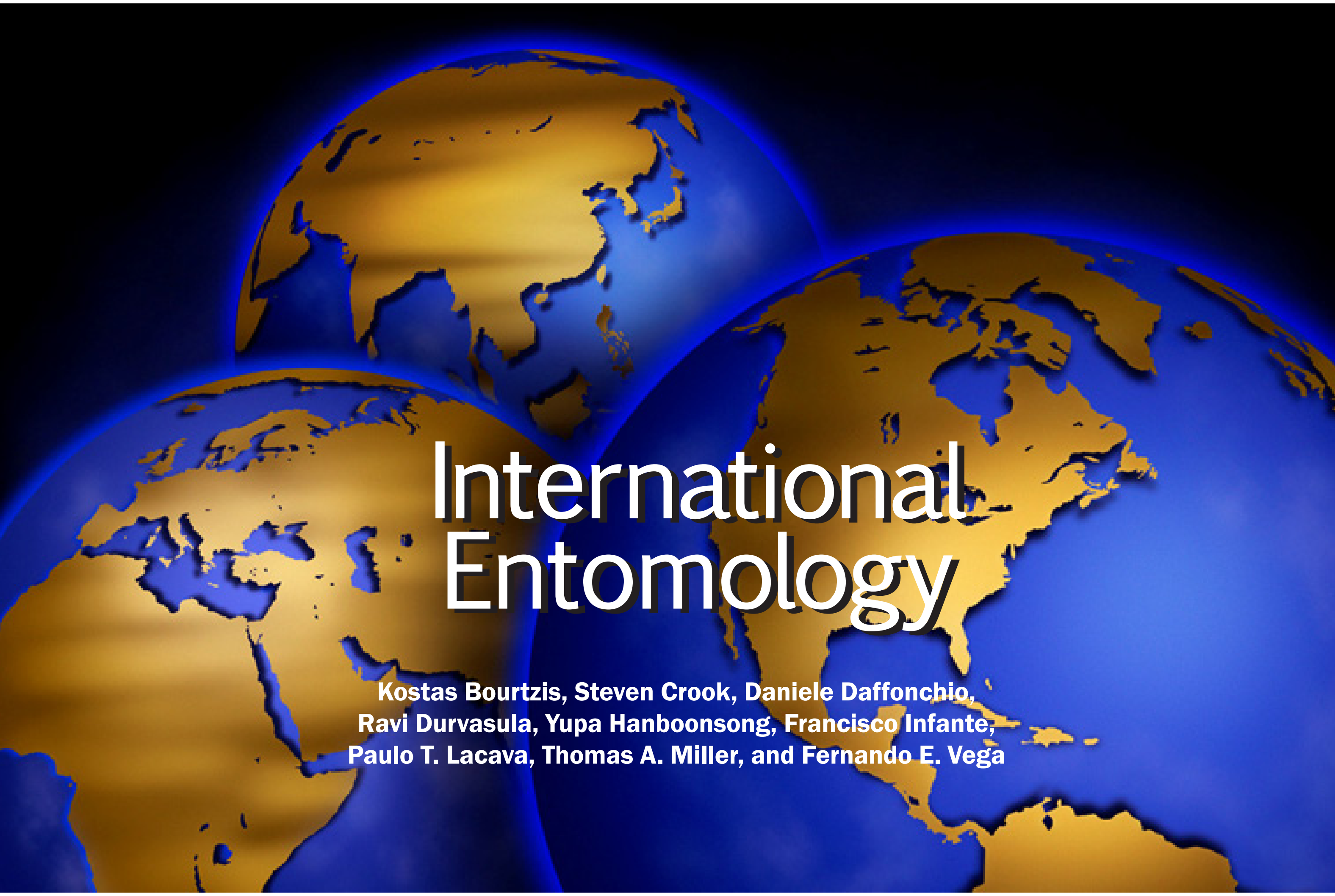

International Entomology

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Pests and diseases of plants are cosmopolitan, and have been from the beginning of agriculture. Domestication of plants and breeding for increased yields allowed civilizations to develop, but often created new problems associated with monocultures and displaced native flora and fauna. In the modern era, inorganic fertilizer in some cases has eliminated crop rotation and led to pollution of rivers and aquifers. The following descriptions of several international collaborators' attempts to manage pests and diseases represent the beginnings of a practical international entomology, and are a form of science diplomacy. A common theme in each of the examples is the urgent need for new tools in pest and disease management.

Pink Bollworm

First recorded as a cotton pest in India about 1843, the pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is thought to have originated in the Indo-Pakistan region, based on the richness of parasites and predators found there (Naranjo et al. 2002). The larval stage infests fruit and flower buds of the Malvaceae family of plants, including okra, mallow, hibiscus, and cotton.

The pink bollworm has from three to six generations a year depending on prevailing weather conditions; earlier generations feed on cotton squares or flowers in the spring, while later ones infest bolls. A certain number of larvae in field populations enter diapause; the percentage is lowest near the equator and higher farther away. As a tropical moth, the pink bollworm can survive freezing winter nights in diapause only at non-freezing temperatures underground. Diapause is triggered by low temperature, short days, and food quality. Older literature claimed that diapause could last two years or more (see F. C. Willcocks 1916, in Naranjo et al. 2002; Anonymous 1984). Diapausing pink bollworm larvae tend to associate with cotton seeds, spinning a modified cocoon and often knitting seeds together. It can survive shipment from infested areas to other cotton-growing regions in this condition.

Pink bollworm was the object of a Sterile Insect Technique (SIT) program starting a few years after introduction to California from Arizona around 1965. While suppression of infestations in eastern Arizona starting in 1927 were considered successful using early plow-down and other sanitation measures, a court decision to lift these restrictions lead quickly to a population explosion that spread through Arizona to California (Naranjo et al. 2002). This incident clearly shows how human intervention and lack of vigilance can exacerbate pest problems.

The advent of Bollgard® cotton developed by Monsanto provided a powerful new tool to control pink bollworm. Insertion of one of the delta endotoxin genes from a strain of *Bacillus thuringiensis*, an entomopathogenic bacterium, into cotton offered 100% protection against most chewing Lepidoptera, including larvae of the budworm and bollworm complex (Lepidoptera: Noctuidae) and pink bollworm itself.

Resistance was readily demonstrated by feeding pink bollworms on a Bollgard® diet for several generations (Bartlett 1995). Despite these published studies and anecdotal infestation reports, resistance of pink bollworm to the Bollgard® plants was not reported from introduction around 1994 until 2011, when resistance was confirmed in India by Tim Dennehy, then with Monsanto (Dhurua and Gujar 2011). Perhaps this outstanding record of holding off resistance in the United States was due to the resistance management strategy followed by the U.S. Environmental Protection Agency, which insisted that a non-Bollgard® harborage be included in every Bollgard® cotton field. Because resistance is a recessive trait, the intent was to maintain a large enough supply of susceptible genes to prevent the occurrence of homozygous resistant strains (Tabashnik et al. 2000). The Indian resistance incident may have been caused by relaxing this rigid resistance management strategy.

Like the boll weevil (*Anthonomous grandis grandis* Boheman), the pink bollworm is the object of eradication attempts, the latest one starting in 2005 (http://tinyurl.com/cjhh5bk). The main elements of the program are a special waiver of the EPA rule requiring non-Bollgard® harborage plants, early-season pheromone treatment, SIT releases when the field populations are reduced to small enough numbers to make SIT practical, and extensive pheromone trap monitoring. The waiver was granted by EPA because sterile insect releases were said to satisfy the harborage requirement.

No pink bollworms have been found in California, Arizona, New Mexico, or most of Chihuahua, Mexico for the past few years. The eradication program is now focusing on west Texas and Mexico in the Colorado River drainage areas of San Luis, Sonora and Mexicali, Baja California Norte. During the 2011 cotton season, 83,202 acres of cotton were grown in the Mexicali area just over the California border from El Centro. Of this acreage, 137 fields out of 2,310 were non-Bollgard® cotton, and fewer than 12 larvae were found in the tens of thousands of cotton bolls that were randomly sampled. Forty-five million SIT moths were released each week in this area and San Luis during the 2011 growing season. Twenty-five larvae were found in the San Luis-Mexicali area at the end of the 2012 season.

Eradication programs like those for boll weevil and pink bollworm are expensive, requiring intensive labor and unprecedented cooperation. Therefore, they are only feasible when the costs are outweighed by overwhelming financial incentives.

Coffee Berry Borer

The most devastating insect pest of *Coffea arabica* L. and *C. canephora* Pierre ex A. Froehner worldwide is the coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae: Scolytinae) (Fig. 1). This bark beetle has disseminated from Africa to most coffee-producing countries, causing severe damage to the coffee seed, which is the marketable product. Yearly losses due to the coffee berry borer have been estimated at over $500 million (Vega et al. 2002a).

The insect poses a formidable control challenge due to its cryptic nature inside the coffee berry (Fig. 2). Infestation begins when a female bores a hole in the coffee berry and deposits up to 300 eggs (Jaramillo 2008) in galleries within the seed. Once the larvae hatch, they start consuming the seed, reducing both quality and yield. Two factors make the insect even more problematic: the sex ratio of the species is skewed, favoring females 10:1 (likely due to the presence of the maternally inherited intracellular alphaproteobacterium *Wolbachia* or another male-killing agent [Vega et al. 2002b]); and sibling mating inside the berry means that females are inseminated and ready to oviposit as soon as they exit the berry.

Fig. 1. Coffee berry borer, *Hypothenemus hampei* Ferrari. Left photo by Eric Erbe, colorized by Chris Pooley, USDA; right photo by Peggy Greb, USDA.

Fig. 2. Coffee berries ready for picking. Photo by Fernando E. Vega.

Research aimed at developing pest management strategies against the coffee berry borer have focused on the use of biological control agents, such as parasitoids and fungal entomopathogens. Unfortunately, even though some mortality might ensue, use of these biocontrol agents is not sufficient to control the insect. Furthermore, long-term studies after introduction of both parasitoids and fungal entomopathogens are lacking (Vega et al. 2009). A novel aspect of the use of fungal entomopathogens against the coffee berry borer was the development of a technique that was effective in introducing *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) as a fungal endophyte in coffee plants (Posada and Vega 2006; Posada et al. 2007), although establishment was short-lived, probably due to the presence of other fungal endophytes in the plant (Vega et al. 2008a, 2010). Further research aimed at permanent and systemic establishment of fungal entomopathogens as endophytes (Vega 2008; Vega et al. 2008b) might result in an effective pest management strategy against the coffee berry borer.

One of the interesting aspects in the biology of the coffee berry borer is the insect's ability to survive on a food source that contains the alkaloid caffeine (1,3,7-trimethylxanthine; Vega et al. 2003). Caffeine levels in the coffee seed are on average 1.1-1.7% (on a dry weight basis) in *C. arabica* and 2-3% in *C. canephora* (Mösli Waldhauser and Baumann 1996). Nathanson (1984) hypothesized that the role of caffeine in plants might be as a defense against herbivores. Therefore, the ability of the coffee berry borer to successfully exploit a food source containing caffeine is likely due to detoxification mechanisms that are absent in species that cannot survive on caffeine-containing food sources. Research aimed at elucidating the role of the coffee berry borer microbiome in caffeine detoxification is part of an ongoing project between scientists at the U.S. Department of Agriculture–Agricultural Research Service and the U.S. Department of Energy–Lawrence Berkeley National Laboratory. When this aspect of the biology of the insect is better understood, it might be possible to develop new pest management strategies by interfering with microorganisms that might be contributing to caffeine breakdown.

Improving attractants used in traps might provide another possible mechanism to manage the insect (Vega et al. 2009). The ethanol:methanol mixture currently used in different trap designs results in capture of high numbers of insects, but this capture has not been shown to significantly reduce infestation levels or yield losses. The development of a repellent that could be used when the berry becomes suitable for infestation, i.e., when dry weight becomes 20% or higher (Bustillo et al. 1998), could be a revolutionary pest management strategy. Finally, determining whether the insect produces and responds to a pheromone could also result in a novel and effective pest management strategy against the insect.

Tools to Control Pests and Diseases in Agriculture

As shown by the two examples above, some insects can be inaccessible to surface insecticide treatments. The adult pink bollworm spends the day in cracks in the ground in arid growing areas, and at night, it feeds on nectar or searches for a mate. The egg, pupal, and larval stages are respectively spent hidden under boll calyxes, in the soil, or inside the cotton boll, where they consume the seed. Thus, the most efficient method of delivering an insecticide is via incorporation of a toxin into a transgenic plant so that all tissues contain lethal doses. The alternative of leaving an insecticide residue on the cotton plant for the adult to encounter does work, but is much less efficient in the face of continuous emergence of moths from diapause over spring and early summer. The other successful control method for pink bollworm is to saturate the cotton field with pheromone early in the season to disrupt attraction for mating. One such treatment can last a month when applied in slow-release formulations.

The coffee berry borer spends considerable time inside the coffee berry, and due to sibling mating in this cryptic environment, opportunities for mating disruption appear limited. Some success has been reported in recruiting local entomopathogenic fungi as a biological control method that is applied like an insecticide. However, as in the case of the pink bollworm, delivery is the problem. A new delivery paradigm is needed.

Tools for pest control fall into one of the four categories shown in Table 1. Of these, neurotoxic chemicals are the most frequently used and the only ones on the list that give results immediately. In the case of broad-spectrum neurotoxic insecticides, well-established agrochemical companies enjoy a $30 billion global market that maintains the industry. The current trend to find local fungi or other microbial control agents is hampered by a narrow market dictated by the very attractive selectivity that this approach offers. While biopesticides are more sustainable and environmentally friendly than neurotoxic insecticides, they still suffer from development of resistance and the delivery problem faced by neurotoxins.

The California Cotton Pest Control Board spent significant funding on a ten-year effort to find natural enemies for pink bollworm, but did not succeed. The Curly Top Virus Control Program funded a similar effort to find natural enemies for the key vector, beet leafhopper, *Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae), also without success. The current emphasis on biodiversity and natural resource protection has had a negative side effect on attempts at biocontrol: countries of origin are increasingly reluctant to approve

Table One. Pest control categories with examples.

Cultural (Agronomic):
- Crop rotation; intercropping to avoid monoculture
- (Resistant crop varieties)

Physical:
- Barriers; Farming in remote areas; Quarantines
- Greenhouse crops
- Heat; cold

Chemical:
- Neurotoxic insecticides; growth regulators
- Systemic insecticides
- Pheromones; biopesticides
- Inorganics (arsenates; Paris Green; boric acid)

Biological:
- Natural enemies
- Entomopathogens (viruses, bacteria, fungi)
- (Biopesticides)
- Sterile Insect Technique
- Genetically modified insects
- Natural product fumigants (cassava leaf extract)
- Symbiotic control:
  - *Wolbachia*-based population replacement
  - *Wolbachia*-based population suppression
  - Paratransgenesis
- Transgenic crop plants (with weed or insect resistance)

Biopesticides appear in two places on this list. Entomopathogens are also biopesticides, but some biopesticides are broad-spectrum neurotoxic natural product chemicals, such as avermectin (from *Streptomyces avermitilis*) and the spinosyns (from *Saccharopolyspora spinosa*). Thus their exact categorization is not entirely clear.

shipment of natural enemies for testing abroad, and local officials are less inclined to approve releases locally without evidence that native fauna will be unaffected.

Sterile Insect Technique (SIT)

Daily field release of mass-reared and sterilized insects to swamp out reproduction of pest populations has been conducted in different countries and continents by international consortia of entomologists. These efforts guide scientific societies towards true "internationalization" of entomology, while stressing environmentally friendly and sustainable methods of pest control.

Invented by Edward F. Knipling (USDA) and collaborators, SIT has become a key tool of several area-wide control programs (Tan 2000; Vreysen et al. 2007). Some insects (famously, the aforementioned boll weevil) are not suitable for SIT protocols. Doses of radiation low enough to allow the survival of boll weevils for at least ten days did not induce sterility (Abdul Matin et al. 1980). The successful U.S. national boll weevil eradication program (http://www.txbollweevil.org/) (http://tinyurl.com/8czdnbx) did not rely on classical SIT. Instead, the program relied on monitoring ~~using aggregation~~ pheromone ~~or lures and spot treatments~~ with malathion or other neurotoxic insecticides.

To inflict the desired fitness costs on a target pest, large numbers of mass-reared and irradiated insects must be released in order to compete with wild types. New variations on the SIT method, Autocidal Biological Control (ABC; Fryxell and Miller 1995) and Release of Insects carrying a Dominant Lethal (RIDL; Thomas et al. 2000), employ a single lethal gene in a strain that is capable of being mass-reared, but passes on lethal genes to field populations when released. This new strategy allows the strain to be reared under permissive conditions, out-crossed to wild types, and then back-crossed to the homozygous condition for the lethal gene with increased fitness. The lethal gene must be dominant so that the lethal condition is expressed with only one copy as heterozygote. Although this strategy is one of the most sustainable and environmentally friendly ever invented (Miller 2012), it has been delayed or even prohibited in the United States by regulatory strangulation.

The efficiency of SIT can be sustained by proper management of the insect gut microbiome (Crotti et al. 2012) that is essential for maintaining host fitness following irradiation. For instance, the microbial gut symbionts of Mediterranean fruit fly (medfly) *Ceratitis capitata* (Diptera: Tephritidae) have been shown to be extremely important in maintaining the reproductive fitness of irradiated males (Ben Ami et al. 2010). In a standard SIT application, the sterile *C. capitata* males are less competent in mating than wild males, decreasing the efficiency of the field treatment. Ben Ami et al. (2010) showed that the decreased mating competence was associated to a profound change in the structure of the microbiome associated to the medfly. The irradiation treatment, besides sterilizing the males, decreased the levels of certain probiotic members of the symbiotic microbiome of the medfly (namely *Klebsiella* species) while favoring the growth of *Pseudomonas* sp. that have no beneficial effects on the host. By feeding the males after irradiation with cells of a *Klebsiella oxytoca* strain isolated from the medfly prior to irradiation, Ben Ami et al. (2010) demonstrated higher mating fitness associated with a stable colonization of *K. oxytoca* and decreased presence of the potentially pathogenic pseudomonads.

The study of Ben Ami et al. (2010) indicates that in insects, as in other animals, maintaining a stable gut microbiome is essential for the host fitness. Dysbiosis (i.e., an imbalance of the gut microbiome) is one of the first factors that allow pathogens to invade the host body and trigger diseases or decrease general host fitness (Hamdi et al. 2011).

BIODESERT

Symbiotic control is a general strategy that aims at exploiting specific members of the insect host microbiome (e.g., *Wolbachia*) for various aspects of insect management, including population suppression of agricultural pests (Zabalou et al. 2004, 2009, Apostolaki et al. 2011) and disease vectors (Atyame et al. 2011; Brelsfoard and Dobson 2009, 2011) or interference with pathogen transmission by insect vectors (Hoffmann et al. 2011, Walker et al. 2011). A novel, currently developing approach is the application of symbiotic control for the protection of useful insects such as pollinators or parasitoids (Hamdi et al. 2011, Crotti et al. 2012).

One recent case study is the exploitation of honeybee symbionts for protection from a widespread and dangerous honeybee disease, American Foulbrood Disease (AFB). AFB is caused by a pathogenic bacterium, *Paenibacillus larvae*, that fatally infects bee larvae (Hamdi et al. 2011). In a European Union-funded project (GA-245746), three teams from Tunisia, Italy, and Greece are cooperating to assess the potential of bacterial symbionts in protecting the honeybee from AFB. The project, BIODESERT, is generally framed under the concept of evaluating microbial resources in the arid ecosystems of the Saharan and pre-Saharan lands in southern Tunisia. Using approaches from molecular ecology and metagenomics coupled with biochemical and physiological characterization of honeybee symbionts, the BIODESERT project selected a set of natural microbial probiotics for improving honeybee health. *In vivo* tests showed the feasibility of the approach for protecting the major pollinator from AFB (Hamdi and Daffonchio 2011).

Sugarcane White Leaf Disease

Sugarcane white leaf disease (SCWL) is one of the most harmful diseases of sugarcane, making it a threat to sugarcane industry in the Asia region, especially in Thailand, Taiwan, Lao PDR, Vietnam, and Sri Lanka (Nakashima and Murata 1993, Kumarasinghe and Jones 2001; Nakashima et al. 2001, Thein et al. 2012). The disease is caused by plant-pathogenic phytoplasma, a bacterium without a cell wall belonging to the class *Mollicutes* that lives in the phloem of host plants and is vectored by insects (Chen 1974; Wongkaew et al. 1997). The SCWL phytoplasma belongs to the 16Sr XI-B genetic group of the rice yellow dwarf category (Lee et al. 1998) and shows symptoms of stunt, leaf chlorosis, and profuse tillering (formation of aboveground shoots from a node at the base of the principal shoot in grasses and some other plants) on infected sugarcane plants (Fig. 3).

The infected sugarcane plants show disease symptoms at an early growth stage (1-6 months old), and in the case of severe infection, plants die at a young age. Infected plants with no symptoms show shortened internodes and cannot prolong growth until the second ratoon (multiple seasons of yield) crop as the disease symptoms will appear and the plant dies, with high yield loss. The SWCL phytoplasma is transmitted to the plant by the leafhoppers *Matsumuratettix hiroglyphicus* (Matsumura) and *Yamatotettix flavovittatus* Matsumura (Hemiptera: Cicadellidae) (Matsumoto et al. 1968, Chen 1974, Hanboonsong et al. 2002, 2006).

The disease is spread through the use of infected cane stalks for planting material and by insect vectors. The SCWL phytoplasma still

Fig. 3. A field of sugarcane in northeast Thailand heavily affected with white leaf disease. Photo by Yupa Hanboonsong.

cannot be cultured, and so far there is no resistant sugarcane variety or effective method to control SCWL disease, except elimination of infected plants to decrease the amount of phytoplasma inoculums. Approaches such as tissue culture technique or hot water treatment have been applied to ensure disease-free cane stalks, but these strategies have not been able to effectively control or prevent the disease.

Integrated pest management practices are aimed at both insect vectors and sugarcane host plants. Currently, no alternative host plants are known as reservoirs of SCWL phytoplasma (Wongkaew et al. 1997). Insect vectors can transmit the pathogen from generation to generation through eggs (Hanboonsong et al. 2006); the insect vector is therefore one of the most important factors contributing to the spread of SCWL disease. The transmission efficiency of *M. hiroglyphicus* (55%) is higher than that of *Y. flavovittatus* (45%) (Hanboonsong et al. 2006). The estimated natural dispersal distances for these insect vectors (*M. hiroglyphicus* and *Y. flavovittatus*) are approximately 8 and 20 meters per day, respectively (Thein et al. 2011, 2012).

Insect vector transmission efficiency and dispersal are relevant not only in disease epidemiology, but also in developing vector models and strategies to prevent the spread of the pathogens by vector insects. In addition, using a symbiotic control approach for interruption of disease transmission in insect vectors is under investigation. It was revealed that the insect vector *M. hiroglyphicus* harbors the bacterial symbiont "Bacterium Associated with *M. hiroglyphicus*" (BAMH). BAMH is present in the midgut and ovaries of the leafhopper and is found in all developmental stages including eggs, nymphs, and adults. Moreover, it appears to be specific to the vector of the pathogen causing SCWL (Wangkeree et al. 2012). This symbiont is a candidate for symbiotic control of sugarcane white leaf disease.

Implementing good farming practice is also important for controlling this disease. Crop rotation between sugarcane and upland rice is likely to reduce the prevalence of SCWL, and may cut down the growth cycle of the insect vector population and consequently reduce the presence of the disease pathogen. In addition, improving plant health by providing essential and appropriate nutrients to infected plants could prevent the appearance of symptoms in plants with a low phytoplasma load and allow them to reach maturity. The threshold of phytoplasma inoculum that leads to symptoms has yet to be determined.

SCWL disease remains a major challenge to the sugarcane industry of Southeast Asia. However, the multiple control approaches described here offer the hope, if not of eradicating the disease, at least of diminishing its impact.

Xylella fastidiosa and Pierce's Disease

Pierce's disease (PD) of grapevines (Fig. 4) was first detected in Southern California in 1884, where it destroyed approximately 40,000 acres of grapes in Anaheim, CA, during a five-year outbreak (Pierce 1892, Goodwin and Purcell 1992). Following this devastating experience, which was restricted to Orange County, PD became only an occasional concern to West Coast viticulture for many decades because of the preference of native vector leafhoppers for riparian habitats. This all changed when the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae; Takiya et al. 2006) invaded California at some point during the 1980s. By the late 1990s, it had caused new epidemics centered in Temecula, where about one-third of the vineyards were lost. The GWSS (Fig. 5) is the most widespread sharpshooter insect vector of *X. fastidiosa* in the United States. The insect is a major concern for horticultural industries beyond viticulture due to its ability to transmit *X. fastidiosa* strains that cause scorch diseases in a number of host plants, including *X. fastidiosa* subsp. *fastidiosa*, which causes PD in grapevines (Purcell, 2005).

Fig. 4. Pierce's disease symptoms in Chardonnay grapevines, Temecula, California, 2004. Leaf scorch characteristic is clearly evident and the grapes themselves slightly shriveled. Photo by José M. Ramírez.

As with other sharpshooter insects, GWSS is a xylophagous insect that feeds on hundreds of plant species (Purcell and Hopkins 1996, Purcell and Saunders 1999), with citrus being one of its preferred hosts (Blua et al. 2001). Perring et al. (2001) demonstrated a relationship between PD incidence in grapes and the proximity of vineyards to citrus orchards. The leafhopper has the capacity to survive winter temperatures as low as -6°C (Park et al. 2006).

Moreover, compared with other *X. fastidiosa*-carrying insects associated with PD and native to California, GWSS has a longer flight range (up to 1/4 mile). These traits make the GWSS a very serious

Fig. 5. One week of glassy-winged sharpshooters, *Homalodisca vitripennis*, on yellow sticky cards in research vineyard at UC Riverside, 7 July 2009. The lines are one inch apart (2.54 cm). Photo by Candice Sanscartier.

threat to the wine industry in Southern to Central California (Castle et al. 2005). Indeed, since the first identification of GWSS in the California vineyards, programs aimed at controlling the dissemination of this insect as a strategy to prevent PD outbreaks have involved more than $160 million of direct investments (http://www.cdfa.ca.gov/phpps/pdcp/). So far, GWSS has not become established in Northern California because of an aggressive quarantine program.

Xylella fastidiosa is a complex of fastidious Gram-negative xylem-limited bacteria, rod-shaped with distinctively rippled cell walls. The strains are non-flagellate, do not form spores, and measure 0.1-0.5 × 1-5 micrometers (Nyland et al. 1973, Bradbury 1991). These Gram-negative bacteria were formally named only in 1987 (Wells et al. 1987), and are extremely slow-growing in culture. These traits have made the pathogen difficult to study, contributing to its previous obscurity. Natural transmission occurs via insects feeding suctorially on xylem sap. Transmission efficiency varies widely among vector species. The bacterium overwinters in the xylem of the host plant as well as in weeds.

The main control method for PD in the quarantine zone of Southern California is one early season treatment with imidacloprid systemic insecticide applied via drip irrigation. This was extremely effective in most vineyards but was not used in organic vineyards, which continue to sustain losses. Citrus that provides overwinter harborage for GWSS is also treated where possible. These measures stimulated a rebound in new plantings and vineyards as the Temecula area recovered, and allowed researchers and the industry to buy time to search for other remedies. One new method is a transgenic grapevine with factors preventing PD symptoms from developing (Dandekar 2012), but of course, this cannot be used by organic growers either.

Besides PD, other specific strains of *Xylella fastidiosa* are known to cause scorch diseases in many fruit trees and ornamental species, especially in North and South America (Hopkins 1989). Control of any of the GWSS-transmitted pathogens causing diseases of horticultural crops in California by a symbiotic control (SC) or paratransgenic approach would be of immediate interest to other industries as well. The technique of paratransgenesis was developed as a novel method to create conditions that render insect vectors incompetent. The strategy of SC employs both paratransgenic and non-recombinant methods to control disease or health problems. In some cases, these solutions may result in competitive displacement of the pathogen with a more benign microbe.

The strategy, paratransgenesis, was intended to prevent the transmission of pathogens by insect vectors to humans (Beard et al. 1998, 2001, 2002, Rio et al. 2004) using symbiotic microbes as delivery vehicles. However, in principle it can be applied to displace any pathogen, including a plant pathogen. This overall strategy of disease prevention is an example of SC and is a variation on the theme of symbiotic therapy (Ahmed 2003). Genetic manipulation has fitness costs that must be factored into the application (Durvasula et al. 1997, Miller 2007).

The key to SC, and therefore paratransgenesis, is to find a local candidate microbe that has an existing association with the pathosystem that includes the problem or condition at hand. The local candidate microbe should occupy the same niche as, or have access to, the target pathogen or condition (Durvasula et al. 1997). The local origin of the biocontrol microbe in SC differs from classical biological control, in which microbes, herbivores, parasites, or predators are sought from outside of the local ecosystem to control a pest (Miller 2007). In SC, all elements originate at the local site and are already co-evolved with and established in the pathosystem; foreign exploration is not only unnecessary, but also most likely counter-productive. Because of these strict requirements, a suitable symbiotic candidate may not always be found or may not be amenable to practical manipulation (Miller 2007).

Microbes chosen for symbiotic control must be able to pass subsequent regulatory scrutiny (Miller 2007). Once a candidate symbiont is identified as a control agent for paratransgenesis, all genetic or other manipulations can be local. Indeed, a symbiotic control or paratransgenic solution developed for a specific location may not be suitable for another site or condition elsewhere (Durvasula et al. 1999, 2003, Miller 2007).

Azizi et al. (2012) recently presented a simple, robust approach for the generation of panels of recombinant single-chain antibodies against the surface-exposed elements of *X. fastidiosa* that may have potential use in diagnosis and/or disease transmission blocking studies. In vitro combinatorial antibody ribosome display libraries were assembled from immunoglobulin transcripts rescued from the spleens of mice immunized with heat-killed *X. fastidiosa*. The libraries were used in a single round of selection against an outer membrane protein, MopB, resulting in the isolation of a panel of recombinant antibodies. The potential use of selected anti-MopB antibodies was demonstrated by the successful application of the 4XfMopB3 antibody in an enzyme-linked immunosorbent assay (ELISA), a Western blot assay, and an immunofluorescence assay (IFA). These immortalized in vitro recombinant single-chain antibody libraries generated against heat-killed *X. fastidiosa* are a resource for the Pierce's disease research community, and may be readily accessed for the isolation of antibodies against a plethora of *X. fastidiosa* surface-exposed antigenic molecules. These authors (Azizi et al. 2012) concluded that recombinant antibodies against MopB and other abundant surface-exposed molecules on *X. fastidiosa* could also be engineered in a similar way to agglutinate the bacteria and evaluated in the GWSS via paratransgenic organisms such as engineered commensals or symbionts, providing novel alternative platforms to investigate in the control of PD.

Citrus Variegated Chlorosis (CVC).

Citrus variegated chlorosis (CVC) (Fig. 6) is a disease of the sweet orange [*Citrus sinensis* (L.)], which is caused by *Xylella fastidiosa* subsp. *pauca* (Chang et al. 1993, Hartung et al. 1994, Schaad et al. 2004), a phytopathogenic bacterium that has been shown to infect all sweet orange cultivars (Li et al. 1997a). CVC was first reported in Brazil in 1987 and has rapidly become one of the most economically important diseases affecting sweet orange production in Brazil (Rossetti et al. 1990, Lee et al. 1991). CVC rapidly became widespread in most major citrus-growing areas through unregulated movement of infected nursery stock due to a previous lack of certification programs and high CVC infection rates in Brazil. CVC can be found in at least 90% of the orchards in Brazil (Lambais et al. 2000), where it causes losses of $100 million per year (Della-Coletta et al. 2001).

CVC occurs on trees propagated on all commonly used rootstocks in Brazil: *C. limonia*, *C. reshni*, and *C. volkameriana* (Li et al. 1997c). The disease has not been observed on limes (*C. latifolia)* or mandarins (*C. reticulata*), even when the trees were planted in severely affected orange groves (Li et al. 1997 b).

Citrus plants with symptoms of CVC show a brilliant leaf chlorosis, similar to zinc deficiency, as the initial symptom (Laranjeira et al. 1998, Anonymous 2000). Later symptoms include wilting, canopy dieback, necrotic leaf lesions, and undersized, hard fruit (Derrick and Timmer 2000; Hopkins and Purcell 2002). The causal agent of CVC has been found to be transmitted in Brazil by sharpshooter leafhoppers (Cicadellidae) (Lopes et al. 1996; Almeida and Purcell 2003). CVC has been experimentally transmitted by 11 different sharpshooter species (Fig. 7) tested in Brazil (Fundecitrus 2005). The pathogen can be also transmitted through seeds (Li et al. 2003).

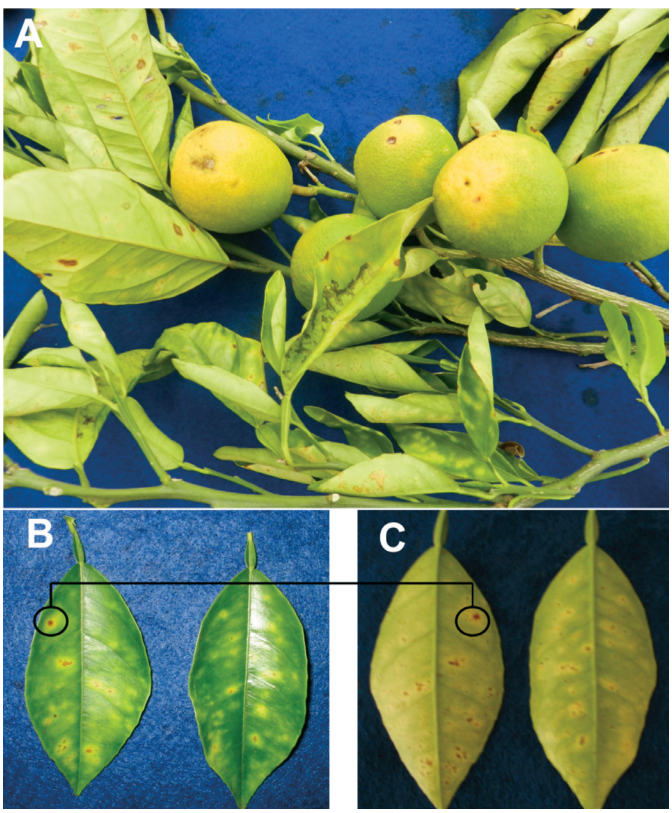

Fig. 6. Citrus Variegated Chlorosis symptoms. A. Advanced symptoms with small, yellowed fruits. B and C. Leaf symptoms showing chlorotic spots on the upper leaf surface corresponding to gummy brown spots on the underside. Photo by H.D. Coletta-Filho.

Although *X. fastidiosa* subsp. *pauca* was the first plant pathogen to have its genome sequenced (Simpson et al. 2000), there is still no effective control. The pathogen is known to have an extraordinary host range among higher plants in New World ecosystems (Freitag 1951). Interestingly, within the majority of native host plants, *X. fastidiosa* does not damage the host plant and behaves as an endophyte (Purcell and Saunders 1999). In contrast, the horticultural crops that suffer from diseases caused by *X. fastidiosa* are those that have been introduced into New World ecosystems (Chen et al. 2000). The observation that a few asymptomatic trees persist in some infected orchards may lead to new approaches to the investigation of the control of CVC. These asymptomatic plants have the same genotype as diseased plants and are located in the same grove under similar climatic and edaphic conditions, suggesting that some other factor is responsible for resistance to CVC. One factor that may influence resistance to CVC is the nature of the endophytic microbial community colonizing individual *C. sinensis* plants (Araújo et al. 2002).

The key to symbiotic control is finding a candidate microbe having an existing association with the ecosystem that includes the problem or condition at hand, and that occupies the same niche as or has access to the target pathogen (Miller, 2007). Bacteria of the genus *Methylobacterium* are known to occupy the same niche as *X. fastidiosa* subsp. *pauca* inside citrus plants (Araújo et al. 2002, Lacava et al. 2004). During feeding, insects acquire not only the pathogen, but also endophytes from host plants. Gai et al. (2009) reported the localization of the endophytic bacterium, *M. mesophilicum*, in a *C. roseus* model plant system and the transmission of this endophyte by *Bucephalogonia xanthophis,* a sharpshooter insect vector of *X. fastidiosa* subsp. *pauca*.

Methylobacterium mesophilicum, originally isolated as an endophytic bacterium from citrus plants (Araújo et al. 2002), was genetically transformed to express green fluorescent protein (GFP; Gai et al. 2007). The GFP-labeled strain of *M. mesophilicum* was inoculated into *C. roseus* (model plant) seedlings and was observed colonizing its xylem vessels. The transmission of *M. mesophilicum* by *B. xanthophis* was verified with insects feeding on fluids containing the GFP-labeled bacterium. Forty-five days after inoculation, the plants exhibited endophytic colonization by *M. mesophilicum*, confirming this bacterium as a nonpathogenic, xylem-associated endophyte (Gai et al. 2009).

These data demonstrate that *M. mesophilicum* not only occupies the same niche as *X. fastidiosa* subsp. *pauca* inside plants, but also that it may be transmitted by *B. xanthophis*. The transmission, colonization, and genetic manipulation of *M. mesophilicum* is a prerequisite to examining the potential use of paratransgenic SC to interrupt transmission of *X. fastidiosa* subsp. *pauca*, the bacterial pathogen causing CVC, by insect vectors. We propose *M. mesophilicum* as a candidate for a paratransgenic SC strategy to reduce the spread of *X. fastidiosa* subsp. *pauca*. It is known that *X. fastidiosa* subsp. *pauca* produces a fastidian gum (da Silva et al. 2006), which may be responsible for the obstruction of xylem in affected plants (Lambais et al. 2000), so the production of endoglucanase by genetically modified

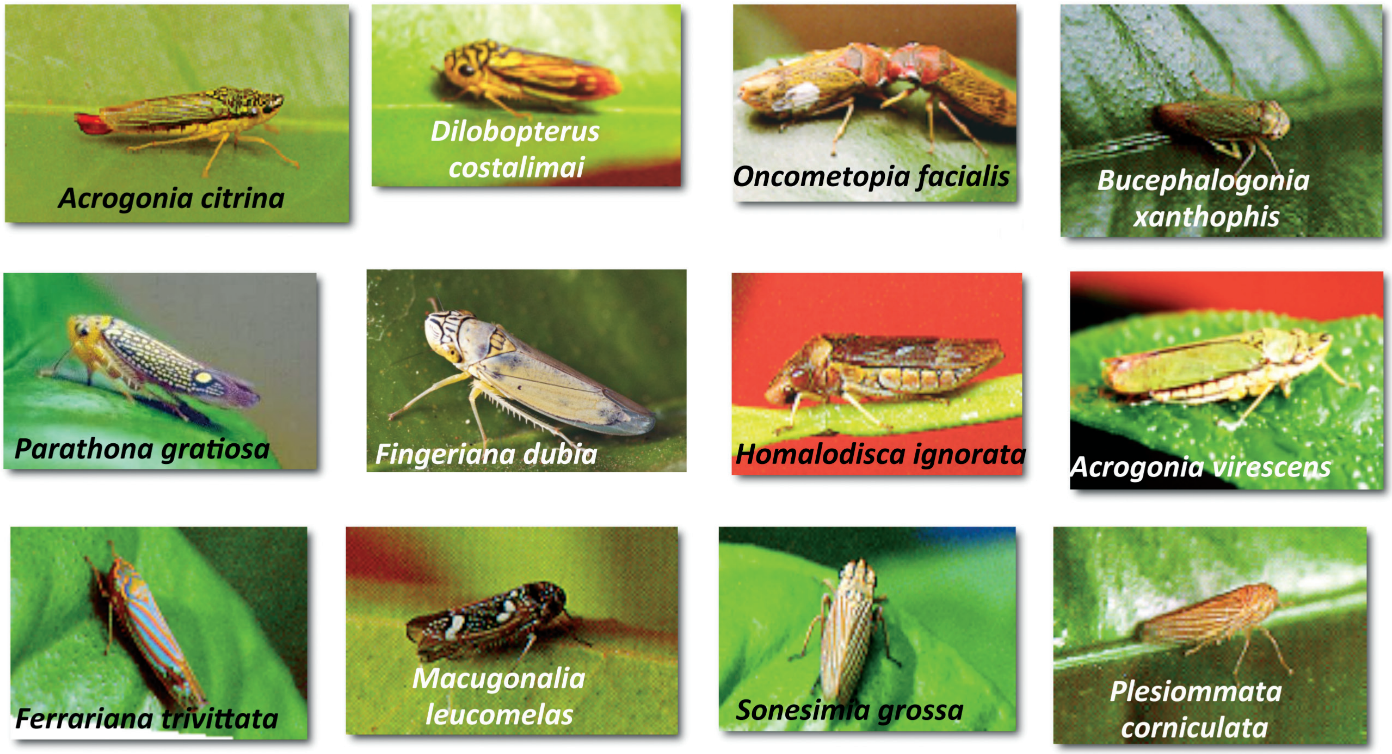

Fig 7. Brazilian leafhopper vectors of Citrus Variegated Chlorosis. Photos by Pedro T. Yamamoto. Authorized by FUNDECITRUS (http:// www.fundecitrus.com.br/Home/Default.aspx).

endophytic bacteria may transform the endophytes into symbiotic control agents for CVC.

Azevedo and Araújo (2003) used the replicative vector pEGLA160 to produce genetically modified *Methylobacterium* expressing antibiotic resistance and endoglucanase genes. Furthermore, other strategies can be evaluated, such as a production of genetically modified *Methylobacterium* to secrete soluble anti-*Xylella* protein effectors in citrus, such as Lampe et al. (2006) suggested in the *Escherichia coli* α-hemolysin system for use in Axd to secrete soluble anti-*Xylella* protein effectors in grapevine and GWSS. Also, Lampe et al. (2007) suggested the evaluation of proteins secreted from the grapevine bacterial symbiont *Pantoea agglomerans* for use as secretion partners of anti-*Xylella* protein effectors. One strategy that can serve as the next step for SC of CVC is producing a genetically modified endophytic bacterium, like *Methylobacterium*, to secrete anti-*Xylella* protein effectors.

According to Gai et al. (2011) the bacterial communities associated with vector insects and plants differ in abundance through the yearly season. Endophytic bacteria could influence disease development by reducing the insect transmission efficiency due to competition with pathogens in host plants and also in insect foreguts. In addition, the bacterial communities in the foregut of insect vectors of *X. fastidiosa* subsp. *pauca* changed with time, environmental conditions, and in different insect species. However, members of the genus *Curtobacterium* were consistently detected in the sharpshooters' foreguts and are commonly isolated from the xylem of citrus plants (Araújo et al. 2002), and may therefore be candidates for biological control.

Barriers to Interdisciplinary Collaboration

The nature of symbiotic control is interdisciplinary, though it is not easy to initiate collaborations across interdisciplinary lines. One of the more successful examples occurred when Nancy Moran started working with Paul Baumann. Moran's beautiful dedication to Professor Baumann in Volume 3 of *Insect Symbiosis* (Bourtzis and Moran 2009) is pertinent:

> "I still remember very clearly my first conversation with Paul Baumann. He had phoned to ask if I might be interested in collaborating on a study on the bacterial endosymbionts of aphids; the year was 1990. His excitement was infectious. I had read much of Paul Buchner's book as a graduate student but had never considered working on symbionts and knew almost nothing of microbiology."

This introduction covers several issues germane to the present context. It illustrates the degree of isolation between disciplines at the time, that a major source of conceptual information may be found in a book, and that collaboration starts by reaching out. Nancy Moran was at the University of Arizona, Tucson and Paul was at the University of California, Davis: two different states, but they could just as well have been in different countries.

Once interdisciplinary collaboration starts, it is difficult to pick a journal for the publications. There are myriad outlets, including entomology, microbiology, and plant pathology journals, but publications that combine all three disciplines include the *International Journal of Pest Management* and *Symbiosis*, the journal of the International Symbiosis Society (ISS). The founders of ISS understood instinctively that symbiosis, besides being interdisciplinary, was global.

Most agriculture-related societies are national, and their only international connection is when international congresses are held. These are typically held every four years. Thus, while it is difficult to know where to publish interdisciplinary work, it is equally challenging to know which society meetings to attend. If you are working on a plant disease caused by a microbial pathogen transmitted by an insect, do you attend an entomology, plant pathology, or microbiology meeting? Do you attend the Congress of Plant Protection, the

International Congress of Entomology, ISS, Integrated Pest Management, or all of them?

Despite the national character of agriculture-based sciences, there is an urgent need to become international. The Entomological Society of America (ESA) took the first step in this direction by establishing an International Branch to join the regional national branches. Everyone in ESA should belong to the International Branch, but, oddly, they don't. When they do, ESA will become the first truly international entomology organization.

It is in the vested interest of every country, developed or developing, to ensure that colleagues are adequately trained and actively working in all countries that have pests or diseases likely to travel and invade others.

International Connections

Several advances serve to support the feasibility of international collaborations. The first is air travel, which has enabled the world's population to become mobile. The second is the revolution in communication that has brought us e-mail and cellular telephones. The third is open-access publication. It is now less expensive and faster to place results into print than ever before.

There are also more of us. Each professor in a major research institution has produced more than enough potential replacement faculty over the past 50 years. Every major research program now has a Web site. Most of these things were not possible 20 years ago. Today, we take them for granted.

The American Association for the Advancement of Science (AAAS) has just opened a new venture, Science Diplomacy (http://diplomacy.aaas.org/). This will greatly increase our ability to reach out to colleagues.

International Education

In 2011, Brazil initiated the Science Without Borders program (http://www.cienciasemfronteiras.gov.br/web/csf-eng/), in which eventually 100,000 of the top students in the country will be sent ~~to study abroad in top research laboratories. The intent is t~~o solve a skilled labor shortage and empower the country's growing economy.

Recognizing a shortage of science and technology talent, Thailand launched the Golden Jubilee Program in 1996 (http://rgj.trf.or.th/eng/rgje11.asp). This was intended to increase the number of Ph.D. students in science and technology and ensure ~~that they receive the latest training in~~ advanced research laboratories around the world. Their intent was to produce 5,000 new Ph.D.s in science and technology in the first 15 years of the program.

The Kingdom of Saudi Arabia (KSA) launched a very similar initiative by founding the King Abdullah University of Science and Technology (KAUST) just outside of Jeddah, KSA. The new campus, opened in September 2009, is a graduate research university and presently has some 800 graduate students and an international faculty. All teaching is in English. The four core areas of emphasis are Resources and Energy; Bioscience and Bioengineering; Materials Sciences and Applied Mathematics and Computation. The standards set are equivalent to Massachusetts Institute of Technology or California Institute of Technology. The state-of-the-art campus is intended to be a catalyst for economic development in the region and around the world. Foreign graduate students are encouraged to apply.

The Korean Advanced Institute of Science and Technology (KAIST) in Daejeon, Korea was founded in 1971 to achieve the same goals of KAUST in KSA. (http://www.kaist.edu/edu.html) Again, all instruction is in English, the students are from around the world and Korea, and the faculty is international. In 2009, about 8,000 students were enrolled as undergraduates and graduates. The placement rate of KAIST graduates is nearly 100%. A Science and Technology Belt is being expanded adjacent to the campus and the major science and technology companies in Korea eagerly recruit the KAIST graduates. Similar Science and Technology education and research centers were started in China over ten years ago, all teaching in English, and a new one opened in North Korea two years ago.

Global Knowledge Initiative (GKI)

The Global Knowledge Initiative (GKI) is a non-profit organization based in Washington, DC. It arose from the 2008 Higher Education Summit for Global Development convened by the U.S. Secretaries of State and Education and the administrator of the U.S. Agency for International Development. Attended by more than 200 university presidents, heads of technology firms, and philanthropists, the summit identified the need for a "clearinghouse for resources and information to help build knowledge partnerships that can tackle development challenges." With a leadership team that includes the Prime Minister of India's Advisor on Innovation and Public Information Infrastructure, Sam Pitroda, the Director of the Library of Alexandria, Ismail Serageldin, and the former Science and Technology Advisor to the U.S. Secretary of State, Nina Fedoroff, GKI responds to this call.

The Global Knowledge Initiative has the unique ability to create a bridge between the needs and resources of scientists and agronomists, so that they can access the assets and expertise needed to understand and address the biology of a pest or disease. Although much of GKI's work has been in the areas of food security and climate change, their mandate stretches across all areas of science, technology, and innovation that support economic development and poverty reduction. Much of GKI's work thus far has been in building collaborations between researchers in the United States and Europe and individuals in East and Southern Africa and Central Asia, and they are rapidly expanding their geographic reach. By forging, enabling, and sustaining knowledge partnerships, GKI hopes to solve development challenges pertinent to science, technology, and innovation worldwide. For more information on GKI, see: www.globalknowledgeinitiative.org.

International Invasive Species Surveillance

The United States Department of Agriculture (USDA) Animal and Plant Health Service (APHIS), Plant Protection and Quarantine's (PPQ) mission is to safeguard U.S. agriculture and natural resources from the risks associated with the entry, establishment, or spread of exotic plant pests. For a proactive and effective safeguarding approach, PPQ needs early warning systems regarding plant pests that are not yet established in the U.S.

To address this need, PPQ established various systems to monitor and report occurrences of significant pest events in other countries. The Exotic Pest Information Collection and Analysis (EPICA) system conducts plant pest surveillance by continuously collecting, analyzing, distributing, and archiving relevant open-source information for PPQ programs. EPICA monitors mostly English-language sources worldwide for relevant pest information. EPICA searches more than 90 scientific journals and uses nearly 250 queries to search the Internet for relevant pest news. The information is then filtered and analyzed, and the articles are supplemented with background research and information. The EPICA notification is e-mailed to PPQ programs and other plant safeguarding officials throughout the U.S. once a week.

Future Pest Populations

The state entomologist of California, Robert Dowell, surveyed records of new pests and diseases in California from historic times until 2000 and found a new occurrence on average of one every 60 days, or 6 times a year. His more recent data indicates that between 2000 and 2010, the rate of arrival has increased to one every 41 days. Dowell also reports that the rate of arrival of new pests and diseases is on the increase elsewhere (Aukema et al. 2010, Kirkendall and Faccol 2010, Roy et al. 2011).

Attributing this increase to a specific cause is difficult, but predictions of a 10% decrease in crop yield have already been tied to every degree of temperature rise in climate change (Fedoroff and Cohen 1999, Fedoroff 2010, Fedoroff and Brown 2004). There is debate about climate change, but Climate Central, a non-profit research and news organization, concluded that

> "The science clearly shows that climate is changing largely as a result of greenhouse gas emissions. The science is equally clear that without rapid and drastic cutbacks in greenhouse gas emissions, the changes are likely to threaten life, property and Earth's biosphere in all sorts of ways ... some of these changes are already happening." (M. D. Lemonick 2012) (http://www climatecentral.org/)

Given this prognosis, and the increase in pest occurrence coupled with few new tools and lack of enthusiasm for genetic modification as one of the new technologies, the apparent decrease in investment in agricultural research seems to be happening at exactly the wrong time—all the more reason to develop international entomology and coordinate efforts globally.

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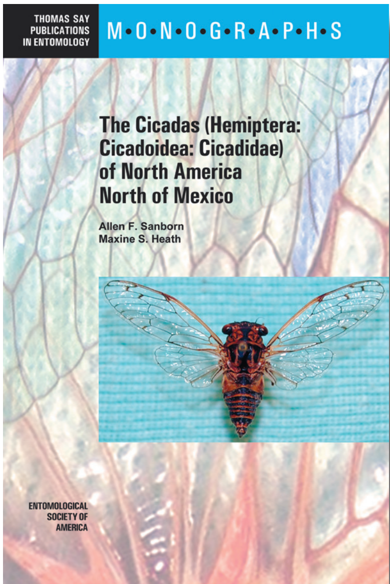

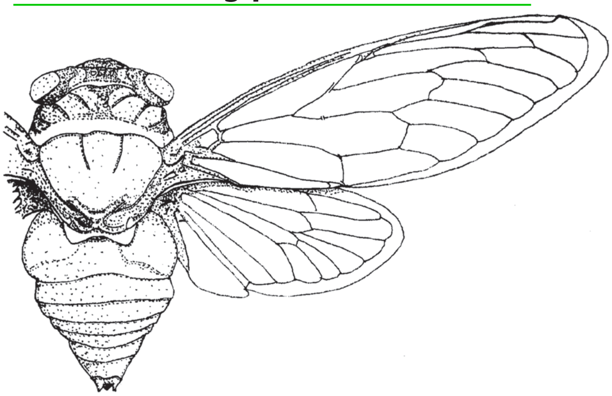

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What is it? answer:

The images are of an Old World epipaschiine moth (Pyralidae: Pyraloidea) in typical resting posture, viewing the animal from the front. This group of moths is characterized by a highly modified and greatly extended scape at the base of each antenna in the male, which together resemble a pair of ballerina arms curving above the head. This posture has been observed in nature by MAS, but it has never before been photographed. The behavior is presumably involved in chemical and/or physical short-range courtship; the morphology of the structures is species-specific. Epipaschiinae and related subfamilies are also characterized by elaborate tufts of upraised scales on the forewing as seen in the photo. This first photograph of *Stericta*, n. sp., from Hong Kong, was taken by Paul Pratt, Invasive Plant Research Laboratory, ARS, USDA.

This photo was submitted by M. Alma Solis (MAS), Systematic Entomology Laboratory, ARS, USDA. SEL, USDA, Smithsonian Institution, P.O. Box 37012, National Museum Natural History, E-517, MRC 168, Washington, DC 20013-7012, alma.solis@ars.usda.gov.

If you have a color photograph of an insect, insect part, or entomological apparatus that you would like to submit for the "What is it?" feature, please e-mail a 300-dpi TIFF and a description of the image to the editor at cdarwin@aol.com.

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