SESSION 4B

PERSPECTIVES IN THE BIOLOGICAL CONTROL OF WEEDS

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Papers	4B-1 to 4B-4

The commercial realisation of biological herbicides

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ABSTRACT

The effective control of weeds is continually being claimed by the application of a wide variety of biological species, particularly fungi. The level of biocontrol achieved, either in the laboratory or in a controlled environment, is often sufficient to suggest that they could provide reliable, effective alternatives to chemical pesticides. Industry is constantly driven by the need to quickly develop more effective, safer and more highly differentiated products. Many advantages are to be gained from the sole use or synergistic action of a biological herbicide. There are, however, as yet relatively few successfully commercialised products. Several significant hurdles exist which need to be overcome to develop such biocontrol agents, most notably the technology required to produce and apply living organisms. This paper will address the potential of biological herbicides from a commercial viewpoint.

INTRODUCTION

Biological control is achieved by the deliberate use of natural living enemies to reduce the density of a particular pest to a tolerably low level (Boyetchko, 1997). A wide range of species have been successfully employed in the control of weeds. Insect-based control, in particular, has had a long history. Over the last few decades fungi, plant viruses, bacteria and nematodes have also been increasingly exploited (Julien & Griffiths, 1998). There are two major types of biological control (Boyetchko, 1997; Mortensen, 1998). Classical biological control involves the import and release of host-specific exotic enemies to control an introduced non-native pest. Augmentation involves regular action to increase populations of biocontrol agents. This may be through either inundative supplemental release of natural enemies or, to a much lesser extent, by environmental manipulation of the natural enemy.

Classical biocontrol of weeds has typically involved phytophagous insects and one of the most cited examples is the control of the prickly pear cactus (*Opuntia* spp.). This has been achieved in Australia by an alien moth (*Cactoblastis cactorum*) on over 24 million hectares, for the last 70 years. Comparable success has also been achieved with *Puccinia chondrillina* for the control of skeleton weed (*Chondrilla juncea*), a major weed of wheat. This fungal rust pathogen, collected in Italy, was introduced into Australia in 1971 and as a result no other herbicides have been required (TeBeest, 1996). The ideal target for this approach is an aggressive, introduced weed which infests large areas where the use of herbicides is often financially prohibitive. Successful control relies on the selection and introduction of an agent which, upon release, is self-perpetuating and provides long term control of the weed population. It is an irreversible, persistent and cost effective process. Since there is also little to prevent the agent spreading to untreated areas, there is low commercial incentive for product investigation and this area is mostly funded by the public sector.

The inundative approach involves mass production of a host's specific control agent and application at high inoculum levels over a localised area infested with the target weed. Weed control by this method is relatively short-term and the biological agent is not expected to be self sustaining. There are some examples using insects, but extensive literature only exists for inundative control using pathogens, particularly fungi (Weidemann *et al.*, 1995; Templeton, 1992). Fungi are the most commonly encountered pathogens of plants: many are destructive, most can be mass cultured and formulated and are capable of actively penetrating the host and therefore have immediate advantages over viral and bacterial bioherbicides. Compared to insects, mycoherbicides are restricted to the area of treatment (with limited capacity for dispersal), are often more host-specific and can be applied with conventional spray equipment at a time when the weed is most susceptible. Such attributes render their development as bioherbicides interesting to the agrochemical industry, yet despite this there remain relatively few commercial products (Table 1).

Table 1. Examples of pathogens investigated as commercial bioherbicides

Pathogen	Product	Target	Status	Territory
Phytophthora palivora	DeVine	*Morrenia odorata* (strangler-vine)	In use	USA
Colletotrichum gloeosporioides f.sp. *aeschynomene*	Collego	*Aeschynomene virginica* (northern jointvetch)	In use	USA
Colletotrichum gloeosporioides	Luboa	*Cuscuta* spp. (Dodder)	In use	China
Colletotrichum gloeosporioides f.sp. *malvae*	BioMal	*Malva pusilla* (Round-leafed mallow)	To launch	Canada
Chondostereum purpureum	Biochon	*Prunus serotina* Ehrh (American black cherry),	In use	Holland
	Ecoclear	*Betula lutea* Michx. (yellow birch) and *Populus* spp. (poplar) (maple and alder)	To launch	Canada
Xanthomonas campestris	Camperico X-Po	*Poa annua* (annual meadow grass)	In use To launch	Japan USA
Sclerotinia sclerotiorum	-	*Cirsium arvense* (California thistle) *C. nutans*, *C. tenuiflorus*, *C. vulgare*	To approve	NZ
Pseudomonas syringae	*X-Tend*	All major broadleaf and grass weeds	Stopped	USA
Epicoccosorus nematosporus	-	*Eleocharis kuroguwai* (water chestnut)	Field trials	Japan
Exserohilum monoceras B-026	-	*Echinochloa crus-galli* (barnyard grass)	Field trials	Japan
Drechslera monoceras MTB-951	-	*Echinochloa crus-galli* (barnyard grass)	Field trials	Japan
Colletotrichum truncatum	-	*Sesbania exaltata* (Rydb) ex. A. W. Hill (hemp sesbania)	Field trials	USA

CONSTRAINTS ON BIOHERBICIDE DEVELOPMENT

For the development of an industrial product, either chemical or biological, several criteria must be adhered to (Cross & Polenko, 1996). There must be sufficient customer demand and market size to ensure a financial return on the research and development investment within a reasonable time frame. There should be a cost effective manufacturing process which leads to a stable, effective and easy to use formulation. The formulated product should be compatible with existing distribution systems and agriculture practices, be highly efficacious, competitive, safe, reliable and give reproducible field performance. There should exist the ability to gain patent protection and product registration. In addition, there should be sufficient sales revenue generated to support continued marketing and sales promotion and enable further product development. While problems can be envisaged in meeting these criteria for a synthetic herbicide, there are further factors in the development of a bioherbicide and these may be considered in four categories (Mortensen, 1998).

Biological

Pathogens are generally specific only to a single, or limited, number of target host species and, while being advantageous environmentally, there may be limited commercial potential. Most pathogens lack the aggressiveness to achieve desired weed suppression and have low residual activity. They are often slow to act with a lag effect as the pathogen becomes established. High inoculation levels are required for the organism to reach the desired target, establish adequate infection, overcome a weed's inherent defence systems and finally control the weed. Plant factors such as morphology of the target weed, low susceptibility of the host, growth habit, growth rate and population dynamics often limit disease development. Bioherbicide development requires a comprehensive understanding of the pathogen, the target weed and their complex interactions to determine the optimum requirements for disease initiation and development. In addition, the level of disease control must be acceptable to the farmer, ideally equalling the level of control achieved by other measures. Compatibility with other pesticides may also be advantageous, particularly as part of an integrated pest management programme.

Novel methods of overcoming a weed's defence system have been implemented for two commercial products. Biochon, *Chondostereum purpureum*, presently sold as a suspension of fungal mycelium in water, prevents regrowth of undesirable forest weed, American black cherry (*Prunus serotina*), when applied to cut stumps. Japan Tobacco has launched the first bacterial herbicide in Japan based on a vascular phytopathogenic bacterium, *Xanthomonas campestris*, for post-emergent control of *Poa annua* on golf courses. Since the bacterium can only infect damaged leaves it must be used in areas which are regularly mown.

Environmental

Pathogens are generally fastidious about varying environmental conditions, resulting in unacceptable levels of control and unpredictable reliability. The optimal efficacy of foliar pathogens is highly dependent on temperature, moisture and dew period, although soil-borne pathogens are generally less susceptible to short term fluctuations in environmental conditions. Appropriate timing of application is a major consideration and must take advantage of the appropriate environmental conditions and/or the most susceptible plant

growth stage. Compatibility of a pathogen with other microflora in the ecosphere must be considered. Such interactions may contribute to the difficulty of translating from the greenhouse to the field situation where bioherbicides must give good reliable performance under the conditions of commercial usage. Bioherbicides are, however, considered environmentally safe, with negligible residual effects or pollution and cause minimal impact on non-target organisms, including humans.

USDA-ARS, for example, is seeking to control hemp sesbania in a range of crops including soybean and cotton. The pathogen used, *Colletotrichum truncatum*, is highly virulent, highly specific and can be mass produced, but is particularly sensitive to lack of moisture.

Technological

Mass production of abundant, viable, infective and genetically stable propagules of a biocontrol agent at an acceptable cost is a major requirement to ensure product consistency. Production may be difficult owing to the lack of viable techniques for *in vitro* large scale culturing. Submerged liquid fermentation techniques are available, and solid state fermentation is becoming more accessible, and economically feasible, particularly for fungi. The development of reliable and efficacious bioherbicides is also reliant on formulation which must be compatible with the agent, maintain stability during storage, distribution and application and offer protection from environmental fluctuations. It should allow unit activity to avoid high dose rates and enhance performance. During production and formulation, spore viability must be maintained by ensuring optimal nutritional conditions. Advantages are to be gained from compatibility of the formulation with normal agriculture practice with regard to the method and timing of application (Green *et al.*, 1998). The choice of delivery system is also based on attributes of the host plant and biocontrol agent. Optimising spray application parameters (droplet size, spray volume, concentration, droplet retention and distribution for example) and formulation for each pathogen-weed combination might be required for reliable field efficacy of microbial herbicides (Greaves *et al.*, 1998). Bioherbicides may be more difficult to apply in the field and education may be required in associated new techniques and in the level of control achieved. The production, formulation and delivery techniques are not as well developed as for chemical pesticides.

DeVine, based on the soil borne fungus *Phytophthora palivora* was developed for effective control of *Morrenia odorata* (strangler-vine) in Florida citrus groves. Sales are made to order owing to the six week expiry of the formulation and distribution relies on constant refrigeration from time of manufacture to use, limiting its market potential. This product was sold from 1981 to 1991 and was reintroduced by Abbott laboratories in 1995 as a result of its efficacy (Cross & Polenko, 1996). Luboa, a Chinese government product, suffered a rapid decline in its use in the early 1980s owing to the deterioration of the *C. gloeosporioides* strain and technical problems associated with fermentation and commercialisation. A new strain obtained in 1985 is presently used (Mortensen, 1998).

Commercial

The small, niche markets of most bioherbicides have often discouraged commitment from the mainstream crop protection industry owing to limited opportunities for the recovery of investment. As a result several potentially efficient products have not been developed.

Although significant differences exist between countries, bioherbicides generally have fewer regulatory requirements, leading to potentially faster registration and rapid market entry. If time savings could be made in other aspects of product development which are often dependent on the complexity of the agent, the entire process has the potential to be faster and cheaper and may allow exploitation of small profitable markets. Patent protection of intellectual property has now also been greatly strengthened by the availability of DNA profiling and other diagnostic tools which allow precise identification of microbial strains.

The ban on 2,4,5-T initially stimulated the market for Collego (*Colletotrichum gloeosporioides* f.sp. *aeschynomene*) which was sold from 1982 to 1992, but then withdrawn owing to the introduction of an alternative chemical herbicide, oxyfluorfen, and the limited market in rice. Its effectiveness and ease of application by conventional sprayers has, however, lead to its reintroduction and sales records are being broken each year (Greaves & TeBeest, personal communication). BioMal, a dry powder formulation based on the foliar pathogen *Colletotrichum gloeosporioides* f.sp. *malvae*, gives excellent weed control of round-leafed mallow. After registration in Canada in 1992, difficulties with mass production and potential market size had held up further development (Cross & Polenko, 1996). The product is, however, due to be launched by Agriculture Canada after re-evaluation of market potential. Japan Tobacco is also developing two new mycoherbicides (*Epicoccosorus nematosporus* and *Exserohilum monoceras*) for the control of economically favoured rice weeds. Mitsui is targeting *Echinochloa crus-galli* in rice using the fungal pathogen *Drechslera monoceras* and AgResearch is seeking approval of selected strains of *Sclerotinia sclerotiorum* in New Zealand for thistle control (TeBeest, 1996). It remains to be seen whether any of these new potential bioherbicides can become commercially viable.

THE FUTURE OF BIOHERBICIDES

The two most successful commercial products, Devine and Collego, resulted from research initiated several decades ago. Development of methodologies to overcome the critical biological and technological constraints are required to produce a significant increase in the utilisation of bioherbicides.

Genetically modified bioherbicides

It has been suggested that biological limitations may be addressed by genetic engineering. Molecular methods allow the identification of traits and genes responsible for weed inhibition, thus allowing the construction of genetically altered, superior biocontrol agents (Kennedy, 1996). The scope of microbial herbicides could be greatly increased, for example, by extending the host range of existing pathogens or enhancing the ability to overcome a weed's defence system. Resistance to other pesticides may be addressed, such as the manipulation of *Colletotrichum gloeosporioides* to overcome resistance to Bilanofos (Brooker *et al.*, 1994). Alternatively, a non pathogenic microbe chosen for the ability to rapidly colonise a weed may be modified to deliver novel natural product active ingredients.

The expectations from these products have not yet been realised. This has largely been as a result of new problems relating to the release of genetically modified organisms, which are considered artificial thus leading to greater caution. Genetic modifications must be evaluated

in relation to ecological and environmental issues and there are particular concerns regarding genetic stability, alteration of host range and the effect of large scale release into a balanced ecosystem. There is concern that biotechnology may overshadow traditional biological control, because it produces patentable products that may yield relatively high profits. Genetic manipulation to improve bioherbicide performance is still open to scientific and political debate and problems regarding product registration have to be resolved. However, even after identification of a genetically modified strain with desired biocontrol properties, its further development is subject to many of the remaining constraints of traditional bioherbicides.

Chemical and microbial synergists

In contrast to genetic manipulation, non-permanent strategies may have greater advantages for the future of bioherbicides (Gressel *et al.,* 1997). The addition of a low dose chemical, or adjuvant to immobilise targets or to increase target range could have a dramatic effect on bioherbicide potential. The use of synergists has the potential to increase bioherbicide efficacy and lower inoculum levels rendering the bioherbicide more cost effective. They may also reduce chemical application rates and, therefore, the reliance on chemical pesticides delaying the onset of possible resistance. By combining the rust *Puccinia canaliculata* with the herbicide paraquat almost complete control of nutsedge was obtained, compared to 60% control with the rust alone and 10% with paraquat alone (Boyette *et al.*, 1996). The potential bacterial bioherbicide, *Pseudomonas syringae*, in conjunction with conventional herbicides (e.g. glyphosate) allowed use of the chemical herbicides at 10% of the normal field dose rate. Synergistic effects have also been observed between microbes. The efficacy of *Colletotrichum coccodes* to control velvetleaf (*Abutilon theophrasti*) was improved when phyllopane *Pseudomonas* spp. was co-inoculated with the fungus, resulting in development of the disease symptoms 4 to 5 days earlier.

The complexity of this approach arises from two living and dynamic systems (weed and microbe) which each act or react physically and biochemically not only to the environmental conditions, but also to each other and to the potentially synergistic chemical. Several general factors can influence chemical-bioherbicide interactions such as the toxicity of a chemical and its residues to the pathogen, the concentrations of chemical and inoculum and timing of chemical and pathogen applications in relation to each other and to weed age. Environmental conditions, elicitation of weed defence by chemical treatment, weed resistance/susceptibility and the effect of chemicals on metabolism in weeds may also be factors (Hoagland, 1996). Special techniques, such as safeners, slow release formulations allowing co-application or timed applications, could be developed to allow a successful combination of chemicals and bioherbicides to achieve practical and selective weed control.

Formulation

Formulation holds the key to the future development of successful bioherbicides as it may be used to address many of the present limitations (Weidemann *et al.*, 1995). It has the potential to produce a standard, stable, viable product with optimal protection from disease limiting environmental conditions which produces consistent field results. It enables incorporation of additives to increase pathogen aggressiveness or decrease host resistance and aid compatibility with chemical synergists and effective delivery systems (Boyette *et al.*, 1996).

Invert emulsion formulations of mycoherbicides, for example, have been used to address problems of dew period, thus aiding infectivity and lowering the inoculum levels required. Encapsulation technology traps the agent in a matrix such as alginate, agarose, or polyurethane to help control rates of release and protect the organism from desiccation, microbial competition, and adverse environmental conditions. Alginate formulations retain activity after periods of storage and are ideal for application of soil borne fungal pathogens. Wettable powder formulations have found much success with foliar bioherbicides such as Collego and BioMal and may be applied through conventional sprayers. The fungal spores are able to withstand the processes of drying, storage and rehydration without appreciable loss of viability. Granular formulations have been found to generally have a longer shelf life than liquid-based formulations and also allow controlled release or growth of the organism from the formulation. These therefore have a greater chance of commercialisation.

Various adjuvants have been included to formulations to address typical problems such as shelf life and stability, propagule germination, pathogen virulence and tolerance to environmental stress. Others may address the requirement to overcome the host resistance factor and assist in delivery by improved adhesion and distribution of propagules on the host surface. These encompass a wide range of compounds including surfactants, wetters, stickers, inert carriers, antifreezing compounds, humectants, sun screening agents, antievaporants, UV protectants, feeding stimulants, micronutrients and herbicides.

CONCLUSIONS

A bioherbicide needs to be fast-acting, predictable and easy to use, and must provide an effective level of control before it will have general acceptance from industry and users as a replacement for a chemical herbicide. Bioherbicides are still unproven as practical, economically viable alternatives to chemical weed control.

Bioherbicides however, do have a strong market advantage owing to their environmental compatibility and safety record. Their increasing demand is being driven largely by the consumers' preference for naturally sourced agents. The interest of industry stems from the high cost of developing new synthetic herbicides and the problems with herbicide resistant weed populations and unfavourable environmental profiles. As a result, biological agents are seen as increasingly attractive in markets where chemical pesticides have been withdrawn or restrictions placed on their use or where chemical control is too expensive or no longer effective. Narrow weed spectrum chemical herbicides exist (e.g. fluroxypyr for the control of *Galium aparine* and difenzoquat for control of *Avena* spp.) and are successful because their effectiveness outweighs the inconvenience of their additional use. Situations where a single economically important weed species of a major crop escapes control are ideal for bioherbicide application. Synergistic application with a low dose herbicide may be exploited to achieve broad spectrum control. Bioherbicides should be developed to complement, rather than compete, with chemical herbicides.

To increase the chance of success, co-operation is needed between academia where a wealth of knowledge is available, and industry, where there is expertise in product development, marketing, and distribution. Critical research is required into production, formulation and delivery to improve performance. Integrated strategies may increase bioherbicide

effectiveness and/or reduce control costs by reduced rates of application of chemical herbicides with a concomitantly favourable impact on the environment. As a result this should lead to increased industrial commitment and the production of economically viable bioherbicide solutions.

REFERENCES

Boyetchko S M (1997). Principles of biological weed control with microorganisms. *HortScience* **32(2)**, 201-205.

Boyette C D; Quimby P C; Caesar A J; Birdsall J L; Connick W J; Daigle D J; Jackson M A; Egley G H; Abbas H K (1996). Adjuvants, formulations, and spraying systems for improvement of mycoherbicides. *Weed Technology* **10**, 637-644.

Brooker N L; Lydon J; Mischke C F (1994). Genetic and phenotypic variability in bialaphos resistant transformants of *Colletotrichum gloeosporioides* f.sp. *aeschynomene*. *Phytopathology* **84(5)**, 541.

Cross J V; Polonenko D R (1996). An industry perspective on registration and commercialization of biocontrol agents in Canada. *Canadian Journal of Plant Pathology* **18(4)**, 446-454.

Greaves M P; Holloway P J; Auld B A (1998). Formulation of microbial herbicides. In: *Formulation of microbial biopesticides: Beneficial microorganisms, nematodes and seed treatments,* ed H D Burges, pp. 203-233. Kluwer, Dordrecht.

Green S; Stewart-Wade S M; Boland G J; Teshler M P; Liu S H (1998). Formulating microorganisms for biological control of weeds. In: *Plant-microbe interactions in biological control*, eds G J Boland & L D Kuykendall, pp. 249-281. Dekker, New York.

Gressel J; Amsellem Z; Michaeli D; Sharon A (1997). Synergized mycoherbicides for resistance management. In: *Weed and crop resistance to herbicides*, eds R De Prado, J Jorrin & L Garcia-Torres, pp. 267-274. Kluwer, Dordrecht.

Hoagland R E (1996). Chemical interactions with bioherbicides to improve efficacy. *Weed Technology* **10**, 651-674.

Julien M H; Griffiths M W (1998). *Biological control of weeds: A world catalogue of agents and their target weeds*, eds M H Julien & M W Griffiths. CAB International Wallingford, Oxford.

Kennedy A C (1996). Molecular biology of bacteria and fungi for biological control of weeds. In: *Molecular biology of the biological control of pests and diseases of plants*, eds M Gunasekaran & D J Weber, pp. 155-172. CRC Press Inc.

Mortensen K (1998). Biological control of weeds using microorganisms. In: *Plant-microbe interactions in biological control*, eds G J Boland & L D Kuykendall, pp. 223-248. Dekker, New York.

TeBeest D O (1996). Biological control of weeds with plant pathogens and microbial pesticides. *Advances in Agronomy* **56**, 115-137.

Templeton G E (1992). Use of *Colletotrichum* strains as mycoherbicides. In *Colletotrichum: Biology, pathology and control,* eds J A Bailey & M J Jeger, pp. 358-380. CAB International, Wallingford, Oxon, UK.

Weidemann G J; Boyette C D; Templeton G E (1995). Utilization criteria for mycoherbicides. *ACS Symposium Series* **595**, 238-51.

Formulation and spray application-forgotten factors in the development of microbial herbicides

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ABSTRACT

This paper discusses the role formulation and, especially, spray application characterisation play in determining microbial herbicide efficacy. In particular, the requirements to allow proper identification of plant pathogenic organisms with real potential as reliable microbial herbicides for field application are discussed. In order to avoid false identification of potential, it is recommended that tests should use sprays at realistic application volumes (not to exceed 500 l ha^{-1}) with a practical spray application system, not an aerosol sprayer.

INTRODUCTION

Despite extensive research over the last 35 years, which has examined large numbers of pathogen-weed combinations, there are very few microbial herbicides in practical use. For example, Boyetchko (1999) refers to 33 bacteria and fungi that have been examined for potential as foliar- or soil- applied microbial herbicides. Of these 8 have been registered with the US Environmental Protection Agency but only 6 have entered the market place.

Clearly, this is not a notable return on the research and development of the last 35 years and several reasons have been offered to account for it (Weston, 1999). Obviously, commercial reasons such as uneconomic market size are important, though a large crop protection company's idea of acceptable market size may be considerably lower than that perceived by a smaller specialist company. More frequently, unreliable field performance in the wide range of environmental conditions under which the agent must work is cited as the reason that a potential control organism is not developed to a product. This can arise from a wide variety of causes, most of which have solutions. For example, low virulence, necessitating very high inoculum levels, can be redressed by selection of high virulence strains. Most often unreliability is seen as a result of adverse environmental factors, particularly lack of moisture during the critical phase between inoculation of the target and establishment of infection (Figure 1). As Figure 1 illustrates, there is a highly complex series of factors, many of which interact, which are operative between preparing the spray suspension (inoculum) of a microbial herbicide and its ultimate ability to express disease and, so, control the target weed. Virtually all of these factors will respond to formulation. Although much work, some partially successful, on formulation development to overcome environmental constraints has been done, and will be reviewed briefly here, little has been done on relationships between formulation and spray application aspects. Even the most effective formulations will not function as intended if they are not applied effectively and this aspect will also be addressed.

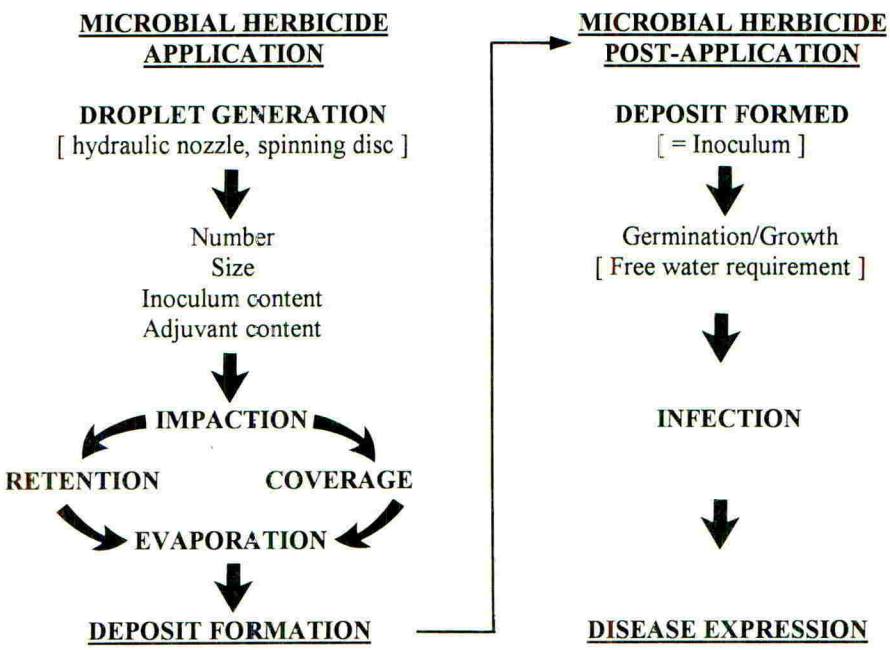

Figure 1. Factors interacting in the application of microbial herbicides and their biological efficacy.

FORMULATION OF MICROBIAL HERBICIDES

Most research to date has concerned foliar applied pathogens as microbial herbicides and, thus, most of the agents in practical use fall in this category. This paper will also, for the same reasons, but also because this type of agent presents particularly difficult problems of formulation and application focus on foliar applications. Formulations for application to soil are described in detail by Greaves *et al.* (1998).

It is commonly reported that potential microbial herbicides have been tested by application to the target weed as suspensions in water or with a surfactant such as Tween 40. This ignores the wealth of information showing the need for more robust formulations to ensure efficacy outside the optimised environments of laboratory research.

Greaves *et al.* (1998) have reviewed the current status of microbial herbicide formulation covering foliar applications and formulations for application to soil. They show clearly, that there is considerable scope for protecting organisms from damage by desiccation during storage and after application and by sub-optimal temperature and UV irradiation after application. It has proved more difficult, in the hours immediately after application to provide the agent with sufficient free water to allow full infection and disease expression. Some

success has been met with oil emulsions and invert emulsions but these must be tailored to each microbial agent. This is a major factor contributing to unreliable efficacy in the field, where free water on the leaf surface is not as common as might be thought in the spring/early summer, when microbial herbicides need to be applied.

In addition to the research into microbial herbicide formulation, there is a wealth of information regarding formulation of other organisms for use in crop protection (Burges, 1998). It is clear that there are many common principles in work on formulation of agents for control of insects, diseases or weeds whether the agents are fungi, bacteria, viruses or nematodes. Unfortunately, it is equally clear that these common principles have not always crossed the boundaries between the different research areas. Much improvement in formulation of microbial herbicides would be gained from such technology transfer. At the same time it has to be recognised that there will inevitably be a need to custom designed formulations for each biocontrol agent. This will be determined for example, by the sensitivity of the organism to the effects of UV irradiation or to possible toxic effects of the co-formulants, and the extent to which the organism has to be protected from desiccation or its dew period requirement has to be reduced.

SPRAY APPLICATION

Even the most effective formulations will not express their benefits fully if they are not applied to their target efficiently. Regrettably, this aspect of the development of microbial herbicides has been almost completely ignored.

The selection of spray application parameters such as application volume and droplet size is always a compromise between what is optimal and what is practical. Thus the optimum retention is achieved with small, slow-moving droplets (as produced by an air-brush) whereas in practice larger, faster droplets (with high kinetic energy – as produced in the standard hydraulic nozzle) are needed to penetrate the crop canopy and resist drift. The optimum size varies with the target (Table 1).

In the case of microbial agents, compromise must again be made as it has been shown that large proportion of very small droplets may not contain any inoculum (spores). The optimum size for carrying appropriate spore numbers is between 150 and 250 µm (Lawrie *et al.*, 1997).

Table 1. Optimum droplet sizes for application of crop protection chemicals to different targets.

Target	Droplet size (µm)
Flying insects	10 - 50
Insects on foliage	30 - 50
Foliage	40 - 100
Soil and drift reduction	250 - 500

(Matthews, 1979)

Droplets reach the target surface mainly by sedimentation. On striking the surface they may be retained, reflected (bounce) or shatter depending on their size and velocity, liquid characteristics (density, viscosity, dynamic surface tension) and the nature of the target surface (angle of inclination, waxes, trichomes). Clearly retention could be maximised, using droplets with low kinetic energy (small and slow). Retention can be increased by formulation with appropriate surface active compounds or stickers. Such formulation is particularly important when the target leaf surface has e.g. abundant rough wax deposits. Smooth surfaces without crystalline waxes or trichomes are most easily wetted, retention is high and largely unaffected by application factors and formulation. Crystalline waxes and trichomes tend to maintain a layer of air between the surface and the droplet and will require small droplets and/or solutions of low surface tension to wet the leaf. Obviously, then vertical leaves (grasses) are harder to hit, and retain less spray, than large, flat (herb) leaves and so require fine sprays. Typical droplet spectra produced by different application systems are shown in Table 2.

Table 2. Droplet size spectra produced by a range of application systems and normal application volumes used.

Application System	Application volume ($l\ ha^{-1}$)	Droplet size range (μm)
Airbrush (aerosol)	1000 – 3000	4 – 150
Hydraulic nozzle	100 - 200	4 – 523
	200 - 500	15 – 654
	500 - 1000	19 – 916
Spinning disc	20 - 100	Controlled

From Table 2 it is immediately obvious that the airbrush completely misrepresents practical field applications. The volume applied is excessive and so applies an artificially high level of inoculum, with many propagules being excluded from the very small droplets (Lawrie *et al.*, 1997) and, so, arriving at the target in a "dry" state. Their viability is assured only because the high volume of liquid applied (up to 3000 $l\ ha^{-1}$) completely wets the leaf surface. In fact, it wets it so much that many spores are moved by liquid flow to drip points where they cause the large necrotic areas that are typical of this application system. In general, this system achieves a level of inundation of the target that cannot be achieved in the field by conventional spraying (max. 500 $l\ ha^{-1}$). Thus, by delivering approx. 6 or more times more inoculum than a field sprayer, it identifies a level of potential efficacy in candidate microbial herbicides that is extremely unlikely to be seen in the field and, so, may be responsible for much wasted research and development.

As has been said above, by affecting retention, the droplet spectrum produced by the application system has an important impact on the efficacy of the applied agent. It also has a marked effect on distribution of the retained spray on and around the target. Droplets less than 67 μm volume median diameter (VMD) will not reach the target but be trapped in turbulent air. Droplets larger than circa 400 μm VMD will be poorly retained on the target and go to waste in the soil. So, as a compromise between optimum retention and minimal

drift droplets in the range 100 to 400 μm are often recommended (Knoche, 1994). As stated earlier, spore loading in droplets depends on droplet size, with 20 to 78%, (depending on spore size), of droplets of 150 μm VMD or less containing no spores.

Recent work (Lawrie and Greaves, unpublished) using sodium fluorescein as a tracer has examined spray deposition on the weed *Amaranthus retroflexus* (Table 3). Using this data, expected spore depositions were calculated, assuming an application concentration of 1 x 10^6 ml^{-1}.

Table 3. Effect of application system on spray deposition and distribution of conidia on *Amaranthus retroflexus.*

	Application system			
Plant target Zone	Airbrush 800 l ha^{-1}	Spinning disc 40 l ha^{-1}	Hydraulic nozzle 100 l ha^{-1}	Twin-fluid nozzle 100 l ha^{-1}
Cotyledons	4.819* (4819)**	0.225 (225)	0.485 (485)	0.339 (339)
Leaf surfaces	2.941 (2941)	0.324 (324)	0.775 (775)	0.734 (734)
Stem	2.316 (2316)	0.021 (21)	0.095 (95)	0.111 (111)
Apex	6.166 (6166)	0.158 (158)	0.431 (431)	0.604 (604)

* Data are μl cm^{-2} of fluorescein solution retained.

** Figures in ( ) are numbers of conidia cm^2 calculated from fluorescein retention data assuming 10^6 conidia ml^{-1} of spray.

It is clear from Table 3 that the Airbrush system will always be more likely to produce effective infection of the target weed, as it delivers so many more conidia especially to stems and apices. All the other application systems favour deposition and retention on the horizontal surfaces of the plant, that is the leaves and cotyledons. Infection of stem and apex targets are more likely to cause weed death than leaf infection, unless the pathogen produces a systemic toxin. The higher efficacy of the Airbrush is due to several factors. It needs to be set at an angle to the plant (usually 45°) as it will not spray vertically downwards like the nozzles. It produces small droplets in a turbulent airstream. It sprays a high application volume and so encourages run-off on to stems. All this suggests that efficacy in the field could be improved by angling the nozzle, placing it nearer to the soil and spraying the largest volume rate possible. Small spray droplets are, unfortunately, prone to drift. A solution may be to use air-assist sprayers (Hislop *et al.*, 1995) or twin-fluid nozzles (Rutherford *et al.*, 1989) which reduce drift and increase the velocity of the droplets.

In practice the spray application volume is unlikely to exceed 500 l ha^{-1}. Potyka (1995) has shown that at such application volumes spore retention is principally along leaf veins (i.e. in the troughs on the leaf) or at descending areas of the leaf (edges and tips, i.e. drip points). These findings have been confirmed in current research (Lawrie, Down & Greaves, unpublished). This pattern of retention results from spray liquid movement during and after spraying and so, the accumulation of large volumes of liquid at these "drip points" will reduce conidial desiccation and encourage and maintain germination and infection. This accounts for the large necrotic patches at leaf edges and tips commonly observed in microbial herbicide experiments.

Another aspect of spray application which can significantly affect efficacy of microbial herbicides, and which has been largely ignored is conidial density in droplets. Practical application volumes produces discrete deposits on the leaf and, thus, the number of conidia contained in the deposit is important with regard to establishment of infection. Too many conidia is economically wasteful and may be counterproductive as there is evidence that some conidia may inhibit each other's germination if they are too dense. Conversely, too few conidia may reduce the chances of infection by reducing autostimulation of germination which is also known to occur. The ideal situation of 1 spore per droplet giving successful infection has been achieved (Amsellem *et al.*, 1991) but is unlikely to be achievable outside the laboratory.

The question of spore loading in droplets is further compounded by calculations, based on the fluorescein retention data, given above, show that as few as 18% of the conidia leaving the spray nozzle may be retained by the plant. Many are, of course, intercepted by the crop in which the target weed is growing, especially dense cereal crops. Others reach the soil or never reach the top of the plant canopy. Currently, direct measurements of spray retained on leaves of *Amaranthus retroflexus* growing in wheat, maize and sugar beet crops (Table 4), appear to show that, as expected, only about half the applied spray is intercepted and retained by the weed, even in the absence of a crop. In a wide-row crop such as maize or sugar beet, weeds between the rows show no further reduction in this retention. However, weeds growing within the rows retain less than 33 to 42% of the spray. In dense crops, such as wheat, retention is markedly further reduced to 15 to 17% of the applied spray. Direct measurements of retention of the conidia in these sprays (Lawrie, Down and Greaves, unpublished) show that in dense crops a maximum of 18% of the conidia are retained on the leaf.

Table 4. Volume of spray (l ha^{-1}) retained on *Amaranthus retroflexus* grown in different crops and sprayed at 86 l ha^{-1} using a hydraulic nozzle.

Crop	Growth Stage	Vol. Spray retained on *A. retroflexus**	
		in the row	between rows
-	-	42	42
Maize	5 leaf	28	56
Sugar beet	6/7 leaf	36	50
Wheat	6 leaf (4/5 tillers)	13	15

* at the 4-leaf growth stage.

Obviously, if an organism is identified as a potential microbial herbicide on the basis of laboratory/glasshouse tests using high volumes of inoculum applied from an aerosol sprayer, it is less likely to perform in a similar or reliable way when applied by more practical means in the field. Even the most sophisticated formulation is unlikely to overcome the small number of spores retained on the target in, possibly, not the most appropriate sites. Equally obviously then, researchers must be persuaded of the essential need to do their initial assessments of potential by spraying it at realistic application volumes with a standard spraying system chosen as appropriate to the cropping system in which the weed is a problem. This must be followed by careful matching of the spraying system to the formulation developed to maximise biological efficacy taking into account spore size/types in relation to droplet size. This is, of course, an oversimplification of a complex interactive process but, nonetheless, it illustrates the approach that must be taken in developing a microbial herbicide research programme. Only then will we avoid spending time and money researching candidate agents that produce excellent results in the laboratory but never perform acceptably in the field. This will allow us to more regularly identify, and subsequently develop, the organism with true potential as microbial herbicides for use in practical circumstances which are, without doubt, waiting to be discovered and developed.

ACKNOWLEDGEMENTS

The unpublished research quoted is funded by the Ministry of Agriculture, Fisheries and Food. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

REFERENCES

Amsellem Z; Sharon A; Gressel J (1991). Abolition of selectivity of two mycoherbicidal organisms and enhanced virulence of avirulent fungi by an invert emulsion. *Phytopathology* **81**, 985-988.

Boyetchko S M (1999) Innnovative applications of microbial agents for biological weed control. In: *Biotechnological approaches in biocontrol of plant pathogens*, eds Mukerji *et al.*, pp. 73-97. Kluwer Academic/Plenum Publishers: New York.

Burges H D (1998). *Formulation of microbial biopesticides, beneficial microorganisms, nematodes and seed treatments.* Kluwer Academic Publishers, London.

Greaves M P; Auld B A; Holloway P J (1998). Formulation of microbial herbicides. In: *Formulation of microbial biopesticides, beneficial microorganisms, nematodes and seed treatments,* ed H D Burges, pp.203-233. Kluwer Academic Publisher: London.

Hislop E; Western N M; Butler R C (1995). Experimental air-assisted spraying of a maturing cereal crop under controlled conditions. *Crop Protection* **14**, 19-26.

Knoche (1994). Effect of droplet size and carrier volume or performance of foliar-applied herbicides. *Crop Protection* **13**, 163-178.

Lawrie J; Greaves M P; Down V M; Chassot A (1997). Some effects of spray droplet size on distribution germination of and infection by mycoherbicide spores. *Aspects of Applied Biology* **48**, 175-182.

Matthews G A (1979). *Pesticide application methods.* Longman Group Ltd., London.

Potyka I (1995). *Emulsion formulation of microbial herbicides.* PhD Thesis, University of Bristol, UK.

Rutherford I; Bell G J; Freer J B S; Herrington P J; Miller P C H (1989). An evaluation of chemical application systems. *Proceedings of the Brighton Conference – Weeds* **2**, 601-613.

Weston V C M (1999). The commercial realisation of biological herbicides. *Proceedings of the 1999 Brighton Conference – Weeds, BCPC* (in press).

Dutch case studies showing the success and limitations of biological weed control

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ABSTRACT

This paper analyses critical success factors for development of mycoherbicides by reference to two Dutch case studies.

Chondrostereum purpureum, a pathogen of several hardwood species, attacks the introduced black cherry (*Prunus serotina*) which is a weed in Dutch forests. It is highly efficacious under diverse environmental conditions if mycelium fragments are applied to fresh wounds in the wood. The risk for non-target species was shown to be acceptable for most situations, and *C. purpureum* is sold as a wood decay promotor in the Netherlands under the name Biochon. The market size is too limited to justify the costs of registration as a mycoherbicide.

For *Chenopodium album*, the potential mycoherbicide market is much larger, those *Chenopodium* populations resistant to triazine herbicides alone being economic. *Ascochyta caulina* is a leaf and stem pathogen of *Chenopodium* and *Atriplex* species. In maize and sugar beet, application of the fungus caused up to 70% mortality and considerable growth reduction of surviving *Chenopodium* plants. Whilst encouraging, the results are too much dependent on weed growth stage and suitable weather conditions for practical use. Solutions are being sought in fungus strain selection, formulation, and combinations of the fungus with its own phytotoxins or a low dose of a herbicide in and EU-sponsored collaborative project.

FACTORS DETERMINING THE SUCCESS OF MYCOHERBICIDES

The control of a native weed by spraying it with spores of an indigenous plant pathogenic fungus (Daniel *et al.* 1973) introduced a new concept of weed control, the mycoherbicide tactic (Templeton *et al.*, 1979). This means a pathogenic fungus inoculated inundatively to reduce weed population density at a specific locality.

Many projects on control of weeds with mycoherbicides have been done, motivated by the public's concern about chemical herbicides, the lack of other controls for key weeds, scientific interests and commercial opportunities. But few projects have yielded practical mycoherbicides. So far, approximately ten mycoherbicides have been used in practice (Müller-Schärer & Scheepens, 1997; Wall, 1997).

To focus future research prperly, the critical success factors for development must be known. Four categories of such factors (biological, technical, commercial and legal) can be distinguished. Biological factors concern the weed, the pathogen or their interaction. Technical factors aim at increasing the efficacy of the control agent and mass production

of infective units, development of stable formulations that ensure efficacy under diverse environmental conditions. Technical factors also include integration of biological control in management systems. Legal factors include patenting procedures or products, and registration procedures. Commercial interest depends on the cost-benefit analysis, determined by biological factors (market size), technical factors (ease of production, shelf life and ease of control), and legal aspects that guarantee a monopoly position.

This paper analyses critical success factors for two Dutch mycoherbicide projects.

CASE STUDIES

Biochon

Prunus serotina (*Rosaceae*) was introduced from North America into the Netherlands to improve the understory of forests and to ameliorate forest soils by its litter (Bakker, 1963). Both in its native range and in the Netherlands, it is an opportunistic species that rapidly colonises open spaces (Auclair & Cottam, 1971; Eijsackers & Oldenkamp, 1976). It has become a noxious weed because it competes with newly planted forest trees and with the natural vegetation in the understory of forests. It used to be controlled by cutting and treatment of the stumps with a broad-spectrum herbicide, usually glyphosate. Because *P. serotina* is the only weed species involved, chemical control in forests could be completely replaced by selective biological control. *P. serotina* has few natural enemies in the Netherlands and none are selective against this species. From the beginning of the project, investigations were aimed at the use of a native plant pathogen as a mycoherbicide (Scheepens & Van Zon, 1982), and *Chondrostereum purpureum* seemed a likely candidate. As a pathogen of fruit trees, its life history had been studied comprehensively.

C. purpureum is a wood-inhabiting fungus, growing saprophytically in logs and stumps of many deciduous tree species. In autumn, it can be recognised by its purple fruiting bodies on the wood. It can also be a parasite, but to fewer species. The most susceptible species belong to the genus *Prunus* within the *Rosaceae*, where it causes silver-leaf disease. Trees are infected by basidiospores through fresh wounds in the wood. The presence of mycelium in the sap wood causes interruption of the transpiration stream to the leaves, and is probably responsible for the ultimate death of branches and whole trees (Butler & Jones, 1949). In the Netherlands, silver leaf disease used to be of great economic importance to plum (*P. domestica*) but is much less importance to fruit trees nowadays (Van der Scheer & Wondergem, 1981).

Silver leaf disease is present in *P. serotina* at a low frequency (less than 1 %). During the period from 1980 until 1986, field experiments were done by cutting back *P. serotina* and treating stumps with agar cultures or suspensions of fragmented mycelium of *C. purpureum*. On average, nearly 90 % of stumps treated with the fungus died. The lowest dose tested (20 µg mycelial dry weight per stump) was as effective as ten times that dose. Mycelium was much better as an inoculum then spores. Applications in spring or autumn were about equally effective (Scheepens & Hoogerbrugge, 1988). A comprehensive risk analysis was done to assess possible consequences of biological

control with *C. purpureum* for non-target trees (DeJong *et al.*, 1990). It was shown that added infection pressure outside forests was of the same order of magnitude as, or less than, natural infection pressure. The Dutch Plant Quarantine Authority approved this conclusion by stating that risk to non-target fruit trees is acceptable, unless the distance between an orchard and a treated forest is less than 500 m.

C. purpureum is sold, as fresh mycelium under the name Biochon, as a wood decay promotor by the company Koppert Biological Systems since 1997. It is not officially registered as a pesticide, but, in practice, can be recognised as the first European mycoherbicide. Research in Canada suggests that *C. purpureum* offers the potential to control the growth of deciduous hardwood species such as alder, birch, maple, and poplar in pine forests (Wall, 1997). After pasting of stumps with *C. purpureum* a proportion of the cut stumps appeared to be dying slowly, while the remainder exhibited stunted and unhealthy growth of the regenerating shoots. Registration under the name Ecoclear is under way in Canada and the US (Shamoun & Hintz, 1998).

Critical success factors for Biochon

In the case of *P. serotina* the weed problem is caused by a single species and selective removal of that species solves the problem. Previous knowledge on *C. purpureum* as the cause of silver leaf disease of fruit trees focussed attention immediately on this fungus as a potential biocontrol agent. It helped, also, that the current control method, painting a systemic herbicide onto cut stumps, was ideally suited to inoculation with mycelium of the wound pathogen *C. purpureum* (Scheepens & Hoogerbrugge 1988). Furthermore, success of control at a high rate was always ensured, irrespective of environmental conditions. The control of re-sprouting of other hardwood species, even species that were previously believed to be non-hosts, shown by Wall (1997) and Shamoun & Hintz (1998) has added to commercial success.

Because *C. purpureum* is very common, mostly as a saprophyte, it was believed that its use as a mycoherbicide would not pose an additional threat to susceptible fruit trees. This was proved by a multi-disciplinary risk assessment that convinced a much broader public, including the Dutch Plant Quarantine Authority (De Jong *et al.* 1990).

Commercially, Biochon is not yet a successful mycoherbicide product. Despite the fact that all the basic research was paid for by the Dutch government, and the costs for production are low, the manufacturer claims that the Dutch market for Biochon is too small to justify the high costs for registration as a mycoherbicide. It is therefore, sold only as a stump rot promoter, which does not need registation. Also, estimated costs of developing a more stable product based on dried mycelium were regarded as too high. Extension of the market to other European countries is not envisaged yet by the company. In Canada, with a wider range of species needing control in a much larger area, the opportunities for commercialisation are better, especially as the government has announced a further reduction of pesticide use in forestry (S. Shamoun, Pacific Forestry Centre, personal communication).

Ascochyta caulina/Chenopodium album

Chenopodium album is world-wide a very important weed of sugar beet, maize, potatoes, cereals and vegetables (Holm *et al.*, 1977). Problems with chemical control of this weed (e.g. triazine resistant populations), economically justify the development of a mycoherbicide against it. *Ascochyta caulina* is a leaf and stem pathogen of *Chenopodium* and *Atriplex* species. Spores of *A. caulina* can be mass produced on various solid substrates, and applied to plants by standard spray equipment for herbicides. In maize and sugar beet crops, application as a post-emergence mycoherbicide resulted in up to 70% mortality and significant growth reduction of surviving *C. album* plants (Kempenaar *et al.* 1996a). In the greenhouse, application of *A. caulina* to non-sterile soil also yielded high levels of control of emerging *C. album* seedlings (Kempenaar et al., 1996c). Furthermore, seed production by *C. album* was prevented by controlling flowering plants with *A. caulina* (Kempenaar *et al.*, 1996b). Host specificity tests showed *that A. caulina* does not affect important arable crops including beet (*Beta vulgaris*) and spinach (*Spinacea oleracea*). On the basis of these results it was concluded that the pathogen could be developed into a successful mycoherbicide (Kempenaar *et al.*, 1996a; Scheepens *et al.*, 1997). *A. caulina* was tested in the field by Novartis in 1996 and 1997, but it was decided not to develop it further as a commercial product.

Critical success factors for *A. caulina*

In contrast to Biochon, economic justification is not an issue as the potential market is very large. *A. caulina* can kill *C. album* plants in the field within two weeks, which is sufficient to prevent competition with the crop. However, the effect is very dependent on occurrence of long dew periods or rain, and on growth stage of the weed (Kempenaar, 1995). These are major limitations for successful development of *A. caulina* as a mycoherbicide. They are currently being tackled in an EU-sponsored project with 7 European countries including the Novartis company. Solutions are being sought via selection of strains with higher virulence and less dependence on long dew period, formulation and combinations of the fungus with its own phytotoxins or low doses of a herbicide.

Mass production technology, shelf life and application technology do not seem to limit the development of *A. caulina* into a mycoherbicide. Integration in weed management systems may be of some concern, because *C. album* is almost never the only weed to control, and integration with other weed control methods needs to be optimised to ensure effective control of all weeds.

To develop *A. caulina* into a commercial mycoherbicide product, some major investments in R&D are still required. As a few commercial mycoherbicides have been developed so far, there is a high level of uncertainty on how large these investments have to be. This is a major limitation to the commercial development of *A. caulina*.
For the registration of a mycoherbicide in most countries, a large dossier has to be provided to pesticide registration offices. Though in the EU, there is a tendency to allow smaller dossiers for biological control agents (e.g. mycoherbicides) than for chemical pesticides, costs are still high and are considered to be a limitation to the development of *A. caulina* into a mycoherbicide.

ACKNOWLEDGEMENTS

The authors thank Dr Meindert De Jong and Drs Wolter Van Der Zweerde for critically reading the manuscript.

REFERENCES

Auclair A N; Cottam G (1971). Dynamics of black cherry (*Prunus serotina* Erhr.) in Southern Winconsin oak forests. *Ecological Monographs* **41**, 153-177.

Bakker G (1963). De ontwikkelingsgeschiedenis van *Prunus serotina* Erhr. in Nederland. *RIVON-mededeling* **147**, 201-206.

Butler E J; Jones S G (1949). Silver leaf of plum, *Stereum purpureum* (Fr.) Fr. In: *Plant Pathology*, p. 763-767. MacMillan & Co: London.

Daniel J T; Templeton R J; Smith Jr R J; Fox W T (1973). Biological control of northern jointvetch in rice with an endemic fungal disease. *Weed Science* **21**, 303-307.

DeJong M D; Scheepens P C; Zadoks J C (1990). Risk analysis for biological control: a Dutch study on biocontrol of *Prunus serotina* by the fungus *Chondrostereum purpureum*. *Plant Disease* **74**, 189-194.

Eijsackers H; Oldenkamp L (1976). Amerikaanse vogelkers: aanvaarding of beperking? *Landbouwkundig Tijdschrift* **88**, 366-375.

Holm L G; Pluckett D L; Pancho J V; Herberger J P (1977). *The World's Worst Weeds; Distribution and Biology*, p. 84-91. University Press of Hawaii: Honolulu.

Kempenaar C (1995). Studies on biological control of *Chenopodium album* by *Ascochyta caulina*. PhD-thesis, Wageningen Agricultural University, the Netherlands.

Kempenaar C; Horsten P J F M; Scheepens P C (1996a). Growth and cometitiveness of common lambsquarters (*Chenopodium album*) after foliar application of *Ascochyta caulina* as a mycoherbicide. *Weed Science* ***44***, 609-614.

Kempenaar C; Horsten P J F M; Scheepens P C (1996b). Effects of application of *Ascochyta caulina* to flowering *Chenopodium album* plants. In: *Challenges of Weed Science in a Changing Europe*, p. 535-542. Proceedings of the ninth Symposium of the European Weed Research Society, Budapest, 1996.

Kempenaar C; Wanningen R; Scheepens P C (1996c). Control of *Chenopodium album* by soil application of *Ascochyta caulina* under greenhouse conditions. *Annals of Applied Biology* **129**, 343-354.

Müller-Schärer H; Scheepens P C (1997). COST 816. A coordinated European research programme: biological control of weeds in crops. *Integrated Pest Management Reviews* **2**, 45-50.

Scheepens P C; Hoogerbrugge A (1988). Control of *Prunus serotina* in forests with the endemic fungus *Chondrostereum purpureum*. In: *Proceedings VII International Symposium Biological Control of Weeds, 6-11 March 1988, Rome, Italy*, ed. E S Delfosse, p. 545-551. Istituto Sperimentale Patologie Vegetale (MAF), Italy.

Scheepens P C; Kempenaar C; Andreasen C; Eggers Th; Netland J; Vurro M (1997). Biological control of the annual weed *Chenopodium album*, with emphasis on the application of *Ascochyta caulina* as a microbial herbicide. *Integrated Pest Management Reviews* **2**, 71-76.

Scheepens P C; Van Zon J C J (1982). Microbial herbicides. In: *Microbial and Viral Pesticides*, ed. E Kurstak, p. 623-641. John Wiley and Sons: New York..

Shamoun, S F; Hintz W E (1998). Development and registration of Chondrostereum purpureum as a mycoherbicide for hardwood weeds in conifer reforestation sites and utility rights of way. In: *IV International bioherbicide workshop, Glasgow 6-7 August 1998*, eds M N Burge; D S Main, p. 14 (abstract). The University of Strathclyde, Glasgow, UK.

Templeton G E; TeBeest D O; Smith Jr R J (1979). Biological weed control with mycoherbicides. *Annual Review of Phytopathology* **17**, 301-310.

Van der Scheer H A Th; Wondergem H J (1981). Het optreden van loodglans en mogelijkheden tot bestrijding. *Fruitteelt* **71**, 242-245.

Wall R E (1997). Bioherbicides - promise and prospects. *Pesticide-Outlook* ***8****, 29-32.*

A perspective after 40 years research at the USDA/ARS European biological control laboratory

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ABSTRACT

This paper examines more than 40 years of classical biological control research on Eurasian weeds invading North America and includes information about target weeds, the biological control (BC) agents discovered, characterized, and released in the U.S., and their impact. It discusses the new EBCL lab completed in September 1999 in France which will contain modern equipment that will enable us to genetically characterize target weeds and their BC agents. The future work on plant pathogens for biological control of weeds will be strengthened as the new lab is fully equipped with approved quarantine and complete microbiological equipment. Explorations for BC agents will be based on informed ecological decisions. The future of biological control of weeds research will depend on support from consortia. By joining forces and pooling scarce resources, partner agencies and institutions would focus on problems and arrive at solutions rapidly.

INTRODUCTION

The European Parasite Laboratory, established in France in 1919, and the Biological Control of Weeds Laboratory – Europe, established in Italy in 1958, were combined in 1991 as the European Biological Control Laboratory (EBCL) in Montpellier, France. EBCL is the leading overseas biological control laboratory in the Agricultural Research Service (ARS), United States Department of Agriculture.

Many of the weeds in the United States are of Eurasian origin, accidentally introduced, free of the natural enemies that control them in their homeland. (Maddox & Mayfield, 1985) Many have become problems of national importance, (Frasier & Johnsen, 1991). Weeds invade millions of hectares of range, pasture, cropland, and recreational/natural areas and their control costs the U.S. millions of dollars each year. For example, Yellow Star Thistle, *Centaurea solstitialis*, in California covers 22 million acres, (Pitcairn *et al.*, 1998). Leafy spurge, *Euphorbia esula*, and the knapweeds, *C. diffusa* and *C. maculosa*, cover millions of acres of rangeland in the northwest and rocky mountain States (Bangsund *et al.*, 1993). Invasive weeds are considered to be important problems in recreational and natural lands because they displace native species. Thus *Tamarix* sp., a weedy tree in the southwest US, has been responsible for the displacement of several native trees which serve as breeding sites for an endangered flycatcher (DeLoach *et al.*, 1996). Biological control is recognized as a first option control measure in some sensitive areas. EBCL has contributed with some success over the last 40 years to solving these problems, and will continue to respond to stakeholder needs from its new facility in Montpellier, France. The mission of EBCL is to find and characterize natural enemies for control of invasive Eurasian weeds (Sobhian & Zwolfer, 1985 ; Turner *et al.*, 1994 ; Sobhian *et al.*, 1999).

The work is done in cooperation with various U.S. and international partners. EBCL, which has 1800m² of laboratory space and 20,000 m² of land staffed with 7 scientists, 6 support personnel and 3 administrative support staff, is a member laboratory of the Complexe International de Lutte Biologique Agropolis (CILBA) and comes under the umbrella of AGROPOLIS, a non-profit consortium including CSIRO, INRA (CGBP), and CIRAD, educational institutions, and agribusiness interests ; its purpose is to serve as a catalyst for the development of collaborative synergy. The current President of CILBA is a USDA scientist (Dr Alan Kirk) and the secretary an Australian CSIRO scientist (Dr. John Scott).

METHODS

Foreign exploration for natural enemies of a pest or weed, and the resulting biocontrol organisms discovered, drives a biocontrol programme from taxonomy through evaluation to release and impact studies. Each biocontrol programme is interdisciplinary in nature and, typically, is run by a team from USDA/ARS/APHIS, Universities , State Depts. of Agriculture and Industry.

RESULTS

Since 1960 EBCL and its collaborators have been responsible for the establishment of insects, mites and a fungal pathogen for the biological control of weeds from 5 families in a wide range of habitats (Table 1). Impact evaluations are rarely carried out and only a general assessment of success is possible (Table 1).

Table 1. Agents for biological control of weeds discovered by EBCL and subsequently established in the USA

No. of US States in which agents are established	47
Habitats involved	Rangelands, Recreational areas, Crops, pastures
Weed targets*	9 species
Biocontrol agents established	34 Insects, 2 mites 1 fungal pathogen
Origins of agents:	Eurasia
Impact	Range : Too early to estimate to very effective

*Asteraceae : *Carduus nutans Centaurea diffusa, C. maculosa, C. solstitialis, Chondrilla juncea.* Convolvulaceae: *Convolvulus arvensis.* Euphorbiaceae: *Euphorbia esula.* Scrophulariaceae: *Linaria genistifolia.* Zygophyllaceae: *Tribulus terrestris*

Eleven weed species from 7 families are currently targeted, some in collaboration with international and national partners (Table 2).

Table 2. Current Research Targets

US States concerned	Entire USA
Habitats invaded	Rangeland, natural areas, Recreational areas, pastures, Catchment areas for urban water resources
Weed targets*	13 species
Agents	24 insects 8 mites 8 fungal pathogens
Origins	Eurasia

*Asteraceae: Acroptilon repens, Carduus nutans, Centaurea diffusa, C. solstitialis, Crupina vulgaris. Brassicaceae; Lepidium latifolium, Cardaria draba, Isatis tinctoria;. Chenopodiaceae: Salsola kali. Euphorbiaceae: Euphorbia esula. Plantaginaceae: Plantago major. Rubiaceae; Galium aparine. Tamaricaceae: Tamarix ramosissima

DISCUSSION

For control of invasive exotic (alien) weeds in rangelands and natural areas in North America there is little alternative to biological control (Julien, 1992). A recent example is the Team Leafy Spurge approach which consists of the USDA/ARS (including EBCL)/APHIS, AgCanada, CABI Bioscience and the US States concerned with *Euphorbia esula* as a weed. Thirteen organisms have been collected, tested and, after evaluation, released into the field.

Recently, certain environmental concerns have arisen in regard to possible effects of biological control agents on closely related indigenous species. These issues are being addressed in programme planning by ARS and its collaborators. However there are no examples to date of monophagus biological control agents having extended their range to non-target plant species after introduction.

The construction of a new building for the USDA/ARS/European Biological Control Laboratory symbolizes strong support for biological control work. It is the only USDA laboratory constructed outside of the USA. The new facility and staffing (including, entomologists and plant and insect pathologists) have been established in the expectation of EBCL's contribution to solving the problems caused by invading exotic weeds worldwide.

ACKNOWLEDGEMENTS

We thank the many collaborators it has been our privilege and pleasure to work with in more than 40 countries and in nearly every State of the USA.

REFERENCES

Bangsund, D A ; Baltezore J F ; Leitch J A ; Leistritz F L (1993). *Economc impact of leafy spurge on wildland in MT, SD and WY. Agricultural Economic Report* ***304***, Department of Agricultural Economics, Agriculture Experiment Station, North Dakota State University : USA.

DeLoach C J ; Gerling D ; Fornasari L ; Sobhian R ; Myartseva S ; Mityaev I D; Lu Q G ; Tracy J L ; Wang R ; Wang J F ; Kirk A ; Pemberton R W ; Chikatunov V ; Jashenko R V ; Johnson J E ; Zheng H ; Jiang S L ; Liu M T ; Liu A P ; Cisneroz J (1996). Biological control programme against saltcedar (*Tamarix* spp.) in the United States of America: progress and problems. *In:* (Moran VC, Hoffmann JH, Proceedings Ninth International Symposium in Biological Control of Weeds, 19-26 January 1996, eds. VC Moran & JH Hoffman, pp. 253-260). University of Cape Town, Stellenbosch, South Africa.

Frasier G W ; Johnsen T N (1991). Saltcedar (Tamarisk : Classification, distribution, ecology and control. In : *Noxious range weeds*, eds. F F James, J Evans ; M H Ralf ; R D Child pp 377- 386. Westview Press : Oxford.

Julien M H G (1992). *Biological control of weeds : a world catalogue of agents and their target weeds, 3r^d edition*, (CABI International), Wallingford, UK.

Maddox D M; Mayfield A (1985). Yellow starthistle infestations are on the increase. California Agriculture (11-12) :10-12.

Pitcairn M J; O'Connell R A; Gendron J (1998). Yellow starthistle : survey of statewide distribution. In: *Biological control Program Annual Summary, 1997*, ed. D M Woods,: California Department of Food and Agriculture, Division of Plant Industry, Sacramento, USA.

Sobhian R ; Tunç I ; Erler F (1999). Preliminary studies on the biology and host specificity of *Aceria salsolae* DeLillo and Sobhian (Acari, Eriophyidae) and *Lixus salsolae* Becker (Col ; Curculionidae), two candidates for biological control of *Salsola kali*. *Journal of Applied Entomology* **123**, 205-209.

Sobhian R ; Zwölfer H (1985). Phytophagous insect species associated with flower heads of yellow starthistle (Centaurea solstitialis L.). Zeitschrift Angewandte Entomologia **99**, 310-321

Turner C E ; Sobhian R ; Joley D B ; Coombs E ; Piper G L (1994). Establishment of *Urophora sirunaseva* (Hering) (Dipt., Tephritidae) for biological control of yellow starthistle in the western United States. *Pan-Pacific Entomologist* **70,** 206-211.