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Perspectives on Mycoherbicides Two Decades After Discovery of The Collego® Pathogen

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Abstract

In 1989 two decades will have passed since the discovery of the endemic anthracnose disease of northern jointvetch caused by *Colletotrichum gloeosporioides* and seven years since its commercialization as the mycoherbicide COLLEGO® in 1982. The continuing commercial success of COLLEGO in a limited market of rice and soybeans in Arkansas affirms early assertions that: a) indigenous plant pathogens have potential as biological control agents but require the bio-pesticide or inundative inoculation tactic; b) fungal plant pathogens can be sufficiently virulent, environmentally compatible, host-specific, genetically stable, fermentable and non-persistent to be produced, dried and used safely as mycoherbicides in annual crops; c) mycoherbicides can be successfully applied in mixtures with chemicals in pest management systems; d) environmental perturbation from use of mycoherbicides is essentially nil, merely that caused by the removal of the target weed; e) growers and applicators understand and accept the few critical requirements for handling and storing a living product and can accurately identify weed species; f) the high specificity of a mycoherbicide is particularly beneficial where pesticide sprays may drift to non-target crops grown in contiguous fields; and g) mycoherbicides can be marketed profitably. Much research is currently underway in the U.S.A. and worldwide to determine the extent to which mycoherbicides may be employed, but a discussion on the problems and prospects of selecting candidate pathogens with high biological and economic potential should be beneficial.

Introduction

Almost two decades of research and experience with mycoherbicides have accumulated since the discovery of an endemic anthracnose disease of northern jointvetch (*Aeschynomene virginica* [L.] Britton, Stern & Poggenburg; Fabaceae) caused by a strain of the fungus *Colletotrichum gloeosporioides* (Penzig) Penzig & Saccardo (Coelomycetes) (*Cga*), which was commercialized as the mycoherbicide COLLEGO® in 1982 (Bowers 1982, 1986, Daniel *et al.* 1973, Smith 1986, Templeton 1986, 1987, Templeton *et al.* 1984, 1986). Commercial success with this pathogen affirms earlier assertions about biological weed control with plant pathogens which can be instructive for developing the mycoherbicide concept for other pathogen and weed combinations. Most of these assertions deal with biological and technical aspects derived from a fundamental understanding of pathogen and weed biology at the organismal and ecosystem levels (Templeton and Smith 1977, Templeton *et al.* 1979, Templeton and Trujillo 1981). In retrospect, it is now apparent that factors in addition to host and pathogen biology also contributed to development and commercial success of COLLEGO (Bowers 1986, Templeton 1987, Templeton *et al.* 1980, 1986). In particular, the interdisciplinary collaboration of weed scientists, plant pathologists and fermentation scientists was essential for successful development of COLLEGO along with technology transfer between the public and private organizations during development (Churchill 1982, Templeton 1986, Templeton *et al.* 1980). It is clear that neither group would have been successful alone, nor is it likely that any single discipline could have achieved success without cooperation of the others. Continuing success of COLLEGO in a relatively small market still relies upon sustained interaction of the private sector with cooperation from a public agency, the Cooperative Extension Service (Huey and Boston 1983).

We will examine some of the assertions about the biology of weed diseases, interactions of disciplines and the transfer of technology between public and private organizations that contributed to the development of COLLEGO. These discussions may be useful for applying the mycoherbicide concept to development of other fungi as mycoherbicides.

Indigenous Plant Pathogens And The Inundative Inoculation Tactic

Although indigenous plant pathogens generally incite disease in a natural population of weeds at endemic levels and are often inconspicuous, occasionally they occur at moderate or severe levels throughout the geographic range of the host (Burdon 1987). As endemic diseases, they are generally present every year and are frequently irregularly distributed in host populations. An ecological advantage for a pathogen that would consistently destroy its host plant by epidemics is not evident. When epidemics occur sporadically in annual weed populations, they generally reach their highest intensity as the host matures. Therefore, epidemics usually occur too late in the growing season to control weeds before interference has already reduced crop yields. The failure of indigenous pathogens to develop epidemics early requires an alternative to the classical biological control tactic.

Use of the COLLEGO pathogen as a classical biological control agent for control of northern jointvetch, an annual weed in an annual crop, was not considered a viable strategy. This pathogen could be successfully used as a biological control agent only if it were possible to overcome two major constraints to epidemic development: 1) limited sources of primary inoculum and, 2) restrictive dissemination of the pathogen on individual host plants or from plant to plant subsequent to initial infection (Smith *et al.* 1973, Templeton and Smith 1977, Templeton *et al.* 1979). The use of the pathogen as a mycoherbicide would compensate for the two deficiencies. Applying a mycoherbicide in the same manner as a chemical pesticide would inundate each plant with inoculum at a susceptible plant growth stage before the weed reduced crop yield and quality.

Ultimate success of the mycoherbicide concept might have been predicted by circumstances that occurred during discovery of the disease in weed research plots (Daniel *et al.* 1973, Templeton *et al.* 1984). The disease reached an epidemic quickly on the weed plants that had been transplanted in the field from seedlings grown from mechanically scarified seeds in the greenhouse (Smith 1986). This suggested that the fungus was highly virulent and that the field environment was favorable for infection and development of the disease after plants had been uniformly inoculated during the process of scarification, seeding, and growth of seedling in the greenhouse, and after transplanting in the field. Success might also have been predicted by information available from previous research on other anthracnose pathogens (Walker 1952). Many anthracnose pathogens overwinter poorly, are often host-specific, are culturable and do not have fastidious environmental requirements which might prevent infection and epidemic development.

All fungal pathogens do not have equal potential as mycoherbicides. Characteristics of the COLLEGO pathogen may be instructive in the search for and selection of other fungal pathogens as biological control agents. One of the most important of these characteristics is selection of pathogens that are constrained from epidemic development primarily by poor dissemination capacity within plant colonies rather than by some environmental requirements such as long dew period or restrictive temperature regimes.

Seed borne pathogens with high mycoherbicide potential can perhaps be located by special survey techniques which emphasize collecting weed seeds over large geographic areas and growing plants from mechanically scarified seed in environments favorable for disease development. Growing seedlings in moist environments at temperatures representative of where the weed is a problem increases the potential of discovering new mycoherbicides. This approach emphasizes diseases of individual plants rather than epidemics in large populations of weed plants. Low percentage of carryover in seeds usually occurs and restricted dissemination is a trait of many weed pathogens. Although seedling blights caused by soil-borne pathogens may kill plants, most from this source are generally considered to have low potential as mycoherbicides because they lack specificity, are genetically variable, or present formulation difficulties.

Biological and Technical Potential of Fungi for Mycoherbicide Development

The assertion that indigenous pathogens, such as the northern jointvetch anthracnose fungus, were biologically and technically sound approaches to weed control also recognized the uniqueness of each disease; that it is the result of the tripartite interaction of host, pathogen and environment or the disease triangle. Absence of disease may be attributed to constraints on one or more components of the disease triangle. This may explain why disease is generally an exception rather than the rule or why most plants are healthy most of the time. Efforts in plant pathology to control diseases of desirable plants, are directed at understanding the interactions of each component of the disease triangle to devise controls that are inimical to completion of this triangle. A similar understanding of disease is needed for developing a mycoherbicide, but emphasis is on implementing components that are conducive rather than restrictive to completion of the triangle. Fundamental knowledge of diseases of economic crops can be applied to diseases of weeds and may be useful in mycoherbicide development.

For example, when the pathogen of northern jointvetch anthracnose was identified as *C. gloeosporioides*, a firm research base was provided by analogy to anthracnose diseases of economic crops, including *C. lindemuthianum* and *C. orbiculare* on legumes and cucurbits, respectively (Walker 1952). These two fungi are host-specific, genetically uniform, produce primary inoculum from infected seeds, require rain dispersal of secondary inoculum, and are nonpersistent in the soil. Laboratory and field plot research confirmed the close analogy of the northern jointvetch anthracnose, and the two crop diseases, and encouraged efforts to explore the technical feasibility of commercial use (Daniel *et al.* 1973). Other members of this pathogen group are found on other important weeds and may also be good mycoherbicide candidates, such as, anthracnose on dodder (*Cuscuta chinensis* Lam.; Cuscutaceae) (Y. Li, pers. comm., 1986), roundleaf mallow (*Malva pusilla* Sm.; Malvaceae) (K. Mortensen, pers. comm., 1987), spiny cocklebur (*Xanthium spinosum* L.; Asteraceae) (Auld, B.A., pers. comm., 1987) and velvetleaf (*Abutilon theophrasti* Medicus; Malvaceae) (Gotleib *et al.* 1984).

The wide range of weedy plants affected by diseases incited by certain species of *Colletotrichum* provides excellent opportunities for research on mycoherbicides. Other imperfect genera that produce spores in a matrix that reduces dispersal, are potential candidates. Low inoculum dispersal also provides a margin of safety if there are susceptible host species that have economic importance. The COLLEGO pathogen disseminates so poorly that northern jointvetch in fields adjacent to treated fields are usually not diseased. Discovery of several additional hosts of the COLLEGO pathogen (TeBeest 1988) since initial host range research, therefore, has not been of particular concern. Safety zones of one km between growers with conflicts-of-interest would seem more than ample for pathogens of this type.

The biology of anthracnose pathogens and related fungi is not the only criterion that is required for a pathogen to have mycoherbicide potential. It must also be technically and economically feasible to produce, dry and apply inoculum. For COLLEGO, the fact that northern jointvetch was genetically uniform, suggested that the entire weed population would be vulnerable to a virulent strain of the pathogen. This assertion is valid for COLLEGO and probably is also valid for many weed disease. Genetic diversity in natural plant populations would seem to be the rule with respect to phenotypic characters, but genotypic traits such as disease reaction may be quite uniform in natural populations. Also, weed species often have passed through one or more ecological or physical bottlenecks in becoming weedy and consequently may be more genetically uniform than the population from which they originated.

Environmental parameters controlling disease development by the COLLEGO pathogen were of less concern than the genetic characteristics of the host and pathogen. The weed occurs in flooded rice fields that provide consistently favorable moisture and temperature conditions suitable for infection and development of the disease. Research verified that the fungus was infectious in a rice microclimate. The spores germinated over a wide range of temperatures and infection and disease development occurred over a wide range of environmental conditions (TeBeest *et al.* 1978). Similar research will be required to establish optimum environmental requirements for each pathogen.

Temperature and moisture are often key constraints to spore germination and infection. However, if a disease persists at an endemic level, environments favorable for the disease must occur sometime. If this time corresponds to when weeds should be controlled, then control by inundative inoculation will not be limited by environment as long as applications are made during periods favorable for infection. Control of roundleaf mallow in dry prairie environments of Canada or control of spiny cocklebur in relatively dry climates of Australia support this contention (Auld, B.A., pers. comm., 1987, K. Mortensen, pers. comm., 1987).

The technical feasibility of producing and applying inoculum of the COLLEGO pathogen was demonstrated soon after the fungus was isolated. It sporulated in liquid media when aerated by vigorous shaking and spores applied to small plots in field tests controlled weeds (Daniel *et al.* 1973). Only 1-2 ml of culture liquid were required to produce enough spores to control the weeds in one square meter plot. The fungus could be dried for storage and shipment by adapting existing technology used for other dry fungus products. Scale-up of spore production from small culture techniques to large fermentation was readily accomplished (Churchill 1982). Although developing the techniques for drying of the spore product was initially difficult, technology eventually was developed with conventional drying equipment and has been subsequently applied to other pathogens.

Other fungi, which have high field potential for development as mycoherbicides, differ considerably from the COLLEGO fungus in their technical requirements and require alternative spore production techniques. For example, *Alternaria cassiae* Jurair & Khan (Hyphomycetes), which is being developed as a mycoherbicide for sicklepod (*Cassia obtusifolia* L.; Caesalpiniaceae), requires a solid substrate for production of spores (Walker 1982, Walker and Boyette 1985). More research is needed on production techniques with fungi to better utilize those with good potential as mycoherbicides. Further research on formulation of fungi is needed.

Successful application of the COLLEGO pathogen in the field has been accomplished without particular difficulty. Applications are made routinely with standard aerial or ground equipment that has been cleaned to remove chemical residues. Generally, a custom aerial applicator cleans the equipment and applies COLLEGO to several rice fields before using the equipment again for chemical pesticides. Although most applications are made in late afternoon, the timing is not critically important. Nor is it necessary to avoid applications immediately before a rain because spores do not wash from plants easily.

Occasional problems have occurred, however, when the weed Indian jointvetch (*A. indica* L.) which is very resistant to infection was misidentified as northern jointvetch.

Growers are also aware of the necessity to keep the fungus in cool storage. Only one case has been reported to date that required re-treatment because of a heat damaged product.

Occasional failures of weed control have occurred because chemical fungicides have been applied to control rice diseases shortly after application of COLLEGO. This problem could increase as shorter season rice cultivars are grown and more widespread use of fungicides is required for rice disease control. Fungicide-tolerant strains of the COLLEGO pathogen have been successfully developed and the tolerant strain is expected to be registered after regulatory review (TeBeest 1984).

Treated rice or soybeans or non-target crops and native plants or animals have not been injured by COLLEGO applications. The environmental perturbation has been essentially nil, except for the removal of the target weed that received the treatment. No evidence of spread beyond treated areas has occurred. COLLEGO has been proven to be safe to humans and animals. No allergies or skin irritations have been reported. These experiences with COLLEGO reaffirm the early belief that the fungus would be safe to the environment and to humans. It is likely, too, that other fungal pathogens can be used safely as mycoherbicides after host-specificity and animal challenge experiments indicate no adverse responses (Beasley *et al.* 1975).

Most applications of COLLEGO are made singly to rice fields to control only northern jointvetch, but COLLEGO has also been applied in tank mixtures with acifluorfen (5-[2-

chloro-4-(trifluoromethyl)phenoxy]-2-nitro-benzoic acid) to control northern jointvetch and hemp sesbania (*Sesbania exaltata* [Raf.] Cory; Papilionaceae) (Smith 1986). Mixing these biological and chemical herbicides avoids dockage assessments and reduced crop value caused by the two major black-seeded weeds in rice. COLLEGO and acifluorfen also control both weeds in soybeans, but economic incentives are lower. Other mycoherbicides and chemical herbicides applied in tank mixtures may be developed in the future to increase the number of species of weeds controlled or to provide synergistic activity on weeds that my not be controlled by herbicides alone. Application of several pathogens in mixtures may be used to control a single, hard-to-kill weed (Boyette *et al.* 1979).

COLLEGO has been a profitable product every year it has been used. Its use is expected to increase because it is now the only product available for control of northern jointvetch and market saturation has not yet occurred. The weed constitutes a very small market potential compared with typical markets for broad-spectrum chemical herbicides, but costs of producing and marketing COLLEGO are inexpensive with a favorable return on investment. Profitability of this low-use product was also aided by transfer of technology developed by public organizations to private industry which used the data for registration and commercialization. Current registration requirements for mycoherbicides are less than those required for registration of COLLEGO. Therefore, the cost of developing future mycoherbicides may be reduced with subsequent greater profit potential (Anonymous 1982, 1983, Charudattan 1982).

Presently, the single greatest constraint to development of mycoherbicides is inadequate field exploration for suitable new mycoherbicide candidates. Increased interest worldwide in mycoherbicides and increased concern regarding high dependence on chemical pesticides in agricultural production suggest that greater efforts will be made to discover and develop disease pathogens as mycoherbicides.

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