Biological control of Bemisia argentifolii
(Homoptera: Aleyrodidae) Silver leaf white fly;
a crucifer pest in the southern USA

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Abstract
Since 1991 Bemisia argentifolii, the silverleaf whitefly (SLWF), has caused annual crop losses of $500 million in the USA including crucifer crops in California and Texas. Foreign exploration for (SLWF) natural enemies has been carried out in 25 countries including SE Spain where the agricultural system is similar to parts of Texas and California where (SLWF) is a pest. Parasitized (SLWF) were sent to the APHIS/PPQ quarantine, Mission Biological Control Center, TX, (MBCC) from Murcia Spain in 1991. The Eretmocerus sp. which emerged was identified using a RAPD-PCR technique and given a unique number (Eretmocerus nr. mundus M92014 - Spain ) which distinguishes it from native and other exotic species of Eretmocerus. Evaluation in both the laboratory and in the Lower Rio Grande Valley on kale, and broccoli showed that this species parasitized more (SLWF) than the native Eretmocerus spp. and other exotic parasitoids tested. It has been recovered from (SLWF) infested cole crops at several release sites through 94–96. In addition M92014 is more tolerant of selected pesticides than native US Eretmocerus spp.

Key words: Bemisia, Eretmocerus, Spain, USA, Biocontrol, Crucifers

Introduction
Bemisia argentifolii Bellows & Perring, 1994, the Silverleaf Whitefly (SLWF) (Perring et al, 1992) is a highly polyphagous, insecticide resistant Bemisia species recognised in the USA since 1988/89. Both B. argentifolii and the Diamondback moth Plutella xylostella are very major, region-wide introduced pests often occurring together in the southern USA where crucifers are an important component of the agricultural system. Both pests exhibit strong invasive potential; wide host plant ranges which make them a very serious threat to a broad range of crops; resistance to pesticides and in the case of P. xylostella resistance to Bacillus thuringiensis; migratory tendencies, leading to mass migrations of clouds of SLWF between crops; and to annual movement of Plutella northwards. Both have spread widely due to the intervention of man in moving infested cuttings and seedlings from one part of the country to another. B. argentifolii has been recorded from over 900 plant species in 74 families, (Cock, 1986, 1993). Outbreaks of B. argentifolii in Arizona, California, Florida, and Texas, caused estimated crop losses in excess of $500 million in 1992 (Faust & Coppedge, 1995). B. argentifolii was rarely seen on cruciferous crops before 1990 in the agricultural region of southern California (Perring et al. 1991) and did not constitute an economic threat. In 1990 reports indicated that cole crop growers were using multiple pesticide applications against whiteflies for the first time (Perring, et al. 1991) and that there was particularly severe damage to cauliflower and broccoli, Brassica oleracea L. In southern Texas severe damage to cabbage was also noted at the same time (Elsey & Farnham, 1994). In addition to considerable direct damage, cole crops act as overwintering reservoir plants for whitefly populations, which move onto cotton in spring when the crucifers are harvested. A number of physiological disorders associated with the presence of immature whitefly stages leading to weight loss have been recorded; in Hawaii yellowing and stem blanch in Kai Choy, (Costa, et al. 1993); and in 1990 in Arizona white streaking disorder of broccoli and cauliflower, (Brown et al, 1992). The appearance of an apparently new gemini virus, Cabbage leaf curl virus followed the arrival of B. argentifolii in southern Florida (Abouzid et al, 1992) and Bedford et al,(1994a, b) report leaf yellowing on B. argentifolii infested cabbage and cauliflower. B. argentifolii is resistant to most insecticides, and despite massive pesticide spraying its numbers continue to increase. Besides causing rapid development of resistance to insecticides, massive pesticide treatments impact on non-target organisms leading to a severe reduction or elimination of natural enemies, further compounding the problem. Foreign exploration for natural enemies has been carried out by the USDA/ARS European Biological Control Laboratory (EBCL) based at...
Montpellier, France since 1991 (Kirk et al., 1993; Lacey et al. 1993). This paper reports on the collection, shipment, rearing, release and preliminary evaluation of insect parasitoids for the biological control of B. argentifolii in cole crops in the USA.

Materials and Methods

Natural enemy collections and shipments

Collection sites were selected, based on climate matching with whitefly infested areas in Arizona, California, Florida and Texas, Walter and Lieth (1967), Anonym. (1963). The diverse landscapes and agricultural systems present worldwide suggested a potential for many suitable habitats for whiteflies and natural enemies. Extensive collections were made twice in the same areas when possible, in spring and fall to cover the widest range of conditions. Parasitized whitefly populations were searched for on crop plants, weeds, trees, and ornamental gardens. Whitefly (parasitized) infested leaves were carefully removed from plants, air dried for 12 hours, wrapped in tissue paper, and placed in paper bags. Each sample bag was placed into another bag and finally a third, which was stapled shut. All samples were collected and stored separately, based upon host plant and locality. Collections were shipped in insulated boxes by airfreight to the USDA/APHIS/PPQ quarantine at Mission, TX (MBCC) where parasitoids emerged.

Rearing of natural enemy cultures

Natural enemies of B. argentifolii imported into the MBCC, were placed in cages containing B. argentifolii infested plants. Rearing conditions were 24–29 CX, 50–70% RH and a light regime of 14:10. A maximum number of unique species or biotypes of natural enemies were isolated into separate emergence containers by date, geographic location, and host plant. Individuals from each candidate population are collected from the emergence containers and used to start separate cultures. Cohorts are also collected for identification using classical taxonomic, biochemical and molecular genetic approaches.

Quarantine screening of natural enemies

Quarantine screening was initiated to estimate the potential effectiveness of exotic Eretmocerus and Encarsia spp. parasitoids in order to prioritize which species are mass reared for field release and evaluation (Goolsby et al. 1996). Screening for potential effectiveness is accomplished by measuring the reproductive potential and attack rate of individual female parasitoids on B. argentifolii infesting cole crops.

Identification and genetic markers for insect parasitoids

The identification of the hymenopterous parasitoids using PCR techniques has not previously been attempted and is an important tool in: maintaining the quality of parasitoid species colonies in quarantine; evaluation experiments; and the following up of the natural enemies released into the field. The DNA of individual hymenopteran parasites was amplified in a GeneAmp™ PCR System 9600 thermal cycler with AmpliTaq® DNA polymerase. The following primers were synthesized by Operon Technologies Inc. for use in this study: C04: 5’-CCGCATCTAC-3’, C01: 5’-TTCGAGCCAG-3’, BAM: 5’-ATGGAATCCGC-3’, and ECO: 5’-ATGAATTGC-3’. Insect DNA isolation and purification, PCR reaction parameters, and electrophoresis of PCR products were according to Black et al. (1992). Representative gel patterns of the voucher specimens are stored on computer disks in the MBCC.

Classical identifications by systematists

Microscope slides of adult parasitoid specimens were prepared using the technique described by Noyes (1982), and of whitefly nymphal case remains using Martin’s (1987) method. The whitefly were sent to S. Nakahara at the Systematic Entomology Laboratory, Beltsville, MD, USA and R. Gill, California Department of Agriculture, Sacramento, CA. The Encarsia were sent to M. Schauff at the Systematic Entomology Laboratory, Beltsville, MD, USA and J. B. Woolley at Texas A&M University, College Station, TX. Eretmocerus species were sent to M. Rose and G. Zolnerowich at Texas A & M University, College Station, TX.

Field evaluation

Releases of the exotic parasitoids in broccoli at the Mission Biological Control Demonstration Farm totalled: 52,000 (M92014 Eretmocerus sp. - Spain), 54,000 for (M95012 Eretmocerus sp. - Pakistan) and 60,000 for (M94055 Encarsia sp. nr. pergandiella - Brazil ) and 6000 of the (M95001 Encarsia sp. novo - Dominican Republic).

Results

Natural enemy collections and shipments

Between 1992–1996 more than one hundred shipments of SLWF natural enemies were sent to the MBCC by ARS European Biological Control Laboratory (EBCL) scientists and US and overseas collaborators (Lacey et al., 1993). Silverleaf whitefly infested plants in arid to wet climatic areas within different agricultural systems have been surveyed for SLWF natural enemies in 25 countries in Africa, S. America, the Mediterranean, Indian sub-continent and SE Asia once or several times. More than 98% of parasitoids emerged from SLWF and Trialeurodes vaporariorum (Westwood) (GHWF), infesting 50 plant hosts; 17 crops (mainly Cucurbitaceae/Solanaceae), 13 Ornamentals (no dominant family), 20 weeds (mainly Compositae/Solanaceae).

Identification and genetic markers for insect parasitoids

Collaborators biotyped Bemisia populations from these diverse collections using esterase electromorphs. B. argentifolii was identified from Cyprus, France, Italy,
Spain, and Egypt. Fourteen Encarsia spp. of the 19 spp. recorded worldwide plus 7 new spp.; I confirmed Eretmocerus spp. of the 5 recorded plus 15 new spp., and 1 Aimitus sp. were collected. The parasitoids at Mission were identified using a RAPD diagnostic method and given unique M numbers.

Releases and recoveries
More than 6 million Eretmocerus and Encarsia spp. from 24 cultures with 14 distinct DNA patterns were released in CA, AZ, TX, F, NH, NY. Three species have been chosen by the MBCC as key exotic natural enemies in the Rio Grande Valley TX Demonstration of Biological Control Based IPM program in collaboration with ARS, Weslaco: Eretmocerus sp. from southern Spain, Eretmocerus sp. from Pakistan and Encarsia nr. pergandiella from Brazil. Recoveries of these species have been made in AZ, CA, and TX.

Field Evaluation
The combined results of two broccoli trials indicate that there are significant differences in the numbers attacked from the species tested (F = 12.2; df = 17,302; P < 0.0001). Of the species tested, Eretmocerus sp. nr. mundus (M92014) attacked significantly more B. argentifolii than the other species tested. The combined results of the two trials also confirm that many of the exotic parasitoids reproduce significantly better on crucifers than do the native species, which may afford the exotic species an advantage in the field. This is important because B. argentifolii overwinters in the field primarily on crucifers. In AZ and CA the native Eretmocerus sp. does not readily accept or develop in Bemisia on crucifers (Hoelmer, unpublished data). The field efficacy of Eretmocerus sp. nr. mundus (M92014) in crucifers was further evaluated in a field demonstration of Biological Control Based IPM of B. argentifolii in Mission, TX. Percent parasitism was highest in the broccoli plots on the demonstration farm, followed by the kale refuge strip in the control farm. Densities of B. argentifolii immatures at the end of the season were significantly lower in the broccoli plots on the demonstration farm compared to the kale refuge strips. Parasitized B. argentifolii from the plots were held for emergence of the adult parasitoids. In the control kale, all of the parasitoids reared from the samples were the native Encarsia pergandiella Howard. In comparison, the parasitoids reared from the demonstration broccoli plots were a mix of native E. pergandiella and exotic Eretmocerus. The exotic Eretmocerus were identified by Vacek using RAPD-PCR as E. nr. mundus (M92014-Spain). Although equivalent numbers of the exotic Eretmocerus sp. (M95012-Pakistan) were released in the broccoli, none were recovered. This result is consistent with prior quarantine screening and field cage tests which showed the Eretmocerus from Spain had significantly higher fecundity on broccoli than the Eretmocerus sp. from Pakistan.

Discussion
B. argentifolii overwinter on cruciferous crops in southern California and Texas, moving onto other crops such as melons rather quickly in spring after the crucifers are harvested. Pest control in The Lower Rio Grande Valley and southern Californian agroecosystems is predominately driven by pesticide applications. The Eretmocerus sp. from Spain was collected in an area where if anything chemical applications are more intense. Eretmocerus mundus from southern Spain was more tolerant of selected pesticides than Eretmocerus spp. from the USA (Jones et al. 1996). Because of this tolerance M92014-Spain can be introduced into these agroecosystems. Releases of exotic parasitoids in the demonstration broccoli plots increased levels of parasitism as compared to the kale refuge strips where no releases were made. Eretmocerus sp. M92014 from Spain appears to be largely responsible for the increase in parasitism and control of B. argentifolii in broccoli. Recovery data shows that the Eretmocerus sp. from Spain successfully overwinter on cole crops and field evaluation indicates that it is more effective in destroying B. argentifolii than the native Eretmocerus sp. in cole crops. The long-term beneficial impact of this species in the Lower Rio Grande Valley agroecosystem, its dispersion and impact on B. argentifolii will be monitored using a RAPD molecular technique to detect it in field collected natural enemy populations.

References


