MYCOHERBICIDES - ACHIEVEMENTS, DEVELOPMENTS AND PROSPECTS

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Summary. Three indigenous fungi are currently used routinely in the U.S.A. as mycoherbicides for control of individual weed species. Another is expected to be registered for sale in 1988. They are sold either as a durable dry spore formulation or as a wet labil spore suspension. Their use is in either rangelands, orchards or annual crops and they provide acceptable profit margins in small markets because of the comparatively low cost of development and registration. One is distributed free by a non-profit Foundation. The economic disadvantages of high specificity/low market size can be overcome by mixing two or more mycoherbicides or by tank-mixing with compatible chemical herbicides. They appear to have greatest potential for weed control in non-agricultural areas, waterways, public use areas, rangelands and pastures. However, they can also be integrated successfully into intensively cultivated agricultural systems that rely heavily upon chemical pesticides. Mycoherbicides are, thus, consistent with the current trend to control weeds with more active, more selective, environmentally safe herbicides and to do this at costs that are lower than required for organic chemicals.

MYCOHERBICIDES

Mycoherbicides is the term coined to characterize fungal plant pathogens that are applied as inundative inoculum for specific, post-emergence control of their weed hosts (25). Only indigenous pathogens have been developed as mycoherbicides thus far (24, 26, 27). Their development is a biological control tactic that compensates for poor natural dispersal of a pathogen, relies upon a high level of specificity for the target host and utilizes strong necrotrophic ability at elevated inoculum levels (10). They are termed mycoherbicides to denote their composition and use pattern; to distinguish them from classical biological control tactics of introduction, self perpetuation and passive dissemination.

Mycoherbicides are developed from pathogens that normally incite diseases at endemic levels in specific weed populations. Endemic diseases are defined as those that are more or less constantly present from year to year in a moderate to severe form (29). They usually are constrained from epidemic development by innate deficiency of the pathogen to disseminate. It is also implied that environmental conditions are usually favourable for inoculum to develop, infection to take place and disease development to ensue. When the pathogen is applied as inundative inoculum, it infects and kills the weed within three to five weeks, and after death of the host plant, is reduced to background levels by natural constraints and deterioration. Consequently there is little or no residual weed control from season to season after mycoherbicide application, particularly if it is an aerial pathogen. Some carry-over may occur with mycoherbicides produced from soil-borne pathogens (13, 17).

There are three mycoherbicides routinely used in the U.S.A. at this time, and a fourth is expected to be ready for commercial use in 1987 or 1988. They were all developed from indigenous fungi that occur naturally at endemic levels and are as effective or more effective than chemical herbicides on their specific weed host.

ACHIEVEMENTS

Persimmon wilt. The persimmon wilt fungus, *Acremonium diospyri* (Crandell) Gams, (= *Cephalosporium diospyri* Crandell), is routinely provided to ranchers in south-central Oklahoma to control thickets of the woody perennial, *Diospyros virginiana* L., in rangelands (11). It is supplied free by The Samuel Roberts Noble Foundation, Inc., a non-profit educational foundation at Ardmore, Oklahoma, established to assist agricultural development in the region. Fresh spore suspensions are provided to individual ranchers in the early spring just as dormancy is broken, leaves emerge and transpiration commences. Individual trees must be inoculated about 1 m above ground level by wounding the trunk with a hand-axe and applying the spore suspension to the wound with a plastic squirt bottle. One or more wounds per tree are inoculated depending upon the trunk diameter which many range from 5 to 25 cm. Wilt symptoms can be seen in the fall of the same year but it may take as much as three years to kill all the trees in a grove. Rate of kill depends upon nutrient and moisture level of the site and mean diameter of the tree trunk, larger trees on poorer sites require longer incubation periods for complete kill. There is some spread from tree to tree within a grove through root grafts so complete kill may be achieved after inoculation of only 80% of the trees in a grove, however, this is slower than that achieved by inoculation of every tree. More rapid kill is desirable to encourage replacement of the grove with native grasses rather than other woody species such as red cedar, *Juniperus virginiana* L., post oak, *Quercus stellata* Waugh, and redbud, *Cercis canadensis* L.

Approval for use of the fungus for persimmon control was obtained from the State Legislature in Oklahoma in 1965 and it was researched in the field for several years before that. This mycoherbicide gives excellent control of treated trees in rangelands and has proven safe. It is naturally constrained by environment and spatial separation from cultivated persimmon in urban areas or forest where it is an economic species. The hand labour requirement for inoculation is a major restraint to its more widespread use in the U.S.A. A novel alternative to wounding with the hand-axe is being evaluated and this may prove to be more practical. The pathogen is coated on to gun shot, loaded into shells and shot into groves with 12 guage shotguns. A large number of trees are wound-inoculated simultaneously with one round. This has been effective for dense groves of small trees.

Other restraints to more widespread use are the pathogen's propensity to lose virulence in culture and low market potential, a distinct disincentive to commercialization.

Another wilt inducing fungus, *Cephalosporium* spp., can be used to control kolomona weed, *Cassia surrattensis* L., a woody plant which infests the high rangelands of Hawaii (27). In this case also, the major restraint to its more widespread use is the requirement for hand inoculation of plants, either individual plants if growing isolated or a few plants of a colony if growing in a dense population.

DeVine^R. DeVine^R is a liquid formulation of chlamydospores of the soil fungus, *Phytophthora palmivora* (Bulter) Butler, that has been used commercially since 1981 to control stranglervine, *Morrenia odorata* (H. & A.) Lindl. (Asclepiadaceae), in certain counties of Florida where it is a serious weed in citrus groves. It was developed in a collaborative effort by the Department of Plant Industries, Florida Department of Agriculture, the University of Florida, and Abbott Laboratories. It is marketed as fresh chlamydospore suspensions (13, 17).

The weed is believed to have been introduced into Florida as an ornamental plant from South America in the 1950's. By 1960 it was recognized as a serious weed pest, which canopies citrus trees, competes for sunlight, water and nutrients, girdles tree limbs and interferes with spraying, harvesting and irrigation practices. By 1970 the vine which is spread by wind-borne seeds, and persists as a perennial, was distributed throughout most of the citrus growing areas in Florida (17).

DeVine[R] was registered by the United States Environmental Protection Agency for use in Florida citrus groves with the restriction that it not be used within 1.6 km of specific susceptible ornamental and crop plants. It is marketed by Abbott Laboratories on a custom basis because it must be handled like fresh milk throughout its distribution system; shelf life normally is about six weeks. The restricted, well defined market area has made it possible to market DeVine[R] as a wet labile formulation. Had it been a product to be sold over a large area it would not have been feasible.

Treatments are made with DeVine[R] to wet soil surface and plants under citrus trees by spray application at rates of 2 to 8 chlamydospores per cm^2 of soil surface. Seedling plants are killed more rapidly than older plants and 96 to 100% control is routinely achieved after 10 weeks. The pathogen may persist in the soil and provide residual control for periods of 2 to 4 years from a single treatment, thus re-establishment of the weed from wind-borne seeds is prohibited (13).

The safety of this mycoherbicide has been borne out in field research and commercial use dating back to 1973. No damage to citrus or non-target plants has been noted, confirming the early assertion that biotic and abiotic constraints on indigenous organisms in the field can be sufficient to negate apparent hazards revealed empirically in controlled conditions of the laboratory and growth chamber (18). Judgement of hazard thus has been based not entirely on what the pathogen is capable of, rather what it actually does when used in the field as a mycoherbicide. After six seasons of use this judgement appears to be a prudent one and constitutes a significant step forward in the mycoherbicide concept. Now, a much broader spectrum of pathogens may be considered for their potential and evaluated for possible development as mycoherbicides.

<u>Collego</u>[R]. Collego[R] is a dry formulation of conidia of the anthracnose fungus, *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* that has been used commercially since 1982 as a selective post-emergence mycoherbicide to control northern jointvetch, *Aeschynomene virginica* (L.) B.S.P. (Leguminosae), in rice and soybean fields in Arkansas (5, 6, 9, 19, 23). It has been termed the "archetypal" mycoherbicide example by some, perhaps because it illustrates the biological, technical and commercial feasibility of using specific indigenous fungal pathogens to kill native weeds in annual crops; crops that are intensively managed and rely heavily upon chemical pesticides in comprehensive pest control strategies. It is a superior substitute for, and has in fact replaced a chemical herbicide for control of this specific weed. It was developed in a collaborative effort by the U.S. Department of Agriculture, the University of Arkansas Agricultural Experiment Station and the TUCO Division of the Upjohn Pharmaceutical Company. The product is now marketed by Nor-Am Chemical Company which purcahsed the Plant Health Products of TUCO, but the product is still produced and packaged by the Upjohn Company (21).

The weed is believed to be indigenous to the rice growing area of Arkansas and has become weedy as a result of rice cultivation. It competes with rice for space, nutrients and water; interferes with harvest and reduces the value of rough and milled rice because the hard black seeds are difficult to remove. It was previously controlled by application of 2,4,5-T, but this herbicide can damage rice if applications are not properly timed, or if spray drifts onto neighbouring fields in which susceptible crops such as soybeans are growing (21).

Collego[R] is a two-component product. Component A is a water-soluble spore rehydrating agent, while Component B is a water suspendible dried spore preparation of the fungus (5). The dried spore component is packaged by the number of viable spores rather than by weight. Consequently, the weight of each package will vary from lot to lot, but each package contains a minimum of 75.7×10^{10} viable spores, enough to treat 4 ha of rice or soybeans when mixed with 380 L of water. Viable spore count in the spray solution should be 2×10^6 spores per ml. It is critical that the spray system be thoroughly cleaned prior to using Collego[R] because chemical pesticide residues that remain in the spray tank and boom may kill the spores. Applications are made to rice near mid-season when weeds are just emerging through the crop canopy. Plants are affected in the first week after inoculation and are killed within 4-6 weeks. The results of efficacy tests with Collego[R] in rice and soybean fields are presented in Tables 1 and 2 (19).

Table 1. Control of northern jointvetch in rice fields with Collego[R] from 1972 to 1982[a] (Source: Smith (19))

Year	Number of Tests	Area treated (ha)	Average control (%)
1972	1	1	95
1973	4	32	97
1974	8	181	76
1975	17	234	94
1976	20	76	93
1977	19	218	98
1978	13	112	94
1979	17	157	96
1980	22	214	86
1981	15	248	98
1982	2	18	90

[a] Collego[R] applied aerially to flooded rice fields in non-replicated trials.

No residual control in subsequent seasons has been detected. Because the active ingredient is a fungus, fungicides should not be applied to the crop closer than one week before, or less than three weeks after application of Collego[R]. Also since Collego[R] contains a living organism it should not be stored for prolonged periods at temperatures below 4.4°C or above 26.7°C (5).

Table 2. Control of northern jointvetch in soybean fields with Collego[R] from 1976 to 1981[a] (Source: Smith (19))

Year	Number of Tests	Area treated (ha)	Average control (%)
1976	4	15	100
1977	6	48	99
1978	2	20	100
1979	5	39	100
1980	7	67	91
1981	5	58	100

[a] Collego[R] applied aerially to soybean fields in non-replicated trials. Fields were wet from irrigation or rain just before application.

Collego[R] has been used commercially for five seasons. The experience can be summarized as follows (6, 21):

1. In the hands of growers it provides greater than 90% control of northern jointvetch.
2. Apparent failures have been due to misidentification of the weed, *A. indica*, as northern jointvetch.
3. Growers are often concerned about the slowness of kill compared to that experienced with chemical herbicides.
4. The majority of growers using it do so repeatedly.
5. The product is making a profit for the company.

Although many chemical pesticides are damaging to Collego[R], some are not and have been used experimentally in tank-mixes to control more than one weed (14). Acifluorfen can be tank-mixed with Collego[R] to control hemp sesbania, *Sesbania exaltata* (Raf.) Rybd. ex A.W. Hill, and northern jointvetch. Bentazon is another chemical herbicide that can be tank-mixed with the mycoherbicide Collego[R] to broaden the spectrum of weeds controlled with one application. Mixtures of two mycoherbicides is another possibility for broadening the spectrum of weeds controlled by one application. This has been demonstrated by control of northen jointvetch and winged waterprimrose, *Ludwigia decurrens* Walt., with Collego[R] and another host specific strain of *Colletotrichum gloeosporioides* (7).

DEVELOPMENTS

The success of mycoherbicides has engendered increased interest in research, commercialization, and regulation of biological control agents in the U.S.A. and abroad. They come at a time when there is heightened concern about problems associated with chemical pesticides. These problems include: (i) the development of resistance in pest populations, (ii) pest population shifts, (iii) toxicity to non-target organisms, (iv) harmful toxic residues in the environment, and (v) contamination of ground water. The consequences of these problems is reflected in a general reduction in the number of new chemical pesticides and increased interest in development of alternative pest control strategies including biological pesticides.

A regional research project has been established in the Southern Region of the U.S. to encourage interstate, interdisciplinary cooperation on biological control of weeds with plant pathogens. Plant pathologists and weed scientists from 12 states are actively engaged in discovery, evaluation and commercialization of pathogens, principally fungi, for control of such weeds as yellow nutsedge, *Cyperus esculentus* L., johnsongrass, *Sorghum halepense* (L.) Pers., morningglory, *Ipomoea* spp., cocklebur, *Xanthium strumarium* L., prickly sida, *Sida spinosa* L., sicklepod, *Cassia obtusifolia* L., hemp sesbania, *Sesbania exaltata*, alligatorweed, *Alternanthera philoxeroides* (Mart) Griseb, waterhyacinth, *Eichornia crassipes* (Mart.) Solms, and hydrilla, *Hydrilla verticulata* (L.f.) Royle. There is close interaction with private industry during development of individual pathogens. The pathogen nearest commercialization is *Alternaria cassiae* Jusair and Khan, for control of sicklepod. The Mycogen Corporation, San Diego, California is involved in EPA registration and commercialization of the mycoherbicide and expects to have a product by 1988. The most formidable technical barrier has been development of solid-state fermentation procedures for spore production, since this fungus has not been induced to sporulate in submerged fermentation.

A substantial number of mycoherbicide research projects are also being undertaken outside the continental United States. A partial list of these projects is in Table 3. It is believed that others are active, especially in Eastern Europe, but are not widely publicised.

Other research areas that appear to hold considerable promise are strain improvement and formulation. Strains of the Collego[R] fungus, for example have been mutated with chemical mutagens to obtain strains resistant to the fungicide benomyl (20). These strains, if approved by EPA, can be used in rice and soybeans without regard to timing application of this fungicide for control of rice blast, *Pyricularia orzyae* L., and various foliar diseases of soybeans. Protoplast fusion techniques are being researched to combine strains that do not have sexual stages or are incompatible sexually, in order to improve their potential (22). Strain improvement with genetic engineering techniques is expected to give added incentive to mycoherbicide research but as yet, specific projects have not been revealed. Government regulations are now being promulgated to cover release of genetically engineered organisms in the environment (3).

Improvement in formulation of mycoherbicides is progressing with the development of gel pellets and use of invert emulsions (16, 28). Mycelium of fungi can be incorporated into gel pellets fortified with nutrients, dried and stored for use as needed. As the gel rehydrates, sporulation occurs on the surface and provides recurring inoculum for epidemic development. They can be soil incorporated or made floatable and used for aquatic weed control. They appear to have greatest potential for aquatic weeds or rosette type weeds in rangelands and lawns.

Invert emulsions applied to weeds inoculated with aqueous spore suspensions can compensate to some degree for lack of adequate dew periods for some fungi (16). This technique appears to be practical and certainly holds great promise for use in areas where dews are erratic and irrigation not feasible.

New regulations for registration of mycoherbicides greatly enhances their attractiveness (1, 2, 8). These requirements are significantly less than for chemical pesticides or for the first two mycoherbicides. Tests required are arranged in tiers of increasing complexity and duration. If the data of Tier I tests fall within acceptable levels than further tests are not required. EPA Data Requirements for Tier I Biorational Pesticides are summarized in Table 4.

Table 3. Partial list of mycoherbicide research outside the continental U.S.A.

Country/location	Research leader	Major weed	Pathogen
AUSTRALIA			
Orange, N.S.W.	Bruce Auld	Spiny cocklebur	*Colletotrichum*
BRAZIL			
Embrapa Londrina, Parana	J T Yorinori	Wild poinsetta	*Helminthosporium*
CANADA			
McGill, Univ.	Alan Watson	Velvetleaf	*Colletotrichum*
Regina	Knud Mortensen Peter Harris	Roundleaf mallow	*Colletotrichum*
CHINA			
Nanking, Univ.	Li, Yan Han	Dodder	*Colletotrichum*
DENMARK			
Copenhagen (Novo Chem.)	Vibeka Leth	Canada thistle	*Colletotrichum* *Phomopsis*
ENGLAND			
Long Ashton	Mike Greaves	Itch grass	unknown
Oxford, Univ.	Robert Hall	Wild oats	*Pyrenophora*
HAWAII			
Univ. of Hawaii	E Trujillo	Hamakua Poi-MaKani *Clidemia* spp.	*Cercosporella* *Colletotrichum*
ISRAEL			
Hebrew Univ. of Jerusalem	Robert Kenneth	Cocklebur	*Puccinia*
Aro-newe-ya'ar Exp. Station	Y Kleifeld R Revveni	*Datura*	*Alternaria*
ITALY			
Rome	Paula Serrone	Velvetleaf	*Alternaria*
NETHERLANDS			
Wageningen	Piet Scheepens	Wild cherry	*Chondrosterium*
PHILIPPINES			
I.R.R.I.	Keith Moody	Gooseweed	*Alternaria*
SCOTLAND			
Strathclyde Univ.	Mike Burge	Brachen fern	*Ascochyta*
SOUTH AFRICA			
Ft. Hare Univ.	John Mildenhall	Tiger pear cactus	*Fusarium*
SWITZERLAND			
ETH Zurich	Defago	Dock	*Ovularia*

PROSPECTS

A great deal of research and evaluation must be done to determine the extent to which mycoherbicides can serve to supplant chemical herbicides. They appear to have greatest potential for weed control in non-agricultural areas, waterways, public use areas, rangelands and pastures. Application can be made to these areas during periods when environmental conditions and host physiological state are optimum for infection and disease development. However, they can also be integrated successfully into intensively cultivated agricultural systems that rely heavily upon chemical pesticides. The comparatively low development/registration costs, their high activity and specificity, and lack of adverse environmental effects suggest that they may become widely used (i) for specific weeds that represent small market sizes, or (ii) for specific weeds in multiple crops (23). Mycoherbicides are, thus, consistent with the current trend in herbicide development to control weeds with more active, more selective, environmentally safe herbicides, and to do this at costs that are lower than required for conventional organic chemicals (4, 12).

Table 4. Synopsis of EPA data requirements (Tier I) biorational pesticides

Category	Data Required
Product analysis	Product identity Manufacturing process Discussion of formulation of unintentional ingredients Analysis of samples
Product analysis	Certification of limits Analytical methods Physical and chemical properties Submittal of sample
Toxicology	Acute oral Acute dermal Acute inhalation IV, IC, IP injection Primary dermal Primary eye Hypersensitivity study Hypersensitivity incidents Cellular immune response Tissue culture (for viruses)
Residue data	Required only if Tier II or III toxicology are required
Nontarget organism - fate and expression	Avian oral Avian injection Wild mammals Freshwater fish Freshwater invertebrates Estuarine and marine animal Nontarget insect Honey bee

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