Current biological weed control agents - their adoption and future prospects.

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Summary
Traditional agricultural practices have generally focused on herbicides, mechanical, and cultural methods as the main tools for weed management. Although these methods have served crop production well, it is important to recognize that there are scientists in Canada and around the world testing the potential of using living organisms like insects, fungi, and bacteria as biological control agents for weed management. Awareness of the need for increased environmental stewardship combined with the expanding organic food industry is stimulating the need for new technologies to assist with weed control. Certainly, the re-evaluation and the potential banning of certain herbicides in Canada, the development of herbicide-resistant weed populations, and the continued threat of invasive weed species are influencing the way weed management is researched. This article will showcase the novel approach offered by living organisms as agents for biological weed control and describe how this weed management tool is evolving as an alternative to herbicides.

Introduction
Weeds are a major contributing factor to crop yield loss on the Prairies through competition for water and nutrients. The prolific seed production of most weeds also increases the weed seed bank which contributes to problems in subsequent years, lowers crop grades, increases dockage, and raises costs associated with harvesting and seed cleaning. Several crop management tactics are used to control weeds including cultural (e.g. seeding rates, crop rotation, time of seeding, etc.), tillage, chemical, and biological methods, yet there is a strong and continued reliance on chemical herbicides as the main weed control method, especially since no-till is being practised on more area every year. In more recent years, crop protection practices have come under greater scrutiny because of environmental issues related to off-target spray drift, chemical residues in the soil, water and food, and development of herbicide-resistant weed populations[17]. The application of biological weed control agents offers alternatives to the use of herbicides that provide new environmental options and more-varied weed management tools[1,2,3,10].

The general definition of biological weed control is the intentional use of living organisms to control or suppress weeds[2,10]. The two major approaches to biological weed control are classical and inundative (bioherbicides). Classical biological control is used for the control of exotic and invasive plant species using their natural enemies from the plant's place of origin (usually Europe and Eurasia for Canada's weeds)[11,14]. Although fungal or bacterial pathogens can be used in classical biological weed control, typically the natural enemy is an arthropod (i.e. plant-feeding insect or mite), which is carefully screened for its host specificity before release as a biological control agent. Once released, the agent is expected to establish, increase and spread on its own, thus providing low cost, long-term weed control. This classical approach is widely recognized as a key strategy for managing weed species in natural areas (e.g. riparian zones, forests) and low-input agriculture (e.g. grasslands and managed pastures for grazing), where habitat disturbance is minimal. However, the classical approach does not aim to eradicate the invasive plant, but when successful, achieves low, stable populations of both the plant and its biological control agent, with the former being held below an economic or environmental threshold. On the other hand, the inundative approach relies on the annual application of microorganisms in high doses to a target weed species or group of related weeds[2,3]. The microorganisms are often naturally-occurring plant pathogens of weeds that are mass-produced artificially and applied during the growing season in a similar manner as herbicides, and are not expected to persist and survive in the environment beyond the season of application. The majority of microbes used as inundative biological control agents are fungal pathogens, but there are a growing number of examples of foliar and soil-applied bacterial agents being explored and developed as well.

A brief history of biological weed control research in Canada

Classical biological weed control

Classical biological control of weeds in Canada using arthropods began in 1951 with the release of two leaf beetle species to control St. John's wort (*Hypericum perforatum* L.) on rangelands in British Columbia (BC) by Agriculture Canada and Agri-Food Canada. Since the program's inception, about 75 arthropod agents have been screened, studied and released in Canada against 24 targeted weed species. About a third of these agents have been successful to some degree in weed control[11]. The majority of these weed biological control agents were tested and released in the late 1960s to early 1990s when funding for biological control research was at its height and there were few public concerns over the potential non-target risks of these agents. In the past 10 years by comparison, only two new insect agents have been approved for release by the Canadian Food and Inspection Agency (i.e. the regulators of classical biological control agents in Canada), mainly due to increased diligence and time spent in pre-screening the foreign insects for their host specificity. A close relationship has existed throughout the history of classical biological control in Canada between AAFC and CAB International in the foreign exploration and screening phases of these biological control programs[14]; with researchers at the collection sites and in Canada working together to provide and present the detailed scientific data required to make informed decisions on the releases of these new biological control agents in Canada.

Early biological control projects and their start dates include: yellow toadflax (*Linaria vulgaris* Miller) (1957), tansy ragwort (*Senecio jacobaea* L.) (1961), Canada thistle (*Cirsium arvense* (L.) Scopoli) (1963) and nodding thistle (*Carduus* spp.) species (1968). Post-release monitoring showed early success with some of these projects including nodding thistle control with the seed weevil, *Rhinocyllus conicus*, and St. John's wort with the *Chrysolina* leaf beetles[11]. Other projects such as Canada thistle and yellow toadflax are still proving difficult with no effective insect agents available. Two other major, historic biological control programs were developed for diffuse and spotted knapweed (*Centaurea diffusa* Lamarck and *C. maculosa*Lamarck) in BC (1970), and for leafy spurge (*Euphorbia esula* L.) (1965) in the Prairie provinces, for which 12 and 18 insect species, respectively, were introduced to Canada. Part of the current scientific process is to make improvements through retrospective examination of past projects dealing with classical biological weed control. For instance, we now know that control of leafy spurge is only successful if habitat conditions are matched with specific species of *Aphthona* root-feeding flea beetles[14]. After more than 20 years, we now observe successful reductions in populations of knapweed due to biological control agents. Part of this success is because some of the more recently released agents have been more effective than those released earlier in the project. Of these, the root feeders (e.g. weevil, *Cyphocleonus achates*), and those that kill plants outright through their feeding (e.g. seed weevil, *Larinus minutus*) appear to be the most damaging to knapweed populations[6,14]. Post-release monitoring experiments continue today on many past biological control agents so that we can be more predictive of success for new projects.

Biological herbicides

Utilization of plant pathogens for weed control was first reported in the early 1900s, but developing biological herbicides for commercial application came much later with the first registrations occurring in the USA in the 1980s[1]. In Canada, the first biological herbicide registered was the fungal pathogen *Colletotrichum gloeosporioides* f.sp. *malvae* as BioMal® in 1992[4]. This resulted from the fortuitous discovery of the disease-causing fungus on round-leaved mallow (*Malva pusilla* Smith) and more than 10 years of research to characterize the fungus, demonstrate its field efficacy and host range, and develop the knowledge to mass-produce and formulate it into a commercial product. The importance of this discovery was that it provided a model for developing new protocols and guidelines on human health and safety, environmental toxicology, and residue/persistence for future biological pesticide registrations in Canada[1,4]. Philom Bios (Saskatoon) was selected as the industry partner for registering BioMal; unfortunately, commercial production was halted in 1994 because changes in the marketplace over the 10 year development period resulted in the market potential being too small to justify further commercialization costs and production expenses. Four new biological herbicides have been registered in

Canada, with Chontrol® (fungal organism *Chondrostereum purpureum)* and Sarritor® (fungal organism *Sclerotinia minor*) being the most recent examples. Chontrol® is comprised of a saprophytic fungus that, upon wounding or injury, will invade the cambium of hardwood tree species such as red alder, black cherry, white birch, and aspen. In the Netherlands, the fungus is sold by Koppert B.V. under the name BioChon® and it was successfully registered in Canada and the U.S. under the name CHONTROL by MycoLogic Inc. (Victoria, B.C.) for vegetation management (of hardwood tree species) in reforestation sites and industrial rights-of-ways. Although the fungus has a very broad host range, studies provided evidence of the safe application and low risk of dispersal of the fungus onto non-target plants[1,3,4,14].

Sclerotinia minor is a fungus that infects many broadleaved plant species but not grasses, thus making it suitable for biological control of dandelion (*Taraxacum officinale* Weber) in turfgrass[2,14]. The fungus was successfully registered in Canada in 2007 as a dry, granular product (Sarritor®), which is applied to actively growing weeds in turfgrass using a drop spreader or spot applicator. The fungus destroys susceptible plant tissues thus providing top growth suppression. The fungus does not survive for more than 11 days and other reproductive structures are rarely formed. As a newer, reduced-risk product, Sarritor® enables the use of sustainable turf management practices in municipalities where the application of conventional herbicides is not desirable or banned.

Current research activities

<u>Classical biological weed control</u>

Current applied research in classical weed biological control in Canada follows two main streams; 1) the search for and development of new agents for both long-established and emerging invasive plants, and 2) learning how to better utilize the biological control agents we have already released. With respect to the search for new agents, Canada is currently facilitating the overseas screening of candidate agents by CAB International Europe for toadflax (*Linaria* spp.), houndstongue (*Cynoglossum officinale* L.), Russian knapweed (*Acroptilon repens* (L.) DC.), dog-strangling vine (*Vincetoxicum* spp.), Japanese knotweed (*Polygonum* spp.), tansy ragwort (*Senecio jacobaea* L.), common tansy (*Tanacetum vulgare* L.), oxeye daisy (*Leucanthemum vulgare* Lam.), and hawkweed species (*Hieracium* spp.).

Figure 1. Root-feeding Weevil (*Mogulones cruciger)*. Photo credit: Henri Goulet

Research on agents already established on target weeds focuses mainly on documenting and understanding the mechanisms for recent successes and then developing strategies for improving their introduction as biological control agents. Examples include studies of the significant and predictable impacts of the weevil root feeder (*Mogulones cruciger*) (**Figure 1**) and the stem borer (*Mecinus janthinus)*, (**Figure 2**) on populations of their rangeland weed hosts, houndstongue and Dalmation toadflax (*Linaria dalmatica* (L.) Mill.)[14]. Both projects have been extremely successful in BC[8,14].

Figure 2. Houndstongue infestation: Dried burr stalks on rangeland in B.C. Photo credit: Rosemarie De Clerck-Floate.

Studies on the attributes of successful agents for leafy spurge, knapweed, houndstongue and toadflax (e.g. dispersal abilities, insect population increase, interactions with other organisms) are being used to generally improve the predicted outcomes of biological control as a tool in weed management[6,8,13]. A better understanding of what to expect upon the release of new agents will lead to a more reliable way of integrating biological control with other methods of weed control.

Once an agent has proven successful against a weed, and is in demand by those who want biological control options, the final step in its development is determining a method for its mass-production. Ongoing research by AAFC has been in developing novel and cost-effective methods for insect rearing such as using agronomic techniques for growing houndstongue as a crop for mass-producing *M. cruciger*[7]. This rancher-supported project, along with efforts to mass-produce *Aphthona* beetles on leafy spurge using field nurseries, has enabled operational scale implementation of biological control of these weeds, thus promoting the use of classical biological control as a viable and predictable tool for weed management.

<u>Biological herbicides</u>

Broadleaved weeds such as dandelion and Canada thistle cause problems in both urban and rural areas. The fungus, *Phoma macrostoma* exhibits control of several broadleaved weeds while showing no effect on grasses or cereal crops and is now being developed as a biological herbicide for weeds in turfgrass (lawns, golf courses, public grounds), agriculture (cereal crops) and agro-forestry (reforestation nurseries) (**Figure 3**)[19]. When applied to the soil as a granule, the susceptible weeds turn white and die. With limited mobility in the soil combined with a non-competitive nature, *Phoma* does not persist, and cannot be detected the year after application. Efforts are underway by Agriculture and Agri-Food Canada to bring this biological herbicide to the market in collaboration with The Scotts Miracle-Gro Company for use in turfgrass. Research continues to refine the processes required to expand the application of this biological herbicide to agriculture and agro-forestry.

Untreated **Treated with *Phoma macrostoma***

Figure 3. Bioherbicide for control of dandelion in turfgrass. Photo credit: Karen Bailey.

Scentless chamomile (*Matricaria perforata* Mérat)is a noxious weed in western Canada with a natural tolerance to many herbicides. Mechanical and cultural methods are only partially effective due to the vigor of the weed and the prolific seed production. Yield reductions of 55% have been observed in wheat infested with as low as 25 plant m^{-2} and reductions can be even greater in less competitive crops like lentil[1,16]. The fungus *Colletotrichum truncatum* was isolated from scentless chamomile in Saskatchewan. Applied as a foliar spray, the fungus causes girdling of the stem and petioles resulting in reduced foliar biomass, but usually does not kill the plants. However when *Colletotrichum* was tank mixed with certain herbicides like metribuzin, the level of weed control surpassed the effect of either the fungus or the herbicide when applied alone. Researchers are exploring fermentation conditions and foliar spray formulations to further increase efficacy and field consistency to attract an industry partner for registration.

Research is being conducted to develop soil bacteria as pre-emergent biological control agents against annual grassy weeds. Several bacterial strains have been identified and field tested, and up to 85 to 90% control of green foxtail (*Setaria viridis* (L.) P. Beauv.) and wild oat (*Avena fatua* L.) was achieved using a granular formulation called "pesta"[2,3,14]. The leading bacterial candidate for biological control of the grass weeds is a *Pseudomonas fluorescens*, strain BRG100, which delays emergence of the weeds and significantly inhibits root growth (**Figure 4**). Detailed studies indicate that there are two or more modes of action of this bacterial organism that are novel and different from current herbicides. They have been shown to control herbicide-resistant green foxtail and wild oat populations. A small Canadian company has been identified as an industry partner and the current research plans are to evaluate a pilot-scale production system for the manufacture of this new biological herbicide. In addition, field studies are being planned to determine methods and rates of application under various cropping situations such as varying crop densities and rotations, row spacing, and soil type.

Untreated **Treated with bacteria**

Figure 4. Bioherbicide for control of green foxtail in a wheat crop. Photo credit: Susan Boyetchko .

One of the major challenges in developing biological herbicides has been the difficulty and costs of mass-production (often referred to as fermentation) and arriving at a suitable formulation for the storage and delivery of the agent[12]. Researchers continue to develop appropriate methods for fermentation for biological herbicides in either liquid or solid-state systems. In addition, there are interesting formulation techniques that will provide an extensive shelf-life of more than 1-2 years using the appropriate system such as various adjuvants or granules and new application technology tools to deliver these microorganisms either as pre-emergent or post-emergent bioherbicides.

Rationale for using biological control

Global herbicide sales in 2006 were valued at $31.2 billion, down by 12% from the previous five years. This declining trend is expected to continue largely due to increased health and environmental hazards associated with many conventional pesticides[5,9,15,17]. Biological pesticides are classed by Health Canada as reduced risk products which are deemed less hazardous to human health and safer to the environment than synthetic chemical pest control products like herbicides[1,12].

Classical weed biological control using introduced arthropods is also considered more environmentally friendly relative to herbicides despite recent concerns about the risks to non-target plants by agents released in the past[18]. Overall, there is a long history of safe use worldwide, with few reported cases of negative impacts on non-target plant species of economic value (e.g. horticultural plants), or on native plants within natural areas[18]. In those instances where there has been non-target feeding, it always has been on plants closely-related to the target weed. The attack was predicted based on pre-release testing of those agents and the damage has often been more immediate than long-term and usually very limited in scope. There is also a growing need to accurately assess the environmental impact of not releasing the agents to control important invasive weeds. There needs to be a proper weighting of the benefits and risks of biological control agents[18]. There is no such thing as zero risk, regardless of the decisions taken in weed management.

Entering the decision of whether to use biological control versus herbicides is the recent ban of synthetic herbicides in many urban municipalities in Canada and several of these are being de-registered due to risk and economic considerations. Certain commonly used pesticides are currently under regulatory review by Health Canada Pest Management Regulatory Agency[1]. There are also situations in sensitive natural areas, including along waterways, where herbicide use is not an option, thus biological control is the only available tool for weed management.

The development of biological pesticides using bacterial or fungal agents represent new pest control products with novel modes of action that can be used in rotation with registered herbicides to help slow down weed populations becoming resistant to herbicides. This will extend the current life of registered herbicides. Research is still required to develop ways to integrate classical and inundative weed biological control agents with other methods of weed control which include herbicides, mechanical weed removal and various other cultural practices[3].

Many economic, environmental, and social benefits can be realized with the adoption of biological weed control technologies. The expanding "organic" and health food industries are major advocates for biological control, with sales of organic food expected to increase by 25-30% per annum[1]. The introduction of new modes of action for herbicides has slowed down due to rising research and development costs, increasingly stringent regulatory requirements, and the commercial availability of genetically modified crops[5,9,17]. Biological control can be used as a tool for the management of invasive weed species in natural areas and for weed populations that have developed resistance to herbicides. Societal concerns for the environment and greater public demand for fewer pesticides in the food chain have stirred political debate and legislative amendments to Canada's Pest Control Products Act, requiring even greater pesticide risk reduction and the introduction of new, lower-risk pest control products. Biological control will have a positive environmental impact through the reduction of herbicides for use at the farm level and in urban areas like parks, lawns and gardens. Furthermore, biological control is a weed management tool that addresses issues related to food safety and environmental stewardship, such as soil, air and water quality and preservation of biodiversity.

Conclusions

The potential that biological control agents as a weed management tool can offer is often overlooked because of: i) perceptions about its novelty and unproven track record, ii) the perceptions about the performance of biological control agents relative to herbicide weed control, iii) the familiarity of using traditional weed control methods, or iv) the concerns over its environmental safety (i.e., non-target impacts of classical biological control agents). However, it should be noted that only a small fraction of newly tested herbicide chemistries ever make it to market as new herbicides for numerous reasons (only 0.007 %) versus the relatively large proportion of successes in classical weed biological control (i.e. 25-30% of all agents released). It is not always easy for producers and consumers to change their weed control and spending habits and adopt these new technologies. However, significant advancements have been made in the selection of biological control agents, their mass-production and method of delivery[1,2,3,12]. Furthermore, it should be remembered that biological control agents, herbicides, and various other weed management tools are always more effective and sustainable when they form part of an integrated weed management approach.

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