BIOLOGICAL CONTROL OF THE CITRUS PSYLLID *DIAPHORINA CITRI* KUWAYAMA (HEMIPTERA: PSYLLIDAE)

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ABSTRACT

Citrus psyllid *Diaphorina citri* and the two primary parasitoids, *Tamarixia radiata* Waterson and *Diaphorencyrtus aligarhensis* Shafee (Alam and Agarwal) were successfully mass reared separately in outdoor screencages using *Murraya paniculata* (L.) Jack one of the preferred hosts of *D. citri* .

The total developmental period of the two parasitoids were determined. Development time from egg to adult emergence for *T. radiata* took 9.57±0.38 for females and 9.6±0.25 days for males with a sex ratio of 1:3.19 (male:female) while for *D. aligarhensis* took 17.88±1.08 days. *T. radiata* is arrhenotokous parasite while *D. aligarhensis* is thelytokous.

Preference test on the different nymphal instar of *D. citri* was determined in the laboratory. *T. radiata* showed preference on the third, fourth and fifth nymphal stages. *D. aligarhensis* preferred the second, third, and fourth nymphal stages.

Parasitization capacity under laboratory condition showed that *T. radiata* significantly parasitized more number of *D. citri* nymphs than *D. aligarhensis*

The incidence of *D. citri* and parasitism in the field in 3 sites was monitored before and after release of the two primary parasitoids and proved at least significant result.

After parasite release parasitism obtained for *T. radiata* was 68% and 34% *for D. aligarhensis.* Persistence of *T. radiata* is a promising sign and could be used in IPM for *D. citri.*

Keywords: *Eulopidae, Tamarixia radiata, Encyrtidae, Diaphorencyrtus aligarhensis, Psyllidae, Diphorina citri, huanglongbing,*

INTRODUCTION

Calamansi *(Citrofortunella microcarpa)* is one of the important citrus species in the country. This marvelous fruit crop has contributed greatly in the export market at a value of US$238.85 thousand for the year 2005, either in the form of fresh fruit, juice, or concentrate. Citrus growing was flourishing industry especially in Batangas during the 1950's but the industry declined due to several factors, one of which is the citrus greening disease or huanglongbing.

The disease is transmitted by an insect vector the Asian citrus psyllid *Diaphorina citri* Kuwayama. Chemical control of the pest includes the use of foliar and or systemic applications of different insecticides. However, environmental consequences of the use of insecticides disrupt the occurrence of natural predators and other citrus pest.

An area of concern that could likely serve as natural alternative to limit or control *D. citri* is through biological control. This measure proved successful when insect was successfully controlled by an introduced ectoparasite *Tamarixia radiata* in the Reunion Island (Quilici, 1988) and in Taiwan (Chen, 1988). In the Philippines, the parasitoid was first introduced in November 1988 in Lipa and Davao and was then recovered in the field. Prior to the introduction of *T. radiata*, a single primary endoparasite was identified as *Diaphorencyrtus aligarhensis* Shafee et al. (1975) and was considered efficient and appeared to be the dominant species accounting to 43 per cent parasitism observed. However mass rearing and release of these parasites were not sustained, hence this study.

OBJECTIVES

1. To mass rear the primary parasitoids of Diaphorina citri.
2. To determine the efficacy of the primary parasitoids in the regulation of D. citri population.

MATERIALS AND METHODS

Cultivation of host plants

Murraya paniculata commonly known as "kamuning" were propagated through cuttings and seeds and were maintained inside the screenhouse. Eight hundred plants were maintained at a low size (1-1.5 ft. high) by regular pruning. The plants were, fertilized and exposed to natural light to stimulate the growth of new flushes since adult psyllids lay eggs only on newly sprouted leaf buds.

Stock Culture of *D. citri*

. The initial population of *D. citri* adults were collected in the field and were released in outdoor rearing screencages about (2.5 meter x 1.5meter) with pots of

Murraya plants having new flushes inside and allowed to develop their population. Emerging adults were collected and transferred to new plant host and were then allowed to deposit eggs to maintain the cultures.

From the initial population gravid females *D. citri* were introduced on *Murraya* plant and allowed to deposit their eggs for 48 hours inside screencages Exposed plants were transferred to another cages (150cmx71cm) for preimaginal development and provide *D. citri* nymphs for the culture of the primary parasitoids.

Stock Culture of the primary parasitoids

The psyllid mummies parasitized by *T. radiata* and *D. aligarhensis* were initially collected from the field and were placed on rearing jars separately for adult emergence. Emerging adult of the parasites were collected confined in test tubes provided with honey and allowed to mate. Mature adults were introduced on new batch of *Murraya* seedlings infested with *D. citri* nymphs for egg deposition and development of the parasitoids. New batches of *Murraya* seedlings previously infested with *D. citri* nymphs were offered daily. Developmental time of the primary parasitoids were also noted.

Host preference of the *T. radiata* and *D. aligarhensis* on *D. citri*

The preference of the two primary parasitoids to the different instar of of *D. citri* was conducted. Ten of each instar nymphs were placed in 2-3 weeks old *Murraya* seedlings covered with mylar cage and were exposed to mated females of *T. radiata* and virgin females *D. aligarhensis* in separate set up. Ten replicates were conducted for each 2 parasites. After 24 hours, parasite were removed and exposed nymphs were observed until mummies of each parasitoids appears. Parasitized nymphs were counted using a magnifying glass (10x) and recorded.

Parasitization capacity of T. radiata and D. aligarhesis under laboratory condition

Parasitization capacity of the two primary parasitoids were determined under laboratory condition. *Murraya* seedlings infested with 10 and twenty (20) third and fourth instar nymphs of *D. citri* were exposed to mated adults of *T. radiata* and virgin females of *D. aligarhensis*. After 24 hours parasites were removed and exposed *D. citri* nymphs were observed until mummies appeared and number of parasitized nymphs recorded.

Parasitism of T. radiata and D. aligarhensis under field condition

Incidence of *D. citri* and level of parasitism by the two primary parasitoids was determined in the field. Three calamansi field located in Mankilam, San Miguel and Magdum both in Tagum city and at DNCRDC-BPI, Davao City were monitored. Calamansi trees were monitored monthly for the presence of *D. citri* and its parasitoids. The total counts of the psyllid and the percent parasitism of each parasitoid were counted and recorded on 20 randomly selected plants. Other biological control agents found associated in colonies of *D. citri* were also recorded.

Released of the parasitoids was also done to evaluate their efficiency in the field. Effect of the parasitoid were assessed by determining the degree of parasitism. Test tube with adult parasitoids were placed in the branches of infested

trees and allow them to search for the host. Calamansi trees were monitored after the released of the parasitoids. Percent parasitism was determined by counting the number of parasitized over the total number of nymphs collected. Weather data were also noted in relation to the occurrence of the pest and its two primary parasitoids.

RESULTS AND DISCUSSION

Mass rearing *Tamarixia radiata* and *Diaphorencyrtus aligarhensis* :

The two primary parasitoids of *D. citri* were mass reared at the same time in separate outdoor rearing cages protected with plastic roofing to protect the culture from rainfall. *Murraya* seedlings were placed in raised benches to prevent ants. The rearing of the insect host and the parasitoids was carried out on a continual basis.

Murraya seedlings was the most suitable host plant for mass rearing of *D. citri* because psyllid feeding is less detrimental to the plant than to citrus and it can maintain higher psyllid population and when regularly pruned making flush readily available for the host psyllid, likewise regularly available for the parasitoids. From the stock culture *Murraya* seedlings were exposed to 20 gravid females of *D. citri* for egg deposition and preimaginal development (nymphal stages) An average of 10 *Murraya s*eedlings about 1.5 foot high with 2 to 6 flushes per seedling produced 360-1194 *D. citri* nymphs.

Adult *T. radiata* were introduced per *Murraya* plant. Ten seedlings of *Murraya* infested with 1,074 psyllid nymphs generated 523 *T. radiata* adults at 27-28^{0}C and 75-85% RH. The surviving adult psyllids were used to produce the next generation of psyllid nymphs. A female parasite:host ratio of 1:20 was used in the production of *T. radiata* and *D. aligahrensis* In separate cage, 466 *D. citri* nymphs generated 148 *D. aligarhensis adults* .

Development of *T. radiata* and *D. aligarhensis*

Table 1 shows the total developmental period of the 2 primary parasitoids. On *T. radiata* total developmental period from egg to adult ranges from 9-11 days ($\bar{x}$= 9.57$\pm$0.38) for female and 9-10 days for males ($\bar{x}$=9.6$\pm$0.25). The females usually deposited their eggs singly on the ventral side of the host between the thorax and the abdomen. The parasitized nymphs can be recognized 5-7 days after exposure. Mummified psyllid became flatten and dark brown. The mature parasite larvae spinned around to stick on the leaves of branch of the host plant. The emerging adult parasitoid emerges from the dorsal thorax of the parasitized nymphs with a mean sex ratio of 1:3.19 (male:female). Population of *T. radiata* is arrhenotokous wherein male and female are present. Adult female and male parasitoid lived for 4-20 days and 2-14 days, respectively.

On *D. aligarhensis* the total development period from egg to adult ranges from 16-23 days ($\bar{x}$=17.88$\pm$1.08) *D. aligarhensis* laid their eggs inside their host body. The nymphs were not dissected to see the stages of development of the parasitoid. Nine to 10 days after exposure the psyllid nymphs became less active and the dorsum swelled gradually the color changed from yellow to brownish red mummy and stick on

the substrate. New adult *D. aligarhensis* emerged through an exit hole in the dorsal abdomen of the parasitized nymph. Adult *D. aligarhensis* lived for 8-33 days ($\bar{x}$ = 21.29±3.32). The parasitoid produced only female progeny. No males were also observed on field collected samples, hence the parasitoid can be considered thelytokous .

Table 1. Duration of the different life stages of *T. radiata* and *D.aligarhensis* under laboratory condition.

Developmental stages	*Tamarixia radiata*				Virgin female *D. aligarhensis*	
	Female		Male			
	Range	Mean±SE	Range	Mean±SE	Range	Mean±Se
Egg	2	2	2	2	_	_
Larva	3-4	3.39±9.81	3	3	_	_
Prepupa	1	1	1	1	_	_
Pupa	3-4	3.29±9.81	3-4	3.5±0.55	_	_
Total Development time	9-11	9.57±0.38	9-10	9.6±0.25	16-23	17.88±1.08
Longevity	4-20	9.7±2.87	2-14	8.2±2.10	8-33	21.29±3.32

Host preference of *T. radiata* and *D. aligarhensis*

Table 2 showed the preference of *T. radiata* and *D. aligarhensis* on the different nymphal stages of *D. citri*. *T. radiata* was able to parasitized the 3rd, 4th, and fifth nymphal stage of *D. citri* but showed more preference on the 4th nymphal stage. For *D. aligarhensis*, they preferred the 2nd 3rd and the 4th nymphal stages with more preference on the third. This corroborate findings of Tang and Huang (1991) reported that *T. radiata* oviposited on the 3rd, 4th and 5th instar nymphs while *D. aligarhensis* oviposited on the 2nd, 3rd and 4th instar nymphs. Chien et al. (1989) used only the fifth instar nymphs in rearing *T. radiata* parasitoid.

Table 2. Preference of *T. radiata* and *D. aligarhensis* on the different nymphal stages of *D. citri.*

Nymphal stage	Percent parasitized	
	T. radiata	*D. aligarhensis*
First	0	0
Second	0	10
Third	14	43
Fourth	60	30
Fifth	37	0

Based on 425 field collected nymphs of *D. citri*, 17 third instar nymphs, 61 fourth instar nymphs and 52 fifth instar nymphs were parasitized by *T. radiata*. Under natural field condition host preference of the female parasitoid varies which probably depends also on the availability of the different host stages in the field.

Parasitism of *D. aligarhensis* and *T. radiata* under laboratory condition

Parasitization capacity of the two primary parasitoids were determined under laboratory condition when introduced with two densities of 3rd and 4th and 5th instar nymphs of *D. citri* (Table 3). When provided with 10 and 20 *D. citri* nymphs a single female of *T. radiata* parasitized 5.4 nymphs 9.1 nymphs, respectively. Similarly virgin females of *D. aligarhensis* parasitized 4.1 nymphs at density of 10 and 4.9 nymphs at density of 20 nymphs. *T. radiata* parasitized morenumber psyllid nymps compared to *D. aligarhensis* which exhibit a high searching capacity and high reproductive rate.

Table 3. Parasitization capacity of *D. aligarhensis* and *T. radiata* on *D. citri* Nymph under laboratory condition.

D. citri nymphs density	Parasitoid	Number of *D. citri* nymphs parasitized		% Parasitism
		Total	Mean	
10	*D. aligarhensis*	41	4.1b	41
	T. radiata	54	5.4a	54
20	*D. aligarhensis*	49	4.9b	25
	T. radiata	91	9.1a	46

Based on 10 females for each parasitoid.

Based on longevity and fecundity a single virgin female of *D. aligarhensis* when offered with 20 third and fourth nymphal stage daily parasitized 65.80±20.50 at a daily rate 1.0-4.77 psyllid nymphs with 5-24% parasitism while mated *T. radiata* female parasitized 98.80±22.28 at daily rate of 4.42 -8.57 nymphs with 22-70 % parasitism throughout its life.

Parasitism of *D. aligarhensis* and *Tamarixia radiata* under field condition:

The population of *D. citri* and the level of parasitism of the two primary parasitoids, the indigenous *Diaphorencyrtus aligarhensis* and the imported *Tamarixia radiata* on the pest population were monitored in Tagum City, Davao del Norte and DNCRDC, Bago-Oshiro, Davao City from 2009 to 2010.

In Mankilam, Tagum City, *D. citri* was recorded at low numbers during the months of February and March 2009 (n=88 and 38) and levels off on the succeeding months until August. *Tamarixia radiata* and *D. aligarhensis* were observed parasitizing *D. citri* nymphs at 10% and 50%, respectively. (Figure 1). Population started to build up again in month of October and peak in November 2009 and February 2010. Low population or absence of *D. citri* was attributed to the scarcity or absence of flushes and increased when tree produced great amount of young new flushes which is most favorable for egg deposition by *D. citri* adult. Generally, population fluctuation was also affected by the amount of rainfall where high rainfall caused the population to drop.

Release of the primary parasitoids was initiated on October 2009 when *D. citri* was apparent (n=84) and second release on the month of February where highest population number of *D. citri* (n= 605) was recorded A total of 1,400 (900 *T. radiata* and 500 *D. aligarhensis)* parasitoids were released in the area. Parasitism recorded after the release were 43-68% for *T. radiata* and 12-31% for *D. aligarhensis. D. citri* disappear in the succeeding months of observation and appear during the later part of the year when limited amount of flush exist with a parasitism of 20% and 60% by *D. aligarhensis* and *T. radiata,* respectively. Hyperparasitism on *T. radiata* and *D. aligarhensis* was also observed during the period of observation and recorded at 2%.

Several general predators were also observed in the field such as spiders, coccinellid beetles, lacewings and syrphid flies during the period.

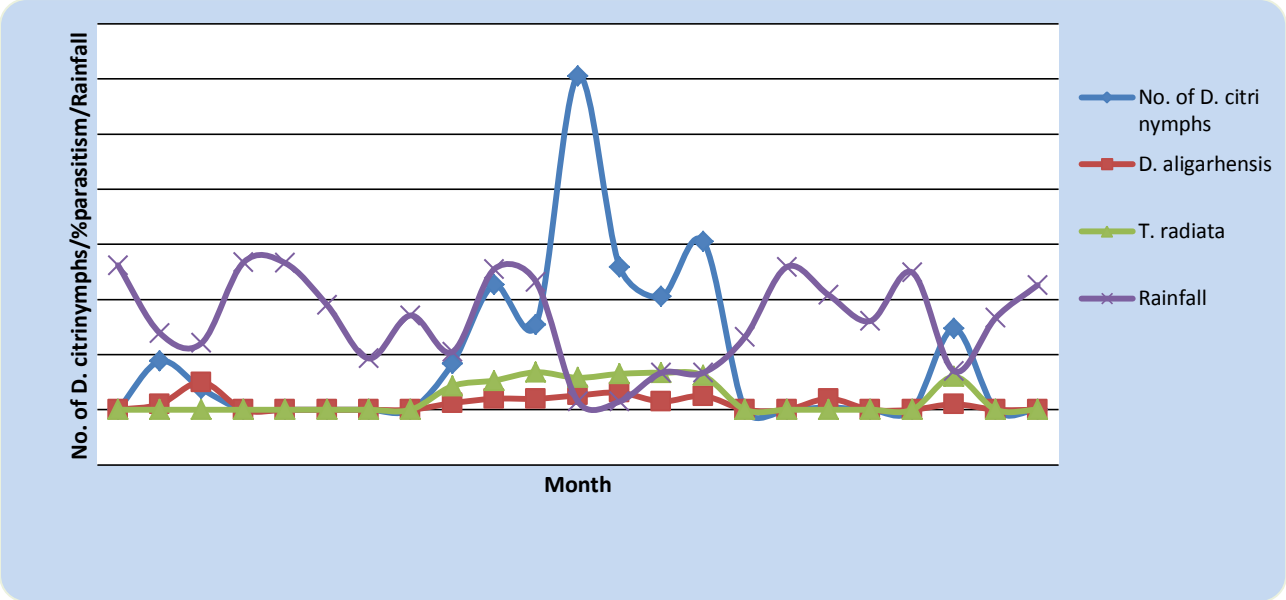

Figure 1. Incidence of *D. citri* and percent parasitism of *T. radiata* and *D. aligarhensis* at Mankilam. San Miguel, Tagum City (2009-2010).

In Magdum, Tagum City, *D. citri* were recorded during the months of Oct-November 2009. (n= 84 and 209) Only *D. aligarhensis* were recorded parasitizing the nymphs of *D. citri* during this periods with 6-10% parasitism. . The area was considered a neglected orchard (no inputs applied and no proper maintenance).

A total of 200 adults *T. radiata* and 100 *D. aligarhensis* were initially released in the orchard in November 2009. Parasitism by *T. radiata* and *D. aligarhensis* were observed at 62% and 30 % parasitism, respectively. No *D. citri* was observed in the succeeding months of observation which was attributed to the absence of new flushes and heavy rainfall during the period. *D.citri* population was observed and recorded only in March 2010 where population are all adults (n=7). In the succeding months no *D. citri* were recorded.

At the BPI-DNCRDC, *D. citri* population were observed during the months of February to May 2009 (n=27,66,90) when calamansi started to flush which is stimulated by rain during the period and levesl off from July to September when flushes matures and there is relatively large amount of rainfall during the period (Figure 2). Low population of *D. citri* were also observed on months of October to November 2009 (n=16, 20) with little flush exist. . High population were noted on the months of January to May 2010 (n= 236, 292, 464 and 218) when there is relatively low rainfall and abundant flushes (Figure 2).

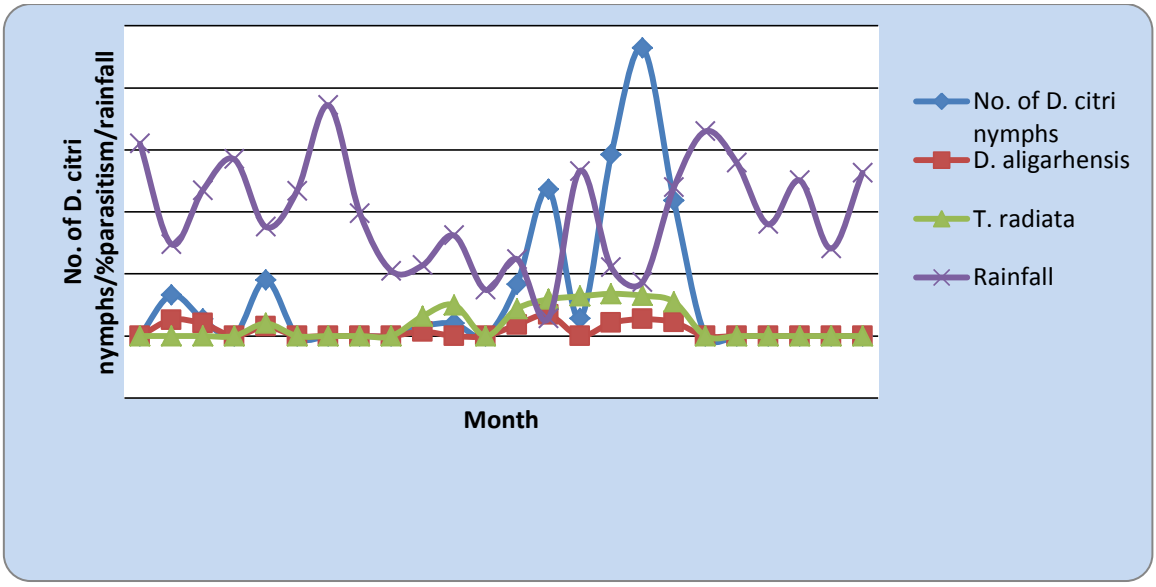

Figure 2. Incidence of *D. citri* and parasitism of *T. radiata* and *D. aligarhensis* at BPI-DNCRDC, Bago-Oshiro, Davao City (2009-2010).

T. radiata and *D. aligarhensis* was found parasitizing *D. citri* with 20 and 15% parasitism, respectively. A total of 200 adult *T. radiata* and 100 *D. aligarhensis* were released in the area. Parasitism recorded for *T. radiata* was 32-68% and 7- 34% for *D. aligarhensis*.

All three sites evaluated for the parasitism of the two primary parasitoids proved at least significant results. The two parasitoids perform differently at different psyllid densities. *Tamarixia radiata* exerted higher rate of parasitism over D. aligarhensis, however, *D. aligarhensis* appears better adapted to low host densities. The presence of *T. radiata* and *D. aligarhensis* is expected to complement each other in the field.

Several generalist predators were also recorded such as species of coccinellid ladybeetles, larva of syrphid flies and spiders and could be supplementing the action of the parasitoids and or probably feeding on the previously parasitized nymphs of *D. citri*. (Aubert, 1987) reported that predators such as syrphid fly and coccinellid beetle were recorded from Reunion Island and Nepal and in Taiwan (Chen, 1998).

CONCLUSIONS

The citrus psyllid *Diaphorina. Citri* Kuwayama and its two primary parasitoids *Tamarixia radiata* Waterson and *Diaphorencyrtus aligarhensis* Shafee et al. were successfully reared in outdoor screencages using “Kamuning” *Murraya paniculata*.

T. radiata significantly parasitized more number of *D. citri* nymphs than *D. aligarhensis*

Population build-up of *D. citri* correspond to flushing activity of the tree which is stimulated by rainfall however, heavy rainfall also affected *D. citri* population.

Both the endemic endoparasite, *D. aligarhensis* and the introduced ectoparasite *T. radiata* were found to be present in the field exerting control on citrus psyllid *D. citri* nymphs even at low levels before parasite release.

With the combined effect of parasitism by the presence of the two parasitoids it is expected to complement each other in the suppression of *D. citri* in the field.

Persistence of *T. radiata* in the field is a promising sign and could be used in the formulation of IPM for *D. citri*

RECOMMENDATIONS

- Biological control of the *D. citri* by the two parasitoids could be used as one of the component in formulating an effective pest management of *D.citri* on citrus.
- The persistence of the introduced parasitoid was a promising sign and requires additional input of mass reared parasitoids and further monitoring on other calamansi and citrus growing areas.
- Evaluation of the impact of the generalist predators in the suppression of *D. citri* population can be studied further.
- Mass rearing and release of the imported parasitoid to enhance the biological control of *D. citri*.

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