FUNGICIDES AS INADVERTENT DRIVERS OF INSECTICIDE RESISTANCE

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Abstract

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is an agricultural pest of solanaceous crops which has developed insecticide resistance at an alarming rate. Up to this point, little consideration has been given to unintended, or inadvertent effects that non-insecticide xenobiotics may have on insecticide susceptibility in *L. decemlineata*. Fungicides, such as chlorothalonil and boscalid, are often used to control fungal pathogens in potato fields and are applied at regular intervals when *L. decemlineata* populations are present in the crop. In order to determine whether fungicide use may be associated with elevated levels of insecticide resistance in *L. decemlineata*, we examined phenotypic responses in *L. decemlineata* to the fungicides chlorothalonil and boscalid. Using enzymatic and transcript abundance investigations, we also examined modes of molecular detoxification in response to both insecticide (imidacloprid) and fungicide (boscalid and chlorothalonil) application to more specifically determine if fungicides and insecticides induce similar metabolic detoxification mechanisms. Both chlorothalonil and boscalid exposure induced a phenotypic, enzymatic and transcript response in *L. decemlineata* which correlates with known mechanisms of insecticide resistance [Clements, 2018].

Objectives

1) Determine whether field relevant rates of fungicides can have a selection pressure on Colorado potato beetles.
2) Characterize the genetic mechanisms that are activated in response to both fungicide and insecticide exposure and determine whether fungicides can activate similar detoxification mechanisms as insecticides.
3) Determine whether fungicide and insecticide susceptibility vary between different geographic populations of *L. decemlineata* and whether genes which are up-regulated after fungicide exposure in imidacloprid susceptible populations are constitutively up-regulated in either population.

Background

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a key agricultural pest causing significant crop loss and direct damage to commercial potatoes (*Solanum tuberosum*), tomatoes (*S. lypcopersicum*), eggplants (*S. melongena*) and peppers (*S. annuum*) [Hare 1990]. The global impact of *L. decemlineata* direct damage to crops is far ranging,

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and these beetles have significant pest status throughout the world, inclusive of over 16 million km2 [Alyokhin, 2008; Hare, 1990]. According to the United Nations Food and Agricultural Organization, the United States (US) produced 19.8 million tons of potatoes in 2013, and it is the leading vegetable crop in the country [FAOSTAT, 2014]. The impact of *L. decemlineata* on individual state agricultural markets is also significant, especially in Wisconsin, where potato production accounts for more than $310 M annually [Kashian, 2014]. To keep this vital crop safe from these pests, we estimate farmers in Wisconsin annually expend $10 M for pesticide inputs (based upon producer surveys) [Huseth, 2014]. The history of insecticidal inputs for control of *L. decemlineata* is a story retold in many potato production areas of the country, where many classes of insecticides have been effective for short periods of time before the beetles become resistant. Recent estimates suggest that populations of beetles have now become resistant to more than 52 insecticides [Alyokhin, 2008] over most of the potato production regions of the US, with the notable exception of far western production areas (Idaho, Washington, and Oregon) where susceptibility remains elevated. In 1995, the registration and introduction of a new insecticide class (Group 4A, neonicotinoid insecticides) resulted in the use of active ingredients which include, imidacloprid, thiamethoxam, clothianadin, and dinotefuran [Alyokhin, 2008; IRAC, 2016]. Since their initial introduction in the mid-1990s, *L. decemlineata* populations have steadily developed resistance to this class, but it remains the principal insecticidal tool used for potato protection [Alyokhin, 2008; Clements, 2016; Huseth, 2013; Mota-Sanchez, 2006]. Multiple studies have attempted to uncover the mechanisms by which this insect rapidly develops resistance [Alyokhin, 2006; Clements, 2016; Mota-Sanchez 2006], but no study to date has fully elucidated the answer.

In addition to heavy and repeated insecticide application, many other environmental factors may lead to, or assist in, the development of insecticide resistance. One notable factor may be cross-resistance between insecticides and fungicides that facilitates rapid evolutionary change. Cross-resistance refers to a situation whereby an insect develops tolerance to a usually toxic, insecticidal substance as a result of exposure to a different, sub-lethal substance that may be less toxic. This can be the product of nonspecific enzymes which attack functional groups rather than the specific molecules [Yu, 2015]. While cross-resistance has historically been examined between multiple insecticides [Alyokhin, 2006; Mota-Sanchez, 2006], few studies have explored the potential cross-resistance between insecticides and fungicides, which are frequently co-applied to potato crops. A comparison between fungicide and insecticide application in different geographic regions (USDA National Agricultural Statistics Service (NASS)) [USDA, 2016] has shown that in Eastern (ME) and Midwestern (MI, WI, MN, and ND) potato production regions, it is common to have 5 to 7 foliar applications of fungicides in a single season, while in the Northwestern region (Washington, Idaho, and Colorado), far fewer foliar applications are required. During seasons with high disease potential, Eastern and Midwestern crops receive 10 to 12 applications of fungicides [Guenthner, 1999; Stevens, 1994]. While there is a clear trend in increased applications of fungicides in Midwest and Eastern regions, the overall amount of neonicotinoid insecticide applications remains relatively constant across the US. At different times during the growing season, measured levels of resistance within populations of *L. decemlineata* varies widely. More specifically, past research in both Michigan [Szendrei, 2012] and Wisconsin [Clements, 2016] has revealed markedly higher levels of resistance in the 2nd generation of *L. decemlineata* (e.g., July to September) when compared to the 1st generation present during May and June. Mancozeb®, a dithiocarbamate, is regularly used as a broad-spectrum protectant for control of the late blight pathogen

Phytophthora infestans (deBary) in potato fields, with applications beginning around the first week of July and continuing throughout the entire growing season. We further hypothesize that the marked increase in insecticide resistance observed in 2nd generation *L. decemlineata* may be partially explained by frequent reapplication of fungicides during this specific portion of the growing season.

We hypothesize that cross-resistance in beetles induce a detoxification response to one chemical pressure (fungicide) that could promote development of more rapid resistance to another chemical stressor (insecticide). If such cross-resistance does occur between select fungicides and insecticides in potato crop culture, the genes activated could lead to considerable insecticide resistance in geographic areas where disease pressure is consistently elevated compared to arid regions where disease pressure is lower, and measured levels of insecticide resistance concomitantly remains low.

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