FINAL REPORT

Citrus Replanting Project
Ministry of Agriculture, Jamaica

Research Services – Entomology

Submitted by

CARDI

Caribbean Agricultural Research and Development Institute

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INTRODUCTION

The Jamaican citrus industry is a major component of the island's agricultural economy. This industry is threatened by the presence of citrus tristeza virus (CTV) which causes decline and death of infected trees. The Virus, CTV, is spread by the brown Citrus aphid (BrCA) (Plate 1) which is the most efficient vector of the virus, CTV.

Plate 1: Brown Citrus Aphid *Toxoptera citricida*

Certain aspects of the biology and behaviour of BrCA contribute to its superiority as a vector of CTV.

The aphid

- is adaptable to a wide range of climatic conditions
- has a very high reproductive rate
- has a narrow host range, tending to feed primarily on citrus thereby enhancing the opportunity to perpetuate the disease
- has a higher transmission efficiency than other CTV vectors
- transmits CTV strains which are not transmitted by other vectors

In response to the presence of the disease, the Government of Jamaica (GoJ) through the Ministry of Agriculture (MINAG) executed a citrus replanting project (CRP) which involves the replanting of Jamaican citrus on to resistant rootstock supported by research services in the disciplines of Agronomy, Entomology and Plant Pathology.

The Caribbean Agricultural Research and Development Institute (CARDI) successfully tendered for the contract to provide research services in Entomology. This contract was signed 9 December 2002

The team which executed this contract comprised:

- Mr Llewellyn Rhodes, Lead Entomologist
- Mrs Dionne Clarke-Harris, Team Leader and Associate Entomologist
- Mr Bruce Lauckner, Biometrician
- Mr Adlai Blythe, Accountant
- Research Assistants, Carlton Allen (2 June 2003 to 31 December 2004), Donald Simpson (1January 2005 to 31 January 2006)

The terms of reference of the contract were:

- to survey the project area to determine the seasonal and spatial distribution of the brown Citrus aphid
- to investigate the importance of indigenous natural parasites and predators of the brown Citrus aphid
- to investigate the potential use of chemical or biological control methods for brown Citrus aphid in the project area
- to determine the presence and significance of other insect vectors of CTV and other economic diseases

EXECUTIVE SUMMARY

In responding to the terms of reference, the consultant conceptualized the scope of works as comprising two components:

Component1

Understanding BrCA ecology through studies on spatial and temporal distribution and the role of key factors

Component 2

Investigating and integrating management options namely biocontrol and the use of pesticides

The scope of works was then disaggregated into discrete studies under each of the terms of reference, involving surveys, laboratory experiment, field trials etc. Specific IPM outputs would become components of a decision support system for citrus pest/crop management. It is envisaged that these outputs will ultimately be incorporated into a citrus production and management database which can be made available in various formats for direct access and for the development of information products.

An outline of the plan of work, experimental protocols and the proposed time frame for executing the various activities were presented in the inception report and was accepted.

The studies and findings in response the terms of reference were as follows:

(i) Survey the project area to determine the seasonal and spatial distribution of the brown Citrus aphid

Determination of spatial distribution

A survey to determine the geographical distribution of BrCA was conducted throughout Jamaica (June to September 2003) taking into consideration the effects of altitude and other geographical features, farm size, rootstock and scion variety. This was done to address the term of reference requiring the determination of the spatial distribution of the insect in the project area.

The execution of the islandwide survey for brown citrus aphid was completed by the end of September 2003 despite numerous constraints (bad weather and transportation, in the main). A total of 124 farms in 13 parishes was surveyed. Data collation was completed by mid November 2003 and analysed. From the data, lowest aphid densities (1.3-1.5 aphids/cm flush) occurred in St. James, Westmoreland, Hanover, Trelawny and Portland. The highest densities were recorded in St. Thomas and Manchester of 5 and 6.7 aphids/cm flush, respectively.

Correlation analyses were conducted on a data set of 83 farms of which 81.9% were infested with BrCA. There was very high variability in observations (especially the number of BrCA recorded) among farms within a given parish. There was a significant correlation between flush length and number of BrCA ($P<0.01$) as well as between the flush density and number of BrCA ($P<0.01$).

In collaboration with the MINAG Databank and Evaluation Division, the survey data was used to generate GIS maps to elucidate geographical factors influencing distribution and population levels of BrCA. GIS maps illustrating, the incidence of infested trees and aphid density at the individual farms were produced. Farms with greater than 50% of its trees infested occurred throughout the island while farms with greater than 76% infested trees occurred in St Catherine, Clarendon, Manchester Trelawny and Hanover. However, the highest aphid densities (5.1-18.0 per cm flush) were recorded on fewer farms and parishes (St Catherine, Clarendon, Manchester and St Elizabeth). It is also noteworthy that farms which had high aphid densities had correspondingly lower dispersal throughout the field although there were a few exceptions.

Based on the high incidence of infested farms (100%), the high incidence of infested trees (55.7%) and the high aphid density (4.1 aphids / cm flush) it may be concluded that the parish of St. Catherine is the most severely infested with BrCA.

Other GIS maps were generated as layers to display the survey data as individual overlays for a better visual appreciation of the correlation between BrCA infestation levels and geographical factors e.g. elevation, rivers, etc. Due to the large size of these files, electronic copies of these GIS layers are omitted from the electronic copy of this report and are submitted separately as an addendum.

Data on BrCA populations from 121 farms were analyzed to determine whether there was any relationship between the number of BrCA and number of flushes per tree, cumulative flush length, age of trees, location of farm (parish) and rootstock variety.

Of these 121 farms, 21 were excluded due to the absence of flush. For the remaining 100 farms from 12 parishes data were subjected to ANOVA and Regression techniques.

Generalized linear models showed that aphid count was related to flush density; length of flush from which the counts were made; age of the trees with trees younger than 5 years being more infested. There were significant differences due to scion variety with Valencia being more heavily infested than Ortanique. There was no relationship between aphid counts and elevation (above sea level), parish and rootstock.

Development of sampling protocol with respect to spatial distribution

Data on the dispersion of pest populations (between tree and within tree) were recorded from two farms to develop an understanding of the spatial distribution infestation dynamics of BrCA, and for the development of sampling protocols for population assessment and pest management.

The following conclusions were drawn:

- Sampling can be conducted to determine presence / absence of BrCA or estimate its population and infestation indices.
- For both objectives, sequential sampling plans are more robust than random sampling due to the high levels of aggregation of the aphid.
- Sampling for presence/absence can be terminated when the first aphid is detected or when a fixed number of trees (25 trees maximum) has been examined using the tree selection protocol presented herein.

- The number of samples required to estimate BrCA populations can be calculated using Taylor's Power law coefficients.
- Large samples of about 50 trees are required to estimate BrCA populations in cases where the proportion of infested trees is between 10 and 25%
- Values of '*D*', the precision level of the estimate, are usually set at 0.01 to detect small population changes in detailed ecological studies and at 0.25 which allows detection of a doubling or halving of the population. Very precise estimates require very large samples. For routine population monitoring, a precision level, '*D*', of 0.25 is adequate.

Determination of seasonal distribution

Determination of the seasonal distribution of BrCA was achieved by monthly monitoring of aphid populations in citrus orchards selected to represent a range of agro ecological conditions.

The relationship between tree and flush infestation is an indication of the aggregation and dispersal behaviour of BrCA in response to flush production. In general, cases where tree infestation levels are high in relation to flush infestation indicate a situation in which many trees are in flush and the aphid is widely dispersed among the abundant flush. The opposite scenario of high flush infestation but low tree infestation indicates a situation where the aphid is aggregated on the relatively fewer trees which are in flush.

During our investigations, the presence of newly expanding flush coincided with the timing of the onset and peak periods of infestation by BrCA. Overall, the flush stage newly expanding/newly hardening (NE/NH) had the highest incidence of brown citrus aphids. The model fitted was highly significant with the accumulated analysis of variance showing highly significant effects for site and stage of hardening but the interaction was not significant. The data obtained during this study did not show any correlation between the quantity of rainfall and BrCA infestation although it is widely recognized that rainfall/soil moisture is the main factor influencing flush production. Additional long term studies are required to develop a fuller understanding of the effects of weather and other abiotic factors on BrCA populations and could form the basis of a research project in a university graduate studies programme.

(ii) Investigate the importance of indigenous natural parasites and predators of the brown Citrus aphid

Distribution of BrCA predators in Jamaica

An islandwide survey of natural enemies of BrCA was conducted concurrently with the survey for the distribution of BrCA. The predator complex of BrCA in Jamaica is dominated by coccinellids. These beetles are distributed across the island. Overall, 52% of the sites surveyed had ladybird beetles. The highest incidence was in St. Thomas (100%), Hanover (86 %) and St. Ann (80%). In Manchester and St. Elizabeth the incidence was 20% and 25%, respectively. Other predators were sparsely distributed and occurred in low numbers on each farm.

Association between predator activity and BrCA presence

During the BrCA population monitoring activities at the four study sites (previously described) data were collected on the presence of predators (coccinellids, lace wings and assassin bugs) found associated with the aphid populations or foraging among citrus foliage. Coccinellids and lacewings were present in the field throughout the year. Chi square analyses indicate that the presence and periods of activity of the coccinellid population is significantly correlated with the presence and abundance of BrCA. The presence of lacewings in relation to presence of BrCA was not quite significant.

Predation tests

The aphidophagous coccinellid, *Cycloneda sanguinea,* is one of the most dominant predators associated with colonies of the BrCA. This species may be acting as an important regulator of BrCA populations. Studies were therefore undertaken to determine the efficacy of these predatory insects against BrCA, specifically, the effect of various densities of the BrCA on predation by various life stages of *C. sanguinea*. The number of aphids consumed by *Cycloneda sanguinea* increased with increasing density of the BrCA. The larval stage consumed larger numbers of aphids than the adult life stage. The results demonstrate that *Cycloneda sanguinea* can potentially impact the growth of BrCA populations; in particular at small aphid population densities where the consumption rate is higher. Providing conditions conducive to the survival of these predators will therefore be important for conserving local populations within citrus orchards.

Survey to determine the distribution of parasitoids

Surveys were also conducted to determine the presence and distribution of parasitoids of BrCA. In 2003, and throughout 2004, field observations did not reveal much parasitoid activity. In 2005, the sampling methods were modified based on recent findings coming out of the University of Florida, and samples collected from 26 farms (318 flushes) in six parishes: Manchester and St Mary (3 farms each); Clarendon and St James (4 farms each) and St Catherine and Hanover (6 farms each) were collected and observed in the laboratory. Parasitoid species, *Lipolexis oregmae* and *Lysiphlebus testaceipes* were recorded on 16 and 4 farms, respectively. However, whereas *L. oregmae* was found in all parishes *L. testaceipes* was only found in four, the exceptions being, St James and Clarendon. In addition to being the more widely dispersed, *L. oregmae* was also the dominant species at all four farms where they were both found to occur.

The presence of *Lipolexis oregmae* in Jamaica which was indicated by PCR analysis by Dr Marjorie Hoy, University of Florida was confirmed morphologically by Dr Keith Pike, Washington State University and later by CARINET. The confirmation of the presence and the wide distribution of *L. oregmae* are significant findings as, in the Western Hemisphere, this parasitoid was only known to occur in Florida and Bermuda, where, in both cases they were introduced as part of classical biocontrol programmes against BrCA (Hoy and Nguyen, undated). How and when this natural enemy entered Jamaica is still unknown but it cannot be assumed to be after the 2003 survey since it would not be easily discerned by the initial method of observing mummies used in the field.

Field monitoring for parasitism

From October to December 2005, parasitism by *L. oregmae* was monitored at two of the four sites used for population monitoring of BrCA, Four Paths and Highgate. However, the levels of parasitism were variable. *L. oregmae* was the only parasitoid recorded.

At Highgate, parasitism levels averaged >14% at low aphid infestation levels and were < 6% at higher infestation levels. During high infestation at the site at Four Paths, Clarendon, parasitism levels were also relatively low (<7%). Levels of parasitism were also monitored at Clarendon Park, a designated release site, and the observations were consistent with the observations made for Four Paths and Highgate i.e. higher parasitism rates occurred at lower aphid densities. The observed rates ranged from 0 to >61%.

The field investigations on natural enemies informed the strategy for incorporating natural enemy manipulation in BrCA management especially as it related to (i) augmentative or innundative releases and (ii) the introduction of exotic natural enemies.

Importation and laboratory rearing of Lipolexis oregmae

In view of the low incidence of parasitoids encountered during the 2003 survey and the absence of BrCA populations during most of 2004, it was decided to import *Lipolexis oregmae* from Florida during 2005. The objectives of this exercise were

to develop and fine tune procedures for international transport of *L.oregmae*

to develop and fine tune procedures for rearing *L. oregmae*

to maintain a nucleus colony of *L. oregmae*

Prior to importation there was much consultation and dialogue with experts from the University of Florida including a one day seminar given by Dr Marjorie Hoy a renouned Entomologist in this field.

During five attempts to import batches of the parasitoid there were initially very high levels of mortality. Subsequently, two methods of packaging the parasitoids (as adults and as mummies on agar plates) were assessed. Upon arrival in Jamaica, the mortality of adult parasitoids was approximately 30% due mainly to drowning in the condensate within the tubes but the survivors were very active. Emergence of adult parasitoids from agar plates was generally very high ranging from 72 to 90 % per plate with a total 80.9% emergence from 110 mummies. This indicates that shipment of mummies is a feasible method for transporting the insects. This information will be valuable for future importations of the parasitoids because although there is a local population it may at times become necessary to out source cultures if local populations are not readily available when required.

Emergence of progeny from the laboratory cultures established using the imported parasitoids, was first observed on 25 October 2005. Parasitoids of three successive generations emerged during rearing activities.

Field releases

Prior to the release of the 90 adult parasitoids at Clarendon Park on 21 December 2005, a sample of 300 BrCA was collected. In the laboratory 183 mummies formed from which 116 *L. oregmae* adults emerged. In the follow up visit post release, there were no BrCA observed and all flush were newly hardened which prevented a post release assessment of parasitism. The efficacy of field releases must however be assessed to establish the benefit-to-cost of augmentation before it can be finally recommended on a large scale.

(iii) Investigate the potential use of chemical and biological control methods for brown Citrus aphid

Screenhouse trials to determine susceptibility of BrCA to candidate pesticides

Studies on pesticide susceptibility of BrCA involved screenhouse and field trials with particular bias to biorational pesticides to minimize any deleterious impact on natural enemy populations.

In the two screenhouse trials conducted, similar trends were observed in the relative efficacies of the treatments. At all intervals after treatment, the highest levels of mortality were effected by Safer Insecticide Concentrate® and NeemX® with Bioneem® being the least efficacious.

Effect of pH on efficacy of two neem-based pesticides

In a subsequent screenhouse trial, the mortality of the two neem-based products was shown to be improved by acidifying the mixture using the adjuvant, pH Plus®, especially with the product Bioneem®. At 3 and 7 days after treatment (DAT), levels of mortality induced by these treatments were all over 90%. In contrast, the percentage mortality for Bioneem® without the adjuvant was only around 20% during the entire post treatment period of observation.

Field trials to determine susceptibility of BrCA to candidate pesticides and evaluation of spay equipment

In field trials conducted at two sites, in Christianna, Manchester and Bog Walk, St Catherine, similar levels of mortality were effected by Safer Insecticide Concentrate® and NeemX®. However, although the pH was adjusted for the mixture of the two neem-based products as was indicated by screenhouse trials, the mortality observed in field plots treated with Bioneem® was very low even at 1 DAT, 5.4% and 37% at Christianna and Bog Walk, respectively. The disparity between the performances of the Bioneem® in the screenhouse versus the field was not explained by coverage differences due to spray equipment when this was measured in a field evaluation.

Assessment of the impact of insecticides on BrCA natural enemies

Bioassays were conducted on Coccinellid, *Cycloneda sanguinea*,-larvae and adults, and parasitoid, *L. oregmae*. The mortality of the test insects in each Petri dish was noted at intervals (1, 3, 6, 12, 24, 48 and 72) hours after treatment.

There was no apparent adverse effect of any of the test pesticides on coccinellid adults and although there were observed differences in mortality of coccinellid larvae in response to the different treatments (Orchard Oil® and Bioneem® having highest mortality after 1 hour) these were not significant. Treatment differences in mortality of parasitoids were more apparent (Orchard Oil® = Bioneem®>NeemX®>Safer Soap®).

The planned bioassays to determine the residual effect of the pesticides on recolonising parasitoids 72 hours after spray application, were however, not possible as sufficient test insects were never available at the period required or at any meaningful period after application. This study would indicate how soon after application these natural enemies could safely recolonise a tree (ie. the residual effects of the pesticides). This study is recommended for follow up studies as this will ultimately determine the suitability of the candidate pesticides.

(iv) Determine the presence and significance of other insect vectors of CTV and other economic diseases

Geographical and seasonal distribution

Concurrently with the population studies on the BrCA, data on the presence of other citrus pests and vectors of diseases were collected, particularly the Asian Citrus psyllid, vector of citrus greening disease, whose presence in Jamaica was confirmed during our course of investigations. Adult psyllid populations in the four groves were variable and displayed no discernible seasonal pattern. There was no significant correlation between adult psyllid populations with rainfall nor were the populations synchronized with the presence or stage-distribution of citrus flush. The key factors regulating the fluctuations of this insect therefore remain unclear.

Assessment of the impact of candidate pesticides on Asian Citrus psyllid

Since the Asian Citrus psyllid was confirmed as having a chronic presence in citrus groves, the four test pesticides Bioneem®, NeemX®, Orchard Oil® and Safer Insecticide Concentrate® were tested against psyllid adults to measure the benefits of the use these pesticides in the management of multiple citrus pests. Treatment differences in mortality of pysillids were Orchard Oil® = Safer Insecticide Concentrate® >Bioneem®>NeemX®, however, the observed differences were only significant for observations 1 hour after treatment. Further studies are however, warranted.

Major constraints relating primarily to the weather (prolonged droughts, active hurricane seasons) long periods of absence of BrCA in the field, availability of test insects and some delays in the procurement of supplies and facilities delayed the implementation of some activities and technical constraints also delayed the recognition and identification of the parasitoid *Lipolexis oregmae (scutellaris)* during early surveys. These delays necessitated two extensions which when approved deferred the completion date to 31 January 2006.

The terms of the contract stipulated that work be conducted in close association with relevant personnel of the Research and Development Division of the Ministry of Agriculture as part of the succession plan. The Principal Research Director was our primary liaison and was responsible for providing on the ground support for the project while relevant research personnel were involved in observations and discussions on technical aspects.

Regular interaction between the Consultant and MINAG staff was also logistically facilitated by the provision of a furnished office in the Plant Protection wing of the Bodles Research Station for the use of the Consultant. In addition space in the general Entomology Laboratory was made available to the Consultant for routine work. This space was shared with the staff Entomologists of the Research Station. A dedicated laboratory space for insect rearing, including the rearing of natural enemies and a purpose-built, insect-proof screenhouse was also made available by the research station

The Ministry of Agriculture was responsible for the procurement/provision of the equipment and supplies specified in the Inception Report. Major items of equipment were shared with the staff at Bodles while general laboratory supplies and consumables were purchased from project funds.

An attendant benefit was derived during the course of this consultation as interaction between personnel from the Plant Protection and Post Entry Quarantine Divisions at Bodles Research Station and the CARDI project team, developed the competence of all the participating scientists in the various aspects of the management of BrCA. In addition to physical proximity, the staff of the Ministry participated in planning, review and training activities associated with the consultancy for research services in entomology. This provided opportunities for cross fertilization and set the stage for smooth transitioning at the end of the contracted services. The training provided by visiting Scientist Dr Marjorie Hoy of University of Florida, in biological control of a number of citrus pests including BrCA and Asian Citrus psyllid also helped enhance the knowledge base of local scientists.

The principal strategic objective of this project was to improve the state of knowledge so as to remove a critical constraint to the citrus sector. The consultant believes that this objective has been achieved and that the knowledge generated constitutes an original contribution to the scientific understanding of brown Citrus aphid and the options for its management in Jamaica.

The recommendations include scouting guidelines, identified key factors affecting BrCA, a list of key natural enemies and suggestions for their conservation, mass rearing protocols for parasitoids and pesticide options deemed most compatible with biological control. In summary the recommendations are,

Citrus replanting

Establishing plants on resistant rootstock is a major component in the management of citrus tristeza virus. The survey conducted in 2003 showed that the aphid is present in all parishes and that its distribution is not correlated with any apparent geographical feature. Therefore BrCA infestations are likely on orchards throughout the island.

With the current replanting exercise large acreages of citrus will be in a highly vegetative phase which predisposes trees to attack by pest groups which favour citrus flush. In this scenario, control of BrCA is indicated in circumstances where their populations are sufficiently high so as to cause feeding damage and the associated leaf deformation and sooty mould production.

Synchronising the period of replanting as much as possible instead of the very staggered transition would shorten this period of prolific vegetative growth within orchards.

Forecasting

Based on general agronomic knowledge that periods of rainfall which induce flush will be coincident with periods of high aphid populations, it is recommended that farmers and grove managers be sensitized to the need to pay particular attention to rainfall and flush production patterns so as to develop the ability to predict the onset of BrCA outbreaks.

Consideration should also be given to the development of a national database in which are input records of rainfall and flush production of all citrus varieties in representative agro ecological zones. An analysis of these patterns may eventually contribute to an early warning system.

Monitoring

Regular inspection of citrus groves using the tree sampling procedure developed for this study (Figure 1) will provide early warning of BrCA presence. More intensive monitoring will however be required during high risk periods when the trees are producing flush. The critical point of intervention is at flush stages (NE to NE/NH).

Biological control

Among the predators of BrCA the coccinellid predators in particular consume large quantities of aphids as larvae and adults and can effect significant control at low BrCA levels but their consumption rate declines as the aphid population increases.

Given the known parasitoid host relationship, despite the widespread distribution of parasitoid *Lipolexis oregmae* a logical strategy is to maintain cultures and intensify mass rearing of this parasitoid for augmentative releases in groves early in the aphid infestation cycle in order to suppress BrCA population growth. It is recommended that the Ministry of Agriculture maintains and expands laboratory cultures established at Bodles to conduct follow up studies on the potential impact of augmentative releases on field parasitism levels in order to guide the further development/expansion of rearing facilities for increased mass rearing of this parasitoid.

Chemical control

Pesticide applications are therefore recommended during outbreaks or to suppress chronically high aphid populations which have outpaced the natural enemies or during high risk periods (periods of widespread flush) to limit the spread of aphids from initial nucleus colonies.

Safer Insecticide Concentrate® is very effective at reducing BrCA numbers and did not exhibit significant adverse effects on natural enemies. Orchard Oil®, although it reduces natural enemy populations is also useful in biointensive management strategy (given its low persistence which would allow early recolonisation) but would require more frequent applications for the effective reduction of aphid numbers. Safer Insecticide Concentrate® also shows potential to reduce populations of psyllids.

These recommended components form the basis for an integrated management strategy for BrCA. Findings to come out of the other yet to be concluded research services in agronomy and pathology with respect to the management of CTV will lend to a more holistic strategy.