

## Assessment of Efficacy of Mycoherbicide Candidates

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### *Abstract*

Efficacy and host-specificity are the two overriding concerns in the development of a mycoherbicide agent. Efficacy, for practical reasons, must be defined as the ability to yield a satisfactory amount, speed, and ease of weed control. All of these three aspects should be criteria for determination of efficacy and selection of most promising mycoherbicide candidates. Different steps in efficacy measurement, such as greenhouse and field evaluations; appraisal at relevant phenological stages of the weed and/or the crop; and suitable measures of weed control, such as reduction in weed numbers, biomass, competitive ability, growth rate, or seed set; increased crop yield; and favorable cost/benefit from the mycoherbicide must comprise efficacy determinations. Among the most frequently used measures is the reduction in weed numbers resulting from a complete and fairly rapid killing by the pathogen. Disease assessments, including visual ratings and direct measurement of disease on individuals and populations, can help to quantify the destructiveness of the mycoherbicide and provide an indirect measure of its efficacy. However, since mycoherbicide technology is regarded as being comparable to chemical herbicide technology, it is my opinion that the public expectation of mycoherbicide efficacy is conditioned by that of the chemical herbicides. The latter are characterized by cost/efficiency, effectiveness, ease of application, speed of control, and predictability of results. Therefore, in the final analysis, mycoherbicides will be expected to compare favorably, equal, or out-perform the available chemical herbicides and only those that fare well in this respect are likely to reach the marketplace. Hence, pathogens that can provide quick, complete, and easy weed kill should be chosen as mycoherbicide candidates.

### **Introduction**

Several scientific, technological, and practical considerations impinge upon the selection, development, and marketing of mycoherbicide candidates. Among these, host-specificity and efficacy are the two primary concerns affecting early decisions on the choice of candidates. Before a pathogen can be given serious consideration as a candidate, it must be determined to be safe in its host range and it must be capable of providing a satisfactory level of weed control. The latter, the aspect of efficacy, is a complex issue; it is not merely the ability of a pathogen to cause disease or kill the weed under a set of experimental conditions. It includes other traits that assure a satisfactory level of weed control in users' hands.

Mycoherbicides, unlike the classical agents, are intended to be used in an inundatory inoculation strategy to achieve a specified level of weed control over a defined time in targeted area (Charudattan 1988). Everything about their use is predetermined and specified. Not only is there a regulatory requirement in the United States, and possibly other countries, to specify an anticipated amount of control for a mycoherbicide and how to achieve it (Charudattan 1982), even the scientific and commercial motivation for development of a candidate will ultimately depend on its efficacy. Moreover, mycoherbicides are intended to be used and regulated in a manner similar to the chemical herbicides (Charudattan 1982, Charudattan TeBeest and Templeton 1982); therefore, the mycoherbicide technology is much like that of the chemical herbicides. Although it is generally assumed that mycoherbicides will be less costly to develop than conventional chemical herbicides, there will nonetheless be a cost associated with mycoherbicides which must be borne by the users. Because of this, mycoherbicides can be used only in situations such as crops where the expense of weed control is justified. Under this strongly economic demand, it is imperative that a candidate targeted for development be highly efficacious and cost-effective. The purpose of this paper is to define efficacy and discuss how to assess it.

### Definition of Mycoherbicide Efficacy

Keeping in mind perspectives of the manufacturer and the user of mycoherbicides, who are the final judges of the mycoherbicide's marketability and performance, efficacy should be defined as the ability to provide a satisfactory amount, speed, and ease of weed control (Fig. 1). In practice, all of these three aspects must favor the mycoherbicide's use. Thus, the level or amount of weed control should be satisfactorily high; the control should be rapid depending on the weed situation; and it should be easy to use and obtain satisfactory performance of the mycoherbicide.

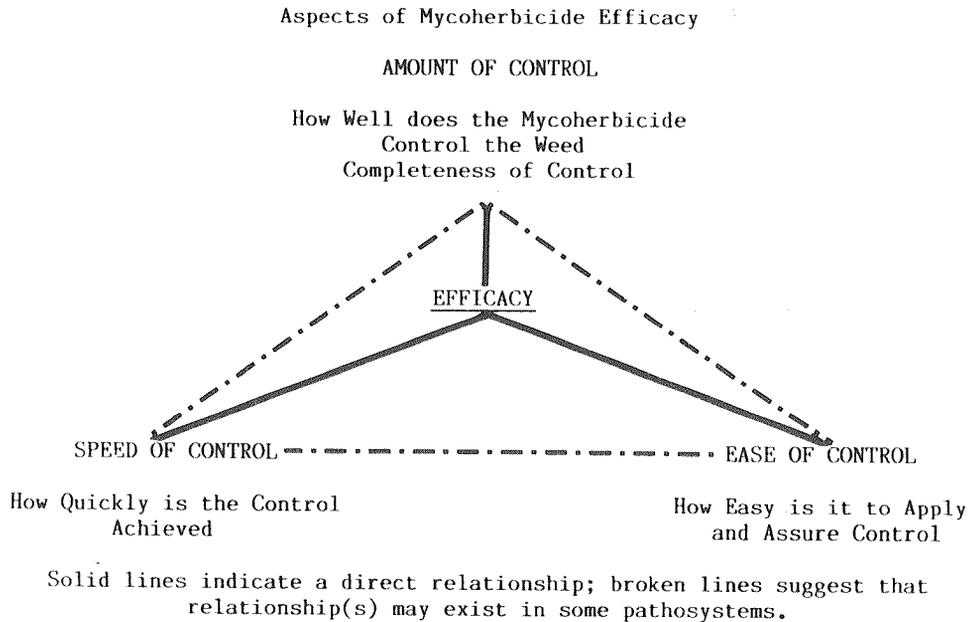


Figure 1. Aspects of mycoherbicide efficacy.

#### Amount

The level of weed control considered satisfactory will vary with each weed problem. Generally in crops, which, for the economic reasons stated above, are perhaps the only situations where mycoherbicides are likely to be used, complete or nearly complete control is likely to be demanded since the users are accustomed to such control from chemical herbicides. However, with proper educational efforts and considering the fact that even chemical herbicides do not always provide complete control, the public may be convinced to accept less than complete or total weed kill. Nonetheless, the level of control will be expected to be fairly high to justify the mycoherbicide's use.

#### Speed

During a cropping season, there are limited number of windows (periods of suitable time) when weed control must be achieved. This is true for annuals that have short life-spans as well as for perennials like tree and shrub crops that are under strict annual management schedules. Unlike other slow-acting biocontrol agents such as insects and rust fungi, the *raison d'etre* of a mycoherbicide in this situation is to provide fairly quick control. Therefore, speed of control, circumscribed by crop phenology, is an important part of efficacy. A mycoherbicide that does not yield fairly rapid control cannot be fitted into the growers'

cultural and pest management schedules and therefore may not find acceptance even if its other aspects are satisfactory. With mycoherbicides, speed should be measured in terms of 2- to 6-wks following application; at the least, during this period disease development and early signs of successful control should be evident although complete or total control may ensue several weeks later. It is biologically unrealistic to expect results sooner than a week. It may also be impractical to have to wait more than six weeks for disease development and weed control because during this period competition from the weed would have generally exceeded a critical level.

### Ease

The ease with which a mycoherbicide can be used is also an important part of efficacy. This is not only in regards to application tools and technique but also in the ability to use the agent over a fairly broad range of environmental conditions and with ongoing pesticide schedules. For example, a mycoherbicide agent that has very stringent environmental requirements for infection and disease development is likely to fail more often than one that is less stringent. It will be difficult to find acceptance of a mycoherbicide that needs strictly controlled applications which are difficult to manage in practice. Likewise, a mycoherbicide that needs special tools and radical shifts from the existing agronomic and pest control practices is less likely to be accepted than one that can be integrated with existing equipment and practices. Any additional cost, due to unusual tools or added steps in management practices, may discourage mycoherbicide use.

### Assessment of Mycoherbicide Efficacy

All three aspects of efficacy (amount, speed, and ease) discussed above should be criteria in the selection and evaluation of a candidate; in fact they can facilitate the choice. This is illustrated with our work (Charudattan and DeValerio, unpubl. data) on showy crotalaria, *Crotalaria spectabilis* Roth (Leguminosae), a hard-seeded, poisonous weed in soybeans in certain regions of the southern United States. We found two highly virulent fungi on this weed. Following greenhouse evaluations, we were able to characterize the relative efficacy of each on the basis of the amount and speed of weed kill as well as the amount of inoculum and the type of inoculum application needed to achieve a satisfactory level of control (Table 1). One of them, *Colletotrichum dematium* (Pers. ex Fr.) Grove f. sp. *crotalariae* (proposed common or trivial name: codecro) yielded 90 to 100% kill within 6 to 14 d when applied to 6- to 80-day-old plants under strictly controlled conditions in the laboratory. However, in the field, the ability to kill decreased as the seedlings aged: the optimum age of seedlings for 100% kill was approximately one week after emergence (i.e., cotyledonary stage of growth). The pathogen also required optimally about 6 h of dew after application of inoculum. The other pathogen, *Fusarium udum* Butler f.sp. *crotalariae* (Kulkarni) Subramanian (trivial name: fuducro), provided 60 to 80% control, but the speed of control, measured by the lag time between the first kill and maximum kill over 50 d, was 17 d as compared to 4 d for codecro. However, being a soil-borne pathogen, fuducro could be sprayed on the soil surface without regard for a dew period, and it was capable of killing older plants that are resistant to codecro. Fuducro could also be applied as foliar spray, but the inoculum level needed to obtain a satisfactory level of control (arbitrarily  $\geq 70\%$ ) was approximately 90 times that of codecro ( $90 \times 10^6$  vs.  $1 \times 10^6$ , respectively). Although applications of fuducro as a soil drench and as inoculum pellets are feasible methods, foliar applications are more practical and preferable. Thus, based on the relative amounts of foliar inoculum needed to obtain satisfactory levels of control, codecro is superior to fuducro.

In the above situation it would seem that the right choice between the candidates on the basis of ability to provide total and rapid control is codecro. However, codecro's dependence on a specific dew period for infection and decreasing field efficacy against older plants are factors limiting its efficacy. Fuducro on the contrary would appear to be more suitable by its ability to kill older plants and independence from the dew requirement. The slowness and incompleteness in killing the weed are its discouraging features.

Showy crotalaria can germinate over several weeks during the growing season, with each cultivation and each rain event initiating a seedling flush. In this situation, codecro can be used against young, emerging seedlings. Because fuducro can kill older seedlings, it can be used against plants that escape the first pathogen. In view of this potential for complementation between the two pathogens, both may be deployed as a mycoherbicide through a tank-mix combination.

**Table 1. Comparison of mycoherbicidal efficacies of two pathogens of showy crotalaria (*Crotalaria spectabilis* Roth), based on the amount and speed of control and the method of application.**

Pathogen <sup>1</sup>	Weed kill (%)	Number of days inoc. to max. kill		Inoculum level <sup>2</sup> and method
			first kill to max. kill	
<i>Colletotrichum dematium</i> (Pers. ex Fr.) Grove f.sp. <i>crotalariae</i> (codecro)	99 ± 3	11 ± 3	4 ± 1	1 x 10 <sup>6</sup> /ml foliar spray
<i>Fusarium udum</i> Butler f.sp. <i>crotalariae</i> (fuducro)	79 ± 30	40 ± 2	15 ± 1	90 x 10 <sup>6</sup> /ml foliar spray
<i>Fusarium udum</i> f.sp. <i>crotalariae</i>	66 ± 31	39 ± 1	17 ± 2	1 x 10 <sup>6</sup> /g soil drench

<sup>1</sup> Speed of control was determined from the time it took to proceed from inoculation to the maximum level of kill and from the first kill to maximum kill. Inoculum quantity and method of application needed to obtain nearly equal levels of control by fuducro were used to determine ease of control. Amount of control was the percent weed kill.

<sup>2</sup> Inoculum consisted of aqueous suspension of conidia (soil drench) or conidial suspension and 0.05% surfactant (foliar spray).

It is noteworthy that the two registered mycoherbicides, Collego® and DeVine®, provide high levels of control, act speedily, and are easy to use (Charudattan 1985, TeBeest and Templeton 1985). Collego, *Colletotrichum gloeosporioides* (Penzig.) Penzig & Saccardo f.sp. *aeschynomene*, and DeVine, *Phytophthora palmivora* (Butler) Butler, provide typically ≥85% control of their respective weed targets, northern jointvetch (*Aeschynomene virginica* [L.] B.S.P.; Leguminosae) and milkweed vine (also called strangler vine; *Morrenia odorata* [Hook. & Arn.] Lindley; Asclepiadaceae). Usually control is obtained within 4 to 6 wk, and both can be easily applied with conventional equipment as post-emergent aerial, over-the-top, or directed sprays. Although both are sensitive to certain fungicides and other pesticides, it is possible to integrate their use with ongoing pest management schedules (Smith 1986). It is therefore noteworthy that the two archetypal mycoherbicides satisfy the three aspects of efficacy, namely amount, speed, and ease, and their success may have been largely due to this fact.

### Methods

Efficacy can be assessed on the basis of weed control, the level of disease stress, or increase in crop yield resulting from reduced weed competition. In the former two cases the assessment can be based on lethal or sublethal effects on the weed. Although pathologists may tend to emphasize the disease aspects, no matter how severe or widespread the disease may become, the only characteristic that the user is likely to appreciate is the amount of weed control. Also, because mycoherbicides are comparable to chemical herbicides in their application and weed control methodologies, in my view, the public expectation of mycoherbicide efficacy is already conditioned by the experience with chemical herbicides. Chemical are known for their cost-efficiency, effectiveness, ease of application, speed of control, and predictability of results. A demand for a comparable type of efficacy for mycoherbicides would mean that only a limited number of pathogens can succeed as mycoherbicides in the marketplace because only a few pathogens may be capable of providing weed kill.

Of course, not all mycoherbicide candidates can yield weed kill; in fact from the efficacy perspective it is convenient to divide pathogens into those that can kill plants (e.g., those capable of causing anthracnoses, blights, rots, and wilts) and those that produce sublethal damage (leaf-spots, rusts, and smuts). The latter might cause subtle effects on the physiology of the infected weed, resulting in reductions in its growth, reproduction, and competitiveness. Such effects may have practical significance to weed control, and as the public becomes more educated about mycoherbicides, weed control objectives may change from complete weed kill to weed suppression. Therefore, while evaluating mycoherbicide candidates, sublethal as well as lethal pathogenic effects should be considered.

#### *Weed Control Assessments*

A number of weed control parameters can be chosen to assess mycoherbicide efficacy: a partial list of examples is provided in Table 2. Among these, assessments based on weed kill, which can be quantified as percent kill or reduction in weed counts (a 'before and after' view of the scene), are the most commonly used. Although assessing the efficacy of a pathogen capable of killing a weed is fairly straight forward, there are cases where complete kill is not achievable. In these pathosystems, disease may merely reduce growth and/or reproduction of the weed, resulting in reductions in biomass, competitiveness, flowering, seed set, etc. For example, in weeds with indeterminate growth habit, such as many aquatic weeds, perennial herbs, shrubs, vines, and certain trees, complete kill is often not possible and regrowth from surviving vegetative parts is common.

Waterhyacinth (*Eichhornia crassipes* [Martius] Solms-Laubach; Pontederiaceae) is a good example to illustrate the above point. The fungus *Cercospora rodmanii* Conway (cerodman) causes severe leaf-spotting, foliar necrosis, and reduction in growth rate and biomass of waterhyacinth (Charudattan *et al.* 1985). Weed kill from cerodman occurs only under very severe and sustained disease pressure over several months (Freeman and Charudattan 1984). Typically, waterhyacinth is able to compensate for disease-caused losses to its photosynthetic tissues by a rapid senescence and death of diseased leaves accompanied by the production of new healthy leaves, provided growth is not slowed by nutrient limitation (Charudattan *et al.* 1985) or biotic stress beyond a critical threshold. Because the disease does not affect the meristematic tissues, the plant is able to produce numerous healthy leaves and daughter plants. The increase in healthy tissue accompanying the accelerated loss of diseased tissue helps to lower the disease pressure by reducing the inoculum level. Thus, in this example efficacy can be measured only in terms of plant growth and reproduction, typically expressed as biomass reductions and reduced surface coverage by the weed, but not as weed kill (Charudattan 1986).

Several other researchers, working with different weed-pathogen systems, have used parameters other than kill or reduction in weed stand to quantify efficacy (Table 2). In addition, recent literature on the synecology of natural plant populations has several excellent examples of the effects of disease on plant survival and reproduction (Alexander and Burdon 1984), plant density (Alexander and Kelly 1984), genetic composition of populations (Alexander *et al.* 1984), and breeding systems of plants (Clay 1984). Studies of this type which measure the subtle, nonlethal effects of diseases on natural plant populations are likely to be of considerable importance in the future in suggesting innovative means of deploying pathogens against weeds.

#### *Disease Assessments*

The severity (amount per unit area of the plant), incidence (frequency of occurrence), and chronicity (severity and persistency) of disease can be used to quantify the destructiveness of disease and thereby derive an indirect measure of the efficacy of a pathogen (Charudattan *et al.* 1985). Although it is unwise to prejudge the efficacy of a pathogen, its destructiveness (ability to attack and destroy plant) can be presumed from its taxonomic identity and the type of disease it causes. Diseases such as blights, rots, wilts, and anthracnoses, which can be termed necrotrophic diseases, are capable, as opposed to the biotrophic smuts and rusts or several relatively innocuous leaf-spots, of causing plant death.

Table 2. A partial list of parameters used by researchers for the assessment of weed control by plant pathogenic fungi.<sup>1</sup>

Weed control parameter	A pathosystem in which the parameter was used	A reference <sup>2</sup>
Biomass	Waterhyacinth ( <i>Eichhornia crassipes</i> [Mart.] Solms.) - <i>Cercospora rodmanii</i> Conway	Conway <i>et al.</i> 1979
Competitive ability	Johnsongrass ( <i>Sorghum halepense</i> [L.] Pers.) - <i>Sphacelotheca holci</i> Jack.	Massion and Lindow 1986
Growth rate	Waterhyacinth - <i>C. rodmanii</i>	Charudattan <i>et al.</i> 1985
Specific leaf area	Groundsel ( <i>Senecio vulgaris</i> L.) - <i>Puccinia lagenophorae</i> Cooke	Paul and Ayres 1985
Plant height	Sicklepod ( <i>Cassia obtusifolia</i> L.) - <i>Pseudocercospora nigricans</i> (Cooke) Deighton	Hofmeister and Charudattan 1987
Post-winter growth, reproduction, and survival	Groundsel ( <i>Senecio vulgaris</i> ) - <i>Puccinia lagenophorae</i>	Paul and Ayres 1986
Water relations (water-use efficiency)	Groundsel - <i>Puccinia lagenophorae</i>	Paul and Ayres 1984
Weed kill	Sicklepod - <i>Alternaria cassiae</i> Jurair and Kahn	Walker 1982

<sup>1</sup> The list is not meant to be exhaustive; it is to complement the discussion in the text.

<sup>2</sup> An example where the particular parameter has been used to assess weed control.

While it is simple to quantify disease on the basis of weed kill, it is more difficult to assess diseases that result in sublethal effects. In these cases, several phytopathometric methods can be used. For example, in our work on cerodman, a fungus that commonly causes sublethal damage on its host, waterhyacinth, we have measured biological control efficacy in terms of the incidence, severity, and rate of progress of the disease, the latter measured as the area under the disease progress curve (AUDPC) (Charudattan *et al.*, 1985). AUDPC can provide a measure of the chronic stress imposed by the pathogen and may also be used to predict the levels of disease needed to achieve the desired weed control effects.

Pictorial keys, such as those provide by James (1971) and Freeman and Charudattan (1984) are commonly used to rate foliar diseases. The latter was developed specifically for the waterhyacinth-cerodman pathosystem. On the contrary, Mortensen (1985) has proposed a standardized rating of damage based on the type of plant reaction to plant pathogens and other biological control agents. McRae *et al.* (1988) have devised a disease assessment technique for analyzing the anthracnose disease of Bathurst burr (*Xanthium spinosum* L., Compositae) caused by *Colletotrichum orbiculare* (Berk et Mont) v. Arx by accounting for the special pathological features of this host-parasite system. Based on a numerical disease rating scale of 1 (no disease) to 6 (dead), which was weighted to reflect the relative importance of leaf, stem, and basal lesions to plant death, the authors developed mean daily ratings until 50% of the plants in the most effective treatment were dead. This rating, termed  $MR_{50}$ , was used to distinguish between three disease progress patterns and the effects of three dew periods and for comparing actual time to plant death with time to death calculated from  $MR_{50}$ . Alternatively, Lindow and Webb (1983) have used a computer-assisted video-image analyzer for a direct, quick, and reliable measurement of disease on individual weeds and populations. For measurements over large areas, aerial remote sensing may be used to assess diseased as well as total populations of weeds in relation to the crop (Jackson 1986).

### Importance of Weed and Crop Phenology

While evaluating candidates, it is important to relate to the objectives of the primary (greenhouse) and secondary (field) evaluations as well as the phenologies of the weed and the crop. Greenhouse evaluation, from the standpoint of efficacy, should be intended to provide proof of pathogenicity and a preliminary assessment of efficacy. However, greenhouse studies have limitations: greenhouse-grown weeds tend to be more susceptible and therefore may overestimate pathogenicity. Also, unless a carefully controlled dew period is assured during incubation of inoculated plants, the microenvironment in the greenhouse is usually not suitable for disease development. Hence, following a critical assessment of the performance of a mycoherbicide candidate in the greenhouse, field evaluations under epidemiologically optimal conditions should be done. Only field performance under appropriate conditions should be taken as the final determinant of efficacy.

Weed and crop phenologies are also important factors. There are example of pathogens that are nonlethal to mature weeds, which when used against seedlings cause death. *Alternaria cassiae* Jurair and Kahn (aecass), a mycoherbicide candidate for sicklepod, *Cassia obtusifolia* L. (Charudattan *et al.* 1986, Walker 1982) is an example. Mature plants incur extensive blighting from aecass, but are rarely killed. But when used as a post-emergent spray against young seedlings (cotyledonary to 2- to 4-leaf stage) aecass provides nearly 100% weed kill. In this weed control system, the biology of the weed allows the use of this mycoherbicide against young seedlings, especially following cultivation. The weed typically germinates in flushes and the resultant population tends to be of fairly uniform age, resulting in a continuous susceptible target for the attacking pathogen. If populations of this weed emerged in a unsynchronized continuum during the entire growing season, it might not be possible to obtain a high level of efficacy with this mycoherbicide.

Among the influences crop phenology may have on mycoherbicide efficacy, its effect on the windows available for weed control is perhaps the most important. Generally, the windows for infection and weed control must coincide with proper growth stages of the crop. For example, if crop growth and cultural practices are not suitable for aerial or tractor-based spraying of mycoherbicides, other application methods or alternative weed control methods may be necessary.

### An Assessment Protocol and A Ranking Scheme

Although minor modifications and refinements may be needed for each pathosystem, a commonly acceptable scheme is needed to assure proper selection, evaluation and reporting of efficacy. In the absence of such, there is a danger of under- and overestimating the potential of candidates, resulting in costly losses in time and effort and a lack of progress in this field. I have proposed a protocol for efficacy assessment (Table 3) and a scheme for ranking candidates on the basis of efficacy (Table 4) with the view to stimulate the use of standardized evaluation and ranking. I hope the proposal will invite scientific discussion on this important subject of mycoherbicide efficacy and lead to further refinements in the scheme (Table 4). I also suggest that the ranking scheme be used for comparison and selection of different pathogens of a particular host, for comparison of strains of pathogens, and to relate the efficacy (or safety) of a candidate against different hosts. The scheme can also be applied to assess the effectiveness of candidates under discriminating environmental conditions.

In this scheme, I have included only those intrinsic characteristics of candidates that, in my view, affect the mycoherbicial performance. There are several other aspects, such as the economics of particular weed control situations, problems associated with production, formulation, and delivery of the mycoherbicide, and market- and regulatory concerns that also impact the decision to develop or abandon a candidate. However, these are not part of "efficacy" *per se* and therefore are not included here. For example, although the ability to produce commercial quantities of inoculum under technologically and economically feasible conditions is essential to the successful development of a mycoherbicide, this aspect does not influence the candidate's intrinsic potential for performance. On the other hand, the ease of application of inoculum, lack of epidemiological constraints to disease development, and

ability to integrate the mycoherbicide with other pest control and crop management practices are largely or totally dependent on the biology of the candidate; hence they are considered here as part of efficacy.

**Table 3. A protocol for assessing the efficacy of mycoherbicide candidates.**

Step 1<sup>1</sup> *Choice*: Determine the pathogen's destructiveness from literature and pathogenicity trials; in general, those causing anthracnoses, blights, rots, and wilts are more likely to kill the weed than leaf-spotters, obligate parasites (e.g., rust fungi), and facultative saprophytes (smut fungi). If a choice can be made, select those capable of killing the weed; if not, select the most destructive. Caveat: A pathogen's real potential may not be obvious under laboratory conditions due to inadequate expression of pathogenicity. A rating system for relative efficacy (see Table 4) is useful at this stage.

Step 2 *Determination of destructiveness*: Quantify under controlled conditions (growth chamber or greenhouse) disease damage and weed control efficacy on the basis of the amount and speed of control. In general, spores are preferred for inoculum since they are the infective structures in most fungi. A minimum of  $10^5$  (ideally  $10^6$ ) spores should be used to guarantee that inoculum was not a limiting factor. Pictorial keys (Freeman and Charudattan 1984, James 1971) are useful for quantifying disease levels; weed control assessments should complement disease assessments. Reduction in numbers (due to weed kill), biomass, growth, competitive ability, and reproduction of weeds are preferred and easily-studied criteria for this purpose; others are listed in Table 2 and the text.

Step 3 *Determination of moisture, temperature, and inoculum requirements for infection*: Under controlled conditions, determine the amount and duration of dew, temperature optimum, and type and amount of inoculum (spores or mycelial preparation) needed to incite an epidemic in the field. This should give clues to the third aspect of efficacy, namely how easy it is to use the pathogen as a mycoherbicide.

Step 4 *Field evaluations*: Applying principles from steps 1 through 3, evaluate the pathogen's efficacy under field conditions that are relevant to weed and crop biology. In addition to the criteria for weed control assessment listed under step 2, it is essential to derive a consensus with weed scientists and agronomists on what constitutes a satisfactory level of control for a given weed problem. At this point, disease assessment may be based on video-image analysis (Lindow and Webb 1983) to facilitate direct assessment.

Step 5 *Further development*: If the level of efficacy based on the amount, speed, and ease of control appears promising, industrial collaboration may be warranted for large-scale studies. If not, attempts may be made to determine if efficacy could be improved by removing any obvious constraints to the destructiveness of the pathogen and the development of the epidemic.

<sup>1</sup> It is assumed that Koch's postulates have been applied and proof of pathogenicity has been established at this stage.

The scheme (Table 4) when applied to the mycoherbicide agents discussed in the text provided the following efficacy scores: Collego, 46; DeVine, 46; aecass, 42; codecro, 42; fuducro, 38; pinigri (*Pseudocercospora nigricans* [Cooke] Deighton), 31; and cerodman, 30. Based on these calculations and published accounts, I suggest that a score of 40 to 50 would represent a highly desirable level efficacy in a candidate. A range of 30 to 39 may be useful if better candidates are not available.

Table 4. A scheme for rating efficacy of mycoherbicide candidates. In this scheme, amount, speed, and ease are weighted equally since all three aspects are considered equally important to the assessment of efficacy. A candidate must be rated (extreme right column) by proceeding to a lowest level of the applicable choice of category or subcategory denoted by the same capital and lower case letters.

Aspect of Efficacy	Assessment categories and subcategories	Importance Factor	Rating Scale
Amount	A.a. Able to kill the weed	2 or 1	
	A.a.1. Able to kill only seedlings <sup>2</sup>	2	
	A.a.1.a. Capable of >85% kill		5
	A.a.1.b. Capable of 50 to 85% kill		4
	A.a.1.c. Capable of < 50% kill		3
	A.a.2. Able to kill older plants <sup>2</sup>		
	A.a.2.a. Capable of >85% kill		5
	A.a.2.b. Capable of 50 to 85% kill		4
	A.a.2.c. Capable of <50% kill		3
	A.b. Unable to kill the weed	1	
A.b.1. Relative damage to the weed is mild to severe but the candidate causes measurable reductions in weed growth, reproduction, and/or competitive ability		3 to 5	
A.b.2. Effects are subliminal but results in stress to the weed or increase in crop yield		1 or 2	
Speed	B.a. Able to kill quickly in 2-6 weeks post-inoculation	2 or 1	
	B.b. Unable to kill quickly; takes > 6 weeks to kill	2	3 to 5
	B.b.1. Effects of disease fall in the subcategories under A.a.1. or A.a.2.	1	3 to 5
	B.b.2. Effects of disease fall under A.b.1.		2
	B.b.3. Effects of disease fall under A.b.2.		1
Ease	C.a. Candidate can be easily applied as a mycoherbicide	2 or 1	
	C.a.1. Can be applied as postemergent sprays	2	3 to 5
	C.a.2. Specific formulation and application tools are needed		1 or 2
	C.b. Difficult to apply as a mycoherbicide	1	0 or 1
	D.a. Candidate can be used over a range of environmental, host, and crop conditions	2	5
	D.b. Candidate can be used only under specific but obtainable conditions	2	3 or 4
	D.c. Candidate needs highly specific conditions that are difficult to obtain	1	1 or 2
	E.a. Mycoherbicide can be easily integrated with ongoing pest- and crop-management practices	2	3 to 5
E.b. Integration difficult in practice	1	0 to 2	

<sup>1</sup> Importance Factor and Rating Scale should be multiplied to derive the total score; perfect score is 50. The higher the total score, the higher will be the ranking of efficacy. The more number of categories and subcategories a candidate can be assessed on, the more valid will be its assessment. However, if information on any category is unavailable or inconclusive, the candidate may be rated on the remaining categories but the total number of categories on which assessment is made should be specified.

<sup>2</sup> Depending on the weed and the crop, these subcategories may be important or unimportant; hence the same rating scales. Rating of categories A and B should be based on greenhouse and/or field performance of the candidate; categories C to E must be based on field performance.

## Conclusions

Several scientific, technical, practical, and economic factors must be considered in the development and commercialization of a mycoherbicide candidate. Among these, efficacy is one of the most important, and assessment of efficacy, according to standardized testing and rating schemes, is therefore of paramount significance. Several methods of assessment are possible, but one based on weed kill is preferred since it is easily quantifiable, readily recognizable, and convincing to the users. Although very few diseases might be capable of killing a targeted weed, the prevailing weed control needs and the realities of the marketplace

dictate that only those pathogens capable of killing, not merely stressing, weeds be considered for development at this stage in the history of mycoherbicide research.

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