Commensalism to synergism: the potential role for biological combinations in bioherbicides.

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Summary

The normal routine in the quest for a potential candidate for a mycoherbicide is to locate diseased plants and attempt isolation of an organism from leaf/stem/petiole lesions. The causal relationship between a suspected pathogen and host is established by Koch's postulates leading to the concept of specific aetiology. Pathogens, however, do not exist alone on leaf or root surfaces and isolation from necrotic tissue invariably yields a number of organisms, each of which may be tested singularly for pathogenicity to the host plant.

Mixtures of organisms are rarely, if ever, used to establish contributory roles in disease expression. The relevance of likely interactions should be established early in the assessment procedure. The scenario of a primary pathogen modifying the infection court or breaching host defences and compensating for the diminished virulence of the subsidiary pathogen needs to be examined. However in many cases, the subsidiary pathogen, as a co-isolate from the field-infected plant, may be discarded in an initial screening of single isolates in pathogenicity testing. The potential use of subsidiary pathogens to improve disease progression in mycoherbicides is discussed with some examples. Whilst biotroph/necrotroph interactions are receiving some attention, it is argued that benefits may be gained by greater examination of synergistic responses in necrotroph/necrotroph combinations.

Introduction

Koch's Postulates are procedural steps for proving pathogenicity of an organism. They are necessary to define the specific aetiology of a disease and must be performed using pure cultures of a single organism. However, in natural plant ecosystems, pathogens do not exist in an axenic state but rather exist with a multifarious epiphytic microflora.

In the initial step of proving causality, pathogenicity is rarely if ever established using two or more organisms simultaneously. As a research strategy, simple hypotheses and simple experiments are preferred to complex ones and multi-factor hypotheses are usually avoided (Hilborn and Stearns 1982). This approach can delay recognition of the real situation.

Whilst infection may be initiated by one organism alone, the speed and severity of disease progression may be influenced by the biota surrounding the infection court.

Infection by a pathogen alters host physiology leading to, amongst other things, electrolyte leakage and reduction in structural defences. Such changes could alter the plant's subsequent response to other, perhaps less virulent, pathogens.

The existence of natural synergistic interactions between organisms should be established early in the assessment procedures for likely candidate pathogens. The scenario of a primary pathogen breaching the host defences and compensating for the diminished virulence of a subsidiary pathogen needs to be examined. However, in many cases, the subsidiary pathogen, as a co-isolate from the field-infected plant, may be discarded in an initial screening of single isolates in pathogenicity testing because one pathogen alone is necessary and sufficient to cause lesions.

The term "subsidiary" is used intentionally here in place of the more usual adjective "secondary". Common usage of "secondary" has connotations of *inconsequential* or *unimportant*. My argument here is that neither meaning is appropriate when the subsidiary organism contributes to disease expression and development.

In the majority of diseases, single entity pathogens are the norm. The addition of another pathogen to the biological spray mixture however, considerably increases the potential for manipulating phylloplane ecosystems and subsequent disease initiation.

Breaching host defences

Fungi overcome the physical barrier of cell walls by enzymatic and mechanical means and evidence tends to support the former as the more important (Misaghi 1982). A common feature of infections are alterations to cell walls and the cell components initiated by an array of enzymes. Most bacteria and fungi, whether specific pathogens or not, produce a variety of cell wall-degrading enzymes which attack pectin, cellulose, hemicellulose, lignin and protein. Pectin-degrading enzymes are the first formed by plant pathogenic organisms (Bateman and Basham 1976), but, because they macerate middle lamella, thus facilitating bacterial movement in tissues, they are much more determinative in bacterial infections than in fungal infections (Ouchi 1983). After the pectic enzymes have exposed other cell wall polymers to enzymatic action, fungal pathogens produce cell wall-degrading enzymes such as arabinase, xylanase, cellulase, etc., in sequence, suggesting that each substrate is exposed in turn.

The sugars released by such macerations and loss of cell membrane integrity could serve as effective inducers of more specific carbohydrate-degrading enzymes (Cooper and Wood 1975) produced by pathogenic fungi. Protoplast death caused by pectolytic enzymes is preceded by increased membrane permeability. The leakage of electrolytes constitutes a source of utilizable carbon (energy) and nitrogen for nutrition of both the pathogen and the subsidiary invader.

The *Erwinia* soft-rotting group of bacteria are particularly good producers of a battery of pectinases, polygalacturonases, pectin methylesterases, etc. Although *Xanthomanas campestris* has been utilized as a biocontrol agent against *Poa annuus* (D. Schisler, personal communication), the potential use of bacteria in combination with fungal pathogens for weed control remains largely untapped.

Benefits need not arise from affects on the host plant directly. Other possible mechanisms of interaction are production of stimulatory compounds, improved leaf wetness or utilizing phylloplane nutrients which may otherwise have encouraged vegetative growth of the pathogen rather than formation of infective structures.

Diseases initiated or compounded by one or more organisms are termed "disease complexes". These occur particularly with soil-borne pathogens, and complexes of *Fusarium, Pythium, Rhizoctonia* etc., with or without nematode involvement, are well documented (Powell 1971, Magnusson 1986). Although foliar disease complexes are apparently less numerous, some studies serve as examples of the synergistic interaction under discussion.

Fungal interactions

An example of synergy where a biotrophic rust renders a host susceptible to a necrotrophic fungus, which is not normally a pathogen, has been recently described. Hallett *et al.* (1990a) showed that whereas *Botrytis cinerea* killed rust-infected groundsel (*Senecio vulgaris*) the fungus was unable to kill mechanically-wounded plants. The stimulatory effects of the rust (*Puccinia lagenophorae*) on germination of *Botrytis* conidia demonstrates a synergistic interaction (Hallett *et al.* 1990b). Several similar biotroph/necrotroph interactions have been studied (Brockenshire 1974, Yarwood 1977) and the potential for biocontrol of *Xanthium* spp. based on such an interaction has recently been reported by Morin *et al.* (1992).

Hasan and Ayers (1990) differentiate between the biotroph/necrotroph interaction which occurs at the infection site of the biotroph, and predisposition where infection by one pathogen makes the host more susceptible to secondary (sic) infection because of change in host vigour or growth habit. Synergism, however, can occur from an interaction very early in the initiation of disease and a time lapse between inoculations for one infection to benefit the other need not be mandatory.

Of greater immediate potential for use in inundative biocontrol is necrotroph/necrotroph synergism. Closely related necrotrophs such as *Cochliobolus heterostrophus* and *C. carbonum* may interact (Pascholati and Nicholson 1983). *Monographella maydis* can be isolated from symptomless green maize leaves but in the presence of *Phyllachora maydis* it develops virulence to the plant. Lesions in which both fungi are present are larger than those caused by *P. maydis* alone (Muller and Samuels 1984).

Although a wound pathogen is not the primary virulent invader traditionally sought as a mycoherbicide candidate, the desired result (a severely debilitated or dead host) can be ultimately achieved. Dutch elm wilt (*Ceratocystis ulmi*, Pirone 1978) and midge blight of raspberry (Williamson and Hargreaves 1979) are examples of natural interactions between insects and plant pathogenic fungi. The deliberate combined use of arthropods and pathogenic fungi has had some success (Hasan and Ayers 1990) in biocontrol of weeds.

Bacterial interactions

Bacteria survive best at the leading edge of infected tissue. The membrane-bound vacuole in mature plant cells is a reservoir of hydrolytic enzymes and phenolic substances. Damage to the tonoplast surrounding the vacuole releases these substances, which alone or after hydrolysis by plant enzymes, can be toxic to bacteria. Thus to maximize their potential, pathogenic bacteria must colonize tissue rapidly and multiply rapidly before the consequences of tissue maceration limit their growth. Massive cell death is not required to provide the level of nutrients needed by the number of bacteria usually found in infected leaves (Hancock and Huisman 1981) and nutrient availability per se should not restrict disease development.

Interactions between fungi and bacteria, particularly on the phylloplane, have been examined from the point of view of antagonism especially where such interplay decreases the incidence or severity of infection (Blakeman 1982). The possible stimulatory effects of these interactions are less well understood although increased appressoria formation in *Colletotrichum* (Blakeman 1982, Slade *et al.* 1986) and increased disease levels (Schisler *et al.* 1992) have been noted.

Unlike the fungi, bacteria have no means of penetrating the plant surface and rely on natural openings or wounds. The pathogenicity of *Erwinia stewartii* to maize is naturally increased by its association with the corn fleabettle *Chaetocnema pulicaria* (Bradbury 1967) which provides the necessary entry points.

Fungal/bacteria interactions can occur where both pathogens infect the plant independently. A *Pseudomanas* sp. was found not to be a serious pathogen of Easter Lily stems but the combined damage of the bacterium and a *Fusarium* sp. was much more severe than the sum of the damage caused by each organism alone (Bald and Solberg 1960, Bald *et al.* 1979).

Bacterial/bacterial synergism in disease has been reviewed by Ouchi (1983) and is not confined to nutrient leakage stimulating avirulent or heterologous pathogens. Synergism is also expressed at the levels of multiplication and symptom expression.

In natural systems bacteria are subject to the usual fluxes of nutrient, temperature, light and moisture. Populations fluctuate greatly with wetting and drying of the leaf. Both pathogens and saphrophytes will multiply on leaf surfaces, substomatal cavities and small wounds. Not all multiplication of a pathogen will cause cell death and not all cell death will be macroscopically obvious. Presence on the plant surface may be transient and normally be too brief for any positive interaction with a fungal pathogen. Manipulation of this population, however, could be achieved by spray adjuvants added to the formulation of the principal pathogen. This formulation technology which is so essential to the fine-tuned application of a mycoherbicide is equally applicable to the effectiveness of the subsidiary pathogen which may already reside on the leaf surface.

Bacteria as bioherbicides are a neglected commodity but they have much to offer. They multiply rapidly and are easy to harvest, many survive for extended periods in sterile distilled water (shelf life), and when applied, they are easy to track by antibiotic resistance genes and other markers. The DNA profiles of native and introduced strains are also easy to compare and monitor.

Addition of a second organism to a biological spray would increase the cost of production and may be economically unviable. However in the short term, interactions between two organisms may allow control of serious weeds in the "public domain" whilst studies on the mode of interaction provide a better understanding of the infection processes. For example, in the commercial product X-Tend®, synergistic necrotrophism of two organisms has been replaced by the use of a single organism (*Pseudomonas* sp.) assisting a chemical herbicide to modify host physiology and achieve cheaper control.

Genetic manipulation of fungal plant pathogens is often considered a possible solution to problems of increasing the pathogenicity or host specificity of a bioherbicide candidate (Bailey 1990, Sands *et al.* 1990). However, Templeton and Heiny (1989) also acknowledge the need for a greater understanding of the biology of the pathogen at the organism and ecosystem levels. This improved knowledge should encompass the microbial environment, especially in natural vegetation, and the microflora of the infection court. This information may provide more opportunities than genetic manipulation for increasing the range and complexity of bioherbicides. This would be especially so if the release of genetically modified organisms is indefinitely delayed by legal and environmental debate.

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