MEETING

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Biological Control of Plant Diseases and the Environment

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An alternative to chemical control of plant diseases is biological control based on application of antagonistic micro-organisms. It is often stated that biological control would be safer for the environment than chemical control. However, one can argue that, on the contrary, releasing high inoculum densities of micro-organisms in order to modify the microbial balance could be dangerous for the environment. In fact, there is no general answer to this concern; it obviously depends on the biological control method proposed. If the method consists in mimicking natural control that exists in certain situations, it can be regarded as safe. For example, biological control of fusarium wilts based on applications of non-pathogenic strains of *Fusarium oxysporum*, associated with strains of *Pseudomonas fluorescens*, tends to reproduce in conducive soils the mechanisms by which suppressive soils naturally control these diseases.

Suppressive soils are those in which disease incidence or disease severity remains low in spite of the presence of the pathogen, a susceptible host plant and climatic conditions favorable to disease expression. The soil is not a neutral milieu in which the pathogens interact freely with the roots of the host plant. On the contrary, it is a very complex milieu interfering in several ways with the relationships between and among micro-organisms, pathogens and plants.

Soils suppressive to some of the most important diseases have been described in different areas of the world, and studies of the determinism of suppressiveness constitute a solid approach to the development of new disease control strategies. In most examples of suppressive soils, the saprophytic microflora plays an important role, and suppressiveness has been related to the activity of the whole microbial biomass or to the specific activity of an antagonistic population of micro-organisms. Although the most important, these biotic interactions are not independent of the abiotic characteristics of the soil, and factors such as pH, organic matter content and nature of the clay minerals have been involved in the mechanisms of suppression.

Suppressive soils constitute good reservoirs of potential biocontrol agents, and several strains of fungal and bacterial antagonists have been isolated from them. Competition for nutrients, competition for infection sites, antibiosis and induced systemic resistance are the main mechanisms involved in soil suppressiveness and the main modes of action of biological control agents. Based on the knowledge acquired from the study of suppressive soils, two approaches can lead to the management of soilborne plant pathogens: either enhancement of the natural suppressiveness that exists in every soil, or application of biological control agents previously isolated from suppressive soils. However, to succeed with applications of biological agents it is necessary to know not only their modes of action but also the environmental conditions required for their establishment and the expression of their antagonistic activities. The production and formulation processes must also be chosen with respect to the modes of action to ensure the efficacy of the biocontrol. Lastly, according to European legislation, biological control agents have to be registered as chemical pesticides. Registration is based on efficacy and risk assessment in relation to toxicity for man, animal and the environment.

Commercial applications of biological control agents are limited to a few examples, and biological control has to be considered as a component of an integrated control strategy combining every possible method of control.

Novel Approaches to Biocontrol of Plant Diseases

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Isolates of *Trichoderma harzianum* antagonistic to *Sclerotium rolfsii, Rhizoctonia solani, Fusarium* spp. and *Pythium* spp. were isolated and selected from field soils. Studying the mechanisms involved in disease reduction revealed that *Trichoderma* acts as a mycoparasite. Apparently it detects its host from some distance and a chemotropic growth can be observed. Later the *Trichoderma* recognizes and attaches to the pathogenic fungus by sugar–lectin linkage and begins to excrete extracellular lytic enzymes such as β-1,3-glucanase, chitinase, protease and lipase. This recognition mechanism is the basis for the specificity of the antagonist. Studying this mechanism showed that *S. rolfsii* produced a D-glucose, D-mannose specific lectin that can agglutinate several kinds of bacteria. This is a soluble lectin, strongly associated with the extracellular polysaccharide of the fungus. When the mycoparasitic, biocontrol fungus *T. harzianum* was allowed to grow on nylon fibers treated with concanavalin A or *S. rolfsii* lectin, it coiled around the nylon fibers and produced hooks in a pattern similar to that observed with the real host hyphae. Biomimics of the recognition provide direct evidence for the role of lectins in mycoparasitism. Evidence for chitinase in natural control of plant pathogenic fungi is becoming more and more convincing. Chitin is one of the main cell wall constituents of many plant pathogenic fungi. Addition of the cloned chitinase gene from *Serratia marcescens (chiA)*, which is known to have biocontrol activity, into the *Trichoderma* is an excellent tool for studying the capability of the *Trichoderma*. The foreign gene, which was transformed into the *Trichoderma*, was integrated into the genome. Our new *Trichoderma* transformants express the *chiA* gene under the 35S promoter from CFMV. The capability of those transformed fungi to survive in the rhizosphere was tested and after 4 months the transformants were found to be stable. Those transformants are now being tested as improved biocontrol agents and should enhance antagonistic activity. The same gene was also cloned into tobacco plants and enhanced the resistance of the seedlings to *R. solani.*

Difficulties in Developing Nonchemical Approaches for Pest Management

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The use of pesticides, improved cultivars and other means enables sufficient food production to avert a worldwide food crisis. However, pesticides confront us with a dilemma: they play a major role in food production, yet they are, to various degrees, toxic and polluting substances. The incorporation of nonchemical approaches into pest control programs enables us to sidestep this dilemma. Numerous potential nonchemical approaches are available. These include the use of resistant cultivars and scions, biological control, cultural methods (*e.g.* crop rotation and sanitation), manipulation of pest or host response, etc. Integrated pest management (IPM) aims to integrate, in an optimal economic, environmental and technological manner, all available approaches, including pesticides at reduced doses.

There are many difficulties in introducing nonchemical approaches for pest control: (i) Pesticides are effective agents, making it difficult to find an equivalent replacement. (ii) Frequently, research into the development of nonchemical control is conducted with simulation systems which are not representative of real conditions. This may lead to nonreproducible (and disappointing) results. (iii) Economic factors are not taken into consideration. (iv) Implementation, extension and application procedures are not adequately developed. (v) Biocontrol organisms are living agents and are therefore influenced by environmental conditions. When developing a new, nonchemical control method, the new method should be tested under realistic conditions, using an upscaling program. Its mode of action should be studied in order to avoid failures under unusual conditions. Finally, we should aim to incorporate all pest management methods into IPM programs.

Examples of introducing alternative methods of pest management were described, including the development of alternatives to methyl bromide.

Advantages and Disadvantages of Recombinant Baculoviruses in Insect Pest Control

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Recombinant DNA technology is having a major impact on agriculture. The new collection of tools provided by recombinant methods is certain to benefit international agriculture. However, like any new technology it also is certain to be disruptive to aspects of agriculture. Most of the recombinant approaches have involved plant genetic engineering. However, for integrated pest management to function, we need a collection of tools of varying specificity and persistence. Biological pesticides represent a promising class of tools, and the baculoviruses are one group of biologicals where recombinant DNA technology has increased their utility as pesticides. Nonrecombinant viruses have been used for many years for insect control due to their efficacy and specificity, but their utility is limited by their slow speed of kill, of pest insects. We and others have used several types of genes in viruses specific for noctuiid larave to increase the speed of kill including insect juvenile hormone esterase. However, the most successful viruses have been developed using neurotoxins from scorpions isolated by Eli Zlotkin and co-workers. By placing genes for selective neurotoxins under viral promoters, viruses have been developed in several laboratories which kill noctuiid larvae very quickly. We have utilized genes coding for insect specific scorpion toxins both alone and in synergistic combinations. It is likely that other arthropod venoms also will yield valuable peptides for use in insect control. The toxins used have no effect on vertebrates and baculoviruses are not known outside of the arthropods. There are many layers of selectivity with these recombinant pesticides, making human health issues minimal. The viruses have the advantages of very high specificity and lack of environmental and human health problems, yet they can be applied like a standard pesticide.

The recombinant viruses are efficacious in the laboratory, greenhouse and field and dramatically reduce damage caused by insect feeding. The recombinant viruses synergize and are synergized by classical pesticides such as pyrethroids. Thus they offer potential as resistance management tools. This also brings up the possibility that the field application rate of pyrethroids could be dramatically reduced if mixed with baculoviruses, since high levels of pyrethroids used in the field often are driven up by the presence of relatively insensitive noctuiid larvae. Because the parent viruses are so selective, the recombinant viruses appear to kill only pest caterpillars, and thus can be used without disrupting biological control. Since the recombinant viruses produce fewer progeny in infected larvae than wildtype viruses, they are rapidly out-competed in the ecosystem. The viruses can be used effectively with crops expressing endotoxins of *Bacillus thuringiensis*. They can be produced industrially but also by village industries, indicating that they have potential to deliver sustainable pest control in developing countries. The success of recombinant baculoviruses suggests that both recombinant and wildtype forms of other insect viruses can be used effectively in agriculture. It remains to be seen if the current generation of recombinant viruses will be competitive with the new generation of synthetic chemical pesticides. However, current research clearly indicates that the use of biological vectors of genes for insect control will find a place in agriculture.

Benefits of New Technologies for Safe and Sustainable Food Production

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The world's population has already reached 6 billion and is forecast to double in the next 50 years. This, together with the requirement for greater food quality and variety, means that technologies such as chemicals, improved seeds, fertilizer use and good husbandry will continue to play a crucial role in food production. The integration of crop protection effects delivered *via* genes and chemicals will be critical in providing agricultural outputs safely and sustainably.

Radical change is evident within the crop protection world. The supremacy of organic chemistry in the last 50 years is now being challenged by exciting developments based upon biotechnology. The organic chemists have responded by exploiting advances in combinatorial chemistry and robotics to increase vastly the input to high throughput 'smart' screens dedicated to unearthing chemistries which are effective at low use rates and which are environmentally and toxicologically benign. Formulation and application technology is also changing, for example by provision of sophisticated solid formulations with consequent benefits of controlled release and improved operator safety. One of the most spectacular advances has been in the area of precision agriculture in which accurate maps of individual fields allow inputs such as fertilizers and pre-emergence herbicides to be adjusted to suit the specific conditions, with consequent economic and environmental benefits.

Alongside the progress in chemical-based technologies for crop protection, are impressive advances in the use of biotechnology to meet agricultural needs. In the mid 1990s the first transgenic crops were introduced into a few major markets with significant success. Whereas biotechnology-based solutions will not be relevant to all pest, disease and weed problems, there will be many applications which will find favor with farmers and growers. In addition, transgenes will be employed to improve both the quality (*e.g.* flavor) and agronomics (*e.g.* cold tolerance) of crop plants. Farming of transgenic crops will dramatically change the basis of competition within the crop protection industry. Traditional price structures will change, as will marketing strategies.

Public perception of genetically modified crops continues to inspire lively debate. Several groups hail biotechnology-based agriculture as the next green revolution whereas others oppose its use as an unnatural process which unnecessarily tampers with Nature. Processors and retailers beyond the farm gate reflect a nervousness over the outcome of this issue, and in Europe presently favor food labeling to provide the consumer with choice. The identity preservation required to support labeling presents logistical problems but these can, and will, be solved. The public debate on genetically modified crops will undoubtedly impinge upon the regulatory framework, but it is crucial that science-based risk assessment be taken as the key input to legislation.

The early years of the new millennium promise to be fascinating. The imperative of providing a safe supply of food will dictate that pragmatic solutions will be reached on a worldwide basis, albeit with some countries adopting outlying positions. Integrated crop management systems which optimize technology solutions will ensure that the global population is fed safely and sustainably.

Long-Term Effects of Rational and Irrational Use of Chemical Weed Control

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Weeds cause severe yield losses worldwide particularly in the developing world, where losses in some crops amount to more than 25% of the yield. Weed removal at the proper time may result in reduced competition of the weed with the crop plant and increased yield. Adequate weed control can be achieved by cultural methods including soil cultivation and hand weeding (performed mainly by cheap labor, *i.e.*, women and children), or by chemical methods using herbicides. Unfortunately, in most countries biological control of weeds is not yet an option for the farmers. Proper use of herbicides, integrated within the crop management, reduces the need for manpower and increases crop yields and farmers' incomes. Misuse of herbicides, however, may result in a significant impact on the environment: it may result in carry-over, and contamination of water resources by surface run-off and leaching to the groundwater. It also may cause damage to non-target organisms, change the soil microbial population, reduce biodiversity and modify the structure of weed populations. In Israel, the impact is well represented by the dramatic increase in infestation of irrigated crops with purple nutsedge (*Cyperus rotundus*) and dryland wheat fields with grass weeds such as wild oats (*Avena sterilis*) and canarygrass (*Phalaris* spp.).

Repeated use of a herbicide, particularly in monoculture and minimum tillage, imposes high selection pressure on the weed population, and has inevitably resulted in the evolution of herbicide-resistant weeds. Worldwide, more than 150 weed species, grasses and broadleaves, annuals and perennials have evolved resistance to virtually all groups of herbicides due to improper use of herbicides. The economic impact of these herbicide-resistant biotypes is not yet fully recognized, but in Australia and Canada – where small-grain cereals are important – the damage is significant. In order to prevent or at least delay the evolution and spread of herbicide-resistant weeds, the farmer should be aware of and adopt alternative weed control methods, whereby herbicides are used rationally. Modern and sustainable agriculture should supply the world's needs for sufficient food, and this goal cannot be accomplished without chemical pest control. However, farmers should be alert to both the positive and the adverse implications of these indispensable tools on the environment and use them wisely.

Habitat Management for Biological Control: Lessons from Conservation Biology

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Ecological theory has been used as a basis to manage populations of organisms in agroecosystems and natural systems. Since the 1970s applied ecology in agricultural systems has developed as the sub-discipline agroecology, and a prominent sub-discipline of applied ecology in natural systems has developed as conservation biology. Both sub-disciplines are concerned with managing species populations in their habitats. However, the fields of agroecology and conservation biology have advanced separately, with few attempts to adapt lines of inquiry laterally from one field to the other. This lecture explores how ecological theory aimed at the conservation of threatened or endangered species can be transferred to foster new concepts in the conservation of natural enemies in agroecosystems. To set the stage, I have developed two arguments. First, landscape trends in natural and agricultural lands have parallels with respect to loss of habitat. Whether target animal species are predatory birds or primary parasitoids, some species are declining because of changes in the amount and quality of critical habitat. Second, the aims and objectives in conservation biology are comparable, in many ways, to those in conservation biological control. A series of lessons developed in conservation biology may be useful conceptually or practically in the conservation of natural enemies in agroecosystems. Specifically, these lessons are:

- Habitat loss, fragmentation, isolation and degradation are the most common causes of species extirpation.
- In addition to absolute loss of a species through extinction, a species can become locally extinct or ecologically extinct. A species is considered ecologically extinct if it persists at such low numbers that its effects on other species in its community are negligible.

- Many kinds of disturbances greatly influence biodiversity, and often the effects are strongly influenced by scale, intensity, and the frequency of disturbance regimes.
- Spatial fragmentation of natural areas will result in: (*a*) smaller areas with reduced species richness and (*b*) increased boundary/interior ratios so that edge effects will play an important role in determining the fate of different species in the habitat.
- Population dynamics are influenced strongly by the abundance and location of suitable habitats, and the process of maintaining functional populations and communities may necessitate the identification of critical sub-populations and habitat patches that may act as source pools for re-colonization.
- The conservation of biodiversity involves not only preservation but also enhancement through active programs of ecological restoration of habitat quality.

These lessons have been presented with the caveat that theoretical ecology, conservation biology and agroecology are dynamic fields of study, full of controversy and debate; thus, for every lesson or principle mentioned, there are challenges and exceptions. Challenges to these operating principles produce a constellation of corollary lessons that may also apply to the conservation of natural enemies to foster pest control in agroecosystems.

Plant Protection in the Era of Precision Agriculture

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Precision agriculture, also known as precision or site-specific farming, is a set of management practices that vary inputs spatially within a field. A wide range of technologies, which have recently become available, provide the tools to make precision agriculture a realistic farming practice. There are two approaches for implementing precision agriculture: (i) a map-based method, in which data obtained by grid sampling are used to generate a site-specific map, which is then coupled with a variable-rate applicator in the field; and (ii) a sensor-base method that utilizes real-time sensors to measure the desired variable and immediately uses the information to control a variable-rate applicator. To date, a growing number of farm operators have adopted precision agriculture methodologies, primarily for crop management purposes; information on soil and plant characteristics is used to apply fertilizer, gypsum, lime, and water differentially to various parts of the field. Clearly, these site-specific agricultural inputs are cost effective and greatly reduce the release of contaminants into the environment.

The application of precision agriculture to plant protection has been slow because of technological constraints stemming from the dynamic and cryptic nature of disease agents and insect pests. Yet, the clustered distribution of crop pests (pathogens, insects and weeds) and the toxic effects of pesticides upon the environment, call for a careful examination of ways by which precision farming methods could be adapted for plant protection.

The map-based method may be suitable for the control of weeds and honey dew-secreting insects, since changes in their distribution in the field are relatively slow and may be detected through remote sensing. However, these methods do not allow for species identification and thus do not contribute to the selection of target-oriented control measures.

Neither map- nor sensor-based methods are suitable for disease or insect control; their distribution changes rapidly over the growing season and plants show detectable symptoms of attack only when it is too late to take corrective measures. Nonetheless, site-specific control of insect pests and diseases may be possible based on (i) pest biology (*e.g.* typical within-field distribution patterns) and (ii) parameters correlated with pest infestation (*e.g.* soil/plant nitrogen levels, climatic factors). Finally, precision agriculture methodologies such as the global positioning system (GPS), remote sensing, and a geographical information system (GIS) may be used for area-wide management of pests. These approaches were discussed using examples from recently conducted entomological studies.