Industrial Potential of Plant Pathogens as Biocontrol Agents of Weeds

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Abstract

Two major strategies can now be recognized in the use of fungal plant pathogens for weed biological control: (a) the classical strategy — aimed at the introduction of an exotic pathogen into a weed infestation thereafter relying on its ability to self perpetuate and reduce the host population, and (b) the inundative/bioherbicide strategy — using virulent strains of native or exotic pathogens and enhancing their destructive action. This latter method of weed control is under extensive study and alone or in combination with other control measured e.g. compatible chemical herbicides, presents considerable potential for private industry.

Keywords: Biological weed control, weed pathogens, microbial herbicides

1. Introduction

The use of plant pathogens in weed biological control is relatively new, but in the last decade has developed tremendous importance. Several programs currently underway around the world have been described in recent reviews (Freeman et al., 1978; Hasan, 1974a, 1980, 1983; Scheepens and Van Zon, 1982; Templeton, 1982). This paper summarizes aspects of this increasing activity and considers the potential of plant pathogens for industrial development.

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2. Development of Plant Pathogens in Weed Control

Selection of suitable pathogens

Plant pathogens belonging to various groups, including viruses, bacteria, fungi and nematodes, which cause reduction in plant growth or reproduction can be considered as biological control agents for weeds. Among these, fungi have so far received most attention, probably as they outnumber other groups and, in several cases, their host range is well known. Moreover, though fungal spores are often dispersed by visiting or host-feeding invertebrates, in general they do not depend on this mode of dispersal. Many fungi, especially Deuteromycetes, grow readily on standard mycological media and these can be suitable for commercial production.

To locate suitable pathogens, whether exotic or endemic, information from the literature about the occurrence of a weed and its diseases and their geographical distribution can help in the selection of areas to be surveyed. Field surveys at different times of the year enable the collection of pathogens attacking different plant stages. A pathogen infecting various plant parts at different stages from seedling to mature plants should be given priority, along with such considerations as virulence and widespread occurrence.

Glasshouse and field effectiveness

The biological control effectiveness of exotic pathogens can be evaluated in their native range, both in the field and the glasshouse. Host plants of different age groups are inoculated in the glasshouse with the pathogen and evaluations made of the nature and degree of infection. Field observations are made tc evaluate the relationship between changes in the weed population and variation in attack by the pathogen throughout the season. This criterion was used to demonstrate the damaging action of *Puccinia chondrillina* (Hasan and Wapshere, 1973).

Where endemic pathogens are under study, field assessments are more oriented to the relation between damage and quantity of inoculum and the means of inoculation (Tebeast and Templeton, 1985).

Host specificity determination

It is important that plant pathogens selected for biological weed control should not be harmful to plants other than the host weed. Evidence of specificity can be obtained mainly from glasshouse tests, but also from field observations and literature surveys.

The detailed host range of the pathogen is determined by exposing it to a wide range of important cultivated and wild plants under optimum conditions of inoculation and incubation. As it is not possible to test all plant species, methods have been developed to select representative species which could be most at risk. One of the most appropriate methods is based on the phylogenetic relationships of the target weed (Wapshere, 1974). This method is at present commonly used in most testing programs and includes other forms of the target species, and in descending order of importance, other species of the same genus, other members of the tribe, sub-family, family and order.

3. Biological Control Strategies Involving Plant Pathogens in Weed Control

Currently two main biological control strategies are in use for the application of plant pathogens in weed control: (a) the classical strategy — aimed at the control of introduced weeds by exotic plant pathogens. Effective pathogen strains are discovered in the weed's native range and introduced into the target area, thereafter relying on their ability to self perpetuate and reduce the host population below the economic threshold; and (b) the inundative/bioherbicide strategy — this is a more recently developed technique where virulent strains of pathogens are manipulated to enhance their destructive action. The pathogens are mass-produced and applied as biological herbicides or "mycoherbicides" (Templeton et al., 1979). the method is suitable for both endemic and exotic pathogens, though so far mostly endemic pathogens have been used (Hasan, 1980, 1983; Tebeast and Templeton, 1985; Templeton et al., 1979).

In addition, biological weed control with plant pathogens can also be used in pest management systems. The strategy of integrated weed control is still in its infancy. Attempts are underway to combine the destructive effect of pathogens with that of other biotic agents, compatible herbicides and mechanical or cultural practices.

4. Weed Control with Exotic Plant Pathogens — the Classical Strategy

Many plants have been accidentally or deliberately introduced from one part of the world to another where they have become serious weeds. They may not be problem weeds in their native ranges, often due to the presence of natural enemies. A search is made for the latter and, if found effective and host-specific, they are imported and released into the target area to control their host weeds. Phytophagous arthropods have traditionally been used in such projects, but some plant pathogens, especially rust fungi, known for their host specificity have also been used in this classical technique.

One of the foremost examples is the successful introduction into Australia of *Puccinia chondrillina* Bub. & Syd. for the biological control of skeleton weed, *Chondrilla juncea* L. This composite weed of Mediterranean and Middle Eastern origin was a serious problem in the wheat growing areas of south-eastern Australia (McVean, 1966). The rust was found to occur commonly in the Mediterranean area, where it caused serious damage to the plant, reducing its reproductive capacity and playing an important role in the reduction of skeleton weed populations in situations ecoclimatically homologous to those infested in Australia (Hasan and Wapshere, 1973). A strain of *P. chondrillina* from Italy was found to be aggressive to the most common form (narrow-leaf) of the three Australian forms of skeleton weed. This strain was demonstrated to be very host-specific and was introduced into Australia in 1971 (Hasan, 1972, 1974b). Shortly after its release in the field the pathogen became well established and widespread through skeleton weed infestations, thereafter steadily reducing the populations of the narrow-leaf form of the weed (Cullen, 1978; Cullen et al., 1973). This action has continued and in several areas the narrow-leaf form of skeleton weed is no longer a problem (Hasan and Cullen, 1984). However, the other two forms (intermediate- and broad-leaf) have remained unaffected and have been increasing. Two more strains of the rust have recently been introduced into Australia for the biological control of the intermediate-leaf form (Hasan, 1981, 1984). One of these strains IT-36 again from S. Italy has apparently established in the field. A few strains infecting the broad-leaf form have also been discovered and these are at present under investigation.

Chondrilla rust has also been recently introduced on the Pacific coast of USA where it has established in skeleton weed infestations (Emge et al., 1981).

In Chile two other rust fungi, *Phragmidium violaceum* (Schultz) Wint. and *Uromyces galegae* (Opiz) Sac., have been introduced from Europe to control respectively blackberries (*Rubus constrictus* Lef. and M. and *R. ulmifolius* Schott.) and *Galega officinalis* L. (Oehrens and Gonzalez, 1974, 1975). Encouraged by this success, *P. violaceum* has recently been studied in Europe for its biological control potential to control European blackberries (*Rubus fruticosus* L. agg.) naturalized in Australia. A very large selection of cultivated and wild *Rubus* species and other members of Rosaceae existing in the Australasian region were tested against the pathogen, which showed

a high level of specificity (Bruzzese and Hasan, 1986). Application for its introduction into Australia has been made to the Australian Plant Quarantine authorities.

The fungal pathogen *Cercosporella ageritinae* (nomen nudum) from Jamaica has recently been introduced into Hawaii for the biological control of hamakua pamakani, *Ageratina (Eupatorium) riparia* (Regel) K. & R., a composite weed of range land and pasture (Trujillo, 1976). Several other pathogens are also currently under investigation in Europe for the biological control of Australian weeds originally from the Mediterranean region (Hasan and Cullen, 1984).

5. Pathogens Currently in Commercial Use or Under Consideration for Use — the Bioherbicide Strategy

Production and storage

Several species of fungal pathogens, especially Deuteromycetes, grow well on standard mycological media and may also be produced in existing commercial fermentation facilities. These fungi may be cultured as mycelium, asexual conidia and/or resting spores. For some species all these stages develop in liquid media; for others mycelium grown by agitating liquid cultures have to be transferred to solid media for sporulation. Mycelia enclosed in agar-gel pellets may also be helpful in producing abundant spores as and when required e.g. immediately prior to aerial spraying or after application of pellets to weeds (Walker and Connick, 1983).

Although spores of many aerial plant pathogens, including Deuteromycetes remain viable in nature for several months, their mass storage in the laboratory is a problem. Many of these fungi can be lyophilised and others can be stored in liquid nitrogen for an indefinite period. However, these methods are expensive and unsuitable for normal commercial use i.e. storage for shorter periods in more convenient conditions. Methods have now been developed by appropriate formulation of the products, especially for storing dried propagules at temperatures somewhat lower than ambient. Conidia and ascorpores of some fungi have remained viable for several months when stored at room temperature in an alginate-clay matrix as dry pellets (Fravel et al., 1985). Aquatic fungi including Phycomycetes can be commercialized as liquid suspensions procurable just before application.

Registration of pathogens as bioherbicides

Till quite recently, the requirements for the registration of bioherbicides were the same as those for chemicals, but regulatory agencies in some countries, including USA, now accept a separate set of safety criteria significantly less than for chemical pesticides (Charudattan, 1982). Guidelines for the handling of weed biocontrol agents and safety testing have been prepared recently by the European Weed Research Society's Workshop on Biological Control of Weeds and will soon be submitted through such agencies as the European Plant Protection Organisation in Paris as a possible basis for future legislation (Schroeder, 1984).

A short description follows of the pathogens already being commercialized as mycoherbicides or with potential for future commercialization:

Colletotrichum gloeosporioides (Penz.) Sacc. f. sp. *aeschynomene*

This fungal pathogen was isolated from northern jointvetch, *Aeschynomene virginica* (L.) B.S.P., a leguminous weed mainly in the rice fields of Arkansas, USA (Daniel et al., 1973). Under natural conditions the fungus infects all stages of the host from seedling to fully developed plants, but the infection is never sufficiently severe to kill plants or significantly reduce vigor or seed production. However, the efficacy of *C. gloeosporioides* f. sp. *aeschynomene* as a biological control agent against northern jointvetch was demonstrated in the field. Thus when 2 million spores/ml water were applied at the rate of 94 l/h on weed infested rice fields early and mid-way through the season, 99% of the *A. virginica* plants were killed (Daniel et al., 1973). The safety of the pathogen was demonstrated by testing against crop plants and laboratory animals (Smith et al., 1973; Beasely et al., 1975). The fungus has recently been commercialized as a dry powder of 15% spores and 85% inert ingredients under the trade name "Collego" and successfully used for the selective control of northern jointvetch in rice and soybean fields of Arkansas, Louisiana and Mississippi (Tebeast and Templeton, 1985). The powder is rehydrated and resuspended in a sugar solution before being mixed with water for application. It is normally applied only once each season when the weed is just above the rice plants.

Phytophthora palmivora (Butler) Butler

This fungus, originally identified as *P. citrophthora* (R.E. Sm. and E.H. Sm.), was isolated from dying milkweed vine, *Morrenia odorata* Lindl., a problem species in the citrus groves of Florida (Burnett et al., 1974). The pathogen was evaluated to determine its effectiveness as a mycoherbicide. A water suspension of chlamydospores sprayed onto infested fields at the rate of 8 chlamydospores/cm^2 of soil surface killed approximately 60% of the vine within 3 weeks and approximately 95% after 10 weeks (Ridings et al., 1978). *P. palmivora* was also studied for its host range by subjecting 58 representatives of crop plants from 12 families to pre-emergence, post-emergence and foliage inoculation tests. About 30% of the plants were found to be infected but only with the inoculum levels higher than necessary for infection of milkweed vine (Ridings et al., 1978). A liquid formulation of chlamydospores has recently been commercialized as "Devine". It must be ordered before the season when it is applied to control milkweed vine (Tebeast and Templeton, 1985).

Other pathogens

A few other fungi have also been studied in detail and are nearly ready to be commercialized. Thus *Cercospora rodmanii* conway has been studied as a potential mycoherbicide for the control of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.) infesting rivers and lakes of Florida. The pathogen has been found to be host-specific and effective to kill host plants especially when sprayed in low nutrient areas. However, in recent experiments it has been demonstrated that *C. rodmanii* could also be effective in high nutrient areas when used in combination with arthropod parasites (Conway et al., 1978). Similarly *Alternaria alternata* has recently been evaluated as a mycoherbicide for the control of spurred anoda, *Anoda cristata* (L.) Schlecht. in cotton fields in USA. The fungus is not only effective by aerial applications but also when spore and mycelium are mixed with vermiculite and supplied as a granular formulation in the soil (Walker, 1981; Walker and Sciumbato, 1979).

6. Potential of Microbial Herbicides for Private Industry

Among conventional methods to control herbaceous weeds in annual cropping systems, chemicals have played a major role. However, in recent years, several of these weeds have developed tolerance or resistance to herbicides due to their constant and long-term use. Microbial herbicides present a suitable alternative. Such weeds may have endemic or exotic diseases which are not necessarily well known, but whose biology could possibly be studied in publicly funded research, while the private sector could develop such aspects as formulation, safety testing and marketing. The pathogens may be formulated as a wetable powder of conidia or as a liquid as for the already commercialized products, Collego and Devine, respectively. Survival of spores may be greatly improved by mixing with refined clay or refrigeration. They could be applied as an aerial spray or as granules in the soil, along with fertilizers.

Moreover, recently other possibilities of bioherbicide use have developed and these may further attract private industry. For example specific mycoherbicides have been combined with each other and/or chemical herbicides to increase the spectrum of action, destroying more than one weed species in a crop at the same time (Boyette et al., 1979; Klerk et al., 1985). Practical use of the product is further assured by integrating its use into the whole management system including the use of other control measures. Devine was found to be inhibited by herbicide treatments such as diuron, glyphosate and paraquat. However, when the pathogen was applied 3 weeks after spraying glyphosate, these together were effective in controlling the weed (Smith, 1982).

In another case efficiency has been increased by combining pathogens with low doses of chemical herbicides. Research is currently underway in the Netherlands where the combination of the fungus *Cochliobolus lunatus* Nelson & Haasis (*Curvularia lanata* (Wakker) Boedijn) and 10% of a normal dose of chemical herbicide has effectively controlled *Echinochloe crus-galli* (L.) Beauv. Even the most virulent strain of the pathogen on its own caused only limited damage to the weed (Scheepens and Van Zon, 1982). Similarly, in the USA the possibility of using the endemic rust *Puccinia canaliculata* (Schw.) Lagerth. in conjunction with a herbicide against yellow nutsedge (*Cyperus esculentus* L.) has been suggested (Phatak et al., 1983). Charudattan (1986) working on the effective use of *C. rodmanii* for the biological control of waterhyacinth in Florida points out that apart from the combined action of the pathogen and arthropods, two herbicides are compatible with the pathogen. Water hyacinth plants treated with 6.4% and 0.3% of the recommended dose respectively of 2,4-D and diquat were highly susceptible to *C. rodmanii* attack.

There is as yet no information available regarding the compatibility of microbial herbicides with insecticides or fungicides in the same mixture, but Collego has been shown to be compatible in successive applications. Thus spore suspensions of *C. gloeosporioides* applied in rice fields in midseason controlled northern jointvetch after carbofuran or isofenphos had been applied in the early season for the control of rice water weevils. Similarly, the pathogen was also found effective when applied 2–3 weeks before the application of benomyl or tricyclazole (Smith, 1982).

7. Discussion

Successful use of plant pathogens in biological control of weeds has been demonstrated in various parts of the world. In some areas including North and South America, Australia and New Zealand, where weed have been introduced from elsewhere, notably Eurasia, classical biological control is being applied. This strategy has the great advantage of economy, control continuing after the initial introduction and establishment without the need for recurring expense. The example of *Chondrilla* rust is typical in this respect and has also demonstrated the efficacy of the method and its applicability to cropping systems as well as undisturbed areas. In the restricted classical sense such a system is not readily amenable to industrial development, except by combination with other control systems if efficacy requires to be enhanced.

Possible disadvantages of the classical strategy using phytopathogens are that it is normally only applicable to exotic weeds and spread is not controllable. Thus the target weed should not be of benefit anywhere, or any potential conflicts of interest are resolved before implementation.

The more recently developed inundative/bioherbicide strategy, being dependant on man's intervention, can be restricted to those areas where control is required. This requirement for periodic application also makes it a more attractive proposition for industrial development. Further, the strategy is applicable to both exotic and endemic weeds, the latter representing a large market in intensive cropping systems as well as often not requiring the same level of investment in overseas exploration and obtaining authorization for importation. Most of the progress in this field has been in the USA, but several projects are now underway in Europe where many indigenous weeds are serious problems in agriculture. Worldwide, Collego and Devine are as yet the only two bioherbicides actually on the market, but several more are close to commercialization or under evaluation (Hasan, 1986).

The development of weed management systems incorporating both plant pathogens and herbicides, either separately applied or in special formulations is also expected to develop as the advantages of control by pathogens are further appreciated and their use more commonly accepted.

Extensive research is necessary to discover further weed pathogens with potential as bioherbicides. They have the advantages of being host-specific and thus harmless to non-target plants. Also, they have no residues in plants, in the soil or in underground water, have long-term control action and do not cause environmental pollution. With increasing willingness of industries to develop safe and selective herbicides, microbial herbicides can be suitable alternatives, especially by employing advanced techniques for production and formulation.

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