A LARCH CANKER CAUSED BY LEUCOSTOMA KUNZEI (FR.) MUNK EX KERN[1]

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Abstract

A branch and stem canker disease, affecting up to 40% of exotic larch trees in plantations and a number of native larch trees in natural stands, was investigated in Quebec. The disease was found to be associated with the imperfect stage of *Leucostoma kunzei* (Fr.) Munk ex Kern. The taxonomic position of this fungus is reviewed.

Monoascosporic isolates obtained from different sources yielded various cultural patterns but these could not be related to hosts or to ascospore size. Isolates from exotic larches developed less rapidly than those from native larch on various media.

Results of field inoculations indicated that exotic larches are more susceptible than the native species. Susceptibility of a number of other coniferous species was also demonstrated. Unfavorable environmental factors, such as persistent summer drought, seemed necessary for the establishment and development of the disease.

Introduction

Recently, a canker disease was observed on many planted exotic larch trees: Japanese larch (*Larix leptolepis* Sieb. and Zucc.) and European larch (*L. decidua* Mill.), and on native larch (*Larix laricina* (Du Roi) K. Koch) in natural stands in Quebec. Since the constantly associated fungus, a "*Cytospora*" species, was previously unreported on these hosts in Canada, studies were undertaken to determine its identity and its relationship with the disease. The incidence and importance of the disease were also considered.

Materials and Methods

The study material was collected from the following locations: Harrington Forest Farm (Argenteuil County), Morgan Arboretum (Jacques-Cartier County), Proulx Provincial Nursery (Laviolette County), Duchesnay (Portneuf County), Quebec City and vicinity (Quebec County), and Parke Reserve (Kamouraska County). For morphological studies, fruiting structures were obtained from exotic and native larch branches and stems between $\frac{1}{2}$ in. and 3 in. in diameter. Structure measurements were made on freehand sections dipped in lactic acid, or on microtome sections permanently mounted in Canada balsam. In processing for permanent mounts, preparation of the sections by the tertiary butyl alcohol method of Johansen (6) was adopted. China ink smears were used for the study of asci, ascospores, and conidia. Cultures obtained from ascospores were grown on 2% malt extract agar (M.E.A.) in daylight at room temperature (20 to 24 °C). A barkmeal was prepared by grinding small pieces of bark from fresh twigs with dry ice in a Waring blendor. The barkmeal medium contained 1.8% Bacto-agar by

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weight, and 5% barkmeal (net weight). To test sugar utilization, a synthetic medium, consisting of 2 g asparagin, 1 g KH_2PO_4, and 0.5 g $MgSO_4$ in a liter of distilled water, was prepared, to which was added 10 g of the sugar to be tested. Sterilization was performed in an autoclave without steam pressure for 20 minutes on 3 successive days. For field inoculations, the fungus was also grown on a sterile 3 to 1 mixture of wheat and oat grains, which was found to stimulate pycnidia formation (15).

Four-year-old seedlings of European and native larch were used for inoculation tests in a greenhouse at 20 °C and a cold room at 4.5 °C. In both cases, half of the seedlings received approximately 1000 ml of tap water once a week and the other half about 350 ml every day.

In field trials, the following types of inoculum were used: (*a*) suspension of ascospores; (*b*) suspension of conidia; (*c*) one seed of the grain mixture on which pycnidia had developed; (*d*) a 4-mm plug of M.E.A. with the mycelium. Inoculations were carried out on trees between 8 and 12 years old, growing under similar conditions, by placing the inoculum into deep bark wounds made with a sterile scalpel at three 2-ft intervals, beginning at ground level; the cuts were diametrically opposite and differed in orientation at each level.

Disease Incidence and Symptoms

Cankers occuring naturally were observed on branches and stems. Branch cankers were found only on trees more than 13 years old. Ordinarily they occurred on the lower branches in groups of two or three, each successively girdling the branch closer to the stem. Typical symptoms appeared only on branches older than 3 years, but infection can take place and extend in younger twigs causing a dieback. Such cankers were observed on ornamental European larch at Berthierville, Valcartier, and on border trees in a 20-year-old plantation at Drummondville.

Stem cankers are often found on larch trees less than 15 years old. Individual cankers develop slowly, especially on native larch (Fig. 3), but, when numerous on one single host, their combined action may girdle and kill the trees quickly (Fig. 1). At Proulx, 40% of 5000 6-year-old European larches transplanted in 1961 showed cankers near pruning wounds by the end of the same year. One year later, at least 10% of the trees had died from girdling by coalesced cankers. On the other hand, unpruned planted native larch of the same age had very few stem cankers. At Harrington, 20% of 2200 Japanese larches, planted in 1956 as a 2-3 stock, were attacked in 1960. Cankers occurred about 3 ft above ground and apparently developed in wounds made during weeding operations. Two years later, however, less than 5% mortality could be attributed to the disease while the remaining affected trees seemed

FIG. 1. Small cankers on a stem of young European larch, a few months after the beginning of infection.

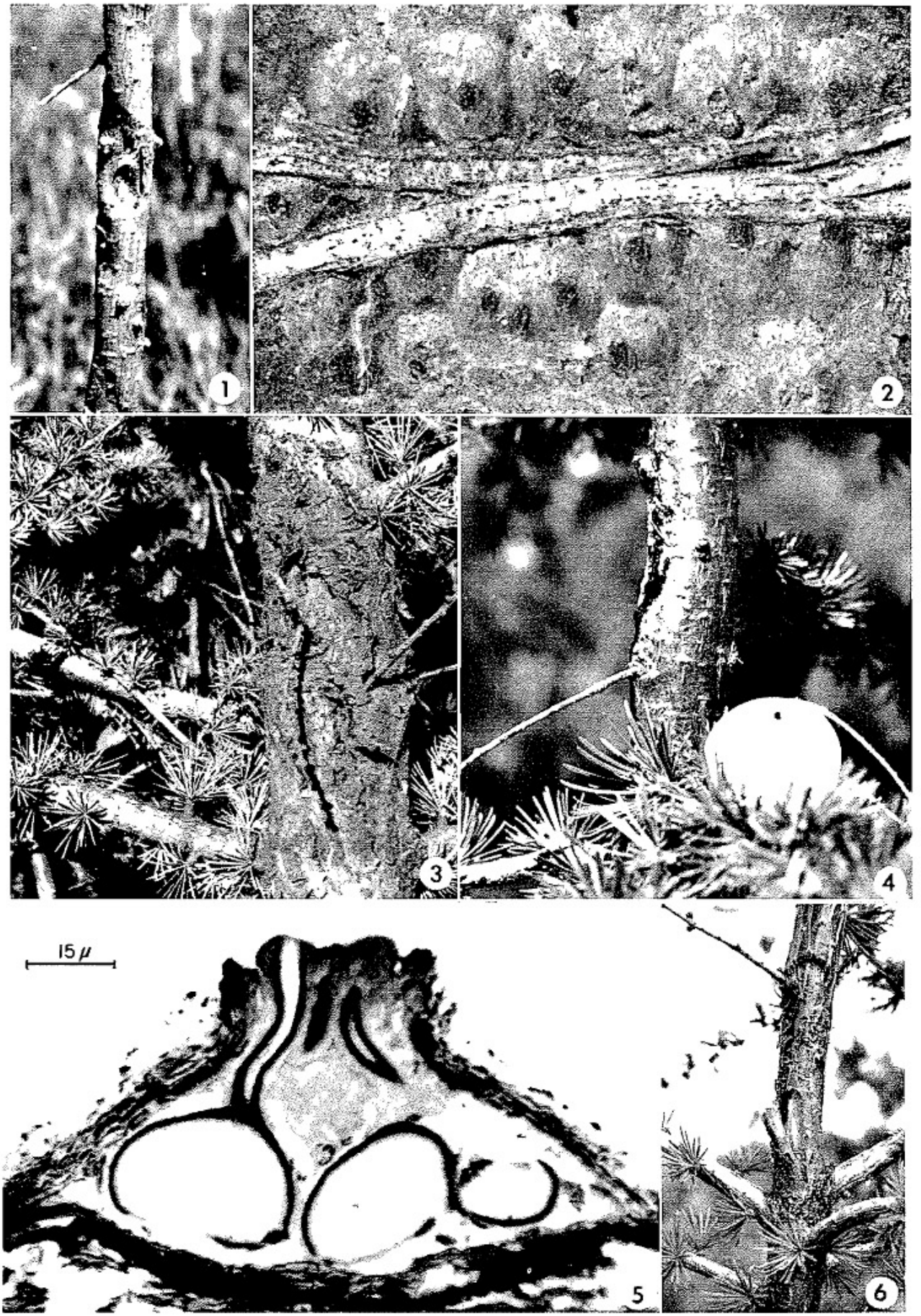
FIG. 2. Dead bark of native larch with fruiting structures of *L. kunzei*.

FIG. 3. Stem canker on native larch. Note slower development 4 years after infection.

FIG. 4. Canker on European larch 7 months after inoculation. Stem deformation is already noticeable.

FIG. 5. Transverse section through a stroma of the perfect stage, *Leucostoma kunzei* on European larch.

FIG. 6. Canker on European larch, 7 months after artificial inoculation. Resinosis is not abundant but extensive girdling has already killed a branch.



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to have recovered. In a plantation of European larch at the Morgan Arboretum, no diseased trees were observed. In contrast to the situation at Proulx, pruning lateral branches of 10-year-old trees in 1960 did not favor the establishment of cankers, although inoculum was present on numerous dead branches on the ground. Traces of stem infection were also observed on 7-year-old European larches in the Parke Reserve and in Acton Vale (Bagot County).

In natural stands, infected native larch is exceptional. Such an exception was observed near Laurier Station (Lotbinière County) in 1961 and 1962, where a number of 25-year-old trees had severe stem and branch infections.

Inoculation Tests

Tests on seedlings (10 to 20 per test) with isolates from native and European larch trees yielded from 10% to 20% infection in a greenhouse (20 °C) and 30% to 60% infection in a cold room (4.5 °C). Each isolate gave a higher proportion of infection on its original host. The fungus was recovered from infected parts of both species of larch. During this experiment, the relation between the percentage of infection and air or soil moisture was not clearly established, although more infections were produced on seedlings of European larch which received a low water supply.

Periodic field inoculations on larch at Proulx, Quebec City, and Saint Etienne, in 1960 and 1961, also yielded positive although inconsistent results (Figs. 4 and 6). Some differences were due to the time of year when inoculations were made, others probably resulted from climatic conditions especially those of October 1960 following a 3-month dry period (Fig. 8). In most cases, inoculations with isolates from exotic larch caused twice as many cankers as those from native larch. Furthermore, cankers on European larch were consistently nearly twice as long as on native larch. In the spring of 1963, signs of recovery such as termination of resinosis and complete healing of small cankers, were noticeable on almost every cankered host.

In addition to the above tests on larch material, inoculations with isolates from exotic and native larch, balsam fir (*Abies balsamea* (L.) Mill.), and eastern white pine (*Pinus strobus* L.), were carried out on each of the following hosts (two inoculations on each of eight trees):

coniferous trees:
Abies balsamea (L.) Mill., balsam fir,
Picea glauca (Moench) Voss, white spruce,
Pinus strobus L., eastern white pine;

broad-leaved trees:
Acer rubrum L., red maple,
Betula papyrifera Marsh., white birch,
Crataegus spp., hawthorn,
Fraxinus americana L., white ash,
Populus tremuloides Michx., trembling aspen,
Prunus pensylvanica L.f., pin cherry,
Quercus rubra L., red oak,
Salix longifolia Mukl., sand-bar willow,
Sorbus americana Marsh., American mountain-ash,
Tilia americana L., basswood,
Ulmus americana L., white elm.

In this experiment, isolates from fir and pine were not pathogenic on either species of larch. Except for one isolate from European larch which was specific to larches (on which it also seemed the most virulent isolate tested), isolates from coniferous species all produced cankers on fir and spruce. Cultures from controls were negative. Among hardwoods, 7 months after inoculation, the fungus was reisolated only from pin cherry and hawthorn. Regardless of the original cultural aspects of the isolates used as inoculum, isolates from these inoculated hosts all appeared similar on M.E.A. slants. Pycnidia were larger and more numerous than on any isolate from larch.

The Causal Organism

Morphological and Taxonomical Studies

The fungus constantly associated with the disease described above is characterized by a conical (Fig. 5), usually solitary (Fig. 2) fruiting structure.

TABLE I

Dimensions (in microns) of various parts of the perfect and imperfect stages of *Leucostoma kunzei* on larches

Structures	On exotic larch	On native larch	No. measured on each host
	Leucostoma stage		
Stroma			
Height	450–910 (650)*	550–1070 (750)	60
Diameter	800–1650 (1100)	940–1670 (1300)	60
Disc			
Diameter	200–520 (325)	327–810 (422)	60
Perithecium			
Diameter	170–400 (280)	162–617 (422)	75
Thickness of wall	11–32 (22)	24–66 (53)	75
Ostiole			
Length	195–520 (250)	260–546 (351)	75
Conceptacle			
Thickness	40–130 (70)	13–95 (46)	70
Ascus			
Length	12–23 (18)	25–61 (46)	500
Ascospore			
Length	5–16 (8)	7–14 (11)	1000
	Leucocystospora stage		
Pycnidium			
Height	350–600 (500)	525–676 (624)	45
Diameter	550–1000 (750)	800–1100 (886)	50
Disc			
Diameter	200–400 (300)	208–390 (307)	55
Conidiophore layer			
Height	10–22 (16)	10–26 (16)	50
Conceptacle			
Thickness	30–100 (60)	39–91 (66)	45
Conidium			
Length	3–7 (5)	2–7 (5)	200

*These figures represent, in order of listing, the low and high extremes and the average in each series of measurements.

The perfect stage, which ordinarily develops in dead bark outside the cankered areas, appears as a stroma in which monostichous perithecia, containing hyaline asci with eight allantoid and hyaline ascospores, are predominantly arranged distichously. The imperfect stage, usually present around the cankered areas, also appears as a stroma in which pycnidial locules form a labyrinth lined with conidiophores bearing allantoid and hyaline conidia. Both stages are characterized by the presence of a conceptacle which limits the base of the stroma.

In general, the structures of the fungus are noticeably larger on native than on exotic larch (Table I). However, as shown by ascospore measurements (Fig. 7), variations exist which prevent recognition of separate entities on exotic and native larch. These curves show different peaks and various degrees of overlapping for ascospore size from each series of collections. Furthermore, the overall extremes in spore length are both represented by collections from exotic larch. It was at first thought that differences in bark thickness could lead to a difference in ascospore size, but this was not substantiated.

Von Höhnel (4) was the first to consider the genus *Leucostoma* separate from the genus *Valsa* originally described by Fries (2), although Nitschke (12), Saccardo (14), and Winter (19) had earlier classified *Leucostoma* as a subgenus of *Valsa*.

The controversy on the validity of the genus *Leucostoma* which persisted for a number of years, as exemplified by the work of Gillman *et al.* (3), who did not consider von Höhnel's distinction to be valid for the Iowa collections studied, seems to have been settled by recent workers (5, 9, 11, 16, 18) who

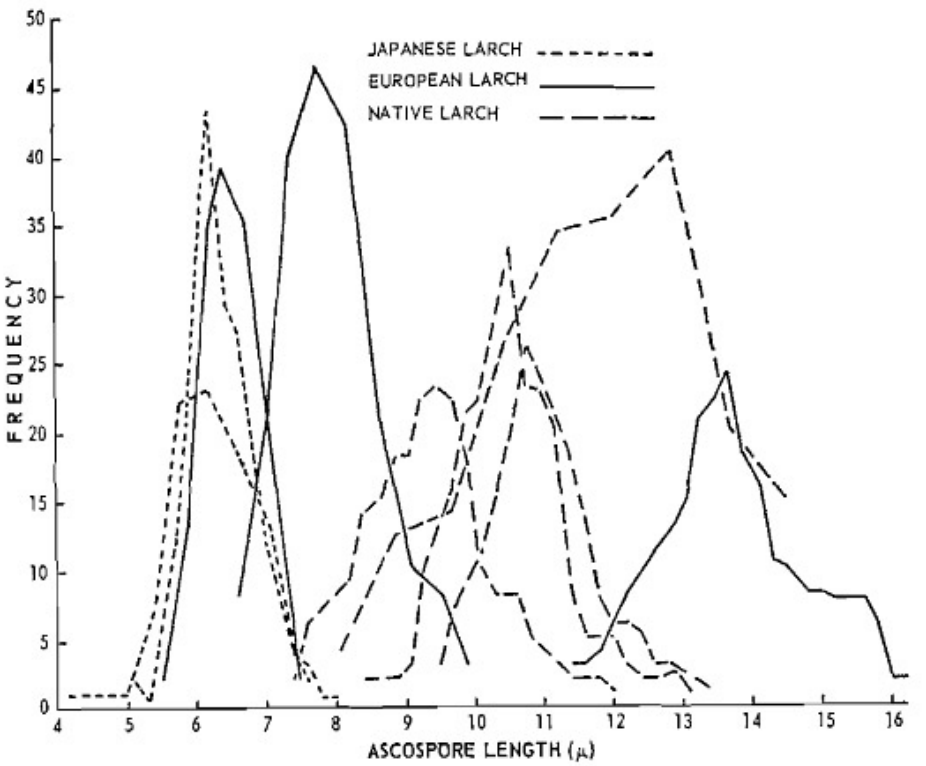

FIG. 7. Comparative length of ascospores of different collections of *Leucostoma kunzei* on various larch trees.

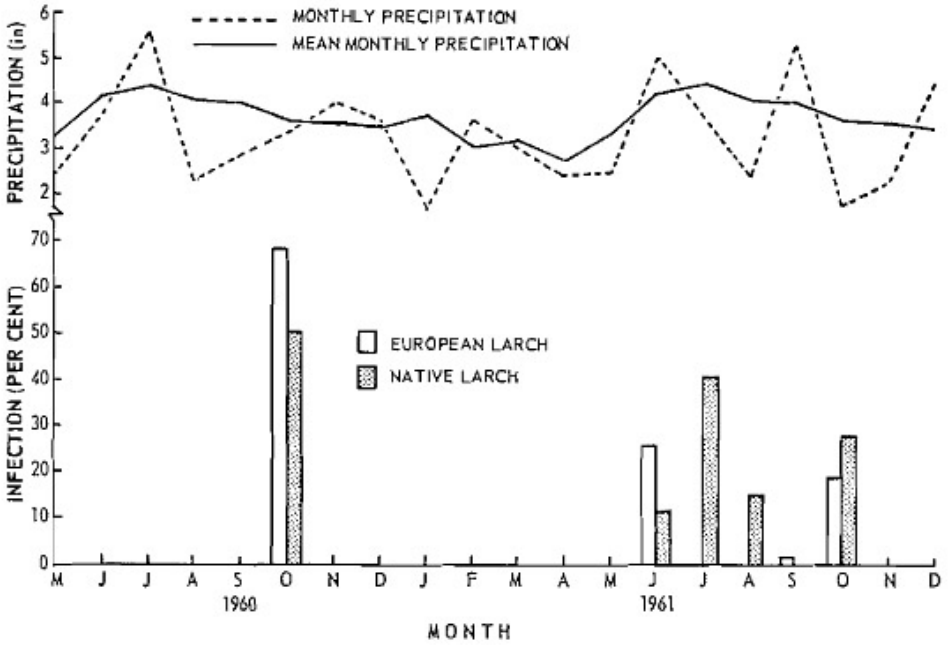

FIG. 8. Relation between the percentage of infection on native and exotic larches, and the deviation from mean monthly precipitation (over a 90-year period).

adopted two distinct genera. The genus *Leucostoma*, as compared to *Valsa*, is characterized by the presence of a conceptacle, and more conspicuous entostroma (5, 9, 13, 18).

The fungus found in Quebec is distinguished from *L. curreyi* (Nit.) Defago, a species occurring on European larch (1), by structure differences. It has, however, the characterization of *L. kunzei* (Fr.) Munk ex Kern (8) as revised by Urban (16) and, on the basis of the present study, it is concluded that this species is associated with the cankers on various larches in Quebec. In agreement with other authors (1, 5), the variations observed in spore size and the diversity of hosts are not recognized as specific features of *Leucostoma* in this study. Kern (8) considered as *L. kunzei* all collections of *Leucostoma* which he encountered on conifers in Michigan, but larch is not mentioned as a host in his list. However, Waterman (17) mentions that *Valsa kunzei* var. *kunzei* is found on European larch, but since her description corresponds to that of *L. kunzei*, it seems an improper connotation.

The imperfect stage of both *Valsa* and *Leucostoma* has been placed, traditionally in the genus *Cytospora* Ehrenb. However, the author favors the adoption of the generic name *Leucocytospora* proposed by Defago (1) for the imperfect stage (like that of the fungus considered in this paper) with pycnidia developing in a stroma within conceptacles, as separate from those without conceptacles, which he places in *Cytospora*.

Cultural Studies

The 120 monoascosporic isolates from Japanese, European, and native larch yielded three main types of cultures differing in pycnidia formation and color of the colony. These differences could not be related to hosts or spore size. On the other hand, constant differences in growth rates were obtained between morphologically similar monoascosporic isolates from exotic and native larch. When grown on 2% M.E.A. in Petri dishes at 20 °C, recent or 2-year-old periodic transfers of isolates from exotic larch, covered

TABLE II

Mycelial growth of different isolates on barkmeal of various trees and water agar during a 7-day period

	Diameter* in mm of isolates					
	Exotic larch			Native larch		
Barkmeal of:	16	95	110	28	58	81
Larix decidua	46	48	50	53	60	53
L. laricina	50	52	—†	—†	66	—†
Pinus rigida	45	31	58	49	66	52
P. strobus	44	32	63	42	52	43
Prunus pensylvanica	54	12	65	90	86	90
Quercus rubra	62	51	61	75	90	86
Salix longifolia	48	44	—†	—†	13	—†
Water agar (2%)	21	20	19	25	24	27

*Average of five replicates.
†Not tested.

10 cm plates in 11 days as compared to 7 days for isolates from native larch. Both groups of isolates grew best on M.E.A. and P.D.A. at a temperature of 25 °C. Minimal and maximal temperatures were near 0 °C and 30 °C, respectively, for both groups of isolates. Studies on carbon utilization showed that maximal growth of the fungus was obtained with dextrose, and minimal growth with pentoses (ribose, arabinose, and xylose). Galactose, mannose, and levulose yielded intermediate growth. On barkmeal media, all but two isolates grew equally well. Growth of an isolate from native larch was stimulated on barkmeal of red oak and pin cherry, while apparently inhibited on sand-bar willow. An isolate from European larch was inhibited on barkmeal of pin cherry (Table II). Since these isolates, especially Nos. 58 and 95, are not morphologically similar to the others, this reaction indicates possible varietal differences. The use of these media, however, did not permit a definite separation as for other species of *Leucostoma* (10).

Discussion

The results of this study indicate a pathogenic relation between *Leucostoma kunzei* (Fr.) Munk ex Kern and a canker disease of exotic and native larch. Most successful inoculations were obtained in the greenhouse with seedlings under low temperature and low water supply, and in the field after a period of greatly reduced precipitation. Wright (20) and Jorgenson and Cafley (7) also mentioned a similar effect of drought on the development of this type of canker on Douglas fir and on white and Norway spruce trees. However, under apparently more favorable climatic conditions, as in 1962 and 1963, larch trees heal over the cankers.

The fact that isolates from exotic larch produced more and faster-developing cankers on both hosts than isolates from native larch suggests pathogenic strains. This is further substantiated by the different growth rate of pathogenic isolates in culture. It is possible that these strains resulted from passage to exotic species on which they acquired more virulence. Therefore, this fungus, considered as a weak parasite, may eventually become an important pathogen and cause serious damage, particularly in plantations.

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Résumé

Ce travail résume une étude, effectuée dans le Québec, d'un chancre des branches et du tronc qui a attaqué jusqu'à 40% des mélèzes exotiques en plantation et un certain nombre de mélèzes indigènes dans les peuplements naturels. Le rapport constant entre la maladie et le stade imparfait du *Leucostoma kunzei* (Fr.) Munk ex Kern a conduit à une étude taxonomique de cette espèce.

Les souches monoascosporiques de diverses origines présentent en culture des variations qui ne dépendent pas de la provenance ou de la taille des spores. Cependant les souches isolées des mélèzes exotiques croissent, sur divers milieux, plus lentement que celles provenant des mélèzes indigènes.

L'inoculation des arbres à l'extérieur a montré que les mélèzes exotiques sont plus sensibles à l'attaque de cette maladie que le mélèze indigène et aussi que plusieurs autres espèces de conifères sont infectées par ce champignon. Il semble que l'inoculation et le développement de la maladie soient liés à certaines conditions du milieu, notamment les sécheresses estivales prolongées.