Biological Control of Red Alder (*Alnus rubra*) with the Fungus *Nectria ditissima*[1]

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Abstract. With few exceptions, north temperate North American weeds are native species that have proliferated following site disturbance. Social pressures are rapidly eroding the availability of essential silvicultural tools such as chemical herbicides and prescribed burning. Biological control with microorganisms in forestry is at an early stage of development. An inoculation strategy involving a fungus pathogen (PFC-082: *Nectria ditissima* Tul./ALDERKILL™), its formulation for biological control of *Alnus rubra* Bong. in the form of the PFC-MYCOCHARGE™ and a newly devised instrument for bioherbicide delivery into woody stems (PFC-ALDERWAK™) are described. The overall strategy and implements are potentially useful for the delivery of any biological or translocatable material into amenity or orchard trees as well. PFC-082 was the single isolate of *N. ditissima* tested that produced 100% infection and incited the formation of red alder cankers longer than 0.5 m in 30 mo when inoculated by the method described herein. Natural infections by this pathogen occur on fewer than 1% of red alder stems in the forest.

Additional index words: Augmentative, inundative, pathogen, Ascomycotina, mycoherbicide, patent, vegetation management.

INTRODUCTION

The technology involved in biological control of agricultural weeds with microorganisms is well advanced after only 20 to 30 yr of research (6, 7, 8, 9, 12). The classical approach, involving the application of living agents imported from the area to which the weed was indigenous, is generally preferred for noxious or intrusive weeds (14). Techniques termed variously the inundative, augmentative, and enhancement procedures of formulation and application are ordinarily applied where indigenous weeds are controlled with native biocontrol agents (3, 6, 7, 8, 9, 12, 14) or mycoherbicides (11).

Noxious or intrusive weeds cause occasional difficulties in forest renewal in north temperate North America (10). The most important forest weeds in Canada and the northern U.S.A. are native species that proliferate following complete forest harvest, which accounts for 90% of all Canadian logging (1). Deforestation also accompanies industrial activities such as mining, transmission line installation, etc., and can occur as a consequence of natural disturbances (2). The generalized forest weed is a by-product of cultural/industrial treatment or similar alteration of the forest biosphere such as harvest of a less competitive species designated as the crop. Harvest of forests as individual crop plants eliminates the common agricultural problem of product contamination by weeds. Indigenous forest weeds are a part of the natural forest community, even in the industrial forest, and particularly so when they occur at endemic population levels. The underlying philosophy behind the Pacific Forestry Centre (PFC) Forest Weed Biocontrol Program is constraint rather than elimination of forest weeds. Any non-crop plant not in intensive and direct competition with the designated crop species is a legitimate component of the forest biosphere and a contributor to biodiversity.

Research in forest weed biocontrol was initiated to generate an alternative to chemical control in the event primary herbicides were deregistered or their use discouraged, as occurred with 2,4,5-T [(2,4,5-trichlorophenoxy)-acetic acid]. An increasing number of countries and forest areas within North America are effectively herbicide-free, either by imposition of prohibitory statutes or because special interest groups have made the use of pesticides impractical or effectively impossible. Similarly, some degree of weed control is achieved as an ancillary benefit of prescribed burning of logging waste. Increasingly organized public reaction has resulted in the reduction or termi-

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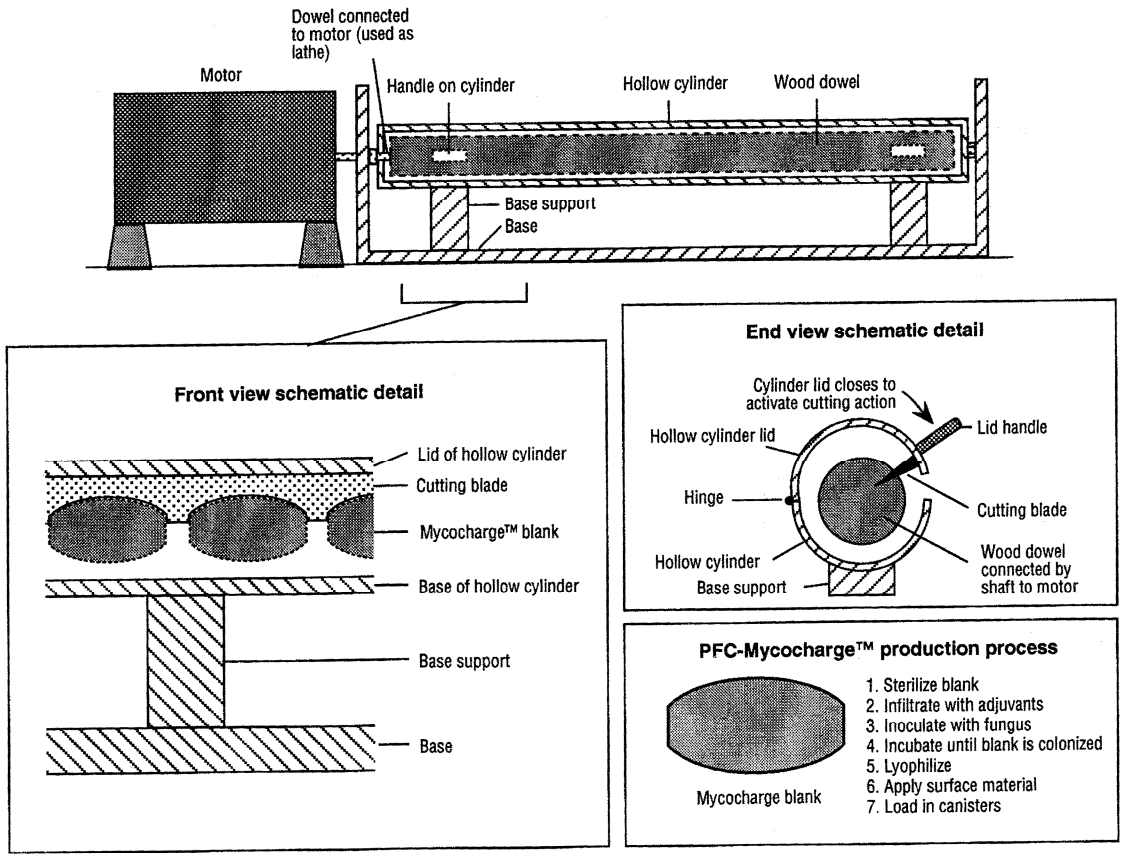

Figure 1. Schematic of equipment and the process used to produce the PFC-MYCOCHARGE™, an inoculation pellet intended for inoculation of hardwood stems with biocontrol fungi.

filla[3]. Stems were selected at random from those in a red alder stand located within a restricted entry forest (The Victoria Watershed, Lat. 23°42′N; Long. 48°46′W), as specified in Canadian Federal Regulations for testing of biological herbicides.

Data records. This was intended as a screening test of a variety of Ascomycotina. Only the most successful isolate, being that intended for commercialization, is reported here, along with a second isolate of the same species which is included for comparison (Table 1). Pathogenicity of the isolates was determined as capacity to infect the host. Relative virulence of isolates was estimated as capacity to colonize host tissue, recorded as length and width of cankers after 30 mo of incubation in the stems. All data were subjected to analysis of variance by SAS[4] (Table 1).

DISCUSSION AND CONCLUSIONS

Two tools designed to achieve stem injection with liquid herbicides were described by Gilmour (5), neither of which is used extensively in its original form. Monsanto Chemical Co.[5] has marketed the EZ-JECT™ Lance and EZ-JECT™ capsules (these being herbicide-loaded .22 caliber firearm cartridge cases) which have proved successful in single and multiple injections of hardwood stems with herbicides. Similarly, Pace Chemicals Ltd.[6] has marketed the GEL CAP™ Application Tool and the GEL CAP™ herbicide cartridge. The GEL CAP™ cartridge is literally screwed into the tree with the aid of the applicator mounted on a portable hand drill, releasing a liquefied preparation of herbicide into the stem. Larger stems require multiple

[3]Polyfilla, M. C. Lepage Lte., Boucherville, Quebec J4B 1V5.
[4]SAS Institute Inc., Box 8000, Carey, NC 27511 U.S.A.
[5]Monsanto Canada Inc., P.O. Box 1159, Delta, B.C. V4M 3T3.
[6]Pace Chemicals Ltd., 8321 Willard St., Burnaby, BC V3N 2X3.

nation of burning in parts of both Canada and the U.S.A. (14).

A vacuum has been created thereby wherein the development of new vegetation management strategies such as intensive forest culture or biocontrol is essential. The chemical companies are reluctant to support such research, thereby placing the responsibility for the development of biological control strategies within the public sector. As a consequence, forest biocontrols are being developed almost entirely as replacements for chemical herbicides, based upon the assumption that chemical herbicides *will* become increasingly unavailable within a decade or less. Biological controls might otherwise and *far more logically* be viewed as one of several components of integrated forest vegetation management.

The majority of indigenous microorganisms that are prospective biocontrol agents are in balance with their host plants and do not ordinarily cause widespread epidemics. Success of inoculations is often as much a function of the method of formulation and deployment of such biocontrol agents as it is of the virulence of the pathogen.

Attempts by the author over several years to achieve hardwood tissue colonization with such opportunistic fungi using standard pathology methods, e.g., deposition or insertion of spores and/or mycelium, with or without adjuvants and agar medium, were unsuccessful, whether performed in the field or within moist chambers. Success was gained with a modification of a technique employed earlier to permit infection of pines with another species of Ascomycotina (2) whereby pathogens were permitted to colonize wood dowels which were inserted directly into stem tissues. Substratum colonized by one organism is often somewhat or totally resistant to colonization by a second microorganism, at least during the initial stages of infection or colonization. Further, the substratum will serve as a nutrient base from which the intended biological control agent can proliferate into adjacent tissues.

In the present case, the fungus *Nectria ditissima* Tul. was chosen as the primary test subject, being a known pathogen of red alder (4). Previous tests with several isolates of *N. ditissima* had failed to achieve stem infection except where colonized substratum was used. Objectives were two: 1) Test the candidate biological control agent in the field for efficacy, and 2) develop a formulation and inoculation strategy that would facilitate production and deployment of the biocontrol agent at commercial levels.

RESEARCH PROTOCOL

Candidate biological control agents. All of the fungi used in this study were of the class Ascomycotina. Only a single example—*Nectria ditissima* Tul., a narrow spectrum pathogen specific to the target weed tree red alder (*Alnus rubra* Bong.) (4)—will be considered here. The other fungi yielded only minor or erratic host responses. The isolate of *N. ditissima* which proved to be an effective pathogen is PFC-082/ATCC 74260 (patent protected deposition—U.S. Patent 5,340,578; Canadian Patent applied for); planned for commercialization as PFC-ALDERKILL™. The essential formulation (PFC-MYCOCHARGE™) and inoculation strategy, and the invention for inoculation (PFC-ALDERWAK™) are included in the patent. For comparison, results of inoculation with a second isolate of *N. ditissima* (PFC-065), also isolated from red alder in British Columbia, are included.

Formulation. This experiment involved the development of a technique that would permit formulation of the biocontrol agent at experimental or industrial levels (Figure 1). Production of the PFC-MYCOCHARGE™, an inoculum pellet containing a fungal element, may be achieved by milling a long piece of wood or alternative material—the "substratum"—in a modified lathe. The lathe body is a metal cylinder cut in half longitudinally. A cutting blade attached to the upper half of the cylinder serves to cut the substratum into solid double-nosed pieces to produce PFC-MYCOCHARGE™ blanks. Production can be adjusted to requirements by altering the length and cross-sectional dimensions of the substratum block and the dimensions of the machining lathe according to the projected market.

In the simplest formulation, the blanks are twice-autoclaved for 30 min. at a temperature of 121 C and a pressure of 1.05 kg/cm^3. The second autoclaving is accomplished with the blanks immersed in a nutritive medium appropriate to the fungus under production. The charges are inoculated by any appropriate means (placement of mycelia, distribution of spores upon the surfaces, etc.) and incubated at the optimum growth temperature for the fungus used (~20 C for *N. ditissima*).

Inoculation. Two isolates of *N. ditissima* were incubated aseptically in separate Erlenmeyer flasks for 90 d on twice-autoclaved red alder dowels (2 by 3 cm) in the dark at 20 C ± 2 C. Six red alder stems in each of five diameter classes (Table 1) were drilled to yield holes of approximately 2 by 3 cm into which the pathogen-colonized dowels were inserted by hand. The openings were capped with Poly-

Table 1. Percentage of stems infected and dimensions of cankers produced by *Nectria ditissima* Tul. PFC-065 and PFC-082 following stem inoculation. Each value is the average of measurements of six stems.

Isolate used for inoculation	Alder diam class	No. stems cankered[a]	Canker length[a]
	cm	%	cm
PFC-065	15–19	67	12
Nectria ditissima	20–24	50	5
	25–29	50	17
	30–34	33	9
	35–40	17	4
PFC-082	15–19	60	70
Nectria ditissima	20–24	100	60
	25–29	100	61
	30–34	100	65
	35–40	100	50
Sterile alder dowels[b]	15–19	0	0
	20–24	33	4
	25–29	0	0
	30–34	17	1
	35–40	0	0

[a]Values (averages) for PFC-065 and Sterile Alder Dowels were statistically equivalent and both differed from values for PFC-082 at F = 0.01.

[b]Any gross stem reaction emanating from the inoculation point was recorded as a canker.

injections with either the EZ-JECT™ or GEL CAP™ to achieve success.

Although applications of pesticide or biocontrol agents to individual trees are labor-intensive, the value of commercially mature (~100 yr) conifers on medium to high-quality sites on Vancouver Island and coastal British Columbia is CAN $80 000 to 120 000/ha. Red alder in particular will outgrow and suppress or kill coastal conifers when it proliferates at the time of plantation establishment. Even if growth of the crop species is merely retarded, crop value per hectare, amortized to year one, is approximately CAN $1000/yr (1994 values) and a great deal of hand labor is justified thereby.

Both of the injection devices above have advantages; however, neither of them was designed to place a large block of colonized nutrient substratum in intimate contact with the host cambial tissues. The actual utility of these devices as part of an inoculation strategy with biological control agents needs to be tested. The general method involving the PFC-MYCOCHARGE™ has been verified with European pines (2) and, in this study, a North American hardwood.

The PFC-MYCOCHARGE™ (Figure 1) may be used directly from the sterile containers if desired, or they may be frozen. Shelf life depends upon the fungus used. Depending upon the fungus employed, the PFC-MYCOCHARGE™ might also be lyophilized, treated with antibacterial agents or anti-oxidants (or chemicals appropriate to requirements), coated with a biodegradable and possibly nutritive material to render the surfaces smooth and thereby facilitate dispensing and insertion, and stored in dry containers. The formulation of the PFC-MYCOCHARGE™ must depend upon specific tests of each fungus in order to define shelf life and ensure maximum efficacy. The charges may also be stored in capped disposable plastic cylinders for use in a delivery instrument devised to facilitate multiple field inoculations (PFC-ALDERWAK™).

The PFC-ALDERWAK™ (Figure 2) is essentially a hammer with a 70-cm hollow handle on which is positioned a detachable 1-kg or 2-kg head. Dimensions and weights may be altered to meet requirements. The head is fitted with a threaded pin of appropriate diameter (1 cm in this case), adjusted by means of a screw-knob to the required depth of the MYCOCHARGE™ insertion hole (dependent largely upon the depth of the phelloderm). For use with *N. ditissima,* a cambial necrotroph, it is essential that the charge be in contact with the cambium of the tree.

The handle contains a cylinder loaded with the PFC-MYCOCHARGES, backed by a spring, to discharge the pellets through the base of the handle. The handle of the PFC-ALDERWAK is equipped with a trigger which elevates a movable gate at the base of the handle, thereby allowing one charge to be dispensed into the MYCOCHARGE™ insertion hole. The tip of the handle is fitted with guides which permit positioning of the handle at the pre-formed hole. Release of the trigger permits the gate to drop back in position, assuring that a single charge is dispensed.

The handle, trigger assembly and spring are removable for quick field replacement or cleaning. The head of the device is also removable such that the handle alone can be used for PFC-MYCOCHARGE™ insertion by a working party of two persons. One person can prepare stems with a portable, rechargeable drill and carry a quiver of PFC-MYCOCHARGE™ loaded cylinders while the second performs inoculations. Whether or not the charge inserted in the stem must be sealed off in every instance with a non-fungitoxic substance such as Polyfilla™ has not been determined nor has the utility of multiple stem-inoculations for larger trees been investigated. Presumably this device and the associated PFC-MYCOCHARGE™ could be used with only minor modification to deliver almost any biological material or translocatable chemical into the

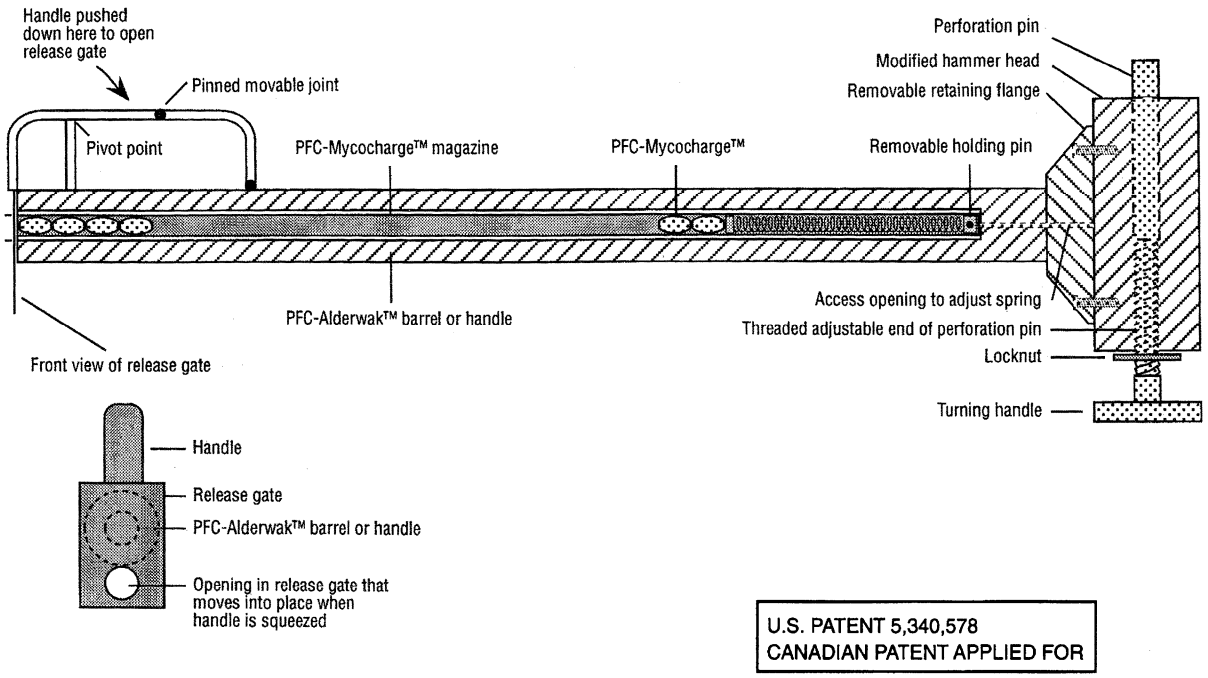

Figure 2. Schematic view of the PFC ALDERWAK™: a hammer-like tool designed to perforate hardwood stems and to release PFC-MYCOCHARGE™ pellets, one at a time, from a built-in multiple charge cassette.

stems of amenity or orchard trees as well, and additional applications can be envisioned.

As a means of killing weed trees, the invention, the formulation strategy, and (for red alder) the biocontrol agent were selected through trials for maximum efficiency. They do not possess utility greater than or possibly equal to the more successful chemical herbicides. The general wish to introduce methods perceived by the public as more nearly environmentally benign than herbicides in response to the increasingly militant stance taken by parts of the population provides a part of the rationale for biocontrol development. The public is often inclined to view the farmer as a free agent pursuing an activity that is necessary and, by its nature, environmentally benign. The general population is far more inclined to view the forest as a public trust, irrespective of ownership, and the activities of forest companies as irresponsible regardless of the care taken by such firms. Vegetation management and particularly weed abatement is an essential element of forest renewal and yet 71% of Canadians interviewed were opposed to the use of chemicals in the forest (13). The Pacific Forestry Centre intends to have in place alternatives to chemical pesticides before they are required by deregistration or *de facto* losses of chemicals, and the process of forest renewal is interrupted.

There is a tendency among research personnel to avoid discussions of environmental acceptability of pesticides. This may be necessary to maintain the objectivity of such workers but it ignores the raison d'être for their existence. *In the general sense,* we are distributing chemical formulations developed largely over the past 50 yr onto an ecosystem(s) which has evolved over hundreds of millions of years, and with no proper idea of the long-term effects to be expected. *In the specific sense,* more people than ever are aware of that fact and are prepared to object. Extrapolating directly, *in the general sense,* we can expect changes of as yet unknown types and magnitudes when we apply entirely new chemical constants upon an ecosystem(s) which possesses the inherent capacity to respond by change. *In the specific sense,* the general public is unwilling to accept the use of pesticides based entirely on the generalized assurance that such chemicals are essential to provide for our needs and wants. With regard to the alternatives reported here:

Advantages of the PFC-ALDERKILL System

1. The PFC-ALDERWAK™ will be especially useful in niche areas, as along flowages of potable water, where use of herbicides is prohibited.

2. Slow-killing of trees (3 to 5 yr with *N. ditissima*/PFC-082) will permit a gradual interposition of crop trees into the soil and aerial living space. This would alleviate the sudden changes in micro-climate due to rapid tree kill which occurs following applications of most herbicides.

3. In the case of red alder, slow-killing would provide time for progressive incursion of conifer crop tree roots into the alder root mass. Debilitation of alder roots over an extended period would, presumably, result in slow release of nodular nitrogen. This could prevent the explosion of soil microflora which accompanies sudden additions of N_2 and accompanying volatilization of a large part of same. Further conjecture on that point is unwarranted without accompanying experimental data.

Disadvantages of the PFC-ALDERKILL System

1. An industrial partner must be found to produce and distribute the product as public funds are diminishing. Most chemical companies have shown no interest in forest mycoherbicides.

2. Chemical herbicides, used according to manufacturers' specifications, are usually efficient weed killers. A biocontrol agent such as PFC-ALDERKILL™ is more a vegetation management aide for forest husbandry than a weed killer. It is intended for skillful integration into forest management plans and is, in the industrial sense, less efficient than chemical herbicides.

3. Biocontrol agents such as *N. ditissima*/PFC-082 do not necessarily offer a cost-benefit advantage over chemicals. An interaction involving two living organisms (the target weed and the biocontrol agent) involves greater opportunities for error than does one in which the target weed and an organic herbicide are used.

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