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IMPORTATION AND LABORATORY PRODUCTION OF TWO SPECIES OF PARASITIC WASPS FOR BIOLOGICAL CONTROL OF THE CITRUS BLACKFLY (*ALEUROCANTHUS WOGLUMI* ASHBY) (HOMOPTERA: ALEYRODIDAE) IN TRINIDAD

V. F. Lopez and M.T.K. Kairo, CABI Bioscience Caribbean & Latin American Regional Centre, Gordon Street, Curepe, Trinidad & Tobago
J. Seales, P. Ram, Ministry of Food Production and Marine Resources, Central Experimental Station, Centeno, Trinidad & Tobago

ABSTRACT: Two exotic parasitic wasps, *Amitus hesperidum* Silvestri and *Encarsia perplexa* Huang & Polaszek, were introduced from Florida into Trinidad during 2000 and 2001, respectively, for the biological control of the citrus blackfly (CBF), *Aleurocanthus woglumi* Ashby. The importation was carried out following guidelines under the FAO Code of Conduct (1996) and the two species were reared in the laboratory for at least one generation to eliminate contaminants prior to being released in the field. Procedures followed during the importation are described. Details are provided of the protocols developed for the laboratory rearing of the CBF and the two natural enemy species.

INTRODUCTION

The citrus blackfly, *Aleurocanthus woglumi* Ashby (Homoptera: Aleyrodidae), a native of Asia, was first reported in the Caribbean from Jamaica in 1913. It subsequently spread to many other countries in the region (IIE, 1995) and was first reported from the Port of Spain area of Trinidad in 1998. Over the next two years, the pest spread rapidly, initially on citrus and other plants in backyard gardens, and later moved to areas of commercial citrus production. This rapid spread was attributable to a lack of effective, host specific natural enemies. The citrus blackfly (CBF) has the potential to cause severe losses in fruit production, ranging from 25% to almost complete crop failure (Smith *et al.*, 1964; Watts and Alam, 1973). This has serious implications for Trinidad, where citrus is an important commercial crop.

CBF is an excellent candidate for classical biological control since a number of successful biological control programmes have been launched against this pest in the Caribbean (Caltagirone, 1981; Browning, 1992; Martin, 1999). In January 2000, the Ministry of Food Production and Marine Resources (MFPMR), Trinidad & Tobago embarked upon a project aimed at the implementation of biological control for CBF. Two parasitic wasps selected for introduction were *Amitus hesperidum* Silvestri (Hymenoptera: Platygasteridae) and *Encarsia perplexa* (=*E. opulenta* (Silvestri) of authors, misidentified) (Hymenoptera: Aphelinidae). This was based on the fact that in all the countries where CBF was introduced, biological control using one or both natural enemies was found to be the most economical, long-term and sustainable method of control (Browning, 1992). In Florida, for example, *A. hesperidum* and *E. perplexa* successfully kept the pest under good control for more than 10 years, resulting in millions of dollars in savings and engendering an increased credibility in biological control of alien pests (Tefertiller *et al.*, 1991).

MFPMR funded the importation programme and contracted CABI Bioscience's Caribbean and Latin American Regional Centre (CLARC) to undertake the importation on its behalf. CABI was also required to assist with capacity building within MFPMR through the provision of training in laboratory production of CBF and the introduced natural enemies and in the identification of the two natural enemy species. Other partners in the importation programme were Caroni (1975) Ltd and the Cooperative Citrus Growers' Association (CCGA). The introduction was carried out following guidelines outlined in the Code of Conduct for the Import and Release of Exotic Biological Control Agents (FAO, 1996). The parasitoids were reared in the laboratory at CLARC for at least one generation prior to being released in the field. This paper outlines procedures followed during the introduction, and protocols developed at CLARC for the laboratory rearing of CBF and the two natural enemies.

IMPORTATION OF NATURAL ENEMIES

The natural enemies were obtained through collaboration with Entomologists in a laboratory in Florida, USA. The parasitoids were collected in the field in Florida and reared under laboratory conditions for 2-3 generations to eliminate any contaminants before being shipped to Trinidad.

One of the requirements of the Code of Conduct is the preparation of dossiers on the potential natural enemies being considered for introduction. Thus, prior to the importation, dossiers were prepared on *A. hesperidum* and *E. perplexa* and submitted to MFPMR for appraisal (Lopez *et al.*, 2000a, b). Each dossier consisted of three sections:

1. summarized information on CBF and its control, including biological control
2. summarized information on the potential natural enemy and
3. potential risks and their minimization, and procedures for elimination of contaminants

Relevant authorities within MFPMR reviewed the dossiers. Following this, the introduction of the two natural enemies was approved and the Plant Quarantine Division of MFPMR issued the necessary import permits.

One shipment of *A. hesperidum* and two shipments of *E. perplexa* were imported from Florida. Procedures followed during the importation were similar for all three shipments. Entomologists from CLARC and MFPMR and the Plant Quarantine Officer received each shipment at the airport. The shipment was then transported to CLARC's laboratories in Curepe and opened in the quarantine area in the presence of the Entomologist from MFPMR. All the insects in the shipment were carefully examined under the microscope and compared with voucher specimens to confirm their identity prior to being released on CBF-infested citrus plants under cages in the laboratory.

The first shipment, which arrived on the 5th of April 2000, was hand-carried by Dr. A. Polaszek, CABI Bioscience's expert taxonomist. It contained 240 adults of *A. hesperidum* and 200 mummies (pupae of CBF containing pupal stages of the parasitoid). After the shipment was opened, Dr. Polaszek prepared the voucher specimens by collecting some male and female *A. hesperidum* from the shipment vials and placing them in alcohol in small glass vials after confirming their identity under a microscope. The vouchers were deposited with MFPMR. The 200 mummies received in the shipment were carefully transferred into clear glass vials, covered with a mesh cap and placed on moistened paper towels in a clear plastic container. This prevented desiccation of the mummies and facilitated the emergence of a further 232 adults.

The first shipment of *E. perplexa*, containing 125 adults, arrived on the 5th of January 2001. The second shipment, which was received on 24th January, consisted of 200 adults. Of these, only 3 and 4 adults were males in first and second shipments, respectively, and the remaining were females. The Entomologists in Florida sent a glass vial containing about 6 specimens in alcohol with the shipments. This material was deposited with MFPMR as vouchers for the shipments.

Throughout the duration of the project, a total of six persons, including staff members from the Research Division of MFPMR, Caroni (1975) Ltd and CCGA, were attached to CABI Bioscience and received hands-on training in the production of CBF and the two natural enemies. They were thus exposed to the day-to-day problems encountered during the rearing process and the methods used to solve the problems.

During his stay in Trinidad, Dr. Polaszek conducted a one-day training on the taxonomic identification of parasitic Hymenoptera for all persons involved in the rearing and field release programmes. He elucidated the main characteristics used for distinguishing various parasitic genera that attack Aleyrodidae. The participants then examined alcohol-preserved specimens of *A. hesperidum* and female *Encarsia perplexa* (obtained by Dr. Polaszek during his visit to Florida) to familiarize themselves with various morphological features of each species. They also examined prepared slides of *E. perplexa* and other *Encarsia* spp.

IDENTIFICATION OF NATURAL ENEMIES

The morphological features used to diagnose *A. hesperidum* included the entirely dark (black) body, lack of wing venation (except for a short vein along the margin) and long, transparent wings, which extended beyond the length of the adult. Males were generally larger than females. All the antennal segments in males were almost similar in size and shape, and loosely attached to each other so that they were bead-like in appearance. The antennal segments in the female were more tightly packed and ended in a club-shaped clava.

Variations in the size, shape of abdomen and colour were among the main features that distinguished the two sexes of *E. perplexa*. Females were almost entirely yellow in colour, except for some dark markings on the thorax and the sides of the abdomen. The tip of the abdomen was black and pointed due to presence of a short, sharp ovipositor. The males were much smaller than females with body almost entirely black with occasionally lighter patches and the tip of the abdomen was not pointed but slightly rounded. There was a distinct fringe of hairs along the margin of the wings in both sexes.

LABORATORY PRODUCTION

Host plants: Citrus plants with 5-6 expanded new flush leaves were used for culturing CBF. The plants were potted in 15-cm diameter plastic bags in a 2:1 topsoil, manure mixture. They were maintained under caged conditions outdoors to prevent them from becoming infested with various pests. The plants were watered three times a week and a foliar fertilizer (Nutrex® N, P, K 20:20:20) was applied once a month.

Rearing conditions: Plants destined for use in laboratory production of CBF and the parasitoids were brought into the Controlled-temperature laboratories (CT Rooms) 1-2 days prior to being used in order to acclimatize them. All rearing rooms were fitted with a double door entrance and an ultra-violet light (uv light or insecutor) to prevent contaminants from getting in or out of these areas.

CT rooms: CT Rooms were used to obtain oviposition by CBF as well as for CBF and parasitoid rearing. Temperatures of 28±2° C and relative humidity (RH) 70±10% were maintained in the laboratory in which citrus plants were placed for oviposition. This room did not have any lights because better oviposition was obtained under dark conditions. In the other CT rooms used for rearing CBF and the parasitoids, the temperature and RH were 26°C and 60±10%, respectively. Artificial lighting was provided in the form of banks of 4-6 fluorescent lights and 2-4 incandescent bulbs, suspended 10-20 cm above the cages. The lighting regime used was 12 h light : 12 h dark.

Greenhouse conditions: The greenhouse was maintained under ambient conditions, with a wall-mounted fan for air circulation. Several steps were taken to prevent contaminants from getting into the rearing room. Along with the double door entrance and uv light, the room was sealed with on all sides with a nylon mesh. Additionally, a 20-cm wide moat around the greenhouse prevented ants and other contaminants from getting in.

Cages: Metal frame cages, 52 cm x 52 cm x 90 cm or 1 m^3, covered with thin, white nylon mesh and open at one end, were used for rearing both the CBF and the parasitoids.

Culture establishment and maintenance: Plants with new flush growth were examined for the presence of pests and diseases. They were washed with a very dilute soap solution, then with water and allowed to dry before being used for culturing CBF. Once the plants had developed 4-6 generations of CBF or 2-3

generation of parasitoids, they were completely depleted of nutrients. They were clipped back and moved out. The plants were placed under outdoor cages, fertilized and allowed to reshoot.

CBF

Five to nine potted plants were placed in each cage, depending on the size of the plants and the cage. Large numbers (400-1000) of field-collected CBF adults were released in each cage. A few days later, the plants were examined for oviposition, and for the presence of contaminants such as pests like mealybugs as well as natural enemies like coccinellids, parasitoids and spiders, which were promptly removed. Plants were labeled with date(s) of infestation and moved into cages in the outdoor rearing facility. When the allocated space in the outdoor facility became insufficient, they were placed in CT Rooms. CBF took about 7-8 weeks to complete one cycle outdoors and an additional 1-2 weeks in the CT Room. A total of 25-40 plants were infested each week until about 400-500 plants with moderate to heavy infestations were obtained.

For culture maintenance, emerging adults were allowed to continue to develop on the same plant(s) for another generation if new flush growth is present on the plant at this time. If new flush was not present, the adults were collected and released on clean plants with new flush placed in cages in the dark room for oviposition. Plants that harboured mainly 1st / 2nd instars were used for parasitoid rearing and the remaining plants were used for culture maintenance.

Amitus hesperidum

A total of 20 citrus plants harbouring mostly 1st instars of *Aleurocanthus woglumi* Ashby were set up in four small cages. All the adults received in the shipment as well as those emerging from the mummies were released in the various cages. Development of the parasitoid from egg to adult stage took 60-75 days. When the adult parasitoids began to emerge, the date(s) of emergence were recorded. The parasitoids were collected, sexed and allowed to mate. From an initial population of 472 adults, a total of 1983 parasitoids were produced in the first generation during June and July. The number of males produced was 606 and females 1492, with a male to female ratio of 1:2.3. A total of 491 adults (112 males, 379 females) were used for culture maintenance. The remaining 1492 adults (494 males, 998 females) were used for field releases.

Subsequently, routine culture maintenance was carried out by releasing about 50-100 mated females per cage containing 4-5 moderate- to heavily-infested plants. At least two generations of parasitoids could often be reared on most plants. This is because CBF adults usually emerged from unparasitized pupae about 2-3 weeks before parasitoids emergence occurred. Once the plants in the cage had new flush growth, the adults moved to these points and oviposited. Two-three weeks later, the eggs had hatched and mostly 1st / 2nd instars were present on the plants. The emerging parasitoids thus found these stages and oviposited on them. The parasitoids were subsequently collected using a brush or an aspirator and allowed to mate before being released in a new cage or in the field.

Amitus hesperidum was very prolific and a total of over 2000 parasitoids were produced at CLARC between August and November despite lower scales of production. More than half of these insects was used for augmenting field populations. Transfer of *A. hesperidum* cultures to MFPMR's Central Experimental Station, Centeno (CES) which began with the first laboratory generation in June/July, was completed in December 2000.

Encarsia perplexa

For the two shipments, a total of 18 and 15 citrus plants harbouring all stages, but predominantly 2nd/3rd instar of *Aleurocanthus woglumi* Ashby were set up in a total of 8 cages, 4 cages per shipment. Adults from the shipments were released on the plants within a few minutes after opening the shipment and examining the vials with parasitoids for contaminants.

Development from egg to adult stage of the parasitoids took 25–30 days. Production of first generation *E. perplexa* occurred in only one cage from the first shipment and two cages from the second shipment. A total of 237 female *E. perplexa* were collected for about six weeks during February and March 2001. A total of 176 females were sent for field releases between 6th February and 13th March. On the remaining, 32 unmated females were released in second shipment cages @8 females per cage. This was done to ensure male production in these cages. The remaining insects, together with those emerging after the 13th of March, were used for maintenance of laboratory cultures as follows: Plants on which production of parasitoids occurred were placed in two 1m x 1m x 1m cages together with 5-10 new plants harbouring mixed stages of CBF. Adult *E. perplexa* that were already present or emerged later thus had additional CBF to parasitize. The cages were observed twice a month but were otherwise left undisturbed to provide near-natural conditions for the developing parasitoids.

During the 1st week of May, at least one male and fairly large numbers of females were seen in the cages. This meant that production of males and a new generation of parasitoids had occurred in the cages. From nearly 150 parasitoids that were found in the two cages, 110 females were collected and sent for field releases on the 8th of May. On the 16th of May, two *E. perplexa* females emerged from mummies collected at the site of the 1st release, confirming its establishment in the field. Culture maintenance in the large cages has continued. Transfer of cultures to CES has already begun and is expected to be completed in a few weeks.

Procedures for eliminating contaminants

Constant vigilance was of utmost importance to maintain clean, contaminant-free cultures. To deal with specific problems of contamination, the following routine practices were carried out:

- only healthy, contaminant-free plants were used in culturing CBF and parasitoids
- after CBF oviposition and before the introduction of parasitoids, plants were thoroughly examined for contaminants, which were promptly removed
- all infested plants were examined regularly and dead insects and other debris was removed
- when necessary, leaves with older infestations were wiped with a clean moist cloth to remove honeydew deposits and minimize the development of sooty mold
- cage meshes were changed regularly and dead insects and other debris removed
- the introduction process was overseen by experienced Entomologists at CABI Bioscience who had conducted extensive work on classical biological control of Aleyrodidae and other Homoptera

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