49.3%, 3 days later). In contrast, the combination *B. bassiana* - *E. formosa* showed that mortality increased as the time interval increased (52.6% to 61.9%, 3 days later). In both cases, mortality from the effect of the two control agents was greater than that caused by either of the natural enemies acting independently. In a second experiment, each fungus was applied to whitefly nymphs previously exposed to *E. formosa*. The greatest mortality was observed when fungi were applied 2 days after nymphs were exposed to the action of the parasitoids (75.4% in the combination *E. formosa* - *P. fumosoroseus*, and 84.5% in the combination *E. formosa* - *B. bassiana*). Mortality decreased as the time between parasite exposure and infection increased. The results suggest that the combination of *E. formosa* and one of the two entomopathogenic fungi has potential use in the control of *T. vaporariorum* nymphs under greenhouse conditions.

Microsatellite Markers in the Entomopathogenic Fungus Paecilomyces fumosoroseus for Monitoring of Isolates Introduced against Bemisia tabaci, Epidemiological and Population Genetics Studies

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One of the greatest challenges for improving the use of fungi, not only as microbial control agents but also for all epidemiological and population genetics studies, is the ablity to identify the isolates used and analyzed. The haploid entomopathogenic hyphomycete *Paecilomyces fumosoroseus* (*Pfr*) used for biological control of economically serious agricultural pests is a geographically widespread fungus infecting various orders of insects. In particular, it is naturally associated with the whitefly *Bemisia tabaci* (Homoptera Aleyrodidae) in various geoclimatic regions. Ongoing difficulties in applying morphological approaches to isolate-recognition *Pfr* have spurred the search for discriminant markers. Based on rDNA-ITS RFLP approach, all isolates collected from *B. tabaci* clustered in 1 group although they originated from diverse geographical locations. Microsatellite markers were therefore developed and characterized in *Pfr*. Upon the 9 isolated, 8 were revealed as polymorphic. Amplification success and length and sequence variability of these markers were investigated on a set of 34 *Pfr* sampled from various insect-host species and geographical locations. First, PCR amplifications at the 9 previously designed loci were only successful with *Pfr* from Homoptera hosts (25 *B. tabaci* and 1 *Phenacoccus sp*. hosts) which has suggested existence of genotype host-associations. Second, allele size and above all sequence analyses (flanking microsatellite-regions and/or microsatellite regions) improve our ability to discriminatamong *Pfr* isolates and provide a means to subdivide isolates obtained from *B. tabaci*. Indeed, from a unique *Pfr* rDNA-ITS RFLP haplotype, 7 patterns based on specific microsatellite allelic-sizes and 14 haplotypes based on their sequencing were resolved from *B. tabaci* which emphasizes their value for further epidemiological studies and use in biological control. Finally, their value for genetic relationships inference was also proven and a phylogeographic framework was henceforth established providing evidence for two distinct lineages of *Pfr,* American-Cuban and Asian, with a putative Indian origin of the American-Cuban *Pfr* group from an Indian group. Consequently, the microsatellite markers described represent a potent tool for *Pfr* population diversity studies and open a new and informative window on the use of well-discriminated isolates in epidemiology and biological control strategy against *B. tabaci*.

Novel Candidates for the Development of Biopesticides to Control Whitefly Pests

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The sweet potato whitefly, *Bemisia tabaci* (Biotype B) causes hundreds of millions of dollars in crop losses each year both in field and greenhouse settings. Reports of pesticide resistance in whiteflies and environmental concerns related to pesticide usage have made the reduction of pesticide application a primary goal for agriculture and have increased the emphasis on the use of cost-effective biological control strategies and insect specific biopesticides in IPM programs. Characteristics associated with the designation biopesticide include: greater safety than conventional pesticides and usually no residue problems. The two classes of biopesticides are biochemical and microbial. The use of biochemical and bacterially-produced insecticidal compounds as control agents for whiteflies and other sucking insects are worthwhile alternative strategies that should be examined. Recently, several monosaccharides as well as toxins produced by

Photorhabdus luminescens and a newly discovered bacterium, *Chromobacterium substugae*, were found to be toxic to whiteflies. Arabinose, mannose, ribose and xylose, when added to artificial diet, were found to kill sweet potato whitefly (SPWF) nymphs and adults. Results showed that there was no substantial inhibition of alpha glucosidase (converts sucrose to glucose and fructose) or trehalulose synthase (converts sucrose to trehalulose) activity by arabinose, mannose or xylose. When adult *B. tabaci* were fed for 24 h on diets containing U- ^{14}C sucrose, L-[methyl-^{3}H] methionine or inulin-[^{14}C]-carboxylic acid, and one or none (control) of the toxic sugars, the four insecticidal sugars, significantly reduced (by 70 – 80%) the amount of radioactivity incorporated and excreted in honeydew. Thus, we conclude that the four sugars act as antifeedants. When U- ^{14}C sucrose and either arabinose, xylose, or mannose were fed to *B. tabaci* adults, less radiolabeled carbon dioxide was excreted; thus, respiration also appears to be reduced. Interestingly, melibiose and trehalose, two sugars that are not toxic to whiteflies, also reduced (by 30 – 40%) U- ^{14}C sucrose uptake but not carbon dioxide release. In other experiments, a high molecular weight insecticidal protein complex (Tca) produced by the entomopathogenic bacterium P. luminescens, has been reported to be orally toxic to *B. tabaci*. When artificially fed Tca, newly emerged nymphs developed poorly, or not at all. Tca concentrations of between 0.1 and 0.2 ppm reduced the number of nymphs reaching the second instar by 50%. In addition, a preparation of Tca missing two prominent subunits, TcaAii and TcaAiii, was found to be at least as toxic to *B. tabaci* nymphs as Tca itself, indicating that the activity of Tca is not dependant on the presence of these subunits at the time of ingestion. Tca (−Aii and Aiii) administered at 4 ppm to adult whiteflies also reduced mean day survival from 6.7 to 2.6 days. In addition, a recently discovered species of *Chromobacterium* (*C. subtsugae*) was found to produce toxins that are insecticidal to whiteflies and other insect pest species. When fed on a diet containing 10% of a *C. subtsugae* extract, the number of 2nd, 3rd and 4th instar nymphs and emerged adults was significantly lower (15 –34%) in experimental groups as compared to control groups. When adult *B. tabaci* were fed on artificial diet containing 5% of a cell-free bacterial preparation, 100% mortality was observed after 4 days. Upon fractionation through molecular weight cut-off filters, activity was present in the 3–10 kDa and greater than 300 kDa fractions. Thus, data support the existence of at least two toxins, and although the identities of these toxins have not yet been determined, results show that the toxins are not protein in nature.

Bemisia tabaci research: Past -present – future

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Bemisia tabaci was described as a pest in 1889 and has maintained that status ever since. Damage ranges from direct plant sucking to honeydew production and plant contamination through virus transmission. Research followed damage-related developments. We review it in relation to the pest's identity, plant relationships, natural enemies and management, pointing out past research directions and possible directions for future research. Principal research on the identity of *B. tabaci* began with the recognition of more than one biotype differing in life history parameters, host plant associations, plant-related damage and insecticide resistance. Following mapping of the world situation, we have reached a phase in which finer meanings of the biotype identity can serve as landmarks for their affinity and pestiferous characteristics. Plant relationships, combined with the study of insect behavior, probably constitute the most meaningful subject on which research can bring about better management of *B. tabaci*. The discovery that plant metabolism is manipulated by whiteflies and the existence and action of PR proteins and of other plant defensive chemicals, are landmarks that should be followed. Their mode of action should be better understood and serve as bases for future plant manipulation and breeding. The initial production of plant stimulators and their utilization for overcoming whitefly-caused problems are steps in this direction. Study of ecological parameters such as influences of field sizes and shapes and the behavior of the organisms involved, may also help to improve whitefly management. Natural enemy exploitation has moved from utilization of spontaneous whitefly decimation by local fungi, parasitoids and predators through the manipulation of available biological control agents to the screening and selection of globally occurring organisms. This has provided excellent greenhouse control and should encompass more of the available unstudied organisms and broaden its scope to all facets of *B. tabaci* pest conditions. Natural enemy utilization should take into account the host plant relationships as well as the available insecticidal solutions. Controls strategies presently take into account the available enemies and insecticides. Plant resistance-inducing materials and plant stimulators are just coming into use. With proper research, the integration of molecular technology, proper plant breeding and utilization of additional natural enemies, better *B. tabaci* management can be achieved.