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The effect of host plant species on the detoxifying enzymes of the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae)

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Diaphorina citri Kuwayama (Hemiptera: Liviidae) is a phytophagous insect that transmits 'Candidatus' *Liberibacter asiaticus*, a Gram-negative α -proteobacterium (Capoor et al. 1967). This pathogen causes Huanglongbing (HLB), which is a disease that severely reduces fruit yield and eventually kills citrus trees (Grafton-Cardwell et al. 2013). *Diaphorina citri* feeds on a broad range of plants within the Rutaceae (Halbert & Manjunath 2004; Yang et al. 2006), including *Murraya paniculata* (L.) Jacq. (orange jasmine), *Berberis koenigii* L. (Indian curry leaf), and commercially grown citrus (Aubert 1990). Plants produce a battery of allelochemicals, with a broad diversity of chemical structures that are metabolized by detoxification enzymes in phytophagous arthropods (Li et al. 2007; Chronopoulou et al. 2012). Host plant species can passively affect the biochemistry of arthropods and one of the most important effects is on the detoxification enzymes involved in insecticide metabolism, including general esterases, glutathione S-transferases, and cytochrome P450 monooxygenases (e.g., Dermauw et al. 2013).

Given that *D. citri* has a fairly broad host range within the Rutaceae, we postulated that detoxifying enzyme levels may vary as a result of rearing *D. citri* on different host plants. Further, we hypothesized that this may have an impact on insecticide resistance management protocols for this pest. We investigated the effect of host plant species used to rear *D. citri* on detoxification enzyme production.

Three host plant species were used as treatments: *Citrus sinensis* L. (sweet orange), *M. paniculata*, and *B. koenigii*. We measured the response of 3 detoxifying enzyme systems: general esterase (EST), glutathione S-transferase (GST), and cytochrome P450 monooxygenase (P450). The main culture of *D. citri* was established from psyllids collected in Polk County, Florida, USA, in 2000. This culture is maintained without exposure to insecticides on *Citrus aurantium* L. at the University of Florida, Citrus Research and Education Center (Lake Alfred, Florida, USA). Three separate cultures of *D. citri* were established from the main culture on *C. sinensis*, *B. koenigii*, or *M. paniculata*. These new cultures were established with 200 adults and maintained in a greenhouse at 27 to 28 °C, 60 to 65% RH, and a 14:10 h L:D photoperiod. It was assumed that these 3 subcultures were relatively homogenous in genetic background in comparison with one another.

After 12 generations, *D. citri* were collected and protein isolations were prepared according to established protocols (Tiwari et al. 2011a). Ten psyllids of mixed age and gender were collected for each protein isolation, with 3 technical replicates for each experiment. Each experiment was replicated twice. Protein concentration for each isolation was determined using the bicinchoninic acid method according to the

manufacturer's protocol (Pierce™ BCA Protein Assay Kit; Fisher Scientific, USA; Cat. # 23221). A SpectraMax 250 microtiter plate reader (Molecular Devices; Sunnyvale, California, USA) was used for all assays. One-way analysis of variance (ANOVA) was used to determine if EST, GST, or P450 activity differed between *D. citri* reared on the 3 host plants. If AVOVAs were significant, Fisher's Least Significant Difference (LSD) tests were used to determine differences between means.

General esterase activity was measured with a kinetic assay using pNPA (p-nitrophenol acetate; Sigma, USA; Cat. # N8130) as a substrate. The reaction product, p-nitrophenol, was monitored at 405 nm every 20 s at 25 °C. Mean general esterase activity was calculated and standardized per mg of protein as described in Tiwari et al. (2011b). No significant difference in general esterase activity was observed between psyllids reared on the 3 different host plant species ($P > 0.05$; Fig. 1A).

For glutathione S-transferase activity, CNDB (1-chloro-2,4-dinitrobenzene; Sigma, USA; Cat. # 237329) was used as the substrate in a kinetic assay. The reaction was monitored every 30 s at 344 nm for 30 min at 25 °C. Change in absorbance per min was converted into micromoles of CDNB conjugated per min per mg of protein using the extinction coefficient ($9.5 \text{ mM}^{-1} \text{ cm}^{-1}$) of the product 5-(2,4-dinitrophenyl)-glutathione (Habig et al. 1974). The mean (\pm SE) GST activities in *D. citri* reared on *B. koenigii*, *M. paniculata*, and *C. sinensis* were 159.7 ± 8.2 , 174 ± 3.7 and $129.1 \pm 1.2 \mu\text{mol}/\text{min}/\text{mg}$, respectively (Fig. 1B). The activity of GST in *D. citri* reared on *M. paniculata* was significantly higher ($F = 8.5$, $df = 5$, $P = 0.023$) than in those reared on *C. sinensis*, but not higher than in those reared on *B. koenigii* ($F = 9.5$, $df = 5$, $P = 0.266$).

P450 activity was determined using a heme-peroxidation method with TMBZ (3,3',5,5'-tetramethylbenzidine; Sigma, USA; Cat. # MKB-K4137V) as a substrate in an endpoint assay. Reactions were read at 650 nm at 25 °C. To quantify heme peroxidase activity, a 2.5-fold serial dilution of cytochrome C from horse heart (Sigma, USA; Cat. # SLB-D7905V) was prepared and P450 activity was expressed as equivalent units of cytochrome P450 per mg of protein using the standard curve of cytochrome C. The mean (\pm SE) P450 activities for *D. citri* reared on *B. koenigii*, *M. paniculata*, and *C. sinensis* were 0.72 ± 0.15 , 0.87 ± 0.20 , and 0.68 ± 0.17 equivalent units of P450/mg of protein, respectively (Fig. 1C). The activity of P450 in *D. citri* reared on *M. paniculata* was significantly higher than for those reared on *B. koenigii* ($F = 5.4$, $df = 5$, $P = 0.022$) and *C. sinensis* ($F = 10.7$, $df = 5$, $P = 0.007$).

Preliminary toxicological investigations revealed no differences in mortality at the LD₅₀ (49.4, 52.5, and 48.2 $\mu\text{g}/\text{mL}$ for *D. citri* reared on *B. koenigii*, *M. paniculata*, and *C. sinensis*, respectively) estimate for fen-

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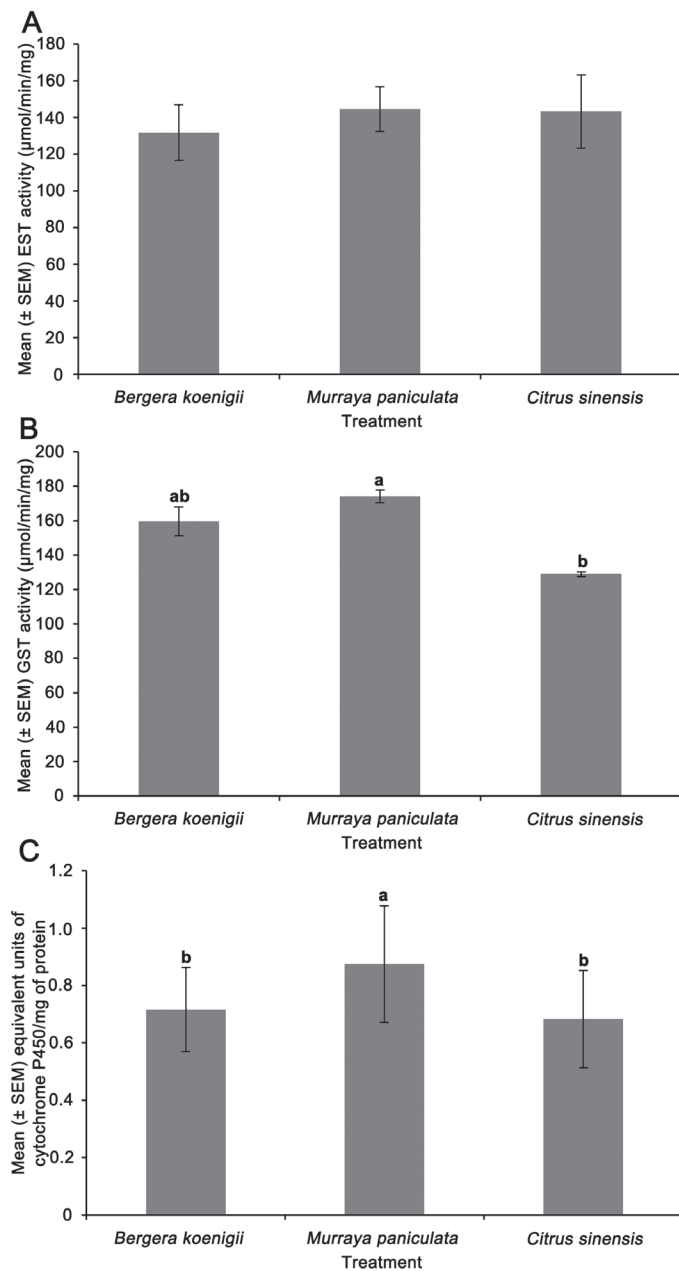


Fig. 1. Enzymatic activity of (A) general esterase (EST), (B) glutathione S-transferase (GST), and (C) cytochrome monooxygenase P450 from *Diaphorina citri* reared on *Citrus sinensis*, *Murraya paniculata*, and *Bergera koenigii*. Means with the same letter are not significantly different from each other ($P < 0.05$, Fisher's protected LSD test).

propathrin between *D. citri* reared on the 3 different host plants. This may be due to an insufficient duration of selection pressure to affect insect mortality, or the enzymes that were affected by host plant species are not involved in the metabolism of fenprothrin. However, these activity changes could potentially metabolize other classes of insecticides that were not tested (Yorulmaz & Ay 2009; Gong et al. 2013). Future investigations should include testing of different insecticide classes, as well as a comparison of allelochemical content of the plant species tested.

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Summary

Diaphorina citri Kuwayama (Hemiptera: Liviidae) is a phytophagous insect and the vector of the bacterium '*Candidatus*' Liberibacter asiaticus. This is the likely causal pathogen of Huanglongbing, which results in decline and possible death of citrus trees. It has been shown that host plants can affect the detoxification enzyme profile of arthropods. Here, we examined the effect of rearing *D. citri* on various host plant species with respect to activity of general esterases (ESTs), glutathione S-transferases (GST), and cytochrome P450 monooxygenases (P450s). These enzymes were selected because they are known to metabolize a wide diversity of insecticides and are known to directly contribute to resistance in *D. citri*. We reared *D. citri* on *Citrus sinensis* L., *Bergera koenigii* L. and *Murraya paniculata* (L.) Jacq. (Sapindales: Rutaceae). Following 12 generations of rearing, the activities of EST, GST, and P450 enzymes were compared between the colonies raised on the different host plants. The GST activity level was significantly higher in *D. citri* reared on *M. paniculata* than in those reared on *C. sinensis*. The P450 expression level was significantly higher in *D. citri* reared on *M. paniculata* than in those reared on either *B. koenigii* or *C. sinensis*. There was no significant difference in EST activity between treatments. These results suggest that host plant allelochemicals may alter the detoxification enzyme system in *D. citri*. However, these changes did not correlate with changes in mortality of *D. citri* when treated with fenprothrin.

Key Words: allelochemical; detoxification enzyme; general esterase; glutathione S-transferase; cytochrome monooxygenase P450

Sumario

Diaphorina citri Kuwayama (Hemiptera: Liviidae) es un insecto fitófago y el vector de la bacteria '*Candidatus*' Liberibacter asiaticus. Este es el patógeno causal probable de Huanglongbing, que resulta el declive y posible muerte de los árboles de cítricos. Se ha demostrado que las plantas hospederas pueden afectar el perfil de enzimas de desintoxicación de los artrópodos. Aquí, hemos examinado el efecto de criar *D. citri* sobre varias especies de plantas hospederas con respecto a la actividad de las esterasas generales (ESTs), glutatión S-transferasas (GST), y monooxigenasas del citocromo P450 (P450s). Estas enzimas fueron seleccionados porque son conocidos para metabolizar una amplia diversidad de insecticidas y se sabe que contribuyen directamente a la resistencia de *D. citri*. Se crió *D. citri* sobre *Citrus sinensis* L., *Bergera koenigii* L. y *Murraya paniculata* (L.) Jacq. (Sapindales: Rutaceae). Después de 12 generaciones de cría, se compararon las actividades de EST, GST y las enzimas P450 entre las colonias criadas sobre las diferentes plantas hospederas. El nivel de actividad GST fue significativamente mayor en *D. citri* criados sobre *M. paniculata* que en aquellos criados sobre *C. sinensis*. El nivel de expresión P450 fue significativamente mayor en *D. citri* criados en *M. paniculata* que en ambos *B. koenigii* o *C. sinensis*. No hubo una diferencia significativa en la actividad EST entre los tratamientos. Estos resultados sugieren que los aleloquímicos de plantas hospederas pueden alterar el sistema de enzimas de desintoxicación en *D. citri*. Sin embargo, estos cambios no se correlacionaron con cambios en la mortalidad de *D. citri* cuando son tratados con fenprotrina.

Palabras Clave: aleloquímicos; enzima de desintoxicación; esterasa general; glutatión S-transferasa; P450 monooxigenasa citocromo

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