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POTENTIAL MITIGATION OF THE THREAT OF THE BROWN CITRUS APHID, *TOXOPTERA CITRICIDA* (KIRKALDY), BY INTEGRATED PEST MANAGEMENT

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Abstract. **Studies were initiated on the biocontrol of the brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy), the efficient vector of citrus tristeza virus (CTV). Six parasitoid wasp species were found attacking the BrCA in Asia during 2 surveys conducted in 1991 and 1992. *Aphelinus gossypii* Timberlake and *Aphelinus* nr. *gossypii* (both Hymenoptera: Aphelinidae) were found attacking the BrCA and the spirea aphid (SA), *Aphis spiraecola* Patch, in Malaysia, Taiwan, China, and Hong Kong and were imported to Florida for study. Greenhouse tests indicated that *A.* nr. *gossypii* from China readily attacked the SA and the black citrus aphid (BlCA), *Toxoptera aurantii* (Boyer de Fonscolombe), but not the melon or cotton aphid (MA), *Aphis gossypii* Glover. *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) was found parasitizing the BrCA in Venezuela and Puerto Rico, sometimes at high numbers. In Florida, indigenous *L. testaceipes* were found attacking citrus aphids in grove surveys in 1993. A solitary, endoparasitoid gall midge, *Endaphis maculans* (Barnes) (Diptera: Cecidomyiidae), was discovered which parasitized up to 50% of some SA populations in dooryards in central Florida. The gall midge also parasitized the MA and the BlCA. Our study indicated that *Aphelinus* nr. *gossypii* may be able to be established as a "preemptive" biocontrol agent in citrus environs on alternate hosts before the BrCA arrives and could, therefore, help reduce populations of the BrCA to tolerable levels sooner than with our indigenous parasitoids alone. We speculate that integration of biocontrol with cross protection, limited pesticide use, and other control measures have potential to mitigate the threat posed by the BrCA and severe strains of CTV.**

The brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae), is an important citrus pest because it is the most efficient vector of citrus tristeza virus (CTV) (Meneghini, 1946; Costa and Grant, 1951). The BrCA is native to Asia and is distributed throughout the citrus-growing regions except in North America (exclusive of Central America and the Caribbean) and the Mediterranean region. The rapid spread of CTV that lead to the destruction of the citrus industry on sour orange (*Citrus aurantium* L.) rootstock in Argentina and Brazil in the 1930's and 1940's (Knorr and DuCharme, 1951) has been attributed to the BrCA. The BrCA has subsequently spread to northern South America (Knorr et al., 1960), and was followed by the spread of severe CTV strains.

The BrCA is continuing this northward establishment in Central America (Lastra et al., 1992), and its establishment throughout most of the Caribbean Basin (Yokomi et al., unpublished data) in the past 2 years increases the likelihood that it may soon be in Florida. It is desirable, therefore, to develop control measures for the BrCA that may slow its establishment or reduce its populations enough to prevent rapid spread of severe CTV isolates. Pesticides can be used in limited areas, but are not long-term solutions. If effective biocontrol agents of aphids are developed, it would help protect existing plantings and provide more time to develop new cultivars with CTV resistance.

Predators are general feeders but are not well suited for regulation of aphid populations because they arrive too late and leave too early to be reliable biocontrol agents unless inundative measures are used (Carver, 1989). Their increase lags behind that of the aphid host (Frazer, 1988). Parasitoids, in contrast, are more host specific and can increase rapidly as hosts become available. For example, Nearctic *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) was introduced into southern France to control the spirea aphid (SA), *Aphis spiraecola* Patch and the black citrus aphid (BlCA), *Toxoptera aurantii* (Boyer de Fonscolombe), in 1973. It quickly became established throughout the Mediterranean area and became an effective biocontrol agent for the BlCA and some other pest aphids (Tremblay, 1984; Starý et al., 1988). Introduction of some of the BrCA's native parasitoids into Florida's indigenous aphid populations has potential to bring BrCA (once introduced) populations to a lower equilibrium more rapidly than in their absence. Such a "preemptive" biocontrol program has been developed for the Russian wheat aphid in Australia (Aeschlimann and Hughes, 1992). For these reasons, we explored parts of Malaysia, Taiwan, China, Hong Kong, and Venezuela for BrCA parasitoids in 1991 and 1992. In this paper, we discuss progress on the biocontrol of citrus aphids and suggest ways it could be used in an integrated management strategy to mitigate or suppress the threat of the BrCA and severe CTV strains.

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Methods and Materials

Collection of exotic parasitoids of the BrCA. In late August to September 1991, the senior and fourth authors collected citrus aphids and parasitoids in Malaysia and Taiwan, and in 4 southern provinces of China and Hong Kong in July 1992 (Table 1). In September 1992, the senior author surveyed the area around Maracay, Venezuela, and San Juan and Utardo, Puerto Rico, for parasitoids of the BrCA (Table 1). The emphasis was placed on parasitoids of the BrCA and the (SA). Parasitized aphids were collected and placed in containers covered with clear plastic film (food wrap) or organdy and checked daily for adult emergence. Adult parasitoids were collected in gelatin capsules with a small amount of tissue paper to prevent specimen breakage or in 70% alcohol and returned to the laboratory for identification. All live material was introduced under permits issued by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) to quarantine in the Biocontrol Laboratory, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida.

Parasitoid Identification. Specimens of the aphid parasitoids found were sent to the USDA, Agricultural Research Service, Systematic Entomology Laboratory in Beltsville, Maryland, and Washington, D.C. (R. Gagné, P. Marsh, and M. Schauff) and M. Hayat, Aligarh Moslem University, Dept. of Zoology, Aligarh, India, for identification. Additional parasitoid identifications were made by F. D. Bennett and G. A. Evans, Department of Entomology and Nematology, University of Florida, Gainesville, Florida.

Parasitoid colonies. Parasitoid adults were caged with the SA and the melon or cotton aphid (MA), *Aphis gossypii* Glover, in separate cages in attempts to establish live cultures of the parasitoid. Adult parasitoids were given a honey/water diet to assist their subsistence. The SA was reared on *Chenopodium ambrosoides* L. in 1991, and later on sweet viburnum, *Viburnum odoratissimum* Awabuki (Rubiales: Caprifoliaceae), and red tip photinia, *Photinia* × *Fraserii*. The MA was reared on okra (*Hibiscus esculentum* L. var. Clemson spineless). Parasitoid colonies were maintained in ventilated cylindrical plexiglass cages (15.2 cm OD × 41.9 cm) in the insectary under artificial light, and in the greenhouse in the Phase I quarantine (maximum security) at the FDACS, DPI in Gainesville. Additional parasitoid colonies were established in an air-conditioned insectary greenhouse at the U.S. Horticultural Research Laboratory (USHRL), Orlando, Florida, upon receiving the proper permit. This greenhouse was equipped with a screened vestibule entry and was covered by sealed, insulated lexan panels. The temperature in the insectary was maintained by an air conditioner at an average temperature of 25 to 27°C with a maximum of 32°C and a minimum of 20°C.

Surveys for indigenous natural enemies. Commercial citrus groves were surveyed from April to June 1993 in 5 locations including Orlando, Winter Garden, Clermont, and Dundee in central Florida. In September 1993, a survey was made of citrus groves in the Indian River, Indiantown, Labelle, Immokalee, and Lake Placid areas. Blocks of 10 trees with tender shoots were examined carefully for aphids and aphid parasitoids. Aphids were collected in plastic bags and kept in a cooler and examined in the laboratory for parasitoids. Thirty aphids per sample were dissected to determine percent parasitism. Additional aphid and parasitoid samples were taken from August to November 1993 from *Viburnum suspensum* and *V. odoratissimum* at Leu Botanical Gardens, Orlando, Florida.

Results

Foreign exploration. We found BrCA population levels to be fairly low in our Asian surveys, even when flush conditions were good. General aphid predators were common. These included many species of ladybird beetles (Coleoptera: Coccinellidae), larvae of syrphid flies (Diptera: Syrphidae), green lacewings (Neuroptera: Chrysopidae), brown lacewings (Neuroptera: Hemerobiidae), and larvae of chamaeymids (Diptera: Chamaeymiidae). We collected 6 different species of parasitoids from the BrCA. These included

Table 1. Areas surveyed for exotic biological control agents of the brown citrus aphid and collection record.

Date	Collection site (Province or region)	Parasitoids collected
	MALAYSIA	
August 1991	Titi (Negri Sembilan); Serdang, Kuala Lumpur, Ulu Langat (Selangor); Tanah Rata, Kuala Terla (Pahang); Johore Baru, Segamat (Johore)	*Lipolexis scutellaris*, *Lipolexis* sp., *Lysiphelbus* sp., *Aphelinus* sp.
	TAIWAN	
September 1991	Kutanshi area (Hsin Chu Perfecture)	*Aphelinus* sp.
	CHINA	
July 1992	Chengdu, Jinyan, Dujiangyan, Chongqing, Bei Bei, Henchuan (Sichuan); Changsha, Hengshang, Shaoshan (Hunan); Xiamen, Putian, Fuzhou, Minqing (Fujian); Fushan City, Guangzhou (Guangdong)	*Aphelinus gossypii* *Aphelinus* nr. *gossypii* *Lysiphlebus* sp., Aphidiidae
	HONG KONG	
July 1992	Hong Kong, Kowloon, Aberdeen, New Territories	*Aphelinus gossypii*
	VENEZUELA	
September 1992	Maracay	*Lysiphlebus testaceipes*
	PUERTO RICO	
September 1992	San Juan, Utardo	*Lysiphlebus testaceipes*

Lipolexis scutellaris, Lipolexis sp., *Lysiphlebus* sp. (Hymenoptera: Aphidiidae); and *Aphelinus gossypii, A.* nr. *gossypii*, and *Aphelinus* sp. (Hymenoptera: Aphelinidae) (Table 1). In Malaysia, parasitoids from the Aphidiidae were more common than the Aphelinidae, whereas in China, they were similar in abundance. Although care was taken to collect newly parasitized aphids to prevent hyperparasitization (condition where the parasitoid is parasitized by another parasitoid), 5 of 14 collections (36%) from China had hyperparasitoids. These included *Tassonia* sp. (Encyrtidae), and other species from the Eulophidae, Proctotrupidae, and Pteromalidae. BrCA and SA (mummies) parasitized by *L. scutellaris* were shipped from Kuala Lumpur, Malaysia, to Gainesville in September, 1991; SA parasitized by *Aphelinus gossypii* and *A.* nr. *gossypii* were hand-carried to the same facility from Guangzhou, China, in August 1992. Both *Aphelinus gossypii* and *A.* nr. *gossypii* were established in quarantine on the SA. These tiny wasps are difficult to differentiate and were mixed in the same cage. After 2 generations, *Aphelinus* nr. *gossypii* apparently displaced the other species and was the only parasitoid which survived under the crowded conditions in quarantine. Preliminary studies of *A.* nr. *gossypii* indicated that it readily attacked and completed its life cycle on the SA and the BlCA and temperature tolerance tests indicated that it may have good promise as a biocontrol agent in Florida (Yokomi and Tang, unpublished).

We found *Lysiphlebus testaceipes* attacking the BrCA in San Juan and Utardo, Puerto Rico, in September 1992 (Table 1). Inspectors from the USDA, APHIS, PPQ also collected this parasitoid in Puerto Rico in 1992. A high rate of parasitism by *L. testaceipes* was observed in BrCA populations in citrus groves near Maracay, Venezuela, in September 1992 (Table 1). In several fields, nearly all BrCA were parasitized and numerous adult *L. testaceipes* were seen searching leaves for BrCA in which to oviposit. Fifty BrCA mummies were collected from 2 different fields and adult aphidiids were reared. All parasitoids emerged and no hyperparasitoids were present in this cohort. Although the parasitism rate was high, the abundance of aphid mummies indicated that there were once good aphid population levels in the grove. This area is in a severe CTV endemic area and the citrus were planted on a CTV-tolerant rootstock and appeared to be vigorous and productive under their local conditions. What role biocontrol played in this situation is unknown. *Lysiphlebus testaceipes* was imported from Maracay but was not established due to the limited facilities available in quarantine in Gainesville.

Indigenous parasitoids. The principal parasitoid in Florida citrus groves was found to be *L. testaceipes*, and it attacked the MA and the BlCA. Occasionally, we observed the SA with aphidiid-induced mummies but no adult parasitoid emerged indicating that they could not complete their life cycle in this species. No aphelinid parasitoids were found in these surveys. Dissections of citrus aphids in fall 1993, indicated that parasitism by aphidiids ranged from 2-5% in citrus groves except in one instance in Winter Garden when parasitism rose to 33% on October 14, 1993. Numerous MA on hibiscus were found parasitized by *L. testaceipes* in a commercial nursery in Eustis, Florida, and a parasitoid colony was established from this source at the USHRL. Studies are now underway to determine its host range and temperature requirements.

In August 1993, we discovered a solitary, endoparasitoid gall midge, *Endaphis maculans* (Barnes) (Diptera: Cecidomyiidae), readily attacking the SA on *V. odoratissimum* and *V. suspensum* in Orlando. This is the first record of *E. maculans* in the U.S. It was previously recorded only in Trinidad (Barnes, 1954; Kirkpatrick, 1954) and was identified as *Pseudendaphis maculans*. After taxonomic comparison of our specimens with part of the type series loaned to us by the Natural Museum of Natural History, London, and a series found from Costa Rica in the U.S. National Museum, Washington, D.C., we concluded that the genus *Pseudendaphis* is a junior synonym of *Endaphis* (Tang et al., unpublished). *Endaphis maculans* was found to parasitize up to 50% of some SA populations on *V. odoratissimum* in dooryards in central Florida. The gall midge was also found attacking the SA, the MA, and the BlCA in dooryard and abandoned citrus but was absent in commercial citrus groves where pesticides were used. Studies are now underway to determine its potential as a biocontrol agent of aphids.

Discussion

Biological control of homopteran pests of citrus is among the most successful examples of biocontrol in agriculture. A recent example is the control of the citrus blackfly, *Aleurocanthus woglumi* (Ashby) (Homoptera: Aleyrodidae), by the importation of *Encarsia opulenta* (Silvesteri) and *Amitus hesperidum* (Silvesteri) in Florida beginning in 1976 (Dowell et al., 1979). In California, control of the California red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspidae), is obtained by yearly field augmentation by a parasitoid complex including *Aphytis melinus* Debach, *Encarsia perniciosi* Tower, and *Comperiella bifasciata* Howard (Graebner et al., 1984). Other well known examples of biocontrol in citriculture include that of the cottony cushion scale, *Icerya purchasi* Maskell (Homoptera: Margarodidae), and the citrophilus mealybug, *Pseudococcus fragilis* Brain (Homoptera: Pseudococcidae).

Examples of biocontrol successes involving aphids in other crops include that of the wooly apple aphid (Clausen, 1936), the spotted alfalfa aphid (van den Bosch et al., 1959), and the walnut aphid (van den Bosch et al., 1962). In citrus, the success of *L. testaceipes* in the Mediterranean area (Starý 1988) has been discussed earlier in this text. We must, however, be realistic and not to overestimate the degree to which biocontrol can contain the BrCA problem. In Israel, for example, Rosen (1967) found as many as 15 species of hymenopterous parasitoids attacking citrus aphids but still concluded that, in general, those parasitoids were secondary to flush conditions as regulators of aphid populations. Hence, biocontrol should be considered one component, albeit the foundation, of an integrated pest management approach to mitigate the problem anticipated with the introduction of the BrCA to Florida.

In Florida, biocontrol of aphids was first conducted by the USHRL in 1969 with the release of *Aphelinus gossypii* and *Binodoxys indicus* Subbs, Rao, & Sharma (Hymenoptera: Aphidiidae) from India (Denmark and Porter, 1973). No data exist which indicate if these biocontrol agents ever became established. Our Florida surveys did not detect the presence of either of these parasitoids nor *A.* nr. *gossypii*. *Lysiphlebus testaceipes* was found attacking the MA and the BlCA in Florida. We observed that the *L. testaceipes* attacking the BrCA in Venezuela were larger than those found in the Florida surveys. This may indicate strain differences but size is likely due to the larger size the BrCA host compared to our indigenous MA or BlCA. Strain differences between populations of parasitoids will be considered later in our studies. The finding of *E. maculans* parasitizing the SA is very significant. Until now, there were no known parasitoids that effectively attack the SA in the U.S. (Cermeli, 1964; Cole, 1925; Miller, 1929). SA has a wide host range and is extremely abundant in Florida. With such an excellent food supply, it is not surprising that we found *E. maculans* capable of inflicting up to 50% parasitism on populations of this aphid and was exerting control of the aphid population (Tang et al., unpublished data).

Greenhouse and laboratory tests with *A.* nr. *gossypii* indicate that the SA and the BlCA are excellent hosts and show good potential as biocontrol agents. We are currently expanding host spectrum tests and examining the taxonomy of this species to collect data required to obtain a permit for field release. Other parasitoids of the BrCA are known (Carver, 1978; Lo and Chiu, 1986; Starý and Cermeli, 1989; Takanishi, 1990; Yasumatsu and Watanabe, 1965) and will be imported for study at a later date as resources permit.

Most successful biocontrol programs, historically, have involved controlling a non-native pest by the introduction of an exotic natural enemy. This is defined as classical biological control and is the approach being taken in our BrCA program. In biocontrol eradication is never achieved. Periods occur when the pest resurges before the natural enemy populations rise to levels that provide control. If the pest is a vector such as the BrCA, its economic threshold level always remains low and is a limiting factor in the use of biocontrol to mitigate severe CTV/BrCA problems. Therefore, we propose to combine biocontrol with other control strategies. CTV is controlled by the use of a clean stock and budwood certification program (Calavan et al., 1978), resistant or tolerant rootstocks in the case of CTV decline (Wallace, 1978), and mild strain cross-protection for severe stem pitting strains of CTV (Costa and Muller, 1980).

Cross-protection is used commercially in Brazil (Costa and Muller, 1980) and South Africa (von Broembsen et al., 1978; van Vuuren and Collins, 1993). Yokomi et al. (1991) obtained preliminary field data that preinfection by certain mild Florida isolates may delay infection of a severe CTV isolate in cross-protection tests in Dundee, Florida. Two isolates, T49 and T50a, delayed severe CTV isolate infection in Hamlin. In the same test, 11 of 14 mild isolates appeared to delay infection by the severe CTV in Redblush grapefruit. Unfortunately, a devastating freeze destroyed the plot after 3 years and long term evaluation was not possible. Yokomi et al. (1991) concluded, however, that there was some merit in cross-protection, even for citrus planted on sour orange rootstock.

Breakdown of cross-protection after significant challenge in time and inoculum pressure has been shown with CTV decline on sour orange (Powell et al., 1992) and papaya ringspot (Wang et al., 1987). In greenhouse tests, we have shown that vector pressure (number of viruliferous aphids per tree) and the severe isolate selected as the challenge isolate both contribute to cross-protection breakdown (Yokomi, unpublished data). We speculate that reduction of vector populations by biological or limited chemical control may reduce challenge levels of vector populations to levels that may prolong cross-protection as suggested by Gonsalves and Garnsey (1989).

Florida has a significant inoculum reservoir of CTV decline strains on tolerant rootstock (Yokomi et al., in press) and at least 18,000,000 citrus trees on CTV-sensitive sour orange rootstock. Therefore, when the BrCA enters, we predict that CTV decline incidence will increase rapidly. "Preemptive" biocontrol by native BrCA parasitoids may help to mitigate the threat of this aphid by bringing the aphid's populations to a tolerable balance sooner than with our indigenous parasitoids alone. A similar strategy of vector/virus control was recommended by Mackauer (1976) who suggested biocontrol of the sowthistle aphid, *Hyperomyzus lactucae* (L.), to suppress production of winged forms and reduce the incidence of the luteovirus it transmits, lettuce necrotic yellows virus. This was carried out in Australia by Carver and Woolcock (1986) with the introduction of 2 aphidiids for the control of the sowthistle aphid with limited success. Another example is the introduction of parasitoids to control *Cavariella aegopodii* Hottes and Frison, the vector of carrot motley dwarf virus complex (luteovirus) (Hughes et al., 1965; van den Bosch, 1971; Waterhouse, 1985) which was successful.

Limited use of an effective systemic insecticide such as the experimental compound, imidacloprid (Mullins, 1993), may also be incorporated into the integrated pest management program with biocontrol, cross-protection, and a budwood certification program to mitigate the threat of severe CTV/BrCA complex. Such systemic insecticides are relatively safe to nontarget organisms such as aphid parasitoids. Experiments are now being planned to evaluate the potential of chemical control of the BrCA to suppress CTV spread in Puerto Rico.

In summary, biocontrol may be useful in an integrated pest management approach combining other control strategies such as cross protection, tolerant- and resistant rootstocks and scions, limited insecticide use, and transgenic plants with resistance/tolerance to mitigate the problem associated with the BrCA and severe CTV strains.

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POTENTIAL FOR SPREAD OF CITRUS TRISTEZA VIRUS AND ITS VECTOR, THE BROWN CITRUS APHID

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Abstract. **The recent discovery of citrus tristeza virus (CTV) and its most efficient vector, the brown citrus aphid (BCA), in new countries in the Caribbean Basin has heightened fears of the U.S. citrus industry that the aphid will be introduced into the continental U.S. and further CTV-related losses will occur. Florida has a high incidence of mild and decline-inducing strains of CTV, but stem-pitting strains are rare. Stem-pitting**