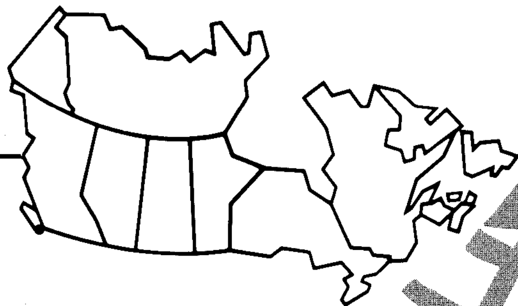
Government of Canada
Gouvernement du Canada

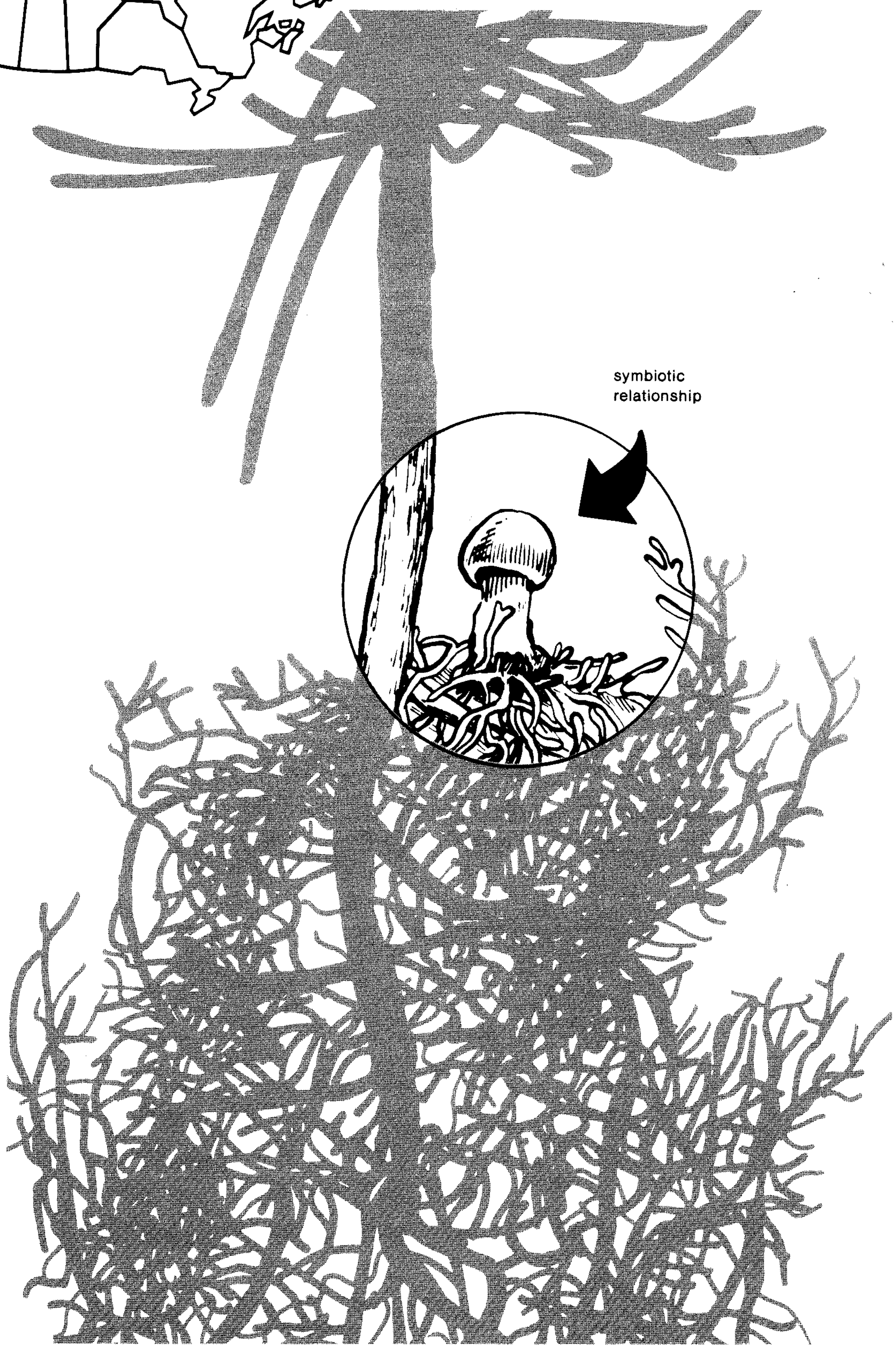
Canadian Forestry Service
Service canadien des forets



Effect of pH and temperature on *in vitro* growth of ectomycorrhizal fungi

J.J. **Dennis**

Information Report BC-X-273
Pacific Forestry Centre



Cover: Mycorrhizal *Hebeloma* fruiting on
container lodgepole pine seedling

Effect of pH and temperature on *in vitro* growth of ectomycorrhizal fungi

J.J. Dennis

Canadian Forestry Service
Pacific Forestry Centre

BC-X-273

1985

Canadian Forestry Service
Pacific Forestry Centre
506 West Burnside Road
Victoria, B.C.
V8Z 1M5

ISSN 0705-3274
ISBN 0-662-14337-X
Cat. No. Fo46-17/273E

Abstract

Forty species (67 isolates) of ectotrophic mycorrhizal fungi were grown at various pH levels and temperatures on agar medium. Optimum pH for growth ranged from 4 to 7; eight isolates grew over a range of seven pH units. None of the fungi grew at a pH greater than 9. Optimum temperature for growth ranged from 15 to 30°C with 45 isolates growing best at 25°C. Only *Sphaerosporella brunnea* grew at 35°C.

Resume

Quarante espbces (67 isolats) de champignons mycorhiziens ectotrophes ont ete cultivés sur gélose à des pH et temperatures varies. Leur pH optimal de croissance variait entre 4 et 7; huit isolats se sont, toutefois, développés dans un intervalle de pH de sept unites. Aucun champignon n'a pousse a un pH supérieur à 9. Par ailleurs, la temperature optimale de croissance variait entre 15 et 30 "C; elle était de 25 "C pour 45 isolats. Seul *Sphaerosporella brunnea* s'est développé a 35 °C.

Introduction

Ectomycorrhizal fungi are beneficial symbionts of forest trees in that they transfer nutrients and water to plants (Skinner and Bowen 1974; Melin and Nilsson 1950). Some fungi are more efficient in obtaining nutrients and water for the plant (Marx 1980), perhaps because they have rhizomorphs which are transporting vehicles (Brownlee *er al.* 1983). Another possibility is that certain fungi are better suited to grow or survive in a particular forest soil.

Until recently, selection of mycorrhizal fungi for nursery inoculation and subsequent outplanting trials was limited because the growth requirements for many of the fungi were not known. Improved growing and culturing techniques have increased fungus availability and selection could be based on the physiological characteristics of candidate fungi that may improve seedling survival or growth. Few physiological studies have been made on large numbers of ectomycorrhizal fungi. Mexal and Reid (1973) reported variation in growth under water stress for *Suillus luteus* (Fr.) S.F. Gray and *Thelephora terrestris* (Ehrh.) Fr. and confirmed tolerance to low water potential of *Cenococcum graniforme* (Sow.) Ferd. and Winge. Marx and Zak (1965) showed how laboratory manipulation of pH affected root colonization and subsequent enhancement of seedling growth by several fungi. Littke *et al.* (1984) examined the nitrogen physiology of *Hebeloma crustuliniforme* in culture and Rygiewicz *et al.* (1984 a, b) examined uptake of nitrogen by ectomycorrhizae formed by this fungus on three conifers. Studies such as these were done *in vitro* because of the complexity of natural soil, but the results might be used to select fungi suited to particular planting sites. Recently Hung and Trappe (1983) studied pH preferences of ten fungi and noted differences not only among species but also among fungus strains. Hacskaylo *et al.* (1965) studied the preferred temperature range of six species of mycorrhizal fungi and again found wide variations.

About 1978, studies were begun at the Pacific Forestry Centre to determine the effect of ectotrophic mycorrhizae on survival and growth of outplanted conifer seedlings. Physiological information was needed on a wide range of mycorrhizal fungi to determine the candidate that would be most beneficial to outplanted stock. The purpose of the research reported here was to determine the pH and temperature requirements for growth of a large number of mycorrhizal fungi which could be used in subsequent experiments on seedling growth and survival,

Materials and Methods

Sixty-seven cultures representing 40 species of fungi were obtained by isolation from sporocarps collected in British Columbia, or they were obtained from other researchers (Appendix I). Cultures were maintained by transferring monthly and incubating in darkness at 20°C. The medium used in both the pH and temperature growth range experiments was a modified Melin-Norkrans agar (Marx and Zak 1965) which was modified further by adding 1.5 g of tryptone per litre of medium.

To determine the effect of pH on growth of the fungi, pH of the medium was adjusted from 2 to 10 at intervals of approximately one pH unit by adding the required amounts of either 1N HCl or 1N NaOH to 1 litre amounts of cooled (45°C) medium immediately before pouring it into 9-cm diameter petri plates. Each plate was center inoculated with a 6-mm agar plug cut from 4-week-old, actively growing (same medium as above) cultures of the test fungus. The pH of the medium was determined using a flat-membrane combination electrode before the plates were inoculated, and again when the final growth measurements were made. Growth (mm) was determined by measuring the colony diameter of five replicate plates per treatment either after 4 weeks incubation in darkness at **20°C** or when the fungus growth in any particular treatment completely covered the medium.

The procedures for the temperature range experiment were the same as for the pH experiment except that the pH of the medium *(5.7)* was not adjusted and cultures were incubated at **5°C** intervals between **5** and 35°C. Data for both experiments were subjected to analysis of variance and the significance of treatment differences was determined using the Student-Newman-Keuls multiple range test (Steel and Torrie 1980).

Results

Detailed results including the significance ($p = 0.05$) of treatment mean differences are given in Appendixes II and III for the pH and temperature experiments, respectively. Table 1 summarizes the results of both experiments.

Growth of all the fungi was affected by pH to varying degrees; the optimum pH ranged from 4 to 7 (Table 1) . Three groups were recognized: fungi that grew best at only one pH, over a range of two adjacent pH units, or over a range of three adjacent pH units. Included in the latter were three *Suillus lakei* isolates and one isolate each of *Suillus tomentosus* and *Suillus breuipes* which had a double optimum (pH 4 and 6). The ability to grow, even slowly, over a range of pH levels also varied. Most of the fungi (45 isolates) grew over a range of four to five pH units. Only eight fungi grew over a range of seven pH units. Numerous isolates were tolerant to extreme acid or alkaline conditions. Of the six isolates that grew at pH 2, four were strains of *Suillus tomentosus*. The others were a strain of *S. breuipes* and *Rhizopogon vinicolor*. None of these fungi grew at a pH greater than 7. Six fungi grew at pH 9, including two isolates of *Hebeloma crustuliniforme*, both isolates of *Clitocybe nebularis* and isolates of *C. suaveolens* and *Lactarius uvidus*. No fungi grew at a pH greater than 9.

The optimum temperature for growth of the fungi ranged from 15 to 30°C (Table 1). Most isolates (46) grew best at either 20 or 25°C. Only one isolate each of *Armillaria ponderosa* and *Cortinarius violaceus* grew · equally well at three temperatures. There were also differences in the ability to grow over a range of temperatures. Thirteen isolates grew over the wide range of six temperatures while eight grew over a range of only three temperatures. Only *Hygrophorus agathosmus* did not grow above 20°C. and only *Sphaerosporella brunnea* grew at 35°C.

Discussion

Using a solid medium in petri dishes allowed many fungi to be tested quickly for growth at various pH levels and temperatures. Fast growing fungi had to be assessed a few days after inoculation because they soon reached the edge of the plates while very slow growers had very little measurable differences after 4 weeks. Extending the incubation period was of no benefit because the medium began to dry out. Both very fast growing and very slow growing fungi, however, could be evaluated.

Results of the pH experiment agreed with results reported by Hung and Trappe (1983). Many isolates grew equally well over a range of pH levels and the double peak (at pH 4 and 6) reported for *Suillus lakei* occurred with five *Suillus* isolates, i.e., three *S. lakei*, one *S. tomentosus* and one *S. brevipes*. Results of the temperature experiment agreed with those reported by Hacskaylo (1965) and also demonstrated growth variability among and within species. Optimum and extreme temperatures varied as much as 10°C. and could not be related to origin of the fungus. The results should help in selecting fungi for experiments on planting sites with extremes in temperatures.

Criteria for the selection of symbiotic fungi have not been well defined. It is logical to select a fungus well adapted to the extremes of a particular site. Observations by Parke *et al.* (1983) have shown the importance of temperature to indigenous mycorrhizal fungi and survival of outplanted seedlings. Fungi that can grow, even slowly, over a broad range of pH levels and temperature may be particularly useful in situations where soil conditions are unknown or may change during the growing period. The results of these experiments should be useful in selecting candidate fungi for mycorrhizal synthesis and subsequent outplanting trials.

Acknowledgements

I thank R.M. Danielson, J.M. Theron, and C.S. Bledsoe for cultures, J.R. Sutherland and J.A. Dangerfield for advice and K. Randle for technical assistance.

References

BROWNLEE, C.J., A. DUDDRIDGE, A. MALIBARI, and D.J. READ. 1983. The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. Plant and Soil 71: 433-443.

HACSKAYLO, E., J.G. PALMER, and J.A. VOZZO. 1965. Effect of temperature on growth and respiration of ectotrophic mycorrhizal fungi. Mycologia 57: 748-756.

HUNG, L.L. and J.M. TRAPPE. 1983. Growth variation between and within species of ectomycorrhizal fungi in response to pH *in vitro*. Mycologia 75: 234-241.

LITTKE, W.R., C.S. BLEDSOE, and R.L. EDMONDS. 1984. Nitrogen uptake and growth *in vitro* by *Hebeloma crustuliniforme* and other Pacific Northwest mycorrhizal fungi. Can. J. Bot. 62: 647-652.

MARX, D.H. 1980. Ectomycorrhizal fungus inoculations: a tool for improving forestation practices. *In* Tropical Mycorrhiza Research. Ed. P. Mikola. pp 13-17. Clarendon Press, Oxford.

MARX, D.H. and ZAK, B. 1965. Effects of pH on mycorrhizal formation of slash pine in aseptic culture. For. Sci. 11: 66-75.

MELIN, E. and H. NILSSON. 1950. Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. Physiol. Plant. 3: 88-92.

MEXAL, J. and C.P.P. REID. 1973. The growth of selected mycorrhizal fungi in response to induced water stress. Can. J. Bot. 51: 1579-1588.

PARKE, J.L., R.G. LINDERMAN and J.M. TRAPPE. 1983. Effect of root zone temperature on ectomycorrhiza and vesicular-arbuscular mycorrhiza formation in disturbed and undisturbed forest soils of southwest Oregon. Can. J. For. Res. 13: 657-665.

RYGIEWICZ, P.T., C.S. BLEDSOE, and R.J. ZASOSKI. 1984a. Effects of ectomycorrhizae and solution pH on [^{15}N] ammonium uptake by coniferous seedlings. Can. J. For. Res. 14: 885-892.

RYGIEWICZ, P.T., C.S. BLEDSOE, and R.J. ZASOSKI. 1984b. Effects of ectomycorrhizae and solution pH on [^{15}N] nitrate uptake by coniferous seedlings. Can. J. For. Res. 14: 893-899.

SKINNER, M.F. and D.G. BOWEN. 1974. The uptake and translocation of phosphate by mycelial strands of pine mycorrhizae. Soil Biol. Biochem. 6: 53-56.

STEEL, R.G.D. and J.H. TORRIE. 1980. Principles and procedures of statistics. 2nd Ed., pp. 137-187. McGraw-Hill Book Company Inc. New York, Toronto, London.

Table 1. Minimum, maximum and optimum temperatures and pH levels for growth of ectomycorrhizal fungi.

Fungi	pH			Temperature (°C)		
	minimum	optimum	maximum	minimum	optimum	maximum
Agaricus augustus	4	5	6	10	25	25
A. silvaticus	4	5-6	6	5	15-20	25
Amanita muscaria	3	6	I	5	25	25
A. porphyria	4	5	6	10	15-25	25
A. silvicola						
Isolate 596	3	6	6	15	25	25
Isolate 5268	4	5	6	15	20	25
Armillaria ponderosa						
Isolate 5107	3	5-6	7	15	20	25
Isolate J120	3	6	6	10	20	25
Isolate 5169	3	6	8	5	15-25	25
A. zelleri						
Isolate J89	3	5	7	5	20	25
Isolate 5144	3	6	7	5	20	25
Isolate 5259	3	5-6	7	5	20	25
Bankera fuligineo-alba	3	5	I	10	15-20	25
Boletus chrysenteron	3	6	6	15	20	25
B. zelleri	3	5	6	5	20	25
Cenococcum geophilum						
Isolate 5223	3	5	6	10	25	30
Isolate 5329	3	5	6	10	20	25
Clitocybe nebularis						
Isolate 5185	3	6	9	5	20	25
Isolate 5291	4	6	9	5	20	25
Clitocybe suaveolens	4	6	9	5	20	30
Cortinarius violaceus	3	6	7	5	15-25	30
Hebeloma crustulinijorme						
Isolate 518	3	6	9	5	20-25	30
Isolate 590	4	6	8	10	25	25
Isolate 5168	4	6-1	9	10	25	30
Hygrophorus agathosmus	5	6	7	5	20	20
Hygrophorus eburneus	4	I	8	5	20	25

Table 1. (continued)

Fungi	pH			Temperature (°C)		
	minimum	optimum	maximum	minimum	optimum	maximum
Laccaria laccata						
Isolate 545	3	7	8	5	20	25
Isolate J68A	3	7	8	5	20-25	30
Isolate J68B	3	6	8	5	20-25	25
Isolate 5233	4	6	7	5	20-25	25
Isolate 5330	4	7	8	10	20-25	25
Lactarius chelidonius	4	6	7	10	25	30
L. deliciosus						
Isolate 529	3	6	7	10	25	30
Isolate J 103	3	5	7	15	20-25	25
L. rufus	3	5	6	5	20-25	25
L. sanguifluus	4	5	6	15	20	25
L. uvidus	3	5-7	9	5	20	25
Lycoperdon marginatum	4	4-6	7	5	25	30
L. perlatum						
Isolate 5167	3	6	7	5	25	30
Isolate 5209	4	5-6	6	5	25	30
Rhizopogon rubesceris	3	5-6	6	15	25	25
R. vinicolor	2	4-5	6	5	20-25	25
R. vulgaris	3	5-6	7	5	25	30
Russula brevipes	3	6	8	5	25	25
Scleroderma bovista	3	6	6	5	25	25
Sphaerosporella brunnea						
Isolate 5228	3	4-5	8	5	30	35
Isolate 5328	3	5-6	8	5	30	35
Stropharia ambigua	3	6	8	5	20	25
Suillus albidipes						
Isolate 5229	3	6	7	15	25	25
Isolate 5237	3	3-5	6	10	25	25
S. brevipes						
Isolate 572	3	4-6	6	15	20	25
Isolate 5222	2	6	8	5	25	25
Isolate 5227	3	4-5	6	10	25	30
Isolate 5256	3	4-6	6	5	25	**30**
S. granulatus	3	6	6	10	25	25

Table 1. (continued)

Fungi	pH			Temperature (°C)		
	minimum	optimum	maximum	minimum	optimum	maximum
Suillus lakei						
Isolate 577	3	4-6	7	5	20	25
Isolate 578	3	4-6	6	5	20	25
Isolate 585	3	4-6	7	10	20	30
Isolate 5193	3	4-6	6	5	15-25	25
Isolate 5213	3	4-6	6	5	20	25
S. luteus	3	6	6	15	20-25	25
S. tomentosus						
Isolate 5138	2	4	7	10	25	30
Isolate 5249	2	6	6	5	25	30
Isolate 5275	2	4-6	7	5	25	25
Tricholomapessundatum						
Isolate 5207	3	4-6	7	5	20	30
Isolate 5244	3	5-6	7	5	25-30	30
Truncocolumellacitrina	3	4-6	6	10	20	25

Appendix I

Identity and origin of the fungi tested for growth response to pH and temperature

Fungi	Isolate number	Year isolated	Origin and tree association (s)*
Agaricus augustus Fr.	5172	1979	Vancouver Island, Goldstream Park Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco)
A. silvaticus Vitt ex Fr.	584	1978	Vancouver Island, Parksville Douglas-fir
Amanita muscaria (Fr.) S.F.Gray	587	1978	Vancouver Island, Victoria watershed Douglas-fir/western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) mix
A. porphyria (Alb. and Schw. ex Fr.) Secr.	547	1976	Vancouver Island, Sooke western hemlock
A. silvicola Kauf.	596	1978	Vancouver Island, Sooke mixed conifers
	5268	1980	Williams Lake lodgepole pine *(Pinus contorta* Dougl.)/Douglas-fir
Armillaria ponderosa	5107	1978	Vancouver Island, Sooke lodgepole pine
	5120	1978	Vancouver Island, Sooke lodgepole pine
	5169	1979	C.S. Bledsoe, locality and tree unspecified
A. zelleri Stuntz and Smith	589	1978	Vancouver Island, Victoria watershed mixed conifers
	5144	1975	Alberta, *R.M.* Danielson #2479 jackpine (*Pinus contorta* Dougl. var. latifolia Engelm.)
	5259	1976	Alberta – *R.M.* Danielson #2491 jackpine
Bankera fuligineo-alba (Schmidt) Coker and Beers	5234	1976	Alberta, *R.M.* Danielson #2192 jackpine
Boletus chrysenteron Fr.	5171	1979	Vancouver Island, Goldstream Park western red cedar (*Thuja plicara* Donn)
B. zelleri Murr.	576	1976	Vancouver Island, Beaver Lake mixed conifer forest
Cenococcum geophilum Fr.	5223	1975	Alberta jackpine
	5329	1981	Oregon, **U.S.A.**

Appendix I (continued)

Clitocybe nebularis (Fr.) Kummer	5291	1980	Vancouver Douglas-fir
	5185	1979	Vancouver Island Douglas-fir solitary
Clitocybe suaveolens (Fr.) Kummer	5206	1979	Vancouver Island – Campbell River Douglas-fir
Cortinarius violaceus (Fr.) S.F.Gray	512	1974	Vancouver Island western hemlock
Hebeloma crustulinijorme (Bull. ex Saint-Amans) Quel.	518	1974	Vancouver Island – Sooke Douglas-fir
	590	978	Vancouver Island – Victoria watershed Douglas-fir
	5168	979	C.S. Bledsoe Locality and trees unspecified
Hygrophorus agathosmus Fr.	5116	978	Vancouver Island – Goldstream Douglas-fir
H. eburneus (Fr.) Fries	J190	979	Vancouver Island – Spectacle Lake mixed conifers
Laccaria laccata (Scop.: Fr.) Berk. & Br.	545	1976	Vancouver Island – Sooke western hemlock
	J68A	1976	Vancouver Island – Cowichan mountain hemlock-(*Tsuga mertensiana* (Bong.) Carr.)
	J68B	1976	Vancouver Island – Cowichan mountain hemlock-fir
	5233	1978	Alberta jackpine
	5330	1978	Oregon, U.S.A. western larch (*Larix occidentalis* Nutt.)
Lactarius chelidonius Peck	5239	1976	Alberta jackpine
L. deliciosus (Fr.) S.F. Gray	529	1975	Vancouver Island – Victoria watershed Douglas-fir
	5103	1978	Vancouver Island – Victoria watershed Douglas-fir
L. rufus (Scop. ex Fr.) Fries	5226	1979	Alberta jackpine
L. sanguifluus Fr.	5204	1979	Vancouver Island – Campbell River Douglas-fir
L. uvidus (Fr.) Fries	J95	1978	Vancouver Island – Sooke Douglas-fir

Appendix I (continued)

Lycoperdon marginatum Vitt.	5254	1980	Alberta jackpine
Lycoperdon perlatum Pers	3167	1979	Vancouver Island – Campbell River spruce (*Picea sitkensis* (Bong.) Carr.) lodgepole pine
	5209	1979	Vancouver Island – Campbell River Douglas-fir
Rhizopogon rubescens Tul.	5240	1976	Alberta jackpine
R. vinicolor Smith	537	1976	Oregon, U.S.A. Douglas-fir
R. vulgaris (Vitt.) Lange	5338	1982	South Africa Monterey pine (*Pinus radiata* D. Don)
Russula brevipes Pk.	5279	1980	Vancouver Island – Victoria lodgepole pine
Scleroderma bovista Fr.	549	1976	Vancouver Island – Victoria lodgepole pine
Sphaerosporella brunnea (Alb. and Schw. ex Fr.) Svrcek and Kubicka	5228	1980	Alberta jackpine
	5231	1980	Alberta jackpine
Stropharia ambigua (Pk.) Zeller	523	1975	Vancouver Island – Victoria Douglas-fir
Suillus albidipes (Pk.) Singer	5229	1979	Alberta jackpine
	5237	1976	Alberta jackpine
S. brevipes (Pk.) Kuntze	572	1976	Vancouver Island – Victoria lodgepole pine
	5222	1979	Alberta jackpine
	5227	1979	Alberta jackpine
	5256	1978	Alberta jackpine
S. granulatus (Fr.) Kuntze	5235	1977	Alberta jackpine

Appendix I (continued)

S. lakei (Murr.) Smith and Thiers	577	1976	Vancouver Island – Beaver Lake mixed conifers
	578	1976	Vancouver Island – Thetis Lake mixed conifers
	585	1978	Vancouver Island Douglas-fir
	5193	1979	Vancouver Island – Gold River Douglas-fir
	5213	1979	Vancouver Island – Victoria Douglas-fir
S. luteus (Fr.) S.F. Gray	5211	1979	Vancouver Island – Victoria western white pine *(Pinus monticola* Dougl.)
S. tomentosus (Kauf.) Snell, Singer and Dick	5138	1979	Alberta lodgepole pine-Douglas-fir
	5249	1979	Alberta jackpine
	5275	1980	Williams Lake lodgepole pine
Tricholoma pessundatum (Fr.) Quel.	5207	1978	Vancouver Island – Campbell River Douglas-fir
	5244	1978	Alberta jackpine
Truncocolumella citrina Zeller	533	1975	Vancouver Island – Victoria watershed Douglas-fir/western hemlock

* Predominant tree species in the forest stand and nearest to the isolation sporocarp.

Appendix II

Growth diameter (mm) of ectomycorrhizal fungi on a modified Melin-Norkrans medium adjusted to various pH levels

Fungi	pH 2	3	4	5	6	7	8	9
Agaricus augustus	0	0	7a	9b	7a	0	0	0
A. silvaticus	0	0	4a	8b	8b	0	0	0
Amanita muscaria	0	3a	6b	7c	8d	4a	0	0
A. porphyria	0	0	4a	7b	4a	0	0	0
A. silvicola								
Isolate 596	0	3a	7b	8c	10d	0	0	0
Isolate 5268	0	0	9a	9a	6b	0	0	0
Armillaria ponderosa								
Isolate J 107	0	2a	7b	9c	9c	3a	0	0
Isolate 5120	0	4a	6b	8c	10d	0	0	0
Isolate J 169	0	3a	6b	7b	8c	1d	1d	0
A. zelleri								
Isolate 589	0	3a	16b	20c	11d	3a	0	0
Isolate J 144	0	7a	10b	10b	12c	3d	0	0
Isolate 5259	0	6a	13b	16c	15c	3d	0	0
Bankera-fuligineo-alba	0	7a	11b	13c	10b	3d	0	0
Boletus chrysenteron	0	1a	5b	6c	7d	0	0	0
B. zelleri	0	11a	16b	18c	14d	0	0	0
Cenococcum geophilum								
Isolate 5223	0	5a	7b	9c	6d	0	0	0
Isolate 5329	0	3a	7b	10c	7b	—	0	0
Clitocybe nebularis								
Isolate 5183	0	7a	25b	38c	41d	15e	15e	15e
Isolate 5291	0	0	20a	24b	34c	15d	18e	12f
C. suaveolens	0	0	16a	27b	36c	33d	24e	18f
Cortinarius violaceus	0	8a	14b	23c	42d	7a	0	0
Hebeloma crustulinijorme								
Isolate J 18	0	3a	11b	11b	14c	12b	10d	8e
Isolate 590	0	0	3a	6b	9c	8d	7b	0
Isolate 5168	0	0	5a	7b	10c	10c	7b	6a
Hygrophorus agathosmus	0	0	0	6a	8b	3c	0	0
H. eburneus	0	0	6a	13b	24c	27d	12d	0

Appendix II (continued)

Fungi	pH 2	3	4	5	6	7	8	9
Laccaria laccata								
Isolate 545	0	5a	12b	18c	25d	29e	18c	0
Isolate J68A	0	4a	11b	24c	31d	38e	26f	0
Isolate J68B	0	6a	16b	23c	37d	34e	18f	0
Isolate J233	0	0	3a	13b	17c	5d	0	0
Isolate 5330	0	0	7a	15b	28c	30d	14b	0
Lactarius chelidonius	0	0	5a	7b	10c	5a	0	0
L. deliciosris								
Isolate 529	0	3a	8b	12c	16d	8b	0	0
Isolate J103	0	3a	6b	9c	6b	3a	0	0
L. rufus	0	10a	20b	21c	13d	0	0	0
L. sanguifluus	0	0	1a	8b	5c	0	0	0
L. uvidus	0	13a	18b	21c	21c	22c	19d	17e
Lycoperdon marginatum	0	0	12a	18b	19b	7c	0	0
L. perlatum								
Isolate J167	0	3a	17b	21c	27d	12e	0	0
Isolate 5209	0	0	14a	22b	21b	8c	0	0
Rhizopogon rubescens	0	16a	20b	22c	23c	0	0	0
R. vinicolor	4a	17b	20c	21c	17b	0	0	0
R. vulgaris	0	18a	20a	26b	27b	3c	0	0
Russula brevipes	0	20a	29b	33c	39d	8e	3f	0
Scleroderma bovista	0	1a	9b	12c	15d	0	0	0
Sphaerosporella brunnea								
Isolate 3228	0	40a	42b	42b	42b	35c	6d	3e
Isolate 5231	0	27a	39b	42c	42c	14d	2e	0
Stropharia ambigua	0	9a	18b	25c	36d	29e	8f	0
Suillus albidipes								
Isolate 5229	0	20a	23b	27c	31d	3e	0	0
Isolate 5237	0	16a	17a	16a	10b	0	0	0
S. brevipes								
Isolate 372	0	11a	12b	11ab	12b	0	0	0
Isolate 5222	3d	17a	22b	25b	28c	3d	3d	0
Isolate 5227	0	19a	20ab	22b	18a	0	0	0
Isolate J256	0	12a	17b	15c	18b	0	0	0

Appendix II (continued)

Fungi	pH 2	3	4	5	6	7	8	9
S. granulatus	0	15a	20b	23c	27d	0	0	0
S. lakei								
Isolate 577	0	13a	16b	14a	17b	1c	0	0
Isolate 578	0	5a	8b	9b	8b	0	0	0
Isolate 585	0	16ab	18a	15b	18a	4c	0	0
Isolate J 193	0	14a	18b	18b	16ab	0	0	0
Isolate 5213	0	12a	15b	14c	15b	2d	0	0
S. luteus	0	13a	17b	18b	20c	0	0	0
S. tomentosus								
Isolate 5138	4a	12b	14c	12b	13b	3a	0	0
Isolate 3249	3a	12b	13bc	14c	16d	0	0	0
Isolate 5275	3a	17b	13c	14bc	16bc	2a	0	0
Tricholomapessundatum								
Isolate 5207	0	3a	14b	21b	24b	3a	0	0
Isolate 5244	0	10a	11a	16b	17b	4c	0	0
Truncocolumella citrina								
Isolate 533	0	15a	18ab	20b	16ab	0	0	0

* Reading across, means followed by a common letter do not differ significantly ($p = 0.05$)

Appendix III

Growth diameter (mm) of ectomycorrhizal fungi on a modified Melin-Norkrans medium at different incubation temperatures

Fungi	Incubation temperatures (°C) 5	10	15	20	25	30
Agaricus augustus	0	4a	8b	12c	13d	0
A. silvaticus	2a	2a	8b	9b	5c	0
Amanita muscaria	2a	3ab	2a	3ab	4a	0
A. porphyria	0	1a	4b	4b	5b	0
A. silvicola						
Isolate 596	0	0	6a	10b	12c	0
Isolate 5268	0	0	5b	8b	1c	0
Armillaria ponderosa						
Isolate 5107	0	0	3a	5b	5c	0
Isolate 5120	0	3a	6b	6c	4d	0
Isolate 5169	2a	3a	4b	6b	6b	0
A. zelleri						
Isolate 589	2a	6b	13c	18d	3a	0
Isolate 5144	2a	3ad	7b	11c	4d	0
Isolate 5259	6a	10ab	16bc	20c	8a	0
Bankera fuligineo-alba	0	2a	4b	4b	3a	0
Boletus chrysenteron	0	0	7a	13b	10c	0
B. zelleri	2a	5b	11c	13d	4b	0
Cenococcum geophilum						
Isolate 5223	0	3a	7b	11c	13d	7b
Isolate 5329	0	3a	6b	8c	6b	0
Clitocybe nebularis						
Isolate 5185	1a	5b	21c	25d	5b	0
Isolate 5291	14a	19b	37c	39d	11e	0
C. suaveolens	6a	16b	30c	34d	24e	3f
Cortinarius violaceus	6a	14b	35c	40c	37c	2a
Hebeloma crustulinijorme						
Isolate 518	1a	3b	11c	14d	13cd	1a
Isolate 590	0	3a	6b	6c	7d	0
Isolate 5168	0	3a	2b	3ab	4c	3a
Hygrophorus agathosmus	3a	3a	1b	7c	0	0
H. eburneus	4a	14b	15b	20c	11d	0

Appendix III (continued)

Fungi	Incubation temperatures (°C) 5	10	15	20	25	30
Laccaria laccata						
Isolate 545	2a	9b	17c	23d	22e	0
Isolate J68A	2a	11b	22c	30d	29d	1a
Isolate J68B	1a	9b	18c	23d	22d	0
Isolate 5233	1a	4b	7c	11d	10d	0
Isolate 5330	0	11a	22b	31c	32c	0
Lactarius chelidonius	0	3a	6b	11c	15d	9bc
L. deliciosus						
Isolate J29	0	3a	4a	8b	10c	1d
Isolate J103	0	0	2a	4b	4b	0
L. rufus	3a	4b	8c	12d	12d	0
L. sanguifluus	0	0	1a	6b	1a	0
L. uvidus	5a	9b	14c	20d	1e	0
Lycoperdon marginatum	3a	8b	14c	21d	24e	8b
Lycoperdon perlatum						
Isolate J167	3a	6b	14c	20d	30e	16f
Isolate 5209	8a	12b	18c	35d	45e	3f
Rhizopogon rubescens	0	0	4a	8b	17c	0
R. vinicolor	2a	5b	9c	14d	13d	0
R. vulgaris	2a	7b	17c	25d	28e	27de
Russula brevipes	8a	14b	24c	35d	41e	0
Scleroderma bovista	1a	2b	4c	5c	6d	0
*Sphaerosporella brunnea***						
Isolate 5228	1a	4b	12c	16d	26e	31f
Isolate 5231	1a	5b	10c	13d	19e	23f
Stropharia ambigua	3a	16b	26c	30d	17b	0
Suillus albidipes						
Isolate 5229	0	0	11a	22b	31c	0
Isolate 5237	0	1a	4b	7c	14d	0
S. brevipes						
Isolate 572	0	0	6a	15b	3a	0
Isolate 5222	3a	3a	3a	16b	29c	0
Isolate 5227	0	3a	8b	17c	23d	2a
Isolate 5256	3a	3a	8b	17c	23d	2a
S. granulatus	0	2a	4b	10c	26d	0

Appendix III (continued)

Fungi	Incubation temperatures (°C) 5	10	15	20	25	30
S. lakei						
Isolate 577	3a	3a	8b	18c	13d	0
Isolate 578	1a	1a	6b	9c	5d	0
Isolate 585	0	2a	3a	6b	2a	2a
Isolate 5193	1a	4a	15b	20b	17c	0
Isolate J213	3a	5b	9c	14d	3d	0
S. luteus	*0*	*0*	11a	24b	27b	0
S. tomentosus						
Isolate J138	0	3a	3a	9b	13c	5d
Isolate 5249	3a	5b	8c	12d	19e	3a
Isolate 5275	3a	9b	11c	18d	30e	0
Tricholomapessundatum						
Isolate 5207	3ab	3ab	4a	6c	2b	3ab
Isolate 5244	1a	1a	7b	1oc	14d	18cd
Truncocolumella citrina	0	2a	3a	5b	3a	0

* Reading across, means followed by a common letter do not differ significantly (p = 0.05)

** Both isolates of *S. brunnea* grew at 35°C (**5228** – 5h and **5231** – 7g) hut would not grow at 40°C.