Proc. VII. Int. Symp. Biol. Contr. Weeds, 6-11 March 1988, Rome, Italy.
Delfosse, E.S. (ed.). Ist. Sper. Patol. Veg. (MAF), pp. 559 - 63 (1989).

Factors in the Infection Process of Fungal Pathogens for Biological Control of Weeds

C. Gerald Van Dyke

Department of Botany, Box 7612, North Carolina State University, Raleigh, NC 27695 USA

Abstract

Mycoherbicides have gained much attention in recent years. These indigenous fungi cause diseases on weeds after inundative application; evaluation procedures combine techniques used by plant pathologists and weed scientists. Plant pathologists have studied the processes by which fungal propagules (usually spores) interact with plants and eventually cause disease. There is a paucity of research with these principles as they relate to mycoherbicides. Laboratory and field testing, combined with light and scanning electron microscopy, suggest that many factors need to be considered and optimized in mycoherbicide evaluations. Variability in spore size and internal nutrients, spore hydration rate, spore adhesion to plant parts, the number of germ tubes/spore, germ tube lengths, infection structures produced by germ tubes, penetration of plant parts, toxin production, inoculum adjuvants such as surfactants, interaction with other phylloplane microbes, etc., can effect the success of a particular fungal isolate. This is a critical time in the development of mycoherbicides for addition to the arsenal of weed control methods. Potential candidate fungi cannot be fully evaluated before the basic factors involved in the fungal-weed, disease process have been studied and the resultant knowledge used in optimization of mycoherbicide use strategy.

Introduction

The mechanisms and processes by which fungi interact with plant surfaces and subsequently gain entrance into the plant have been of much interest. Several authors have reported on the survival, germination, and adhesion of fungi to leaf surfaces (Lapp and Skoropad 1987, Hyde *et al.* 1986, Murray and Maxwell 1974, Parberry and Emmitt 1977, Park 1982, Potter *et al.* 1980, Wheeler and Gantz 1979). It has been especially difficult to distinguish what part the microenvironment, the genetics of each organism, chemical and physical interactive processes and other factors play in this drama. Most of the investigations in this area are concerned with understanding why a particular fungus does or does not gain entrance to the host for the purpose of understanding fungal virulence or host resistance. Phytopathologists usually focus on preventing fungal entrance or understanding why the fungus does not enter. In recent years there has been increased interest in the use of fungal pathogens for biological control of weeds. Fungal propagules, particularly conidia of imperfect fungi, are applied to weeds, usually leaves and stems, with the expectation that the fungus will cause disease. The obvious goal is to provide every opportunity for the fungus to incite sufficient disease and weed damage to reduce or eliminate weeds or their competitive advantage over some economic plant.

Fungal-host interaction studies usually provide a better understanding of the disease process and subsequent methods for controlling disease. In biological control procedures it is important to understand the fungal-host interactions, but in this case for purpose of enhancing the disease process.

In recent years, improved light microscope techniques and the addition of electron microscopy for better resolving of structural detail has provided tools to answer host-parasite questions (Van Dyke *et al.* 1982). It is particularly important that we understand these disease processes, as we attempt to formulate and maximize the efficacy of mycoherbicides.

Spore Characteristics

Fungal spores are of many shapes and sizes. Some are single celled, whereas others are multicelled. Apparently spores differ in their ability to adhere to plant surfaces, depending on physical and chemical characteristics and the concomitant interaction with the plant surface and its physical and chemical characteristics (Hyde *et al.* 1986). Some spores have endogenous nutrients sources and need only free water or high humidity to germinate, others require an external nutrient source. Moisture and temperature are often critical factors in the germination process. Bacteria are rather tenacious in their adherence to plant surfaces (Huang and Van Dyke 1978, Spurr and Van Dyke 1980), whereas fungal spore tenacity begins primarily with germ tube attachment (Van Dyke, unpublished data). Since each fungal species has its unique spore characteristics, those characteristics must be given careful consideration when mycoherbicide research is being conducted. It may be necessary to apply sticking material(s) to ensure that fungal spores adhere to plant surfaces, supply nutrients for spore germination, or apply spores in solutions that will provide adequate water for germination.

Germ Tubes and Appressoria

Fungal spore germination and appressorial formation has been the subject of many studies and has been reviewed (Nicholson 1983). Nicholson (1983) suggests that attachment of fungal spores to plant surfaces should be considered to include all mechanisms, both mechanical and chemical. He then defines adhesion as "a more suitable term to describe phenomena of attachment requiring either the presence of adhesive materials or at least a modification of the cuticle by the fungus". Various kinds of adhesion materials (AM) have been described (Nicholson 1983).

Considerable research has been done on the germination and appressorial formation of *Colletotrichum graminicola* (Coelomycetes) (Lapp and Skoropad 1978). Conidia are produce in an AM and appressoria are attached to surfaces by this same material. The AM protects conidia from desiccation. It is an antidesiccant and in its presence conidia may be dried to 45% relative humidity and stored for months with only nominal loss of viability. "In contrast, conidia die within hours, regardless of the relative humidity in the absence of matrix and liquid water" (Nicholson 1983).

The AM also contains at least two enzymes which may aid in the formation of appressoria by the fungus. One of the enzymes of the AM appears to alter the surface texture of corn leaf cuticle and change its wettability, suggesting that the enzyme acts on the cuticle or epicuticular area. Rate of disease development may be increased by 3 d when AM is included with spore inoculum. The AM protects spores from desiccation, but when spores are released by liquid water, AM enzymes may speed the infection process by providing nutrients, hastening appressorial formation, and perhaps altering the host cuticle (Nicholson and Moraes 1980, Nicholson 1983).

Wynn (1981) has reviewed the occurrence of AM associated with germ tubes and appressoria. He reports that AM is found only along certain parts of the germ tube, other times the entire length of the germ tube has AM. In still other cases AM is primarily associated with the appressorium. In all of these examples, indirect evidence has suggested that the AM is the result of a host stimulus or a host response as a result of interaction with the fungus.

Two primary spore types used for biological control of weed are urediniospores of rust fungi and conidia of imperfect deuteromycete fungi. Urediniospores germination has been done on leaves as well as on artificial surfaces. Their response to stomata is well known. Wynn (1981) has shown that some germ tubes respond to tactile stimuli in the appressorial formation and subsequent penetration through a stoma. In contrast, appressoria of basidiospores of the same fungus do not form over stomata but penetrate the cuticle directly.

Perhaps most intriguing in the germ tube-appressorial formation sequence is the question: What triggers the change from one to the other? It has been suggested that nonspecific

environmental factors may initiate the appressorial response (Parberry 1981). Other studies support the idea that germ tube-plant surface interactions chemically initiate the appressorial response (Blakeman 1981). In the case of rust fungi it has been well documented that tropic and taxic response of the fungus to the plant are responsible for appressorial formation (Wynn 1981). In all these examples it is clear that not all fungi respond by the same mechanisms and perhaps several factors are responsible for these responses. It has also been shown that some fungi respond to the age of plant tissue; younger tissue is often more resistant to infection, whereas older tissue is often more susceptible (Muirhead 1981). We have found that specific fungi respond very differently through time on leaf surfaces as seen with scanning electron microscopy (Spurr and Van Dyke 1980, Van Dyke and Trigiano 1987). In our preliminary study of *Bipolaris* species (Hyphomycetes), as potential mycoherbicides on johnsongrass, germ tube growth, and appressorial formation was highly variable (Van Dyke and Winder, unpublished data).

All of these findings strongly suggest that a better understanding of germ tube growth and subsequent appressorial development could provide clues about methods for enhancing infection processes.

Fungal Toxins

Dunkle (1984) suggested that if microbial "toxins" includes enzymes, plant growth regulators, and low molecular weight metabolites, then "toxins" are important in the disease process. The host cuticle, composed of cutin and waxes, can probably be degraded or altered by the fungus. The role of cutinase in the penetration of plants by fungi has been reviewed (Kolattukudy 1981). Virulence of different fungal isolates is probably related to their ability to produce cutinase. Specific studies have shown that cuticle-degrading enzymes are synthesized and released at specific stages in the development of the fungus on the host surface (Nicholson *et al.* 1972). We have shown that the necrotrophic fungus *Alternaria cassiae* Jurair & Khan (Hyphomycetes), which is being developed as a mycoherbicide (Bannon and Walker 1987), causes host mesophyll tissue to become nectrotic prior to penetration, suggesting necrosis was due to a diffusable toxin (Van Dyke and Trigiano 1987).

Fungal "toxins" are quite diverse and apparently significant in the infection process, therefore the selection of fungal biotypes that accentuate these beneficial "toxin" effects should be considered in mycoherbicide research.

Leaf Surface Microbe Interactions

There have been four international symposia on the *Microbiology of the Phyllosphere*. Other reviews have also considered the importance of microbe-microbe interactions on the surfaces of plant parts. Cullen and Andrews (1984) have stated that there are major gaps in our basic knowledge of the leaf surface and its relationship to pathogenesis. They further indicate that much research needs to be done relative to host-antagonist interactions, including mechanisms of microbial adhesion, spatial patterns of colonization, and the nature of resource utilization on leaf surfaces. These need to be compared for native organisms and for those cultured and reapplied. Others have emphasized the importance of understanding the interaction between bacterial, fungal, and applied chemical components of the phylloplane (Fokkema and Schippers, 1986). Fokkema (1981) also states that "there are numerous reports that common fungal leaf saprophytes antagonize plant pathogens. Further research, therefore, is urgently needed to quantify the role of phylloplane fungi in field situations (Fokkema 1981)." Reinecke (1981) also stressed the importance of the "buffering capacity of natural microflora" on plant surfaces.

Our knowledge of microbe-microbe interactions is sparse; however it appears to be a factor which has great potential for augmenting disease processes. These interactions should be considered in future mycoherbicide research.

Conclusions

It is clear from previous investigations of microorganism-plant interactions that much information has been gained, but that many of the interactions are, at best, poorly understood. It is also clear that although in-depth study has been done for certain fungal-plant interactions, that this information is not necessarily transferable to other fungal-plant situations (e.g. weed pathosystems).

There are many questions which must yet be answered. What is normal for a particular fungal-host interface? For some there is considerable variation and this makes investigation of manipulated sequences difficult. How does virulence of the pathogen being used as a mycoherbicide influence various other factors in the infection process or the application procedures? If we attempt to control younger tissues of weeds, are these tissues more resistant to the fungus? Many mycoherbicides are being applied to seedling weeds in high dosage to initiate demise or stunting of the weed. If these tissues are more resistant, perhaps pathogenesis can be enhanced by manipulation of the fungus and/or environmental factors.

The use of microorganisms to stimulate fungal virulence or to alter the microenvironment is only recently being considered as another approach to mycoherbicide strategies.

Two areas of mycoherbicide application that warrant consideration for detailed evaluation are first the use of adjuvants to aid inoculation and to enhance disease, and secondly the mixing of mycoherbicides with chemical herbicides or other mycoherbicides. Several studies have already shown that choice of adjuvants is an important consideration in mycoherbicide application (Bannon and Walker 1987, Winder and Van Dyke 1990). Wymore and Watson (1988) have demonstrated the positive effect of mycoherbicide and chemical herbicide combinations for the control of velvetleaf. Collego® (a commercial mycoherbicide for control of northern jointvetch) has been successfully combined with herbicides for several years (Templeton 1987). As we better understand how disease progresses under "natural" circumstances, then perhaps we can better determine more use strategies, and also possibly explain efficacy failures.

Presently there is much interest in the development of mycoherbicides; however, there is also a tendency to look for the "silver bullet", that ideal control agent comparable to chemical demise. Future weed control strategies may have to settle for something less than demise if regulations and restrictions on chemical herbicides continue to proliferate. Therefore, it is imperative that the mode of action of mycoherbicides be studied as intensively as possible and manipulated in every way possible to achieve the best understanding of their behavior. This should provide a basis for future decisions in determining the biological control potential of particular fungi. If we abandon certain fungi at present because they do not "perform" according to presently prescribed standards, we may lose valuable potential additions to our weed control arsenal.

References

Bannon, J.S. and H.L. Walker. 1987. Influence of non-ionic and non-phytotoxic crop oils on control of sicklepod by *Alternaria cassiae*. *Proc. S. Weed Sci. Soc.* **40**:288 (abstract).

Blakeman, J.P. and P. Atkinson. 1981. *In: Microbial Ecology of the Phylloplane*. Blakeman, J.P. (ed.). Academic Press, pp. 245-64.

Cullen, D. and J.H. Andrews. 1984. Epiphytic microbes as biological control agents. *In: Plant-microbe Interactions*. Kosuge, T. and E.W. Nester (eds.). Macmillan Publishing Co., pp. 381-99.

Dickinson, C.H. 1986. *In: Microbiology of the Phyllosphere*. Fokkema, N.J. and J. Van Den Heuvel (eds.). Cambridge Univ. Press, pp. 77-100.

Dunkle, L.D. 1984. Factors in pathogenesis. *In: Plant-microbe Interactions*. Kosuge, T., and E.W. Nester, (eds.). Macmillan Pub. Co., pp. 19-41.

Fokkema, N.J. 1981. Fungal leaf saprophytes, beneficial or detrimental? In: *Microbial Ecology of the Phylloplane*. Blakeman, J.P. (ed.). Academic Press. pp. 433-454.

Fokkema, N.J. and B. Schippers. 1986. Phyllosphere versus rhizosphere as environments for saprophytic colonization. *In: Microbiology of the Phyllosphere*. Fokkema, N.J. and J. Van Den Heuvel (eds.). Cambridge Univ. Press, pp. 137-59.

Huang, J.S. and C.G. Van Dyke. 1978. Interactions of tobacco callus tissue with *Pseudomonas tabaci*, *P. pisi* and *P. fluorescens*. *Physiol. Plant Path.* **13**:65-72.

Hyde, K., E.B.G. Jones and S.T. Moss. 1986. How do fungal spores attach to surfaces? *Proc. Int. Biodeteriation Symp.* (*in press*).

Kolattukudy, P.E. 1981. Structure, biosynthesis and degradation of cutin and suberin. *Annu. Rev. Plant Physiol.* **32**:539-67.

Lapp, M.S. and W.P. Skoropad. 1978. Nature of adhesive material of *Colletotrichum graminicola* appressoria. *Trans. Brit. Mycol. Soc.* **70**:221-23.

Muirhead, I.F. 1981. The role of appressorial dormancy in latent infection. *In: Microbial Ecology of the Phylloplane*. Blakeman, J.P. (ed.). Academic Press, pp. 155-68.

Murray, G.M. and D.P. Maxwell, D.P. 1974. Ultrastructure of conidium germination of *Cochliobolus carbonum*. *Can. J. Bot.* **52**:2335-40.

Nicholson, R.L. 1983. Adhesion of fungi to the plant cuticle. *In: Conference Report: Infection Processes of Fungi*. Roberts, D.W. and J.R. Aist (eds.). The Rockefeller Foundation, pp. 74-89.

Nicholson, R.L., J. Kuc and E.B. Williams. 1972. Histochemical demonstration of transitory esterase activity in *Venturia inaequalis*. *Phytopathology* **62**:1242-7.

Nicholson, R.L. and W.B.C. Moraes. 1980. Survival of *Colletotrichum graminicola*: Importance of the spore matrix. *Phytopathology* **70**:225-61.

Parberry, D.G. 1981. Biology of anthracnoses on leaf surfaces. *In: Microbial Ecology of the Phylloplane*. Blakeman, J.P. (ed.). Academic Press, pp. 135-54.

Parberry, D.G. and R.W. Emmett. 1977. Hypotheses regarding appressoria, spores, survival and phylogeny in parasitic fungi. *Revue de Mycologie* **41**:429-47.

Park, D. 1982. Phylloplane fungi: tolerance of hyphal tips to drying. *Trans. Brit. Mycol Soc.* **79**:174-8.

Potter, J.M., Tiffany, L.H., and C.A. Martinson. 1980. Substrate effect on *Helminthosporium maydis* race T condidium and germ tube morphology. *Phytopathology* **70**:715-9.

Pugh, G.J.F. and N.G. Buckley. 1971. The leaf surface as a substrate for colonization by fungi. *In: Ecology of Leaf Surface Micro-organisms*. Preece, T.F. and C.H. Dickinson (eds.). Academic Press, London, pp. 431-45.

Reinecke, P. 1981. Antagonism and biological control on aerial surfaces of the Gramineae. *In: Microbial Ecology of the Phylloplane*. Blakeman, J.P. (ed.). Academic Press, pp. 382-95.

Spurr, H.W., Jr. and C.G. Van Dyke. 1980. Morphological changes in the germination of plant pathogenic *Alternaria alternata* conidia induced by bacteria. *2nd Int. Symp. of Microbial Ecol.* Coventry, England (*abstract*).

Templeton, G.E. 1987. Mycoherbicides - achievement, developments and prospects. *Proc. 8th Australian Weeds Conf.*, 21-25 Sept. 1987, Sydney, Australia, pp. 489-97.

Van Dyke, C.G., P.M. Beckman and G.A. Payne. 1982. A simple technique for correlating light and scanning electron microscopy of fungal-host interactions. *J. Elisha Mit. Sci. Soc.* **98**:130-4.

Van Dyke, C.G. and R.N. Trigiano. 1987. Light and scanning electron microscopy of the interaction of the biocontrol fungus *Alternaria cassiae* with sicklepod (*Cassia obtufisolia*). *Can. J. Plant Path.* **9**:230-5.

Wheeler, H. and D. Gantz. 1979. Extracellular sheaths on hyphae of two species of *Helminthosporium*. *Mycologia* **71**:1127-35.

Wiinder, R.S. and C.G. Van Dyke. 1990. The pathogenicity, virulence and biocontrol potential of two *Bipolaris* species on johnsongrass (*Sorghum halepense*). *Weed Sci.* **38**:89-94.

Wymore, L.A. and A.K. Watson. 1988. Interaction between the mycoherbicide (*Colletotrichum coccodes* [Wallr.] Hughes) and selected herbicides for velvetleaf (*Abutilon theophrasti* Medik.) control under greenhouse conditions. *Weed Sci. Soc. Amer. 28*:50 (*abstract*).

Wynn, W.K. 1981. Tropic and taxic responses of pathogens to plants. *Annu. Rev. Phytopathol.* **19**:237-55.