
Application of Biological Control to Vegetation Management in Forestry

SIMON F. SHAMOUN

Natural Resources Canada
Canadian Forest Service, Pacific Forestry Centre
506 West Burnside Road, Victoria BC V8Z 1M5 Canada

Abstract

It has been well documented that some plant pathogenic fungi can be developed as inundative biological control agents (mycoherbicides) to suppress native competing forest vegetation in conifer regeneration sites. Biological control agents need to be sufficiently virulent to mitigate the aggressiveness of competing vegetation, while allowing crop trees to compete successfully to the free-to-grow stage. To manage hardwood weeds in conifer regeneration sites and utility rights-of-way, an experiment was conducted to test the efficacy of the wound pathogen *Chondrostereum purpureum* (Pers.:Fr.) Pouzar as compared to the herbicide Vision®. Results indicate that *C. purpureum* is as effective as Vision® for control of red alder. In another pathosystem, weedy *Rubus* spp. are being targeted due to their capacity to rapidly invade reforestation and riparian sites, effectively reducing the growth and survival of young planted and naturally regenerating conifer seedlings. A potential candidate, *Fusarium avenaceum* (Fr.) Sacc. was selected and applied inundatively on target weeds under greenhouse conditions. Test plants receiving formulated *F. avenaceum* combined with 0.4% Silwet L-77® induced significant foliar necrosis. These two pathosystems are presented as examples for an applied biocontrol strategy for vegetation management in forestry and will be discussed in detail.

Keywords: mycoherbicides, *Chondrostereum purpureum*, *Fusarium*, competing forest vegetation, hardwood weeds, *Rubus*.

With rising demands for forest products, there is a need for intensification of forest management to raise productivity. Interference by non-commercial or competing vegetation (forest weeds) will continue to be a serious problem in management of young conifer plantations. Control of competing vegetation can take many forms, including removal by mechanical or manual brushing and chemical herbicides (Wall *et al.* 1992). These methods have distinct disadvantages such as environmental concern, non-target effects, and cost-effectiveness. This has necessitated a more intensive search for alternative management strategies for invasive forest weeds that are cost effective, efficacious, environmentally safe and sustainable (Jobidon 1991, Watson and Wall 1995).

The idea of using plant pathogens for management of weeds is not new. However, the concept of the development of a bioherbicide technology as a specialized field of study is very young. The use of a biological control strategy in natural ecosystems entails the enhancement of naturally occurring plant pathogens, thus quickening the decline of competing vegetation through the manipulation of these pathogens. The expected result should be an increase in early conifer growth rate and a shorter rotation age of commercially

valuable crop trees (Wall and Hasan 1996).

As in other ecosystems, three major biological control strategies are being used with respect to management of competing forest vegetation: classical, inundative (bioherbicide or mycoherbicide), and augmentative (silvicultural manipulation). The classical biocontrol strategy has been used to control exotic forest weeds. The inundative strategy using indigenous plant pathogens is one of the more promising approaches for management of native forest weeds. The augmentative strategy of forest stands has been promoted by sound research based on knowledge of the biology of plant pathogens, autecology of the target weeds and the ecology of forest ecosystems (Wall 1984, Wall *et al.* 1992). Markin and Gardner (1993) and Wall and Hasan (1996) and have reviewed in detail numerous examples of forest weed biocontrol initiatives worldwide.

A focussed program on development of biological control agents for forest weeds was established at the Canadian Forestry Service-Pacific Forestry Centre (CFS-PFC) in 1986 (Dorworth 1990). Among the target weeds considered were *Acer macrophyllum* Pursh, *Alnus rubra* Bong., *Calamagrostis canadensis* (Michx.) Beauv., *Epilobium angustifolium* L., *Gaultheria shallon* Pursh, *Populus tremuloides* Michx., and *Rubus* spp., including wild red raspberry [*Rubus strigosus* Michx. = *R. idaeus* var. *strigosus* (Michx.) Focke], thimbleberry (*R. parviflorus* Nutt.), and salmonberry (*R. spectabilis* Pursh). Plant pathogens from these weeds have been isolated, identified and tested for their potential use as biological control agents (Dorworth 1990, Wall and Shamoun 1990, Oleskevich *et al.* 1998). To date, researchers at CFS-PFC have successfully secured four U.S. patents on different biological control pathosystems, including biological control of *C. canadensis* with *Colletotrichum* sp. and *Fusarium* sp. (Winder 1995), control of *A. rubra* with *Nectria ditissima* Tul. (Dorworth 1994), biological control of weed trees with *C. purpureum* (Wall *et al.* 1996) and biological control of weedy *Rubus* spp. with *Fusarium avenaceum* (Shamoun and Oleskevich 1999).

The use of *Chondrostereum purpureum* has been tested on hardwood weed species and efforts are directed towards commercialization of the bioherbicide "ECOclear™", according to an agreement between CFS-PFC and MycoLogic Inc., University of Victoria, British Columbia (BC), Canada. Recently, a promising biological control agent *Fusarium avenaceum*, for weedy *Rubus* spp. and *C. canadensis* has been tested, and further research is underway for its development as a bioherbicide (Oleskevich *et al.*, 1998, Winder 1999). A combined action of *F. avenaceum* and rhizobacteria has been proposed as a potential biocontrol strategy for *C. canadensis* (Winder and Macey 1998). Research efforts are underway to elucidate the interaction of various endophytic fungi with *Alnus* spp. and *Rubus* spp., and to assess their potential use as mycoherbicides (Sieber *et al.* 1991, Shamoun and Sieber 1993, Shamoun and Sieber 2000).

The objective of this article is to illustrate two case studies: 1) *C. purpureum* as a potential biological control for *A. rubra* in conifer regeneration and utility rights-of-way sites, and 2) *F. avenaceum* as a potential control agent for invasive *Rubus* spp. in conifer regeneration and riparian sites.

Case study I: *Chondrostereum purpureum* - *Alnus rubra* pathosystem.

Traditionally, periodic manual brushing of competing tree species or spraying with chemical herbicides in conifer regeneration sites and within rights-of-way (ROW) has been used. These approaches have serious disadvantages, including high labour requirements, resprouting from cut stumps, and concerns over soil and water contamination.

Recently, biological control of invasive hardwood trees using the fungus *C. purpureum* to suppress regrowth has been suggested (Scheepens and Hoogerbrugge 1989, Wall 1994, Shamoun *et al.* 1996, Dumas *et al.* 1997). This strategy could considerably increase intervals between repeat cutting operations in conifer regeneration sites and ROW, particularly if sufficient effectiveness is attained and automation of simultaneous brushing and stump treatment operations was developed. A biological control strategy using *C. purpureum* would have a low likelihood of soil or water contamination and minimal risk to non-target plant species (de Jong *et al.* 1996, Ramsfield *et al.* 1996, Shamoun and Wall 1996)

A research program was established to develop and register *C. purpureum* as the first mycoherbicide for use in Canadian forests as an essential component of an integrated forest vegetation management in conifer reforestation sites and utility ROW (Shamoun and Hintz 1998a).

Case study II: *Fusarium avenaceum* - *Rubus* spp. pathosystem.

Biological control strategies that utilize microbial control organisms or their secondary metabolites are receiving greater consideration for use within conifer regeneration sites. A research project focusing on the biological control of *Rubus* spp. using indigenous fungi has been established. Three *Rubus* spp., wild raspberry, thimbleberry and salmonberry are being targeted due to their capacity to rapidly invade reforestation sites, effectively reducing the growth and survival of many conifer species in Canada and the northern United States (Oleskevich *et al.* 1996). The study has thus far assessed fungi associated with *Rubus* stem and leaf diseases and selected a candidate pathogen, *F. avenaceum*. The biological control strategy utilizes inundative levels of fungal inoculum applied as a foliar spray to incite leaf damage and to temporarily suppress *Rubus* growth. Inoculum production methods, amendment of inocula with adjuvants, and co-application with low doses of glyphosate have been investigated to increase fungal pathogenicity (Abbas *et al.* 1995, Oleskevich *et al.* 1998).

Materials and Methods

Case study I: *Chondrostereum purpureum* - *Alnus rubra* pathosystem.

The site for this field experiment was established under a utility ROW near Duncan, BC (48°49'N, 123°50'W) encompassing healthy red alder (*A. rubra*) of 5-10 cm diameter, in September 1994. Within a randomized block design containing 30 plots, six treatments were compared: two fungal formulations (*C. purpureum* isolates PFC 2139, PFC 2140), a control formulation treatment, two chemical treatments (12% Vision® spray and a carbopaste formulation of Vision®), and manual cutting (slash). Alder trees were cut with a brushing chain saw and the appropriate treatment was applied manually to the cut wood surface. *Chondrostereum purpureum* was grown on nutrient base, formulated, and dried in the laboratory, and subsequently resuspended and applied as a paste to cut stumps (Wall *et al.* 1996). During the following two growing seasons, data collected included occurrence of resprouting from stumps, the number of living sprouts per stump, and stump mortality based on the presence and absence of living sprouts. The presence of fruiting bodies of *C. purpureum* and other basidiomycetes on treated stumps was assessed 18 mo post-treatment.

Case study II: *Fusarium avenaceum* - *Rubus* spp. pathosystem.

F. avenaceum was collected and purified from diseased foliage and stems of wild raspberry from central (49° to 54° latitude) and coastal British Columbia, between May to September, 1990-1994. *F. avenaceum* caused foliar damage in pathogenicity tests on detached *Rubus* leaves. The optimum temperature for *F. avenaceum* growth and germination was determined through testing. As well, agar, liquid, and grain media were evaluated for their ability to promote *F. avenaceum* growth and sporulation.

Inundative applications of conidial inoculum were made to *Rubus* plants in shade-house trials. Plants were rated for up to 3 wk after inoculation and compared to control plants, and experiments were repeated. In efforts to enhance pathogenicity, amendments to *F. avenaceum* inoculum included nutrients (sucrose, neopeptone, malt, sodium alginate), humectants (starch, psyllium hydrophilic muciloid), dispersants (Tween 80, wetting agents), stickers/surfactants (Silwet L-77® - Loveland Industries, Greeley, CO, USA), and formulation into an invert emulsion. The strategy of combining *F. avenaceum* with low doses of glyphosate (Round-Up® - Monsanto Canada, Sardis, BC, Canada) to increase host susceptibility was assayed, after determining the effect of the herbicide on fungal growth and germination. The presence of phytotoxins produced by *F. avenaceum* grown in a rice medium was also investigated (Oleskevich *et al.* 1998).

Results and Discussion

Case study I: *Chondrostereum purpureum* - *Alnus rubra* pathosystem.

Resprouting of cut alder stumps occurred throughout the six treatments by spring 1995, reaching a maximum height of 50 cm among resprouts within the slash treatment. Resprout mortality occurred on many stumps by mid-summer, resulting in 65-100% mortality (Table 1). Alder stumps treated with *C. purpureum* and with herbicides showed significantly less living sprouts than other treatments, with a mean of less than 1 living resprout per stump. Analysis of first year data by planned contrasts revealed that *C. purpureum* and herbicide treatments resulted in similar levels of stump mortality and

Table 1.
Mortality and number of living sprouts on cut stumps of red alder treated with *Chondrostereum purpureum* or chemical herbicides.

Treatment	1995		1996	
	Mortality (%)	Living sprouts (no. per stump)	Mortality (%)	Living sprouts (no. per stump)
Slash control	65.00b	4.45a	86.00ab	1.18a
Formulation control	70.00b	3.49ab	72.00 b	0.37b
PFC 2140	83.00ab	0.95bc	96.00a	0.02b
PFC 2139	92.00a	0.45c	100.00a	0.00b
Vision® (spray)	97.00a	0.35c	99.00a	0.01b
Vision® (Carbopaste)	100.00a	0.01c	100.00a	0.00b

Treatments with the same letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test).

resprouting of alder, and were statistically different from the formulation control and slash treatments. Both fungal treatments gave similar results. At 2 yr post-treatment (1996), > 95% stump mortality was recorded on stumps treated with fungal and herbicide treatments, with PFC 2139 and Vision® reaching 100% mortality. In comparison with 1995, all treatment plots had less resprouting and higher stump mortality. Analysis of 1996 data the trend showed a trend similar to 1995, that the overall *C. purpureum* treatments were not significantly different from herbicides but were different from the formulation controls and slash treatment.

Fruiting bodies of *C. purpureum* were observed about 18 mo after *C. purpureum* inoculation of red alder stumps. The peak of *C. purpureum* fruiting bodies was found in spring 1996, on 66% and 84% of the stumps treated with PFC 2139 and PFC 2140 respectively, on about 19% of stumps treated with herbicides, and on 43% of stumps which had received the formulation control and slash treatment (Table 2). Fruiting bodies of *Trametes (Coriolus) versicolor* (L.:Fr.) Pil. and *Schizophyllum commune* Fr. and other

Table 2.
Occurrence (percentage) of fruiting bodies of basidiomycetes on red alder stumps.

Treatment	<i>Chondrostereum purpureum</i>	<i>Schizophyllum commune</i>	<i>Coriolus versicolor</i>	Others
Slash control	42bc	19a	28a	37a
Formulation control	43bc	17a	13ab	21b
PFC 2140	84a	15a	9b	11b
PFC 2139	66ab	13a	18ab	19b
Vision® (spray)	15c	3a	6b	11b
Vision® (Carbopaste)	23c	13a	4b	8b

Treatments with the same letter are not significantly different ($P \leq 0.05$; Duncan's multiple range test).

basidiomycetes were observed on many stumps in all treatment plots.

Results of these tests, and similar large scale field trials conducted in the conifer reforestation sites of BC interior (Harper *et al.* 1998), Ontario (Dumas *et al.* 1997) and in the Netherlands (de Jong *et al.* 1990), indicate that *C. purpureum* is quite effective as a biological control agent of stump sprouting of alder, aspen and American black cherry, respectively.

Case study II: *Fusarium avenaceum* - *Rubus* spp. pathosystem.

Fusarium avenaceum, maximum colony growth and spore germination was observed between 10-30°C and 15-25°C, respectively, A formulation of *F. avenaceum* was developed by growing the fungus on rice grain (Abbas *et al.* 1995), and subsequent inoculum combined with an organosilicone surfactant at a concentration 0.4% Silwet L-77®, enhanced greater foliar damage than other formulations (data not shown). Extensive foliar necrosis occurred with this formulation within 24-48 h on wild *R. strigosus* and *R. parviflorus*, resulting in large areas of necrotic leaf tissue, leaf curl and death. *Rubus strigosus* was the most susceptible to the formulated spray, followed by *R. parviflorus* and *R.*

Table 3.
Foliar necrosis of *Rubus* plants resulting from inundative applications of *Fusarium avenaceum* inoculum, originating from infested rice cultures, and combined with an organosilicone surfactant (Silwet L-77®), means \pm SEM.

Treatment	Foliar injury*		
	<i>Rubus strigosus</i>	<i>Rubus parviflorus</i>	<i>Rubus spectabilis</i>
control-water	0.44 \pm 0.18d	0.20 \pm 0.13c	0.20 \pm 0.13b
surfactant (Silwet L-77®)	1.89 \pm 0.26b	2.17 \pm 0.31b	0.67 \pm 0.21b
<i>F. avenaceum</i>	1.33 \pm 0.17c	1.38 \pm 0.38b	0.75 \pm 0.25b
<i>F. avenaceum</i> + surfactant	3.89 \pm 0.11a	3.31 \pm 0.18a	2.00 \pm 0.26a

*Foliar injury rating index with <2 = slight injury, 2-3.5 = moderate injury, and >3.5 = severe injury. Within a column, values followed by the same letter are not significantly different ($P = 0.05$; Student-Newman-Keuls test).

spectabilis, respectively. Analysis of variance showed significant differences between *F. avenaceum* and Silwet L-77® treatment and all other treatments for *R. strigosus* ($F=61.39$, $P<0.001$), *R. parviflorus* ($F=38.43$, $P<0.001$) and *R. spectabilis* plants ($F=12.39$, $P<0.001$) (Table 3). All treated *Rubus* spp. flushed new leaves by 3 weeks, and the new foliage and stems were free of damage symptoms. A preliminary host- range study showed no effects on major conifer species when sprayed with *F. avenaceum* and Silwet L-77®. . The incorporation of low- doses of glyphosate was not further pursued, as the combined action of *F. avenaceum* with glyphosate did not exceed that of glyphosate alone (data not shown). Phytotoxin extraction and analysis of *F. avenaceum*- infested rice filtrates revealed a single toxin, moniliformin at levels of 3 300 p.p.m. (Oleskevich *et al.* 1998). The enhancement of foliar necrosis by the combined action of *F. avenaceum* and Silwet L-77® may have been achieved by stomatal egress and through the maximum uptake of *Rubus* plants of the phytotoxin, moniliformin (Stevens 1993, Shamoun and Oleskevich 1999). Similar results were demonstrated on *Ascochyta pteridis* Bers. for biocontrol of bracken [*Pteridium aquilinum* (L.) Kuhn.] (Womack and Burge 1983), and most recently, by using *F. avenaceum* for biocontrol of *C. canadensis* (Winder 1999). The biorational strategy for management of weedy *Rubus* spp. and other agricultural weeds is a promising approach (Abbas *et al.* 1991, Jobidon 1991). Ongoing research is underway to screen other phytotoxins associated with *F. avenaceum* isolates collected from *Rubus* spp. by using biochemical and tissue culture techniques (Hollmann *et al.* 1999). Based on the research results by Oleskevich *et al.* (1998) and Shamoun and Oleskevich (1999), ongoing research activities are being focused on using biorational applications of the formulated *F. avenaceum* on invasive weedy *Rubus* spp. in conifer and riparian regeneration sites.

Conclusions and general prospects

Biological control strategy for management of competing vegetation is poised to become an essential component of forest management practices. Plant pathogenic fungi are presently considered the most promising biological control agents. Research and

development on this subject was a result of public pressure and demand for alternative management strategies that are cost-effective, environmentally safe and sustainable (Wagner 1993).

Recent advances in formulation technology, phytopathology, molecular biology and silviculture have accelerated the commercialization and production of three biological control products for management of invasive and competing forest vegetation in South Africa, The Netherlands and Canada, respectively (STUMPOUT®, BioChon®, and ECOclear™) (Morris *et al.* 1998, Ravensberg 1998, Shamoun and Hintz, 1998a, 1998b). Traditionally, classical biological control approach has been used for control of introduced weeds. Development of bioherbicide/mycoherbicide is more promising strategy for management of indigenous forest weeds. The augmentative biological control has special relevance for forestry and therefore could be termed as “silvicultural manipulation” strategy which can be promoted by sound research programs based on ecology of forest ecosystems, biology of plant pathogens and autecology of target weeds. Biological control agents will likely provide alternatives to some chemical herbicides and other unpractical vegetation management tools. The enhancement of the effectiveness and safe use of biological control agents can be achieved by integrating them with manual brushing practices, such as application of ECOclear™ or STUMPOUT® on cut stump of hardwood weeds, or combining foliar pathogens such as *Fusarium avenaceum* with adjuvants/surfactants or low-doses of registered herbicides for foliar applications onto target weeds.

The main concern to both regulatory authorities and to the public in general, in using fungal pathogens for control of forest weeds, is their potential threat to non-target plants. This is especially relevant to classical biological control strategy, where exotic pathogens are introduced into new ecosystems. In contrast, risk analysis of indigenous fungal pathogens used as mycoherbicides (e.g. *C. purpureum*) is extremely low, according to the investigations by de Jong *et al.* (1990). Recently, results based on advanced epidemiological modeling systems and molecular analyses and monitoring (e.g. PCR-DNA technology: RAPD, RFLPs, rDNA and mtDNA) studies, have revealed the safe use of native fungal pathogens (de Jong *et al.* 1996, Becker *et al.* 1999, Gosselin *et al.* 1999, Ramsfield *et al.* 1996 Ramsfield *et al.* 1999). In contrast, most of the plant pathogens that have caused serious losses to forest tree species in the new world were introduced accidentally in forest products and nursery stocks, such as Dutch elm disease, chestnut blight, and white pine blister rust disease (Manion, 1981), and not through using native plant pathogens via planned biological control programs (Cook *et al.* 1996). The potential use of biotechnological techniques to enhance the efficacy of biological control agents is very promising strategy for development of bioherbicides (Watson and Wall 1995). From a practical, sociological, economical and ecological viewpoint, bioherbicide technology should be viewed as an essential component of an integrated forest vegetation management that will be employed in combination with manual brushing, mechanical removal, adjuvants/surfactants, plant growth regulators, and reduced doses of chemical herbicides. Current research on forest weed biocontrol should yield several improvements in forest management, including new commercial products and more widely acceptable approaches to forest management.

References

- Abbas, H.K., C.D. Boyette, R.E. Hoagland, and R.F. Vesonder. 1991. Bioherbicidal potential of *Fusarium moniliforme* and its phytotoxin, fumonisin. *Weed Sci.* 39: 673-677.

-
- Abbas, H.K., C.D. Boyette, and R.E. Hoagland. 1995.** Phytotoxicity of *Fusarium*, other fungal isolates, and of the phytotoxins fumonisin, fusaric acid, and moniliformin to jimsonweed. *Phytoprotection* 76:17-25.
- Becker, E.M., L.A. Ball, and W.E. Hintz. 1999.** PCR-based genetic markers for detection and infection frequency analysis of the biocontrol fungus *Chondrostereum purpureum* on sitka alder and trembling aspen. *Biol. Contr.* 15: 71-80.
- Cook, R.J., W.L. Bruckart, J.R. Coulson, M.S. Goettel, R.A. Humber, R.A. Lumsden, R.D. Maddox, J.V. McManus, M.L. Moose, L. Meyer, P.C. Quimby, J.P. Stack, and J.L. Vaughn. 1996.** Safety of microorganisms intended for pest and plant disease control: a framework for scientific evaluation. *Biol. Contr.* 7: 333-351.
- de Jong, M.D., P.C. Scheepens, and J.C. Zadocks. 1990.** Risk analysis for biological control: A Dutch case study in biocontrol of *Prunus serotina* by the fungus *Chondrostereum purpureum*. *Plant Dis.* 74: 189- 194.
- de Jong, M.D., E. Sela, S.F. Shamoun, and R.E. Wall. 1996.** Natural occurrence of *Chondrostereum purpureum* in relation to its use as a biological control agent in Canadian forests. *Biol. Contr.* 6: 347-352.
- Dorworth, C.E. 1990.** Mycoherbicides for forest weed biocontrol- the PFC enhancement process, pp.: 116-119 In: Bassett *et al.* [eds.], *Alternatives to chemical control of weeds*, Proc. Int. conf. At the Forest Research Inst., Rotorua, New Zealand, FRI Bull. 155.
- Dorworth, C.E. 1994.** Method for controlling red alder using *Nectria ditissima* ATCC 74260. U.S. Patent No. 5,340,578.
- Dumas, M.T., J.E. Wood, E.G. Mitchell, and N.W. Boyonoski. 1997.** Control of stump sprouting of *Populus tremuloides* and *P. grankikentata* by inoculation with *Chondrostereum purpureum*. *Biol. Contr.* 10: 37-41.
- Gosselin, L., R. Jobidon, and L. Bernier. 1999.** Genetic variability and structure of Canadian populations of *Chondrostereum purpureum*, a potential biophytocide. *Molec. Ecology* 8: 113-122.
- Harper, G.J., P.G. Comeau, W.E. Hintz, R.E. Wall, R. Prasad, and E. M.Becker. 1998.** Second-season efficacy results of *Chondrostereum purpureum* applications on aspen and sitka alder in British Columbia. Pp.:121-123, In: Wagner, R.G. and D.G. Thompson (Compilers). *Third Int. conf. On Forest Vegetation Management: Popular summaries*, Ont. Min. of Nat. Resour., Ont. For. Res. Inst., For. Res. Info. Paper No. 141.
- Hollmann, P.J., S.F. Shamoun, and S.P. Lee. 1999.** Establishment and characterization of weedy *Rubus* tissue cultures for *in vitro* bioassays of *Fusarium avenaceum* phytotoxins. *Phytopathology* 89 (6): S 34 (Abstract).
- Jobidon, R. 1991.** Some future directions for biologically based vegetation control in forestry research. *For. Chronicle* 67: 514-519.
- Manion, P.D. 1981.** *Tree Disease concepts*. Prentice- Hall, Inc., Englewood Cliffs, New Jersey, 389 pp.
- Markin, G.P., and D.E. Gardner. 1993.** Status of biological control in vegetation management in forestry. *Can. J. For. Res.* 23: 2023-2031.
- Morris, M.J., A. R. Wood, and A. Den Breeyen. 1998.** Development and registration of a fungal inoculant to prevent re-growth of cut wattle tree stumps in South Africa, and a brief overview of other bioherbicide projects currently in progress. P. 15, *In IV International Bioherbicides Workshop- Programme and Abstracts*. August 06-07, 1998, University of Strathclyde, Glasgow, Scotland. (Abstract)
- Oleskevich, C., S.F. Shamoun, and Z.K. Punja. 1996.** The biology of Canadian weeds. No. 105. *Rubus strigosus* Michx., *R. parviflorus* Nutt., *R. spectabilis* Pursh. *Can. J. Plant Sci.* 76:187-201.
- Oleskevich, C., S.F. Shamoun, R.F. Vesonder, and Z.K. Punja. 1998.** Evaluation of *Fusarium avenaceum* and other fungi for potential as biological control agents of invasive *Rubus* species in British Columbia. *Can. J. Plant Pathol.* 20:12-18.

- Ramsfield, T.D., E.M. Becker, S.M. Rathlef, Y. Tang, T.C. Vrain, S.F. Shamoun, and W. E. Hintz. 1996. Geographic variation of *Chondrostereum purpureum* detected by polymorphisms in the ribosomal DNA. *Can. J. Bot.* 74:1919-1929.
- Ramsfield, T.D., S.F. Shamoun, Z.K. Punja, and W.E. Hintz. 1999. Variation in the mitochondrial DNA of the potential biological control agent *Chondrostereum purpureum*. *Can. J. Bot.* Ramsfield *et al.* 1999, 77(10): 1490-1498.
- Ravensberg, W.J. 1998. BioChon® effective biological and environmentally friendly product. Koppert Biological Systems (The Netherlands) Pest leaflet, 2 pp.
- Scheepens, P.C., and A. Hoogerbrugge. 1989. Control of *Prunus serotina* in forests with the endemic fungus *Chondrostereum purpureum*, pp. 545-551. In E.S. Delfosse [ed.], Proceedings, 8th International Symposium on Biological Control of Weeds, 6-11 March, 1988, Rome Italy.
- Shamoun, S.F., and T.N. Sieber. 1993. Isozyme and protein patterns of endophytic and disease syndrome associated isolates of *Melanconium apiocarpum* and *M. marginale*. *Mycotaxon* 49: 151-166.
- Shamoun, S.F., and R.E. Wall. 1996. Characterization of Canadian isolates of *Chondrostereum purpureum* by protein content, API ZYM and isozyme analyses. *Europ. J. For. Pathol.* 26:333-342.
- Shamoun, S.F., T.D. Ramsfield, G. Shrimpton, and W.E. Hintz. 1996. Development of *Chondrostereum purpureum* as a mycoherbicide for red alder (*Alnus rubra*) in utility rights-of-way. Page 19, In Comeau, P. and G. Harper [eds.], Proceedings, Expert Committee on Weeds National Meeting, December 09-12, 1996., BC Min. of Forests, Res. Branch, Victoria, BC.
- Shamoun, S.F., and W.E. Hintz. 1998a. Development and registration of *Chondrostereum purpureum* as a mycoherbicide for hardwood weeds in conifer reforestation sites and utility rights-of-way. Page 14, In IV Int. Bioherbicide workshop programme and Abstracts. August 06-07, 1998, University of Strathclyde, Glasgow, Scotland.
- Shamoun, S.F., and W.E. Hintz. 1998b. Development of *Chondrostereum purpureum* as a biological control agent for red alder in utility rights-of-way. Pages: 308-310, In Wagner, R.G. and D.G. Thompson (Compilers). Third Int. Conf. on Forest Vegetation Management. Popular summaries, Ont. Min. Nat. Resour., Ont. For. Res. Inst., For. Res. Info. Paper No. 141.
- Shamoun, S.F., and C. Oleskevich. 1999. *Fusarium avenaceum* and its use as biological control agent for *Rubus* species. U.S. Patent No. 5,598,648.
- Shamoun, S.F., and T.N. Sieber. 2000. Colonization of leaves and twigs of *Rubus parviflorus* and *Rubus spectabilis* by endophytic fungi in a reforestation site in British Columbia. *Mycol. Res.* (In press).
- Sieber, T.N., F. Siber-Canavesi, and C.E. Dorworth. 1991. Endophytic fungi of red alder (*Alnus rubra*) leaves and twigs in British Columbia. *Can. J. Bot.* 69: 407-411.
- Stevens, P.J.G. 1993. Organosilicone surfactants as adjuvants for agrochemicals. *Pestic. Sci.* 38:103- 122.
- Wagner, R.G. 1993. Research directions to advance forest vegetation management in North America. *Can. J. For. Res.* 23: 2317-2327.
- Wall, R.E. 1984. The role of disease in removal of weed species from developing forest stands. PP. 673-676. In Delfosse, E. [ed.]. Proceedings VI Int. Sympos. Biol. Contr. of Weeds, 19-25 August, Vancouver, BC, Canada.
- Wall, R.E., and S.F. Shamoun. 1990. Experiments on vegetation control with native pathogenic fungi in the southern interior of British Columbia. *Can. For. Serv. and BC Min. of Forests, Forest Resources Development Agreement Rep.* 134, Victoria, BC, 18 pp.
- Wall, R.E. 1990. Biological control of red alder using stem treatments with the fungus *Chondrostereum purpureum*. *Can. J. For. Res.* 24:1527-1530.
- Wall, R.E., R. Prasad, and S.F. Shamoun. 1992. The development and potential role of

mycoherbicides for forestry. For. Chronicle 68:736- 741.

- Wall, R.E., and S. Hasan. 1996.** Management of plant pathogens for vegetation management control in forestry. PP. 1-19, *In* Raychaudhuri, S.P. and K. Maramorosch [eds.]. Forest Trees and Palms –Diseases and control., Oxford and IBH Publishing Co., PVT, Ltd. New Delhi, India.
- Wall, R.E., R. Prasad, and E. Sela. 1996.** Biological control for weed trees. U.S. Patent No. 5,587,158.
- Watson, A.K., and R.E. Wall. 1995.** Mycoherbicides: their role in vegetation management in Canadian forests. *In* Recent progress in forest biotechnology in Canada, pp. 74-82. P.J. Charest and L.C. Duchesne [eds.], Canadian Forest Service Information Report PI-X-120, Victoria, British Columbia.
- Winder, R.S. 1995.** Mycoherbicide and method for controlling *Calamagrostis canadensis*. U.S. Patent No. 5,472,690.
- Winder, R.S., and D.E. Macey. 1998.** Biological control of grasses in reforestation areas: problems and prospects.. Pages 360-362. *In* Wagner, R.G. and D.G. Thompson (Compilers). Third Int. Conf. on For. Vegetation Management: Popular summaries, Ont. Min. Nat. Resour., Ont. For. Res. Inst., For. Res. Info. Paper no. 141.
- Winder, R.S. 1999.** Evaluation of *Colletotrichum* sp. and *Fusarium* spp. as potential biological control agents for marsh reed grass (*Calamagrostis canadensis*). Can. J. Plant Pathol. 21:8-15.
- Womack, J.G., and M.N. Burge. 1993.** Mycoherbicide formulation and the potential for bracken control. Pestic. Sci. 37:337- 341.