Biocontrol of forest weeds

proceedings of a workshop held at the Western International Forest Disease Work Conference in Vernon, British Columbia, August 9, 1991

Edited by
Charles Dorworth and S.G. Glover

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Forestry Canada
Pacific Forestry Centre

1992

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ISBN 0-662-19640-6
Cat. No. Fo 18-18-1991E
Printed in Canada

Canadian Cataloguing in Publication Data
Main entry under title:
Biocontrol of forest weeds: proceedings of a workshop held at the Western International
Forest Disease Work Conference in Vernon, British Columbia, August 9, 1991

ISBN 0-662-19640-6
DSS cat. no. Fo18-18/1991E

1. Weeds–British Columbia–Biological control–Congresses.
2. Weeds–British Columbia– Control–Congresses.
I. Dorworth, C. E. II. Glover, S. G. (Stephen Gerard), 1954-
III. Pacific Forestry Centre. IV. Title: Proceedings of a workshop held at the Western
International Forest Disease Work Conference in Vernon, British Columbia, August 9, 1991

SB764.C3B56 1992 634.9'658'0971 C92-099701-5

Contents

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Foreword

Biological control, though seemingly a new innovation, has been practiced for decades in various forms under the umbrella title of "good plant husbandry." Quite often, particular agricultural practices which were conducive to crop improvement proved to be effective through modification of the soil microflora. Such successful efforts are manifested in increased crop yield and quality, usually through attenuation of the extant population of plant pathogens. In general, two primary biocontrol mechanisms were activated in the course of good plant husbandry practice:

> Case I - Certain practices led to the establishment of ecological microsites unsuitable to the growth or propagation of plant pathogens. Such alteration in microsite was effected through changes in soil nutritional base, aeration, pH, or available water.
>
> Case II - In the second instance, perhaps closer to the heart of the microbiological ecologist, good plant husbandry practices favoured the development of microorganisms that were antagonistic toward primary plant pathogens.

With respect to Case II, Curl and Truelove (The Rhizosphere, Springer Verlag, N.Y., 1986) described an earlier practice wherein wheat stubble was left to become colonized by moulds (notably Actinomycetes, and *Trichoderma* spp. and allied genera) which proved antagonistic toward plant pathogens once they were ploughed into the soil prior to planting. Earlier bits of historical notation can be coupled together to yield still another story: 'Sloppy farmers' — those who did not clean and burn their crop residues in autumn — occasionally produced better crops than farmers nearby who were more tidy. This effect was a direct consequence of the stimulation of antagonistic soil fungi and bacteria that were antagonistic to plant pathogens by the incorporation of cellulose crop waste into the soil. The picture was never quite clear, however, as some crop waste provided a nutrient substratum wherein particular plant pathogens could overwinter and propagate. Further, in certain soils, excessive crop waste exacerbated soil deficiencies of some nutrients, particularly nitrogen.

Many of these practices are now part of contemporary farming practice, though perhaps with the basis for chemical and biological activity better understood.

All of the above practices concern the attenuation of virulence of plant pathogens. "The cursed weed" was another problem altogether. Generally, though not always, both agriculture and forestry involve attempts to establish one or more favorable species per unit area as near-monocultures in the presence of highly successfully alternative species termed weeds. Before the introduction of chemical herbicides, which now represent greater than 50% of the annual North American pesticide market, weeds were dealt with largely through cultivation. Although cultivation is usually cost-effective with high-value row crops that can be machine-worked, it becomes increasingly less feasible with non-row crops and those with minimum post-harvest value per unit area. Whether or not additional methods of agricultural and forest vegetation management, coupled to good plant husbandry, might have been found is a moot point. Regardless, the development of chemical herbicides provided an excellent means of weed control and one that could be applied more or less successfully by persons with a minimum of formal training in pesticide application. The search for alternative methods of vegetation management was aborted and land managers in general began to depend almost entirely on chemical herbicides for weed control.

Consequently, chemical herbicides provided an extraordinary opportunity to gain maximum yield in agriculture from field crops such as corn, where a pre-plant and post-plant (at most) application of chemical herbicides could generate a nearly weed-free crop.

Forest crops are notable examples of crops with a low value per unit area, because harvest value is usually amortized over growth intervals approaching or exceeding one century, even in modern forest culture. In modern forest practice, especially on the west coast of North America, a post-harvest burn of logging waste is sufficient to destroy weeds, weed seeds, and often roots and rhizomes of weeds when sufficient fuel of adequate moisture content is present at the time of burning. Post-planting application of a chemical herbicide is often sufficient to guarantee the establishment of the desired forest crop, even on the west coast of North America where species such as red alder *(Alnus rubra)* and salal *(Gaultheria shallon)* are highly effective competitors of forest trees in forest renewal areas. Consequently, controlled burning and chemical herbicides are primary silvicultural tools in the forest manager's bank of technology. Once a conifer plantation reaches the stage of crown-closure it becomes its own best weed control agent.

We in forestry are fully aware that these techniques do not have the full approval of the public and that this attitude is becoming increasingly prevalent. Such lack of confidence is frequently translated into political and statutory prohibitions against the use of even the most useful and environmentally benign silvicultural techniques. In B.C., our primary vegetation management chemical is glyphosate, marketed as Vision®. Although this chemical seems entirely compatible with good forest environmental practice, it is coming under increasing attack by concerned citizens groups merely because it fall into the category of "a chemical".

The recent article by C.A. Lévesque and J.E. Rahe (Ann. Rev. Phytopathology, in press) reviews the argument to the effect that glyphosate is effectively a natural herbicide, by virtue of its mode of action in plants. Rather than simply killing weeds, which we can accomplish with a number of lethal chemicals, glyphosate may render weeds more susceptible to colonization by native plant pathogenic fungi which are then the effective agents of weed death. Unfortunately, this information will probably reach relatively few of the people who make decisions on pesticide registration.

Finally, as forest workers and forest scientists, we must deal with the problems that limit our ability to meet the urgent requirement for successful forest renewal. Chemical herbicides, no matter how nearly environmentally benign they might be, are only one element in the forester's bag of silvicultural tools. Further, nature is too diverse to permit us to place such great dependence upon a single tool to the extent that we now depend upon glyphosate in forest vegetation management. The discussions which follow will explain alternative means of forest vegetation management now in the planning or operational stages whereby the forest can be renewed after inadvervant or advertant loss. These presentations will be made by leaders in each of the specialties represented and represent many years of training, research and development by the people who have volunteered to present their work and philosophy. The aim of all contributors herein is the solution of the problem of forest vegetation management to the benefit of all involved and the perpetuation of the forest resource.

We merely ask that you, the reader, examine these transcripts with an open mind, value them accordingly and criticize them positively and negatively to our mutual advantage.

With our best regards,

Charles Dorworth
Organizer

The Pacific Forestry Centre Mycoherbicide Program: principles and practice

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Abstract

The Pacific Forestry Centre initiated research in biological control of forest weeds in order to provide environmentally benign alternatives to chemical herbicides and controlled burns, especially in sensitive areas. Present efforts are divided between first-order and second order studies. First-order studies are directed toward cultivating living agents and isolating metabolic fractions of these agents that might be applied to target weeds; Second order studies are directed toward stimulating the resident endophytic microflora to serve as an innate biocontrol set. Biological controls must employ native microorganisms, be reasonably target-specific, be under operator control, and be cost-effective and competitive.

Introduction

The Pacific Forestry Centre in Victoria, British Columbia (B.C.) initiated research and development in microbiological control of forest weeds in 1986. Although such research has proceeded for more than two decades in agriculture, this was one of the first attempts to extend this type of work to forestry. Since that time, the program has expanded from one to five permanent research staff, one visiting scientist, and one research technician.

The requirement that we in B.C. develop an alternative to our primary silvicultural tools of chemical control and controlled burning is becoming urgent. More than 90% of the chemical control in B.C. is accomplished with the use of a single chemical (Vision®) and controlled burning is employed routinely in many cutover blocks. Although foresters believe that neither of these vegetation management tools is damaging in such a way as to be considered ecologically unacceptable, the climate of public reaction makes their continued use uncertain, especially in more settled areas.

Biological control is not expected to be a final answer to forest vegetation management. Living organisms such as plants are too variable to be treated uniformly with a single tool or strategy. Rather, biocontrol must be included as an essential element in overall integrated forest management systems. This will not only provide an alternative or complement to present vegetation management strategies, it will also take the pressure off those strategies by removing them somewhat from public scrutiny, especially in sensitive situations. A vegetation management option that is essential for forest renewal over thousands of hectares of northern forest could be lost because of public reaction in settled areas, where it may be used on relatively small blocks. We, as foresters, must protect our most useful tools or we will not have the use of them in the future.

Biological controls fall generally within one of two major categories: second-order biocontrol and first-order biocontrol.

Second-order biocontrol

This strategy is defined as Manipulation of environmental conditions, the targeted hosts, the indigenous microflora, or all of these in order to induce pathogenicity or stimulate virulence of the native microflora and, thereby, to yield biocontrol.

Second-order biocontrol was practiced for decades in agriculture for purposes of crop improvement under the general headings of plant and land husbandry, long before the biological basis for its success was fully understood. Techniques employed for control of (in this case) disease-causing microorganisms included crop rotation, fallowing, soil incorporation of plant residues, and area flooding. Each of these practices either placed indigenous plant pathogens at a disadvantage or encouraged the development of microorganisms which were antagonistic to plant pathogens.

For purposes of second-order biocontrol of forest weeds, an approach that is nearly opposite in effect must be developed. In this case, it is desirable to stimulate elements of the resident microflora to an extent that they become effective biocontrol agents.

At the Pacific Forestry Centre we are now working with the native endophytic fungi of *Alnus rubra* Bong. and attempting to enhance their activity to the point they become actively pathogenic toward the targeted host. This has been a cooperative research effort involving the Pacific Forestry Centre, the Swiss Federal Research Institute in Zürich, Switzerland, and the University of Firenze (Florence) Italy.

First-order biocontrol

This strategy is defined as direct application of living agents or their metabolites which reduce target pest populations either in number, or in vigour, or both.

First-order biocontrol agents may be applied with or without the addition of enhancers such as adjuvants that stimulate or facilitate the activity of the biocontrol agent. A variety of herbivorous and parasitic insects and mites, as well as herbivorous mammals, microorganisms, and even fish (for control of aquatic weeds) have been employed in unidirectional or integrated pest management efforts. In forestry, the bacterium *Bacillus thuringiensis* Berliner has been used with notable success, particularly against the spruce budworm. Similar though less widespread success was realized with the larch sawfly virus.

Using first-order Biocontrol, we at the Pacific Forestry Centre are attempting to control *Alnus rubra, Calamagrostis canadensis* (Mich.) Beauv., *Gaultheria shallon* Pursh, *Populus tremuloides* Michx. and *Rubus* spp. with fungi and with bacteria. In this category of biocontrol, we are using only native pathogens and attempting to enhance their efficacy by means of various strategies that stimulate their activity or, conversely, that diminish the physiological efficiency of the target host.

A final important last step in this type of work involves testing of the primary candidate biocontrol agents to determine whether they produce toxins detrimental to the target hosts. Most biocontrol microorganisms are effective by virtue of toxins. We are making progress in this area although further research is needed. Natural toxins have several advantages over industrially synthesized compounds because of the relative ease with which they can be registered for use.

These are the general avenues of research we are following at the Pacific Forestry Centre. My colleagues will go further into these matters and clarify the progress they have made in the past several years.

Most important among our premises for research is our insistence that only native microorganisms be employed in this research. In one sense, this places our research at a disadvantage. Such microorganisms, often termed "opportunists," are generally weak pathogens that have co-evolved with the target hosts. As such, the two organisms have developed some measure of mutual genetic tolerance. Consequently, our job is to elaborate the means whereby such microbes can be enhanced in activity for use in weed control.

On the positive side of the story, there is a built-in safety factor with these microbes. In the case of imported microorganisms, it is always necessary to insure that their activity does not spread to other species of hosts or exceed the desired degree of weed control. Using native microorganisms, which are in balance with the environment and must be temporarily enhanced to be predictably effective, the biosphere serves as a buffer to reabsorb these pathogens once the specified task is completed.

In forest vegetation management, it is not necessary or even desirable to kill all vegetation that competes with the commercial crop, as is usually the standard procedure in agriculture. Rather, any plant that is constrained in growth or numbers to the point that it no longer competes with the crop species is, by definition, no longer a weed. This makes vegetation management in forestry easier to justify in cost-benefit terms, since control efforts in excess of those required represent dollars wasted. On the other side of the question, destruction of plants which are not harming the crop is to be avoided in order to limit site degradation and provide nurse species, as a minimum value. Nitrogen "contributors", such as *Alnus* spp., are obviously beneficial when they do not compete excessively with the intended commercial crop. Other non-crop vegetation may well form a bank of wildlife feed or cover. Other species may provide local employment, such as *Epilobium angustifolium* (fireweed Honey) or *Gaultheria shallon* (ornamental greens) or an alternate rangeland grazing species (*Calamagrostis rubescens* Buckl.). Nearly every species of plant that is termed a weed by one person has an advocate somewhere else, and that must be respected.

Our research is guided largely by a tight set of criteria whereby we judge acceptability of a candidate biological control agents:

1. Native microorganisms must be used
2. Operator control must be built into the application strategy
3. Target specificity of the candidate biocontrol agents must be assured
4. The biocontrol agents and their use strategies must be cost-effective

Overall, it is our intent to generate biocontrol agents and strategies for their use which are environmentally benign and yet are industrially useful. As mentioned, these are not necessarily intended to replace other elements of overall vegetation management, except perhaps in local cases. In the largest sense, they are intended as complements to other vegetation management strategies. Our work is geared toward development of biocontrol as an effective element for incorporation within the overall integrated vegetation management strategy for British Columbia.

Integrating forest protection with silvicultural planning and practice

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Abstract

Forest management has become increasingly complex and challenging over the last several decades in response to the dwindling acreage of natural forests and increasing demands for a variety of resource values. The general changes in forest resource management have been accompanied by a shift in philosophy toward forest protection. Historically, forest managers responded to pest problems when they arose, often with direct controls. The current emphasis in forest protection is on the development and implementation of integrated forest pest management programs. This approach relies on a thorough knowledge of the ecological relationships among pests and host trees to prevent the occurrence of pest problems. Silvicultural treatments and systems are designed to meet management objectives while simultaneously maintaining or enhancing natural control of pest populations. Vegetation management activities are one component of an integrated pest management program and must be compatible with the total forest resource management system. Findings from vegetation management research are used to illustrate the need for an interdisciplinary approach to understanding and solving forest pest problems.

Introduction

The last several decades represent a period of rapid change in natural resource management in North America and current indications suggest that even more dramatic shifts in management policy and practice will occur in the near future (Behan 1990). Until recently, most of our forest resources were supplied by natural forests that developed with little or no direct input from humans. The abundant natural forests were capable of supplying a variety of forest resources with few conflicts among alternative uses. However, with the pressures of an increasing human population the abundance of natural forests has declined drastically and most of our forestlands now have been affected to some degree by human activities. With the decline in natural forest ecosystems and the increased demand for a variety of resources, the task for forest managers has become increasingly complex (Carroll *et al.* 1989). The traditional emphasis on timber production is being replaced by more diverse approaches for the sustainable production of multiple forest resources (Behan 1990; Heilman 1990; Wood 1990). It is not surprising that along with the general changes that are occurring in forest management there have been numerous appeals for a new approach to forest protection (Branham and Hertel 1984; Dahlsten and Rowney 1983; Dahlsten and Dreistadt 1984; Norris 1988; Schowalter 1986; Stark 1977; Waters and Stark 1980).

Prior to the 1970s, the prevailing attitude that had developed among forest managers for dealing with pests was to ignore them until there was an obvious problem. Periodic tree mortality and defoliation caused by insect outbreaks were particularly alarming to forest managers and the general public. The typical reaction to pest outbreaks was to prescribe direct controls, often pesticides (Keen 1952; Anderson 1960; Ciesla 1978; Stipe 1987). One noteworthy exception was the early application of risk rating systems in the logging of old-growth ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) stands to reduce bark beetle losses (Keen 1936; Salman and Bongberg 1942). Direct control was viewed as a reasonable strategy because the primary objective usually was to protect timber resources until they could be utilized (Burke 1990; Keen 1952). Unfortunately, this approach ignores the causes of pest outbreaks which are the forest conditions that allow pest populations to increase to damaging levels. In the absence of management activities that alter forest stand conditions, direct controls do nothing to prevent the recurrence of pest outbreaks and may even increase their frequency (Blais 1974). With the increasing intensity and complexity of forest management the policy of responding to pest problems when they occur is no longer acceptable in most cases. This paper briefly discusses some of the principles of integrated pest management in relation to forestry and uses specific examples from forest vegetation management research to illustrate the need for an interdisciplinary approach in the development and application of forest pest management programs.

Integrated pest management

The concept of integrated pest management should by now be familiar to everyone involved in forest protection and so I will only briefly review it here. The philosophy of integrated pest management was originally developed as an alternative to heavy reliance on insecticides for protecting agricultural crops (Stern *et al.* 1959), but it has since been applied to other pest groups and management systems, including forests (Stark 1977). There are several fundamental concepts that form the basis for integrated pest management in forestry. First, integrated pest management is based upon a recognition of the holistic nature of forest ecosystems. Because forest ecosystems are complex assemblages of interrelated organisms any management activities that alter the structure or composition of the forest will affect potential pest populations (insects, pathogens, weeds, and vertebrates) and, conversely, any treatments designed to reduce the density of a pest population will affect other components of the ecosystem as well. Therefore, effective pest management must be incorporated into the total forest resource management system to optimize production of resource values while satisfying biological, social, and economic constraints. Second, although potential pest organisms serve important ecological functions in natural forests, they can have negative impacts on the production of desired resources in managed forests. Organisms become pests when their populations reach densities that interfere with forest management objectives. In the case of some weeds, these densities are also related to the size of individuals. These population densities are called economic-injury levels. Finally, the primary goal of integrated pest management is to prevent pest populations from reaching economic-injury levels by maintaining forest conditions that promote natural controls. Direct control of pests should only be applied when their populations reach or threaten to reach economic thresholds. Furthermore, this tactic should be combined whenever possible with silvicultural treatments to provide long-term solutions to pest problems.

Waters and Stark (1980) provide a thorough description of the general structure of an integrated forest pest management system. The four major components or subsystems that they identified are: (1) pest population dynamics and epidemiology, (2) forest stand dynamics, (3) impact of pests on resource values, and (4) treatment strategies. The information from these four general areas is synthesized through benefit/cost analyses to guide decision-makers in the planning and implementation of management activities. The successful development of an integrated pest management program will necessarily involve the input and cooperation of specialists from a multitude of both basic and applied, biological and social sciences. Throughout the remainder of my discussion, I will emphasize the treatment strategies component of this model system which I feel is the key to implementing successful pest management programs. All silvicultural treatments, whether they are specifically directed toward pest populations or not, must be considered as treatment strategies because they will affect pest populations to some extent. The goal of forest resource managers should be to prescribe silvicultural treatments that create forest conditions that are consistent with management objectives and simultaneously minimize the probability of pest-caused losses.

Many of our past and current forest pest problems have resulted from a failure to adequately anticipate the response of potential pests to the forest conditions created by management activities. For example, there are currently outbreaks of the western spruce budworm (*Choristoneura occidentalis* Freeman) and Douglas-fir tussock moth (*Orgyia pseudotsugata* (McD.)) of unprecedented severity occurring in eastern Oregon (Clapp 1991). The intensity and extent of these outbreaks has been attributed, in part, to fire suppression policy and selective logging practices which have greatly increased the abundance of the preferred host trees, true firs (*Abies* spp.) and Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco), at the expense of ponderosa pine and other fire-adapted species (Schmidt 1985). Although stands composed of a high percentage of true firs and Douglas-fir were recognized as highly vulnerable to spruce budworm at least 40 years ago (Keen 1952), silvicultural prescriptions prior to 1977 largely ignored the budworm (Stipe 1987). We are now paying the price for not applying knowledge that was available decades ago that could have prevented or reduced the severity of the present outbreaks. Throughout the 1980s, large sums of money have been spent on annual applications of chemical and biological insecticides to prevent defoliation in eastern Oregon. In 1988 alone, the USDA Forest Service spent approximately $17 million for the planning and application of *Bacillus thuringiensis* to about 950 000 acres in the Pacific Northwest (Hadfield 1988). Although silvicultural treatments are recognized as the long-term solution to the present problem, insecticide applications are considered to be justifiable in areas where insect populations have apparently reached densities equivalent to economic thresholds (Hadfield 1988).

Several other examples of important pests which have been favored by forest management activities include fusiform rust

(*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) (Powers 1984), pine tip moths (Berisford 1988), and reproduction weevils (Nord *et al.* 1982, 1984) in the southeastern U.S., root diseases and dwarf mistletoes (*Arceuthobium* spp.) in the western U.S. (Baker 1988; Reaves *et al.* 1989; Zobel *et al.* 1985), and the eastern spruce budworm (*C. fumiferana* (Clemens)) in eastern Canada (Blais 1985). We must begin to do a better job of including pest management considerations in the planning and application of silvicultural prescriptions if we hope to create forested landscapes that are resistant to future pest outbreaks (Perry 1988). Some of our current problems are the result of inadequate information in the past, but many others can be attributed to not fully utilizing available information. Consequently, there is not only a need to gather new information about current and potential pests, but also a need to ensure that pest related information is available to forest managers in a form that they can use and will accept. The development of new and improved hazard/risk rating systems, more reliable population models, better quantitative forecasts of impact, and more responsive detection and monitoring programs for a variety of current and potential pests would provide valuable information that could help forest managers to anticipate and prevent future pest problems (Hedden *et al.* 1981; Waters 1986; Hicks *et al.* 1987).

Forest vegetation management

The history of the development of forest vegetation management provides another example of foresters responding to a pest problem rather than anticipating and preventing the problem from occurring. The detrimental impacts of undesirable vegetation in managed forests were recognized as early as 1902, but by 1973 millions of hectares of forestland across the U.S. were dominated by low-quality trees, brushfields, and herbaceous vegetation (Walker 1973). These nonstocked and poorly stocked stands were the result of many types of natural and human-caused disturbances which were not all related to forest management activities. However, the problem was at least partly due to poor timber harvesting practices and unsuccessful reforestation efforts. The lack of tools for suppressing competing vegetation prior to the 1940s contributed to the severity of the problem. Since the 1940s there has been a steady increase in the variety and effectiveness of tools available to the forester for control of competing vegetation. Recently, there has been a recognized need to learn more about basic weed biology and the response of weeds to silvicultural treatments to develop strategies for problem prevention (Stewart 1987). Vegetation management is now recognized as an essential element in a program for prompt, successful regeneration of conifer forests (Walstad and Kuch 1987).

Vegetation management is one component of an integrated pest management program and must be compatible with the total forest resource management system (Walstad and Kuch 1987). Viewed in this way vegetation management is much more than simply removing undesirable vegetation. Although associated vegetation may reduce the survival and/or growth of crop trees through competition for limited site resources, it may also provide benefits such as maintaining or enhancing site productivity, wildlife habitat, or habitat for natural enemies of insect herbivores. Consequently, all of the potential impacts of alternative treatments must be evaluated when planning and prescribing vegetation management activities. I will use the results of my own research to illustrate the need for an interdisciplinary approach to the development and implementation of vegetation management practices.

Many studies have demonstrated the negative effects of plant competition on the survival and growth of ponderosa pine (Ross and Walstad 1986). A comparison of mechanical and chemical site preparation treatments in southcentral Oregon found that survival and growth of planted ponderosa pines were directly related to the level of vegetation control provided by the treatments, with one obvious exception (Ross *et al.* 1986). The brushblade treatment provided thorough vegetation control which resulted in good survival of planted seedlings. However, seedling growth following the brushblade treatment was less than expected compared to other treatments that resulted in similar levels of vegetation control. The slow growth of seedlings following the brushblade treatment was apparently due to effects of the treatment on soil properties. This treatment moved most of the vegetation, organic debris, and surface soil (about 2-5 cm) into windrows outside of the plots. As a result, the concentrations of total carbon (an index of organic matter), total nitrogen, extractable phosphorus, and total sulfur in the upper 25 cm of soil were lower and bulk density was higher following the brushblade treatment than following all of the other treatments. Consequently, although the brushblade treatment provided the benefit of reduced competing vegetation, the potential growth gains by conifer regeneration were partly negated by the detrimental effects that the treatment had on soil properties.

Vegetation management activities have the potential to affect other pests in addition to competing vegetation. Both decreases and increases in herbivore damage to pine seedlings have been observed following the removal of competing vegetation. In southcentral Oregon, herbicide applications that removed herbaceous vegetation significantly reduced mortality of planted ponderosa pine seedlings caused by pocket gophers (*Thomomys mazama* Merriam) (Crouch 1979). However, other herbivores may be favored by vegetation control treatments. In the site preparation study discussed previously, damage caused by the western pine shoot borer (*Eucosma sonomana* Kearfott) was directly related to the intensity of vegetation control and tree growth (Ross 1989). As a result, the short-term conifer growth gains from reductions in competing vegetation were partly offset by increased growth losses to the shoot borer. The long-term implications of the increases in shoot borer damage during the first 8 years of plantation establishment are unknown.

In the southeastern U.S., damage caused by the Nantucket pine tip moth (*Rhyacionia frustrana* (Comstock)) often increases following treatments that reduce the amount of associated vegetation in pine plantations (Berisford 1988; Ross *et al.* 1990). Several hypotheses have been proposed to explain this response. It has been suggested that reducing the abundance and diversity of associated vegetation may create a less favorable habitat for natural enemies of the tip moth (Berisford 1988). Although this is a strong possibility, it is one which is difficult to test and there is little specific data to support it. However, most data for other plant-herbivore systems indicate that vegetation diversity can have a significant effect on natural enemy populations (Russell 1989). Other factors that could contribute to the increases in tip moth damage following vegetation control treatments are increases in the quantity, quality, and/or accessibility of food associated with the increased survival and growth of pines.

Increasing the survival and growth of planted loblolly pines (*Pinus taeda* L.) through vegetation control treatments will also increase the density of growing shoot tips which are the required food for tip moth larvae (Ross *et al.* 1990). The increased abundance of food provides the resources to support higher tip moth population densities, provided that some other environmental factor is not limiting. In addition to providing a greater abundance of food for tip moths, the rapidly growing seedlings may also represent a more nutritious food source. Ross and Berisford (1990) found that vigorous loblolly pine seedlings were more attractive to ovipositing tip moths than were seedlings growing under some degree of water or nutrient stress. Furthermore, the larvae that fed on the vigorous seedlings developed into heavier pupae which presumably would become adults with higher reproductive capacities than the larvae that fed on the stressed seedlings. The vigorous seedlings had higher concentrations of nitrogen and lower concentrations of defensive compounds than the stressed seedlings and apparently were a better nutritional source for the developing tip moth larvae. In summary, a higher density of heavier insects developed on the vigorous seedlings than on the stressed seedlings. Despite higher levels of tip moth damage, seedlings developing in relatively weed-free environments still grow at more rapid rates than their counterparts in unweeded areas (Ross *et al.* 1990). Thus, pest management in this situation must consider the relative impacts of plant competition and herbivory.

Another factor which compounds the impact of higher tip moth damage following vegetation control treatments is the associated increase in fusiform rust and pitch canker (*Fusarium moniliforme* Sheld. var. *subglutinans* Wollenw. & Reink.) infections that can occur. The incidence of infection by both of these fungi has been shown to be positively correlated to tip moth damage (Cade *et al.* 1986; Powers and Stone 1988), although the specific causes for these responses are unknown. Furthermore, pitch canker development is more rapid on seedlings with high nitrogen content (Solel and Bruck 1989). Vegetation control is likely to increase the availability of nutrients to planted pines, resulting in seedlings which are highly suitable for the pitch canker fungus. Because of the tip moth and disease problems that are likely to occur following vegetation management activities, long-term studies are currently underway to evaluate the possibilities for combining tip moth control with vegetation control (Berisford *et al.* 1989). Considering the known relationships between tip moth damage and stand characteristics (Berisford and Kulman 1967; Berisford 1988), there are also opportunities to develop silvicultural systems that would create stands that are less susceptible to infestations.

This brief discussion has illustrated just a few of the many interactions that can potentially be influenced by vegetation management activities. With the broadening focus of forest management objectives, increasing questions about the sustainability of forestry practices, and increasing concerns about forest health, there is a need for more interdisciplinary and comprehensive evaluations of silvicultural treatments and systems, particularly in relation to potential forest pests. In response to that need, a new Integrated Forest Protection Program has been developed in the Department of Forest Science at Oregon State University. The research, teaching, and extension components of this program will emphasize prevention of pest problems through well-planned silvicultural operations. However, there will always be occasions when direct control of pest populations will be necessary and justifiable. Consequently, we need to continue developing environmentally sound and economically efficient methods of pest suppression. The papers that follow will discuss in detail a relatively new interdisciplinary effort to develop one such alternative, the use of mycoherbicides in forest vegetation management.

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Forest vegetation management in British Columbia

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Abstract

Site preparation and brushing treatments are used to improve the survival and growth of crop trees in British Columbia. In 1989 site preparation treatments were applied to 147 500 ha of Crown forest land. These treatments were used for debris removal, vegetation management, and other purposes. Mechanical site preparation, prescribed burning, and chemical site preparation can all be useful in achieving initial control of competing vegetation. Grass and legume cover crops may also be seeded to reduce future competition, to provide forage for livestock and for erosion control.

Brushing activities have increased substantially over the past decade. In 1981, 3000 ha of Crown forest land were brushed. In 1989, 61 000 ha were brushed to improve the performance of established conifers. Herbicides were used on 62% of the area brushed in 1989, 36% of the area was treated manually, and sheep browsing was used in less than 2% of the area brushed. Vision® herbicide was used on 92% of the area that was chemically brushed.

In the future site preparation and brushing activities are expected to remain close to current levels. To support operational programs there is a need for continued testing and evaluation of available and promising new treatments. The British Columbia Ministry of Forests, Forestry Canada, and other agencies are supporting research aimed at developing techniques for predicting and diagnosing vegetation management problems and testing promising new treatment options.

Introduction

Vegetation management is essential to the successful establishment of forest plantations and the achievement of acceptable levels of tree survival and growth on many sites in British Columbia (B.C.).

Following forest harvesting or other disturbances, a variety of different seral plant communities can develop. The major seral communities found in B.C.'s forests are roughly classified into 22 major complexes (Table 1). The type of community that develops following disturbance is influenced by the species present prior to disturbance, the species which occur in adjacent areas, and the nature, intensity, and timing of the disturbance. The rate at which vegetation develops, the species involved, and their cover, height and net effect on crop seedling performance is influenced by environmental factors and by the nature and intensity of the disturbance (Eis 1981; Hamilton and Yearsley 1988).

Effect of vegetation on crop performance

Non-crop vegetation can have beneficial and/or detrimental effects. Beneficial effects include: a) providing thermal cover; b) protecting soils from erosion; c) enhancing soil chemical and physical properties (e.g. through symbiotic nitrogen fixation); d) reducing pest damage; e) acting as alternate hosts for desirable microorganisms; and, f) providing forage for wildlife and livestock.

Table 1. Major seral vegetation complexes found on forest land in British Columbia (from Newton and Comeau 1990)

Complex	Major species	Biogeoclimatic zone
Cottonwood	*Populus balsamifera*	IDF,ICH,MS,SBS,BWBS
	Lonicera involucrata	
	Cornus sericea	
	Rubus parviflorus	
	Alnus spp.	
	Calamagrostis canadensis	
Cottonwood - alder	*Populus balsamifera*	CDF,CWH
	Alnus rubra	
	Rubus spectabilis	
	Cornus sericea	
	Rubus parviflorus	
Bigleaf maple	*Acer macrophyllum*	CDF,CWH
Mixed hardwood	*Populus tremuloides*	IDF,ICH,MS,SBS,BWBS
	Populus balsamifera	
	Betula papyrifera	
	Salix spp.	
	Alnus spp.	
Aspen	*Populus tremuloides*	IDF,ICH,MS,SBS,BWBS
Boreal poplar	*Populus balsamifera*	SBS, BWBS
	Populus tremuloides	
Red alder - shrub	*Alnus rubra*	CDF,CWH
	Acer circinatum	
	Rubus parviflorus	
	Rubus spectabilis	
	Sambucus racemosa	
	Polystichum munitum	
Salmonberry	*Rubus spectabilis*	CDF,CWH
	Rubus parviflorus	
Salal	*Gaultheria shallon*	CDF,CWH
Mixed shrub	*Rubus parviflorus*	ICH,MS,ESSF,SBS
	Rubus idaeus	
	Lonicera involucrata	
	Acer glabrum	
	Alnus spp.	
	Epilobium angustifolium	
Ericaceous shrub	*Rhododendron albiflorum*	ICH,ESSF,MH
	Menziesia ferruginea	
	Vaccinium spp.	

Table 1. cont.

Complex	Major species	Biogeoclimatic zone
Dry alder	*Alnus viridis* *Epilobium angustifolium* *Calamagrostis rubescens*	IDF,ICH,MS,SBS
Wet alder	*Alnus viridis* *Alnus incana* *Rubus parviflorus* *Lonicera involucrata* *Athyrium filix-femina*	ICH,MS,SBS,BWBS,ESSF
Dry shrub	*Amelanchier alnifolia* *Ceanothus* spp. *Paxistima myrsinites* *Shepherdia canadensis* *Symphoricarpos albus* *Holodiscus discolor* *Physocarpus malvaceus*	IDF,ICH,MS,SBS
Willow	*Salix* spp.	IDF,ICH,MS,ESSF, SBS,BWBS
Pinegrass	*Calamagrostis rubescens*	PP,IDF,ICH,MS,ESSF, SBS,BWBS
Reedgrass	*Calamagrostis canadensis*	SBS,BWBS
Fern	*Athyrium filix-femina* *Dryopteris assimilis*	ICH,ESSF,SBS,BWBS
Bracken	*Pteridium aquilinum*	CDF,ICH,ESSF,SBS
Fireweed	*Epilobium angustifolium*	CDF,CWH,IDF,ICH, MS,ESSF,SB PS,SBS,BWBS
Subalpine herb	*Valeriana sitchensis* *Senecio triangularis* *Veratrum viride* *Luzula* spp. *Epilobium angustifolium*	ESSF
Introduced grasses	Domestic grasses	all

Neighboring non-crop vegetation can reduce survival and growth of tree seedlings by: a) competing for light, water, and nutrients; b) causing physical damage; c) affecting soil and air temperatures (e.g. Hogg and Lieffers 1991); or, d) harboring organisms that damage crop species.

As the cover and height of competing vegetation increase, growth and survival of conifers declines (Brand 1986; Simard 1990; Wagner *et al.* 1989). Figure 1 illustrates observed relationships between growth of Engelmann spruce and the amount of competing vegetation for fireweed and mixed shrub communities in the Interior Cedar Hemlock zone of southern B.C. The curve depicting survival in Figure 1 is based on information from Oregon presented by Wagner *et al.* (1989) and field observations in B.C.

Several studies have been initiated in B.C. to develop and test methods for diagnosing brush problems in the field. Results from research completed to date suggest that a simple competition index based on percent cover and relative height of neighboring vegetation is as effective as more detailed measurements of neighboring plant communities. Under some circumstances, such as in patch scarified areas, it may be necessary to incorporate measures of distance between crop and non-crop species into such indexes.

Treatment options

Preventative or early treatments generally provide better survival and growth responses than treatments applied after competition has already seriously affected tree vigour (Newton and Preest 1988; Petersen *et al.* 1988). Prompt planting of healthy, vigorous conifer seedlings of the appropriate stock type and species can significantly reduce the need for treatments. Delaying planting or using planting stock

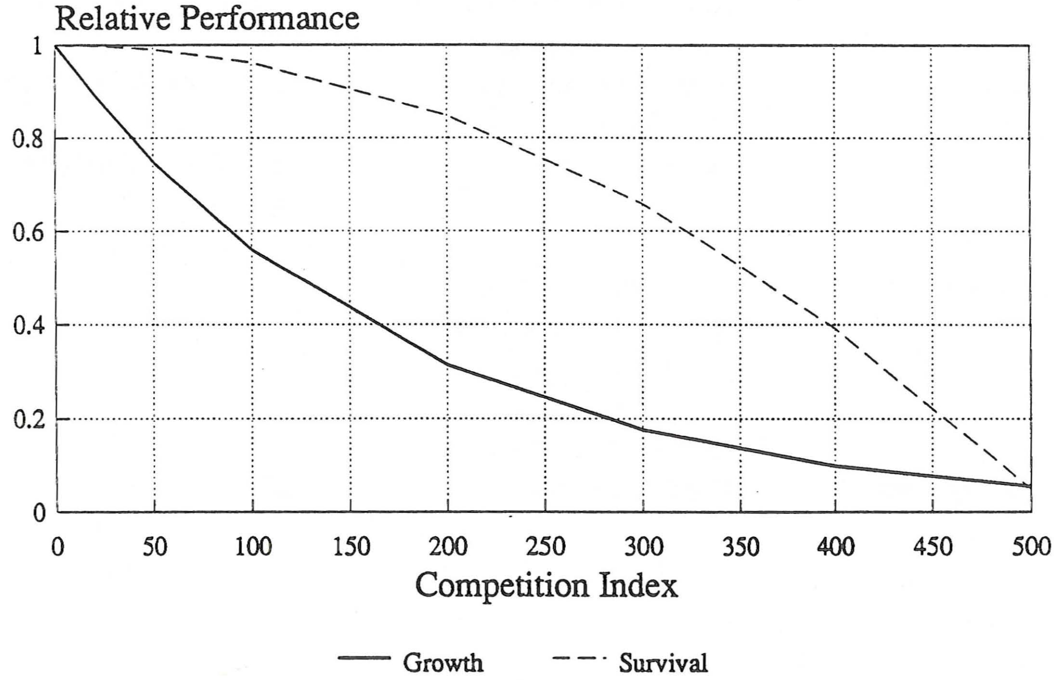

Figure 1. Relationships between performance of Engelmann spruce and competition index. (Competition index was calculated as: CI = sum (%cover x height)/ seedling height).

with poor vigour can place crop seedlings at a competitive disadvantage.

Treatments can be applied before planting (e.g. site preparation) or after planting (e.g. brushing).

Site preparation

Following harvesting, site preparation treatments can be used to remove debris, to improve microsite conditions for seedlings, or to manage vegetation. Using site preparation for vegetation management involves creating conditions having minimal competition and which are conducive to seedling performance.

Prescribed fire can give effective control of many shrubs. However, on moist rich sites in the ICH and ESSF zones of B.C., moderate intensity slash fires can create very dense cover of fireweed. Mechanical site preparation can give some control of vegetation by removing existing vegetation and seed stored in the forest floor. Exposure of mineral soil, however, can create seedbeds which favor the establishment of species such as red alder, Sitka alder, poplar, and willow.

Cover crops of grasses and legumes can be seeded following site preparation to provide forage for livestock, reduce soil erosion, and to reduce the invasion of native vegetation. When successfully established, cover crops can be effective in reducing invasion of plantations by species such as red alder.

Site preparation treatments were applied to 147 500 ha of Crown forest land in B.C. in 1989 (Table 2). Prescribed fire was used on 60 000 ha (41%), mechanical site preparation was used on 77 000 ha (52%), and herbicides were used to site prepare 6 000 ha (4%). In 1989/1990 approximately 10 000 hectares of forest land were seeded in B.C.

Site preparation activities have increased from 56 000 ha in 1981 to current levels (Figure 2).

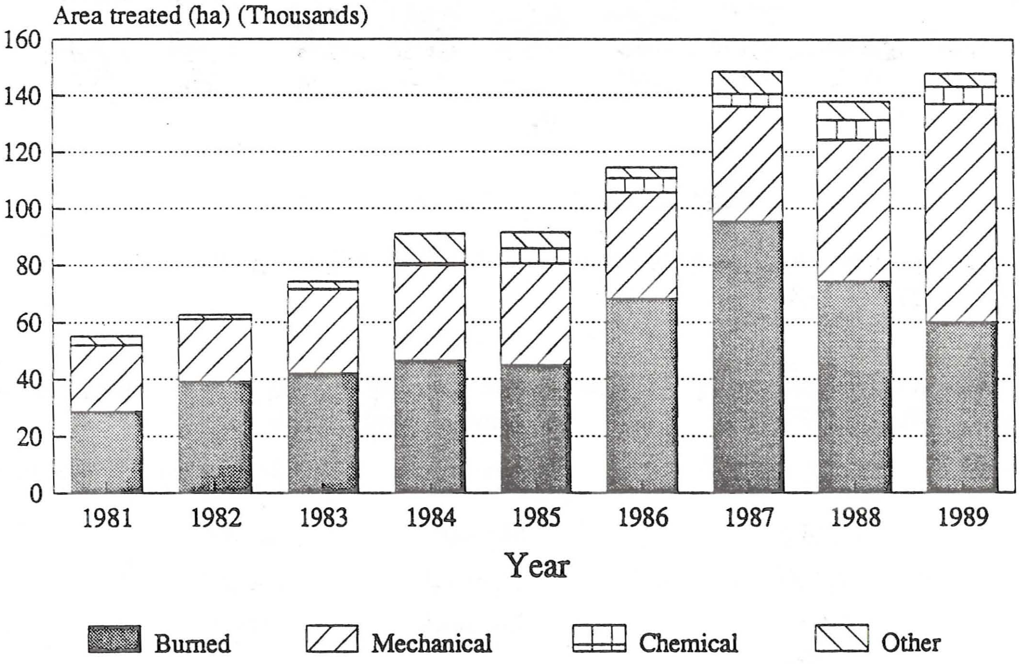

Figure 2. Site preparation activities on Crown forest land in British Columbia 1981-1989 (From Ministry of Forests Annual Reports).

Brushing

Manual, chemical and biological methods are used for brushing in young forests. Manual methods include cutting with brushsaws or machetes, girdling with tools such as the Vredenber girdler, and physically bending vegetation by hand. Chemical methods involve the use of herbicides such as Vision®, Velpar®, or Release®. Biological methods include the use of sheep or cattle, mycoherbicides, or natural pests.

In 1989, 61 000 ha of Crown forest land were brushed in B.C. (Table 3). Herbicides were used to treat 62% of this area, manual treatments were used on 36%, and sheep were used to brush less than 2% of this area.

Average costs for manual brushing were $550/ha in 1989. Costs for chemical brushing averaged $350/ha. Based on these average costs, the brushing of Crown forest land in B.C. cost approximately $25 million.

The total area of Crown forest land brushed in B.C. has increased from 3000 ha in

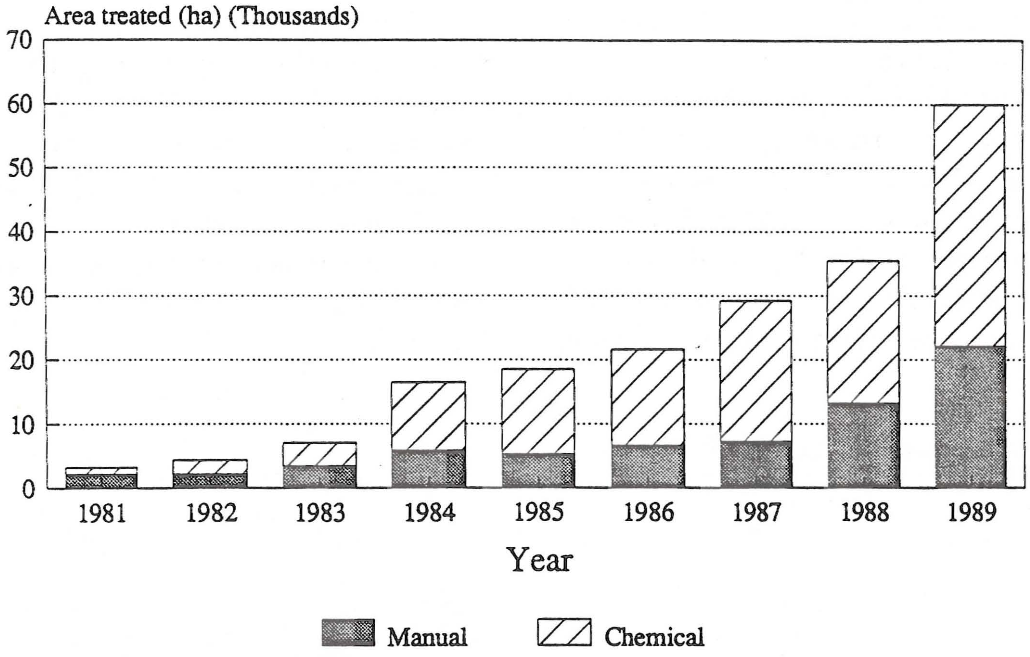

Figure 3. Brushing activities on Crown forest land in British Columbia 1981-1989 (From Ministry of Forests Annual Reports).

Table 2. Site preparation activities on Crown forest land in British Columbia (1989/1990)

Method	Area treated (ha)
Mechanical	77 000
Fire	60 000
Chemical	6 000
Other	4 500
TOTAL	147 500

Table 3. Brushing activities on Crown forest land in British Columbia (1989/1990)

Method	Area treated (ha)	% of Total
Manual	22 000	36
Chemical	38 000	62
Livestock	<1 000	<2
TOTAL	61 000	

1980 to 61 000 ha in 1989 (Figure 3). Ministry of Forests projections suggest that 80 000 ha should be brushed annually to achieve reforestation and free-growing targets.

Only a few herbicides are used operationally in B.C.'s forests. In 1989 Vision® was used to treat 92% of the area that was brushed chemically, products containing 2,4-D were used on 5%, and products containing hexazinone were used on 3%. During 1989 ground application was used to treat 30% of the area and aircraft were used to apply herbicides over 70% of the area brushed.

What does the future hold?

At present the forestry sector is under substantial pressure from the public and from a variety of special interest groups. There is growing public concern about the potential impacts of treatments such as prescribed slash burning, herbicides, and livestock browsing on forest lands. Growing pressures to meet non-timber resource management objectives will have an increasing role in how forest lands are managed in the future. The vegetation communities that we are attempting to manipulate in order to grow our crop trees are a forage resource for wildlife.

Opportunities for commercial utilization of hardwood species such as aspen, cottonwood, poplar, paper birch, red alder, and bigleaf maple will probably continue to expand over the next decade. This will increase the need for highly selective brushing treatments that do not damage desirable hardwood crops.

In addition, the growth of mixtures of coniferous and hardwood species within individual stands or in separate stands within forests or watersheds will increase in response to interest in managing for biodiversity. We need to improve our understanding of the nature and outcome of interactions between species in mixed stands.

For successful reforestation field staff require a wide range of treatment options. This provides flexibility in selecting effective treatments that achieve reforestation or timber production objectives while keeping impacts on non-timber resources to a minimum. Consequently, continued testing of promising treatment options is essential.

With increasing pressures on the forest land base, it is likely that substantial efforts will be made to maximize sustainable yields on lands that are designated primarily for timber production. Vegetation management will be a key ingredient to our success in this area. While several methods are currently available, opportunities still exist to explore new treatment options such as biocontrol for forest vegetation management.

To support operational programs there is a need for continued testing and evaluation of available and promising new treatments. The B.C. Ministry of Forests, Forestry Canada, and other agencies are supporting research aimed at developing techniques for predicting and diagnosing vegetation management problems and testing promising new treatment options.

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Sheep grazing — a biological tool for controlling competing vegetation in spruce plantations

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Abstract

The objective of this report was to evaluate domestic sheep grazing as a biological tool for controlling competing vegetation in spruce plantations. Two sheep grazing trials were established in the Cariboo Forest Region of British Columbia: the Hendrix Lake Pilot Trial established in the 100 Mile House Forest District in 1984, and the Doreen Creek Provincial Trial established in the Horsefly Forest District in 1986. The pilot trial measured an operational sheep grazing program, whereas the provincial trial was designed to test five treatments - a June grazing, a July grazing, an August grazing, a Glyphosate herbicide ground application, and a control. In both trials target and total vegetation was assessed for percent cover, height, damage, and control. The crop seedlings were assessed for total height, annual height growth, stem diameter, vigor, degree of overtopping, and any seedling damage including damage due to sheep grazing.

Preliminary 5-year results from both trials indicate that sheep grazing can effectively reduce fireweed, thereby reducing vegetation competition and press damage which results in improved seedling condition. However, other vegetation will re-invade and completely occupy the site to pretreatment levels. Damage to seedlings from sheep grazing varied considerably from 7 to 50%, but on average has decreased over time with better sheep management. Sheep grazing has not increased seedling height and only slightly increased seedling diameter.

Introduction

Using sheep to graze competing vegetation from recently established conifer plantations is not a new concept. Published accounts of grazing impacts on timber regeneration date back to 1898. In forests of the United States, Europe, New Zealand, Australia, Sweden, and Ireland, sheep have been used as a biological means of controlling competing vegetation.

The British Columbia (B.C.) sheep industry has gone through many changes since its inception in the 1860s during the Cariboo gold rush when the influx of 20 000 gold seekers provided a huge demand for fresh meat. The number of sheep fluctuated with the changes in the market, but reached a high of 145 000 head in the 1930s (Kindt 1936). Between 1950 and 1960, the number of sheep in B.C. had dropped to only 19 000, most of which were found on only 30 commercial ranches in the North Thompson and Nicola areas (Weir 1953).

By the 1970s, the sheep production in B.C. had declined significantly because of high production costs and poor returns. Only one commercial sheep ranch remained in the province, and the majority of sheep were owned by hobby farmers (Cruikshank 1978). Today, the B.C. sheep flock is estimated at 57 000 sheep, which in 1986 were produced on a total of 1536 farms. Almost half of the province's producers are concentrated near the lower mainland and on Vancouver Island (British Columbia Ministry of Agriculture and Food 1990). Profitability must be increased through various management and marketing techniques before the sheep population will experience any real growth (Downes 1990).

In 1984 a pilot trial was established near Hendrix Lake. The objective of this trial was to test whether domestic sheep could be used to graze unwanted competing vegetation (mainly fireweed) from spruce plantations, and also to reduce the demand for additional pasture land requested by the B.C. Sheep Breeders Association. The preliminary results were promising. The sheep adequately grazed the plantations, gained weight, and only caused minimal damage (5%) to the spruce seedlings. As a result the pilot trial was expanded in 1985. Some 1600 ewes and lambs were grazed for the summer on 260 ha of spruce plantations. That year, major operational problems were encountered. There was a 10% loss of sheep to predators, a high frequency of foot rot, and high costs associated with transportation, equipment, and personnel. Although the B.C. Sheep Breeders' Association ended with a deficit, the pilot trial was still considered a silvicultural success since the competing vegetation was adequately removed with less than 5% seedling damage.

Consequently, the Chief Forester of the B.C. Ministry of Forests convened a task group which produced a discussion paper that was later approved by the Executive of the B.C. Ministry of Forests (Anon. 1986). The first and foremost recommendation in the discussion paper was to continue investigations in sheep grazing before proceeding with an operational program.

In June of 1986, two trials were established: 1) to continue the pilot trial at Hendrix Lake, and 2) to establish a more in depth provincial trial in the Horsefly Forest District near Doreen Creek.

The objectives of the Cariboo sheep grazing trials were to determine:

1) the susceptibility of conifers to sheep grazing,
2) the optimum timing of grazing,
3) the level of utilization or control of vegetation,
4) the target vegetation species sheep will graze,
5) the crop seedling growth response attributed to grazing, and
6) to refine operational sheep grazing.

Part a - Hendrix Lake pilot trial

Methods

In 1986, exclosures were established on five plantations scheduled for yearly grazing. At least 20 permanent monitoring plots were established within each exclosure and in the adjacent grazing areas. A planted spruce seedling was located at the center of each plot for yearly assessment of height, annual height increment, stem diameter, seedling condition, damage, and vegetation cover. Percent cover of fireweed (the target species) and all species combined was also assessed within each plot. Percent vegetation cover, and seedling condition and damage, were assessed shortly after the plantations were grazed while seedling growth measurements were collected after seedling budset.

Results

Vegetation cover

In 1987, 20 to 30% of the seedlings in the control were classified as free-to-grow. Two years later, no free-to-grow seedlings were reported in the control and 43 to 70% of the seedlings were overtopped by competing vegetation. While the majority of seedlings on the grazed areas were classified as free-to-grow in 1987 and threatened in 1989, the percentage of overtopped seedlings on the grazed area remained about 50% lower than on the comparable control.

In 1987, the vegetation was grazed fairly heavily; vegetation cover was reduced by more than 50% in comparison to the control (Figure 1). After grazing, total vegetation cover averaged less than 7%, and fireweed cover had been reduced to less than 2%. In 1989, fireweed cover was reduced to approximately 7% while total vegetation cover was approximately 12% after grazing (Figure 2). These different grazing intensities may explain why many of the seedlings on the grazed area classified as free-to-grow in 1987 were subsequently classified as threatened in 1989.

Vegetation press damage and seedling condition

On two of the blocks that were grazed yearly (blocks F and G), vegetation press damage to the seedlings in the control was extensive (43 and 35%, respectively). This is compared to 0 and 13% vegetation press damage reported on the grazed areas of these blocks, respectively. On these blocks, grazing reduced vegetation press damage - damage that may be more detrimental to seedlings than sheep damage resulting from grazing. Block F, for example, had the highest total vegetation cover in the control and 43% of the seedlings were damaged by vegetation press. On the grazed area, no vegetation press damage was reported, and only 17% of the seedlings were damaged by sheep; most of the damage was abraded stems.

Sheep grazing damage

In 1987, sheep damage to seedlings in the form of browsing and stem damage (trampling and abrasions) was very high on blocks E and F (50 and 30%, respectively); only 17 percent of the seedlings on block G

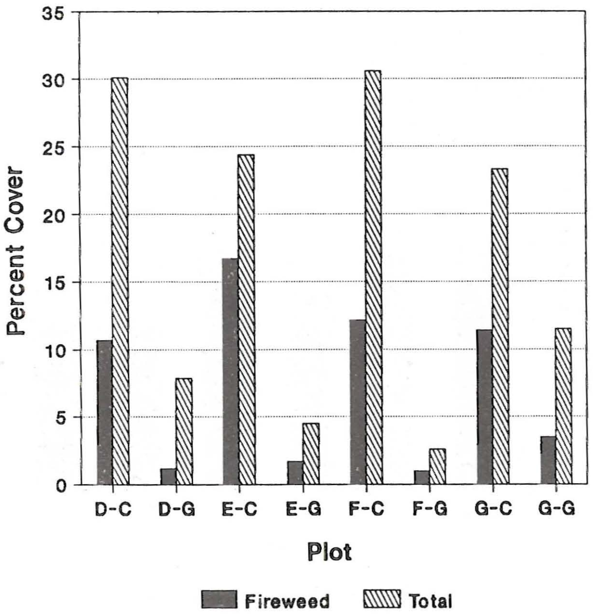

Figure 1. Hendrix Lake sheep grazing trial, fireweed and total vegetation cover - 1987. (D-C = D block, control; D-G = D block, grazed; etc.)

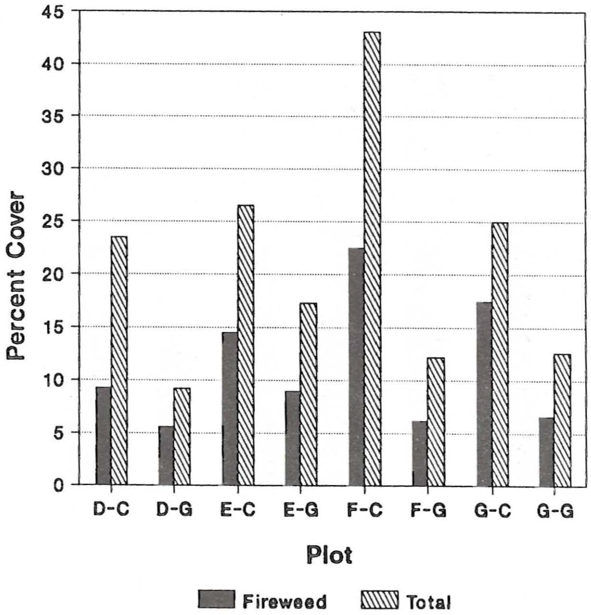

Figure 2. Hendrix Lake sheep grazing trial, fireweed and total vegetation cover - 1989. (D-C = D block, control; D-G = D block, grazed; etc.)

sustaining sheep damage. In 1989 sheep damage was reduced considerably on all blocks, with damage ranging from 7 to 17%. This reduction in damage could be attributed to reduced grazing intensity and better sheep management practices. On block F, most seedling damage was due to sheep grazing, while on blocks E and G the incidence of sheep damage was low relative to other forms of seedling damage.

Seedling height and diameter

Differences in seedling total height and diameter between grazed and ungrazed areas were not consistent or very large (Figures 3 and 4). Sheep management techniques were gradually improved from 1986 to 1989 but initial management problems could have resulted in the erratic seedling data. On block F where the sheep management problems were minimal, the seedlings are showing a growth response. Both seedling diameter and seedling height are greater on the grazed versus the ungrazed sections in this block.

Part b - Doreen Creek provincial trial

Although the pilot trial near Hendrix Lake showed good potential, many questions could not be answered quantitatively so a provincial trial was established near Doreen Creek in the Horsefly Forest District to help determine whether sheep grazing is a biologically viable vegetation management tool. Grazing was concentrated on two spruce plantations for a combined total of 69 ha. The blocks were planted in 1984 which gave the seedlings two growing seasons to become established prior to grazing.

Methods

Experimental design

The experimental design was based primarily on a protocol developed by

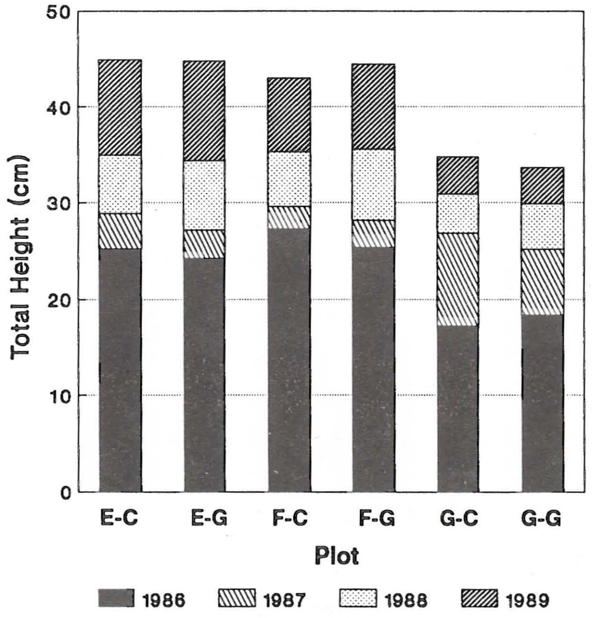

Figure 3. Hendrix Lake sheep grazing trial, total seedling heights. (E-C = E block, control; E-G = E block, grazed; etc.)

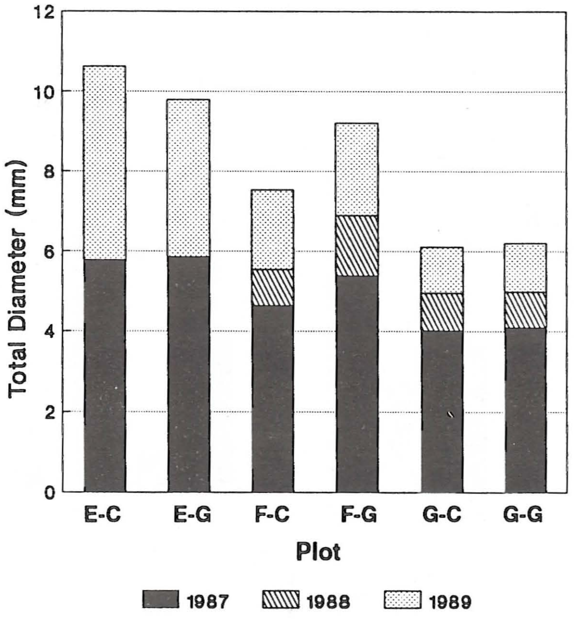

Figure 4. Hendrix Lake sheep grazing trial, total seedling diameters. (E-C = E block, control; E-G = E block, grazed; etc.)

Herring and Pollack (1985). This provincial sheep grazing trial was established as a randomized complete block design with three replications. Five treatments were originally planned; a June, July and August grazing, a Glyphosate herbicide ground application, and a control. However, the June grazing treatment was omitted and the July grazing treatment consisted of the last week of July and first one or two weeks of August due to a late start up. Grazing was continued for 3 years, but the original design was not used in the last 2 years due to lack of funding and sheep management problems.

Treatment plot size and layout

The six grazing treatments (two grazing times x three reps.) were, on the average, 5 ha in size. The three herbicide treatments and three control blocks were each 0.25 ha in size (50 x 50 m exclosures).

Subplot layout and intensity

Within each of the six grazing treatments, 30 circular subplots were established at 25-m intervals along randomly located transect lines (Herring and Pollack 1985). A planted spruce seedling was located at the center of each subplot. A labelled 1.5-m-tall stake was placed 2 m north of each subplot center. This was done to ensure that a bias was not created by attracting sheep to the plot center. In the case where crop trees were not located on a transect line, the subplot center was offset to the nearest planted spruce seedling. Twenty subplots were similarly located at 10-m intervals within each of the three herbicide and three control exclosures. The stakes were placed 50 cm north of the seedling to prevent the stake from shading the seedling.

Measurement procedures

Baseline assessments were not conducted since sheep grazing occurred before the fireweed had developed to maturity. Treatment response was determined through comparison to the control treatment. Seedling assessments included total height, annual height growth, stem diameter, vigour, degree of overtopping, condition and damage. Vegetation assessments included percent cover, height, condition, damage, and a control rating. This data was collected one, two, three and five growing seasons after the initial treatments. The sheep grazing continued for three seasons, i.e., years one to three.

Results

Vegetation dry weight and cover

Utilization, which was determined by clipping the vegetation after each treatment, varied considerably from year to year. In 1986, less than 40% of the fireweed was removed due to a late start, but utilization increased in to 52% in 1987 and 89% in 1988.

The longevity of treatment response also affected utilization over time. In 1987 sheep grazing removed approximately 50% of the fireweed, whereas the herbicide eliminated over 90% of the vegetation. This was the first year after herbicide treatment. The following year, sheep grazing was more effective at controlling the vegetation than the herbicide treatment since there were no subsequent herbicide applications.

As evident in the 1990 assessment, both the herbicide and grazing treatments controlled the re-invasion of fireweed. The dry weight of fireweed on these treatments was less than 50% of that found on the control (Figure 5). This 50% reduction was also reflected in the percent cover of fireweed (Figure 6). Fireweed cover on the grazed and herbicide treatments was only 5 and 7% respectively, as compared to almost 15% on the control.

Although grazing and the herbicide treatments have effectively controlled fireweed, the dry weight of total vegetation for both treatments has recovered to pretreatment levels is comparable to the control (Figure 5). Similar trends are seen in the percent vegetation cover (Figure 6).

Vegetation species shift
Both treatments have created a species shift in the vegetation: the grazing treatment has resulted in increases in raspberry, woody species such as thimbleberry, and other vegetation such as dandelion and pearly-everlasting; the herbicide treatment resulted in increases in thistle, dandelions, and grasses.

Seedling height and diameter
Seedlings that were monitored throughout the trial were on average similar in height and diameter between treatments. Although after 5 years there are no significant differences in height and diameter growth between treatments, there is a trend of improved height and diameter growth on the herbicide treatment (Figures 7 and 8). On the grazed treatment seedling height growth is slightly lower, probably due to browsing damage to leaders sustained in the first season.

Discussion

In order for foresters to establish a free growing crop of healthy trees, the competing vegetation must be controlled. This is the basic requirement of any vegetation management prescription. When using sheep to control vegetation, there is a fine line between the amount of vegetation that has to be grazed to minimize seedling damage from vegetation press and promote seedling survival and growth, and the amount of

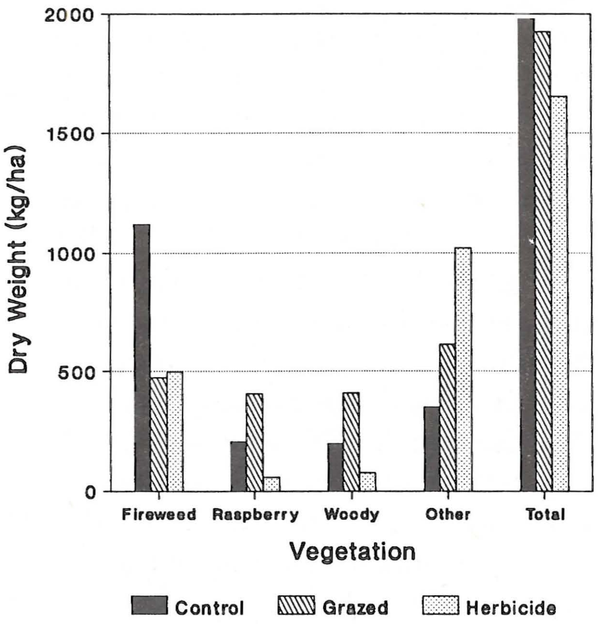

Figure 5. Doreen Creek sheep grazing trial, vegetation dry weights from clipped plots - 1990

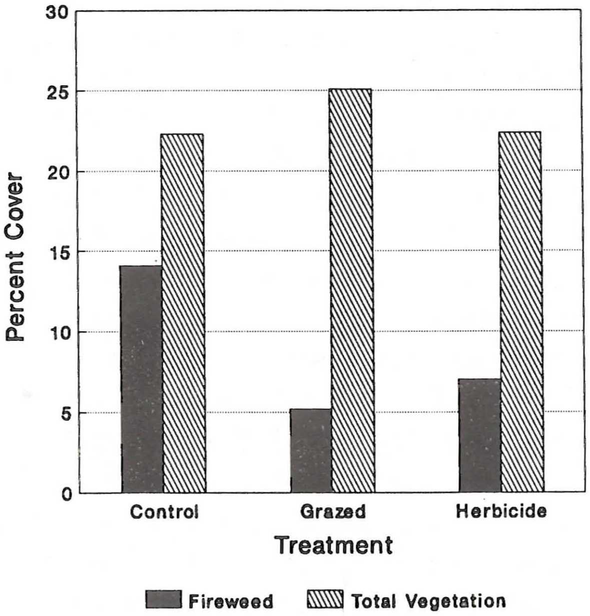

Figure 6. Doreen Creek sheep grazing trial, percent vegetation cover - 1990

vegetation that has to be left ungrazed to minimize seedling damage from over grazing.

Even though sheep grazing can effectively control fireweed it must be realized that within 3 to 5 years of treatment the site will be completely re-occupied by other species of vegetation. One concern with any vegetation management treatment, including sheep grazing, is that there will be significant shifts in vegetation types which will result in seedlings competing with another vegetation community. This new vegetation may be as damaging as the original target species, fireweed. In these studies, fireweed was replaced primarily by raspberry and other species such as thimbleberry, dandelion, and pearly-everlasting. Fortunately, these species on these ecosystems are considered less competitive and are less damaging than fireweed. Also, given the height of the seedlings, these shifts in vegetation should not be much of a threat to the free growing status of these plantations.

Overall, there has been a reduction in seedling damage caused by sheep grazing during the last 5 years on these trials mainly because of improvements in grazing management techniques. The six most important sheep management techniques to consider to reduce sheep grazing damage are 1) site selection, 2) type of sheep, 3) time of grazing, 4) forage quality and quantity, 5) herding, and 6) bedding and corral placement.

In order to ensure the success of any sheep grazing project, one must first select the proper sites suitable for sheep to graze. The site must have suitable forage, adequate stocking of seedlings that are at least 2 years old, minimal slash, less than 50 to 60% slope, and have adequate amounts of water and salt (Newsome and Sutherland 1989).

Sheep should be conditioned to the variety of forages available on the sites to minimize seedling damage. Sheep with previous forest grazing experience are the best suited to plantation grazing (Kistner and Smith 1983; Leininger and Sharrow 1983).

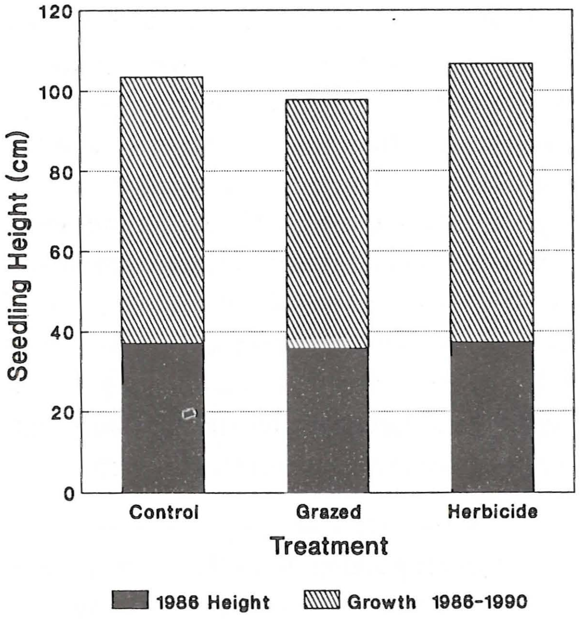

Figure 7. Doreen Creek sheep grazing trial, seedling height growth

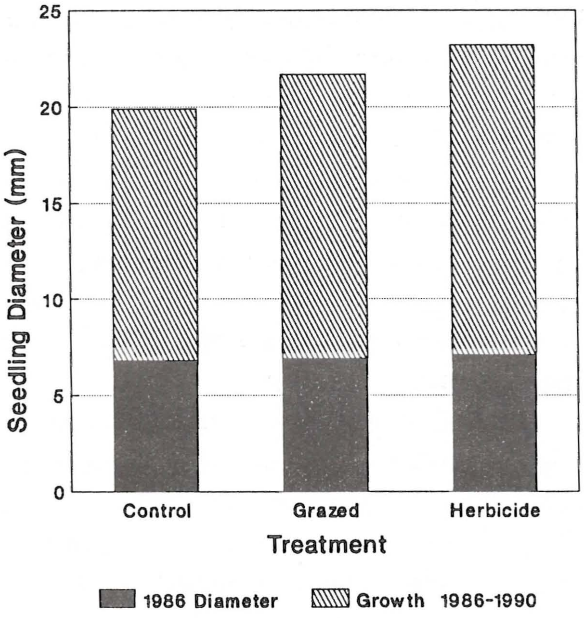

Figure 8. Doreen Creek sheep grazing trial, seedling diameter growth

Additionally, Phelps (1979) observed that if an animal is not familiar with a species by previous conditioning, they may even avoid plants which are readily palatable.

Time of grazing is also important to minimize damage to seedlings. Sharrow and Leininger (1983) found that in the spring, when the seedlings are just flushing, they are more susceptible to damage from browsing and trampling. Other mechanical damage was also noted in the Cariboo where the sheep could occasionally cause the leader to drop off just by brushing up against a seedling.

Ingram (1928) found forage quality and quantity may influence both sheep dietary preferences and the incidence of seedling damage. Quality and quantity of the forage are related to the time of grazing. Quantity of forage tends to be more of a problem in the spring when the vegetation is more sparse. Careful herding techniques are required to ensure adequate forage is available for the sheep so they do not graze the seedlings. The seedlings are flushing in the spring and are more prone to damage at this stage. However at any time of the year limited quantities of forage usually caused by over grazing can lead to seedling damage. Quality of the forage becomes more important in the late summer when frosts begin to damage the vegetation. In the Cariboo an early summer frost made the fireweed less palatable and within 48 hours of the frost the sheep damaged up to 80% of the seedlings by browsing.

A well experienced sheep herder is very important to the success of a grazing operation on plantations. The herder should be able to quickly recognize unacceptable levels of seedling damage and alter the grazing plan to prevent future damage. Young *et al.* (1942) reported that pushing sheep onto rough ground or into heavy slash, or even moving them too quickly through an area will not only increase the chance of an animal injury but also may result in "retaliatory" conifer browsing.

Proper placement of bedding grounds and night corrals is critical if seedling damage is to be minimized. Damage is reported to increase if sheep are bedded down for more than one night on the same area (Sparhawk 1918). Pearson (1950) stated that bedding grounds should be placed a minimum of one-half mile apart on forested range to minimize seedling damage. If night corrals are used they should be moved to the next block when the sheep are moved, unless the blocks are close together and the route of travel back to the corral does not endanger the seedlings (Newsome and Sutherland 1989).

The preliminary results from these trials indicate that sheep grazing can be a viable biological tool in controlling unwanted vegetation; however, there still is little evidence that sheep grazing has improved seedling growth, which is a primary objective of any vegetation management prescription. The high degree of variability in the results from year to year and between trials shows a need to continue sheep grazing in British Columbia on a small, well controlled basis with extensive monitoring until the complexity of this issue is more fully understood. There are still many biological and operational questions that need to be addressed and the following list identifies some of the current problems:

1) What sites are most suitable for sheep grazing and what is the extent of these areas in British Columbia?
2) What is a desirable level of vegetation control that will minimize

seedling damage yet improve seedling survival and growth?

3) How many years of repeat grazing are required to successfully remove the effects of the competing vegetation and improve seedling survival and growth?

4) What is the net effect of vegetation species shifts?

5) What are the long-term effects of browsing and trampling damage on seedling survival, growth, and pathological resistance?

6) How will domestic sheep grazing integrate with the mandate of the British Columbia Ministry of Fish and Wildlife, specifically in regard to interactions between sheep and wildlife.

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The potential for biological control of bluejoint *(Calamagrostis canadensis* [Michx.] Beauv.) in reforestation areas in British Columbia[1]

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Abstract

Bluejoint *(Calamagrostis canadensis* [Michx.] Beauv.) causes problems in northeastern British Columbia reforestation areas that range from competition to snow press. Where it is dominant, it is a weed that can delay spruce development for up to a decade and seriously impair timber production. Bluejoint primarily competes for light and nutrients. In view of the fact that chemical controls such as glyphosate are increasingly unavailable due to public pressure, alternative methods such as biocontrol are needed. At least 90 species of fungi are reported on bluejoint, 30 of which appear to cause some type of epidemic or disease on the weed. It is therefore likely that a fungal or bacterial biocontrol agent could be found using the bioherbicide approach.

The primary objective of biocontrol in this situation would be to reduce competition without eliminating desirable vegetation. About thirty percent of the approximately fifty major bluejoint pathogens have been collected and identified for further testing at the Pacific Forestry Centre in Victoria, British Columbia. Using a standard approach to mycoherbicide development, pathogenicity and virulence tests should lead to preliminary field trials by next year.

What is bluejoint?

Bluejoint *(Calamagrostis canadensis* [Michx.] Beauv.) is a grass species in the Graminae consisting of a complex of at least eight subspecies. Bluejoint varieties have a drooping panicle or seedhead, and also form creeping rhizomes. This highly variable northern grass is very hardy and thrives in arctic environments, in a wide range of moisture and nutrient regimes (Haeussler and Coates 1986). As bluejoint is an important arctic species, it has been keenly studied by taxonomists, with the perhaps inevitable result that there are many unanswered questions regarding the taxonomy of bluejoint subspecies. Regarding the diverse phenotypic and genotypic variability in this complex, Hultén (1968) said that "...variation within the genus is considerable... To construct a universally adequate key is hardly likely". The subspecies are also variable in terms of distribution, ranging from circumpolar to very localized. Although some authors refer only to *C. canadensis* var. *canadensis* as bluejoint, this paper will follow the trend of more recent authors (Hitchcock and Cronquist 1973) in applying the common name to the entire complex.

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[1] This research was funded by a grant from the British Columbia Ministry of Forests, Prince George Region, 1011-4th Avenue, Prince George, B.C., V2L 3H9.

What kind of problem is bluejoint to forestry?

Bluejoint infestation is a serious problem in reforestation areas in Canada, particularly in northeastern British Columbia (B.C.) and specifically in the Peace River and Fort Nelson regions. After logging opens up a site, bluejoint may form continuous mats within 3 to 4 years (Haeussler and Coates 1986). Originally found mostly on the prairies, bluejoint has been able to move into northern BC forests, taking advantage of the disturbed, open sites that land-clearing activities made available. It is now probably the primary obstacle to the success of spruce plantations in the region (Cal Wilson, B.C. Ministry of Forests, Dawson Creek Forest Region, personal communication, 1991). The grass can become quite tall (about 2 m or more) and profuse, leading to several problems for young tree seedlings. The main adverse effects in plantations are due to very strong competition for nutrients and light, in which young conifers are usually overwhelmed. The dense litter from this weed can cause snow-press and smothering problems, and can delay spring soil thaw. The litter also halts natural regeneration by preventing conifer seeds from reaching the ground before desiccation (Haeussler and Coates 1986). Empirical field observations are indicating further problems with the litter - it can cause seedlings to pitch-over and grow laterally, and it can provide a safe haven for seedling-eating mammals such as rabbits. Bluejoint populations seem to decline after 10 years, which may correspond with the build-up of litter (Les Herring, B.C. Ministry of Forests, Prince George Forest Region, personal communication, 1991). Bluejoint competition problems are similar to those created by pinegrass (*C. rubescens* Buck.), a related species concentrated in southeastern and central British Columbia that competes mostly for nutrients and water (Haeussler and Coates 1986).

Why biological control?

Biological control of bluejoint, versus chemical control with glyphosate, is a viable option in B.C. forests, and fits well with the political climate in B.C. Although it is safe and effective, the use of Vision®, a glyphosate formulation used to control bluejoint and other forest weeds (Haeussler and Coates 1986), could ultimately be curtailed due to political pressure (Campbell 1990). The biological control option is viable for several reasons. The principal objective in dealing with weeds is to reduce competition and to allow the desired plants to grow freely. Complete weed eradication may be counterproductive, leading to wide-ranging problems including soil erosion, high soil temperatures, and other phenomena (National Research Council 1975). Part of the philosophy of biocontrol is that reduction of chemical use leads to greater diversity, hence greater stability or "homeostasis" and more secondary, indirect biological control of all pests in the system. Weakening weeds in a chemical-free way without elimination is, therefore, highly desirable in forestry from a technical point of view. Inundative biological control agents certainly have the potential to achieve this, based on the experience in agro-ecosystems with a variety of organisms. Inundative or inoculative biocontrol of several reforestation weeds has been either studied or proposed (Wall 1986, 1990; Wall and Shamoun 1990; Wall *et al.*

1991; de Jong *et al.* 1990; Riendeau 1991). Inundative methods can achieve results ranging from partial to complete weed control or mortality, depending on the nature of the particular pathosystem, the formulation, and the virulence and concentration of inoculum (Winder 1990). For this perennial weed, it will be important to devise a strategy to deplete carbohydrate reserves in the rhizomes in order to prevent rapid regeneration (Winder and Van Dyke 1990; Hogg and Lieffers 1990). From a public standpoint, this way of controlling weeds would address concerns about the effects of chemicals on environmental integrity and human health (de Jong *et al.* 1990).

What is an inundative biological control agent?

Also known as "bioherbicides" or "mycoherbicides", inundative biocontrol agents are endemic pathogens which are applied to the pest population in quantity, in the same general manner as a chemical agent. This is distinct from the classical approach, in which a pathogen is introduced from another location to establish a persistent, weed-supressing epidemic or "epiphytotic". The precise details of these approaches will be forgone here; they can be found in recent reviews (Charudattan 1988).

What kinds of diseases are reported on bluejoint?

Approximately 90 species of fungi, some causing important diseases, have been reported on *Calamagrostis* species in Canada and the United States. A synopsis of the major diseases which have been reported on *Calamagrostis* species, including bluejoint, appears in Table 1.

What has been done so far?

At the Pacific Forestry Centre, research is in the exploratory or "discovery" phase. Extensive collections were made during the summer of 1991 to obtain a diverse collection of the weed and its pathogens. Collections were made primarily in the Peace River and Fort Nelson areas of B.C. The sampling areas, accessible by logging roads or helicopter, consisted mostly of typical reforestation sites heavily infested with bluejoint. To date, we have isolated about 34 different fungi and several bacteria from the diseased shoots and roots of bluejoint. Among these organisms, perhaps a dozen have been identified as potential pathogens; these represent about one-third of the major diseases reported on bluejoint. Collections also include seeds and potted *Calamagrostis* spp. from throughout northern British Columbia.

Where do we plan to go from here?

I. Discovery phase. The pathogens mentioned above will be screened for pathogenicity on the target weed. The most promising pathogen or pathogens will be selected during this phase.

II. Development phase. The candidate pathogens will be tested for pathogenicity and safety on conifers and other hosts. The virulence of these pathogens will be quantified in relation to parameters such as temperature, dew period, growth stage, and inoculum concentration. If necessary, adjuvant will be formulated to enhance virulence in the field. Such formulations can be used, for example, to lessen the need for a dew period or improve adhesion to the target weed (Winder 1990). When a deployment strategy has been developed, and when a technique is available to generate sufficient quantities of inoculum, the candidate pathogens will be tested in preliminary field trials in northern B.C.

III. Deployment phase. The pathogen will be tested rigorously in the field on a larger scale in preparation for public use. If the pathogen is effective, safety and efficacy tests would have to be conducted to assess environmental impact and register the final product in Canada.

What are the problems and prospects?

A technical problem that will be probably be encountered in this research is the limited taxonomic understanding of *Calamagrostis* species and bluejoint subspecies. Ultimately, the pathogens will decide the differences or lack of differences among *Calamagrostis* spp. for themselves. There is a potential conflict of interest with range operators who wish to keep bluejoint as a grazing resource for their cattle. Although we do not believe that a native pathogen of the type we work with is capable of significant spread into non-target areas, there will probably be some need to resolve the issue of rangeland vs. forest areas in the political arena if this weed is successfully controlled. Because so many diseases have been reported on bluejoint, and because our objective is to temporarily suppress rather than eliminate the plant, the prospects for finding an inundative, endemic biological control for bluejoint appear to be excellent.

Acknowledgements

This study is being funded in part by the B.C. Ministry of Forests and Forestry Canada. We gratefully acknowledge the financial and technical assistance of those agencies involved in this project.

Table 1. A synopsis of some major fungal diseases reported on *Calamagrostis* spp. in North America, including Alaska, Canada, Greenland, and the northern continental United States.

Fungus name(s)	Disease name(s)	Host(s)[a]	Cited[b]
Acremoniella spp.	Leaf spot	Cs	1,3
Ascochyta spp.	Leaf spot	Cs	1,4
Balansia epichloe (Weese) Diehl.	Black ring, Sterility dis.	C,S	1,2,4,5
Cercosporidium graminis	Leaf spot, leaf streak, brown stripe	C,Cs,S	1
Cheilaria agrostis (= Septogloeum)	Blotch spot, char spot	C,N,R,S,X	1,2,3,4
Claviceps spp.	Ergot	C,Cs,Cl,G,N, S,X	1,2,3,4,5
Colletotrichum graminicola (Ces.) Wils.	Anthracnose	C,N,P,R,S,X	1,2,3,4,5
Conithyrium psammae Oud.	Leaf spot	Cs,N,X	1,4
Cylindrosporium calamagrostidis Ell. & Ev.	Leaf spot	C,S	1,2,4
Dilophospora alopecuri (Fr.) Fr.	Twist	C,E	1,2,4,5
Epichloe typhina (Pers.) Tul.	Choke	C,X	1,2,3,4,5
Erysiphe graminis D.C.	Powdery mildew	C,R	1,4
Fusarium spp.	2ndary root rot,	C,M	1,2
	snow mold	C,X	4
Helminthosporium spp.	Leaf spot	C,X	1,4
Hendersonia spp.	Leaf mold	C,Cs,Ph,S	1,2,3,4,5
Herpotrichia spp.	Leaf spot	C,Cx,R,X	1,2,3,4,5
Mastigosporium rubricosum (Dear. & Bart.) Nannf.	Red eyespot, purple eyespot	C,Cs,D,N,R,X	1,2,3,4,5
Microdochium spp.	Root necrosis,	E,M	1
	Snow mold	B,C,X	1
Mollisia spp.	Leaf mold	C,L,P,Ph,S,X	1,3,4,5
Passalora graminis (Fckl.) Hohn	Brown stripe	Cs	3,4
Phyllachora graminis (Prs. ex Fr.) Fckl.	Tar spot	C,Cl,Cs,E,S, X	1,3,4,5
Psuedoseptoria everhartii	Speckle, eyespot	C,Cs,K,P,Pa,	1,2,3,4 R,S,X
Puccinia coronata Cda.	Oat crown rust	C,Cl,E,K,Lp, N,P,S,X	1,2,3,4,5
" var. *calamagrostidis*	Crown rust	C,R,S,X	2,3,4
Puccinia spp.	Crown, stem, & other rusts	C,M,N,R,S,X	1,3,4
Ramularia pusilla Ung.	Leaf spot	C,Cs,R	1,3,4
Rhizoctonia solani K.	Root rot	M	1,4

Table 1 cont'd.

Fungus name(s)	Disease name(s)	Host(s)[a]	Cited[b]
Rhyncosporium orthosporum	Scald	C,Cs	1,3,4
Sclerotium rhizoides Avers (= *Rhizoct.* or *Typhula?*)	Leaf rot	C,Cs,S	1,4,5
Septoria spp.	Leaf spot	C,Cs,N,Ph, S,X	1,3,4,5
Spermospora subulata (Sprague) Sprg.	Leaf blast	R,X	1,4
Staganospora spp. (summer form = *Ascochyta* spp?)	Leaf mold	C,Cs,S,X	1,2,3,4
Typhula incarnata Lasch ex. Fr.	Snow mold	Cs	3
Urocystis agropyri	Flag smut	C	1,4,5
Ustilago calamagrostidis (Fckl.) Clint.	Stripe smut	C,Cs,P,I,X	1,2,3,4,5
Ustilago spp.	Smuts	C,M,S,Pi,R,X	1,2
Wojnowicia hirta ?	2ndary foot rot, straw rot, leaf spot	Cs,R	1

[a] Abbreviations for species of *Calamagrostis* are: B = *C. breweri* Thrub., C = *C. canadensis,* CL = *C. c.* var. *lactea* (Beal) Hitch., CS = *C. c.* var. *scabra* (Kunth.) Hitch., D = *C. deschampsiodes* Trin., E = *C. elongata* Rydh., G = *C. groenlandica* Kunth., K = *C. koleriodes* Vasey, L = *C. lanceolata* Roth, LP = *C. lapponica,* M = *C. montenensis* Scrib., N = *C. nutkaensis* (Presl.) Steud., P = *C. purpurascens* Buckl., PH = *C. phragmitoides* Hartm., PI = *C. pickeringii* Gray, X = Unidentified *Calamagrostis* sp.

[b] Abbreviations for citations are: 1 = Ginns (1986), 2 = Conners (1967), 3 = Farr *et al.* (1989), 4 = United Stated Department of Agriculture (1960), 5 = Seymour (1929).

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Protein and esterase patterns of endophytes and disease syndrome associated isolates of *Melanconium* spp.

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Abstract

Esterase and non-SDS protein patterns of five disease-syndrome-associated (DSA) isolates and five isolates of symptomless endophytes (SE) of *Melanconium apiocarpum* as well as of 10 DSA *Melanconium marginale* isolates from red alder (*Alnus rubra*) were determined by polyacrylamide gel electrophoresis. Isolates of *M. marginale* differed from those of *M. apiocarpum* in their non-SDS protein and esterase patterns, protein content, and yield of acetone powder. None of these methods used showed differences between SE and DSA isolates of *M. apiocarpum*. The use of esterase and non-SDS protein patterns as potential biochemical markers is proposed as a taxonomic tool to differentiate *M. apiocarpum* (SE and DSA) from *M. marginale* isolates. These findings imply that DSA and SE isolates of *M. apiocarpum* are similar if not identical. Future research should be directed toward manipulation of the endophytic fungi to promote them to the pathogenic phase so that they can function as biological control agents (mycoherbicides) for red alder.

Introduction

Industrially valuable conifers planted on forest renewal sites must compete with unwanted vegetation, or forest weeds. One such forest weed is red alder (*Alnus rubra* Bong.) which is a deciduous tree indigenous to the Pacific Coast Mesothermal forest region of British Columbia and the United States and which often grows in pure stands as an early occupant on cleared land (Haeussler and Coates 1986).

Traditionally, chemical herbicides and burning have been used to control the growth of forest weeds, but increasing public opposition to these methods has caused much research to be directed toward development of biological control agents or mycoherbicides (Dorworth 1990; Wall and Shamoun 1990; Wall *et al.* 1992).

Biochemical characterization of biocontrol agents is required to satisfy the requirements for registration and labelling of the mycoherbicides (Byrne 1991; Wall 1990; Shamoun *et al.* 1991b).

So far, the search for candidate biocontrol agents has been limited to fungi from diseased plants. There exists also a distinctive community of fungi, called endophytes, that live within apparently healthy plants; disease symptoms are not manifested during part or all of the life cycles of these endophytic fungi (Sieber *et al.* 1991a; Carroll *et al.* 1977). Some are potential pathogens, whereas others may be mutualistic symbionts.

Melanconium apiocarpum Link (Synonym: *Melanconium sphaeroideum* Link: Fr., anamorph of *Melanconis alni* Tul. & C. Tul.) is known to occur in *Alnus rubra* either as a symptomless endophytes (SE) or as a disease syndrome associated (DSA) microorganism, associated with cankers and necrosis of stems and twigs (Sieber *et al.* 1989). Among DSA fungi of *A. rubra* in coastal British Columbia 55% of the *Melanconium* spp. isolates were *Melanconium marginale* Wehmeyer [anamorph of *Melanconis marginalis* (Peck) Wehmeyer] and 45% were *M. apiocarpum* (Dorworth 1990). Currently, these fungi are under intensive investigation at the Pacific Forestry Centre to assess their usefulness as potential biocontrol agents (or mycoherbicides) for red alder in British Columbia.

The electrophoretic technique for analysis of proteins and enzymes has been used extensively in fungal taxonomy in the last 15 years (Franke 1973; Shecter 1973; Snider 1973; Stipes *et al.* 1982; Micales *et al.* 1986). To a lesser extent, electrophoresis of isoenzymes has also been utilized to differentiate and characterize strains, formae speciales, or isolates of an organism varying in pathogenicity (Alfenas *et al.* 1984; Bernier *et al.* 1983; Morrison *et al.* 1985; Shamoun *et al.* 1991a, 1991c). Similar results have been obtained with polyclonal antibodies and polyacrylamide gel electrophoresis in separating different strains of *Gremeniella abientina* (Lagerb.) Morelet (Dorworth 1974; Benhamou *et al.* 1984).

The objectives of this study were to determine if non-SDS protein and esterase patterns could be used as biochemical markers to: (1) differentiate between SE and DSA isolates of *Melanconium apiocarpum*; and (2) differentiate and clarify the taxonomic relationships between *Melanconium apiocarpum* and *Melanconium marginale* isolates collected from red alder.

Materials and methods

Isolates of *Melanconium* spp. and culture conditions

Twenty isolates of *M. apiocarpum* and *M. marginale* were studied. The origin, type (SE or DSA) and host of the isolates are given in Table 1. Following incubation at 20°C for 14 days on acidified potato dextrose agar, 1-cm^2 squares were cut from the periphery of fungal colonies and used to inoculate sterile V8 juice medium (Benhamou *et al.* 1984). Each isolate was grown in 100-ml aliquots of medium, and each isolate was grown in five 250-ml Erlenmeyer flasks. Inoculated flasks were grown at room temperature for 14 days. Following incubation, the mycelium from each isolate was harvested by suction filtration through Whatman no. 4 qualitative filter paper, removing any inoculum agar squares in the process. The remaining mycelium was rinsed with distilled water, weighed, and frozen in vials at -20°C. The frozen samples were then freeze dried for 48 hours. The resulting mycelial cakes were homogenized on ice using a cold mortar and pestle (previously stored at -20°C) to which 5 g of dry ice and 10 ml of cold acetone were added. The homogenate was subsequently suction filtered through Whatman no. 1 filter paper and the resulting "acetone powder" was scraped off with a razor blade, weighed and stored at -20°C.

Protein extraction and determination

Proteins were extracted by placing 100 mg of the acetone powder and 1.0 ml of extraction solution (0.004 M $NaHCO_3$) into 1.8-ml microcentrifuge tubes. These were left overnight at 4°C and centrifuged in an Eppendorf microcentrifuge (model 5415) at 14 000 rpm (RCF = 16 000 x g) for 30 minutes. The resulting clear supernatant was collected and, by using Bradford's method (1976), the protein content of each isolate sample was determined.

Protein supernatant solutions of *Melanconium* spp. were then diluted in 0.004 M $NaHCO_3$ to a protein content of 500 µg/sample. Each 400 µl volume of standardized supernatant was amended with 40 µl of 0.25% bromophenol blue and 60 µl of 20% glycerol. The resulting solutions were dispensed into 50 µl aliquots and frozen at -20°C in 1.8-ml microcentrifuge tubes. Samples were kept on ice when not frozen during the aforementioned procedures to minimize any possible protein degradation prior to electrophoresis.

Polyacrylamide gel electrophoresis

Samples were thawed on ice and 25 µl of each sample solution were applied per well. All 20 isolates were separated during a single electrophoretic run. A support

Table 1. Hosts and origins of *Melanconium apiocarpum* and *Melanconium marginale* isolates.

Isolate	Species/Type	Host	Origin
PFC 043	*M. apiocarpum*/DSA	*Alnus rubra*	Victoria, B.C.
PFC 047	*M. apiocarpum*/DSA	*A. rubra*	De Courcy Island, B.C.
PFC 050	*M. apiocarpum*/DSA	*A. rubra*	N.W. Bay (Mac/Blo), B.C.
PFC 053	*M. apiocarpum*/DSA	*A. rubra*	Sombrio Beach, B.C.
PFC 055	*M. apiocarpum*/DSA	*A. rubra*	Jordan River, B.C.
PFC 067	*M. apiocarpum*/SE	*A. rubra*	Jordan River, B.C.
PFC 068	*M. apiocarpum*/SE	*A. rubra*	Cowichan Lake, B.C.
PFC 071	*M. apiocarpum*/SE	*A. rubra*	Campbell River, B.C.
PFC 147	*M. apiocarpum*/SE	*A. rubra*	Jordan River, B.C.
PFC 155	*M. apiocarpum*/SE	*A. rubra*	Gold Stream, B.C.
PFC 022	*M. marg.*/DSA	*A. rubra*	Metchosin, B.C.
PFC 025	*M. marg.*/DSA	*A. rubra*	Kennedy Lake, B.C.
PFC 049	*M. marg.*/DSA	*A. rubra*	Galiano Island, B.C.
PFC 051	*M. marg.*/DSA	*A. rubra*	Mesachie Lake, B.C.
PFC 078 (ATCC 56907)	*M. marg.*/DSA	*A. tenuifolia*	Idaho (U.S.A.)
PFC 013	*M. marg.*/DSA	*A. viridis*	Kootenay, B.C.
PFC 083	*M. marg.*/DSA	*A. viridis*	Nakusp Hot Springs, B.C.
PFC 084	*M. marg.*/DSA	*A. viridis*	Prophet River, B.C.
PFC 091	*M. marg.*/DSA	*A. viridis*	N.W.T.
PFC 092	*M. marg.*/DSA	*A. viridis*	Nakusp Hot Springs, B.C.

backing (Gel Bond polyacrylamide gel support medium) was used to enable easy handling of the gels. Protein separation was performed using 7.5% acrylamide slab gels (0.75 mm x 12 cm x 17 cm) in a vertical gel electrophoresis system (Bethesda Research Laboratories model V16). A constant current of 5 mA (using a Searle Buchler 3-1500 constant power supply) was applied while samples migrated through the stacking gel and subsequently increased to 15 mA as movement through the separating gel occurred. Electrophoresis was carried out at 1°C until the bromophenol blue solvent front was approximately 1.0 cm from the bottom of the gel.

Standardized protein samples were then subjected to electrophoresis and stained for non-SDS total protein esterase according to Cheliak and Pitel (1984). Following the staining procedure, gels were rinsed, fixed and preserved in a 30% ethanol - 10% acetic acid - 10% glycerol solution (V/V) for approximately half an hour. Gels were then covered with cellophane preserving sheets (Pharmacia LKB 1850-221) and left overnight to dry at room temperature and later photographed. Relative migration (R_f) values were determined as the distance of the band migration from the origin divided by the distance travelled by the tracking dye front.

Results

Yield of mycelium, protein content and patterns of *Melanconium* spp. isolates

There were no differences in the yield of acetone powder between SE (mean = 238 mg; s.d. = 33.10 mg) and DSA (mean = 252 mg; s.d. = 35.44 mg) isolates of *M. apiocarpum*, but *M. marginale* isolates yielded more acetone powder (mean = 460 mg; s.d. = 48.99 mg) than those of *M. apiocarpum* (Table 2). The protein contents of *M. marginale* isolates (mean = 4020.9 µg/mg; s.d. = 543.78 µg/mg) were consistently higher than those of both SE (mean = 1544.4 µg/mg; s.d. = 520.77 µg/mg) and DSA isolates of *M. apiocarpum* (mean = 1585.6 µg/mg; s.d. = 500.87 µg/mg) (Figure 1).

Detection of protein and isozymes patterns on gels

The non-SDS electrophoretic protein patterns revealed no major qualitative differences between DSA and SE *M. apiocarpum* isolates. However, isolates of *M. marginale* can readily be distinguished from *M. apiocarpum* by three fast moving protein bands at R_f 0.82 in isolates PFC 051, PFC 078 and PFC 013, and slow moving protein bands at R_f 0.21 (Figure 2).

Esterase (β-esterase; E.C.3.1.1.1)

a) *Melanconium apiocarpum* (SE and DSA) isolates:

Six bands of esterase were detected among DSA and SE isolates of *M. apiocarpum*. Bands at R_f 0.65, 0.73 and 0.80 were present in all isolates. Bands at R_f 0.64 were present in all isolates except PFC 043 and PFC 047. Bands at R_f 0.52 were present only in isolates PFC 050, PFC 053 and PFC 068; while bands at R_f 0.60 were detected only in isolates PFC 050, PFC 053 and PFC 068 (Figures 3 and 4).

b) *Melanconium marginale* isolates

Isolates PFC 049, PFC 051, PFC 078 and PFC 013 had common bands at R_f 0.63 and 0.65 as well as at R_f 0.42 (except PFC 078). Isolate PFC 022 had two bands at R_f 0.37 and 0.70; PFC 025 had four bands at 0.32, 0.35, 0.65 and 0.67; PFC 083 had three bands at R_f 0.34, 0.46 and 0.73; PFC

Table 2. Mycelial fresh weight and acetone powder weight of mycelium of *Melanconium* spp. isolates.

Fungal isolate	Species/Type	Mycelial fresh weight per 500 ml medium (g)	Acetone powder of mycelium per 500 ml medium (mg)
PFC 043	*M. apiocarpum*/DSA	11.95	230
PFC 047	*M. apiocarpum*/DSA	7.25	190
PFC 050	*M. apiocarpum*/DSA	11.91	280
PFC 053	*M. apiocarpum*/DSA	19.19	220
PFC 055	*M. apiocarpum*/DSA	19.91	270
PFC 067	*M. apiocarpum*/SE	16.48	220
PFC 068	*M. apiocarpum*/SE	10.66	200
PFC 071	*M. apiocarpum*/SE	15.21	280
PFC 147	*M. apiocarpum*/SE	15.16	290
PFC 155	*M. apiocarpum*/SE	14.99	270
PFC 022	*M. marg.*/DSA	22.52	490
PFC 025	*M. marg.*/DSA	11.05	500
PFC 049	*M. marg.*/DSA	3.30	400
PFC 051	*M. marg.*/DSA	4.27	430
PFC 078 (ATCC 56907)	*M. marg.*/DSA	3.22	370
PFC 013	*M. marg.*/DSA	4.83	420
PFC 083	*M. marg.*/DSA	15.71	500
PFC 084	*M. marg.*/DSA	4.44	480
PFC 091	*M. marg.*/DSA	6.62	480
PFC 092	*M. marg.*/DSA	16.21	530

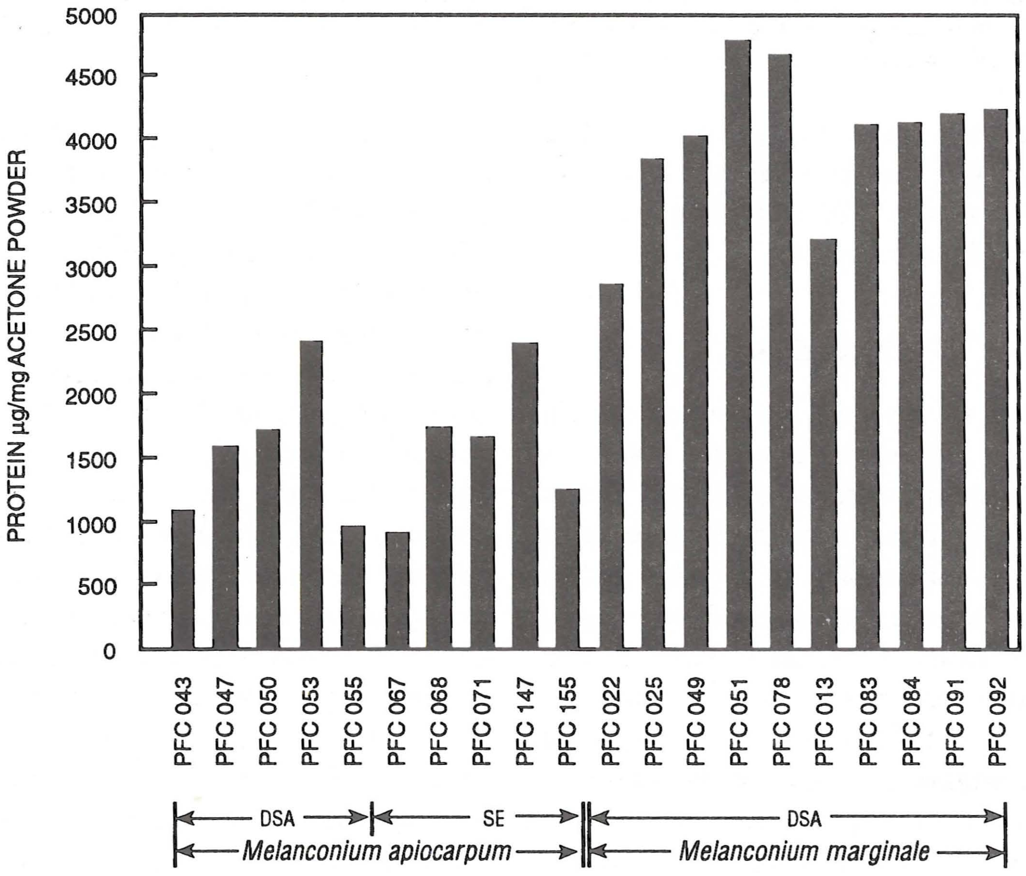

Figure 1. Protein content of *Melanconium* spp. isolates.

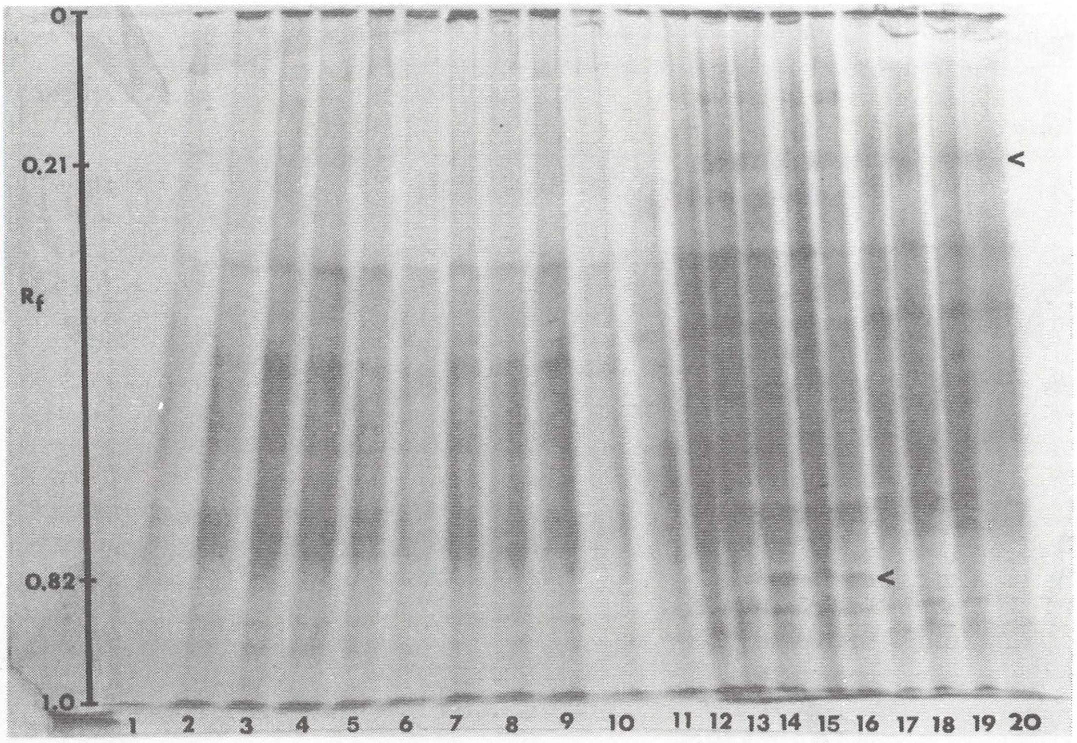

Figure 2. Non-SDS general protein profiles of *Melanconium* spp. isolates: Lanes (1-5), *M. apiocarpum* (DSA); and Lanes (6-10) *M. apiocarpum* (SE); Lanes (11-20) *M. marginale* (DSA). <, protein bands that are different between *M. apiocarpum* and *M. marginale*. Note the presence of three fast moving protein bands at R_f 0.82 (lanes 14, 15 and 16); and slow moving bands at R_f 0.21.

084 had three bands at R_f 0.31, 0.34 and 0.70; PFC 091 had three bands at 0.34, 0.48 and 0.69; and PFC 092 had 2 bands at 0.34 and 0.73 (Figures 3 and 4).

Discussion

The results of this study demonstrate certain differences and similarities among isolates of *Melanconium* spp. with respect to their protein and esterase patterns. Similar results were also obtained by Alfenas *et al.* (1984); their studies on Eucalyptus canker caused by *Cryphonectria cubensis* (Bruner) Hodges isolates showed better differentiation of the isolates on the basis of seven isozyme systems, namely aspartate aminotranferase, β-esterase, hexokinase, malate dehydrogenase, peroxidase, phosphoglucomutase and polyphenoloxidase.

By using SDS-polyacrylamide gel electrophoresis, Sieber *et al.* (1991b) showed no differences between SE and DSA isolates of *M. apiocarpum* from *A. rubra*, but they were able to differentiate *M. apiocarpum* from *M. marginale* isolates. Although the treatment of proteins with negatively charged SDS is widely used in polyacrylamide gel electrophoresis for determining the molecular weights of proteins, the binding of SDS to the polypeptide chains of protein also causes them to unfold and dissociate (Payne 1976). Lin *et al.* (1989) suggested that by keeping the proteins in their natural forms, better differentiation between four biological species of *Armillaria* was possible. Although an extensive quantitative analysis was not performed on the non-SDS total

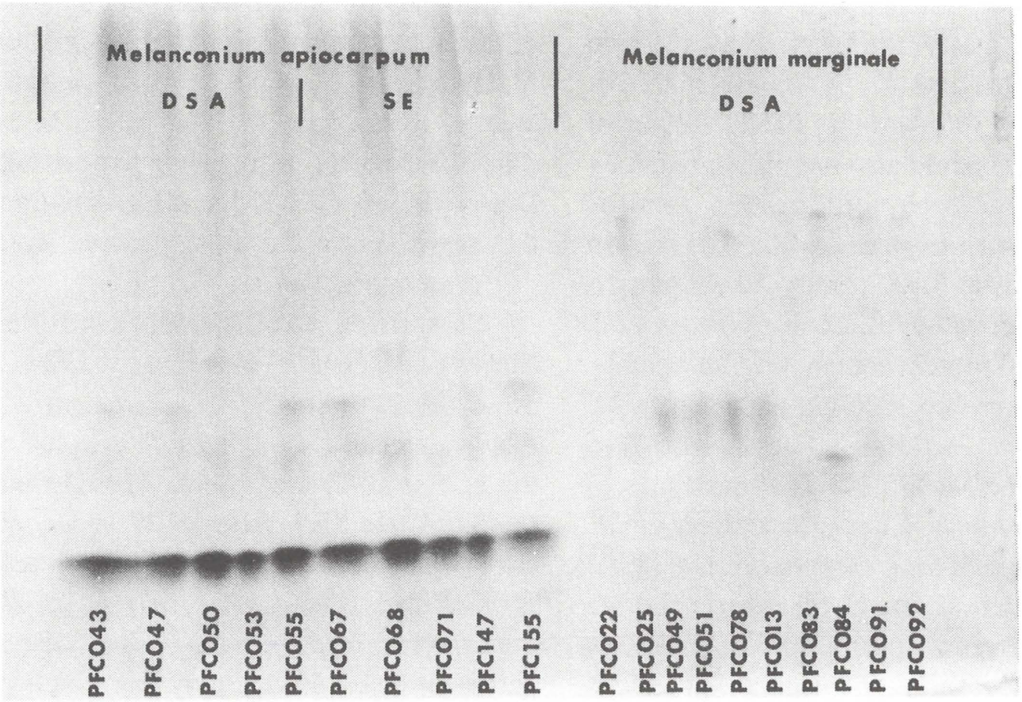

Figure 3. Isozyme banding patterns of β-esterase of *Melanconium* spp. isolates.

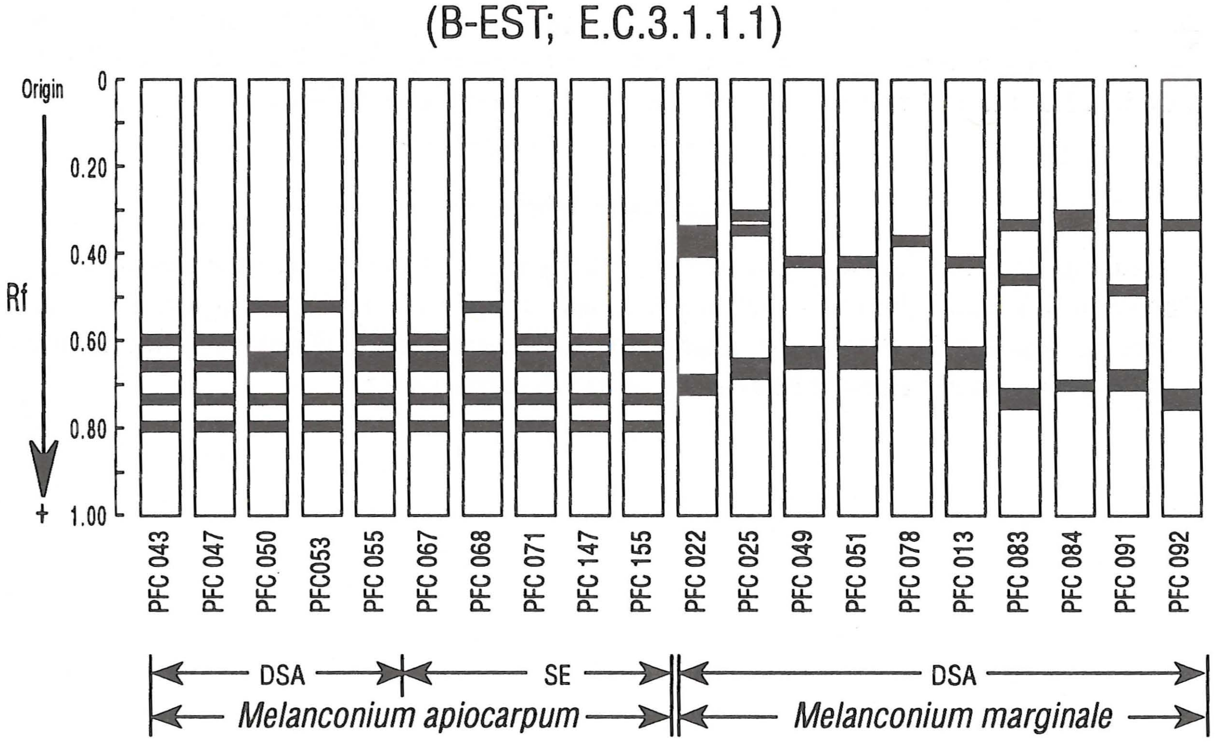

Figure 4. Zymogram and R_f values of the β-esterase patterns of *Melanconium* spp. isolates.

protein assay in the present study, no obvious qualitative differences were found between SE and DSA isolates of *M. apiocarpum*. On the other hand, isolates of *M. marginale* could also be differentiated by non-SDS electrophoretic protein patterns from *M. apiocarpum* isolates based on protein bands at R_f 0.21 and 0.82 (Figure 2).

The mean values of acetone powder yield (Table 2) and protein content (Figure 1) of *M. marginale* were consistently greater than *M. apiocarpum* isolates. There were no differences between SE and DSA isolates of *M. apiocarpum*. Characterization of *M. marginale* and *M. apiocarpum* (SE and DSA) isolates with respect to their relative virulence by inoculating golden delicious apples and measuring the diameter of lesions suggested that *M. marginale* isolates were more virulent than those of *M. apiocarpum* (SE and DSA). No obvious differences were noted among SE and DSA isolates of *M. apiocarpum* (Shamoun, S.F., unpublished data).

Our data on acetone powder yields, protein content, and relative virulence on apples, may indicate biochemical markers to differentiate among SE and DSA isolates of *M. apiocarpum* and between *M. marginale* and *M. apiocarpum* (SE and DSA) isolates. Similar results were also proposed by Bernier *et al.* (1983) for differentiation of the aggressive and non-aggressive isolates of *Ceratocystis ulmi.*

This study confirms the findings of Sieber *et al.* (1991b) and Jensen (1984) that *M. apiocarpum* and *M. marginale* are distinct species. This research coupled with the earlier studies of Sieber (1991b) imply that DSA and SE isolates of *M. apiocarpum* are similar if not identical. Further research is warranted to learn how the species might then be promoted from the endophyte to the pathogen phase so that they can function as mycoherbicides. It must be pointed out that even the most pathogenic fungi on red alder, as for example *Melanconium* and *Nectria* species, are still weak fungi which have to be used in conjunction with specific predisposing factors (mechanical, chemical or environmental) to permit effective biocontrol of red alder.

Acknowledgements

The authors gratefully acknowledge the support by a grant from the Science and Technology Opportunities Fund, Forestry Canada, Ottawa, the technical support of M. Pollard, D. O'Gorman and T.A.D. Woods, the graphics of D. Dunaway and J. Wiens, the photography of G. Jensen, and finally Heather Matson for typing the manuscript.

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Field experiments in forest weed biocontrol

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Abstract

Small field trials were established to test the following possibilities in forest weed biocontrol: (1) stressing of thimbleberry *(Rubus parviflorus)* through defoliation and inoculation with the foliar pathogen *Septoria rubi;* (2) prevention of hardwood regrowth in cut stumps and girdled trees by treatment of fresh wounds with *Chondrostereum purpureum.* Both defoliation of thimbleberry and inoculation with *S. rubi* resulted in increased stress as indicated by increased foliar disease and better growth of underplanted Douglas-fir seedlings, but did not measurably reduce thimbleberry cover in the two years following treatment. Treatment of cut stumps and frilled trees with *C. purpureum* resulted in less resprouting and earlier mortality of treated hardwoods. Evidence was found of seasonal variation in response to *C. purpureum* treatment and of variation in virulence among isolates. Both lines of study indicate that it is feasible to develop biological controls using native plant pathogenic fungi but that improvements in efficacy and consistency are needed.

Introduction

Biological control of unwanted forest vegetation offers a vast array of possibilities. In considering these possibilities, we need to focus on primary needs in forest management to see where major benefits may be derived. Replacement of chemical herbicides is not the only goal in our search for suitable biologicals; a major goal is to provide new management tools for presently unsolved problems.

There are many ways in which biologicals could improve forestry. In vegetation management, there are a number of needs that are only partly filled by conventional practices — manual, mechanical, chemical, or incendiary. We need tools for timely release of conifers before significant growth losses or mortality occur. Chemical herbicides, because of their toxicity to young, growing conifers, usually do not provide us with this option. We need a means of suppressing regrowth of competing species after top-kill by chemicals or fire, or after manual felling. We require less drastic methods than those currently in use — methods which are aesthetically more pleasing as well as less threatening to wildlife, water supplies, soils and vegetation.

The following paper is a review of some field studies undertaken over the past 10 years in both eastern and western Canada. In retrospect, the goals of these experiments appear to address some of the requirements for better forest management. In any case I will present them as if they had been so planned and try to point out how improvements can be made.

Defoliation of weed species

If we could completely defoliate dominant hardwoods or shrubs, especially during the early to middle stage of the growing season when leaf area has almost reached a maximum, we could accomplish two things: temporary release of conifers from overtopping vegetation, and reduction of the competitive advantage of the target species. The degree to which the competitive advantage of the target species is reduced will vary, depending on the target species and timing, and is caused by depletion of stored food reserves in the stems and roots (Coleman 1986; Wargo 1981). We know of several pathogenic fungi and defoliating insects that cause severe defoliation of hardwoods and shrubs, such as *Pollaccia* shoot blight of aspen (*Populus tremuloides* Michx.) caused by *Venturia macularis* (Fr.) E. Muller & Arx and the forest tent caterpillar (*Malacosoma disstria* Hbn.), but methods of managing them as weed biocontrols have yet to be developed.

The obvious approach to biocontrol using foliar disease fungi is to spray the foliage with spore suspensions developed in artificial culture. I have tried this numerous times with little success (Wall 1977, 1983) and undoubtedly there have been many unreported trials. Recently, working with Septoria leaf spot of thimbleberry (Rubus parviflorus Nutt.), successful inoculations were achieved in greenhouse and field trials. Leaf spot severity was noticeaby higher than controls or surrounding unsprayed thimbleberry foliage was observed after applying macerated cultures of *Septoria rubi* West. (Table 1). Furthermore, underplanted Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings showed a positive response to this treatment (Table 1, Figure 1). The growth response was slight and needs to be evaluated over a longer time frame to determine its full significance.

Table 1. Response of thimbleberry and underplanted Douglas-fir to inoculation with *Septoria rubi* and artificial defoliation

	Thimbleberry	Underplanted Douglas-fir[b]		
Treatment	foliar disease rating[a]	Height (cm.)	Terminal shoot length (cm.)	Number of buds[c]
Inoculated	2.6a	41.7a	7.6a	3.8a
Defoliated	2.3a	37.7a	6.9ab	3.5a
Untreated	1.6b	34.6a	5.3b	2.3a

[a] Rated August, 1989, 2 months after inoculation with *Septoria* and 13 months after defoliation. Ratings based on a 0 to 5 scale: 0 = no disease symptoms, 1 = scattered leaf spots or mild chlorosis, 2 = readily detectable leaf spots or other disease symptoms, 3 = about 50% of leaf area diseased, 4 = 60-90% of leaf area diseased, 5 = plants dead or completely defoliated due to disease. Figures followed by the same letter not significantly different at P = 0.05 according to Duncan's multiple range test.

[b] 1-0 stock, size 415, planted in May of 1988 prior to any treatments. Data recorded in November, 1990. Figures within a column followed by the same letter not significantly different at P = 0.05 according to Duncan's multiple range test.

[c] Number of interwhorl buds on current year's terminal shoot in November, 1990.

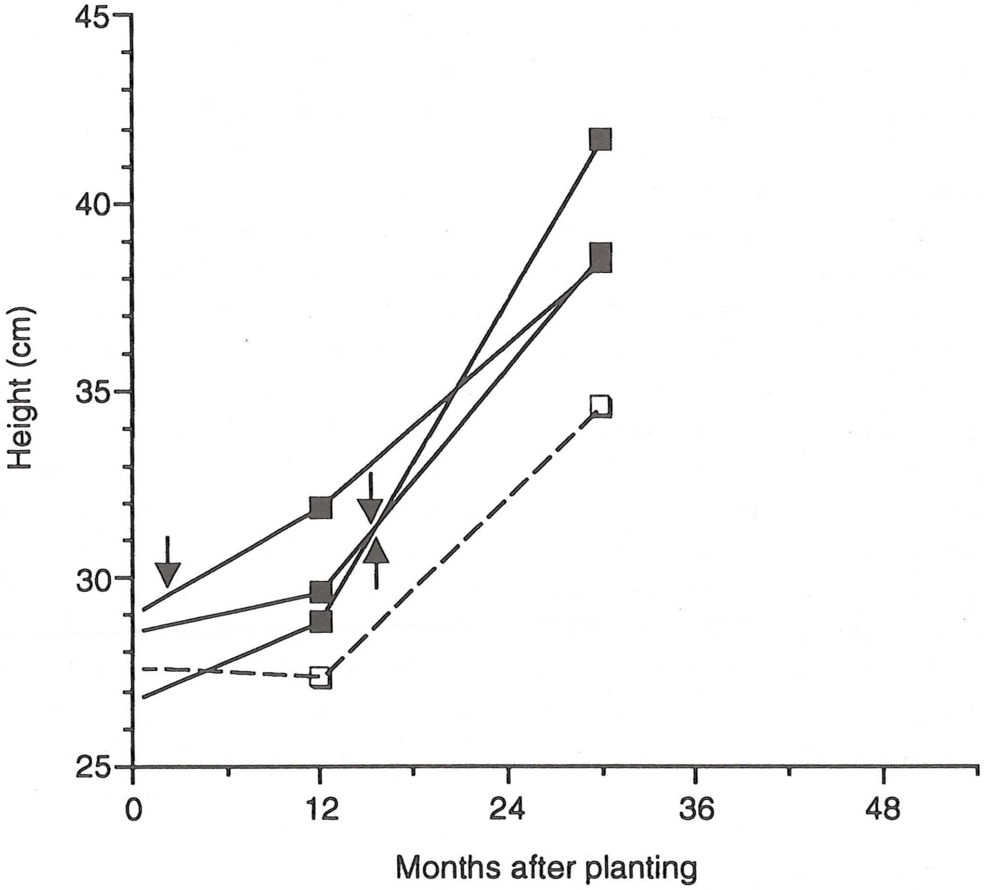

Figure 1. Height growth of Douglas-fir in plots where thimbleberry had been inoculated with *Septoria rubi* (solid lines). Time of inoculation indicated by arrows. Broken line depicts growth in untreated checks.

How effective is defoliation in a vigorous and regenerative shrub like thimbleberry? We defoliated thimbleberry by pulling the leaves off each stem in a large number of plots during early July. Refoliation occurred in two weeks and there was no reduction in cover, height or density. However, the treated plants showed more foliar disease symptoms during the following growing season and there was a slight but positive response of underplanted Douglas-fir seedlings (Table 1).

The results of these two experiments do not provide a new biocontrol, but they do indicate that defoliation is feasible and worthwhile, even for woody perennials with substantial carbohydrate reserves. For effective crop tree release we need more prolonged or repeated defoliation than that achieved in this study, but this should be feasible biologically. There are better defoliators than the *Septoria* diseases and some may be relatively easy to manipulate.

Control of regrowth in woody plants

When hardwoods and shrubs are cut or girdled, they often respond by producing new shoots at the base of the stem. Stump sprouting is a major constraint to the use of manual stand cleaning as a means of employing people in silviculture. Use of chemicals presents a potential hazard to both workers and adjacent crop trees. Use of a pathogenic fungus to prevent regrowth seemed logical since it should be relatively easy to establish such a fungus in cut stumps or frills. A fungus known to possess many of the desired characteristics - establishment in fresh wounds, invasion of sapwood and cambium, and pathogenicity to adventitious shoots - is *Chondrostereum purpureum.* This information was available because of extensive research on this fungus as a cause of silver leaf disease of fruit and ornamental trees.

Cut stumps of a variety of weed hardwoods treated with cultures of *Chondrostereum* often were killed, as evidenced by their failure to resprout or subsequent death of stump sprouts (Wall 1990). Also, young red alder (*Alnus rubra* Bong.) trees, frilled as they would be in a hack-and-squirt treatment and immediately treated with an oil slurry of *C. purpureum* mycelium, died within the next 2 years without resprouting (Figure 2). Many of the controls also died, as might be expected from treatment of frills with oils, but basal resprouting was more frequent in controls than in stems treated with the fungus. Oils seemed necessary to provide a slurry which would remain on the stump or in the frill and retard dessication and direct exposure to ultra-violet light. Motor oil was routinely used, but in some tests other mineral and vegetable oils have been tried and so far appear to be equally satisfactory.

Trees or stumps treated with *Chondrostereum purpureum* often gave rise to abundant basidiocarps 1 or 2 years after treatment. There appeared to be a relationship between the presence of

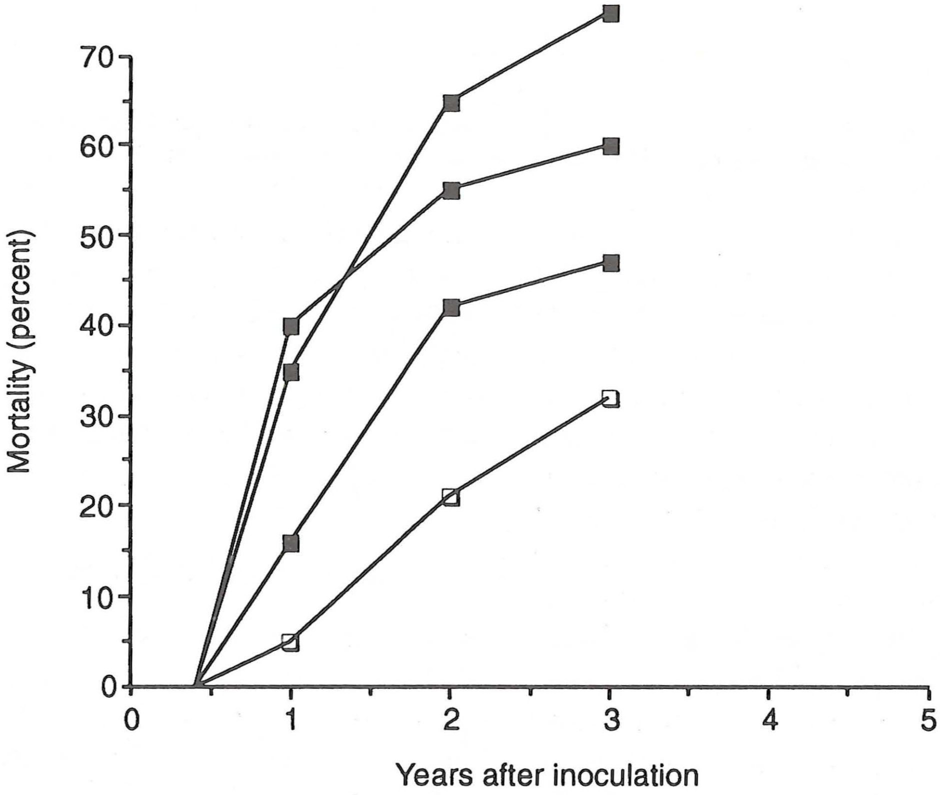

Figure 2. Mortality of red alder frilled and treated with oil slurries of three isolates of *Chondrostereum purpureum* cultures in June of 1988 (solid lines). Broken line depicts mortality in the controls (frills treated with sterile oil slurry).

basidiocarps and efficacy; in tests on Vancouver Island, regrowth occurred in only 2% of the red alder stumps on which basidiocarps were recorded while stump sprouts developed on 19% of the stumps on which no basidiocarps had been recorded; similarly, mortality has so far occurred in 67% of the frilled alder on which basidiocarps were observed while only 16% of the remaining trees have died. After two years, other fungi replaced *C. purpureum,* confirming the accepted view of this fungus as a ruderal species that neither causes extensive decay nor persists for long periods in nature (Coates and Rayner 1985).

Chondrostereum purpureum offers great potential as a mycoherbicide. In the Netherlands, Scheepens and Hoogerbrugge (1988) have demonstrated this in the control of unwanted *Prunus serotina* Ehrh. DeJong *et al.* (1990) and Wall (1991) have addressed some of the perceived risks of exploiting this fungus. The fungus may become registered in the Netherlands as a mycoherbicide—a first for forestry. In the meantime, its potential in Canadian forestry should be considered seriously, given that millions of dollars are spent annually on manual and mechanical brush removal and much of this effort is lost through regrowth. In the meantime however, we should not stop looking for better mycoherbicide candidates, such as fungi that are more selective, produce fewer spores, or invade rhizomes and root systems.

General conclusions

A common feature of the above trials is that they were all undertaken with well known fungi. *Chondrostereum purpureum* and *Septoria* species have been studied for decades as pathogens of economic crops. Choice of these fungi is an advantage in that there is available background information that will assist in their deployment. A disadvantage is that their use will be restricted to areas some distance from susceptible economic crops. De Jong *et al.* (1990) have addressed this concern with respect to *C. purpureum* and concluded that its use as a forest mycoherbicide would not significantly increase the risk to susceptible fruit crops at distances greater than 500 m.

The above ideas and experiments are only a beginning and are intended to stimulate rather than circumscribe further efforts. Hopefully, research in forest vegetation management will evolve beyond the "silver bullet" or "one-on-one" approach and incorporate biological controls into integrated management. This can only come with better understanding of the ecosystems in which we are working. Furthermore, the fixation on development of commercial products for what may be small markets should not inhibit research, since there are other means of deployment than the commercial route.

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Biochemical characterization of the potential mycoherbicide *Chondrostereum purpureum*

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Abstract

Proteins were extracted from acetone powders of fungal mycelia and were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The detection, quantitation and matching of protein bands among the isolates were carried out by a laser-based scanner interfaced with appropriate software. In a typical experiment, the number of bands that could matched among the isolates was as high as 56. An average linkage cluster analysis of protein patterns of isolates showed that while isolates could be grouped, there is no apparent association of this grouping with geographic origin.

Introduction

It is evident from the previous presentations of my colleagues in the mycoherbicide program at the Pacific Forestry Centre that if a fungus has to be registered as a mycoherbicide, there is a need for a clear understanding of the fungus. How does it work? How are its various isolates related? And is there any variation among isolates from different areas? The isolates of *Chondrostereum purpureum,* which are now being actively examined by R. Wall for ability to control vegetation, are morphologically indistinguishable. Biochemical methods such as comparisons of zymograms or total mycelial protein profiles of fungi are widely being used to distinguish fungi. In this presentation, I will describe the analysis of the total protein profile of *C. purpureum* rather than a group of selected enzymes. Using this approach, we have shown previously that the isolates of a fungus pathogenic to insects, could be distinguished. Similarly, work in collaboration with Charles Dorworth and Thomas Sieber showed that the isolates of endophytic fungi could also be distinguished by their protein profiles.

Methods

Geographic origin of isolates

In this study we examined a total of 18 isolates provided by Ron Wall of the Pacific Forestry Centre. Two isolates were from New Brunswick, four were from southeast corner of British Columbia, and twelve were from Vancouver island.

Culture of *Chondrostereum purpureum*

Isolates were first grown in agar plates, a small section from the periphery of the actively growing culture was used to inoculate liquid culture. Mycelia were harvested and lyophilized. Acetone powder was prepared from the lyophilized mycelia. To extract protein, 5 mg of acetone powder was extracted with a buffer consisting of sodium dodecyl sulphate (SDS) and mercaptoethanol. The extraction procedure included boiling the sample in the extraction buffer for 5 minutes, and stirring for another 5 minutes at room temperature. (Heating the sample would inactivate proteolytic enzymes that might have activated during the extraction and degraded proteins which might have given rise to an artificial protein pattern.) After centrifugation, the supernatant was stored frozen.

Protein analysis

Prior to electrophoresis, the protein content of each extract was determined by a method developed at the Pacific Forestry Centre. Thus, to allow comparative analyses of the isolates, each sample on an equal protein basis was subjected to SDS-polyacrylamide gel electrophoresis. Although it is an electrophoretic separation, because of the presence of SDS, proteins were indeed separated based on their size. Since we used a small protein sample for maximum possible resolution, we used silver stain which detects proteins in the nanogram range. Stained gels were scanned with a laser-based scanner and quantitated, and the molecular weight of each band was determined from comparison with standard marker proteins.

Results and discussion

Each isolate showed a complex mixture of proteins of molecular weights ranging from 14 to 114 kDa. In a typical gel pattern, a total of 56 matched protein bands were observed. A majority of these proteins are common to all isolates, as reflected by their high similarity coefficients. Similarity coefficients were calculated by dividing the number of matched bands in two isolates by the total number of bands detected in the two isolates, and multiplying the result by 200. The highest similarity coefficient (93.1) was found for isolates 2065 and 2047; the lowest similarity coefficient (74.0) was shown for the pair of isolates 2075 and 855. In another series of experiments, triplicate cultures of a given isolate were compared on the same gel. Because of the limitation of the gel size, only six isolates were compared at a time. For the calculation of similarity coefficients in these experiments, a band detected in at least one of the triplicate cultures of a given isolate was taken into consideration in the the matching of the bands. Consequently, higher similarity coefficients were observed.

For cluster analyses, the similarity coefficients obtained in three different experiments in which all 18 isolates were compared on the same gel were averaged. The variation of similarity coefficient between these three experiments is indicated by the standard deviation which ranged from 1% for isolate pair 855 and 2090 to 15% for the isolate pair 2064 and 2075. A dendrogram based on the average linkage cluster analysis of the similarity coefficients showed that there were two major clusters, and that isolate 855 was outside these two clusters. The cluster analyses revealed that isolates could be grouped and this grouping is independent of their sources or geographic origin. (Detailed procedures and results will be published elsewhere.)

Integrating plant pathogens into weed management systems

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Abstract

The successful integration of plant pathogens (as biocontrol agents) into weed management systems will require progress in research to ensure product efficacy and stability. Inconsistency in achieving economic weed control levels is a bottleneck currently constricting commercialization of a number of promising fungi as mycoherbicides. The inconsistency in efficacy can be attributed largely to unfavorable environments at periods critical for infection. To overcome this problem, one systemized approach is to provide a favorable micorclimate for the fungi through formulation, the use of specialized diluents or carriers, and specialized application techniques. The favorable microclimate can be provided, at least for some fungi, by use of an invert (water-in-oil) emulsion as a carrier to protect the propagules against dessication. However, the invert-emulsions are mayonnaise-like in consistency and so must be applied with air-atomizing nozzles. Although technologically successful, the invert emulsions have some disadvantages that offer opportunities for further research: they create problems with cleanup of spray rigs; sometimes they are phytotoxic to desireable plants; and the treatments may require too much volume/unit area for practicality. None of these problems is insurmountable. A slow-drying granule sprayable with conventional equipment has been developed and is being evaluated in our lab; preliminary results indicate that these granules may provide at least a partial improvement in this technology. Additional research is ongoing in our lab and elsewhere to evaluate possible combination of mycoherbicides with low rates of various herbicides.