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Biological Control and the Management of *Calamagrostis canadensis* (Bluejoint Grass)

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Strategic Importance

Calamagrostis canadensis, commonly called bluejoint or marsh reed grass, has become a serious competitor of forest regeneration in western boreal Canada. Bluejoint is a perennial, rhizomatous grass native to North America, growing up to 2 m tall from creeping rhizomes. It is a pioneer species and can increase greatly in abundance following disturbances such as fire, flooding, insect outbreak, windfall or timber harvesting. Harvesting often results in the establishment of an herbaceous community dominated by *C. canadensis*, particularly on moist rich sites characteristic of the region. In the absence of shade, this grass can form continuous dense stands and develop a heavy, deep thatch. A bluejoint-dominated disclimax community may persist for 25 to 100 years or more, limiting re-establishment of other plant species and altering forest succession. However, without further disturbance, *C. canadensis* more often loses dominance of sites within 10 to 20 years. With the development of a woody shrub and tree canopy, the grass will die back and eventually become fairly inconspicuous as a forest understory species.

Established, mature bluejoint grass,* Calamagrostis canadensis, *can suppress conifer seedlings (near Fort St. John, British Columbia).

Calamagrostis canadensis is found throughout Canada in wetlands, open uplands, and forests of spruce, pine and mixed hardwoods. Although the grass can be found in a wide variety of habitats, its best growth occurs under moist to wet, nutrient-medium to nutrient-rich, and open (high light) conditions. It is associated with wet-temperate and cool-mesothermal climates, and is particularly successful in the boreal forest zone of western Canada and southern Alaska. As a native plant, it is important in many boreal, arctic and wetland ecosystems. *Calamagrostis canadensis* furnishes browse for bison, moose, elk and other ungulates and provides food and habitat for bears, small mammals and birds. Bluejoint is also used as a forage crop for domestic livestock, although this is somewhat limited due to the high silica content of the grass. *Calamagrostis canadensis* helps to control erosion, provides stability to streambanks, maintains water quality by filtering runoff, and reduces flooding through increased evapotranspiraton. Bluejoint has been used to successfully rehabilitate wetlands and to revegetate areas disturbed by industrial activity.

Although *Calamagrostis canadensis* is a natural part of many ecosystems, large clearings caused by disturbance have enhanced the success and spread of this grass. The tall, dense stands of grass that develop following disturbance can significantly inhibit regeneration of tree species such as white spruce and trembling aspen, exclude agricultural and range crops, and reduce woody browse species important to moose and other ungulates. Because of its characteristics on disturbed sites, *C. canadensis* has become a problem weed species in white spruce plantations in the boreal forest of western Canada following harvesting.

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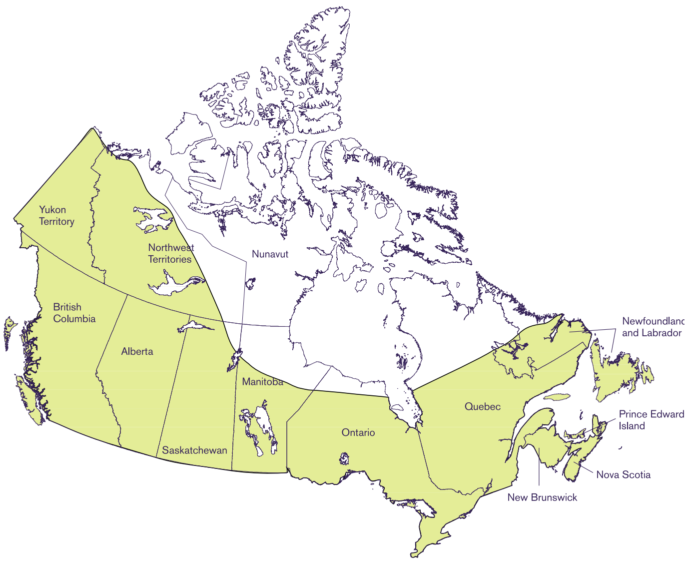
A number of techniques have been used to control this grass in forest regeneration including chemical herbicides, mechanical site preparation and livestock grazing. Biological control using native plant pathogens provides another option.



Distribution of* C. candensis *in Canada.

Mechanism of Calamagrostis canadensis *competition with crop species*

A highly invasive species, *C. canadensis* colonizes and dominates disturbed sites through rapid rhizome growth, seed dispersal, and sprouting of seeds buried in the soil. Warm soils, abundant nutrients and moisture, soil disturbance, and high light levels allow the grass to grow profusely in short periods of time. Wind-blown seeds can readily colonize other sites. The seeds are light and easily dispersed, even in winter conditions, when the tall seed heads are often exposed above the snow, and full occupation of a site can take place in as little as three years.

Calamagrostis canadensis competes with regenerating seedlings for limited site resources including space, nutrients, and light. The heavy accumulation of litter that results from the dieback of shoots and leaves each year also causes several problems for regenerating seedlings. Snow can compress the litter, smothering and crushing tree seedlings. The litter can cause seedling growth deformities (exaggerated lateral growth and pitch over), and inhibit seed germination. Accumulated litter can insulate the soil, delaying spring thaw and keeping soil temperatures cool throughout the growing season.

Management methods for Calamagrostis canadensis

Forest managers have a number of vegetation control options to chose from during harvesting and regeneration. Chemical herbicides, including glyphosate (Vision®) and hexazinone (Velpar®), have been used to temporarily control *C. canadensis* infestations, but potential for damage to crop species, public pressure, and concerns about impacts on non-target species have reduced herbicide use. Prescribed burning can be useful in controlling the grass if the soil is burned to the mineral layer and the burn is hot enough to destroy grass rhizomes; however, lighter burning will stimulate growth of *C. canadensis*. Mechanical site preparation methods that scalp the soil surface must also be deep enough to remove the rhizomes in order to be effective. Plowing has been used to effectively control *C. canadensis*, but, as with deep burning and scalping, there is concern that the resulting soil disturbance may have long-term impacts on site productivity. Mechanical site preparation methods that mix the soil are usually unsuccessful and may even encourage the grass by stimulating rhizome sprouting from cut segments. Creating planting mounds capped with mineral soil (inverted mounds) can effectively reduce competition from *C. canadensis* and reduce snow press damage. Manual cutting and sheep grazing can control competition from the grass when treatments are repeated throughout the growing season and for several years following planting. Applying biodegradable mulch mats around seedlings before the grass is established may provide effective control, but this method has not received much attention to date.

A principal objective in vegetation management is to reduce competition and allow desired plants to grow freely. In forestry, this means effectively channeling site resources into forest products rather than non-crop species. Suppression of non-crop species is often preferable to eradication, which can result in additional problems including soil erosion, nutrient loss, and disruption of normal ecosystem function. Alternative practices that control *C. canadensis* by suppressing growth or reducing abundance are being studied. One alternative control method is planting replacement vegetation, which generally consists of low-growing herbaceous plants that effectively compete with the grass without significantly affecting the growth of the crop trees. Replacing clearcutting with alternative harvesting methods such as partial cutting or variable retention is also being tested. *Calamagrostis canadensis* is a moderately shade-intolerant species; thus growth and flower production is less vigorous under a forest canopy and plants are less likely to proliferate following partial overstory removal. Planting larger stock and increasing stocking densities may also reduce losses from grass competition. Biological control (biocontrol) is another alternative method that may be used to suppress the growth of this invasive grass.

The biological control option

Biocontrol can be divided into two types: classical and inundative. Classical biological control refers to a pathogen or insect introduced from another location to establish a persistent weed-suppressing epidemic. The classical approach is more often used to control introduced pests and can sometimes have unintended negative effects on ecosystems. Inundative biocontrol uses endemic or native pathogens applied to a pest population in quantity in much the same manner as traditional control agents. Inundative biocontrol organisms are also known as “bioherbicides” when applied to weeds, or more specifically as “mycoherbicides” when the biological control agent is a fungal pathogen. Inundative biocontrol has been used with success in agricultural systems and is being studied for control of several reforestation weeds in Canada. A number of fungal and bacterial pathogens native to western forests are being investigated for control of *C. canadensis*.

Using fungal pathogens to limit competition

Approximately 90 species of fungi, some causing important diseases, have been reported on *Calamagrostis* species in Canada and at least 30 fungi are known to cause disease in *C. canadensis*. Over 240 different fungal isolates have been collected from diseased *C. canadensis* plants in northern British Columbia, Alberta and the Northwest Territories. An extensive library of the fungal isolates has been established by the Canadian Forest Service with many isolates deposited in long-term (cryogenic) storage at Pacific Forestry Centre. A searchable computer database has been developed for the fungal collection.

Several potential fungal biocontrol agents for *C. canadensis* have been identified from the fungal collection. Isolates were compared for virulence on *C. canadensis* seedlings in repeated trials over three years, and several isolates have proven pathogenic to the grass. *Colletotrichum* caused up to 54% leaf area damage, while *Fusarium* isolates caused as much as 78% leaf area damage when applied as foliar sprays. While some of the *Fusarium* isolates infected a range of herbaceous plants, *Colletotrichum* was much more host specific. None of the potential biocontrol fungi infected spruce trees.

When produced in large-scale bag cultures,* Fusarium avenaceum *can effectively limit shoot and root growth of* C. canadensis *enabling conifer seedlings to become established.

Virulence of the fungal isolates varied with the way the inoculum was formulated and the environment in which the fungi and grass were grown. The presence of allelopathic chemicals released as leachate from *C. canadensis* litter, and interactions between different fungi, also influenced the virulence of the biocontrol pathogens. In field trials using *Colletotrichum* and *Fusarium* isolates, both fungi caused foliar symptoms, but the plants were able to recover from the infection. However, significant growth reduction occurred in plots with grass mulch, particularly when fungi were applied in winter where snow was compacted on the plots. Formulation of the fungal inoculum used in the field trials appeared to limit the potency of the fungal treatments. Research has continued to improve formulation and delivery of biocontrol organisms, and has led to the development of a micro-encapsulation technique that results in fungal inocula with improved viability and virulence.

Similar studies to uncover microbial control agents for management of *Calamagrostis canadensis* are being conducted at the Northern Forestry Centre of the Canadian Forest Service, in Edmonton, Alberta. These studies focus on the use of a low-temperature basidomycete (LTB) fungus that is native to western forests and causes cottony snow mold disease in the grass. In addition to controlling bluejoint on newly harvested sites, the LTB may be an effective option for remediation of cutblocks where accelerated decline of established perennial grass populations is desired.

Using deleterious rhizosphere bacteria to limit competition

Deleterious rhizosphere bacteria (DRB) are non-parasitic, root-associated plant pathogens that inhibit root or shoot growth by producing phytotoxic or plant suppressive compounds that are absorbed by plant roots. Deleterious rhizosphere bacteria can also impair seed germination and delay plant development. These bacteria aggressively colonize plant roots as well as plant residues but generally do not produce visible disease symptoms. In agricultural systems, naturally occurring deleterious rhizosphere bacteria have been used to reduce specific weed infestations without reducing crop yields.

Numerous rhizobacteria have been isolated from *C. canadensis* plants collected at boreal forest sites throughout British Columbia, northwestern Alberta and the southern Northwest Territories. Over 500 isolates have been identified and characterized using fatty acid methyl ester (FAME) analysis and carbohydrate utilization profiles. The rhizobacteria from dif-

White spruce planted on inverted mound in bluejoint infested site near Ft. St. John, British Columbia

ferent sites have been compared and related to soil and site characteristics. Similar to the fungal collection, the bacterial isolates are in cryogenic storage at the Pacific Forestry Centre and a searchable computer database has been developed for the collection.

Over 500 rhizobacteria isolates have been screened for biocontrol activity against *C. canadensis* as well as container-grown spruce, pine and aspen. Of the rhizobacteria collected from *C. canadensis* in British Columbia, 20% suppressed growth. In laboratory tests, root growth was reduced by 32-54%, shoot growth by 16-61%, and germination by 26-70% when *C. canadensis* was grown on media containing the cell-free filtrates of growth-suppressive rhizobacteria. Live cell cultures of several isolates were found to completely inhibit germination of *C. canadensis* seed. In greenhouse tests, the rhizobacteria caused various responses ranging from slight stimulation to 30% reduction in seedling biomass. Again, culture conditions, nutritional factors and inoculum formulation had significant influence on biocontrol performance of the bacterial pathogens. Bacteria suppressive to *C. canadensis* caused no damage or growth reduction when applied to white spruce, lodgepole pine or aspen. Deleterious rhizosphere bacteria are aggressive colonizers of plant roots, can selectively suppress *C. canadensis*, and function as direct delivery systems for the suppressive compounds they produce. Research continues to improve formulation and application technology for rhizosphere organisms.

Co-inoculation strategy using fungal pathogens and deleterious rhizobacteria

Calamagrostis canadensis is a difficult target for both chemical and biological control agents. Leaf sheaths protect the shoot apical meristem and leaves can regenerate from the basal meristems following defoliation. Even when outer, older leaves are killed or succumb to disease, the arrangement of separate vascular bundles allows continued translocation within the plant and helps to protect the stem and younger leaves. Additionally, *C. canadensis* has a high regenerative capacity due to underground rhizomes that store energy in the form of carbohydrate and remain protected in the soil. Deleterious rhizobacteria can overcome these defenses by attacking roots and acting on the entire vascular system of the grass. However, the level of growth inhibition produced by rhizobacteria alone may be insufficient, especially because the principal problem caused by *C. canadensis* may relate more to straw accumulation than to competition. Applying deleterious rhizobacteria in combination with the foliar pathogens provides a means to overcome plant defenses and an opportunity for synergistic enhancement of disease.

In greenhouse tests, selected DRB applied in combination with *Fusarium avenaceum* resulted in biomass reductions greater than 75% in *C. canadensis* seedlings. This was highly significant compared to about a one-third reduction in aboveground biomass when either *Fusarium* or the rhizobacteria were applied alone. Similarly, combined foliar and soil treatments reduced shoot height by 70-87%, while suppression of shoot growth was less than 25% in the individual treatments. Mean root length of co-inoculated plants was less than half the mean root length of plants treated with either the fungal or best performing bacterial isolates. Further, when the bacterial inoculum was applied four days after foliar treatments, leaf damage increased 10% and shoot growth was reduced 25% compared to same day co-inoculations. By attacking both the root and shoot system, combined impact of *Fusarium* and DRB was much more effective at suppressing *C. canadensis* than single species inoculations. Co-inoculation with *Fusarium* and DRB did not cause foliar or root damage nor effect growth of white spruce, lodgepole pine or trembling aspen seedlings.

Calamagrostis *reponse to biological control.*

Management interpretations

The biocontrol pathogens would likely be applied as a bioherbicide using an inundative approach and would need to be applied shortly after harvesting and early in the spring as new shoots emerge. Evidence suggests that DRB may reside in grass rhizosphere and straw residues for some time; however, only very low levels were recovered from soils 26 days after treatment. Similarly, *Fusarium* would quickly equilibrate to endemic levels following application, so there are no long-term or residual effects. Alternatively, snow mold may persist in the soil for up to 3 or 4 years, and may be useful for longer term control. It may not be necessary, or even desirable, to kill the majority of grass plants but to simply suppress growth, which will reduce foliar biomass, slow rhizome production, reduce plant vigor, and lower seed production.

The main advantages of inundative biological control with native plant pathogens are:

- it can be used in situations where chemical herbicides are not desired
- it uses endemic organisms and is environmentally benign
- it is effective in high-straw areas where chemicals perform poorly
- it does not injure the tree crops, as herbicides sometimes do
- it does not result in soil compaction as mechanical methods sometimes do
- it suppresses the grass but does not eliminate it, and thus retains the ecological function of *C. canadensis.*

Studies to measure the effects of inoculation on foliar biomass and rhizome growth, the type and amount of inoculum needed to achieve efficacy, and the response to various environmental parameters are underway. Further studies to elucidate the mode of action, measure growth response of trees following release from competition and to integrate biocontrol with other silvicultural practices will follow.

Developing an integrated vegetation management strategy

It is important that forest managers recognize the threat of *C. canadensis* invasion prior to harvest, so that silvicultural prescriptions that prevent post-harvest *C. canadensis* infestations can be implemented. The likelihood of invasion and site colonization can be predicted based on a variety of factors. If bluejoint is growing in the vicinity, or if the grass is well distributed in the forest understory prior to harvesting, then the probability of invasion is high. Because the grass grows best on moist-to-wet, nutrient-medium to nutrient-rich sites, such sites are more prone to invasion. In British Columbia, *C. canadensis* can dominate sites in the Interior Cedar-Hemlock (ICH) and Engelmann Spruce-Subalpine Fir (ESSF) biogeoclimatic zones, but is especially problematic in the Sub-Boreal Spruce (SBS) and Boreal White and Black Spruce (BWBS) biogeoclimatic zones in the northern part of the province.

In situations where grass invasion is deemed likely, practices that minimize invasion and control the spread of *C. canadensis* should be part of silvicultural prescriptions. For example, using partial cutting rather than clearcutting could reduce the risk of invasion or serious infestation. Practices, either during harvesting or site preparation, that mix the soil will likely encourage rhizome sprouting and germination of buried seed, as will light prescribed burning. Such practices should be avoided where grass invasion is a possibility.

Biocontrol, and weed management techniques in general, often work best when used in combination with other silvicultural practices. A major defense against infestation is the immediate planting of a site following disturbance. The use of large caliper stock and higher than minimum stocking levels on inverted mounds can increase the chances of seedling survival and reforestation success. One-year and three-year survival checks are recommended with accompanying fill-in or replanting if necessary. Vegetation control treatments need to be applied soon after disturbance to control *C. canadensis* before it becomes well established.

Although no biologicals are currently registered for use in forestry, an integrated strategy that combines biological control with low-impact silvicultural techniques is envisioned. A strategy to proactively control infestations before they begin will be most successful at reducing plantation failures and increasing productivity of conifers and hardwoods in areas of high risk for *C. canadensis* invasion.

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