NOTE
Forest pathology / Pathologie forestière

Assessment of *Neonectria neomacrospora* (anamorph *Cylindrocarpon cylindroides*) as an inundative biocontrol agent against hemlock dwarf mistletoe

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Abstract: A field trial was conducted on Vancouver Island to evaluate the efficacy of the native fungus *Neonectria neomacrospora* as a biocontrol agent of hemlock dwarf mistletoe (*Arceuthobium tsugense*), a conifer parasite. Treatments consisted in formulated inoculum of *N. neomacrospora* applied to unwounded and wounded *A. tsugense* swellings. After 10 months, the amount of bark necrosis for the wounded, inoculated treatment was significantly greater than for the other treatments. Similarly, sporodochia were more common (present on 50% of swellings) for the wounded, inoculated treatment than the other treatments (present on less than 10% of swellings). *Neonectria neomacrospora* was isolated from 55.0% of unwounded, inoculated swellings and from 73.7% of those wounded and inoculated, while isolated from 20.0% of unwounded, uninoculated swellings and from 35.0% of those wounded and uninoculated. These results suggest that, although some infection does occur when inoculum is applied to unwounded swellings of hemlock dwarf mistletoe, wounding significantly enhances infection. Hemlock dwarf mistletoe swellings with confirmed *N. neomacrospora* infection (isolation of the fungus and (or) presence of sporodochia) had their numbers of healthy mistletoe shoots significantly reduced (by 1.6, or about 36%) when compared with mistletoe swellings with unconfirmed infection ($P = 0.014$).

Key words: hemlock dwarf mistletoe, *Arceuthobium tsugense*, biological control, *Neonectria neomacrospora*, Stabileze formulation.

Résumé : Un essai sur le terrain fut mené sur l'Île de Vancouver pour évaluer l'efficacité du champignon indigène *Neonectria neomacrospora* comme agent de lutte biologique contre le faux-gui de la pruche (*Arceuthobium tsugense*), un parasite des conifères. Les traitements consistèrent en des applications d'une formulation d'inoculum de *N. neomacrospora* à des renflements de l'*A. tsugense* blessés et non blessés. Après 10 mois, la quantité de nécrose de l'écorce blessée et inoculée était significativement plus élevée que pour les autres traitements. Aussi, les sporodochies étaient plus fréquentes (présentes sur 50% des renflements) pour le traitement blessé et inoculé que pour les autres traitements (présentes sur moins de 10% des renflements). Le *N. neomacrospora* fut isolé sur 55,0% des renflements non blessés et inoculés et sur 73,7% de ceux blessés et inoculés, alors qu'il fut isolé sur 20,0% des renflements non blessés et non inoculés et sur 35,0% de ceux blessés et non inoculés. Ces résultats démontrent que, même si l'infection peut se développer lorsque de l'inoculum est appliqué à des renflements de faux-gui de la pruche non blessés, les blessures en augmentent significativement le taux de succès. Les renflements de faux-gui de la pruche avec présence vérifiée d'une infection due au *N. neomacrospora* (isolement du champignon et (ou) présence de sporodochies) avaient un nombre de pousses saines significativement réduit (soit de 1,6, une réduction d'environ 36%) comparativement aux renflements de faux-gui avec une infection non vérifiée ($P = 0,014$).

Mots clés : faux-gui de la pruche, *Arceuthobium tsugense*, lutte biologique, *Neonectria neomacrospora*, formulation Stabileze.

Accepted 10 September 2005.

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Introduction

Hemlock dwarf mistletoe (*Arceuthobium tsugense* (Rosendahl) G.N. Jones) occurs along the Pacific coast from Alaska to California. Primary hosts for *A. tsugense* are western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), shore pine (*Pinus contorta* Dougl. ex Loud var *contorta*), and several fir species (*Abies* spp.) (Hawksworth and Wiens 1972; Geils et al. 2002). Damage caused by *A. tsugense* consists of increment losses, some mortality, and reduced merchantability of wood (Calvin and Wilson 1996).

The most effective and efficient way to control dwarf mistletoe is clear-cutting of the whole contaminated area with removal of all trees contaminated by mistletoe at an advance stage of development (ready to release seeds) in a "safe" perimeter, taking into consideration the dispersal distance of mistletoe seeds. However, current harvesting guidelines require retention of riparian areas in addition to single trees and patches of trees throughout cutblocks. This is expected to result in substantially increased infection by mistletoe because a larger proportion of regenerating trees will be within the range of mistletoe seed dispersal from infected residual trees. Bloomberg and Smith (1982) have shown that the infection level of second-growth trees is proportional to the number of diseased residual trees. As a consequence of these changes in forest management, it has become necessary to develop alternative methods for control of *A. tsugense*.

Chemical control of *A. tsugense* has been sought since the mid 1950s (Gill 1956; Quick 1964; Scharpf 1972). Evidence of possible natural resistance to infection has been observed for healthy *Tsuga heterophylla* in areas heavily infested with *A. tsugense* and for artificially inoculated grafted *Tsuga heterophylla* trees (Smith et al. 1993).

Biological control of *Arceuthobium* spp., using insects or fungi, has also been investigated (see Shamoun and DeWald (2002) for review). Biological control of hemlock dwarf mistletoe would be contemplated for situations where clear-cutting is not possible, as well as in parks and riparian reserves. In addition, it may be employed along the perimeter of a new stand that is growing in proximity of a stand infected with hemlock dwarf mistletoe to reduce the spread of disease. Fungal parasites of *Arceuthobium* consist of two groups: aerial-shoot and fruit fungi and canker fungi (Hawksworth and Geils 1996). Common aerial-shoot fungi include *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., *Cylindrocarpon* (*Septogloeum*) *gillii* (Ellis) J.A. Muir, and *Caliciopsis* (*Wallrothiella*) *arceuthobii* (Peck.) Barr. Canker fungi parasitize the endophytic system and (or) the infected-host bark (Hawksworth and Geils 1996). Several weakly pathogenic fungi have been shown to cause resin-disease symptoms (superfluous resin being secreted from the infected branch or mistletoe swelling), with *Alternaria alternata* (Fries:Fries) von Keissler and *Aureobasidium pullulans* (de Bary) Arnaud most commonly isolated from diseased *Arceuthobium americanum* Nutt.:Engelm. swellings (Mark et al. 1976). Canker fungi are good biocontrol agents because they kill diseased host tissues and, therefore, any obligate parasites that depend on those host tissues. Canker fungi may act perennially and also tend to develop quickly on hosts or host parts that are under stress. Canker fungi that are good candidates for biological control of dwarf mistletoe are *Cytospora abietis* Sacc. (Hawksworth 1972) and *Neonectria neomacrospora* (Booth & Samuels) Mantiri & Samuels (Mantiri et al. 2001), formerly *Nectria neomacrospora* Booth & Samuels (Booth and Samuels 1981).

In early work, Byler et al. (1972a, 1972b) found that the necrotizing fungus *N. neomacrospora* was able to infect and kill galls caused by *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka (= *Peridermium harknessii* J.P. Moore) (Hiratsuka 1969; Ziller 1974) on Bishop pine (*Pinus muricata* D. Don) and Monterey pine (*Pinus radiata* D. Don). Further investigations revealed that this fungus is a virulent pathogen of dwarf-mistletoe swellings on infected pine tissues, but only weakly pathogenic to healthy conifer tissue (Byler and Cobb 1972).

In one study conducted by Funk et al. (1973), an average of 30% reduction in the number of aerial shoots of hemlock dwarf mistletoe was attributed to natural infection of *A. tsugense* swellings by *N. neomacrospora*. Infection by *N. neomacrospora* has been associated with reduced vigor of hemlock dwarf mistletoe, and inoculum (conidia) can be easily mass-produced in vitro (L. Rietman, personal observation).

Establishment of an inoculation procedure is necessary before *N. neomacrospora* can be further assessed as a biocontrol agent against hemlock dwarf mistletoe. While previous research has provided some information on the potential impact of *N. neomacrospora* infection on *A. tsugense* vigor (health, number, and size of shoots of hemlock dwarf mistletoe) (Byler and Cobb 1972; Funk et al. 1973; Smith and Funk 1980), there is still uncertainty as to whether conidia are able to serve as inoculum and whether wounding is required for infection.

The purpose of this study was to investigate the effectiveness of *N. neomacrospora* (anamorph *Cylindrocarpon cylindroides* Wollenw.) as an inundative biocontrol agent against *A. tsugense*. Inundative biological control involves the application of mass inoculum of an indigenous pathogen to target pests with the goal of interfering with their life cycle (Evans 1995). The specific objectives of this trial were: (1) to determine if wounding facilitates infection of hemlock dwarf mistletoe swellings by *N. neomacrospora* and (2) to measure the impact of infection on the number of healthy shoots of hemlock dwarf mistletoe.

Materials and methods

Field-trial site

The field-trial site was established in a young stand of *Tsuga heterophylla* heavily infested with *A. tsugense* near Parksville, Vancouver Island, British Columbia, Canada (latitude, 49°21′00″N; longitude, 124°38′00″W). The field site was distributed across approximately 2 ha in the eastern very dry Maritime variant of the coastal western hemlock zone (CWHxm1) (Meidinger and Pojar 1991). *Tsuga heterophylla* was the primary tree present on the site and ranged in age from 5 to 40 years. Other common tree species included *Arbutus menziesii* Pursh., white pine (*Pinus strobus* L.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), and occasionally, cedar (*Thuja plicata* Donn). Symptoms and signs of *N. neomacrospora* and *Colletotrichum*

gloeosporioides were present on the *A. tsugense* site, as well as on all sites inspected that had a sufficient level of infection on young trees to allow this study. Because the site of this trial was in a dry variant of the coastal western hemlock zone, results may not be applicable to wetter subzones. However, where *Tsuga heterophylla* occurs in the very dry variant and if hemlock dwarf mistletoe is present, infection of *Tsuga heterophylla* is almost always severe. Several sites on southern Vancouver Island were assessed, and there appeared to be a strong correlation between high levels of *Tsuga heterophylla* infection by *A. tsugense* and the presence of *N. neomacrospora*. It was necessary to choose a site with high infection by *A. tsugense* to have a sufficient number of replicates.

Isolate selection and inoculum production

Prior to commencing the field trial, the growth characteristics of six isolates of *N. neomacrospora* were measured. As no *A. tsugense* material was available for inoculation under controlled conditions, the lead isolate of *N. neomacrospora* was selected based on growth, sporulation in culture, and conidial germination over temperatures ranging from 5 to 30 °C. Isolate PFC 2559 was determined to be the most "vigorous" based on these characteristics and was utilized in the field trial. Mass conidia were produced by placing 5–7 mycelial plugs into flasks containing 50 g each of sterile brown rice and 15–30 mL of sterile distilled water. The suspensions were shaken two times daily by hand for 1–2 min and grown at room temperature for 12 days, at which time 100 mL of sterile distilled water was added to the flasks. The flasks were then placed on a rotary shaker at 225 r/min (1 r = 2π rad) for 30 min, and the contents were filtered through sterile cheesecloth into multiple 50 mL centrifuge tubes, followed by centrifugation at 2500 r/min (1083*g*) for 10 min. The supernatant was decanted, and conidia were resuspended in 5 mL of sterile distilled water. The spore concentration of the resulting solution was determined on a haemocytometer and adjusted with sterile distilled water to a concentration of 1.0×10^7 conidia/mL.

Spores were formulated into Stabileze (Quimbey et al. 1999), using a modified method to facilitate storage and transport of the spores to the field site. Five grams of Waterlock B-204 (Grain Processing Corp., Muscatine, Ia.) was mixed with 5 mg of corn (Spectrum Naturals, Inc., Petaluma, Calif.) under sterile conditions. The mixture was then heated on high setting in the microwave oven for 1 min and allowed to cool to room temperature. Once cooled, 20 mL of conidial suspension was slowly blended into the mixture, followed by 7.0 g of Hi-Sil 233 (PPG Industries Inc., Pittsburgh, Penn.) hydrated silica. The mixture was then spread onto foil-covered pans in the flow hood and allowed to air-dry for 2 days. For uninoculated treatments, a sterile Stabileze solution was prepared with sterile distilled water in place of the conidial suspension. Once dry, both solutions were stored in a sealed container at 4 °C.

On the day of the treatment application, 5.0 g of the dried formulation was added to 500 mL of sterile distilled water. The solution was alternatively hand shaken and stirred on a rotary shaker for 30 min or until the formulation was mixed thoroughly. A total of three batches of 500 mL of Stabileze solution with conidial suspension and three batches of 500 mL of the sterile Stabileze solution were prepared. The final concentration of spores was approximately 54 000 conidia/mL. For each treatment, the Stabileze solution was hand sprayed onto the *A. tsugense* shoots and swellings till run-off.

Experimental design

Selection of *A. tsugense* experimental units was based on the following criteria: (1) the *A. tsugense* shoots and swellings were disease free, (2) the *Tsuga heterophylla* host branch was healthy, and (3) the hemlock dwarf mistletoe was accessible and easy to monitor. Both male and female *A. tsugense* plants of various sizes were used in this trial.

A total of 150 infections by *A. tsugense* were selected, tagged, and randomly assigned to one of four treatments or a control, regardless of the *A. tsugense* plant sex, size, or location. Treatments consisted in spraying conidia formulated in Stabileze on unwounded (W–I+) or wounded (W+I+) *A. tsugense* swellings, or spraying sterile Stabileze on unwounded (W–I–) or wounded (W+I–) *A. tsugense* swellings. Wounding of *A. tsugense* involved cutting transversely through the bark of each mistletoe swelling, every 2–3 cm along the entire length of the swelling, using a sterile razor blade (4–10 points depending on swelling size). Wounding preceded inoculum application. A control with unwounded, uninoculated swellings and no Stabileze solution (W–I–S–), served to determine the effect of Stabileze only. There were 30 replicates of treatments W–I+, W+I+, W–I–, and W+I– and 27 replicates of the W–I–S– control. The experimental design was a factorial design with two factors: wounding and inoculation.

Inoculation occurred on 29 August 2002 between 5:00 PM and 8:00 PM (Pacific standard time). The day of the treatment, the temperature ranged from 19 to 24 °C during the day, with sunny and warm weather, and reached 7 °C at night. Relative humidity ranged from 53% to 80% during the day, with the minimum at 3:35 PM. The following week had moderate temperatures, ranging from 7 to 23 °C, and relative humidity ranging from 40% to 100%.

Measurements

The number of healthy and diseased *A. tsugense* shoots was recorded before treatment and then at 2 weeks and 1, 2, 3.5, 5, 6, and 9 months after treatment. At 10 months, two thirds of the experimental units were destructively sampled. The extent of bark necrosis and the occurrence of sporodochia were measured, and isolation of *N. neomacrospora* from the *A. tsugense* swellings was attempted. The extent of bark necrosis for each *A. tsugense* swelling was expressed as the percentage of the circumference killed at the point of greatest necrosis; this can be considered a measurement of how close the swelling is to being girdled. Sporodochia (presence or absence) were recorded on a monthly basis and confirmed at 10 months by spore morphology. Isolation involved cutting 10 small sections from the bark and wood and surface sterilizing them for 2 min in each of 10% bleach (containing 10% sodium hypochlorite), 95% ethanol, and three rinses of sterile distilled water. Preference was given to necrotic bark margins and wood beneath necrotic bark regions. The sterile sections were then plated onto potato dextrose agar, allowed to grow at room temperature, and monitored weekly for 3 weeks.

The extent of bark necrosis, occurrence of sporodochia, and isolation of *N. neomacrospora* from destructively sampled *A. tsugense* swellings were used to determine the requirement of wounding for successful infection by the fungus. The occurrence of sporodochia and isolation of *N. neomacrospora* from *A. tsugense* swellings are useful in that they confirm the successful infection, but failure to detect sporodochia or to isolate *N. neomacrospora* does not necessarily mean that infection has not occurred. It is advantageous to use bark necrosis to quantify infection by *N. neomacrospora* because it is easy to measure and is consistently correlated with infection by the fungus (Funk et al. 1973; Smith and Funk 1980).

Data analysis

Data were analyzed using SimgaStat version 2.03 (SPSS Inc. 1997) with $\alpha = 0.05$. The W–I–S– control provided only observational data and was not integrated in the statistical analysis. The extent of bark necrosis, the occurrence of sporodochia, and the isolation of *N. neomacrospora* were analyzed for each treatment to determine the necessity of wounding to achieve successful infection by the fungus. Data measuring the extent of bark necrosis did not meet the assumptions of the analysis of variance (ANOVA), and arcsine – square root transformation of x did not remedy this problem. Analyses of the extent of bark necrosis versus treatments (Fig. 1) and versus isolation of *N. neomacrospora* were therefore performed with one-way and two-way ANOVAs on ranks, respectively. Differences among treatments were determined by the Tukey test. The occurrence of sporodochia at 9 months and the frequency of isolation of *N. neomacrospora* from the *A. tsugense* swellings at 10 months were analyzed using χ^2. Differences among treatments were determined by the Fisher exact test (Mendenhall 1987).

Results and discussion

Because of concern for secondary infection of the experimental units from naturally occurring inoculum and background inoculum, the field trial was terminated 10 months after treatment. Sporodochia were common at the field site and occurred on experimental units and other *A. tsugense* swellings that were not included in the trial. Perithecia were also observed on *A. tsugense* swellings that were not used in the trial. Early termination of the field trial prevented the study of long-term effects of infection by *N. neomacrospora* on *A. tsugense*.

Comparison of *A. tsugense* swellings on which sporodochia were observed with those from which *N. neomacrospora* was successfully isolated confirmed that neither of these measurements were perfect indicators of infection. Sporodochia were observed on only 21% of the *A. tsugense* swellings from which *N. neomacrospora* was isolated, and *N. neomacrospora* was isolated from only 70% of the *A. tsugense* swellings that had sporodochia.

Bark necrosis

Although there was some bark necrosis in almost all *A. tsugense* swellings, regardless of the treatment, the *A. tsugense* swellings from which *N. neomacrospora* was successfully isolated had significantly more bark necrosis ($P < 0.01$) than the other swellings. The extent of bark necrosis for swellings of *A. tsugense* with successful and unsuccessful isolation of *N. neomacrospora* were, respectively, 73.7% and 38.7%, on total numbers of swellings of 44 and 48, respectively. An effort was made to discriminate between "natural" bark necrosis and that caused by the wound treatment. Necrotic regions outside the wound area were observed and were often darker than wounds caused by treatment. Inoculation plus wounding (W+I+) resulted in a significantly greater extent of necrosis and frequency of sporodochia production than all the other treatments. An interaction between wounding and inoculation was also observed ($P = 0.015$). On the other hand, inoculation without wounding (W–I+) did not significantly increase the extent of bark necrosis or the number of sporodochia observed at 10 months compared with the W–I– and W+I– treatments and the control W–I–S– (Figs. 1 and 2).

Isolation of *Neonectria neomacrospora*

The proportion of swellings from which *N. neomacrospora* was isolated did not differ significantly between the W+I+ (73.7%) and the W–I+ (55.0%) treatments (Fig. 3). The difference between the W+I+ and the W+I– treatments was significant ($P = 0.056$) as well as the difference between the W–I+ and the W–I– treatments ($P = 0.028$).

Success of treatments in establishing infection

Based on these findings, the W+I+ treatment had the greatest impact on infection by *N. neomacrospora*. This treatment is currently not feasible on a large scale, and discovery or development of a wounding mechanism (i.e., wood-boring insects that specifically target the dwarf-mistletoe swelling) is necessary before it can be considered as a control mechanism.

Since *N. neomacrospora* produces both conidia and ascospores, there was also some uncertainty at the start of this study whether conidia could function as an effective inoculum. Previous studies (Funk et al. 1973) used fungal mycelium on agar plugs placed on undisturbed basal cups or young shoots. The results from this trial show that conidia are readily able to cause infection.

The long-term impact of infection by *N. neomacrospora* (i.e., 1 year or longer) was not measured in the present field trial because it was terminated early at 10 months. Previous studies suggest that, in some cases, symptoms may take more time to develop. In the trial conducted by Funk et al. (1973), inoculated *A. tsugense* swellings were monitored for almost 2 years after treatment, and visible symptoms commonly took 1 year or more to develop while some *A. tsugense* swellings did not show signs of infection until as late as 21 months. Isolation of *N. neomacrospora* at 10 months was not a perfect measurement of infection; the extent of bark necrosis in different treatments was, however, significantly different.

Although the extent of bark necrosis for the W+I+ treatment was not significantly greater than for the W–I+ treatment, the W+I+ treatment had 38.7% more *N. neomacrospora* isolation success than the W+I– treatment ($P = 0.048$) (Fig. 3).

Fig. 1. Extent of bark necrosis in conifers, as average percentage of the circumference girdled on hemlock dwarf mistletoe (*Arceuthobium tsugense*) swelling at 10 months after treatment: inoculation (I+) or no inoculation (I–) with *Neonectria neomacrospora*, with (W+) or without (W–) wounding. The bars are standard errors of the mean.

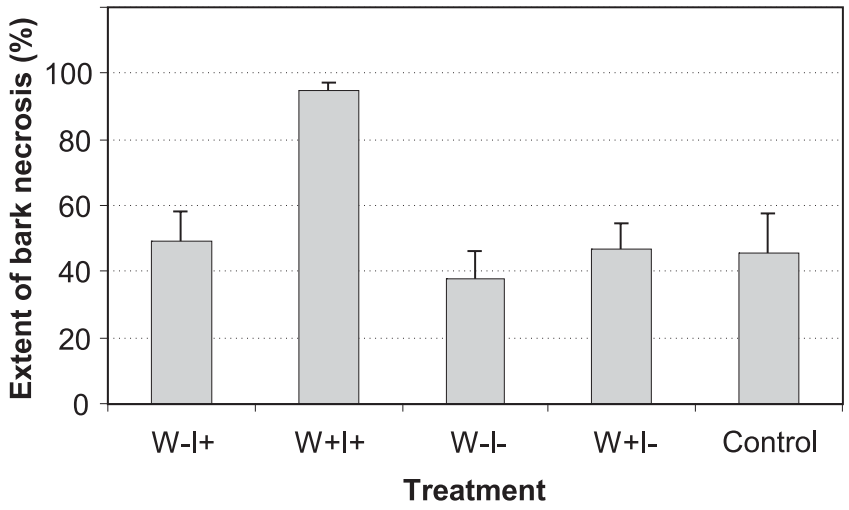
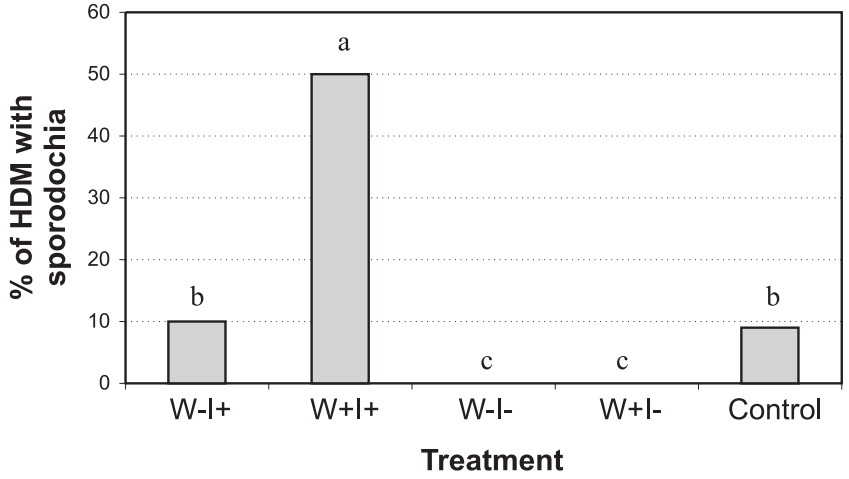


Fig. 2. Percent of *Arceuthobium tsugense* swellings with confirmed sporodochia of *Neonectria neomacrospora* at 9 months after treatment: inoculation (I+) or no inoculation (I–) with *Neonectria neomacrospora*, with (W+) or without (W–) wounding. Letters are used to describe significance between treatments, where treatments with the same letter are not significantly different from one another at $P < 0.05$. HDM, hemlock dwarf mistletoe.



It is possible that development of symptoms and signs of disease (i.e., bark necrosis, sporodochia) in the W–I+ treatment is slower than in the W+I+ treatment. The impact of the W–I+ treatment on *A. tsugense* health may therefore be larger in the long term, when disease has had sufficient time to develop.

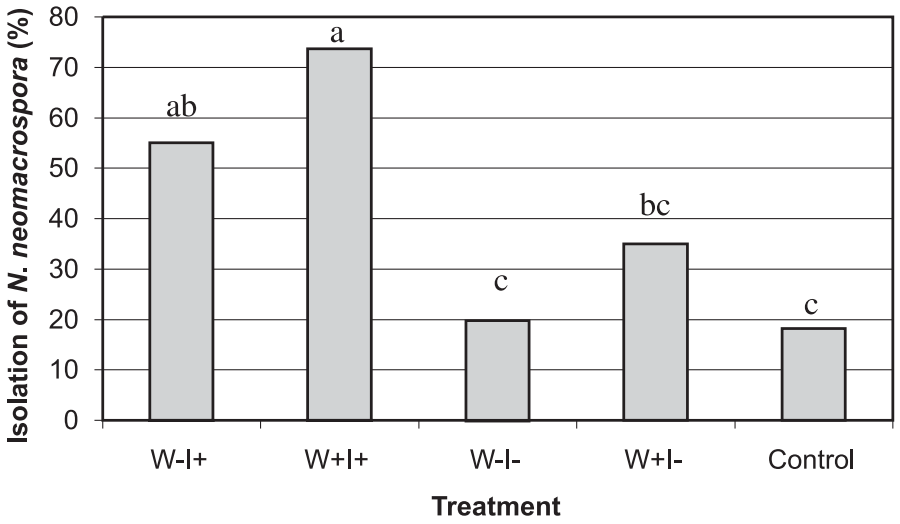
Impact of infection on the number of *Arceuthobium tsugense* shoots

To measure the impact of infection on the number of *A. tsugense* shoots, experimental units were regrouped into two groups (confirmed and unconfirmed infection) regardless of treatment. The confirmed group consisted of *A. tsugense* swellings from which *N. neomacrospora* had been successfully isolated, or which had sporodochia at the end of the trial. The unconfirmed group consisted of all other *A. tsugense* swellings and may have contained swellings infected by *N. neomacrospora* that escaped detection.

The impact of infection by *N. neomacrospora* on *A. tsugense* seed production was estimated by determining the average number of healthy *A. tsugense* shoots per swelling for confirmed- and unconfirmed-infection groups, assuming that seed production by hemlock dwarf mistletoe is roughly proportional to the number of healthy shoots. Since *N. neomacrospora* acts perennially, shoot reduction is expected to continue and negatively impact the ability of *A. tsugense* to produce inoculum in the year(s) following treatment.

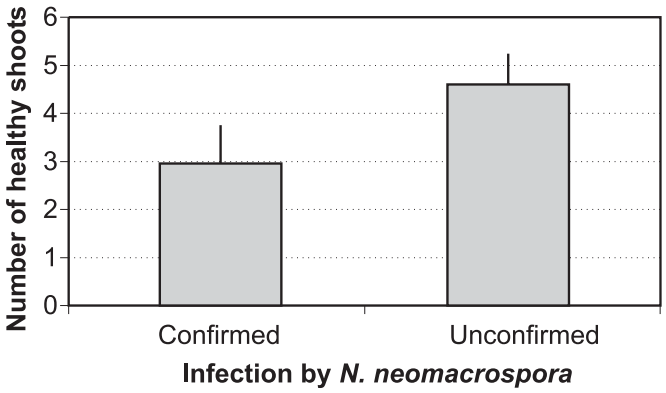
A general trend of decline in the number of *A. tsugense* shoots over the trial period was observed for all experimental units, regardless of treatment. There was no initial difference in the number of shoots for the confirmed group and the unconfirmed group (mean, 16 shoots). One possible ex-

Fig. 3. Percent of *Arceuthobium tsugense* swellings from which *Neonectria neomacrospora* was isolated at 10 months after treatment: inoculation (I+) or no inoculation (I–) with *Neonectria neomacrospora*, with (W+) or without (W–) wounding. Letters are used to describe significance between treatments, where treatments with the same letter are not significantly different from one another at $P < 0.05$.



planation for the decline is the time of year: the trial went through the winter and terminated in early spring. Despite this trend, the confirmed group still lost significantly more *A. tsugense* shoots than the unconfirmed group ($P = 0.025$) (Fig. 4). In addition, more *A. tsugense* swellings from the confirmed group did not have any *A. tsugense* shoots. At 9 months, infection of *A. tsugense* by *N. neomacrospora* reduced the number of healthy shoots by 1.6 shoots, or about 36%. These results were similar to those of Funk et al. (1973) who attributed a 30% reduction in the number of *A. tsugense* shoots to infection by *N. neomacrospora*. As bark necrosis develops in infected *A. tsugense* swellings, the impact of *N. neomacrospora* on the number of healthy *A. tsugense* shoots is expected to increase.

Fig. 4. The mean number of healthy shoots observed for groups of *Arceuthobium tsugense* with confirmed and unconfirmed infection by *Neonectria neomacrospora*. Bars measure standard error of the mean.



Final remarks

In summary, this study suggests that wounding *A. tsugense* swellings facilitates rapid infection by *N. neomacrospora*, resulting, after 9 months, in a greater extent of bark necrosis and sporodochia production than inoculation without prior wounding, and that such bark necrosis is associated with a loss of healthy shoots by hemlock dwarf mistletoe. Unless a wounding mechanism is discovered, applying *N. neomacrospora* inoculum to unwounded *A. tsugense* swellings is the only economically feasible approach to control *A. tsugense*. Although the initial impact of the unwounded, inoculated treatment on infection by *N. neomacrospora* appeared minor, the inoculation of the fungus cannot be dismissed as a potential biocontrol method without further long-term study.

Before a final judgment can be made on the potential of *N. neomacrospora* as a biocontrol agent, it will be necessary to conduct longer studies. Such studies will have to be conducted on sites with rather low levels of *A. tsugense* so that secondary infection from either inoculated or naturally occurring *N. neomacrospora* will not become a confounding factor. In addition, utilizing the molecular markers with polymerase chain reaction (PCR)-DNA technology (Langrell 2002) will allow early detection of infection by *N. neomacrospora*, an important contribution in the study of the host–pathogen interaction.

Acknowledgements

Funding of this research was provided by Forest Renewal British Columbia, Victoria, and Forestry Innovation Investment, Vancouver, British Columbia. The authors would like to thank Sue Askew, Cheryl Konoff, and Holly Williams for their contributions to this research.

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