Biological Control of Weeds Using Plant Pathogens

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Abstract

Changes in program directions at the Saskatoon Research Centre have resulted in the organization of a research team equipped to develop microorganisms for the biological control of important agricultural weeds. This paper presents a brief introduction to the science of inundative biological control and an overview of the research progress made on controlling Canada thistle, wild oats, and green foxtail using fungi and bacteria.

Introduction

Chemical herbicides comprise the largest component of all pesticides used in the world (Bellinder et al. 1994). In Canada, weeds cause annual crop losses of $984 million and 60% of this loss occurs in western Canada (Swanton et *al.* 1993). Current pest management strategies rely heavily on chemical pesticides but repeated use of certain herbicides year after year has led to the development of herbicide resistant populations of weeds (Beckie and Morrison, 1993; Heap et al., 1993). Public concern about environmental and user safety of herbicides, along with the rising costs of agricultural inputs, has pressured governments and industry to develop more sustainable and integrated weed management strategies. Environmentally safe herbicides which are less persistent and more selective than most agrochemicals currently in use are desired. Weed control through biological means such as with plant pathogens, offers an additional approach that can complement existing cultural and chemical control methods.

What is Biological Control?

Biological control may use biotic agents (such as fungi, bacteria, and viruses) or metabolites produced by these agents to kill, suppress, inhibit or cause damage to specific weeds while leaving other plants unaffected. The two main types of strategies for controlling weeds through biological control are the classical and inundative approach (Charudattan, 1991). The classical approach involves an assisted release of a biotic agent followed by natural establishment and dissemination throughout a weed-infested area. The agent reduces the weed population below the socio-economic or ecological threshold and provides long-term control, with no requirement for reintroducing the agent (Boyetchko, 1996).

The inundative approach, often referred to as a bioherbicide or mycoherbicide (when fungi are used), involves the periodic application of a pathogen to the weed-infested area (Boyetchko, 1996). For bioherbicides, a high inoculum level of a host-specific pathogen, artificially produced, are applied to a target weed species. Weed control is usually short-term and not expected beyond one season post-application. The inundative approach parallels the concepts and strategies currently used with chemical herbicides.

A Successful Bioherbicide will.....

For a bioherbicide to be successful, the pathogen must a) produce abundant and durable inoculum in culture, b) be target specific, c) be genetically stable, and d) be capable of infecting and killing or suppressing a significant proportion of the weed population under a variety of environmental conditions (Boyetchko, 1997). For commercial adaptation, bioherbicides must have low costs associated with their production and formulation. They must also be designed for easy use by the majority of people. This means making sure they accomodate common farming equipment and practices.

The "science of inundative biological control" is only about 20 years old and has made substantial progress during that time. To date, four mycoherbicides have been registered in the United States and another one pending registration. Three have been developed and commericalized on a limited scale. One was registered in Canada and the U.S., but not commercialized for use. From these developments we have learned that commercialization of registered agents is often abandoned because of poor pathogen performance, high costs of production, or small markets.

'DeVine' was the first registered mycoherbicide. It is a liquid preparation of chlamydospores of *Phytophthora* palmivora for control of strangler-vine in citrus orchards by foliar spray application (Charudattan, 1991; Boyetchko, 1997). As much as 96% weed kill has been obtained with this mycoherbicide and control can last 2 years. Abbott Laboratories produced and sold the product from 1981 to 1992, and recently, re-introduced 'DeVine' into the U.S. marketplace for sale in 1995 (R. Charudattan, personal communication, 1995).

The fungus *Colletotrichum gloeosporioide* f.sp. *aeschynomene* is was registered for use as a biological control agent under the name 'Collego', for control of northern jointvetch in rice and soybeans (TeBeest and Templeton, 1985). 'Collego' is prepared as dried spores in a wettable powder formulation. It provides more than 90% weed control when applied with conventional herbicide sprayers. Ecogen Inc. produced the product for market from 1982 to 1992. Presently it is not available because the market size could not justify the costs involved in its commercial production. However, new improvements in mass-production methods by a different company have led to 'Collego' being reintroduced to the public in 1997 (D. TeBeest, personal communication, 1996).

Puccinia canaliculata was developed as a bioherbicide, under the name 'DR. BIOSEDGE' for control of yellow nutsedge (Greaves and MacQueen, 1992). Application of this rust in early spring completely inhibits flowering and reduces stand and new tuber formation by 46% and 66%, respectively (Phatak *et al*, 1983). 'DR. BIOSEDGE' was registered, but finding a commercial producer for the rust was difficult because it is an obligate parasite which requires inoculum to be raised on living plants and cannot be artificially cultured. In 1996, the CCT Corporation in Carlsbad, California marketed 'DR. BIOSEDGE' in Texas and **New** Mexico treating approximately 160-200 ha of yellow nutsedge infested land (S.C. Phatak, personal communication, 1996).

The first bioherbicide registered in Canada was 'BioMal', a product derived from fungal spores of ***Colletotrichum gloeosporioides*** f.sp. ***malvae*** for control of round-leaved mallow (Makowski, 1987; Mortensen, 1988). Philom Bios, the industrial partner involved in development of 'BioMal', decided not to manufacture it because they felt it was too expensive to mass-produce for that small market size and sell the product at a reasonable cost to farmers (Cross and Polenenko 1997).

Alternaria cassiae was investigated as a possible biocontrol agent for sicklepod in soybean and peanuts (Walker and Boyette, 1985). It has provided 60 to 100% weed control (stunting and death) in sicklepod seedlings. Improvements in formulation resulted in better bioherbicidal activity by increasing germination and infection (Daigle and Cotty, 1991). Registration of this mycoherbicide under the tradename 'CASST' was pending but because of inconsistent field performance Mycogen Corporation has relinquished their rights to licence the agent (Powell and Jutsam, 1993). It is presently being pursued as a bioherbicide in Brazil (R. Charudattan, personal communication, 1996)

Biological Control of Weeds in Saskatoon

To improve the chances of developing biocontrol agents that can be adapted for commercial application, the problem of developing a commercial biocontrol agent for weeds was approached from a holistic perspective. Firstly, research activities are now concentrated on important agricultural weeds that are difficult to control in field crops and also affect large acreages. Secondly, a significant portion of the research activities are allocated to production, formulation, and delivery systems while maintaining a balance with the more traditional activities of exploration, discovery, and biological assessment. Thirdly, new activities were introduced for application of genetic manipulations and natural metabolite production.

The **Weeds:** Annual grassy weeds represent a problem throughout the world, especially with the adoption of conservation tillage practices. In Saskatchewan, green foxtail was ranked as the most abundant weed in recent weed surveys. These weeds are difficult to control in cereal crops because they are closely related to the crop, may have a similar life cycle, and they are capable of producing large amounts of seed. Herbicide performance in cereal crops may be inconsistent and a high level of herbicide selectivity is required to control the grassy weeds. Repeated use of grass herbicides has led to the rapid development of resistance in wild oats and green foxtail in western Canada, with some weed populations being resistant to several groups of herbicides. Biological agents may provide new modes of action to control these weeds.

Perennial weeds, such as Canada thistle are also difficult to control because they have an aggressive and invasive root system. Producers perceive Canada thistle to be the second most important weed in field crops. Chemical and cultural controls are often costly, and not completely effective. In recent years, Canada thistle has become an even greater problem with the increased practice of reduced tillage (i.e. less mechanical control) and the increased production of broad-leaf specialty crops, such as peas.

Exploration and Evaluation: For the most part, foliar-borne fungi have been investigated for use as mycoherbicides. Annual weed disease surveys involve the collection of specimens from sites with different farming practices (i.e. conventional and reduced tillage, organic producers) throughout the prairie provinces. Weed seed screenings obtained from grain elevators across Canada allow testing for seed-borne pathogens that can inhibit seed germination and cause plant infection. Bacteria and fungi from the rhizosphere (roots and soil) and crowns of plants are also being explored as possible biological control agents of weeds.

All organisms collected must pass the test of “Koch's Postulates”, which determines whether the organisms isolated actually are the causal agent of disease. They are then passed on into various bioassays systems to evaluate their ability to cause disease on the leaves, stems, and roots of the weeds. These tests include foliar applications with and without formulations at specified temperature, light, and humidity levels, stem injection tests with spores or homogenized mycelial pieces, growth pouch tests for inhibition of seed germination and root growth, and soil inoculation tests to evaluate the ability of the biocontrol agents to compete with other soil microflora. Followup work includes host range testing and determining the biology and epidemiology of the pathogen.

Genetic Manipulation: Biotechnology offers several techniques that have useful applications in biological control. The polymerase chain reaction (PCR) has been used to genetically characterize the organisms and may be used in the future to track and monitor the pathogens when released in the environment. Antibiotic resistance genes have been incorporated into the bacteria for this same purpose.

The genetic transformation of fungal and bacterial biocontrol agents is being attempted with genes that may help to overcome entry level resistance mechanisms of the weed hosts. Cutinase is an extracellular enzyme that breaks down cutin which is one of the structural components of the plant cuticle. This enzyme is present in some but not all fungal pathogens; and it is absent in some of our biocontrol agents. The addition of this gene into the biocontrol agents may speed up entry of the pathogen into the host and thus reduce the influence of environmental factors.

Natural Products: The literature reports of the potential to use natural products and phytotoxins produced by biocontrol agents. The fungus ***Gliocladium virens*** produces the phytotoxin viridiol which has herbicidal activity to pig-weed (Howell and Stipanovic 1984). Although viridiol was unstable when introduced into field soil, sufficient quantities of the phytotoxin were produced by the fungus and suppressed weed growth when the fungus was introduced into the soil. This example illustrates how phytotoxins may be the key to understanding the mechanism of pathogenicity. Natural products may also be developed for new naturally derived herbicides with new modes of action. These may be synthesized or produced and extracted from culture. Knowledge on the biosynthetic pathways allows for genetic manipulation of key precursors to increase production of the natural products or by manipulation of the fermentation process. Several bacterial and fungal isolates in the biocontrol collection have shown preliminary evidence of phytotoxin production.

Fermentation and Product Supply A bioherbicide must be able to be mass-produced efficiently to be commercially viable. Bacteria and some fungi are well adapted for liquid fermentation processes. The quantity of cells may be increased and the efficacy enhanced by altering the carbon sources and ratio with amino acids. For example, the addition of a carbon source in the culture of a rhizobacterial isolate showed greater than 50% suppression of green foxtail whereas the addition of a particular amino acid with the carbon source resulted in greater than 95% suppression.

Some fungi, on the other hand, are not well adapted to liquid fermentation processes and will only sporulate on solid substrates. Several of our most promising fungal biocontrol agents require the development of production methods that will make it economically feasible to produce the agents in large quantities under commercial conditions. Mushroom spawn production is an example of a commercial non-liquid production process developed for fungi.

Our research is focusing on developing different types of non-liquid, solid substrate technologies. It is also concentrating on the environmental and nutritional factors affecting production. For one biocontrol agent on Canada thistle, a IO-fold increase in spore production on a solid substrate is triggered by the addition of oxygen to the mycelial growth phase in liquid culture. Also changes in the nutritional components of the medium can induce sclerotial production which does not normally occur in nature.

Formulation and Application Technology: Efficacy of biocontrol agents in the field is challenged by environmental conditions that are not conducive to disease development. Moisture requirement for disease development, usually provided by a dew period, is one such factor that may be altered by formulating the agent with suitable additives like oils, humectants, and penetrants. Other factors that should be considered in formulating biocontrol agents relate to nutrition, ability to adhere, protection from UV light, and placement at optimal points for infection to take place.

Foliar applications may be influenced by water volumes, rates of application, nozzles, droplet size, and shear stress. Many agents prefer to be applied in high carrier volumes, and this requirement must be reduced to make large scale application practical. Some fungal spores may collapse if they are pumped too aggressively with centrifugal force, and special recommendations may be need to be developed.

Granular applications may be the most efficient and effective way to place soil and root pathogens at the site for infection. Granular bases may be made from peat, vermiculite, starch, flour and water dough, and crop residue. The development of simple delivery systems using common farm equipment for easy integration into common farming practices is necessary to make biological control a viable option for weed management.

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