Abdominal color of the Asian citrus psyllid, *Diaphorina citri*, is associated with susceptibility to various insecticides

Siddharth Tiwari, Nabil Killiny, Rajinder S Mann, Erik J Wenninger and Lukasz L Stelinski*

Abstract

BACKGROUND: Color morphs of an insect species are known to vary in activities of detoxifying enzymes and associated susceptibility to insecticides. In *Diaphorina citri*, three color morphs are known to occur. In the present study, susceptibility to four insecticides was compared among gray/brown, blue/green and orange/yellow color morphs of field-collected *D. citri*.

RESULTS: The orange/yellow morph was significantly more susceptible to fenpropathrin than the blue/green morph, and imidacloprid and chlorpyrifos caused higher mortality in the orange/yellow morph than in the blue/green and gray/brown morphs. To confirm the genetic basis of variable levels of susceptibility, the relative expression of five *CYP4* genes was compared among the color morphs. *CYP4C67* was expressed at significantly higher levels in the blue/green than in the orange/yellow and gray/brown morphs. *CYP4DA1*, *CYP4C68*, *CYP4G70* and *CYP4DB1* were expressed at significantly higher levels in the blue/green and gray/brown morphs than in the orange/yellow morph. Lower expression of *CYP4* genes in the orange/yellow morph as compared with the others was correlated with reduced signal of 45 kD cytochrome P450 proteins, as determined by the western blot.

CONCLUSIONS: The results indicate differential susceptibility of *D. citri* color morphs to insecticides, which will need to be accounted for in future insecticide monitoring programs and may affect management programs.

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Keywords: CYP4 genes; cytochrome P450 monooxygenase; gene expression; huanglongbing; citrus greening

1 INTRODUCTION

In insects, individuals of a species commonly have variations in body color. The range of color variation plays an important role in mate selection, deterring predators, regulating body temperature and habitat selection. These color variations are possibly the result of phenotypic plasticity, nutritional status, bacterial symbionts, genetic factors or a combination of two or more of the above factors. The diversity of body color at an individual level within a species – often referred to as color polymorphism – can also be the result of allelic variation at polymorphic loci or environmental factors.

Color polymorphism is common in the Asian citrus psyllid, *Diaphorina citri*; this species occurs in three distinct color morphs: gray/brown, blue/green and orange/yellow. The abdominal color of *D. citri* may reflect sexual maturity or indicate the oviposition period of females. In *D. citri*, gray/brown adults are characterized by lower body mass than other color morphs, and orange/yellow adults exhibit the greatest body mass when transferred to new citrus seedlings, gray/brown adults show an increase in body mass, which suggests that body color may be indirectly related to plant quality.

Color morphs are known to vary in activities of detoxifying enzymes and associated susceptibility to insecticides. For example, red morph tobacco aphids, *Myzus nicotianae* Blackman, exhibit higher carboxylesterase activity than green morphs. In addition, the red morph is characterized by a 1,3 autosomal translocation, which is less common in green morph aphids that exhibit high carboxylesterase activity. The red morph of the green peach aphid, *Myzus persicae* (Sulzer), is more resistant to dimethoate, endosulfan and lambda-cyhalothrin than the green morph. However, susceptibility to acephate, bifenthrin, imidacloprid or mevinphos is comparable among different color morphs in this species. Likewise, the green morph of the tobacco-adapted form of the green peach aphid, *M. persicae*, exhibits lower esterase levels and LC50 values when exposed to acephate and methomyl than red and orange morphs. This highly resistant orange morph was characterized by both *E4* and *FE4* gene amplification. The above results indicate that color polymorphism influences insecticide susceptibility at the gene level.
Previously, the present authors reported that insecticide susceptibility and detoxifying enzymes are influenced by the presence of a bacterial endosymbiont, Candidatus Liberibacter asiaticus, in D. citri. Bacterial endosymbionts are known to influence the development of color polymorphism by facilitating the synthesis of pigments in hemolymph. The present results indicate that color polymorphism is associated with insecticide susceptibility of D. citri. In addition, color-morph-associated insecticide susceptibility was negatively correlated with the expression of five CYP4 genes and 45 kD cytochrome P450 proteins in D. citri.

2 MATERIALS AND METHODS

2.1 D. citri cultures

Three color morphs of D. citri were collected using aspirators from a commercial citrus grove located in Polk County, Florida. Collected adults were transferred to the laboratory in coolers and released onto citrus plants in Plexiglas cages (40 × 40 × 40 cm) until treatment with insecticide, or gene and protein expression assays. Ten adults were randomly chosen from each morph to confirm the absence of Las using qPCR assay methods described by Tiwari et al.

2.2 Insecticides

Three color morphs of adult D. citri were tested for susceptibility to four insecticides from various insecticide chemistry classes and modes of action. Commercially available formulations of each insecticide were used in bioassays, and each insecticide was tested at 5–6 concentrations, which were prepared in distilled water on the day of testing. The insecticides evaluated against D. citri adults included chlorpyrifos (Lorsban 4E; Dow AgroSciences, LLC, Indianapolis, IN), imidacloprid (Provado 1.6F; Bayer CropScience Indianapolis, IN), fenpropathrin (Danitol 2.4EC; Valent USA Corp., Walnut Creek, CA), and 20–25 D. citri adults were transferred into each dish using a camel hair brush following a brief anesthetization with CO2 to facilitate handling and transfer. Petri dishes were wrapped with parafilm (Pechiney Plastic Packaging, Chicago, IL) to prevent adults from escaping. Sealed petri dishes with D. citri were transferred into a growth chamber (Percival Scientific, Inc., Perry, IA) set at 25 ± 1 °C, 50 ± 5% RH and 14:10 (L:D) photoperiod. Each concentration of an insecticide was replicated 3 times (n = 60–75 D. citri per concentration).

D. citri mortality was assessed 48 h after transfer into the growth chamber. D. citri found on their side or back that were unable to move when probed with a camel hair brush were considered dead. Mortality data were corrected for control mortality (<5%) using Abbott’s formula and analyzed separately for each color morph. Mortality data for each concentration and color morph were subjected to probit regression analysis to calculate the LC50 and LC95 for each insecticide with corresponding 95% confidence intervals and slopes of regression lines. The LC50 and LC95 values among color morphs were considered significantly different (P < 0.05) if their 95% confidence intervals did not overlap.

2.3 Leaf-dip bioassay

Insecticide susceptibility was determined using a leaf-dip petri-dish bioassay method. Plastic disposable petri dishes (35 mm diameter; Fisherbrand, Thermo Fisher Scientific, Waltham, MA) containing 3–4 mL of a 1.5% solidified agar solution constituted the bioassay arenas. The leaf discs (35 mm diameter) were made from citrus leaves collected from Valencia orange trees growing in a greenhouse at the Citrus Research and Education Center, Lake Alfred, Florida. Leaf discs were dipped in test (insecticide) solutions for 30 s and allowed to air dry in a fume hood for 1 h prior to bioassays. For the control treatment, leaf discs were dipped in distilled water alone. After 1 h, leaf discs were placed on agar beds, and 20–25 D. citri adults were transferred into each dish using a camel hair brush following a brief anesthetization with CO2 to facilitate handling and transfer. Petri dishes were wrapped with parafilm (Pechiney Plastic Packaging, Chicago, IL) to prevent adults from escaping. Sealed petri dishes with D. citri were transferred into a growth chamber (Percival Scientific, Inc., Perry, IA) set at 25 ± 1 °C, 50 ± 5% RH and 14:10 (L:D) photoperiod. Each concentration of an insecticide was replicated 3 times (n = 60–75 D. citri per concentration). D. citri mortality was assessed 48 h after transfer into the growth chamber. D. citri found on their side or back that were unable to move when probed with a camel hair brush were considered dead. Mortality data were corrected for control mortality (<5%) using Abbott’s formula and analyzed separately for each color morph. Mortality data for each concentration and color morph were subjected to probit regression analysis to calculate the LC50 and LC95 for each insecticide with corresponding 95% confidence intervals and slopes of regression lines. The LC50 and LC95 values among color morphs were considered significantly different (P < 0.05) if their 95% confidence intervals did not overlap.

2.4 Quantitative real-time PCR (qPCR) for quantifying CYP4 gene expression

Field-collected D. citri of each color morph and an insecticide-susceptible gray/brown morph from the authors’ laboratory culture were subjected to RNA isolation and cDNA synthesis. Prior to RNA isolations, ten adults were randomly chosen from each morph to confirm the absence of Las using qPCR assay methods described by Tiwari et al. RNA isolations were performed using the SV total RNA isolation kit (Promega, Madison, WI). RNA isolation was performed on groups of 40–50 psyllids of each color morph. Each treatment was replicated 3 times. The quality and quantity of RNA from each sample was measured on a NanoDrop 1000 spectrophotometer using an A260 and A260/A280 ratio respectively. Additionally, the quality of RNA was assessed by visualizing the RNA samples on 1% agarose gel electrophoresis in TAE buffer with ethidium bromide staining. cDNA was synthesized from the RNA samples using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). In brief, cDNA was synthesized in a 20 µL reaction volume containing 4 µL of 5x iScript reaction mix, 1 µL of iScript reverse transcriptase, 8 µL of nuclease-free water and 7 µL of total RNA (100 ng µL−1). Amplification cycles consisted of 25 °C for 5 min, 42 °C for 30 min and 85 °C for 5 min, and subsequently the samples were held at 4 °C. The PCR products were verified by 1% agarose electrophoresis.

The relative expression of five CYP4 genes in each color morph was detected by qPCR using SYBR Green PCR master mix in an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA). Primers for the five CYP4 genes and the reference gene, Actin, were used as described by Tiwari et al. For all qPCR experiments, the production of gene-specific products and the absence of ‘primer-dimers’ was verified by 1% agarose electrophoresis in TAE buffer with ethidium bromide staining. In brief, qPCR was performed in a 20 µL reaction volume containing 10 µL of SYBR green PCR master mix, 1 µL of cDNA, 1 µL each of forward and reverse primers and 7 µL of nuclease-free water. Amplification cycles consisted of an initial denaturing step at 95 °C for 10 min, followed by 45 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, an extension step at 72 °C for 10 min and the final melting-curve step (90 cycles of temperature reduction from 90 to 50 °C at a rate of 0.5 °C 10 s−1). Three biological replicates were performed for each gene and each color morph.

The relative expression of each gene among the treatments was compared using the 2−ΔΔCt method. Initially, normalization was performed using Actin (i.e. the reference gene) gene expression, followed by normalization to the treatment with the lowest gene expression (insecticide-susceptible greenhouse gray/brown morph). A separate one-way analysis of variance (ANOVA) was performed for each gene to compare the relative gene expression among the various color morphs, followed by Fisher’s protected LSD tests for mean separation (PROC GLM).
**2.5 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis**

The greenhouse (insecticide-susceptible) gray/brown, field-collected gray/brown and field-collected orange/yellow morphs were subjected to extractions and quantifications of subcellular protein fractions. Prior to protein analysis, ten adults were randomly chosen from each morph to confirm the absence of Las using qPCR assay methods described by Tiwari et al.\textsuperscript{23} Protein concentration was determined by the Bradford method\textsuperscript{32} using a protein standard. Subcellular protein fractions were extracted using methods described by Mouchés et al.\textsuperscript{33} Each protein fraction dissolved in potassium phosphate buffer was loaded on a 10% polyacrylamide gel separately and subjected to electrophoresis using methods described by Wheeler et al.\textsuperscript{33} Protein fractions were visualized by staining with colloidal Coomassie blue G250. In order to determine the presence of cytochrome P450 proteins, a western blot was performed on various subcellular protein fractions using the greenmine the presence of cytochrome P450 proteins, a western blot was performed on various subcellular protein fractions using the green

**Table 1. Insecticide toxicity in relation to color polymorphism in Diaphorina citri**

<table>
<thead>
<tr>
<th>Color morph</th>
<th>95% CI at LC\textsubscript{50}</th>
<th>95% CI at LC\textsubscript{95}</th>
<th>Slope ± SEM</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenpropathrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue/green</td>
<td>0.85 – 1.57</td>
<td>29.21 – 306.46</td>
<td>0.90 ± 0.10</td>
<td>3.42</td>
</tr>
<tr>
<td>Gray/brown</td>
<td>0.05 – 6.67E16</td>
<td>11.32 – 1.08E22</td>
<td>0.49 ± 0.15</td>
<td>15.83</td>
</tr>
<tr>
<td>Orange/yellow</td>
<td>9.70E-10 – 0.13</td>
<td>0.57 – 9.26E17</td>
<td>0.77 ± 0.22</td>
<td>22.05</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue/green</td>
<td>0.40 – 0.55</td>
<td>1.84 – 6.36</td>
<td>2.04 ± 0.27</td>
<td>0.72</td>
</tr>
<tr>
<td>Gray/brown</td>
<td>0.41 – 0.52</td>
<td>1.59 – 3.68</td>
<td>2.38 ± 0.24</td>
<td>4.19</td>
</tr>
<tr>
<td>Orange/yellow</td>
<td>0.18 – 0.25</td>
<td>1.09 – 3.25</td>
<td>1.87 ± 0.26</td>
<td>2.40</td>
</tr>
<tr>
<td>Spinetoram</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue/green</td>
<td>2.06 – 32.17</td>
<td>28.82 – 55 3124.00</td>
<td>0.98 ± 0.19</td>
<td>13.01</td>
</tr>
<tr>
<td>Gray/brown</td>
<td>3.47 – 12.47</td>
<td>51.71 – 1856.00</td>
<td>1.12 ± 0.12</td>
<td>6.87</td>
</tr>
<tr>
<td>Orange/yellow*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue/green</td>
<td>3.93 – 4.74</td>
<td>12.12 – 22.48</td>
<td>2.95 ± 0.31</td>
<td>5.98</td>
</tr>
<tr>
<td>Gray/brown</td>
<td>3.62 – 11.17</td>
<td>11.62 – 919.60</td>
<td>2.32 ± 0.47</td>
<td>15.50</td>
</tr>
<tr>
<td>Orange/yellow</td>
<td>0.92 – 1.56</td>
<td>2.67 – 5.36</td>
<td>3.81 ± 0.71</td>
<td>5.37</td>
</tr>
</tbody>
</table>

* Bioassays were not performed owing to the lack of a sufficient number of field-collected D. citri adults.

### 3 RESULTS

#### 3.1 Leaf-dip bioassay

Susceptibility to four insecticides differed among field-collected gray/brown, blue/green and orange/yellow color morphs of *D. citri* (Table 1 and Figs 1B and C). Based on non-overlapping 95% confidence intervals at the LC\textsubscript{50}, the orange/yellow morph was significantly more susceptible to fenpropathrin than the blue/green morph. Likewise, the orange/yellow morph was significantly more susceptible to chlorpyrifos and imidacloprid than the blue/green and gray/brown morphs, and the blue/green and gray/brown morphs were equally susceptible to spinetoram. Moreover, based on non-overlapping 95% confidence intervals at the LC\textsubscript{50}, the orange/yellow morph was significantly more susceptible to chlorpyrifos than the blue/green and gray/brown morphs.

#### 3.2 CYP4 expression levels in *D. citri* color morphs

Relative expression levels for the five CYP4 genes differed among three field-collected morphs and one greenhouse (insecticide-susceptible) morph (Fig. 2). The expression levels of CYP4C67 (\( F = 13.29; df = 3, 8; P = 0.0018 \)), CYP4C68 (\( F = 52.44; df = 3, 8; P < 0.0001 \)), CYP4DA1 (\( F = 876.92; df = 3, 8; P < 0.0001 \)), CYP4G70 (\( F = 6.33; df = 3, 8; P = 0.0166 \) and CYP4DB1 (\( F = 99.91; df = 3, 8; P < 0.0001 \)) were significantly different among the three field-collected morphs and the greenhouse morph. Expression levels of CYP4C68, CYP4DA1, CYP4G70 and CYP4DB1 were significantly lower in the orange/yellow morph than in the blue/green and gray/brown morphs. The expression level of CYP4C68 was 45- and 67-fold lower for the orange/yellow morph than for the blue/green and gray/brown morphs, respectively. The expression level of CYP4DA1 was 70 and 125 times higher in the blue/green and gray/brown morphs than in the orange/yellow morph. Likewise, the expression level of CYP4G70 in both blue/green and gray/brown morphs was 11-fold higher than in the orange/yellow morph. The expression level of CYP4DB1 was 64 and 101 times higher in the blue/green and gray/brown morphs, respectively, than in the orange/yellow morph.
Figure 1. Differential toxicity of four insecticides as a function of color polymorphism in *Diaphorina citri*. (A) *D. citri* color morphs. (B) LC$_{50}$ (mg Al L$^{-1}$) values obtained on the basis of color morphs of *D. citri*. Significant differences in LC$_{50}$ values among the morphs are indicated by different letters, based on non-overlapping 95% confidence intervals. The 95% confidence intervals, slope and $\chi^2$ obtained from probit analysis are presented in Table 1. (C) LC$_{95}$ (mg Al L$^{-1}$) values obtained on the basis of color morphs of *D. citri*. Significant differences in LC$_{50}$ values among the morphs are indicated by different letters, based on non-overlapping 95% confidence intervals. The 95% confidence intervals, slope and $\chi^2$ obtained from probit analysis are presented in Table 1.

3.3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

SDS-PAGE was performed using subcellular protein fractions prepared from the greenhouse (insecticide-susceptible) gray/brown morph of *D. citri* (Fig. 3A). SDS-PAGE indicated varying levels of proteins in various subcellular fractions. Subsequently, western blot was performed with the different subcellular fractions to locate cytochrome P450 proteins (Fig. 3B), which revealed the presence of a band corresponding to a 45 kDa protein in the microsomal protein fraction that cross-reacted with the primary antibody of cytochrome P450 protein. Subsequently, microsomal fractions were used to perform a western blot for comparing cytochrome P450 protein levels among field-collected gray/brown, greenhouse (insecticide-susceptible) gray/brown and field-collected orange/yellow *D. citri* morphs (Fig. 3C). Three quantities of microsomal proteins for each morph were used to perform the western blot. The field-collected orange/yellow morph exhibited lower levels of cytochrome P450 proteins than the field-collected or greenhouse (insecticide-susceptible) gray/brown morphs (Fig 3C).

4 DISCUSSION

Several factors may influence the development of color in *D. citri*, including the contents of the abdomen, mating, developmental stage, sex, feeding, reproductive potential and the presence of various pigments.$^{8,17,18,35}$ In other insect species, color polymorphism is influenced by juvenile hormones, selection pressures imposed by natural predators, symbiotic bacteria and lateral gene transfer related to carotenoid synthesis.$^{11,36–38}$ Irrespective of the role or underlying mechanism responsible for color polymorphism, all insects that display multiple color morphs exhibit the presence or absence of specific pigments. One of the most commonly occurring pigments in insects are carotenoids, which occur in various forms and influence insect color.$^{9,11,36–38}$ However, the mechanism explaining color polymorphism in *D. citri*, including the effect of carotenoids, has not been explored. In general, after mating, *D. citri* females become orange/yellow in color, which indicates the presence of eggs in the female’s abdomen.$^{8,19,35}$ No such change occurs in males after mating. The orange/yellow color morph in *D. citri* may be associated with the presence of carotenoids and blue bile pigments.$^8$

In the present study, insecticide susceptibility was compared among three color morphs of *D. citri*, and expression of five CYP4 genes and proteins related to insecticide susceptibility in this species was analysed. Insecticide susceptibility and activity of related enzyme systems are known to vary among color morphs in other insect species,$^{20–22,39}$ and color morphs also vary in their susceptibility to natural enemies.$^{37,40}$ In the present study, the orange/yellow morph was more susceptible to chlorpyrifos, fenpropathrin and imidacloprid than the blue/green and gray/brown morphs. Similar results were found with *M. persicae*, where the orange morph was characterized by higher esterase activity and LC$_{50}$ values (to methomyl and acephate) than red and green morphs.$^{22}$ However, the red morph of *M. persicae* was
more resistant to dimethoate, endosulfan and lambda-cyhalothrin than the green morph.\(^{21}\) Variation in insecticide susceptibility among color morphs has also been reported for the tobacco aphid, \textit{M. nicotianae}. In this species, the red morph is less susceptible to malathion, monocrotaphos, acephate and the esterase inhibitor \(S,S,S\)-tributyl phosphorothriothioate than the green morph.\(^{39}\) Lower carboxylesterase activity was reported in the green morph of \textit{M. nicotianae} than in the red morph.\(^{20}\) The above results confirm that insecticide susceptibility varies among insect color morphs, and, in a few instances, this is related to variation in enzymatic activity among different morphs.

Resistance conferred by cytochrome P450 monooxygenases is the most frequent form of metabolism-based resistance, followed by resistance conferred by esterases and glutathione S-transferases.\(^{41}\) Cytochrome-P450-dependent monooxygenases have been implicated in resistance to pyrethroid, neonicotinoid, organophosphate and spinosyn insecticides.\(^{42-46}\) In the present study, the expression levels of five \(CYP4\) genes were compared among three field-collected morphs and one greenhouse (insecticide-susceptible) morph to determine whether there was a relationship between insecticide susceptibility levels and expression levels of these \(CYP4\) genes. All five of these \(CYP4\) genes were induced in response to varying doses of imidacloprid and therefore may be associated with insecticide detoxification in \textit{D. citri}.\(^{30}\) Field populations of \textit{D. citri} exhibiting more than 30-fold resistance to imidacloprid are also characterized by greater expression of cytochrome-P450-dependent monooxygenases.\(^{26}\) The present results indicated lower expression levels of \(CYP4C68\), \(CYP4DA1\), \(CYP4G70\) and \(CYP4DB1\) in orange/yellow than in blue/green and gray/brown morphs. These results are consistent with the hypothesis that expression of \(CYP4\) genes is related to insecticide susceptibility levels in \textit{D. citri}.

The western blot indicated lower expression of cytochrome P450 proteins in the orange/yellow morph than in the other morphs examined, which was congruent with the observed expression levels of the \(CYP4\) genes examined. Previously, the partial sequences of these genes in \textit{D. citri} were identified.\(^{30}\) Given partial sequencing, the molecular masses of the corresponding proteins are unknown. As expected, lower expression of \(CYP4\) genes was correlated with lower expression of corresponding protein(s) in the orange/yellow morph than in the other morphs examined. Increased quantity of cytochrome-P450-dependent monooxygenases, corresponding to increased \(CYP\) gene transcripts, is associated with lower insecticide susceptibility.\(^{47-49}\) The present protein analysis suggests a variation in cytochrome P450 protein levels among the three color morphs that relates to their susceptibility to insecticides. Further investigations are needed to synthesize the antibody-based amino acid sequences of \(CYP4\) genes and to conduct a western blot assay for each individual protein.

The present results indicate that insecticide susceptibility of \textit{D. citri} is associated with color polymorphism. This variation in color-related insecticide susceptibility may be explained by differential expression of \(CYP4\) genes, which code for cytochrome P450 enzymes/proteins involved in insecticide detoxification. However, changes in gene expression levels over time as a function of...
insecticide susceptibility will require further investigation. Studies are also needed to determine the role of carotenoids or other pigments in color development of the three *D. citri* morphs. The color trait in various morphs of *D. citri* may be related to the differential expression of several other genes, including additional CYP4 genes, or it may simply be an incidental correlation with insecticide susceptibility. Future microarray analysis should provide a more comprehensive description of gene expression associated with insecticide susceptibility in *D. citri*. The present results indicate that future monitoring of insecticide resistance for *D. citri* will need to account for the color morph trait. It is also possible that these data may have implications for *D. citri* management. If a specific color morph dominates the population during certain points of the season, it may influence the choice of insecticide chemistry applied for *D. citri* management. A specific color morph may influence management. Previously, the present authors reported reduced insecticide susceptibility to chlorpyrifos and spinetoram in Las-infected when compared with uninfected *D. citri* adults. Therefore, both the rate of Las infection and the prevalence of specific color morphs may be factors to consider in management decisions for *D. citri*.

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