BIOLOGICAL CONTROL APPROACH FOR MANAGEMENT OF DWARF MISTLETOES*

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ABSTRACT

Dwarf mistletoes (*Arceuthobium* spp.) are destructive forest pathogens that parasitise commercially important conifer species. Timber losses result from growth reduction, from wood degradation, from increased predisposition to attack by bark beetles, decay, and sapstain fungi, and ultimately from plantation failure. Research and experience in North America have demonstrated the potential use of hyperparasitic fungi as biological control agents for management of dwarf mistletoes. Although much information is available on the mycobiota associated with dwarf mistletoes, significant research and development are required for these to become operational tools. The most promising biological control agents are *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. and *Neonectria neomacrospora* (C.Booth & Samuels) Mantiri & Samuels which attack shoots and berries, and the endophytic systems of dwarf mistletoe. The use of these two hyperparasitic fungi as potential biological control agents for management of dwarf mistletoes is under investigation. The development of an effective and efficient biological control strategy will reduce the impact of dwarf mistletoes on timber production in areas where traditional silvicultural control, such as retention silviculture or partial harvesting systems, is not practical.

Keywords: biological control; retention silviculture; dwarf mistletoe; *Arceuthobium tsugense*; *Arceuthobium americanum*; *Arceuthobium laricis*; *Arceuthobium douglasii*; *Arceuthobium pusillum*; *Colletotrichum gloeosporioides*; *Neonectria neomacrospora*.

INTRODUCTION

Dwarf mistletoes of the genus *Arceuthobium* (Viscaceae) (Fig. 1) are flowering plants that are obligate parasites of conifers within the families Pinaceae and Cupressaceae; they

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rely upon the host for support, mineral nutrients, a portion of their required carbon compounds, water, and possibly other growth factors. Thirty-four New World species and eight Old World species are currently recognised. In North America, the greatest species diversity is located in north-western Mexico and the western United States where 28 of the 34 New World species are present. Ranges of five species of dwarf mistletoe — *Arceuthobium americanum* Nutt. ex Englem., *A. laricis* (Piper) St John, *A. douglasii* Engelm., *A. tsugense* (Rosendahl) G.N.Jones, and *A. pusillum* Peck — extend into Canada. Of these, all but *A. pusillum* are present in British Columbia (Hawksworth & Wiens 1996).

Volume losses due to dwarf mistletoe infection are estimated at 3.8 million m^3 annually in western Canada and 11.3 million m^3 in the western United States. The economic impact of this volume loss is difficult to calculate, but totals of several billion dollars annually have been estimated (Hawksworth & Wiens 1996). Infection of young trees by dwarf mistletoe results in high mortality, while infection of older trees results in decreased needle length, decreased length of needle-bearing branches, decreased needle surface area, and decreased total number of needles. As severity of dwarf mistletoe infection increases, growth in diameter and height decreases, resulting in reduced volume production. In some host-parasite combinations, mortality is increased by dwarf mistletoe infection (Hawksworth & Wiens 1996).

MANAGEMENT STRATEGIES FOR DWARF MISTLETOES — BIOLOGICAL, CHEMICAL, GENETIC RESISTANCE, AND SILVICULTURAL APPROACHES

Constrained silvicultural options for dwarf mistletoe control in partial harvesting systems have re-activated research for alternative dwarf mistletoe management strategies. Chemical control has been investigated, and almost 60 different formulations — mostly mixtures of 2,4-D or 2,4,5-T — have been tested. None controlled dwarf mistletoe without also damaging the host tree, and none affected the endophytic system of dwarf mistletoe. The only chemical that reduces the rate of dwarf mistletoe spread is the plant-growth regulator ethephon (2-chloroethyl phosphoric acid), which causes shoot abscission. The chemical does not affect the endophytic system, and rapid shoot re-sprouting occurs after shoot removal. Ethephon is registered by the Environmental Protection Agency (EPA) in the U.S.A. under the name “Florel” (Rhone-Poulenc AgCo.), but is not registered for forestry use in Canada (Hawksworth & Wiens 1996; Shamoun & DeWald 2002).

Despite relatively limited investigation, field observations, progeny tests, and graft studies point to some degree of resistance to mistletoe in North American conifers (Hawksworth & Wiens 1996; Shamoun & DeWald 2002). Mistletoe-resistance screening programmes have been initiated for *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) and *Tsuga heterophylla* (Raf.) Sarg. (western hemlock) pathosystems (Shamoun & DeWald 2002). Biotechnology tools, including tissue culture and PCR-DNA technologies, can be used to supplement traditional resistance-screening and resistance-breeding programmes (Deeks *et al*. 2001; Marler *et al*. 1999). Trees in which resistance to mistletoe has been confirmed can be searched for molecular DNA markers. These markers then can be used in marker-aided selection for mistletoe resistance to eliminate the long generation times currently needed to confirm genetic resistance (Shamoun & DeWald 2002).

The emphasis of this article is on reviewing the mycobiota associated with dwarf mistletoes and their exploitation as biological control agents. The aim of the research investigation was to describe the pathological effects of hyperparasitic fungi (e.g., *Colletotrichum gloeosporioides* and *Neonectria neomacrospora*) on the berries, shoots, and endophytic systems of dwarf mistletoes to elucidate the biology, mode of action, and epidemiology of these hyperparasitic fungi. In addition, the investigation served to increase our understanding of the role these hyperparasitic fungi play in regulating the life cycle of dwarf mistletoes, and to explore the potential use of the fungi as biological control agents to mitigate impact of dwarf mistletoes on establishment and regeneration of young conifer plantations under retention silviculture treatments (i.e., partial harvesting systems).

BIOLOGICAL CONTROL APPROACH

Many fungi are pathogens and many insects are herbivores of dwarf mistletoes (Hawksworth & Geils 1996; Hawksworth *et al*. 1977; Stevens & Hawksworth 1970). None, however, have been sufficiently studied and developed for operational use as biological control agents (Hawksworth 1972). Some fungal pathogens and insect herbivores (particularly lepidopteran larvae) are highly destructive to dwarf mistletoe reproduction and populations in some areas in some years. The factors, which induce or regulate these outbreaks result from complex and often-indirect interactions of weather and a multi-trophic community of organisms. Dwarf mistletoe pathogens and herbivores are also indigenous organisms, having co-evolved with their hosts into long-term stable (perhaps fluctuating) relationships that are not readily amenable to human control. Nonetheless, given the potential number of agents and advantages of the approach, development of biological control as a management option in the near future appears promising (Hawksworth 1972; Shamoun 1998; Shamoun & DeWald 2002).

Insects as Biological Control Agents

Initial research identifies several destructive insect predators that apparently are endemic to Pakistan (Mushtaque & Baloch 1979), but no steps have been taken to test their applicability for introduction into North America. Other Asian dwarf mistletoes also host potential candidates for biological control of New World dwarf mistletoes (Tong & Ren 1980).

Hyperparasitic Fungi as Biological Control Agents

The extensive literature on biological control of unwanted higher plants or "weeds" has been reviewed by DeBach (1964), TeBeest & Templeton (1985), Shamoun (2000), Wall *et al*. (1992), and Wilson (1969). Mycoherbicides have been developed as practical tools in agriculture. Examples of mycoherbicides include *Phytophthora palmivora* (E.J.Butler) E.J.Butler (DeVine®) for control of strangler vine in citrus (Ridings 1986), *Colletotrichum gloeosporioides* f. sp. *aeschynomene* (Collego®) for control of northern jointvetch in rice and soybean (Daniel *et al*. 1973), and *Colletotrichum gloeosporioides* f. sp. *malvae* (BioMal® and Mallet WP™) for round-leaved mallow in field crops (Jensen 2000; Makowski & Mortensen 1992). *Chondrostereum purpureum* (Pers.:Fr.) Pouzar, a well-known primary-wood invader, is being developed for biological control of woody vegetation in forests and rights-of-way (de Jong *et al*. 1990; Shamoun *et al*. 1996; Wall 1994). *Chondrostereum purpureum* (Chontrol™) may become the first biological control agent in North America used for integrated forest-vegetation management (Shamoun & Hintz 1998). In South Africa, C*ylindrobasidium laeve* (Pers.:Fr.) Chamuris (Stumpout®) is used to clear Australian wattle tree (Morris *et al*. 1998). Mortensen (1998) has reviewed a number of other products in development.

A particular challenge for application of biological control agents for management of mistletoes is that death of the plant is not assured by destruction of the aerial shoots. The endophytic system of mistletoes within the host branch or trunk survives even when the shoots are killed back repeatedly, and may persist long after.

There are many scenarios in which an inundative biological control strategy may prove to be a useful option to the forest managers. If timber production with retained overstorey is the primary stand-management objective, preventing the entry of dwarf mistletoe into regenerating stands from overstorey and adjacent trees by using an inundative biological control approach may allow retention of overstorey trees without risking infection by dwarf mistletoe. Under the forest practices code of the Province of British Columbia, Canada, reduced clearcut size and increased riparian reserves lead to increased dwarf mistletoe spread and intensification; however, inclusion of a biological control strategy in the silvicultural treatment of the stand may reduce spread. In areas such as parks, where large witches' brooms increase the hazard rating of trees, applying a biological control agent to prevent new dwarf mistletoe infections in trees around camp or picnic sites would prevent dwarf mistletoe spread and intensification while retaining infected host trees.

For a hyperparasitic fungus to be effective in biological control, it must possess a number of attributes (Mark *et al*. 1976; Wicker & Shaw 1968):

(1) It should parasitise only the target mistletoe, and not the host or other vegetation;

(2) Its activities should seriously interfere with the life cycle of the mistletoe;

(3) It must produce abundant inoculum for the establishment of a significant infestation on the target mistletoes;

(4) It must have sufficient ecologic amplitude to assure its persistence throughout the range of the target mistletoe;

(5) Its distribution must coincide with that of the target mistletoe;

(6) It must exhibit high infectivity;

(7) It must show high virulence; and

(8) It must have an efficient mode of action for curtailing development of the target mistletoe.

Fungal parasites of dwarf mistletoe are of two general forms — those that attack aerial shoots, and those that attack the endophytic system (called canker fungi). Although a large number of fungal parasites have been found associated with dwarf mistletoes (Hawksworth & Geils 1996), there are no comprehensive reviews of these fungi and their hosts. Some fungi are specific to a single host species; others parasitise different mistletoe species (Hawksworth *et al*. 1977).

Aerial shoot fungi

These fungi usually parasitise shoots, berries, and pistillate flowers of certain spring-flowering species of mistletoes. Three of these fungi — *Colletotrichum gloeosporioides*, *Cylindrocarpon gillii* (D.E.Ellis) J.A.Muir ex *Septogloeum gillii* D.E.Ellis, and *Caliciopsis arceuthobii* (Peck) Barr ex *Wallrothiella arceuthobii* (Peck) Sacc. — are common and widespread in western North America (Hawksworth & Geils 1996).

Colletotrichum gloeosporioides is commonly isolated from dwarf mistletoes in the United States and western Canada (Wicker & Shaw 1968; Kope *et al*. 1997; Muir 1967). Although different isolates of the fungus are distinct in mycelial growth, colony colour, sporulation, and diameter growth, cross-inoculation experiments demonstrate that the isolates are not host-specific (Scharpf 1964). *Colletotrichum gloeosporioides* infections first appear as small brown-to-black necrotic lesions on the nodes of fruits and shoots. Lesions enlarge, coalesce, and cause die-back of the berries and shoots (Fig. 2) (Wicker & Shaw 1968; Parmeter *et al*. 1959). Parmeter *et al*. (1959) observed the endophytic system of *Arceuthobium abietinum* Engelm. ex Munz invaded by the fungus. Wicker (1967) determined that when both sexes of *A. campylopodum* Engelm. are attacked, from 35 to 67% of the plants, or 24% of the shoots, may be destroyed. Although the fungus may persist for several years (Wicker & Shaw 1968), its occurrence is usually sporadic (Hawksworth

FIG. 1 (left)–Female plant of the western hemlock dwarf mistletoe (*Arceuthobium tsugense*).

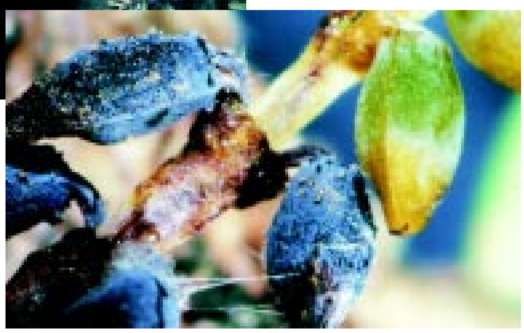

FIG. 2 (right)–*Colletotrichum gloeosporioides* infecting shoots and berries of *Arceuthobium tsugense.*

et al. 1977) and destructive to *A. americanum* and *A. tsugense* subsp. *tsugense* in western Canada (Kuijt 1963; Muir 1967, 1977; Kope *et al*. 1997; Kope & Shamoun 2000; Deeks *et al*. 2001). Muir (1977) concluded that *C. gloeosporioides* can be a significant natural biological control of *A. americanum.*

Colletotrichum gloeosporioides is being developed as a biocontrol agent of *A. tsugense* and *A. americanum*. Successful projects to date include an *in vitro* bioassay system (Deeks *et al*. 2001; Deeks *et al*. 2002) and field trials in British Columbia (Ramsfield *et al*. 1999; Ramsfield 2002; Shamoun 1998). The fungus is easily and inexpensively cultured, and it germinates over a wide range of temperatures (Shamoun 1998; Parmeter *et al*. 1959). Its mode of action is to disrupt development of mistletoe shoots, thereby preventing reproduction. Because it attacks any time after shoot emergence (Parmeter *et al*. 1959), there is a broad window when the agent could be applied.

Cylindrocarpon gillii is a fungal parasite that causes anthracnose in staminate and pistillate shoots of dwarf mistletoes (Muir 1973). The fungus and disease are characterised by white eruptions at the nodes and conspicuous masses of hyaline, and cylindrical-to-fusiform spores. The fungus parasitises most dwarf mistletoes of western North America (Hawksworth *et al*. 1977), including *A. americanum* and *A. tsugense* subsp. *tsugense* in western Canada (Shamoun 1998; Muir 1973). Mielke's (1959) inconclusive results when inoculating an isolate from a warm dry climate to a cool montane site suggest the need for proper climate matching for evaluating and using this potential biocontrol agent (Hawksworth *et al*. 1977).

Caliciopsis arceuthobii is the oldest-known fungal parasite of dwarf mistletoes. It attacks the spring-flowering mistletoes *Arceuthobium pusillum, A. americanum*, *A. douglasii*, and *A. vaginatum* (Willd.) Presl. (Dowding 1931; Kuijt 1969; Knutson & Hutchins 1979). Infection occurs at anthesis when the stigma is inoculated with aeciospores by insects, wind, and rain. Within 2 months, hyphae have penetrated the fruits to the ovary wall; host cells deteriorate and are replaced by black stromatic mass of hyphae. Normal fruit development and seed production are destroyed (Wicker & Shaw 1968). The fungus is widely distributed throughout western Canada, the United States, and Mexico (Hawksworth *et al*. 1977). Its potential as a biocontrol is limited by a large annual variation in infection rate. Although natural infection may be high one year (80% of flowers infected), there may be almost no infection during the following year in the same location (Hawksworth *et al*. 1977; Wicker & Shaw 1968; Dowding 1931; Weir 1915). Parker (1970) demonstrated that the fungus can be germinated and grown on an artificial media.

Other fungal parasites associated with aerial shoots of dwarf mistletoes are *Alternaria alternata* (Fr.) Keissler, *Aureobasidium pullulans* (de Bary) G.Arnaud, *Coniothyrium* sp., *Metasphaeria wheeleri* Linder, *Pestalotia maculiformans* Guba & Zeller, *Pestalotia heteroercornis* Guba, and *Phoma* sp. (Hawksworth & Wiens 1996; Hawksworth *et al*. 1977; Shamoun 1998; Muir 1973; Gilbert 1984). The potential of these species for biological control applications requires additional evaluation.

Canker fungi associated with the endophytic system of dwarf mistletoes

The canker fungi of dwarf mistletoe attack both the cortex and the endophytic system (Hawksworth & Geils 1996). More than 20 species of canker fungi are identified for

Arceuthobium tsugense in British Columbia (Shamoun 1998; Muir 1973; Baranyay 1966; Funk & Baranyay 1973; Byler & Cobb 1972; Funk & Smith 1981). Their potential as biological control agents includes advantages and disadvantages. Because they attack the endophytic system, effects are immediate, pronounced, and likely to kill the mistletoe. Nonetheless, because the host tree may be damaged as well, additional study is required before mass field inoculations are attempted. Three canker fungi are candidates for biological control.

Neonectria neomacrospora (formerly *Nectria macrospora, Nectria neomacrospora*) is characterised by a stroma with dark red perithecia (Fig. 3a) containing eight-spored asci (Booth & Samuels 1981). The conidial sporodochia (*Cylindrocarpon*) appear white and are found most commonly and recently on freshly cankered swellings caused by *A. tsugense* (Fig. 3b, 4) in British Columbia (Shamoun 1998; Muir 1973; Byler & Cobb 1972). Byler & Cobb (1972) reported *N. neomacrospora* (as *N. funkeliana*) as a virulent pathogen of *A. occidentale* Engelm. on *Pinus muricata* D.Don; the fungus is only weakly parasitic on the pine host and is secondarily parasitic on western gall rust cankers caused by *Peridermium harknessii* J.P.Moore syn. *Endocronatium harknessii* (J.P.Moore) Y.Hiratsuka. *Cylindrocarpon cylindroides* Wollenweb is more virulent than *Colletotrichum gloeosporioides* on *Arceuthobium tsugense* (Deeks *et al*. 2002).

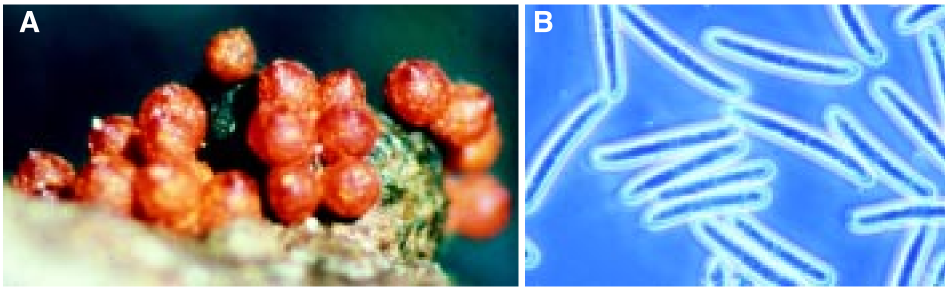

FIG. 3A–Perithecia of *Neonectria neomacrospora* infecting basal cup and swelling (endophytic system) of *Arceuthobium tsugense*.
B–Conidia of *Cylindrocarpon cylindroides* (Anamorph stage of *Neonectria neomacrospora*) infecting swelling (endophytic system) of *Arceuthobium tsugense.*

FIG. 4–*Neonectria* sp. canker of *Arceuthobium tsugense* swellings.
Note: symptoms of the disease are resinosis and girdled branch of western hemlock.

The characteristics which recommend *Neonectria neomacrospora* as a potential biocontrol agent are its selectivity for dwarf mistletoe-infected host tissue, its proven pathogenicity, its ability to invade without host wounding, its rapid canker production and abundant spore production, its reduction of mistletoe shoot growth, and host-tree girdling and branch mortality. Aspects requiring development involve improvement of fungal formulation and delivery technology (Shamoun 1998; Byler & Cobb 1972).

Cytospora abietis Sacc. is the best-known fungus associated with dwarf mistletoe cankers and is common (20%) on *Abies magnifica* A.Murr. and *Abies concolor* (Gord. & Glend.) Lindl. parasitised by *Arceuthobium abietinum*. The fungus also occasionally parasitises non-mistletoe-infected branches. The overall interaction between fungus, mistletoe, and host tree needs to be evaluated. Although the fungus kills mistletoe-infected branches, it is not known by how much the mistletoe population is reduced (Hawksworth 1972).

RESIN DISEASE SYNDROME

Resin disease syndrome is common on *Arceuthobium americanum* infecting *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. in the Rocky Mountains (Mark *et al*. 1976). Symptoms include excessive resinosis of the mistletoe canker, necrotic lesions, and discoloration of host bark, and formation of necrophylactic periderms, resin-filled needles, and dead mistletoe shoots. Numerous fungi are isolated from resin disease cankers. *Alternaria alternata* is the most consistent (recovered from 89% of cankers), but the disease appears to be a complex caused by *Alternaria alternata*, *Aureobasidium pullulans*, and *Epicoccum nigrum* Link (Mark *et al*. 1976; Gilbert 1984). Gilbert (1984) isolated these fungi as well from asymptomatic mistletoe cankers and host wood, suggesting these fungi are not the sole cause of the syndrome. Additional studies needed to assess resin disease include: effects on reproductive potential of the mistletoe, comparisons for systemic and non-systemic mistletoe infections, and the roles of environmental factors and each fungal component in disease development (Mark *et al*. 1976).

INTEGRATING BIOLOGICAL CONTROL STRATEGY WITH SILVICULTURAL ACTIVITIES

Development of an effective biocontrol programme requires technology for mass production of the fungal agent, an efficient delivery system, and sound strategy for deployment. The biocontrol agent does not have to eradicate all the dwarf mistletoe from an entire stand. The control strategy is to reduce mistletoe spread from residual trees bordering or within regeneration areas by timely treatment with biocontrol agents that kill or deflower the parasite. The selection of specific treatment areas and regimes is determined as a silvicultural operation, based on an understanding of the epidemiology of the fungus, the population dynamics of the mistletoe, and the silvics of the host species. The areas requiring treatment will be identified during pre-harvest prescription or during surveys preceding silvicultural treatments. Threshold mistletoe ratings for treatment, in relation to stand attributes such as mean diameter at breast height, age, and density, will be identified during research investigation. Only those patches exceeding the threshold would require treatment. A spatial-statistical computer model can simulate various deployment strategies.

The objective is to protect new plantations from early mistletoe infestation where the constraints of regeneration have retained a significant number of infected residual trees.

DISCUSSION AND CONCLUSIONS

Numerous studies of the mycobiota associates of dwarf mistletoes are complete. The fungal parasites *Colletotrichum gloeosporioides*, *Cylindrocarpon gillii*, *Caliciopsis arceuthobii*, and *Neonectria neomacrospora* are effective in destroying either aerial shoots or the endophytic system, thereby disrupting the mistletoe life-cycle and, consequently, reducing dwarf mistletoe spread, intensification, and damage. Canker fungi are attractive biological control agents because they attack the infection at the swelling stage, before or after shoot emergence, and infect the endophytic system. This group has the potential to kill the mistletoe rather than merely reduce reproduction. The most promising biocontrol agents are *Colletotrichum gloeosporioides* and *Neonectria neomacrospora*. They are easily cultured, virulent fungi that attack dwarf mistletoe aerial shoots, fruits, and endophytic systems. The complex of fungi associated with resin disease needs additional research before its potential can be properly evaluated. The stand areas that exceed the threshold mistletoe ratings and require treatment will be identified during pre-harvest prescription, or during surveys preceding silvicultural treatments.

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