LEUCOSTOMA CANKER OF LARCH IN QUEBEC

by

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INTRODUCTION

The pathology of our native larch, Larix laricina (Du Roi) Koch, has never been carefully investigated in the province of Quebec. One of the explanations of this apparent lack of interest in diseases of this tree species is the very important infestation by the larch sawfly (Pristiphora Erichsonii Htg.), which, at the beginning of the present century, greatly reduced the best stands of larch in Northeast America.

Usually the Eastern larch, commonly called tamarack, provides a hard, heavy and non-porous wood of high technical value. Its resistance to decay outweighs any other conifer of Eastern and Central Canada (2). This straight-stemmed species, found in our natural forests, extends into the West and North, growing together with the black and white spruces. Among other uses, this tree is suitable for railway ties, post and telegraph poles.

Within the past decade, foresters have used an increasing number of exotic species in plantations, mainly because of their rapid growth under certain ecological conditions which are not yet completely defined. For example, European larch (Larix decidua Mill.) and Japanese larch (L. leptolepis Sieb. & Zucc.) can increase to twice the volume of our native larch within fifteen years in some parts of Quebec. Consequently an earlier exploitation of these exotic species is now possible and will avoid a new

infestation by the larch sawfly, the population of which increases with the maturity of the host.

Facultative parasites, such as Leucostoma kunzei (Fr.) Munk, have been neglected because of their common saprophytic occurence in the native forest. However, on exotic species they may become pathogenic, especially if the young trees are not well adapted to the local environmental conditions. Foresters in Eastern Canada, have not associated this fungus with a canker disease on conifers until quite recently, and then mainly on ornamental trees, shelterbelts and plantations.

If this canker disease confined itself to small branches it would not be of significant economic importance, but when it extends to the stem it may kill the smaller trees or lead to malformation of the trunks of larger trees. In such instances it has to be considered of importance in forestry. Since the symptoms actually appear in plantations of young trees, some virulent strains of the pathogen may contribute to a breakage or a rapid girdling of the host stem.

Leucostoma kunzei (Fr.) Munk is a wound parasite and sources of inoculum are widely distributed on dead branches. Injuries by insects, birds, frost etc... provide continuously available points of entry and consequently the disease is potentially serious in all stands of larch.

In observations on the rusticity of the species of trees planted in Quebec, it is necessary to learn about their susceptibility to endemic pathogens. In dealing with any particular disease, the first problem concerns the taxonomy and identification of the causal organism. This contribution includes a study of the variations in the characteristics of the fungus itself in order to discuss an acceptable description and to comment on a review of opinions from taxonomists concerned with the genus Leucostoma and the species included in it.

Under what conditions is the disease observed most frequently? How rapidly can the canker develop? Which isolates are more pathogenic on native and exotic larches? These are some of the questions which the present work will explore.

REVIEW OF LITERATURE

Although the larches are resistant to decay, these species have been reported as hosts to a few diseases. For example, mistletoes, needle casts, needle rusts and certain decay organisms are recorded as not presenting serious problems (5).

The only larch canker disease to have been studied, especially on European larch (L. decidua Mill.), is the one commonly called "European Larch Canker". The causal organism, known as Dasyscypha willkommii (Hart.) Rehm., was observed for the first time in North America in 1927, in the Eastern United States where it was considered to have been introduced from Great Britain (15). Under the name Trichoscyphella willkommii (Hart.) Nannf., its taxonomy and biology have been recently studied by Manners (30, 31). In 1962, Day (10) published his report on certain physiological aspects of the canker forming organism. According to Fowler (15) there were only a few diseased trees left in the United States in 1953. This corresponds to the period of major introductions of European larch into Quebec when the canker had not been reported in this province.

European Larch Canker may be stimulated by the presence of other fungi, such as Cytospora abietis Sacc. and Cytospora curreyi Sacc. in the extension of dead bark. This relation

has been observed at the University of Oxford where it was shown that living bark of European larch contains a microflora of fungi and bacteria and that these include the precited fungi (9).

Very often in the literature the name of the imperfect stage, Cytospora kunzei Sacc., is used to describe the disease because of the absence of the perfect stage or to avoid the difficulties associated with the classification of the perfect stage, which has sometimes been called Valsa and at other times referred to as Leucostoma. This multiplicity of names makes a review of literature very difficult, expecially when two or three different species may be more or less grouped under the two generic names. In the present review, practically any fungus in the genus Leucostoma, as it will be defined later in this study, is being mentioned, provided it can be related to cankers on conifers.

In 1930, Faull (14) in Nova Scotia, attributed the reddening of branches on Balsam fir (Abies balsamea Mill.) partially to insect and frost damage but "largely due to a girdling fungus Valsa Friezii (Duby) Fuck". In the year 1924, Wehmeyer (45) described Valsa kunzei Fr. on the host Thuja plicata Don. and in vitro. He concluded that the type of substratum has a definite influence on the formation of the fruiting structures. A canker on European larch attributed to species of Cytospora and Valsa in New York, Massachussetts and Pensylvania was also reported in 1930 (1).

Infections by Valsa Friezii (Duby) Fuck. on Douglas fir, Pseudotsuga taxifolia (Poir.) Britt., were observed after fire injury in Western British Colombia by Dearness and Hansbrough (11) in 1934.

Cytospora kunzei Sacc. was observed on various species of spruces in 1933 and 1936 by Gilgut (18,19) but he could not find the perfect stage. According to his observations, the fungus causing a dieback of branches initiates infections on the underside of the limbs. This form of the disease is commonnest on trees over fifteen years old in Massachusetts. Cankers were found on branches of 3/4 of an inch in diameter or more. From his inoculation tests, Gilgut concluded that C. kunzei is a virulent wound parasite.

In 1937, Waterman (43) included additional species of spruces as hosts for the fungus or fungi causing Cytospora canker. In the same year, Baxter (4) reported cankers in plantations of Himalayan pine (Pinus wallachiana A.B. Jackson) on which Valsa superficialis Nits. and its Cytospora stage were present in the State of Michigan.

In 1941, a canker, on various species of Cypress up to ten feet high that had been weakened by unfavourable environment, was reported in Southern and Central California by Zentmyer (51). He emphasized that the columnar Italian cypress is losing

favor as an ornamental tree because of this Cytospora canker. As a control measure, he suggested to avoid wounding of healthy trees and to cut the cankered branches six inches closer to the stem than the last cankered area and burn the infected part. The causal organism in that particular case was referred to as Cytospora cenisia Sacc..

Wright (48), in 1942, reported Cytospora abietis Sacc. as the cause of a canker which, in some instances, girdles the branches and causes a dieback on true firs, Abies balsamea (L.) Mill., in California and Nevada. He stated that the pathogen is a semi-parasite requiring weakened tissue and that the factors predisposing to infection include host decline, drought, fire and insects.

In 1948, Marsden (32) observed Valsa kunzei Fr. and its imperfect stage Cytospora kunzei Sacc. on Colorado blue spruce (Picea pungens Engelm. var. glauca Buissm.) and on Norway spruce (Picea abies (L.) Karst.) and provided a good description of both stages of the fungus. Although he could inoculate both stages on steam-sterilized twigs and get pycnidia after thirty days, he did not succeed in producing the perfect stage on seedling trees in the greenhouse. The pycnidia on the dead twigs were slightly larger when inoculated from the Cytospora stage as compared with pycnidia produced by the inoculum from the Valsa stage.

Cytospora spp. have also been related to a brown stain in sapwood of white pine (Pinus strobus L.) by Fritz (17) in 1952 and Zabel (50) in 1953. In this brown-stain form of the disease, the mycelium develops in ray parenchyma and may proliferate in the tracheids but penetrates the cell walls only through the pits. The effect of the fungus on both bending strength and toughness was found to be negligible (17).

In 1953, Strong (41) found various species of spruce as hosts for Cytospora kunzei Sacc.. In this particular reference, the author describes a canker killing the branches at the base of the tree and progressing upwards from one branch to the other. The hosts considered to be mostly affected were from ten to fifteen years old or ten to twenty feet high. He adds that the canker can be on any part of the stem except in small twigs. Among other observations, he noted the appearence of yellowish to orange, thread-like spore tendrils exuding from previously wetted bark.

In 1957, Wright (49) attributed to Cytospora kunzei Sacc. the ability of producing the pitch-girdle canker on Douglas fir in Central Colorado. He stated that diseased trees had poorly developped root systems and that any pronounced reduction in precipitation leads to a slow rate of growth which results in more fungal infection. According to him, the fungus grew better under thin bark than under thick bark.

In 1960, Hawksworth and Hinds (21) reported trunk and branch infections on Engelmann spruces (Picea engelmannii Parry) in Colorado. The resulting cankers were attributed to Cytospora kunzei Sacc. which was commonly seen on trees up to six inches in diameter. They could not identify the perfect stage. On bigger trees, branch cankers were observed and the authors suggest that the disease may be associated with hail damage from a storm in 1955.

In Southern Ontario, a branch and stem canker disease of white and Norway spruces was associated with the wound parasite C. kunzei Sacc var. picea Waterman in 1961. A study of tree growth revealed that stem-cankered trees had been predisposed to attack by drought as indicated by a sudden decrease in the rate of diameter growth one year or more in advance of the infection year. After that study, Jorgensen and Cafly (25) stated that the disease occured mainly on trees more than twenty years old, and that stem cankered trees had shallower root systems than healthy ones. The disease incidence in both species was higher in shelterbelts than in plantations.

Raymond and Reid, in 1961, attributed a dieback of balsam fir in Ontario to the presence of three fungi one of which was Valsa abietis Fr. and its Cytospora stage (35). Successful inoculations were completed only with trees which had

been transplanted prior to inoculations. They observed very few infections in non-transplanted trees, but excessively drained soils contributed to infection. Trees smaller than five feet high were killed in one season.

As can be seen, there exists a number of observations on this canker disease of conifers, but very few workers paid much attention to the morphology or to the taxonomy of the fungus in its perfect stage. To this review, a few other pertinent references will be added in the discussion on the taxonomy of the causal organism.

THE CAUSAL ORGANISM

a) Taxonomic discussion.

Wehmeyer (46) divided the stromatic Sphaeriales into two families, Allantosphaeriaceae and Diaporthaceae, which have asci "with short evanescent stalks". Within the Diaporthaceae, the group of Valseae is differenciated by its characteristic multiloculate pycnidium.

The traditional descriptions of species in the genus Valsa and its relative are far from being well defined because they are based almost entirely on measurements of spores and asci and "these measurements can vary widely according to the position of the fruiting structure on the host" (23). During the pioneer period of description, the host range was used as a differentiating factor. This resulted in the addition of species to the already too complicated group (23, 27). Such additions are confusing and probably improper because it has been demonstrated that no valid separation is possible on this particular basis (12, 26). Wehmeyer (46), in referring to the taxonomy of stromatic Sphaeriales stated that, "one need make only a superficial examination of the pages in Saccardo's 'Sylloge' to be impressed by the numerous inadequate and inaccurate descriptions of species and genera. The scattered conditions in the literature concerning specific descriptions should discourage the description of new species but the result is apparently the opposite".

Nitschke divided, in 1867 (Pyrenomycetes Germanici. Breslau, 320pp.) the genus Valsa, as described by Fries in 1849 (16), into five sub-genera one of which was Leucostoma. In 1882, Saccardo accepted only two sub-genera, Euvalsa Nit. and Leucostoma Nit. in his 'Sylloge Fungorum' (39). In 1887, Winter (47) also recognized the sub-genus Leucostoma Nit. as one of the nine sub-divisions that he distinguished. Von Höhnel (22) considered Euvalsa and Leucostoma as definitely separate genera in 1917 but he placed the latter with the "Diaportheen". Wehmeyer (46) acknowledged this separation suggested by von Höhnel but, in dealing with Leucostoma, he reverted to the older generic name Valsa, referring to it as Valsa (Leucostoma).

The fruiting structure of the species in the genus Leucostoma (Nit.) v. Höhn. is envelopped in a stroma, composed of two distinct portions called ectostroma and entostroma, under which there is a basal layer called conceptacle. This latter may partially surround the locules and extend to the periderm of the host, as illustrated in Figure 1. The entostroma is a complex of fungus hyphae and host cells or remnants of cells, gradually becoming an ectostroma which is distinctly formed with dense hyphae without any host remnant (23, 27, 38). The part of the ectostroma which pierces through the host bark is known as the disk. The disk is often white at the beginning but becomes darker with age.

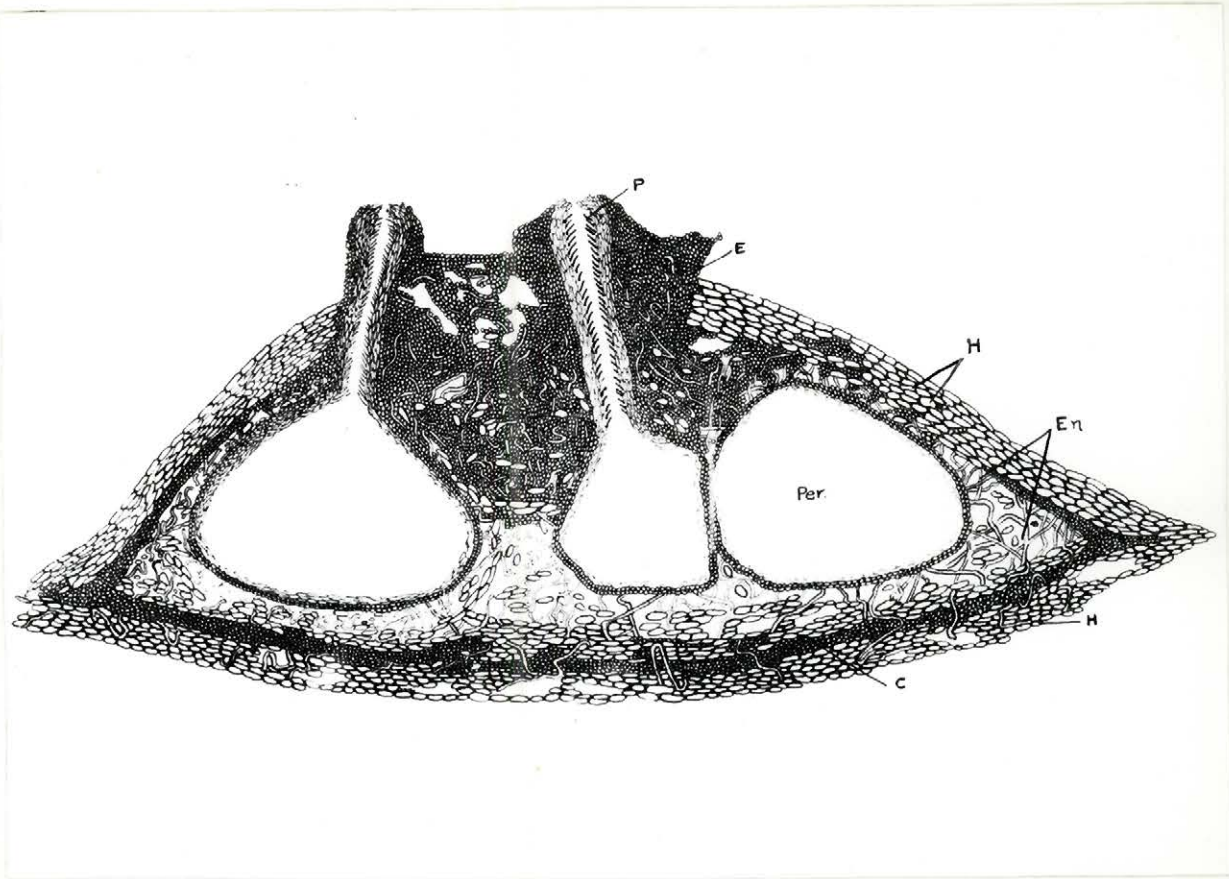

Fig. 1: Schematic drawing of a cross section through a fruiting structure of Leucostoma kunzei (Fr.) Munk on European larch. X 1,500.

P: periphyses,
H: host cells (bark),
Per.: perithecium,
E: ectostroma,
C: conceptacle,
En.: entostroma.

Fruiting structures in the genus Leucostoma are better developped in their entostroma of the sexual stage and their pycnidia of the imperfect stage, than are similar structures in the genus Valsa which lacks a conceptacle. This development "is a differential character and if a species possess such a stromatic development it belongs in Leucostoma" (46).

Defago (12) working on Prunus spp. made Valsa leucostoma (Pers.) Fr. a synonym of Leucostoma Persoonii (Nit.) Togashi, and Valsa cincta Fr. passed to Leucostoma cincta (Fr.) v. Höhnel. After the examination of type material, he also transferred other species of Valsa that he considered to be of the genus Leucostoma.

In a second paper, Defago (13) described a new species, Leucostoma Curreyi (Nit.), on Larix decidua, the imperfect stage of which he called Leucocytospora Curreyi (Sacc.) comb. nov.. This description, which is the only description of such a fungus on Larix up to 1944, is similar in some respects to the organism that has been under study by the present author.

In 1953, Leucostoma kunzei (Fr.) Munk was revised by Urban (42) who considered that there is no real difference in the material collected from firs and that from pines. Urban referred to Dansk. Bot. Arkiv. 15 (2): 80, 1953 as the original description, but when this reference was checked a validating Latin description was not found.

Kern (26) translated Urban's description to English, because he considered it to be descriptive of the specimens collected on conifers in Michigan, but larch is not mentioned as a host. He also claimed this organism to be the same as the one found on Abies alba in Europe.

In United States, Waterman (44) tried, in 1955, to group various species under Valsa kunzei Fr. based on the same principle used by Defago in the previous years. She worked on almost all species of conifers. Her review on the phylogeny of this species is complete up to 1849, but not later. It is very surprising that even if she knows the earlier separation of the genus Leucostoma, she prefers to keep the Valsa name "until a better classification will be available". However, the pictures she used, illustrate very well the characteristics of Leucostoma. On all conifers, she divided Valsa kunzei in three varieties; V. kunzei Fr. var. piceae var. nov., V. kunzei Fr. var. superficialis var. nov., and Valsa kunzei Fr. var. kunzei. In her contribution, this last variety, reported on European larch, is said to agree with V. kunzei Fr. in Saccardo's Syll. Fung. 1: 139, 1882 and also with Cytospora kunzei Sacc. in Syll. Fung. 3: 270, 1884.

Most of Waterman's elaborate studies deal with spruces firs and pines. But concerning Larix decidua, she divided Valsa kunzei Fr. into three types of stromal development which are superficialis, pini and curreyi.

Waterman (44) includes the type of stromal development curreyi in an attempt to include the species already described by Defago who called the organism Leucostoma curreyi but not Valsa kunzei.

Possibly based on a similar analysis, Kern, in his paper of 1957 (27), and in the one of 1961 (28), called the fungus Leucostoma kunzei (Fr.) Defago, without further explanation even though he had already called the organism L. kunzei (Fr.) Munk in 1955 (26).

A paper written by Kern in 1957 characterizes the genus Leucostoma (27). He furthermore explains that the delimitation of various Leucostoma is rather doubtful. As he said, after examination of type material from numerous collections, "this genus is variable in morphology, parasitic and physiological characters". For example, closely related strains of Leucostoma nivea may vary from 5-16 microns in the length of ascospores. If this size range is compared with the 12-14 micron range provided by Saccardo (39) the difference becomes obvious. Kern concluded that the concepts of species in Leucostoma do not represent distinct entities in the classical sense, but rather subjective groups of strains without sharp limits. The studies reported herewith tend to support Kern's conclusions.

However in 1957, Gillman, Tiffany and Lewis (20) did not accept the separation of the genus Leucostoma from

that of Valsa. These workers considered that "the distinctions in stromatic structure pointed out by Wehmeyer (46) are not valuable for the Iowa collections studied". Recent workers (23, 26, 29, 42) accepted this generic separation and made various attempts to characterize species within the many named species, strains or varieties. Kern (28) tried to differentiate, in 1961, various strains of Leucostoma and Valsa species by growing them on bark extracts of various plants. Growth on these extracts helped in some cases to establish taxonomic characteristics between Leucostoma Massariana (de Not.) v. Höhn. and L. Persoonii. Here again, he suggests that Leucostoma should be divided in groups of species because individual species are difficult to characterize morphologically or physiologically.

b) Distribution and symptom expressions in Quebec.

In plantations or in natural forests, the presence of Leucostoma kunzei (Fr.) Munk seems to be a sign of degradation or weakness of the host due to either a deficiency of nutrient or water or to any factor promoting the drying of bark. On larch trees, it is observed that the larch sawfly may sometimes provide the required wound, but in plantations, crowding and mechanical injuries are often the major agents of wounding. Reduction in precipitation or a poor adaptation of exotic species to the environment represent some of the factors concerned with degradation or weakness of the host.

In 1960, 1961 and 1962, surveys of the important plantations of larches in the province were undertaken by the author. These surveys provided the following information.

A plantation of the Canadian International Paper Company, located at Harrington Forest Farm (Argenteuil), was affected in 26% of the 2,200 stems of Japanese larches in 1960. The cankers were often located from one to three feet above the ground on trees of an average height of ten feet. In previous year, the trees had been sprayed in order to eliminate the sawfly. The soil is a well drained, medium to fine sand. Mechanical injuries following the clearing operations in 1957 are suspected of having provided additional points of entry for

the fungus. Two years later, this plantation had about 5% mortality, apparently due to the canker but the remaining trees recovered very well because of their rapid growth.

During the spring of 1961, about five thousand young European larches were transplanted at the Proulx Provincial Nursery (Laviolette). The lower branches were pruned, as may be seen in Figure 2, and within the same year, small cankers developed on 40% of the stems. These cankers were much smaller than those at Harrington and seemed to have resulted from fungal invasion of the bark around a cut branch or the scar from which a bunch of needles had been eaten along young stems (Fig.3). The soil of this area was sandy and very well drained. However, in the summer 1962, new bark covered the cankered areas on most of the infected trees and approximately 15% of them died. Some of the dead trees had been girdled by the canker, but others probably died because of other factors, such as inadaptability of the host and the feeding of hares on bark at the base of the young stems.

Native larches in Proulx nursery were more resistant to infection by Leucostoma kunzei. This was observed very readily in the remaining stock just beside the transplanted European larches. A plot of about 2,000 trees of both species of larches was examined and traces of infections were observed on native larch in comparison to the 20% of infection on the remaining stock of European larch.

In Parke (Kamousraska) a few European larch stems were infected in 1961 but not seriously. The conditions of soil and drainage were similar to those of the Proulx nursery, and the cankers also had the same aspect.

Another plantation of European larches, near Drummondville, was examined in 1961. This twenty year old plantation was healthy although a few border trees had cankers on the underside of the lower branches. The soil of this particular area was not as sandy as in the young plantations under survey and water in the soil was not a limiting factor.

Living trees in the plantations of the Morgan Arboretum, at MacDonald College (Jacques-Cartier), were free of the canker in 1961 and 1962, but many dead branches lying on the ground were covered with the fruiting structures of the canker inducing fungus.

Ornamental European larches were cankered at Berthierville (Berthier) and Valcartier Station (Quebec) in 1961 and 1962. The cankers were mostly located on the larger lateral branches.

The native larch, in forest stands, is usually unaffected by the canker, however one area of more than 1,500 square feet showed more than 50 trees badly cankered, in 1961 and 1962,

near Laurier Station (Lotbiniere). The trees are older than those in the previously mentioned plantations of exotic species. The stem canker in Figure 4 was found 15 feet above the ground.

A plantation in Acton Vale (Bagot) in which the European larches were about seven years old, did not show symptoms of the canker in 1962. However a few trees died very quickly in the preceeding two years and Leucostoma was present on these dead trees.

In general, the cankers on big lateral branches start about two or three feet from the extremities of the branches and sometimes the foliage dries out and the diseased branch can be seen from a certain distance. The following year, another canker develops one or two feet closer to the stem, and the process is repeated until the canker eventually contacts the stem. In most cases, the first or second canker on a branch is already closed by the time other cankers are invading closer to the stem.

On larch trees more than five inches in diameter breast height (D.B.H.), the canker may develop on the stem, as it has been observed on spruces (9), but such cankers were not found during the last three years of survey. Very often the organism passes from a lower branch to a higher one (Fig. 5), presumably with the help of a vector such as insect or bird.

Stem cankers form frequently on young hosts. These stem cankers can be fatal to small trees in a poor site, because if they are numerous on an individual stem, as observed in Figure 4, their complemental work can girdle the stem faster than the rate of growth in diameter of the host. On bigger trees, branch cankers are more common in crowded plantations and on border trees, because wounds are easily formed when branches of neighbouring trees are rubbing one against the other, and mechanical injuries due to the wind, frost and heavy loads of snow are more frequent on border trees.

Fig. 2: Young European larches in the plantation at Proulx. The trees, between 4 to 9 feet high, were pruned in the spring of 1961.

Fig. 3: Cankers on a stem of young European larch (Proulx).

Fig. 4: Stem canker on native larch. The development is slow the second year after infection.

Fig. 5: Defoliation of big trees by numerous branch cankers. (Laurier Station)

MATERIALS AND METHODS

a) Observational methods.

The material used for the comparison of morphological characteristics, especially variations in size and shape of the various parts of the fruiting structure (perfect or imperfect stage) of the fungus, included both exotic and native species of larch collected in six different parts of the province. The branches and stems on which the structures were measured varied in size from half an inch to three inches in diameter.

The collection numbers refer to the following hosts and localities:

1- Larix leptolepis, Harrington Forest Farm, collected in 1960.

2- Idem but collected in 1962.

3- Larix decidua, Proulx Provincial Nursery, collected in 1961.

4- L. decidua, Morgan Arboretum, collected in 1961.

5- L. laricina, St-Etienne de Lauzon, collected in 1962.

6 - Idem but collected in 1961.

7- L. laricina, Duchesnay, collected in 1961.

8- L. laricina, St-Etienne, collected in 1962, on different site.

9- L. decidua, Parke Reserve, collected in 1961.

Free-hand sections were placed directly into a drop of lactic acid on a microscope slide in order to measure the various parts in the fruiting structure. From each structure,

three to five representative transverse sections were retained so that the majority of perithecia within one structure could be examined. The gross measurements were made with a 50x enlargement or higher and the minute structures, such as ascospores and conidia, were measured under oil (970x) with the aid of an ocular micrometer. The dimensions obtainded from specimens dipped in lactic acid were in accordance with those obtained from permanently mounted sections in Canada balsam.

Since the asci, ascospores and conidia are hyaline, they were placed in a mixture of lactic acid and cotton blue for a few minutes. This mixture does not necessarily colour the walls of these structures but it aids in defining their outlined. Later it was learned that "China ink" smears provided a better dark field on which measurements could be made. For the last series of measurements, these smears were in use and provided a very satisfactory microscopic image.

In making permanent mounts, the Tertiary Buthyl Alcohol method of Johansen (24) gave better results for dehydration than did one making use of Ethyl Alcohol. The steps in dehydrating are as follows:

1- Fixation in Formalin-Aceto-Alcohol mixture (F.A.A.) for more than 24 hours under vacuum.

2- Pass to 50%, Tertiary Buthyl Alcohol (T.B.A.) 4 hours.

3- T.B.A. 70% overnight.

4- T.B.A. 85%, 2 hours.

5- T.B.A. 95%, 2 hours.

6- T.B.A. 100%, 2 hours.

7- Three changes in pure T.B.A., the last one overnight.

8- Add chips of paraffin, 48 hours at 35°C.

9- Transfer to the 56°C. oven for 6 to 8 hours.

10- Pour off half of the mixture and replace with melted paraffin, 2 to 4 hours. Repeat once.

11- Pour off all the mixture and replace with melted paraffin, 2 to 4 hours. Repeat once.

12- Pour off all the paraffin and replace with tissuemat, 2 to 4 hours. Repeat once.

13- Embed.

The Flemming's triple stain method of coloration and also the Hematoxylin method, as described in Johansen (24), were tested but both darkened the sections too much. Finally the sections were mounted without staining. The thickness of each section was about 15 microns. Other thicknesses were tried but with the bark tissue, this thickness was almost the best that could be obtained with the sliding microtome used.

b) Cultural media and methods.

1- Agar media.

The standard agar media tested included the following: Malt-extract agar, potato dextrose agar, Czapek's sucrose nitrate solution, Richard's solution, Elliott's agar and Leonian's solution. These were all prepared as described by Riker and Riker (37) except that the solutions were made semi-solid by adding 18g. of Bacto-agar per liter of distilled water. Other media used were; Yeast-extract agar, composed of 18g. Bacto-agar and 20g. yeast-extract per liter of distilled water; Mycological agar, containing 10g. Bacto-soytone, 10g. Bacto-dextrose, 18g. Bacto-agar per liter of distilled water, and finally a mixture of Yeast-extract (20g.) malt-extract (20g.) and bacto-agar (18g.) was prepared in a liter of distilled water.

After this preliminary test, it was decided to use Malt-extract agar as basic medium for most of the studies in vitro. Its composition was:

Difco Malt-Extract	20g.
Difco Bacto-agar	18g.
Distilled water	1,000ml.

The mixture was heated for about five minutes and then autoclaved for 15 to 20 minutes at 15 pounds per square inch of pressure.

A barkmeal medium was prepared from the bark of fresh twigs of different hosts, reduced to powder with a Wearing Blendor. The small pieces of bark were mixed with "dry ice" to avoid heating by the cutter and to reduce enzymatic activity to a minimum. This powder was stored in a cold room (-30°C.) so that it could be used a few days later with almost the same characteristics as fresh powder, when the medium was not used the same day. The percentage of water contained in this barkmeal was determined so that uniform agar plates containing five per cent by dry weight of barkmeal and 1.8 per cent of Bacto-agar could be prepared. The bark tested was from Larix decidua, Larix laricina, Pinus strobus, P. rigida, Salix laurina, Quercus borealis and Prunus pennsylvanicum.

In all the studies completed in Petri dishes, except where otherwise noted, 20cc. of medium was poured in each container in order to eliminate some of the variations in the amounts of nutrients. Uniform 4mm. diameter plugs of agar, obtained by the use of a sterile cork borer from the most active area of the mycelium, were used as inoculum. Colony diameters were measured along two perpendicular axes and the average of five replicates provided the final values, as given in the tables of results.

2- Liquid media.

For each study in liquid medium 25cc. of the nutrient solution was used in a 125cc. Erlenmeyer flask.

Because of problems associated with the solidification of agar under acidic conditions, dry weight of mycelium grown on liquid media provided the measure for optimum pH values. Here again five replicates were weighted each time. A solution of 2% malt-extract (Difco) was used. The solution was fractionally sterilized on each of three successive days at 100°C. without steam pressure for twenty minutes. The first experiment was conducted with Na_2HPO_4 (0.2m.) and citric acid (0.1m.) in the proportions suggested in the Table of McIlvaine apud Kordatski W. 1938, "Taschenbuch der praktiken pH Messung" Müller, Steineke, München, p. 58. The pH was adjusted before sterilizations, checked before inoculations and again after the filtration for weighting the dry weight of mycelium. Since the variations observed were in the order of 0.1 to 0.2 units, the buffer seemed adequate for the experiment.

However, a possible inhibition by the presence of citric acid was suspected. The optimum pH was tested also by using the phosphate buffers obtained with H_3PO_4 (M/3), KH_2PO_4 (M/3) and K_2HPO_4 (M/3). These buffering substances were added after autoclaving, and the results differed from those obtained with the first buffer solution as can be seen in the Tables 9 and 10.

For the utilization of carbon sources, a basal medium contained 2g. Asparagin, 1g. KH_2PO_4, and 0.5g. of $MgSO_4$ in a liter of water to which was added 1% by weight of each of the following sugars; Dextrose, levulose, galactose, mannose, xylose, ribose and arabinose. Three successive fractional sterilizations were performed without steam pressure for 20 minutes. The dry weight process was used for measuring mycelial growth in these solutions.

3- Other media.

In addition to agar and liquid media, the fungus grew very well on a sterile mixture of 3 parts wheat to 1 part oat grains. These particular proportions provided a good consistency after the sterilization. Otherwise the medium was either too hard or too fluid to be easily handled in the field (40).

This medium was used in order to keep the conidia viable for a longer time when inoculated in the field. A great number of pycnidia are formed within one month and remained viable for longer than three months when waiting for favourable conditions. The mixture was prepared and, after adding enough water to fill the spaces between the grains, it was autoclaved for 20 minutes at 15 pounds per square inch of pressure.

Before inoculations in the field, attempts were made to produce infections on twigs in culture tubes. Two types of sterilization were used. First, normal autoclaving of tubes containing half an inch of water in which twigs of 4 to 5 inches in length were placed. The other type of sterilization was with propylene oxide. The material was placed in a dessicator in the presence of 1cc. of propylene oxide per liter of capacity of the container and left there for 18 hours. The gas was then removed by vacuum suction and replaced with fresh filtered air. This latter process was repeated three times.

The inoculum in the experiment on twigs originated from mycelium, conidial suspension and ascospore suspension. Inoculum was placed under the bark after a wound made with a sterile scalpel in some cases and in other cases it was placed on the surface of the uninjured bark.

c) Field methods.

1- Inoculation of seedlings.

During the winter, inoculations were made in a greenhouse (21°C.) and a cold room (4.5°C.) in order to see if the temperature or the water supply had any influence on the conditions for infection of seedlings.

A four year old stock of European and native larch seedlings was used. A very small wound was made with a sterile scalpel either on the terminal shoot or at the base of the seedling. The mycelium placed in the opening was covered with aluminium foil to keep the moisture on the inside and to reflect the radiations of the sun, which could dry the wound and the inoculum too rapidly.

The seedlings placed in a cold room were in an atmosphere of 80% relative humidity and those placed in the greenhouse had an atmosphere of 30 to 65% relative humidity in their environment. In both conditions, some seedlings received very low water supply, (approximately 500-1,000cc. once a week) while others received a reasonable amount of water almost every day.

Seedlings in the greenhouse were in daylight conditions and those in a cold room had not more than two hours of artificial light each day.

2- Inoculation in the field.

A first series of inoculations in the field was conducted on both European and native larches in September, 1960. Two types of inoculum were used: (1) A suspension of ascospores, and (2) Mycelium of the fungus developed on malt-extract agar. These inocula were placed directly in the wound made superficially (not to the cambium) with a sterile scalpel or by breaking a small branch, as it occurs sometimes in nature. In some other cases the inoculum was placed at the junction of the branch and the stem without any injury. These preliminary trials were performed chiefly to learn if a wound is absolutely necessary for infection.

In the other experiment of 1960, inoculum consisted of a 4mm. plug of agar with the mycelium on it or one grain of the mixture of oat and wheat on which pycnidia had developed.

Three conditions were created artificially:

a) Inoculum on the bark without opening.
b) Inoculum in the bark after wounding to the cambium.
c) Inoculum between a broken branch and the stem after a mechanical and partial break of the branch.

The two types of control were; Wounding without inoculum, or sterile culture medium placed in the wound.

All these inoculations were covered with aluminium foil and closed with "Scotch Tuck Tape". The wounds were produced at three different levels and four orientations of the stem. Since it was at an early stage of the research work only one type of isolate was available in the fall of 1960.

In 1961 and 1962, additional isolates were tested periodically by the methods shown to be effective in 1960 i.e. providing wounding to the cambium.

Unfortunately Larix decidua was inoculated less frequently than the native larch because of the difficulty of finding available material for periodical inoculations. Most of the inoculations on the exotic larches had to be done at Proulx, Provincial nursery in June 1961. A few additional trees were also inoculated in the arboretum, near the Forest Biology Laboratory in Ste-Foy, Quebec.

In every inoculation series, uniform groups of host were selected in order to obtain comparable results.

EXPERIMENTAL RESULTS

a) Characteristics of the fungus on the host plants.

The canker organism is almost exclusively found in its imperfect stage around the diseased area. Sometimes it develops with other saprophytes, like Nectria sp., on the bark killed by the canker. In some instances, the only way to obtain it in culture is by plating a piece of the surrounding infected bark.

The perfect stage is easily distinguished from the imperfect stage by the use of a stereoscopic microscope (15x). When the fruiting structure is opened, the conceptacle is observed readily by the same magnification. The ascosporic stage seems to occur almost exclusively in dead bark, and the Cytospora stage is also commonly found among the fruiting bodies of the Leucostoma stage.

The stroma is defined by Ainsworth and Bisby (3) as a "mass or matrix of vegetative hyphae, with or without tissue of the host or substratum, in or on which spores are produced". Applied to Leucostoma, this description includes the structures inside the conceptacle. Usually, the stroma of both stages has a conical shape in transverse section as it may be seen in Fig. 6. Very often, the perithecial stroma is somewhat bigger than the pycnidial one but they are both circular to

fusoid and erumpent. They are mostly characterized by an individual development (Fig. 7) Although they will rarely develop in connected groups.

The epidermis, when present, and the endodermis cover the sides of the stroma which develops within the cortical tissues. Sometimes, the entostroma is very wide and enlarges the base of the conical shape (Fig. 9). In these particular cases, the number of perithecia or pycnidial locules is greater, This shape seems to be more frequent when the branches, larger than one inch in diameter, are in a moist environment (e.g. on the ground).

As it was stated by Wehmeyer (46), the separation between the endostroma and ectostroma is gradual and very vague in most cases. The endostroma stands on loose mycelium and host cells. The ectostroma is filled exclusively with hyphae without host cells.

On all the hosts, young pycnidia are first embedded in the bark and later the disc pierces through the bark. Very often, on L. laricina, a stroma with perithecia around and one pycnidium in the center is observed (Fig. 10). This structure is also observed on other larches, depending on the stage of development when the material is collected.

The white disc was considered characteristic by von Höhnel (22) but Petrak (34) and Wehmeyer (46) pointed out that it does not distinguish Leucostoma from the genus Valsa. In the present study, the disc observed from the outside is rather black, but in a section, longitudinal to the axis of the branch the disc is whitish gray and the ostioles look like black points perforating it.

Inside the fruiting structure, the perithecia are monostichous and surrounded by a dark wall with an internal layer of diamond-shaped cells which are hyaline. Perithecia contain hyaline asci in which the allantoid, and hyaline spores are often arranged in two columns (Fig. 8). There were eight ascospores per ascus. Asci are sessile or subsessile; their wall is thickened at the extremity and a very small opening is left for ascospore liberation. The drop of oil or fatty substances on each side of the aperture is not colored by iodine and this seems to be characteristic (23). Paraphyses are claimed by Defago (13) to be present in the structures of Leucostoma but they were never observed in the specimens examined.

The conceptacle is usually closer to the perithecia in the bark of exotic than of native larches. In the latter this black line is often separated from the perithecia by a layer of two or three cells of host cortex as illustrated in Fig. 8. In the imperfect stage, the conceptacle is often present before the complete formation of the pycnidium. The

conceptacle is sometimes visible through the bark of larches. This phenomenon occurs mostly on young branches of exotic larches because the bark is somewhat reddish and lighter in color than that of native larch.

Pycnidia rarely have more than one central opening through the disc (Fig. 11). The pycnidial locules form a sort of labyrinth in which hyaline conidiophores produce allantoid and hyaline conidia usually smaller than the ascospores.

Spore tendrils are formed on the opening of the pycnidia when the branch is alternately submitted to moist and dry conditions. These tendrils contain a tremendous amount of conidia; they are sometimes cream-buff to pale yellow-orange and sometimes Garnet brown, according to Ridgway's "Color Standards and Nomenclature" (36).

Tables 1 and 2 illustrate the size of various parts of both types of fruiting structures on native and exotic larches. Similarity exists between almost any part measured on all hosts. In general, the structures on native larches are somewhat larger than those on exotic larches but not enough to differentiate them according to their respective hosts. The number of measurements or the frequency is not the same for each structure because when the range of variation was small the measurements were less numerous.

Only one structure is different in size from one host to the other. The perithecial wall is twice as thick on native as it is on exotic larches. No more measurements are required to see the overlapping of the respective size of structures on the three species of hosts. These data come from the collections numbered 1, 3, 4, 5 and 9 which are described on page 25.

The size of the ascospores and asci covers a wide range of variation (Table 3 and 4) and is also usually larger on native larches than on exotic larches. From visual observation, it seemed possible to obtain a relation between the thickness of the bark and the size of the ascospores. Eleven series of measurements were completed on the same host and the Table 5 indicates that too many exceptions exist to say that the relation holds true. The largest spores are observed on the thinnest bark but smallest spores are not necessarily found in the thickest bark.

These dimensions of morphological features are more in accordance with Urban's description of Leucostoma kunzei (42) than with the Leucostoma curreyi described by Defago (13) who mentioned the presence of papillae around the disc. Such papillae were never observed in the organism under study. Defago gives 49-84 microns as the length of asci, 7-21 microns is given as the length of ascospores and 2-7 microns

as the length of conidia. Urban gives respectively for the same structures 18-26 microns, 5-9 microns and 5 microns. The organisms observed on Larix are 12-61 microns long in their asci (Fig. 12), 4-16 microns in their ascospores (Fig. 13) and 2-7 microns in the length of their conidia.

The complete description of Leucostoma kunzei (Fr.) Munk as translated by Kern (26) is the following:

"Stromata generally numerous, covering large areas of twigs or stems, rounded to elliptical, 1-2mm. in diameter, prominent, conical on the outside, often with a clearly visible margin, sometimes flattened or even depressed around the disc; substance pale yellow to light gray or brownish; conceptacle black, about 50-100u thick more or less adnate to the inner bark tissue. Disc rounded to elliptical, flat or convex, erumpent, white to pallid, later brown, 0.2-1 (1.5)mm. diameter; ostioles generally numerous (5-30), often crowded and covering the whole disc, black, small, globose flattened or sometimes elongate. Perithecia globose to irregular with slender necks, about 200-600u in diameter. Asci clavate, 15-25 x 4-6μ. Ascospores, hyaline, allantoid mostly 5-8 x 1-2μ.

Stromata of the conical form (Cytospora kunzei Sacc.) similar, generally smaller with occasionally a very thin conceptacle and with radiate chambers and a central pore. Conidia, allantoid, 4-6 x 0.5-1 micron".

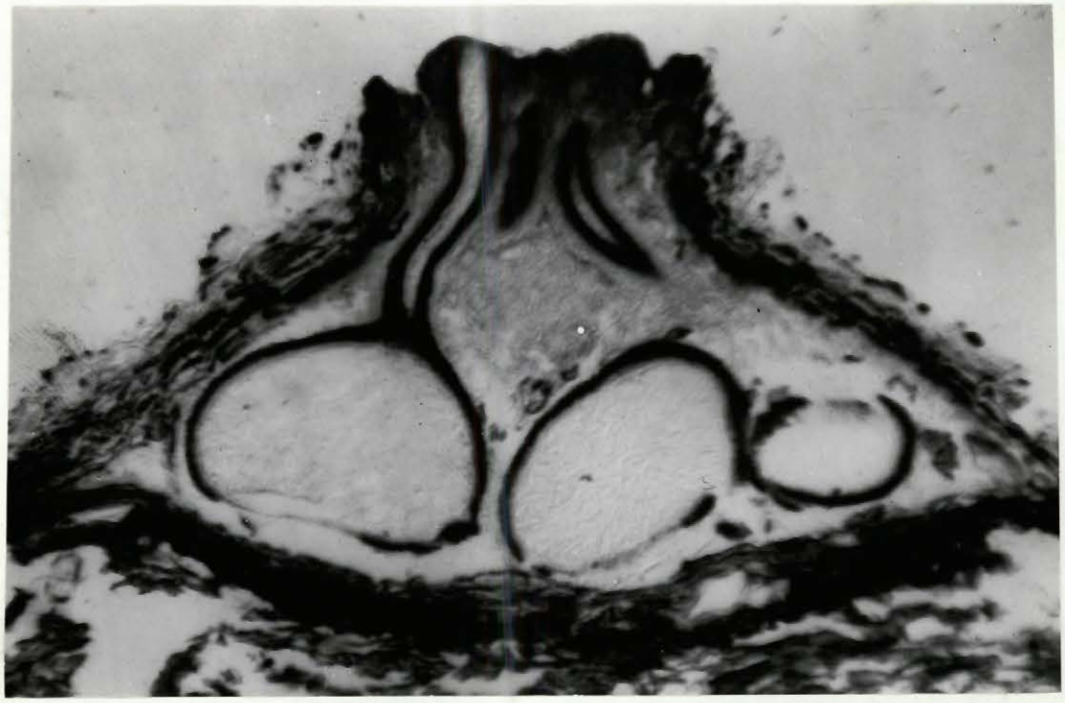

Fig. 6: Transverse section through a stroma of the perfect stage Leucostoma kunzei (Fr.) Munk on European larch. X 1,200.

Fig. 7: Fruiting structures of L. kunzei in the bark of native larch. X 5.

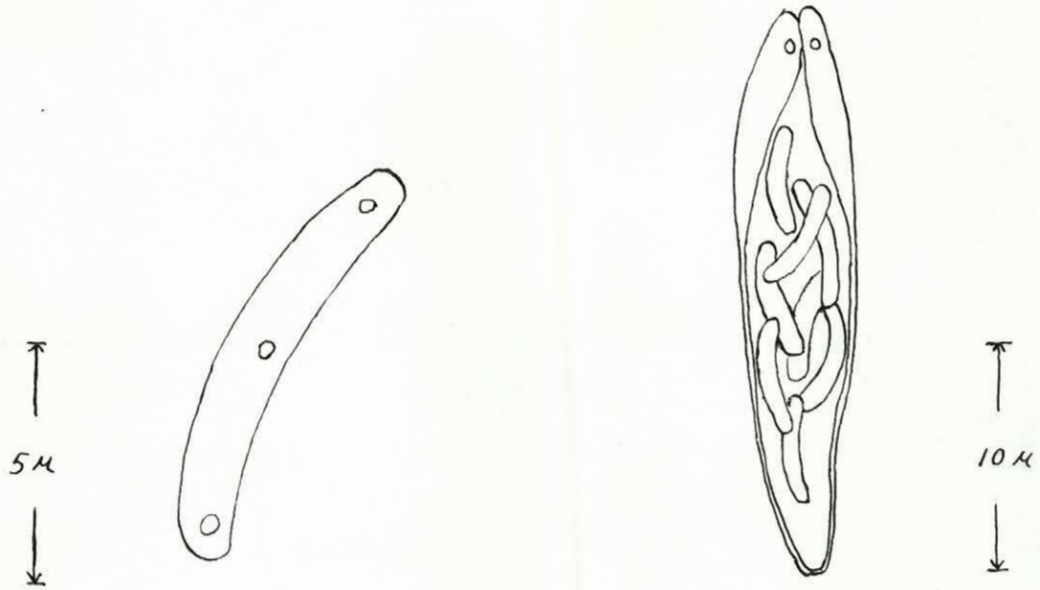

Fig.8: Camera lucida drawing of one ascospore and one ascus of Leucostoma kunzei.

Fig.9: Cross section through the pycnidium of Cytospora kunzei in the bark of native larch. X 1,200.

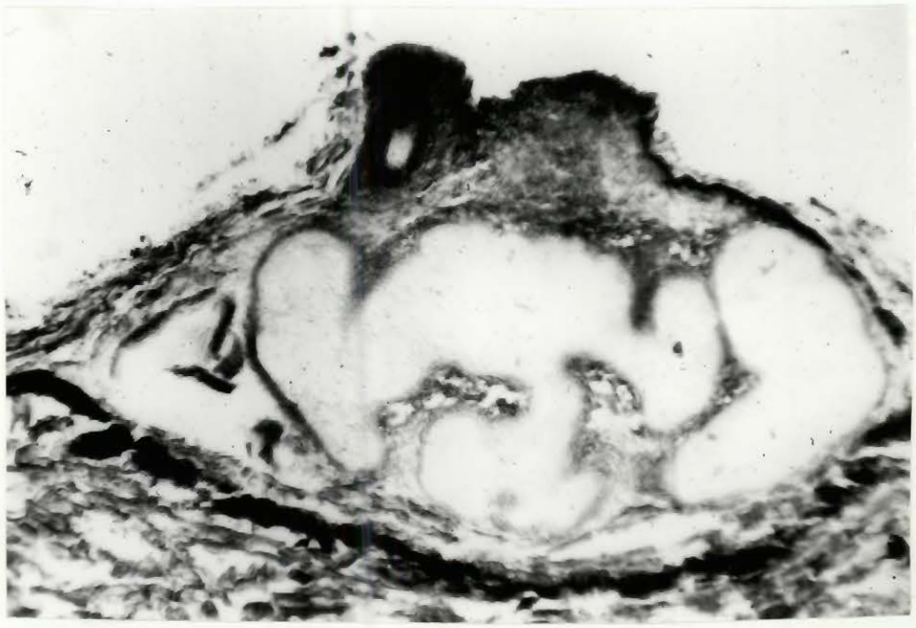

Fig. 10: Cross section through a fruiting structure of Cytospora kunzei Sacc. in the bark of native larch. X 1,200.

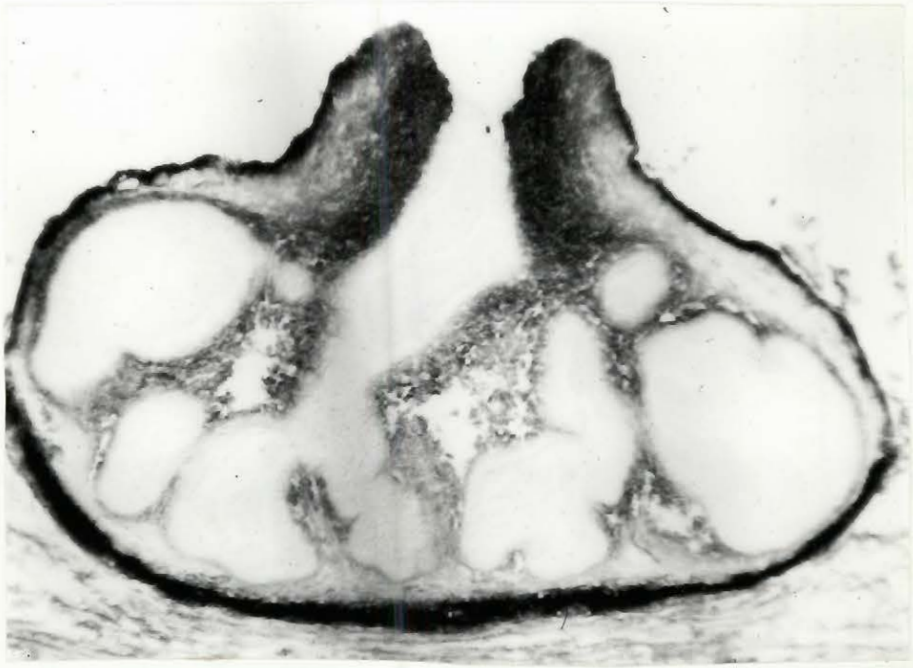

Fig. 11: Cross section through a pycnidium of C. kunzei Sacc. in the bark of European larch. X 1,200.

TABLE 1

DIMENSIONS, IN MICRONS, OF THE VARIOUS PARTS OF THE *LEUCOSTOMA* AND *CYTOSPORA* STAGES ON EXOTIC LARCHES.

Structures	Extremes (Ave.)	Measurements
LEUCOSTOMA KUNZEI		
Stroma		
Height	450-910 (650)	60
Diameter	800-1650 (1100)	60
Disc		
Diameter	200-520 (325)	60
Perithecia		
Diameter	170-400 (280)	75
Thickness of wall	11- 32 (*22*)	75
Ostioles		
Length	195-520 (250)	75
Conceptacle		
Thickness	40- 130 (70)	70
CYTOSPORA KUNZEI		
Pycnidia		
Height	350-600 (500)	45
Diameter	550-1000 (750)	55
Disc		
Diameter	200-400 (300)	55
Conidiophores layer		
Height	10-22 (16)	48
Conceptacle		
Thickness	30-100 (60)	45
Conidia		
Length	3-7 (5)	200

TABLE 2

DIMENSIONS, IN MICRONS, OF THE VARIOUS PARTS OF THE

LEUCOSTOMA AND CYTOSPORA STAGES ON NATIVE LARCHES.

Structures	Extremes (Ave.)	Measurements
LEUCOSTOMA KUNZEI		
Stroma		
Height	550-1070 (750)	60
Diameter	940-1670 (1300)	60
Disc		
Diameter	327-810 (422)	50
Perithecia		
Diameter	162-617 (422)	50
Thickness of wall	24-66 (53)	60
Ostioles		
Length	260-546 (351)	50
Conceptacle		
Thickness	13- 95 (46)	60
CYTOSPORA KUNZEI		
Pycnidia		
Height	525-676 (624)	45
Diameter	800-1102 (886)	40
Disc		
Diameter	208-390 (307)	55
Conidiophores layer		
Height	10-26 (16)	50
Conceptacle		
Thickness	39-91 (66)	45
Conidia		
Length	2-7 (5)	200

TABLE 3

LENGTH OF ASCOSPORES OF

LEUCOSTOMA KUNZEI IN THE BARK OF LARCH.

Hosts	Collection* number	No. spores measured	Length in μ
L. leptolepis	1	200	4.2-7.5
	2	200	5.1-7.6
L. decidua	3	200	5.5-8.0
	4	200	6.6-9.9
	9	250	11.4-16.2
L. laricina	5	225	7.4-12.0
	6	250	8.4-13.1
	7	200	9.5-13.3
	8	200	8.1-14.5

* The origin of each collection is described on page 25 and each collection includes measurements on more than five different branches.

TABLE 4

LENGTH OF ASCI OF

LEUCOSTOMA KUNZEI IN THE BARK OF LARCH.

Hosts	Collection* number	No. spores measured	Length in μ
L. leptolepis	1	175	15-20
	2	175	12-22
L. decidua	3	100	13-23
	4	100	15-23
L. laricina	5	100	25-47
	6	220	34-54
	7	200	45-61

* The origin of each collection is described on page 25 and each collection includes measurements on more than five different branches.

TABLE 5

RELATIONSHIPS BETWEEN THE LENGTH OF ASCOSPORES AND THE THICKNESS OF THE BARK ON NATIVE LARCH.

Branch*	Bark		Spores	
	Thickness(μ)	Measurements	Length(μ)	Measurements
1 A+	530	30	13.1	50
B	647	30	9.7	50
C	651	30	9.3	50
D	666	30	11.4	50
E	675	30	11.8	50
F	694	30	11.4	50
2 A	445	50	14.6	100
B	707	50	10.6	100
3 A	399	30	12.9	50
B	530	30	11.4	50
C	920	30	11.6	50

* Each branch came from a different host tree.

+ The letters represent the areas of measurement which were ten inches apart.

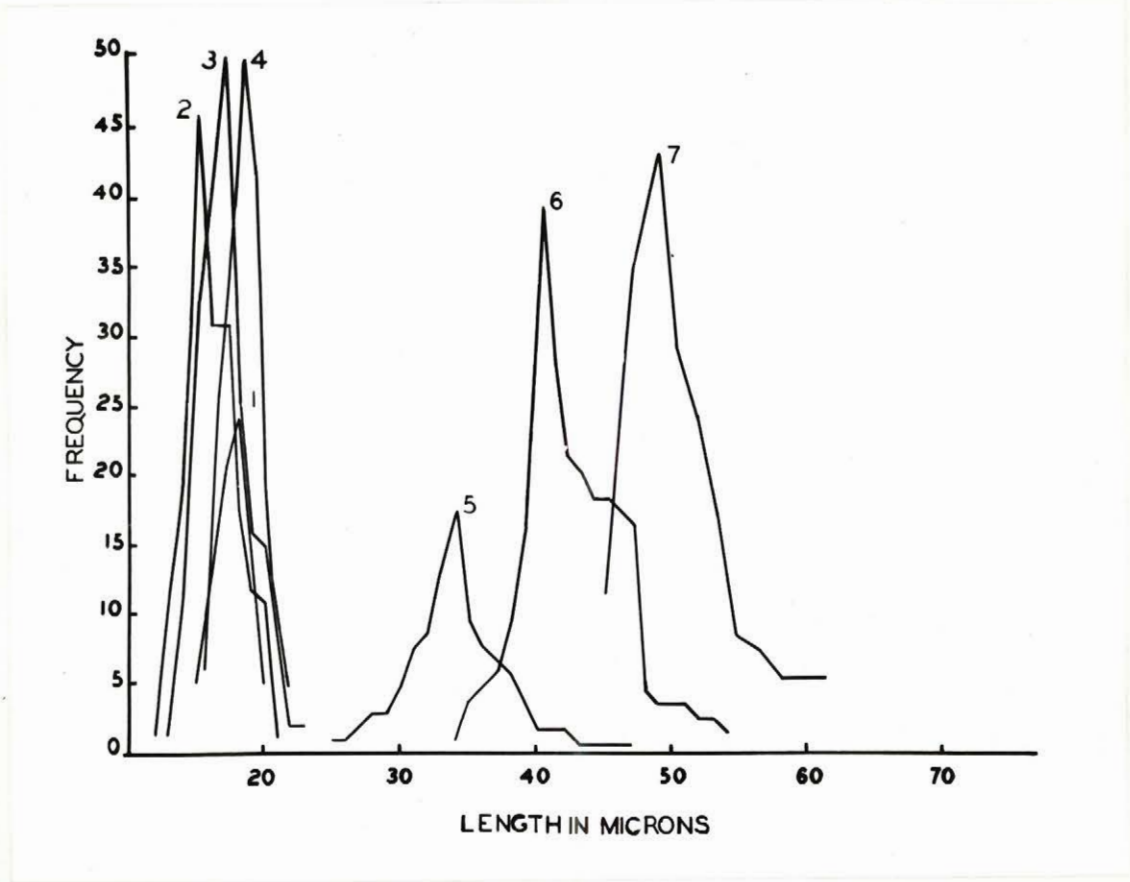

Fig. 12: Comparative length of asci of different collections of Leucostoma kunzei. The identification numbers are explained on page 25.

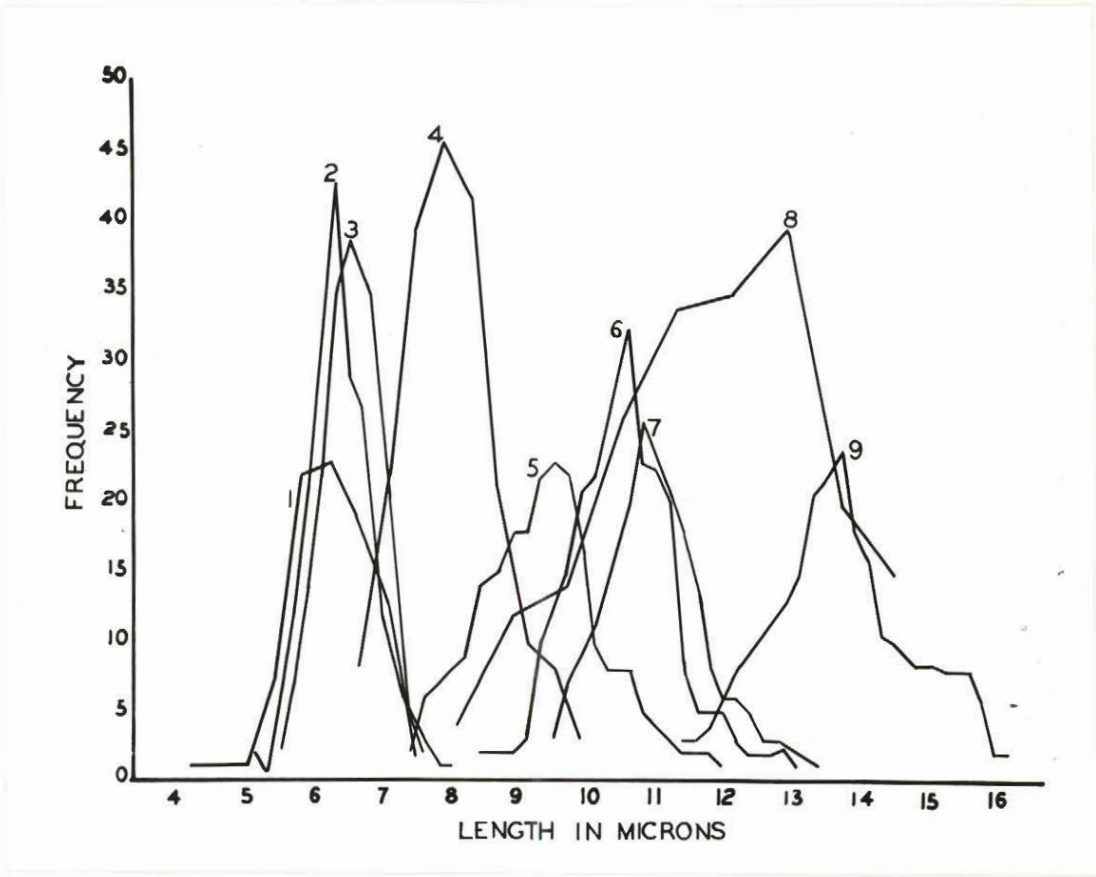

Fig. 13: Comparative length of ascospores of different collections of Leucostoma kunzei. The identification numbers are explained on page 25.

b) Characteristics of the fungus "in vitro".

After the wide variation observed in the morphology of the causal organism in nature, studies in culture were carried out in order to see if a sort of specific or other distinction could be found. Some workers tried this type of study for different species of Leucostoma and according to them (12, 13, 23), the monosporic isolates were variable in appearance, type of growth and other characters.

1- Growth on various culture media.

Tests on various culture media were performed in order to find out which medium would be appropriate for subsequent studies and also to obtain indications on the basic nutritional requirements of the causal organism.

From Table 6, it is obvious that the standard media composed exclusively of mineral salts are not satisfactory to insure a good growth of the mycelium. These results indicate that malt-extract contains a nutritional factor which favors the mycelial growth and that this factor may be inhibited by the presence of an equal amount of yeast-extract. Leonian's and Mycological agar are intermediary because they include peptone, maltose and/or malt-extract in their composition, in addition to mineral salts. Each diameter in Table 6 is an average of five replicates.

TABLE 6

GROWTH OF MONOSPORIC ISOLATES OF LEUCOSTOMA KUNZEI

AFTER SEVEN DAYS ON VARIOUS CULTURE MEDIA.

Media	Diameter(mm.) of the colony		
Isolate*:	16	81	95
Malt Extract Agar	90	90	90
Potato Dextrose Agar	90	65	90
Leonian's and Agar	79	90	81
Mycological Agar	24	28	48
Yeast and Malt Extract Agar	31	28	44
Yeast Extract Agar	0	0	0
Richard's and Agar	10	0	0
Czapek and Agar	0	0	0
Czapek Sucrose and Agar	0	0	0
Elliot's and Agar	0	0	0

*Isolates 16 and 95 are from exotic species of larches and isolate 81 originated from native larch.

2- Appearence of cultures.

The characteristics noted in Table 7 are based on one hundred and twenty monosporic isolations from Japanese, European and native larches in various localities. In this table, the cultures have been placed in three groups according to certain associated cultural characters. Colors refer to Ridgway's 'Color Standards and Nomenclature' (36). These groups could not be separated according to the host range or the size of the original ascospore.

When present, tendrils in the first group ranged in color from white to pale orange-yellow. In the second group they were white to pinkish buff. This characteristic could not be related to the original host.

TABLE 7

CHARACTERISTICS OF MONOSPORIC CULTURES

AFTER 2 MONTHS ON MALT-AGAR SLANTS.

Color of the mycelium	Color of the agar	Density of the mycelium	Pycnidia
1- Saccardo's Umber Olive		Medium	Small or absent
2- Dull-blackish-green	Deep green-blue-gray	Heavy	Mixture of large & small
3- White to olive lake	Unchanged to light buff	Light	Small

3- Diameter growth rate.

The growth rate on malt-extract agar could be one criterion of distinction between isolates. When five isolates from native larches were obtained from each of three different localities and cultured on malt-extract agar there were no appreciable differences in their growth characteristics. Similarly, there were no appreciable differences in the growth characteristics of five isolates from exotic larch obtained in each of three different localities. A comparison of the average growth rates of the fifteen cultures from each host is shown in Figure 14.

Isolates from native larch require seven days to cover the surface of the agar; isolates from exotic larches grow more slowly. The experiment was conducted under identical conditions for each isolate since they were tested all together in daylight and at room temperature (22°C).

These relative rates of growth were unchanged during the subsequent studies in vitro and one year later the experiment was repeated and the same comparative rates were observed.

Growth rate in liquid solution of malt-extract gave the same trends on a basis of dry weight after a period of twelve days. Since this method provided only one reading per flask, the data do not permit the drawing of a graph on a basis of successive daily readings.

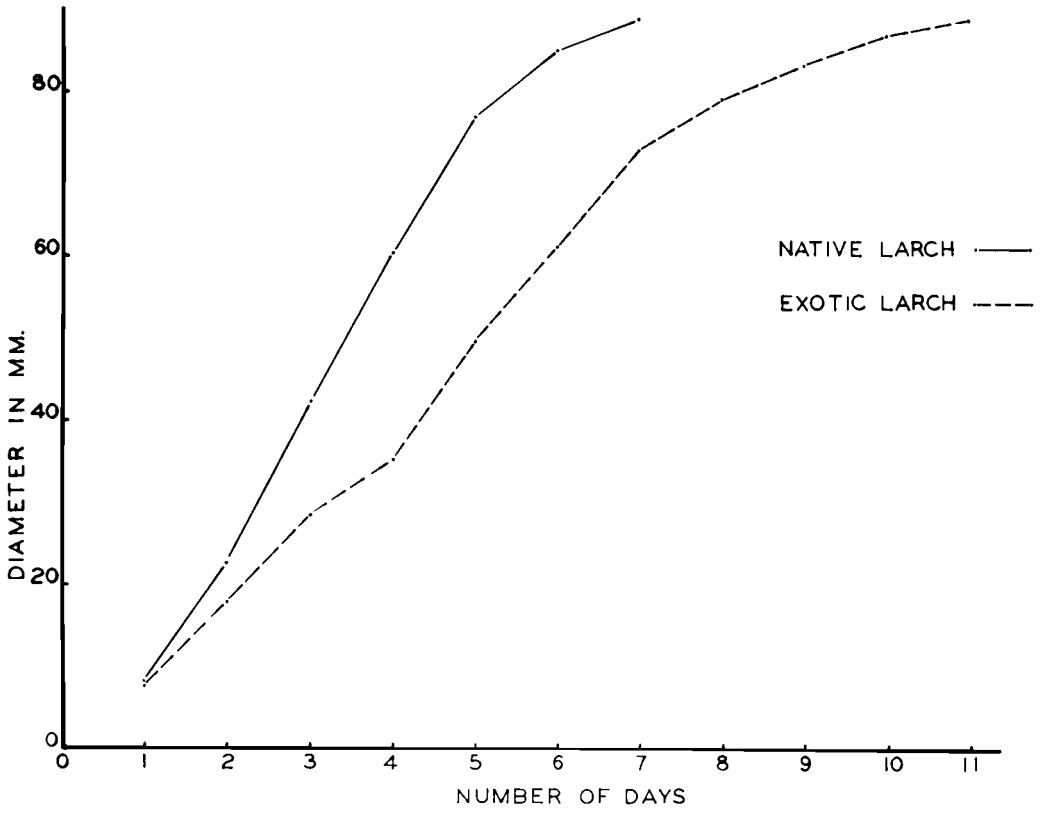

Fig. 14: Comparative diameter growth rates between isolates from native and exotic larches on malt extract agar.

4- Influence of temperature on mycelial growth.

The influence of temperature on mycelial growth was tested by culturing the fungi from both native and exotic larches at various temperatures. Colony diameters of isolate 16 from Japanese larch, 95 and 110 from European larch and 28, 58 and 81 from native larch are shown in Table 8.

TABLE 8

INFLUENCE OF TEMPERATURE ON MYCELIAL GROWTH

AFTER SIX DAYS OF GROWTH ON MALT EXTRACT AGAR.

Temperatures	Diameter*(mm.) of mycelium from					
	Exotic larch			Native larch		
Isolates:	16	95	110	28	58	81
0.0°C	4.0	4.0	4.0	4.0	4.0	4.0
4.4°C	4.0	4.0	9.0	4.0	4.0	4.8
10.0°C	11.8	13.4	43.2	44.8	29.6	52.8
15.6°C	44.4	50.0	71.8	76.0	55.4	85.0
21.1°C	62.8	65.6	84.0	90.0	78.6	90.0
26.7°C	68.4	72.2	87.2	90.0	79.6	90.0
32.0°C	25.0	20.0	13.8	88.4	10.0	85.5

*Each diameter of this Table is the average of five replicates.

The optimum temperature was found to be practically the same for all isolates, the range of which was 21°C. to 26°C. for isolates 28 and 81, and the others have an optimum around 26°C. Isolates from native larches seem to stand a higher temperature than those from exotic larches. These same isolates from native larch grow a little more under very low temperatures. For instance after six weeks under 0°C., some of the isolates from native larch were observed to grow slowly while the others did not.

5- Influence of pH on mycelial growth.

The data, in Tables 9 and 10, indicate that the pH of the medium cannot be used as a basis for the distinction of strains coming from different larch species or for two strains coming from the same host species. Each point on the curves in Figure 14 represents the average of fifteen readings since the isolates were from the same sources as those for the temperature test, and no distinction was drawn between the isolates from the same host. Values in Figure 15 are for the buffer solution 1.

An odor of alcohol was noticed at the end of this first experiment and the possible fermentation by the fungus seemed to be related to the amount of mycelium. To avoid this phenomenon, agitated cultures instead of stable cultures were used, but the buffer did not act normally under these conditions and no optimum values were obtained.

The pH of the bark was measured at the same time, and it was a little higher than the optimum obtained on malt, in each case it ranged between 4.0 and 4.5.

Because of the curious fact that the fungus does not grow at pH 5.0 in malt-extract solution while it grows well on agar of the same nutrient at a pH value as high as 6.0, the experiment was repeated with two strains, but using phosphate buffer. One of the strains gave again the same type of curve while the other had an optimum pH of 5.0.

From these repetitions, it was evident that the fungus can undergo fermentation. This could explain the differences between the growth on agar and in liquid solution of the same nutrient. The strain which had an optimum pH of 5.0 in this second experiment developed very little odor of alcohol, and the mycelium grew on the surface of the medium; the other strain which provided results similar to those in earlier trials grew at the bottom of the flask and produced an odor of alcohol.

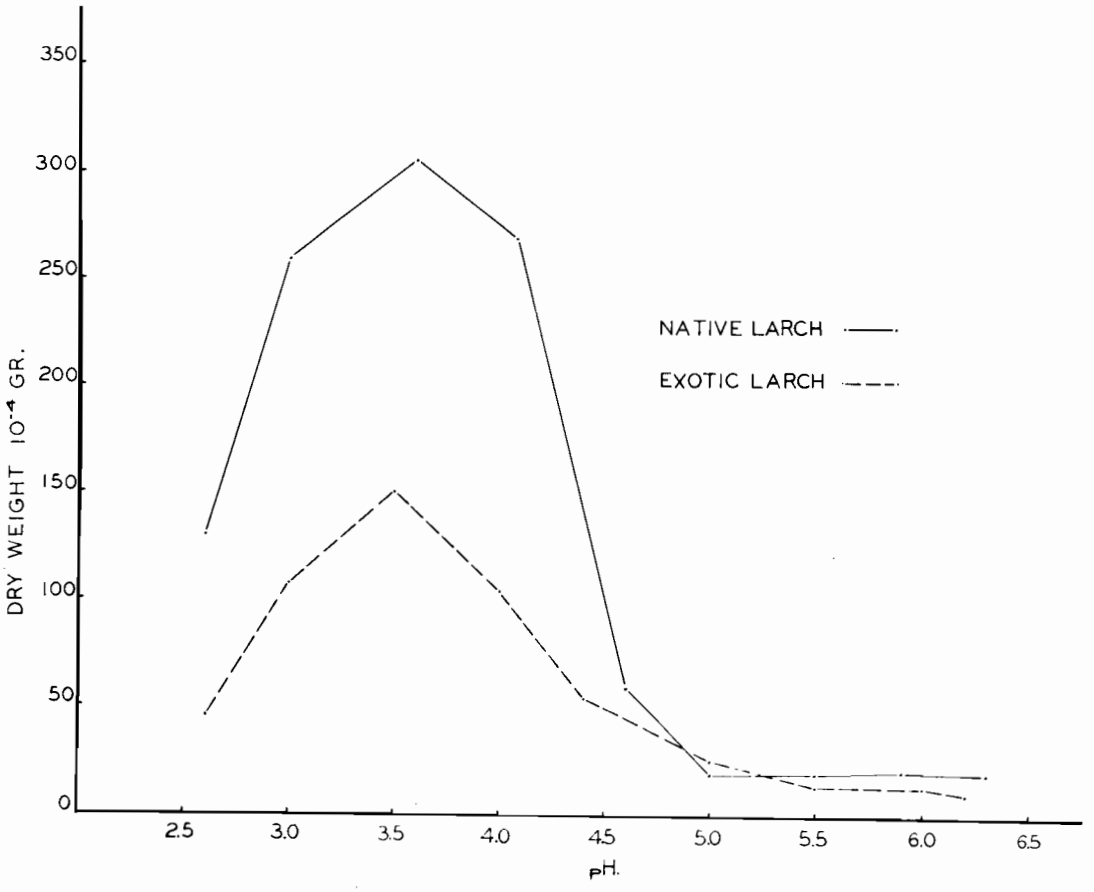

Fig. 15: Influence of pH on growth of mycelium from monosporic isolations in liquid malt extract solution buffered with sodium phosphate and citric acid.

TABLE 9

EFFECT OF THE pH ON THE GROWTH OF HETEROTROPHIC ISOLATES OF LEUCOSPORA NUDIFLORA FROM EXOTIC LARCHES.

pH calculated	Buffer soln.* 1 Measured pH Before Growth	Buffer soln.* 1 Measured pH After growth	Buffer soln.* 1 Dry wt. 10^{-4} g.	Buffer soln.** 2 Measured pH Before growth	Buffer soln.** 2 Measured pH After growth	Buffer soln.** 2 Dry wt. 10^{-4} g.
2.5	2.6	2.7	45.3	2.5	2.6	105.7
3.0	3.0	3.9	117.6	3.0	3.0	312.1
3.5	3.4	3.5	151.7	3.5	3.5	430.0
4.0	4.0	4.0	113.6	4.0	4.0	326.0
4.5	4.4	4.5	54.0	4.5	4.5	251.6
5.0	5.0	5.0	25.6	5.0	5.0	269.0
5.5	5.5	5.3	13.3	5.5	5.4	131.2
6.0	5.8	5.7	13.3	6.0	5.9	73.0
6.5	6.2	6.1	11.3	6.5	6.3	34.0

* Buffer solution numbered 1 is composed with lactic acid and sodium phosphate. Under this section values represent an average of 15 readings.

** Buffer solution number 2 is made of H_3PO_4, KH_2PO_4 and K_2HPO_4. Values under this section are an average of five readings.

TABLE 10

EFFECT OF THE pH ON THE GROWTH OF MONOSPORIC ISOLATES OF

LEUCOSTOMA KUNZEI FROM NATIVE LARCHES.

pH calculated	Buffer soln.* 1 Measured pH Before growth	Buffer soln.* 1 Measured pH After growth	Buffer soln.* 1 Dry wt. 10^{-4}g.	Buffer soln.** 2 Measured pH Before growth	Buffer soln.** 2 Measured pH After growth	Buffer soln.** 2 Dry wt. 10^{-4}g.
2.5	2.5	2.6	152.3	2.5	2.5	267.0
3.0	3.0	3.0	259.0	3.0	3.0	500.7
3.5	3.6	3.5	306.9	3.5	3.6	554.0
4.0	4.1	4.0	268.3	4.0	4.0	669.0
4.5	4.6	4.6	58.6	4.5	4.5	777.6
5.0	5.0	5.0	18.6	5.0	4.9	906.2
5.5	5.5	5.4	19.6	5.5	5.5	360.0
6.0	5.9	5.8	20.3	6.0	6.0	393.5
6.5	6.3	6.3	20.0	6.5	6.4	56.0

* Buffer solution numbered 1 is composed with lactic acid and sodium phosphate. Under this section values represent an average of 15 readings.

** Buffer solution number 2 is made of H_3PO_4, KH_2PO_4 and K_2HPO_4. Values under this section are an average of five readings.

6- Growth of mycelium on different barkmeals.

Kern (28) stated that various bark extracts inhibit or stimulate the growth of races of Leucostoma in a specific manner. He even used this variability in growth as a taxonomic characteristic for two species.

A test was make to see if differences could be observed between isolates of Leucostoma kunzei. Three isolates were tested on all the available barkmeals. Two of these, numbered 16 and 95 in Table 11, were from L. decidua and one, numbered 58, was from L. laricina. Three others were tried on five of these barkmeals, they are numbered 28 and 81, from native larch and 110 from exotic larch.

Each reading in Table 11 is the average of the diameter of five replicates. Here again the mycelium of monosporic isolations from exotic larches (No. 16, 95, 110) grows slowly. Two particular isolates are inhibited in their growth; number 58, from L. laricina, is inhibited by a factor contained in bark of Salix, the other one, 95, from L. decidua, is inhibited by the bark of Prunus pennsylvanicum. The other four isolates grew almost indifferently on barkmeal of different families of trees.

TABLE 11

MYCELIAL GROWTH OF DIFFERENT ISOLATES

ON BARKMEAL OF DIFFERENT HOSTS.

Barkmeal* of:	Diameter in mm. after 7 days					
Isolates+:	16	28	58	81	95	110
Larix decidua	46	53	60	53	48	50
Larix laricina	50	--	66	--	52	--
Pinus strobus	44	42	52	43	32	63
Pinus rigida	45	49	66	52	31	58
Salix laurina	40	--	13	--	44	--
Quercus borealis	62	75	92	86	51	61
Prunus pennsylvanicum	54	90	86	90	12	65
Water agar (2%)	21	25	24	27	20	19

+ Isolates numbered 16, 95 and 110 are from exotic larches.

+ Isolates numbered 28, 58 and 81 are from native larches.

* Two per cent agar was added to the barkmeals.

7- Utilization of carbon sources.

Recently, some workers (27, 33) used the selective utilization of carbon sources as a criterion for characterization of closely related species of organisms. Could some of our isolates have something individual in this regard? The Table 12 illustrates the results obtained.

TABLE 12

UTILIZATION OF CARBON SOURCES BY MONOSPORIC ISOLATES OF LEUCOSTOMA KUNZEI.

Carbon sources	Differences in growth of isolates from					
	Exotic larches			Native larches		
	16	95	110	28	58	81
Dextrose	+++	+++	++	+++	++	+++
Galactose	+++	++	+	++	++	+
Mannose	++	++	++	++	+	+
Levulose	+	++	++	++	+	++
Ribose	+	+	-	+	-	+
Arabinose	+	+	+	+	+	-
Xylose	-	-	-	-	-	-
Control Solution*	0	-	0	0	0	0

+++: Dry weight of 10 mg. or more. ++: Dry weight of 5 to 10 mg.

+: Dry weight of 1 to 5 mg. - : Traces of growth.

0: No growth.

*: Basic nutrient solution as described on page 31, without sugar.

After 24 days of growth, all the isolates grew better on Dextrose; Galactose, Mannose and Levulose were a little less utilized; on Ribose and Arabinose they grew more than on Xylose, and finally, Xylose provided a smaller quantity of mycelium for all the isolates.

8- Compatibility between isolates of the fungus.

The compatibility of the representative isolates from Japanese larch, European larch and native larch resulted in very few valuable indications concerning their separation one from the other according to the type of cultural characteristics already observed under Section 2 on page 54.

In Table 13, the isolate numbered 16 originated from Japanese larch; Those numbered 95 and 110 are from European larch and finally 28, 58 and 81 are from native larches.

In all these confrontations, anastomosis was not observed even though almost any isolate could develop over and under its neighbouring isolate. The lines of opposition which formed after seven days usually delayed the growth for about one days

Isolates numbered 28 and 81 formed a line of separation when they were faced with isolates from exotic larches. When they are faced one against the other, thay do not show any opposition, but when they meet another isolate from native larch,

isolate 28 does not show any opposition, while 81 does. Incidentally these two isolates are representatives of the group numbered 2 (page 54) for their cultural characteristics and this group is very distinct from the others, at least in its cultural appearance.

TABLE 13

OPPOSITION OF DIFFERENT ISOLATES OF

LEUCOSTOMA KUNZEI ON MALT EXTRACT AGAR.

Isolates	Opposition of isolates	
	After 7 days	After 10 days
16- 28	+	-
16- 58	-	-
16- 81	++	+
16- 95	+	-
16- 110	-	-
28- 58	-	-
28- 81	-	-
28- 95	+	-
28- 110	+	-
58- 81	++	+
58- 95	-	-
58- 110	+	-
81- 95	+	-
81- 110	+	-
95- 110	+	-

++: Five mm. or more between the two colonies.

+: Less than five mm. between the colonies.

-: No delay in growth.

c) Pathogenicity and virulence.

1- Inoculation of twigs in culture tubes.

The main objective in making inoculations on twigs was to obtain the perfect stage and to induce the fungus to complete its life cycle. In reaching this objective, the size of ascospores could be measured and the variation found in nature could have been easily compared with any variation obtained under controlled conditions. Frequently in the literature (7, 32) the pycnidial structures obtained on twigs in culture tubes are compared with the same structures on branches from the field collection.

In the present test, no similarity was observed between the structures in the field collections and the structures on inoculated twigs. Beginning of the formation of pycnidia started by an accumulation of mycelial hyphae and later the superficial pycnidia were also surrounded with white hair-like hyphae.

Out of fifty twigs inoculated, only two appeared to have produced the perfect stage at room temperature after one year. The structures were opened and carefully examined for the presence of ascospores but they were completely sterile.

Pycnidia exceptionally formed inside the bark were much smaller than those found in nature and their development was not complete after one year. Some of the inoculated twigs had one end in sterile water, a factor which might explain the superficial growth of mycelium on such twigs. Other twigs became so dry that the fungi could not achieve their development when water was not supplied. After this period of one year, all the tubes were opened and the twigs were examined.

The superficial mycelium, produced on twigs inoculated with a spore suspension from a single perithecium, was similar on all 25 inoculated twigs. In general, the appearence of the mycelium on the twigs was similar to the mycelium produced on culture media.

2- Inoculation of seedlings.

Small infected seedlings do not show visible cankers as do older material. Therefore, since symptoms are very difficult to detect, the best criterion to determine whether infection took place or not is the presence or absence of pycnidial development. Six months after inoculation, every seedling was carefully examined for the presence of pycnidia. The marginal cases were brought to the laboratory and examined under the stereoscopic microscope and samples of bark were plated on nutrient media to make sure of the presence of the fungus and its identity.

Apparently temperature is important in seedling inoculations (Table 14). The seedlings submitted to a temperature of 4.5°C. grew very slowly and a higher percentage of infection was obtained under such conditions than when the seedlings grew faster in the warmer environment of a greenhouse at about 20°C. The results illustrated in Table 14 are from two experiments completed successively in which the same tendency was observed.

These results are also cumulated against water supply in Table 15 but no marked tendency is apparent. The control seedlings remained healthy in all cases, this indicates that the wound was not big enough to weaken the seedlings. This survival of the control trees also tends to prove that the low water supply is not limiting either.

In Figure 16, a group of five seedlings, all infected, can illustrate the type of symptoms obtained on seedlings. The part of the seedling, over inoculation region, is killed. After the host tissue dries out, pycnidia form as illustrated in the close up picture of Figure 17. The control numbered 145 t is healthy, as it can be seen by the condition of the foliage.

TABLE 14

PERCENTAGE OF INFECTIONS PRODUCED ON SEEDLINGS OF LARCHES AFTER ARTIFICIAL INOCULATIONS.

Hosts	Inoculum*98				Inoculum 58			
	Greenhouse		Cold room		Greenhouse		Cold room	
	No. inoc.	Inf. (%)	No. inoc.	Inf. (%)	No. inoc.	Inf. (%)	No. inoc.	Inf. (%)
L. laricina	5	0	-	-	20	20	20	55
L. decidua	15	12	15	60	10	10	10	33

TABLE 15

PERCENTAGE OF INFECTIONS PRODUCED ARTIFICIALLY ON SEEDLINGS OF LARCHES AS RELATED TO WATER SUPPLY.

Hosts	Inoculum*98				Inoculum 58			
	Low water supply		High water supply		Low water supply		High water supply	
	No. inoc.	Inf. (%)	No. inoc.	Inf. (%)	No. inoc.	Inf. (%)	No. inf.	Inf. (%)
L. laricina	-	-	5	0	20	35	20	35
L. decidua	16	43	10	30	10	30	15	7

* Inoculum 98 is an isolate from exotic larch, while inoculum 58 is from native larch.

Fig. 16: Symptoms obtained on seedlings of native larch six months after inoculations in greenhouse.

Fig. 17: Enlarged view of the seedling no. 150 showing young pycnidium in formation.

3- Inoculation in the field.

Many investigators dealing with Cytospora, Valsa or Leucostoma consider these fungi as weak parasites or as saprophytes (6, 7, 12, 29, 35, 48). Inoculations were therefore conducted in order to determine to what extent Leucostoma kunzei is pathogenic.

The first experiment was conducted on healthy trees having a D.B.H. between two and three inches. As explained under field methods (p.34), this experiment, conducted in September 1960, was performed mainly to find out if a superficial wounding is sufficient to produce infection. The results were conclusive; no symptoms appear unless a wound reaching the cambium is made.

The highest levels of infection were produced in October 1960, the symptoms of which were evaluated by May 1961. The fungus was reisolated from the cankered stems. These two inoculation series in 1960 were performed with mycelium of the isolate No. 16 originating from cankered exotic larches in Harrington.

In 1961, periodic inoculations were made and the results for 1960 and 1961 are cumulated in Table 16.

TABLE 16

INFECTIONS FOLLOWING SEASONAL

INOCULATIONS WITH MYCELIUM OF *LEUCOSTOMA KUNZEI*.

Date of inoculation	Locality	Host inoculated	Inoculum isolates	No. of inoculations	Infection %
Oct., 1960	Quebec	L. dec.	16	19	68
	Quebec	L. lar.	16	20	50
June, 1961	Proulx	L. dec.	16	16	13
	Proulx	L. dec.	28	14	7
	Proulx	L. dec.	58	14	21
	Proulx	L. dec.	110	12	67
	St Etienne	L. lar.	16	12	25
	St Etienne	L. lar.	81	12	0
	St Etienne	L. lar.	95	12	8
July, 1961	St Etienne	L. lar.	81	10	50
	St Etienne	L. lar.	95	10	20
	St Etienne	L. lar.	110	10	50
Aug., 1961	St Etienne	L. lar.	16	10	10
	St Etienne	L. lar.	28	20	5
	St Etienne	L. lar.	110	10	40
Sept., 1961	Quebec	L. dec.	114	16	0
Oct., 1961	Quebec	L. dec.	58	16	37
	Quebec	L. dec.	110	16	13
	Quebec	L. dec.	115	16	6
	St Etienne	L. lar.	58	16	12
	St Etienne	L. lar.	110	16	69
	St Etienne	L. lar.	114	16	18
	St Etienne	L. lar.	115	16	12

Isolates numbered 16, 95, 110, 114 and 115 originate from exotic larches and those numbered 28, 58 and 81 are from native larches.

In general, the inoculum from exotic larches produced more cankers than the one from native larches, nevertheless inoculum from either host can produce cankers on both host species. Isolates from both sources could not be tested each month because of the limitations of material and time.

The differences in percentage of infection at different periods of inoculation cannot be related to differences in the age of the inoculum or in the number of times it was transferred in culture, because instances of both high and low percentages of infection were obtained from fresh cultures as well as from older cultures of the fungus. Fresh inocula were isolated during 1961 (No. 114-115) and they produced very few infections in the fall of 1961. On the other hand, a one year old isolate (No. 110) produced a higher percentage of infection during that same period.

The size of the original spores from which the cultures were developed does not help to explain the different results obtained in the inoculations. Unfortunately, up to this time no perfect stage has been observed, so that it was impossible to know if the size of ascospores is constant or variable after their passage through inoculated branches or stems of different sizes and hosts.

The isolate numbered 110 was more virulent than any other on both species of larches. The other isolates

produced, exceptionally, 50% infection during the summer 1961, but in most cases, the percentage of infection ranged from 0 to 25%.

Since no isolate showed a marked specificity for one or the other host species, the percentage of infection, illustrated in Figure 18, is given according to the host inoculated. No marked preference was observed for a particular orientation on the host or a certain level above the soil. Figure 18 also illustrates the relations between monthly precipitation during the test period and the average monthly precipitation based on the ninety previous years. The high level of infection in October 1960 seems to be related to the end of a three month period when the precipitation was much lower than the average. In June 1961, the previous three months were also lower than the average in precipitation but the difference was not as marked as in October 1960. In no other period did we obtain such a high level of infections and in no other period was the precipitation so much below the average.

From the success of growth on various barkmeals and from the non-specificity of isolates on the larch hosts, it was logical to wonder if the host range was limited to conifers or not. In October 1961 strains of the fungus were isolated from European and native larches and also from balsam fir and white pine and inoculated on a wide range

of hosts. The two isolates from fir and pine were of the same type as the cultures in the group 1 (p. 54), and the fruiting structures on the host had also the same appearence and dimensions as the structures commonly found on larches. The hosts tested were: Larix laricina, L. decidua, Pinus strobus, Picea glauca, Abies balsamea, Quercus rubra, Acer rubrum, Ulmus americana, Salix laurifolia, Populus tremuloides, Sorbus americanum, Betula papyrifera, Tilia glabra, Fraxinus americanum, Prunus pennsylvanicum and Crataegus spp. These hosts represent the possible hosts for all the fungi described in Saccardo's 'Sylloge' having equivalent morphological characteristics.

No canker was observed on hardwoods, but seven months later, the fungus was reisolated from the bark of Crataegus and Prunus. A very peculiar fact is that regardless of whether the original cultures had the aspect of number 1, 2 or 3, as noted in Table 7, they all appeared similar to type number 2 when reisolated from these two hosts. Furthermore, more and larger, pycnidia were produced by these cultures than by any other isolate, and sometimes the perfect stage seemed to be in formation.

Isolates respectively from Abies and Pinus, No. 116 and 118, did not produced canker on either species of larch, but even inoculations from larch to larch resulted in low percentage of infections in that experiment. All the inocula produced cankers on fir and spruce except the isolate 110 which behaved as if it was really specific to larch (see Table 17).

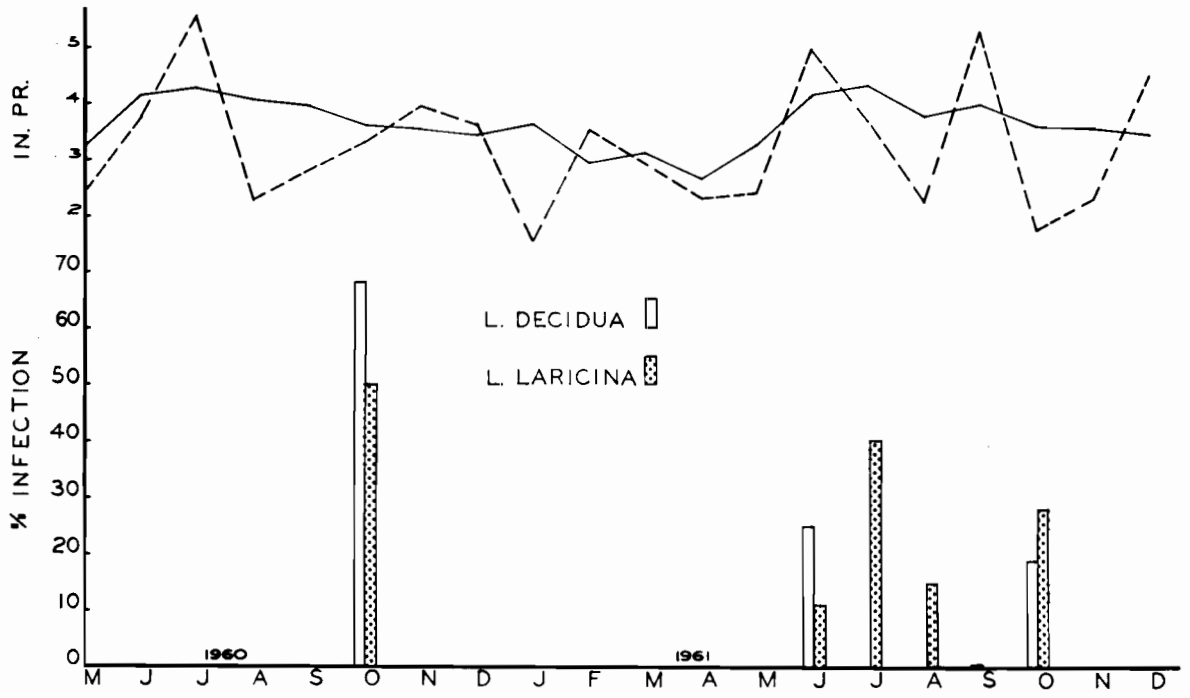

Fig. 18: Relationships between the percentage of infection on native and exotic larches and the deviation of the monthly precipitation from the average.

Broken line: monthly precipitation for the years 1960 and 1961.
Solid line : average monthly precipitation over a 90 year period.

TABLE 17

CONIFER HOST RANGE OF LEUCOSTOMA KUNZEI

AFTER INOCULATIONS OF OCTOBER 1961

Inoculum*	Hosts				
	L. decidua	L. laricina	Picea glauca	Abies bals.	P. strobus
58 (L. lar.)	+	+	+	+	-
110 (L. dec.)	+	+	-	-	-
114 (L. dec.)	+	+	+	+	-
115 (L. lep.)	+	+	+	+	-
116 (Abies b.)	+ -	+ -	+ -	+	-
118 (Pinus)	+ -	-	-	+	-

\+ : Symptoms of a canker.

\- : No infection.

\+ - : Infection but symptoms not as evident.

*Each inoculum is identified according to its original host.

4- Development of the cankers.

Most cankers produced on European larches were larger in size than those on Canadian larches. The long axis of the canker is always in the direction of the stem. After seven months, dimensions of the cankers produced in 1960 on Larix laricina averaged 45 x 33mm. and after twenty months they reached 60 x 55mm. in size. On L. decidua the average was 61 x 35mm. after seven months, and 80 x 52mm. after twenty months. These estimations of the advance of the fungus were based on the inoculations performed with an inoculum from exotic larch.

The cankers produced in 1961 were more numerous when inoculated with isolates from exotic species than from native larch. Also the dimensions of the cankers produced on European larch averaged 49 x 17mm. after ten months while those produced on native larch averaged 28 x 10mm. in size after the same period.

These data indicate very well that the fungus can develop faster in living bark of European larch than in the bark of native larch.

Figure 19.

Figure 20.

Fig. 19 & 20: Canker formed on L. laricina respectively 7 and 20 months after inoculation.

Figure 21.

Figure 22.

Fig.21 & 22: Canker formed on L. decidua respectively 7 and 20 months after inoculation. Pycnidia are present.

Fig 23: Control wounding without inoculum, twenty months after the period of inoculation.

DISCUSSION

From the surveys conducted in the province of Quebec, European larches in plantations as well as those planted as individual trees, are more affected by Leucostoma kunzei than are native larches in natural stands, plantations or ornamental fields. The rapid appearence and the actual distribution of this new disease lead easily to the idea of acquired virulence of facultative parasites on exotic species which are not yet adapted to unfavourable environmental conditions. In fact, this disease on larch was observed for the first time in Quebec in 1960, the same year that this project started.

Water content in the bark of the host, as related to drainage of the soil in infected areas and monthly precipitation, seem to provide logical explanations for the naturally occuring cankers. On big trees, the lower branches, which are the first to become dry under normal conditions, are cankered first. On smaller trees, pruning the lower branches of larches on sandy and well-drained soils makes them more susceptible to stem canker. Observations on the occurence of the Larch Canker in relation to greatly reduced precipitation are also in accordance with the infections produced artificially in the fall of 1960.

The development of this canker can be very serious on trees of a diameter smaller than three inches and when more than one canker is present on the stem. However, larch trees have a rapid growth and they can recover the following year if the climatic conditions are favourable to the host, as it was in the summers 1961 and 1962. These observations from the survey may be related to reports in the literature concerning the importance and occurence of cankers produced by the same causal organism or its relatives (25, 35, 41, 48, 49, 51). According to these reports, transplanted conifers are more susceptible; excessive drainage is closely related to the occurence of the disease; small stems are killed more often than bigger ones; branch cankers are mostly found on lower branches of bigger trees; and finally, drought, fire, and insects can contribute to infections.

The characteristics on the host illustrate without any doubt that the causal organism is of the genus Leucostoma as recognized by Wehmeyer (46), Kern (26, 27 and 28) and others.

The imperfect stage is the only one to appear in the cankered area and one has to wait until a part of the tree dies before the perfect stage is observed. The reason Gilgut (18, 19) and Hawksworth and Hinds (21) did not observe the perfect stage may be that they were more interested in the pathological aspect of the canker disease and its importance, and most likely

the imperfect stage was the only stage present at the time of their observations.

Leucocytospora, suggested bt Defago (13), is a logical name for the imperfect stage corresponding to Leucostoma and this would help to elucidate the situation. But since no morphological distinction exists, Cytospora is still used for the imperfect stage of Valsa and Leucostoma.

It seems obvious that very often the perfect stage is formed on the same stroma but after the pycnidial stage. This is why, very often, perithecia are observed around remnants of pycnidial locules. Defago (25) observed the same phenomenon on L. curreyi. But the papillae formed around the disc and the presence of paraphyses are considered typical by this worker and no similar structures are observed on L. kunzei.

The overlapping in the dimensions of structures inside the stroma is evident on both host species. In this respect, Hubbes (23) made the same observations on willow, and Defago (12, 13) on wild plum and European larch. Because of this overlapping between the morphological characteristics of the species, it is evident that the numerous species names found in Saccardo's 'Sylloge' cannot be maintained any longer on spores and asci,

the overall picture of Figure 11 and 12 will finally give only one summit between the extremes. After 1,200 ascospores were measured, three groups of curves were more or less distinct, but after three additional series of 200 measurements, the distribution presents only two groups. It might take many additional series of measurements to obtain a uniform distribution between the extremes but it might take only one or two more series.

Kern (26) suggested that Leucostoma kunzei (Fr.) Munk can represent the group found on conifers in Eastern United States for the present time. It was noted under the heading of Taxonomic Discussion that almost all the species of the Leucostoma type reported on conifers have some reasons to be classified under the specific name kunzei, therefore Kern's suggestion is logical. Taxonomists need better criteria than the morphological ones before subdivisions can be realized on a logical basis. Examination of other fruiting structures on other conifer host showed us the close relationships between any fruiting structures og the genus Leucostoma on conifers.

The opposite was presented by Waterman (44) who tried to use the name of all the old species for new varieties but this was undertaken in an attempt to keep only one species name. Even if Munk's description does not

provide a wide enough range of variation in its measurements, it is the most logical description for the causal organism of the canker disease on larch.

The conceptacle is the main criterion for the classification of the genus Leucostoma. However the precise nature of this structure has not been determined. It can be formed by a physiological process, depending on the substrate, but since both Valsa and Leucostoma were observed on the same branch of the same host, it is assumed to be distinctive.

Monosporic isolations are variable in the color of their mycelium, type of growth and type of pycnidia formed on malt-extract agar. Since the agar itself is colored differently, there should be a metabolic process involved which produces this difference. The process is not known yet, but indications are that the original individual substrate has something to do with this variation. In fact, mycelium of one color, inoculated on wild plum trees and reisolated on malt-extract agar produced mycelium similar to that of another existing group of isolates. The repetition of these experiments will show whether a passage through other hosts will always produce this interesting effect on the cultural characteristics. Perhaps only one substance is present in one individual tree which is not present in another one of the same species, growing in another ecological site.

To a certain extent, the change in color of tendrils is likely related to the stage of oxidation of the tendril at the time of observation. Usually, they are white or cream colored at the beginning and, with the time, they become darker and darker. The same type of phenomenon is logical for the color of the disc, and white disc is typical for the genus Leucostoma in a fresh section, but this white color is not obvious from the outside.

From a preliminary test (not reported here) with many vitamins, amino acids, sugar and salts, it seems that one or a combination of amino-acids, are concerned with the optimal mycelial growth of this heterotrophic fungus. In the test which shows that Leucostoma kunzei does not grow on exclusively mineral solutions, the original pH is not strictly limiting. In fact, Richard's Agar and Czapek Agar had respectively pH values of 5.2 and 7.4 and the mycelium did not develop on them, but malt-extract agar had a pH of 5.9 and the fungus grew very well.

The rate of growth of isolates from exotic larches is lower than that of isolates from native larches. On the other hand, the organism is considered to grow almost exclusively as a saprophyte in natural stands of native larch, while on exotic larch cankers are numerous on living trees. This respective differental rate stands even after three or four transfers of the mycelium,

so this characteristic can possibly be of use in differentiating pathogenic strains of Leucostoma kunzei on Larix. On the other hand, it is possible to observe that cankers produced with inoculum from exotic larches extend more rapidly in exotic larches than in native larches. So pathogenic races would develop cankers faster in the host, and grow slower on agar. The host response is more vigourous with hosts adapted to environment than with poorly adapted hosts. And this can explain also the faster rate of the development of cankers on exotic hosts than on native hosts.

Optimum temperature for mycelial growth does not provide a good basis for distinction between isolates from various hosts. However the mycelium of isolates from native larches tolerates higher temperatures in culture. During a dry hot summer, when the host grows slowly because of a lack of water, the fungus still develops and can invade any wound. Strains from native larch can also tolerate lower temperatures than those from exotic species and here again potentialities are enlarged as far as the temperature requirements for successful inoculation is concerned. But this ability to widthstand a wider range of temperatures did not seem to influence the results of inoculations in the present work. The inoculum from exotic larch was found to be more virulent than the inoculum from native species. It may be that a passage of the commonly saprophytic stage through new compatible hosts would increase the virulence of the canker organism, but this is still an hypothesis.

The pH values are not better than temperature values for differentiating isolates of the causal organism. so many factors are involved in a pH study that possible relations with inoculation results were not determined. Although distinction between isolates could not be found on a basis of pH, one additional observation in this connection seems worthy of comment.

It was very interesting to observe that the mycelium seems to be able to develop a fermentation process when oxigen is less available. This is not mentioned very often in the literature on Leucostoma kunzei. This observation would be a logical explanation to the presence of Cytospora species in the living bark of European larch in a dormant stage as stated by Day (9). Being able to develop without a direct contact with the surrounding atmosphere, the fungus can be present in the bark for a long time without expressing any symptoms. This phenomenon could also explain the production of cankers closer to the stem without external vector, providing the fact that an old canker is already on the branch and that a mechanical injury helps the expressions of the symptoms.

None of the observations in culture could be associated with the original size of ascospores. This provides another reason to argue that species characteristics in the Leucostoma group cannot be exclusively related to morphology, and that isolates originating from ascospores of various sizes may be grouped under one species name.

Growth of mycelium on different barkmeals is a good indication that the species of Leucostoma are not as host specific as indicated by Saccardo. Indeed this growth on barkmeals does not necessarily imply that the fungus will develop on all the hosts from which the bark originated, but readings from this experiment provided a motivation for testing the organism on a wider range of hosts in the field. From these tests the capacity of the fungus to attack other conifers was observed.

Kern's (28) statement that "bark extracts of plants inhibit or stimulate the growth of races of Leucostoma in a specific way" was not proven but still it cannot be entirely rejected. More experiments in this respect could perhaps provide interesting indications about the variability of cultural aspects and the presence or absence of a specific substances in the bark associated with the disease occurence.

From the survey and the inoculation tests, it seems obvious that canker symptoms will develop only on bark of branches which are more than two years old. Strong (41) came to about the same conclusion with references to spruce in Ontario. Infections can take place on younger bark but no external symptoms will develop unless the branch dies. The succulent parts are preferred by the larch sawfly and consequently it is very difficult to obtain direct correlation between the occurence of the disease and

the insect population. In fact, the population of larch sawfly was building up within the last four years and the passage from a lower branch to a higher one is possibly favoured by the migration of the larvae but wounding is certainly provided by another agent in many instances.

From inoculations of twigs, growth on barkmeals and growth on artificial media, the perfect stage was not produced under controlled conditions. Inoculations in the field will eventually produce this perfect stage if branches are gradually killed. But mechanical injuries are difficult to imitate on larch in the sense that the host branch either recovers rapidly or dies quickly before the establishment of the pathogen. Other attempts are under way and if this objective is reached, it would be the key for explaining many morphological variations by genetics, bark extracts or other means.

Seedlings are more sensitive to a weakening due to low temperatures. Infections were produced more frequently on seedlings in a cold room. Since the problem is mostly concerned with small trees that have passed the seedling stage, studies in that line were not pursued further. However, in the field, even if precipitation seems to be a major limiting factor, high temperatures of the summer can help to reduce the level of infection by favouring the host at the expense of the pathogen.

Various levels of inoculations were tested because of the survey data obtained from Harrington Forest Farm. In that plantation, exotic larches were cankered mostly at the base. However, no relation was obtained between inoculations performed one foot above the ground and six feet above the soil. This is an argument to suspect the clearing operation completed previously as the main cause of wounding.

Leucostoma kunzei is not confined to one host species but can produce infections on various conifer trees. Only one isolate behaved as if the fungus was really specific to larch. Consequently there is no reason to recall a new species name every time the organism is found on a new host plant.

SUMMARY.

Native and exotic larches were found cankered by a fungus named Leucostoma kunzei (Fr.) Munk in its perfect stage and Cytospora kunzei Sacc. in its imperfect stage. After a morphological study of the organism, these names were accepted in spite of the complicated taxonomy of the Leucostoma group. No morphological criteria sufficient to provide specific divisions in this group could be found.

Almost all the standard cultural studies completed with monosporic isolates were of little help in promoting differenciation of species according to the host on which they were collected or to the original size of ascospores. Variations between individual isolates exist in one respect or the other, but the explanation for such variations is not definitely known.

Morphological structures on the same host and even on the same individual branch can vary in size and shape within extremes which could include many species described on conifers. Generally the pathogen is not confined to a particular species of host. Physiological studies did not clarify the situation regarding species delimitations. Therefore it is suggested that, the name applied to the fungus producing canker on larch should be extended to include the causal organisms of Leucostoma canker on all conifers. Host-pathogen relationships must be better understood to explain the wide variation in the expression of this pathogen on various hosts.

This taxonomic problem would be solved more rapidly if one could produce the complete life cycle of the causal organism under controlled conditions. More attempts have to be performed in this respect and time is necessary to complete them because it takes much longer to obtain results from forest tree diseases than from field crop diseases. It also takes longer time for the disease to lead to the death of the host.

Strains commonly found in a saprophytic stage may become pathogenic depending on the host and environmental conditions. Leucostoma kunzei is found everywhere in the forest in the saprophytic stage and its host range can extend to various families of plants.

The occurence of this stem and branch canker during the summer of 1960 was relatively important. Since the host recovered rapidly in the two subsequent years, we can expect severe damage only when small exotic larches suffer from drought in plantations over a period of two or more seasons of vegetation. At present,in the province of Quebec, the severity of this disease is far from being comparable to the severity of the major forest diseases in Eastern Canada, because exotic larches have not been extensively planted. But one has to be aware of the endemic pathogen on all the conifers in plantations because it can acquire a certain level of virulence through exotic species of trees.

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BIBLIOGRAPHY

(1) Anonymous, 1930.
Larch canker.
New York conserv. Dept. Rpt. 20: 204.

(2) Anonymous, 1961.
Native trees of Canada.
Can. Dept. Forestry Bull. 61 (Sixth Edition), 291pp.

(3) Ainsworth, G.C. and G.R. Bisby 1961.
Dictionary of the fungi.
Commonwealth Mycol. Inst. Kew, Surrey.

(4) Baxter, D.V. 1937.
Development and succession of four fungi and diseases in forest plantations.
Univ. Mich. School Forest. and Conserv. Cir. 1: 44pp.

(5) Boyce, J.S. 1961.
Forest pathology.
McGraw-Hill Book Co. Inc. N.Y. Toronto, London.
(Third Edition), 572pp.

(6) Chiarappa, L. 1960.
Distribution and mode of spread of Cytospora canker in an orchard of President plum variety in California.
Plant Disease Reporter 44: 612-616.

(7) Christensen, C.M. 1940.
Studies on the biology of Valsa sordida and Cytospora chrysosperma.
Phytopathology 38: 459-475.

(8) Coppel, H.C. and K. Leius 1955.
History of the larch sawfly with notes on origin and biology.
Canadian Entomologist 87: 103-111.

(9) Day, W.R. 1958.
The distribution of mycelia in European larch bark, in relation to the development of canker.
Forestry 31: 63-86.

(10) Day, W.R. 1962.
The development of canker on European larch, Larix decidua Mill.
Phytopath. Z. 44: 313-323.

(11) Dearness, J. and J.R. Hansbrough 1934.
Cytospora infection following fire injury in Western British Columbia.
Can. J. Research 10: 125-128.

(12) Defago, G. 1935.
De quelques Valsées von Höhnel, parasites des arbres à noyau dépérissants.
Beitr. Krypt. Flora Schweiz. 8:(3), 109pp.

(13) Defago, G. 1944.
Seconde contribution à la connaissance des Valsées von Höhnel.
Phytopath. Z. 14: 103-147.

(14) Faull, J.H. 1930.
Notes on forest diseases in Nova Scotia.
J. Arnold Arboret. 11: 55-58.

(15) Fowler, M.E. 1935.
Surveys for larch canker.
Phytopathology 43: 405.

(16) Fries, E.M. 1849.
Summa vegetabilium Scandinaviae.
Kolmeae and Lipsiae, Upsala. 572pp.

(17) Fritz, C.W. 1952.
Brown stain on pine sapwood caused by Cytospora.
Can. J. Botany 30: 349-359.

(18) Gilgut, C.J. and O.C. Boyd 1933.
Cytospora canker of Picea spp.
(Abs.) Phytopathology 23: 11.

(19) Gilgut, C.J. 1936.
Cytospora canker of spruces.
Natl. Shade Tree Conf. Proc. 12: 113-118.

(20) Gillman, J.C. Tiffany, L.H. and R.M. Lewis 1957.
Iowa ascomycetes II. Diaporthaceae: Valsae.
Iowa State College J. Sci. 31: 623-647.

(21) Hawksworth, F.G. and T.E. Hinds 1960.
Cytospora canker of Engelmann Spruce in Colorado.
Plant Disease Reporter 44: 72.

(22) Höhnel, F. von 1917.
System der Diaporteen.
Ber. Dtsch. Bot. Ges. 35: 631-638.

(23) Hubbes, M. 1960.
Systematische und Physiologische Untersuchungen and Valsaceen auf Weiden.
Phytopath. Z. 39: 65-93.

(24) Johansen, D.A. 1940.
Plant microtechnique.
McGraw-Hill Book Co. Inc. N.Y. Toronto, London. 523pp.

(25) Jorgensen, E. and J.D. Cafley 1961.
Branch and stem cankers of white and Norway spruce in Ontario.
Forestry Chronicle 37: 394-400.

(26) Kern, H. 1955.
Taxonomic studies in the genus Leucostoma.
Papers Mich. Acad. Sci. A.L40: 9-22.

(27) Kern, H. 1957.
Untersuchungen über die Umgrenzung der Arten in den ascomycetengattung Leucostoma.
Phytopath. Z. 30: 149-180.

(28) Kern, H. 1961.
Physiologische und systematische untersuchungen in der gattung Leucostoma.
Phytopath. Z. 40: 303-314.

(29) Leonian, L.H. 1921.
Studies on the Valsa apple canker in New Mexico.
Phytopathology 11: 236-243.

(30) Manners, J.G. 1955.
Studies on larch canker, taxonomy and biology of Trichoscyphella willkommii and related species.
Rev. Appl. Mycology 34: 6-7.

(31) Manners, J.G. 1959.
Relationships between larch canker and Trichoscyphella willkommii.
Rep. For. Res. For. Comm. London p. 111-112.

(32) Marsden, D.H. 1948.
A Valsa associated with Cytospora canker of spruces.
Phytopathology 38: 307-308.

(33) Mix, A.J. 1954.
Differenciation of species of Taphrina in culture. Utilization of carbon compounds.
Mycologia 46: 721-727.

(34) Petrak, F. 1921.
Mycologische notizen II.
Ann. Mycol. 19: 17-128.

(35) Raymond, F.L. and J. Reid 1961.
Dieback of balsam fir in Ontario.
Can. J. Botany 39: 233-251

(36) Ridgway, R. 1912.
Color standards and color nomenclature.
Ridgway (Ed.) A. Hoen & Co. Baltimore.

(37) Riker, A.J. and R.S. Riker 1936.
Introduction to research on plant diseases.
J.S. Swift Co. Inc. St Louis, Chicago, N.Y.

(38) Ruhland, W. 1900.
Untersuchungen zu einer morphologie der stromabildenden Sphaerialis.
Hedwigia 39: 1-79.

(39) Saccardo, P.A. 1882.
Sylloge fungorum omnium hujusque cognitorum. Vol. 1.

(40) Shea, K.R. and J.E. Kuntz 1956.
Inoculations of aspens with Hypoxylon pruinatum (Kl.) Cke.
Univ. Wisconsin Forest. Res. Note 27: 2.

(41) Strong, F.C. 1953.
Spruce branch canker.
Natl. Shade Tree Conf. Proc. 29: 30-35.

(42) Urban, Z. 1958.
Revise ceskolovenskych zastupcu rodu Valsa, Leucostoma a Valsella.
Rozpravy Ceskolovenské Akademie Ved. 68: 1-100.

(43) Waterman, A.M. 1937.
Cytospora canker of spruce.
U.S. Dept. Agr. Pl. Dis. Rpt: 21: 55.

(44) Waterman, A.M. 1955.
The relation of Valsa kunzei to cankers on conifers.
Phytopathology 45: 686-691.

(45) Wehmeyer, L.E. 1924.
The perfect stage of the Valsaceae in culture and the hypothesis of sexual in this group.
Mich. Acad. Sci. A.L. 4: 395-412.

(46) Wehmeyer, L.E. 1926.
A biologic and phylogenetic study of the stromatic Sphaeriales.
Am. J. Botany 13: 575-645.

(47) Winter, G. 1887.
Gymnoascaceen und pyrenomyceten.
Rabenhorsts Kryptogamenflora Bd. 1. Leipzig, 976pp.

(48) Wright, E. 1942.
Cytospora abietis the cause of a canker of true firs in California and Nevada.
J. Agr. Research 65: 143-153.

(49) Wright, E. 1957.
Cytospora canker of Roxky mountain Douglas fir.
Plant Disease Reporter 41: 811-813.

(50) Zabel, R.A. 1953.
Lumber stain and their control in Northern white pine.
Journ. For. Prod. Research Soc. 3: 36-38.

(51) Zentmyer, G.A. 1941.
Cytospora canker of Italian cypress.
Phytopathology 31: 896-906.