Difference in Foliar Fatty Acid Composition in Potato Cultivars over a Growing Season May Influence the Host Location Preference of *Leptinotarsa Decemlineata*

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Abstract

The production of commercial potatoes (*Solanum tuberosum*) for different market classes is of vital agricultural importance in the United States. For the production of chips, fresh-market or processing potatoes, potato producers rely upon different potato cultivars to meet market and consumer demands. Many cultivars possess distinctive traits which make them more or less susceptible to disease and insect pressure. One important and understudied trait that may confer host location preference and population performance include leaf fatty acid composition(s). It is known that leaf fatty acid composition can influence growth, taste, and even insect defense mechanisms. In the current investigation we examined the fatty acid composition of leaf tissue obtained from 8 commercial potato cultivars representing different market classes grown in a controlled field study. We examined colonization preference of *Leptinotarsa decemlineata* among the 8 different potato cultivars over a 45 day period representing the first full generation in Wisconsin. Results of these experiments suggest, i) that fatty acid compositions in leaf tissue vary between potato cultivars and among sample dates throughout the experiment, ii) that *Leptinotarsa decemlineata* may preferentially colonize particular potato cultivars, and iii) that there was a significant correlation between omega-3 fatty acid composition in leaf tissue and *Leptinotarsa decemlineata* cultivar preference.

Resumen

La producción de papas comerciales (*Solanum tuberosum*) para diferentes clases de mercado es de vital importancia agrícola en los Estados Unidos. Para la producción de papas fritas, para el mercado fresco o de procesamiento, los productores dependen de diferentes variedades de papa para satisfacer las demandas del mercado y de los consumidores. Muchas variedades poseen rasgos distintivos que las hacen más o menos susceptibles a las enfermedades y a la presión de los insectos. Un rasgo importante y poco estudiado que puede conferir preferencia de ubicación del hospedante y rendimiento de la población incluye la composición de ácidos grasos de la hoja. Se sabe que la composición de ácidos grasos de las hojas puede influir en el crecimiento, el sabor e incluso los mecanismos de defensa de los insectos. En la presente investigación examinamos la composición de ácidos grasos del tejido foliar obtenido de 8 variedades comerciales de papa que representan diferentes clases de mercado cultivadas en un estudio de campo controlado. Examinamos la preferencia de colonización de *Leptinotarsa decemlineata* entre las 8 variedades de papa diferentes durante un período de 45 días que representa la primera

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generación completa en Wisconsin. Los resultados de estos experimentos sugieren, i) que las composiciones de ácidos grasos en el tejido de la hoja varían entre las variedades de papa y entre las fechas de muestreo a lo largo del experimento, ii) que *Leptinotarsa decemlineata* puede colonizar preferentemente variedades de papa particulares, y iii) que hubo una correlación significativa entre la composición de ácidos grasos omega-3 en el tejido de la hoja y la preferencia de la variedad por *Leptinotarsa decemlineata*.

Key Words Colorado potato beetle · Host preference · Fatty acids · Omega-3

Introduction

The species *Solanum tuberosum* is comprised of more than 5,000 different cultivars, with only a fraction of them being produced commercially (Lutaladio et al. 2009). Growers base cultivar selection on multiple factors including current market demands, yield potential, profitability, and growing region. Additionally, growers that produce potatoes actively protect against a range of biotic organisms including bacteria, fungi, and insects, and cultivar selection can influence plant protection strategies. One particularly problematic pest that growers must manage is the Colorado potato beetle, *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae). *Leptinotarsa decemlineata* is an infamous agricultural pest of solanaceous crops throughout the world (Schoville et al. 2018). Both the larval and adult stages are considered major pests; the damage caused by *L. decemlineata* can result in field failure of solanaceous crops if not properly controlled (Alyokhin et al. 2008). *Leptinotarsa decemlineata* is considered an herbivore specialist, as it feeds on select members of only a single plant family (Solanaceae), including potatoes, tomatoes, peppers, eggplants and other nightshades (Hare 1990). However, within this family, *L. decemlineata* is primarily associated as a pest of cultivated potatoes (Alyokhin et al. 2008), it has been suggested that *L. decemlineata* may prefer to feed on certain potato cultivars (Jansky et al. 1999) and that potato leaf surface compounds including fatty acid methyl can influence *L. decemlineata* host choice (Szafranek et al. 2008). Potato traits are regularly selected by breeders to develop advanced germplasm for cultivar improvement and insect resistance (Spooner et al. 2009, Rodon et al. 2021). One trait that has been examined in-depth is high glycoalkaloid concentrations, which could be bred as a mechanism for insect resistance (Crossley et al. 2018). While laboratory and greenhouse examinations have demonstrated beetles prefer to feed on plants with lower glycoalkaloid concentrations, field trials demonstrated no difference among cultivars in terms of host location and feeding preferences (Lyytinen

et al. 2007, Crossley et al. 2018). Hufnagel also noted that solanaceae chemical plant defense does not correlate to *L. decemlineata* host preferences (Hufnagel et al. 2017). While potato glycoalkaloid concentration has been examined in depth for *L. decemlineata* cultivar preferences, other biological factors may play important roles in host location and cultivar choice.

Insects often locate and subsequently determine host plant suitability through a variety of different mechanisms, including visual, olfactory, gustatory and tactile cues to include probing of plant tissues (Cao et al. 2014, Carrasco et al. 2015, May and Ahmad 1983, Schütz et al. 1997). One cue that insects use involves the interaction with plant volatiles including those derived from the oxidation of fatty acids (Bernays and Chapman 2007, Cingel et al. 2016). Plant tissue is composed of a variety of macronutrients that insects acquire through their diet, including essential fatty acids (Gelman et al. 2001, Schwimmer et al. 1954, Elias 2012). Insects typically acquire essential fatty acids from either a plant source (herbivory) or animal tissue (Behmer 2008). Both omega-3 and omega-6 fatty acids are essential for most insects, including *L. decemlineata*. Unlike some insects, research from Cripps et al. (1986) suggests that Coleopterans are not capable of de novo omega-6 or omega-3 synthesis, and these fatty acids must be obtained from the diet. Omega-3 and omega-6 requirements in *L. decemlineata* have not been determined, although supplementation experiments have shown their benefit on adult fecundity (Krzyszowska 1976). Omega-3 fatty acids are primarily composed of alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acids, and are essential for the biological development and growth of insects (National Institutes of Health 2021). While fatty acids are typically esterified within the foliage of the plant, the oxidation of fatty acids creates volatile organic compounds (e.g. alcohols, aldehydes, epoxides, hydrocarbons, ketones) which can be emitted as green leaf volatiles (GLVs) by the plant as an insect signaling cue (Blomquist et al. 2010, Blomquist et al. 2020).

In the current investigation we examined differences among cultivars in foliar fatty acid compositions throughout the growing season and whether *L. decemlineata* differentially colonized specific potato cultivars in correlation with foliar fatty acids. We hypothesize that potato cultivars have different foliar fatty acid compositions which change over the course of a growing season and that foliar fatty acid composition may influence host location or *L. decemlineata* feeding preference. Differences in foliar fatty acid composition together with variations in *L. decemlineata* feeding preference will further our understanding of potato growth and lipid composition together with insect host location and preference that may prove useful in developing new integrated pest management strategies.

Materials and Methods

Potato Cultivar Field Layout

In the spring of 2019, potato plantings (cv. ‘Adirondack Red’, ‘W9576-11Y’, ‘Goldrush’, ‘Yukon Gold’, ‘Red Norland’, ‘Pinnacle’, ‘Silverton’, and ‘Atlantic’) were established at the University of Wisconsin’s Hancock Agricultural Research Station (HARS) (44.118181°N, -89.549045°W), which has a history of high *L. decemlineata* pressure. Seed potatoes were acquired as year one foundation class seed from the Lelah Starks Elite Foundation Seed Potato Farm, Rhinelander, WI. Four replicate sets of each cultivar were planted (5 plants per replicate) and cultivar location within the field plot was randomized by using a random number generator to assign a location for each treatment group (excel function RAND()).

Fatty Acid Composition Analysis

Fully expanded leaves from the upper canopy of three replicate plots of each cultivar were sampled at 15, 30, and 45 days after emergence. Leaf samples were placed in a cooler (held at 10 °C) and transported to the University of Wisconsin-Madison for fatty acid analysis. Leaf tissue was extracted according to the Folch methodology using dichloromethane as a substitute for chloroform (Folch et al. 1957). Total extracted fatty acids were methylated using methanolic HCl, similar to methods described by Palmquist (Palmquist and Jenkins 2003). Briefly, toluene was added to dried dichloromethane extract (2:1 v/w). Next, methanolic HCl was added to the lipid extracts (100:1 v/w) and samples were heated at 60°C for 20 minutes in a water bath. The methylation reaction was followed by hexane extraction of fatty acid methyl esters (FAME) to yield a final FAME concentration of 10mg/ml for composition analysis by gas chromatography. The relative abundance of FAMES was analyzed using gas chromatography (Agilent 6890N) coupled with flame ionization detection as previously described (Huebner et al. 2010, Sampath and Ntambi 2006). A 100m biscyanopropyl polysiloxane

Fig. 1 Log mean leaf estimates of omega-3 (n-3) and omega-6 (n-6) fatty acid abundance of field-grown potato foliage by cultivar and sampling date. Total n-3 and n-6 fatty acid fraction is shown, as is the n-3:n-6 ratio. Potato cultivar abbreviations include: Adirondack Red

(ARR), Atlantic (ATL), Goldrush (GDR), Pinnacle (PIN), Red Norland (RNL), Silverton (SLV), W9576-11Y (W95), and Yukon Gold (YKG)

capillary column (Rt-2560, Restek Corp, Bellefonte, PA) was used for separation of FAMES. FAMES were identified using a custom qualitative FAME standard (Matreya LLC, Pleasant Gap, PA, #SPL4833).

Insect Counts

Plots were monitored weekly (June 14–August 19) for the colonization of *L. decemlineata* after plant emergence and populations were assessed on all five plants in each plot for the following life stages: adults, egg masses, small larvae (1st–2nd instars), and large larvae (3rd–4th instars). All plots achieved full emergence, so no insect count adjustments were required.

Data Analysis

Data analysis was performed in R version 4.0.4 (R-Core team 2021). Insect counts were log-transformed prior to statistical analysis to satisfy assumptions of normality (formula: $\log(x + 1)$). Main and interaction effects were determined using Analysis of Variance, and means were separated using letter codes generated with Tukey's HSD procedure ($\alpha=.05$, package 'agricolae', (De Mendiburu 2014)).

Results

Potato Cultivar Fatty Acid Composition

Fatty acid compositions differed significantly between potato cultivars and varied significantly within cultivars over time (June 14th, July 1st, and July 16th, which correlates to day 15, 30, and 45 after first emergence). The relative abundance of major fatty acids was examined among cultivars in this investigation (Supplemental Figure S1), with an emphasis on essential fatty acids (omega-3 and omega-6; Fig. 1). Generally, total omega-3 fatty acid composition increased and total omega-6 fatty acid composition decreased over time across all cultivars, though the effect was not significant. The most significant differences in fatty acid composition between cultivars was apparent early in the season, with potential implications for adult beetle preference and (early) larval success. On June 14th, omega-3 fatty acid content varied significantly between cultivars ($F=4.52$, $df=7$, 14 , $P=.0079$), with the highest content found in Pinnacle, Red Norland, and Yukon Gold leaf tissue. Omega-6 fatty acid composition was generally inversely correlated with omega-3 composition and varied significantly between cultivars on Jun 14 ($F=9.27$, $df=7$, 14 , $P=.00025$). Total omega-3 + omega-6 fatty acid composition did not vary significantly between cultivars, comprising around 50% of total fatty acid content in leaf tissue.

Colorado Potato Beetle Cultivar Preference

Patterns of adult colonization were significantly influenced by potato cultivar. Beetle colonization was recorded throughout the experimental interval (Fig. 2), with first colonization of potatoes recorded on June 14th. Adult beetles were observed colonizing potato cultivars at statistically different levels ($F=4.45$, $df=7$, 21 , $P=.0036$). Mean separation analysis suggest that adult colonization preferences could be divided into three categories representing high, intermediate, and low preference. The most heavily colonized cultivars included Red Norland and Pinnacle. Beetles colonized W9576-11Y, Goldrush, Yukon Gold and Atlantic at intermediate levels. Finally, beetles colonized Adirondack Red and Silverton at the lowest level (Fig. 2).

While initial colonization appeared to be influenced by cultivar, by the following week adult beetles had reassorted and adult counts were no longer statistically different between cultivars, and no significant differences in egg mass counts were observed between cultivars. Despite no observed differences in egg mass counts among cultivars, early counts of small larvae (Jun 25) were higher on Red Norland and Pinnacle, supporting the earlier observations of adult beetle colonization preference on those cultivars ($F=2.87$, $df=7$, 21 , $P=.029$). By the following week enough small larvae had developed on all cultivars that no statistically significant differences in small larvae populations were observed. On Jul 1, large larvae were initially observed on Red Norland, Pinnacle, and Silverton, again supporting the earlier colonization of those cultivars by adult beetles, although the counts between cultivars were not statistically significant and large larvae counts were high across all cultivars by the following week. On Jul 16, statistically significant differences in both small and large larvae were observed between cultivars (small larvae: $F=2.87$, $df=7$, 21 , $P=.029$; large larvae: $F=3.30$, $df=7$, 21 , $P=.016$).

Correlation between Fatty Acid Composition and Feeding Preference

Fatty acid composition differences observed among cultivars together with differential beetle colonization rates provided interesting clues about linkages between beetle preference and fatty acid composition. We noted a significant correlation between increased omega-3 fatty acid composition and greater mean adult *L. decemlineata* colonization on 14 June ($R^2 = 0.4$, $P = 0.055$, Fig. 3). We also noted a significant and corresponding lower mean number of small larvae on plants with high omega-3 concentrations on July 16th ($R^2 = -0.48$, $P = 0.01$), which corresponds to the end of the first generation of *L. decemlineata*, and a non-significant correlation of higher large larvae populations at the same timepoint,

Fig. 2 Log mean *Leptinotarsa decemlineata* counts by potato cultivar across dates representing adult colonization, early larval and late larval development periods. Day 15 post-emergence corresponded to peak first-generation *L. decemlineata* adult counts, day 30 corresponded to peak small larvae (1st and 2nd instar) populations, and day 45 corresponded to peak large larvae (3rd and 4th instar)

suggesting that larvae may develop faster on plants with a higher relative abundance of omega-3 fatty acids.

Discussion

Within this investigation we determined that different potato cultivars possess unique fatty acid compositions and that those compositions change over the growing season. We also examined cultivar colonization preference by *L. decemlineata* and noted significant differences in terms of patterns of abundance on potato cultivars. Within commercially cultivated potatoes, *L. decemlineata* is widely recognized as the most economically significant arthropod pest, threatening potato production throughout the world (Schoville et al. 2018). There are more than 5,000 recognized native and commercial potato varieties currently in production (Lutaladio et al. 2009), allowing beetles to adapt to different cultivars. While it remains unknown why this species originally switched host preference from the native to the cultivated host, beetle fitness could be a major factor in

populations. Potato cultivar abbreviations include: Adirondack Red (ARR), Atlantic (ATL), Goldrush (GDR), Pinnacle (PIN), Red Norland (RNL), Silvertown (SLV), W9576-11Y (W95), and Yukon Gold (YKG). Box plots and associated means with different lower-case letters are significantly different ($\alpha=0.05$)

this host switch, including acquisition of macronutrients. One of the macronutrients needed for insect growth is fatty acids, including omega-3, which is vital for growth and development.

Fatty acid composition significantly differed between potato cultivars and over time. The highest fatty acid abundance in the potato foliage for all cultivars was omega-3 fatty acids and, more specifically, 18:3 n-3 (supplemental file S1). However, omega-3 fatty acids significantly varied between cultivars and overtime within each cultivar. Red Norland had the highest proportion of omega-3 fatty acids at day 15, however, by day 30 there was no significant difference in the relative abundance of omega-3 between any cultivars. By day 45, W9576-11Y had the highest relative abundance of omega-3 fatty acids of any cultivar. Omega-6 fatty acid composition was generally inversely correlated with omega-3 composition, with W9576-11Y having the highest composition of omega-6 fatty acids at day 15 and a relatively low composition of omega-6 fatty acids by day 45. Overall fatty acid compositions within cultivars changed over the growing season. No one cultivar had a higher rate of

Fig. 3 Statistical correlation between omega-3 (n-3) and omega-6 (n-6) fatty acid abundance and *L. decemlineata* life stage by sample date. Adult populations peaked on day 15, small larvae populations peaked on day 30, and large larvae populations peaked on day 45.

Correlations are derived from a linear model comparing total fatty acid fraction with the log-normalized *L. decemlineata* count for each life stage

omega-3 or omega-6 fatty acid throughout the entire experimental interval.

Through a controlled field study, we determined that adult beetles preferred to initially colonize the cultivars Red Norland and Pinnacle within our field setting. However, after initial adult colonization the population of adults re-assorted themselves more uniformly among all the potato cultivars and there was no statistical difference at later dates (Fig. 2). Early counts of small larvae (Jun 25) were higher on Red Norland and Pinnacle, supporting the earlier observations of adult beetle colonization preference on those cultivars. By the following week, enough small larvae had developed on all cultivars that no statistically significant differences in small larvae were observed. On Jul 1 large larvae were observed on Red Norland, Pinnacle, and Silverton, again supporting the earlier colonization of Red Norland and Pinnacle cultivars by adult beetles. However, Silverton was not preferred by adults in initial colonization, and the counts between cultivars for large larvae were not statistically significant. Large larvae counts were high across all cultivars by the following week. The findings suggest that beetles may be able to differentiate between potato cultivars, and that cultivar-specific traits or cues are attractive to adult

beetles. Similarly, Szafrank et al. 2008 was able to isolate a compound from potato leaf tissue that had deterrent attributes suggesting that beetles are receiving olfactory cues from potatoes which inform host choice. We noted that beetles preferred potato cultivars with high omega-3 fatty acid abundance. We also noted a significant decrease in small larvae on plants with high omega-3 fatty acids on July 16th and a non-significant correlation of large larvae at the same timepoint, suggesting that larvae are developing faster on plants with high omega-3 fatty acids. We noted no difference in oviposition preference within our experimental plots. Hufnagel demonstrated that *L. decemlineata* oviposition host preference is not significantly correlated to beetle feeding preference, and this could help explain why we did not see more egg masses plants that the beetles preferential colonized (Hufnagel et al. 2017). It has been further suggested that fatty acid composition of adult beetles significantly differs between insecticide resistant and susceptible populations of insects (Clements et al. 2020) and increased fatty acid compositions could lead to a fitness advantage in both reproduction and development of insecticide resistance traits.

Dietary constraints and manipulations of fatty acids within *L. decemlineata* diet have been demonstrated to have significant adverse effects on beetle survivorship and fitness. When conjugated linolic acid was added to *L. decemlineata* diet, a decrease in weight gain and survivorship was observed. Further, Clements, demonstrated that *L. decemlineata* larvae significantly avoided plant material coated with conjugated linolic acid, suggesting *L. decemlineata* are acquiring cues from fatty acids to determine host preference (Clements et al. 2019). These results suggest that *L. decemlineata* will preferentially colonize potato cultivars in a randomized and replicated experimental field trial. The findings of this investigation should be repeated using larger blocks of field-grown potatoes to limit inter-plot interference among experimental potato cultivars and validate findings. In addition, we observed that fatty acid composition in potato foliage is significantly different between certain cultivars and further that these compositions change over the growing season. We also correlated cultivar fatty acid composition, including high omega-3 composition, to increased feeding preference. Unfortunately, the fatty acid analysis performed in this study did not give us total fatty acid mass per leaf mass, so we were unable to determine whether feeding preference and differential larval populations were due to differences in fatty acid composition or to differences in total fatty acid abundance among cultivars. This investigation only examined a limited set of biological factors (fatty acids), and a more comprehensive list of other biological and environmental factors should be further investigated to help explain beetle feeding preferences. Further, we only examined 8, commonly grown cultivars of potatoes, and it is possible that other cultivars have different fatty acid compositions and these could significantly influence beetle preference. Still, we believe that while the statistical analyses presented here are only correlative, it suggests a vital new line of investigation for future work and may influence our future pest management of *L. decemlineata*. We recommend further investigation into the effects of cultivar-specific fatty acid composition and quantity on *L. decemlineata* host preference and growth rates. Similar studies should be repeated over multiple seasons and include more cultivars in future investigations and larger plot sizes to support larger beetle populations and improve statistical power.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12230-021-09857-w>.

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Data Availability All relevant data are contained within the paper and its supporting information files.

Declarations

Ethics Statement No specific permits were required for field collection or experimental treatment of *Leptinotarsa decemlineata* for the study described.

Conflict of Interest The Authors declare no conflicts of interest.

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