The application of mycoherbicides

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Summary

The successful application of a biological control agent in the field is the final step in the development of a mycoherbicide. This aspect, however, is worthy of consideration early in a research program. A knowledge of the epidemiology of a pathogen may suggest an alternative propagule of the pathogen to be applied rather than the standard conidial suspension. This in turn may affect the selection of the manner of application. Primary innocula (e.g., conidia) may not be the most appropriate propagules for the inundation of a problem field with a mycoherbicide. It may be preferable to apply a survival structure (e.g., chlamydospores or hyphae in 'debris') from which secondary spread of the fungus can occur. In this way we can attempt to work with mother nature and ensure that the pathogen is in place when environmental conditions congenial to an epidemic arise.

The use of a conventional boom spray is convenient and undoubtedly appeals to the end users. Despite this, the development of specialized equipment, or combinations of existing equipment, should not be ignored at the expense of the successful application of a mycoherbicide. Novel use of existing equipment should also be considered. Possibilities which may improve application of fungi and subsequent disease establishment include variation in droplet size, wounding of the target and timing of application.

Introduction

The concept of using biological control agents as mycoherbicides has received recognition around the world. In line with the concept, there has been a tendency to concentrate on applying pathogens through standard boom spray equipment. Alternative application techniques are however worthy of consideration. This should not be a limitation because traditionally, where the success of a farming operation depended on specialized equipment, farmers have purchased appropriate machinery.

The major limitation to successful application of a mycoherbicide has been the vagaries of the environment. Mycoherbicides will often be applied during periods of less than optimum environmental conditions. Therefore, there is a need to expand the time period during which an adequate level of disease can be established.

Forms of inocula

An understanding of the epidemiology of a potential mycoherbicide agent may offer a means whereby we can avoid some environmental limitations. For example, the application of a survival propagule which may remain viable for at least several days (if not for several weeks) should allow greater flexibility in timing an application of a pathogen. Butler (1951), who first identified anthracnose on Bathurst burr (*Xanthium spinosum*), recognized that artificial inoculation with spores should be under cloudy, showery conditions. He later forwarded grain cultures of the pathogen to growers which resulted in disease establishment on a number of farms. The fungus naturally survives in infested residue thus the distribution of colonized grain simulates nature and may provide a mechanism whereby the time frame of application is less restricted. Brosten and Sands (1986) broadcast a combination of infested wheat kernels and sclerotia of *Sclerotinia sclerotiorum* onto problem areas of Californian thistle (*Cirsium arvense*), many plants in inoculated plots died prematurely. Similarly Riddle *et al.* (1991) applied *Sclerotinia sclerotiorum* infested ryegrass seed to a turf grass sward and achieved 81% control of dandelion (*Taraxacum officinale*). They applied it several times within a year, but for intensive agriculture the economics of more than one application are justified.

Morris (1989) applied a dried formulation of wheat bran infested with *Colletotrichum gloesporiodes* to areas of *Hakea sericea*. Profuse sporulation and subsequent rain splash of conidia resulted in seedling infection. The bran inoculum retained its viability when exposed to direct sunlight for up to 16 days thus allowing a broad period during which appropriate environmental conditions could occur.

Commercial application of *Phytophthora palmivora* as chlamydospores to control milkweed vine (*Morrenia odorata*) in citrus orchards illustrates the role fungal survival structures may play in the development of a mycoherbicide (Templeton and Heiny 1989). Residual control for up to five years after application is a bonus for growers. Weidemann and Templeton (1988) applied conidia of *Fusarium solani cucurbitae* and colonized alginate granules to soil which resulted in mortality of the majority of plants of Texas gourd (*Cucurbita texana*). Their study demonstrated the advantage of utilizing a soil-borne pathogen as a mycoherbicide. There are associated risks with soil-borne pathogens because of their longevity, especially for species which have a wide host range, but they offer greater versatility in application techniques and timing.

Timely application may also be critical in successful disease establishment. This includes not only environmental considerations but also aspects of plant age and growing conditions. Generally plants tend to be more susceptible at younger growth stages but appropriate growing conditions vary between each host/pathogen combination. Actively growing plants of some species are susceptible whereas in other species stressed or injured plants become infected more readily.

Application of *S. sclerotiorum* to Californian thistle at the vegetative growth stage in early summer resulted in a higher level of dead shoots than when applied to rosettes in late winter or to budding plants in mid summer (Brosten and Sands 1986). Similarly, a delay of two or four weeks in the application of spores of *Colletotrichum orbiculare* to large plants of Bathurst burr in mid to late summer tended to decrease the incidence of dead plants (Auld *et al.* 1990a). It is difficult to separate the effects of plant age and environment on disease development in these studies but they highlight the significance of timely application. For *C. orbiculare* low temperatures following application delays initial disease development but has little effect on ultimate disease levels (Auld *et al.* 1990a). However, moisture stress immediately prior to application reduces the ability of *C. orbiculare* to infect young plants of Bathurst burr (Auld *et al.* 1990b). Hence, at least for anthracnose on Bathurst burr, consideration of timing of applications extends beyond potential leaf wetness periods and growth stage to include any history of stress. Given that initial low temperatures only delay ultimate anthracnose development (Auld *et al.* 1990a) early application to Bathurst burr seedlings of *C. orbiculare* before moisture stress is likely to occur may be desirable.

Riddle *et al.* (1991) applied *Sclerotinia* spp. to dandelion plants eight and twelve weeks old so that post application environmental conditions were the same for both age groups. They did not detect any effect of plant age on disease establishment. In contrast only young plants of velvet leaf (*Abutilon theophrasti*) were killed by application of spores of *Colletotrichum coccodes* in glasshouse trials (Wymore *et al.* 1988). Thus optimum growth stage for timely application will vary between species.

Volume of spray applied

The standard boom sprayer for conventional herbicides uses hydraulic nozzles which have been criticized because of their inaccuracy and their large range of droplet sizes which "means that only part of the spray is actually physically deposited where it is required" (Bals 1987). The same holds for application of mycoherbicides where droplet size and carrier volume have a significant effect on distribution of spores and the time water is retained around the spore. Application in volumes approaching 1000 L ha^{-1} allows coverage to run off (Quimby and Boyette 1987) and may allow the accumulation of spores at leaf axils or the base of plants. In contrast use of invert emulsions can decrease water volume but reduce water evaporation, thereby increasing the number of infective propagules of mycoherbicides (Daigle *et al.* 1990). The successful use of ultra low volume (ULV) or controlled droplet application (CDA) with pesticides and conventional herbicides (Bals 1987) suggest that further investigation in relation to mycoherbicides is warranted. Control of northern jointvetch (*Aeschynomene virginica*) with *Colletotrichum gloeosporioides aeschynomene* was equally as effective whether applied as spores in a high (187 L ha^{-1}) volume boom spray or with a spinning disk at ULV (21.5 L ha^{-1}) in each of three years when applied at high spore concentrations (Khodayari *et al.* 1987). A reduction in spore concentration by 50% resulted in poorer control with ULV.

The advantages of reduced carrier volume are two fold. Invert oil emulsions containing spores of *Alternaria cassia* or *Alternaria crassa* have proved effective in enhancing disease development on *Cassia obtusifolia* and *Datura stramonium* (Amsellem *et al.* 1990) and thus use of CDA and ULV may provide a viable means of utilizing carriers where oil is the sole or majority component without risk of phytotoxicity. In addition small hand held CDA applicators are available in underdeveloped countries whereas conventional sprayers may not be practical (Bals 1987).

Wounds to enhance infection

Many facultative pathogens gain entry into plants via wounds. In terms of mycoherbicides, the novel use of lead pellets coated with spores of *C. gloeosporioides* fired from a shotgun into groves of *H. sericea* (Morris 1991) emphasizes the role that imagination may play in creating appropriate injuries. Popay and Cheah (1990) indicated that farmers believed that mowing Californian thistle before rain resulted in plant death. They suggested that death may be due to invasion of mown plants by pathogens. McElwee *et al.* (1990) observed that mechanical wounding increased infection of bracken (*Pteridium aquilinum*) by *Ascochyta pteridis* and *Phoma aquilina*. Livestock trample and damage bracken (West and Dean 1990) thus it may be appropriate to apply *A. pteridis* and *P. aquilina* after grazing. Mycoherbicides could readily be introduced to weeds in Lucerne (*Medicago sativa*) fields following hay making where mown weed stems may be susceptible to invasion by appropriate pathogens. Similarly, the use of harrows ahead of a boom sprayer in broadacre agriculture may readily aid in the infection process.

Conclusion

We should not ignore conventional herbicide sprayers and application practices in applying mycoherbicides but on the other hand we should not confine research efforts to commercially available equipment.

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