

SURVIVAL OF *DIAPHORINA CITRI* (HOMOPTERA: PSYLLIDAE),  
AND ITS TWO PARASITIDS, *TAMARIXIA RADIATA*  
(HYMENOPTERA: EULOPHIDAE) AND *DIAPHORENCYRTUS*  
*ALIGARHENSIS* (HYMENOPTERA: ENCYRTIDAE), UNDER DIFFERENT  
RELATIVE HUMIDITIES AND TEMPERATURE REGIMES

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ABSTRACT

The ability of an exotic citrus pest, *Diaphorina citri* Kuwayama, and its two parasitoids, *Tamarixia radiata* (Waterston) and *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal), to survive under different relative humidities (7%, 33%, 53%, 75% and 97%) at 25 and 30°C was compared. The data obtained may help to predict potential climatic limitations to their establishment in Florida and indicate whether the pest has different climatic tolerances compared to these parasitoid populations. Adult survival was evaluated under relative humidities maintained by saturated salt solutions. *D. citri* survived longer than the parasitoid populations at all experimental conditions, suggesting it has a lower net water loss rate. The *T. radiata* (Taiwan) population showed the greatest moisture requirement at all temperatures and relative humidities tested. The *T. radiata* (Vietnam) population survived longer than the Taiwan, suggesting that the two populations may perform differently in different geographical regions. *D. aligarhensis* and *T. radiata* (Vietnam) survived similar lengths of time, except at the higher relative humidities, so the moisture requirements for these two populations are comparable.

Key Words: Asian citrus psylla, relative humidity tolerances, saturated salt solutions, biological control

RESUMEN

La habilidad de la plaga exótica del cítrico, *Diaphorina citri* Kuwayama, y sus dos parasitoides, *Tamarixia radiata* (Waterston) y *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) de sobrevivir bajo diferentes humedades relativas (7%, 33%, 53%, 75% y 97%) a 25 y 30°C fueron comparadas. Los datos obtenidos pueden ayudar a predecir las limitaciones potenciales climáticas a su establecimiento en la Florida e indican si la plaga tiene diferentes tolerancias climáticas en comparación a estas poblaciones parasitoides. La supervivencia de adultos fue evaluada bajo humedades relativas mantenidas por soluciones de sal saturada. *D. citri* sobrevivió mas tiempo que las poblaciones parasitoides bajo todas las condiciones experimentales, sugiriendo que tiene una velocidad mas baja de perdida de agua neta. La población *T. radiata* (Taiwán) demostró el mayor requerimiento de humedad en todas las temperaturas y humedades relativas probadas. La población *T. radiata* (Vietnam) sobrevivió mas que la de Taiwán, sugiriendo que las dos poblaciones pueden ejecutar de manera distinta en diferentes regiones geográficas. *D. aligarhensis* y *T. radiata* (Vietnam) sobrevivieron duraciones de tiempo similares, excepto bajo humedades relativas altas, indicando que los requerimientos de humedad para estas dos poblaciones son comparables.

The Asian citrus psylla, *Diaphorina citri* Kuwayama, was found for the first time in June 1998 by personnel in the Division of Plant Industry, Florida Department of Agriculture and Consumer Services (Hoy & Nguyen 1998; Halbert 1998; Mead 1977). It has successfully established in Florida and it will probably colonize all citrus-growing areas in Florida and may spread to Louisiana, Texas, Arizona and California citrus.

*D. citri* damages citrus by depleting sap from the plant and, because its saliva is toxic, growing shoots are distorted and may die. It also causes damage by excreting honeydew, which allows the growth of sooty mold (Chien & Chu 1996). How-

ever, the worst threat is that *D. citri* is an efficient vector of the bacterium, *Liberobacter asiaticum*, which causes greening disease. This is the most serious disease of citrus in the world, causing reduced production and eventual death of the trees (McClellan & Schwarz 1970).

No effective eradication efforts are known for *D. citri*, but classical biological control of the psyllid vector should contribute to suppression of their populations. Two parasitoids have been imported (Hoy et al. 1999; Hoy & Nguyen 2000). *Tamarixia radiata* (Waterston) is an ectoparasitoid imported from Taiwan and Vietnam, while *Diaphorencyrtus aligarhensis* (Shafee, Alam &

Agarwal) is an endoparasitoid of *D. citri* obtained from Taiwan. Approximately 12,000 *T. radiata* were released into Florida between 15 July and 1 December 1999. *D. aligarhensis* was approved for release on 15 March 2000 and 5000 were released during the 2000 growing season (Hoy, unpublished).

The success of classical biological control programs may be determined by the use of the appropriate natural enemy ecotypes. Moisture requirements may be especially important in determining suitability of an imported biological control agent for release and establishment in a new geographic area. Only a few experiments have been conducted to compare the water balance or the temperature-relative humidity relations in a pest and its parasitoids. Yoder & Hoy (1998) found the citrus leafminer and two populations of its endoparasitoid *Ageniaspis citricola* Logvinovskaya both require a moisture-rich environment although the citrus leafminer is less dependent on high relative humidities than its parasitoid, *A. citricola*.

The objective of this study is to compare, under laboratory conditions, the effect of temperature and relative humidity on the survival of an exotic citrus pest, *D. citri* and its two parasitoids *T. radiata* and *D. aligarhensis*. This information will help in mass rearing these parasitoids and may help to predict potential climatic limitations to their establishment in Florida.

## MATERIALS AND METHODS

### Rearing

*D. citri* was reared on *Murraya paniculata* (Chien *et al.* 1989), ornamental orange jasmine, in the quarantine facility at the University of Florida, Gainesville (Skelley & Hoy, unpublished). The plants were pruned, fertilized and held under a photoperiod of 16:8 (L:D) at 21 to 24°C to stimulate the growth of new flushes because adult psyllids lay eggs only on newly sprouted leaf buds. Trees that had aphid or scale infestations were sprayed with an application of oil or pyrethrum (Prentiss, Inc., Sandersville, GA).

*D. citri* was reared in quarantine under a photoperiod of 18:6 (L:D) at 26°C and 60% relative humidity (RH). *T. radiata* colonies from Taiwan and Vietnam and *D. aligarhensis* from Taiwan were reared in separate cages and rooms in quarantine to reduce the risk of contamination. Both parasitoid species were reared at 27°C and 60% RH under a photoperiod of 18:6 (L:D) in cages (76 cm × 46 cm × 66 cm) constructed with 1.27 cm diameter polyvinyl chloride pipe and covered with a fine nylon mesh.

### Specimen Collection

Adults used for experiments were collected at 5 PM on the day of their emergence. To ensure that all four populations emerged on the same

day, the psyllids, *T. radiata* and *D. aligarhensis* were set up 15 days, 12 days and 18 days, respectively, before the start of the experiments. The day before starting an experiment, all old psyllids and parasitoids were collected to ensure that only new individuals would be present in the cages. *D. citri* is collected by aspirating adults off the plant, while *T. radiata* can be collected off the top of the mesh cage. *D. aligarhensis* is aspirated most often from the bottom of the cage. Individual adults were placed in groups of five into 28.35 g condiment cups (Plastics, Inc. St. Paul, MN). The lid of the cup was covered with mesh glued to it with clear nail polish. All containers were cleaned with 70% EtOH before being reused.

### Pretreatment Conditions

After collection, all individuals were pretreated for 21 hours by placing them into bell jars placed in an incubator (Percival I-30B) programmed with a photoperiod of 18:6 (L:D) at 17°C. RH within the bell jars was maintained by a saturated salt solution, Mg(NO<sub>3</sub>)<sub>2</sub> · 6H<sub>2</sub>O, which yields a 56% RH (Winston & Bates 1960; O'Brien 1948; Carr & Harris 1949). Specimens were given no water or honey. Pretreatment was expected to remove adsorbed water from the cuticular surface and to ensure a uniform physiological state. Pretreatment minimizes the effects of ingestion, reproduction, excretion and defecation (Arlian & Ekstrand 1975). The survival rate should reflect the relative changes in water balance of the insects before desiccation (Wharton 1985).

### Experimental Conditions

At 2 PM specimens were transferred into stainless steel chambers (30.5 cm × 30.5 cm × 30.5 cm) (Fisher Scientific, Pittsburgh, PA) held within a single incubator (Percival I-35 series) at either 25 or 30°C with a photoperiod of 18:6 (L:D). Plastic cups containing five adults were placed into a chamber with either 7, 33, 53, 75 or 97% RH maintained by saturated salt solutions. Reagent grade salts (Sigma, St. Louis, MO) dissolved in distilled water were used: 200 ml of saturated salt solution was placed into chambers with the three higher RH, and 100 ml of salt solution was used in the two lower RH because they are hygroscopic substances and should be formulated as a paste to maintain the desired RH. These salts were chosen because they maintain the same RH at 25 and 30°C (Winston & Bates 1960). The chemicals used were sodium hydroxide (7% RH), magnesium chloride hexahydrate (33% RH), magnesium nitrate hexahydrate (53% RH), sodium chloride (75% RH) and potassium sulfate (97% RH). RH in each chamber was confirmed by a hygrometer (Thomas Scientific, Swedesboro, NJ), as well as the salt crystal method outlined by Winston & Bates (1960), which utilizes the fact

that a salt crystal will pick up moisture and deliquesce in relative humidities which are at or above that maintained by its saturated solution. A very small crystal is introduced into the closed chamber and observed under magnification; if the RH is below that for the salt, there will be no change. If the RH is very close, the deliquescence will be only partial; if just above there will be a narrow rim of liquid around the mass. Experimental units were placed randomly into each RH chamber.

#### Survivability Tests

Survival of individuals was recorded every three hours. RH chambers were sampled separately and specimens were out of the chamber for a maximum of ten minutes. Insects found on their dorsal surface that failed to respond to probing were considered dead. All dead specimens were sexed.

#### Statistics

Four replicates were tested at 25°C under each RH on separate dates, while three replicates were run at 30°C and each RH condition. At 25°C, a total of 400 individuals (100 per experiment) were tested for *D. citri* and the two *T. radiata* populations. Only 300 individuals (75 per experiment) of the *D. aligarhensis* population were tested at 25°C. At 30°C, a total of 300 individuals (100 per experiment) were tested for all four populations. Mean percentage survival ( $\pm$ SEM) was calculated for all four populations at each temperature-RH combination.  $LT_{50}$  values were calculated by adding the time it took to get 50% mortality under each condition on each date. A grand mean and SEM was determined for each population at each temperature-RH combination.  $LT_{50}$  values were compared statistically by z test statistics.

### RESULTS AND DISCUSSION

The survival of *D. citri*, *D. aligarhensis* and *T. radiata* populations increased with increasing RH (Figs. 1, 2). When the temperature was increased from 25 to 30°C, adult survival of all populations decreased, as expected (Figs. 1, 2). At 75 and 97% RH at both temperatures, *D. citri* survived longer than the parasitoid populations, suggesting it has a lower net water loss rate (Figs. 1D, 1E, 2D, 2E).

The *T. radiata* (Taiwan) population showed the greatest moisture requirement at all temperatures and RH tested. The *T. radiata* (Vietnam) population survived longer than the Taiwan population at 25°C and 53, 75 and 97% RH as well as all RH tested at 30°C (Figs. 1C, 1D, 1E, 2). This suggests that the two populations may perform differently in different geographical regions.

*D. aligarhensis* and *T. radiata* (Vietnam) survived similar lengths of time under all RH at 25 and 30°C, so the moisture requirements for these two populations appear comparable (Figs. 1, 2).

$LT_{10}$  and  $LT_{90}$  values can be found easily from the figures, but the differences are small and not many inferences about the populations can be made at the ends of the curves. The  $LT_{50}$  values of the pest *D. citri* were always higher than the  $LT_{50}$  values of its parasitoid populations at 25°C (Fig. 1).

$LT_{50}$  values at 25°C for *D. aligarhensis* are different from  $LT_{50}$  values for the *T. radiata* (Taiwan) population ( $P < 0.05$ ) (Table 1). *D. aligarhensis* and *T. radiata* (Vietnam) had different  $LT_{50}$  values, except at 53 and 97% RH ( $z = 0.10, P > 0.05$ ;  $z = 0.75, P > 0.05$ ) (Table 1). The  $LT_{50}$  values of the two *T. radiata* populations are not different at 7 and 33% RH ( $z = 1.55, P > 0.05$ ;  $z = 0.10, P > 0.05$ ) (Table 1).

All parasitoids held at 25°C were dead by 40.5 hours, whereas a few psyllids survived up to 94.5 hours (Fig. 1). There were no differences between survival of males and females of the *D. citri* and *T. radiata* populations, perhaps because there is no apparent difference in size. The *D. aligarhensis* population is made up of females only.

The  $LT_{50}$  values of the pest were equal to or greater than the  $LT_{50}$  values of its parasitoids at 30°C (Table 1). As the RH increased to 97%, the  $LT_{50}$  of the *D. citri* population was different from that of *D. aligarhensis* and the *T. radiata* (Vietnam) population ( $P < 0.05$ ) (Table 1). The  $LT_{50}$  values of the *T. radiata* (Taiwan) population were different ( $P < 0.05$ ) from the *D. citri*, *D. aligarhensis* and *T. radiata* (Vietnam) populations at all RH tested (Table 1). Again, there were no differences in survival rates of males and females. All parasitoids held at 30°C were dead by approximately 40.5 hours, but a few psyllids managed to survive up to 52.5 hours (Fig. 2).

The data suggest that, especially at 75 and 97% RH at 25°C and 97% RH at 30°C, some *D. citri* will have up to two times longer to find food and water in the field compared to its parasitoids (Figs. 1D, 1E, 2E). *D. citri* also survived well at the lower RH of 7 and 33%, suggesting that it could disperse and survive in the more arid conditions found in western citrus-growing regions in the United States (Figs. 1A, 1B, 2A, 2B).

The RH responses of the Vietnam population of *T. radiata* were different from those of the Taiwan population, especially at 30°C, suggesting that the two populations of *T. radiata* may be ecotypes (populations varying in their ability to survive in different climates).

This information is helpful in rearing these parasitoids for release in biological control programs because it indicates there are no large differences in pest and natural enemy responses to the RH and temperatures likely to be encountered in the rearing facility. The ability of *D. citri*

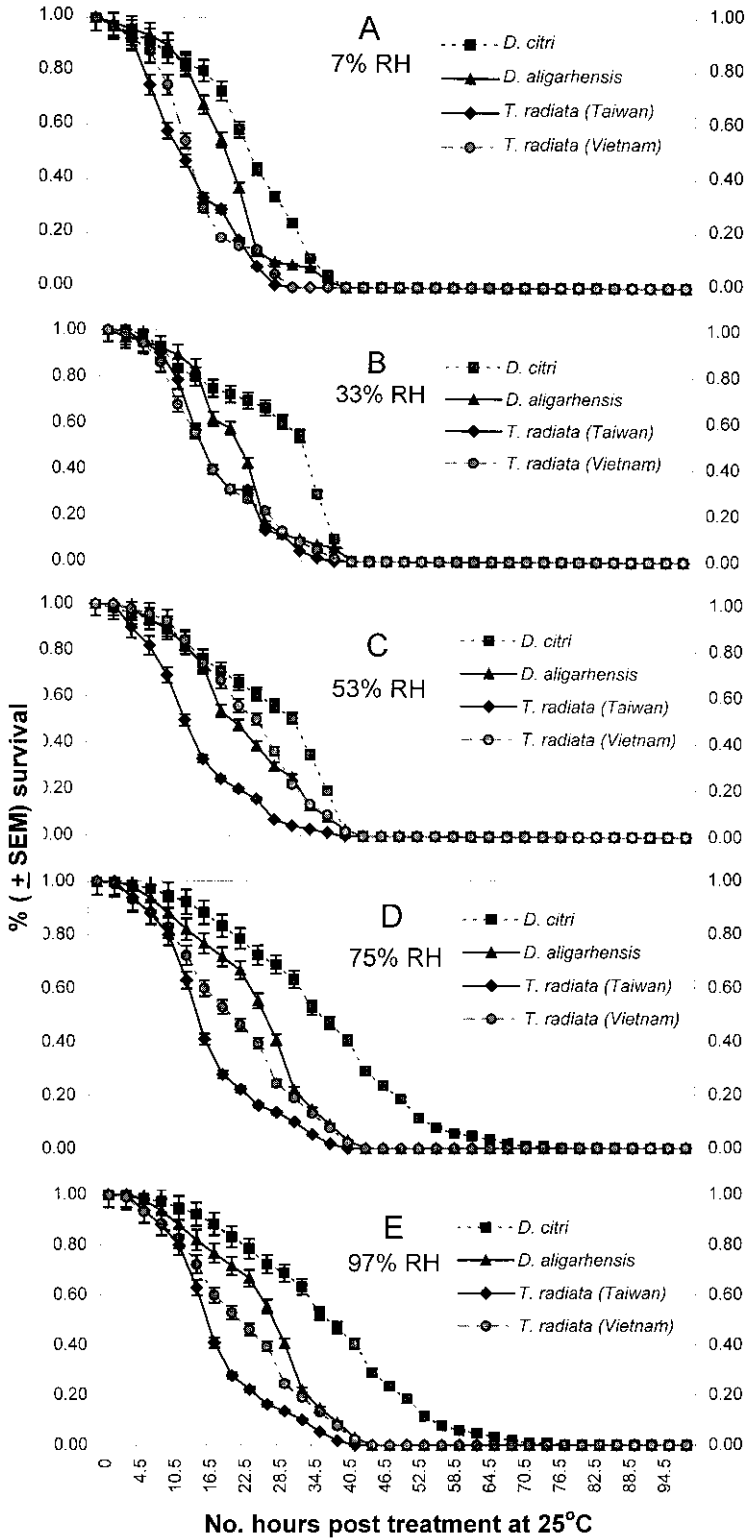


Fig. 1. Survival curves of the Asian citrus psylla, *Diaphorina citri*, and its two parasitoids, *Diaphorencyrtus aligarhensis* imported from Taiwan and *Tamarixia radiata* imported from Taiwan and Vietnam, at 25°C and 7% RH (A), 33% RH (B), 53% RH (C), 75% RH (D), and 97% RH (E).

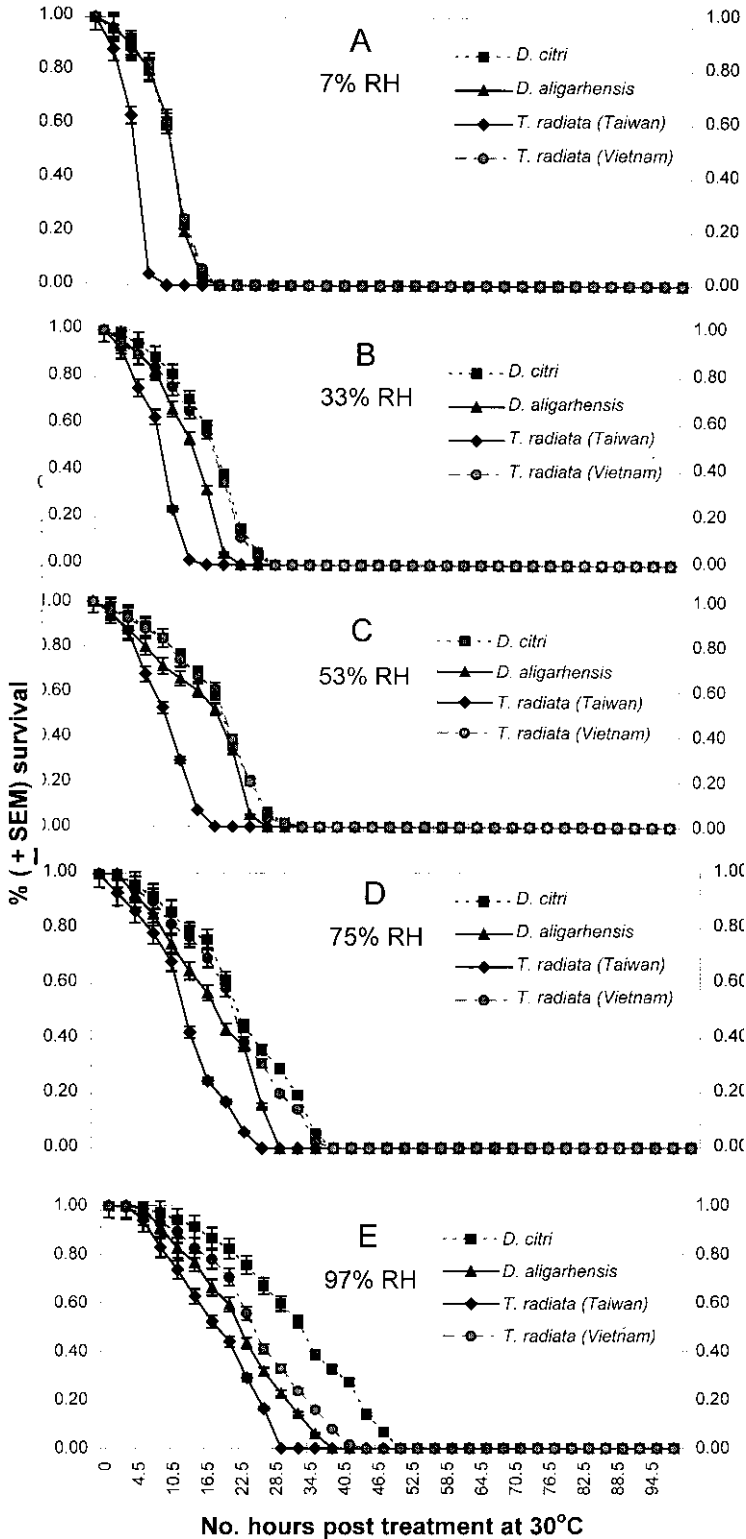


Fig. 2. Survival curves of the Asian citrus psylla, *Diaphorina citri*, and its two parasitoids, *Diaphorencyrtus aligarhensis* imported from Taiwan and *Tamarixia radiata* imported from Taiwan and Vietnam, at 30°C and 7% RH (A), 33% RH (B), 53% RH (C), 75% RH (D), and 97% RH (E).

TABLE 1. SURVIVAL OF *D. CITRI* AND ITS PARASITOIDS *T. RADIATA* AND *D. ALIGARHENSIS* AT 25 AND 30°C AND FIVE RELATIVE HUMIDITIES.

Species	LT <sub>50</sub> values (SEM) in hours at relative humidity (%) of				
	7	33	53	75	97
----- at 25°C -----					
<i>D. citri</i>	24.9 (2.4)	28.3 (2.0)	28.5 (2.4)	37.0 (2.8)	43.2 (3.2)
<i>D. aligarhensis</i>	21.3 (1.8)	21.8 (1.6)	24.2 (1.5)	26.1 (1.8)	25.5 (2.1)
<i>T. radiata</i> Taiwan	15.3 (1.0)	18.1 (1.3)	16.5 (1.0)	18.4 (2.5)	22.5 (1.4)
<i>T. radiata</i> Vietnam	16.2 (1.2)	18.1 (1.0)	25.6 (1.9)	22.4 (1.8)	26.1 (1.5)
----- at 30°C -----					
<i>D. citri</i>	12.1 (1.1)	18.0 (1.4)	20.4 (1.8)	23.2 (1.5)	32.2 (2.5)
<i>D. aligarhensis</i>	12.1 (0.8)	14.3 (1.1)	18.0 (1.2)	18.6 (1.2)	22.3 (1.8)
<i>T. radiata</i> Taiwan	6.2 (0.4)	9.2 (0.8)	11.7 (0.9)	14.0 (0.9)	18.2 (1.1)
<i>T. radiata</i> Vietnam	12.3 (0.8)	17.0 (1.5)	23.3 (2.1)	21.8 (1.6)	25.2 (1.7)

to survive longer at 97% RH than its parasitoids is interesting and difficult to interpret (Figs. 1E, 2E). It could mean that *D. citri* is better adapted to very high RH than its natural enemies. The data obtained compare the intrinsic water loss rates of these four populations under extreme conditions of no food or water, and the ability of *D. citri*, *D. aligarhensis* and *T. radiata* populations to disperse to more optimal sites was precluded. In citrus groves *D. citri* can feed on host plants to obtain water and food. Likewise, the parasitoids can host feed for moisture and nutrients, feed on honeydew, drink droplets of water and disperse to find microhabitats more suitable for survival.

Yoder & Hoy (1998) found two populations of *A. citricola* and their host, the citrus leafminer, both require a moisture-rich environment although the citrus leafminer is less dependent on high RH than its parasitoids. The results of this study were similar, with *D. citri* and its parasitoids requiring a moisture-rich environment, although *D. citri* does not depend on high RH as much as its parasitoids, especially the *T. radiata* (Taiwan) population. *D. citri* survived longer at higher temperatures and lower RH than the *T. radiata* (Taiwan) and the *D. aligarhensis* populations (Figs. 2A, 2B).

Hoy et al. (2000) found slight physiological differences in the Taiwan and Australian ecotypes of *A. citricola*, which indicated genetic differences exist. Subsequent analysis of Random-Amplified-Polymorphic-DNA Polymerase-Chain-Reaction

(RAPD-PCR) and DNA sequence analyses of two *Actin* genes led to the conclusion that the two populations actually are cryptic species, even though these two populations of *A. citricola* can not be separated on the basis of morphological characteristics (Hoy et al. 2000). Additional analysis of the two populations of *T. radiata* could establish whether they are cryptic species.

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