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Seasonal Population Dynamics of the Asian Citrus Psyllid, *Diaphorina citri* Kuwayama in Sarawak

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Abstract: Problem statement: Effective control of phytophagous pests requires a thorough understanding of their seasonal population dynamics, dispersion behavior, natural enemy activity and climate. To date, although very little detail information had been published on the ecology of Diaphorina citri. The objective of this investigation was to test through field experiment the hypothesis that the major factors influencing local D. citri populations particularly their seasonal population dynamics in Sarawak are (a) flushing cycles, (b) climate and (c) the impact of the primary parasitoids namely Tamarixia radiata and Diaphorencyrtus aligarhensis. Approach: Seasonal abundance D. citri was studied weekly from March 1998 to December 2000 in the 1-ha citrus honey mandarin (Citrus aurantium L.) commercial orchard at Jemukan (1° 33'N, 110° 41'E), Kota Samarahan Division, Southwest Sarawak, in Malaysia, **Results:** Field studies on citrus trees showed that the D. citri population fluctuates throughout the year on citrus honey mandarin in Sarawak. Generations overlapped but adult and egg population peaks for a short period generally coincided with three annual flushing cycles, in August-September, February-March and June-July between March 1998 and December 2000. Conclusion: Psyllid population levels are positively related to the availability of new shoot flushes. Psyllid populations are adversely affected by weather conditions and parasitoids. Adult psyllid populations increased exponentially during periods of flush growth and migration and dispersal of the adults was also related to flushing cycles. Dispersal and colonization of new trees is greatest in September-October, at the onset of the rainy season.

Key words: Diaphorina citri, seasonal population dynamics, Citrus aurantium, Diaphorencyrtus aligarhensis, Tamarixia radiate, phytophagous pests requires, citrus honey mandarin, psyllid population, hyperparasitoids, instar nymphs, hymenoptera, natural enemies

INTRODUCTION

Effective control of phytophagous pests requires a thorough understanding of their seasonal population dynamics and dispersion behavior in relation to range of factors including host plant phenology, natural enemy activity and climate. To date, although very little detail information has been published on the ecology of *Diaphorina citri* (Wang, 1981; Tsai *et al.*, 2002; Aubert, 1987), it is known that the three main weather factors influencing the development of its populations are temperature, relative humidity and rainfall. Its two

primary parasitoids *Tamarixia radiata* (Waterson) [Hymenoptera: *Eulophidae*] and *Diaphorencyrtus aligarhensis* (Hayat *et al.*, 1975) [Hymenoptera: *Encyrtidae*] and predators, particularly coccinellids (Michaud, 2004) are important natural enemies but the effectiveness of the parasitoids is limited by an extensive range of hyperparasitoids (Waterhouse, 1998).

Studies on the ecology of *D. citri* elsewhere (Catling and Annecke, 1968; Wang *et al.*, 1996) are only indicative of influences of biotic and abiotic factors on *D. citri* populations in Sarawak. As part of an effort to

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develop an integrated management program for D. citri in Sarawak, field studies of D. citri were therefore undertook. The objective of this investigation was to test through field experiment the hypothesis that the major factors influencing local D. citri populations particularly their seasonal population dynamics in Sarawak are (a) flushing cycles, (b) climate and (c) the impact of the primary parasitoids namely T. radiata and D. aligarhensis. Field studies of D.citri in a citrus orchard were therefore undertook to determine the influences of flushing cycles, climate and the impact of the primary parasitoids on the *D.citri* populations. An understanding of the influence of these factors and interactions between them, is fundamental for the development of reliable pest management strategies, particularly timing of pesticide applications and management of Huanglongbing.

MATERIALS AND METHODS

Study sites: Seasonal abundance of *D.citri* Kuwayama, population was studied weekly from March 1998 to December 2000 in the 1-ha citrus honey mandarin (*Citrus aurantium* L.) commercial orchard at Jemukan (1° 33'N, 110° 41'E), Kota Samarahan Division, southwest Sarawak, in Malaysia. It comprised 200 initially disease-free, bud-grafted non-bearing honey mandarin (*C. aurantium* L.) trees planted in 1996 in 25 rows (N-S) in 8 columns on a 5.3×5.3 m grid. The trees grew rapidly and were 2-3 m tall with relatively open canopies when the studies commenced in 1997.

Data collection: Psyllid populations (adult, egg, nymph per flush) were assessed at weekly intervals throughout the growing season by stratified random sampling of five twigs per tree on the middle canopy and examining each flush (10-15 cm long) with a 10x hand lens. The field was stratified into 10 blocks with 20 trees per block. Initially, when populations were relatively low, psyllids were counted in situ on 5 randomly selected shoots (2-4 cm long) immatured leaves per tree and a total of 100 randomly selected shoots for each sampling. Later, as infestation levels increased to higher levels, 50 shoots at weekly intervals. Assessments were undertaken from March 1998 to December 2000. Psyllids were observed to feed preferentially on young flushes (less than 1 cm long, 4 -6 leaves with light green in colour) and young shoots (6-10 cm long). Base on this young flush was chosen as the sample unit. The total number of psyllids was recorded per flush. Visual counts were taken on the number of psyllids (eggs, nymph and adults) on the five twigs (each 6-10 cm long and had 5 immature leaves with the size range 2-4 cm long) per tree. Insects were counted *in situ* and on plant clippings taken to the laboratory. All other cultured practices were in accordance with standard commercial practice.

Meteorological data (temperature, rainfall and relative humidity) were obtained from Paya Paloh padi testing station in Samarahan Division ($1^{\circ} 27'$ N, $110^{\circ}27'$ E). The monthly daily average temperature was the average of mean daily maximum and minimum temperature and the monthly daily average relative humidity was the average of the mean daily relative humidity recorded at 9.00 h and 15.00 h.

The study of the flushing rhythm of citrus at the study site in the Jemukan citrus orchard comprised 200 citrus trees was carried out from October 1998 to June 2000. All flush points enclosed by the one square feet metal frame and regarded as being suitable for the development of *D. citri* were counted. All the young flush points were expressed as percentage of flushing. Population assessments were made by a direct count of colonies on the lower one metre of the canopy of each sample tree which comprised 40-50% of the canopy area. Assessments were usually made at weekly intervals. Each colony was recorded separately according to its predominant age class, i.e., eggs, nymphal instar and adults.

Study of the primary parasitoid activity at the study site in the Jemukan citrus orchard was carrid out from January 1999-December 2000. Nymphs of D. citri were examined with a 10x handlens for the presence of parasites. Parasitised nymphs were collected and percent parasitism by T. radiata and D. aligarhensis was based on weekly examination of third to fifth instar nymphs collected weekly. The nymphs were examined in the laboratory under a stereomicroscope on a day of collection and then placed in test tubes in which the parasitoids were allowed to emerge. Parasitism was based on the presence of immature or adult parasitoids and exit holes. The exit hole of T. radiata normally occurs on the thorax of third, fourth and fifth instar exoskeletons, while the conspicuous exit hole of D. aligarhensis normally occurs on abdomen of third and fourth instar exoskeletons. Exit holes on the sides of the abdomen are usually associated with hyperparasitoids. The identities of T. radiata and D. aligarhensis were confirmed by Dr. YQ Tang, Biological Control Institute, Fujian Agricultural College, Fuzhou, Fujian, China.

The key-factor analysis was not used to identify the most important agent that regulates the field population dynamics. This is because to do such a study with *D*. *citri* would be very complex and would require a lot of initial planning and experiment for field population

dynnamics. These include plant nutrition and its influence on fecundity, mortality induced by predators and parasitoids then the impact of bud densities on populations and subsequent influences on natural enemy effectiveness. The abundance of plants that might encourage predators and parasitoids to enter an orchard and the influences of the hyperparasitoids should also be considered.

Statistical analysis: The dependent variables in the field experiments were average number of adults, eggs and nymphs per flush on a shoot and the parasitism (the proportion of parasitized 3rd-5th instar nymphs including live, dead and parasitised). For the analysis of seasonal population abundance the data was subjected to multiple correlation and regression analyses with meteorological data and flush cycles as to assess the association and relationship between population level of *D. citri* and flush cycle, temperature, rainfall and humidity using the GENSTAT 5 computer package. A Chi-square Goodness of fit test ($p \le 0.05$) was used to compare the differences between the mean numbers of adult and nymph among months of the year.

The results obtained by standard statistical methods from consecutive sampling occasions were presented in the form of a mean and its standard error. The logarithmic or the arcsine square root transformation when required was made on the psyllid counts before data are analyzed by one-way ANOVA. All differences between means were separated using the Fisher's Least Significant Difference test (LSD) after a significant Ftest at $p \le 0.05$.

RESULTS

Seasonal abundance of D. citri population: The seasonal abundance of D. citri eggs, nymphs and adults from March 1998-December 2000 at Jemukan are presented in Fig. 2 and 3. There were no significant differences between the mean numbers of adult and nymph among months from March 1998 to December 1999 ($\chi^2 = 19.736$, df = 20 and $\chi^2 = 22.140$, df = 20). The psyllids were found on citrus tree throughout the year whenever new flushes are available. In 1998, populations of adult, egg and nymph were generally lowest during the months of July and November-December and the peaked populations occurred during the months of April and August-September (Fig. 1). The peak populations of adult and nymph occurred during February-March, May and August-September for 1998 and 2000 (Fig. 2 and 3).



Fig. 1: Seasonal abundance of *Diaphorina citri* in 1998 Maximum temperature



Fig. 2: Seasonal abundance of *Diaphorina citri* in 1999 Maximum temperature



Fig. 3: Seasonal abundance of Diaphorina citri in 2000



Fig. 4: Seasonal abundance of *Diaphorina citri* population in relation to flushing cycle and climate

From early April in 1999 and 2000, generations of adult and nymph were increased progressively and population density increased exponentially as the dry season (April to September) progressed. Although the second increase in populations started from July month onwards during dry seasons (monthly rainfall between 197 and 207 mm) in 1998, 1999 and 2000, peak number of nymphs and adults occurred in August-September. The highest ovipositor peaks occurred in September/October for 1998 and 1999 at the onset of rainy season, but populations decreased sharply after late September as the rainy season progressed. Annually, there were three adult peaks, two to three egg peaks and three nymph peaks. Higher populations of nymphs and adults were observed in 1999 than in 1998, although rainfall, temperatures and relative humilities in each year were similar.

Influence of flushing cycles and climate: Figure 4 indicates flush incidence and populations of D. citri at the experimental site in the Jemukan citrus orchard. Three main flush cycles were produced during the study period and the three annual well-defined peaks occurred during February-March, July and September-October (Fig. 4). D. citri populations for nymph were generally higher during the rainy season from October to January than during the dry season and population peaks for adult and egg were somewhat coincided with cyclic production of flush growth during February- March, July and September-October (Fig. 4). Psyllid population was gradually increased from May onwards through to August-September during the main flushing periods. However, the study showed that the overall population fluctuations of D. citri breeding on citrus were slightly influenced by the flushing rhythm (r = 0.28, df = 20; P ≤0.05), which generally occurred during February-March, July and September-October.

The multiplecorrelation values showed that the maximum temperature and relative humidity were negatively correlated with the adult and egg populations. The effect of relative humidity was least significant as correlation value was less than 0.1. The minimum temperature was positively correlated with the adult and egg populations. The rainfall had less effect on adult but was negatively correlated with egg population. However, the most favorable condition for increasing incidence of eggs and adults was the gradual rise in daily ambient temperatures that led to nymph peaks during hot and dry season from May to August (Fig. 4) and this also show that maximum temperatures have greater influence on nymph population. The correlation values showed that the temperatures appear to have greater influence on psyllid population than flushing cycles. The linear regression equation for psyllid population = $1232 \times 0.238^{\circ}$ (% Flushing) -15.6*(Max temp) +1.2*(Min temp) -0.0187*(Tot rainfall) -8.84*(Mean RH).



Fig. 5: Percentage parasitism by *T. radiata* and *D. aligarhensis* and abundance of its host *D. citri* at Jemukan for season 1999-2000

Influence of natural enemies: Parasitism by T. radiata and D. aligarhensis ranged from 4.4-22.3% and 2.6-35.3% respectively (Fig. 5). The trend of seasonal incidence of both parasitoids was somewhat similar in both 1999 and 2000 (Fig. 5) and there were three peaks annually for both species, in February/March, June/July and November/December. In June/July 1999. parasitism by both species exceeded 20%. By November/December of the same year, this had increased to 22.3 and 35.4% for T. radiata and D. aligarhensis respectively. There is an indication of density-dependent mechanism operating between D. *citri* population and the parasitoids as both are specialist parasitoids specifically parasitising D. citri.

DISCUSSION

Seasonal abundance of D. citri population: Eggs, nymphs and adults of D. citri occurred throughout the year. The numbers of each life stage fluctuated each year (Fig. 1-3) and were generally higher during the dry season in April and August to September than during the wet season from October to November. During the three years of study, from March 1998-December 2000, there were three adult, two to three egg and three nymphal peaks annually. The peak population for nymph occurred during February-March, May-June and August-September. These results were similar to those recorded by Atwal et al. (1970) and Mercado et al. (1991), in relation to seasonal conditions in the regions where their studies were undertaken. Results of this study confirmed that D. citri populations fluctuate throughout the year, adult and egg population peaks for a short period linked to flushing cycles. Population peaks generally coincided with three annual main flush cycles, in February-March, June-July and August-September (Fig. 4). However, the oviposition resources (new flush growth with lengths ranging from 2-10 mm) that psyllid females can exploit in relatively low abundance. The close link between oviposition preference and nymphal performance occurs because an immature nymph is restricted to a single leaf selected by its female. This close association and the relatively low abundance of resources resulted in quite similar seasonal population trends in the two successive years investigated in this study (Fig. 1 and 2).

In the field studies in South Africa, Catling and Annecke (1968) found that flushing cycles of citrus, weather extremes, the condition and nutritional status of young growth and activity of natural enemies were the key factors regulating the abundance of Trioza erytreae. The present studies showed that psyllid populations were slightly influencd by flushing cycles and higher population occurred due to the flushing periods in March, July and September. Egg incidence was correlated with the availability of new growth flushes and D. citri are strongly attracted to new flushes and sometimes invade entire orchards within days (Samways and Manicom, 1983). It was observed in Fig. 4 that D. citri populations increased from February, which coincided with the first flush cycle of the dry season and relatively low levels of parasitism. Heavy and prolonged flushing of young citrus trees as incicated in this result (during July to September), make them, as noted by Catling (1970), very attractive to citrus psyllids for oviposition.

Aubert (1987) listed three main factors influencing the development of D. citri populations: temperature, relative humidity and rainfall. 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 correlation values from the present studies showed that the temperature and rainfall appear to have greater influence on psyllid population than flushing rhythm. Atwal et al. (1970) suggested that both high and low temperatures were detrimental to the psyllid population increases. The current study suggested that the dry season from March through August favoured increasing populations of D. citri and particularly its nymphs. Lakra et al. (1983) reported similar findings in their studies in India. Catling (1970) also reported that all stages of African citrus psyllid, Trioza erytreae were sensitive to high temperature together with low relative humidity. Its populations were consistently the highest in the cool and moist upland region and were always low in the hot and arid lowlands of South Africa. In current study, nymph population was relatively higher than adults particularly

during the dry periods. This might due to a negative correlation between adults, relative humidity and maximun temperature. In 1999, as ambient temperatures in the orchard at Jemukan became gradually higher with the onset of the dry season, psyllid populations gradually increased before peaking during June.

Gradually these three factors began to work against D. citri to produce a slight decrease in egg population in April but nymphal and adult populations remained relatively high from July through September and started to decline again from October onwards (Fig. 4), when monthly rainfall and the maximum temperature were about 581 mm and 31.3°C respectively, relative humidity was about 83.6% and parasitism was more than 20%. During the rainy season i.e. from October 1999-April 2000, the egg numbers gradually decreased and remained at a relatively low numbers (Fig. 4). There was a negative correlation between egg, relative humidity and rainfall. The populations of eggs and adults declined during the rainy season and were lower during January and April in 2000 (Fig. 4). D. citri is more sensitive to high rainfall and relative humidity than extreme of temperatures. Heavy rainfall reduces populations as it washes eggs and first and second instar nymphs, off young flush from October 1999-January 2000. During such rainfall, adults hide on the lower surfaces of the leaves and twigs. Monthly rainfall increased from 374-458 mm between October 1998 and March 1999, from 581-919 mm between October 1999 and January 2000 (Fig. 4).

The decline in populations of egg and adult from October 1999-January 2000 was probably due to the harmful effects of the rainy weather. It seems most possible that monthly rainfall >150 mm and high relative humidity, are major limiting factors to the abundance and distribution of D. citri in various areas in Malaysia. However, the nymph populations were still high during December 1999-February 2000. This may due to the older instar nymphs are more tolerant to high rainfall and the climates (rainfall and relative humidity) appear to have less effect on older nymph population. However, high temperature combined with low relative humidity is extremely detrimental to psyllid populations resulted in death of eggs and first instar nymph due to desiccation as reported by Aubert (1987), but this factor did not seem to play role in regulating psyllid population in this study.

Nymphal mortality of 60-70% can be expected when relative humidity exceeds 87-90%. Although the older instar nymphs are tolerant to climatic extremes (Catling and Annecke, 1968; Lakra *et al.*, 1983), but very high temperatures also seem to hinder the development of D. citri nymphs (Liu and Tsai, 2000). Such influences may have contributed to population troughs observed in the dry season months from April to July. More recently, Liu and Tsai (2000) found that the optimum range of temperatures for growth of D. citri populations is 25-28°C and the populations failed to complete development at 10 and 33°C. McFarland and Hoy (2001) reported increases in D. citri population survival with increasing relative humidity in Florida. This suggests that nymphal mortality was higher at higher temperature with higher humidities and low at moderate temperatures (20-28°C) with lower humidities (43-75% RH). The results of the present studies found a positive correlation between temperature and D. citri population and a negative correlation with relative humidity and rainfall which was in agreement with the findings by Arora et al. (1997).

Influence of natural enemies: Parasitoids and predators adversely affect D. citri populations (Waterhouse, 1998). Only two species of parasitoids namely T. radiata and D. aligarhensis were considered to play an important role in suppressing D. citri populations in the Jemukan orchard. There are a number of unidentified predators including coccinellids, spiders, mantids and entomopathogenic fungi (e.g., Beauveria bassiana) also found attacking D. citri adults and nymphs but their impacts on D. citri population are not being assessed in this study. However, the level of field parasitism in the present study was variable and at levels < 23% and 36% respectively for both species, relatively lower than some recorded levels elsewhere and possibly not sufficient to reduce spread of HLB. T. radiata was particularly effective with a 80% parasitism being reported in northern India and China (Husain and Nath, 1927; Yuhua et al., 1987) and levels > 90% for *T. radiata* have been reported from Nepal and Bhutan, Réunion Island, Mauritus and Taiwan (Lama et al., 1988; Regmi and Lama, 1988; Etienne and Aubert, 1980; Shui-Chen-Chiu et al., 1987). Likewise, higher levels of parasitism by D. aligarhensis have been reported from India (Hayat et al., 1975), the Philippines and Réunion Island (Etienne and Aubert, 1980) and Vietnam. Mercado et al. (1991) reported from the Philippines that the highest rate of parasitism of D. aligarhensis on D. citri obtained was 62%.

However, the levels recorded in this study were similar to those reported previously in West Malaysia where levels recorded for both parasitoids were <37% (Osman and Quilici, 1991). Although *D. aligarhensis* appears to be a less effective parasitoid than *T. radiata*,

but it is widespread in Asia (Prinsloo, 1985) and it still contributes for the suppression of *D. citri* population without encountering hyperparasitoids. Vector control was achieved in Reunion Island by the total elimination of *T. erytreae* through a biological control program (Aubert and Quilici, 1988). The primary parasitoids would play a significant role in reducing the *D. citri* population and hence to supplement the present chemical control strategies to supplement each other if both establish. Beneficial effects of the establishment of both parasitoids could reduce the *D. citri* populations in citrus orchard, frequency of transmission of HLB, applications of pesticides to control of *D. citri* and secondary pest outbreaks is due to the negative effects of pesticides on other natural enemies of citrus pests.

The impact of both parasitoids can be reduced by the hyperparasites. Although Waterhouse (1998) reported 12 species of hyperparasitoids for D. citri and hyperparasitism could be a limiting factor for the biological control of D. citri (Shui-Chen-Chiu et al., 1987), a thorough evaluation of their impact was beyond the scope of this current study. The two primary parasitoids are very widespread in Southeast Asia (Waterhouse, 1998). Biological control should be encouraged where the insects are beyond the reach of chemical treatments. Therefore, the extent of their impact in Sarawak requires further investigation. Both T. radiata and D. aligarhensis kill psyllids by two mechanisms: parasitism and host feeding. Regular field releases of both parasitoids to complement each other if establish. The two species may perform differently at different psyllid densities and in different climatic zones. In Taiwan, T. radiata had higher populations than D. aligarhensis during part of the year, but D. aligarhensis appears better adopted to low host densities there (Shui-Chen-Chiu et al., 1987).

The prospects for biological control are unpromising but still effective. Once both species are established after regular field release, such insecticides as imidachloprin or preferably Horticultural Mineral Oils (HMOs) could favor the buildup of parasitoids and harmful for the psyllids. Hedgerow planting of jasmine orange or curry leaf plant which is a suitable and nondiseased host plant (Gottwald et al., 1989; Hung et al., 2000) within the citrus orchard could attract more psyllids to colonise and breed on it. This will to some extent also allow some emigrant adult psyllids from the citrus orchard to settle down on these jasmine orange or curry leaf plants. Emigration may be a regular feature of the life cycle of the citrus psyllid as reported by Berg et al. (1991). These psyllids colonise on the alternate

host can be regulated by the parasitoids or controlled by other biological agent or HMOs. Waterhouse (1998) report that in Taiwan, T. radiata performed best in relatively stable habitats (jasmine orange plantings with no pesticides and little large-scale pruning); under these conditions T. radiata and D. aligarhensis were able to maintain psyllids at low levels. These systems can offer alternate prey, nectar sources and suitable microhabitats for sustaining the parasitoid and predator populations within and between seasons, especially during the off season of the main crop. Greater colonization and abundance of natural enemies in flowering trees and shrubs has been reported. In Malaysia, Menochilus sexmaculatus Fabr has been reported as the most common species of coccinellid predator feeding on aphids (Parker and Singh, 1973) and has a good potential for biological control. These predators can enter the existing natural enemy complex against D. citri in Sarawak requires further investigation.

CONCLUSION

In Sarawak, the D. citri populations can colonise and breed on the citrus trees throughout the year. Generation can overlap, but as trees generally have three major flushes a year, there are only three adult peaks, two to three egg peaks and three nymph peaks linked to flushing periods, in February-March, June-July and September-October. However, highest adult numbers occur in September-October. This is a critical point for preventing spread of populations within There is a time lag between the orchards. commencement of flushing and the upsurge of the D. citri population. Therefore, it is likely that the areas where D. citri does not maintain high populations, it requires sometime until the insect vector actually exhibits its high potential of population increase. Population fluctuations of D. citri breeding on citrus in this study were closely related to flushing rhythm because eggs are laid exclusively on new flush point and nymphs developed on immature leaves. The most favourable conditions for the egg hatch and build-up in the numbers of nymph and adult was the gradual rise in daily ambient temperatures. D. citri can adapt better under hot and dry conditions than rainy weather.

Psyllid population levels were positively related to the availability of new shoot flushes. Psyllid populations were adversely affected by weather conditions and parasitoids. Adult psyllid populations increased exponentially during periods of flush growth and migration and dispersal of the adults was also related to flushing cycles. Dispersal and colonisation of new trees was greatest in September-October, at the onset of the rainy season. The primary parasitoids namely T. radiata and D. aligarhensis affect D. citri population and are considered to be one of the major biological control agents of the psyllid in south- west Sarawak that can suppress psyllid populations. The rate of field parasitism of T. radiata and D. aligarhensis ranged from 4.6-22.3 and 20.6-35.3% respectively. Therefore, the use of primary parasitoids as a biological control agent should be further investigated. Since the rate of field parasitism was rather low, there may be useful to augment for mass-produce of both parasitoids for field release into a more favorable environmental habitats to increase their efficiencies. Predators can be important in reducing D. citri population, the role of coccinellids and other potential predators of D. citri in Sarawak should be evaluated.

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