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## MORTALITY OF THE LOBATE LAC SCALE, *PARATACHARDINA PSEUDOLOBATA* (HEMIPTERA: KERRIIDAE), AT NEAR OR BELOW FREEZING TEMPERATURES

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### ABSTRACT

The ability of the invasive lobate lac scale, *Paratachardina pseudolobata* Kondo & Gullan, to survive exposure to near or below freezing temperatures (-25, -15, -10, -5, 0, 5, and 10°C) for extended durations (6, 12, 24, 48, and 72 h) was assessed in the laboratory. Exposure at 10°C for 6 to 72 h did not cause significant mortality in immature and adult lobate lac scales. All nymphs and adults were killed after exposure to very low temperatures (-25 to -10°C) for 6 h. All nymphs were killed within 24 h at -5, 0, and 5 °C, while about 2% of adults were still alive after 72 h at 0 and 5°C. Results suggested that the range expansion of the lobate lac scale in the United States will be limited by winter temperature. The lobate lac scale may have a higher potential to invade areas where the average winter temperature do not drop below freezing for an extended duration (3 or more consecutive days), such as southern Florida, Rio Grande Valley in Texas, and Colorado river Delta between California and Arizona.

Key Words: lobate lac scale, invasive organism, temperature tolerance, low temperature sensitivity, *Paratachardina pseudolobata*

### RESUMEN

La capacidad de la escama lobulada de laca, *Paratachardina pseudolobata* Kondo & Gullan, una especie invasora, para sobrevivir la exposición a temperaturas cerca o bajo la temperatura de congelación (-25, -15, -10, -5, 0, 5 y 10°C) por periodos extendidos (6, 12, 24, 48 y 72 horas) fue evaluada en el laboratorio. La exposición a 10°C por 6 a 72 horas no causo mortalidad significativa en inmaduros o adultos de la escama lobulada de laca. Todas las ninfas y los adultos fueron matados después de exponerlos a temperaturas muy bajas (-25 a -10°C) por 6 horas. Todas las ninfas fueron matadas entre las 24 horas a los -5, 0 y 5°C, mientras que aproximadamente 2% de los adultos todavía estuvieron vivos después de 72 horas los 0 y 5°C. Los resultados sugirieron que la expansión de distribución geográfica de la escama lobulada de laca en los Estados Unidos será limitada por la temperatura de invierno. La escama lobulada de laca puede tener un potencial mas alto para invadir áreas donde el promedio de la temperatura de invierno no cae bajo la temperatura de congelación por un periodo extendido (3 o mas días consecutivos), como en el sur de la Florida, el valle del Río Grande en Texas y el delta del río Colorado entre California y Arizona.

The lobate lac scale, *Paratachardina pseudolobata* Kondo & Gullan, is an invasive species that poses significant threats to the native vegetation and horticultural crops of Florida (Pemberton 2003a, b). Lobate lac scale was first detected in Broward County in 1999, and subsequently in Miami-Dade County in 2000 (Hamon 2001). Its host list includes 160 plant species in 49 families (Pemberton 2003a). Some of the most severely impacted species, such as wax myrtle (*Myrica cerifera* L.) and cocoplum (*Chrysobalanus icaco* L.), are important both as popular ornamental plants and as essential components of natural ecosystems and wildlife habitats (Pemberton 2003a, b). The lobate

lac scale readily infests some popular fruit tree species including mango (*Mangifera indica* L.) and avocado (*Persea americana* Miller) in southern Florida (Howard et al. 2004). The invasion of the lobate lac scale into the subtropical regions of Texas, California, Hawaii, the West Indies, and Mexico (Pemberton 2003a) will undoubtedly affect local crop production, the urban landscape, and natural area and wildlife managements.

Life history information, such as developmental threshold and cold tolerance, are essential to predicting the potential distribution and to determining the regulatory and management responses to the lobate lac scale. The scale insect developed

through 2 nymphal instars in about 20 weeks in the laboratory and the mortality was the highest during the first instar (Chong, unpublished data). The lack of life history and cold hardiness information for the lobate lac scale hinders the ability to predict its potential distribution. This study was conducted to assess the mortality of the lobate lac scale after exposure for specific durations (6 to 72 h) at near or below freezing temperatures ( $-25$  to  $10^{\circ}\text{C}$ ). The results of this study will be useful in predicting the climatic limitations to the establishment and spread of the lobate lac scale in Florida, the United States, and the Caribbean.

## MATERIALS AND METHODS

The mortality of lobate lac scales was determined in the laboratory at constant temperatures of  $-25$ ,  $-15$ ,  $-10$ ,  $-5$ ,  $0$ ,  $5$ , and  $10 \pm 1^{\circ}\text{C}$ . The temperature treatments of  $-5$  to  $0^{\circ}\text{C}$  were conducted in environmental chambers (Environmental Growth Chambers, Chagrin Falls, OH) and the  $-25$  to  $-10^{\circ}\text{C}$  treatments were conducted in programmable ultra-low temperature freezers (New Brunswick Scientific Co., Edison, NJ) at USDA-ARS Subtropical Horticulture Research Station (SHRS), Miami, Florida. The relative humidity within the ultra-low freezers (averaged 87.7%) was lower than those in the environmental chamber (averaged 91.8 to 97.6%).

All lobate lac scales used in this study were collected from a single cocoplum (*Chrysobalanus icaco* L.) shrub located in Kendall, Miami-Dade County, Florida. The samples were taken in Jan 2006 to allow the acclimatization of the scale insects to the lower ambient temperature. Because the highest numbers of live scale insects were found on the distal terminals (Chong, personal observations), only the first 30-cm section (from the tip) of the branch was collected. The branch was stripped of all leaves and cut into 10-cm samples. Each sample branch bore 8-36 lobate lac scale nymphs or adults. All the samples were combined and randomly assigned to the 7 constant temperature treatments. Ten 10-cm samples were assigned to each of the 42 combinations of temperature ( $-25$  to  $10^{\circ}\text{C}$ ) and duration (unexposed, and exposed for 6, 12, 24, 48, and 72 h). The entire experiment with all the temperature/duration combinations (each with 10 samples) was repeated 3 times. At each repetition, new samples were collected and the temperature treatments were alternated among the environmental chambers or ultra-low freezers.

The samples were sealed in plastic bags and kept at the prescribed temperatures for the predetermined durations. After the samples were taken out of the environmental chambers or ultra-low freezers, the samples were immediately inserted into individual glass vials half-filled with tap water. The numbers of dead and live nymphs and adults were determined after maintaining

the samples in the glass vials for 3 d at  $21^{\circ}\text{C}$  and  $\sim 70\%$  R.H. A preliminary study showed that live cocoplum terminals and scale insects could be maintained in tap water for more than 3 d, but the dead scale insects desiccated within 3 d after the exposure (Chong, unpublished data). Dead scale insects are dry and brittle, making them easily distinguishable from the live scales which exude red fluid after being crushed.

Viability of lobate lac scales before and after the low temperature exposure was determined by crushing each individual. Live scale insects exuded red body fluid while dead individuals were empty and the exoskeleton was brittle and easily crushed. This type of destructive evaluation method precluded the determination of the scale insects' viability before low temperature treatment. Therefore, samples assigned to the unexposed treatment were used to estimate the viability of the scale insects before low temperature exposure. The proportion of live lobate lac scales on each sample assigned to the unexposed treatment was determined by dividing the number of live scales by the total number of scale insects (dead or alive) found on the sample. The proportion of live adult or immature scales was estimated separately. The average proportion of live (adult or immature) scales was then calculated for each temperature treatment. We assumed that all samples assigned to a particular temperature treatment had the same average proportion of live scale insects before exposure. Based on this assumption, the estimated number of live scale insects on each sample was the product of the average proportion of live scales and the total number of scale insects found on the sample. The numbers of live adult and immature scales before exposure were estimated separately. The mortality rates of lobate lac scale nymphs and adults were estimated separately as the difference between the estimated number of live scales before exposure and the recorded number of live scale after exposure divided by the estimated number of live scales before exposure.

## Statistical Analyses

A preliminary statistical analysis suggested that the estimated mortality rates from the 3 repetitions of this experiment were not significantly different ( $F = 0.24$ ,  $df = 2$ ,  $207$ ,  $P = 0.6869$ ) (ANOVA; SAS Institute 1999). Therefore, the data from the 3 repetitions were combined, resulting in 30 replicates (or samples) for each temperature/duration combination. The estimated mortality rates were arcsine-transformed to normalize the variance before they were analyzed with a two-way factorial analysis of variance at  $\alpha = 0.05$  (SAS Institute 1999). When significant effect of temperature or exposure duration was detected, a Tukey's test was performed to separate the means (SAS Institute 1999).

RESULTS

Exposure temperature and duration, as well as their interaction, had significant effects on the estimated mortality rate of immature lobate lac scales (temperature:  $F = 17.75$ ,  $df = 6$ , 1008,

$P < 0.0001$ ; duration:  $F = 115.95$ ,  $df = 5$ , 1008,  $P < 0.0001$ ; temperature x duration:  $F = 2.95$ ,  $df = 30$ , 1008,  $P < 0.0001$ ) (Fig. 1). The nymphs exposed to 10°C had the lowest estimated mortality rate among all the temperature treatments. Exposure duration at 10°C did not have a signif-

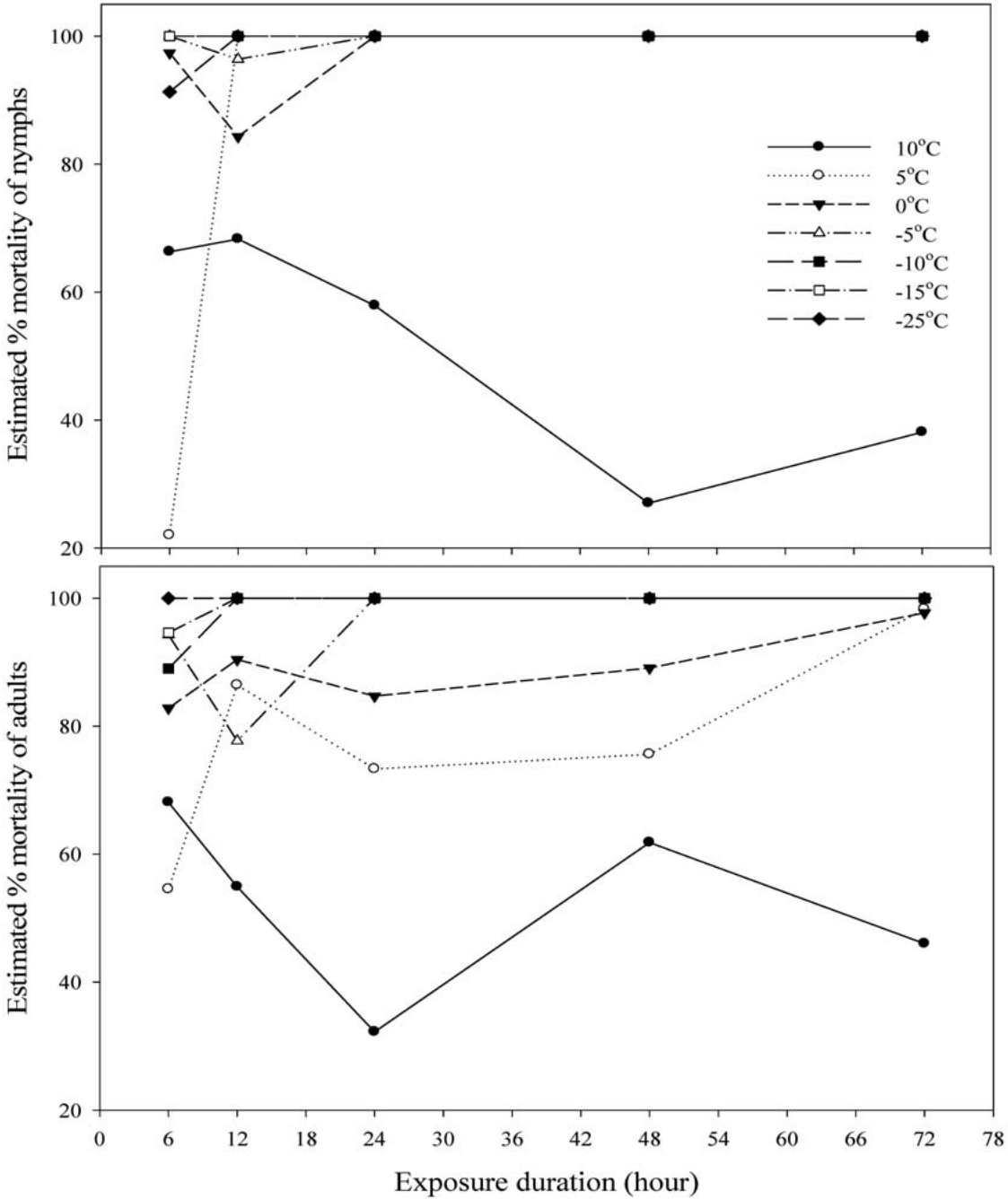


Fig. 1. Estimated mortality rates of immature and adult lobate lac scales after exposing to near or below freezing temperatures (-25 to 10°C) for 6 to 72 h.

icant influence on the estimated nymphal mortality rates, with 68% dying after 12 h and 27% dying after 48 h of exposure. All nymphs were killed after 6 h of exposure at the lowest temperatures (-25 to -10°C). Between -5 and 5°C, the mortality rate of nymphs increased as the exposure duration increased. All nymphs were killed when held for 12 h at 5°C and 24 h at 0 and -5°C.

Temperature, exposure duration, and their interaction significantly affected the survival of adult lobate lac scales (temperature:  $F = 14.69$ ,  $df = 6$ , 1158,  $P < 0.0001$ ; duration:  $F = 101.76$ ,  $df = 5$ , 1158,  $P < 0.0001$ ; temperature  $\times$  duration:  $F = 1.53$ ,  $df = 20$ , 1158,  $P = 0.0353$ ) (Fig. 1). All adults were killed after they were exposed for 6 h at -25°C, 12 h at -10 and -15°C, and 24 h at -5°C. About 2% of the adults were still alive at 0 and 5°C after 72 h. Adult lobate lac scales exposed to 10°C had the lowest estimated mortality rate compared to other temperature treatments. The estimated mortality rates of adults at 10°C were not affected by the exposure duration. The lowest estimated mortality rate at 10°C was 32% after 24 h and the highest was 68% after 6 h.

## DISCUSSION

The results of this study suggested that the range expansion of the lobate lac scale will be limited by winter temperature. Low winter temperature can significantly reduce the size of an established population or prevent the establishment of a new population. Areas in the United States that experience more than 3 consecutive days of near or below freezing average temperature may not be suitable for the establishment of the lobate lac scale. South-central Florida (around and south of Lake Okeechobee), southern regions of the Rio Grande Valley in Texas, and the Colorado River Delta in Arizona and California, and most of the Caribbean may be susceptible to the establishment of lobate lac scales (Vischer 1944; FAWN 2008; NOAA-NCDC 2008). A comparison of the scale life history and cold-hardiness data to the current distribution of the scale insect and the suitable host plants would most accurately predict where the lobate lac scale could establish.

Adult lobate lac scales were more cold-tolerant than the nymphs at 0 and 5°C. However, both adult and immature lobate lac scales were equally susceptible to below freezing temperatures (-25 to -5°C). A higher cold tolerance of adults or older instars is also recorded in western flower thrips *Frankliniella occidentalis* (Pergrande) (Thysanoptera: Thripidae) (McDonald et al. 1997), Mexican fruit fly *Anastrepha ludens* (Loew) (Diptera: Tephritidae) (Hallman 1999), and lesser appleworm *Grapholita prunivora* (Walsh) (Lepidoptera: Tortricidae) (Neven 2004). A cold-tolerant adult stage could potentially protect the eggs within the adult lobate lac scales

and allow the scale insect population to persist in an infested area.

The cold hardiness of insect populations differs among different geographical locations. Skinner et al. (2003) showed that the hemlock woolly adelgids (*Adelges tsugae* Annand) (Hemiptera: Adelgidae) collected from the plant hardiness zone 5a survived a lower temperature for a longer duration than those from the warmer plant hardiness zone 6. Insects that survived the winter were more adapted or acclimatized to the cold winter temperature. The cold hardiness may allow these individuals and their offspring to expand their distribution range northward. The ability and probability of the lobate lac scale to adapt to colder temperature and expand their range is unknown. Individuals surviving on the northern edge of their current distribution may gradually increase their cold tolerance and expand their range. A long-term monitoring program would verify if such adaptation or acclimatization is occurring in the northern populations.

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