BIOLOGICAL CONTROL OF ACER MACROPHYLLUM: OVERVIEW OF HOST BIOLOGY AND THE SCREENING OF FUNGAL ISOLATES WITH POTENTIAL TO CONTROL HOST GROWTH

by

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ABSTRACT

Bigleaf maple, *Acer macrophyllum*, is an economically significant weed on both forest plantations and utility rights-of-way in British Columbia and the Pacific Northwest of the United States. Increasing environmental concerns and public resistance to the use of synthetic herbicides create the need to search for alternative vegetation management systems such as biological control. There is currently no biological control system for the management of bigleaf maple. However, researchers have archived specimens of a native fungus, *Cylindrobasidium laeve*, that appear to be pathogenic to maple species in eastern Canada. The two objectives of this thesis are to compile baseline information on the biology and ecology of bigleaf maple and to challenge bigleaf maple seedlings with *C. laeve* isolates to determine their potential for controlling growth of this weed. An extensive literature review of bigleaf maple consisting of the biology, ecology, current control methods and the potential of biological control is presented. In the second phase of thesis work, four isolates of *C. laeve* were assayed for their capacity to infect bigleaf maple seedlings. Each isolate was applied to the cut stumps of bigleaf maple seedlings in a greenhouse environment. By the ninth week of the trial, none of the test isolates significantly ($\alpha = 0.05$) affected the number of resprouted shoots, the length of the longest shoot, the number of leaves or the size of the largest pair of leaves when compared to the negative control. Only the positive control plants treated with the herbicide Garlon 4 died. Qualitative examination of the host tissue revealed no sign of infection by the isolates. Further experimentation is required to

evaluate *C. laeve* as a potential biological control candidate. Future research should include the use of locally collected isolates, refinement of the application procedure, and the study of *C. laeve* biology.

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1.0. INTRODUCTION

About 3 million hectares of the most productive forest lands in British Columbia require some form of vegetation management "to reach their potential within a reasonable time" (Boateng 1986). In the last 10 years, over 480,000 ha have been brushed using manual cutting or girdling, and the application of chemical herbicides (Shamoun 1997). Brushing is among the top three silvicultural activities and 29% of total brushing involves the use of herbicides (Anonymous 1996). The BC Ministry of Forests notes that brushing levels have tripled since 1986/87 and will remain high due to the need to establish free-growing forests. In addition, BC Hydro spends approximately $25 million annually to control vegetation on its 74,650 km of power line rights-of-way (distribution and transmission). Approximately $468,000 per year is spent on management of bigleaf maple, *Acer macrophyllum* Pursh, on transmission line rights-of-way (Dr. Tom Wells, BC Hydro, 8475 – 128th Street, Surrey, BC V3W 0G1, pers. comm. 2001).

A recent approach to vegetation management is the use of pathogenic biological control agents as replacements for chemical herbicides. One fungal organism that is being considered for biological control of bigleaf maple is *Cylindrobasidium laeve* (Pers.:Fr) Chamuris (=*Corticium laeve*, =*Corticium evolvens*, =*Cylindrobasidium evolvens*), a fungus native to British Columbia and well represented in the Canadian Forest Service - Pacific Forestry Centre herbarium collection and host-parasite index (including specimens collected from bigleaf maple). It has proven to be successful as a biological control agent for black wattle, *Acacia mearnsii* de Wild., in South Africa.

As part of a larger initiative designed to identify and develop biological control agents for bigleaf maple, this thesis project had the following objectives:

1. to compile a review on bigleaf maple biology, ecology and control practices,
2. to establish fungal cultures of *C. laeve* isolates which may have potential as biological control agents, and
3. to conduct a greenhouse trial to determine the potential of this fungus in controlling the growth of bigleaf maple seedlings.

2.0. LITERATURE REVIEW

Bigleaf maple, *Acer macrophyllum*, is an economically significant weed in both forest settings and on utility rights-of-way throughout coastal British Columbia. It responds to most current vegetation management techniques by profusely resprouting and subsequently can shade out conifer seedlings on forest regeneration sites and grow into overhead powerlines. Many of the current control tactics rely on the use of synthetic herbicides. Increasing environmental concerns, public opposition, and strict regulations governing chemical use establish a need to develop alternative methods for managing this species. The fungus, *Cylindrobasidium laeve*, is proposed as a candidate biological control agent for bigleaf maple in British Columbia.

This thesis defines the initial stages of developing a biological control program for bigleaf maple. A literature review was conducted to

define the current status of the biology and ecology of bigleaf maple and the problems associated with its growth attributes. A greenhouse trial was conducted with four isolates of *C. laeve* to test its effects on the growth of bigleaf maple seedlings.

2.1. Biology of Bigleaf Maple

2.1.1. Botanical Nomenclature

Common names for *Acer macrophyllum* include: bigleaf maple, broadleaf maple, Oregon maple, large-leaf maple, white maple, common maple, British Columbia maple, long-leafed maple, and canyon maple (Lyons and Merilees 1995; Haeussler et al. 1990; Black 1981)

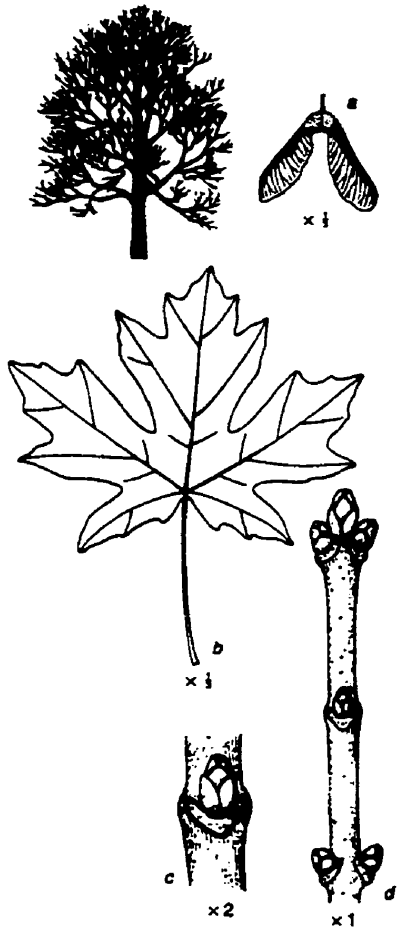
The genus *Acer* was created in 1700 by Tournefort and accepted in 1737 by Linnaeus as distinct (van Gelderen et al. 1994). The word *acer* derives from the Proto-Indo-European word *ac*, meaning "sharp, to be sharp, to sharpen" and possibly refers to the pointed leaves associated with maples. The name *macrophyllum* translates as "big leaf" and refers to the large-sized leaves possessed by this species. In 1814, Frederick Pursh published the official description of bigleaf maple from descriptions of plants from the Lewis and Clark expedition (Pursh 1814). This was nine years after Lewis saw this species "On the great rapids of the Columbia River" (Pursh 1814), most likely the Gorge (Black 1981).

2.1.2 Description and Account of Variation

Acer macrophyllum (Figure 1) is a deciduous, broadleaf tree that spreads mainly by seed. It resprouts readily from the stump following cutting. *Acer macrophyllum* reaches 15-25 m at maturity (Haeussler et al. 1990), although heights of 30 m have been reported (Brayshaw 1996). It has a broad, spreading crown and stout twigs with opposite branching (Haeussler et al. 1990), a trunk up to 60 cm in diameter, and branches into numerous upright limbs (Lyons and Merilees 1995). It grows straight, and has a loose open crown of up-pointing branches surmounting a clear trunk. The trunk is sometimes forked, and often occurs as several trunks growing from a common base. The root system is shallow and wide spreading (Farrar 1995).

Bigleaf maple twigs are stout, reddish-brown, and hairless. The buds are blunt, greenish to reddish, and have 3-4 pairs of scales. The terminal bud is large (approximately 6 to 9 mm long) (Farrar 1995). Leaf scars show 5-9 dot-like bundle scars, the highest number for any Canadian maple (Hosie 1979). The bark is green on young trunks, becoming finely roughened on trees to 15 cm dbh (diameter at breast height = 1.3 m), then becoming a drab gray-brown, and furrowed into narrow, horny ridges on older trees. Older bark is often covered with mosses, lichens and ferns (Pojar and MacKinnon 1994). The wood is fine-grained and fairly dense (Lyons and Merilees 1995).

Figure 1. Bigleaf maple form and structure a. Fruit. b. Leaf. c. Lateral bud and leaf scar. d. Winter twig. (Trees in Canada, Farrar 1995, Natural Resources Canada). Reproduced with the permission of the Minister of Public Works and Government Services, 2001).



Bigleaf maple leaves are 15-30 cm across (occasionally growing to 40 cm), and are palmate with five prominent deep lobes. Leaves are arranged oppositely, are dark green on the upper surface and paler green below, and turn a pale yellow colour in the fall (Lyons and Merilees 1995).

The flowers are yellowish-green, about 3 mm in diameter, numerous on short stalks, and arranged in a raceme at the ends of the twigs. The flowers appear before or during leaf flush. The fruit consists of a winged samara with paired, hairy seeds. The paired wings are about 5 cm long and at a 90° angle to one another (Brayshaw 1996).

In the central part of its range (Oregon and Washington), leaf buds burst in early April. Bud burst can be delayed until May at high elevations. The seeds reach maturity from late August-October and are dispersed by winds in late fall and early winter. The chromosome number is $2n = 26$ (van Gelderen et al. 1994).

Two additional *Acer* species are found in British Columbia (Pojar and MacKinnon 1994). Vine maple, *Acer circinatum* Pursh, is a shrub or scraggly small tree that reaches 7 m in height with round leaves, 5-12 cm across, 7-9 lobes, that turn bright red in autumn. The flowers are white, 6-9 mm broad and the fruits are 2-4 cm long, and widely spreading (not V-shaped as in *A. macrophyllum*). Vine maple is found in the lower and middle elevations of the coastal forest from Knight Inlet southwards but is very rare on Vancouver Island. It is found predominantly in damp places along creeks or meadows where soils are

nitrogen-rich (Klinka et al. 1989). It is tolerant of shade but usually found along forest borders (Lyons and Merilees 1995). The other species, *Acer glabrum* Torr. var. *douglasii* (Hook.) Dipp., Douglas maple or Rocky Mountain maple, is a shrub or small tree growing to 10 m tall, with leaves 2-8 cm across and having 3-5 lobes. The flowers are small and greenish-yellow, and the male and female flowers can occur on separate plants or the same plant. The fruits are 2-3 cm long and V-shaped. Douglas maple is very abundant and widespread east of the Cascades and in the southern two-thirds of British Columbia. It grows at elevations over 1200 m (Lyons and Merilees 1995).

There are no varieties or subspecies of *A. macrophyllum* described in British Columbia (Haeussler et al. 1990). However, Ruth and Muerle (1958) described a variety of *A. macrophyllum*, fairly common in the vicinity of Longview, Washington known as the Kimball maple, *Acer macrophyllum* Pursh *kimballi* var. nov. The leaves of this variety possess very deep indentations between major leaf lobes and have lacerated leaf margins. Harrar (1940) described it as a rare variety of *A. macrophyllum* with dissected, tropical-like foliage. van Gelderen et al. (1994) describes the leaves as dissected into 3-5 leaflets. It was first observed in southern Snohomish County, Washington. The flowers of this maple are often tricarpellate and the fruit are often triple samaras. It grows slowly and is often shrub-like (van Gelderen et al. 1994).

van Gelderen et al. (1994) and Peterson et al. (1998) described three cultivars of *A. macrophyllum*. The Rubrum cultivar, *Acer macrophyllum* Pursh forma *rubrum* E. Murray *Forma Nova* (Murray

1969), which has reddish-bronze leaves when young, is in cultivation in the Blake Gardens, University of California, Berkeley. The Seattle Sentinel cultivar is an erect tree, growing to about 50 feet in height with a crown diameter of about 12 feet, with a similar appearance to the type species (Mulligan 1954). It is not known if this variety is still cultivated. An old German cultivar, *Acer macrophyllum* Cv. 'Tricolor' (Murray 1969), has green to reddish leaves flecked with white, forming a tricoloured effect. These coloured forms are not stable and lose their colour when mature. It is no longer in cultivation.

2.1.3. Ecological and Social Importance

Acer macrophyllum plays an important role in forest ecosystems. This includes cycling nutrients; providing nurse sites on the bole and branches for plants (over 130 species of lichens, liverworts, mosses, and ferns (Nadkarni 1984)) and fungi; providing food, cover, and nesting sites for animals, including birds, small mammals, insects and amphibians; and broadening the diversity of forest structures, forest mixtures, and organisms in forest communities (Peterson et al. 1998). *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* (Mirb.) Franco, by increasing the availability of certain elements to the tree roots. The mull humus that develops where maple litter is deposited is also beneficial to western redcedar, (*Thuja plicata* Donn). Therefore, retaining a minor component of *A.*

macrophyllum during site preparation and stand tending may be beneficial to the stand (Peterson et al. 1998).

Peterson et al. (1998) note that the total weight of epiphytes on a mature *A. macrophyllum* tree is often 4 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.

When planted along stream banks and steep slopes *Acer macrophyllum* resists to erosion because of the soil-binding capabilities of its roots. Dead trees that topple into streams form long-lasting, large woody debris that regulate water flow and are an important component of stream habitats (Peterson et al. 1998).

Acer macrophyllum is gaining recognition as an important species in "mixed wood" plantations. It not only contributes to enhanced nutrient availability, but also to both structural and species diversity and to aesthetics in coastal forests (Petersen et al. 2000). *Acer macrophyllum* also displays resistance to some root rot diseases (Peterson et al. 1998). It is immune to the pathogen responsible for laminated root rot, *Phellinus weirii* (Murrill) R. L. Gilbertson, which attacks conifers in the genera *Pseudotsuga*, *Abies*, *Tsuga*, *Picea* and *Pinus*. Growing maple in disease centres can prevent the spread of this pathogen to conifers by providing a physical barrier to its spread.

The wood of *Acer macrophyllum* is used today for making various specialty products. In British Columbia, bigleaf maple is locally

significant for the manufacture of furniture, musical instruments, interior panelling, veneer, moulding, plywood, and other specialized uses such as the production of large bowls turned from maple burls (Peterson et al. 2000). In the past, coastal First Nations used *A. macrophyllum* wood to make dishes, pipes, and hooks for clothing (Parish and Thomson 1994). The wood was also used to make paddles and *A. macrophyllum* was often referred to as the paddle tree. The inner bark was used to make baskets, rope, and whisks for whipping a foamy concoction from the berries of soopalalie, *Shepherdia canadensis* (L.) Nuttall. The Saanich tribes used preparations from *A. macrophyllum* to make an internal medicine and to treat sore throats, and the leaves were rubbed on a boy's face at puberty so he would not grow whiskers. The wood was also used for spindle whorls and the leaves were good for temporary containers. Sprouted seeds were used as a food source by members of the Nlaka'pamux tribe. Interior BC First Nations ate the young maple shoots raw in spring and also made a form of maple syrup from the sap (Parish and Thomson 1994).

2.1.4. History

The maple family (*Aceraceae*) includes two genera, *Dipteronia* and *Acer* (Peterson et al. 1998; Elias 1980). *Dipteronia* contains two species of small trees, both native to central China, whereas *Acer* contains about 148 species of trees and shrubs that are widely scattered through the Northern Hemisphere, but are most abundant in the eastern Himalayan Mountains and in central China.

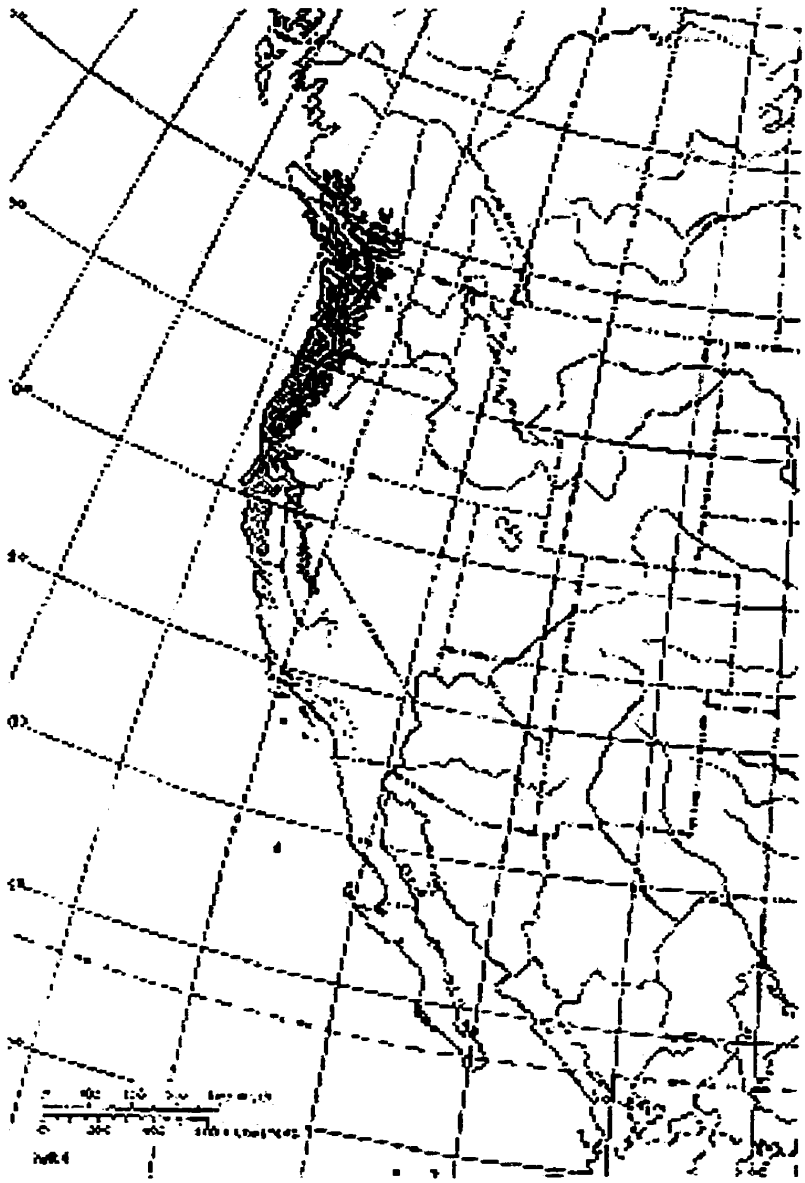
Thirteen species of maple are indigenous to the United States, 10 to Canada, including the three species that occur in British Columbia. Plant biogeographers suggest that because of the isolating effects of Pleistocene continental ice sheets on plant distributions, *A. macrophyllum* is actually more closely related to some of the Asian and European maples than to those in eastern North America. This observation is based on taxonomic features such as chemical production (flavonoids), leaf characteristics (margins, shapes and phyllotaxy), fruit variability (especially the angle between the wings), geographical distribution, and "special" habitat preferences (van Gelderen et al. 1994).

2.1.5. Geographical Distribution

Many immediate relatives of *A. macrophyllum* grow in Europe, but it is the only surviving representative of its phylogenetic series on the North American continent (van Gelderen et al. 1994). A number of ice ages are responsible for the peculiar distribution patterns exhibited by maples throughout the Northern Hemisphere (Hosie 1979). In North America, the range of *A. macrophyllum* (Figure 2) extends from the San Bernardino Mountains (Ruth and Muerle 1958), its southern limit characterized by isolated groves along the southern California coast to about 33°N in San Diego County. From its southern range, it is extends northwest through the western parts of Oregon, Washington, and British Columbia, as far north as Sullivan Bay on Broughton Island near the mouth of Kingcome Inlet at 50° 51' N, 126° 45' W (Haeussler et al. 1990,

Minore and Zasada 1990, Lyons 1995, Brayshaw 1996, Thomas and Comeau 1998, Thomas 1999). In British Columbia, the distribution of *A. macrophyllum* occurs west of the Coast Mountains (Haeussler et al. 1990; Lyons and Merilees 1995). It ranges along the Fraser River to Hope, northward in low elevation valleys to Seton Portage near Lillooet and Siska in the Fraser Canyon, and eastward to the Skagit River Valley near Hope. It occurs on Vancouver Island as far north as Port Hardy (Minore and Zasada 1990, Thomas and Comeau 1998), but not on the Queen Charlotte Islands.

Figure 2. The native range of bigleaf maple (Minore and Zasada 1990)



Acer macrophyllum occurs only in the Coastal Douglas Fir (CDF), Coastal Western Hemlock (CWH), and subcontinental Interior Douglas Fir (IDF) biogeoclimatic zones. However, it is most abundant on southeastern Vancouver Island and adjacent areas of the CDF zone. *A. macrophyllum* is a minor component of most low elevation CWH forests, but it is concentrated in the Fraser Timber Supply Area (TSA) and to a lesser extent in the Sunshine Coast TSA (Peterson et al. 1998).

In British Columbia it is a low elevation species, rarely occurring above 300 m, but has been observed at elevations above 350 m on southeastern Vancouver Island (Haeussler et al. 1990). Ruth and Muerle (1958) describe its lower elevational limits as sea level in the north end of its range and 900 m in the south end of its range. Moving south, its upper elevational limits gradually increase from 450 m on the Olympic Peninsula, to 1000 m in the central part of the Coast Range in California, and finally to 1700 m on the western slopes of the San Gabriel Mountains.

2.1.6. Habitat

Acer macrophyllum is confined to the warmest, mildest climate in British Columbia and temperature limits its northern distribution (Haeussler et al. 1990). Insufficient moisture and humidity combined with seasonal temperature extremes limit its distribution into interior of British Columbia. Humid climates where the mean annual temperature is above 10°C and there is little annual variation in temperature are ideal. *Acer macrophyllum* has low frost resistance and it does not grow where the ground freezes solid before snow falls (Krajina et al. 1982). Its distribution in British Columbia is therefore limited to areas with minimal frost (Peterson et al. 1998).

Bigleaf maple grows in a variety of soils from deep and loamy to shallow and rocky (Ruth and Muerle 1958; Haeussler et al. 1990). It grows best on fluvial sites and at the base of colluvial slopes, but also appears on morainal and marine soils and often appears as pioneering vegetation following landslides. It must have access to adequate soil moisture and therefore grows best on sites with abundant seepage or on fluvial sites along stream banks. It is classified as "intermediate" relative to other Pacific Coast tree species with respect to moisture requirements for optimal growth. Its flood tolerance is very high, and it often occurs on floodplains in "hygric" soils (Krajina et al. 1982).

Soils high in nutrient concentration, cation exchange capacity, base saturation, and nitrogen to carbon ratio are preferred by *A.*

macrophyllum (Peterson et al. 1998). It prefers moderately deep, loamy, very porous (low bulk density) soils with dark brown Ah horizons, classified as Humo-Ferric Podzols or Cumulic Regosols with Vermimulls. *Acer macrophyllum* exhibits the most vigorous growth in nutrient rich soils and it has a high requirement for calcium, magnesium, nitrates, potassium, and phosphorus (Haeussler et al. 1990). The levels of nitrogen, potassium, and calcium in foliage, bark and litter are high relative to other northwest tree species and the levels of phosphorus and magnesium are relatively low.

Open sites disturbed by logging and burning promote the growth of *Acer macrophyllum* (Ruth and Muerle 1958; Pojar and MacKinnon 1994). It is an occasional pioneer on hillsides laid bare by land slides or fire. It is also an important component of Douglas-fir stands, and its common associates in the north are red alder, *Alnus rubra* Bong; Douglas-fir; western redcedar; grand fir, *Abies grandis* (Dougl.) Lindl.; western hemlock, *Tsuga heterophylla* (Raf.) Sarg.; black cottonwood, *Populous trichocarpa* Torr. & Gray; garry oak, *Quercus garryana* Dougl.; and Oregon ash, *Fraxinus latifolia* Benth. In the south, its common associates are California-laurel, *Umbellularia californica* (Hook. & Arn.) Nutt.; coast redwood, *Sequoia sempervirens* (D. Don) Endl.; willows, *Salix* spp.; arbutus, *Arbutus menziesii* Pursh; white alder, *Alnus rhombifolia* Nutt.; California live oak, *Quercus agrifolia* Nee; and California sycamore, *Plantanus racemosa* Nutt.

Most bigleaf maple stands occur on sites of good to medium productivity (Peterson et al. 1998). It is a prominent component in

ecosystems described as the most productive for growth of Douglas-fir on the Lower Mainland of British Columbia and Vancouver Island. It most often grows in a clumped distribution. *Acer macrophyllum* is occasionally found in pure stands in moist soils near streams, but generally is scattered or in small groves with other species (Ruth and Muerle 1958). It is often an important component in riparian habitats.

Acer macrophyllum is the most shade-tolerant deciduous tree in south coastal British Columbia, and is rated as having moderate shade tolerance. This tolerance decreases with age and the best growth occurs where the maple canopy has direct access to sunlight. In Oregon, the survival of seedlings after 2 yr is highly dependent on forest canopy density. Survival is highest in clearcuts where the seedlings are not shaded and is lowest under dense overstoreys where the seedlings are shaded out (Fried et al. 1988).

2.1.7. Growth and Development

Acer macrophyllum is a tall tree with a broad, spreading crown (Haeussler et al. 1990). It has a shallow, wide-spreading root system, a short trunk and large leaves. Its growth is rapid during the first 40-60 yr and maturity is reached between 150 and 300 yr of age. By maturity, it is often >30 m tall with a stem dbh of 2.5 m. Low light conditions lead to a narrow crown and a long, limb-free bole; open conditions lead to broad crown and rounded appearance.

Few studies have been conducted on *A. macrophyllum* physiology. Lei and Lechowicz (1997a, 1997b) studied the photosynthetic response of eight maple species, including *A. macrophyllum*, under light regimes simulating forest gap edge and gap centre. They measured the leaf nitrogen concentration, chlorophyll a:b ratio, photosynthetic rate, stomatal conductance, internal CO_2 partial pressure, and photosynthetic induction. They observed that area-based leaf nitrogen concentration was greatest in gap centre-grown seedlings, whereas area-based [1]chlorophyll was highest in gap edge-grown plants. The gap edge-grown plants also possessed a low chlorophyll a to b ratio. The maximum photosynthetic rate was 60% higher in the gap centre than in gap edge trees. These results were consistent with the hypothesis that shade-acclimated plants will increase radiant-energy harvesting capacity as a result of limited photon input while gap-acclimated plants will operate most efficiently under bright irradiance by increasing carbon fixation.

Hansen et al. (1998) conducted a comparative study to examine gas exchange, water relations, and sap flow in *A. macrophyllum* and red alder in the same stand. From their measurements, they observed that although *A. macrophyllum* had higher transpiration rates during mid day, red alder developed lower water potential. The sap flow data indicated that *A. macrophyllum* was able to supply greater amounts of water to leaves than red alder. They hypothesized that differences in the

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[1] Area-based chlorophyll was determined by measuring the amount of chlorophyll a and b in leaf disks and expressing this amount (g) per unit of leaf area (m) (Lei and Lechowicz 1997a).

root structure or root to shoot ratios may cause differences between the two species in the ability to supply leaves with water. Since red alder is a nitrogen fixer, it is hypothesized that it may have a low root to shoot ratio limiting its ability to supply water under high demand situations, making *A. macrophyllum* a superior competitor under these conditions. For example, T.K. Pavlychenko in the 1930's and 40's demonstrated how rooting patterns of weeds in crop fields can cause crop roots to be smaller when grown with a weed than in weed-free conditions (Radosevich et al. 1997).

Some *Acer* species possess vesicular-arbuscular mycorrhizae (endomycorrhizae) (van Gelderen et al. 1994), but *A. macrophyllum* is not known to harbour any mycorrhizae.

2.1.8. Reproduction

In Oregon, Washington and British Columbia flowering usually occurs in early April-May and the flowers and buds emerge simultaneously from the same bud. Pollination, primarily carried out by insects, occurs within 2-4 weeks of bud burst. Fruit ripening occurs from September-October and seed dispersal takes place from October-January, although some seeds remain on the tree until March. Peak leaf abscission occurred from October 2-23 in a Washington-Oregon study, rapidly followed the first frost, and was hastened by heavy rains (Haeussler et al. 1990).

Acer macrophyllum produces its first flowers at about 10 yr of age with open-grown specimens producing more abundantly and earlier than trees in dense stands (Ruth and Muerle 1958). In the central part of its range buds open in early April but can be delayed until May at high elevations. The seeds reach maturity from late August-October and are dispersed by winds in late fall and early winter. The seeds germinate in spring.

Bigleaf maple flowers occur in scented racemes and insects attracted to these blossoms in great numbers are responsible for pollination (Ruth and Muerle 1958). The flowers are small (about 3 mm in length), greenish-yellow, fragrant, and occur in drooping clusters, 10-15 cm long (Peterson et al. 1998). The floral clusters appear before the leaves and contain both pollen flowers and seed flowers in the same cluster. These flowers usually have five small sepals and petals, four to six stamens in male flowers, and a single pistil in the female flowers (Elias 1980). The fruits are double or occasionally triple samaras, green at first, later turning brown as they ripen. The fruits occur on branches in elongated clusters and have a hairy seed cover.
Acer species do not produce large quantities of pollen compared to *Alnus* and *Betula* species (van Gelderen et al. 1994). The *A. macrophyllum* pollen grain is one of the largest maple pollen grains with a mean width of 55 μm.

The major trends in the evolution of flowering in maples are from insect pollination (entomophily) to wind pollination (anemophily) and from monoecy to dioecy (van Gelderen et al. 1994). *Acer macrophyllum*

flowering is described as protogynous or dichogamous, i.e. it still relies mainly on insect pollination and tends to be monoecious in nature. Cross-pollination is predominant over self-pollination (van Gelderen et al. 1994).

There are approximately 7440 *A. macrophyllum* seeds per kg (Ruth and Muerle 1958). Seed dispersal is primarily by wind during the fall and winter, but some small mammals (mice, woodrats, squirrels, and chipmunks) and birds can also distribute the seed (Peterson et al. 1998). Bigleaf maple possesses one of the heaviest seeds of northwestern US tree species. The seeds' large wings aid in effective dispersal and are observed to descend through air like "little helicopters" (Pojar and MacKinnon 1994). In the Coast Ranges of central Oregon, the dry weight of samaras ranged from 0.25-0.65 g, with embryo dry weight accounting for 30%-40% of the total weight.

In an Oregon study, all *A. macrophyllum* seeds not taken by herbivores either germinated within 1 yr of dispersal or decayed (Haeussler et al. 1990). Germination occurs on both mineral and organic seedbeds and survival depends on presence of adequate moisture. The first year survival of *A. macrophyllum* seedlings is greater in clearcuts (1-2 yr old) than in young (20-40 yr old), pole-sized (41-80 yr old), or old conifer stands (81-250 yr old). In Oregon, seedlings in small forest openings are significantly more abundant and taller than seedlings in adjacent sites under dense canopies. The window for the most successful seedling establishment appears to begin after canopy thinning and end before forbs and shrubs regenerate.

Ruth and Muerle (1958) note that ca. 87% of bigleaf maple seeds are viable. Germination ranges from 32%-90%. Zasada et al. (1990) found that viability of stored seeds is very variable, and is influenced by factors such as seed maturity and moisture content. They found that seed collected in the fall, but before fall rains occurred could be stored at 1°C for 12 months without an appreciable loss in viability.

Seeds are dispersed in the fall and early winter, stratify during winter and spring, and germinate as soon as temperature thresholds are reached (Peterson et al. 1998). Germination and establishment are best under partial shade, and seedling health rapidly declines under either dense shade or in open clearcuts. Natural regeneration is best under a conifer canopy that has been thinned either naturally or silviculturally. Germination before dispersal (vivipary) is quite common. This avoids some of the dangers of herbivory, but risks that the seed may disperse to an inhospitable site or dry out before dispersal.

Acer macrophyllum sprouts vigorously from stumps and reproduction by this "coppicing" or multi-stemmed resprouting is very successful (Ruth and Muerle 1958; Haeussler et al. 1990). The number of sprouts and size of the original stump control the growth of sprouts. Up to 50-60 sprouts can form from one stump and within 30-40 yr, one clump can create a canopy cover as large as 100 m^2. *A. macrophyllum* sprouts arise from dormant buds at the base of the stem following top kill or cutting of the parent tree. However, it does not produce suckers from rhizomes or roots. Layering may occur in some circumstances (P.

Comeau, University of Alberta, Department of Renewable Resources, pers. comm., 1999).

2.1.9. Population Dynamics

Acer macrophyllum is described by Haeussler et al. (1990) as a seedling banker. A bank of persistent seedlings that establishes itself under young coniferous or mixed stands will persist in a stunted or etiolated condition until a disturbance occurs that creates a favourable environment for growth. Rapid growth ensues immediately after a disturbance, but typically slows down as sprouts become shaded again in the understorey. Peterson et al. (1998) classify bigleaf maple as a stress-tolerator because it is able to withstand relatively long periods under highly limiting conditions but retains the capacity to utilize resources rapidly once available. Thus *A. macrophyllum* exhibits the characteristics of a competitor immediately after disturbances, but behaves as a stress-tolerator over the long term.

Fried et al. (1988) suggest that the window for successful establishment of *A. macrophyllum* seedlings occurs in mature stands as the canopy thins or as gaps are formed and ends when shrubby and herbaceous vegetation invades following clearing.

2.2 Management of Bigleaf Maple

Acer macrophyllum is an important competitor of conifer seedlings, especially Douglas-fir, on some of the most productive growing sites in British Columbia (Haeussler et al. 1990). Its presence can decrease successful establishment and growth of young conifers on planted or naturally regenerated sites. The primary source of competition is by coppicing from the stumps of maples already established on the site, especially in areas disturbed by logging, burning and mechanical site preparation. The resulting *A. macrophyllum* resprouts are aggressive competitors and shade out conifer seedlings. Light levels beneath established young *A. macrophyllum* canopies can be as low as 4.6% of full sunlight (Gendron et al. 1998) but are more typically at around 10 to 15% (Thomas and Comeau 1998). Spreading lateral shoots and branches can crush or cause physical abrasion of nearby conifer seedlings, and leaf litter can crush very small seedlings, especially when wet or under snow. Consequently, conifer saplings are rarely found growing beneath the dripline of young *A. macrophyllum*. Because a single tree has the capacity to establish cover over a large area, the potential stocking of desired species can be greatly reduced and this significant negative impact on growth occurs even when bigleaf maples are found in relatively low numbers (Figueroa and Nishimura 1992). As much as a 30% reduction in Douglas-fir height growth after 5 yr for seedlings growing within 1 m of *A. macrophyllum* has been reported (Figueroa and Nishimura 1992).

Acer macrophyllum is also an important species on industrial rights-of-way (G. Shrimpton, BC Hydro, 8475 – 128th Street, Surrey, BC V3W 0G1, pers. comm. 1999). Utility vegetation staff consider *A. macrophyllum* a weed because its rapid height growth and tall stature poses a risk to overhead power lines.

2.2.1. Response to Physical Control Methods

There are three physical control options that can be employed to manage *A. macrophyllum*. First, manual brushing or slashing can be used to cut maple stems. This can be done using hand-held tools such as hatchets, machetes, sandviks, saws or chainsaws. It can also be carried out with mowing equipment such as hydro-axes or excavator mowers, which are used mainly on utility rights-of-way (Table 1). Second is to combine cutting the tree with caping the stump in heavy black plastic or geotextile landscaping fabric, blocking out all light. Third is the use of girdling, but this tactic has proven ineffective and difficult, considering the large number of sprouts that require treatment.

Sprout heights of 5 m and crown diameters of 6.5 m can be reached in 3 yr during which sprouts can grow 1-2 m in height per year (Peterson et al. 1998; Haeussler et al. 1988). In contrast, bigleaf maple seedlings can only attain a maximum eight growth of approximately 1 m per year on ideal sites, and transplanted seedlings often grow slowly during the first year. The height of a cut stump height influences sprout clump size (Tappeiner II et al. 1996). For example, 2 yr after

clearcutting, the sprout clump volume for short stumps (<30 cm above ground) was significantly less than for tall stumps (60 cm). Reducing stump heights reduces the bud bank size. In the same study, the sprout clump volume, area, and number of sprouts were all significantly reduced for trees cut 1 and 2 yr before harvest of associated conifers than for trees cut during harvest. The sprout number, but not clump size, was reduced for bigleaf maples in the understorey compared to those out in the open. Over time, the number of stems declined due to self-thinning and breakage of lateral shoots, and mature trees of coppice origin typically had between three and five stems per clump.

Thinning of maple clumps has been observed to reduce the vigour of resprouting when one sprout is left per 25 cm of stump circumference (P. Comeau, University of Alberta, Department of Renewable Resources, pers. comm., 1999). Preliminary results from field studies in BC suggest that leaving three or more shoots per clump can substantially reduce resprouting of maple (P. Comeau, unpubl. data). This finding suggests that apical dominance of the remaining stems controls lateral bud dormancy.

Table 1. Manual and mechanical brushing techniques available for *A. macrophyllum* control (adapted from Biring et al. 1996, Haeussler et al. 1990, and Anonymous 1995).

Method	**Result**	**Degree of Control**
Manual cutting	• forms multiple sprouts with up to 50X increase in stem number • increases crown area	• temporary • can significantly reduce the crown volume for at least 1 yr
Girdling	• difficult to carry out due to thick bark of mature stems or large numbers of stems in young coppices • effective topkill above the girdle • scars heal over and trees resprout from below the girdle	• temporary
Stump Caping	• labour intensive	• varies (up to 60% control) • effective if geotextile remains in place for at least 2 yr
Burning	• produces top-kill, followed by rapid resprouting from dormant basal buds • sprouts can grow 3-4 m per year	• temporary • light burning produces no significant difference in cover from preburn conditions
Mowing	• forms multiple sprouts with up to 50X increase in stem number • increases crown area	• temporary • can significantly reduce the crown volume for at least 1 yr
Stumping	• stump sprouting is minimal and sprouts are of very low vigour	• high

2.2.2. Response to Herbicides and Other Chemicals

There are a number of herbicides registered for forestry and industrial use, which have varying degrees of efficacy on *A. macrophyllum* (Table 2). Herbicides can be applied by a number of methods including foliar sprays, soil applications, and basal bark sprays. Foliar application of most available herbicides will cause top-kill, but roots are seldom killed as little chemical is translocated downward (Haeussler et al. 1990). Subsequently, treated plants tend to resprout from the base.

Herbicides are also used in an integrated fashion with other types of treatments. One combination involves cutting or slashing *A. macrophyllum* sprouts or stems and then treating the cut surfaces with herbicide (cut stump application). Alternatively, the bark is wounded and the herbicide is injected into the wound for uptake by translocation (hack and squirt or stem/stump injection).

Table 2. Herbicides registered for *A. macrophyllum* control in British Columbia (adapted from Biring et al.. 1996).

Application Method	**Herbicide**	**Active Ingredient**	**Degree of Injury**
Foliar	Roundup®/Vision®	glyphosate	25-90%
Foliar	Arsenal®	imazapyr	Unknown[2]
Foliar	Esteron 600®, For-Ester E.C.®	2,4-D ester	<25%-60%
foliar, soil	Velpar L®, Pronone 5G®, Pronone 10G®	hexazinone	<25%
Foliar	Release®	triclopyr ester	<25%-100%
basal bark, cut stump	Release®, Garlon 4®	triclopyr ester	80%-100%
cut-stump	Carbopaste®	glyphosate	90%-100%
cut-stump	Forestamine 500®, Forestamine 250®, Dow Formula 40F®, Silvamine 500®	2,4-D amine	25-60%
basal bark	Weedone CB®	2,4-D ester + 2,4-DP ester	<25%
stem/stump -injection	EZ-Ject®	glyphosate	Unknown[1]

[2] No formal studies that quantify the efficacy of these products have been published.

Some research has been undertaken in California and Nevada to study the effects of growth regulators for vegetation control. Arron et al. (1997) tested the effect of four growth regulators (paclobutrazol, flurprimidol, dikegulac and uniconazole) on 12 common West Coast species for utility line clearance. These are referred to as second-generation growth regulators that inhibit gibberellin biosynthesis, reducing cell elongation and retarding the growth of trees without the "undesirable phytotoxic effects" observed with first generation plant growth regulators. At one site growth of *A. macrophyllum* injected with uniconazole was inhibited, however, application of dikegulac had no significant effect. Paclobutrazol and flurprimidol were not tested on bigleaf maple in the study.

2.3. Biological Control and its Role in Bigleaf Maple Management

2.3.1. Background

Biological control is the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be (Van Driesche and Bellows 1996). In biological control of weeds consumption of all or part of a plant by a herbivore replaces the activity of a predator on an animal. Pest populations can include insects, mites,

weeds, plant pathogens and vertebrates. Conversely, all of these organisms can be used as biological control agents. Biological control may be used to suppress forest or crop pests or to restore natural systems affected by non-native pests. Two examples in which weeds have been successfully controlled with fungal pathogens include use of *Colletotrichum gloeosporioides* f. sp. aeschynomene for the control of northern jointvetch, *Aeschynomene virginica* (L.) BSP in rice and soybean fields (marketed under the product name Collego™) and *Phytophthora palmivora* (Butler) Butler, for the control of strangler vine, *Morrenia odorata* Hook. & Arn in citrus groves (marketed under the product name of DeVine™) (Charudattan 1988; Hintz and Shamoun 1996).

There are two distinct biological control tactics, classical biological control and the use of bioherbicides (Charudattan and Walker 1982). In the classical approach, exotic organisms are imported and released to control introduced weeds (Harris 1986). The classical tactic is aimed at organisms that have been introduced into a new region and have become pests in the absence of their natural enemies. In this case, insects or pathogens are sought from the region of origin and introduced into the new region in the hope that they will become established and eventually suppress a pest to subeconomic levels and maintain it there. The bioherbicide tactic is a more recent approach that uses native fungi to control native weeds (Wall et. al. 1992). These fungi are targeted to indigenous organisms that have become pests. This could be for two reasons. First, the pest organisms may have reached an equilibrium state with their natural enemies that is above economically acceptable levels. Alternatively, they may have become pests as a result of human

activities such as cultivation, fertilization, selective pesticide use, or some crop, range or pasture management practice that disrupts or eliminates their enemies' life cycles (Charudattan and Walker 1982).

Biological control tactics may be used independently of chemical, physical or cultural controls, but are more commonly used in an integrated approach with these other tactics. For example, the trees can be physically slashed (to aid in infection) and the agent applied to the wound. Biological control may also be used in riparian areas, where the use of synthetic herbicides is prohibited, in combination with synthetic herbicides applied in non-riparian areas.

The concept of using plant pathogens for weed control is documented as far back as 1893 (Wilson 1969). However, this concept has not been put into practice until the last three decades. Canadian research on this subject began in the early 1970s when Dr. Ron Wall, (a forest pathologist formerly of the Maritimes Forestry Centre, Canadian Forest Service, Fredericton, New Brunswick) commenced investigations on controlling weed species with disease agents in Maritime forests (Singh 1988). He noted that in many cases, wounding was necessary for a successful infection to take place. Subsequently, Pacific Forestry Centre (PFC), Canadian Forest Services, Victoria, British Columbia, has been instrumental in researching the use of fungi as biological control agents for hardwood tree species. In 1986, Dr. Charles Dorworth initiated a programme of research on "mycoherbicides" as a forest weed control possibility in British Columbia. Dr. Wall joined him at PFC in 1987. They opted to take the bioherbicide approach with their research,

as they were interested in controlling indigenous tree species such as red alder with native fungal pathogens. Their research was focussed mainly on facultative biotrophic fungi that are able to grow on both living plant tissues and dead material as saprophytes (Sieber et al. 1990). The pathogenicity of these fungi tends to be low as they have co-evolved with their respective hosts, and the hosts' defense mechanisms are well developed. Therefore, the "PFC Enhancement" process was developed to shift the balance of the pathogenic interaction in favour of the fungus. With the enhancement method, the vigour of the biological control agent is promoted through nutritional and other reinforcement means that can increase its virulence (Singh 1988). Concurrently, attempts are made to "pre-condition" the target plant through chemical or physical disturbance of its tissues to increase its susceptibility to infection and colonization by the biological control agent. This can be accomplished by wounding the bark or by excising stems to create a cut surface or by treating the target plant with chemical sublethal doses of herbicides to weaken its defense mechanisms to pathogen attack or decrease its vigor.

This "Enhancement" methodology was implemented in the testing of a biological control agent for red alder. The fungus *Chondrostereum purpureum* (Pers.) Pouzar was 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).

It has taken 14 years from the time when Drs. Dorworth and Wall first set out to find a biological control candidate for red alder, for the

biological control product ECOclear™, a *Chondrostereum purpureum*-based formulation, to reach the verge of registration (G. Shrimpton, BC Hydro, pers. comm. 2001). During those years, extensive screening, assay development and field testing was conducted to identify a candidate fungus with the desired attributes as a biocontrol agent against red alder. Considerable attention was also paid to developing a suitable formulation and conducting environmental and health impact assessment studies required for the registration process. This product will be the first registered biological control agent on the market for hardwood tree control in Canada.

2.3.2. The Response of Bigleaf Maple to Parasites and Herbivores

Several potential biological control candidates for *A. macrophyllum* have been tested (Table 3) in both greenhouse and field trials. *Chondrostereum purpureum*, the biological control agent developed for red alder failed to control growth of *A. macrophyllum*. In a field trial conducted by Wall (1996), all *C. purpureum* isolates tested on *A. macrophyllum* caused some stem cankering, but in most trees, the cankers healed by the end of 20 months. Wall (1996) notes that there is a high level of resistance to this fungus in many hardwood species including *A. macrophyllum*.

Table 3. Biological control methods tested for *A. macrophyllum* (data derived from Biring et al. 1996; Wall 1996; Comeau et al. 1995; Sieber and Dorworth 1994).

Organism	**Type**	**Mode of Action**	**Degree of Control**
Sheep	Mammal	grazing	- can browse 30-50% of leaves - repeated grazing for 2-3 yr may reduce 50-75% of leaves
Chondrostereum purpureum	Fungus	stem disease	- no effect
Diplodina acerina (Pass.) Sutton	Fungus	stem disease	- induces formation of circumferential cankers on 60-80% of inoculated 6-mo-old *A. macrophyllum* seedlings

Few other potential biological control agents for *A. macrophyllum* exist. A nematode, *Rhizonema sequoiae* n. gen. n. sp., has been observed infecting the roots of bigleaf maple, with unknown impact (Cid Del Prado 1982), and there is one record of *A. macrophyllum* being attacked by the striped ambrosia beetle, *Trypodendron lineatum* (Olivier), which normally attacks conifers (Lindgren 1986). Peterson et al. (1998) noted that important damaging agents for *A. macrophyllum* are slugs and rodents which cause major seedling mortality, and wildlife species such as elk, *Cervus elaphus* ssp. *roosevelti* (Merriam), and black-tailed deer, *Odocoileus hemionus* ssp. *columbianus* Richardson, which browse seedlings and saplings year round. However, there are no prominent diseases or insects that limit the growth or distribution of *A. macrophyllum*. Bigleaf maple is susceptible to frost damage and is intolerant to flooding for extended periods, and old or damaged trees commonly have serious defects caused by wood-rotting fungi. Of the insect species that feed on foliage, twigs and wood, the carpenter worm, *Prionoxystus robiniae* (Peck), the roundheaded borer, *Synaphaeta guexi* LeConte, and powderpost beetles, *Ptilinus basalis* LeConte, are potentially the most damaging as they weaken affected trees and make them unsuitable for lumber (Peterson et al. 2000). *Acer macrophyllum* is susceptible to white mottled rot, *Ganoderma applanatum* Persoon, a non-aggressive decay agent of dead tissue in both living and dead trees. In living trees, wounds are key entry points for infection and this results in weakened branches and stems.

2.4. A Potential Fungal-based Control Agent

Very few fungal candidates have been identified to date as potential biological control agents for bigleaf maple. One proposed biological control organism is *Cylindrobasidium laeve*, a basidiomycete fungus that is worldwide in distribution that is also native to British Columbia. It is currently registered in South Africa under the trade name Stump Out® where it is used for control of the hardwood, black wattle, *Acacia mearnsii* (Morris 1995, Morris et al. 1998). Black wattle is an exotic invasive tree in South Africa that forms dense thickets along watercourses, clogging streams and decreasing soil moisture through transpiration. *Cylindrobasidium laeve* has been successful in preventing resprouting from black wattle stumps. Application of this fungus minimizes the need for inefficient mechanical slashing or synthetic herbicides. Black wattle is similar to bigleaf maple in how it responds to mechanical control. When the stems are cut, the stumps profusely resprout or coppice, forming multi-stemmed trees, much in the same way as bigleaf maple. In fact, where black wattle is grown for wood production, e.g. for fuel, it is regenerated through coppice harvesting (Morris 1995). The success of *C. laeve* on this difficult-to-manage species led to the hypothesis that it will be a suitable candidate for management of bigleaf maple (S. Shamoun, Pacific Forestry Centre, Canadian Forest Service, Victoria, British Columbia, pers. comm., 1999).

Cylindrobasidium laeve is a white rot pathogen, attacking both lignin and cellulose in wood (Harry Kope, Contact Biologicals, Victoria, British Columbia, pers. comm. 1999). It is an early colonizer and attacks fresh wounds or recently dead wood. Upon growth, it forms a flat white span across the wood or stem. This fungus has been collected on various occasions on southern Vancouver Island and is well represented in the Canadian Collection of Fungal Cultures (CCFC) and American Type Culture Collection (ATCC). It has been collected mainly from hardwood and shrub species and there is no evidence of widespread infection on conifer species. Recently, new pathogenic isolates have been collected and identified from bigleaf maple tissue on Vancouver Island (Harry Kope, pers. comm, 2001). However, these isolates were not available when my research was conducted.

Kendrick (1992) offers the following hierarchical classification system for this species.

The Fungal Union (Kingdom Eumycota and Kingdom Protoctista)
Eukaryotic, heterotrophic, absorptive organisms that develop a diffuse, branched, tubular body and reproduce by means of spores.

Kingdom: Eumycota
Absence of motile cells from the life cycle, mainly terrestrial life style.

Phylum: Dikaryomycota
Hyphae are narrow, usually septate. Wide ecological range, can use many forms of combined nitrogen, some incorporated into lichens.

Occurrence of the dikaryon in the life cycle (sexually compatible nuclei from different mycelia are brought together, pair off, but don't fuse immediately to form a diploid zygote).

Subphylum: Basidiomycotina

Can digest cellulose and sometimes lignin, chitinous hyphal walls, production of macroscopic sexual fruit bodies, assimilative hyphae fused with one another (anastomosis), presence of dikaryophase in the life cycle, turgor pressure driven mechanism for launching the meiospores into the air, hyphal walls multi-layered, often have an extended dikaryophase.

Class: Holobasidiomycetes

Ten orders, hyphae not subdivided by septa; most develop fleshy, corky or woody basidiomata.

Order: Aphyllophorales

Diverse order with eight families including the club and coral fungi, the tooth fungi, the chanterelles and the horn of plenty, the dry rot fungi, the paint fungi, and the bracket fungi, name translates to "without gills". Most are saprobic on wood, some ectomycorrhizal, some attack structural timbers, or the wood and roots of living trees.

Family: Corticiaceae

Form is effuse or resupinate (spread out) on the surface of decaying wood. Hymenium (fertile layer or basidia) may be smooth, wrinkled, or toothed, basidiospore smooth in outline, colourless or pale, and non-

amyloid (do not turn blue in iodine). Basal tissue usually composed of only one kind of hypha (monomitic), basidial hymenium may also incorporate specialized accessory sterile hyphae.

Genus: *Cylindrobasidium*

Species: *laeve*

3.0. Screening of *C. laeve* Isolates

3.1. Introduction

At present, there are no biological controls for bigleaf maple. Fungi such as *C. purpureum* that have proven successful in controlling other hardwood species like *A. rubra* do not appear to affect the health of bigleaf maple. Therefore, my research constitutes the start of a program designed to identify fungal candidates with potential to negatively impact bigleaf maple growth. The long term goal is to develop one or more sustainable biological control tactics for managing bigleaf maple growth in forestry and industrial settings.

The two objectives of this study were to establish experimental cultures of *C. laeve* isolates provided by the Canadian Collection of Fungal Cultures, and to assess the efficacy of these isolates on bigleaf maple seedling growth in a greenhouse experiment.

3.2. Materials and Methods

3.2.1. Plant Material

Ninety-one 1-year-old *A. macrophyllum* dormant seedlings in 4.5 L pots were obtained from Nat's Nursery in Surrey, BC on April 7, 2000 and placed in a greenhouse at BC Ministry of Forests Green Timbers Nursery, Surrey to break dormancy and to allow for growth. The

seedlings had been pruned back to stump heights ranging from 2.5 cm to 8 cm the previous season. They were fertilized 28 times in a 25 week period with nitrogen ranging from 25 to 100 ppm and grown in the greenhouse under natural light. The seedlings were transplanted on May 29 and August 1 into larger pots, ultimately ending up in 13.5 L pots. Sixty of the healthiest 68 surviving seedlings were chosen for experimentation. These seedlings appeared to be growing the most vigourously, were the least chlorotic and were relatively free of visible pest damage.

3.2.2. Fungal Isolates

Four *C. laeve* isolates (Nos. 1, 2, 3 and 4) obtained from the Canadian Collection of Fungal Cultures (CCFC) in Ottawa (Table 4) were plated onto malt dextrose agar (MDA) (3 plates per isolate). They were maintained in the dark at room temperature and subcultured every 2 weeks by transferring a plug from an established culture with a sterile cork borer to fresh medium.

Table 4. Fungal isolates obtained from the Canadian Collection of Fungal Cultures.

Isolate No.	Catalogue No.	Name	Host/ Substrate	Collection Site	Collector	Collection Date
1	CCFC001944	*C. evolvens*[3]	From fruit body on *Acer* sp.	Cantley, PQ	J.H. Ginns	11/04/76
2	CCFC008882	*C. laeve*	Culture from sporophore on *Acer* stub	Clear Lake, Dorset, ON	M.K. Nobles	02/05/63
3	CCFC010292	*C. laeve*	Isolated from brown stained sapwood and heartwood of sugar maple tree	Saulte Ste. Marie, ON		
4	CCFC003627	*C. laeve*	Culture from sporophore on *Acer*	Maple, ON	E.R. Dearden	03/10/46

[3] *Cylindrobasidium laeve* has been taxonomically re-classified and is listed in literature also under the names *Corticium laeve*, *Corticium evolvens*, and *Cylindrobasidium evolvens* (Chamuris 1984).

3.2.3. Treatments

Each bigleaf maple seedling was assigned a number from one to 60. Thirty seedlings were randomly chosen for destructive sampling (Group A) and the remaining 30 were assigned to a non-destructive sampling group (Group B). Destructive sampling was done to provide tissue that could be dissected and examined for evidence of fungal growth at the end of the treatment. The two groups were mixed and grown together in the greenhouse. Five seedlings were randomly chosen for each treatment. The plants were labeled as illustrated in Table 5.

The positive control treatment involved cutting and treating the seedlings with a herbicide (Garlon 4, Table 2) known to control *A. macrophyllum*. The negative control treatment consisted of cutting the seedlings only. On August 11, 2000 all seedlings were cut to a stump height of 15 cm with pruning shears sterilized with 70% ethanol between cuttings, to minimize microbial cross-contamination. Each seedling was treated immediately after cutting. The positive control seedlings were treated with a 30:70 solution of Garlon 4 in a canola oil solvent immediately after cutting. The herbicide mixture was applied with a squeeze bottle to flood the cut surface. This was intended to simulate a cut surface treatment with Garlon 4 that is currently practiced in the field by BC Hydro vegetation maintenance contractors. Negative controls were cut only.

Table 5. Labelling system for respective treatments.

Label	Treatment
Group A (Destructive Sampling Group)	
A1	*C. evolvens* isolate 1
A2	*C. laeve* isolate 2
A3	*C. laeve* isolate 3
A4	*C. laeve* isolate 4
A+	Positive control (Garlon 4 herbicide)
A-	Negative control (cutting only)
Group B (Non-destructive Sampling Group)	
B1	*C. evolvens* isolate 1
B2	*C. laeve* isolate 2
B3	*C. laeve* isolate 3
B4	*C. laeve* isolate 4
B+	Positive control (Garlon 4 herbicide)
B-	Negative control (cutting only)

For the fungal treatments, an agar plug from the leading edge of a 1 week-old culture was removed using a cork borer (10 mm diam.) sterilized between treatments with 70% ethanol. The agar plug was extracted from the borer using forceps sterilized in 70% ethanol and placed mycelial side down on the cut surface of the seedling. The agar plug and top of each stump was wrapped immediately with Parafilm™ to minimize moisture loss.

Data collected for 9 consecutive weeks on August 18, 25, September 1, 8, 15, 22, 29, October 6, and 13 were: number of shoots per plant; length of longest shoot per plant; number of leaves per plant; and size of largest pair of leaves per plant (base of petiole to tip). Due to time constraints, the leaves were not counted after September 22.

3.2.4. Destructive Sampling

Twelve weeks after treatment (November 2, 2000), tissue samples were collected from all 30 plants in the destructive sampling group (Group A). The treated stump surface was removed from each plant by cutting the stem with pruning shears (sterilized as above) as close to the root crown as possible and trimming all branches and leaves from the stem piece. The cuttings were placed in labeled plastic bags, transported to the laboratory, and each was digitally photographed with the Parafilm™ wrap still intact, and again with the cap removed. Approximately 2.5 cm of bark was peeled back from the treated end of each stump and the exposed end was photographed. The cuttings were

then bagged and held at –20°C. Throughout the above process, the stumps were observed for evidence of infection, i.e. the presence of mycelium, tissue staining and tissue necrosis.

3.2.5 Statistical Analyses

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 for each type of measurement at the end of nine weeks, excluding the positive control seedlings (Garlon 4), which were all dead after one week. The Tukey-Kramer HSD test for All Comparisons was used to determine which means were significantly different from one another when ANOVA p was <0.05.

3.3. Results

None of the fungal-treated stems exhibited any characteristics indicative of a fungal colonization such as presence of mycelium, staining, or tissue necrosis. With the exception of the positive control group (Garlon 4 treated), all stems were alive after 13 weeks and resembled the negative control group in growth characteristics and appearance. All positive control plants, treated with Garlon 4, were dead after 1 week. No plants in any of the other treatment groups died. In many cases, the expanding lateral buds either broke through or pushed

the Parafilm™ cover off of the cut stumps. Many of the leaves became infected with powdery mildew, which did not appear to affect their health.

In no case did ANOVA indicate a significant difference among treatments for any characteristic after 9 weeks (Figures 3-6). Shoot growth appeared to be stimulated by isolate 3 from weeks 3-6, but there was no difference in shoot growth by the end of nine weeks.

Figure 3. Mean number (+SE, n=10) of shoots counted for each treatment group for nine weeks. Positive control seedlings all dead by week 1, and therefore excluded. For Week 9, F=0.8282, df= 4, 46, p=0.5141.

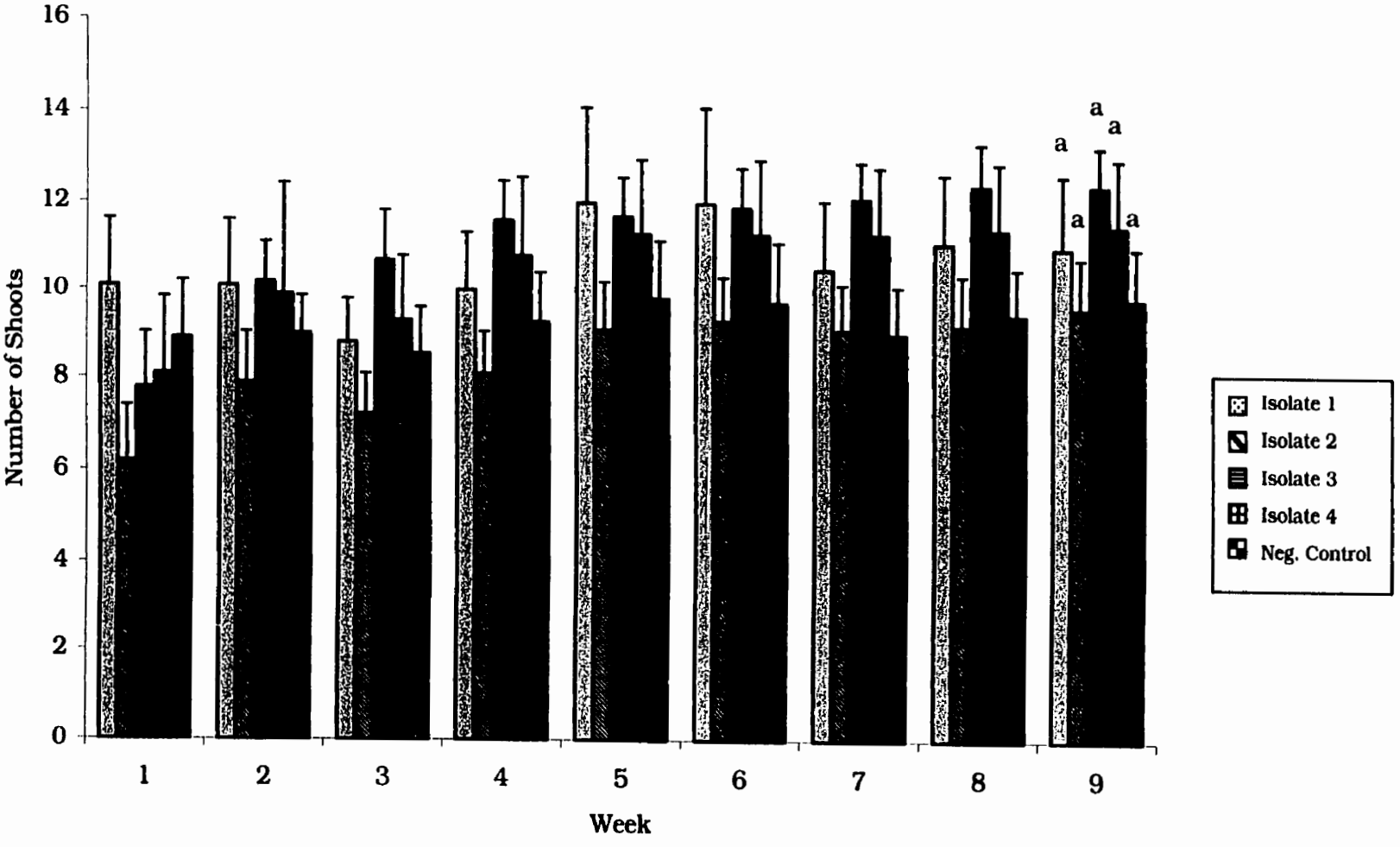


Figure 4. Mean length of shoots (+SE, n=10) measured for each treatment group for nine weeks. Positive control seedlings all dead, and therefore excluded. For Week 9, F=0.9473, df=4, 46, p=0.4454.

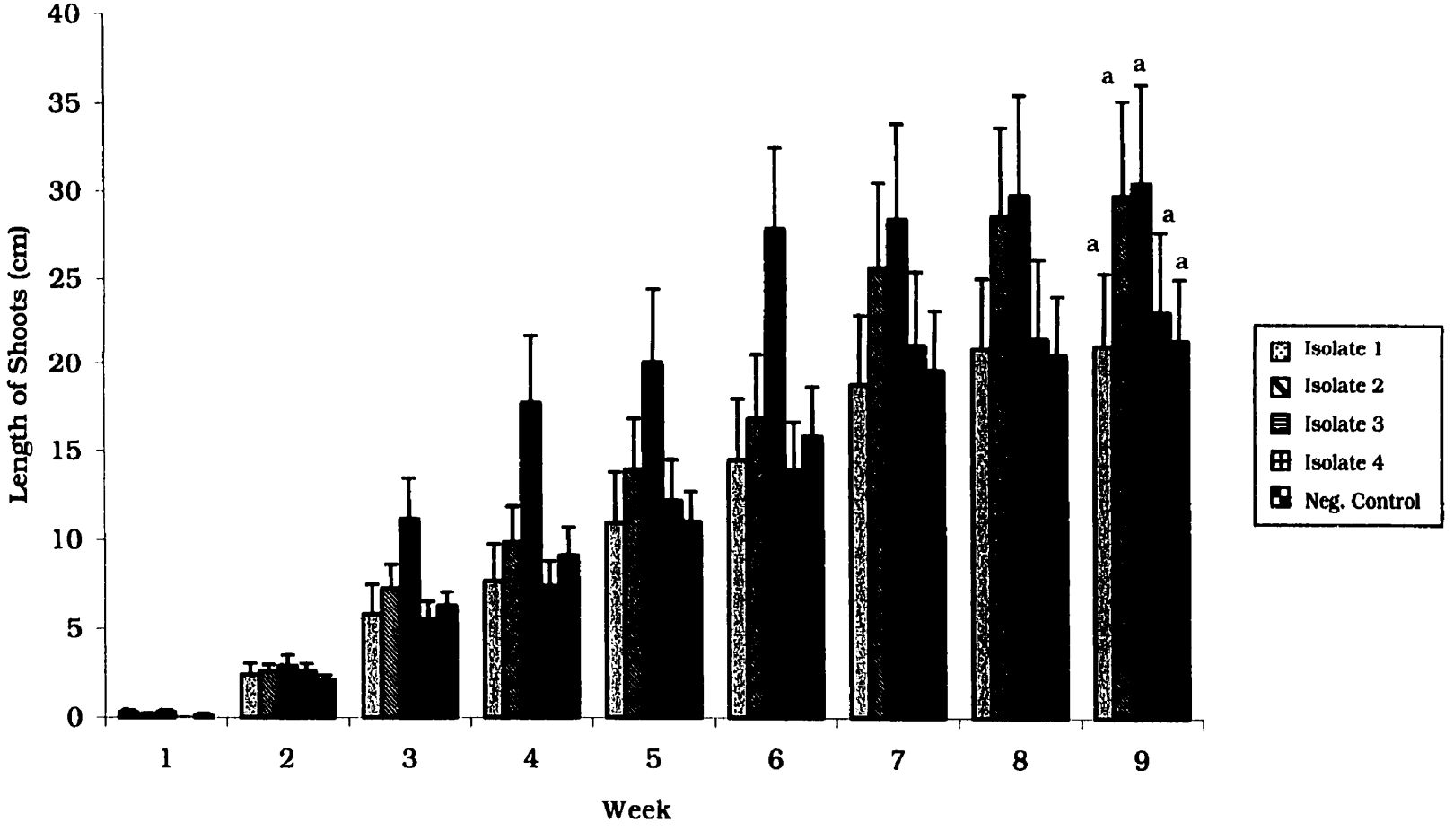


Figure 5. Mean number of leaves (+SE, n=10) counted for each treatment group for nine weeks. Positive control seedlings all dead, and therefore excluded. For Week 6, F=2.1535, df=4, 46, p=0.0893.

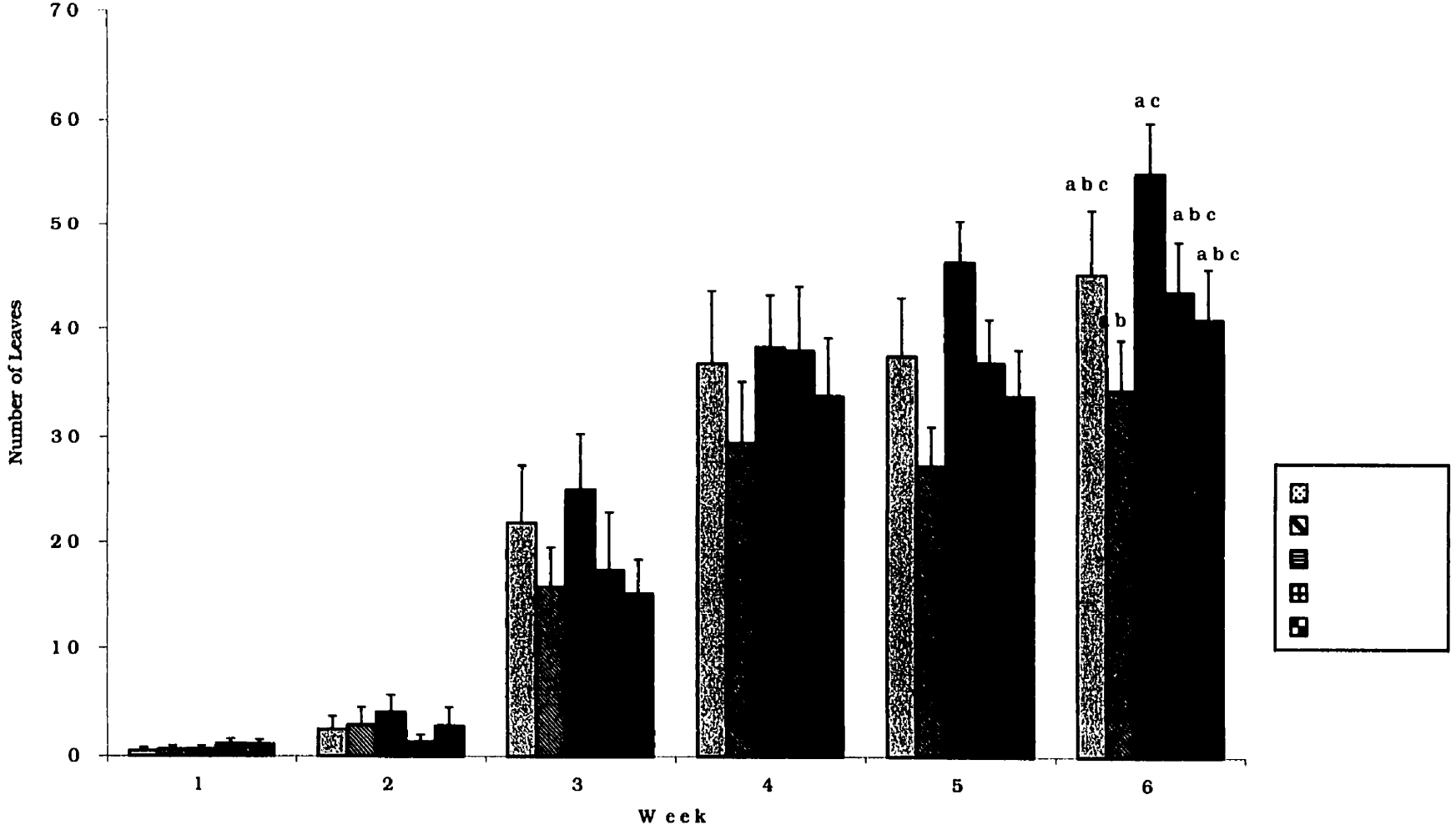
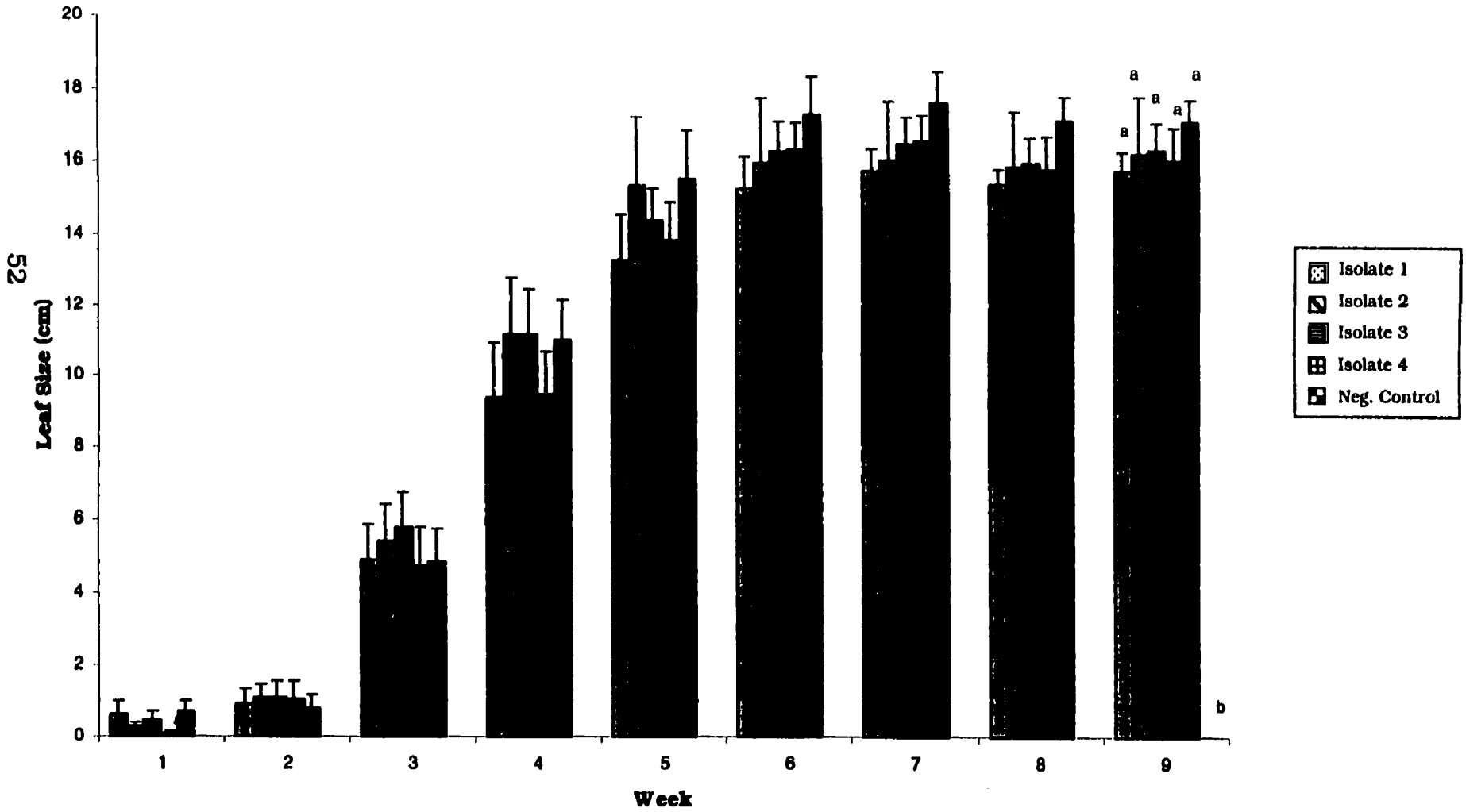


Figure 6. Mean leaf size (+SE, n=10) measured for each treatment group for nine weeks. Positive control seedlings all dead, and therefore excluded. For Week 9, F=0.2966, df=4, 46, p=0.8787.



3.4. Discussion

The search for new fungal biological control agents is difficult and lengthy, often taking up to 15 years to develop a marketable product e.g. the *Chondrostereum purpureum*-based product, ECOClear™. It involves three main phases of research and development.

The discovery phase involves the search for suitable fungal candidates, and is predicated on a thorough knowledge of the biology and ecology of the pest organism (in this case bigleaf maple) to identify stages in its life cycle at which it is more susceptible to control by fungal infection. For example, although bigleaf maple can survive repeated removal of its leaves and stems, it is susceptible to root pathogens such as *Armillaria* and to wood-rotting basidiomycetes. Thus leaf pathogens may not make effective biological control agents, but root pathogens or wood rotting pathogens might have potential. For example, powdery mildew had no apparent effect on seedling health in my experiment. Unfortunately many of the root rot pathogens that attack bigleaf maple are also economically significant disease organisms on most commercial conifer species, and are therefore undesirable choices for biological control. This leaves wood-rotting agents like *C. laeve* as the most attractive candidates for biological control.

The discovery phase also involves conducting surveys in bigleaf maple habitats to determine which fungi are found on healthy bigleaf maple. Those that are opportunistic and only found on dying or sickly

trees may have potential as biological control agents. This was not done prior to my research, and I relied in isolates from maples in eastern Canada. Host-parasite indices and mycological herbaria can also reveal what other researchers have collected from the host of interest.

If potential candidates are found, the discovery phase concludes with testing them on the target species in a controlled setting to determine if they have potential as biological control candidates. The fungal candidates should possess certain desired attributes including fast growth and amenability to easy and cost effective culturing. If the fungi perform as desired in a controlled setting, they must then be tested in a field setting where they are subject to ambient environmental conditions. It may also be necessary to determine in which season they should be applied, as plant growth stages and environmental factors may affect efficacy (Dr. J.E. Rahe, Simon Fraser University, pers. comm. 2000).

The second phase of research involves the development of a product formulation. Formulations allow the fungus to be applied easily in a field setting and will contain a growth medium and nutrients to support the fungus until it infects the host tissue. Formulations must also be cost effective, practical to manufacture and provide reasonable shelf life (Boyette et al. 1991).

The third and final phase is registration of the product as a pesticide by government agencies. Registration relies on studies that ensure that the product does not adversely affect human health or the

environment, that it is specific to the pest organism, and in Canada that it is efficacious. Only when all of these tests are done can the product be considered for registration. Only registered products may be sold and applied in certified procedures.

There are a number of possible reasons why the four *C. laeve* isolates tested may not have had a negative effect on *A. macrophyllum* seedling growth. These can be summarized into six categories.

3.4.1 Isolate Virulence

The first possibility is that the isolates were not sufficiently virulent. They are not known to be associated with bigleaf maple, and are probably not adapted to a west coast environment. I would recommend that additional bioassays only be done with isolates collected specifically from bigleaf maple in British Columbia.

3.4.2 Fungal Ecology and Biology

Little is known of the ecology and biology of *C. laeve* in British Columbia. For example, it is not known if the fungus is common or is rare, what its target hosts are and what its distribution is in a bigleaf maple environment. Answers to these questions require local field surveys specifically on bigleaf maple trees. If local isolates colonize bigleaf maple wood pieces in the lab, then transfer of the fungus from infected wood could prove useful for further experimentation. An important factor that needs to be determined is which tissue type this

fungus attacks in trees (cambium, sapwood, or heartwood). If it prefers one tissue over others, then targeting that specific tissue may improve the chance of successful infection. We are collaborating with Dr. Harry Kope of Contact Biologicals, Victoria, B.C., to further our understanding of the ecology of this fungus, and experimental work on the biology of this fungus is currently being carried out by Ms. Deepraj Purewal under the direction of Dr. Rahe at Simon Fraser University.

3.4.3 Environmental Conditions

The environmental conditions (temperature, light, and relative humidity) may not have been conducive for infection with the isolates tested. For example, the agar plugs may have dried out too quickly. Further experimentation is necessary to assess effectiveness of other coverings (in addition to Parafilm™) and to cut the stems so that expanding buds do not force the Parafilm™ coverings open or tear through them. In addition, a more detailed understanding of *C. laeve* biology would provide useful information to understand the way in which growth conditions may alter virulence of *C. laeve*.

It is possible that sterilization of the cut surface prior to application of the agar plug harmed the fungus or prevented mycelial attachment. Therefore, surface sterilizing only the sides of stems (not the cut surface directly) or use of a different sterilant should be tested.

3.4.4 Types of Inoculum

It is possible that although the isolates were appropriate, the type of inoculum used may not have been suitable. The following forms of inoculum should ideally be tested: a basidiospore-based viscous matrix, mycelial colonies cultured on bigleaf maple wood (hence producing the enzymes characteristic of the wood rotting fungi), and mycelial colonies on wood from other species of hardwood trees. Although some species of basidiomycetes are successfully applied in a mycelial-based inoculum, such as the application of *C. purpureum* on red alder, spore-based inocula have also been used. The original *C. laeve* Stump Out® formulation is comprised of spores in an oil carrier. Conditions for creating successful sporulation of *C. laeve* in culture have been described (M. Morris, ARC Plant Protection Research Institute, Weeds Research Division, P/Bag X5017, Stellenbosch, 7599, South Africa, unpublished data, 1999). *Cylindrobasidium laeve* was first grown on a modified Potato-Marmite-Dextrose medium for 3 days. Small blocks of agar were then transferred to Petri dishes containing small autoclaved discs of *A. mearnsii* wood cut from young saplings onto a water agar. Such a basidiospore-based inoculum warrants testing in future studies with bigleaf maple.

Boyette et al (1991) stated that, generally, the most suitable infective units in biological control programs are fungal spores. They noted that asexually produced spores (conidia) are usually the easiest to produce under controlled conditions and since spores are the most common mechanism for natural disease dispersal they should serve as the best candidates for mycoherbicide formulations.

How the inoculum is presented to the host may play a role in the infection process. The four types of inoculum described above could be presented to wounded bigleaf maple tissue in a number of ways including spores in an oil carrier (assuming that the spores remain viable in oil) and mycelium in wedges of infected wood. In my experiment the fungus was applied with a plug of growth medium that supported mycelial growth of the fungus. However, it is possible that this medium may not have promoted maximum virulence for the isolates tested.

Seasonality may have played an important factor in how the seedlings responded to potential infection, and greenhouse conditions may have overridden seasonal changes in the natural environment. To test the possible effect of seasonality on efficacy, bigleaf maple trees could be inoculated in two distinct seasons, i.e. in May (after the spring growth spurt) and in December (during dormancy). Because many field-collected plants do not grow well in greenhouse conditions, e.g. *Rubus* species (Hollmann, 2001), it may be appropriate to perform future bioassays under field conditions.

It is possible that mature trees may be more susceptible to *C. laeve* than 1 year-old bigleaf maple seedlings. This could be readily tested by establishing a small scale field trial with native fungal isolates on trees at different sites and developmental stages.

3.4.5 Duration of Experiment

It is possible that the fungus may take longer than 9 weeks to infect bigleaf maple wood. However, since none of the stumps appeared to be infected at the end of the sampling period it is unlikely that continuing this study any longer would have yielded a different result.

3.4.6 Other Bigleaf Maple Pathogens

It is possible that even if native isolates of *C. laeve* were used, it may not be a suitable biocontrol agent against bigleaf maple. If this is the case then alternative fungi should be sought. However, given suitable conditions and potentially more virulent isolates, this fungus may have potential as a biological control agent and further experimentation is warranted. Research currently being conducted by Ms. Deepraj Purewal at Simon Fraser University with isolates collected from bigleaf maple tissue in the field, is showing promise. These isolates do appear to affect the health of bigleaf maple seedlings in the greenhouse (S. Lee, pers. comm. 2001).

References

Agriculture Canada. 1985. Seeds Act and Regulations. Queen's Printer, Ottawa, ON, Publs. RE-3153 and YX55-1985-S-8.

Anonymous, 1995. Geotextile for maple resprout. BC Hydro, Safety and Environment, Burnaby, BC.

Anonymous, 1996. Ministry of Forests Annual Report 1995/1996. Ministry of Forests, Victoria, BC. http://www.for.gov.bc.ca/pab/publctns/AN_RPTS/9596AN/AN96_TOC.HTM

Arron, G.P., S. de Becker, H.A. Stubbs, and E.W. Szeto. 1997. An evaluation of the efficacy of tree growth regulators paclobutrazol, flurprimidol, dikegulac, and uniconazole for utility line clearance. J. Arbor. 23:8-16.

Biring, B.S., P.G. Comeau, and J.O. Boateng. 1996. Effectiveness of forest vegetation control methods in British Columbia. FRDA Handbook 011. Canadian Forest Service and BC Ministry of Forests, Victoria, BC.

Black, M.E. 1981. *Acer macrophyllum*: hills of gold. Univ. Washington Arboretum Bull. 44:35-38.

Boateng, J.O. 1986. Estimates of forest brush problems in British Columbia. BC Ministry of Forests, Silvicultural Branch, Victoria, BC.

Boateng, J.O. and G. Ackerman. 1988. Manual and chemical control treatments of bigleaf maple coppices. Expert Committee on Weeds, West. Can. Sect. Meeting, Nov. 29-Dec. 1, 1988, Winnipeg, MB. Res. Rep., p. 192.

Boyette, C.D., P.C. Quimby, Jr., W.J. Connick, Jr., D.D. Daigle, and F.E. Fulgham. 1991. 12. Progress in the production, formulation, and application of mycoherbicides, p.p. 209-222 in D.O. TeBeest, Ed. Microbial Control of Weeds. Chapman and Hall, London, England.

Brayshaw, T.C. 1996. Trees and shrubs of British Columbia. Handbook (Royal British Columbia Museum) No.31. UBC Press, Vancouver, BC.

Chamuris, George P. 1984. Nomenclatural adjustments in *Stereum* and *Cylindrobasidium* according to the Sydney Code. Mycotaxon 20:587-588.

Charudattan, R. 1988. Assessment of efficacy of mycoherbicide candidates, p.p. 455-464 in E.S. Delfosse, Ed. Proc. VII International Symposium on Biological Control of Weeds. CSIRO Publications. Victoria, Australia.

Charudattan, R. and H.L. Walker. 1982. Biological control of weeds with plant pathogens. Wiley, New York.

Cid Del Prado, I. 1982. Taxonomy, morphology, biology and histopathology of *Rhizonema sequoiae* on coast redwood *Sequoia sempervirens*. Ph.D. Dissertation. University of California, Davis.

Cole, E.C. and M. Newton. 1990. Efficacy of different herbicides on bigleaf maple sprout clumps. Proc. West. Soc. Weed Sci. 43:37-43.

Cole, E.C. and M. Newton. 1991. Efficacy of cut stump treatments for controlling bigleaf maple. Proc. West. Soc. Weed Sci. 44:50-53.

Cole, E.C., M. Newton, and D.E. White. 1986. Response of northwestern hardwoods, shrubs, and Douglas-fir to Arsenal® and Escort®. Proc. West. Soc. Weed Sci. 39:93-101.

Cole, E.C., M. Newton, and D.E. White. 1988. Efficacy of imazapyr and metsulfuron methyl for site preparation and conifer release in the Oregon Coast range. Oreg. State Univ., For. Res. Lab., Corvallis, OR. Res. Note 81.

Comeau, P.G., Wall, R. and Prasad, R. 1995. Control of bigleaf maple using cut stump and basal treatments. Expert Comm. on Weeds, West. Can. Sect. Meet. Nov. 28-Dec. 1, 1995, Montreal, PQ. Res. Rep., p. 486.

Conard, S.G. and W.H. Emmingham. 1984a. Herbicides for brush and fern control on forest sites in Oregon and Washington. Oreg. State Univ., For. Res. Lab., Corvallis, OR. Spec. Publ. 8.

Conard, S.G. and W.H. Emmingham. 1984b. Herbicides for clump and stem treatments of trees and shrubs in Oregon and Washington. Oreg. State Univ., For. Res. Lab., Corvallis, OR. Spec. Publ. 9.

Conard, S.G. and W.H. Emmingham. 1984c. Herbicides for forest brush control in southwestern Oregon. Oreg. State Univ., For. Res. Lab., Corvallis, OR. Spec. Publ. 6.

D'Anjou, B.N. 1990. Growth response of several vegetation species to herbicides and manual cutting treatments in the Vancouver Forest Region. BC Min. For. and For. Can., Victoria, BC. FRDA Rep. 135.

D'Anjou, B.N. 1991. Comparison of several alternatives for controlling bigleaf maple sprouts. Expert Comm. on Weeds, West. Can. Sect. Meeting, Nov. 26-28, 1991, Vancouver, BC. Res. Rep., p. 72.

D'Anjou, B.N. 1993. Comparison of several alternatives for controlling bigleaf maple sprouts. BC Min. For., Vancouver For. Reg. Progress Rep.

Elias, T.S. 1980. The complete trees of North America: field guide and natural history. Book Division, Times Mirror Magazines, Inc. New York.

Farrar, J.L. 1995. Trees in Canada. Fitzhenry & Whiteside Limited and Canadian Forest Service. Markham, ON.

Figueroa, P.F. 1989. Bigleaf maple control: triclopyr thin-line and spot-foliar application treatments using imazapyr, metsulfuron, and glyphosate. Proc. West. Soc. Weed Sci. 42:104-119.

Figueroa, P.F. 1995. Reduction of weed competition below the level that impacts conifer growth: another approach to hardwood management. *In* 2nd. Int. Conf. on Forest Vegetation Management, March 20-24, 1995, Rotorura, N.Z. N.Z. For. Res. Inst., Rotorura, N.Z. FRI Bull. 192:173-5.

Figueroa, P.F. and V.F. Carrithers. 1994. Triclopyr for control of bigleaf maple: thinline applications in December, February, and April. Proc. West. Soc. Weed Sci. 47:38-43.

Figueroa, P.F. and T.E. Nishimura. 1992. Bigleaf maple control: basal thin-line applications using imazapyr liquids and ground applications of imazapyr granules. Proc. West. Soc. Weed Sci. 45:65-72.

Figueroa, P.F. and T.E. Nishimura. 1994. Imazapyr for control of bigleaf maple: basal thin-line and ground-applied granules. Proc. West. Soc. Weed Sci. 47:43-49.

Figueroa, P.F., V.F. Carrithers, and R.G. Lym. 1993. Bigleaf maple control: thinline basal applications using triclopyr and triclopyr plus picloram. Proc. West. Soc. Weed Sci. 46:24-30.

Figueroa, P.F., T.E. Nishimura, and R.G. Lym. 1992. Bigleaf maple control: basal thinline applications using imazapyr liquids and ground applications of imazapyr granules. Proc. West. Weed Sci. 45:65-72.

Fraser, R.G., T.S. Mackay, M. Mihajlovich, T. Olson, G. Mercier, and N. Helewa. 1990a. Rates and timing of foliar application of triclopyr ester to control bigleaf maple and red alder. Expert Comm. Weeds, West. Can. Sect. Meeting, Nov. 26-29, 1990, Regina SK. Res. Rep. 3:202.

Fraser, R.G., T.S. Mackay, M. Mihajlovich, T. Olson, G. Mercier, and N. Helewa. 1990b. Rates and timing of basal bark application of triclopyr ester to control bigleaf maple. Expert Comm. Weeds, West. Can. Sect. Meeting, Nov. 26-29, 1990, Regina SK. Res. Rep. 3:201.

Fried, J.S., J.C. Tappeiner II, and D.E. Hibbs. 1988. Bigleaf maple seedling establishment and early growth in Douglas-fir forests. Can. J. For. Res. 18:1226-33.

Gendron, F., C. Messier, and P. Comeau. 1998. Comparison of various methods for estimating mean growing season percent photosynthetic photon flux density in forests. Agric. For. Meteor. 92:55-70.

Haeussler, S., D. Coates, and J. Mather. 1990. Autecology of common plants in British Columbia: a literature review. FRDA Report 158. Canadian Forest Service and BC Ministry of Forests, Victoria, BC.

Hansen, D., T. Hudiberg, and M. Turnock. 1998. Sap-flow, gas exchange and water-relations in red alder (*Alnus rubra*) and big leaf maple (*Acer macrophyllum*). Bot. Soc. Amer. Abstr., Ecology Section 2-6 August. Item 102, p34-35.

Harrar, E.S. 1940. The Kimball maple. J. For. 38(9):726-728.

Harris, P. 1986. Biological control of weeds. Fortschr. Zool. 32:123-138.

Hintz, W. and S. Shamoun. 1996. Environmental fate and risk assessment of a novel forest-weed biocontrol agent, in *"Risk Assessment at the Crossroads": Canada-U.S. Symposium on Biotechnology Risk Assessment.* Proceedings of the 8th Symposium on Environmental Releases of Biotechnology Products: Risk Assessment Methods and Research, Ottawa.

Hollmann, Peter J. 2001. Development of weedy *Rubus* (*R. parviflorus, R. spectabilis,* and *R. strigosus*) based-tissue cultures and assessment of their use for screening *Fusarium avenaceum* phytotoxins. M.Sc. Thesis. Simon Fraser University, Burnaby, BC.

Hosie, R.C. 1979. Native trees of Canada, 8th Ed. Fitzhenry and Whiteside, Ltd. Don Mills, ON.

Kendrick, B. 1992. The fifth kingdom, 2nd ed. Focus Information Group. Newburyport, MA.

Klinka, K., V.J. Krajina, A. Ceska, and A.M. Scagel. 1989. Indicator plants of coastal British Columbia. University of British Columbia Press. Vancouver, BC.

Knowe, S.A. 1995. Evaluation of objective estimates of treatment efficacy for bigleaf maple. Can. J. For. Res. 25:844-850.

Krajina, V.J., K. Klinka, and J. Worrall. 1982. Distribution and ecological characteristics of trees and shrubs of British Columbia. University of British Columbia, Faculty of Forestry. Vancouver, BC.

Lauterbach, P. and L.E. Warren. 1982. Control of resprouting hardwood clumps with applications of triclopyr ester by hovering helicopter. Proc. West. Weed Sci. 35:36-38.

Lei T.T. and M.J. Lechowicz. 1997a. Functional responses of *Acer* species to two simulated forest gap environments: leaf-level properties and photosynthesis. Photosynthetica 33: 277-289.

Lei T.T. and M.J. Lechowicz. 1997b. The photosynthetic response of eight species of *Acer* to simulated light regimes from the centre and edges of gaps. Func. Ecol. 11: 16-23.

Lindgren, B.S. 1986. *Trypodendron lineatum* breeding in bigleaf maple, *Acer macrophyllum*. J. Entomol. Soc. Brit. Columbia 83:44.

Lyons, C.P. and B. Merilees. 1995. Trees, shrubs, and flowers to know in British Columbia and Washington. Lone Pine Publishing. Vancouver, BC.

Minore, D. and J.C. Zasada. 1990. *Acer macrophyllum* Pursh: bigleaf maple, in R.M. Burns and B.H. Honkala, Eds. *Silvics of North America: Volume 2, Hardwoods*. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, WADC.

Morris, M. 1995. Resprouting of black wattle stumps – a solution in sight. Plant Protection News 42:4-5.

Morris, M.J., A.R. Wood, and A. Den Breëyen. 1998. Development and registration of a fungal inoculant to prevent regrowth of cut wattle free stumps in South Africa, and a brief overview of other bioherbicide projects currently in progress. IV International Bioherbicide Workshop Programme and Abstracts.

Mulligan, B.O. 1954. An upright Oregon maple. Univ. Washington Arbor. Bull. 17:112.

Murray, E. 1969. *Acer macrophyllum* forma *rubrum*. Kalmia 1:5.

Nadkarni, Nalini M. 1984. Biomass and mineral capital of epiphytes in an *Acer macrophyllum* community of a temperate moist coniferous forest, Olympic Peninsula, Washington State. Can. J. Bot. 62: 2223-2228.

Newton, M., E.C. Cole, and D.E. White. 1986. What influences control of deciduous brush with glyphosate? Proc. West. Soc. Weed Sci. 39:86-92.

Oleskevich, C. 1996. Autecology and potential biological control of *Rubus strigosus, Rubus parviflorus*, and *Rubus spectabilis*. M.Sc. Thesis. Simon Fraser University, Burnaby, BC.

Parish, R. and S. Thomson. 1994. Tree book: learning to recognize trees of British Columbia. Canadian Forest Service and BC Ministry of Forests. Victoria, BC.

Peterson, E.B., N.M. Peterson, P.G. Comeau, and K.D. Thomas. 1998. Bigleaf maple managers' handbook for British Columbia. Draft Report. BC Ministry of Forests, Victoria.

Pojar, J. and A. MacKinnon. 1994. Plants of coastal British Columbia including Washington, Oregon and Alaska. Lone Pine Publishing. Vancouver, BC.

Pursh, F. 1814. Flora Americæ Septentrionalis; or, a systematic arrangement and description of the plants of North America. Volume 1. White, Cochrane, and Co., London.

Radosevich S., J. Holt, and C. Ghersa. 1997. Weed ecology: implications \for management, 2nd ed. John Wiley and Sons, Inc. New York, NY.

Ruth, R.H. and G.F. Muerle. 1958. Silvical characteristics of bigleaf maple. Pacific Northwest Forest and Range Experiment Station, Silvical Series No. 13. US Department of Agriculture, Forest Science. Portland, OR.

Shamoun, S.F. 1997. Biological control potential to manage hardwood weed species: *Chondrostereum purpureum.* Natural Resources Canada, Canadian Forest Service, Victoria, BC.

Sieber, T.N. and C.E. Dorworth. 1994. An ecological study about assemblages of endophytic fungi in *Acer macrophyllum* in British Columbia: in search of candidate mycoherbicides. Can. J. Bot. 72:1397-1402.

Sieber, T.N., F. Sieber-Canavesi, and C.E. Dorworth. 1990. Identification of key pathogens of major coastal forest weeds. FRDA Report 113. Forestry Canada, Victoria.

Singh, P. 1988. Biological control of forest weeds: Canadian Research Efforts, p.p. 675-683, in E.S. Delfosse, Ed. Proc. VII International Symposium on Biological Control of Weeds. CSIRO Publications. Victoria, Australia.

Staebler, G.R. 1960. Basal injection of 2,4,5-T fails to kill red alder and bigleaf maple. Forestry Research Note 23, Weyerhaeuser Company.

Tappeiner, J.C., J. Zasada, D. Huffman, and B.D. Maxwell. 1996. Effects of cutting time, stump height, parent tree characteristics, and harvest variables on development of bigleaf maple sprout clumps. West. J. Appl. For. 11:120-124.

Thomas, K.D. and P.G. Comeau. 1998. Effects of bigleaf maple (*Acer macrophyllum* Pursh) on growth of understory conifers and the effects of coppice spacing on the growth of maple (MOF EP 1121.02) BC Ministry of Forests, Research Branch, Victoria. Extension Note 24. 4p.

van Gelderen, D.M., P.C. de Jong, and H.J. Oterdoom. 1994. Maples of the world. Timber Press, Portland, OR.

Van Driesche, R.G. and T.S. Bellows. 1996. Biological control. Chapman and Hall, New York.

Wagner, R.G. 1987. First-year results from treatments to control bigleaf maple sprout clumps. Oreg. State Univ., For. Res. Lab., Corvallis, Oreg. CRAFTS Tech Rep.

Wagner, R.G. and M.W. Rogozynski. 1994. Controlling sprout clumps of bigleaf maple with herbicides and manual cutting. West. J. Appl. For. 9:118-124.

Wall, R.E. 1996. Pathogenicity of the bioherbicide fungus *Chondrostereum purpureum* to some trees and shrubs of southern Vancouver Island. FRDA Report 246. Canadian Forest Service and BC Ministry of Forests, Victoria, BC.

Wall, R.E., R. Prasad, and S.F. Shamoun. 1992. The development and potential role of mycoherbicides for forestry. For. Chron. 68:736-741.

Walstad, J.D., M. Newton, and D.H. Gjerstad. 1987. Overview of vegetation management alternatives. *In* Forest vegetation management for conifer production. J.D. Walstad and P.J. Kuch (editors). Wiley, New York, NY.

Warren, L.E. 1980. Control of sprouting hardwoods with basal applications of Garlon 4 herbicide. Indust. Veg. Turf and Peat. Manag. 12:23-8.

Warren, L.E, M. Vomocil, M. Newton, and D. Belz. 1984. Control of bigleaf maple and associated hardwoods in conifer forests with Garlon 3A. Down to Earth 40:8-12.

Wilson, C.L. 1969. Use of plant pathogens in weed control. Ann. Rev. Phytopathology 7:411-434.

Zasada, J.C., J.C. Tappeiner II, and T.A. Max. 1990. Viability of bigleaf maple seeds after storage. West. J. Appl. For. 5:52-55.