AUTECOLOGY AND POTENTIAL BIOLOGICAL CONTROL
OF RUBUS STRIGOSUS, RUBUS PARVIFLORUS,
AND RUBUS SPECTABILIS.

by

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ABSTRACT

Wild raspberry (*Rubus strigosus* Michx.), thimbleberry (*R. parviflorus* Nutt.), and salmonberry (*R. spectabilis* Pursh) are native perennial deciduous shrubs which rapidly invade disturbed forest areas and may effectively outcompete economically valuable regenerating conifers. The biology of these *Rubus* species is reviewed in detail with botanical descriptions, geographical distribution, history, economic importance (detrimental and beneficial), habit, growth and development, reproduction, population dynamics, and response to chemical (herbicide), manual, and biological control.

Three endemic fungi, *Fusarium avenaceum*, *Colletotrichum dematium*, and a *Phomopsis* sp., were investigated as potential biological control agents for *R. parviflorus* and *R. spectabilis*. *F. avenaceum* was selected for further study after inducing foliar necrosis on intact *Rubus* plants when inoculum was grown on a rice medium and applied as a culture filtrate in shadehouse trials. Analysis of *F. avenaceum* filtrate revealed that a single toxin, moniliformin, was present in high concentrations (>3000 ppm). The filtrate combined with a surfactant (0.4% Silwet L-77®) induced 50-100% foliar necrosis in *R. parviflorus* and 25-50% in *R. spectabilis*, without requirement of a dew period. A host-range test revealed that six economically important conifer species showed no visible injury when inoculated with the formulation of *F. avenaceum*.

A first approximation vegetation management field trial was conducted to compare biological, chemical, and manual control of invasive *R. parviflorus* and *R. strigosus* in a suppressed 1-year old spruce plantation in the Sub-Boreal Spruce biogeoclimatic zone. A randomized complete block design was established with seven treatments replicated in three blocks. After 3 weeks post-application, neither the biological control treatment of *F. avenaceum* combined with Silwet L-77® or herbicide treatments demonstrated an immediate reduction in light attenuation measurements at the seedling level or in direct conifer and target vegetation measurements. The biological control treatment did not
induce necrosis previously observed in shadehouse trials except when target plants were predisposed to a low dose of glyphosate (0.356 kg a.i./ha or 1 L/ha Roundup®).

The results from this study demonstrate the potential of *F. avenaceum* as a biological control agent of *Rubus* species when grown on a rice medium and applied in combination with a surfactant or low dose of glyphosate.
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CHAPTER 1

AN AUTECOLOGICAL REVIEW OF *RUBUS STRIGOSUS* MICHX., *R. PARVIFLORUS* NUTT., AND *R. SPECTABILIS* PURSH.

1.0. INTRODUCTION

Wild raspberry (*Rubus strigosus* Michx.), thimbleberry (*R. parviflorus* Nutt.), and salmonberry (*R. spectabilis* Pursh) are native perennial deciduous shrubs which rapidly invade disturbed forest and roadside areas. Through prolific vegetative growth, these shrubs form dense, multilayered, and monospecific stands and form extensive clonal colonies. They create habitat and supply food sources for a variety of forest fauna and are important in nutrient cycling and reducing soil erosion. These *Rubus* shrubs may effectively outcompete economically valuable regenerating conifers and a review of chemical, manual, and biological control methods is presented. Reproductive biology, growth and development, and population dynamics are discussed in detail in an autecological review of these three *Rubus* species.

1.1. Botanical nomenclature

I. *Rubus strigosus* Michx. — In North America, also referred to as *R. idaeus* L. or *R. idaeus* var. *strigosus* (Michx.) Focke (Kartesz 1994). — wild red raspberry, framboisier sauvage (Mulligan 1992).


Rosaceae, Rose family, brambles, Rosacées.

1
1.2. Description and Account of Variation

1.2.1. Botanical description: *Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* are perennial deciduous shrubs spreading principally by the production of root suckers or rhizomes.

*Rubus strigosus* (Figure 1). Erect biennial stems (canes) arising from perennial subterranean branching root suckers and stolons, up to 2 m tall, often arched. First year stems (primocanes) with broadly based prickles, slender bristles and glandular hairs; leaves alternate, pinnately compound, 3-5 or 3-7 foliate, leaflets from broadly ovate to narrowly lanceolate, irregularly serrate-pointed, petioles bristly-hispid, stipules slender. Second year stems (floricanes) armed with weak spines and bristles, often glandular-hairy, bark brownish, exfoliating, older stems smooth striate; leaves approximately ternate, 7-10 cm long, margins evenly double-serrate, green above, white-tomentose beneath. Inflorescence of 2-5 flowers in terminal or axillary racemes or solitary in upper leaf axils, flower stalks and hypanthium bristly and glandular hispid, flowers drooping or in small thyrsoid clusters; flowers white, 1 cm broad, elliptical petals 5-6 mm long, shorter than sepals, carpels numerous. Fruit an aggregate of many small drupelets, mature fruit red, ovoid, sweet, falling intact from dry receptacle, receptacle persistent on plant (Hitchcock et al. 1961; Rouleau 1964; Scoggan 1978; Roland 1983).

Chromosome number differs due to the highly variable nature of this species. Worldwide, most raspberries are reported as diploid with $2n = 14$ (Ellis et al. 1991) although a range of ploidies exists in Canadian populations including $2n = 14, 21, 28, 35,$ and 42 (Moss 1983). Karyotype variation was investigated by Pool et al. (1981), and Nybom and Schaal (1990) reported DNA fingerprinting identified genotypic distribution in natural populations.

*Rubus parviflorus* (Figure 2). Rhizomatous shrub with erect to semi-prostrate stems, reaching 0.5-2.5 m tall, unarmed, glandular hairy, bark shredding. Leaves simple,
large, 12-20 cm long, palmately 3-5 or 3-7 lobed with deep basal sinus, lobes triangular, cordate, twice dentate-serrate, soft, slightly pilose on both surfaces to densely pilose beneath; petioles glandular-pubescent, up to twice as long as blade; stipules narrow, 6-13 mm long. **Flowers** in terminal inflorescences, long peduncled corymbs (Hulten 1974) or cymes (Taylor and MacBryde 1977; Scoggan 1978) of 3-11 white flowers each 4-5 cm across; sepals broadly ovate and 15 mm long, petals ovate, usually 5, and 15-30 mm long; stamens and carpels numerous, ovaries pubescent, style glabrous and club shaped. **Fruit** an aggregate of many small, red, pubescent drupelets, 1.5-2 cm wide, hemispheric, very soft, juicy and palatable. Chromosome number reported as 2n = 14 (Taylor and MacBryde 1977).

*Rubus spectabilis* (Figure 3). Erect or curved stems arising from extensive branching rhizomes, reaching 0.5-5 m tall, young stems strongly bristly especially below with acicular prickles; mature stems woody, weakly armed with scattered spines or prickles, hairless, yellow-brown bark shredding. **Leaves** compound, 12-20 cm long, mostly trifoliate; leaflets thin, glabrous or sparingly pubescent above, biserrate or lobulate-serrate; terminal leaflet largest, acuminate at apex, truncate or cuneate at base; lateral leaflets obliquely ovate, stipules linear or setaceous. **Flowers** usually solitary, 2-4 on short leafy shoots, 2-4 cm across; sepals pubescent and ovate, petals showy, deep pink (reddish-purple), elliptical, and 1.5 times longer than sepals; stamens very numerous. Mature **fruit** an aggregate of small drupelets, yellow to glossy red, ovoid, up to 2 cm long, glabrous or with fine hairs, readily separated from dry receptacle, receptacle persistent on pedicel, palatable (Hitchcock et al. 1961; Taylor 1973). Chromosome number reported as 2n = 14 (Taylor and MacBryde 1977).

**1.2.2. Distinguishing features** — *Rubus strigosus* is distinguished from other raspberry-like *Rubus* species by being an erect shrub without rooting at shoot tips, and having white flowers, canes with numerous bristles and prickles, and a red mature fruit.
falling intact from the receptacle. *Rubus strigosus* can be distinguished from many blackberries (*Rubus* species) mainly by the fruit which separates easily from a receptacle (torus) that remains attached to the raspberry plant (Ellis et al. 1991). *R. strigosus* is distinguished from cultivated raspberry varieties by having more numerous but thinner, shorter canes, thin laterals bearing small flowers, and fruit 2-3 times smaller (Jennings 1988).

*Rubus parviflorus* is easily distinguished from most *Rubus* species by tall, erect, unarmed stems and large, palmately lobed, simple leaves. *Rubus odoratus* L. has a similar growth habit and foliage but characteristically has rose-purple flowers and a dry unpalatable fruit (Soper and Heimburger 1985).

*Rubus spectabilis* is distinguished from other *Rubus* species by having compound leaves and showy, deep pink, solitary flowers, and by often reaching heights of over 2 m.

**1.2.3 Intraspecific variation** — Across Europe, Asia, and North America, red raspberries are highly variable with many geographical and cultivated varieties. Two main ecotypes are described, *R. i. vulgatus* Arrhen. and *R. i. strigosus*, and the two forms readily intercross (Jennings 1988). *Rubus strigosus* (or *R. i. strigosus*) is the diploid form of E. Asia and North America, characterized by glandular inflorescences and round fruit. Many synonyms have been suggested, notably *R. idaeus* ssp. *melanolasius* (Dieck) Focke and *R. i. ssp. sachalinensis* (Lev) Focke (Hulten 1974), although *R. strigosus* remains the prevailing term for wild red raspberry in Canada (H.A. Daubeney, pers. comm. 1994).

Wide variations are found among populations of *R. strigosus* collected from different sites. Collections across British Columbia and Alberta showed significant differences in cane length, number of buds per cane, percent buds growing, number of inflorescences and flowers per bud, number of autumn flowering canes, and fruit weight, size, and number of seeds (van Adrichem 1972). As with studies on wild raspberry in Europe by Jennings (1964) and Rousi (1965), van Adrichem (1972) found little or no
Figure 1. *Rubus strigosus* Michx. (*R. ideaus* var. *strigosus* (Michx.) Focke) (Taylor 1973).
Figure 2. *Rubus parviflorus* Nutt. (Taylor 1973).
Figure 3. *Rubus spectabilis* Pursh (Taylor 1973).
ecotype differentiation and was unable to correlate plant characteristics with climate, location, or elevation.

Rubus parviflorus was previously described as having at least seven varieties and subspecies distributed between its western and Great Lakes ranges (Fassett 1941; Scoggan 1978). For example, R. p. ssp. parviflorus was reported in British Columbia (Taylor and MacBryde 1977) and R. p. var. grandiflorus Farw. throughout the northwest (Hulten 1974). Kartesz (1994) considers R. parviflorus to have only two varieties, namely var. parviflorus and var. velutinus (Hook. & Arn.) Green.

Rubus spectabilis has no reported varieties or subspecies in Canada although var. franciscanus (Rydb.) (syn. var. menziesii (Hook.) S. Wats.(Kartesz 1994) is reported in California and ssp. vernus Focke is reported in Asia (Hulten 1974).

Rubus parviflorus is described as being highly variable, particularly for traits such as degree of pubescence and glandularity (Fernald 1970). Both R. parviflorus and R. spectabilis have shown variations in leaf size reaching >20 cm long when found growing in a moderately shady understory in British Columbia. Rubus spectabilis has shown terminal leaflets reaching 15 cm in length and 13 cm in width (C. Oleskevich, pers. obs. 1994).

1.3. Economic importance
1.3.1. Detrimental — Rubus strigosus, R. parviflorus, and R. spectabilis are important competitors of conifer seedlings and can significantly reduce the successful establishment and growth of young conifers in planted or naturally regenerated forest renewal sites. These Rubus species are aggressive invaders into areas disturbed by logging, burning and site preparation activities, and can impede reforestation efforts by effectively monopolizing site resources such as nutrients, moisture, space, and in particular, light.

Rubus strigosus quickly invades disturbed forest lands and often becomes a competitive factor in reforestation sites within 1-5 yr after logging (Whitney 1982; Reynolds and Roden 1995a). The species can become detrimental due to rapid invasion
of the site, particularly if tree planting is delayed after harvesting practices (Eis 1981). In eastern Canada, *R. strigosus* dominates certain clearcut areas and is referred to as the most unwanted woody weed in NE Quebec (Jobidon et al. 1989). *Rubus strigosus* cover increased from 49% to 75% within 1 yr in cleared *Picea mariana* (P. Mill.) B.S.P. sites in New Brunswick (Reynolds and Roden 1995a). *Rubus strigosus* can effectively reduce height, diameter growth, and survival of conifer seedlings including *Abies balsamea* (L.) P. Mill. (Wall 1983; Fox 1986; Ruel 1992), *Picea glauca* (Moench) Voss (Eis 1981; Adam 1989), and *P. mariana* (Adam 1989). In Maine, the height and diameter of *A. balsamea* seedlings are estimated to be reduced by approximately 22% and 33% respectively, by *R. strigosus* competition (Fox 1986). Most coniferous and deciduous trees are able to overcome the shade-intolerant *R. strigosus* shrubs within 5-12 yr (Whitney 1982; Cromwell and Freedman 1994) although some sites can remain dominated by this weedy species for up to 25 yr (Ruel 1992).

Throughout the coastal ranges and the Interior wet belt of the Pacific Northwest, *R. parviflorus* is a common forest weed and may cause greater conifer mortality than any other brush species (Haeussler et al. 1990). *Rubus parviflorus* can often dominate clearcut and burned sites immediately after a site disturbance in coastal and interior British Columbia (Hamilton and Yearsley 1988). Conifer seedlings are severely inhibited by continuous dense canopies of *R. parviflorus*, which may create a survival threshold for light competition (Comeau 1988) and can reduce photosynthetically active radiation that reaches seedlings by 50 to 100% between early to late June (Spittlehouse and Stathers 1990). LePage and Coates (1994) suggest that a threshold of <5% *R. parviflorus* cover is required for substantial growth to occur in hybrid spruce (*Picea glauca* (Moench) Voss X *Picea sitchensis* (Bong.) Carr.) and lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex Loud.) seedlings. Abundant leaf litter of *R. parviflorus* may also smother seedlings. *Rubus parviflorus* and *R. spectabilis* leaf and litter extracts initially inhibited seed
germination and growth of certain test plants, although no further allelopathic effects were observed under field conditions (del Moral and Cates 1971).

*Rubus spectabilis* is considered to be one of the most severe competitors in many Pacific coastal forest areas and can establish dense, continuous thickets, producing pure stands of >30,000 stems/ha, >2 m tall, within 2-3 yr (Allen 1969). Tappeiner et al. (1991) found *R. spectabilis* communities to maintain 80-100% crown closure over areas of 0.5 ha in a variety of study sites. *Rubus spectabilis* is a major competitor of conifers, including *Pseudotsuga menziesii* (Mirb.) Franco, *Tsuga heterophylla* (Raf.) Sarg., and *Picea sitchensis* (Bong.) Carr. (Barber 1976; Newton and White 1983), and may exclude even shade tolerant conifers (Ruth 1970, Tappeiner et al. 1991; Zasada et al. 1994). Newton and White (1983) found that 11 species of conifers overtopped by *R. spectabilis* required an additional 4.1 yr to reach survival height and further studies showed that conifers less than 60 cm tall were often killed by 4 yr-old *R. spectabilis* (Newton et al. 1993). Dense cover of *R. spectabilis* can substantially inhibit regeneration of trees through shading and smothering with mats of leaf litter.

### 1.3.2. Beneficial

*Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* are important plants in forest ecosystems and play a role in nutrient cycling and conservation, reducing soil erosion on disturbed sites, and reducing the invasion of longer-lived competitive deciduous species in reforestation areas (Haeussler et al. 1990). They are valued for land rehabilitation in avalanche areas, bank stabilization along steep road cuts and streams, and in dune stabilization (Hungerford 1984; Marchant and Sherlock 1984; Minore and Weatherly 1994). *Rubus parviflorus* cover may protect young conifer seedlings from frost damage as hybrid spruce showed increased damage as cover was reduced from 35 to 17% (LePage and Coates 1994). These *Rubus* species provide important habitat and food sources for wildlife, as the fruit and foliage form a significant part of the spring and summer diets of many animals and birds. Leaves and stems provide browse for large
(deer, elk) and small mammals (bears, coyotes, rabbits, squirrels, beaver, raccoons) and fruit are consumed extensively by birds (songbirds, grouse, pheasant, quail) and by other animals. For example, \textit{R. spectabilis} shoots may comprise up to 26\% of spring and summer diets of coastal black and grizzly bears (Lloyd 1979) and form a significant portion of summer diets of Roosevelt elk (Jenkins and Starkey 1991).

These \textit{Rubus} species are valued for their genetic contributions to domestic raspberry breeding programs by providing new sources of resistance to root rot and cane diseases, viruses, weevils and nematodes (Bristow et al. 1988; Knight 1991; Daubeny and Anderson 1993; Davidson 1995). \textit{Rubus strigosus} is also used in breeding programs for other desirable characteristics such as winter hardiness, self-supporting habit, early and late fruiting, and an easily removed, non-darkening red fruit. Flowers and foliage of \textit{R. parviflorus} and \textit{R. spectabilis} are considered to have economic ornamental value (Taylor and McBryde 1977) and fruit of all three species are valued by berry-pickers.

1.3.3. Legislation — \textit{Rubus strigosus}, \textit{R. parviflorus}, and \textit{R. spectabilis} are not listed in the Canada Seeds Act and Regulations (Agr. Can. 1985) or any provincial weed and seeds acts.

1.4. Geographical Distribution

\textit{Rubus strigosus}, \textit{R. parviflorus}, and \textit{R. spectabilis} are native to North America and their Canadian distribution is outlined in Figures 4, 5, and 6.

1.4.1. \textit{Rubus strigosus}. Distributed from Yukon to Newfoundland, occurs in low subarctic/high temperate regions as far north as southwest Mackenzie District to Hudson Bay, northern Ontario (55° N 88° W), northern Quebec (to Ungava Bay and Côte Nord), and Labrador (approx. 56° 30' N) (Scoggan 1978). In British Columbia, common except west of the Coast Mountains, not naturally occurring on Vancouver Island and Queen Charlotte Islands (Taylor 1973; Taylor and MacBryde 1977). In North America, distributed from Alaska south to California, Arizona, northern Mexico, New Mexico, and
North Carolina. Elevation range from inland valley bottoms to subalpine elevations near timberline.

1.4.2. *Rubus parviflorus*. Distributed through British Columbia (to 55° N) and SW Alberta, becoming rarer in the Cypress Hills, Alberta (Moss 1983), also restricted to isolated patches along shores and on islands of Lake Superior, to Bruce Peninsula, Ontario. In North America, from southeast Alaska, restricted to coast to 55° N, south to California, northern Mexico, New Mexico, South Dakota, and in isolated areas in the Great Lakes region (Scoggan 1978). Elevation range from sea level to >900 m on the coast of British Columbia, valley bottoms to >1200 m in Interior British Columbia (Haeussler et al. 1990), common at 1800 m in the western Cascades, WA (Douglas 1972).

1.4.3. *Rubus spectabilis*. Primarily found west of Coast Mts., British Columbia from low subarctic/high temperate regions in the Aleutian Islands and southern Alaska, south to northwestern California. *Rubus spectabilis* is common along west coast of British Columbia and penetrates inland along Skeena and Fraser River drainages (Haeussler et al. 1990). Elevation range from sea level to lower alpine elevations (Hulten 1974), most abundant below approx. 800 m (Barber 1976).

1.5. Habitat

1.5.1. Climatic conditions — The main climatic factors limiting the distribution of *R. strigosus*, *R. parviflorus*, and *R. spectabilis* within their habitat are light and moisture. These species are restricted by their intolerance to moderate or high shade and their preference for moist, water-receiving sites. *Rubus spectabilis* is also limited by cold temperatures.

*Rubus strigosus* is the most widely adapted to environmental conditions among the three *Rubus* species and survives cold temperatures and short growing seasons.
Figure 4. Distribution of *Rubus strigosus* Michx. in Canada (adapted from Hulten 1974; Scoggan 1978; Porsild and Cody 1980).
Figure 5. Distribution of *Rubus parviflorus* Nutt. in Canada (adapted from Moss 1983; Soper and Heimburger 1985; Haeussler et al. 1990).
Figure 6. Distribution of *Rubus spectabilis* Pursh in British Columbia (Haeussler et al. 1990).
(Haeussler et al. 1990). Restrictions to *R. strigosus* distribution include humid, maritime climates with low annual temperature variation (e.g. west of the Coast Mts.), xeric, subxeric, and subhydric moisture regimes (Angove and Bancroft 1983), and extreme wind and rains which may damage canes (Williamson et al. 1979). *Rubus parviflorus* tolerates a wide range of conditions but is limited by cold winters, short growing seasons, and summer moisture stress. It has been found to tolerate low light levels under closed forest canopies, although achieves greater cover under 60-100% full light (Haeussler et al. 1990). *Rubus spectabilis* is the most susceptible of these *Rubus* species to cold temperatures and a short growing season and is restricted entirely to mild maritime climates, and preferring humid water-receiving or -collecting sites including subhydric regimes (Klinka et al. 1989). Ruth (1970) reported that young seedlings are significantly limited by drought conditions. *Rubus spectabilis* has a relatively high shade tolerance and can achieve net photosynthesis at low light levels, reaching maximum photosynthesis at lower light radiation levels (150 μE m⁻² s⁻¹) than *R. parviflorus* (Barber 1976).

1.5.2. Substratum — These *Rubus* species are found on a wide range of soil types, including Luvisol, Brunisol, and Podzol soils with fluvial, morainal, and lacustrine parent material and with optimum growth occurring on soils with high nutrient levels. All three shrubs are nitrophytic species and are indicator plants for nitrogen-rich forest soils (Klinka et al. 1989). Optimum growth may be reached by *R. strigosus* on sandy loams from glacial tills (Whitney 1986), by *R. parviflorus* on fluvial and alluvial soils, and by *R. spectabilis* on floodplains with well-aerated soils near field capacity (Haeussler et al. 1990).

1.5.3. Communities in which the species occur — *Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* prefer open forest sites disturbed by logging, fire, or silvicultural activities and are pioneer invaders which often form monospecific shrub communities. In British


*Rubus spectabilis* is often found growing in moist, disturbed coastal areas, swampy places, along banks of streams at low elevations, and under old- and second-growth forests with plants similar to those associated with *R. parviflorus* and with wetland plants such as *Lysichiton americanum* Hult. & St. John (Klinka et al. 1989).

1.6. History

The genus *Rubus* (Latin *ruber* = red) subgenus *Idaeobatus* is considered to have its centre of origin in eastern Asia. Preserved leaf impressions (ca. 10 000 yr old) in northern
California have indicated that *R. parviflorus* and *R. spectabilis* were part of the closed-cone Pine forest during the Pleistocene period (Mason 1934 (from Barber 1976); Langenheim and Durham 1962). Fassett (1941) suggests that the present distribution of *R. parviflorus* is due to a migration of western colonies across Canada to the Great Lakes region during a postglacial period, with the range later bisected by the aridity of the Great Plains.

Native Peoples of North America have long used these *Rubus* species primarily as food plants since fruits and shoots were gathered in abundance (Pojar and MacKinnon 1994). In British Columbia, the Nuxalk, Tsimshian, and perhaps the Heiltsuk consumed *R. strigosus* fruit fresh, boiled, mashed with other berries, and dried into cakes (Turner 1975) and the Coast Salish also used the berries as a purple stain (Pojar and MacKinnon 1994). Coastal Native Peoples including the Nuu-chah-nulth, the Kwakwaka’wakw, and Nuxalk extensively used young sprouts of *R. parviflorus* and *R. spectabilis* as a green vegetable eaten peeled and raw and canoes were observed laden with shoots (Turner 1975). *Rubus parviflorus* berries were often dried into cakes while *R. spectabilis* berries were mostly eaten fresh, sometimes with salmon. *Rubus spectabilis* patches, like other food plants, were owned by families or individuals, and certain groups, such as the Nuu-chah-nulth, gave permission for communal harvest after the owner had collected enough to hold a feast (Pojar and MacKinnon 1994).

Domestication of raspberries occurred within the last 400-500 yr with cultivated varieties of *R. idaeus* available by the 16th century in Europe and by the 17th century in North America. It was not until the 1850's that *R. strigosus* was selected for cultivation and crossed with European varieties, resulting in great advancements in raspberry breeding (Jennings 1988).

The first botanical records of these plants in North America include *R. strigosus* in *Flora Boreali-Americana* in 1803, *R. parviflorus* in *Generum plantarum* in 1818, and *R. spectabilis* in *Flora Americae Septentrionalis* in 1814 (Hitchcock et al. 1961). In North
America, extensive logging and land clearing practices of the last century have contributed to an increased distribution and abundance of these invasive Rubus species.

1.7. Growth and development

1.7.1. Morphology — The rapid development of extensive foliage and root systems allows these Rubus species to colonize new habitats and survive for many years. The bristly and spiny nature of young R. strigosus and R. spectabilis shoots may enhance survival by discouraging grazing.

1.7.2. Perennation — Perennation of these Rubus species occurs primarily by vegetative reproduction. The biennial canes of R. strigosus normally fruit and senesce in a 2-yr period, with replacement canes arising from buds at the base of the floricane. The perennial R. strigosus stools may produce new shoots for up to 1-2 decades (Whitney 1986). Rubus parviflorus and R. spectabilis generally produce annual shoots and maintain extensive bud banks and rhizome systems, with clones surviving for up to 45 yr (Tappeiner et al. 1991; Zasada et al. 1992; Maxwell et al. 1993). Rubus strigosus and R. parviflorus show bud set and dormancy in the winter months while R. spectabilis may become dormant or continue minimum shoot elongation throughout a mild coastal winter (i.e. mean temperature of 6°C) (Barber 1976).

1.7.3. Physiological data — Few physiological studies have been completed on these Rubus species. Rubus strigosus physiology may be similar to that of cultivated raspberry, of which extensive studies have been completed on leaf pigment content, gas exchange, percent water content, macronutrient concentration and distribution among plant parts, acclimation, onset of dormancy, artificial cultivation of plantlets, germplasm storage, and others (Donnelly and Vidaver 1984; Jennings 1988; Reed 1993; Kowalenko
In studies on nitrate assimilation by brush species in recent clearcut areas, *R. strigosus* was found to show high nitrate reductase activity (Truax et al. 1994).

These *Rubus* species store reserves of carbohydrate energy for the dormant season and several studies were completed on the total nonstructural carbohydrate (TNC) content. From studies on the dichotomy of energy demands in *R. strigosus*, carbohydrate content was shown to be proportionally highest in developing primocanes in spring and fall, in floricanes by midsummer, and in roots by late summer (Whitney 1982). The TNC of *R. spectabilis* rhizome segments was found to reach a high of 13% of dry weight during the winter season and fell to 7% with spring shoot production and summer growth until early fall (Zasada et al. 1994).

1.7.4. Phenology — *Rubus strigosus* canes have differing leaf phenology with peak leaf biomass occurring on floricanes in late June and on primocanes at the end of the summer season. Floricane shoots are active earlier and are shorter lived than primocanes. Floricane leaves flush by early spring, reach full development in May-June, and begin senescing by end-June, coinciding with fruit maturation. Primocane leaves are developed by May and persist through the summer to as late as October (Whitney 1982). Flower buds appear in May and fruit ripens in early June in the southern range of *R. strigosus* (Haeussler et al. 1990). In the northern range, flowering occurs in June-July with fruit set in July-October (Viereck and Little 1972). Seed dispersal generally occurs from July to October.

*Rubus parviflorus* and *R. spectabilis* buds may be active very early in the spring (i.e. February) with bud burst and leaf flush occurring in April-May and March-April, respectively. Leaves are generally fully expanded from May to late August with senescence and leaf drop occurring until late October (Maxwell et al. 1993). *Rubus parviflorus* flowering occurs mainly in June-July but can extend from early May to early August with fruit maturation from early June to mid-September (Haeussler et al. 1990).
Rubus spectabilis flowering occurs between April-June in its southern range, continuing for 1 month longer in its northern range. Fruiting may vary from June-July in the south to July-August in the north and at higher elevations. Rubus spectabilis seeds may germinate by early April with new seedlings appearing in early June (Ruth 1970).

Maximal root growth for R. strigosus and R. spectabilis occurs from August through October (Whitney 1982; Zasada et al. 1994).

1.7.5. Mycorrhiza — Malloch and Malloch (1982) found no mycorrhiza on R. strigosus roots examined from the boreal forest region of Ontario. Fine vesicular-arbuscular mycorrhiza caused by Glomus tenuis (Greenhall) Hall were found to form on cultivated R. idaeus in Europe (Gianinazzi-Pearson et al. 1981). Bioassays (in vitro) by Côté and Thibault (1988) showed leachates from R. strigosus inhibited the growth of ectomycorrhizal fungi found on Picea mariana roots in Quebec. No mycorrhizal associations are reported on R. parviflorus although small, nodule-shaped expansions have been observed on roots (R.E. Wall, pers. comm. 1994). The occurrence of Glomus microcarpus Tul. & Tul. and G. fasciculatus Gerdemann and Trappe vesicular-arbuscular mycorrhiza was reported on R. spectabilis in pot culture (Gerdemann and Trappe 1974).

1.8. Reproduction

1.8.1. Floral biology — Flowers are self-infertile in R. strigosus, R. parviflorus, and R. spectabilis (Keep 1968) and seed is produced through cross-pollination followed by fertilization. Embryo development was found to be normal in R. strigosus (Jennings 1988) and R. spectabilis (Virdi and Eaton 1969a). Apomixis is rare in the Rubus subgenus Idaeobatus, only occurring in a few triploid specimens, and is more commonly found in the subgenus Rubus (i.e. blackberries) (Jensen and Hall 1979; Nybom 1988).

Flowers are pollinated primarily by insects with R. spectabilis also being visited by hummingbirds. Rubus strigosus has an advanced floral structure, attracting mainly
bees (Whitney 1984), while *R. spectabilis* has a more primitive flower suited to unspecialized vectors such as beetles (Barber 1976). Whitney (1984) recorded *R. strigosus* flowers producing an abundant supply of nectar towards the end of the flowering season, reaching 18 kg/ha per day in a 4-yr old site dominated by wild raspberry in northeastern USA. High pollen viability is typical of sexually reproducing *Rubus* species (Nybom and Schaal 1990) and high pollination rates were observed in *R. strigosus* with 85% of the flowering individuals producing seed (Whitney 1986). The fruits are aggregates of small drupelets, with each drupelet producing one hard-coated pyrene containing normally one seed. *Rubus strigosus* fruit may be similar to that of *R. idaeus* which consists mainly of water with 14% solids, of which <1% are pectins. *Rubus idaeus* fruit has a total sugar content (mainly glucose and fructose) between 1.5 - 5.3% w/w and contains relatively high amounts of vitamin C (Jennings 1988). Yellow colouration of fruit produced by *R. spectabilis* may be due to the predominance of pelargonin glycosides rather than the anthocyanidin pigments found in red-coloured fruit.

1.8.2. **Seed production and dispersal** — *Rubus strigosus, R. parviflorus,* and *R. spectabilis* are major seedbank species which annually produce a prolific number of small seeds (average length 2 mm), depending on environmental conditions, stand development, and elevation. *Rubus spectabilis* produces over 300 000 seeds per kg of seeds (Tappeiner and Zasada 1993). Dense populations of *R. strigosus* growing on southeast facing slopes in previously cleared areas produced >26 000 seeds/m² over a 4 yr period (Whitney 1986). At later stages of stand development, *R. strigosus* was found to devote a greater proportion of reserves to seed production (Whitney 1982). Haeussler et al. (1990) reported that *R. parviflorus* seed production decreased at high elevations.

Seeds are dispersed either directly below the parent plant (as ripe fruit falls readily), throughout the soil by burrowing animals, or by fruit consumption and dispersion by birds and mammals. In seedbank analysis of deciduous- and coniferous-
dominated sites in the Acadian forests, Moore and Wein (1977) found *R. strigosus* comprised 90% of the seedlings arising from soil core samples. In early- and mid-seral forest communities, *R. parviflorus* produced >75 seeds/m² with 60% constancy (McGee and Feller 1993) and up to 84 seeds/m² with 75% constancy (Morgan and Neuenschwander 1988). *Rubus spectabilis* sites under timber accumulated from 2-125 seeds/m² (Ruth 1970; Barber 1976) with seed predation appearing to be a minimal factor (Tappeiner and Zasada 1993). Greatest seed numbers for *R. parviflorus* and *R. spectabilis* were found on forest floors in undisturbed and low disturbance areas while the lowest seed numbers were found in rights-of-way and in burned sites (Morgan and Neuenschwander 1988; McGee and Feller 1993; Zasada et al. 1994). Seed dispersal is the primary means by which these *Rubus* species colonize new sites.

1.8.3. Viability of seeds and germination — Seeds can remain viable buried in the soil for many years, an estimated >50 yr for *R. strigosus* and at least 100 yr for *R. spectabilis*. Under artificial conditions, viability may be reduced as domestic raspberry seeds showed only 0-22% germination after 26 yr of dry storage (Clarke and Moore 1993). Both the red and yellow fruit of *R. spectabilis* produce viable seeds (Barber 1976). A gradual decline in seed viability, constancy, and number with soil depth was found for *R. strigosus* (Moore and Wein 1977) and *R. parviflorus* (McGee and Feller 1993).

Seeds must pass through a dormant phase before being stimulated to germinate by increased light and temperature, conditions normally associated with soil disturbances. Germination of buried *R. strigosus* seed is also stimulated by soil nitrates and nitrate-N fertilization (Whitney 1982; Jobidon 1993). The dense impermeable seedcoat of these *Rubus* species inhibits germination and passage through the crop or gut of a bird may enhance germination (Haeussler et al. 1990). Jennings (1988) determined that *R. strigosus* seeds remained dormant due to the presence of an acidic, ether-soluble growth inhibiting substance. To induce germination, dried seeds generally require a lengthy procedure of
chemical scarification, warm stratification, and pre-chilling to break dormancy, followed by an alternating temperature regime (Anon. 1974b; Anon. 1994). Other studies show that raspberry seeds may require only cold stratification at 2°C for 120 days to break dormancy (Hills and Morris 1992) and that germination time can be greatly reduced by halving fresh seeds (Ke et al. 1985) or by nicking or removing the seed coat (Nesme 1985).

For *R. parviflorus* seeds, variable germination results were obtained with a sulfuric acid soak followed by cold stratification for 90 days (Marchant and Sherlock 1984), although cold stratification alone at 3°C for 90 days followed by warm stratification and alternating 5°/15°C temperatures resulted in increased germination (Costanzo 1980).

*Rubus spectabilis* seeds germinated following scarification in sulfuric acid, cold stratification, and alternating temperatures at 2-3°C for 5 months (Barber 1976; Tappeiner and Zasada 1993).

Seed germination decreases with increasing soil depth for these small-seeded *Rubus* species. Reduced *R. parviflorus* germination occurred at depths >1 cm on the forest floor and at 3 cm in mineral soil (McGee and Feller 1993). Soil disturbance may be critical for seed germination as *R. spectabilis* consistently emerged on disturbed mineral soils but showed low emergence rates on undisturbed forest floor (Tappeiner and Zasada 1993).

**1.8.4. Vegetative reproduction** — Once these *Rubus* species seedlings are established on a site, the principal means of spread and perpetuation is by vegetative reproduction through extensive clonal colonies. *Rubus strigosus* primarily spreads by short-lived root suckers, establishing up to 16 suckers/m² and 20 - 50 independent stools/m² in a 3 yr-old plot (Whitney 1982; 1986). *Rubus parviflorus* and *R. spectabilis* spread via an extensive rhizome system with annual ramets arising from a large rhizomal bud bank, or, if the plant has been cut back, from buds associated with the basal stem and root collar (Zasada et al. 1992; 1994). Tappeiner et al. (1991) found *R. spectabilis* clones produced 1-2 new rhizomes/yr with annual rhizomal extensions of 0.1-0.8 m/yr, reaching an average rhizomal
length of <1.7 to 18.3 m depending on the stand type. All three *Rubus* species are readily propagated from dormant root cuttings for research study purposes.

1.9. Hybrids

*Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* can be crossed with cultivated red and black raspberries and blackberry plants (Virdi and Eaton 1969b; Jennings and Ingram 1983; Daubeny and Anderson 1993). Pool et al. (1981) recognized *R. idaeus* chromosomes in F₁ hybrid crosses with cultivated raspberry plants in Europe. *Rubus spectabilis* has been known to naturally hybridize with *R. strigosus* and *Rubus arcticus* L. in Alaska (Viereck and Little 1972).

1.10. Population dynamics

*Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* populations follow two general stages of growth in a new site: i) an initial building phase lasting 1-2 yr which involves seed germination and a rapid increase in stem number, and ii) a growth phase involving vegetative growth and reproduction, increased stand density, and the establishment of extensive clonal colonies. Seed production continues throughout the growth phase, resulting in abundant seedbanks. Whitney (1986) suggests seedbank build-up and the lengthy seed dormancy of *R. strigosus* may be considered as a third phase in population growth.

Within *R. strigosus* stands, seedling establishment from buried seed is soon replaced by the extensive production of root suckers and development of independent stools. With an increase in stand density and in net biomass production under an open canopy, a self-thinning phase may follow which results in a decrease in stool number. As interspecific competition and shading become influential factors, *R. strigosus* may shift energy resources from clonal, vegetative production to prolific seed production (Whitney 1982).
Rubus parviflorus and R. spectabilis become established from seed in new sites with seedling survival rates reaching up to 44% and 32%, respectively (Maxwell 1990). Sprouts arising from buds on stems and rhizomes show greater initial growth rates and greater mean survival rates (100% and 70%, respectively) than seedlings, and thus populations become dominated by ramets and stems. Within 2-3 yr after R. parviflorus and R. spectabilis establishment, extensive rhizomal growth can spread up to 50 m² from the parent plant and canopy closure may be complete (Maxwell 1990; Tappeiner et al. 1991). Mature populations consist mainly of aboveground ramets interconnected by extensive rhizome systems (Maxwell et al. 1993). Rhizome-generated ramets show a high annual turnover and populations are made up of stems decreasing in number from small to large size classes (Tappeiner et al. 1991; Zasada et al. 1992). In work on population simulation models for R. parviflorus and R. spectabilis, Maxwell et al. (1993) found basal stem buds to be the main factor in initiating shoot production in natural populations.

Rubus parviflorus and R. spectabilis population growth is generally regulated by density and interspecific competition, mainly from the growth of overstory trees (Maxwell 1990). Rubus spectabilis can maintain a persistent cover once established, unless a severe disturbance allows for succession of trees and other shrubs (Tappeiner et al. 1991). Rubus spectabilis clone size may be influenced by stand type since larger clones with a greater production of ramets and aerial stems were found in alder stands than in conifer stands. Tappeiner et al. (1991) also determined that R. spectabilis clonal biomass was negatively related to basal area of overstory trees and suggested that rhizome length and biomass could be predicted from the measurement of basal area of clonal stems and of overstory trees.

1.11. Response to Herbicides and Other Chemicals

Herbicide applications offer varying levels of control as even extensive foliar damage may be followed by rapid resprouting as root systems remain unaffected. Late season
applications of glyphosate are most effective in reducing *Rubus* species cover. For *R. strigosus*, foliar applications of 2.14 kg acid equivalent (a.e.) glyphosate/ha in Aug.-Sept. caused only light to moderate injury in several trials in British Columbia (Haeussler et al. 1990). Greater control is often obtained in eastern Canada, and Pitt et al. (1992) demonstrated >60% *R. strigosus* cover reduction with a refined aerial applications of 0.5 kg a.e. glyphosate/ha in a New Brunswick trial. In *R. parviflorus* stands, glyphosate applications of 1.4 kg a.e./ha were shown to be as effective as higher rates of 2.4 kg a.e./ha in reducing cover when applied in early to late August after full leaf expansion was complete (LePage et al. 1991). Glyphosate generally causes moderate to severe injury in *R. spectabilis* (D'Anjou 1990) and July-Sept. applications of 1.4 to 2 kg a.e./ha gave good control (Newton et al. 1986; William 1994).

Hexazinone was shown to be effective in reducing *R. strigosus* cover with aerial applications of 2 kg active ingredient (a.i.)/ha applied in early summer site-preparation treatments over cleared sites in NW New Brunswick (Reynolds and Roden 1995a). Hexazinone is generally ineffective or causes only light injury in *R. parviflorus* and *R. spectabilis*, although a liquid formulation of 4 kg a.i./ha caused 25-60% injury in approximately 4-yr old *R. parviflorus* stands in SW British Columbia (D'Anjou 1990).

Sulfometuron reduced *R. strigosus* cover by 35% and 30% in spring site-preparation when applied at 0.3 and 0.45 kg a.i./ha, respectively (Reynolds and Roden 1995b). Sulfometuron also gave good control of *R. parviflorus* and *R. spectabilis* with a broadcast spray of 0.6 kg a.i./ha in March-April (D'Anjou 1990). Metsulfuron applied as a spot spray at 0.6 kg a.i./ha or as an aerial spray at 0.03 kg a.i./100 L/ha may give excellent control of *R. spectabilis* in site preparation practices (Cole et al. 1988; William 1994). A site-preparation application combining picloram and 2,4-D (0.25 g a.i. + 0.9 kg a.i./ha) gave good control of *R. parviflorus* and *R. spectabilis* in the Pacific NW region (William 1994). Triclopyr esters applied in early summer at 2.9 kg a.e./ha gave up to 60-90% injury of *R. parviflorus* and *R. spectabilis* (D'Anjou 1990).
In studies on herbicide residues in *R. strigosus* fruit in Ontario, Roy et al. (1989) reported <10% of glyphosate sprayed at 2 kg a.e./ha penetrated the fruit within 9 hr and that glyphosate levels dissipated to 50% with 5.55±0.880 ppm residues recorded in fruit after 13 days. Preliminary residue testing by Hoyles and Wilson (1994) showed much lower glyphosate residue levels in *R. strigosus* fruit with 0.27 ppm reported at 10 days post-application in central British Columbia. Frank et al. (1983) found that 2,4-D sprayed at 1.1 - 3.9 a.e./ha initially left residues of 2.6-3.1 mg/kg fruit, with residues decreasing to 0.1-3.3 mg/kg within 2-5 weeks. No glyphosate or 2,4-D residues were found in fruit produced in the following year in the abovementioned studies. In Newfoundland, *R. strigosus* foliage accumulated up to 400 ppm fluoride from phosphorus plant emissions (healthy foliage had 8 ppm), causing up to 70% flower mortality and resulting in reduced fruit dry weight and seed size, foliar injury, delayed leaf fall, and increased vegetative spread (Staniforth and Sidhu 1984).

1.12. Response to Human Manipulations

A variety of manipulations aimed to reduce *R. strigosus*, *R. parviflorus*, and *R. spectabilis* cover, including site preparation (scarification and prescribed burning) and manual cutting often stimulate germination and prolific resprouting, allowing stands to recover to pretreatment levels or greater within 1-3 yr.

Mechanical site preparation can fragment roots, increasing individual stool number for *R. strigosus* (Hudson 1959) and stem density for *R. parviflorus* and *R. spectabilis*. Scarification treatments which expose mineral soil stimulate germination of buried *Rubus* seed. On disturbed sites with the soil organic layer and vegetation removed, *R. strigososus* showed 1.2-1.5 times greater germination and greater seedling survival than on undisturbed sites (Roberts et al. 1993). In not-satisfactorily-restocked sites of *Picea* species and *Pinus contorta*, site preparation treatments such as windrowing and disc trenching stimulated *R. strigosus* and *R. parviflorus* to exceed pretreatment cover levels by the
second growing season (Taylor et al. 1991). In comparison, Oswald and Brown (1992) found that scarification treatments (with brush blade, flex-track forwarder with blade, or dip and dive) successfully reduced *R. strigosus* in *Picea englemannii* Parry ex Engl. plantations.

Prescribed burning, especially low severity burns, may create seedbeds for *Rubus* species and stimulate germination of buried seed and resprouting of the remaining stems, resulting in greater cover on burned sites than that found on unburned sites (Allen 1969; Wright 1972; Lafferty 1972; Delaney and Cahill 1978; Johnson and Woodard 1985; Hamilton and Yearsley 1988). These *Rubus* species are moderately to highly resistant to fire with adaptive traits (i.e. buried seed, buried rhizomes, rapid regrowth) and show decreased vigour only after severe burns, particularly on dry sites (Haeussler 1991). Broadcast burning and spray and burn treatments achieving soil temperatures of >60°C at a 3 cm depth for 3-5 min did not successfully control *R. strigosus* and *R. parviflorus* regrowth (Taylor et al. 1991).

Cutting these *Rubus* shrubs stimulates resprouting as *R. parviflorus* and *R. spectabilis* can regrow to 60-90% of pretreatment height within one year of manual cutting (Hart and Comeau 1992). LePage et al. (1991) found that cutting *R. parviflorus* stands with brush saws at time of full leaf development was ineffective with a single cutting and resulted in only limited control for 1-2 growing seasons. With a disturbance of overstory and understory plants, mature *R. spectabilis* stands rapidly initiated new rhizomes and aerial stems, annually producing 1-2.5 m of rhizomes/m² and 25-50 stems/m² for at least two growing seasons (Tappeiner et al. 1991). *R. spectabilis* stands can be temporarily diminished by cutting in June-July, although plants can recover even after 9 months of intensive, monthly cutting treatments (Zasada et al. 1990).

Grazing trials have demonstrated that sheep will graze on *R. strigosus*, *R. parviflorus* and *R. spectabilis* in reforestation areas, although *R. strigosus* and *R. parviflorus* have only moderate palatability for sheep (Irving and Bailey 1985; Sutherland et al. 1991; Pickering and Richards 1993; Dereshkevich et al. 1994). Net *R. parviflorus* growth in grazed stands in *Pseudotsuga menziesii* plantations was 32% of that on ungrazed sites, although scattered *R. spectabilis* stands showed no decrease in the net annual growth due to grazing (Sharrow et al. 1989).

Seeding of recently scarified sites with legumes, bunchgrasses, and sod-forming grasses diminished the re-establishment of *R. parviflorus* and *R. spectabilis*, allowing *Picea sitchensis* to outgrow these *Rubus* species in northwestern British Columbia (Coates et al. 1993). Applying grass/legume seeds and fertilizer to burned sites substantially reduced the frequency of cover and height of *R. parviflorus* and *R. spectabilis*, especially during the first 3 yr in *Pseudotsuga menziesii* plantations (Kastner and Monthey 1992). Seeding with a grass/ *Medicago sativa* L. mixture controlled *R. strigosus* sucker growth better than spraying and burning among *Populus* species in Alberta parkland (Irving and Bailey 1985).

Cover mulches of barley, oat, or wheat straws or mixtures of all three significantly reduced *R. strigosus* establishment through allelopathy of decomposing straw, and resulted in reduced spring seed germination and height growth of seedlings (Jobidon et al. 1989).

1.13. Response to Parasites

These *Rubus* species may act as reservoirs of plant pathogenic microorganisms. *Rubus parviflorus* is known to harbour apple mosaic, thimbleberry ringspot, and raspberry bushy dwarf viruses which may be transferred through aphids (*Masonaphis* species) and by pollen to cultivated raspberry (Credi et al. 1986; Stace-Smith 1987; Stace-Smith and Martin 1989; Stace-Smith and Shier 1989; Bulger et al. 1990). A single-host aphid,
*Masonaphis maxima* Mason, is reported to emerge with bud break on *R. parviflorus*, although damage to the host was not reported (Frazer and Forbes 1968; Gilbert 1980). A cyanid wasp, *Diastrophus kincaidii* Gillette, causes dieback of *R. parviflorus* due to the formation of numerous stem galls (each containing about 10 parasites) consisting of parenchyma tissue which interferes with translocation of plant materials (Wangberg 1975; Kraft and Erbisch 1990).

*Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* are hosts to several fungal parasites (Ginns 1986; Farr et al. 1989) although these shrubs are often found with few disease symptoms. Wall and Shamoun (1990a) reported *Septoria rubi* West. was the most common leaf spot pathogen found on *R. parviflorus* and *R. spectabilis* with symptoms appearing in early June and continuing throughout the summer. *Phragmidium occidentale* Arth. is also reported to be common on *R. parviflorus* although not associated with a foliar necrosis and dieback frequently observed in mid-summer stems (Wall and Shamoun 1990a). Widespread distribution of leaf spot symptoms on *R. spectabilis* observed by the authors in coastal British Columbia may be attributed to *Phomopsis* species which are frequently present as endophytes in *R. spectabilis* foliage.

Biological control of *Rubus* species have included trials in Australia, New Zealand, and Chile with a rust fungus, *Phragmidium violaceum* (Schultz) Winter, to control naturalized blackberry (Bruzzese and Hasan 1986) and in Hawaii using necrotic and rust fungi on native and non-native *Rubus* species (Gardner 1983). In Canada, biocontrol trials of *Rubus* species have involved mainly bacterial and native fungal pathogens and have generated successful preliminary results. Foliar sprays of bialaphos, a phytotoxin produced from an actinomycete, *Streptomyces viridochromagenes*, applied at 2-2.5 kg a.i./ha in late July-late August, was highly successful in controlling shoot height growth and resurgence in *R. strigosus* shrubs (Jobidon 1991). Wall (1989) evaluated and demonstrated mild disease symptoms with several pathogens on *R. strigosus*, including bacteria associated with fire blight (*Erwinia amylovora* f.sp. *rubi* (Burr.) Winslow et al.)
and fungi associated with leaf and shoot blights. Investigations by Thibault (1989) to control *R. strigosus* in Quebec have included pathogen surveys and identification of potential biocontrol agents such as *Didymella applanata* (Niessl) Sacc. Potential mycoherbicides incorporating *Septoria rubi, Cylindrocarpon destructans* (Zinf.) Schöltlen, or *Hainesia lythri* (Desm.) Höhnel have shown initial suppression of *R. parviflorus* by rendering leaves non-functional through successful inoculations (Wall and Shamoun 1990b; Shamoun and Callan 1992). In shadehouse trials, *Fusarium avenaceum* (Fr.) Sacc. and a *Colletotrichum* species, both isolated from *R. strigosus*, were found to cause extensive foliar lesions on *R. strigosus, R. parviflorus*, and *R. spectabilis* plants when combined with surfactants and applied in inundative doses (Oleskevich et al. 1996a).

Note: Chapter 1 has been published in the Canadian Journal of Plant Science 76:187-201.
CHAPTER 2

EVALUATION OF *Fusariumavenaceum* AND OTHER CANDIDATE FUNGI FOR BIOLOGICAL CONTROL OF INVASIVE *Rubus* SPECIES IN BRITISH COLUMBIA.

2.0. INTRODUCTION

In reforestation sites in Canada and the northern United States, invasive *Rubus* species, particularly wild red raspberry (*R. strigosus* = *R. idaeus* var. *strigosus*), thimbleberry (*R. parviflorus*), and salmonberry (*R. spectabilis*) can effectively outcompete newly planted or naturally regenerated conifer seedlings. These native *Rubus* species are among the top twenty major forest weeds in Canada (Wall et al. 1992) and were shown to significantly reduce the growth and survival of several conifer species, including black and white spruce (*Picea mariana*, *P. glauca*), balsam fir (*Abies balsamea*), Douglas-fir (*Pseudostuga menziesii*), Sitka spruce (*Picea sitchensis*) and western hemlock (*Tsuga heterophylla*) (Eis 1981; Newton and White 1983; Ruel 1992). These *Rubus* species are perennial, deciduous shrubs which are associated with both hardwoods and softwoods, and form monospecific, multi-layered shrub communities with long-lived clonal root systems (Whitney 1982; Zasada et al. 1994; Oleskevich et al. 1996b).

In forest renewal sites, *Rubus* vegetation is frequently managed by manual and chemical means, with more recent investigations into alternative strategies including biological control, cover mulches, and sheep grazing (Oleskevich et al. 1996b). Manual cutting and site treatments (e.g. site scarification, prescribed burn) are often not economically feasible and are ineffective due to vigorous *Rubus* resprouting from rhizomal, basal, and root buds and the stimulation of seedbank germination. Herbicides such as glyphosate and hexazinone may provide adequate *Rubus* control if root systems are affected, but growing public concern over pesticide use in forested areas may minimize their availability in the future (Wagner 1993).
Biological control strategies which utilize microbial organisms and/or their secondary metabolites to control weeds have been deployed in agriculture (Templeton 1982; Charudattan 1991; TeBeest et al. 1992) and similar strategies are now being promoted to reduce competing vegetation in forestry (Wall et al. 1992; Watson and Wall 1995). Compared to agricultural sites, the forest environment presents numerous challenges to the development of biocontrol agents for competing vegetation due to the diversity and density of forest weed populations and to fewer investments in site management. In reforestation areas, biocontrol agents may achieve temporary suppression of competing vegetation, allowing for conifer release, and may be best considered as one component of an integrated vegetation management program.

Previous studies using biocontrol agents to reduce competing *Rubus* species have included classical strategies with two rust fungi, *Phragmidium violaceum* and *Kuehneola uredinis*, to control exotic or naturalized *Rubus* species in Australia, New Zealand, Chile, and Hawaii (Gardner 1983; Bruzzese and Hasan 1986). Field studies with bialaphos, a phytotoxin produced by *Streptomyces viridochromogens*, successfully reduced height and resurgence of *R. strigosus* in *Picea mariana* plantations in eastern Quebec (Jobidon 1991).

The present study is part of an ongoing investigation at the Canadian Forest Service towards the discovery and development of biological control agents for *Rubus* species. Three weakly virulent, indigenous pathogens, *Septoria rubi*, *Cylindrocarpon destructans*, and *Hainesia lythri*, were shown to be potential mycoherbicides of *R. parviflorus* when target plant resistance was weakened through mechanical or chemical wounding or with adequate inoculum formulation (Wall and Shamoun 1990; Shamoun and Callan 1992). The objective of this study was to investigate three fungal pathogens isolated from diseased *Rubus* species, namely *Fusarium avenaceum* (Fr.) Sacc. (syn. *F. roseum* Lk. (Snyd. & Hans.), *Colletotrichum dematium* (Pers.) Grove, and a *Phomopsis* sp., as potential biological control agents. The biological control strategies tested employed inundative applications of the whole organisms and tested the effect of inoculum production methods, of adjuvants, and of glyphosate to
predispose *Rubus* plants to fungal infection.

2.1. MATERIALS AND METHODS

2.1.1. Isolation and selection of candidate fungi

Extensive field samples of foliage and stems of *R. strigosus*, *R. parviflorus*, and *R. spectabilis* showing disease symptoms (anthracnose, foliar and stem lesions, necrosis, shoot blight, dieback) were collected from central (49° to 54° latitude) and coastal (including coastal mainland and Vancouver Island) BC during May to September, 1990-1994. Samples were obtained from the following biogeoclimatic zones: Coastal Douglas-fir, Coastal Western Hemlock, Interior Cedar-Hemlock, Interior Douglas-fir, Montane, and Sub-Boreal Spruce (Meidinger and Pojar 1991). Diseased *Rubus* tissues were excised (ca. 0.25 cm² sections) and surface-disinfested by successive 1 min rinses in 95% ethanol, 0.525% sodium hypochlorite (w/v) and 3 rinses in sterile distilled water. Plant tissues were blotted on sterile filter paper, aseptically plated onto malt extract or potato dextrose agar (MEA, PDA, Difco Laboratories, Detroit MI), and incubated at 20-25°C. Resulting fungal colonies were subcultured from hyphal tips and pure cultures were stored on MEA and PDA slants and in sterile distilled water at 5°C, with periodic testing for viability. For subsequent testing, minimum subculturing was done and fungi were inoculated onto *Rubus* hosts and re-isolated to obtain fresh isolates as needed. *Fusarium avenaceum* identification was confirmed by Dr. P. Nelson, Fusarium Research Centre, Pennsylvania State University.

To select potential biocontrol candidates, fungal isolates were screened for pathogenicity by first inoculating detached leaves of *Rubus* species obtained from shadehouse-grown plants. Test plants were grown from field rootstocks (after cold stratification for 3 months at 0°C) by planting 10 cm-long root segments in a peat-perlite (1:2) medium and placing in a mist chamber. Healthy plants were transplanted and maintained to an average height of 0.5 m in 1 gallon pots in peat-vermiculite-sand (3:1:1)
medium with a low rate of slow release fertilizer (18-7-12 Osmocote, Grace Sierra, Milipitas CA) in an outdoor shadehouse. Additional plants were later propagated from Rubus stem cuttings by dipping 10 cm stem segments with two leaves in 0.4% indole-3 butyric acid rooting powder (Stim-root No. 2, Plant Products Ltd., Bramalea ON) and planting in soil mixtures as above. Mature plants were maintained under greenhouse conditions of 16 h photoperiod, 18-21°C (night and day temperatures, respectively), and ca. 60% relative humidity.

Detached Rubus leaves were inoculated using mycelial plugs (1 cm²), taken from the edge of actively growing colonies, and incubated on moistened filter paper (9 cm diameter) in glass Petri plates at 20-22°C for 7 days. Control leaves were inoculated with sterile MEA or PDA plugs under identical conditions and often remained green for up to 7 days. An isolate each of F. avenaceum, C. dematium, and a Phomopsis sp. were selected as potential biocontrol candidates from these screening tests after causing ≥ 50% of leaf area damaged within 7 days. Percent leaf area damaged was assessed visually by using the area-addition method in which necrosis within leaf quadrants was added cumulatively and the mean percentage was calculated per leaf. Values ≥ 50% were considered to indicate strong pathogenicity.

Conditions for optimal inoculum production for each of the three fungi were established by determining the optimum temperature and medium required for spore germination and colony growth. Temperatures ranging from 0-35°C, in 5°C increments, were used in germination and colony growth tests. For germination tests, conidia were obtained from sporulating colonies on MEA or PDA by flooding plates with sterile distilled water and gently scraping the surface. Conidial suspensions were diluted and spread onto 2% water agar plates and percent germination was recorded at 24 h, with a total of 300 spores counted at each temperature. The effect of temperature on colony growth was determined by measuring colony diameter for each fungus on PDA after 7 days. At each temperature, there were 3-5 replicate plates of each fungus and the
experiments were repeated.

2.1.2. Pathogenicity tests

2.1.2.1. Effect of inoculum production

(i) Agar and liquid media: Several agar and liquid media were evaluated for their ability to promote sporulation, as determined by regular hemacytometer tallies, and the following media were selected. *Fusarium avenaceum* was grown in modified Richard's V-8 broth (Walker 1980), infested with two mycelial plugs (5 mm diameter) per 250 ml broth, and maintained on a continuous shaker at 100 rpm at 20-22°C with a 12 h light/dark regime. *Colletotrichum dematium* and *Phomopsis* sp. were grown on PDA and MEA, respectively. Agar plates were infested with one mycelial plug (5 mm) from actively growing colonies and incubated at 20-22°C with an alternating 12 h light/dark regime.

To evaluate pathogenicity, inundative applications of conidial inoculum were made to *R. parviflorus* and *R. spectabilis* plants in shadehouse trials. Inoculum of each fungus consisted of 10^6 spores/ml combined with 2% sucrose and 0.5% gelatin and was sprayed onto test plants with a hand-held sprayer (Garden Sprayer, Greenleaf Products Inc., Burnaby BC) at a rate of 50 ml/m^2. A 24 h dew period, induced by covering plants with a clear plastic bag, was included to enhance germination and infection. Plants were rated for %necrosis by dividing the plant into quadrants, adding cumulatively the %necrosis per quadrant, and calculating the mean % necrosis per plant. Percent necrosis was rated on a scale of 0-4 where: 0 = no damage, 1 = <1% damage, 2 = 1-10% damage, 3 = 11-50% damage, 4 = 51-100% damage. Plants were rated for up to 3 weeks and compared to control treatments of water, with 2-3 replicate plants per treatment, and the experiment was repeated. Treatment data were combined and subjected to analysis of variance and the Student-Newman-Keuls test at p = 0.05.

(ii) Grain medium: *Fusarium avenaceum* was grown on a rice substrate to induce the production of potential phytotoxic compounds (Abbas et al. 1991). Rice grains (Uncle
Ben's Ltd., Effem Foods Ltd., Bolton ON) were combined with distilled water (100 g:60 ml w/v) in 500 ml Erlenmeyer flasks sealed with foam stoppers, autoclaved at 120°C for 20 min, and inoculated with a mycelial plug (5 mm). Flasks were incubated on the lab bench at 20-22°C under continuous fluorescent light, relative humidity at 40-60%, with vigorous shaking daily for 2 min. Controls consisted of autoclaved rice inoculated with sterile PDA plugs. After 30 days, rice grains were air-dried for 48 h in a laminar flow hood and stored at 5°C in plastic bags. The grains were ground to a coarse powder for 10 sec with an electric grinder (Household coffee mill, Braun Canada Ltd., Vancouver BC), resuspended at 5 g substrate per 50 ml sterile distilled water, sonicated (Sonic Dismembrator 12100, Quigley-Rochester Inc., Rochester NY) for 10 min, and filtered through cheesecloth to obtain culture filtrates for use in testing.

Filtrate inoculum was combined with 0.2% and 0.4% Silwet L-77® (organosilicone surfactant, Loveland Ind., Greeley CO) and sprayed onto R. parviflorus and R. spectabilis plants at 50 ml/m² under shadehouse conditions. Sprayed plants were visually rated for %necrosis and other disease symptoms over 3 weeks and compared to filtrates from non-infested rice grains, 0.2% and 0.4% Silwet L-77®, and water. The experiment was repeated, with 2-3 replicate plants per test, and treatment results subjected to analysis of variance and the Student-Newman-Keuls test at $p = 0.05$.

In a host-range test, several economically important conifer seedlings including Douglas-fir, western hemlock, Amabilis fir [Abies amabilis (J. Forbes)], western redcedar [Thuja plicata D. Don], yellow cedar [Chamaecyparis nootkatensis (Sudworth)], and hybrid spruce [Picea engelmannii Engelm. X P. glauca (Moench) Voss] (BC Ministry of Forests, Green Timbers Reforestation Centre, Surrey BC) were planted (age = 1+0) in 1 gallon pots in a peat-vermiculite-sand (3:1:1) mix under shadehouse conditions. Vigorously growing seedlings were sprayed with F. avenaceum culture filtrates with 0.4% Silwet L-77®, with similar controls and design as above, and two replicate seedlings per treatment. The seedlings were examined for necrotic symptoms over 3 weeks and rated
on a scale from 0-4, as previously described.

To detect the presence of known *Fusarium* toxins, extractions of culture filtrates were conducted at the USDA/NCAUR, Agriculture Research Service, Peoria IL, by Dr. R. L. Vesonder. Briefly, infested rice filtrates were extracted with CHCl₃, followed by CHCN: H₂O (60:40), and analyzed by thin layer chromatography (toluene: acetone: methanol (5:3:2) and ultraviolet spectroscopy (Vesonder 1986).

2.1.2.2. Effect of formulation with adjuvants

The effects of several adjuvants, selected from mycoherbicide studies involving closely related fungi, in enhancing pathogenicity were tested. Adjuvants were screened individually by combining fungal inoculum produced on agar and liquid media as described above. *F. avenaceum* spore suspensions (10⁶ spores/ml) with 1% malt broth (Difco Laboratories, Detroit MI), 1% neopeptone (Sigma Chemical Co., St. Louis MO), and 1% sodium alginate (BDH Inc., Toronto ON) and applying a central, 100 μl drop onto detached *R. parviflorus* and *R. spectabilis* leaves. The leaves were incubated as described for fungal screening tests and evaluated for %necrosis over 7 days. Inoculum viability, when combined with each adjuvant, was verified by plating on MEA or PDA. Control treatments consisted of spore suspensions or adjuvants, and water. Formulations which resulted in ≥50% necrosis, visually rated at 7 days, were further tested on intact plants as described above.

Similar formulation assays were performed with *C. dematium*: adjuvants tested included nutritional amendments (25% aloe, 0.5 % lecithin, 1% apple and citrus pectin, 1% cellulose (Sigma Chemical Co., St. Louis MO), 1% sodium alginate) and formulation in sodium alginate/kaolin clay granules (Walker and Connick 1983). The *Phomopsis* sp. was combined with 0.2% Tween 80, 15% canola oil, and *Rubus* leaf leachate. Both *C. dematium* and *Phomopsis* sp. were also tested after attempted removal of conidial matrices using a 0.01M tannic acid wash or repeated centrifugation to remove matrix
substances that could possibly inhibit germination.

2.1.2.3. Effect of glyphosate

Low doses of glyphosate (Roundup®, Monsanto Canada Inc., Sardis BC) were applied to Rubus plants to determine if combined treatments of glyphosate followed by fungal sprays would enhance pathogenicity compared to either treatment applied alone. To first determine the effect of glyphosate on fungal growth, PDA amended with increasing concentrations (up to 6 mM glyphosate or 0.06% filter-sterilized (0.2 μm) Roundup®) was used. Amended plates were infested with a mycelial plug (5 mm) from actively growing colonies of the three fungi and incubated at 20°C. Colony diameter was measured after 7 days from 4-6 replicate plates per treatment and the experiment was repeated. Colonies were further assessed for sporulation and viability of conidia produced after 2-3 weeks as previously described.

In shadehouse trials, R. parviflorus and R. spectabilis received an application of glyphosate [2 mM or 6 mM glyphosate (0.02% or 0.06% Roundup®)] (10 fold less than the recommended dose Roundup® for Rubus species), followed by conidial inoculum (10⁶ spores/ml with 0.02% Tween 80) after 24 hours, both applied at 50 ml/m². Plants were visually rated for %necrosis as described previously over a 3-week period and compared to control treatments of spore suspensions, glyphosate doses, and water. For each treatment, three replicate plants were included and the experiment was repeated. Treatment data was subjected to analysis of variance and the Student-Newman-Keuls test at $p = 0.05$.

2.2. RESULTS

2.2.1. Isolation of fungi

Fusarium avenaceum and C. dematium were isolated from stem lesions on R.
strigosus in the Sub-Boreal Spruce biogeoclimatic zone. The Phomopsis sp. was isolated from circular leaf spots on *R. spectabilis* and was widespread throughout 7 of twelve collection sites in the Coastal Western Hemlock zone. Repeated subculturing (5 times) of *C. dematium* resulted in sectoring and loss of conidial production, with low sporulation rates and difficulty in inducing sporulation regardless of media or UV light exposure. All three fungi remained viable for at least 1 year in cold storage, and more vigorous growth was derived on fresh agar from colonies stored on agar slants than from those stored in sterile distilled water.

Maximum growth and spore germination was observed at 20°C for *F. avenaceum* (Figure 7) and 25°C for *C. dematium* and *Phomopsis* sp. (data not shown). In Richard’s V-8 broth, *F. avenaceum* produced macro- and microconidia. Both alpha- and beta-spores were obtained from the *Phomopsis* sp. in MEA cultures.

### 2.2.2. Pathogenicity tests

#### 2.2.2.1. Effect of inoculum production

(i) **Agar and liquid media:** In shadehouse trials, inoculum of each of the three fungi combined with sucrose and gelatin failed to induce >10% total plant necrosis on *R. parviflorus* and *R. spectabilis*.

(ii) **Grain medium:** Culture filtrates from *F. avenaceum* grown on rice grains, combined with 0.4% Silwet L-77®, consistently induced 50-100% necrosis on *R. parviflorus* and 25-50% necrosis on *R. spectabilis* plants (Table 1). Treated foliage developed a water-soaked appearance, followed by the development of large areas of necrotic tissue, leaf curl and death. Extensive foliar lesions occurred within 24 h on *R. parviflorus*. *Fusarium avenaceum* was re-isolated from diseased leaf tissue of inoculated plants. Inoculated *Rubus* plants flushed new leaves by 3 weeks, and new foliage and stems were free of necrotic symptoms. In the host range test, conifer seedlings treated with *F. avenaceum* filtrate inoculum did not display disease symptoms and appeared as vigorous as controls.
after 3 weeks.

Extractions of 30 day-old *F. avenaceum*-infested rice and analysis for moniliformin, butenolide, enniatin B and beauvericin showed that a single toxin, moniliformin, was present at levels of 3 074 ppm (Vesonder, pers. comm.).

2.2.2.2. Effect of formulation

When combined with adjuvants, formulations which induced ≥50% necrosis on detached leaves could not be reproduced on intact plants regardless of the formulation tested or incorporation of dew periods (data not shown).

2.2.2.3. Effect of glyphosate

*Fusarium avenaceum* grew on PDA amended with up to 6 mM glyphosate, but developed an irregular colony margin compared to the even mycelial margin observed in the controls. Colony diameters reached 50% of controls at concentrations of >1 mM glyphosate after 7 days. *Fusarium avenaceum* sporulated on PDA with 0 - 2 mM glyphosate and conidia germinated readily at 25°C when suspended on 2% water agar and observed after 24 h. *Colletotrichum dematium* and *Phomopsis* sp. showed similar irregular colony margins and reduced colony diameter when grown on glyphosate-amended PDA.

In shadehouse trails, applications of 2 mM glyphosate followed by *F. avenaceum* caused significantly greater %necrosis on *R. parviflorus* than either fungus or a 2 mM dose of glyphosate alone after 7 days (p = 0.0076) (Table 2). For *R. spectabilis*, up to 50% foliar damage was observed with the 2mM glyphosate-*F. avenaceum* treatment, although %necrosis was not significantly greater than other treatments, except for the water treatment (p = 0.0530). Applications of glyphosate followed by *C. dematium* and *Phomopsis* sp. showed no significant difference than glyphosate treatments alone on both *Rubus* spp. (data not shown). After 3 weeks, no significant difference (p = 0.05) was observed among any treatments incorporating glyphosate in *Rubus* species, and
Figure 7. Effect of temperature on *Fusarium avenaceum* colony growth (after 7 days) and spore germination (after 24 h), with standard error bars.
Table 1. Effect of *Fusarium avenaceum* inoculum filtrates, obtained from infested rice cultures, combined with an organosilicone surfactant (Silwet L-77®) on foliar necrosis of *Rubus* plants.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar necrosis (%)*</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>R. parviflorus</em></td>
<td><em>R. spectabilis</em></td>
<td></td>
</tr>
<tr>
<td>water</td>
<td>0.17 a</td>
<td>0.00 a</td>
<td></td>
</tr>
<tr>
<td>uninoculated rice</td>
<td>0.00 a</td>
<td>0.00 a</td>
<td></td>
</tr>
<tr>
<td>uninoculated rice + 0.2% Silwet L-77®</td>
<td>0.00 a</td>
<td>0.00 a</td>
<td></td>
</tr>
<tr>
<td><em>F. avenaceum</em></td>
<td>1.00 ab</td>
<td>0.50 a</td>
<td></td>
</tr>
<tr>
<td>0.2% Silwet L-77®</td>
<td>0.33 a</td>
<td>0.33 a</td>
<td></td>
</tr>
<tr>
<td>0.4% Silwet L-77®</td>
<td>1.50 b</td>
<td>0.00 a</td>
<td></td>
</tr>
<tr>
<td><em>F. avenaceum</em> + 0.2% Silwet L-77®</td>
<td>2.50 c</td>
<td>2.50 b</td>
<td></td>
</tr>
<tr>
<td><em>F. avenaceum</em> + 0.4% Silwet L-77®</td>
<td>3.44 d</td>
<td>2.56 b</td>
<td></td>
</tr>
</tbody>
</table>

* %necrosis rated at 7 days on a scale of 0-4 where: 0 = no damage, 1 = <1% damage, 2 = 1 - 10% damage, 3 = 11 - 50% damage, 4 = 51 - 100% damage. Within a column, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at $p = 0.05$. 


Table 2. Effect of *Fusarium avenaceum*, applied alone and in a delayed application following low doses of glyphosate (applied as Roundup®), on foliar necrosis of *Rubus* species.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Foliar necrosis (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>R. parviflorus</em></td>
</tr>
<tr>
<td>water</td>
<td>0.33 a</td>
</tr>
<tr>
<td><em>F. avenaceum</em></td>
<td>1.00 ab</td>
</tr>
<tr>
<td>0.02% Roundup®</td>
<td>1.33 ab</td>
</tr>
<tr>
<td>0.06% Roundup®</td>
<td>3.00 b</td>
</tr>
<tr>
<td><em>F. avenaceum</em> + 0.02% Roundup®</td>
<td>3.33 b</td>
</tr>
<tr>
<td><em>F. avenaceum</em> + 0.06% Roundup®</td>
<td>3.00 b</td>
</tr>
</tbody>
</table>

*% necrosis rated at 7 days on a scale of 0-4 where: 0 = no damage, 1 = <1% damage, 2 = 1 - 10% damage, 3 = 11 - 50% damage, 4 = 51 - 100% damage. Within a column, means followed by the same letter are not significantly different according to the Student-Newman-Keuls test at *p* = 0.05.
glyphosate-plus-fungal applications did not increase foliar damage levels compared to glyphosate alone. All plants receiving glyphosate showed increasing necrosis over time with symptoms of chlorosis (particularly in young leaves), wilting, and low vigour, reaching ≥50% necrosis after 3 weeks.

2.3. DISCUSSION

In this study, neither *C. dematium* or the *Phomopsis* sp. produced significant disease symptoms when applied as inundative doses to healthy *Rubus* plants under controlled conditions, regardless of the incorporation of adjuvants or low doses of glyphosate. To date, numerous *Colletotrichum* species have been researched as biological control agents on over twenty target weeds in agricultural systems and several have demonstrated significant weed suppression (Templeton 1982; Charudattan 1991; TeBeest et al. 1992). Some of the difficulties that were found in using *Colletotrichum* species as biocontrol agents include dependence on a dew period or free moisture (Charudattan 1991; TeBeest et al. 1992), problems with inoculum production, storage, and formulation (Auld et al. 1990; Schisler et al. 1991; Silman et al. 1991), genetic instability of cultures (Daigle and Cotty 1994), and interaction with other microorganisms (Schisler et al. 1991). While the *Phomopsis* sp. was widely recovered on *R. spectabilis* among collection sites, our efforts to develop *Phomopsis* sp. as a potential mycoherbicide were unsuccessful. In other biological control studies, constraints in the development of candidate *Phomopsis* species include requirement for dew or free moisture (Ormeno-Nuñez et al. 1988), requirement for a high spore concentration (Morin et al. 1989), conidial matrix inhibitors to germination (Sparace et al. 1991), lack of sufficient virulence (Nikandrow et al. 1990; Shivas and Scott 1993), and adoption of an endophytic stage in healthy *Rubus* spp. leaves and twigs (Shamoun and Sieber, unpublished data). Latent infection was reported to occur with both *Colletotrichum* and *Phomopsis* species (Cerkaukas 1988; Sinclair 1991).
and may account for the lack of symptom expression in our tests on *Rubus* species. Considerable efforts may yet be required to determine optimum conditions and formulations of *C. dematium* and the *Phomopsis* sp. to enhance pathogenicity to *Rubus* species. The manipulation of endemic pathogens to be successful biocontrol agents in forest areas is dependent on the interaction of many variables, which may be host-related (i.e. vigour, phenology), pathogen-related (i.e. virulence, survival), and abiotic.

*Fusarium* species have been investigated as potential mycoherbicides and several are being considered for biological control of annual and perennial crop, rangeland, aquatic, parasitic, and forest weeds (Charudattan 1991). Development of certain *Fusarium* species into potential mycoherbicides has been restrained by requirements for high spore concentrations (Abbas et al. 1991), an adequate dew period duration and temperature, and susceptible plant growth stage (Boyette and Walker 1985; Weidemann and Templeton 1988). In our tests, inundative applications of *F. avenaceum* inoculum produced on agar and liquid media, formulated with various adjuvants with and without dew periods, did not induce sufficient necrosis to control *Rubus* test plants. Shadehouse trials with applications of low doses of glyphosate followed by *F. avenaceum* initially showed greater damage on *Rubus* spp. than by chemical or fungal treatments alone, although these results were not sustained over a 3-week period. The development of extensive damage in all treatments incorporating glyphosate within 3 weeks may be due to a lack of carbohydrate reserves in potted *Rubus* plants, normally provided by extensive root systems in field sites.

When produced on a rice substrate and applied to target plants, *F. avenaceum* caused greater foliar damage, particularly on *R. parviflorus*. In other studies, *Fusarium* species tested as biocontrol agents were found to be less virulent when grown in liquid media then on grain substrates due to 1) the grain substrates supplying residual nutrients after fungal application and aiding in anchoring the fungus to the plant (Boyette et al. 1993) or 2) the fungus failing to produce or producing sub-lethal doses of phytotoxic
compounds (Abbas et al. 1991). The use of fungal metabolites in controlling competing vegetation is under investigation as an alternative to employing whole microbial organisms (Hoagland 1990; Duke et al. 1991). Fusarium species are known to produce a number of phytotoxins, including fumonisins, fusaric acid, moniliformin, enniatin, and trichothecenes (Abbas et al. 1995), many of which are determinants in the development and severity of plant diseases. The isolation and identification of large quantities of a single phytotoxin, moniliformin, from our F. avenaceum-rice cultures suggests that a phytotoxin may be responsible for the necrosis observed on Rubus test plants. Abbas et al. (1991; 1995) reported that moniliformin, extracted from F. moniliforme cultures, reduced plant height and dry weight and caused up to 100% injury to jimsonweed (Datura stramonium L.). Other secondary metabolites extracted from F. avenaceum cultures, namely acetamido-butenolide and enniatin B, acted synergistically in causing foliar lesions on detached leaves of spotted knapweed (Centaurea maculosa L.) (Hershenhorn et al. 1992). Symptoms observed on Rubus species from applications of the F. avenaceum-rice filtrates formulated with Silwet L-77® indicate a phytotoxic effect, with rapidly forming irregular lesions, leaf margin necrosis, leaf curl and death. Silwet L-77® likely acted as a carrier for phytotoxic compounds by reducing the surface tension of the liquid formulation to below the critical level for wetting leaf surfaces (Stevens 1993), allowing for increased stomatal infiltration. Silwet L-77® was found to enhance stomatal egress and increase infectivity of Pseudomonas syringae pv. phaseolicola (Burk.) Young, Dye & Wilkie, in causing halo blight of bean (Phaseolus vulgaris L.) (Zidack et al. 1992) and of Ascochyta pteridis Bres. for biological control of bracken (Pteridium aquilinium (L.) Kühn.) (Womack and Burge 1993).

From this research, F. avenaceum, when grown on rice, demonstrated several suitable characteristics for further evaluation as a biological control agent, including simple production of large amounts of inoculum, a formulation requiring no dew period, a spray method for application, extensive foliar damage to R. parviflorus, and a lack of disease.
symptoms, as visually assessed, on the economically important conifers tested. Results from this study implicate biologically active herbicidal activity of a toxin produced by *F. avenaceum* grown on rice substrates. Further bioassays using pure moniliformin are underway to quantify electrolyte leakage and chlorophyll loss from *Rubus* leaf disks, to elucidate mode of action, and to evaluate the potential use of moniliformin for biological control of invasive *Rubus* species in reforestation sites.
CHAPTER 3

FIELD EVALUATION OF A POTENTIAL BIOLOGICAL CONTROL AGENT, *Fusarium avenaceum*, AGAINST COMPETING *Rubus* SPECIES IN A SUPPRESSED WHITE SPRUCE PLANTATION.

3.0. INTRODUCTION

The objective of this field trial was to evaluate the efficacy of a biological control agent, the native fungal pathogen *Fusarium avenaceum* in suppressing invasive *Rubus* species in a young conifer plantation. The biological control treatment was compared with other forest vegetation control methods, including chemical (herbicide) and manual treatments. The field trial was categorized as a first approximation trial and followed the experimental design protocol for a stand establishment trial (one to several years after planting) (Herring and Pollack 1985). The purpose of a first approximation trial is to compare vegetation management treatments which encourage early seedling establishment and growth by minimizing vegetative competition. The basic goals are to identify treatments that temporarily suppress vegetation to favour conifer seedling establishment and growth and to determine the impact of treatments on conifer seedlings. Treatments are applied when seedlings can best respond to a reduction in vegetative competition and cause little damage to the seedlings.

In this trial, treatment efficacy was based on (i) increased light attenuation at the individual-tree seedling level, (ii) by visual estimates of percent cover and vigour of *Rubus* species, and (iii) by measurements of both conifer seedlings and target vegetation. In part, the biological control treatment was based on *Rubus* suppression assays by Wall and Shamoun (1990) and Jobidon (1991); the chemical treatments were based on recommendations by D'Anjou (1990) that late summer glyphosate applications are more
effective than in early summer for suppressing thimbleberry (*Rubus parviflorus*); and the manual treatment was based on recommendations by Hart and Comeau (1992) and LePage et al. (1991) that mid-July cuttings are the most effective in suppressing thimbleberry regrowth. The primary treatment evaluation technique was based on light transmission recorded through the target vegetation canopy to the seedlings. The photosynthetically active radiation (PAR) reaching spruce seedlings was measured using a Sunfleck ceptometer, a handheld device measuring fluxes of 400-700 nm. This technique has been used in other studies on *Rubus* control (Comeau et al. 1993; Jobidon 1992; 1994), and, for example, in estimation of forest leaf area index and in forest canopy studies. The study was established in a 1-year old spruce plantation in the Prince George Forest Region, British Columbia, which was invaded by thimbleberry, *R. parviflorus* and wild red raspberry (*R. strigosus*). These *Rubus* spp. outcompete young conifer seedlings by monopolizing resources, particularly light, since these shrubs may develop an extensive canopy cover (Comeau 1988; LePage et al. 1991; Oleskevich et al. 1996b). A first assessment of treatment effects was completed 3 weeks after treatment application to observe any immediate, short-term effects. Data collection in subplots focused on changes in live canopy cover of the target vegetation, specific treatment effects on target vegetation, and post-treatment growth response and/or damage to crop seedlings (Herring and Pollack 1985).

### 3.1. MATERIALS AND METHODS

#### 3.1.1. Field site

The field site selected was a 1 year-old white spruce (*Picea glauca*) plantation, established in 1994 (at 1200 trees/ha, min. spacing 1.5 m) after harvesting and site preparation (broadcast burn and windrow piling, mound, screef) activities. The site was located in the Prince George Forest District, British Columbia, subzone Sub-Boreal Spruce e2 to Sub-Boreal Spruce j1 (-08 to -07), at 54°39' and 122°53', and was situated
at an elevation of 550 - 750 m with a north-facing slope. Soil substrates were a silty loam, loam, and silty clay soils with 10-60% coarse fragments. The target vegetation species, *R. parviflorus* and *R. strigosus*, were in full leaf stage with mid- to late-fruiting. Associated plant species included *Epilobium angustifolium*, *Oplopanax horridus*, *Sambucus racemosa* ssp. *pubens* (Michx.) House, *Lonicera involucrata*, *Veratrum viride* Ait., *Calamagrostis canadensis* (Michx.) Beauv., *Athyrium filix-femina*, *Streptopus roseus*, *Equisetum* spp., *Ribes* spp., *Anaphalis margaritacea* (L.) Benth. & Hook., and *Urtica* spp.

### 3.1.2. Experimental design

A randomized complete block design was assigned, incorporating seven treatments with three replicates per treatment. Three homogeneous blocks were established with each treatment plot completed once per block. Blocks were set up perpendicular to any fertility gradients and were ca. 10 m x 50m in size. Treatment plots of 5m x 5m, with a minimum buffer of 2 m between plots, were established within blocks using a table of random numbers (Gomez and Gomez 1976). Treatment plot corners were marked with permanent, 1 m tall orange-painted posts, labeled with metal tags for block and plot identification, and azimuth readings were taken. Each treatment plot had a predetermined number of subplots for both conifer seedling and target vegetation measurements.

### 3.1.3. Treatments

Vegetation management treatments included i) fungal application of *Fusarium avenaceum*; ii) a combined application of a low dose of glyphosate (0.356 kg a.i./ha or 1 L Roundup®/ha) (Monsanto Canada Inc., Sardis BC) followed by a fungal application after 6 hr lag time; iii), iv), and v) chemical applications of low-, mid- (1.05 kg a.i./ha or 3 L Roundup®/ha), and high- (1.78 kg a.i./ha or 5 L Roundup®/ha) dose of glyphosate; vi) manual cutting with hand-held shears; and vii) control plots. The inoculum of *F.*
*avenaceum* was prepared as follows: the fungus was grown on autoclaved rice substrate for 30 days, dried and ground to a coarse powder, suspended in distilled water (4.5 g infested rice: 50 ml distilled water), and filtered through cheesecloth (Oleskevich et al. 1996c). The filtrate was combined with 0.4% Silwet L-77® (organosilicone surfactant, Loveland Ind., Greeley CO) and applied to *Rubus* foliage by a Solo backpack sprayer (20 L) with No-Drift AN 5.0 nozzle at 10 -15 psi, with 1.25 L filtrate per 25 m² treatment plot or 500 L filtrate/ha. Glyphosate treatments were applied with a hand-held pump sprayer (Monsanto Roundup® Grass and Weed Killer Applicator, Monsanto Canada Inc., Sardis BC)) with a cone-shaped mist, 45 psi pressure, and 60 L/ha delivery rate. Treatments within one block were applied on the same day. Treatments were applied on July 27, 1995 with average midday conditions of 15.15 - 18.40 °C, 45.5-62.5% R.H., winds of 12.08 - 19.65 km/hr, and followed by 5.6 mm rain within 24 hr. Daily weather conditions were consistent for 36 hr post-application, followed by increasing rainfall.

### 3.1.4. Measurements

Treatment effects were evaluated through pre- and post-treatment measurements in subplots for both conifer seedlings and target vegetation. For conifer seedlings, light attenuation was measured at four permanent subplots (of 1 seedling each) per plot, recording the average measurement of three ceptometer readings taken perpendicular to the stem, at the terminal bud and at mid-stem height. The total incoming PAR was also recorded at every plot and measurements were taken on a cloudless day between 10:00 - 14:00 (daily peak in solar irradiance). The percent transmission (PT) was calculated as follows:

\[ PT = \left( \frac{I_g}{I_o} \right) \times 100 \]

where \( I_g = \) transmitted PAR and \( I_o = \) total incoming PAR. Height and diameter of conifer seedlings were recorded as well as the free-to-grow status (overtopped = leader below
surrounding vegetation, threatened = leader at same height as surrounding vegetation, and free-to-grow = leader well above surrounding vegetation). Post-treatment visual assessments of seedling tolerance to treatment applications were made according to the ECW Western Canada Section Rating Scale (0-100%), where >10% damage is unacceptable injury.

Treatment response evaluations of target Rubus species included visual estimates of percent vegetation cover per plot and direct measurements of target vegetation and seedling characteristics (no. of Rubus stems/m² and average Rubus shoot height/m²) recorded at two random subplots of 1 m² per plot. Percent cover of Rubus species was calculated using the area-addition method in which the percent area covered by target vegetation within plot quadrants is added cumulatively and averaged to give a plot estimate. A collection of post-treatment diseased Rubus foliar and stem tissues from fungal treatment plots was made for re-isolation of the fungal pathogen to fulfill Koch's postulates (Agrios 1988). During measurements and treatments, trampling of plots and subplots was avoided as much as possible.

3.2. RESULTS

Baseline vegetation assessments of conifer and target vegetation recorded prior to treatment applications are presented in Tables 3 and 4. Analysis using the Student-Newman-Keuls test showed that no significant difference in Rubus species cover were found between pre-treatment plots at $p = 0.05$ ($F = 2.389$). Spruce seedlings also showed little significant difference at $p = 0.05$ ($F = 0.705$) for percent transmission recorded among pre-treatment plots. Seedlings were rated as threatened with regard to overtopping by competing vegetation, and appeared free from mechanical, grazing, or other damage. Field trial inoculum was viable when plated on nutrient agar immediately following field applications.
Table 3. Pre-treatment characteristics of competing *Rubus strigosus* and *R. parviflorus* in a vegetation management field trial.

<table>
<thead>
<tr>
<th>Treatment plot</th>
<th>Rubus species vegetation</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>stems/m²</td>
<td>shoot ht. (cm)</td>
<td>vigour$^\dagger$</td>
<td>% cover</td>
</tr>
<tr>
<td>control</td>
<td>16.83</td>
<td>53.50</td>
<td>3.50</td>
<td>36.67 a</td>
</tr>
<tr>
<td>manual cutting</td>
<td>9.33</td>
<td>33.83</td>
<td>3.00</td>
<td>11.67 a</td>
</tr>
<tr>
<td>low-chemical dose</td>
<td>17.67</td>
<td>64.83</td>
<td>3.33</td>
<td>35.00 a</td>
</tr>
<tr>
<td>mid-chemical dose</td>
<td>15.00</td>
<td>61.67</td>
<td>3.67</td>
<td>66.70 a</td>
</tr>
<tr>
<td>high-chemical dose</td>
<td>12.17</td>
<td>32.40</td>
<td>3.00</td>
<td>20.33 a</td>
</tr>
<tr>
<td>combined application</td>
<td>15.83</td>
<td>60.67</td>
<td>3.33</td>
<td>43.33 a</td>
</tr>
<tr>
<td>fungal application</td>
<td>14.33</td>
<td>63.17</td>
<td>3.50</td>
<td>66.70 a</td>
</tr>
</tbody>
</table>

Note: each value represents the mean of three blocks. Within a column, means followed by the same letter are not significantly different by the Student-Newman-Keuls test at $p = 0.05$ level of significance.

$^\dagger$ vigour: scale of 0 - 4 where 0 = dead and 4 = good vigour and growth rate.
Table 4. Pre-treatment characteristics of 1-yr old white spruce (*Picea glauca*) seedlings in a vegetation management field trial.

<table>
<thead>
<tr>
<th>Treatment plot</th>
<th>diam. (mm)</th>
<th>ht. (cm)</th>
<th>vigour†</th>
<th>PT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>81.91</td>
<td>34.18</td>
<td>2.73</td>
<td>41.17 ab</td>
</tr>
<tr>
<td>manual cutting</td>
<td>89.58</td>
<td>45.08</td>
<td>3.67</td>
<td>56.45 ab</td>
</tr>
<tr>
<td>low-chemical dose</td>
<td>83.00</td>
<td>40.75</td>
<td>3.42</td>
<td>57.04 ab</td>
</tr>
<tr>
<td>mid-chemical dose</td>
<td>79.73</td>
<td>41.82</td>
<td>2.64</td>
<td>39.91 ab</td>
</tr>
<tr>
<td>high-chemical dose</td>
<td>83.00</td>
<td>47.00</td>
<td>3.58</td>
<td>57.44 ab</td>
</tr>
<tr>
<td>combined application</td>
<td>85.00</td>
<td>39.92</td>
<td>3.25</td>
<td>67.16 b</td>
</tr>
<tr>
<td>fungal application</td>
<td>72.92</td>
<td>33.83</td>
<td>2.75</td>
<td>30.77 a</td>
</tr>
</tbody>
</table>

Note: each value represents the mean of three blocks. Within a column, means followed by the same letter are not significantly different by the Student-Newman-Keuls test at $p = 0.05$ level of significance.

† vigour: scale of 0 - 4 where 0 = dead and 4 = good vigour and growth rate.

* PT (percent transmission) = (transmitted photosynthetically active radiation/total incoming photosynthetically active radiation) x 100.
Table 5. Selected post-treatment characteristics (after 21 days) of *Rubus* species and white spruce (*Picea glauca*) seedlings in a vegetation management field trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Rubus</em> species</th>
<th>Spruce seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vigour†</td>
<td>%cover</td>
</tr>
<tr>
<td>control</td>
<td>3.17</td>
<td>30.00</td>
</tr>
<tr>
<td>manual</td>
<td>2.00</td>
<td>8.33</td>
</tr>
<tr>
<td>low-chemical dose</td>
<td>2.33</td>
<td>33.33</td>
</tr>
<tr>
<td>mid-chemical dose</td>
<td>1.33</td>
<td>43.33</td>
</tr>
<tr>
<td>high-chemical dose</td>
<td>2.00</td>
<td>23.33</td>
</tr>
<tr>
<td>combined appl.</td>
<td>1.50</td>
<td>40.00</td>
</tr>
<tr>
<td>fungal application</td>
<td>2.83</td>
<td>58.33</td>
</tr>
</tbody>
</table>

Note: each value represents the mean of three blocks. Values in bold are significantly different from pre-treatment measurements according to the Student-Newman-Keuls test at $p = 0.05$ level of significance.

† vigour: scale 0 - 4 where 0 = dead and 4 = good vigour and growth rate.

* PT (percent transmission) = (transmitted photosynthetically active radiation/total incoming photosynthetically active radiation) x 100.
Analysis of the data collected 3 weeks post-application, a first assessment of immediate treatment effects, showed no significant decreases in either percent *Rubus* cover or increases in percent light transmission reaching conifer seedlings, except for the manual control treatment, when compared to baseline assessments (Table 5). The most significant treatment effect was a decrease in *Rubus* spp. vigour for all treatments, except the untreated control, when compared to baseline measurements.

All treatments involving a chemical application showed foliar damage (chlorosis, mottled leaves, leaf curl) to *Rubus*, although complete mortality was not observed 3 weeks from application. Seedling tolerance to all treatment applications was rated as acceptable (0-9% foliar damage) for 97.6% of seedlings, indicating that no detrimental, short-term impact occurred.

3.3. DISCUSSION

This first approximation trial was conducted to fulfill the goals of establishing a field trial to test biological, chemical, and manual control methods for invasive *Rubus* spp. and evaluating the immediate response to these methods. By establishing a permanent field site, evaluations can be re-assessed yearly to observe the long-term impacts of each of the control methods. The use of a handheld ceptometer appeared to be an accurate and rapid method for measuring light transmission at the seedling level, although cloud conditions are crucial and delays due to weather must be expected when using this methodology.

In previous shadehouse trials, *F. avenaceum* demonstrated potential as a biological control agent by rapidly inducing extensive foliar lesions in *R. parviflorus* when grown on a rice medium and applied as a culture filtrate combined with the surfactant Silwet L-77® (Oleskevich et al. 1996c). In this field trial, the biological control treatment did not induce necrosis previously observed in shadehouse trials on *Rubus* spp. Biological control agents often demonstrate lower efficacy in field trials when compared to greenhouse assays.
(Yang and TeBeest 1993) due to varying environmental (e.g. temperature, free moisture availability) and host plant characteristics. In the field trial, the temperature during and following the fungal application (18.4°C average) was within optimal colony growth and spore germination range (15-25°C) of *F. avenaceum*. During application, the relative humidity of ca. 50% combined with a wind factor of ca. 20 km/hr and a low rainfall for ca. 12 hr. may have created suboptimal conditions for fungal survival.

Host plant characteristics which may have influenced the fungal infection process include the phylloplane environment, plant phenology, and plant defenses. Phylloplane conditions (chemical and biotic) can influence spore survival, germination, and infection due to the presence of available nutrients, toxins, and antagonistic and competing microorganisms. Plant phenology may have influenced infectivity as the fungal inoculum was applied during the late, full *Rubus* leaf stage. Mature foliage with thickened leaf cuticles may have presented a physical barrier to spore establishment and penetration. As well, optimal carbohydrate reserves may have been available from the extensive clonal root systems of the *Rubus* spp., allowing inoculated plants to draw on energy reserves and withstand infection through production of chemical (phytoalexins) and physical barriers.

In the combined fungal/chemical application, the fungal treatment caused greater damage to target *Rubus* species, particularly *R. strigosus*, as determined by visual estimates. The combined application may have caused sufficient chemical wounding to *Rubus* species to allow for subsequent infection by the pathogen. Low doses of herbicide are known to render plants more susceptible to subsequent disease by interfering with biochemical pathways related to the production of compounds used in plant defense systems. Laboratory tests demonstrated that *F. avenaceum* remained viable when combined with up to 0.5% Roundup® (data not shown).

The use of *F. avenaceum* as a biological control agent has focused on inducing primary infections on *Rubus* foliage. The importance of secondary infections and
multiple applications in enhancing disease and suppressing Rubus growth can be more closely evaluated during successive growing seasons. As well, investigating the optimal infection window for F. avenaceum on Rubus species can be tested through timing trials (early-, mid-, and late-summer applications). While this trial was based on established chemical and manual vegetation control methods, researchers suggest that biological control may have to involve new concepts and approaches for control of the target organism (Yang and TeBeest 1993). New control methods for Rubus species which focus on long-term impacts by reducing carbohydrate reserves, thus reducing resistance to natural pathogens and increasing susceptibility to environmental factors (climate, available nutrients), may result in sufficient suppression to allow for conifer release. Further studies may identify factors limiting the infection and build-up of F. avenaceum on Rubus species and evaluate the role of this native pathogen in a broader ecological context.
An in-depth autecological review of *Rubus strigosus*, *R. parviflorus*, and *R. spectabilis* was conducted to provide current knowledge on the botanical description, distribution, habitat, growth and development, reproduction, and population dynamics. These *Rubus* species are native, perennial, deciduous shrubs which are associated with both hardwoods and softwoods and form monospecific, multi-layered shrub communities with profuse seedbanks and long-lived clonal root systems. The species often have a strong economic impact in forest renewal sites and a review of chemical, manual and other control methods revealed the hardiness and regenerative capabilities of these species following herbicide applications, cutting, burning, site preparation activities, grazing, seeding, and the use of cover mulches. Chemical and manual vegetation management practices are often ineffective due to inconsistent levels of control and vigorous resprouting from rhizomal, basal, and root buds.

Biological control strategies are being promoted to reduce competing forest vegetation and this thesis investigated the potential use of several native pathogenic fungi as biological control agents to suppress *Rubus* species. Fungi, isolated from naturally diseased *Rubus* tissue and cultured on artificial media, were applied in inundative, foliar doses to healthy *Rubus* plants in shadehouse trials. Of three candidate fungi screened for pathogenicity, namely *Fusarium avenaceum*, *Colletotrichum dematium*, and a *Phomopsis* sp., only *F. avenaceum* demonstrated sufficient virulence. Neither *C. dematium* or the *Phomopsis* sp. produced sufficient disease symptoms, regardless of formulation with numerous adjuvants or after exposing host plants to low doses of glyphosate. Inundative doses of *F. avenaceum* inocula (conidia and mycelia) combined with low doses of glyphosate showed greater damage on *R. parviflorus* than fungal or chemical treatments alone.
Under laboratory investigations, *F. avenaceum* was found to produce large quantities of a single phytotoxin, moniliformin, when grown on rice grains. Culture filtrates, when combined with an organosilicone surfactant Silwet L-77®, induced rapid formation of necrotic lesions on *Rubus* test plants, particularly *R. parviflorus*. Repeated shadehouse trials indicated that the *F. avenaceum*-rice-surfactant formulation demonstrated several suitable characteristics for further evaluation as a potential mycoherbicide.

The biological control strategy was evaluated in a small field trial, employing the *F. avenaceum* formulation to suppress invasive *Rubus* spp. in a young white spruce plantation. The biological control treatment was compared to herbicide applications of varying doses, combined applications of biological and low doses of glyphosate, manual cutting, and control plots in a randomized complete block design. Three weeks after treatment, no significant differences were found between treatments (except for those manually cut), as evaluated by light attenuation ceptometer readings at the individual-conifer seedling level, %cover and vigour ratings of target *Rubus* spp., and direct measurements of conifer seedlings and target vegetation. By establishing permanent field plots, evaluations can be re-assessed yearly to observe the long term impacts of the biological control methods.
5.0. LITERATURE CITED


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