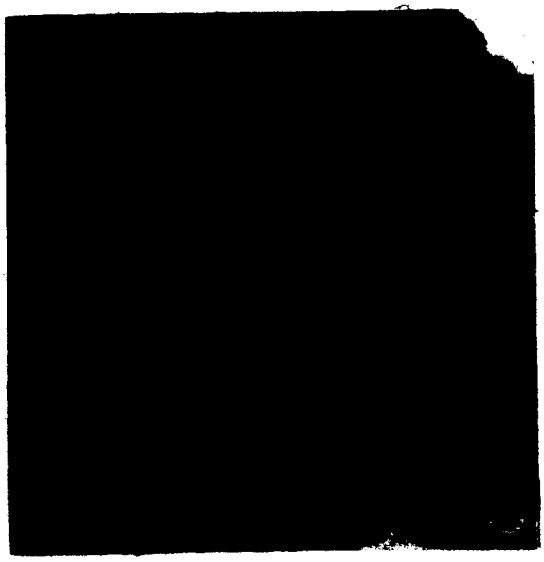












spore whose branched germ tube is entering a stoma on a cotyledon of a seedling inoculated when 11 days old. The epidermal cells around this stoma showed the characteristic red discoloration (Figures 25, 27) suggesting that penetration of host tissues had taken place. Microdissection allowed internal examination of this specimen. Figure 20 views the same stoma as Figure 19 from the inside of the sub-stomatal chamber. The germ tube and branch can be seen entering the stoma and growing into the surrounding tissues. This provides evidence that E. harknessii can infect young pine seedlings through entry into stomata on the needles. Germ tubes were seen to enter stomata on young hypocotyls (Figures 21, 22). Fungal entry by this method or by direct penetration (Figure 23) resulted in the successful infections that were observed on hypocotyls (Figures 24, 25, 26).

Location of Penetration

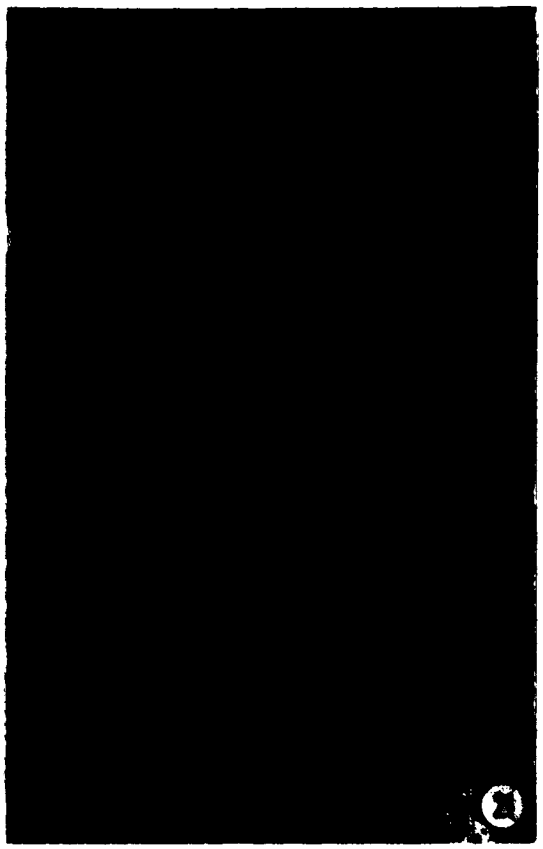
The location of infection seemed to be determined by the age at which the seedlings were inoculated. Figures 25, 26, and 27 show a lodgepole pine seedling that was inoculated when 11 days old. At the time of inoculation the only plant parts present were the hypocotyls and the cotyledons. The areas of red tissue discoloration mark the location of successful infections on these organs. Microscopic examination of these areas showed the fungus to be present. Haustoria were observed in the epidermal cells

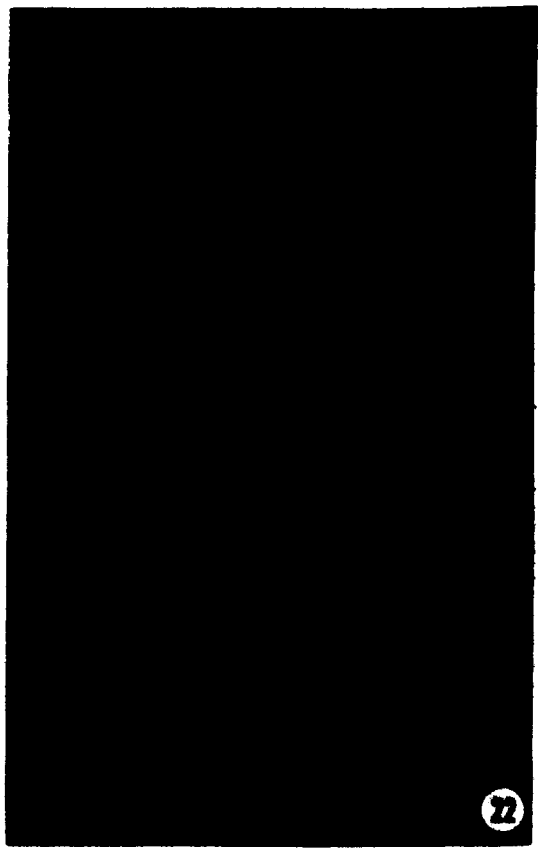
Figure 21 - Germinating spore of E. harknessii entering a stomate on the hypocotyl of a 20-day-old lodgepole pine seedling (magnification 2100X).

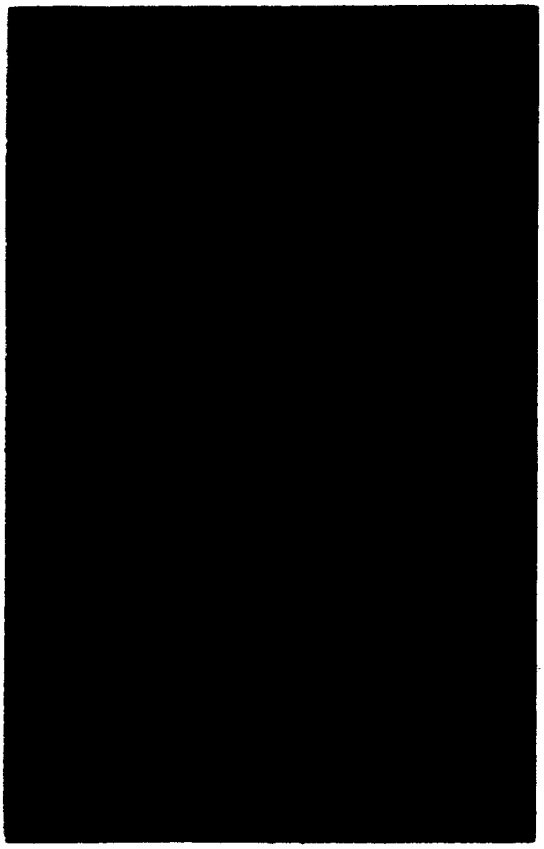
Figure 22 - Germ tube of E. harknessii entering a stomate on the hypocotyl of a 20-day-old lodgepole pine seedling. Hand cross section stained with cotton blue (magnification 1600X).

Figure 23 - Germinating E. harknessii spore entering the hypocotyl of a 20-day-old lodgepole pine seedling by direct penetration. Epidermal peel stained with cotton blue. Arrow indicates point of entry (magnification 1900X).

Figure 24 - Haustorium and mycelium of E. harknessii in the hypocotyl of a 20-day-old lodgepole pine seedling. Fungal tissues lie immediately below the epidermal cell. Epidermal peel stained with cotton blue (magnification 1600X).







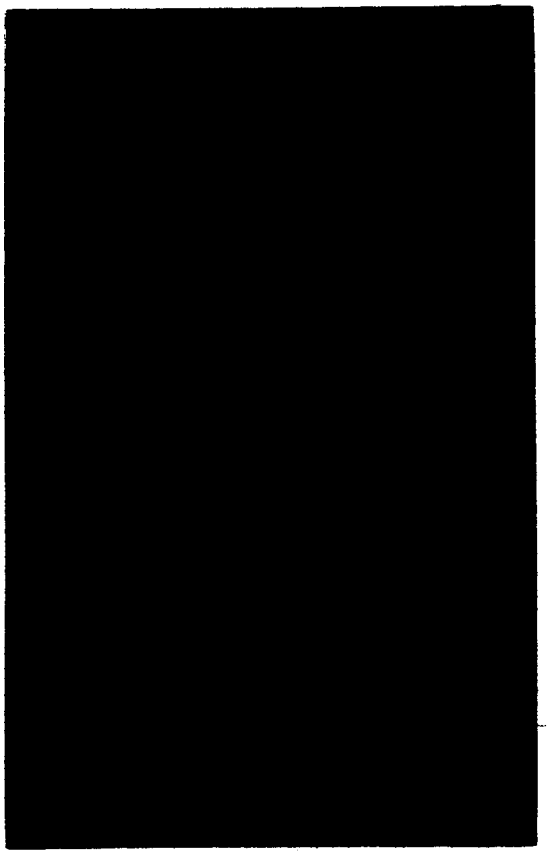
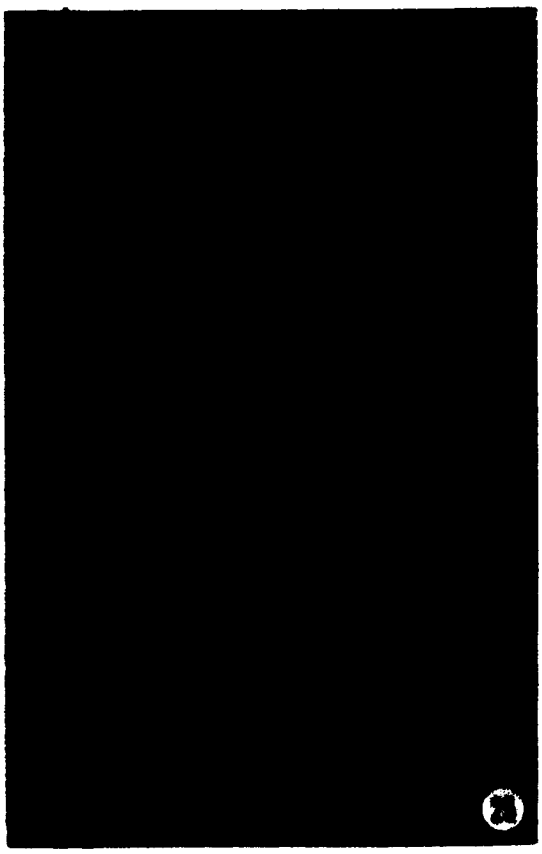


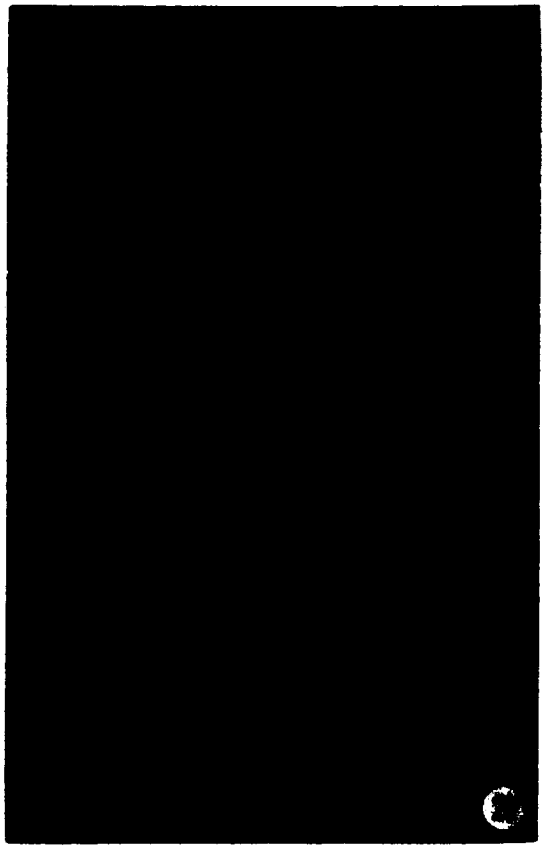
Figure 25 - Tissue discoloration caused by E. harknessii on the hypocotyl and cotyledons of a 35-day-old lodgepole pine seedling, 24 days after inoculation (magnification 3.5X).

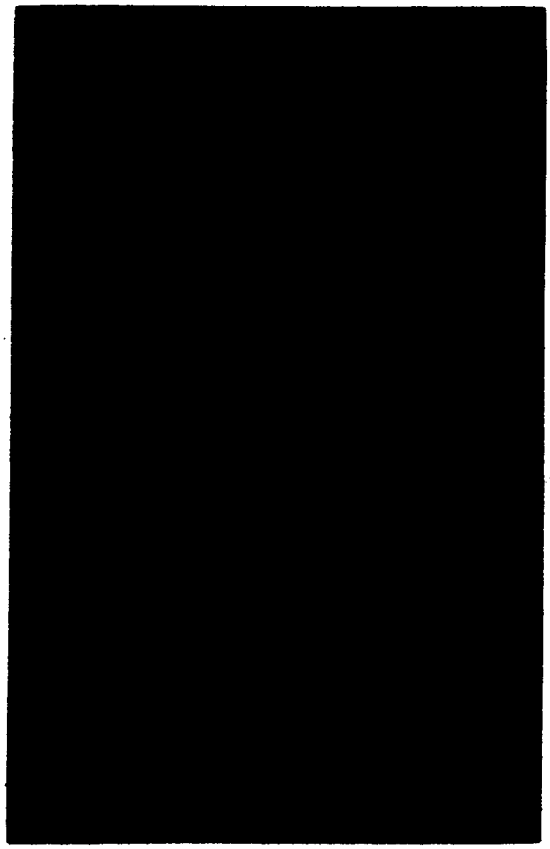
Figure 26 - Same as Figure 25. Close up of hypocotyl discoloration (magnification 17X).

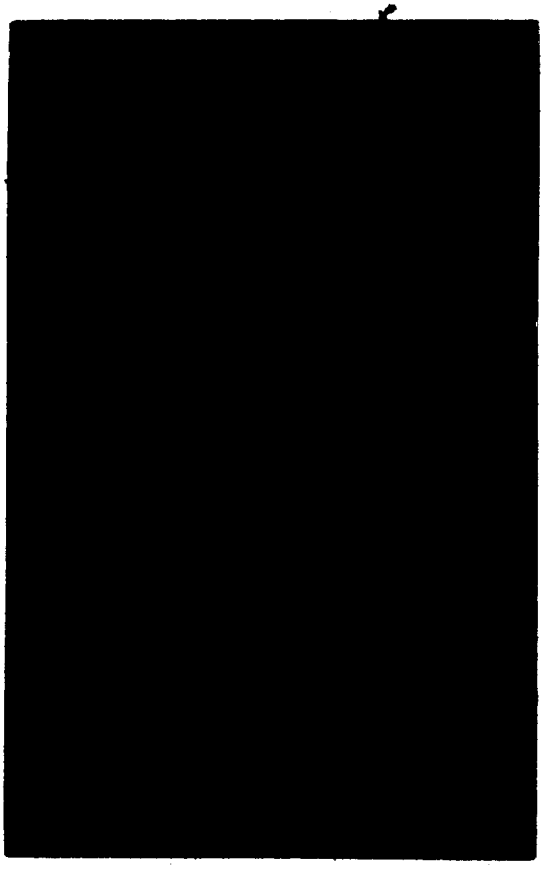
Figure 27 - Same as Figure 25. Close-up of discoloration on cotyledons (magnification 17X).

Figure 28 - Tissue discoloration caused by E. harknessii infection at the base of the primary needles of a 37-day-old lodgepole pine seedling, 21 days after inoculation (magnification 17X).









of hypocotyl infections (Figures 24, 26), and hyphae were seen proliferating in the infected areas of needle tissue. Figure 28 shows an infection at the base of the primary needles in the axil of the cotyledons. This was the most common site of infections on seedlings inoculated when 25-35 days old. As these seedlings grew, they developed symptoms similar to those shown in Figure 29.

Inoculations on older seedlings were also successful, but the location of the infections differed from those on younger plants. Figure 30 illustrates the red discoloration symptom produced on a seedling inoculated when it was 54 days old. This infection developed high on the epicotyl, suggesting that the area of susceptible tissue was no longer near the cotyledons but closer to the growing tip. Figures 31 and 32 also show seedlings which were inoculated at an older age of 63 days. Again, the infections developed high on the stem, or on the needles surrounding the growing tip (Figure 32). These particular infections showed this striking reaction 7 days after inoculation. None of the tissues on the lower part of these stems showed any sign of infection.

C. Colonization

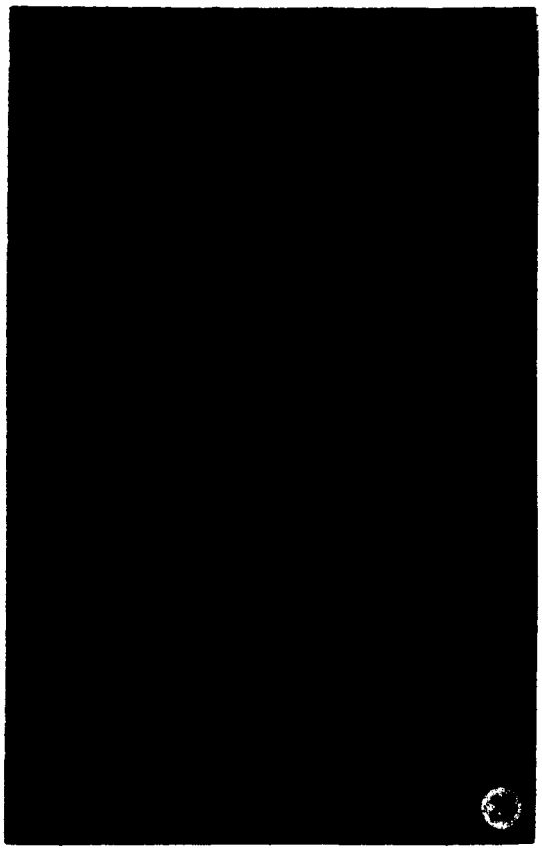
This section describes the events in the colonization of lodgepole pine by E. harknessii after the germ tube had entered the host plant. The manner in which the fungus developed depended to a large extent on the stage of

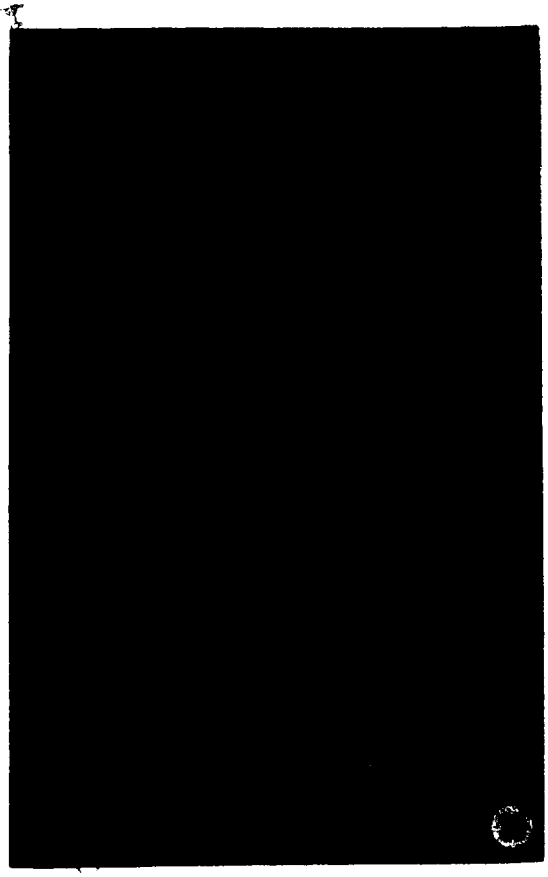
Figure 29 - Tissue discoloration on lower epicotyl of a 65-day-old lodgepole pine seedling resulting from an infection by E. harknessii , 43 days after inoculation (magnification 17X).

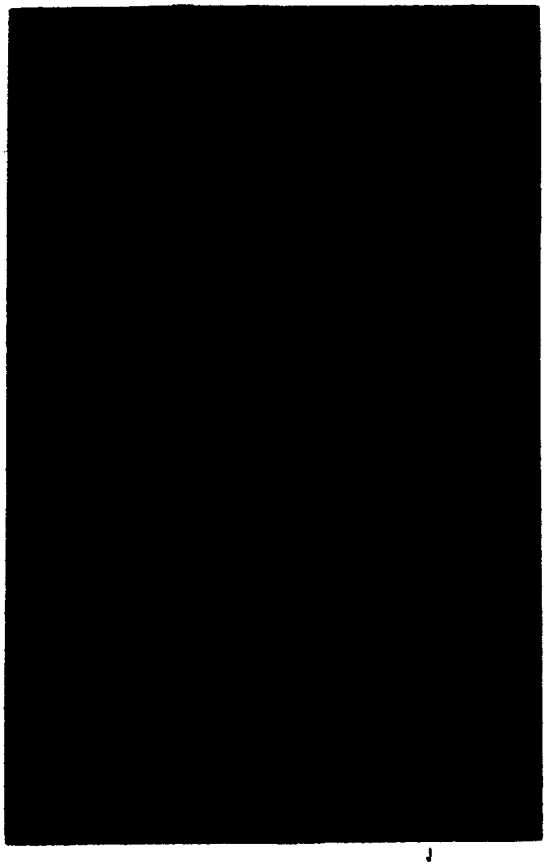
Figure 30 - Tissue discoloration caused by E. harknessii infection on the upper stem of an 85-day-old lodgepole pine seedling, 31 days after inoculation (magnification 20X).

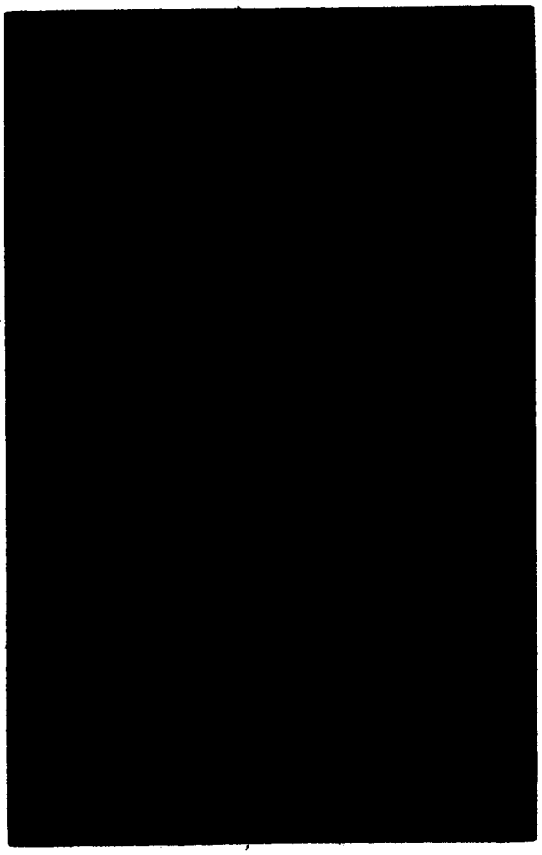
Figure 31 - Tissue discoloration caused by E. harknessii infection on the upper stem of a 63-day-old lodgepole pine seedling, 7 days after inoculation (magnification 17X).

Figure 32 - Same as Figure 31, but on upper needles (magnification 8X).









development that the seedling had attained when it was inoculated.

Shortly after entry, the fungus moved into the cortical cells immediately underlying the epidermis and established haustoria (Figure 33). In some cases, particularly in upper stem infections, the fungus showed rapid vertical growth in a narrow column of cells in the outer cortex. This was visible externally as a reddish colored vertical strand of cells (Figure 34). Within the stem, the fungus showed very little tangential or radial growth. One such strand, in a seedling sectioned serially 8 days after inoculation, affected approximately 30 cells near the infection site, narrowing to approximately 10 cells more than 2 mm down the stem (Figure 35). Haustoria were consistently produced in these cells (Figure 36).

The cells in the outer cortex of an elongated stem segment allowed unobstructed movement along the vertical axis. In contrast, infections on younger epicotyl tissue occurred where little internodal tissue was present (Figure 12). These showed less initial vertical movement of mycelium, due perhaps to the complex orientation of the host cells at this site. The further development of infections at this age of tissue showed the fungus proliferating in the cortex, growing radially toward the vascular cambium, and circumferentially around the stem. The mycelium in the cortex followed the convoluted path that the spaces between the cells allowed (Figure 33). Deeper intrusions into the

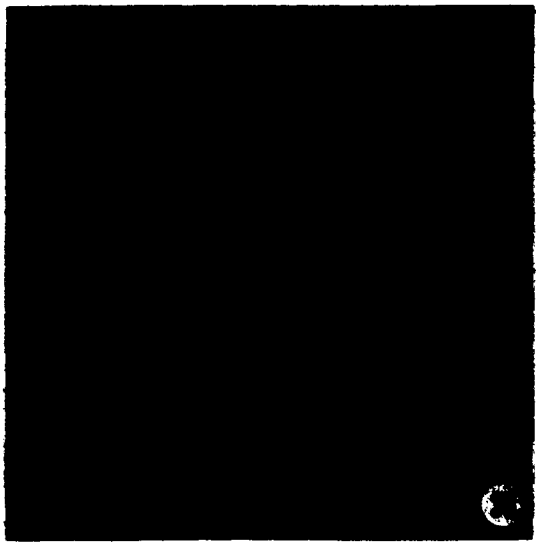
Figure 33 - Hyphae (Hy) and haustorium (Ha) of E. harknessii growing in cortex of 39-day-old lodgepole pine stem (magnification 640X).

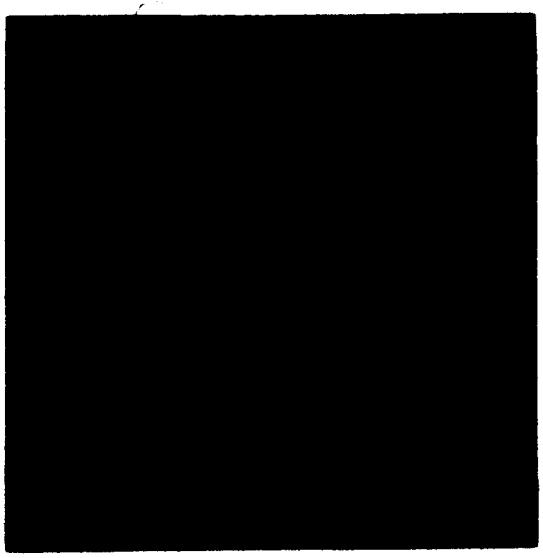
Figure 34 - Upper epicotyl of 72-day-old lodgepole pine, 7 days after inoculation with E. harknessii . Streaks of red tissue discoloration indicate the presence of the fungus (magnification 20X).

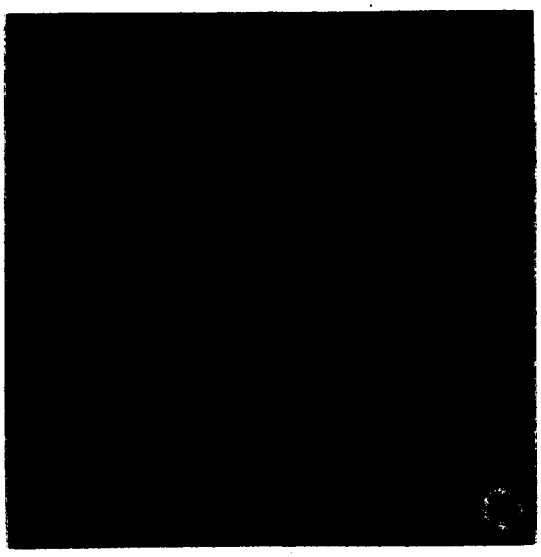
Figure 35 - Cross section of 37-day-old lodgepole pine stem, 21 days after inoculation with E. harknessii . Arrow indicates infected area (magnification 42X).

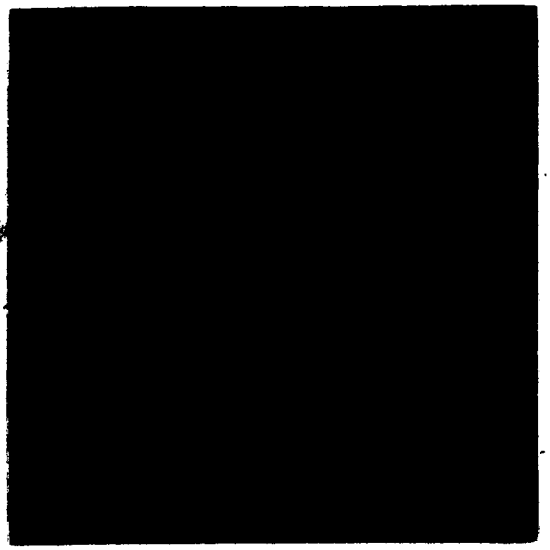
Figure 36 - Higher magnification of Figure 35 showing hyphae and a haustorium (magnification 1470X).

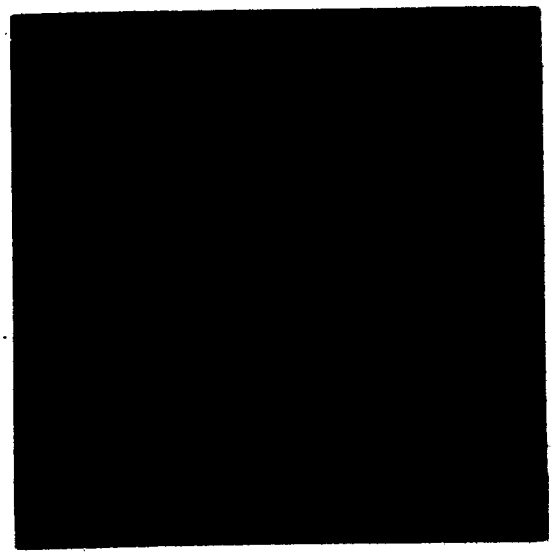
Figure 37 - Stunted haustoria of E. harknessii growing from a xylem ray into xylem cells. Longitudinal section of lodgepole pine stem (magnification 1600X).











stem were made through the xylem and phloem rays where the cell orientation permitted ease of radial growth. Similarly, the vertically oriented phloem cells and resin ducts seemed to allow more rapid growth along that axis.

Hyphae that grew through xylem rays often produced haustoria that penetrated into xylem cells (Figure 37). These were much smaller and rounder than the haustoria observed in other tissues.

As the infected seedlings grew older, the amount of infected tissue increased, with the fungus moving longitudinally, radially, and vertically in the stem. Vertical development occurred in two ways. The bulk of the mycelial growth was associated with normal stem elongation, where the fungus grew in phase with the elongating stem. Infections that occurred in internodal areas on seedlings prior to elongation, developed with the stem in this fashion and the fungus occupied all of the internodal tissue when elongation was complete (Figure 29).

Infections that occurred on the stem in axils of needles or lateral buds were seen to grow into the base of these structures. Here too, the fungus grew in phase as elongation took place in these tissues. Observations made one to two months after inoculation showed that needles that were young and undeveloped at the time of infection contained the fungal tissues further up from the base than did needles that were fully developed when infected. For example, cotyledons which were nearly mature at the time of

most inoculations were never found to have mycelium more than 0.5 mm from their base. The mycelium in primary needles however, often penetrated more than 2 mm up from the base. In most cases, these needles died.

The second method of vertical growth occurred in the absence of stem elongation. In stems of seedlings observed 2 months after inoculation, upward mycelial growth was occurring in the vascular tissues and resin ducts. In needles, such growth was restricted to the vascular tissues.

The fungus was also observed to move downward and cause swelling in the hypocotyl (Figure 38). This was a common occurrence, especially in infections occurring at the base of the epicotyl, and resulted in the formation of fusiform galls.

E. harknessii was observed to enter through the stomata of cotyledons and young primary needles. After entry, the mycelium proliferated and moved easily through the loosely packed mesophyll cells (Figure 39). The extent of fungal growth along the long axis of the needle was not ascertained but it appeared to be limited. Discrete areas of reddish discoloration were observed (Figure 40) which did not coalesce.

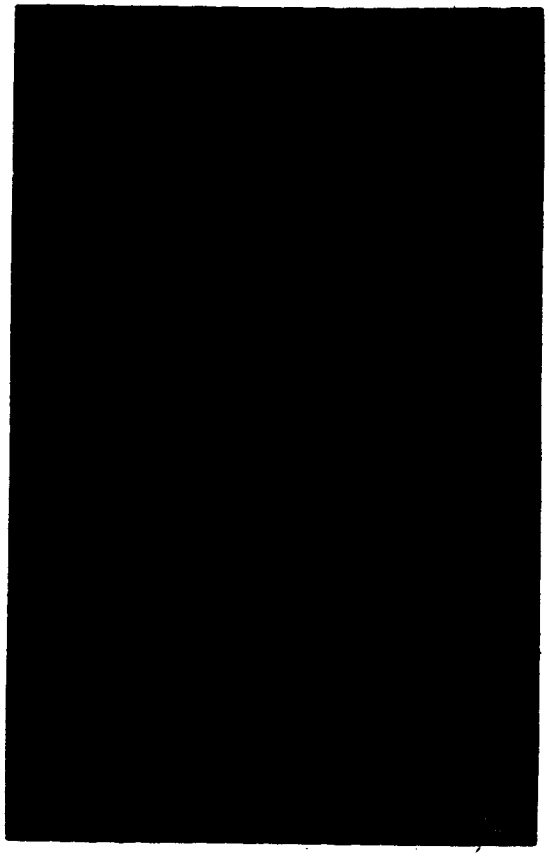
D. Host Response

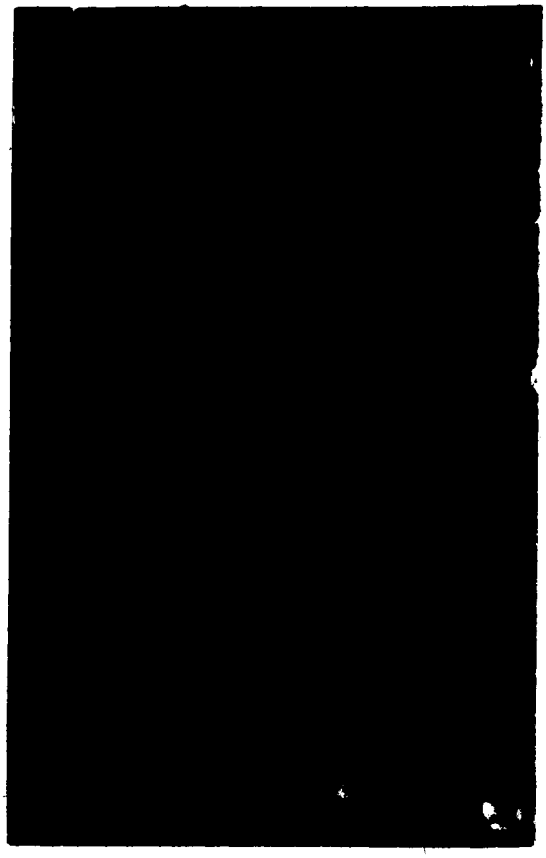
Lodgepole pine response to E. harknessii infection of juvenile tissues varied among individual plants. Upon initial fungal penetration, the host tissues turned red at

Figure 38 - 198-day-old lodgepole pine inoculated when 29 days old. Fungal growth has moved down the hypocotyl and swelling has occurred (magnification 4.2X).

Figure 39 - E. harknessii hyphae growing in needle tissue of a 41-day-old lodgepole pine seedling, 30 days after inoculation (magnification 800X).

Figure 40 - Red discolored spots on 77-day-old lodgepole pine cotyledons, 66 days after inoculation with E. harknessii (magnification 6X).





the site of infection. In one case of direct penetration, a single host cell was initially affected (Figure 17). As the fungal hyphae grew into and along the vertical axis of the stem, contacting more cells, further pigmentation occurred (Figure 34). Not all cells that changed color were penetrated by haustoria, but the response seemed restricted to cells that were in contact with hyphae (Figures 35, 36). Some individual seedlings showed large areas of discoloration within 7 days of inoculation. No initial responses to infection other than tissue pigmentation were observed on inoculated seedlings, and only seedlings that developed this symptom were observed to produce galls. However, not all pigmented seedlings developed galls. A small number of seedlings became externally discolored and were later shown to be infected, but fungal development was arrested and the seedlings developed normally.

Internal observations of infected stems showed a host response in these tissues. As the fungus grew radially into the stem, the cells of the cortex that came into contact with the hyphae were modified. Transverse hand-sections made of infected stems at this stage showed the affected cells to be filled with a yellowish-brown material. In paraffin sections prepared with safranin-fast green, these areas stained heavily, ranging in color from red to purplish-blue (Figures 35, 36, 41). Such stained areas were always found to be associated with fungal mycelium and were therefore useful in rapidly identifying areas of infected tissue. This

stain combination has been used to identify the presence of tannins and tannin-like substances in plant cells (Gurr 1965).

As the infected seedlings continued to develop, areas of necrotic tissue were observed near the site of infection, primarily in the outer cortex and epidermal tissues of the stem. These areas contained fungal material and stained bright red with safranin-fast green. In some cases, a layer of periderm tissue was observed between the necrotic zone and the healthy tissue (Figure 42). When the infected stem increased in diameter as gall formation began, necrotic outer tissues split and were sloughed off (Figure 47). When necrotic tissue included needle bases, the affected needles usually died (Figure 43). On some seedlings only one or two needles were killed and stem growth continued normally. At the other extreme, all needles were affected and the seedling died within a few weeks of inoculation (Figure 44). Such mortality only occurred when seedlings were infected at an early stage of development (less than 30 days).

Some seedlings exhibited limited needle death, but were severely stunted (Figure 45). These had roughly the same number of needle traces as plants of an equivalent age, but showed no internodal elongation.

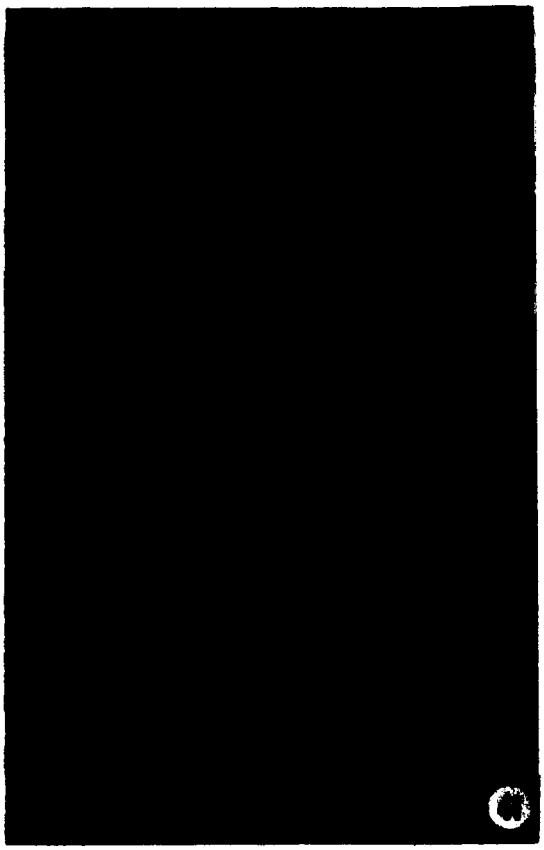
Another growth anomaly was observed on seedlings that were inoculated when about 30 days old (Figure 46). Above the point of infection, the epicotyl seemed to be normally elongated, but the stem tissue was swollen and the needles

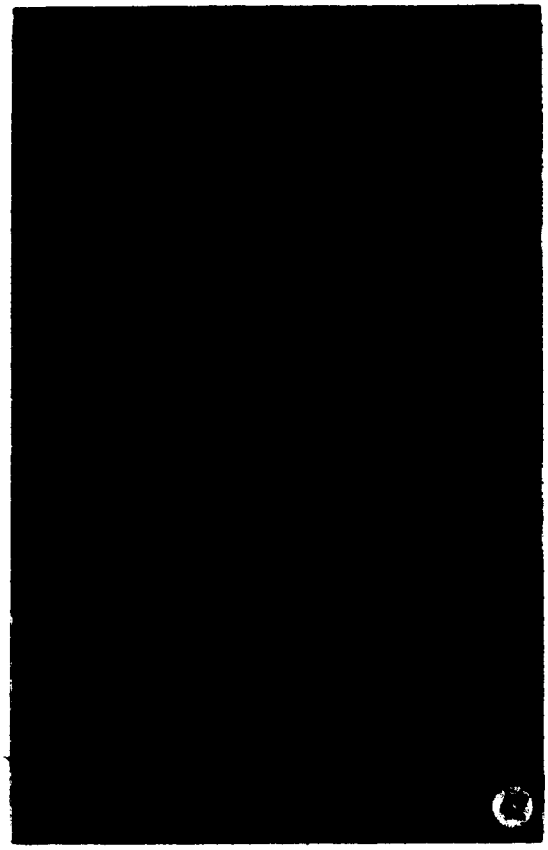
Figure 41 - Haustoria in tannin-filled cells at the base of a secondary needle in a 117-day-old lodgepole pine seedling infected with E. harknessii when 41 days old. Longitudinal section, (magnification 1600X).

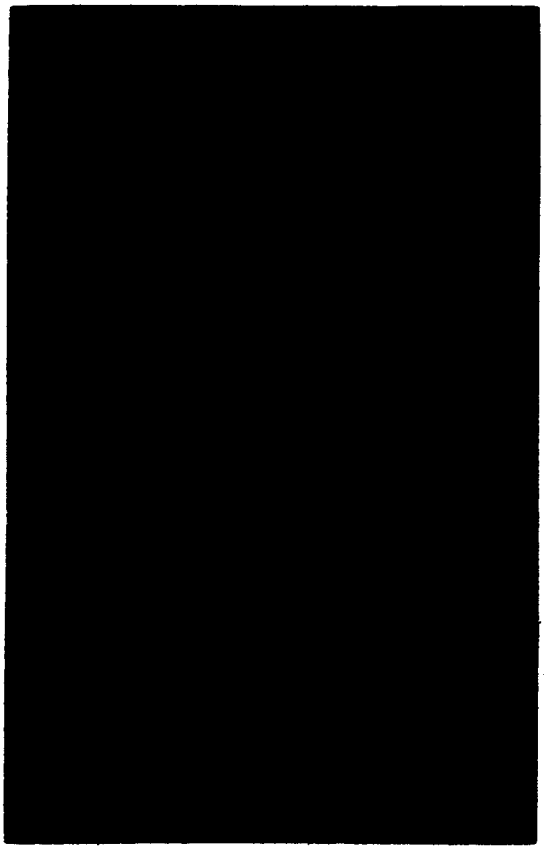
Figure 42 - Periderm cells bordering necrotic tissue in a cross-section of a 117-day-old lodgepole pine stem, inoculated with E. harknessii when 41 days old (magnification 400X).

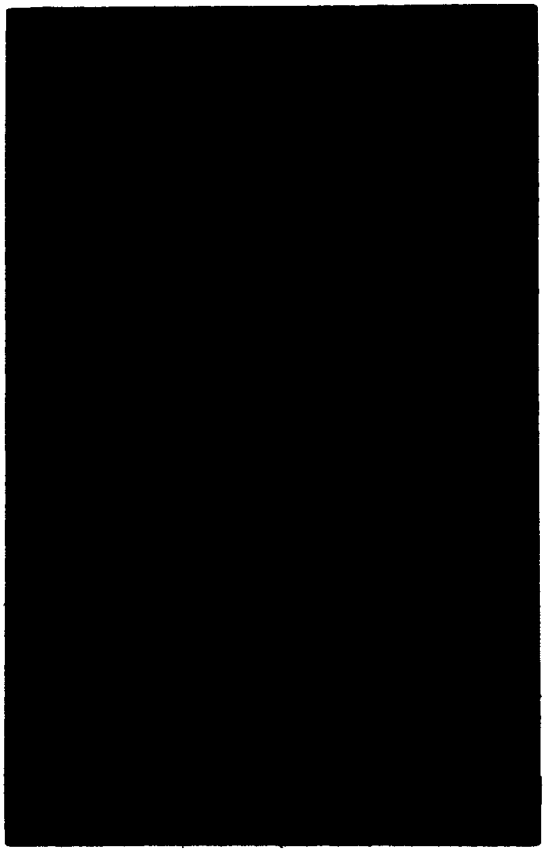
Figure 43 - 142-day-old lodgepole pine seedlings, both inoculated with E. harknessii when 29 days old. Note difference in growth (magnification 1X).

Figure 44 - Mortality of lodgepole pine seedling, 30 days after inoculation with E. harknessii (magnification 2.4X).









were discolored. In contrast, the hypocotyl was poorly developed with little or no additional diameter growth. These seedlings generally collapsed, bending near the cotyledons as the underdeveloped hypocotyl was unable to support the swollen epicotyl. This phenomenon is thought to be due to a complete blockage of the phloem at the infection site, preventing the flow of carbohydrates from reaching the hypocotyl and roots.

Some seedlings which initially showed pigmentation symptoms were able to recover from the infection and resume normal growth (Fig 43). In these seedlings fungal hyphae were present in necrotic lesions near the infection site, and had not spread to the surrounding tissues. Most of the seedlings that were successfully inoculated developed stem swellings within 4 months. These varied in shape, from fusiform to globose, and in position on the stem, as determined by the seedling age at the time of inoculation.

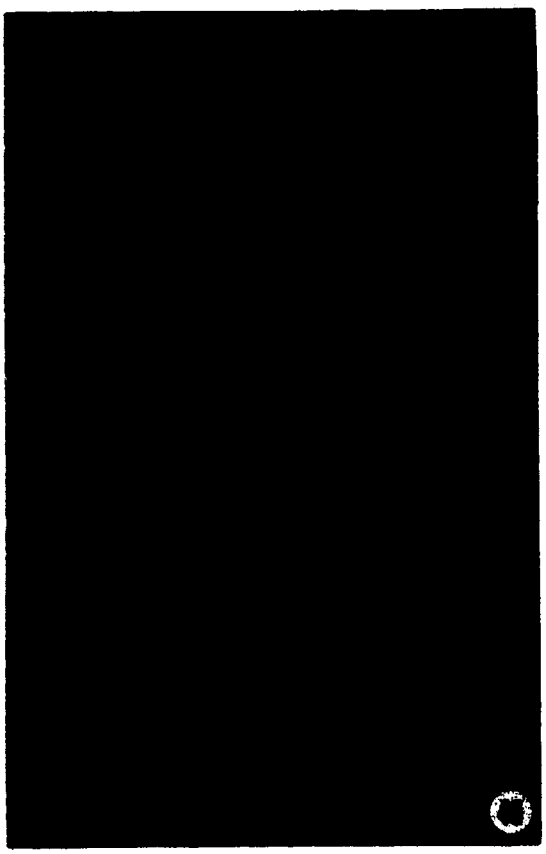
Figures 47 and 48 show cross-sections of 138-day-old pine stems, taken from equivalent positions of infected and uninfected stems, respectively. Figure 47 exhibits the increased size and number of xylem cells characteristic of gall development. Within 6 months the swellings had become more globose, and showed the typical external appearance of an E. harknessii gall (Figure 49).

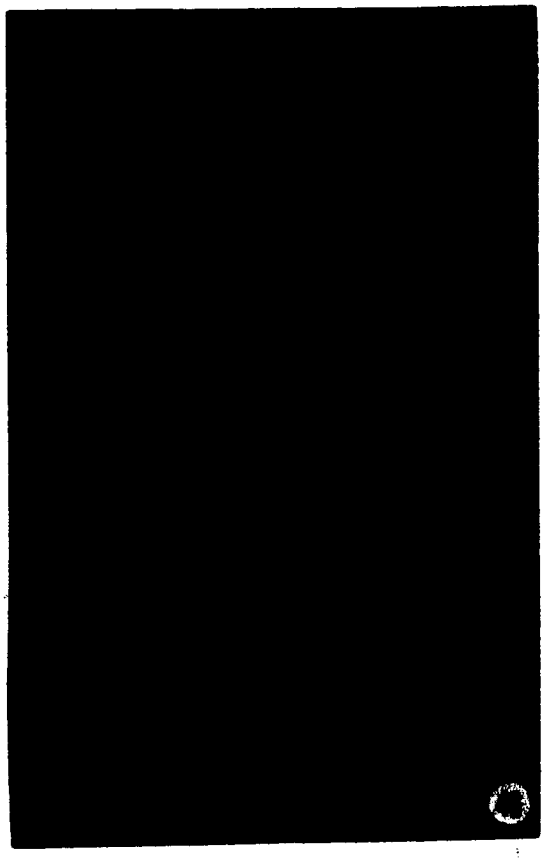
Figure 45 - 198-day-old lodgepole pine seedling showing severe stunting. Inoculated with E. harknessii when 29 days old (magnification 1X).

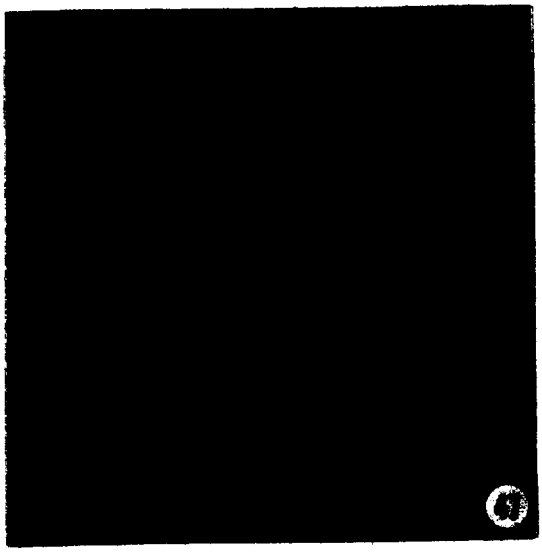
Figure 46 - Epicotyl swelling and needle discoloration caused by phloem blockage on lodgepole pine seedling infected by E. harknessii (magnification 3.5X).

Figure 47 - Cross section of a 131-day-old lodgepole pine stem, inoculated with E. harknessii when 22 days old (magnification 77X).

Figure 48 - Cross section of an uninfected 131-day-old lodgepole pine stem, taken from roughly the same position as Figure 47 (magnification 77X).







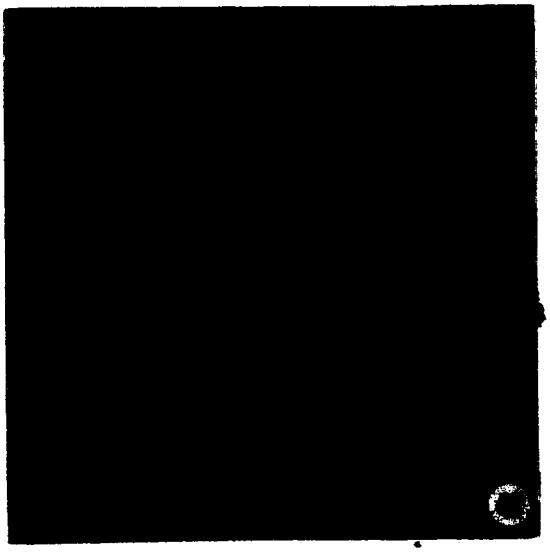
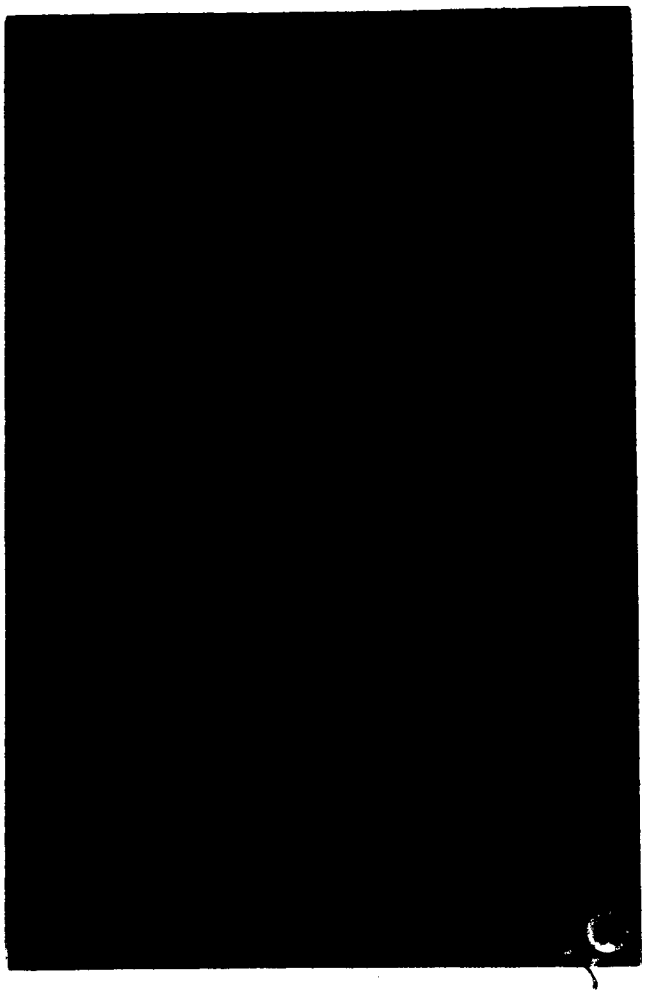


Figure 49 - Gall development on a 206-day-old lodgepole pine seedling, inoculated with E. harknessii when 20 days old (magnification 1.8X).



IV. Discussion

The successful penetrations by Endocronartium harknessii suggest that tissues on all above ground parts of young lodgepole pine seedlings are susceptible to infection at some time during their first year of growth. However, as the seedlings develop, tissues mature and change, preventing fungal entry and infection. This is seen in the maturing hypocotyl (Figures 11-14). Hypocotyl tissues are susceptible to infection when the seedlings are less than 20 days old, that is until the formation of the periderm and subsequent rhytidome development. Once the periderm is well developed, fungal penetration does not occur.

Similarly, young epicotyl tissue is susceptible to infection, but becomes resistant to penetration as it matures. Maturation begins at the base of the epicotyl and continues up the stem as elongation takes place. However, new tissue is being produced near the growing tip, so there is always some susceptible tissue on the seedling until elongation is complete and the bud is set. As a result, galls are formed on the lower portion of seedlings infected at a young age, whereas seedlings infected when elongation is further advanced produce galls higher on their stems. This is a similar situation to that observed on the elongating shoots of older trees.

The development of E. harknessii mycelium in the host seems to be affected by the location of the infection on the stem. Fungal tissues were observed to move primarily by

growing with dividing host cells in elongating stems. By this method of movement, infections occurring on rapidly elongating tissue would be expected to produce galls with a longer vertical axis than those infections occurring where the growth rate was less. This could help to explain the variation observed in the shapes of galls, from fusiform to spherical.

Needle and hypocotyl infections by E. harknessii on lodgepole pine seedlings have not been described previously. The development of the fungus in such infections was limited to discrete lesions. It is doubtful that needle infections would ever grow into the stem unless they occurred very close to the base of the needle. Consequently, it is unlikely that needle infections result in gall formation.

A wide range of reactions to infection by E. harknessii was observed on the lodgepole pine seedlings. This variation in response could be due to a number of factors. First, the density of spores applied to each plant during the inoculation procedure was uncontrolled making the number of infections per plant variable. The severity of the host response could also be affected by spore density. Studies on pine seedlings infected with fusiform rust showed that high inoculum density and high host resistance increased the rate and magnitude of pigmentation following infection (Lundquist and Luttrell 1982). In addition, the distribution of infections differed among plants. Some may have had many infections in a small area which could have resulted in a

more severe host reaction.

Another major cause of variation in host response could be due to differences among spore lots used for inoculation. Different spore sources were used and tests revealed germination rates that ranged from approximately 30% to 100%. Some of the spores had been frozen for several months prior to use in inoculation, whereas some were used the same day that they had been extracted. Natural variation in fungal virulence could be another contributing factor.

If the differences in host response due to such factors could be reduced, differences in host resistance to the fungus might be more easily identified. Field observations of disease-free lodgepole pine growing in stands heavily infected with western gall rust suggest that such resistance mechanisms are present in the host population. Such mechanisms could be operating in the seedlings at this stage and could be used in the development of disease resistant stock.

Some of the host responses observed in the diseased seedlings may be linked to resistance mechanisms. The accumulation of tannins in host cells, for example, has been thought to retard the growth of rust hyphae in pine tissue (Hutchinson 1935). In this study, hyphae and haustoria were observed in tannin-filled cells (Figure 41), but whether or not they were functional remains in question. Walkinshaw (1978) reported that tannin did not limit the development of fusiform rust hyphae in pine seedlings. He felt that the

deposition of tannin in slash pine (Pinus elliottii var. elliottii Engelm.) was a normal response to infection, but was not responsible for resistance to the fungus.

The observed development of periderm tissue around some infected areas may be linked to a resistance mechanism. Further study of this response is indicated, particularily as it relates to the normal process of stem maturation.

V. Conclusions

Western gall rust is an important disease of forest trees in North America. As the needs of forest regeneration programs increase, more demand is being placed on nurseries to produce healthy planting stock.

This study was a preliminary examination of the early stages of the infection of lodgepole pine by E. harknessii . The results have shown that western gall rust poses a significant threat to pine seedlings in their first year of growth. Seedlings infected at this time generally develop main stem galls which are invariably lethal. The recognition and control of the disease in forest nurseries is therefore imperative in order to prevent loss and to control the spread of infected seedlings.

Potential control strategies are influenced by the results of this study. The observation that young epicotyl tissue is the primary infection court for E. harknessii directs our attention to that tissue, and to its physical and phenological characteristics, for the development and timing of chemical or biological controls. The tissue pigmentation symptom was observed to be a reliable indicator of infection and a useful method of identifying diseased seedlings. Such an easily recognizable symptom will be useful in culling infected seedlings during greenhouse production.

The development of western gall rust resistant seedling stock may be greatly facilitated through the use of juvenile

seedlings. These respond quickly to infection, reducing the time required to identify resistant genotypes. Further study is required to more clearly identify resistance mechanisms and to determine the relationships between these mechanisms and visible host responses. For example, refinements in the inoculation technique may allow the use of the pigmentation response as a selection character.

The next phase of research in this area must quantify the observations made in this study in order to contribute to the assessment of the host-parasite relationship in seedlings of this age.

Literature Cited

Anderson, G.W. 1965. The distribution of eastern and western gall rusts in the Lake states. Plant Dis. Rep. 49:527-529.

Anderson, G.W.; French, D.W. 1965. Differentiation of Cronartium quercuum and Cronartium coleosporioides on the basis of aeciospore germ tubes. Phytopathology 55:171-173.

Arthur, J.C. 1922. New species of Uredinae. XIX Bull. Torrey Bot. Club 49:189-196(191).

Ayer, W.A.; Lee, S.P.; Tsuneda, A.; Hiratsuka, Y. 1980. the isolation, identification and bioassay of the antifungal metabolites produced by Monocillium nordinii. Can. J. Microbiol. 26:766-773.

Baker, F.E. 1950. Principles of silviculture. McGraw-Hill. London 414 p.

Bingham, R.T. 1983. Blister rust resistant western white pine for the Inland Empire: the story of the first 25 years of the research and development program. Gen. Tech. Rep. INT-146. Ogden, UT. U.S.D.A. For. Serv. Intermountain For. and Range Exp. Sta. 46 p.

Bissing, D.R. 1974. Haupt's adhesive mixed with formalin for affixing paraffin sections to slides. Stain Tech. 49:116-117.

Borger, G.A.; Kozlowski, T.T. 1972. Early periderm ontogeny of Fraxinus pennsylvanica, Ailanthus altissima, Robinia pseudoacacia, and Pinus resinosa seedlings. Can. J. For. Res. 2:135-143.

Boyce, J.S. 1957. The fungus causing western gall rust and Woodgate rust of pines. For. Sci. 3:225-234.

Byler, J.W. 1970. Non-rust fungi associated with galls caused by Peridermium harknessii. Ph.D. thesis, Univ. Calif, Berkeley, Calif. 81 p.

Byler, J.W.; Cobb, F.W., Jr. 1969. Fungi associated with galls caused by Peridermium harknessii. Phytopathology 59:1020. (Abstract).

Byler, J.W.; Cobb, F.W., Jr.; Parmeter, J.R., Jr. 1972. Occurrence and significance of fungi inhabiting galls caused by Peridermium harknessii. Can. J. Bot. 50:1275-1282.

Carlson, L. 1979. Guidlines for rearing containerized conifer seedlings in the Prairie Provinces. Environ. Can., Can. For. Serv., North. For. Res. Cent. Edmonton, Alberta. Inf. Rep. NOR-X-214.

Forbes, R.S.; Underwood, G.R.; van Sickle, G.A. 1972. Env. Can., Can. For. Serv. Annual Report of the Forest Insect and Disease Survey. 1971. pp.19-33.

Gill, L.S. 1925. Peridermium harknessii J.P. Moore in western yellow pine tops. Phytopathology 15:615.

Gill, L.S. 1932. Notes on the pycnial stage of Peridermium cerebroides. Mycologia 24:403-409.

Gross, H.L. 1983. Negligible cull and growth loss of jack pine associated with globose gall rust. For. Chron. 61:308-311.

Gurr, E. 1965. The rational use of dyes in biology and general staining methods. Leonard Hill. London. 422 p.

Hare, R.C. 1972. Physiology and biochemistry of resistance to pine rusts. p 465-478. In: Biology of rust resistance in forest trees. U.S.D.A., For. Serv., Misc. Publ. No. 1221 681 p.

Harkness, H.W. 1884. New species of Californian fungi. Calif. Acad. Sci. Bull. 1:29-47.

Hedgcock, G.G.; Hunt, N.R. 1920. Notes on Peridermium harknessii. Phytopathology 10:395-397.

Hildreth, S.C. 1969. Pine susceptibility to western gall rust. M.Sc. thesis, Colo. State Univ. 78 p.

Hiratsuka, Y. 1969. Endocronartium, a new genus for autoecious pine stem rusts. Can. J. Bot. 47:1493-1495.

Hiratsuka, Y. 1973. The nuclear cycle and the terminology of

spore states in Uredinales. Mycologia 65:432-443.

Hiratsuka, Y.; Allen, E. 1980. Pine stem rust survey at the Pine Ridge Tree Nursery, Smoky Lake, Alberta. Can. For. Serv., North. For. Res. Cent. Unpublished report for the Alberta Forest Service.

Hiratsuka, Y.; Maruyama, P.J. 1968. Identification of Peridermium harknessii in eastern Canada on the basis of nuclear condition of aeciospore germ tubes. Plant Dis. Rep. 52:850-851.

Hiratsuka, Y.; Maruyama, P.J. 1983. Resistant reactions of two Asian pines to western gall rust, Endocronartium harknessii. Phytopathology 73:835. (Abstr.)

Hiratsuka, Y.; Morf, W.; Powell, J.M. 1966. Cytology of the aeciospores and aeciospore germ tubes of Peridermium harknessii and P. stalactiforme of the Cronartium coleosporioides complex. Can. J. Bot. 44:1639-1643.

Hiratsuka, Y.; Powell, J.M. 1976. Pine stem rusts of Canada. Can. For. Serv., North. For. Res. Cent., For. Tech. Rep. 4. 103 p.

Hiratsuka, Y.; Tsuneda,A.; Sigler, L. 1979. Occurrence of Scytalidium uredinicola on Endocronartium harknessii in Alberta, Canada. Plant Dis. Rep. 63:512-513.

Huber, R.F. 1980. An attempt to control western gall rust using fungicides. Can. For. Serv., North. For. Res. Cent., File Rep. NOR-10-039. 4 p.

Hutchinson, W.G. 1935. Resistance of Pinus sylvestris to a gall forming Peridermium. Phytopathology 25:819-843.

Jewell, F.F. 1958. Stain technique for rapid diagnosis of rust in southern pines. For. Sci. 4:42-46.

Jewell, F.F.; Speirs, D.C. 1976. Histopathology of one and two-year-old resisted infections by Cronartium fusiforme in slash pine. Phytopathology 66:741-748.

Kistler, B.R.; Merrill, W. 1978. Seasonal development and control of pine-pine gall rust (Endocronartium harknessii). Am. Christmas Tree J. 22:21-23.

Krebill, R.G. 1970. Autoecious gall rusts of pines in southern Michigan and New York. Plant Dis. Rep. 54:853-855.

Lundquist, J.E.; Luttrell, E.S. 1982. Early symptomology of fusiform rust on pine seedlings. Phytopathology 72:54-57.

Martinsson, O. 1980. Stem rusts in lodgepole pine provenance trials. Silvae Genet. 29:23-26.

McKenzie, M.A. 1942. Experimental autoecism and other biological studies of a gall-forming Peridermium on northern hard pines. Phytopathology 32:785-798.

Meinecke, E.P. 1916. Peridermium harknessii and Cronartium quercuum. Phytopathology 6:225-240.

Meinecke, E.P. 1920. Facultative heteroecism in Peridermium cerebrum and Peridermium harknessii. Phytopathology 10:279-297.

Meinecke, E.P. 1929. Experiments with repeating pine rusts. Phytopathology 19:327-342.

Merrill, W.; Kistler, B.R. 1976. Phenology and control of Endocronartium harknessii in Pennsylvania. Phytopathology 66:1246-1248.

Meyer, T.R.; Walla, J.A.; Stack, R.W. 1982. Wave year infection of shelterbelt pines in North Dakota by Endocronartium harknessii. Phytopathology 72:979.

Nelson, D.L. 1970. Ecology and pathology of pine gall rust in California. Ph.D. thesis, Univ. Calif., Berkeley, Calif. 160 p.

Parmeter, J.R., Jr.; Newhook, F.J. 1967. New Zealand Pinus radiata is susceptible to western gall rust disease. N.Z. J. For. 12:200-201.

Peterson, G.W. 1973. Dispersal of aeciospores of Peridermium harknessii in central Nebraska. Phytopathology 63:170-172.

Peterson, R.S. 1960. Development of western gall rust in lodgepole pine. Phytopathology 50:876-881.

Peterson, R.S. 1967. The Peridermium species on pine stems. Bull. Torrey Bot. Club 94:511-542.

Peterson, R.S.; Shurtleff, R.G. Jr. 1965. Mycelium of limb rust fungi. Am. J. Bot. 52:519-525.

Peterson, R.S.; Jewell, F.F. 1968. Status of American stem rusts of pine. Annu. Rev. Phytopathol. 6:23-40.

Powell, J.M. 1971. Occurrence of Tuberculina maxima on pine stem rusts in western Canada. Can. Plant Dis. Surv. 51:83-85.

Powell, J.M. 1982. Rodent and lagomorph damage to pine stem rusts, with special mention of studies in Alberta. Can. Field Nat. 96:287-294.

Powell, J.M.; Hiratsuka, Y. 1973. Serious damage caused by stalactiform blister rust and western gall rust to a lodgepole pine plantation in central Alberta. Can. Plant Dis. Surv. 53:67-71.

Powell, J.M.; Wong, H.R.; Melvin, J.C.E. 1972. Arthropods

collected from stem rusts cankers of hard pines in western Canada. Can. For. Serv., North. For. Res. Cent., Inf. Rep. NOR-X-42. 19 p.

Rhoads, A.S.; Hedgcock, G.G.; Bethel, E.; Hartley, C. 1918. Host relationship of the North American rusts, other than gymnosporangiums, which attack conifers. Phytopathology 8:309-352.

Sass, J.E. 1958. Botanical microtechnique. Iowa State University Press. Ames, Iowa. 214 p.

Scott, C.W. 1960. Pinus radiata. F.A.O. Forestry and Forestry Products Study No. 14. Rome. 14 p.

Smith, F.H. 1958. Anatomical development of the hypocotyl of Douglas-fir. For. Sci. 4:61-70

True, R. 1938. Gall development on Pinus sylvestris attacked by the Woodgate Peridermium and morphology of the parasite. Phytopathology 28:24-50.

Tsuneda, A.; Hiratsuka, Y. 1979. Mode of parasitism of a mycoparasite, Cladosporium gallicola, on western gall rust, Endocronartium harknessii. Can. J. Plant Pathol. 1:31-36.

Tsuneda, A.; Hiratsuka, Y. 1980. Parasitization of pine stem rust fungi by Monocillium nordinii. Phytopathology 70:1101-1103.

Tsuneda, A.; Hiratsuka, Y.; Maruyama, P.J. 1980. Hyperparasitism of Scytalidium uredinicola on western gall rust Endocronartium harknessii. Can. J. Bot. 58:1154-1159.

Tsuneda, A.; Hiratsuka, Y. 1981. Biological control of pine stem rusts by mycoparasites. Proc. Jpn. Acad. 57 (Ser. B):337-341.

Tsuneda, A.; Hiratsuka, Y. 1981. Scopinella gallicola a new species from rust galls of Endocronartium harknessii on Pinus contorta. Can. J. Bot. 59:1192-1195.

Wagener, W.W. 1958. The question of heteroecism in some western tree rusts. Pages 41-46 in Proc. 6th West. Int. For. Dis. Work Conf., Vancouver, B.C.

Wagener, W.W. 1964. "Facultative heteroecism". Was it demonstrated in Peridermium harknessii in 1919-1920? Mycologia 56:782-785.

Walkinshaw, C.H. 1978. Cell necrosis and fungal content in fusiform rust infected loblolly, long-leaf, and slash pine seedlings. Phytopathology 68:1705-1710

Waterman, A.M. 1955. A stain technique for diagnosing blister rust in cankers on white pine. For. Sci. 1:219-221.

Weir, J.R.; Hubert, E.E. 1917. Recent cultures of forest tree rusts. Phytopathology 7:106-109.

York, H.H. 1926. A Peridermium new to the northeastern United States. Science N. S. 64:500-501.

York, H.H. 1929. The Woodgate rust. J. Econ. Entomol. 22:482-485.

Zalasky, H. 1976. Xylem in galls of lodgepole pine caused by Endocronartium harknessii . Can. J. Bot. 54:1586-1590.

Zalasky, H.; Riley, C.G. 1963. Infection tests with two caulicolous rusts of jack pine in Saskatchewan. Can. J. Bot.41:459-465.

Ziller, W.G. 1970. Studies of western tree rusts. VII. Inoculation experiments with pine stem rusts (Cronartium and Endocronartium). Can J. Bot. 48:1313-1319.

Ziller, W.G. 1974. The tree rusts of western Canada. Can. For. Serv., Pac. For. Res. Cent., Publ. No. 1392. 272 p.