Status of bioherbicide research in Florida

R. Charudattan and **T.A. Bewick**, Departments of Plant Pathology and Vegetable Crops, University of Florida, Gainesville, Florida 32611, USA.

Abstract

Several bioherbicide candidates are under evaluation, available for licensing, or undergoing commercial development in Florida and elsewhere, the latter under cooperative arrangements with sponsors and other researchers. The list includes *Colletotrichum dematium* f. sp. *crotalariae* and *Fusarium udum* f. sp. *crotalariae* for showy crotalaria (*Crotalaria spectabilis*); *Alternaria eichhorniae* for waterhyacinth (*Eichhornia crassipes*); an unidentified hyphomycete for pigweeds (*Amaranthus* spp.); *Fusarium tricinctum* and an *Alternaria* sp. for dodders (*Cuscuta* spp.); *Puccinia canaliculata*, *Curvularia lunata* var. *aeria*, and *Fusarium oxysporum* for purple and yellow nutsedges (*Cyperus rotundus* and *C. esculentus*); *Dothidea puccinioides* for groundsel bush (*Baccharis halimifolia*), and a Brazilian strain of *Alternaria cassiae* for sicklepod (*Cassia obtusifolia*).

Colletotrichum dematium f. sp. *crotalariae* and *Fusarium udum* f. sp. *crotalariae* are capable of controlling showy crotalaria at different growth stages. Based on host range studies and greenhouse and field trials of efficacy, both fungi are considered suitable bioherbicide candidates. A two-component formulation consisting of both fungi is envisioned.

Cultural characteristics, pathogenicity, in vitro toxin production, and formulations of *Alternaria eichhorniae* strain No. 5 from Egypt were studied in Gainesville by Dr. Yasser Shabana. It was confirmed that this strain has bioherbicidal potential against waterhyacinth. This pathogen could be utilized in countries where it is native.

A foliar blight-inducing hyphomycete was capable of inciting severe disease on many species and accessions of pigweeds. Host range and field evaluations are planned.

Fusarium tricinctum and an *Alternaria* sp., reported to be effective bioherbicides for dodders (Bewick *et al*. 1986, Weed Sci. Soc. Am. Abstr. 26:27), are undergoing field evaluations in Wisconsin and Massachusetts with support from HACCO, Inc., IR-4, and the Wisconsin Alumni Research Foundation. Results indicate that over 90% control is achieved within two weeks of application. Based on these results, HACCO has initiated the registration process.

Three bioherbicide candidates are being evaluated for control of purple and yellow nutsedges. In field experiments, *Puccinia canaliculata* has been shown to be as effective as chemical treatments in controlling yellow nutsedge in snap bean, peanut, and soybean. Both *Curvularia lunata* var. *aeria* and an isolate of *Fusarium oxysporum* have been shown to infect both nutsedge species. Experiments on host range, dose response, dew period conditions, and climatic requirements are currently being conducted.

Life-cycle, epidemiology, culturing, host range, and biocontrol potential of *Dothidea puccinioides*, incitant of the black pustule disease of groundsel bush, are being investigated. The disease is systemic on infected branches and/or entire plants, and a high proportion of infected plants die over a period of several months. The disease could be initiated with conidia collected from the pycnidiostroma but so far not with the *Dothidea*-type ascospores gathered from the ascostroma. Further studies are in progress.

Alternaria cassiae has been found to occur in Brazil. This discovery may stimulate interest in this bioherbicide agent in Brazil and other South American countries.

Some preliminary evaluations of *Sclerotinia sclerotiorum* as a mycoherbicide against Californian thistle (*Cirsium arvense*) in New Zealand

Ian Harvey and **Graeme Bourdôt**, Ministry of Agriculture and Fisheries, PO Box 24, Lincoln, New Zealand.

Abstract

Twelve wild isolates of *Sclerotinia sclerotiorum* from six hosts were tested for in vitro pathogenicity against leaf laminae, leaf mid ribs, soft and hard stems, underground stems and creeping roots of Californian thistle (*Cirsium arvense*). The isolates were ranked into high, medium and low pathogenicity groups on each tissue type. The pathogenicity of some isolates was found to be tissue-type dependent whereas others were equally pathogenic on all tissues. Strains that demonstrated the latter characteristics will be further field tested.

One strain from *C. arvense* was tested in a grass/clover pasture against this host using a single dose applied in October to the spring cohort of vegetative rosettes. In the first 30 days, the treatment showed a much higher mortality rate than the untreated. In the following 60 days, the mortality rates were equal. Some shoots killed by the mycoherbicide regrew from basal axillary buds, but in most cases these soon died.

New shoots from the root system occurred in equal density on treated and untreated plots indicating that the mycoherbicide did not stimulate this recruitment. Furthermore, these recruits had equal probability of dying in treated and untreated stands, the monocyclic nature of the pathogen apparently restricting it to the treated shoots and their immediate regrowths. The high mortality of the treated shoots caused by the mycoherbicide, and of the new recruits due to "natural" causes, jointly resulted in a very high level of weed control.

Evaluation of *Stagonospora* sp. as a mycoherbicide for the control of *Calystegia sepium*

S. Hasan[1], C. Beglinger[2], M. Buehler[2], B. Daclinat[1], L. Sedlar[2] and G. Defago[2].
[1]CSIRO Biological Control Unit, 335, Av. Paul Parguel, 34090 Montpellier, France.
[2]Institute of Phytomedicine, Swiss Federal Institute of Technology ETH-Zentrum, 8092 Zurich, Switzerland.

Abstract
Greater bindweed (*Calystegia sepium*) is a problem weed in agriculture in Europe, being most troublesome in vineyards and maize crops. Two isolates of *Stagonospora* sp. (214 ca a and MW 299) have been obtained from diseased *C. sepium* collected in Europe. The fungus caused brown lesions on all aerial parts of the plant with serious damage. It grew well on V8-agar medium producing pycnidia with conidia. In a controlled greenhouse environment the two isolates reduced growth and severely injured or killed young seedlings.

To evaluate the efficacy of the isolate in the field, an experiment was carried out at Montpellier (S. France) in 1990-91. Plots of mixed *C. sepium* and maize were used. Two plots were inoculated with distilled water suspension of conidia (10^6 conidia mL^{-1}) of the isolates 214 ca a, and MW 299. The control plot was kept free of the fungal infection by the alternate use of the fungicides, Benlate® and Dithane M45®.

All plants developed disease symptoms one week after inoculation, showing brown lesions especially on leaves. In another two weeks the infection became severe, with increased lesion size covering the majority of the leaf area, and also spreading to newly formed leaves. Both isolates caused early defoliation, reduced growth and caused death of infected shoots (40–50% mortality) and even whole plants. During the experiment in 1990 isolate 214 ca a was slightly more damaging than MW 299. The two isolates were found to be less effective in 1991, probably due to excessive hot and dry conditions. Moreover, a similar field experiment at Zurich (Switzerland) with the isolate 214 ca a gave a reduction in ground cover of 82% in 1990 and 63% in 1991.

Mass production of spores was attempted on sterilized cereal grains such as wheat, maize, barley and millet. Flasks with 25 g of grains were inoculated with 3 mL of conidial suspension (10^3 conidia mL^{-1}) of the isolates 214 ca a or MW 299. Production of conidia was estimated after an incubation period of 16 and 23 days at 19°C with a photoperiod of 16 h. Although conidial production did not vary greatly between media, those based on wheat, barley and maize seemed to be more suitable (1.5×10^7 conidia g^{-1} vs 1.1×10^7 and 0.12×10^7 conidia g^{-1} for millet and V8-agar media, respectively).

Commercialization of bioherbicides: from the university/research institute perspective

Alan K. Watson, Department of Plant Science, McGill University, 21, 111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, H9X 1C0, Canada.

Abstract
Discussions concerning commercialization of bioherbicides are usually led by persons from industry. They provide detailed information and documentation on patenting, licensing, and general aspects of the commercializing process from the industrial perspective. (Is there any other?). Factors such as production, formulation, toxicity, quality assurance, marketing and sales, product efficacy, and regulatory aspects are carefully examined to determine whether or not the investment is financially justified. Bioherbicide development worldwide is guided by return on investment. Many projects have faltered (stalled) because they are not economically justified. Issues of secrecy, patenting, disclosure and licensing agreements, etc. are central to successful commercialization of bioherbicides and it is essential for those of us involved in discovery and lead development research to be acutely familiar with and knowledgeable of these aspects. Often we cannot rely on others to bring our bioherbicide technology into the hands of the users. Most universities and research institutes now have offices of technology transfer (fledgling as they may be) which can provide a certain amount of assistance. However, the bioherbicide researcher must be actively involved in patenting, licensing, and similar issues to "champion the cause".

Commercialization of bioherbicides can follow three routes: partnership with a large company (agrochemical or otherwise), partnership with a small, venture capital, niche market company, or establishment of a spin-off company. Each approach has advantages and limitations. With a decade of experience and the associated "highs" and "lows", one occasionally questions the sincerity of potential industrial partners, the functioning of our institutional technology transfer offices, the inner workings of government patent offices, and so on. Understanding the factors involved in the commercialization process of bioherbicides will help us to interact positively with industrial partners to advance bioherbicide technology without compromising our principles of advancing knowledge and publishing our findings. Dialogue between the public and the private partners is extremely important. Not all bioherbicides will need to be commercialized, especially those being initiated in the developing world. The use of *Striga* pathogens in West Africa or the use of *Cyperus rotundus* pathogens in upland rice in Southeast Asia will not be bound by the economic values of the developed world. Most of the recipients of this technology in the developing world, the subsistence farmers, simply do not have the resources to purchase inputs. Adoption and use of bioherbicides in these areas will depend largely on the development of low input, on-farm technology to produce and apply specific weed pathogens. Likewise, very efficacious pathogens (cf. DeVine®) will not be commercially developed because they are too effective.

Partial characterization of progeny from a cross between *Colletotrichum gloeosporioides* f.sp. *aeschynomene* and a *C. gloeosporioides* from *Carya*

D.O. TeBeest[1], C.R. Cisar[1] and F.W. Spiegel[2]
[1]Department of Plant Pathology and [2]Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701, USA.

Abstract

A fungus, *Colletotrichum gloeosporioides* f. sp. *aeschynomene*, has been used since 1982 in the USA to control the weed, northern jointvetch (*Aeschynomene virginica*), in rice and soybean fields (Bowers 1986). The fungus has been described as lacking a *Glomerella*, or sexual, stage (Templeton *et al.* 1979). Results of recent studies in our laboratories indicate that *C. gloeosporioides* f. sp. *aeschynomene* is capable of mating with other isolates of *C. gloeosporioides* with different host specificities. Perithecia with fertile ascospores have been produced in crosses between *C. gloeosporioides* f. sp. *aeschynomene* and *C. gloeosporioides* isolates from winged water primrose (*Ludwigia decurrens*) and pecan (*Carya illinoensis*) (Brenneman and Reilly 1989) in culture and on plant tissue. Progeny from a single perithecium from a cross between *C. gloeosporioides* isolates from northern jointvetch and pecan have been partially characterized. The ribosomal RNA (rDNA) RFLP patterns of the parents and progeny have been compared. Most of the progeny have the rDNA RFLP patterns of one or the other parent but two of nine progeny exhibit the rDNA RFLP patterns of both parents, indicating that recombination may have occurred. Even though the rDNA RFLP patterns of several of the progeny are the same as that of the *C. gloeosporioides* f. sp. *aeschynomene* parent none of the progeny are pathogenic to northern jointvetch. In addition, the progeny have exhibited varying levels of pathogenicity on apples. The observation of mating between isolates of *C. gloeosporioides* from distantly related hosts adds a new element to risk analysis of biological control agents.

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Bioherbicides: an industrial perspective

F. Dan Hess, Biological and Biochemical Research, Sandoz Agro Inc., Palo Alto, California 94304, USA

Abstract

Considering the size of the worldwide herbicide market and the environmental safety of bioherbicides, the potential for their development and use for weed control is of significant interest to industrial research and development groups. Although there are numerous niche markets where bioherbicides could be used, the most important economic application of bioherbicides is in agronomic crop production (for example, corn, soybeans and small grains in the USA). For many industrial companies other markets are of less interest. For example, whereas weed control in range and forestry markets are important, the permissible cost per unit land area is low when compared to agronomic markets. Other markets such as organic farms and home lawn and garden are important potential markets, but are small when compared to traditional agronomic markets.

As long as chemical herbicides are readily available for weed control in agronomic crops, bioherbicides will have to compete for market share. Assessing the performance of chemical herbicides reveals three general characteristics necessary for bioherbicides to gain significant farmer acceptance in agronomic crop production. First is price. In many major crops, the cost of complete weed control ranges from US $30 – 40 per hectare; bioherbicides will have to be similarly priced to be competitive. Additional research on production technology and scale up will no doubt have the largest impact on establishing price for bioherbicides. Second is handling characteristics. Bioherbicide applications need to be compatible with conventional sprayers and spray volumes (200 L ha^{-1} or less), have application rates of 3 to 4 kg ha^{-1} or below, and have a shelf life approaching at least one year. Research on strain selection and optimization, as well as bioherbicide formulation can help to satisfy these requirements. Third is performance. Individual or mixtures of bioherbicides need to have a reasonably broad spectrum of weed control. Research should focus on finding organisms that control more than one weed species without harming the crop, and on the concept of limiting the spread of broad spectrum bioherbicides by mutagenic disarming (i.e., production of auxotrophs). To have flexibility in controlling the diverse spectrum of weeds found in different geographical regions, bioherbicides should be spray-tank mixable with environmentally sound chemical herbicides. The consistency of bioherbicide performance needs to match that of current chemical herbicides. Tank-mix compatibility and consistency can be largely addressed by formulation research. A major formulation research need is to significantly reduce, or preferably eliminate, the association of dew period with performance.

Toxicological risks associated with potential bioherbicides

R.G. Shivas, Plant Pathology Branch, Department of Agriculture, South Perth, W.A. 6151, Australia.

Abstract

One of the perceived advantages of bioherbicides over chemical herbicides is that the environment is not polluted with harmful and persistent chemical residues. In many countries, including Australia, there are regulations concerning the registration and release of plant pathogens for weed control. One of the purposes of these regulations is to ensure safety to the public. It is unlikely that these regulatory bodies would allow the commercial development (including field trials) of bioherbicides that produce mammalian toxins.

Phomopsis emicis has been investigated as a potential mycoherbicide candidate for spiny emex *(Emex australis)* in Australia. *E. australis* is an introduced polygonaceous winter annual which has become a serious weed of crops and pastures in southern Australia. *P. emicis* was found to produce comparatively large quantities of a mammalian toxin and this has important implications in the possible use of this fungus as a mycoherbicide. The main concern is that livestock will graze *E. australis* under certain conditions. The amount of toxin that occurs in pastures can be expected to be proportional to the biomass of *P. emicis*. Pastures treated with massive doses of inoculum of *P. emicis* might pose a serious health threat to grazing stock.

Risk assessment of exotic plant pathogens in the United States and development of bioherbicides

W.L. Bruckart, G.L. Peterson and **N. Shishkoff,** USDA-ARS-FDWSRU, Building 1301, Fort Detrick, Frederick, MD 21702, USA.

Abstract

Risk assessment begins at home, whether the plant pathogen is exotic or endemic. Scientists must be convinced of safety and virulence of the candidate biocontrol agent before a proposal is made to use it. Such judgement is based on the available information and knowledge about the organism. Even with the latest in knowledge and technology, judgements are made on the basis of incomplete information. Our major obstacles in evaluating biocontrol agents include: propagative material for host range studies (particularly from endangered, threatened, or rare species), taxonomy (identification) of biocontrol agent and host, and the difficulty interpreting available knowledge (especially greenhouse data) for risk assessment. Approaches used at FDWSRU to address these issues include study of surrogate species, molecular approaches to taxonomy, and attempts to quantify damage in realistic greenhouse experiments.

Communication also is an obstacle which affects both the regulatory process and our public image. Good communication is a very important part of risk assessment, particularly when non-target infections occur. This is a more difficult area in which to pin-point issues and solutions. Ideas will be put forth for discussion.

Identification of *Colletotrichum gloeosporioides* f. sp. *malvae* for registration and patenting

Roberte M.D. Makowski, Agriculture Canada, Research Station, Regina, SK, Canada.

Abstract

The indigenous fungal pathogen *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *malvae* (C.g.m.), isolated from anthracnose lesions on stems of round-leaved mallow (*Malva pusilla*), is the first mycoherbicide registered for use in Canada. It is marketed under the tradename BioMal®. Agent specification which includes identification of the organism and its metabolites is required for registration. Differentiation from other similar species and forma speciales is essential for patenting.

Numerous differences between various *Colletotrichum* species and between a number of forma speciales of *Colletotrichum gloeosporioides* were found when comparisons were made of: a) growth and spore production at 100 different temperature combinations on a thermogradient germinator and b) polypeptide patterns using two-dimensional (2-D) polyacrylamide gel electrophoresis. However, few differences were observed between isolates of C.g.m. These findings were sufficient to satisfy present patenting and regulatory needs. With the dynamic state of the regulatory and patenting environments, the proposed requirements for agent specification of future products, future directions and their implications are discussed.

Formulation of a bioherbicide for an invasive shrub, *Hakea sericea*

M.J. Morris[1] and **C. Ziniades**[2]
[1]Plant Protection Research Institute, Private Bag X5017, Stellenbosch 7600, South Africa.
[2]National Chemical Products (Research and Development), Private Bag 1067, Germiston 1400, South Africa.

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Hakea sericea, a shrub native to south-eastern Australia, has invaded many of the coastal mountain ranges of the southern and south-western Cape Province of South Africa. It forms dense thickets over large areas excluding the indigenous vegetation. A strain of the fungus, *Colletotrichum gloeosporioides*, causes shoot-tip dieback and fatal gummosis cankers on the stems and branches of *H. sericea* in South Africa. Seedlings of *H. sericea*, which occur in dense infestations following fire, to which the mountains are prone, are readily killed by the fungus.

Various methods of applying the fungus to seedlings, including colonized wheat bran, alginate pellets, agar pellets, soybean granules, attapulgite powder and spraying with a spore suspension in water were tried. Spraying with spore suspension was successful only if followed soon after application by favourable moist weather conditions. The wheat bran, alginate, agar and soybean granules rely on the copious production of conidia on the granule when wet by rain in the field and the natural dispersal of these conidia, by rain, to the seedlings. Wheat bran applications were 93 – 100% effective when applied early- to mid-winter in small and large scale field trials. Alginate and agar pellets gave equally good control in plot trials but were considered too expensive for large scale use. Soybean granules, produced on a small scale, sporulated well, but yeast contamination and poor viability hindered large scale production and resulted in ineffective weed control. However, large scale trials with these granules showed that field application of granules using a specially adapted monsoon fire-fighting water bucket suspended below a helicopter is feasible. An attapulgite powder formulation is currently under trial. A paste, containing the fungus, and a hand-held applicator effectively inoculate adult plants and are being considered for field use.