Proceedings of the Eighth International Symposium on Biological Control of Weeds
2-7 February 1992, Lincoln University, Canterbury, New Zealand.
Delfosse, E.S. and R.R. Scott (eds.). DSIR/CSIRO, Melbourne, pp. 641-8 (1995).

Regulating Microbial Pest Control Agents in Canada: The First Mycoherbicide

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Microbial pest control agents are regulated under the *Pest Control Products Act* and Regulations since these are products that are to be manufactured and sold. The *Guidelines for Registration of Naturally Occurring Microbial Pest Control Agents* and the *Requirements for Field Trials* are just now being finalized. The main areas for data requirements are: Agent Specification, Manufacturing Methods and Quality Control, Human Health Safety Testing, Food and Feed Residue Studies, Environmental Fate, Environmental Toxicology, and Efficacy. The implications of these guidelines to research are discussed. The indigenous fungal pathogen, *Colletotrichum gloeosporioides* f. sp. *malvae* (*C.g.m.*), was first isolated from anthracnose symptoms on round-leaved mallow *(Malva pusilla)* in Saskatchewan in 1982. Its potential as an effective mycoherbicide for control of round-leaved mallow was established and *C.g.m.* is being commercialized under the tradename "BioMal"® by Philom Bios Inc. The development and testing of protocols that addressed concerns by regulatory agencies in the areas of Environmental Toxicology, Crop Tolerance, and Food Residue were undertaken. *C.g.m.*, being the first mycoherbicide registered for use in Canada, has set the precedence for the development of future microbial agents.

Introduction

The growing concern over the use of chemical pesticides to human health and the environment has brought about an increasing interest in the use of biological alternatives (Hoagland 1990, Templeton 1990). The inundative approach to biological control is of increasing interest in the research community not only for control of weeds, but for control of other pests such as plant diseases and insects (McClay 1991). This approach provides greater opportunity for integration into existing weed management systems in cultivated crops than other biocontrol strategies (Watson and Wymore 1990). As more microbial agents are being investigated, regulations, protocols, and guidelines are emerging to assess potential risk in plant protection, human health and safety, environment, etc. The unique properties of microbial pest control agents challenge the regulatory agencies to develop appropriate, reasonable, science-based requirements to assess the safety, merit, and value of the products (Agriculture Canada 1990*a*). Requirements for the registration of microbial agents in Canada have been drafted but are still evolving. The first mycoherbicide has finally been registered for use in Canada which indicates a bright future for development of these products.

The purpose of this paper is to give an overview of the legislation responsible for regulating microbial biocontrol agents in Canada, the present guidelines and regulations for registration, as well as to explain how the registration system works, and to briefly describe the history of the registration of the first mycoherbicide in Canada.

Legislation

In Canada, there are no Acts of Parliament that include biological control *per se* as in Australia (Cullen and Delfosse 1985). Potential risks are dealt with by existing legislation. Microbial pest control agents are regulated under the *Pest Control Products Act* and Regulations. Certain

components of the requirements for registration are also regulated under the *Food and Drugs Act* and Regulations and the *Canadian Environmental Protection Act* (Canada 1988).

The mandate of the *Pest Control Products Act* is to regulate the manufacture, sale, and use of pest control products which is defined under the *Act* as "any product, device, organism, substance or thing that is manufactured, represented, sold or used as a means of directly controlling, preventing, destroying, mitigating, attracting or repelling any pest." A pest is defined under the *Act* as "any injurious, noxious or troublesome insect, fungus, bacterial organism, virus, weed, rodent or other plant or animal pest, and includes any injurious, noxious or troublesome organic function of a plant or animal" (Canada 1988). This mandate is very broad covering chemical pesticides as well as organisms. However, to date, organisms other than microbials have not been regulated under this *Act*. Microbial organisms are defined as bacteria, algae, fungi, protozoa, viruses, mycoplasmae or rickettsiae and related organisms. This situation will soon change since guidelines for regulating other biocontrol agents besides microbials are being proposed. Draft guidelines will be available for comment in April and a workshop to discuss them will be held in May.

Under the *Food and Drugs Act* and Regulations, dietary exposure to pest control products must be evaluated and residue limits set. The *Canadian Environmental Protection Act* has the mandate to conserve and protect national treasures; i.e., wildlife, water and the environment in general. Therefore, the environmental impact of pest control products must also be evaluated (Canada 1988).

Industry becomes involved in the commercialization of microbial pest control products. For the protection of their research investment, patent laws are applicable.

Present Regulations for Registration

The requirements for registration are presented in 2 memoranda: *Guidelines for Registration of Naturally Occurring Microbial Pest Control Agents* (Agriculture Canada 1990*a*) and *Requirements for Field Trials of Naturally Occurring Microbial Pest Control Agents* (Agriculture Canada 1990*b*). The main areas for data requirements for registration are: 1.) Agent Specification, Manufacturing Methods and Quality Control; 2.) Human Health Safety Testing; 3.) Food and Feed Residue Studies; 4.) Environmental Fate; 5.) Environmental Toxicology; and 6.) Efficacy. A brief summary, based on the aforementioned memoranda, of the purpose and description of the tests required in each area is presented as well as how field trials should be carried out when required.

Agent Specification, Manufacturing Methods and Quality Control

The main requirements for agent specification involve providing information on the taxonomy, morphological description, geographical distribution, known or suspected relationship to any known pathogen, biological properties, etc. Information on infectivity and potential production of toxins or metabolic byproducts, and on methods of isolation and culture procedures are also required. A full description of the manufacturing method and quality control procedures are required.

Human Health Safety Testing

The purpose of safety testing is to determine potential effects of a microbial product on human health and to quantify any responses seen. Different tests are used to evaluate routes of exposure and the potential for pathogenicity, infectivity, toxicity and irritation. The general areas of testing are: infectivity/toxicity, acute intravenous (IV)/interperitoneal (IP)/intercerebral (IC) infectivity, irritation, hypersensitivity and immunological effects, tissue culture (viral agents only), genotoxic potential, and exposure assessment.

It is highly recommended that all protocols be approved before starting any of these tests as these are costly. If the results from these tests demonstrate no effects, then no further tests are required. However, if concerns arise, further investigations will be necessary.

Food and Feed Residue Studies

The purpose of these studies is to estimate the exposure of humans and livestock to potential residues in food and feed, and to set maximum residue limits. Residues relate to the number of microbial organisms and, where appropriate, may relate to a representative chemical component or metabolic byproduct of the microbial agent. Food and feed residues are also regulated under the *Food and Drugs Act* and Regulations. Data are required, when applicable, in the areas of crop residue (food and/or feed); livestock, poultry, egg and milk residue (if uptake from dermal application and/or from feeding treated crops); processing; and storage stability.

Environmental Fate

The purpose of these tests is to evaluate the population growth characteristics of the microbial pest control agent and its ability to survive or propagate after it has been introduced into the environment.

A very controversial concept that is a major component of both the Environmental Fate and the Environmental Toxicology requirements is the *Ecozone* concept. An ecozone is defined as "large and very generalized ecologically distinctive area based on the interplay of landform, water, soil, climate, flora, fauna, and human factors." The controversy relates to the practicality of defining regions within a country for microorganisms because mass transportation systems and the ubiquitous nature of most microorganisms. Whether an organism is indigenous (i.e., occurs naturally in the ecozone of intended use) or is non-indigenous (i.e., occurs naturally in an ecozone different from ecozone of intended use) determines the extent of the requirements. For example, for non-indigenous organisms, environmental fate studies are required. However, for an indigenous microbial agent, if in the *Tier I* Environmental Toxicology tests, demonstrates absolute host specificity or affects a limited number of closely related species, then no environmental fate studies would be required.

If fate studies are required, these would mainly consist of determining the optimum and range of physical and chemical factors relating to survival and multiplication of the organism and its response to variations in salinity, antagonists, and adverse conditions. Microcosm studies may be necessary to assess the interaction of the microbial agent with the 'natural' microbial population. Field studies are required when microbials demonstrate poor host specificity or effects on important non-target organisms. These must address aspects of persistence, multiplication and dispersion in both aquatic and terrestrial ecosystems.

Environmental Toxicology

Environmental Toxicology testing is used to determine possible infectivity, toxicity and pathogenicity of microbial agents to non-target organisms. Testing for effects on non-target organisms is done on a tier system. Initial submissions should include all *Tier I* tests. If results from the initial tests present no hazard (negative results), then further testing in not needed. If positive results are found in the first tier, then appropriate follow-up tests are required with progression to higher tiers as necessary. There are specific requirements for each tier and each group of organism (i.e. birds, mammals, fish, invertebrates, microorganisms, and plants). The selection of the non-target organisms is based initially on a centrifugal taxonomic approach similar to the approach used in classical biological control (Zwölfer and Harris 1971, Wapshere 1974). In addition, a number of other non-target organisms must be tested which meet various criteria (i.e. susceptible to pathogens closely related taxonomically to the microbial agent, etc.). For example, in the case of testing non-target plants, *Tier I* tests should determine the plant host range. These are carried out under conditions favourable for disease development in the target species utilizing the maximum challenge concentration (equal to 10^3 x the maximum expected concentration to which the non-target organism would be exposed to in the environment). *Tier II* tests deal with the maximum environmental concentration. *Tier III* relates to the effect of the microbial throughout

the life cycle of the non-target organism and distinctions are made for indigenous and non-indigenous microbial agents. *Tier IV* involves field studies.

Efficacy

There is a guarantee on the label of any product registered under the *Pest Control Products Act* that it is efficacious. Therefore, efficacy data are required from both laboratory and Canadian field trials. Data from other countries may be used to supplement Canadian data.

Field Trials

Research permits are required to perform field trials for all microbial pest control agents that involve new uses for registered products, new formulations and new sources of registered active ingredients, and new active ingredients (Agriculture Canada 1990*b*). There is an exemption for the requirement of a research permit for small-scale field trials (<10 ha) utilizing indigenous organisms on property owned by a research institution and which do not involve cooperators. For non-indigenous organisms, host specificity tests and non-target tests are required as specified in the Environmental Toxicolgy section to obtain a research permit even for small-scale field plots. This raises the ecozone concept controversy once again. It seems unreasonable to request completion of all the Environmental Toxicology components before finding out if the product works in the field. As the scale of the field plot increases, more tests are required similar to a full registration package.

The Registration Process

The *Pest Control Products Act* falls under the jurisdiction of the Pesticides Directorate of Agriculture Canada. The Pesticides Directorate ultimately grants registration of products to be manufactured and sold in Canada. It is responsible for the coordination of the evaluation of the data package. It evaluates mainly the efficacy component and calls on experts in other government departments to evaluate the other components. These governmental consulting agencies are Health and Welfare Canada which evaluate the Human Health Safety tests and the Food and Feed Residue studies; Environment Canada, Canadian Wildlife Service and Fisheries and Oceans Canada which evaluate the Environmental Fate and the Environmental Toxicology components; and the Laboratory Services of Agriculture Canada which evaluate the Manufacturing Methods and Quality Control.

The registrant or company has to guide the data submission through each consulting agency to ensure timely review and address concerns as they arise. There is approximately a 2-yr backlog on any new submission.

Except for *Bt (Bacillus thuringiensis)* for control of Lepidopteran pests, microbial pest control products are fairly new to the registration process. As regulators and evaluators become more familiar with microbials and enlarge their knowledge base, requirements and protocols will certainly change and evolve. Due to the novel nature of microbials in Canada, the guidelines are presently very dynamic. This can be difficult for the researcher or the company in that there are uncertainties in what is required exactly for registration. It is extremely important to consult with the regulators early in the research and to have all protocols approved prior to undertaking major testing. Since the guidelines encompass all possible microbial pest control agents, each submission will be dealt with on a case-by-case basis. Waivers of certain data requirements may be possible when supported by sound scientific rationale. The guidelines outlined above will serve as a basis for the requirements for genetically modified microbial pest control agents.

The entire registration process has just undergone a major review. The final report of the review team was published and submitted to the Minister of Agriculture in December 1990 (Pesticide Registration Review Team 1990). Some of the recommendations suggest changes to the Department responsible for the Act and reorganization of the evaluation within the consulting agencies. Public involvement in the process was highly recommended. An appeal process and a more timely review of the submissions were also suggested. Which

recommendations and their implementation has yet to be finalized.

Products may have to undergo another level of review at the provincial level. Some provinces in Canada (i.e., Ontario) must also approve products before they can be available on the market in those provinces, even though federal registration has been granted.

To develop the guidelines for registration of microbial agents, the Pesticides Directorate organized 2 workshops (1989-90) to discuss the draft proposals with all parties with vested interest in the process; i.e., government and university researchers, private sector representatives, etc. Many concerns were raised particularly the *ecozone* concept as mentioned above and the Human Health Safety testing section specifically endpoints (measured toxicology parameters) and the 30-d feeding test. Environment Canada is presently reviewing its requirements and another workshop will be held in the summer of 1992 to discuss the implications. This is a step forward which will hopefully prevent overregulation and unreasonable requirements. The continued input of specialists in the development of guidelines is a tremendous opportunity to ensure a future for biological control in Canada.

The First Mycoherbicide Registered in Canada

The indigenous fungal pathogen, *Colletotrichum gloeosporioides* (Penz.) Sacc. *f. sp. malvae* (*C.g.m.*), was first isolated from anthracnose lesions on stems of round-leaved mallow (*Malva pusilla* Sm.) by Dr. Knud Mortensen in 1982 at the Agriculture Canada Research Station in Regina, Saskatchewan. Host specificity tests were conducted to determine the host range of *C.g.m.* Initial findings showed a restricted host range to species in the Malvaceae, mainly *Malva* species and velvetleaf (*Abutilon theophrasti* Medic.) (Mortensen 1988). The optimum requirements for successful control (i.e. inoculum concentration, stage of growth of plant, temperature, dew, etc.) were determined under controlled and field conditions (Makowski 1987, Makowski and Mortensen 1990, Mortensen and Makowski 1990). These findings established that *C.g.m.* had potential as a bioherbicide.

Company involvement was sought early. Philom Bios Inc. (Innovation Place, Saskatoon, Saskatchewan) signed an agreement with Agriculture Canada in 1985 for the rights to commercialize the organism (tradename BioMal®). Philom Bios initiated the patenting in Canada, the U.S., and Europe. Patents for the use of the organism have been obtained in Canada, 27 November 1990, in Agriculture Canada's name; and in Europe in December 1989, but individual National patents were not pursued. A joint patent with Agriculture Canada and Philom Bios is pending in the U.S. Philom Bios was responsible for the manufacturing process (large scale production, fermentation, drying, and packaging), for guiding the registration through regulatory agencies, and for marketing and sale of the product. A royalty agreement with Agriculture Canada was signed in 1989.

An initial data package was submitted to the Pesticides Directorate in October 1987. At that time, there were no guidelines or protocols for the registration of microbial agents in Canada. A major portion of the early submission was based on the initial host specificity testing, efficacy and optimum requirements studies. This submission forced the Pesticides Directorate and the consulting agencies to develop guidelines and requirements for the registration of microbials. Similar guidelines developed in other countries and by international organizations were considered during the preparation of the Canadian guidelines and expanded upon. Protocols had to be developed to address concerns of the regulators and were instrumental in the evolution of the guidelines. Unfortunately, the guidelines in Canada and in the U.S. are still not harmonious.

An example of one of the concerns raised was the issue of latent infections, based on the claim that the development of bioherbicides without investigating latent infections may potentially lead to infection of commercial crops especially with *Colletotrichum* species (Cerkauskas 1988). To address this, an extensive set of protocols and experiments were developed as part of the environmental

toxicology testing on non-target crop species. The standard visual rating for disease symptoms and disease development were not adequate in the eyes of the regulators. Plating of plant material at various time intervals following inoculation to determine whether the fungus was present in addition to microscopic examination of spore germination and possible penetration after inoculation on the various non-target species were required. Resolution of this issue took 3 yrs of experiments for 2 scientists and 3 technicians. Even though there was a greater percentage of latent infections than originally anticipated, no adverse effect on crop yield or development was observed except for safflower under controlled or field conditions. The presence of latent infections by *C.g.m.* was not surprising as several other plant pathogens and saprophytic fungi have been reported to produce latent infections (Norse 1972, Mortensen and Bergman 1983, Spur and Welty 1975). Since *C.g.m.* is a facultative parasite it is not surprising that it grew on senescing cotyledons and leaves, which are similar to artificial media which provide nutrients for growth. Selection towards a more pathogenic fungus on crops as suggested by Cerkauskas (1988) is unrealistic, since the environment supports saprophytic growth. Selection, rather, would be towards a saprophytic organism. The regulators were satisfied with these findings and may not raise this concern again with future products.

A large portion of the Environmental Fate, Environmental Toxicology (non-target plant testing, crop tolerance), Food Residue, Efficacy, Agent Specification components were conducted by the Agriculture Canada Regina Research Station. The human health safety testing and the bird and fish components of the Environmental Toxicology were contracted to third parties. The final complete data package was submitted in December 1990 and registration was granted 16 January 1992.

This entire process was a learning experience. The evaluators needed to become familiar with dealing with a mycoherbicide as opposed to a chemical herbicide, requiring a drastic change in mind set. Researchers needed to become familiar with which concerns might arise and how to properly develop protocols and experiments from the start. In some cases, experiments needed to be repeated with more stringent standards (some of the host range tests), in other cases, questions were anticipated and addressed early in the experimentation (environmental fate).

Conclusion

BioMal® set the precedent for bioherbicide registration in Canada. It instigated the development of guidelines. As the level of confidence and the knowledge base of the regulators increases, the requirements for the next bioherbicide to be submitted for registration should be more straightforward. The protocols which were developed will serve as a template for research requirements. Early involvement and discussions with regulators is crucial for the effective use of financial resources or time and because each submission is dealt with on a case-by-case basis. Although this is the first bioherbicide registered in Canada, the time from discovery to registration was excessive at 10 yrs. It would have been fortuitous had the political will and the working reality coincided for the availability of guidelines. Hopefully, this will be the case for products of biotechnology. The success of the development of BioMal® rested on the commitment of both the public and private sector players, but also on the close collaboration of this multidisciplinary team.

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