TESTING NATIVE ISOLATES OF

Cylindrobasidium laeve FOR SUPPRESSION OF

REGROWTH OF *Acer macrophyllum*

by

Deepraj Purewal
B.Sc. Ag. (Hons.), Punjab Agricultural University 1992
M.Sc. (Plant Pathology), Punjab Agricultural University 1995

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Name: Deepraj Purewal

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Examining Committee:

Chair: Dr. L. Lesack

Dr. J.E. Rahe, Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. A.R. Kermode, Associate Professor
Department of Biological Sciences, S.F.U.

Dr. S.P. Lee, IT Systems Consultant
Richmond, B.C.

Dr. J.M. Webster, Professor Emeritus
Department of Biological Sciences, S.F.U.
Public Examiner

April 7, 2004

Date Approved

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Abstract

Bigleaf maple (*Acer macrophyllum*) produces large numbers of sprouts after the main stem is removed and the resultant large crowns interfere with growth of young conifers and constitute a hazard on hydroelectric rights of way. Management mainly relies on use of herbicides in combination with physical and manual control methods. However, recent research on preventing sprouting of stumps by inoculation with naturally occurring fungi has shown promise. The research described in this thesis was undertaken to evaluate the potential of *Cylindrobasidium laeve* for suppressing regrowth from cut stumps of *A. macrophyllum*. Three local isolates of *C. laeve* were applied to cut stumps of *A. macrophyllum* seedlings in June and September of 2001. Growth characteristics of isolates in axenic culture, and when inoculated into cut stems of selected native hardwood and softwood species were examined. Seedlings of *A. macrophyllum* treated in June of 2001 with two of the three isolates from culture media and all three isolates as colonized wood inoculum did not show any significant difference in number of shoots when compared with the negative control at the end of the nine weeks ($P>0.05$). Seedlings treated with one of these isolates from culture medium showed significant reduction in number of shoots as compared to the negative control treatment ($P<0.05$). However, all of the plants treated with *C. laeve* in June 2001 were alive and appeared healthy one year after treatment. None of the isolates suppressed regrowth of *A. macrophyllum* when applied in September of 2001. Growth of *C. laeve* on defined media was much less than on natural media. *C. laeve* grew comparably on three hardwood and three softwood species.

Acknowledgements

DEDICATION

To my Father

"Malkiat Singh Hehar"

Who waited so long for this!

And to the memory of my mother

"Ranjit Kaur Hehar"

Who inspired me to continue my studies!
Who worked very hard together to raise me!
Their impression on me will last forever!

Their unselfish love, their gentleness, their
understanding, and their wisdom that shaped me into the
person that I am today!

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1.0. Introduction and review of literature

1.1. Weeds and woody plants in context

"Weeds are defined as plants that originated under natural environments and, in response to imposed and natural environments, have evolved and continue to do so as organisms that interfere with our desired plants and activities" (Aldrich and Kremer 1997). Plants may be weeds for a variety of reasons. They can be poisonous to humans (e.g., poison oak) or toxic to livestock (e.g., tansy ragwort). They can interfere with crop establishment or production (e.g., quack grass in soybeans). They can create hazardous conditions by encroaching along roadsides or power lines (Walstad and Kuch 1987). Brush or woody plants become weeds whenever they cause problems in forest and rangeland or problems around industrial buildings and structures, railroads and overhead power lines where they may interfere with safety, visibility, transportation, recreation and other human activities (Bovey 2001).

Woody plants are plants that produce secondary growth in the form of wood. The mechanical support provided by the wood allows them to grow taller and effectively compete for available sunlight. They are perennial, and the wood is produced over the lifetime of the plant (Rudin 1997). Control of undesirable woody vegetation is sometimes difficult. Woody plants are adapted to survive

injury from frost, fire, cutting and other disturbances. By removing top growth, apical dominance may be suppressed and dormant buds may be activated, producing new shoots that can grow to produce mature plants unless they are further treated by cutting or other means. Woody plants may be strong competitors of other vegetation as they may grow tall and deprive lower-stature plants of light (Bovey 2001). Negative influences of woody plants on other species may result from competition for water, nutrients and space, rainfall interception, litter accumulation, shading, and/or root competition (Fuhlendorf et al. 1997). The result of this competition is often reduced growth of regenerating conifers, therefore undesirable hardwood plant species are considered to be weeds in forestry (Biring et al. 1996)

Undesirable competing vegetation in forestry is different from weeds in agricultural settings. The main difference between forest and agricultural weeds is that forest weeds are often perennial and native and they resprout vigorously from cut stumps or roots. Undesirable woody plants in forests are mostly primary colonizers of disturbed sites and have important ecological roles, such as contribution of organic matter and nitrogen to soils. They also suppress the commercial species, necessitating some vegetation management (Watson and Wall 1995). A number of different characteristics enable undesirable woody plant species to establish in relatively open environments and thus limit the success of management practices (Fuhlendorf 1999). Many of the woody plants on rangeland or in forests are capable of surviving in stressful environments (water

and nutrient-limited). Plant traits that promote stress tolerance include nitrogen fixation and extensive root systems. Dispersal mechanisms are effective in transporting seeds and depositing them in sites where they can establish and survive. Most undesirable woody plants are at least partially dependent upon animal dispersal of seeds. Dispersal of woody plant seeds by free-roaming wildlife is difficult to control suggesting that management of such plants should focus on stages other than dispersal (Brown and Archer 1987).

Vegetation management in forestry settings is also implemented by utility companies in the maintenance of their rights-of-way. In British Columbia, the Provincial power authority has 70,000 km of power lines located on 11,000 km of designated rights-of-way which must remain clear of vegetation to ensure efficient power service and to prevent electrical hazards (de Jong et al. 1996; Shrimpton et al. 1996)

1.1.1. Biological control of weeds

The application of chemical herbicides has proven to be effective in suppressing resprouting of undesirable woody species as most brush species resprout from cut stumps. Weedicide/herbicide use is encountering increasing public opposition. Where herbicide use is prohibited, the lack of stump treatment can lead to extremely high stem densities. Mechanical means of woody plant control require labor and persistence to achieve control. Woody plants must be treated repeatedly before they will succumb to mechanical control. Equipment

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used for mechanical control is also dangerous (Motooka et al. 1999). With increasing regulation and costs, use of chemical and mechanical means for weed and brush control becomes less attractive, and alternatives such as biological control are receiving more attention (de la Bastide et al. 2000). By definition, "biological control of weeds is the intentional use of living organisms (biotic agents) to reduce the vigor, reproductive capacity, density, or impact of weeds" (Koul and Dhaliwal 2002). The strategies of biological control of weeds can be classified in two broad categories: (i) classical or inoculative, and (ii) inundative or mass exposure.

The classical strategy is based on the introduction of host – specific organisms (e.g. insects, pathogens or nematodes) from a weed's native range into regions where the weed has established and become a widespread problem. The classical approach may require several years to achieve adequate control while the agent population builds up to levels sufficient to impact the weed population. It is most effective against introduced weeds that form dense, stable stands on uncultivated land. Rust and other self dispersing agents which cause epidemics after their release, such as *Puccinia chondrilla* which resulted in a 79% reduction of skeleton weed (*Chondrilla juncea*) in Australia, are examples of this biological control method. Before imported pathogens are released, however, they must undergo a thorough screening process to ensure host specificity and no effect on economically important crop species (Mortensen 1986). In Canada, 20 weed species have been targeted so far, control agents have been

established on 18, and the density of 11 has been reduced in at least some habitats (Harris and Shamoun 2003).

The inundative strategy attempts to overwhelm a weed infestation with massive numbers of a biotic agent in order to attain weed control in the year of release. In contrast to classical biological control, inundation involves timing of agent release to coincide with periods of weed vulnerability to the agent, and formulation of the agent to provide rapid attack of the weed host. The inundative strategy is sometimes called the *bioherbicide* approach, since it involves application of microorganisms where and when the weed is a problem, in a manner similar to herbicide applications. Microbes used as control agents include bacteria, viruses, and fungi. The most useful of the microorganisms in the biological control of weeds are the fungi (OTA 1995, Strobel 1991). Because many bioherbicides are formulated from fungal spores, they are often called "mycoherbicides" (Koul and Dhaliwal 2002). Mycoherbicides typically have high virulence and can be mass-produced. Pathogens identified as potential biological control agents may be found in a weed species' original range or in the region into which the weed was introduced.

Many fungi have fairly narrow host-ranges and thus low risk of detrimental effects on non-target species. On the other hand, specificity may make production, storage and application of certain mycoherbicides more difficult. Fungi have several characteristics that hinder their use as mycoherbicides. Most

will only infect from spores and require moist conditions to infect (OTA 1995, Auld 2000). Compared with chemical pesticides, mycoherbicides take longer to produce control in most cases (Strobel 1991).

In a recent review, TeBeest (1996) states, "the science of using plant pathogens to control weeds is almost as old as the science of plant pathology". The use of plant pathogens as biological control agents was reported as early as 1893 (Wilson 1969). In 1960s the wilt fungus *Acermonium diospyri* was first used to control persimmon trees (*Diospyros virginiana*) in the Oklahoma rangelands (Burges 1998). The first commercial microbial herbicides were marketed in the early 1980's. DeVine™, registered with the US Environmental Protection Agency in 1981, contains *Phytophthora palmivora* and is used to control strangler vine (*Morrenia odorata*) in citrus plantations in Florida. It is an aqueous suspension of live mycelium and chlamydospores and is applied directly to the soil (Kenney 1986). Collego™, a wettable powder formulation of spores of *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, registered in 1982, controls the legume weed northern jointvetch, *Aeschynomene virginica,* in rice and soybeans (Burges 1998). Several other plant pathogenic fungi have been developed as potential herbicides, but none has yet appeared on the market since the introduction of DeVine and Collego (Burges 1998). DeVine and Collego were both withdrawn in 1994, because their limited sales were unable to sustain the costs of production and marketing. DeVine has been re-introduced, and Collego may reappear in the

near future (Burges 1998). In 1989, there were at least 109 projects researching 105 fungal taxa on 69 target weeds (Charudattan 1991).

1.1.2. Biological control of undesirable vegetation in forestry

Considerable research has been conducted on biological control of agricultural weeds in Canada during the past few decades but little has been directed towards the control of forest weeds and undesirable vegetation through the use of plant pathogens. In forestry, native plants may increase rapidly after logging and suppress tree regeneration. Mechanical and herbicidal control methods are expensive and have non-target effects of environmental concern. For forest vegetation management the use of mycoherbicides appears to have much potential (Markin and Gardner 1993, Harris and Shamoun 2003). Research on the potential of biological control for management of forest weeds in Canada started in the early 1970's when Dr. R.E. Wall of the Maritime Forestry Center initiated investigations on the possibility of using pathogens for controlling major weed species in plantations and young forests in New Brunswick, Nova Scotia and Prince Edward Island (Singh 1988). Interest in the use of biological controls in forestry is increasing markedly, and far greater emphasis is expected in the future.

The Pacific Forestry Center at Victoria, B. C., has initiated studies to identify native facultative biotrophic fungi that might be used as mycoherbicides

to suppress forest weeds (Sieber and Dorworth 1994). Research was initiated to generate alternatives to chemical herbicides or silvicultural methods, due to rising environmental concerns about use of chemical control methods. An increasing number of forest areas within North America are effectively herbicide-free, either by imposition of prohibitory statutes or because special interest groups have made the use of pesticides impractical or impossible (Dorworth 1995). Some degree of weed control is achieved by prescribed burning of logging waste but increasingly, organized public reaction has resulted in the reduction or termination of burning in parts of both Canada and the U.S.A. (Dorworth 1995).

Dorworth (1995) has developed a novel inoculation strategy to control red alder (*Alnus rubra*) by inserting a pellet loaded with the fungus *Nectria ditissima* into the stem of the tree using a novel spring loaded 'hammer'. The need for wound inoculation limits the widespread use of this microbial herbicide. *Chondostereum purpureum* may offer a cost effective, environmentally safe alternative to chemical herbicides for use in reforestation sites and utility company rights-of-way. *Chondrostereum purpureum* is registered in eastern Canada by MycoForestris Inc. under the trade name Myco-Tech™ (Information Forestry Newsletter – 2003). Research was started by Drs. Ron Wall and Simon Shamoun of the Canadian Forest Service and continued by Dr. Will Hintz at the University of Victoria. Research has resulted in the development of a formulation of *C. purpureum* that can be used to maintain clearings by the prevention of resprouting from undesirable trees. *Chondrostereun purpureum* invades the

cambium and prevents resprouting thus offering an effective control. This research was supported by federal and provincial agencies including the B.C. Hydroelectric Power Company, the Canadian Forest Service, the B.C. Ministry of Forests, and the Forest Pest Management Institute. It has taken more than 14 years from the time when Dr. Wall and others first set out to find a biological control candidate for red-alder and other undesirable tree species.

The procedure in developing biological control consists of determining the suitability of the weed problem for biological control which includes the study of biology and ecology of the weed, surveying the weed for natural enemies in both naturalized and native habitats, studying the biological characteristics and host relations of the natural enemies to determine how they may be exploited, evaluating the effectiveness of the natural enemies, and implementing the natural enemies in weed control (Bovey 2001).

1.2. Bigleaf Maple – *Acer macrophyllum*

Bigleaf maple (*Acer macrophyllum* Pursh), also called broadleaf or Oregon maple, is a deciduous, broadleaved tree native to the Pacific coastal forest region of southwestern British Columbia and the northwestern United States. There are no other varieties or subspecies of *A. macrophyllum* in British Columbia (Lohbrunner 2001). *Acer macrophyllum* commonly grows on stream banks and in moist canyon soils where it sometimes forms pure stands (Sieber and Dorworth 1994).

1.2.1. Uses of *Acer macrophyllum*

Bigleaf maple is locally significant in British Columbia for the manufacture of furniture, musical instruments, interior paneling, and other select uses such as large bowls turned from maple burls (Peterson et al.1999). The wood of maples is weather resistant (van Gelderen et al.1994). Among maple species, bigleaf maple is considered a softer wood, and is used to make wooden tools, kitchen utensils, furniture, veneer, moulding, pallets, pulpwood, and hardwood plywood, as well as for firewood (Peterson et al. 1999).

1.2.2. *Acer macrophyllum* - Role in the ecosystem

Acer macrophyllum plays an important role in forest ecosystems. This includes cycling nutrients; providing nurse sites on the bole and branches for over 130 species of lichens, liverworts, mosses, and ferns (Nadkarni 1984) and fungi; providing food, cover, and nesting sites for birds, small mammals, insects and amphibians; and broadening the diversity of forest communities (Peterson et al.1999). The total weight of epiphytes on a mature *A. macrophyllum* tree may be four times the weight of the host tree's foliage, and epiphyte mats up to 30 cm thick have been reported. These epiphytes are composed of bryophytes, lichens, club mosses and ferns (Peterson et al. 1999).

Acer macrophyllum litter provides a rich nutrient reserve for forest sites. The rapid cycling rates of this litter can benefit surrounding Douglas-fir trees, *Pseudotsuga menziesii*, by increasing the availability of certain elements to the tree roots. The mull humus that develops where maple litter is deposited is beneficial also to western red cedar (*Thuja plicata*) (Peterson et al. 1999).

Acer macrophyllum is gaining recognition as an important species in "mixed wood" plantations. It not only contributes to enhanced nutrient availability, but also to structural and species diversity and to aesthetics in coastal forests (Peterson et al.1999). *Acer macrophyllum* displays resistance to some root rot diseases. It is immune to the pathogen responsible for laminated root rot, *Phellinus weirii*, which attacks conifers in the genera *Pseudotsuga, Abies, Tsuga, Picea and Pinus*. Growing maples in disease centers can prevent the spread of this pathogen to conifers by providing physical barriers to its spread (Lohbrunner, 2001).

When planted along stream banks and steep slopes, *A. macrophyllum* provides resistance to erosion because of the soil binding capabilities of its roots. Dead trees that topple into streams regulate water flow and are an important component of stream habitat (Peterson et al. 1999).

1.2.3. *Acer macrophyllum* – A weed

Foresters often consider bigleaf maple as a weed because it produces

large quantities of coppice after the main stem is removed and the resultant large

crowns interferes with growth of young conifers (Dorworth 1989). It is an

important competitor of conifer seedlings especially Douglas fir on some of the

most productive sites in British Columbia (Lohbrunner 2001). Competition from

fast growing hardwood trees is a major problem endemic to conifer regeneration

sites that results in conifer mortality, reduced growth, delays in harvesting time,

increased costs related to forest management and decreases in annual allowable

cut (Wall et al. 1992).

Acer macrophyllum is considered a high hazard weed on hydroelectric

rights of way. Its rapid regrowth from cut stumps and weakly anchored stems can

contribute to power outages and poses a risk to workers and public (BC Hydro

Vegetation Management Manual 1997).

1.2.4. Control options for *Acer macrophyllum*

Bigleaf maple resprouts vigorously after cutting of mature trees and is

generally difficult to control. Management mainly relies on use of herbicides in

combination with physical and manual control methods. Manual and mechanical

brushing techniques for *A. macrophyllum* include cutting, girdling, stump capping,

burning, mowing and removal of stumps. Most of these techniques are labor

intensive and give only partial or short-term control. Cutting maple sprouts during mid- to late-summer results in a slight reduction in vigor of resprouting bigleaf maple. Girdling resprouted bigleaf maple is difficult, considering the large number of sprouts that require treatment and is generally not successful because vigorous resprouting often follows the treatment (Hart and Comeau 1992).

A number of herbicides that are registered for forestry and industrial use have varying degrees of efficacy on *A. macrophyllum*. Glyphosate (Roundup®/Vision®), imazapyr (Arsenal®), hexazinone (Estron 600®), Velpar L®/Pronone 5G®/Pronone 10G®) and triclopyr ester (Release®) provide <25-90% control when they are used as foliar treatments. Foliar herbicides mostly cause top kill (Lohbrunner 2001). Hack and squirt treatments, involving injecting herbicide into slits in the bark created with an axe, can provide effective control of bigleaf maple. Total eradication of undesirable vegetation is seldom feasible without chemical herbicides (Burges 1998).

Treatment of cut-stumps of big leaf maple with glyphosate (Carbopaste®), trichlopyr ester (Garlon®/Release®), 2, 4-D amine (Forestamine 500®/ Forestamine 250®/ Dow Formula 40F®/Silvamine 500®) provides 25-100% control. Trichlopyr ester (Garlon®/Release®) and 2, 4-D ester (Weedone CB®) used as basal bark treatments provide <25-100% control. Glyphosate (EZ-Ject®) is used for stem/stump injection of big leaf maple (Lohbrunner 2001).

There is potential for use of fungi and microbes to control bigleaf maple, but actual use does not yet occur. *Chondrostereum purpureum* was successful in controlling red alder but showed no effect on growth of *A. macrophyllum* when applied in a nutrient-rich paste formulation to either cut stump surfaces or to wounds in the tree bark with the intention of infecting the wound and overcoming the tree's defenses (Wall 1996). However, preventing resprouting of stumps of *A. macrophyllum* by inoculation with other naturally occurring fungi may have potential. Cut and treat methods using inoculation with fungi could become a physical/biological control option in the future (BC Hydro Vegetation Management Manual 1997).

1.2.5. Fungi associated with *Acer macrophyllum*

Fungi can cause considerable damage in bigleaf maple but decay is not usually a serious problem in young healthy trees (Hepting 1971). Fungi such as *Heterobasidium annosum*, *Fomitopsis pinocola*, *Polyporus berkeleyi* and *Inonotus dryadeus* can invade wounds in the stem and branches, reducing the tree to a hollow shell (Hepting 1971). In a survey of endophytic fungi of aerial tissues of *A. macrophyllum*, Sieber and Dorworth (1994) identified *Cryptodiaporthe hyterix* (Tode) Petrik (teleomorph of *Diplodina acerina* (Passerini) Sutton) and *Glomerella cingulata* (Stoneman) Spaulding and H. Schrenk (teleomorph of *Collectotrichum gloeosporides* (Penzig) Penzing and Saccardo) as potential candidates for biological control. Stem wound inoculations with endophytic *C. hysterix* induced circumferential cankers in 6-month old *A.*

macrophyllum. Seedlings and growth of host callus was inhibited in dual culture
(Sieber et al. 1990).

Sieber and Dorworth (1994) examined species composition, species
frequency, and density of the endophyte assemblages in leaves and twigs of
bigleaf maple at seven sites that are with one exception, situated on Vancouver
Island. They reported that *Phomposis* sp. and *Glomerella cingulata* were found
commonly in Jordan River area and Courtenay, and *Phomopsis* sp. was the
second most frequently isolated endophyte of *A. macrophyllum* in Vancouver.
Cryptosporiopsis abietina occurred on *A. macrophyllum* most frequently at Port
Renfrew, where the amount and frequency of rain was highest. *Diplodina acerina*
was found to be the main colonizer of twigs of *A. macrophyllum* at most sites but
occurred rarely in leaves. Stem wound inoculations with endophytic *D. acerina*
isolates induced the formation of circumferential cankers on 60%-80% of
inoculated 6-month-old *A. macrophyllum* seedlings (Sieber and Dorworth 1994).

Phomposis sp. was also isolated from a severely cankered coppice of
A. macrophyllum by Dorworth (1989). The use of *Phomopsis* spp. as
mycoherbicides would pose problems in quality control, in patenting, and in
registration because identification of *Phomposis* species that is based on
morphology alone is difficult if not impossible (Sieber and Dorworth 1994). Maple
(*Acer*) species are known to be the best shade tree hosts of *Verticillium* spp.
(Stipes and Hensen 2000). Verticillium wilt (*Verticillium albo-atrum*) is very

severe on ornamental bigleaf maples and occasionally kills young forest trees (Hepting 1971).

Other fungi that have been reported to be associated with *A. macrophyllum* are *Alatoapora acuminate* and *Articulospora tetracladia* (Bandoni 1972), *Armillaria mellea* (root rot) (Anderson and Ullrich 1979), *Aleurodiscus* sp., *Bjerkandera fumosa*, *Bondarzewia berkeleyi*, *Botryobasidium pruinatum*, *Calocera cornea*, *Chlorociboria aeruginascens*, *Ciboria herbarum*, *Coriolus hirsutus*, *Crepidotus mollis*, *Crustomyces subabruptus*, *Cylindrobasidium laeve* (*C. evolvens*, *Corticium evolvens*) (Lowe 1977). Overmature bigleaf maples are often decayed by root-rot (*Armillaria* spp.) and butt rots (*Ganoderma applanatum* and *Oxysporum populinus*) (Hepting 1971).

1.3. *Cylindrobasidium laeve* – a potential mycoherbicide

Watson (1991) reviewed the use of phytopathogenic fungi for biological control purposes. *Cylindrobasidium laeve* (Pers. Fr) Chamuris has been proposed for use as a biocontrol agent to suppress the regrowth of cut stumps of bigleaf maple. This fungus is native to British Columbia and is well represented in the Canadian Forest Service – Pacific Forestry Center herbarium collection and host parasite index. It has been reported from Europe, North America, South Africa and other continents (Nakasone 1990). *Cylindrobasidium laeve* is a

pioneer colonizer of various kinds of recently dead deciduous and coniferous wood, especially fresh cut surfaces (Vasiliauskas and Stenlid 1998). It causes white rot on woody gymnosperms and angiosperms throughout Canada and in the USA throughout the northern states, south to Missouri and Washington, D.C., and Arizona. White rot fungi degrade cellulose, hemicellulose and lignin, making the wood stringy, spongy or mottled (Nakasone 1990). Although *C. laeve* causes white rot, the fungus itself is regarded as a weak decayer, and of little importance in living trees (Roll Hansen and Roll Hansen 1980).

Cylindrobasidium laeve is used in South Africa for the treatment of cut stumps of black and golden wattles (*Acacia mearnsii* and *A. pycantha*) (Morris 1995). The product Stumpout™ was registered South Africa in 1997 for use as a proprietary formulation of *C. laeve* to treat and kill wattle stumps. Stumpout™, produced in a small factory on the premises of the Plant Protection Research Institute, Weed Pathology Unit, Stellenbosch, consists of live basidiospores in an oil formulation. The product is diluted with sunflower oil, and is painted onto a freshly cut surface of tree stumps. The stumps die within a year of treatment. Currently, tests are being carried out in South Africa to determine the efficacy of the product against various alien weed species (Lennox et al. 1999). The success of *C. laeve* on *A. mearnsii* and *A. pycantha* and its association to bigleaf maple in B. C., led to the hypothesis that it may be a suitable candidate for management of bigleaf maple (Lohbrunner 2001).

Stumpout™ is called a 'fungal inoculant' rather than a 'bioherbicide' for several reasons:

(a)   The word herbicide implies an agent that kills plants whereas *C. laeve* is a weak pathogen only effective on cut stumps and not standing trees.

(b)   The term 'inoculant' stresses that the agent is applied in a manner that is different from modes of application that are used for herbicides.

(c)   Many farmers are conversant with the use of Rhizobium seed inoculants and the specific handling that these living organisms require (Morris et al. 1998).

Host specificity of an agent is a prime consideration in any biological control program. Host specificity and efficacy are two concerns that affect early decisions in the screening and development of candidate agents (Charudattan 1988). In the United States, *C. laeve* has been found on species of *Acer, Betula, Fagus, Magnolia, Morus, Pinus, Populus, Psuedotsuga* and *Salix* (Far 1995). It has been collected mainly from hardwood and shrub species. In British Columbia, *C. laeve* has been reported to occur on *Abies lasiocarpa, Acer macrophyllum, Alnus rubra, Cytisus scoparius, Osmaronia cerasiformis, Picea glauca, Populus trichocarpa, Salix scouleriana, Tsuga heterophylla* and *Sambucus* species (Lowe 1991). There are no reports of presence of *C. laeve* on conifers on Vancouver Island (Harry Kope[1], Personal Communication, 2001).

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[1] Harry H. Kope Ph.D., P.Ag. Contact Biologicals 17 Jedburgh Road, Victoria, British Columbia, V9B 1K7 Phone/Facsimile (250) 727-0514 Email hkope@islandnet.com

1.3.1. Nomenclature and hierarchical classification of *Cylindrobasidium laeve*

Cylindrobasidium laeve (Pers. Fr) Chamuris has also been called *Corticium laeve, Corticium evolvens* and *Cylindrobasidium evolvens* (Chamuris 1984). The genus *Cylindrobasidium* was erected in 1974 to accommodate *Thelephora evolvens* Fr. (= *Corticium laeve* Pers.). Julich (1974) argued that *T. evolvens* and *C. laeve* were synonyms (Julich 1974). Both names were sanctioned but the earlier name *Cylindrobasidium laeve* is generally given priority over *Thelephora evolvens.*

The current classification of *C. laeve* is as follows (Kendrick 2000):

Kingdom – Eumycota

Absence of mobile cells in the life cycle. Phyla Chytridiomycota, Zygomycota and Dikaryomycota belong to this kingdom.

Phylum – Dikaryomycota

Phylum includes subphyla Ascomycotina and Basidiomycotina. Hyphae have chitinous walls and perforate septa, dikaryotic phase is present in the life cycle, i.e., sexually compatible nuclei from different mycelia pair off but do not fuse immediately to form diploid zygotes.

Sub Phylum – Basidiomycotina

Exogenous meiospores (basidiospores) are produced on basidia.

Class - Holobasidiomycetes (=Homobasidiomycetes)

Basidiomycetes in which the basidia are not divided by septa belong to this class. Most holobasidiomycetes develop fleshy, corky or woody basidiomata. The Holobasidiomycetes comprise two interrelated series – Hymenomycetae and Gasteromycetae

Order – Aphyllophorales

Aphyllophorales has 400 genera and 1,200 species. Its name is translated as 'without gills'. Aphyllophorales include eight families with conspicuous but different basidiomata – the club and coral fungi, tooth fungi, the dry rot fungi, the paint fungi, the 'split-gills' fungi and the bracket fungi. Most members of this order are saprobic on wood, some ectomycorrhizal, some attack structural timbers and the wood or roots of living trees.

Family – Corticiaceae

Hymenium may be smooth or wrinkled or toothed, basidiospores smooth in outline, colorless or pale and non-amyloid. Fungi of this family have monomitic hyphal system (basal tissue usually composed of only one kind of hypha). Basidiomata are effuse/resupinate (spread out) on the surface of decaying wood.

Genus – *Cylindrobasidium*

Hymenial surface even, cream coloured. Basidia 40 – 80 um long, cylindrical to narrowly clavate, with clamps at the base, the basal parts not abruptly constricted. Spores hyaline, thin walled, non-amyloid, four per basidium (Julich, 1974).

Species – *laeve*

1.3.2. Characteristics of *Cylindrobasidium laeve* in axenic culture

Most wood inhabiting basidiomycete fungi can be cultured on agar media. These fungi have been studied in culture for about a century. The published literature is mainly focused on species in the Agaricales and Polyporaceae. In culture, most wood inhabiting basidiomycete fungi grow vegetatively but only occasionally develop fertile hymenia or teleomorph. Only a few species of the Corticiaceae are described in culture (Nakasone 1990). The first descriptions of about 130 species of Corticiaceae in culture were published in 1958 (Nakasone 1990). Since then, many more species have been studied and their cultural descriptions have been published, but still there is no information published about *C. laeve* with regard to its sporulation, temperature and light requirements.

The major growth needs of wood inhabiting fungi are water, oxygen, a favorable temperature range, a digestible substrate, a favorable pH range,

essential minerals and, in many instances, preformed growth factors such as organic nitrogen compounds and vitamins (Zabell and Morrell 1992). Most wood inhabiting basidiomycetes grow vegetatively in culture, and produce fertile hymenia only occasionally (Nakasone 1990). In the development of Stumpout™, a range of media and environmental conditions were tested to induce production of basidia and basidiospores. Optimal basidiospore production was obtained by first growing *C. laeve* on a modified potato-marmite-dextrose medium for 3 days at 25 °C. Then small blocks of this agar were transferred to Petri dishes containing small autoclaved discs of *A. mearnsii* (2-3 cm diameter, 2mm thick). These plates were incubated at 19 °C and a 12-hour photo phase under fluorescent and near UV light (M. Morris)[2].

Nakasone (1990) reported that colonies of *C. laeve* on malt extract agar medium were white, moderately thick and downy to cottony toward margins at 2 weeks. Colonies of *C. laeve* were 79-86 mm diameter at 1 week and 90+ mm at 2 weeks. Aerial hyphae were thin walled, septate, sparsely branched and 3-5 um in diameter. Submerged hyphae were moderately to frequently branched and 2 - 6.5um in diameter. By 6 weeks, fungal mats showed moderately thick growth with raised or even margins. Aerial hyphae were similar to submerged hyphae except that they were sparsely to moderately branched. Cultures of *C. laeve* often degenerate when maintained on MEA or malt syrup agar. Degenerated cultures were sodden and slow growing (Nakasone 1990).

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[2] Agricultural Research Council, Plant Protection Research Institute, Weeds Research Division, P/Bag X5017, Stellenbosch, 7599, South Africa, unpublished data, 1999.

Davidson et al. (1938) reported that some wood-decaying fungi that cause white rots formed dark diffusion zones or "coronas" under the fungus mats on media containing small quantities of gallic or tannic acid. In contrast, species that cause brown rot gave no reaction. The "corona" was considered to be the result of oxidation of gallic and tannic acids. It was suggested that the production of a corona could be used for identification purposes (Davidson et al. 1938). Nakasone (1990) recorded the oxidase reaction of *C. laeve* on gallic and tannic acids after 1 and 2 weeks of incubation. *Cylindrobasidium laeve* gave a negative or weakly positive reaction on gallic acid and a strong, positive reaction on tannic acid.

1.3.3. Effect of *Cylindrobasidium laeve* on regrowth of *Acer macrophyllum*

Lohbrunner (2001) evaluated the ability of *C. laeve* to suppress regrowth of cut stumps of bigleaf maple seedlings under greenhouse conditions. The tests used 2nd leaf potted seedlings and four isolates of *C. laeve* that were obtained from the Canadian Culture Collection[3]. The seedlings were cut to an approximate stump height of 15 cm in August and mycelium of the fungal isolates was applied immediately to the cut surfaces and covered with Parafilm™ to minimize moisture loss. None of the isolates of *C. laeve* appeared able to

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[3] Canadian Collection of Fungal Cultures (CCFC), Ottawa.

colonize the cut stumps, and none of the isolates suppressed sprouting from the cut stumps in comparison with the untreated controls. In discussing these results, Lohbrunner pointed out that the isolates of *C. laeve* that were evaluated were from eastern Canada, and suggested that in any future work local isolates should be evaluated using different methods of inoculation and at different times of the year.

1.4. Objectives of the present research

The research described in this thesis was undertaken to continue evaluation of the potential of *C. laeve* for suppressing regrowth from cut stumps of bigleaf maple. The primary objective of this research was to evaluate the potential of local isolates of *C. laeve* to suppress regrowth from cut stumps of bigleaf maple following application made early and late in the growing season and using two different methods of inoculation. Secondary objectives were to evaluate growth characteristics of local isolates of *C. laeve* in axenic culture, and when inoculated into cut stems of selected native hardwood and softwood species.

2.0. Methods and Materials

2.1. Effect of *Cylindrobasidium laeve* on regrowth of cut stumps of *Acer macrophyllum*

2.1.1. Fungal isolates and fungal inoculum

Cylindrobasidium laeve was isolated from fallen *A. macrophyllum* at several locations on Vancouver Island, British Columbia during December 2000 by Dr. Harry Kope[4]. Isolates provided by Dr. Kope were maintained in Petri plates on malt extract agar (MEA) medium at room temperature in the dark. Subcultures of the isolates were made approximately every 2 weeks.

The effects of three different isolates of *C. laeve* on regrowth of cut stumps of *A. macrophyllum* were compared. Inoculum for these experiments consisted of pieces of colonized MEA, and pieces of colonized stem segments of *A. macrophyllum*. The colonized stem segments were prepared from 1-year-old growth obtained from a bigleaf maple tree growing on the Simon Fraser

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[4] Harry H. Kope Ph.D., P.Ag. Contact Biologicals 17 Jedburgh Road, Victoria, British Columbia, V9B 1K7 Phone/Facsimile (250) 727-0514 Email hkope@islandnet.com

University campus. This was cut into segments approximately 4 cm in length and wound-inoculated with pieces of colonized MEA taken from the edges of 8-day old cultures of *C. laeve*. The inoculated segments were then placed in plastic bags along with pieces of moist seed germination paper (Anchor Paper, Chicago IL) and kept in the dark at room temperature for approximately 4 weeks prior to use.

2.1.2. Bigleaf maple trees

One hundred and fifty, 1-year-old *A. macrophyllum* seedlings in 4 - L pots were obtained from Linnaeus Nursery, Langley B.C. on April 26, 2001. They were kept in a greenhouse at the B.C. Ministry of Forests Green Timbers Nursery, Surrey, B.C. Plants were repotted into larger pots twice during the experiments, ultimately ending up in 11-L pots. Seventy-two plants were used for the June - 2001 experiment and the remaining plants were kept for the September - 2001 experiment. Plants were kept in an unheated greenhouse with unsupplemented ambient light, at Green Timbers Nursery, Surrey, B.C. Plants were watered and fertilized on a regular basis with the standard regime used at the green house.

2.1.3. Experimental design and treatments

Experiments to evaluate the ability of isolates of *C. laeve* to prevent regrowth from cut stumps of *A. macrophyllum* were initiated on June 1 and

September 8, 2001. The shoots of potted seedlings were excised 15 cm above the crowns and the cut stumps were treated immediately. Treatments consisted of negative control, positive control (Garlon 4: Canola oil, 3:7 v/v), *C. laeve* mycelium in MEA agar (12-day old cultures, three isolates), and *C. laeve*-colonized stem segments (three isolates). Inocula were inserted into slits made in the freshly cut stump surfaces with a sterilized blade and covered immediately with plastic film (Saran Wrap) to conserve moisture. Negative control plants were cut at 15 cm and received no additional treatment. There were eight treatments in total with nine replicate trees for each treatment, arranged in a completely randomized design. Data on growth parameters including number of new shoots, average length of the shoots, and number of leaves and visible fungal growth for the summer experiment were collected weekly for 9 weeks starting from June 1, 2001.

The remaining *A. macrophyllum* plants were repotted into 11 - L pots and kept for the September experiment. Treatments for the September experiment were the same as described for June experiment. The number of new shoots, average length of shoots and number of leaves for plants treated in the early September experiment were measured on May 20, 2002.

2.1.4. Analysis of data

Data were analyzed using JMPIN version 4.0.3 (Academic) statistical software (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513).

Analysis of variance (ANOVA) was used to compare means between treatments at the end of 9 weeks. The Tukey-Kramer HSD test for all comparisons was used to determine which means were significantly different from one another, with $P< 0.05$.

2.2. Growth characteristics of local isolates of *Cylindrobasidium laeve* in axenic culture

2.2.1. Growth on three undefined media

Growth of three isolates of *C. laeve* was compared on sterile MEA, potato dextrose agar (PDA) and V-8 agar (150 ml V8 juice, 3 g $CaCO_3$, 15 g agar, 850 ml water) in 9 cm Petri plates. Each plate, containing 18-20 ml of medium, was inoculated in the center with a piece (approximately 4 mm diameter) of colonized agar cut from the edge of a 2-week old colony growing on malt extract agar. The inoculated plates were placed upside down in the dark at 22 ^{o}C. There were three replicate plates for each isolate on each medium. Colony diameters were measured and recorded every 2 days until the growing colonies reached the edges of the plates.

2.2.2. Effect of temperature and carbon source in defined media on rate of growth of *Cylindrobasidium laeve*

Growth of the three isolates of *C. laeve* was compared at 5 °C, 15 °C and 22 °C on MEA and on defined media containing basic salts and 1% glucose or 1% maltose as carbon sources. The basic salts consisted of $(NH_4)_2SO_4$ - 2.64 g, KH_2PO_4 – 2.38 g, K_2HPO_4 – 5.65 g, $MgSO_4 \cdot 7H_2O$ – 1 g, $CuSO_4 \cdot 5H_2O$ – 0.0064 g, $FeSO_4 \cdot 7H_2O$ – 0.0011 g, $MnCl_2 \cdot 4H_2O$ – 0.0079 g, $ZnSO_4 \cdot 7H_2O$ – 0.0015 g, added to 1 litre of distilled water along with 15 g of agar. Plates were prepared and inoculated as described in 2.2.1., with three replicates for each combination of isolate, medium and temperature of incubation. Colony diameters were measured and recorded every 2 days.

Data on fungal growth rates in culture were analyzed by comparing regressions of colony diameters over time, using JMPIN version 4.0.3 (Academic) statistical software (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA 27513).

2.3. Growth of *Cylindrobasidium laeve* in cut stem segments of selected native hardwood and softwood tree species

2.3.1. Collection, inoculation and incubation of stem segments

The colonizing ability of *C. laeve* on wood of different tree species native to British Columbia was tested under laboratory conditions. Terminal growth of three hardwood species (big leaf maple, *Acer macrophyllum*; red alder, *Alnus rubra*; and cottonwood, *Populus trichocarpa*) and two conifer species (Douglas fir, *Pseudotsuga menzesii* and hemlock, *Tsuga heterophylla*) was collected from trees growing on the Simon Fraser University campus on July 17, 2002 and cut into 15 cm long segments. Ten-day-old cultures of three isolates of *C. laeve* growing on MEA were used for inoculation of the stem segments. Inoculation was done by insertion of colonized MEA into wounds made at the midpoint of each segment with a sharp, sterile blade. Twenty-four stem segments were inoculated for each species, 12 of which were used for non-destructive observation and 12 for destructive observation. Inoculated stem segments were kept at 5°C, 10°C and 15°C in ambient laboratory light in sealed plastic bags along with wet germination paper. Observations of visible fungal mycelium and discoloration of tissue were taken weekly for 7 weeks after inoculation. The rating scale shown in Table 1 was used for recording fungal growth.

Table 1. Rating scale for fungal growth and discoloration of outer surface of inoculated cut stem segments.

Fungal Growth Rating	Observation
0	No visible mycelium around the inoculated wound.
1	Mycelium visible on and around the inoculated wound, but not extending from the wound.
2	Mycelium visible on the inoculated wound and extending $\leq$ 2.5 cm from the wound.
3	Mycelium extending > 2.5 cm from wound but not covering the entire segment.
4	Mycelium completely covering the inoculated stem segment.

Destructive observations were taken by cutting 1-2 cm long sections starting from the point of inoculation. These cut sections were evaluated for the presence of mycelium and discoloration inside the bark, using the rating scale in Table 2.

Table 2: Rating scale for fungal growth and discoloration of tissue on the inner surface of inoculated cut stem segments.

Discoloration Rating	Description
0	No mycelium and discoloration visible.
1	Mycelium and/or brown discoloration covering 0-25% surface area inside the bark.
2	Mycelium and/or brown discoloration covering 26-50% of surface area inside the bark.
3	Mycelium and/or brown discoloration covering 51-75% of surface area inside the bark.
4	Mycelium and/or brown discoloration covering > 75% of surface area inside the bark.

3.0. Results

3.1. Effect of *Cylindrobasidium laeve* on regrowth of cut stumps of *Acer macrophyllum*

3.1.1. June - 2001 treatments

All of the plants treated with Garlon 4 (positive control) on 1st June appeared dead within 1 week of treatment, and none of these resprouted during the period of observation. In contrast, resprouting occurred in all of the negative control plants within 2 weeks of cutting. One of the treatments with *C. laeve* significantly reduced resprouting (Figure 1). Only one of the plants treated with isolate #1 using colonized agar medium showed growth after 9 weeks of treatment. Some resprouting occurred in each of the other treatments with *C. laeve*. However, all of the plants treated with *C. laeve* had 7 – 10 sprouts and appeared indistinguishable from untreated controls when observed one year later in May, 2002 (Figure 2).

Figure 1. Effects of *Cylindrobasidium laeve* isolates applied in June 2001 as mycelium in agar (M) and as colonized wood (W) to cut stump surfaces on resprouting of bigleaf maple (*Acer macrophyllum*) seedlings at 9 weeks after treatment.
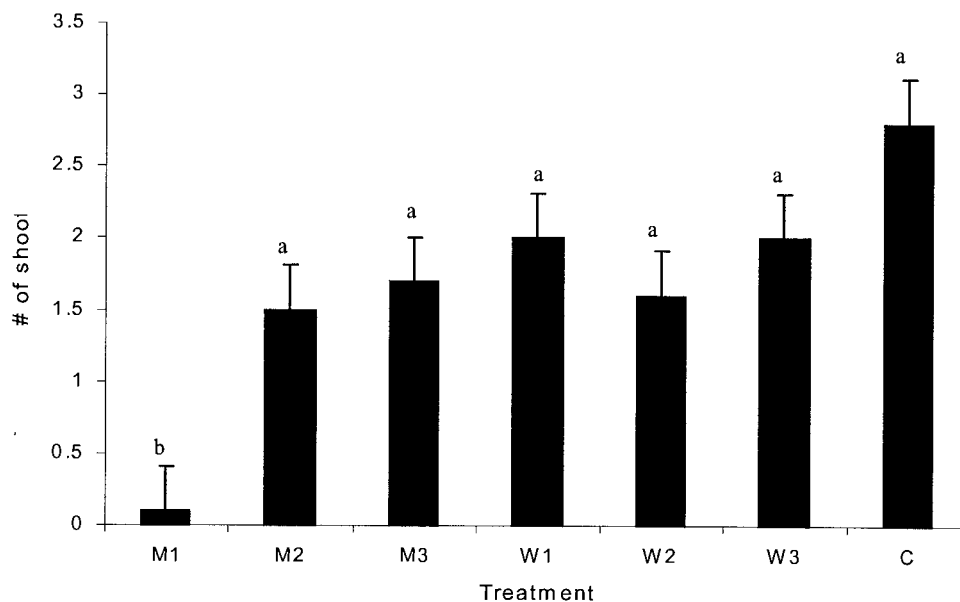
Figure 2. Effects of *Cylindrobasidium laeve* isolates applied in June 2001 as mycelium in agar (M) and as colonized wood (W) to cut stump surfaces on resprouting of bigleaf maple (*Acer macrophyllum*) seedlings one year later (May 2002).
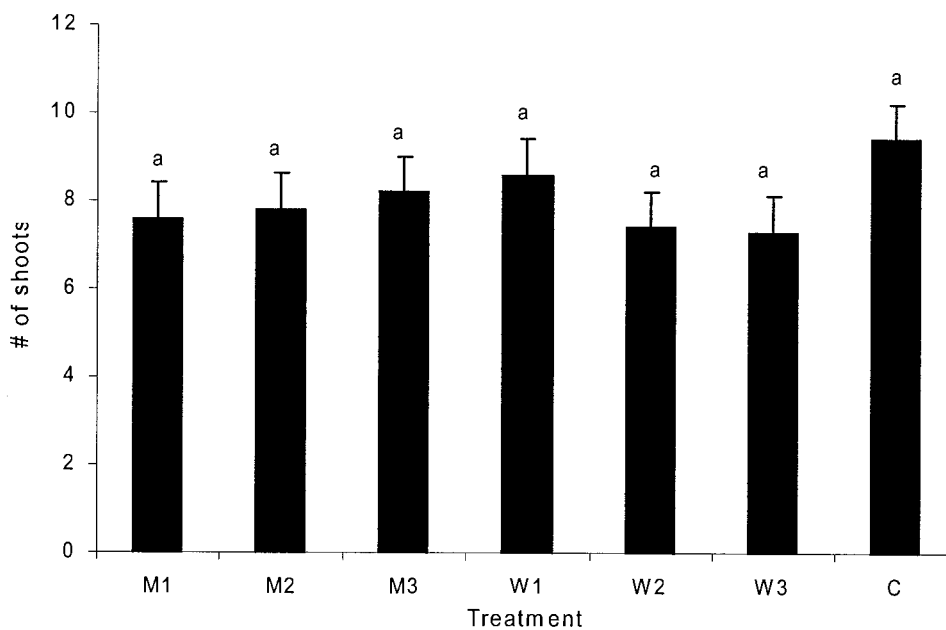
3.1.2. September - 2001 treatments

All positive control plants appeared to be dead on September 15, 2001,

1 week after this treatment was done. No regrowth was observed on any of the

treated plants at 7 weeks after treatment (October 30th 2001) and all treated

plants were completely dormant. Regrowth from the stumps of *A. macrophyllum*

seedlings cut and treated with *C. laeve* on 8 September, 2001 was observed

beginning on April 04, 2002. Observations taken in early 2002 (April 4 to June

10, 2002) indicate that none of the cut seedlings treated with *C. laeve* showed

significant suppression of regrowth (Figure 3). None of the fungal treated plants

died. Regrowth on plants treated in September - 2001 appeared to be healthy

and fast.

Figure 3. Effects of *Cylindrobasidium laeve* isolates applied in September 2001 as mycelium in agar (M) and as colonized wood (W) to cut stump surfaces on resprouting of bigleaf maple (*Acer macrophyllum*) seedlings in May 2002.
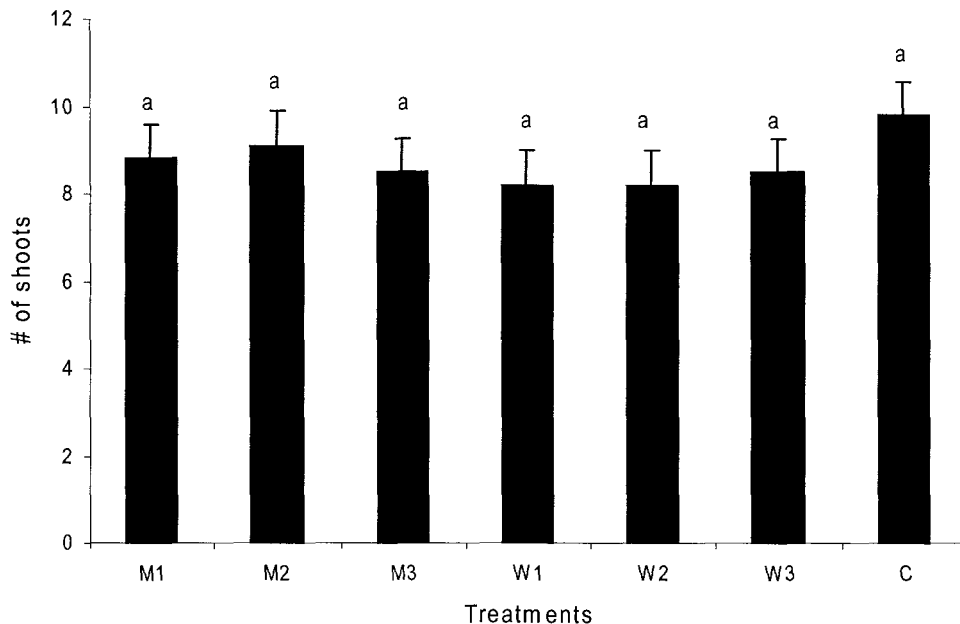
3.2. Growth characteristics of local isolates of *Cylindrobasidium laeve* in axenic culture

Isolates of *C. laeve* grew more rapidly at room temperature on MEA than on PDA or V-8 agar. No significant differences in growth rates among the three isolates were observed. Growth rates on MEA, PDA and V-8 agar were 5.2 mm/day, 3.0 mm/day and 1.4 mm/day, respectively (Figure 4). Mycelium of colonies of *C. laeve* growing on MEA was white in color, and thick and fluffy cottony in texture. Colonies growing on PDA and V-8 agar were also white in color, but with comparatively thin and downy to downy-cottony texture.

Figure 4. Rates of growth (colony diameter) of *Cylindrobasidium laeve* growing in axenic culture on three media (pooled data for three isolates)

$R^2 = 0.9902$

$R^2 = 0.9878$

$R^2 = 0.9981$

colony diameter (mm)

Time (days)

△ Malt Extract Agar Medium   ◻ Potato Dextrose Agar Medium
◇ V8-Agar Medium
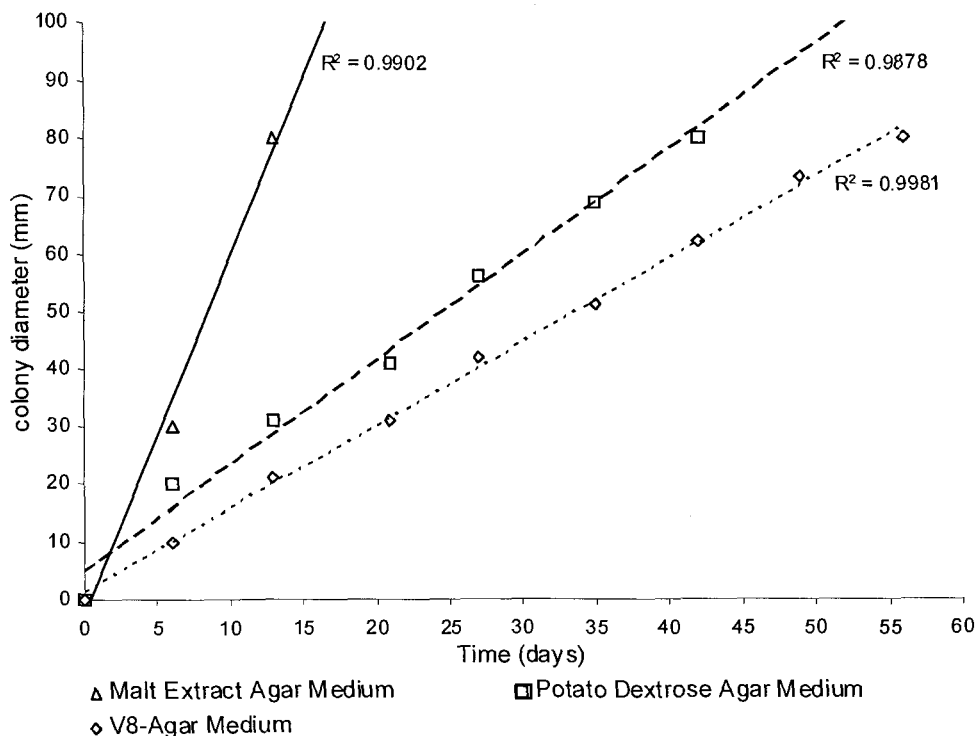
3.2.1. Effect of temperature on growth of *Cylindrobasidium laeve*

No significant differences in growth rates were observed among the

three isolates, therefore data for the three isolates were pooled for analysis.

None of the isolates showed growth on defined media, whereas slow growth was

observed on MEA at 5 °C (Figure 5) . Growth was observed on all of the media at

15 °C and 22 °C, but was much slower on the defined media than on MEA

(Figures 6,7). Growth rates on MEA at 5 °C, 15 °C and 22 °C were 1.1 mm/day,

2.3 mm/day and 5.2 mm/day, respectively.

Figure 5. Growth of *Cylindrobasidium laeve* in axenic culture on MEA and defined media at 5 °C (pooled data for three isolates).

Colony diameter (mm) vs Time (days)

R² = 0.9931

△ Malt Extract Agar Medium   □ Basal Salt Medium

◇ Basal Salt Medium + 1% Maltose   ○ Basal Salt Medium + 1% Glucose
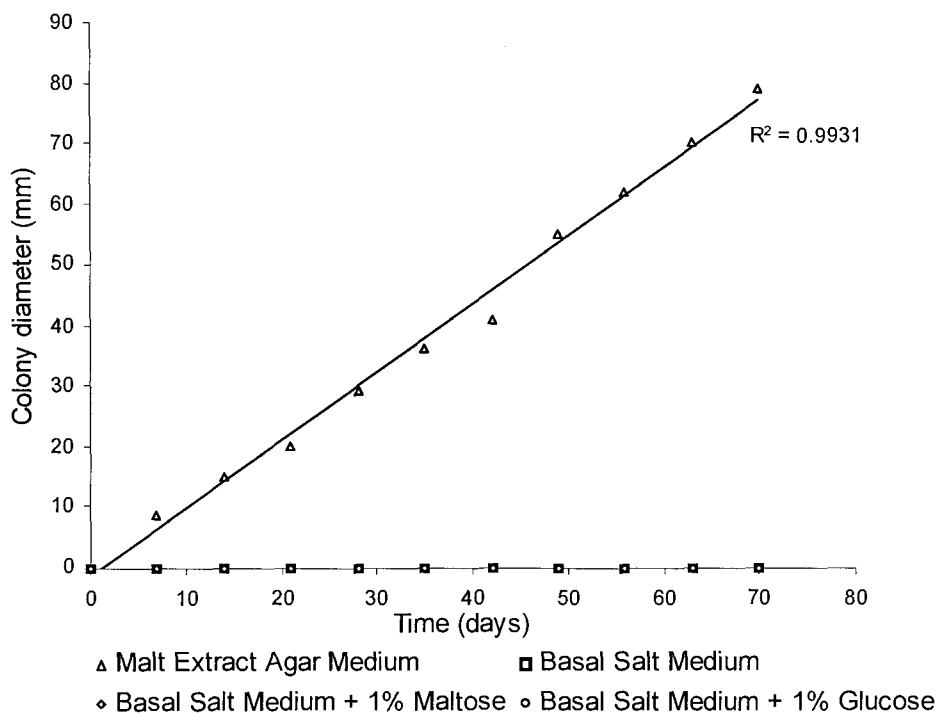
Figure 6. Growth of *Cylindrobasidium laeve* in axenic culture on MEA and defined media at 15 °C (pooled data for three isolates).

Chart axes: y-axis "Colony diameter (mm)" from 0 to 9; x-axis "Time (days)" from 0 to 40.

$R^2 = 0.9912$
$R^2 = 0.9338$
$R^2 = 0.9589$
$R^2 = 0.9125$
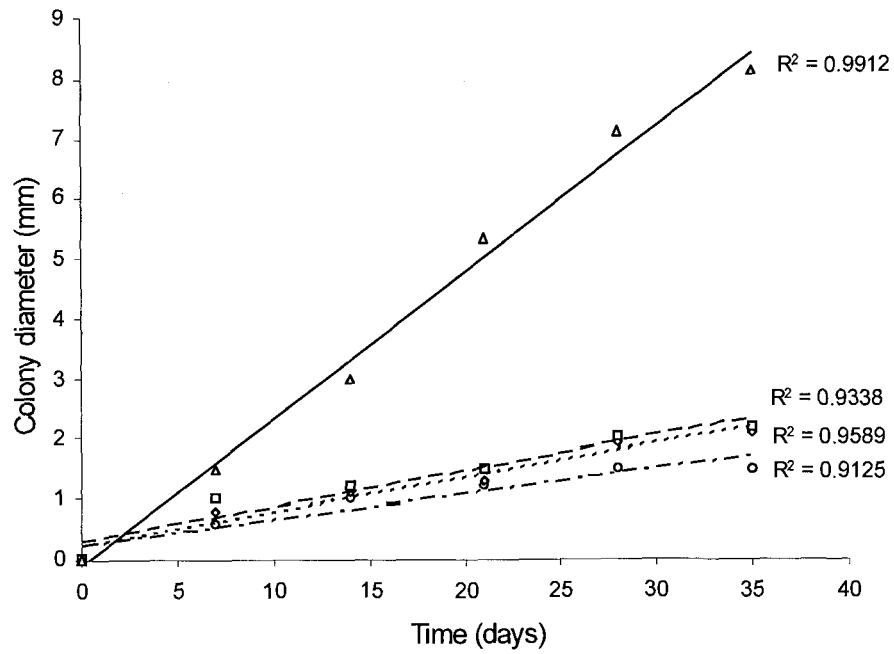
o Basal Salt Medium   ◊ Basal Salt Medium + 1% Maltose

◻ Basal Salt Medium + 1% Glucose   ▵ Malt Extract Agar Medium

3.2.2. Growth on natural and defined media

C. *laeve* grew better at 22 °C on MEA medium than on basal salts media amended with 1% glucose and 1% maltose. Growth rates on MEA, and on basal salts media amended with glucose and maltose were 5.2 mm/day, 1.6 mm/day and 1.4 mm/day, respectively (Figure 7).

Figure 7. Growth of *Cylindrobasidium laeve* in axenic culture on MEA and defined media at 22 °C (pooled data for three isolates).

△ Malt Extract Agar   o Basal Salt Medium

□ Basal Salt Medium + 1% Maltose ◇ Basal Salt Medium + 1% Glucose
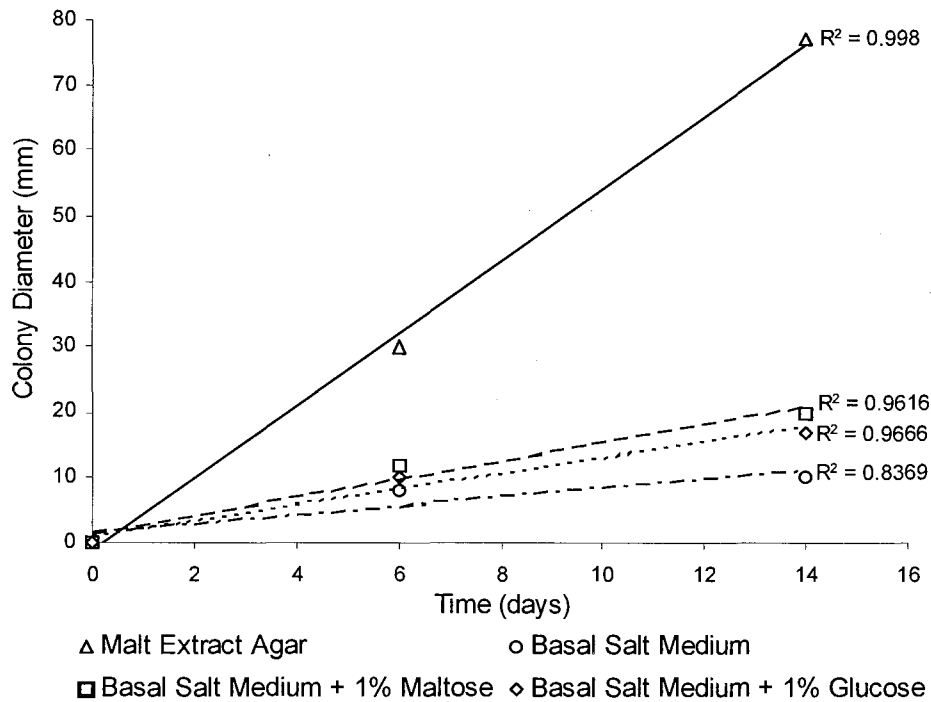
3.3. Growth of *Cylindrobasidium laeve* on and in cut stem segments of selected native hardwood and softwood tree species

3.3.1. Growth of *Cylindrobasidium laeve* on outer surface of inoculated stem segments of selected native hardwood and softwood species at 5 °C, 15 °C and 22 °C

At 5 °C, mycelium extended approximately 2.5 cm around the wound of inoculated segments (rating 2) of bigleaf maple, red alder, Douglas-fir and hemlock after 7 weeks (Table 3), whereas on cottonwood, mycelium was visible only on the wound surface (rating 1) in the same time period. No further growth of mycelium was observed on the outer stem segment surfaces of any of the species after 7 weeks time period. At 15 °C, mycelium completely covered the outer surface of inoculated segments (rating 4) of bigleaf maple, red alder, Douglas-fir and hemlock after 7 weeks. Mycelium covered only 75 percent of the inoculated surface area of cottonwood segments (rating 3) after 7 weeks (Table 3). At 22 °C, mycelium completely covered the outer surface area of inoculated twigs (rating 4) of bigleaf maple, cottonwood, red alder, Douglas-fir and hemlock by 4 weeks after inoculation (Table 3). Fungal growth was faster at 22 °C as compared to 5 °C and 15 °C on all the inoculated species.

Table 3: Mean ratings[a] for growth of *Cylindrobasidium laeve* on outer surface of wound inoculated stem segments of selected native hardwood and softwood species at 5 °C, 15 °C and 22 °C.

Time after Inoculation (wks)	Species[b]				
	BM	CW	RA	DF	WH
5 °C					
1	0	0	0	0	0
2	0.75	0	0	0.92	1.00
3	0.83	0	1.00	1.00	0.92
4	1.00	1.00	1.00	1.00	1.00
5	1.00	1.00	1.00	1.00	1.00
6	1.83	1.00	1.00	1.83	2.00
7	2.00	1.00	2.00	2.00	2.00
15 °C					
1	0.92	0.83	1.00	0.92	1.00
2	1.83	0.92	1.00	1.00	1.00
3	2.00	1.00	1.00	1.83	2.00
4	3.00	1.00	1.83	2.00	2.00
5	3.66	1.83	2.00	2.75	2.00
6	4.00	2.00	2.75	4.00	4.00
7	4.00	2.75	4.00	4.00	4.00
22 °C					
1	1.00	0.92	1.00	1.00	1.00
2	2.75	2.00	2.75	3.00	3.00
3	3.66	2.75	2.75	4.00	4.00
4	4.00	3.66	4.00	4.00	4.00

[a] growth rated from 0 (none) through 4 (covers entire stem segment). n=12.
[b] BM=bigleaf maple, *Acer macrophyllum*; CW=cottonwood, *Populus trichocarpa*; RA=red alder, *Alnus rubra*; DF=Douglas fir, *Pseudotsuga menzesii*; WH=western hemlock, *Tsuga heterophylla*.

3.3.2. Growth of *Cylindrobasidium laeve* on the inner bark and cambial surfaces of wound inoculated stem segments of selected native hardwood and softwood species at 5 °C, 15 °C and 22 °C

At 5 °C, no visible mycelium or discoloration (rating 0) was observed on the inner bark and cambial surfaces of any of the species except bigleaf maple (rating 1) at 4 weeks after inoculation. At 7 weeks after inoculation, mycelium and discoloration was evident on <25% area inside the bark (rating 1) of inoculated segments of all the species (Table 4). At 15 °C, fungal growth and discoloration was visible (rating 1) at 2 weeks after inoculation on all the species except cottonwood (Table 4). At 7 weeks after inoculation, mycelium and discoloration covered >75% surface area on the inner bark (rating 4) on all the species except cottonwood in which case mycelium covered 50%-75% of the inner bark area (rating 3). At 22 °C, mycelium and discoloration covered >75% of the inner bark area (rating 4) in bigleaf maple, red alder, Douglas-fir and hemlock by 4 weeks after inoculation. In cottonwood, mycelium and discoloration covered 50%-75% of the inner bark (rating 3) at 4 weeks, and >75% area of the inner bark (rating 4) at 5 weeks (Table 4).

Table 4: Mean ratings[a] for growth of *Cylindrobasidium laeve* on inner bark and cambial surfaces of wound inoculated stem segments of selected native hardwood and softwood species at 5 °C, 15 °C and 22 °C.

Time after inoculation (wks)	Species[b]				
	BM	CW	RA	DF	WH
			5 °C		
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0.75	0	0	0	0
5	0.83	0	0.75	0.75	0.75
6	1.00	0.75	0.83	0.83	1.00
7	1.00	0.83	1.00	1.00	1.00
			15 °C		
1	0	0	0	0	0
2	1.00	0	0.83	1.00	0.83
3	1.83	0.83	1.00	1.83	2.00
4	2.00	1.00	1.83	2.00	2.00
5	2.75	1.00	2.00	2.75	2.00
6	3.00	1.75	2.75	3.00	3.00
7	4.00	2.66	4.00	4.00	4.00
			22 °C		
1	1.00	0	1.00	1.00	1.0
2	2.75	0.83	1.83	1.83	2.00
3	3.66	1.75	3.00	3.66	4.00
4	4.00	3.00	4.00	4.00	4.00

[a] growth rated from 0 (none) through 4 (covers > 75% of inner bark surface). n=12.

[b] BM=bigleaf maple, *Acer macrophyllum*; CW=cottonwood, *Populus trichocarpa*; RA=red alder, *Alnus rubra*; DF=Douglas fir, *Pseudotsuga menzesii*; WH=western hemlock, *Tsuga heterophylla*.

4.0. Conclusions

4.1. Evidence that *Cylindrobasidium laeve* has insufficient potential for biological control of resprouting of *Acer macrophyllum*

Lohbrunner reported that none of the *C. laeve* isolates obtained from

Canadian Culture Collection, Ottawa suppressed the regrowth of bigleaf maple

when applied as mycelium to stumps of freshly cut seedlings in the fall of 2000

(Lohbrunner 2001). There are several possible reasons for the observed lack of

efficacy.

Cylindrobasidium laeve may simply lack the ability to suppress regrowth of

big leaf maple. On the other hand, it is possible that the mode of application or

type of inoculum used by Lohbrunner did not enable *C. laeve* to establish

sufficiently to suppress the regrowth. The season/time of application may have

played an important role in limiting the efficacy of the isolates. The particular

tested isolates in Lohbrunner's experiments were ineffective but perhaps local

isolates might have greater potential to suppress regrowth of big leaf maple.

In my experiments, three isolates of *C. laeve* obtained from big leaf maple

growing in natural habitats on Vancouver Island, BC, were applied to the stumps

of big leaf maple seedlings immediately after cutting, at two times of application and using two modes of application. None of the isolates applied by either mode of application at the beginning of September suppressed regrowth of the cut seedlings. When applied at the beginning of June as a mycelial treatment, one of three isolates gave incomplete but significant suppression of regrowth when observed 9 weeks after treatment, compared with regrowth that occurred on untreated control plants. None of the isolates was effective when applied by insertion of colonized stem segments into freshly cut stumps. All of the plants treated with *C. laeve* in June 2001 were alive and appeared healthy one year after treatment. To be used operationally, a biological agent applied to cut stumps of big leaf maple would have to provide substantial to complete suppression of regrowth consistently over an extended time period and when applied at any time over most or all of the cutting season. Evidence that *C. laeve* has this potential has not been obtained in two studies. Both Lohbrunner (2001) and I found that applications made near the end of the growing season (mid- to late August) were without effect and the plants were also observed to be growing well one year after treatment in 2003.

What are the possibilities that this conclusion might be premature, and that *C. laeve* might have the potential to suppress regrowth of big leaf maple? What hasn't been tested?

It is suggested that three additional lines of research that could be followed before accepting the conclusion that further research on the biological control potential of *C. laeve* is not warranted. One is to obtain StumpOut®, the commercial formulation of *C. laeve* that is reportedly successful in preventing resprouting of *Acacia* spp. in Africa, and assess its efficacy for suppressing regrowth of big leaf maple in British Columbia. A second suggested line of research is to evaluate the efficacy of local isolates of *C. laeve* when applied as inoculum consisting of basidiospores in various carriers. This would require development of methods to induce sporulation of local isolates of *C. laeve* in axenic culture. Finally, it is suggested that a small-scale field trial to evaluate the efficacy of *C. laeve* for preventing resprouting should be done on *A. macrophyllum* of variable ages growing under natural conditions. It is possible that resprouting of older trees growing in natural conditions may be more susceptible to *C. laeve* than is resprouting on second leaf potted seedlings maintained under green house conditions as employed in Lohbrunner's research and in the research presented in this thesis.

4.2. Conclusions regarding other aspects of research

The present research showed that *C. laeve* prefers to grow at 22 °C on MEA as compared to 5 °C and 15 °C under laboratory conditions. It is also inferred that fungus needs preformed organic factors in addition to an organic carbon source for optimal growth. Minimum fungal growth on the media containing the carbon sources suggests that the growth could be limited by the

absence of other organic growth factors and not by the absence of trace minerals. There is still more to know about essential minerals, preformed growth factors and a favourable pH range.

The experiments on wound inoculated cut stem segments shows that *C. laeve* grow superficially at the same rate on three selected native hardwood (big leaf maple, cottonwood and red alder) and two softwood tree species (Douglas-fir and hemlock) at 5 °C, 15 °C and 22 °C. My research does not specify the host range of *C. laeve* however; it indicates that the wood of all the tested tree species (hardwood and softwood) provides a digestible substrate for the tested isolates of *C. laeve* when introduced via wounds. Fungus appears to be growing equally on cut stem segments of all the tested plants.

4.3. Concluding Remarks

The use of biological agents for management of competing vegetation could become an essential component of forest management practice as a result of changing societal perspective. Endemic plant pathogenic fungi are presently considered to be promising candidates for biological control. The main concern to regulatory authorities and to the public about using fungal pathogens as mycoherbicides is their potential threat to non-target plants/trees. This is very important in the classical biological control strategy, where exotic pathogens are introduced into new ecosystems. In contrast, risks posed by indigenous fungal

pathogens used as biological control agents (e.g. *C. purpureum*) are generally low (de Jong et al. 1996, Gosselin et al. 1999).

Such may not always be the case, however. Endemic *Verticillium* sp. is a pathogen that kills maples and might be considered as a candidate for biological control. However, *Verticillium* spp. typically have broad host ranges and approval for use of *Verticillium* as a biological control agent in forest ecosystems, even if effective, would likely require extensive research on host range showing negligible risk to other plant species in forest ecosystems. Even with such evidence, its use would likely be controversial. Similarly, most of the other fungi associated with bigleaf maple (e.g., butt rots and root rots) are also associated with other trees of economic importance.

Cylindrobasidium laeve has also been reported to be present on a few tree species other than *A. macrophyllum* in British Columbia or in North America (Lowe 1991). It is mainly present on dead trees as it is reported to be a weak competitor. It is most likely not able to infect a healthy growing tree without presence of wounds. There is no documentation of *C. laeve* in British Columbia or in North America where it has damaged a native tree species or a forest. *C. laeve* is not present on conifers in Vancouver Island (Harry Kope[5], Personal Communication, 2001) where it was collected and where *A. macrophyllum* is a

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[5] Harry H. Kope Ph.D., P.Ag. Contact Biologicals 17 Jedburgh Road, Victoria, British Columbia, V9B 1K7 Phone/Facsimile (250) 727-0514 Email hkope@islandnet.com

severe problem for the British Columbia Hydro rights of way. A better understanding of the biology of *C. laeve* is required. Poorly understood aspects include growth factors, distribution and variability of the fungus and its effects on different native tree species. There is a need for research regarding the biology of *C. laeve*.

The question arises that even if a *C. laeve* based protocols was effective in preventing resprouting of bigleaf maple, would it be safe for use in a forest? If this fungus can grow well on the wound inoculated tree species segments then similar findings may come from studies under field conditions. Another point that needs to be considered is that the host range should be thought about only after an effective mode of inoculation has been established. The particular mode of application of *C. laeve* that is effective, if there will be any, should be used for testing the host range. However, the research done so far indicates that this fungus does not have the potential as a mycoherbicide and further research should be conducted on testing the efficacy of StumpOut® from Africa and on the biology and sporulation of the fungus.

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