NEW DIRECTIONS IN BIOLOGICAL CONTROL

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Poster Sessions

New Directions in Biological Control

Keynote Address

CB 001 PLANNED RELEASE OF AN ENGINEERED BACULOVIRUS, David H.L. Bishop, NERC Institute of Virology, Mansfield Road, Oxford OX1 3SR, U.K.

Naturally occurring baculoviruses have been used as insecticides since the last century to control insect pests of agriculture and forestry. Although slower and more specific than chemical insecticides, baculovirus insecticides have found particular favour where the area to be treated is environmentally sensitive (e.g., in forest catchment areas), or where the cost of chemical insecticides is prohibitive, or where the pest species has developed resistance to chemical insecticides. The host range specificity of baculoviruses, although commercially unattractive, is of value when non-target insects need to be conserved.

The objective of the programme of research and development at the Institute of Virology in Oxford (UK) is the improvement of baculovirus insecticides through the use of genetic engineering procedures. It is anticipated that through the incorporation of toxin genes, insect-specific hormones (etc), improvements can be made to the efficacies of these insecticides. Between 1986 and 1988, licences to prepare and release in a field facility genetically altered baculovirus insecticides were given by the appropriate U.K. authorities. The release studies have demonstrated that an innocuous genetic marker can be inserted into the genome of the virus without altering the phenotype (host range, virulence, ability to survive in the environment, etc.). Field trials have shown that marked viruses are suitable for following the fate of engineered viruses in the environment. It has also been demonstrated that a genetically crippled virus can be made that cannot, and does not persist in the environment. Such viruses are substrates for future generations of custom-designed virus insecticides that will be environmentally safe to deploy for pest control and for crop protection.

Foundations of Biological Control

CB 002 **INVESTIGATION OF MECHANISMS: THE KEY TO SUCCESSFUL USE OF BIOTECHNOLOGY,** Robert R. Schmidt, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611. Biocontrol strategies are being developed to control such diverse organisms as viruses, bacteria, fungi, insects/arthropods, and even weeds. The application of many of these strategies will involve the use of biotechnology, particularly molecular biology procedures for insertion of new genes or modification of existing genes (or their regulation) to provide organisms with resistance to pathogens/pests or to expand the host range and/or effectiveness of organisms used as biocontrol agents. Recent examples of single gene transfers that have resulted in formation of transgenic plants with viral or insect resistance include the transfer of genes encoding viral-coat proteins to give resistance against tobacco and cucumber mosaic viruses, a "gene" encoding a small viral satellite RNA to give resistance against tobacco ringspot virus, and the gene for ***Bacillus thuringiensis*** toxin to confer resistance against certain lepidopteran insects. Although the transfer of single genes (or their cDNAs), under the control of new promoter regions, has yielded these recent successes, many biocontrol traits of future importance will be encoded by eukaryotic genes whose expression is normally dependent upon or regulated by post-transcriptional or co-/post-translational processes involving RNAs/proteins encoded by other genes. For example, alternative splicing of precursor mRNAs, processing of precursor-proteins targeted for secretion or for import into specific subcellular-organelles, covalent modifications of proteins/enzymes to activate/inactivate or tag them for degradation are cellular processes that will have to be considered if genes are to be expressed after transfer to a new organism. Moreover, although genes (or their cDNAs) can be placed under the control of new regulatory regions to have them transcribed in their transgenic hosts, it may be more desirable in some cases to have genes remain under control of their natural promoter/enhancer regions to have them respond to the same environmental stimuli as in their natural hosts. The genetic engineering task becomes even more difficult as traits are identified which are encoded by multiple genes which affect multiple metabolic pathways. Thus, if biotechnology is to be successfully applied to modify organisms by gene manipulation, a significant portion of our effort and resources should be focussed on basic research aimed at elucidation of the molecular mechanisms underlying expression of genes of importance to biocontrol. This lecture will include a discussion of different types of mechanisms that can potentially affect expression of genes in transgenic organisms and experimental approaches for identifying and studying them in different organisms.

Theoretical Basis for Biological Control

CB 003 ECOLOGICAL THEORY AND BIOLOGICAL CONTROL,
William W. Murdoch, Department of Biological Sciences, University of California, Santa Barbara, CA 93106
Pest control is an attempt to manipulate the dynamics of one or more populations and is thus ultimately an exercise in applied population ecology. An understanding of population dynamics should thus increase the effectiveness of control measures. This paper reviews and evaluates mathematical theory aimed at describing the population dynamics underlying successful classical biological control. Success implies maintenance of the pest species by the controlling agent below the level at which it causes economic damage, and thus requires a low mean pest density and low temporal variability. The models suggest a range of mechanisms and processes that can lead to low pest density. Maintenance of low temporal variability, and its relation to stability, is a more complex matter, though several useful results are available. The paper will relate theoretical insights to field situations.

CB 004 THE FUNCTIONAL RESPONSE OF ARTHROPOD PREDATORS AND ITS ROLE IN THE BIOLOGICAL CONTROL OF INSECT PESTS IN AGRICULTURAL SYSTEMS. ROBERT J. O'NEIL, DEPARTMENT OF ENTOMOLOGY, PURDUE UNIVERSITY, WEST LAFAYETTE, IN 47907. The responses of predators to changes in prey density are broadly characterized into the functional and numerical responses. The numerical response relates the changes in predator density to prey density, and is influenced by differential predator reproduction, development, survival, and aggregation at higher prey densities. The functional response relates the number of prey attacked per predator to prey density. As an attribute of predator-prey dynamics, the functional response is seemingly well-understood and widely documented. The predator behaviors that influence the magnitude and form of the functional response have been identified and verified with experimental studies, mostly conducted under laboratory conditions. The relative importance of the functional response to prey dynamics and the stability of predator-prey systems have been described. Various modifications to the basic models have incorporated the effects of predator density, alternative prey, nonrandom search, and patterns of prey dispersion. For the field of biological control, the relationship between the number of prey attacked and prey density is a commonly measured aspect of predator biology, and it is conceivable that it will be a target opportunity for genetic engineering of natural enemies. Recently, however, there have been several criticisms of our understanding of the functional response and its role in predator-prey dynamics and biological control. While there are important statistical difficulties, the principal critiques have focused on the differences in the functional response measured under laboratory and field conditions, and the relative importance of specific predator behaviors in determining its form. In general, there appears to be little relationship between the magnitude and dynamics of predation measured in the laboratory and similar measures in the field. These criticisms illustrate that there is much to be learned from even "well-understood" attributes of predator-prey dynamics. Manipulating predators toward desired endpoints will require not only the techniques to institute changes in predator biology, but also a specific understanding of the attributes that influence the nature of predator-prey interactions in the field.

Viruses in Biological Control

CB 005 APPROACHES TO STUDYING VIRAL CROSS PROTECTION. J. A. Dodds, Department of Plant Pathology, University of California, Riverside, CA 92521.

Interactions between related strains of plant viruses are often studied by establishing an infection with a strain producing mild symptoms and challenge inoculating at a later date with a strain which produces characteristic symptoms. At a minimum, cross protection is evaluated by the degree to which the timing or effects of the challenge strain is delayed and minimized by the protective strain, compared to effects in non-protected plants. Assays which can further evaluated the accumulation of virus products, such as virions, structural and non-structural proteins, and viral replicative and genomic nucleic acids add to the understanding of the interaction. The nature and magnitude of the protection or interference obtained is dependent on the group of viruses being studied, the relatedness of the virus strains used, the delay and site between protection and challenge inoculation, the nature of the host/challenge strain interaction, and the part of the plant analyzed, especially the distinction between challenge inoculated leaves and those which develop after challenge inoculation. Experimental variabilities make it difficult to compare studies and have contributed to confusion over probable mechanisms for cross protection. Many of these problems are minimized by using transgenic plants to study the antagonistic effects of selected viral genes on challenge inoculation.

CB 006 ENGINEERING BACULOVIRUSES FOR BIOLOGICAL CONTROL OF INSECT PESTS, Lois K. Miller, Departments of Entomology and Genetics, University of Georgia, Athens GA 30602

Recombinant DNA technology offers many new avenues to understanding the biology and ecology of insect pathogens as well as providing a means of manipulating and improving the characteristics of these microorganisms for more efficient and effective control of specific pest populations (1). Insect baculoviruses have a long history of use as insect pest control agents but their commercial development has been impeded by a lack of commercial interest which is often ascribed to the narrow host range of the viruses, their sensitivity to UV light, and the delay between pesticide application and visible indications of control (ie. the lack of a rapid knockdown affect). Genetic manipulations which would enhance these viruses as pest control agents could bridge the road to commercial feasibility. The possibility of introducing foreign genes into the baculovirus genome under the control of broad host range promoters, thereby utilizing the virus as a gene vector to effect more rapid and broader insect control has been proposed (1,2). Useful foreign genes which are being tested include insect hormone genes and insect-specific neurotoxin genes. A view of baculoviruses and the potential for manipulating their genomes will be presented.

(1) Miller, L.K., Lingg, A.J., and Bulla, L.A., Jr. Science, 219: 715-721 (1983). Bacterial, Viral and Fungal Insecticides.
(2) Carbonell, L. and Miller, L.K. J. Virology, 56: 153-160 (1985). Baculovirus-mediated Expression of Bacterial Genes in Dipteran and Mammalian Cells.

CB 007 THE RESOLUTION OF CONFLICTS AND STRATEGIES IN FUTURE DEVELOPMENT OF MYCOHERBICIDES. David O. TeBeest, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.
Mycoherbicides, commercial formulations of fungal plant pathogens, are used to control or eliminate specific weeds in agricultural production systems. Two mycoherbicides, DEVINE and COLLEGO, have been used successfully since 1981 and 1982, respectively, without the report of any significant problems. Several additional fungal plant pathogens are now under development and nearing commercialization. However, a significant number of fungal plant pathogens have been studied and rejected as potential candidates for development. The cited explanations for rejection as suitable mycoherbicides include: lack of sufficient virulence to a specific weed host, environmental limitations on disease development, insufficient sporulation in axenic culture limiting inoculum production, host ranges either too broad or too narrow to justify commercial development, sensitivity to agricultural chemicals, and potential for genetic variability and instability. Several strategies which have been proposed to resolve these conflicts in future development of mycoherbicides will be discussed.

Entomopathogenic Bacteria

CB 008 DELTA ENDOTOXIN STRUCTURE AND TOXIN SPECIFICITY, Arthur I. Aronson and Dong Wu, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907
A clone of the *Bacillus thuringiensis* (*B.t.*) subsp. *kurstaki* HD73 protoxin gene in *E. coli* did not have the selectivity for *Heliothis virescens* over *Trichoplusia ni* found for crystals isolated from the *B.t.* strain of origin. Protoxin from kurstaki HD73 solubilized in the presence of mercaptoethanol had also lost the selectivity in contrast to protoxin solubilized under nonreducing conditions. Given the reducing condition in *E. coli* cells, it is likely that the protoxin produced from the cloned gene was in the sulfhydryl form. Removal of the cysteine residues by converting protoxin to toxin with trypsin or with subclones encoding only the toxin portion resulted in selectivity for *H. virescens*, so while disulfides are not essential for toxin specificity, they do play a role in protoxins. Another possible function for disulfides is in conferring stability to certain protoxins. A plasmid-cured derivative of *B.t.* subsp. *kurstaki* HD1 containing only a single protoxin gene on a 45 MD plasmid was conditional for protoxin accumulation due to the instability of this species of protoxin in cells grown at temperatures greater than 25°C. In contrast, the immediate parent of this cured strain, which contains three protoxin genes, produced stable inclusions at all temperatures containing predominantly this species of protoxin. The synthesis of a second protoxin in this strain, even though at lesser amounts, somehow stabilized the inclusions, possibly by the formation of heterodimers. The extensive similarity of the cysteine-rich carboxyl halves of many 130-140 kd protoxins (cryA gene products) may permit disulfide-linked heterodimer formation. Since many *B.t.* subspecies contain multiple protoxin genes, heterodimer formation may be important for stability and perhaps novel specificities. Toxins *per se* are contained within the amino half of protoxin molecules and have some conserved hydrophobic domains as well as highly variable regions. The latter presumably contribute to specificity whereas the former may be involved in the interaction with larval cell membranes. Both regions have been extensively altered by employing mutagenic oligonucleotides. After eliminating clones containing nonsense or frameshift mutations, others were sequenced at random. About 25% contained at least one amino acid change and these were analyzed for protoxin stability as well as LD_{50} values for *M. sexta*, *T. ni* and *H. virescens*. Those with alterations in stability or specificity have been found among toxins with single amino acid changes in one ten-amino acid stretch in a variable region of the *B.t.* subsp. *kurstaki* HD73 toxin. Some assessment of the importance of certain regions within the toxin for stability, specificity or toxicity is now possible.

CB 009 MICROBIAL CONTROL OF VECTOR INSECTS, Elizabeth W. Davidson, Department of Zoology, Arizona State University, Tempe, AZ 85287-1501.
Microbial control of vectors moved dramatically from the status of possibility to operational fact with the discovery and development of Bacillus thuringiensis var. israelensis (BTI or BT H-14). This organism exhibits a high level of insecticidal activity for larvae of mosquitoes and black flies, and has little or no activity toward nontarget insects or other organisms. The activity of BTI against vectors which are resistant to chemical pesticides and its environmental safety are among its most attractive features. Products based on BTI have proven very useful against black fly vectors in the West African Onchocerciasis Programme, against mosquito larvae in the Upper Rhine Valley of West Germany, and in a number of mosquito and black fly abatement programs in North America and elsewhere.

Several other microorganisms with promising activity toward vector insects are currently under study. These include another sporeforming bacterium, Bacillus sphaericus, and several fungi, nematodes, and protozoa. The problems limiting development of these organisms include inadequate techniques for mass production, long term storage, formulation, and delivery into the aquatic environment. The potential benefits of the use of microbials for control of public health disease vectors are great; reduced reliance on and exposure to chemical insecticides, avoidance of widespread insecticide resistance, little environmental alteration, and the availability of a number of ecologically diverse organisms with a variety of modes of action.

CB 010 ALTERNATIVE HOSTS FOR BACILLUS THURINGIENSIS DELTA-ENDOTOXIN, Malcolm Finlayson and Frank Gaertner, Deptartment of Biochemistry and Molecular Genetics, Mycogen Corporation, San Diego, CA 92121.

Bacillus thuringiensis (BT) is widely used as a microbial insecticide for control of lepidopteran, coleopteran and dipteran pests. The active component has been identified as a parasporal protein also termed a crystal that is released along with a spore upon lysis of the BT during stationary phase. In general, agricultural application is limited to the use of formulated spore-crystal mixtures. These preparations are not persistant, degrading within one to three days following application. This appears to be a consequence of a number of factors including cycles in temperature and moisture, proteolytic activity, photooxidation, chemical interactions, and microbial activity. We have developed a novel insecticide delivery system (MCAP™) that adresses these concerns by effectively micro-encapsulating the insecticidal protein within a stabilized Pseudomonas fluorescens (PF) cell. Biotoxin genes isolated from BT are introduced into PF with the appropriate vector. The biotoxin expressed in PF forms a crystalline array similar to that seen in BT with expression levels up to 30%. Unlike BT, the cells of PF do not lyse during stationary growth and PF cells do not form a spore. A chemical fixative is added to the complete fermentation broth to rapidly kill the biotoxin containing PF and to simultaneously stabilize the cells (MCAP). This stabilization process strengthens the cell wall by cross-linking and inactivates biotoxin degrading proteolytic enzymes. The process results in an active, stable biotoxin encapsulted within a non-viable cell. The MCAP cells exhibit enhanced field persistance and are environmentally acceptable as the microorganism will not spread from the site of application. The MCAP delivery system is potentially applicable to a variety insecticidal proteins.

CB 011 INSECT RESISTANCE TO BACILLUS THURINGIENSIS DELTA-ENDOTOXIN, Wm. H. McGaughey, U.S. Grain Marketing Research Laboratory, ARS, USDA, 1515 College Avenue, Manhattan, KS 66502.

Plodia interpunctella, a major lepidopteran pest of stored grain and cereal products, is the first insect species found to develop resistance to the delta-endotoxin of Bacillus thuringiensis. Under laboratory selection pressure using a commercial formulation of the HD-1 strain of B.t., resistance progressed rapidly to very high levels in five insect strains collected from different regions of the United States. Only very low levels of resistance have been observed to date under field conditions, but field studies have been limited. The resistance may be due primarily to a single gene and appears to be inherited as a recessive trait. Resistance is stable when selection pressure is discontinued. All of the resistant Plodia strains are resistant to both spores and crystals, but remain susceptible to beta-exotoxin. They are cross-resistant to many but not all isolates of B.t. The physiological mechanism(s) of the resistance has not been elucidated. Potential mechanisms are discussed, as well as the practical implications of B.t. resistance in stored grain and other pest management programs, including those involving gene transfer technologies.

Antagonism Exterior to the Plant

CB 012 MYCOPARASITISM: RECOGNITION PHYSIOLOGY AND ECOLOGY. Ilan Chet. Hebrew University, Otto Warburg Center for Biotechnology. Faculty of Agriculture, Rehovot 76100 Israel.

Mycoparasitism occurs in different groups of fungi. Trichoderma spp is one of the most common mycoparasites. It attacks several pathogenic fungi in soil and favors low pH. The minimal population level to be effective was found to be 10^4 cfu/gr soil. Studying the mechanism involved in reduction of disease caused by Sclerotium rolfsii and Rhizoctonia solani, revealed that the mycoparasite apparently detects its host from some distance and a chemotropic growth can be observed. The second step in this process is the rather specific "recognition" of the host by the mycoparasite Trichoderma harzianum. Rhizoctonia solani produces a L-fucose specific agglutinin which agglutinates cells of Escherichia coli B. The agglutination was inhibited by L-fucose, L-galactose and their derivatives. A correlation was found between this inhibition by (-methyl L-fucoside, and the prevention of Trichoderma coiling around its host. Sclerotium rolfsii produces a lectin specific to D-glucose and D-mannose which can agglutinate several kinds of bacteria. The ability of different isolates of Trichoderma to attack S. rolfsii was correlated with the agglutination of Trichoderma conidia by S. rolfsii lectin. This may indicate the possible role of lectins in the specific fungal-fungal cell interaction. (Chet 1987) Antiserum against the purified lectin was raised and the location of the lectin on the fungus surface was determined. Only after recognition the Trichoderma attaches to its host and begins to excrete extracellular lytic enzymes. These enzymes are capable of degrading the main polymers consisting the fungal cell walls such as β 1-3 glucon and chitin. Indeed the activity of chitinase as well as β 1-3 glucanase could also be detected in soil.

Chet.I. (ed) 1987. Innovation approaches to Plant Disease Control. John Wiley & Sons N.Y. pp.

CB 013 BIOCHEMICAL AND ECOLOGICAL ASPECTS OF COMPETITION IN BIOLOGICAL CONTROL.
Timothy C. Paulitz, USDA-ARS, Horticulture Crops Research Lab, 3420 NW Orchard Ave, Corvallis, OR 97330

The biological control of some plant diseases is attributed to competition between biological control agents and plant pathogens. Consistent with the role of competition in biocontrol is the observation that numerous soil-borne and aerial pathogens exist as saprophytes in nutrient-limiting environments, and require exogenous nutrients for successful pathogenesis. By depriving the pathogen of nutrients needed for germination and penetration, competitive biocontrol agents can reduce plant disease. The most essential factors are probably iron, nitrogen, and carbon. Microbial competition for iron involves siderophores, which are secondary metabolites with a high affinity for Fe^{3+} and which transport iron from the environment into the microbial cell. Siderophores produced by fluorescent pseudomonads are implicated in the biocontrol of Fusarium wilt and Pythium damping-off diseases. Competition for nitrogen appears important only when nitrogen in soils limited by microbial immobilization. Competition for carbon substrates is probably the most widespread of the three, especially in soil. Evidence for competition is supported by studies of population dynamics, by addition of carbon to remove the limitation, by measurements of carbon in soil, and by measurements of respiration and biomass. In studies where mutants of biocontrol agents deficient in the production of antimicrobial compounds still give a degree of control, competition has been proposed as a mechanism by default. Competition for carbon might explain the biological control of Pythium damping-off by Pythium nunn and by fluorescent pseudomonads and the suppression of pathogenic isolates of Fusarium oxysporum by saprophytic isolates. Despite this literature, competition is still the least understood mechanism implicated in biological control. With the exception of iron competition, little is known about the physiological, biochemical and molecular basis of competitive interactions. Competition is probably not the sole mechanism in some biocontrol systems, as mycoparasitism and antibiosis may act in conjunction with competition. In the future, molecular techniques might be used to develop methodologies to determine the importance of competition in biological control. The wider use of mathematical tools, such as replacement series, to describe competition in natural environments might also be useful. A greater understanding of competition will facilitate the selection and enhancement of competitive biocontrol agents.

CB 014 ANTIBIOTICS: EVIDENCE FOR THEIR OPERATION AND SITES WHERE THEY MIGHT BE PRODUCED,
David M. Weller and Linda S. Thomashow, USDA-ARS Root Disease and Biological Control Research Unit, Pullman, WA 99164.

Antibiotics are microbially produced, low molecular weight organic compounds that inhibit the growth or metabolism of other microorganisms (1). Several lines of indirect evidence support antibiotic production as a mechanism of disease suppression by some biocontrol agents (2). Many microorganisms that provide biological control produce antibiotics effective against target pathogens in vitro. Some of these antibiotics when purified and applied to plants duplicate the disease suppression provided by the biocontrol agent. Thus, pyoluteorin and pyrrolnitrin produced by Pseudomonas fluorescens Pf-5 provided the same protection of cotton against damping-off caused by Pythium ultimum or Rhizoctonia solani as did the bacterium. Some antibiotic-producing biocontrol agents are more suppressive of disease than their nonproducing mutant derivatives. For example, Tn5 mutants of P. fluorescens 2-79 deficient in the production of the antibiotic phenazine-1-carboxylate were significantly less suppressive of take-all of wheat, caused by Gaeumannomyces graminis var. tritici, than the parental strain; a mutant of P. fluorescens Hv37a, deficient in the production of an antifungal compound, was less effective than the parental strain in protecting cotton against Pythium ultimum. A β-galactosidase gene fusion into one of the genes responsible for antibiotic synthesis in Hv37a showed that the gene was expressed in the cotton spermosphere. Although this indirect evidence is very supportive of a role for antibiotics in biological control, direct evidence for the presence of antibiotics in natural soil has been lacking (3). However, we have recently isolated phenazine-1-carboxylate from the rhizosphere of wheat colonized by P. fluorescens 2-79 and grown in a growth chamber or in the field in natural soil. The presence of the antibiotic was associated with protection of wheat against take-all. These results provide direct evidence that antibiotics are produced in the rhizosphere and substantiate their importance in biological control.

1. Fravel, D. R. 1988. Annu. Rev. Phytopathol. 26:75-91.
2. Weller, D. M. 1988. Annu. Rev. Phytopathol. 26:379-407.
3. Williams, S. T. and Vickers, J. C. 1986. Microb. Ecol. 12:43-52.

Fungi in Biological Control

CB 015 FUNGI AS NATURALLY OCCURRING ENTOMOPATHOGENS, Raymond I. Carruthers, U.S. Department of Agriculture; Agricultural Research Service; Plant Protection Research Unit; Federal Plant, Soil and Nutrition Laboratory; Cornell University, Ithaca, NY 14853 --

Entomopathogenic fungi are known from practically all insect taxa and are commonly seen causing extensive epizootics in nature. They are know from most habitats including: aquatic, terrestrial, subterranean and foliar environments. Fungi are unique as insect pathogens, as they infect their hosts through the cuticle rather than per os as do bacteria, viruses and protozoa. This mode of entry allows fungi to infect insects with sucking mouthparts (aphids, leafhoppers, planthoppers and their allies), a resource from which other pathogen groups have essentially been excluded. Insect pathogenic fungi grow vegetatively as hyphae or protoplasts in the haemocoele of their host and induce mortality either through the production of toxins or by digesting away critical host tissues. Disease incubation is temperature-dependent, with host death typically occurring within a few days of infection. Soon after host death, fungal spores are formed and released into the environment where they act either as dispersal and infection agents or as resistant structures capable of withstanding harsh environmental conditions. Depending on the exact life-cycle of the pathogen, monocyclic or polycyclic infection processes partially regulate disease development and spread in host populations. In many situations, fungal pathogens cycle through several generations during a single generation of its host. Secondary infection of the host populations is extremely important in the development of disease epizootics. Fungi are thought to be limited as insect pathogens primarily by their necessity for high moisture levels for spore germination and infection. Although free water is typically required for spore germination, even under very dry conditions some microhabitats have been shown to provide enough moisture to allow infection and even epizootics to occur. Many other factors are important in understanding disease dynamics and include host and pathogen interactions, abiotic and biotic effects on these populations and the temporal, spatial and genetic characteristics of both the host and pathogen population. Fungi as natural biological control agents are known from many different environments including the managed ecosystems of agriculture, rangeland and forestry. The use of fungi for biological control has been furthered by introduction of exotic species and enhancement of native species using many different tactics, yet there is still a tremendous knowledge gap that must be bridged before fungi can be routinely used in pest management systems. Biotechnology may provide some of the tools necessary for expanding the use of fungi for biological control by helping researchers solve some of the many problems still limiting their controlled use.

CB 016 FUNGI AS ANTAGONISTS, J.M. Lynch, AFRC Institute of Horticultural Research, Littlehampton, West Sussex, BN17 6LP, UK

A substantial literature has accumulated indicating that a wide range of fungi can antagonise plant pathogens in vitro and in vivo. Such antagonism can occur by the production of antibiotic metabolites or cell wall-degrading enzymes. However the ecological success of the antagonist on the plant can also be governed by its ability to colonize and utilise substrates on plant surfaces, allowing it to compete effectively with pathogens. Such actions of antagonists on pathogens are not necessarily mutually exclusive and successful antagonists may exhibit more than one action. However, the lack of understanding of the mode(s) of action of angatonists is limiting their exploitation as biological control agents because: (1) it is difficult to optimise their action phenotypically or genotypically, (2) formulation can be more difficult to prescribe, and (3) regulatory authorities can justifiably present more obstacles to their registration for on-farm use irrespective of whether the strains have been derived by genetic engineering. The state of knowledge for bacteria as antagonists is better. This has allowed, for example, the failure of the Agrobacterium system because of transfer of the bacteriocin-coding plasmid from the antagonist to the pathogen to be overcome by producing a strain with a deficient plasmid transfer system. Analysis of opportunities for gaining a better understanding of fungal systems will be presented with emphasis on Trichoderma spp. as antagonists. Different strains of Trichoderma spp. can act against a very wide range of pathogens by the production of antibiotics and cell-wall-degrading enzymes as well as being good competitors in the rhizosphere. They can also decrease the susceptibility of host plants to pathogens by increasing plant vigour. Novel methods, including the use of polyclonal antisera have been used to investigate population dynamics of introduced organisms. Trichoderma spp., and closely related Gliocladium spp., are ideal targets for a concerted international effort at the molecular, physiological and ecological levels to bring credibility to the potential of fungi as antagonists generally.

CB 017 FUNGI AS MICROBIAL INSECTICIDES. Clayton W. McCoy, University of Florida, Institute of Food and Agricultural Sciences, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850
About 750 fungal species representing 56 genera are known parasites or pathogens to arthropod pests inhabiting terrestrial and aquatic ecosystems. Some entomopathogenic fungi contribute significantly to seasonal natural mortality; mycoses sometimes being so severe to eliminate a host population in a given habitat. Observations of fungal epizootics have been a motivating force in research and development of some fungal species as microbial control agents. This is even true in view of limiting biological features such as microclimate dependency and slow mode of action via germinating spores.

Ten fungal genera, all amenable to semisolid fermentation, have been used in large-scale mass production by industry and governmental agents in different parts of the world. Projects, focusing on the control of specific pests, supported by governmental agencies of various countries have been relatively successful. Two fungal species have been commercially produced, formulated and approved by regulatory agencies as registered microbial pesticides. None are available today! Numerous pitfalls unique to the genetic characteristics of each fungal pathotype, their in vitro production, formulation and field application have been identified in the development of these organisms as microbial control agents. In addition, industry has established overall criterion to evaluate fungi as potential candidates. Research progress relating to some key factors important to the utilization of fungi as microbial insecticides will be addressed in light of past failures.

Parasitoid-Host Interaction

CB 018 PARASITOID EFFECTS ON HOST DEVELOPMENT, Nancy E. Beckage, USDA/ARS and Department of Entomology, University of Wisconsin, Madison, WI 53706. Parasitism drastically alters the developmental fate and reproductive capacity of host insects by a variety of mechanisms. Ectoparasites inject venoms that either induce immediate paralysis and cessation of feeding (e.g. Bracon), or act more subtly to inhibit the onset of subsequent larval molts, as does Euplectrus. Endoparasites likewise induce an array of developmental abnormalities including an arrestment of embryogenesis, precocious or suppressed metamorphosis, and in social insects, the formation of intersexes and intercastes. In adult female insects, parasitism interferes with oocyte maturation via inhibition of vitellogenin biosynthesis or its uptake by oocytes, which may or may not be linked to hormonal factors depending upon the species. Metabolic changes occur to facilitate transfer of nutrients from host to parasite, and to some extent developmental disruption may be attributed to derangements in metabolism and behavior, i.e. the decreased rate of feeding observed in many parasitized insects. The agents causing the observed developmental alterations are hypothesized to include parasite polydnaviruses, non-paralyzing venoms, and teratocytes, as well as the developing endoparasites and their secretions. Parasite polydnavirus genes are rapidly expressed once transferred to the host, thus generating the hypothesis that their action is required for successful infection and "redirection" of the host. In Heliothis virescens larvae parasitized by Campoletis sonorensis, viral mRNAs appear within a few hours after parasitization, and in Manduca sexta parasitized by Cotesia congregata, new proteins are produced. Endoparasite venoms appear to facilitate uptake of polydnavirus particles by host cells but may or may not be required for expression of viral genes. In fifth instar H. virescens, C. sonorensis virus induces degeneration of host prothoracic glands, but earlier stages appear refractory and the glands remain intact. A variety of other parasites, including leishmania and amoebae, have viral or extrachromosomal DNAs associated with them, suggesting their occurrence may be common. Entomopathogenic nematodes are similarly associated with third party elements - bacteria - that elicit fatal septicemia and arrest of the host while serving as a food source for the parasites. Thus, a variety of strategies are exploited by parasites to regulate host development.

CB 019 PHYSIOLOGICAL INTERACATIONS BETWEEN PARASITOIDS AND HOSTS AND THEIR INFLUENCE ON REPRODUCTIVE STRATEGIES, Michael R. Strand, Department of Entomology, University of Wisconsin, Madison, WI 53706
Two important features of progeny allocation by parasitoids is the clutch and sex ratio to produce for each host attacked. While much of the interest in parasitoid clutch and sex ratio evolution has been in a theoretical context, both factors are relevent to several applied problems in biological control. An evolutionary perspective to progeny allocation has improved worker's understanding of this variable life history character, but physiological and developmental data are also important to understanding progeny allocation because they define the parameters, limitations and constraints involved in the clutch and sex ratio biology of particular parasitoid species. In this presentation I will illustrate the importance of developmental considerations in understanding progeny allocation patterns by contrasting the reproductive strategies of mono- and polyembryonic parasitoids. For monoembryonic species such as the braconid larval parasitoid *Bracon hebetor* and scelionid egg parasitoid *Telenomus heliothidis* shifts in clutch size and sex ratio are due to female wasps facultatively adjusting the number of eggs they lay and fertilize on hosts of differing quality. Understanding the mechanisms by which females perceive host quality is critical to being able to manipulate progeny production for biological control. For polyembryonic species such as the encyrtid *Copidosoma floridanum*, individual eggs mitotically divide to produce multiple progeny. Several thousand genetically identical progeny may be produced from a single polyembryonic egg. Development of polyembryonic wasp broods are highly synchronized with host development, and host endocrine factors directly or indirectly appear to regulate wasp development. Thus, the number of parasitoid embryos per host and, in effect, clutch size are determined by the interactions between host and parasitoid progeny development.

CB 020 MOLECULAR BIOLOGY OF THE CAMPOLETIS SONORENSIS POLYDNAVIRUS IN HOST-PARASITE RELATIONSHIPS. M.D. Summers, Department of Entomology, Texas A&M University, College Station, TX 77843.

The molecular biology of the polydnavirus of Campoletis sonorensis and its relationship to the parasitic wasp host and host insect, Heliothis virescens, is the most thoroughly studied of this family of unique insect DNA viruses. The segmented DNA genome consists of at least 28 double-stranded covalently closed DNAs, each with superhelical configuration. The expression of transcripts has been shown to be organized in a multipartite motif within the multiple SH-DNAs. The virus replicates in the calyx cells of the female wasp reproductive system and is transmitted to the lepidopteran host along with the parasitic egg. The virus does not apparently replicate in the parasitized host of Heliothis virescens but several viral genes are expressed. The virus is required for the successful development of the endo-parasite. The physical organization of the multipartite viral DNA genome and genes that are expressed in the wasp and parasitized insect will be described. The viral genome is also apparently integrated into the chromosomal DNA of every male and female wasp.

Biological Control of Foliar Pathogens and Weeds

CB 021 USE OF ALLELOPATHY AS A BIOLOGICAL WEED CONTROL STRATEGY. Alan R. Putnam, Department of Horticulture, Michigan State University, East Lansing, MI 48824.

Plants exert interference against neighboring plants through competition for resources and through chemical influences generally referred to as allelopathy. In addition, plant residues exert selective phytotoxic action on succeeding plants. The objective of our work has been to use allelopathic attributes of plants in an integrated weed control approach.

One approach has been to screen for allelopathic types in germplasm collections of crops, the idea being to ultimately transfer this character into cultivars by either conventional breeding or other genetic transfer techniques. Superior weed suppressing types have been reported from searches of cucumber, oat, sunflower, and soybean collections. When thoroughly researched, this idea may have potential for crop plants that are maintained in high density monocultures i.e. turf grasses, forage grasses, or legumes.

Another approach has been to utilize allelopathic rotational crops or companion plants in annual or perennial cropping systems. Living rye (Secale cereale L.) and its residues have been shown to provide nearly complete suppression of a variety of agroecosystem weeds. Similarly, residues of sorghums, barley, wheat, and oats can provide exceptional suppression of certain weed species. Rye residues are known to suppress weeds through release of two hydroxamic acids and transformation of these compounds to more phytotoxic compounds by soil microbes.

CB 022 THE PHYLLOPLANE, Harvey W. Spurr, Jr., Crops Research Laboratory, USDA-ARS, P. O. Box 1555, Oxford, NC 27565. What is the phylloplane? It is the leaf surface of plants surrounded by a microenvironment called the phyllosphere wherein dynamic biological interactions occur. It is on the phylloplane where many pathogens begin the infection process, where insects may establish, where dew and frost form, where nitrogen may be fixed, where bacteria and fungi reside, where pollen and dust collect, where pollution and acid rain react, and where leaf hair exudates stick. The interactions are driven by factors such as temperature and humidity and altered by mass introductions of new microorganisms deposited from the air. The microorganisms per square centimeter of leaf surface vary with conditions and may range from 1,000 to 1,000,000 or more. Usually, less than five percent of this population is comprised of pathogens. In addition to microorganisms, numerous leaf surface chemicals stimulate or inhibit pathogen growth. Biological control of foliar pathogens depends on a sufficient knowledge of interactions on the phylloplane to enable the development of management strategies. Studies of the introduction and survival of microorganisms on the phylloplane have demonstrated the practical potential for controlling disease, reducing frost injury and fixing nitrogen for plant nutrition. A detailed review of biological disease control on the phylloplane is found in the following publication.

Windels, C. E. and Lindow, S. E. 1985. Biological Control on the Phylloplane. The American Phytopathological Society, St. Paul, MN. 169 pp.

CB 023 MYCOHERBICIDES, George E. Templeton and Dana K. Heiny, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.
Interest in research and development of indigenous fungal pathogens as mycoherbicides is increasing at an accelerating pace for several reasons. Increasing costs of chemical herbicide research and development, persistent lack of confidence in synthetic chemicals by a discerning society and presence of successful commercial examples have been major motivators. Four mycoherbicides are available for commercial use and five are in advanced stages of development. Only naturally occurring strains of fungi have been tested for mycoherbicide potential and only a meager portion of those found have been deemed promising enough to seek regulatory approval. Ideal weed targets are species derived from transient weed communities in temporate regions that have passed through one or more genetic bottlenecks while becoming weedy. Ideal fungal pathogens are those whose capacity to disseminate is their single most important epidemiological constraint. Important barriers to commercialization include absence of spore production and formulation technology, loss of virulence in culture, innate low virulence, fastidious environmental requirements for spore germination, host penetration or disease development, and excessively narrow host-range with consequent low market potential or excessively broad host-range with non-target hosts of economic or ecological importance. Several advances have been made in the technology of solid state spore production and formulation of dry and wet spore preparations. These include spore production on mycelium in trays or gel encapsulated mycelium, and formulation as dry mixes with inert carriers, or as wet preparations containing antibiotic or fungistatic compounds. Efforts to overcome innate biological deficiencies of weed pathogens with genetic modification have been modest and DNA transformations have yet to yield improved strains. In most cases, lack of fundamental knowledge of disease at the molecular level limits application of recombinant DNA techniques to enhancement of mycoherbicide effectiveness.

Obligate Pathogens of Insects

CB 024 POLYMORPHIC MICROSPORIDIA OF MOSQUITOES: POTENTIAL FOR BIOLOGICAL CONTROL, Theodore G. Andreadis, Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, CT 06504
Microsporidia belonging to the family Amblyosporidae are a large group of obligate intracellular parasites that infect a wide variety of mosquitoes in nature. To date, they have been isolated from more than 100 different host species in 10 genera from 5 continents. These parasites are highly specialized and possess complex life cycles involving polymorphism with the formation of several different spore types, aspects of transovarial and horizontal transmission, well synchronized development with the host, and in some species, obligatory development in an intermediate copepod host. Most species cause delayed density dependent mortality and kill their respective hosts slowly, by destroying the normal functions of organs and/or by depleting the host of essential reserves. Some species may additionally depress adult fecundity. They have high rates of direct reproduction within the host and rely heavily on the biotic environment for their survival and dispersal. These characteristics facilitate parasite persistence within a mosquito population by ensuring that they will not cause their own extinction and are compatible with other natural regulatory processes. Most, but not all isolates presently appear to be host specific. The levels of infection observed in the field have mostly been low (usually no more than 1%), however, some species do produce seasonal epizootics (with up to 100% infection) and help to regulate mosquito populations in nature. Few, if any species hold much promise as agents for immediate reduction of mosquito populations via inundative releases. Their greatest potential lies in: (1) periodic inoculative releases of established species to increase infection rates and/or augment a weak link in the transmission cycle, (2) environmental manipulation and conservation to improve the effectiveness of naturally occurring species, (3) applied epizootiology and the recognition of seasonal epizootics, and (4) the introduction and permanent establishment of exotic species with wide host ranges through inoculative releases. In addition to mass culture techniques and continued life cycle studies, we need more foreign exploration and basic epizootiological studies to identify the various environmental factors that play a dominant role in the ecology of these microsporidian parasites.

CB 025 NEW DIRECTIONS FOR INSECT CONTROL WITH BACULOVIRUSES,
James R. Fuxa, Department of Entomology, Louisiana State University, Baton Rouge, LA 70803.
There have been four approaches to suppression of insect pest populations with baculoviruses: inundative augmentation of the virus, inoculative augmentation, introduction-establishment, and environmental manipulation. There have been 12 successful cases of introduction-establishment, seven of inoculative augmentation, five of unknown types of augmentation, one of environmental manipulation, and none with inundative augmentation. The reasons for success or failure relate to the type of habitat, whether the attempt is made in a developing or industrial nation, biological and ecological factors, economics, and the level of population suppression that must be achieved. Future success will depend heavily on choosing the appropriate approach to control in each host-virus system with particular attention to the peculiarities of each ecosystem. In this regard, the factors that must be evaluated will include the speed of kill by the virus, host specificity relative to pest complexes, production costs, viral persistence in storage and in the environment, virulence, efficiency of transmission, dependence of viral population dynamics on the density of the host population (including host population growth characteristics), frequency of natural epizootics, and the pest's economic injury level. The best chance for successful biological control in the near future with natural isolates of virus will be through introduction-establishment in certain situations, including unstable agroecosystems. Inoculative augmentation also has promise, particularly through production by cottage industry for niche markets. Inundative augmentation is unlikely to be successful except perhaps in developing nations. Genetic engineering is most likely to contribute to the success of the two types of augmentation in the near future and has almost unlimited potential to contribute significantly to all approaches, particularly introduction-establishment, in the more distant future.

CB 026 PROTOZOA, J. E. Henry, USDA/ARS and Entomology Research Laboratory, Montana State University, Bozeman, MT 59717.Most of the entomophilic protozoa occur in the phyla Apicomplexa and Microspora with fewer in Sarcomastogophora. All types of symbiotic relationships occur between insects and protozoa which indicates a long evolutionary coexistence. Accordingly, relatively few species of protozoa function as highly virulent pathogens to their insect hosts. Generally protozoa, and particularly the Microsporida, cause debilitative diseases that result in subtle regulation of host populations. From the point of applied insect control, protozoa function more in the manner of classical biological control agents than as biological insecticides which suggests potential for introduction or augmentation of microbial agents. Exploration and introduction of exotic parasite protozoa should be emphasized. Genetic selection and alteration of isolates for increased efficacy is a viable approach, as is the concept of new associations. Overall there are many protozoa that could be manipulated in some manner to increase their activity against noxious insects.

CB 027 ENTOMOPATHOGENIC NEMATODES IN BIOLOGICAL CONTROL OF INSECTS, Harry K. Kaya, Department of Nematology, University of California, Davis, CA 95616

Entomopathogenic nematodes in the genera Steinernema and Heterorhabditis occur naturally in soil and are logical biological control agents for many soil-inhabiting insect pests. These nematodes possess a wide host range and are motile, attracted to their insect hosts, easy to mass produce and apply, and exempted from registration by the Environmental Protection Agency. Although the nematodes have many positive attributes, results from field trials against a number of coleopterous and lepidopterous soil insects have been inconsistent (1). Soil factors such as water potential, texture, porosity, alkalinity, salinity, presence of roots, and temperature may affect the nematodes' ability to find their insect host and account for the inconsistencies (2). Moreover, effective field persistence of these nematodes is relatively short, lasting no more than a few weeks. This lack of persistence has been attributed to a number of abiotic factors such as insufficient moisture, ultraviolet light, temperature extremes, and pesticides, or biotic factors such as predators, pathogens, competitors, and absence of suitable hosts.

Our laboratory is investigating biotic factors affecting survival of the nematode's infective stage in the soil. Studies include the recycling ability of the nematode in the presence and absence of a host, the impact of interspecific competition between heterorhabditid and steinernematid nematodes or between a nematode and another biotic agent for a common insect resource, and the effect of nematophagous fungi on nematode persistence. These studies have demonstrated that some biotic factors play a major role in nematode persistence in the field.

1. Kaya, H. K. 1985. Entomogenous nematodes for insect control in IPM systems, pages 283-302 in Biological Control in Agricultural IPM Systems, M. A. Hoy and D. C. Herzog (eds.), Academic Press, New York. N.Y.
2. Akhurst, R. A. 1986. Controlling insects in soil with entomopathogenic nematodes, pages 265-267 in Fundamentals and Applied Aspects of Invertebrate Pathology, R. A. Samson, J. M. Vlak, and D. Peters (eds.), Foundation of the Fourth International Colloquium of Invertebrate Pathology, Wageningen, The Netherlands.

Application of Strategies for Enhancement - I

CB 028 ENHANCING EFFICIENCIES OF BIOCONTROL AGENTS BY USE OF BIOTECHNOLOGY, Neal Gutterson, Advanced Genetic Sciences, 6701 San Pablo Avenue, Oakland, California 94608

The tools of biotechnology may be used to improve performance of biocontrol agents. However, major advancements are still needed before the full benefits of this approach will be seen. Focusing on the control of soilborne fungal pathogens, issues and approaches for efficiency enhancement will be presented.

Enhancement in two general properties is being approached through genetic technologies: rhizosphere competence and stress tolerance, elaboration of antifungal substances. In most cases the genetic bases of these properties is unknown, making direct approaches to strain improvement difficult.

Genetics of the biosynthesis of fungal inhibitory substances by fluorescent pseudomonads has received the most intensive effort. In one example, that of Pseudomonas fluorescens strain Hv37a, genes for biosynthesis of the disease suppressive antibiotic oomycin A have been cloned, and regulation of antibiotic biosynthesis has been altered. The impact of altered regulation on strain performance is being studied.

One of the most significant uses of biotechnology will be the development of genetic resources to control useful properties. Specific tools have been developed, such as transposon mutagenesis in pseudomonads and transcriptional fusions using reporter genes, which facilitate isolation of useful genes and understanding mechanisms of action. Appropriate assays and strategies which enable the isolation of genes determining root colonization and stress tolerance must be developed in the future.

CB 029 GENETIC APPROACHES TOWARDS STUDYING RHIZOSPHERE COMPETENCE, Stephen T. Lam, Daniel M. Ellis, James M. Ligon, and Nancy R. Torkewitz, CIBA-Geigy Biotechnology Research, P.O.Box 12257, RTP, NC 27709.

Colonization of plant roots by bacteria is a complex process and likely involves many bacterial traits, most of which are unknown. We have initiated studies to identify bacterial genes which play significant roles in this process. A Pseudomonas-wheat system is being used as the model. The general approach involves the use of transposons to construct collections of insertion mutants, each of which is then screened for alterations in their interactions with the host plant.
In one example, a Tn*5* derivative which carries a constitutively expressed beta-galactosidase (*lacZ*) gene was used to generate a collection of insertion mutants, all of which can now be distinguished from the wild-type parent on X-gal plates. Each mutant was examined for its ability to colonize wheat seedlings in the presence of the wild-type parent (competitive colonization assay). Roughly equal mixtures of mutant and wild-type bacteria were used to inoculate wheat seeds, which were then allowed to germinate. Five days later, bacteria were recovered from roots of the seedlings and the ratio of wild-type to mutant bacteria were determined. Competition defective mutants which gave wild-type : mutant ratio of 20:1 or greater were examined further. The competitive colonization assay is also being used to determine the relative competitiveness of different bacterial isolates and the factors involved.
In a second example, a Tn*5* derivative which carries a promoterless *lacZ* gene located near one end of the transposon was constructed. Expression of the *lacZ* gene depends on the presence of an active promoter outside of the transposon in the correct orientation. Insertion mutants generated with this transposon were examined for changes in beta-galactosidase expression in the presence and absence of plant root exudate. A number of mutants which showed differential *lacZ* expression have been identified and are being characterized. Bacterial genes which respond to plant root exudate may play significant roles in colonization. Characterization of such genes will contribute to the understanding of the process.

CB 030 MOLECULAR AND BIOCHEMICAL BASES FOR ACTIVITIES OF BIOLOGICAL CONTROL AGENTS, Joyce E. Loper, USDA-ARS, 3420 N.W. Orchard Ave., Corvallis, OR 97330

The central challenge of mechanistic studies in biocontrol is to bridge the conceptual and technical chasm separating the laboratory and the field. Molecular approaches have had a primary role in enabling scientists to meet this challenge. The application of such approaches has evolved during the past decade from the first use of chemically-induced mutants of biocontrol agents to the present use of reporter gene systems for detecting the activity of biocontrol genes *in situ*. Certain mechanisms underlying biocontrol have been elucidated primarily because of advances in the molecular biology of biocontrol organisms. Examples include mechanisms based on preemptive exclusion of epiphytic phytopathogenic or ice nucleation active bacteria, and on production of metabolites with activity against bacterial or fungal phytopathogens. Many of these metabolites are readily detected and quantified in culture, but are difficult to detect and quantify in natural environments. Indirect approaches, such as the construction of mutant strains impaired in metabolite production, have been useful in assessing the production and possible role of such compounds in natural habitats.

Field variability associated with biological control strategies remains the single greatest obstacle limiting their agronomic application. This variability may result from any factor influencing either the population size of a biocontrol agent or its expression of biocontrol activity. Identification of the key characteristics contributing to the activity of a biocontrol agent and of chemical and physical factors determining the expression of such characteristics may enable scientists to predict the range of expected efficacy for a given biocontrol agent. The availability of reporter gene systems, in which a phenotype that is readily detected and quantified in nature is placed under the regulatory control of a gene involved in biocontrol, now allows us to identify those conditions which limit expression of biocontrol genes *in situ*.

The potential enhancement of biocontrol agents with recombinant DNA technology is the hope and expectation of biocontrol proponents. However, the identification of genes determining biocontrol activity is a prerequisite to genetic enhancement. To date, the immense contribution of recombinant DNA technology to biocontrol has been in basic research, in identifying such genes and providing scientists the tools to bridge the gap between the petri dish and the plant surface.

Application of Strategies for Enhancement - II

CB 031 SPECIAL PROBLEMS ASSOCIATED WITH AQUATIC WEED CONTROL, Raghavan Charudattan, James T. DeValerio, and Joan D. Prange, Plant Pathology Department, Center for Aquatic Plant Research, University of Florida, Gainesville, FL 32611.

From an ecological perspective the presence of a balanced and diversified assemblage of aquatic macrophyte species is preferable to the total absence of macrophytes or the existence of one or two dominant species at nuisance levels. The latter tend to be exotic, invasive species, and for practical reasons it has been justifiable to seek microbial controls only for those species and not for the varied native species that may occasionally become problematic due to man-induced changes in their niches. However, invasive aquatic macrophytes have proved to be more difficult to control with pathogens than terrestrial weeds primarily because of specific attributes that make these plants successful invaders in the first place, and due to public preferences in aquatic weed control. In nature, there is a scarcity of virulent and destructive pathogens on these aggressive plants, perhaps a reason for their invasiveness. The high rates of indeterminate vegetative growth enable them to escape disease pressure through compensatory mechanisms. There are technical limitations to producing, applying, and maintaining efficacy of inoculum. User demand for quick and complete weed elimination usually limits the choice of control options to chemical herbicides. The paucity of information on the etiology and epidemiology of diseases of submerged plants and the concerns about possible adverse side-effects caused by using inundative doses of microbial inoculum on underwater plants also compound the problems. Despite these limitations, we have made considerable progress towards mycoherbicidal control of the emergent weed Eichhornia crassipes (waterhyacinth) with Cercospora rodmanii. This indigenous mycoherbicide candidate, which yields practical levels of control when used in combination with insect biocontrol agents or chemical plant growth retardants, has undergone extensive field testing and is awaiting commercial development. Similar attempts at microbial control of the submerged weeds Hydrilla verticillata (hydrilla) and Myriophyllum spicatum (eurasian watermilfoil) are in progress. Our results with Fusarium culmorum, a promising mycoherbicidal candidate for hydrilla, will be discussed to illustrate the underwater pathosystem involved. Results from ongoing research on genetic engineering of host specific microorganisms for production of herbicidal metabolites, enhancement of efficacy of mycoherbicides through adjuvants or synergists, and field trials with selected pathogens will be presented to define potential solutions to some of these problems.

CB 032 FORMULATION OF BIOCONTROL AGENTS FOR USE IN PLANT PATHOLOGY, William J. Connick, Jr., SRRC, USDA-ARS, P.O. Box 19687, New Orleans, LA 70179; Jack A. Lewis, BARC-W, USDA-ARS, Beltsville, MD 20705; and Paul C. Quimby, Jr., SWSL, USDA-ARS, P.O. Box 350, Stoneville, MS 38776

Numerous organisms with potential to be biological control agents are discovered each year. Formulating these organisms effectively will be the key to their successful use. Because these organisms are living, they must be carefully handled in order to maintain viability throughout processing, storage, and application. Unlike chemical pesticides that begin to degrade after application, biocontrol agents must survive and begin to proliferate. This paper will review experimental and commercial formulations of microbial agents that have been used in plant pathology. These include formulations of fungal pathogens of weeds (mycoherbicides), weed parasitic nematodes, and plant disease antagonists. The versatile alginate process, invert emulsions, and other formulation techniques will be discussed.

Development of Biocontrol Agents for Agriculture

CB 033 GENETIC IMPROVEMENT OF ARTHROPOD NATURAL ENEMIES, Marjorie A. Hoy, Department of Entomological Sciences, 201 Wellman Hall, University of California, Berkeley, CA 94720

Genetic improvement of arthropod natural enemies is an old concept, but only recently has a genetically-manipulated natural enemy been implemented in an agricultural cropping system. Genetic manipulation projects are based on the assumptions that traits suitable for manipulation can be identified, and genetically-manipulated beneficial species can be more effective in artificial agroecosystems. Genetic improvement of arthropod natural enemies has received serious attention only within the past few years. Projects involving a diverse array of species are currently underway in laboratories around the world. A review of these present and past genetic improvement projects indicates that a number of questions have been answered, but more remain. Critical issues remaining include how to identify species suitable for genetic manipulation. What attribute(s) should be manipulated? How were they identified? What is the most appropriate improvement method for a specific project: selection, hybridization, or recombinant DNA methods? Should mutagenesis be employed to develop the desired variability? Can traits determined by multiple genes be manipulated successfully? What is the success rate? Is the manipulated strain of high quality? Has it been evaluated in the laboratory, greenhouse, or field? What fitness attributes should be evaluated? Are they predictive of field success? Is the natural enemy being released inoculatively or inundatively? How will the strains be implemented? What are the economic benefits of the improved strains? The potential role that recombinant DNA techniques may play in genetic manipulation of arthropod natural enemies remains to be determined. Recombinant DNA methods could improve efficiency of genetic manipulation should it be possible to insert cloned genes into an array of beneficial species. This could reduce the time involved in conducting surveys for variability and conducting selections. Because many beneficial species are difficult to rear in the laboratory, such a technique could make genetic manipulation feasible for a wider array of species. Methods for transformation remain to be developed, however; inserted genes must be shown to be expressed appropriately, be expressed in a stable fashion, and have little impact on fitness. Regulatory issues surrounding releases of arthropods that have been genetically manipulated through recombinant DNA techniques remain to be resolved.

CB 034 NUTRITIONAL CONSIDERATIONS IN THE PROPAGATION OF ENTOMOPHAGOUS SPECIES. S. N. Thompson, Division of Biological Control, University of California, Riverside, California, U.S.A., 92521. **The nutritional and dietary requirements of entomophagous insects are summarized. The role of supplemental adult feeding in biological control is discussed. The present status of in vitro culture and development of continuous artificial mass culture are outlined. The importance of quality control and the potential of genetic manipulation in in vitro culture are emphasized.**

The Take-Home Lessons

CB 035 CROP MANAGEMENT SYSTEMS AND THEIR EFFECTS ON BIOLOGICAL CONTROL OF INSECTS, Jerry L. Stimac, Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611
The primary objective of crop management systems is to produce net profit, not to merely to manage populations of insects and other pest species through use of biological control tactics. Yet, biological contol of insects and other pest species can play an important role in pest management if crop systems are engineered in ways which allow biological agents to realize their potential without major interferences from other control tactics (chemical pesticides) and other crop production practices (cultivation and irrigation). To accomplish this goal, crop management system models which incorporate the effects of biological control agents in the context of the crop production system can be constructed and computer simulation can be used to evaluate alternative crop production strategies. Computer simulation offers a means to explore a large number of combinations of biological control agents and predict how they might perform in the crop system environment. Also, simulation models may be used to evaluate the characteristics that biological agents should have to be successful in the crop system environment, thus the models might be used to help identify the goals of genetic engineering of biological control agents. The use of two highly technological tools, computer simulation and genetic engineering, could provide new and unique opportunities for biological control experimentation and perhaps allow the agricultural research community to move from classical biocontrol into the new age of biological control. Some of the challenges and obstacles we will face in attempting to incorporate the effects of biocontrol agents into crop system models are identified and discussed. A serious challange will be to gain a better understanding of how crop management systems effect biological control of insects and others pests.

CB 036 PLANT MANAGEMENT IN RELATION TO BIOLOGICAL CONTROL OF PATHOGENS, R. James Cook, USDA-ARS, Root Disease and Biological Control Research Unit, Pullman, WA 99164
Plant pathogens are vulnerable to biological control at each step of the disease process, including 1) during infection, 2) during take-over or destruction of the host-plant cell or tissue, and 3) during reproduction or saprophotrophy on or within the diseased or dead host. Since the plant is involved either passively or actively in each of these episodes, plant management can be critical to the success of any attempt at biological control. The approach may involve the use of A) nonpathogenic epiphytic or endophytic microorganisms supported by the plant and antagonistic to the pathogen during infection, disease progress, or reproduction/saprophotrophy and B) defense (resistance) mechanisms under direct genetic control of the plant. Typically, plants have been managed to maximize biological control by "self defense," achieved through cultural practices and conventional breeding. These approaches have led to disease suppression through relief of predisposing environmental and nutritional stresses and introduction of genes for disease resistance. Fusarium foot rot of wheat caused by F. culmorum has come under biological control in the Pacific Northwest through a combination of cultural practices and better-adapted wheat cultivars that minimized plant water stress and permitted expression of the natural (constitutive) resistance of wheats to this disease. Cultural practices and conventional breeding continue to offer opportunities for large gains in biological control of pathogens responsible for stress-related diseases, especially as we learn more about plant genotype x environment interactions and the biological limits of agroecosystems. There is evidence that plants can also be managed culturally or modified genetically to make better use of biological control by the plant-associated microorganisms. Thus, some wheat cultivars are significantly more supportive than others of fluorescent pseudomonads introduced for defense of wheat roots against take-all caused by Gaeumannomyces graminis. Research is needed to understand the genetic, molecular, and nutritional basis for plant-microbe associations having potential for biological control. Future gains through introduction of genes for disease resistance will depend increasingly on recombinant DNA technology because the usual sources of resistant germplasm used in conventional breeding are now nearly all in use. As other approaches, today's cultivars with resistance to only certain biotypes of the pathogen can be grown as mixtures or in sequences, or genes for resistance can be pyramided by breeding so as to regulate the pathogen population (or the mixture of virulent biotypes of the pathogen population) at or below some economic threshold.

Molecular Biology and Biological Control

CB 100 CLONING, SEQUENCING AND EXPRESSION OF THE TOXIN GENES OF *BACILLUS SPHAERICUS*. Colin Berry, Coreen Oei, John Hindley, Jeannette Jackson-Yap, Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511.
The toxin genes from several strains of *B. sphaericus* have been cloned in pUC plasmids and transformed into *E. coli*. Sequencing of these cloned genes has revealed a high degree of sequence conservation between the genes of highly toxic strains of *B. sphaericus*. Experiments are being undertaken to over-express in *E. coli* both the 51 kDa and 42 kDa proteins (identified in the work of Baumann *et al.*) from *B. sphaericus* 2297 in order to clarify the role of both proteins in toxicity.

CB 101 PGPR STRAIN IDENTIFICATION BY RESTRICTION FRAGMENT LENGTH POLYMORPHISMS, G. Brown, Z. Khan, H. Guilmette, R. Lifshitz, and J. Kloepper, Agricultural Microbiology, Allelix Inc., 6850 Goreway Dr., Mississauga, Ontario, Canada, L4V 1P1. Plant growth-promoting rhizobacteria (PGPR) are receiving increased attention for both biocontrol of plant pathogenic organisms and enhanced growth and development of young crop species. Limited routine laboratory procedures for the identification of specific, beneficial strains and the considerable potential for their genetic manipulation, necessitates development of DNA-based identification systems. Partially digested genomic DNA from Pseudomonas putida strain GR12-2 was cloned into the vector pTZ18R. Recombinants were screened for their ability to hybridize with genomic DNA from related Pseudomonas spp. and Serratia spp. Southern analysis with one clone, containing an 8 kb insert from GR12-2 (pAM141), clearly distinguished between restricted genomic DNA from nine strains of Pseudomonas putida, four strains of P. fluorescens, four strains of Serratia liquefaciens and one unidentified Pseudomonas sp.. Strain-ID cards have been prepared for 18 in-house PGPR strains using the restriction enzymes Eco RI, Pst I and Pvu II and the clone pAM141. Comparisons will be made with similar preparations from plant pathogenic Pseudomonas spp. strains. RFLP maps of the ATCC strains and pAM141 will be made available for use in the development of a universal RFLP-based PGPR catalogue.

CB 102 FURTHER STUDIES ON THE MODE OF ACTION OF THE VENOM FROM THE ECTOPARASITOID EUPLECTRUS PLATHYPENAE (HYMENOPTERA: EULOPHIDAE), Thomas A. Coudron, USDA, ARS, BCIRL, P.O. Box 7629, Columbia, MO 65205-5001, USA.
The molting process in lepidopteran hosts is arrested after parasitism by Euplectrus plathypenae. An active substance has been located in: a) hemolymph of parasitized hosts; b) a gland/reservoir complex isolated from the female ectoparasitoid; and c) in crystalline structures found within the gland/reservoir complex. Hemolymph of parasitized host insects, extracts of the tissues containing the active substance and solutions of the crystalline material have been tested on larvae of Trichoplusia ni (Lepidoptera: Noctuidae). Dose-response and inactivation measurements were determined. Treated larvae responded to physiological doses and the substance remained active within the hemolymph of the parasitized larvae for several days. Treatment of parasitized larvae with exogenous juvenile hormone, 20-hydroxyecdysone, and several hormone analogs did not restore the normal molting process.

CB 103 CHARACTERS OF MICROORGANISMS UTILIZING CROWN-GALL OPINES: IMPLICATIONS FOR THE BIOLOGY AND CONTROL OF AGROBACTERIUM, P. Dion,[1] C. S. Nautiyal,[1] , C. Beauchamp,[1] and W. S. Chilton,[2] (1) Département de phytologie, FSAA, Université Laval, Québec, Canada G1K 7P4, (2) Department of Botany, North Carolina State University, Raleigh, NC 27695-7612.

Microorganisms capable of opine catabolism were isolated from pear crown-gall tumors, soil, or potato tubers. Most were biotype 1 or 2 agrobacteria, fluorescent or nonfluorescent pseudomonads, or else coryneform bacteria. Fungi identified as Cylindrocarpon heteronema and Fusarium dimerum utilized succinamopine or mannopine as the sole carbon source. The bacterial isolates obtained on octopine were fluorescent pseudomonads. Use of mannopine as the selective substrate yielded mainly agrobacteria and coryneform bacteria; the few pseudomonads which grew on this opine were nonfluorescent. Of the 30 agrobacterial, opine-utilizing isolates, none was tumorigenic. Whereas these agrobacteria, but not the other Gram-negative isolates, hybridized to probes for Agrobacterium chromosomal genes involved in virulence (chvA and chvB genes), no isolate exhibited homology to Ti plasmid-encoded virulence genes (virB and vir D). Although none of the opine substrates considered here is specific for Agrobacterium, succinamopine and mannopine are more specific for agrobacteria than octopine or nopaline. Oncogenicity is an uncommon character, even among opine-utilizing agrobacteria. This suggests that efficient biocontrol of crown-gall by Agrobacterium radiobacter strain K84 is based on virtually absolute exclusion of the pathogen from the plant rhizosphere.

CB 104 SEMI-PERMISSIVE REPLICATION OF A NUCLEAR POLYHEDROSIS VIRUS OF THE ALFALFA LOOPER AUTOGRAPHA CALIFORNICA (AcNPV) IN A FAT BODY CELL LINE (IPLB-Ld-FB) OF THE GYPSY MOTH LYMANTRIA DISPAR, E. M. Dougherty[1], D. Guzo[1], K. Shields[2], D. E. Lynn[1] and S. Braun[1], IPL, ARS, USDA, Beltsville, Md.[1] and FS USDA, Hamden, Ct.[2]

The host range of insect viruses can be studied under in vitro conditions when appropriate tissue specific cell lines exist. Although AcNPV does not readily infect L. dispar larvae via the oral route, AcNPV injected into the larval hemocoel is able to initiate infection of several larval tissues (i.e., hemocytes and epidermis). Pathological conditions are evident in several other tissues (fat body and midgut) although there is an absence of both occluded and non-occluded forms of progeny virus. The L. dispar fat body cell line IPLB-Ld-FB was subsequently utilized to investigate the molecular pathology of AcNPV infection in the L. dispar fat body. Overall, synchronous AcNPV infection induced changes in IPLB-Ld-FB cells which reflected events occurring in infected L. dispar fat body tissue in vivo. No progeny virus were present by 96 hr as determined by end point dilution (budded virus assay) or phase and electron microscopies (polyhedral inclusion body assay). Dot-blot hybridization of DNA extracted from AcNPV infected fat body cells with ^{32}P nick translated AcNPV DNA probes revealed virus specific DNA synthesis originating at 1-4 hrs PI and increasing in intensity until 8-12 hrs. Protein synthesis profiles of AcNPV infected IPLB-Ld-FB cells were also novel as determined by SDS-PAGE analysis of ^{35}S methionine pulse labeled cells. Synthesis of a virus specific polypeptide of 38k was initiated at 4-8 hr PI and reached maximum expression at 12-16 hr. Quantitative changes in several cellular proteins were observed. By 48 hr a total cessation of both host and viral protein synthesis occurred.

CB 105 VIRULENCE INHIBITION FACTORS ASSOCIATED WITH CHESTNUT RECOVERY IN MICHIGAN, Christine M. Durbahn and Dennis W. Fulbright, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

Double-stranded RNA (dsRNA) molecules in hypovirulent strains of Endothia parasitica function as "virulence inhibition factors" (VIFs). Hypovirulent strains are associated with chestnut trees recovering from chestnut blight in Michigan and Italy. The dsRNA molecules are frequently found as multi-segmented genomes upon polyacrylamide gel electrophoresis. Little is known about the role the various dsRNA segments play in virulence reduction or how they interact. Fungal isolates with multiple segments of dsRNA may be infected with one multi-segmented VIF, two or more single-genomic VIFs or both. To determine if dsRNA segments from various VIFs can reassort to form a new VIF, two dsRNA segments from a hypovirulent strain were transferred to a recipient hypovirulent strain with three dsRNA segments. Asexual segregation patterns showed that the dsRNA segments from each hypovirulent strain assorted independently. In another experiment, variants of a hypovirulent isolate in which dsRNA segments were lost or altered in size were compared in virulence to determine which segment of the VIF carried the "virulence inhibiting gene" (VIG).

CB 106 **DIFFERENTIAL EXPRESSION OF THE BACILLUS THURINGIENSIS SUBSP. KURSTAKI HD1 δ-ENDOTOXIN GENES.** Martin Geiser and Helen M.Parkinson, Department of Biotechnology, CIBA-GEIGY Ltd, K 681.4.46, P.O.Box, CH-4000 Basel (Switzerland)

Bacillus thuringiensis (B.t.) subsp. kurstaki is an entomopathogenic gram positive microorganism. Three genes coding for the toxic proteins have been identified, cloned and sequenced. The promoters for the different genes are identical in their nucleotide sequences up to 156 base pairs upstream the initiation codon. SDS-polyacrylamide gel electrophoresis analysis of the protein crystals obtained in the cells after sporulation shows that the relative amount of the individual proteins can differ in function of the cell growth conditions. We wondered whether this observation could also be confirmed at the RNA level by using oligonucleotides specific for each of the three mRNAs. In our studies we demonstrated transcriptional differential regulation of the single genes in B.t. subsp. kurstaki HD1. We have constructed and introduced into B.t. a fusion between the protoxin promoter and the β-galactosidase gene and have demonstrated that the β-galactosidase activity is sporulation-dependent. This reporter gene will allow us to identify putative regulation factors affecting the expression of the δ-endotoxin genes.

CB 107 ENZYMATIC HOST/PATHOGEN INTERACTIONS OF SICKLEPOD (*CASSIA OBTUSIFOLIA*) AND *ALTERNARIA CASSIAE*, Robert E. Hoagland, USDA, Southern Weed Science Laboratory, Stoneville, MS 38756

Various enzymes have been implicated to play major roles in infectivity mechanisms and in plant defense responses against pathogens. To examine these mechanisms applied to weed biocontrol, extractable activities of a proteolytic enzyme (leucine-p-nitroanilide), peroxidase (guaiacol-H_2O_2), and β-glucosidase (*p*-nitrophenyl-β-glucopyranoside) were examined in extracts of sicklepod (*Cassia obtusifolia*) infected with the pathogenic fungus (*Alternaria cassiae*). Seeds were germinated in paper toweling and grown hydroponically in continuous darkness. Four days after planting, seedlings were inoculated with *A. cassiae* spores and plants were returned to darkness. Enzyme activities were monitored in extracts of untreated and inoculated plant over a 96 h dark period. β-Glucosidase (U/g fr wt) was not appreciably different in control and infected seedlings throughout the time course. Peroxidase activity (U/g fr wt) was greater in infected seedlings at nearly all sample times, and highest activity was found early in the time course (i.e. 3-fold above control level 17 h after inoculation). Proteolytic activity (U/g fr wt) increased with time in uninfected seedlings until peak activity at 45 h, after which activity declined. Proteolytic activity in infected seedlings peaked at about 30 h and then was significantly reduced (20%) compared to control throughout the remaining time course. Results suggest that in dark-grown plants, *A. cassiae* (rather than the host plant) produces a proteolytic enzyme inhibitor and that peroxidase may in some way be involved in host plant resistance.

CB 108 AEROBACTIN PRODUCTION BY A STRAIN OF ERWINIA CAROTOVORA SUBSP. CAROTOVORA, C.A. Ishimaru and J.E. Loper, USDA, ARS Horticultural Crops Research Laboratory, Corvallis, OR 97330

Soft rots, caused by Erwinia carotovora subsp. carotovora, occur worldwide resulting in serious losses as seedling diseases in the field and as post-harvest diseases in storage. Certain Pseudomonas fluorescens strains are known to control both the seedling and post-harvest phases of these diseases, presumably by iron-starvation due to the production of fluorescent siderophores. However, the possible role of siderophore production by E. carotovora subsp. carotovora in biological control has not been explored. Aerobactin is a hydroxamate siderophore produced by certain members of the Enterobacteriaceae and is one of several virulence factors in Escherichia coli. Pathogenic strains of E. carotovora were screened for aerobactin production by bioassay and by homology to aerobactin biosynthesis genes of E. coli. Of 23 strains tested, E. carotovora subsp. carotovora CC105 provided iron to an E. coli mutant (LG1522) that grows under iron-limiting conditions only when given exogenous sources of aerobactin. The genes encoding for aerobactin synthesis were cloned from a cosmid library of CC105 constructed in pLAFR3. Subcloning of these cosmids has defined a 6 kb region of DNA which contains the aerobactin synthesis genes of CC105. Aerobactin production by soft rot erwiniae is probably not common nor is it a requirement for pathogenicity. However, cloning and subsequent inactivation of aerobactin synthesis genes will aid in determining the role of this siderophore in biological control and disease progress of soft rot erwiniae.

CB 109 ANTICARSIA GEMMATALIS BACULOVIRUS REPLICATION IN CELL CULTURES AND MOLECULAR ANALYSIS OF THE VIRAL DNA, James E. Maruniak and David W. Johnson, Entomology, IFAS 0711, University of Florida, Gainesville, FL 32611; Peggy J. Sieburth, ARS, USDA, ABADRL, Box 3965, University Station, Laramie, WY 82071 .
The velvetbean caterpillar, A. gemmatalis is a serious pest in soybeans in the southeastern United States and in South America. The A. gemmatalis baculovirus (AgNPV) is an effective biological pesticide against larvae when applied at a proper dose and time. To characterize this virus we developed a cell line from A. gemmatalis embryos. The cell line was characterized for doubling times (5.9-6.7 d). Restriction enzyme profiles of cell and larvae mitochondrial DNAs were indistinguishable. The cell line, UFL-AG-286, was susceptible to AgNPV. AgNPV extracellular virus titer peaked to 10^6 $TCID_{50}$ at 3 d postinfection and polyhedra were also forming at this time in UFL-AG-286 cells. The wild type virus was plaque-purified 4 times and the predominant genotype was used to construct a physical map of the viral DNA. Restriction fragments were ordered for BamHl, BglII, EcoRl and HindIII for a total of 51 restriction sites. DNA-DNA homology between Autographa californica DPV and AgNPV was used to orient the polyhedrin gene on the physical map.

CB 110 INSECT RESISTANCE IN PLANTS BY TRANSFORMATION WITH BACILLUS THURINGIENSIS GENES, Marnix Peferoen, Henk Joos, Plant Genetic Systems, J. Plateaustraat 22, 9000 Gent, Belgium. One of the most interesting applications of the plant transformation technology is the engineering of insect resistance through expression of Bacillus thuringiensis (B.t.) insecticidal proteins. From all different B.t. insecticidal proteins, three major pathotypes have been described : strains toxic to Lepidoptera, Diptera and Coleoptera. Within the group of Lepidoptera toxins, different types can be discriminated. There is a good correlation between a certain crystal protein type and its toxicity for some Lepidoptera species. It is essential to select for each crop, the B.t. toxin with the best activity against its major insect pests. We selected a B.t. toxin, Bt2, which is active against Lepidoptera such as Manduca sexta and Heliothis virescens, both pests on tobacco. Chimaeric genes were constructed with the entire Bt2 coding sequence or with truncated bt2 genes, fused to the neo gene, a selectable marker gene. These chimaeric genes were transferred to tobacco plants by leaf disk infection with the Agrobacterium tumefaciens vector system and shoots were selected with kanamycin. Plants transformed with the truncated bt2 genes expressed higher levels of Bt2 toxin and proved to be highly toxic to Manduca sexta larvae. The insecticidal trait is stably inherited and several rounds of field trials showed protection of transformed tobacco against feeding damage by Lepidoptera larvae. The transformation experiments with tobacco have been repeated with tomato and potato plants, resulting in plants resistant to both Lepidoptera. These results clearly exemplify the feasibiltiy of using genetic engineering techniques to generate plants resistant to certain insect pests. Transfer of different Bacillus thuringiensis genes into a whole range of crops and vegetables may provide agriculture with a new and environmentally superior method of controlling destructive insect pests.

CB 111 ROLE OF VIRUS-LIKE PARTICLES IN INSECT PARASITOID-HOST INTERACTION Otto Schmidt, Institut für Biologie III, Schänzlestr. 1, D-7800 Freiburg, West-Germany. In a hymenopteran parasitoid Venturia canescens virus-like particles are found on the egg surface, which are responsible for the protection of the parasitoid against the encapsulation reaction of the host Ephestia kühniella. Some of the particle proteins are structurally and probably functionally related to a host protein (p42), which appears to play an important role in insect immune system. The p42-protein is found in hemolymph and appears to accumulate in the basal lamina of the fat body. The protein is induced to higher levels of synthesis by a number of treatments, including wounding or osmotic changes in hemolymph of the larva. The most drastic increase of protein synthesis is observed after bacterial infection. The p42-protein in Ephestia corresponds to the P4-protein in Hyalophora cecropia, a protein induced together with antibacterial proteins.

CB 112 Efficient Transformation of Bacillus thuringiensis and Bacillus cereus via Electroporation: Transformation of Acrystalliferous Strains with a Cloned Delta-Endotoxin Gene. Walter Schurter, Martin Geiser and Danièle Mathé, Biotechnology Department, CIBA-GEIGY Ltd., 4002 Basel (Switzerland)
Electroporation was used as a method to transform intact cells of B. thuringiensis and B. cereus. With our optimized method a range of plasmid vectors could be transformed into strains of B. thuringiensis at frequencies of up to 10^7 transformants per µg DNA. This high transformation frequency allows cloning experiments to be done directly in B. thuringiensis. A bifunctional vector capable of replicating in E. coli as well as in Bacillus spec. was constructed. The kurhd1 protoxin gene was cloned into this shuttle vector to produce plasmid pK93 and transformed into B. thuringiensis HD1 cryB and B. cereus 569 K. The cloned protoxin gene was expressed in sporulating cultures of both strain HD1 cryB (pK93) and 569 K (pK93) producing crystal protein active in biotests against larvae of Heliothis virescens. This example shows the usefulness of the electroporation method for the introduction of cloned toxin genes, either in their native form or modified, into a variety of host strains.

CB 113 PLANT-CONTROLLED ATTRACTION AND ACTIVATION OF PROTECTIVE MICROBIAL INOCULANTS. Charles H. Shaw, Catherine Lilley, Alison M. Ashby and Martin D. Watson. Department of Biological Sciences, University of Durham, Durham, DH1 3LE, England.
Plant exuded phenolic compounds have a dual effect upon *Agrobacterium tumefaciens*: at low concntrations they trigger chemotaxis; and at higher concentrations effect *vir* gene induction. For both processes *virA* & G are required. A pesticidal protein is placed under Ti-plasmid *vir*-promoter control, on a broad host range plasmid carrying *virA* & G, and transferred to avirulent *A.tumefaciens*. This organism is chemotactically attracted towards wounded plant cells, and at the wound site expresses the pesticidal protein. During the crucial post-wounding phase this will protect the plant from pathogen attack. Pesticide production is controlled and localised, avoiding an energy drain on the bacterium. Engineering a plant-associated bacterium to confer pesticidal properties, will allow protection of monocots, which at present is difficult by direct gene transfer to plants.

CB 114 TRANSFORMATION OF THE ENTOMOPATHOGENIC FUNGUS, METARHIZIUM ANISOPLIAE USING THE benA3 GENE FROM ASPERGILLUS NIDULANS, Mark S. Goettel, Raymond J. St.Leger, Srirama Bhairi, Donald W. Roberts, and Richard C. Staples. Boyce Thompson Institute, Cornell University, Tower Road, Ithaca, NY 14853.
Mycelial cultures of the insect pathogenic hyphomycete, M. anisopliae, currently used for biological control of insects, were transformed to benomyl resistance using the pBENA3 plasmid supplied by Drs. Beryl Oakley and K. Jung of The Ohio State University. Protoplasts were prepared from mycelia grown in shake cultures by digestion with 0.8% Novozym 234 in 1.2 M sorbitol, mixed with the pBENA3 plasmid (50 µg in 100 µl Tris-EDTA), and incubated on ice for 30 min. PEG 3000 (50 µl of 60% PEG) was then added and incubated on ice for 10 min, 1 ml more of PEG was added, and after 10 min incubation at rt, the protoplasts were diluted in 4 ml of regeneration medium (1.2 M sorbitol, 0.05% $MgSO_4$, 0.3% $NaNO_3$, 0.1% K_2HPO_4 in 10 mM Tris, pH 7.5), and mixed with 10 ml of molten regeneration medium and incubated for 18 h at 27°C. The plates were then overlayered with 10 ml of agar-solidified regeneration medium containing 10 µg/ml benomyl. Benomyl-resistant colonies appeared after 12 to 23 days of incubation at 27°C. These were then subcultured onto Czapek-Dox agar containing 2.5 µg/ml benomyl. The transformation rate was 9 transformants/50 µg DNA/2 x 10^6 viable protoplasts. To test for mitotic stability, three transformants were selected randomly and grown on benomyl-free medium for successive generations. All single-spore isolates from the transformants were still resistant after 15 successive passages on benomyl-free media. Southern hybridization studies demonstrated that the plasmid was stably integrated into the genome of the transformants.

CB 115 BIOCONTROL OF FUSARIUM OXYSPORUM WITH A CHITINASE ENCODING GENE FROM SERRATIA MARCESCENS ON A STABLE PLASMID IN PSEUDOMONAS. Leif Sundheim, Norwegian Plant Protection Institute, Department of Plant Pathology, 1432 Ås-NLH, Norway
A chitinase encoding gene was cloned from the soil bacterium Serratia marcescens in the cosmid vector pLAFR3. The chitinase gene was subcloned into the plasmid pHI1, which is a derivative of the plasmid pRSF1010 with kanamycin resistance. By conjugational transfer the construct was transferred to a Pseudomonas sp.. The transconjugant expressed chitinase activity on a double layer chitin medium in vitro and the plasmid remained stable in the Pseudomonas sp.. In biocontrol experiments the plant pathogenic fungus Fusarium oxysporum was controlled by Pseudomonas sp. containing the construct.

CB 116 ISOLATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST *Campoletis sonorensis* VENOM GLAND PROTEINS. Bruce A. Webb and Max D. Summers, Department of Entomology, Texas A&M University, College Station, TX 77840. The role of the venom gland in hymenopteran endoparasites is currently unclear. In some endoparasite species the venom gland is apparently essential for endoparasite survival while in others such as *Campoletis sonorensis* surgical removal of the venom gland does not alter the viability of the endoparasite. To characterize the role, if any, of the venom gland in this system, monoclonal antibodies against soluble venom gland proteins have been produced, identified and characterized. A class of monoclonal antibodies has been isolated which reacts with a family of immunologically related proteins in the 29-33 kD molecular weight range. These proteins are not only among the most abundant proteins in the venom gland but are also found in the soluble, non-viral, fraction of the calyx fluid. Proteins with similar molecular weights and antigenic characteristics have also been identified in gradient purified virus but it is currently unclear whether these proteins are virally encoded or are contaminants from the soluble calyx fluid. Venom gland proteins have frequently been identified and studied because of their toxic activities and functions. The presence of relatively abundant soluble proteins in the calyx fluid as well as in the venom resevior indicates that the family of proteins in the calyx may normally enhance the function of venom gland proteins and could maintain immuno-suppressive or toxic effects associated with parasitization in the surgically-induced absence of the venom gland.

Enhancing Biological Control

CB 200 ROLE OF CHEMOTAXIS IN ESTABLISHMENT OF THE RHIZOBIUM-LEGUME SYMBIOSIS Wolfgang D. Bauer, Gustavo Caetano-Anolles, Masroor Khan and Amitha Dharmatilake, Department of Agronomy, Ohio State University, Columbus, OH 43210. Non-motile and non-chemotactic mutants of rhizobia can infect and nodulate the roots of host legumes, but are at a serious competitive disadvantage in doing so. Model studies conducted in growth pouches show that chemotaxis enhances the efficiency of nodule initiation during the first few hours after inoculation by 10- to 30-fold. This can be attributed in part to enhanced contact of bacteria with the root and in part to movement on the root surface to infectible microsites. We find that the host root phenolics which serve as inducers of nodulation gene expression in R. meliloti and Bradyrhizobium japonicum are also potent and specific chemoattractants for these bacteria. Chemotactic responses to luteolin, the inducing flavonoid secreted by alfalfa roots, are maximal at 10^{-9} M, about 1000 times lower than the concentration required for maximal induction of nod gene expression Mutations in certain nod genes were found to selectively abolish chemotaxis of the bacteria to the inducing phenolics, suggesting that the primary function of these nod genes may be to implement or regulate inducer-specific chemotaxis. Our studies indicate that inducer-specific chemotaxis may be of considerable relevance to both rhizosphere colonization and specific plant-microbe interactions.

CB 202 IMPROVING THE EFFICIENCY OF *Metarhizium anisopliae* SOROKIN AS BIOCONTROL AGENT OF PASTURE SPITTLEBUGS IN CENTRAL BRAZIL. Fontes, Eliana G. Luna-Alves Lima, Elza A. Contr. Biologico, EMBRAPA/CENARGEN, CP 102372, Brasília DF 70.770, Brazil. The fungy *M. anisopliae* has been successfully used against sugarcane leaf spittlebug in Northeasthern Brazil. Its use against pasture spittlebugs, however, presents some limitations, specially due to the high genetic variability and regional specificity of this fungy. Aimming to improve the efficiency of *M.anisopliae* as biocontrol agent of *Deois flavopicta* (Homoptera: Cercopidae) in pastures of Central Brazil, basic studies with several isolates has been developed. More than fifty isolates from this region showed high uniformity regarding their morphological and genetic characteristics. The ones more pathogenic, stable and persistent against eggs and ninphs of *D.flavopicta* are being selected for field studies to determine the ecological limits of this host/parasite complex.

CB 203 ENHANCEMENT OF THE PERSISTENCE AND THE EFFICACY OF ENTOMOPARASITIC NEMATODES AGAINST *Heliothis armigera* (Lepidoptera: Noctuidae) ON THE PLANT FOLIAGE, Itamar Glazer, Dr. of Nematology and Amos Navon, Dept. of Entomology, ARO, The Volcani Center, Bet Dagan, 50250, ISRAEL. The pathogenicity of entomoparasitic nematodes from the genera *Steinernematidae* and *Heterorhabditis* against *Heliothis armigera* was tested under laboratory conditions. Complete mortality was achieved with 200 infective juveniles IJ's of *S. feltiae* All strain; the LD_{50} was 45 IJ's per insect. Similar results were obtained with other nematode strains. The youngest insect larvae were to be the most susceptible to nematode infection. Eight hours of insect exposure to the nematode, in the laboratory, were needed to induce more than 80% mortality. However, foliage application of *S. feltiae* against *H. armigera* larvae under glasshouse conditions (50-70% RH, 25°C, natural illumination) had failed due to rapid mortality of the IJ's. Nematodes survival on the foliage of bean plants was reduced to 20% within 4 h after 8 h none surivived. Under the same conditions 20% of the *S. feltiae* All strain survived for 12 h when applied in 0.1 w/v glycerol or fructose solution. A comparative bioassay of the survival of Steinernematid and Heterorhabditid nematode on foliage of bean plant under glasshouse conditions had indicated that *S. feltiae* Mexican and Pye strains had greater capability to withstand the experimental conditions than *S. feltiae* All strain. Foliage application of the *S. feltiae* Mexican strain, mixed in 0.1% w/v fructose solution resulted in 65% control of *H. armigera* larvae.

CB 204 COMPETITION FOR INFECTION SITES IN BIOCONTROL OF FUSARIUM WILT DISEASES, Qaher Mandeel, Department of Plant Pathology and Weed Science, Colorado State University, Fort Collins, CO 80523. Nonpathogenic species of Fusarium oxysporum added to soil reduced diseases induced by pathogenic formae specialis of F. oxysporum. The nonpathogens penetrated and infected roots of hosts but induced no symptoms. Methods were developed to determine the number of successful infections in roots by either pathogenic or nonpathogenic isolates. This afforded an opportunity to quantify the interactions involved in competition for infection sites which leads to biological control induced by nonpathogens.

CB 205 BIOLOGICAL CONTROL OF COTTON SEEDLING DISEASES USING ROOT COLONIZING BACTERIA, Shona E.McKnight and Stephen Rossall, Department of Physiology and Environmental Science, Nottingham University School of Agriculture, Sutton Bonington, Loughborough LE12 5RD, UK.
Potential bacterial antagonists of Pythium ultimum and Rhizoctonia solani were obtained from soil and cotton root samples collected from Texas A & M University. Pseudomonas spp. were isolated onto a selective medium (Oxoid CM559/SR102) and Bacillus sp. after heat treatment at 80°C for 10 min. In vitro activity on agar media was determined against Pythium and Rhizoctonia for these isolated bacteria, and for a novel isolate of B.subtilis. Bacterial cells were applied to cotton seed in sterile, milled peat. A range of commercially available adjuvants were tested to aid bacterial adhesion and survival in storage and to enhance the level of disease control. Growth room trials on control of the cotton seedling diseases were used to narrow the list of potential antagonists to be included in future field trials. Selected Bacillus isolates were transformed using plasmids from Staphylococcus aureus, encoding resistance to kanamycin, streptomycin and tetracycline. These marked strains were used to estimate the extent and duration of root colonization after plating out root segment homogenates onto antibiotic amended agar. The feasibility of improving control by using combinations of bacteria will be determined by using such markers.

CB 206 SIMPLE ECOLOGICAL EXPERIMENTS HELP PREDICT THE SUCCESS OF THE BIOLOGICAL CONTROL OF WEEDS, Andrew W. Sheppard, CSIRO Biological Control Unit, 335 Ave Abbé Paul Parguel, 34090 Montpellier, France.
The choice of insect or pathogen control agents for the biological control of weeds always incorporates ecological assumptions concerning limitations and regulatory mechanisms in the dynamics of weed populations, however, for a variety of reasons, these assumptions are rarely tested under field conditions. This is poor science. Such tests can be simple, and together with sensible specificity evaluation, go a long way to predicting the effectiveness of agents and enhancing the number of successful control attempts. Using examples from biological control projects against thistles, I demonstrate that well conceived and implemented field tests have a high benefit-cost ratio in both time and money and give biological control a better scientific basis.

CB 207 BIOLOGICAL CONTROL OF PHYTOPHTHORA ROOT AND CROWN ROT OF APPLE BY *TRICHODERMA* SPP. AND POTENTIAL FOR USE AGAINST OTHER PATHOGENS. V. L. Smith, W. F. Wilcox, and G. E. Harman, Depts. of Plant Pathology and Hort. Sci., N. Y. Agr. Exp. Sta., Cornell University, Geneva, 14456.
Over 70 isolates of *Trichoderma* spp. were screened for ability to reduce root and crown rot of apple caused by *Phytophthora cactorum*. Forty-four isolates from local orchard and *Aphanomyces*-suppressive pea field soil were selected for ability to grow and sporulate at 10 C, for evidence of antibiotic production against *P. cactorum*, and for tolerance to metalaxyl *in vitro*. In greenhouse trials, apple seedlings were planted into soil mix artificially infested with *P. cactorum*, both with and without the addition of individual *Trichoderma* isolates (ca 10^6 CFU/g, added as colonized peat/wheat bran); disease control was assessed after 72 hr flooding periods and subsequent infection by *P. cactorum*. Significant (P=0.01) increases in plant weight and reductions in root rot severity were obtained with 8 isolates of *Trichoderma* spp.; the remaining isolates provided moderate or no control. The population, as determined by dilution plates, in the soil mix of effective isolates of *Trichoderma* increased over the course of the greenhouse trials. Radial growth of effective isolates on corn meal agar was not influenced by metalaxyl. To determine the efficacy range of *Trichoderma* isolates most active against *P. cactorum*, isolates were tested in greenhouse trials against *Fusarium graminearum* (on wheat), *Rhizoctonia solani* (on radish) and *Sclerotium rolfsii* (on snapbean and cucumber). Isolates effective against *P. cactorum* generally did not reduce disease caused by the other pathogens, but did result in significant increases in total plant weight. Effective isolates exhibited some degree of specificity for controlling *P. cactorum* on apple seedlings. Biocontrol of *P. cactorum* on a deciduous fruit crop by *Trichoderma* spp. has not previously been demontrated.

CB 208 MECHANISM OF BIOLOGICAL CONTROL OF FIRE BLIGHT (*ERWINIA AMYLOVORA*) INFECTION OF HAWTHORN BLOSSOM WITH *ERWINIA* AND *PSEUDOMONAS* SPP. Mark Wilson, Harry A. S. Epton and David C. Sigee, Microbiology Group, Department of Cell and Structural Biology, University of Manchester, Manchester M13 9PL, U.K.
Saprophytic bacteria from the phylloplane of hawthorn were screened for their ability to reduce blossom-blight. Effective isolates were then examined to determine their mechanism of action. All *Erwinia* and *Pseudomonas* isolates effective in reducing blossom-blight produced antibiotics *in vitro* and all the *Pseudomonas* isolates produced fluorescent siderophores *in vitro*, but in neither case was there any correlation between the level of *in vitro* antagonism and *in vivo* biocontrol activity. Interactions between populations of the pathogen and biocontrol agents were studied on the stigmatic and nectarial surfaces. *Erwinia* WHL9 and *Pseudomonas* HL99 both excluded *E.amylovora* when applied to the stigma in advance of the pathogen and outcompeted *E.amylovora* when applied simultaneously with the pathogen. Light and electron microscopy and FITC-immunofluorescence microscopy showed that the biocontrol agents occupied the same site on the stigmatic surface, as did *E.amylovora* in the early stages of infection. These results indicate that site competition is probably the primary mechanism operating in this system, but that antibiosis may also play a role.

CB 209 THE POTENTIAL OF SPIDERS AS BIOLOGICAL CONTROL AGENTS IN FIELD CROPS OF THE UNITED STATES, Orrey P. Young, Southern Field Crop Insect Management Laboratory, USDA-ARS, P. O. Box 346, Stoneville, MS 38776. An analysis of 28 faunal surveys of 9 field crops indicated the occurrence of 618 species of spiders in 191 genera and 26 families. This represents 19% of the ca. 3311 species occurring in North America. Five families included 61% of the species occurring in field crops: Salticidae (90 spp.), Araneidae (79), Linyphiidae (78), Theridiidae (64) and Lycosidae (63). Cotton (308), soybean (262) and alfalfa (233) contained the most species, guar (52), rice (75) and grain sorghum (88) contained the fewest, with peanuts (131), corn (136) and sugarcane (138) intermediate. The North American spider fauna is estimated at the species level to be 59% web-spinners and 41% wanderers, whereas field crop spiders are estimated to be 44% web-spinners and 56% wanderers. These differences may be attributable to guild characteristics associated with ability to disperse to, and survive in, disturbed habitats such as field crops. Wandering spiders in general are less specialized in food choice than web-spinners, less affected by agricultural disturbances, and thus more likely to obtain food and reside in field crops. Spiders were frequently cited as the most abundant group of predators in various field crops, sometimes exceeding 90% of all predators. Other investigations cited them as occurring in very low densities. Spiders have the potential to kill many more prey than are eaten, can readily disperse into crop fields, frequently develop high densities in crop fields and in adjacent habitats, and thus have the potential to exert considerable pressure on crop pest populations both in the crop field and in adjacent habitats. Spiders are prime candidates for augmentation and/or conservation in field crops or adjacent habitats as part of a strategy to increase predation on crop pests.

Late Addition

CB 210 PRODUCTION AND FORMULATION OF PYTHIUM OLIGANDRUM INOCULA, Mark P. McQuilken, Roderic C. Cooke, Department of Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK. and John M. Whipps, Plant Pathology and Microbiology Department, AFRC IHR Littlehampton, West Sussex, BN17 6LP, UK. Small scale experiments have demonstrated conclusively that P.oligandrum is an effective antagonist of a number of damping-off, seedling-blight and other soilborne pathogens. Further advancement in the biological control research of this mycoparasite has been limited. This has been due to the lack of reliable systems for producing and formulating bulk inocula of oospores; the propagules upon which biocontrol properties depend. A study of systems for producing oospores of P.oligandrum will be described with particular reference to simple liquid and solid fermentation. Examples of the formulation of oospores and the use of these under controlled environmental conditions will be presented.

Field Tests Involving Biological Control

CB 300 FUNGICIDAL MICROORGANISMS FOR THE BIOCONTROL OF FOLIAR PATHOGENS, Raphael Hofstein, Mordechai Keren-Zur and Bertold Fridlender, FASP, Jerusalem 91042; Zahir Eyal, Tel-Aviv University; A. Steinberg and I. Chet, Faculty of Agriculture, Hebrew University, Rehovot, Israel. In vitro antagonism of leaf-rust (Puccinia recondita) and leaf blotch (Septoria tritici) was best with a strain of Pseudomonas putida (isolate 6/1) and with a strain of Agrobacterium radiobacter (isolate 2/3). A cost effective procedure was established for industrial production of all fungicidal bacteria and they were subjected to field trials. Five annual sprays were provided during the season of winter wheat (10^8 cells/ml) and significant suppression of the disease symptoms were monitored. Similarly, powdery mildew (PMD) hyperparasytic isolates of Ampelomyces quisqualis were collected. The screening gave rise to an exceptional isolate, namely AQ_{10} which is efficiently, produced by industrial submerged and semi-solid fermentation. It was used for control of PMD on cucumber, marrow and mango. In all instances, the disease symptoms were suppressed, and yields harvested throughout the season were comparable to plots treated chemically. Isolate 2/3 of A. radiobacter and AQ_{10} of A. quisqualis were then used for the control of Alternaria dauci and PMD, respectively. Alternaria symptoms were reduced by 50-70% and PMD was suppressed and entirely hyperparasitized with AQ_{10}. The annual yields were comparable to those measured in chemically treated plots (over 60MT/hectar).

CB 301 BIOCONTROL OF BARLEY POWDERY MILDEW, Inge M.B. Knudsen, Agricultural Research Department, Risø National Laboratory, DK-4000 Roskilde, Denmark

The ability of *Tilletiopsis* sp. (*Sporobolomycetaceae*) to inhibit development of the epidemic spread of barley powdery mildew (*Erysiphe graminis* f.sp. *hordei*) was investigated in a field experiment in 1988.

Barley plots were sprayed with a suspension of *Tilletiopsis* propagules in the beginning of June. Growth of *Tilletiopsis* was measured by the spore-fall method, and of powdery mildew by counting the developing colonies on the leaves. Both measurements were made weekly until the beginning of August.

Up to 87% reduction of powdery mildew colonies was obtained in the beginning to mid-July. During this period growth of *Tilletiopsis* culminated with an average of 70% of the leaves covered. The peak growth of the biocontrol organism was correlated with the rainfall.

CB 302 APPLICATION OF ENTOMOPATHOGENIC FUNGI FOR BIOLOGICAL CONTROL OF AGRICULTURAL PEST IN TAIWAN, Shan-Da Liu, Department of Plant Protection, National Pingtung Institute of Agriculture, Pingtung, Taiwan, Rep. of China. A strong virulent strain of Metarhizium anisopliae var. anisopliae (MA-1) was isolated and screened from naturally infected coconut leaf beetle, Brontispa longissima. The spray of homogenous biomass harvested from Sabouraud dextrose broth culture or granule application of fungus harvested from rice media culture to heavily infested coconut palms effectively eliminated the pest. The extension program of coconut leaf beetle biocontrol is being developed. MA-1 also showed high virulence to rhinoceros beetle, corn borer, diamond back moth and banana skipper. M. anisopliae combined with Beauveria bassiana, Nomuraea rileyi were applied to green onion successfully controlled Spodoptera exigua in fields. The evaluation of biocontrol effect of Peacelomyces javanicus, B. bassiana and MA-1 against leucaena psyllid is conducting in east coast of Taiwan. Development of fungicidal resistant strain and formulation of microbial insecticide for commercial purpose are focused on recent research works.

CB 303 FIELD PERFORMANCE OF EPICOCCUM PURPURASCENS FOR BIOLOGICAL CONTROL OF WHITE MOLD OF BEAN. R.D. Reeleder and Ting Zhou, Department of Plant Science, Macdonald College of McGill University, Ste-Anne-de-Bellevue, Quebec, Canada, H9X 1C0.
The effectiveness of Epicoccum purpurascens (EP) in control of white mold of bean, caused by Sclerotinia sclerotiorum (SS), was assessed in two field trials in 1988. Treatments were applied during the flowering period and were followed with one application of ascospores of SS (10^6 spores / ml). In the first trial, three applications of EP (10^6 spores / ml) in 1% malt extract (ME) significantly reduced disease incidence (DI), disease severity, and percentage of diseased pods. DI for the EP treatment was 42.5%, compared to 78.0% and 70.2% in control plots receiving applications of water or 1% ME, and 64.5% in plots receiving one application of iprodione (1 kg / ha). In the second trial, plots receiving three applications of EP plus ME and those treated with two applications of iprodione (1kg / ha) had significantly lower DI values (52.1% and 48.5%, respectively) than control plots (81.2% and 73.8%). Yield was significantly higher in Epicoccum and iprodione treatments. The addition of ME to EP appeared to increase the efficacity of the treatment. ME by itself, however, did not provide significant control. The mechanism of action of Epicoccum appears to be the production of compounds which reduce ascospore germination and inhibit germ tube elongation. Epicoccum was observed to aggressively colonize senescing bean flowers.

CB 304 BIOLOGICAL CONTROL OF FOLIAR DISEASES USING AN ISOLATE OF BACILLUS SUBTILIS, Stephen Rossall, Jennifer M.Browne, Shona E. McKnight and Michael G.Entwistle, Department of Physiology and Environmental Science, Nottingham University School of Agriculture, Sutton Bonington, Loughborough LE12 5RD, UK.
A strain of Bacillus subtilis, which produces a novel antifungal agent has been used in trials to assess activity against a range of pathogens. In vitro assays on agar media have shown wide spectrum activity. Extensive glasshouse trials have demonstrated effective control of Botrytis fabae infection of faba bean and shown that direct antagonism can occur on the leaf surface. Larger scale trials were carried out in a polythene tunnel, to provide high relative humidity necessary to ensure disease. Significant disease reduction occurred when Bacillus cultures were applied prophylactically or 7d after inoculation with Botrytis. A rifampicin resistant derivative of the parent bacterial strain was used to study survival on leaves; the population stabilized at about 15% of that applied. Preliminary glasshouse experiments failed to control powdery mildew infection of wheat or barley as the Bacillus inoculum alone failed to wet the leaves sufficiently. After incorporation of a range of surfactants the level of mildew infection was reduced by about 95% in glasshouse trails. Small-scale field trials on barley mildew have shown that disease reduction and associated yield benefits obtained using two applications of B.subtilis are similar to those obtained when the fungicide Bayleton is applied at the field rate.

CB 305 **BIOLOGICAL CONTROL OF POWDERY MILDEW BY INDUCED RESISTANCE UNDER FIELD CONDITIONS.** **F. Schönbeck, Ulrike Steiner and E.-C. Oerke. Institut für Pflanzenkrankheiten und Pflanzenschutz, Universität Hannover, Herrenhäuser Straße 2, 3000 Hannover, Fed. Rep. Germany.**
Microbial metabolites produced by a selected isolate of Bacillus subtilis, which induce resistance were used for biological control of powdery mildew on barley. The plant protecting effect of induced resistance and its significance for the epidemiological spreading of the pathogens were examined in field trials with various winter barley cultivars differing in disease susceptibility and productiveness. Not only the infection densities of Erysiphe graminis hordei were decreased on induced resistant plants, but pathogens especially generated less conidia per leaf and cm^2 colony area and formed even fewer cleistothecia per leaf and colony, comparable to the development on partially resistant plants. Indicating a lower degree of disease severity leaf segments of induced resistant plants showed higer photosynthesis and smaller dark respiration rates than untreated plants with equal disease level. Although protection was incomplete induced resistant plants yielded partially higher than fungicide treated ones. This derived from an additional increase of grain weight, due to an elevated starch content. The yield response of induced resistant plants to disease reduction did not fit the yield loss models derived from investigations with fungicides. Grain yields of induced resistant plants indicate an increased tolerance to powdery mildew and/or an improved yield formation.

CB 306 A PRIMARY STUDY ON BIOLOGICAL CONTROL OF RHIZOCTONIA DAMPING-OFF, ROOT AND CROWN DECAY OF SOYBEANS, Zonglin Liu and James B. Sinclair, Department of Plant Pathology, University of Illinois at Urbana-Champaign, 1102 S. Goodwin Avenue, Urbana, IL 61801-4709.
Rhizoctonia root and crown rot of soybeans [Glycine max (L.) Merr.], caused by Rhizoctonia solani Keuhn [teleomorph Thanatephorus cucumeris (Frank) Donk], is worldwide in distriubtion and occurs wherever soybeans are grown. A collection of suppressive isolates of Bacillus subtilis (Ehrenberg) Cohn was isolated from soybean leaves, stems, crowns, and root, as well as from soil from a field in soybean monoculture for over 20 years. Five isolates were obtained which were antagonistic to R. solani and not phytotoxic to soybeans. Isolate B153-2-2 showed the greatest ability to suppress R. solani both in the greenhouse and in the field. It was capable of rapid growth and rapid colonization of soybean roots and the rhizosphere either in the presence or absence of R. solani. A significant ($P=0.05$) reduction of the disease index, as well as the size and number of lesions per plant, caused by R. solani, were obtained following application of this isolate. Root volume and plant dry weight also showed increases. Field application of B153-2-2 resulted in a significantly increased seedling stand when compared with plants infected with R. solani alone. Significant stimulation of root growth and nodulation by Bradyrhizobium japonicum also was oberved in plants treated with our isolate of B. subtilis. Suppression of R. solani by our isolate of Bacillus B153-2-2 may be due to the production of antibiotic(s) by the bacterium.

CB 307 BIOLOGICAL CONTROL WITH LOW TEMPERATURE ADAPTED ISOLATES OF TRICHODERMA HARZIANUM. Arne Tronsmo. Department of Microbiology. Agricultural University of Norway. N-1432 AAS-NLH. NORWAY.

Gray mold (Botrytis cinerea) may cause serious damage on several plants by infection during the flowering period. In Norway the average temperature during flowering of some crops are around 10°C, so to be able to perform biological control in this period, isolates antagonistic at low temperature had to be used. Selection among Trichoderma spp. gave isolates from 6 species that were able to show antagonistic activities at low temperature. Some of these were tested in field trials, and they were able to control gray mold on both strawberries and apples. Further selection for fungicide resistanse, gave an isolate of Trichoderma harzianum, that performed better control than the parent strain of Botrytis cinerea on apple, both as a single biocontrol agent and in combination with low dosage of vinclozolin.

This isolate has also been used for post harvest treatment of fungal rot on cold stored carrots. In addition to reducing the damage caused by Botrytis cinerea, it also significantly redused the rot caused by Rhizoctonia carotae and Sclerotium sclerotiorum.

Late Additions

CB 400 CHARACTERIZATION OF STRAINS OF THE INSECT-PATHOGENIC FUNGUS ENTOMOPHTHORA MUSCAE, Jorgen Eilenberg*, Anne Beauvais#, Jose Bresciani* and Jean-Paul Latge#, *Dept. Zool., Royal Vet. Agric. Univ., Bulowsvej 13, DK-1870 Frb C. Denmark, #Unite de Mycol., Inst. Past., 28 Rue du Dr. Roux, 75724 Paris, France
The fungus Entomophthora muscae is a potential agent for biological control of flies. It is, however, a complex of strains or even species. Methods for characterization of seven Danish strains of E. muscae are presented: 1) host species and field ecology, 2) spore morphology in vivo, 3) isolation and growth in vitro, 4) primary spore and resting spore formation in vitro, 5) API-ZYM test of enzyme activity from protoplasts in vitro, 6) serology based on antiserum produced in a rabbit. Results were as follows: 1) Host species were pest species from the families Muscidae, Anthomyiidae and Psilidae. 2) Primary spores vary with respect to size and number of nuclei pr. spore. 3) All strains can be isolated in vitro, growth efficacy varies from strain to strain. 4) Several strains can be induced to produce both primary spores and resting spores in vitro. 5) Test of enzyme activity showed a very great similarity, strains from Anthomyiid flies do, however, also show activity of glucosaminidase. 6) Antigens originating from the various E. muscae strains were all recognized by antibodies directed against one strain (from Psilidae) using ELISA. The conclusion is that strain characterization involves use of several biological and biochemical methods.

CB 401 MECHANISMS INVOLVED IN THE ANTAGONISTIC ACTIVITY OF TALAROMYCES FLAVUS TOWARD SOILBORNE PLANT PATHOGENS, Lea Madi, Tzion Fahima and Yigal Henis, The Hebrew University of Jerusalem, Faculty of Agriculture, P.O.B. 12, Rehovot 76100, Israel. Thirteen isolates of Talaromyces flavus were examined for their capacity to produce lytic enzymes, antibiotics and toxic metabolites that might be involved in their activity as biocontrol agents of soilborne plant pathogens. Lytic enzymes found to be excreted by these strains included cellulase, β, 1-3 glucanase, and chitinase. Antifungal antibiotics produced in culture by these strains included talaron. In addition, all the isolates showed a glucose oxidase activity, which leads to the production of hydrogen peroxide as a byproduct of glucose oxidation. However, the addition of catalase to the culture filtrate resulted in a loss of only 50% of its toxic activity toward Verticillium dahliae. Transmission electron micrograghraphs of V. dahliae microsclerotia parasitized by T. flavus, revealed a unique cell-to cell invasion by the mycoparasite's hyphae, lysis taking place only at the contact sites between the host's cells and T. flavus hyphal tips. It is concluded that the antagonistic activity of T. flavus isolates toward V. dahliae is possibly due to a combined effect of lytic enzymes, antibiotics and toxic metabolites.

CB 402 DEVELOPMENT OF A HIERARCHICAL SIMULATION ENVIRONMENT FOR RESEARCH BIOLOGISTS, Timothy S. Larkin and Raymond I. Carruthers, USDA Agricultural Research Service, Federal Plant, Soil, and Nutrition Laboratory, Cornell University Ithaca, NY 14853. SERB, a Simulation Environment for Research Biologists, supports the creation of hierarchical simulation models on Symbolics computers. A graphical interface enables a researcher interactively to construct, validate, and operate models of aggregate populations simply and powerfully using both keyboard and mouse commands. SERB facilitates creation of the complex models because a SERB model is hierarchical and can imitate the structure of a biological system; and it facilitates modeling of populations because it uses components and terminology which are familiar to biologists. Simulation results are maintained in databases that can be accessed, viewed, and plotted interactively. Higher level facilities allow the user to conduct sensitivity analysis or optimization, or explore the global behavior of the system.

CB 403 INTRA AND INTERSPECIFIC TRANSFER OF ISOLATED NUCLEI IN TRICHODERMA HARZIANUM.

Alex Sivan, Gary E. Harman and Thomas E. Stasz, Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY, 14456.

Nuclei were isolated from protoplasts of an auxotrophic mutant of Trichoderma harzianum T95. Nuclei were transferred into protoplasts obtained from another auxotroph of the same strain. This nuclear transfer gave rise to stable and balanced prototrophic heterokaryons. Interstrain transfer was also done with nuclei from a wild-type T. harzianum T12 and protoplasts from a lysine deficient mutant of T95. Progeny obtained from this nuclear transfer contained 11%-17% donor type nuclei. Single spore isolates were variable in morphology and phenotype. These included strains similar to the T12 parent in isozyme phenotype and morphology, and other strains similar in all respects to the T95 recipient. From some nuclear transfers, progeny were obtained that were identical to the T95 recipient strain in isozyme phenotype but which varied from it markedly in morphological, growth and nutritional characteristics.