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Increased Gene Expressions and Metabolic Detoxifications, a Major Resistance Mechanism to Organophosphate and Neonicotinoid Insecticides in Tarnished Plant Bug

Short Communication

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During the last decade, widespread adoptions of transgenic Bt cotton and altered chemical control schemes have allowed sucking insect populations to increase. Of these pests, the tarnished plant bug (TPB), *Lygus lineolaris*, has become the most damaging insect to cotton and other field crops. Management of TPB relies almost exclusively on chemical control. Commonly used insecticides include pyrethroids, organophosphates, carbamates, and neonicotinoids. Cotton is sprayed more frequently than other major crops in the South to suppress feeding damages from TPB and bollworm/tobacco budworm.. Over the years, TPB has become increasingly resistant to several chemical insecticides (Zhu and Snodgrass, 2003; Zhu et al., 2004; Zhu et al., 2007). By using spray tower and discriminating dose, we found that TPB populations collected from the Delta regions of Mississippi and Arkansas showed variable survival rate with a wide range from 10% to 95% to acephate and imidacloprid (data not shown). Reduced susceptibility was associated with crop systems and cotton growing history because cotton receives more insecticide sprays than corn and soybean (data not shown). Pigweeds around cotton field attract TPB and allow the insect to build its population quickly around the cotton field. Frequent exposures to sprays/drift prompt resistance development in field populations of TPB, especially in cotton growing areas.

To understand why TPB become less susceptible to many insecticides, we applied major detoxification enzyme inhibitors in bioassays and enzyme activity assays. We found that many enzyme inhibitors could effectively synergize the toxicity of different insecticides against TPB, including pyrethroids, organophosphates, and neonicotinoids. Examinations of esterase, glutathione S-transferase (GST), and acetylcholinesterase activities showed substantial variations among more than 40 field populations collected (Zhu et al, 2011). Enzyme activities fluctuated/increased over a season and closely synchronized and correlated with susceptibility changes (Zhu et al., 2004; Zhu et al., 2007; Zhu and Luttrell, 2012). Higher esterase and GST activities were also associated with crop systems and cotton growing history. Additional insecticide treatment could increase resistance ratio and detoxification enzyme activities as well. All of these data indicated that TPB in Delta has developed metabolic resistance to chemical insecticides (Zhu et al, 2012).

To further analyze how gene regulations altered in resistance populations, we developed gene chip, which contained more than seven thousand of unique cDNA probes. To obtain resistant samples, thousands of TPB were collected from resistant field populations, and were selected with insecticides at concentrations 10-20 times higher than LC50, including 2 pyrethroid, 2 organophosphate, 2 neonicotinoid, and one carbamate insecticide. Double stranded (ds) cDNAs from survivors' gene transcripts were used to hybridize microarray gene chips simultaneously to reveal gene expressions of more than seven thousand genes. Microarray analysis revealed 329 up- and 333 down-regulated (≥2-fold) genes in acephate-selected TPB. Six esterase, three P450, and one glutathione S-transferase genes were significantly up-regulated, and no such genes were down-regulated in resistant TPB (Zhu et al., 2012).

Pathway analysis showed more than twice the number of catalysis genes and more than 3.6-fold of metabolic genes were up-regulated, indicating a substantial increase of metabolic detoxification. Significant increase of acephate resistance, increases of esterase activities and gene expressions, and variable esterase sequences in resistant strain consistently demonstrated a major esterase-mediated resistance in acephate-selected TPB, which was functionally provable by abolishing the resistance with esterase inhibitors (Zhu et al., 2012). In addition, significant elevation of P450 gene expression and reduced susceptibility to imidacloprid in acephate-resistant population indicated a concurrent resistance risk that may impact other classes of insecticides. Microarray analysis of imidacloprid-selected TPB revealed 955 up- and 1277 down-regulated (≥2-fold) genes. Five P450 and 9 esterase genes were significantly up-regulated, and only one esterase gene and no P450 genes were down-regulated (Zhu and Luttrell, 2014). Other up-regulated genes include helicases, phosphodiesterases, ATPases, and kinases. Pathway analyses identified 65 up-regulated cDNAs, which encode 51 different enzymes involved in 62 different pathways, including P450 and esterase genes for drug and xenobiotic metabolisms. This study indicated a significant change of gene expression related to metabolic processes in imidacloprid-selected TPB, resulting in over-expression of P450 and esterase genes for potential excess detoxification and cross/multiple resistance development. The identification of these and other enzyme genes demonstrated a complicity of imidacloprid resistance in TPB (Zhu and Luttrell, 2014).

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