RECENT ADVANCES IN THE BIOLOGICAL CONTROL OF INSECT PESTS

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It is a great honor to be included among the plenary lecturers addressing the first International Plant Protection Congress of the 21st century, and I am grateful to the Secretariat and to Prof. ZHOU Da-rong for inviting me to make a presentation here today.

The word "recent" means different things to different people, but for the purposes of this address, its scope will be limited to advances reported since the 14th IPPC held in Jerusalem, Israel, during July, 1999. The postponement of the 15th IPPC from 2003 to 2004 has afforded me a number of additional developments upon which to consider reporting, but has likewise made it difficult to choose among many fine accomplishments. In this presentation, I have chosen several conceptual advances and several successful implementation projects for your consideration. In a 40-minute lecture, this gives me about 5 minutes per topic, but I won't necessarily spend that much time on each one. Instead, I will give greater attention to those for which the primary contributors were unable to attend this conference, because there will be ample opportunity to hear from those involved with others first hand.

Recent Recognition

Before I get into the main thrust of my talk, however, I would like to applaud the selection of Dr. Hans R. Herren, President of the International Association for the Plant Protection Sciences (IAPPS) and Director General of The International Centre of Insect Physiology and Ecology as a recipient of the 2003 Tyler Prize for environmental achievement. The Tyler Prize is widely recognized as "the premier award for environmental science, energy, and medicine conferring great benefit upon mankind." This award was made in recognition of Dr. Herren's contribution as leader of the team of scientists who brought about classical biological control of the exotic pest, cassava mealybug, in Africa during the 1980's through the introduction of a natural enemy, *Anagyrus lopezi,* from their common area of origin in South America. This prestigious award not only served to recognize a great scientist, but also increased the visibility of plant protection research in general and biological control of arthropod pests in particular, so in a way, it can be numbered among the recent advancements in that subject area. Please join me in recognizing Dr. Herren at this time.

Conceptual Advances

Biotechnology Related

Genetically Modified Predator.

While biotechnology has opened up a virtually limitless number of opportunities to improve and protect the world's food supply, the prime consideration is that any products developed be both effective and safe. For the most part, this research has focused on improving the crops themselves, but consideration has and is being given to improving the efficacy of biological control agents of insect pests. This necessitates evaluating the potential risk of releasing a transgenic parasitoid or predator into the environment. In order to test the feasibility of using genetic engineering to enhance the performance of arthropod predators, Dr. Marjorie Hoy and her colleagues at the University of Florida developed a transgenic strain of *Metaseiulus* (=*Typhlodromus* or *Galendromus*) *occidentalis,* a phytoseiid predator of spider mites, using the technique of maternal microinjection. Briefly, this involved trapping the mites between layers of tape and using tiny needles to inject a bacterial marker gene (lacZ) into their abdomens. The DNA was transported into the mites' eggs and some offspring then carried the gene. Iso-genetic lines of the offspring were bred to obtain lines in which all progeny carried the gene. Before the resultant transgenic strain could be released into an experimental field site on the University of Florida campus at Gainesville, extensive reviews of the request were made by a host of regulatory bodies: the Florida Biosafety Committee, Florida Department of Agriculture and Consumer Services, United States Department of Agriculture Animal and Plant Health Inspection Service, and the United States Fish and Wildlife Service. In addition, a non-transgenic strain of *M. occidentalis* had previously been released into field plots of soybean plants infested with the two-spotted spider mite, without successful establishment, indicating that there was minimal risk of permanent establishment of the genetically modified strain and that harm to the environment was extremely unlikely (McDermott and Hoy 1997). Following these reviews, releases of the transgenic strain were made at a secured area on the University of Florida campus. These releases established a precedent for releasing a transgenic arthropod, although a number of scientific, environmental, and policy issues must be settled before genetically modified beneficial arthropods can be used routinely in applied pest management programs (Hoy 2000). Nevertheless, the mere feat of passing multiple reviews and making the release constituted noteworthy first steps in developing a process that can be used to improve the efficacy of entomophagous arthropods with genetic engineering.

DNA Fingerprinting.

This is perhaps the area which has had the greatest impact on biological control of insect pests since the last congress, because of the multiplicity of techniques that have been developed and the different questions that crop protection scientists are asking. Most frequently the latter involve (1) definitive identification of natural enemies—and in some cases, the pest, (2) determination of where a pest originated and the scope of its distribution, or (3) acquisition of information on the relationships between a natural enemy and the target pest, as well as other species related to it.

By way of example, I am going to cite a number of accomplishments that were made during the course of recent work on biological control of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) [Homoptera: Aleyrodidae]. Over the past 20 years, it has become an important pest worldwide, due to its high degree of polyphagy, ability to transmit viral diseases, and elevated fecundity. During the early 1990s, an especially robust population, first noted in the U.S.A. that was shown to vector a novel array of viruses, quickly spread throughout the New World, Europe, Australia, Southeast Asia and the Pacific. It was soon named *B. tabaci* biotype-B due to esterase profiles that distinguished it from the indigenous populations (biotype-A) occurring in the southwestern U.S. and adjacent areas of Mexico, but by 1994, had been elevated to species status, as *B. argentifolii* following of allozyme and random amplified DNA – polymerase chain reaction (RAPD-PCR) analyses as well as detailed studies on its morphology, mating and viral transmission (Perring et al. 1993, Bellows et al. 1994). Whether the "new" *Bemisia* was a distinct biotype or species generated a certain amount of controversy that persists to the present. Numerous investigations have shown broad differences in many characteristics among various *B. tabaci* populations, perhaps more than might be expected within a single species. Nevertheless, establishment of a robust phylogeny has proved difficult. Independent studies on populations of *B. tabaci* from different localities in the world using mitochondrial DNA markers by Frolich et al. (1999) and ribosomal internal transcribed spacers (ITSI) by DeBarro et al. (2000) offered the initial molecular confirmation of significant genetic divergence among geographically isolated populations. Both studies pointed toward recognizing *B. tabaci* as a species complex, instead of describing each unique population as a separate species.

DNA analyses also played an important role in clarifying the taxonomic relationships among the substantial numbers of overseas collections of whitefly parasitoids made as part of an interagency biocontrol program directed at the whitefly by the U.S. Department of Agriculture's Agricultural Research Service and Animal and Plant Health Inspection Service, state agencies, and university researchers. Classical taxonomy and RAPD-DNA analyses were combined in studies on *Encarsia* and *Eretmocerus* at Mission, Texas, where the quarantine work on this project was conducted (Kirk et al. 2000, Goolsby et al. 2000). The RAPD technique turned out to be especially useful for making initial separations of different strains of *Eretmocerus* from geographically isolated areas, even when morphological characters were not available. Sequencing data have also been useful in detecting undescribed species in whitefly parasitoids that were difficult to distinguish morphologically. In addition, when molecular analyses of *Bemisia* were combined with morphological and RAPD-PCR analyses of parasitoids, it was possible to draw inferences as to which geographic areas were likely to yield the most highly adapted parasitoid strains (Kirk et al. 2000).

DNA probes are being used to monitor the presence and efficacy of natural enemies in post release research (Kirk et al. 2000). The use of DNA techniques had a major impact on the *Bemesia* biocontrol program, because it was used in so many aspects and stages: target pest identification, foreign exploration, setting release priorities, and field evaluation (Goolsby et al. 1998). In addition to RAPD-PCR, satellite DNA and amplified fragment length polymorphism (ALFP) approaches are being used to distinguish parasitized hosts and the identity of the host within, eliminating the need to rear out the parasitoids, which in some groups such as mirid plant bugs, can be a lengthy process (Tilmon et al. 2000).

Sex Determination Gene in Hymenoptera.

Male-biased sex ratios hinder applied biological control efforts using parasitic Hymenoptera, because (1) a reduced number of female founders decreases the likelihood of establishment of imported species used in classical biological control, or (2) they make the production of females too costly for economical use in an augmentative approach. One factor known to contribute to male-biased sex ratios is complementary sex determination (CSD). This system involves multiple alleles at a specific locus. Heterozygotes at this sex locus develop into females, whereas hemizygotes (haploids) and homozygotes (diploids) develop into males (Whiting 1945). Diploid males are sterile or have low fertility, and their frequency is likely to increase with small populations or inbreeding (Stouthamer et al. 1992). Using a variety of molecular techniques including chromosome walking and fine mapping, Martin Beye and colleagues located and characterized the CSD-gene in the honeybee (Beye et al. 2003). It fit the theoretical model in that it was always heterozygous in females and homozygous in males. Even though the honeybee is not a biological control agent, the discovery of the CSD gene means that biocontrol workers might be able to genetically test parasitic wasp lines before mating to ensure that fertilized eggs will end up with two different types of the CSD genes, eliminating the wasteful production of diploid males.

Theory and Practice

Native natural enemies to control exotic pests.

<u>*Hyphantria cunea* in Eurasia</u>. The great majority of classical biological control projects for insect pests have been conducted in North America (Canada and the United States), which likely has the highest number of introduced insect pest species, primarily of European origin, though we have seen a recent influx of pests from Asia. However, a number of North American species have found their way to other continents. One species of interest is the fall webworm or American white moth, *Hyphantria cunea* (Drury) [Lepidoptera: Arctiidae], which has become a problem in both Europe and Asia.

In 1999, Sergei Izhevskii and Miriam Mironova published the conclusions of their long-term studies on this species in different parts of Russia, where heretofore, it had been considered a quarantine pest, routinely receiving compulsory chemical pest control treatments. They found that the use of such treatments against this introduced species did not achieve eradication but rather resulted in the so-called "pesticide syndrome"—that is, it became necessary to apply the chemicals permanently and suffer the negative consequences. They further established that *H. cunea* entering new areas soon became prey or host of indigenous predators or parasites, respectively (Izhevskii and Mironova 1999). By replacing chemical pesticides with bacterial preparations, the complex of native natural enemies was preserved and their action reduced populations of *H. cunea* below threshold levels. This allowed land managers to break the vicious cycle caused by the "pesticide syndrome" and to eliminate *H. cunea* from the list of quarantine species. This approach also might be used to control other adventitious pests.

A somewhat different approach to the *H. cunea* problem was taken in China, where the pest was first found in Liaoning province during 1979. Because the fall webworm is very adaptable, it became resistant to chemicals, and spraying failures have been reported in some coastal areas of China. In 1984, Dr. Yang Zhong-qi of the Chinese Academy of Forestry commenced studies on the indigenous natural enemies that were moving over to *H. cunea*. One of the natural enemies found was a previously unknown pupal parasitoid that he described as *Chouioia cunea* Yang (gen. & sp. nov.) [Hymenoptera: Eulophidae]. This endoparasitic wasp develops gregariously, and an average of 245 individuals can develop successfully in a single host pupa (Yang and Xie 1998). Recognizing its potential as a candidate for inundative releases against the pest, Dr. Yang developed methods for mass-rearing and releasing the wasp. In a two year study encompassing four consecutive generations of the moth, a life table approach was used to demonstrate the efficacy of parasitoid releases, which were made at the beginning and peak of moth pupation at a 1300-ha test site in Shandong Province. Population trend indices of the first and second generations of the fall webworm at the test site averaged 0.29 and 0.14 respectively, and were significantly lower than those at the control site, 8.74 and 4.48, respectively (Wei et al. 2003). At the test site, levels of parasitization by *C. cunea* averaged 68% with a maximum of 88%; total parasitization by *C. cunea* and 18 other parasitoid species averaged 90% with a maximum 96%. These high levels of parasitization because the pesticides were eliminated in the test sites, and the natural enemies were protected and their populations increased (Yang et al. 2004a). Follow-up investigations after releasing the parasitoid for six consecutive years showed that fall webworm has been put under sustained control (Yang et al. 2004b).

<u>Amber Marked Leaf Miner in Canada</u>. The amber marked leaf miner, *Profenusa thomsoni* [Hymenoptera: Tenthredinidae], is one of five European species of birch leaf-mining sawflies, that invaded North America during the twentieth century. Both it and the birch leaf miner, *Fenusa pusilla* [Hymenoptera: Tenthredinidae] had become especially destructive in Alberta during the 1960's, delivering a one-two punch to birches leading off with the first generation of *F. pusilla* in May followed by the second generation of *F. pusilla* and univoltine *P. thomsoni* in June-July. In the 1990's, forest and shade tree entomologists were amazed to observe a spectacular decline in leafminer damage to birch trees in Edmonton, Alberta, but even more astounded to learn that an indigenous parasitoid was a key factor in reducing populations of the second species, *P. thomsoni*. The parasitoid implicated, *Lathrolestes luteolator* (Gravenhorst) [Hymenoptera: Ichneumonidae], apparently is native to both the Old and New Worlds (Digweed 1998). Though not reported from *P. thomsoni* up to that time, it was known to attack tenthredinid leafmining sawflies in the genus *Caliroa* in Europe and North America as well as *Profenusa alumna* MacGillivray in eastern Canada and the U.S. (Krombein et al. 1978). There is nothing extraordinary about discovering indigenous parasitoids that have opportunistically expanded their host range to include an introduced host, but in most cases, these are generalists that have little impact on the host populations. After all, classical biological control of exotic insect pests is predicated on the assumption that the specialized natural

enemies most capable of controlling the target pest exist in the region where the pest originated. In this case, a native oligophagous parasitoid was able to exert a practical level of control on an alien invasive species.

Exotic Natural Enemies to Control Indigenous Pests.

Lygus bugs in North America. The tarnished plant bug, *Lygus lineolaris* (Palisot), and western tarnished plant bug, *L. hesperus* Knight, important pests of numerous crops, are widely distributed east and west of the Rocky Mountains, respectively. Native natural enemies attacking these pests are ineffective. Nymphal parasitoids of *L. rugulipennis* were imported from Europe. Candidates for control of the *Lygus* pests included two polyvoltine species, *Peristenus digoneutis* (Loan) and *P. stygicus* (Loan), and a univoltine species, *P. rubricollis* (Thomson). Although earlier attempts to establish parasitoids were unsuccessful, intensive efforts in the 1980s resulted in the establishment of *P. digoneutis* in New Jersey. Successful biocontrol of *L. lineolaris* in alfalfa was achieved (Day 1996), and *P. digoneutis* had spread throughout much of the northeastern U.S. by 2000 (Day et al. 2000). Recent studies suggest that its action decreases damage by *L. lineolaris* in apples and strawberries. Beginning in 1998, *P. stygicus* and *P. digoneutis* were released in California, where both species became established. Though it is too early to claim successful biocontrol, substantial annual increases in parasitism were noted in 2000-2002. Obviously, the potential for an exotic natural enemy to have an adverse impact on non-target species is an important consideration in attempting a "new association" approach to biological control, so Day made a long-term study on the impact of *P. digoneutis* on non-target mirids in the northeastern U.S. concluded in 2002, indicated that the parasitoid occasionally did attack some other mirids, but that the levels of parasitization were consistently low.

Forest Pests in Canada. In a paper entitled "Prospects and problems in classical biological control against native species: some recent case studies in forestry" presented at the 2004 Eastern Branch Meeting of the Entomological Society of America, Marc Kenis provided some interesting insights using the importation strategy against indigenous pests. All involved efforts to capitalize upon the "new association" principle to control indigenous forest pests in Canada with natural enemies from Europe. Details of each project have been documented in a recent book by Mason and Huber (2002). The first case reviewed was that of conifer bark beetles (*Dendroctonus ponderosae* and other *Dendroctonus* spp.). The source insects in Europe were *Ips* spp., and the primary natural enemies included the clerid predator *Thanasimus formicarius* and parasitoids of the genera *Coeloides*, *Dendrosoter*, *Rhopalicus* and *Tomicobia*. Most of the candidate species had broad host ranges or would likely interaction with other bark beetle competitors (most species). In addition, *T. formicarius* could potentially crossbreed with native species, and *Tomicobia* was too host specific. Consequently, no releases were made. The second case was hemlock looper (*Lambdina fiscellaria fiscellaria*). Various conifer-infesting geometrids were investigated and a number of parasitoids were evaluated, but they were either too host specific or too polyphagous. Again, no releases were made. The third case was spruce budworm (*Choristoneura fumiferana*), one of the worst forest pests in Canada. The source species in Europe was *C. murinana*, and studies focused on its parasitoid complex. The parasitoid complexes were found to be highly similar comprised of different species in the same genera (*Apanteles*, *Cephaloglypta*, etc.). Some of the European species were specific at the generic level, but believed to represent a threat of competitive interactions with their North American congeners. Consequently, only one species, *Apanteles muninanae*, was released, but without a follow-up study. The remaining cases included spruce budmoth (*Zeiraphera canadensis*), white pine weevil (*Pissodes strobi*), spruce seed moth (*Cydia strobilella*) and spruce cone maggot (*Strobilomyia neanthracina*). In all, there were seven cases, but only two resulted in natural enemy releases, neither of which is known to be established (Mason and Huber 2002). Kenis concluded that this led to a loss of confidence in biological control among Canadian Forest Service managers. The major problems were that the insects used as sources were either too different from the target pest (e.g. hemlock looper) or too similar (e.g., spruce budworm). In the first case, parasitoids were either too specific or too polyphagous to be useful. In the second, natural enemy complexes were very similar to those of the target pest, in which case no empty niches appeared to be available and competition with native natural enemies seemed likely. In some cases, it appeared that the natural enemies would not adapt to the biology or ecology of the target pest. Finally, Dr. Kenis concluded that native insect pests are good targets for classical biological control only if taxonomically and ecologically related pests exist elsewhere, if there are empty ecological niches that can be filled by exotic natural enemies, and if these natural enemies are sufficiently specific.

If we contrast the experience in Canada, which involved forest pests having a large complex of natural enemies, with that of lygus bugs in the U.S., which involved agricultural pests having a sparse complex of natural enemies, we see that the likelihood of an empty ecological niche was relatively low in the former and high in the latter. Moreover, at least one empty ecological niche was apparent in the latter in the case of lygus bugs--the hosts were polyvoltine, but the native parasitoids were univoltine.

SUCCESSFUL IMPLEMENTATION PROJECTS

Pre-emptive biological control of mealybugs in Caribbean.

Pink hibiscus mealybug, *Maconellicoccus hirsutus*, known to attack over 200 economically important host plants, became established in the Caribbean region in the early 90's and occurs in most of the Island nations (Sagara and Peterkin 1999). It later spread into Mexico, the U.S. Southwest, and Florida. The potential economic impact on the citrus industry prompted an accelerated program to locate and identify biological control agents to control the existing populations and to slow the spread of the pest. Several agencies were involved including USDA-APHIS-PPQ, USDA-ARS, CABI Bioscience, the Inter-American Institute for Cooperation and the Belize Ministry of Agriculture and Fisheries. From 1994 through 2000, 37 potential parasites and 7 predators from 14 different counties and 3 continents were evaluated in the ARS quarantine at Newark, DE. Five parasites and one predator were selected, which we successfully cultured and shipped to 3 mass rearing facilities in the Caribbean. These natural enemies were released in heavily infested islands where biological control has resulted (Michaud and Evans 2000). Surveys through 2002 indicated establishment of all six biological agents with control percentages averaging between 70-95%. By the time the pest was discovered in southern California, rearing techniques for the natural enemies had been developed and several rearing facilities were in operation. Moreover, host range studies on most enemies had been completed, so USDA-APHIS-PPQ was able to liberate *Anagyrus kamali* within three weeks of its discovery there. Within a month of the pest's discovery in Florida, liberations of *A. kamali* and another wasp *Gyranusoidea indica* were being made. Post release evaluation of non-target mealybugs at selected localities in the Caribbean indicated that there were no adverse effects on any of non-target species found (Dale Meyerdirk, person.

Biocontrol of Diamond-back Moth in St. Helena.

The diamond-back moth (DBM) *Plutella xylostella*, is a pest of crucifers in many parts of the world, and has proved very difficult to control because it has become resistant to many insecticides. During the late 1990's, surveys in St. Helena, a small island in the South Atlantic where *P. xylostella* is a problem, showed that the only parasitoid present was the larval-pupal parasite *Diadegma mollipla*, which occurs on the African mainland. During 1999-2000, releases of a larval parasite *Cotesia plutellae* and *Diadromus collaris* were made in fields where farmers had replaced chemical treatments with Bt sprays to increase the likelihood of parasitoid establishment. Both parasitoids became established and were apparently responsible for a marked decline in the populations of DBM observed on St. Helena in 2001 (Kfir 2001).

Biological control of coconut whitefly in Caribbean.

The coconut whitefly, *Aleurodicus pulvinatus*, is an important pest of coconuts and ornamentals. Native to some areas of the Neotropics, it recently became established into several Caribbean islands, and has the capacity to spread elsewhere in the region in much the same fashion as the pink hibiscus mealybug. Kairo et al. (2001) reviewed the progress of a project focused on biological control of this pest in Nevis. Initially, the pest itself was confounded with *Aleurodicus cocoas*, but once the correct identification was obtained, biocontrol efforts could be placed on a solid footing. Explorations for natural enemies were conducted in Trinidad and Tobago, where the coconut whitefly occurs but is not a severe pest. These studies showed that the *Aleurodicus* spp. present there had a number of natural enemies in common. This complex of natural enemies consisted of several aphelinids in the genera *Encarsia* and *Encarsiella*, an encyrtid, *Metaphycus* sp., and several species of coccinellids in the genus *Nephaspis*. Dossiers were prepared according to FAO Code of conduct (FAO 1996) for the two most promising parasitoids, *Encarsiella noyesi* and *Encarsiella* species D despite the fact that the latter was unnamed, and submitted to the Nevis Department of Agriculture. Approval for release of species D was promptly received and releases were implemented shortly thereafter. Approval of release of *E. noyesi* was denied in order to facilitate the recovery of the first species. Kairo et al. (2001) concluded that *Encarsiella* species D had become established, but that there was a need for in depth post-release evaluation of the natural enemy's effectiveness. This project was noteworthy in that a careful pre-release evaluation and analysis was conducted, but it is disappointing that a corresponding program for monitoring the effectiveness of the natural enemy has not been mounted.

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