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Impact of different temperatures on survival and energy metabolism in the Asian citrus psyllid, *Diaphorina citri* Kuwayama

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ABSTRACT

Temperature influences the life history and metabolic parameters of insects. Asian citrus psyllid (ACP), *Diaphorina citri* is a tropical and subtropical pest. ACP invaded new regions around the world and threatened the citrus industry as a vector for Huanglongbing (HLB) disease. ACP is widely distributed and can survive high (up to 45 °C) and low temperatures (as low as −6 °C). The precise mechanism of temperature tolerance in ACP is poorly understood. We investigated adult survival, cellular energy balance, gene expression, and nucleotide and sugar-nucleotide changes under the effect of different temperature regimes (0 °C to 45 °C with 5 °C intervals). The optimum temperatures for survival were 20 and 25 °C. Low temperatures of 0 °C and 5 °C caused 50% mortality after 2 and 4 days respectively, while one day at high temperature (40 °C and 45 °C) caused more than 95% mortality. The lowest quantity of ATP (3.69 ± 1.6 ng/insect) and the maximum ATPase enzyme activities (57.43 ± 7.6 μU/insect) were observed at 25 °C. Correlation between ATP quantities and ATPase activity was negative. Gene expression of hsp 70, V-type proton ATPase catalytic subunit A and ATP synthase α subunit matched these results. Twenty-four nucleotides and sugar-nucleotides were quantified using HPLC in ACP adults maintained at low, high, and optimum temperatures. The nucleotide profiles were different among treatments. The ratios between AMP:ATP and ADP:ATP were significantly decreased and positively correlated to adults survival, whereas the adenylate energy charge was increased in response to low and high temperatures. Exploring energy metabolic regulation in relation with adult survival might help in understanding the physiological basis of how ACP tolerates newly invaded regions.

1. Introduction

Citrus is one of the most economically important horticulture crops in the world. Citrus grows within a wide strip of about ±40° latitude of the equator (Gottwald, 2010). Citrus trees are attacked by wide varieties of pests and pathogens such as mites, insects, nematodes, bacteria, viruses, and viroids that can severely affect the productivity of citrus trees (Tirtawidjaja et al., 1965). Recently, the citrus industry is severely declining in many countries as a result of citrus greening disease, called also Huanglongbing (HLB) (Gottwald, 2010). HLB is caused by a phloem-limited bacterium (Jagoueix et al., 1994). Three species of the bacterium, *Candidatus* Liberibacter asiaticus (CLas), africanus (CLaf), and americanus (CLam) (Sagaram et al., 2009) have been identified as the causal agents for HLB. Both CLas and CLam are transmitted by the Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae) and located in Asia and Americas (Halbert and Manjunath, 2004), while CLaf is transmitted by the African psyllid, *Trioza erytreae* (Del Guercio) (Triozidae) in African countries (Aubert, 1987; Halbert and Manjunath, 2004; Gottwald, 2010).

Asian citrus psyllid (ACP) is a phloem sap-sucking insect that belongs to superfamily Psylloidea and family Psyllidae. Its direct damage occurs by nymphs and adults feeding on the phloem sap, while the indirect effects arise from accumulation of honeydew which coat the leaves encouraging sooty mold to grow. However the most serious threat to citrus worldwide comes from its ability to transmit CLas bacterium (Garnier et al., 2000; Bové, 2006). Therefore, the spread of HLB mainly depends on the distribution and population size of ACP (Halbert and Manjunath, 2004).

Temperature has a great impact on the development of ACP populations (Aubert, 1987). ACP is native and widely distributed in southern Asia (Grafton-Cardwell et al., 2006). The discovery of ACP in many other tropical and subtropical regions such as Asian countries, the Indian subcontinent, African mainland, Near and Middle East, Arabian Peninsula, North and South America has been reported (Gottwald et al., 2007). In the USA, ACP was first found in Palm Beach County, Florida, in June 1998 and by 2001, it had spread to 31 counties in Florida (Halbert et al., 2002). Since then, ACP has invaded many states included Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, South Carolina and Texas (Mead and Fasulo, 2010). Although some of these states have freezing events during the winter, cold stress did not significantly constraint ACP invasion. The ability of

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ACP to establish in new regions could be explained by its temperature tolerance especially in low temperature environment. Additionally, global climate changes may help the tropical and subtropical insects to colonize temperate regions.

In temperate regions, for overwintering, insects tolerate cold via physiological and biochemical changes (Lee, 1989). Field observations and controlled studies showed that ACP adults can survive sub-zero (as low as −6 °C) temperatures for several hours (Ashihara, 2004; Hall et al., 2011; Hall and Hentz, 2014). However, little is known about how ACP adults survive freezing or low temperature events (Hall et al, 2011). On the other hand, severe freeze is lethal to ACP and can prevent its spread to northern area (Hall et al., 2011) The rapid cold hardening response may function to allow insects to enhance cold-tolerance in response to unexpected seasonal decreases in environmental temperature (Czajka and Lee, 1990).

The mechanism of heat and cold tolerance in ACP is poorly understood. Studying the effects of different temperature treatments on the ACP survival, energy profile, ATPase activity, and the gene expression of genes implicated in energy metabolism and insect response to temperature stress may lead to the development of genetic tools and innovative strategies for ACP population management. Understanding the mechanism by which ACP tolerates temperature changes will help in controlling this vector and subsequently limit the spread of the HLB disease.

The exact cause of insect mortality following a heat treatment is not clear yet (Neven, 2000). Whether the death can be caused by a single event (i.e. breakdown of the mitochondria, disruption of cellular membranes, denaturation of proteins and: or nucleic acids), or as a sequence of the overall events remains to be tested (Neven, 2000). It is also not clear whether the drop in the heat rate at temperature above 40 °C is a protective mechanism (energy conservation) or inability to maintain enough ATP supply (Neven, 2000). A recent study on the effect of heat on (ACP) showed that adults ACP cannot survive at 50 °C for more than few minutes (Hall and Hentz, 2014). ACP adapted to long-term exposure to temperature higher than 27 °C was more heat-tolerant than those exposed to cooler temperatures (Hall and Hentz, 2014). ACP adults exposed to 42 °C increased their transcriptional activity of their heat-shock gene, hsp 70 (Marutani-Hert et al., 2010). Marutani-Hert et al. (2010) indicated that hsp 70 may play a role in response of ACP to heat stress. The hsps are a family of chaperones proteins that are induced in cells after exposure to stressful conditions including heat and toxins (Feder and Hofmann, 1999). The hsps recognize and bind to other non-native (denatured, not fully synthesized, folded, assembled, or secreted in incorrect place) proteins to minimize inappropriate interactions between them (Feder and Hofmann, 1999).

On the other hand, low temperature is one of the significant challenges facing insects in cold regions (Teets and Denlinger, 2013). Low temperature may result in chilling injury in insects (Dollo et al., 2010). Although the exact mechanism behind chilling injury is not clear yet, it is believed that chilling injury results from membrane damage caused by phase transition (Teets and Denlinger, 2013). Chilling injuries may also reduce the rates of protein synthesis, increases production of free radicals, and disrupts ion homeostasis and membrane potential (Dollo et al., 2010). In addition, chilling injury may cause neuromuscular injuries and excessive thermoelastic stress (Dollo et al., 2010). Dollo et al. (2010) hypothesized that indirect chilling injury was linked to a shortage in ATP and suggested that insect exposure alternating pulses of high temperature allows it to regenerate ATP by the activation of ATP synthesis. Colinet (2011) rejected the above assumption because cold did not result in ATP depletion in tested insects. Colinet (2011) also suggested that ATP accumulation under cold stress might result from production/consumption imbalance.

Exploring energy metabolic regulation and expressions of energy-related genes of ACP adults exposed to different temperature regimes, might reveal the mechanism by which ACP adults survive in hot and cold weather. In addition, nucleotide pathways could be potential targets for RNA interference, which might reduce adult survival and temperature tolerance (e.g. AMPK RNAi reduced *Drosophila* lifespan, Stenesen et al., 2013). In the current study, we hypothesized that the effect of temperature on ACP survival is due to an alteration in energy metabolism. In addition, although high and low temperatures decreased ACP survival, we hypothesize that the mechanisms of energy metabolism change are different. To examine this hypothesis, we studied the effect of different temperature on the ACP survival, energetic nucleotide profile, ATPase activity, and gene expression of ATPase, ATP synthase, and nucleotide diphosphate kinase (NDPK). The data presented in the current study may lead to development of genetic tools and innovative strategies for ACP management, which could consequently limit the spread of the HLB disease.

2. Materials and methods

2.1. Insect colonies

Asian citrus psyllid (ACP) adult colonies were maintained in cages on 'Valencia' sweet orange trees in *C*Las-free controlled growth rooms set at 25 ± 2 °C temperature, 60 ± 5% RH, and a 16:8 (L:D) photoperiod (Skelley and Hoy, 2004). Originally insects were collected in 2000 from citrus groves in Polk City, Florida.

2.2. Survival assay under different temperature degrees

The survivals of ACP adults were examined at ten temperatures starting from 0 °C to 45 °C with 5 °C intervals. Temperature-controlled incubators were used. For each treatment, 15 ACP adults were released on six to eight true leaves of 'Valencia' sweet orange seedlings. Seedlings were covered with plastic cylindrical shaped containers (15 cm diameter and 30 cm high). This cylinder was covered with mesh screen for ventilation and its bottom opening was slipped over the soil around the seedling. Furthermore the humidity and photoperiod were adjusted as stated above. Adults were counted daily until 100% death or up to 16 days. For all temperature treatments, four replicates were performed.

2.3. ATP quantification assay

ACP adults were maintained as described above under five different temperatures (5, 15, 25, 35, 40 °C). Insects were collected after 24 h and kept at −80 °C until ATP extraction. Twenty-four adults from each temperature were taken and divided into eight replicates. Each group of three adults was homogenized with 150 μl of ice-cold 5% perchloric acid for 2 min in 1 ml tube. The samples were centrifuged at 10,000 rpm and 4 °C for 10 min. Supernatant was filtered through 10,000 molecular weight cutoff membranes (Millipore, Bedford, MA) and kept at −80 °C until analysis.

The ATP assay was performed using ENLITEN® ATP kit (Promega, Madison, WI). Briefly, a 10 μl aliquot of the sample was mixed with 7 μl of 1 M sodium carbonate (Na_2CO_3) for neutralization and the volume was adjusted to 100 μl using ATP-free water. One hundred microliter of luciferase reagent, provided with the kit, was added to the sample in 12 × 75 mm polypropylene test tubes (Fisher Scientific, Pittsburg, PA) and the intensity of the emitted light was measured for 10 s using an Optocomp I luminometer (MGM Instruments). The standards included the same amounts of 5% perchloric acid and 1 M Na_2CO_3 to correct possible inhibition of light output. Set of ATP standard concentrations were also prepared as described above and were used for the standard curve. A blank containing all the components above except ATP was used to determine the amount of background to be subtracted from the sample and standard relative luminescence unit (RLU). Eight replicates from each treatment were analyzed and each replicate was measured in triplicate.