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Mycoherbicides: Progress in the Biologic

Ideally, the materials used to control weeds should be easy to produce and store, inexpensive to use, reliable at a high and predictable level of control, and safe for the user and the environment. Many of these characteristics are exhibited by plant-pathogenic fungi that infect plants we consider to be weeds in modern-day agriculture.

Research on biological control of weeds with fungal plant pathogens has been extensive enough in the past decade to identify two strategies for their utilization: the classical and the inundative. In the classical strategy, a fungus is simply introduced or released into a weed population to establish, in time, an epiphytotic requiring no further manipulation. A well-known example of the classical strategy is the introduction of *Puccinia chondrillina* Bubak & Syd. into Australia from Europe to control rush skeletonweed (*Chondrilla juncea* L.) (2). This rust fungus has recently been introduced into the western United States in an effort to control the same weed. The inundative strategy, on the other hand, employs the massive, usually annual release of a fungus into specific weed-infested fields or groves to infect and kill susceptible weeds (3). These applications, or inoculations, can be made as often as necessary and can be timed to favor subsequent disease development.

In this article, we discuss important aspects of research, development, and use of two mycoherbicides now registered with the U.S. Environmental Protection Agency (EPA) and available as products for selective weed control. These topics are organized according to the questions most frequently asked of researchers by growers, industry, extension agents, and the public.

What Are Mycoherbicides?

Mycoherbicides are simply plant-pathogenic fungi developed and used in the inundative strategy to control weeds the way chemical herbicides are used. Two fungi have been registered as mycoherbicides (Fig. 1). DeVine, a formulation of *Phytophthora palmivora* (Butler) Butler, was registered in 1981 for the control of strangler (milkweed) vine (*Morrenia odorata* Lindl.) in Florida citrus groves and is marketed by Abbott Laboratories, North Chicago, IL. *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* was registered in 1982 as Collego, a formulation for the selective control of northern jointvetch (*Aeschynomene virginica* (L.) B.S.P.) in rice and soybean fields of Arkansas, Louisiana, and Mississippi; it is marketed by the TUCO division of the Upjohn Company, Kalamazoo, MI. A significant distinction between the fungi used as mycoherbicides and the fungi used in the classical strategy is that mycoherbicides must be registered by the EPA and fungi used in the classical strategy do not require registration but are regulated by the U.S. Department of Agriculture.

How Do They Work?

C. gloeosporioides f. sp. *aeschynomene* incites an anthracnose of northern jointvetch, infecting leaflets, petioles, and stems as well as seedpods and seeds (Fig. 2A). After inoculation, spores germinate, produce appressoria, and penetrate the weed epidermis within 24 hours. The mycelium rapidly ramifies within the tissue, and lesions are visible within 3 days in a greenhouse or 7–10 days in the field. Stem lesions lengthen and encircle the hollow stems, effectively girdling the plant and killing all tissue above the lesions. Leaflet lesions cause the leaflet to abscise, and petiolar lesions cause the death of the leaf above the lesion. In field tests conducted in Arkansas in which inoculum (spores) was applied aerially, one application killed plants 30–40 cm tall within 4–6 weeks.

P. palmivora incites a root and stem rot of strangler vine and girdles the stems of infected plants near the soil surface, resulting in the death of seedlings and older plants (Fig. 2B). Young seedlings can be killed within 1 week after inoculation, and larger vines, which often overgrow mature trees, can be killed within 4–6 weeks. Inoculum (zoospores or chlamydospores) incites the disease whether applied to seedlings or the soil.

How Are They Applied?

Collego is a dry powder, 15% spores and 85% inert ingredients, that is rehydrated and resuspended in a sugar solution before being mixed with water in an applicator's spray tank. Normally, Collego is applied aerially to rice and soybeans at dusk (Fig. 3), although application in soybean fields is also by tractor-mounted equipment. With aerial equipment, Collego is applied at a rate of 10 gal/acre (94 L/ha) containing 2×10^6 viable spores per milliliter. The weight of actual product used per acre depends on the viability of each production lot and ranges from 25 to 40 g (0.06 to 0.09 lb). Collego is normally applied only once each season, during July or August, when the weed has just emerged above the rice canopy.

DeVine is available as a liquid suspension ("wet-pack") consisting largely of chlamydospores of the fungus and must be ordered prior to the season in which it will be used. One pint contains 6.7×10^5 viable chlamydospores per milliliter and is applied to 1 acre (0.4 ha) in at least 50 gal of water. DeVine is normally applied with boom and nozzle sprayer systems to the soil surface under tree canopies; the soil surface must be wet at the time of application. DeVine may be applied from May through September,

Published with approval of the director of the Arkansas Agricultural Experiment Station.

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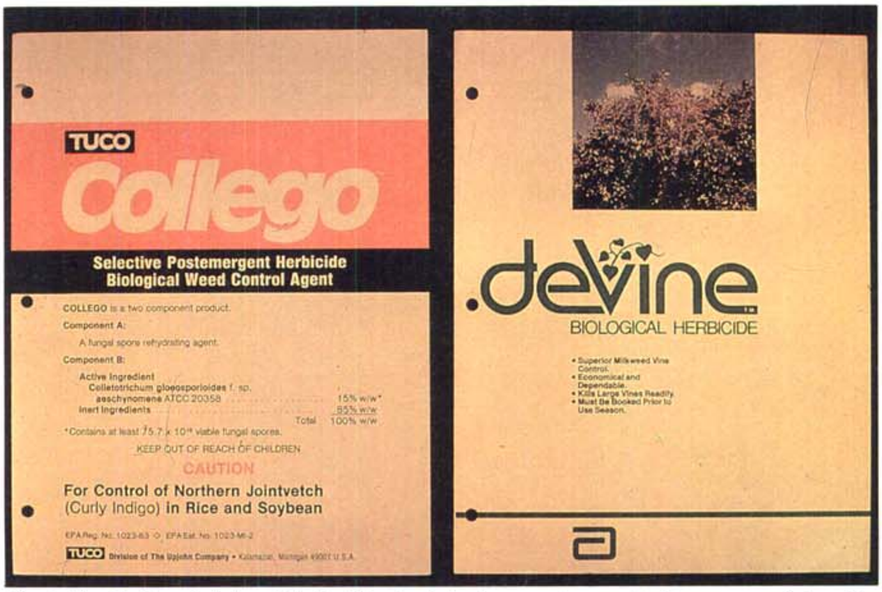
ontrol of Weeds



TUCO

Collego

Selective Postemergent Herbicide
Biological Weed Control Agent

COLLEGO is a two component product.

Component A:

A fungal spore rehydrating agent.

Component B:

Active Ingredient Colletotrichum gloeosporioides f. sp. aeschynomene ATCC 20358	15% w/w*
Inert Ingredients	85% w/w
Total	100% w/w

*Contains at least 75.7 x 10[illegible] viable fungal spores.

KEEP OUT OF REACH OF CHILDREN

CAUTION

For Control of Northern Jointvetch
(Curly Indigo) in Rice and Soybean

EPA Reg. No. 1023-63 ○ EPA Est. No. 1023-MI-2

TUCO Division of The Upjohn Company • Kalamazoo, Michigan 49001 U.S.A.

deVine

BIOLOGICAL HERBICIDE

- Superior Milkweed Vine Control.
- Economical and Dependable.
- Kills Large Vines Readily.
- Must Be Booked Prior to Use Season.

Fig. 1. The Collego label and the DeVine technical manual cover. Collego was registered in 1982 to control northern jointvetch in rice and soybeans, and DeVine was registered in 1981 to control strangler (milkweed) vine in Florida citrus groves.

when the weed is actively growing, and only one treatment is necessary for control. Mitchell (6) has found that 0.2–0.9 chlamydospores of *P. palmivora* per gram of soil are required for 50% infection.

Care must be taken not to expose either *C. gloeosporioides* or *P. palmivora* to wetting agents, fertilizers, or chemical pesticides that are detrimental to the viability of spores. In addition, *P. palmivora* must not be applied where susceptible plants such as watermelon and periwinkle are grown or within 100 ft of cucumber, squash, begonia, *Bougainvillea* spp., boxwood, *Hibiscus* spp., oak, areca palm, *Pittosporum* spp., snapdragon, Washington and cocoanut palm, and hybrid *Rhododendron* spp.

Do Mycoherbicides Persist After Application?

C. gloeosporidoides has been shown to persist for only a short time and only on plant refuse and within infected seed, not in soil. In experiments conducted in the field and laboratory, after 4–6 weeks *C. gloeosporioides* was not reisolated from soil artificially infested with spores (9). Similarly, the fungus was not reisolated from lesions on infected stems of jointvetch buried in soil for more than 4 weeks during the winter months. Survival of the fungus in rice irrigation water was also limited to a few weeks.

C. gloeosporioides overwinters by persisting within and on jointvetch seeds. Infected seeds appear to be the source of seedling infection, since seedlings grown from contaminated seed often develop lesions on stems at the point of attachment of cotyledons. Second, the fungus persists on lesions on jointvetch stems left standing over the winter. Approximately 20% of such lesions yield *C. gloeosporioides* when samples are plated on agar medium. Neither method of overwintering is sufficient to provide

Fig. 2. Symptoms associated with the mycoherbicides: (A) Anthracnose of northern jointvetch caused by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. (B) Root and stem rot of strangler (milkweed) vine caused by *Phytophthora palmivora*.

Table 1. Control of northern jointvetch in rice by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*

Year	Hectares treated	Average percent control
1974	191	76
1975	254	94
1976	76	93
1977	218	98
1978	112	94
1979	157	96
1980	214	86
1981	248	98
	Total 1,470	
	Av. 184	Av. 92

Table 2. Control of northern jointvetch in soybeans by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*

Year	Hectares treated	Average percent control
1976	15	100
1977	48	99
1978	20	100
1979	39	100
1980	67	91
1981	58	100
	Total 247	
	Av. 41	Av. 98

enough inoculum for natural control of jointvetch without augmentation each year.

As a soilborne plant pathogen, *P. palmivora* can be expected to persist in soil. Although the persistence of formulated *P. palmivora*, as described in technical literature, is quite short, the inoculum resulting from infections of *M. odorata* is highly persistent. In field efficacy trials, reduction of strangler vine population exceeded 90% within 1 or 2 years and control continued for 2 years after a single treatment (15).

P. palmivora and *C. gloeosporioides* cannot be effectively used as preventive control measures where hosts are absent.

Do They Spread?

Although *C. gloeosporioides* has been shown to spread to plants within contiguous field plots, interplant spread is severely limited, first by a requirement for splashing water, ie, rain, and second by the widely separated distribution of jointvetch plants within a dense crop population. Insects may also aid in interplant dispersal; several species have been observed feeding on sporulating lesions. Intraplant dispersal is facilitated by mycelial growth within the plant and by dew or rain that washes spores from lesion surfaces down the stems to collect at branch nodes or on stem hairs.

P. palmivora does not disperse well in soil or air. However, Burnett et al (1) examined infected leaves of noninoculated milkweed vines near inoculated vines and found sporulating lesions of *P. palmivora* (= *P. citrophthora*). They suggested that this indicated wind dissemination of sporangia.

Are Mycoherbicides Effective in Controlling Weeds?

C. gloeosporioides has been field-tested as a mycoherbicide since 1973. From 1974 through 1981, the EPA granted experimental use permits to enable large-scale tests in growers' fields. During those 8 years, an average of 16 rice fields totaling 184 ha were treated aerially with spore suspensions of *C. gloeosporioides* (94 L/ha, 2×10^6 spores per milliliter). Weed control averaged 92% (Table 1). Since control is measured as the proportion of plants killed by the fungus, *C. gloeosporioides* is very effective as a mycoherbicide and is as good as or better than chemical herbicides. The remaining plants (approximately 8%) were heavily infected and their seed production was minimal. The fungus was even more effective in soybeans during field tests conducted from 1976 through 1981. In soybean, control of northern jointvetch averaged 98% on 247 ha treated in 29 fields (Table 2). Control is usually achieved in 4–6 weeks in both rice and soybeans.

Infection and, therefore, control is affected by the environment (11). Infection is favored particularly by the high moisture conditions maintained by flooded rice paddies and in soybeans by irrigation immediately before or after treatment with *C. gloeosporioides*. For example, in 1980, control was reduced in both rice and soybeans by the very hot and dry weather in Arkansas that season.

P. palmivora has also proved to be an effective mycoherbicide. In greenhouse studies, 100% of inoculated 1-month-old seedlings died within 20 days after infestation of soil (7,8). In field tests, approximately 62% of the plants, from seedlings to plants a few inches tall, were killed within 2 weeks after soil infestation; after 10 weeks, approximately 96% of the vines were dead (1). Soil moisture is reported to be the primary factor for the good weed control obtained by *P. palmivora*. Control of strangler vine by *P. palmivora* has persisted for 2 years following a single application (15).

Are Mycoherbicides Specific to the Weeds They Control?

C. gloeosporioides was originally described as specific to *A. indica* L. and *A. virginica*, both weeds in Arkansas rice fields (3). Alfalfa, black-eyed pea, cotton, cucumber, grain sorghum, green bean, jack bean, lespedeza, lima bean, okra, rice, soybean, tomato, white clover, and white lupine were immune to infection in greenhouse tests (3), and soybean inoculated during field tests was also immune (3). Recently, however, several additional susceptible species were found in greenhouse tests, including other *Aeschynomene* spp., English pea (*Pisum sativum* L.), *Lupinus densiflorus* Benth., broad bean (*Vicia faba* L.), and *Lathyrus* spp. Although the host range is now considerably wider than originally determined, host specificity is not perceived as a significant problem, since none of these susceptible plants is grown in the vicinity of rice or soybeans and since *C. gloeosporioides* does not persist in soil or disseminate far from application sites.

The host range of *P. palmivora* includes a number of plant species in several families. In preemergence inoculation tests, onion, strangler vine, cantaloupe, watermelon, okra, and tomato showed less than 50% emergence, and endive, cucumber, English pea, and carrot emergence was reduced to near 50%. In postemergence inoculation tests, root infection was detected in strangler vine, squash, watermelon, and English pea. In foliage inoculation tests, strangler vine, English pea, Irish potato, and tomato were infected, resulting in death of strangler vine and English pea. *P. palmivora* was also reisolated from root rot on taproots of carrizo (*Phragmites* sp.) seedlings and was pathogenic to carrizo and *Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf. rootstocks in greenhouse preemergence tests (4,7). Pathogenicity of *P. palmivora* to citrus could not be demonstrated in the field, and isolations from roots of citrus trees treated with *P. palmivora* in the field never revealed infection by *P. palmivora*.

Are Mycoherbicides Safe?

Although plant pathologists emphasize or equate safety with host range, registration of *C. gloeosporioides* as Collego required that environmental and human safety also be assessed. Thus, an extensive battery of tests was conducted by a veterinary pathologist to determine if unreasonable risks were involved. Various animals, including rats, mice, rabbits, guinea pigs, dogs, turkeys, quail, chickens, ducks, crayfish, perch, catfish, frogs, and earthworms, were challenged via oral, nasal, dermal, ocular, or interperitoneal injection. Autopsies were conducted on all animals, and no infections or chronic symptoms were observed in treated animals. Safety to plants and survival in the environment had also been demonstrated (3,10,11). *P. palmivora* was also subjected to toxicology tests, including eye, skin, inhalation, oral, and interperitoneal tests with mice, rabbits, hamsters, and rats. No adverse results were obtained that prevented registration.

Can They Be Integrated with Chemical Pesticides?

Recently, laboratory and field studies have shown that mycoherbicides can be integrated with agricultural pesticides. Klerk (5) has shown that *C. gloeosporioides* can be tank-mixed with the herbicides acifluorfen (Blazer) and bentazon (Basagran) without reducing the activity of *C. gloeosporioides* or affecting the herbicides. In greenhouse tests, malathion, carbofuran, acifluorfen, and bentazon did not significantly reduce infection of jointvetch by *C. gloeosporioides*, whereas similar tank mixtures with propanil, fentin hydroxide, or benomyl (Benlate) severely inhibited *C. gloeosporioides*. Field tests have shown, however, that *C. gloeosporioides* can be used with these pesticides when applied in sequence. In addition, benomyl-tolerant strains of *C. gloeosporioides* have been produced by mutation. These strains may be particularly effective in reducing the inhibitory effects of benomyl on control of the weed and in minimizing problems associated with the timing of sequential application of chemical and biological pesticides.

P. palmivora cannot be mixed with chlorinated water or tank-mixed with wetting agents, fertilizers, or other pesticides.

Are Others Being Developed?

Numerous plant pathogens are being studied throughout the world for possible development as mycoherbicides or biological control agents (12). In the United States, in particular, *Alternaria cassiae* Jurair & Khun, *A. macrospora* Zimm., *Ascochyta pteridium* Bres., *Cercospora rodmanii* Conway, *Colletotrichum coccodes* (Wallr.) Hughes, *C. dematium* (Pers. ex Fr.) Grove, *C. malvarum* (A. Braun & Casp.) Southworth, *C. gloeosporioides* f. sp. *jussiaeae*, *Fusarium lateritum* Nees ex Fr., *F. solani* (Mart.) Sacc. f. sp. *cucurbitae*, and *Sclerotinia sclerotiorum* (Lib.) de Bary are being evaluated for potential use against sicklepod, spurred anoda, bracken fern, water hyacinth, velvetleaf, morning glory, prickly sida, winged water primose, spurred anoda and prickly sida, Texas gourd, and Canada thistle, respectively.

A. cassiae, discovered by Walker (13) in Mississippi, is currently undergoing third-year evaluations as a selective mycoherbicide for sicklepod control throughout the southeastern United States by Regional Research Project S-136. The results have been very encouraging; control of emerging seedlings has exceeded 85% in most tests. *C. gloeosporioides* f. sp. *jussiaeae* has also been tested as a formulated material similar to Collego and has been shown to be very effective alone or in combination with Collego or tank-mixed with herbicides (5).

State of the Art and the Future

Considering the comparatively small initial investment, considerable progress has been made since the discovery of *C. gloeosporioides* in 1969 and of *P. palmivora* in 1972. Numerous plant-pathogenic fungi are now being investigated in 13 states and other locations throughout the world. It is doubtful that Collego and DeVine will remain the only registered mycoherbicides. We cannot

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Fig. 3. Aerial application of Collego to rice. *C. gloeosporioides* is normally applied aerially with standard agricultural equipment at a rate of 10 gal/acre (94 L/ha) containing 2×10^6 spores per milliliter.

assume, however, that with the registration of two organisms all problems associated with registration, eg, toxicology, have been solved. The *Alternaria* spp. are known to be allergenic and will likely require additional toxicology tests that may add substantially to their development costs. Also, *Fusarium*, *Alternaria*, and *Cercospora* spp. produce toxins that will likely also require additional testing to establish safety levels. These examples simply illustrate that each new organism poses new questions that have not been addressed by earlier registrants and that may diminish the organism's potential use as a mycoherbicide or make development too costly.

The "safety" of mycoherbicides also relates, pathologically, to their host specificity and host range. Several procedures have been discussed to establish the host range of a plant pathogen with certainty but without the necessity of screening all plant species (14). These procedures need to be rigorously tested and methods developed to completely answer the simple question, "What is the host range?" Furthermore, can mycoherbicides be used indefinitely without fear of changes in pathological specificity, or will new races develop or be discovered as a result of their continued use?

The apparent specificity of pathogens under study is both an advantage and a disadvantage to their use and development. It is an advantage because pathogens can be safely used to eliminate weeds from closely related crop species without fear of crop damage. Most chemical herbicides are not nearly as selective. But this high level of specificity also requires that numerous fungi be discovered and developed. Development costs may be assessed to each organism for an individual weed rather than across a spectrum of weeds, as in the case of chemical herbicides. This could significantly increase control costs for mycoherbicides. At present, most work with mycoherbicides is concentrated on species that escape standard chemical weed control programs. These "hard-to-control" species, spread across a wide area, present an economic opportunity that could fit into an established chemical control program.

More work will be required in the area of integrating biological and chemical control measures. The development of pesticide-resistant strains seems to be a logical approach provided the chemicals for which resistance is developed are not replaced too frequently. Programs of pest control that properly select specific pesticides and time their application to minimize interference may be more durable over time.

The application of protoplast fusion and recombinant DNA technologies to the development and improvement of mycoherbicides appears more feasible as time passes. Although not yet attempted for mycoherbicides, intraspecific and interspecific protoplast fusions with fungi have already been demonstrated to be feasible with fusants possessing characteristics of both parents. Thus, protoplast fusions may present a method to combine the pathogenicity of two separate plant-pathogenic fungi. The genetic engineering of toxin production also seems to be a fruitful area of research.

Finally, the two mycoherbicides now available have been shown to be effective and dependable. That alone does not guarantee other fungi will be developed as mycoherbicides. It seems logical and practical, however, that mycoherbicides will be developed not as replacements for chemicals but because the benefits extend beyond economics.

Acknowledgments

We thank J. Norland, D. Kenney, W. Ridings, and R. Charudattan for their comments and W. Ridings for Figure 2B.

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