Project Report

project	**Biological Control and Seasonal abundance of the Asian citrus psyllid (*Diaphorina citri*) in three agro-ecological zones in Jamaica**
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1) Acknowledgments

The author wishes to acknowledge the Senior Research Directors of the Orange River Research Stations and Mr. Ken Newman of Cambria Farms, St Catherine and also Mr Roger Newman of Good Hope farm in Trelawny for allowing us to conduct the study in their citrus fields. Thanks to my supervisors Mrs. Michelle Sherwood for their guidance and encouragement. Thanks also to Miss Tavia Gordon and Mr. Worrel Diedrick (Research Assistants) for their assistance with data collection on the field visits and conducting the laboratory and greenhouse experiments.

2) Executive summary

The citrus industry is very important to Jamaica's economy in terms of employment, exports and local production. It is estimated that the total value of the industry to the Jamaican economy is approximately J$4 billion. Ninety-six percent (96%) of total citrus production is utilized locally for the fresh fruit market or in processing, while 4% of citrus is exported. On-farm employment was estimated at 5,460 individuals. Employment at the industry level (including on-farm operations, processing, packaging plant, wholesale and retail trades) is estimated at 19,500 persons. However, since October 2009, the industry has been under threat of devastation from Citrus huanglongbing (HLB) /greening disease. In response to the outbreak the stakeholders of the industry including growers, government and processors have undertaken actions to halt the spread of the bacteria. Assistance has been sought from the FAO to provide technical support to fill gaps in the areas of detection and diagnostic capabilities, and the development and implementation of a holistic and cost effective management strategy.

The future of the citrus industry in Jamaica is challenged by the detection of citrus huanglongbing more popularly known as citrus greening disease two years ago. This is considered one of the most debilitating diseases of citrus. The causal agent of the disease, a bacterium, *Liberibacter asiaticus* is vectored by the psyllid, *Diaphorina citri*. This psyllid has been reported in Jamaica since 2001 and has established itself all over the island on citrus and Murraya plants. The presence of the vector in Jamaica will contribute significantly not only to field spread of the disease but also long distance movement via citrus psyllids carrying the bacterium on infested host plants. The management of citrus greening in established orchards is therefore dependent on management of the vector. This however must be supported by the provision of clean uncompromised planting material for replanting.

Little is known locally regarding the population dynamics of the citrus psyllid vector except that the psyllid is attacked by local predators such as lacewings and ladybird beetles and a parasitoid wasp *Tamarixia radiata* that was fortuitously introduced into Jamaica. The need to obtain local baseline data to determine their contribution to psyllid mortality is therefore critical. This information will be used in identifying IPM approaches that are suitable to Jamaica's climatic and socio – economic conditions. A seasonal abundance study is being conducted for a period of at least one year at three locations having different climatic conditions. By-Weekly visits were made to each site to collect data on population changes and the activities of the natural enemies in the system.

Augmentation of existing natural enemy populations is also being investigated. This tactic is designed to reconstruct the natural enemy complexes of the citrus psyllid. *Tamarixia radiata*, a species specific ectoparasitoid wasp of *Diaphorina citri*, native to India has been identified as a potential biocontrol agent for mass rearing and augmentation of existing field populations. Females of *T. radiata* host feed on the younger instars of *D.citri* and prefer to oviposit underneath the later instars. The newly hatched parasitoid larva sucks hemolymph from the host which is eventually killed and consumed.

Biological control though an important defence against invasive pests for long term and sustainable management, if a disease agent is involved other tactics to support biological control will be required. Hence another aspect of this investigation will be to assess and determine the timing of

environmentally friendly treatments that suppress the psyllid populations to facilitate more effective control by the biocontrol agents.

3) Background

The Jamaican citrus industry valued at approximately $4-billion dollars could be adversely impacted by the establishment of citrus greening disease in light of the presence of its vector the Asian citrus psyllid *Diaphorina citri* in the island. The Asian citrus psyllid *Diaphorina citri*

is a key pest of sweet orange, *Citrus sinensis* in its native range in Southeast Asia and the Indian sub-continent primarily because of its role as a vector of the debilitating citrus greening disease. In the absence of the citrus greening agent, *D. citri* poses very little threat for newly established citrus orchards.

D. citri was present in the Western Hemisphere in Brazil for over 30 years until it was reported in neighbouring countries in , Central, North and South America, , and the Caribbean. With the introduction of the causal agent of greening, the bacterium *Liberibacter asiaticus* into the Western hemisphere spread of the disease has been greatly facilitated by the presence of the vector. This has made management of the disease more difficult given the fastidious nature of the bacterium in that it cannot be cultured outside a plant host.

The citrus greening disease is very devastating, plant yield and fruit quality are greatly reduced and citrus production is severely debilitated within 5 to10 years. The chronic decline in plant health associated with the pathogen leads to yellow mottled leaves with green banding along the major veins and lopsided fruits with aborted seeds.

In order to mitigate the spread and impact of this disease management of the vector is critical. Investigations locally have shown that the citrus psyllid is being parasitized by its natural enemy *Tamarixia radiata* which was fortuitously introduced into Jamaica and local generalist predators such as ladybird beetles and lace wings; but although the levels of parasitism and predation are high in the *Murraya* (a preferred host of the psyllid) system, how much they contribute to psyllid mortality in citrus is unknown. Hence knowledge of the population dynamics of *Diaphorina citri* is important to understand the seasonal trends of the population of the species and the role of mortality factors. Information on seasonal abundance under different climatic conditions will therefore form the basis for the development of a reliable integrated pest management programme.

4) Objectives

a. To conduct an assessment of the seasonal abundance of citrus psyllid, *Diaphorina citri* on *Citrus* sp. in three agroecological zones

b. To assess the levels of parasitism of *D. citri* by the parasitoid *Tamarixia radiata* in the system

c. To implement an integrated biological control programme for the management of the citrus psyllid, *Diaphorina citri*.

5) Methodology

Population dynamics study

Studies were conducted within three different agroecological zones: Good Hope farm in Trelawny which represents a dry cool area in the western side of Jamaica; Orange River Research Station in St. Mary which is on the northeastern side of the island and is in a cool wet area; Cambria Farms, which is privately owned, is located in Bogwalk, St. Catherine in the south central area of Jamaica and is hot and wet. This hot wet area has the largest acreage of citrus on the island.

All sites were visited from February2010 to February 2012 biweekly and 10 trees were randomly selected and sampled. A metre squar apparatus was thrown on to the canopy from a distance of 1.8m (6ft). The apparatus was held at chest height in a vertical position and thrown. All flush then all infested flush within the square was counted and recorded. For each tree the percentage flush infested was noted. Rainfall data were collected from all three sites.

Samples of young flush infested with 4^{th} to 5^{th} instars of psyllids were collected from different locations on each tree and at each site and brought back to the Entomology laboratory to assess for *Tamarixia radiata* parasitism.

The nymph infested flushes were placed in a container, dated, and placed into a growth chamber at 25^{o}C. The number of *T. radiata* and adult psyllids were counted and the rate of parasitism obtained by calculating the percentage of wasps and adult psyllids that emerged after 14 days. After 14 days the container was transferred to a freezer to kill any living insects.

Biocontrol

Maintainance of psyllid host plants (Fig 1-10)

Murraya paniculata seedlings were maintained within a screenhouse. Plants were pruned and fertilized in order to induce the growth of new flush. Four plants were placed in each cage and when new shoots emearged adult psyllids obtained from *M. paniculata* at Bodles Research Station were introduced at a ratio of one psyllid to every shoot. The plants remained in the cage until the fourth to fifth instar nymphs appeared after which they were placed in a sleeve. This was to reduce the time for the parasitoids to locate the psyllid nymph.

Tamarixia radiata was introduced at a ratio of one wasp to every 20 psyllids. If the adult wasps were still alive up to the time when the first mummified nymphs were observed i.e.7-8 days later they were removed. Strips of paper or small cotton balls with honey solution were placed in each cage to feed the newly emerged parasitoids; water was applied to the plant using a spray bottle every three days. All new wasps were collected, counted and released. The plants were removed and allowed to grow for 3-4weeks and then cutback for reuse.

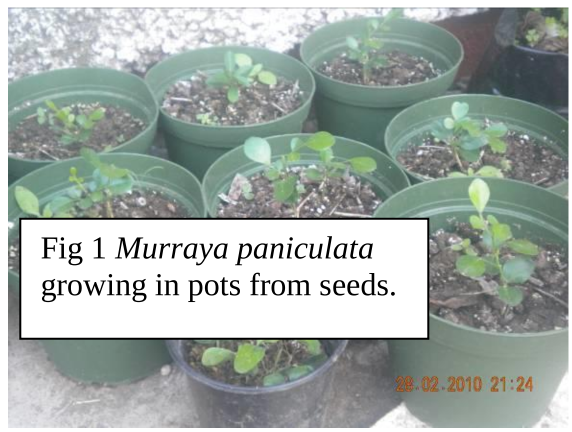


Fig 1 *Murraya paniculata* growing in pots from seeds.

Fig2 Seedlings are covered after the *Tamarixia radiata* are introduced.

Fig. 3 Cages where plants are kept to prevent any other insect inference.

Fig 4 Parent *Tamarixia radiata* is introduced as the first fourth instars appear.

Fig 5 Adult *T. radiata* ovipositing under a fifth instar psyllid. Note the ovipositor is between the thorax and the upper part of the abdomen.

Fig 6 Lime plant covered with psyllid nymphs.

Fig 7 Mummified psyllid nymphs along the stem of lime seedling.

Fig8 Jars showing how the parent Tamarixia radiata wasp was harvested from Murraya.

Fig9 Collecting wasps that has emerged from psyllids.

Fig10 other method of collecting wasp.

6) Achievements against activities and outputs/milestones

a. Objective 1: To conduct an assessment of the seasonal abundance of citrus psyllid, *Diaphorina citri* on *Citrus* sp. in three agroecological zones.

no.	activity	outputs/ milestones	completion date	comments
1.1	Conduct by-weekly field visits to each location.	Psyllid population trend determined for each climatic zone	March 2013	This activity began in February 2010.
1.2	Collect samples of parasitoids.	Determined parasitism level and impact of *T. radiata* in each agroecological zone.	March 2013	Percentage parasitism by *Tamarixia radiata* ranged from 0-20% avarage across the three zones. The St Mary having the lowest.

Objective 2: To implement an integrated biological control programme for the management of the citrus psyllid, *Diaphorina citri*.

no.	activity	outputs/ milestones	completion date	comments
2.1	Maintaining seedlings with psyllid population.	Information supplied to major stakeholder in the citrus industry re mass rearing of *T. radiata* and protocol to be adopted. One thousand parasitoids reared and released monthly.	January 2011	Nine thousand (9000) wasps produced during reporting period to date.

7) Key results and discussion

Population dynamics study

Rainfall Pattern

In the period February 2010 to February 2012 the rainfall levels peaked in all three parishes during the traditional rainy season of May/June and September/October while January to March were the driest months at all sites. The highest rainfall levels were recorded in St. Catherine which recorded levels ranging from 10 – 169.5 mm followed by St. Mary, 11 -139 mm and then Trelawny 5 - 120.5mm for the period monitored (Figure 1).

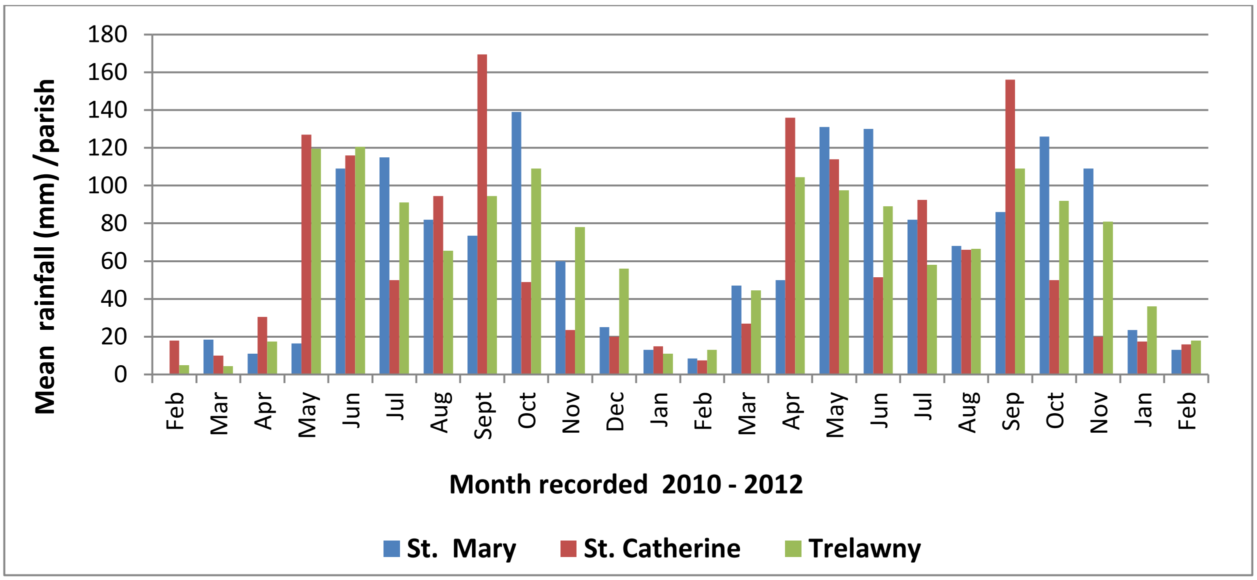


Figure 1: Rain patterns at three locations in three parishes during the monitoring period

Flushing Pattern

The flushing pattern for all three locations was similar overall.Overall flush density was greatest at St Catherine followed by Trelawny and then St. Mary for the period monitored. The highest level of flushing occurred during spring and the lowest during the dry periods of winter which conforms to the usual flush patterns. The highest flushing levels were recorded in St. Catherine (17 -302 mean flush/m2) followed by Trelawny (0 -230.5 mean flush/m2) and then St. Mary (1 – 201.5 mean flush/m2). However, for two consecutive years the flushing pattern in Trelawny exceeded that of St. Mary and St. Catherine during the August to October period in Trelawny (Figure 2). In all three locations there was a peak in flushing patterns after each period of rainfall.

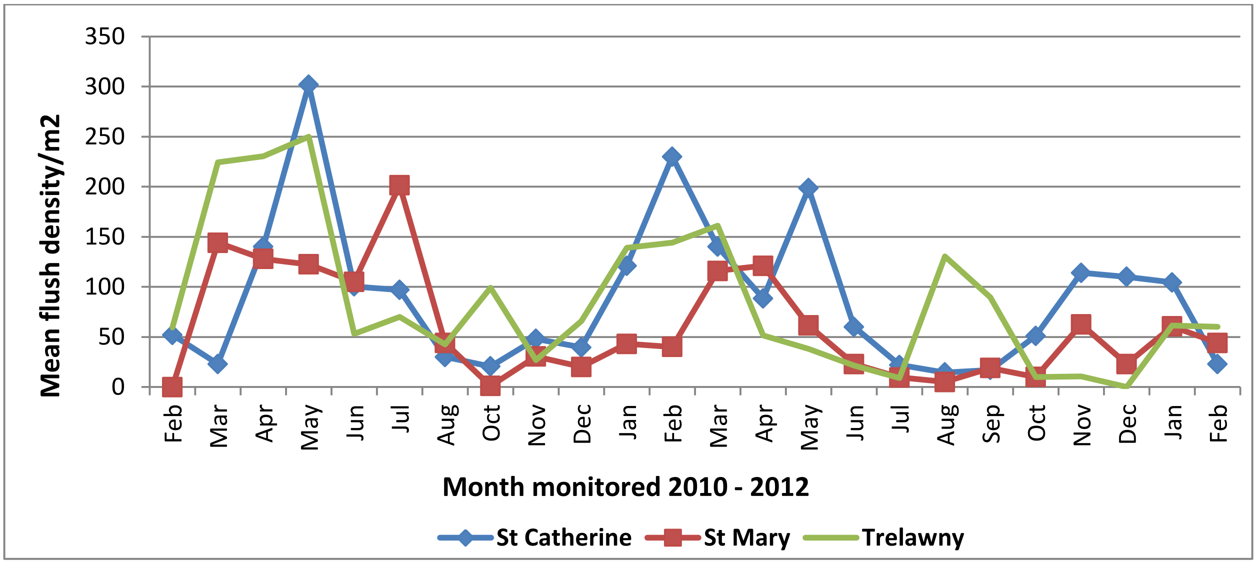
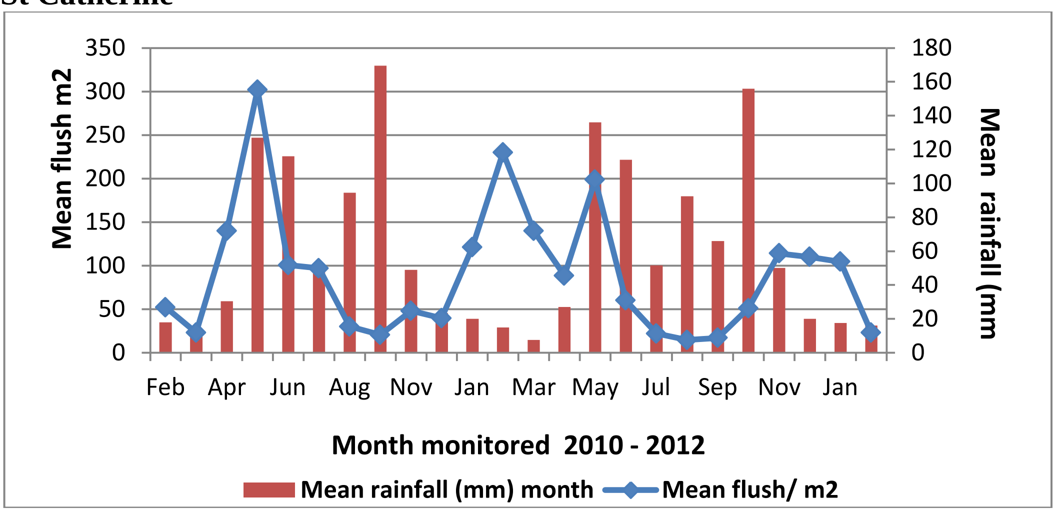
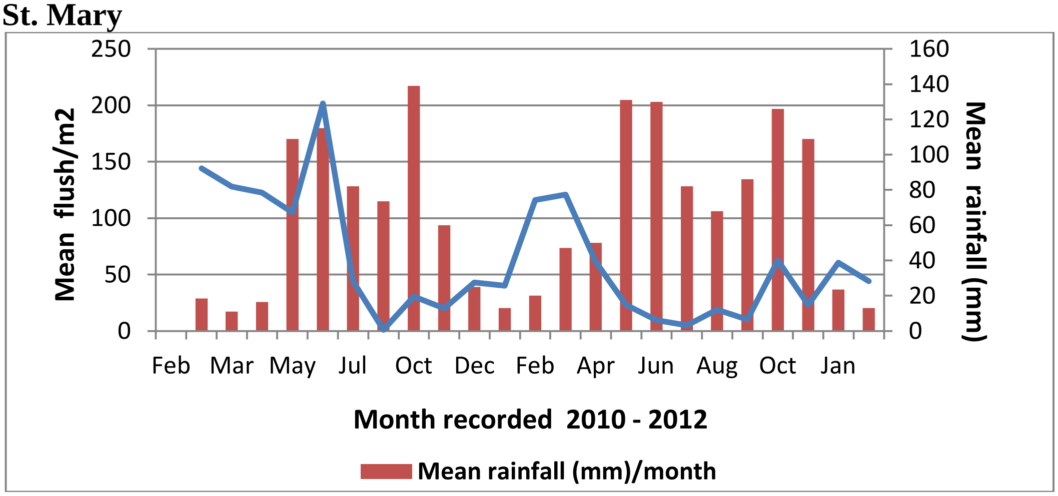


Figure 2: Rain patterns at three locations in three parishes during the monitoring period

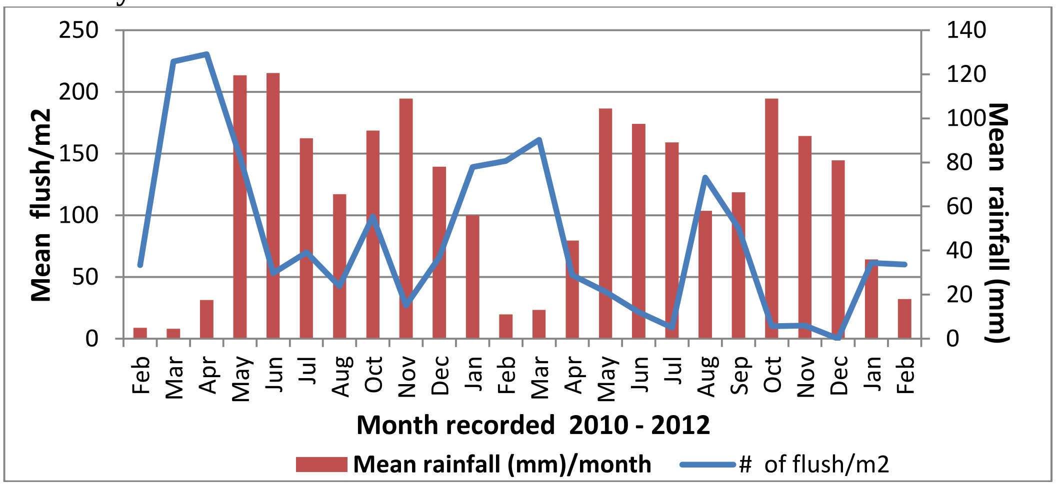
St Catherine



St. Mary



Trelawny



Figures 3, 4 and 5: Flushing and rainfall pattern in three parishes during the monitoring period

Relative Infestation Rate pattern of Asian Citrus Psyllid

Diaphorina citri was present at all locations. The percentage of infested flush and relative infestation rate varied during the year for each location. The percentage of infested flush was highest in Trelawny ranging between 0 – 73 infested flush/m2 followed by St. Catherine 0 – 41.5 infested flush/m2 and then St. Mary with 0 – 30.5 infested flush/m2. The percentage of infested flush was greatest in January at Trelawny.

The percentage infestation was significantly higher at Trelawny in December, July and January than in St Catherine and St Mary. St Catherine and St Mary showed similar trends in infested flush except for May 2010, March 2011 and January 2012 when St Catherine had increased infestation compared to the following month while St. Mary's infestation decreased.

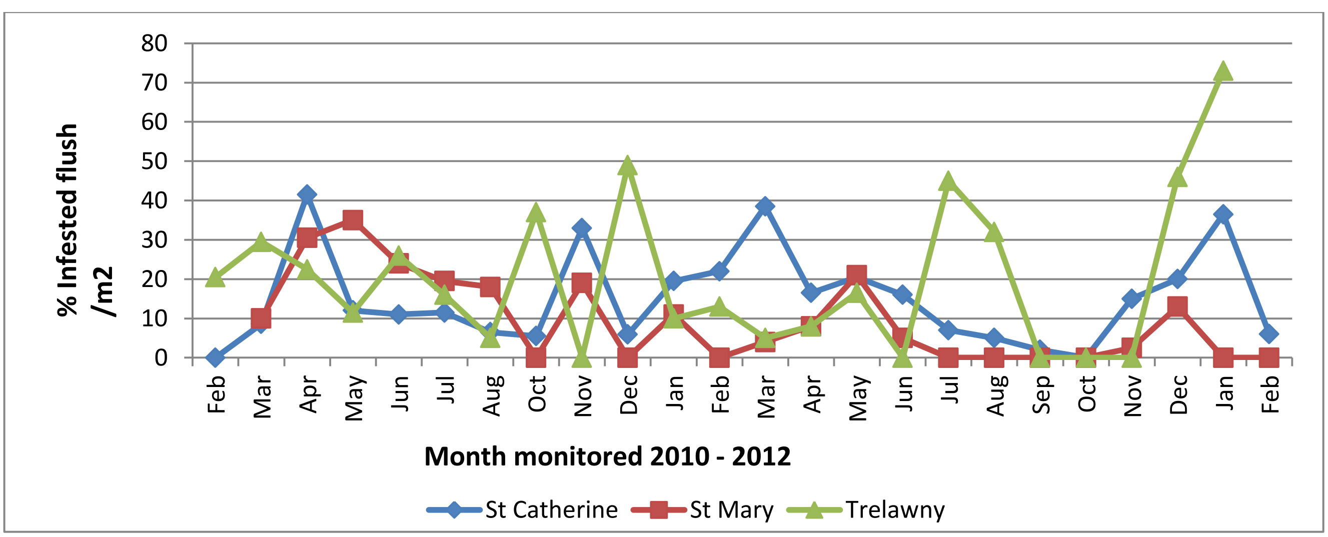
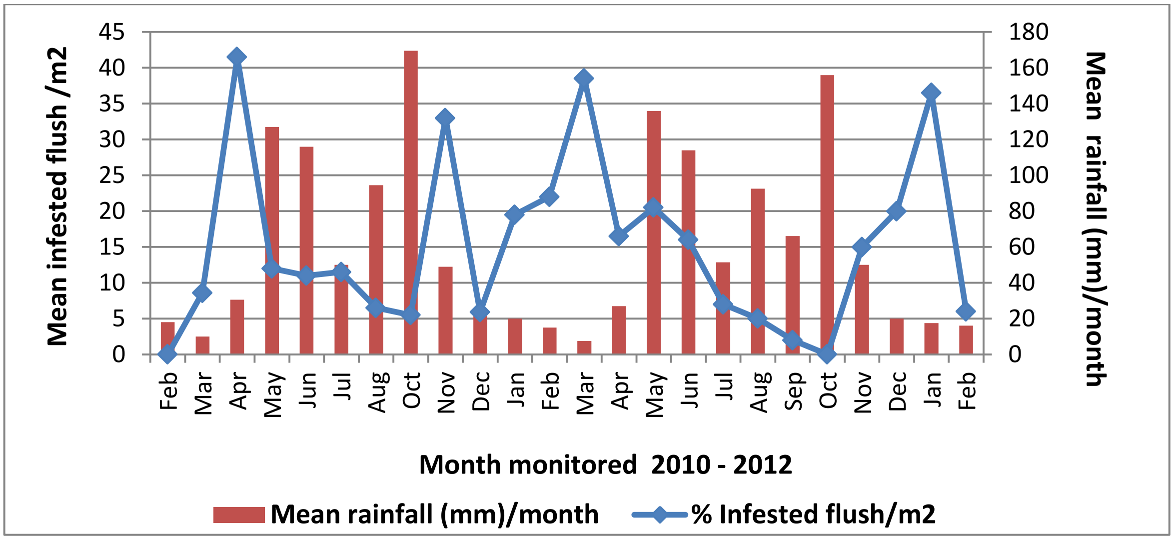
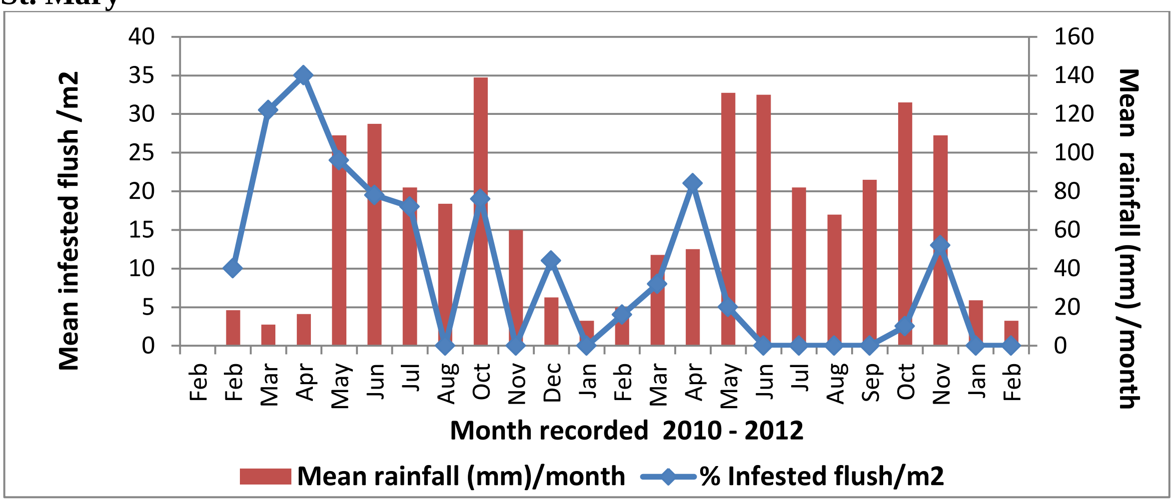


Figure 6: Percentage infested flush/m^2 with *Diaphorina citri* in citrus fields of three parishes

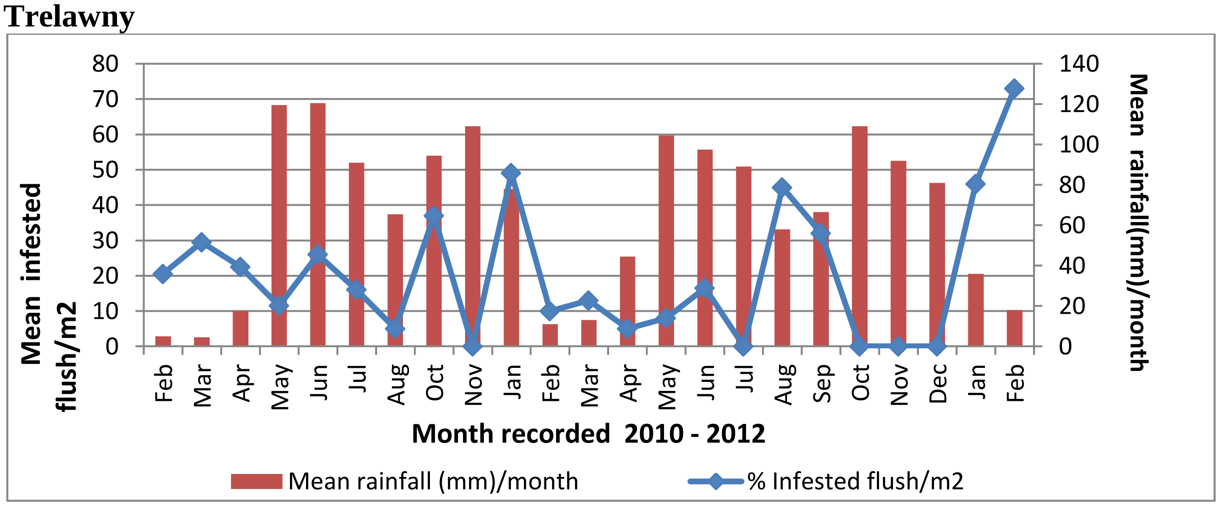
St. Catherine



St. Mary



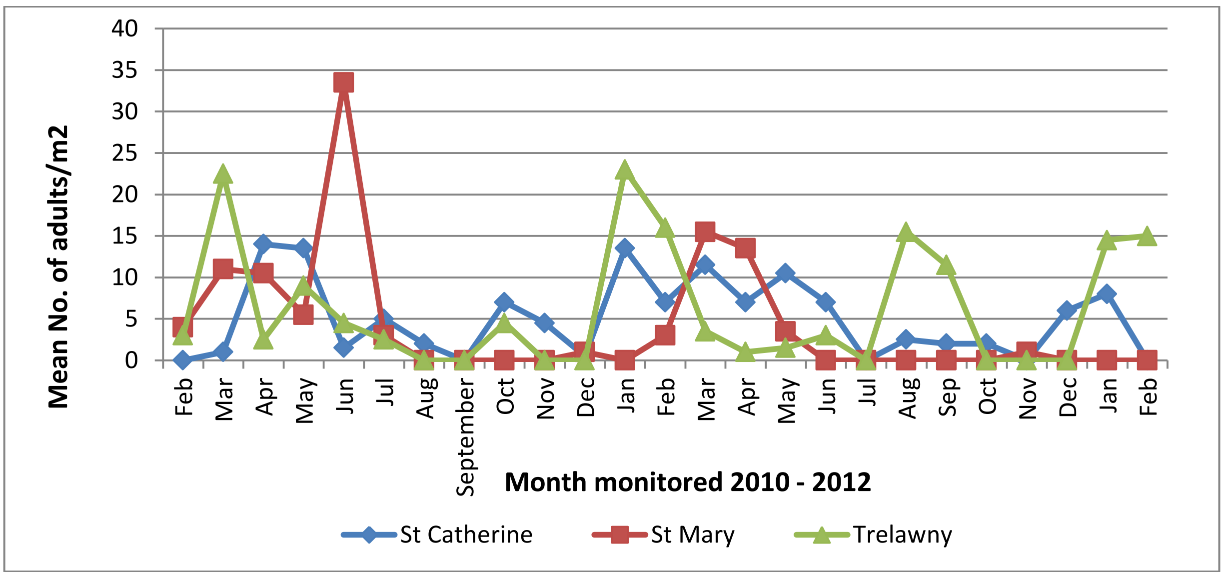
Trelawny



Figures 7, 8 and 9: mean infested flush and rainfall pattern in three parishes during the monitoring period

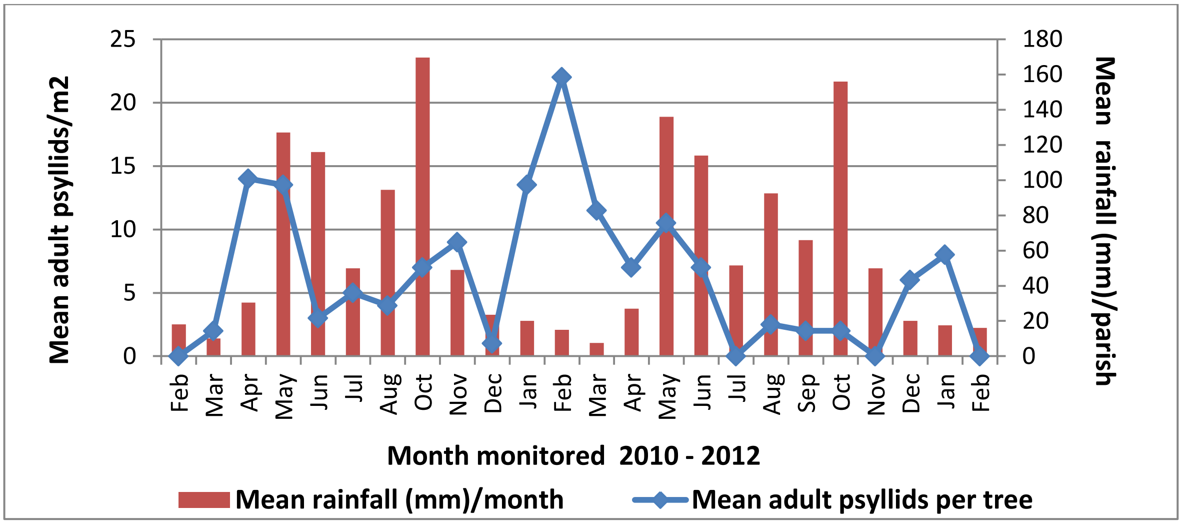
Pattern of population changes in adult Asian Citrus psyllid at three locations

The pattern of the population of psyllid adults was similiar in the three parishes for most of the year except for Trelawny which experienced an increase in the adult population in the August period compared to the other two parishes. The adult population was determined to be highest in St. Mary followed by Trelawny and then St. Catherine (Figure 10). The adult population for the period ranged from 0 – 14/m2, 0 – 33.5/m2 and 0 – 22.5/m2 in St. Catherine, St. Mary and Trelawny respectively. The peaks in population were experienced consistently first in Trelawny, followed by St. Catherine and then St.Mary. The population of the adult psyllids was reduced after periods of rainfall at each location (Figures 11, 12 and 13).

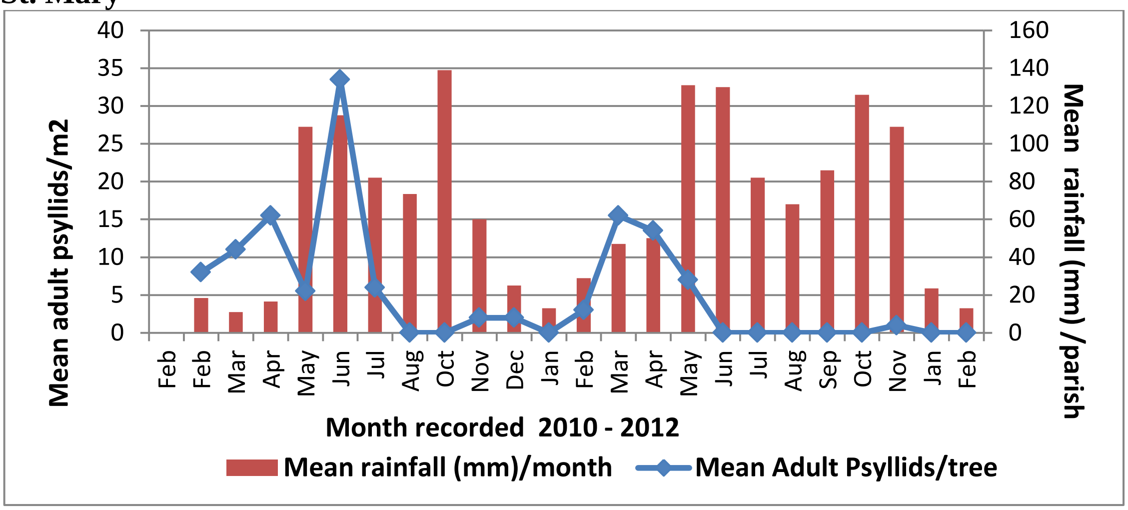


Figures 10: Population of psyllid adults in three parishes during the monitoring period

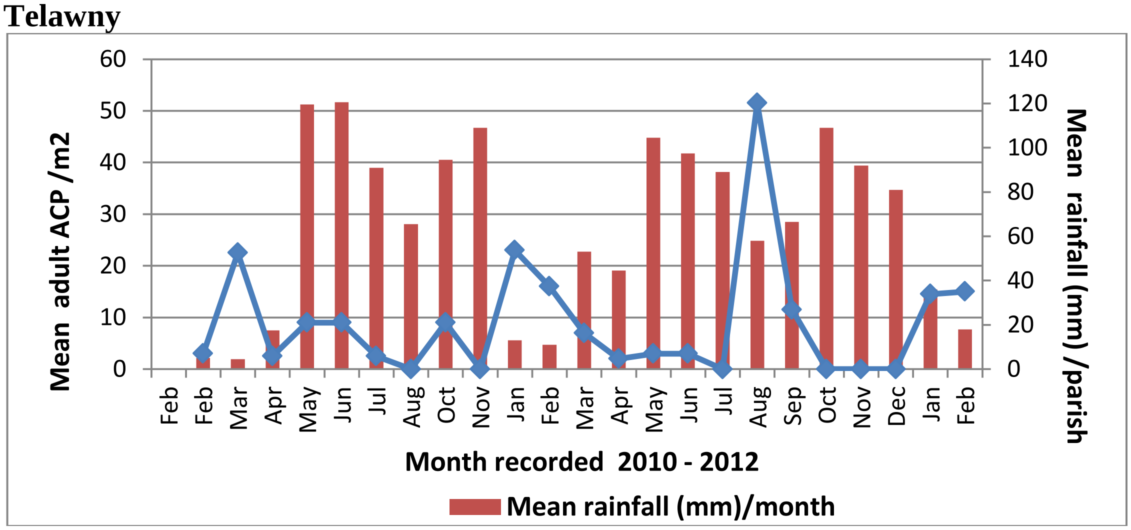
St. Catherine



St. Mary



Telawny

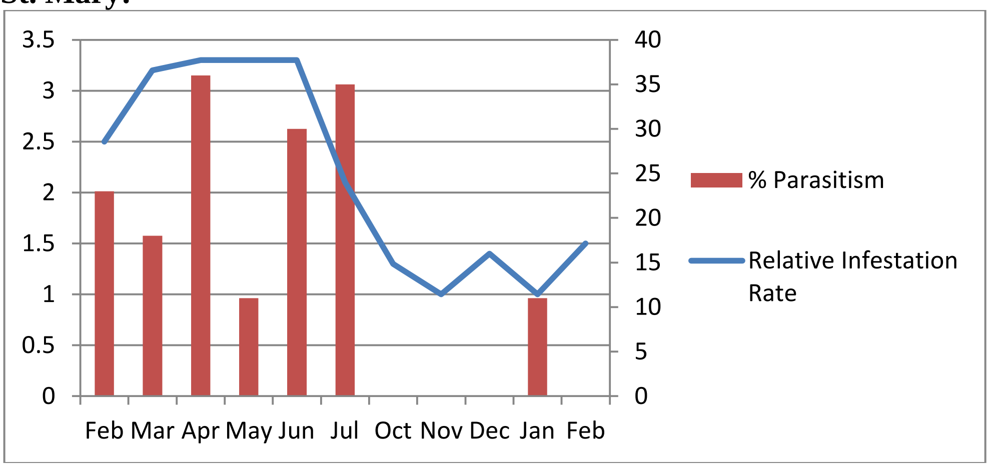


Figures 11, 12 and 13: Population of adult psyllids in three fields across three parishes and the rainfall (mm) levels experienced during the monitoring

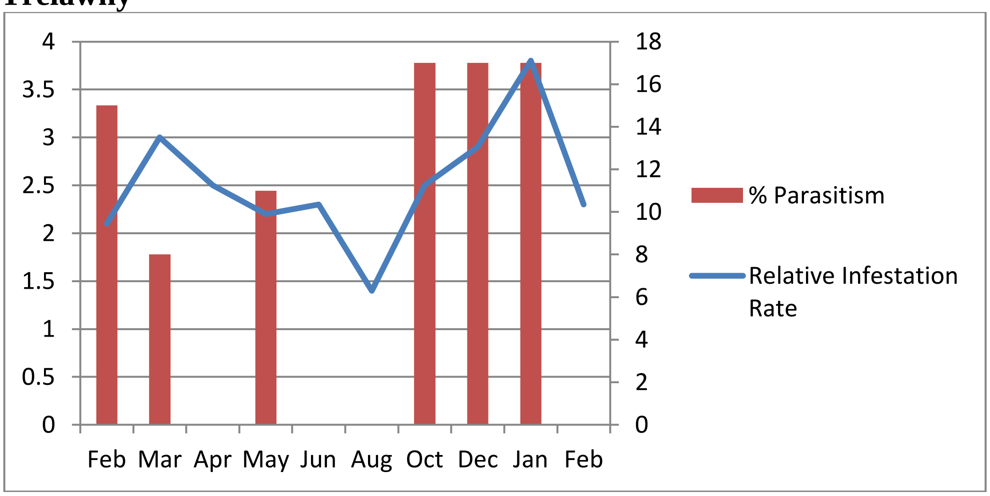
Parasitism levels of Asian Citrus Psyllid

Tamarixia radiata was found parasitizing *D .citri* nymphs on citrus at all 3 sites. Tamarixia radiata was found parasitizing D .citri nymphs on citrus at all 3 sites. There was evidence of the impact of the parasitoid with a corresponding fall in population during periods of high parasitism levels at all sites. The parasitism levels ranged from a low of 8% - 38 % in St Mary, 8 – 17 % in Trelawny and 10 – 34 % in St. Catherine. In St. Mary the parasitoids were present from February to July and Janaury and absent for the October to November months. In St. James/ Trelawny the parasioids were present from October 2010 to January 2011 while in St. Catherine the parasioid was present for most of the year.

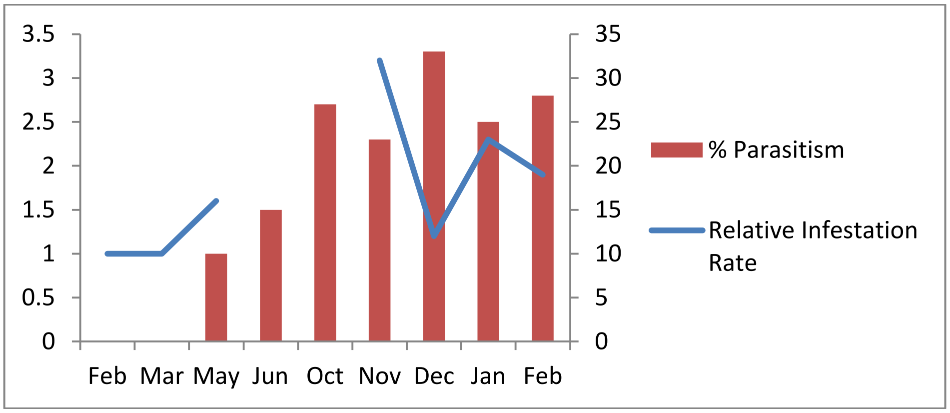
St. Mary:



Trelawny



St. Catherine



Figures 14, 15 and 16: Parasitism levels of *D. citri* by *T.radiata* within an orange field in St Mary and st. Catherine and Lime field in Trelawny.

Discussion

Most new foliage production at all three sites occurred mainly during the spring and to a lesser extent in summer, which conforms to the traditional flush patterns. Spring is the time of the year when trees produced greatest amount of flush and winter the least. St. Mary which had the least amount of flush had the lowest infested flush and the second lowest number of psyl lids, when compared to St. Catherine which had the highest infested flush and adult counts per m2. At all locations, greatest density of flush and psyllids occurred during the spring.The observed differences in flush density may be as a result of the traditional rain pattern which is associated with the May/June and August/September rains.

The results also show that rainfall contributed to *D. citri* mortality which is observed mainly after heavy rains. Aubert (1988) stated that *D. citri* mortality increases with higher rainfall and relative humidity, but it is very low under hot and dry climates. The current study suggested that the dry season from December through to March or the winter season favoured decreasing populations of *D. citri* which is similar to observations seen in Florida (Mead, 2011). The peaks in *D. ctri* population were experienced consistently first in Trelawny, followed by St. Catherine and then St.Mary which may prove useful in designing the implementation of strategies within a national areawide management programme.

Psyllid populations declined in St. Mary in June/July and November in St. Catherine after 33 % parasitism by *T. radiata* while in Trelawny it fell after 16 % of parasitism. The inability of psyllid populations to continue in creasing in response to the increased flush in each location suggested that *T. radiata* may have played a significant role in regulating *D. citri* there. These parasitism levels are comparable to those recorded in Malaysia in 2000 ranging from 23 to 36 % (Chan Teck *et al.*, 2011). This may be as a result of the presence of hyperparasitoids which can negatively impact parasitoid activity. However no hyperparasitoids were detected in this study. Several other factors such as fungus and predators particularly ladybeetles are known to contribute to psyllid mortality (Halbert & Manju nath 2004). In Jamaica records ranging between 80 – 100 % have been recorded at other citrus fields in, St. Catherine which is comparable to levels reported in Florida (Pluke et al., 2008). However, psyllid adults have been observed killed by other natural enemies inJamaica including a fungus (undetermined) as well as coccinellids which have been observed abundant in the fields but as generalist feeders may also be feeding on other pests such as the brown citrus aphid (*Toxoptera citricida*).

The results of this study may prove useful in providing two years of data, a basic requirement to develop a forecasting system to predict changes in the population of *D. citri*. This can be incorporated into a management programme for this pest to timely apply treatments before the pest reach undesirable levels. The use of compatible treatments would prove useful in order to maintain the impact already being made naturally by the *T. radiata* in an area-wide integrated programme.

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8) Impacts

a. Scientific impacts – now and in 5 years

Increased knowledge on what factors impact on the psyllid population in various agro ecological zones and provide baseline data which will assist in formulating an IPM management strategy.

Increased knowledge on rearing and release methods of biocontrol agents. The project will also be helpful in identifying where to release and under what conditions to apply this control measure and where they are most likely to be effective.

b. Capacity impacts – now and in 5 years

Training of farmers and extension officers in the identification of pest and beneficial insects for citrus.
To develop a management programe for psyllid control and how an IPM strategy could be tailored to fit in the control of this vector.

c. Community impacts – now and in 5 years

i. Economic impacts

The management of the citrus psyllid will serve to protect the investment and income of citrus farmers and safeguard national food security. Benefiting also is the fresh fruit market and fast food chains that are supplied by this industry. It is envisaged that if production increased sufficiently it may reduce the importation of some citrus concentrate and open the doorway for the export of such commodities to the region thus contributing to an improvement in Jamaica's GDP.

ii. Social impacts

The preservation of the income of citrus growers in the industry ensures the protection of the welfare of their families who are dependent on this source to provide for daily health, educational, material, entertainment and other needs. The jobs of persons employed in the industry will also be retained

d. Communication and dissemination activities

i. Fact sheet and posters on citrus greening.
ii. Participation in three public awareness seminars in St. Catherine, Clarendon and St.Mary.
iii. Transfer of rearing protocol to the major citrus producer.

9) Conclusions and recommendations

a. Conclusions

- The data shows that the psyllid population in all three areas fluctuates with flushing pattern and rain fall.
- The parasitoid *Tamarixia radiata* was found to be active in fields that had over 30% of inspected flush infested.the ladybird beetles are very good predators and will consume up to 50% of the psyllid nymphs before they reach the fourth instar.
- Our temperature did not show any impact on the population trends, however, rainfall did.
- Heavy rainfall dislodges eggs that are not in flode leaves and even adults get washedoff or drown.

b. Recommendations

- The approach to managing the citrus psyllid can now be more environmentally friendly with the use of biocontrol agents.
- Biocontrol agents can be effective both under protective cover or in open fields and will cause no harm to air or water quality and can remain sustainable so long as the host exists.
- The use of chemicals should be a last resort, but just before flushing would be a good time to knock adults that are waiting for new flush to deposit their eggs.
- Being an observant farmer is the key for early detection of any new pest in the field. This will allow for you to apply your solutions to the problem early and avoid a potential disaster.
- Biocontrol agents are most effective within the murraya hedges or where the citrus can maintaine a dense canopy.