

## COLORADO POTATO BEETLE - EVIDENCE FOR THE EMERGENCE OF METABOLIC RESISTANCE<sup>1/</sup>

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Management of agricultural crops relies on integrated pest management strategies to mitigate production losses due to insect infestation. Producers of field and vegetable crops depend on local and regional guidelines for pesticide applications to limit insect infestation and minimize associated damage (1). Within an agroecosystem, a producer may need to manage multiple insect, pathogen and plant species resulting in the application of several insecticide, fungicide and herbicide mode of action groups, respectively (2). Pesticide applications used for the same cropping system can vary among producers and can vary between different geographic regions. One pest of solanaceous crops which is often controlled using a diverse set of cultural and chemical management practices, is the Colorado potato beetle (*Leptinotarsa decemlineata* Say). If not properly managed, this specialist herbivore can cause significant defoliation of the potato canopy that can result in yield loss and economic injury for the producer. In addition to its ability to rapidly defoliate plants, *L. decemlineata* is considered a major pest species because of its ability to develop resistance to insecticides at a rapid rate (3).

Insecticide resistance develops and propagates within insect populations through the selection of individuals that possess genetic attributes that, when passed onto offspring, result in resistant phenotypes (4). Insecticide resistance can develop through multiple mechanisms, including enhanced metabolic breakdown of insecticides, reduced cuticular penetration, target site insensitivity, and behavioral resistance or avoidance (4). Previous *L. decemlineata* investigations have classified possible mechanisms of insecticide resistance within select populations, including the detoxification and removal of insecticides from the insect's body through phase 1 (detoxification through breakdown) and phase 2 (removal through excretion) enzymes (3, 6-8). These studies have examined the over-expression of transcripts corresponding to xenobiotic resistance mechanisms within select populations of *L. decemlineata* classified as insecticide resistant through traditional dose response bioassays. Crossley et al. 2017 examined short nucleotide polymorphisms and these studies suggest that a combination of detoxification and excretion enzymes play a significant role in insecticide resistance.

Defining the mechanisms of insecticide resistance development within *L. decemlineata* is difficult due to the differences observed between geographically distinct populations of the insect. *Leptinotarsa decemlineata* populations are found within all major potato production areas of the United States. However, insecticide resistance to common insecticide mode of action groups, has predominantly been associated with potato production areas on the East Coast and in the Midwest, but not in the Pacific Northwest region. While estimates of total insecticide applications (kg active ingredient ha<sup>-1</sup>) are similar across the United States, the amount of other pesticides applied, including fungicides, differs dramatically in different geographies. Clements *et al.* (2018) hypothesized that the geographic disparity in insecticide resistance could partially be explained by the increased application of fungicides (8). The transcriptomic response and classification of the enzymatic detoxification mechanisms revealed that both insecticides and fungicides induced similar detoxification mechanisms within individuals from the same population. This result suggests that the geographic disparity in observed resistance levels can be partially explained by the development of cross-functional detoxification pathways driven by chronic exposure to both insecticides and fungicides (8).

Geographic location is often related to the patterns of pesticide management strategies and chemical inputs which are required for crop protection, and consequently may play a significant role in insecticide resistance development. Examining the patterns of transcript expression of previously classified detoxification mechanisms through targeted RNA sequencing provided insight into transcript regulation corresponding to putative insecticide resistance that were linked to geographic location.

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Additionally, differences between the over-expression of detoxification mechanisms within resistant populations of *L. decemlineata* may suggest that insecticide resistance can develop in different ways. We investigated transcript expression of 38 previously classified detoxification enzymes induced by imidacloprid (an insecticide) and chlorothalonil (a fungicide) within 5 discrete populations of *L. decemlineata* obtained from areas in the U.S. representing eastern, midwestern and western production regions. We found unique patterns of transcript expression in different geographic locations, including overexpression of transcripts related to insecticide metabolism within insecticide resistant populations. The results suggest the genetic response of these populations may be partially linked to geographic location and corresponding management practices.

By examining the transcript expression of multiple populations of *L. decemlineata*, we determined the similarities and dissimilarities between transcript expression of known xenobiotic detoxification mechanisms. By establishing the response of populations to insecticides through LD50 estimates and examining transcript expression through targeted RNA sequencing, we determined that the two populations deemed as imidacloprid-resistant over-express unique detoxification mechanisms in different proportions. We further validated the previous induction studies conducted by Clements et al. (2018) in field populations (6,8). The experiments conducted within this study demonstrate a diverse set of transcripts that appear to be over-expressed within different field populations with different resistant phenotypes. The findings of this research further suggest that mechanisms of insecticide resistance in *L. decemlineata* most likely involve multiple metabolic resistance mechanisms, and further that these mechanisms differ among geographic region.

#### References Cited

1. Huseth AS, Groves RL, Chapman SA, Alyokhin A, Kuhar TP, Macrae IV, Szendrei Z, Nault BA. Managing Colorado potato beetle insecticide resistance: new tools and strategies for the next decade of pest control in potato. *Journal of Integrated Pest Management*. 2014 Dec 1;5(4):A1-8.
2. Hare JD. Ecology and management of the Colorado potato beetle. *Annual review of entomology*. 1990 Jan;35(1):81-100.
3. Alyokhin A, Baker M, Mota-Sanchez D, Dively G, Grafius E. Colorado potato beetle resistance to insecticides. *American Journal of Potato Research*. 2008 Dec 1;85(6):395-413.
4. Yu S. *The Toxicology and Biochemistry of Insecticides*. CRC Press, Boca Raton, FL. (2015)
5. Clements J, Olson JM, Sanchez Sedillo B, Bradford B, Groves RL. Changes in emergence phenology, fatty acid composition, and xenobiotic metabolizing enzyme expression is associated with increased insecticide resistance in the Colorado potato beetle. *Archives of insect biochemistry and physiology*. 2020 Mar;103(3):e21630.
6. Clements J, Sanchez-Sedillo B, Bradfield CA, Groves RL. Transcriptomic analysis reveals similarities in genetic activation of detoxification mechanisms resulting from imidacloprid and chlorothalonil exposure. *PloS one*. 2018 Oct 25;13(10):e0205881.
7. Crossley MS, Chen YH, Groves RL, Schoville SD. Landscape genomics of Colorado potato beetle provides evidence of polygenic adaptation to insecticides. *Molecular Ecology*. 2017 Nov;26(22):6284-300.
8. Clements J, Schoville S, Clements A, Amezian D, Davis T, Sanchez-Sedillo B, Bradfield C, Huseth AS, Groves RL. Agricultural fungicides inadvertently influence the fitness of Colorado potato beetles, *Leptinotarsa decemlineata*, and their susceptibility to insecticides. *Scientific reports*. 2018 Sep 5;8(1):1-0.

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