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Genetically Designed Biological Pesticides

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The use of genetic techniques including both conventional mutagenesis and genetic engineering to enable the safe use of broad host range biological control agents. One motif of such broad host range agents should be to limit them to certain targets (e.g., weeds). Alternatively, genetic containment systems can be devised that disallow survival of biological control agents after completing their task. The use of broad host range biological control agents, including pathogens, insects, and vectoring systems, greatly augments the likelihood that a biological control agent can be successfully developed. We have developed a model system using *Sclerotinia sclerotiorum* as a biological control of weeds. Morphogenic and nutritional mutants of this fungus appear to retain virulence while failing to persist. Such genetic containment systems may resolve the schism between ecological and industrial considerations on releasing organisms into the environment.

Introduction

There are at present, few effective biological controls for pests. So while the kinder and gentler biological control specialists are singing praises of their field, they are somewhat myopic if they are not also seeing the damages of uncontrolled pests, unwanted chemical residues, and non-target effects of conventional pesticides. Even with intensive searches for new biological control agents, there may not be a sufficient number of naturally occurring biological control agents that fit the current requirements of specificity, lethality, and ease of culture and application. Given these rather narrow confines of biological control, perhaps it is time to assume a "Neo-Nero-ist" approach: torch the current unworkable structures to the tune of "Paradigms Lost" and from the ashes rebuild the field from a new foundation. We propose just such a transition into a new, more manipulative-genetic-biotechnical field of biological control. With this approach perhaps biological control agents can be delivered with more lethality, specificity, and field efficacy. This paper outlines where our own work has taken us in development of genetic containment systems for broad host range pathogens along with a review of other work in this area. In addition, speculations are given as to what the guiding principles are–"Paradigms Gained"–that might transform this field from the hunting-gathering-testing phase to the concerted genetic design phase (Greaves *et al.* 1989, Miller *et al.* 1989*a*, Miller *et al.* 1989*b*, Sands *et al.* 1990).

Biological Control of Weeds with Fungi

There are some 60 potential fungal products for biological control of weeds currently in the development phase (Charudattan 1990, Templeton *et al.* 1979). Most are host-specific or more accurately, nearly host-specific. Few are lethal unless applied in high doses (inundative mycoherbicides), and few are being developed commercially because their limited host range and cost benefit for this limited market precludes their development and registration. Currently there are 2 on the market: *Collego* (*Colletotrichum gloeosporioides* [Penzig] Penzig & Saccardo; Coelomycetes) and *Devine* (*Phytophthora palmivera* [Butler] Butler; Peronosporales) that are used to control northern jointvetch and strangle-vine, respectively (Charudattan 1990, TeBeest and Templeton 1985). *Collego* is not as specific as originally described, and *Devine* is

truly effective and long-lasting, hence few repeat commercial sales.

To summarize the problems encountered in developing mycoherbicides, only public funds can be expected for research and development unless lethal pathogens can be found that are capable of killing a broad spectrum of weeds, yet unable to spread beyond the area of application or survive to the next cropping season.

Sclerotinia sclerotiorum

S. sclerotiorum (*S.s.*) is a broad host range plant pathogen, endemic throughout temperate climates, and capable of causing serious crop losses in beans, sunflower and numerous other crops (Purdy 1979). It has also been found to infect some 40 genera of weeds (Table 1).

Table 1. Weed-Hosts of *Sclerotinia sclerotiorum.*

Dandelion	Lambsquarter
Blackberry	Oxeye Daisy
Broomrape	Poison Hemlock
Cocklebur	Shepherd's Purse
Field Milk-Thistle	Tall Hedge Mustard
Hemp	Yellowstar Thistle
Nettle	Knapweeds
Pigweed	Black Mustard
Ragweed	Chickweeed
Spurges	Common Sowthistle
Yellow Dock	Groundsel
Canada Thistle	Lupine
Black Medic	Pennycress
Bull Thistle	Prickly Lettuce
Common Purslane	S. Galinosa
Goldenrod	Toadflax

Genetic System

S.s is soil-borne where it infects from mycelium. In addition, the fungus forms hard survival structures (sclerotia) that can survive for several years before either producing mycelia or trumpet-shaped apothecia that emerge above ground from the sclerotia. Ascospores are produced in apothecia after meiosis, presumably giving this fungus the advantage of genetic recombination. This latter conjecture has not yet been proven and some evidence suggests that the genetic system excludes recombination which we refer to as the "Eunuch" Hypothesis.

Field Experiments

Brosten and Sands (1986) reported that wild type endemic strains of *S. sclerotiorum* were successful in significantly reducing populations of *Cirsium arvense* (L.) Scopoli (Asteraceae) in pasture. The fungus was applied as dried mycelium on an infested food base (autoclaved wheat or oat kernels) at high rates of 500-1,000 kg/ha. Strain differences were observed and the most efficacious strains (84.1B and K-1) were preserved for mutation experiments, described later. These strains were also proven effective in the field on other asteraceous weeds, *Centaurea maculosa* (L.) Scopoli and *Taraxacum officinale* L. These strains also attacked sunflower, rape, lettuce, and beans in the greenhouse.

Mutation

Miller *et al.* (1989*a*) exposed strains 84.1B and K-1 of *S.s.* to UV light or chemical mutagen as ascospores and as protoplasts. In all cases, the mutagenesis of multinucleate cells presented difficulties in that non-mutated nuclei could mask the mutant phenotype. After several years of experimentation and development of mutation and selection methodology, several thousand putative mutants were obtained. These were checked for virulence on sunflower and/or screened for auxotrophy (nutritional dependence greater than the wildtype). The mutants were classified into several groups for the purpose of virulence testing: sclerotialess; auxotrophs; and altered virulence or avirulence. The sclerotialess mutants were unable to produce sclerotia on commonly used culture media, but some either reverted or were able to form sclerotia on carrot slices. The auxotrophs required either pyrimidines, amino acids or vitamins for growth. These nutrients, when supplied in ample amounts, sometimes enabled normal growth and near normal virulence. These could be considered as contained pathogens in that they can kill plants until they deplete the exogenous supply of limiting nutrient. Similarly, the sclerotialess are

contained in that they are less able or unable to survive the winter, at least in Montana. Additional work on the "genetically contained" mutants to determine field efficacy, formulation optimization, survival stochastics, and long-term genetic stability is continuing. The premise is that this is a way to use broad host range pathogens in a manner analogous to broad spectrum herbicides, non-selectively killing many plant species and then dissipating.

Natural Containment Systems

Most pathogens and other biological control agents are limited genetically and environmentally to a certain host and/or ecological ranges. Examples within our field are: *Sclerotium rolfsii,* a broad host range pathogen that cannot survive freezing; *Phymototrichum,* a root rotting fungus that has clear limitations in soil pH and temperature; stem rust of wheat, with extreme nutritional fastidiousness and requiring an alternate host for overwintering; *S. sclerotiorum*, a pathogen that cannot grow above 30°C; and *Ophiostoma* and *Endothia*, both requiring insect vectors.

Designed Containment Systems

There are advantages for biological control systems if they lack sexual recombination, fail to survive indefinitely, and/or can be selectively turned on or off by an exogenous environmental condition such as a nutrient. Given these constraints, novel biological control agents are more amenable to regulatory approval and commercialization. It can be surmised that most regulatory agencies (and attorneys) would like to see new biological control agents that self-destruct at the end of each year, at least for the first few years of testing. Secondly, most corporations would desire products that do not persist both to reduce liabilities inherent to residue problems and for sales with required reapplication. The intrinsic nemesis of a containment system is that it may revert to the wildtype. Also, such systems are considered genetically altered and are by necessity not very diversified. We favor containment of endemic pathogens because the wildtype is already present in nature, so reversion to wildtype is inconsequential. Similarly, endemic pathogens are already adapted to the vagaries of the environment.

Design Parameters for Biological control Agents–Indigenous Genes

The *S.s.* case described earlier demonstrates how useful mutants can be obtained from within the organisms genetic system. Such an approach can yield mutants with different traits including altered host range, auxotrophs, fungicide resistance, high or low temperature sensitivity, enhanced virulence, altered vector compatibilities, altered survivability, and genetic neuterization. The appropriate screening system can sometimes enable positive selection for each of these traits.

Design Parameters with Exotic or Altered Genes

We have arrived at a general paradigm that may enhance the rapid design of greatly improved biological control agents. Named after the cartoon character Pogo ("We have met the enemy... and he is us"), the Pogo Hypothesis suggests that the most sophisticated way to kill a pest is to use its own genes. Examples abound including, toxin resistance, pheromone and hormone production, fungicide resistance, host specific promoters, sexual attractants to lure vectors, etc., all derived from the host itself and "cloned" into biological control agents. Additional genetic sources (non-Pogo) for such genes include host-specific and non-specific parasites and pathogens.

In addition to conventional genetic manipulation, we present here an unconventional and as yet hypothetical manipulation (referred to as "Brute"). A generalized microbe is used as a recipient of numerous genetic cassettes containing traits for specificity, lethality, survival, etc. In this way a microbe can be used for numerous different hosts, depending on which traits are transferred to it. This approach might offer the efficiency of modular construction enabling a genetic research lab to turn out a wide range of biological control agents from a single transformable pathogen.

Future Development

We have presented our views of the future of biotechnology in biological control. One of the biggest obstacles to the development of successful biologically based pest control will be the conservative approach of many present practitioners. Perhaps the added safety of genetic containment systems will provide that bridge to modern biotechnology. It seems clear that a biotechnical approach alone cannot provide solutions to biological control problems. Only a fusion of efforts with traditional biological control will enable successes.

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