Étude des effets de la fertilisation azotée sur les concentrations foliaires en sucres, en acides aminés et en glycoalcaloïdes des plants de pommes de terre

Thèse

Guoqi Wen

Doctorat en sols et environnement
Philosophiæ doctor (Ph. D.)

Québec, Canada

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Sous la direction de :

Mohamed Khelifi, Ph.D., directeur de recherche
Athyna N. Cambouris, Ph.D., codirectrice de recherche

Résumé

Le doryphore de la pomme de terre (DPT), *Leptinotarsa decemlineata* (Say), est l'insecte nuisible qui cause les dommages les plus importants aux cultures de pommes de terre. La feuille de pomme de terre est le principal aliment du DPT et les composants chimiques foliaires jouent un rôle prépondérant dans la croissance de cet insecte. À titre d'exemple, les sucres et les acides aminés foliaires pourraient favoriser le développement du DPT, tandis que les glycoalcaloïdes sont généralement utilisés pour réprimer les comportements du DPT. En général, le processus métabolique de ces composants chimiques est étroitement lié à l'azote. Ce travail de recherche avait pour objectif d'étudier en conditions contrôlées (en pots) et au champ les effets de la fertilisation azotée sur les concentrations en sucres, en acides aminés et en glycoalcaloïdes dans les feuilles des plants de pommes de terre. Des expériences au champ ont été réalisées sur le cultivar Russet Burbank avec des doses d'azote de 0, 60, 120, 180 et 240 kg N ha^{-1}. Vingt plants de pommes de terre ont été sélectionnés au hasard dans chaque unité expérimentale et la quatrième feuille du haut de chaque plant a été collectée à 40, 54, 68 et 82 jours après la plantation (JAP). Pour l'expérience en pots, les mêmes doses d'azote ont été utilisées avec les cultivars Russet Burbank et Goldrush. Les troisième, quatrième et cinquième feuille du haut de chaque plant de pommes de terre ont été collectées au hasard dans chaque pot à 62, 75, 89 et 103 JAP. Les concentrations en sucres (saccharose, glucose, et fructose), glycoalcaloïdes (α-solanine et α-chaconine) et acides aminés (une vingtaine) ont été déterminées. Les résultats obtenus dans les essais au champ ont démontré que la fertilisation azotée diminuait significativement les concentrations en sucres, mais favorisait grandement l'accumulation de glycoalcaloïdes et d'acides aminés dans les feuilles des plants de de Russet Burbank. Dans l'expérimentation en pot, la fertilisation azotée a considérablement affecté les concentrations en sucre et en acides aminés plutôt que les teneurs en glycoalcaloïdes. De plus, différentes concentrations de ces produits chimiques foliaires ont été observées entre les deux cultivars de pomme de terre. Les résultats obtenus ont révélé que les concentrations en produits chimiques foliaires de la pomme de terre sont sensibles au climat, à la dose d'azote et aux cultivars. En se basant sur ces résultats, la fertilisation azotée pourrait être considérée comme une approche potentielle et complémentaire à la lutte intégrée contre les ravageurs pour contrôler les populations de DPT en modifiant la composition chimique des feuilles de pommes de terre et une dose d'azote de 180 kg N ha^{-1} est recommandée. Par ailleurs, des études complémentaires sont nécessaires pour évaluer les effets des doses d'azote et des cultivars de pommes de terre sur le comportement du DPT sous des conditions de terrain.

Abstract

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is the insect pest that causes the most important damage to potato crops. Potato leaf is the major food source of CPB, and the foliar chemical components play important roles in this beetle growth. For example, foliar sugars and amino acids could promote CPB development while glycoalkaloids are usually used to suppress the CPB behaviors. Generally, the metabolic process of these chemicals is closely related to nitrogen (N). The objective of this research was to investigate the impacts of N fertilization on sugar, amino acid, and glycoalkaloid concentrations of potato leaves under field and controlled conditions. Field experiments were conducted with N rates of 0, 60, 120, 180, and 240 kg N ha^{-1} and potato cultivar "Russet Burbank" was used. Twenty potato plants were randomly selected from each experimental unit and the 4^{th} leaf from the top of each selected plant was collected at 40, 54, 68, and 82 days after planting (DAP). For the pot experiment, the same N rates were used and potato cultivars "Russet Burbank" and "Goldrush" were planted. The 3^{rd}, 4^{th}, and 5^{th} potato leaves from the top of a randomly selected plant from each pot were collected at 62, 75, 89, and 103 DAP. Concentrations of sugars (sucrose, glucose, and fructose), glycoalkaloids (α-solanine and α-chaconine), and twenty amino acids were determined. Field experimental results showed that N fertilization significantly decreased sugar concentrations. However, it greatly increased the accumulation of glycoalkaloids and amino acids in Russet Burbank leaves. In the pot experiment, N fertilization significantly altered sugar and amino acid concentrations rather than glycoalkaloid contents. Additionally, different concentrations of these foliar chemicals were observed between two potato cultivars. The obtained results indicated that the concentrations of potato foliar chemicals are climatic, N, and genotype sensitive. Based on these findings, N fertilization could be considered as a potential and complementary approach of integrated pest management to control CPB populations by altering the chemical composition of potato leaves and the N rate of 180 kg N ha^{-1} is recommended to potato growers. However, further studies are still required to evaluate the effects of N fertilization and potato cultivars on the CPB behavior under field conditions.

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Liste des abréviations

HPLC	High-Performance Liquid Chromatography
DPT	doryphore de la pomme de terre
JAP	jours après la plantation
N	nitrogen
DAP	days after planting
CPB	Colorado potato beetle
GABA	γ-Aminobutyric acid
AABA	α-Aminobutyric acid
Gly	glycine
Asp	aspartate
Ala	alanine
Glu	glutamate
Pro	proline
Ser	serine
Asn	asparagine
Gln	glutamine
Arg	arginine
Val	valine
Ile	isoleucine
Leu	leucine
Met	leucine
Phe	phenylalanine
His	histidine
Lys	lysine
Thr	threonine
Tyr	tyrosine
DM	dry matter

Cette thèse est dédiée à mon épouse Yuexia Jin et ma fille Nancy Wen pour leurs énormes sacrifices, amour et soutien tout au long de ce processus. Je remercie également mes parents de m'avoir permis d'étudier au Québec. Je vous aime tous!

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Avant-propos

This thesis is composed of four chapters in addition to an introduction, a general discussion and conclusion of this research, and several perspectives for future works. The four chapters include an extensive literature review on the knowledge background related to the research topic, field and greenhouse experimental protocols, and results. The thesis was entirely written by the Ph.D. candidate under the supervision of Prof. Mohamed Khelifi, Dr. Athyna N. Cambouris, and Dr. Noura Ziadi. The Ph.D. candidate is the main responsible for conducting the experiments, collecting and analyzing the data, interpreting the results, and writing the conclusions. The Ph.D. candidate is the first author of all articles inserted in this thesis. The co-authors made significant contributions in the form of comments and suggestions throughout the laboratory and field experiments as well as the preparation of the articles.

The introduction includes the research hypotheses and objectives on which this doctoral thesis is based with regard to the current knowledge and issues.

The first chapter is an exhaustive literature review, which summarizes the previous scientific researches carried out on the chemical concentrations of potato leaves and their possible alteration to control the Colorado potato beetle. This chapter was published as a research article in the journal of Potato Research.

The second and third chapters present the results of a two-year field experiment. Chapter 2 mainly focuses on the responses of potato foliar sugar and glycoalkaloid concentrations to the nitrogen fertilization. It was published in the journal of Field Crops Research. Chapter 3 mainly investigates the effects of nitrogen fertilization on potato foliar amino acid composition for an eventual control of the Colorado potato beetle. This chapter was published as a scientific article in the American Journal of Potato Research.

The fourth chapter presents a greenhouse pot experiment. This chapter mainly investigates the impacts of nitrogen fertilization and potato cultivar on the variation in foliar sugar, glycoalkaloid, and amino acid concentrations. This chapter was published as a scientific article in the American Journal of Potato Research.

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Scientific publications

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Wen, G., Cambouris, A. N., Bertrand, A., Ziadi, N., Li, H., Khelifi, M., 2019. Nitrogen fertilization effects on the leaf chemical concentrations in Russet Burbank potato. *Field Crops Res.* 232, 40–48. https://doi.org/10.1016/j.fcr.2018.12.006

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Wen, G., Cambouris, A. N., Ziadi, N., Bertrand, A., Khelifi, M., 2020. Effects of nitrogen fertilization on the leaf chemical composition of two potato cultivars under controlled conditions. *Am. J. Potato Res.* https://doi.org/10.1007/s12230-020-09765-5

Scientific communications

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Wen, G., Cambouris, A. N., Khelifi, M., Ziadi, N., Bertrand, A., 2018. Nitrogen fertilization effect on potato leaf chemicals: an alternative control method of Colorado potato beetle. Poster presented at the 102nd conference of Potato Association of America (PAA). Boise, Idaho, U.S.A., July 22-26.

Wen, G., Cambouris, A. N., Khelifi, M., Ziadi, N., Bertrand, A., 2018. Impact of nitrogen fertilization on potato leaf chemical composition – an alternative control method of Colorado potato beetle. Poster presented at the Journées de la Recherche en Santé Conférence (JRSC). Université Laval, Québec, QC, Canada, May 23-24.

Wen, G., Cambouris, A. N., Ziadi, N., Bertrand, A., Khelifi, M., 2017. Effects of N fertilization on potato leaf chemical composition for the eventual control of Colorado potato beetle. Poster presented at the 20th Triennial conference of the European Association for Potato Research (EAPR). Versailles, France, July 9-14.

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is widely considered as the most important insect pest of potato crops (*Solanum tuberosum* L.) throughout the world (Hare, 1980). If left uncontrolled, this beetle can induce tremendous tuber yield losses by completely defoliating potato plants. Many physical and biological control approaches have been developed to control this insect pest. Nevertheless, none of them has been successful at large scale in potato production since the complicated and diverse life history of CPB makes it well-suited to various agricultural environments and a challenging pest to control (Alyokhin et al., 2008). For decades, CPB populations have been mainly suppressed by chemical insecticides, which are likely to be the dominant approach for the foreseeable future (Scott et al., 2015). The issue is that heavy applications of chemical insecticides can lead to serious human health and environmental problems, and greatly increase the rate of CPB resistance as well (Alyokhin et al., 2008).

Many studies have been focused on the feeding characteristics of CPBs. Karley et al. (2002) reported that the feeding behaviors of herbivorous insects were frequently attributed to the nutrient quality of host plants and the absence of specific nutriments in plant leaves could slow down the CPB growth (Cibula et al., 1967). Similar results were obtained by Hsiao and Fraenkel (1968) who pointed out that the sucrose and several amino acids (alanine, ascorbic acid, and thiamine) can significantly stimulate the CPB feeding behavior. However, some secondary metabolites in potato foliage, such as glycoalkaloids, are considered as inhibitors for the CPB growth (Lachman et al., 2001).

Nitrogen (N) is one of the most important macronutrients for potato yield and quality. A good N strategy is a critical component of successful potato production (Zebarth and Rosen, 2007). In addition, N fertilization can affect the foliar nutritional composition and distribution because their metabolic processes require N-containing enzymes. The effects of N fertilization on the nutritional composition of potato tubers are already well known. For example, increasing N fertilizer rate increases amino acid concentration of potato tubers (Eppendorfer and Bille, 1996). However, the opposite pattern was observed for sugars. De Wilde et al. (2006) reported that sugar concentrations in potatoes increased in response to N deprivation compared with adequately fertilized. To date, only very few studies have been conducted to investigate the impacts of N fertilization on the potato foliar chemical composition for an eventual CPB management. This thesis presents the results of several experiments conducted in Quebec on the variation in chemical concentrations of potato leaves using different N rates. The hypothesis and objectives of this research were as follows:

Hypothesis and objectives

1. Hypothesis

The foliar chemical concentrations of different potato cultivars mainly depend on N fertilization.

2. Objectives

2.1 General objective

Investigate the impacts of N fertilization on the potato leaf chemical concentrations.

2.2 Specific objectives

Investigate the effects of N rates on sugar, glycoalkaloid, and amino acid concentrations of potato leaves (cv. Russet Burbank) under field conditions.

Investigate the effects of N rates on sugar, glycoalkaloid, and amino acid concentrations of potato leaves (cv. Russet Burbank & Goldrush) under controlled conditions.

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Chapter 1 Responses of the Colorado potato beetle (Coleoptera: Chrysomelidae) to the chemical composition of potato plant foliage

Guoqi Wen, Mohamed Khelifi, Athyna N. Cambouris, Noura Ziadi

This chapter presents an exhaustive literature review related to the effects of volatile chemicals, carbohydrates, amino acids, glycoalkaloids, and mineral elements in potato foliage on the feeding behaviors of the Colorado potato beetle (CPB). In general, chemical components in potato leaves could enhance or reduce the CPB feeding. Therefore, altering the chemical composition of potato foliage may represent an interesting alternative to reduce the use of chemical insecticides to repress CPB populations in potato crops. This chapter was published in the journal of Potato Research.

Wen, G., Khelifi, M., Cambouris, A. N., Ziadi, N., 2019. Responses of the Colorado potato beetle (Coleoptera: Chrysomelidae) to the chemical composition of potato plant foliage. *Potato Res*. 62, 157–173. https://doi.org/10.1007/s11540-018-9405-0

1.1 Résumé

Le doryphore de la pomme de terre (DPT), *Leptinotarsa decemlineata* (Say), est largement considéré comme le principal insecte ravageur des plants de pommes de terre (*Solanum tuberosum* L.). Le DPT peut complètement détruire les cultures de pommes de terre et causer d'énormes pertes de rendement s'il n'est pas contrôlé. Pendant des décennies, les populations de DPT ont été principalement contrôlées à l'aide d'insecticides chimiques. Cependant, la diversité et la souplesse de son cycle biologique, conjuguées à sa remarquable capacité d'adaptation à divers facteurs de stress, font du DPT un ravageur très difficile à contrôler. Le feuillage des plants de pommes de terre qui contient de grandes quantités de produits chimiques volatiles et non volatiles constitue la principale source alimentaire du DPT. Certains chercheurs ont indiqué que les variations dans la défoliation des plants de pommes de terre et l'abondance de ce coléoptère sont imputables à la qualité et à la quantité des composants chimiques présents dans le feuillage de la plante hôte. Cette revue de littérature a examiné les effets des substances chimiques volatiles, des glucides, des acides aminés, des glycoalcaloïdes et des éléments minéraux dans le feuillage des plants de pommes de terre sur la défoliation des plants et le comportement alimentaire du DPT. En général, les composants chimiques contenus dans le feuillage des plants de pommes de terre pourraient améliorer ou réduire l'alimentation du doryphore. La modification de la composition chimique du feuillage pourrait être une alternative intéressante pour la réduction de l'utilisation des insecticides chimiques afin de contrôler les populations de DPT dans les cultures de pommes de terre.

Mots clés: Acides aminés, Glucides, Glycoalcaloïdes, Contrôle des insectes, Minéraux, Produits chimiques volatiles

1.2 Abstract

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is widely considered as the most serious insect defoliator of potato plants (*Solanum tuberosum* L.). The CPB can completely destroy potato crops and cause tremendous yield losses if left uncontrolled. For decades, CPB populations have been suppressed mainly by chemical insecticides. However, this insect's diverse and flexible life history, combined with its remarkable adaptability to a variety of stresses, make the CPB a very challenging pest to control. Potato foliage, which contains high amounts of volatile and non-volatile chemicals, is the CPB's main food source. Some researchers indicated that variations in the feeding performance and abundance of this beetle are due to the quality and quantity of chemical components in the host plant foliage. This review investigated the effects of volatile chemicals, carbohydrates, amino acids, glycoalkaloids, and mineral elements in potato foliage on the feeding behavior and performance of the CPB. In general, the chemical components in potato foliage could enhance or reduce the feeding of the CPB. Altering the chemical composition of potato foliage could be an interesting alternative to reduce the use of chemical insecticides to manage CPB populations in potato crops.

Keywords: Amino acids, Carbohydrate, Glycoalkaloids, Insect control, Minerals, Volatile chemicals

1.3 Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is one of the most destructive insect pests of potato crops (*Solanum tuberosum* L.) (de Ladurantaye et al. 2010). Approximately 40 cm^2 of potato leaves can be consumed by a single beetle during the larval stage, and close to 10 cm^2 of foliage is consumed per day during the adult stage (Ferro et al. 1985). In North America, potential potato yield losses have been estimated at 30 to 50% due to uncontrolled CPB populations (Boiteau 2010). Although the scientific community and commercial producers have paid a great deal of attention to the CPB, it remains a real threat to the potato industry in already colonized potato growing areas and continues to expand its geographic range into new regions of the world.

Colorado potato beetle populations are usually suppressed by means of chemical insecticides, which are likely to remain the predominant approach for the foreseeable future (Alyokhin 2009). Over the years, the CPB has rapidly developed resistance to most registered chemical insecticides. Many other control approaches have also been attempted in the past decades. For example, crop rotation is an effective and easily implemented cultural practice for controlling this pest (Wright 1984). However, the lack of annual rotation in North America has been the primary limiting factor for managing this beetle (Alyokhin et al. 2015). Moreover, Khelifi et al. (2007) have reviewed many physical control methods, including physical barriers and thermal, pneumatic, and electromagnetic control. However, none of these approaches can be applied alone to successfully manage the CPB on a large scale. In recent years, genetic technology was applied as an alternative to chemical pesticides. Zhang et al. (2015) used long double–stranded RNAs to trigger a lethal RNA interference and disorder the essential gene expression in the body of the CPB. The drawback of this technology is that it is difficult to implement under real-field conditions because the targeted RNAs are hampered by the presence of the endogenous plant RNAi pathway. Another frequently used method is to make host plants express toxic proteins in their leaves in order to reduce CPB populations. Cingel et al. (2017) transformed three potato cultivars (Desiree, Dragacevka, and Jelica) through adding rice cystatin oryzacystatin I and II genes and observed that the co-expression of the proteinase inhibitors oryzacystatin I and II could decrease plant damage caused by CPB. So far, transgenic crops are not yet popular with customers because it is unknown how safe they are.

During its life cycle and development process, the CPB requires a huge quantity of nutrients for its growth from larva to adult. All the required nutrients come from host plants via the CPB's feeding behaviors. Previous studies have determined that the feeding behavior of the CPB is attributed to the nutrients' quality and quantity although other factors, such as air temperature, are also important. Hsiao and Fraenkel (1968) carried out a lab test using agar media to evaluate the effects of different nutrients on the CPB's feeding behavior and observed that sucrose and several amino acids (alanine, γ-aminobutyric acid, and serine) elicited marked feeding behavior in CPB larvae. Furthermore, the lack of specific food materials in the tissues of some resistant plants may explain why

those plants have a detrimental effect on the CPB growth (Cibula et al. 1967). Except for foliar nutrients, there are also some toxic chemicals, such as glycoalkaloids and non-protein amino acids, that can inhibit CPB feeding. Therefore, the chemical composition (nutrients and toxic chemicals) of host plants can affect the CPB's growth and behavior. The objective of this review was therefore to provide an in-depth look at the impact of foliar chemicals (volatiles and non-volatiles) on CPB growth and development.

1.4 Life cycle of the Colorado potato beetle

The complete life cycle of the CPB consists of four stages (Figure 1-1). At the end of summer, a considerable number of adults move outside potato fields and prepare for overwintering diapause (De Kort 1990). They burrow themselves into the soil to an average depth of 20 to 25 cm (Casagrande 2014), even reaching as far as 120 cm in severe climates in Canada (Khelifi et al. 2007). In the spring, the overwintered adults emerge from the ground when the air temperature exceeds 10°C and begin searching for host plants in order to obtain the essential nutrients for their development (Jansson and Smilowitz 1985). After colonizing host vegetation, the overwintered adults begin feeding. Once well fed, the females lay orange eggs on the underside of potato leaves. The eggs are generally laid in clusters of 20 to 30 eggs each (Khelifi et al. 2007). The CPB is very prolific, and a single female could lay approximately 300 to 800 eggs over its lifespan (Harcourt 1971). The eggs normally hatch within 1 week, and red-colored larvae with some blackish spots emerge. These larvae go through four stages during a 3-week period, reaching their full development at a length of 0.5 to 1.25 cm. When the fourth instar larva ceases feeding, it drops on the ground and burrows into the soil to pupate. Adult beetles emerge from below ground 1 to 2 weeks later to complete their life cycle. Depending on the climate conditions and food availability, one to four generations of CPB can occur during a single year.

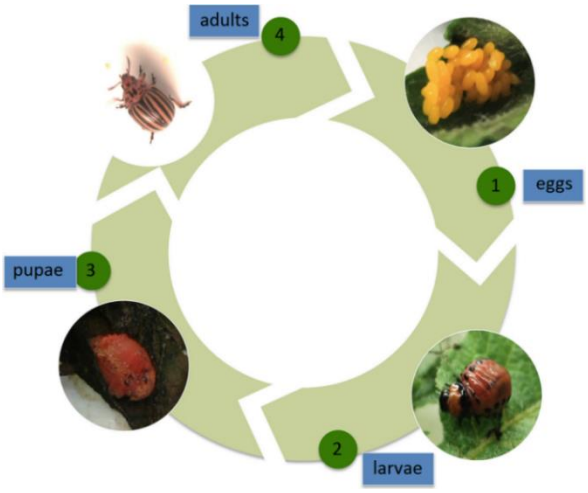

Figure 1- 1 Life cycle of the Colorado potato beetle: (1) eggs, (2) larvae, which go through four stages, (3) pupae, and (4) adults (Maharijaya and Vosman 2015)

1.5 Chemical composition of potato foliage

After emerging from the ground in spring, CPBs begin searching for host plants in order to find the nutrients essential for their development. In this context, volatile chemicals from the host plants play an important role in host plant recognition and acceptance. Once they inhabit the plants, the CPBs begin feeding and require a huge quantity of nutrients for their growth and development. The feeding behaviors of the CPB are attributed to the quality and quantity of the chemical composition of the host plants (Hsiao and Fraenkel 1968). Therefore, it would be worthwhile to review the chemical composition of host plant foliage, including volatile and non-volatile chemicals. Characterizing and quantifying these chemicals is the starting point for the eventual repression of the CPB. Altering the chemical composition of potato leaves without affecting the quality and yield of tubers could therefore represent an interesting alternative to reduce the use of chemical insecticides to manage the CPB populations.

Some volatile chemicals of potato leaves have been identified as the main components of potato odor to attract the CPB by stimulating its olfactory receptors, namely (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, 1-hexanol, (*E*)-2-hexenal, hexanal, and (*Z*)-3-hexenyl-acetate (Visser 1979; Dickens 2002). The concentrations of these volatile chemicals in potato leaves are closely related to many factors. Agelopoulos et al. (1999) observed that the variation in volatile chemicals was dependent on the mechanical damage to potato leaves. The volatiles of (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol were released in high amounts after potato leaf damage when compared to intact leaves. The emission of volatile compounds was also affected by the potato cultivar. Vancanneyt et al. (2001) reported that 187.4 nmol of (*E*)-2-hexenal was released from 24 plants in 30 min for cultivar Desiree but no (*E*)-2-hexenal was released from potato cultivar Granola (Schütz et al. 1997). However, very little information related to the variation of these components in potato leaves during the entire growing season between different cultivars was found in the literature.

The carbohydrates in potato foliage vary significantly according to the leaf age. As a major carbohydrate, sucrose concentrations reach their highest point in the oldest potato foliage (Figure 1-2). However, glucose and fructose concentrations peak at about 60 days after potato plant emergence in the fruit development stage (Kolbe and Stephan-Beckmann 1997). For each growing day, the sugar content reaches its peak value in the afternoon and drops to its lowest point before sunrise (Kolbe and Stephan-Beckmann 1997). This variation is mainly due to the sugar metabolism process, which depends on photosynthesis and respiration in higher plants. Additionally, the carbohydrates concentrations in potato leaves were affected by cultivation practice, such as nitrogen (N) fertilization and cultivar. Braun et al. (2016) reported that more sugars accumulated in potato leaves when N rate increased from 0 to 300 kg N ha^{-1}. Potato cultivar is also an important factor to affect the carbohydrates concentrations in leaves. Lafta and Lorenzen (1995) found that potato cultivars Norchip and Up-to-Date have

different carbohydrates accumulation capacity because they have different sensitivity to climatic conditions, such as air temperature.

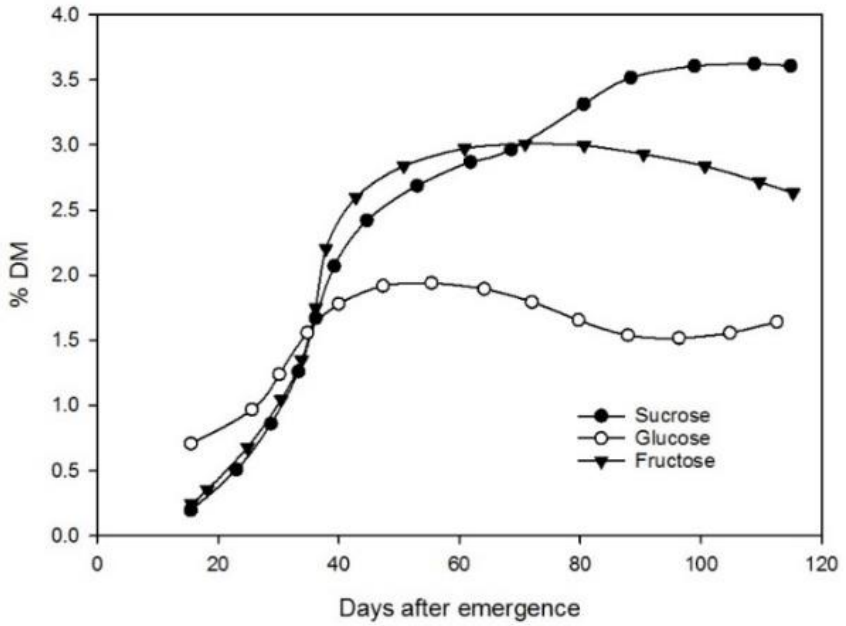

Figure 1- 2 Variations in sucrose, glucose, and fructose in potato foliage as a function of days after emergence (Kolbe and Stephan-Beckmann 1997). DM, dry matter

The quantity of amino acids in potato foliage varies seasonally. According to Cibula et al. (1967), the content of amino acids is one to five times higher in young potato plant foliage than in senescent foliage. A similar result was obtained by Domek et al. (1995), who indicated that concentrations of amino acids are higher in young than in older foliage. However, Kolbe and Stephan-Beckmann (1997) showed that the contents of amino acids increase at the end of the growing season owing to the catabolism of protein in the leaves. To explore the variation in amino acid content, Karley et al. (2002) compared young and old potato leaves and indicated that young plants are mainly composed of non-essential amino acids (glutamine, asparagine, serine, and threonine), while old foliage is dominated by the essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine). In practical terms, the metabolism of amino acids in potato foliage is a complex process that depends on various elements, such as weather conditions and mineral fertilizers (Miflin and Lea 1977; Foyer et al. 2003; Muttucumaru et al. 2013).

The major glycoalkaloids found in potato plants are a mixture of α-chaconine and α-solanine (Friedman 2004) and their concentration ratios range from 2:1 to 7:1 (Speijers 1998; Bejarano et al. 2000). In potato leaves, the glycoalkaloids reach their maximum concentration early in the growing season and decreases markedly thereafter in older leaves (Peferoen et al. 1981). The highest levels of glycoalkaloids are found in the tissues of

new leaves, fruits, flowers, and sprouts because they have the highest metabolic activity (Friedman et al. 1997). The concentration of glycoalkaloid in plants is partially sensitive to stress, such as mechanical wounding and light exposure (Petersson et al. 2013).

Inorganic salts are a crucial part of plant nutrition and their presence plays an important role in the healthy growth of plants. An excess or deficiency of inorganic salts in potato tissues may disturb the metabolism or favor plant pathogens. Taking potassium as an example, Subhani et al. (2015) investigated three potato cultivars (FD 8-1, N-22, and SH788) and found a decreasing tendency over time in all potato cultivars. Although other mineral elements are also important in potato plant resistance, there is not much related information from previous research.

1.6 Roles of chemical composition in the management of the Colorado potato beetle

1.6.1 Leaf surface chemicals

Many kinds of volatile chemicals can be biosynthesized in plant leaves, releasing different odours. These odours play an important role for herbivorous pests searching for food sources. Regarding the CPB, this insect pest begins searching for food immediately after emergence from the ground in spring. It identifies the different odours through its olfactory system and then locates the preferred plants. It is well established that these odours come from volatile chemicals in the plants (Wilde et al. 1969).

It is well known that the CPB prefers potato crops, followed by eggplants and tomatoes (Hitchner et al. 2008). The main reason is probably that different plants contain different volatile chemicals and release different odours. Some odours can enhance the interest of the beetles, and others may be repulsive to them. Mitchell and McCashin (1994) studied the response of galeal sensilla to volatile chemicals (Figure 1-3) and found that the primary alcohols (hexanol and heptanol) and other components, such as the monounsaturated (Z)- and (E)-isomers of hexen-1-ol and (*E*)-2-hexenal strongly appeal to the CPB. However, some other volatiles, such as β caryophyllene and β-selinene, produced little response in the CPB (Visser 1979; Dickens 2002). On the other hand, the quantity of volatiles in plants is also important during host plant recognition. It is well known that the CPB shows different degrees of attraction depending on the age and damage conditions even in the same plants. Bolter et al. (1997) found that the CPB prefers potato plants of 5 to 6 weeks old over those of 2 to 3 weeks old, and the damaged plants become more attractive to CPB than undamaged plants (Schuetz et al. 1997). This could be explained by the larger quantity of volatile chemicals in injured plants, which is seven to ten times higher than in healthy plants (Bolter et al. 1997). Overall, the relationship between volatile chemicals and the CPB's feeding behavior is complex. Thiery and Visser (1995) indicated that starved beetles showed more sensitivity to potato volatile chemicals compared with fed beetles, and some starved CPBs were even

attracted to undamaged potato plants (Visser 2011). This phenomenon suggests that hunger is also an important factor in determining the final food source selection for the CPB. Further studies should be conducted to consider all aspects of climate, plant cultivation, and the life habits of the CPB related to the search for food.

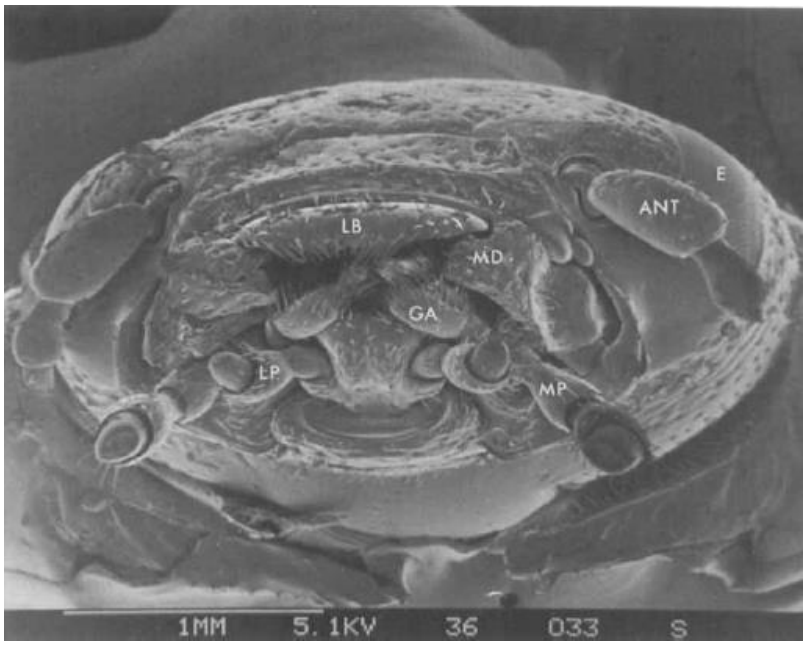

Figure 1- 3 Scanning electron micrograph showing the front view of the head of an adult Colorado potato beetle, with mandibles and antennal flagella removed. ANT, antennal scape; LB, labrum; MD, base of mandible; MP, maxillary palp; LP, labial palp; GA, galea; E, compound eye (Mitchell and Harrison 1984)

After CPB arrives on the host plants, its feeding begins to be closely related to the cuticular waxes on the leaf surface. Szafranek et al. (2008) stated that cuticular waxes may be involved in host plant recognition by the CPB. Prüm et al. (2013) reported that cuticular waxes covering the plant leaf surface have strongly reduced the ability of insects to cling to them. The possible reasons are (1) the cuticular waxes may make the leaf surface smooth, preventing the beetles from gripping firmly; and (2) the waxes have anti-adhesive properties and they can interact with the insect's adhesive fluid, resulting in a slippery leaf surface. However, Harrison (1987) indicated that some cuticular waxes, such as sterol of cholesterol, b-sitosterol, and stigmasterol, stimulate CPB feeding. Szafranek et al. (2008) studied the cuticular wax composition of potato leaves and found that alkanes, sesquiterpene hydrocarbons, wax esters, benzoic acid esters, fatty acid methyl, ethyl, isopropyl and phenylethyl esters, aldehydes, ketones, methyl ketones, fatty acids, primary alcohols, b-amyrin, and sterols did not affect adult CPB feeding. Also, alkanes, sesquiterpene hydrocarbons, wax esters, methyl ketones, sesquiterpene alcohols, and secondary alcohols had no effect on larval CPB feeding.

Overall, foliage chemicals, including volatiles and cuticular waxes, are the critical components in the host plant search and recognition process of the CPB. These chemicals are also influenced by many factors, such as the cultivar (Szafranek and Synak 2006), the circumstance conditions (temperatures, water stress, and photosynthetic radiation), and plant damage (Shepherd and Griffiths 2006).

1.6.2 Carbohydrates

The CPB adults begin feeding on the leaves immediately after successfully damaging the cuticular waxes on the leaf surface. During this process, the nutrients in host plant foliage are essential for CPB growth. Hsiao and Fraenkel (1968) showed that nutrients can elicit marked feeding responses in CPBs and that the absence of specific food elements in plant tissues can inhibit the beetles' growth. Sugars, a series of carbohydrate nutrients in potato foliage, play a significant role in CPB development and growth (Weeda et al. 1979). This significant role was mainly referred to as (1) promoting feeding behaviors (Mitchell 1974); (2) serving as an energy source during flight (Arrese and Soulages 2010); (3) providing energy for CPB diapause (Lefevere et al. 1989); and (4) tolerating thermal stress, particularly in high latitudes (Storey 1997). The importance of sugars to CPB feeding was studied decades ago. A summary of the effects of sugars on the CPB feeding is presented in Table 1-1.

Table 1- 1 Effects of sugars on the feeding of the Colorado potato beetle.

Chemical composition	CPB stage	Role	Activity	Source
Glucose	Larvae	Stimulation of feeding	0.01 mol/L[a]	Hsiao and Fraenkel 1968
Sucrose				Hsiao and Fraenkel 1968, Mitchell 1974
Maltose				
Arabinose			0.1 mol/L	Hsiao and Fraenkel 1968
Raffinose				
Rhamnose		No stimulation	—	
Ribose				
Xylose				
Fructose				

[a]Hsiao and Fraenkel (1986) used 9 mL of agar medium (4% bacto-agar and 4% cellulose) mixed in a 5.5-cm petri-dish with the test substance dissolved in 1 mL distilled water while Mitchell (1974) used a final concentration of 2% agar-cellulose in 0.08 M NaCl instead of 4% agar-cellulose.

The quantity and quality of sugar-related compounds in potato foliage have a significant effect on the feeding behavior of the CPB. Hsiao and Fraenkel (1968) conducted an agar-medium culture test to examine the response of CPB feeding performance to various sugars and related compounds. They reported that sucrose can stimulate and promote feeding behavior in the CPB, while fructose and glucose have little effect on the development of the CPB. These results may be attributed to the sensitivity of the sensilla, present on the galeae of the CPB (Figure 1-3). There are many sensilla and all of them are sensitive to sucrose (Mitchell and Harrison 1984). Therefore, some sugars can enhance the feeding of the CPB and provide energy for the beetle's growth.

A previous study on the variation of sugars in potato foliage showed that the sucrose content increases throughout the potato growing season, with a sharp increase in the first 40 days after potato plant emergence (Kolbe and Stephan-Beckmann 1997); that increase could be related to the rapid development of CPB populations from the larval stage to adulthood (Hsiao and Fraenkel 1968). However, fructose and glucose were found to have little effect on CPB development (Hsiao and Fraenkel 1968).

For the CPB, flight is more important than walking for the colonization of new habitats and also for escaping from hostile environments (Weber and Ferro 1994). It is well established that flight muscles meet their energy requirements by carbohydrate consumption. Weeda et al. (1979) reported that carbohydrates such as glycogen and glucose are decreased in the flight muscles of the CPB, which indicates that these carbohydrates provide energy for flight. Furthermore, the glucose concentration in the flight muscles of the CPB was found to be reduced during starvation as well, which supports the idea that CPB flight muscles have the capacity to utilize carbohydrates when there is a lack of food (Weeda et al. 1979). Lack of necessary carbohydrates may incapacitate the CPB during flight for food searches (Arrese and Soulages 2010). Moreover, insufficient energy sources may shorten the diapause period and result in death under unfavorable conditions (Lefevere et al. 1989). Further studies should be conducted to clarify the impacts of potato leaf carbohydrates on CPB growth for the purpose of reducing its population.

1.6.3 Amino acids

1.6.3.1 Protein amino acids

Protein amino acids refer to the basic 20 standard genetic code amino acids and are the building blocks of protein biosynthesis in organisms (Lu and Stephen 2006). It is well known that protein amino acids are essential for the CPB as well (Dortland and de Kort 1978). The actual concentration of amino acids reflected the steady state among protein synthesis, proteolysis, and transport processes to and from the organs involved. Tomlin and Sears (1992) reported that CPB populations vary greatly when they receive different categories and concentrations of amino acids. If amino acids are deficient, some problems could occur in terms of protein metabolism and some protein deficiency diseases in beetles may appear subsequently (Akram et al. 2011). The lack of essential amino acids in the tissues of potato leaves may have detrimental effects on CPBs growth, including lengthening of the pupation period (Cibula et al. 1967).

Protein amino acids also affect the feeding behavior of CPBs. Hsiao and Fraenkel (1968) reported that protein amino acids could serve as effective feeding stimulants for the CPB. Several aliphatic amino acids (glycine, alanine, serine, and valine), the sulfur-containing amino acid cysteine, and the heterocyclic amino acid proline can elicit marked feeding responses and promote CPB development (Hsiao and Fraenkel 1968). Some amino acids (leucine and isoleucine) could serve as stimulants to promote the beetle's growth when their content in

potato foliage reaches a relatively high level (Hsiao and Fraenkel 1968; Domek et al. 1995). In addition, protein amino acids are beneficial for the CPB during diapause and flight. For example, proline might have a cryoprotective function during diapause (Lefevere et al. 1989). It can also provide energy during the CPB's flight in search of food. Brouwers and de Kort (1979) observed a significant decrease in the proline and glutamate content during flight with a concomitant accumulation of alanine. This indicates that proline and glutamate stored in the muscles may represent two energy amino acids for the CPB, and they form alanine subsequently through the transamination pathway. However, not all amino acids can induce such positive stimulation. The amino acids lysine, arginine, and histidine were found to have little or no effect on the feeding behavior of the CPB (Hsiao and Fraenkel 1968). It was concluded that protein amino acids are essential for CPB growth, influencing feeding and flight behaviors. Although the CPB has been evolving and adapting to diverse climates, it also requires a considerable amount and high quality of protein amino acids for its health. A better way to control the CPB may be to find a cultivar that accumulates more protein amino acids in the tubers and less in the host plant leaves.

1.6.3.2 Non-protein amino acids

Generally, the non-protein amino acids are analogs or derivatives of genetic code amino acids. They are common in plants and are usually used to protect plants against insects (McSweeney et al. 2008). The toxicity of non-protein amino acids is related to their ability to replace a protein amino acid in a metabolic pathway or biological process after being absorbed by insects. They are easily misincorporated into proteins, making them non-functional or toxic, although the protein synthesizing machinery can discriminate between the protein and non-protein amino acids. Some non-protein amino acids can even block the synthesis or uptake of protein amino acids (Singh 2018).

Canavanine, an arginine analog, plays a pivotal role in plant chemical defense against herbivorous insects (Rosenthal 2001). Nakajima et al. (2001) stated that canavanine is highly toxic to a wide range of organisms including bacteria, fungi, algae, and insects. Incorporating canavanine instead of arginine is considered as a major mode of action and would produce structurally aberrant proteins and then bring toxicity to insects, such as Manduca sexta and Heliothis virescens (Rosenthal and Dahlman 1986; Berge et al. 1986). However, little information was found in the literature about the toxicity of canavanine to the CPB. Due to its toxicity to many other insects, more studies should be carried out to investigate the relationship between canavanine and the CPB growth and feeding behaviors.

The GABA is another important non-protein amino acid. Huang et al. (2011) reported that the presence of GABA in plants could reduce the growth and survival of herbivorous insects. The deleterious effects of GABA on insects result from the inhibition of GABA-gated chloride channels that are important in the peripheral nervous system of insects (Hosie et al. 1997). However, this inhibition may not be applicable to the CPB. Mitchell (1974)

compared the responses of fourth instar larva to GABA and found that it was slightly more stimulatory than other amino acids in the same concentrations. This stimulation was caused by the presence of amino acid-sensitive cells in the sensilla on the galea and palpal tips (Figure 1-3) (Mitchell and Harrison 1984). Over time, the CPB had already succeeded in developing resistance to toxic proteins. Rivard et al. (2004) studied CPB resistance to proteinase inhibitors. These inhibitors can damage the digestive proteolytic functions, which can be recovered after several generations. The development of such a resistance suggests that using non-protein amino acids to control the CPB may be limited under natural conditions. The effects of protein and non-protein amino acids on the feeding of CPB are presented in Table 1-2.

1.6.4 Glycoalkaloids

The α-chaconine and α-solanine are two major components of glycoalkaloids, which are a series of secondary metabolites and are produced in every plant organ. Generally, glycoalkaloids serve as stress metabolites or phytoalexins for protection against insects (Singh 2018). Sablon et al. (2013) mentioned that glycoalkaloids in plant foliage can inhibit the feeding behavior and performance of the CPB. A similar result was obtained by Jonasson and Olsson (1994), who indicated that the high levels of glycoalkaloids in host plant foliage are the key factor in preventing larval feeding and leaves with lower glycoalkaloid levels are more susceptible to attack by beetles. However, Kowalski et al. (1999) reported that neither α-chaconine nor α-solanine at concentrations commonly found in potato foliage impaired CPB feeding performance. When they reach a high level (about 7 mg g^{-1} fresh weight), the glycoalkaloids can act as feeding deterrents for the CPB (Sinden et al. 1986; Sablon et al. 2013).

Compared with α-chaconine and α-solanine, another glycoalkaloid of leptine I is more toxic. When the concentration reaches 0.55 mg g^{-1} fresh weight, leptine I can reduce the CPB feeding at about 60%, and the fatal concentration was 1 mg g^{-1} fresh weight (Kowalski et al. 1999). Another alkaloid of α-Tomatine reduced adult feeding by 50% at 2.0 mg g^{-1} fresh weight.

Table 1- 2 Effects of amino acids on the feeding of the Colorado potato beetle.

Chemical composition	CPB stage	Role	Activity	Source
Protein amino acids				
Essential amino acids				
Valine	Larvae	Stimulation of feeding	0.01 mol/L[a]	Hsiao and Fraenkel 1968, Mitchell 1974
Threonine	Larvae, adults		0.01 mol/L, —, —, 0.0008 mol/L	
Isoleucine				
Histidine			0.0004 mol/L	
Lysine			0.0007 mol/L	
Arginine			0.0006 mol/L	
Leucine			—, 0.0008 mol/L	
Phenylalanine	Larvae	No stimulation	—	Hsiao and Fraenkel 1968
Methionine				
Non-essential amino acids				
Alanine	Larvae	Stimulation of feeding	0.001, 0.0008 mol/L	Hsiao and Fraenkel 1968, Mitchell 1974
Asparagine			0.0007 mol/L, —	
Tyrosine			0.0004 mol/L, —	
Serine				Hsiao and Fraenkel 1968
Glycine			0.01 mol/L	
Proline				
Aspartic acid			—	
Cysteine				
Glutamic acid			0.001 mol/L	Mitchell 1974
Non-protein amino acids				
L-Canavanine	—	Formation of aberrant toxic protein	—	Nakajima et al. 2001
GABA	—	Reduction of growth and survival	—	Huang et al. 2011
	Larvae	Stimulation of feeding	0.01 mol/L	Mitchell 1974

[a]Hsiao and Fraenkel (1986) used 9 mL of agar medium (4% bacto-agar and 4% cellulose) mixed in a 5.5-cm petri-dish with the test substance dissolved in 1 mL distilled water while Mitchell (1974) used a final concentration of 2% agar-cellulose in 0.08 M NaCl instead of 4% agar-cellulose.

Glycoalkaloid-related compounds can affect insects at all levels of biological organization by disturbing cellular and physiological processes, such as by altering the redox balance, hormonal regulation, and neuronal signalization, or reproducing in exposed individuals (Chowński et al. 2016). Glycoalkaloids can act as a cell membrane-disrupting factor to inhibit the activity of beetles (Friedman et al. 1997). Furthermore, these glycoalkaloid-related compounds can disorder the neurons of the chemosensory hairs on the galeae of the CPB and result in the inhibition of the CPB's feeding behavior (Hollister et al. 2001). Sinden et al. (1986) believed that

the toxicity of α-chaconine and α-solanine to CPB adults and larvae was due to the acetylation after being absorbed by the beetles. Leptine is transformed into leptinine through the loss of the acetyl group after entering the insects' digestive system, reducing its feeding activity (Kowalski et al. 1999). The approach of using glycoalkaloids for CPB management is limited because the CPB would develop resistance to glycoalkaloids. Lyytinen et al. (2007) studied the glycoalkaloid concentrations in three potato varieties and observed no significant difference in beetle performance related to the glycoalkaloid content of the potato. Moreover, Armer (2004) showed that fourth instar CPB larvae and adults neither sequester nor metabolize glycoalkaloids (solanine and chaconine). However, many attempts at increasing glycoalkaloid contents of potato foliage have been made in order to reduce CPB populations. Lafta and Lorenzen (2000) found that high air temperature of 32°C could enhance potato foliar glycoalkaloid concentration up to 169% of that at 27 °C. Additionally, N fertilization also increased glycoalkaloid concentration in potato plants (Mondy and Munshi 1990). Thus, air temperature and mineral fertilization may influence the CPB behavior through altering glycoalkaloid content of potato leaves. As with chemical insecticides, glycoalkaloids have to be carefully managed, and their activities against various target and non-target species should be further studied. Table 1-3 summarizes the effects of glycoalkaloids on the feeding of the CPB.

Table 1- 3 Effects of glycoalkaloids on the feeding of the Colorado potato beetle.

Chemical composition	CPB stage	Role	Activity	Source
Atropine	—	Inhibition of feeding	7.38 μg cm^{-2}	González-Coloma et al. 2004
Leptine	—	Inhibition of feeding	8.2 mg g^{-1} dry weight of leaf	Rangarajan et al. 2000
Leptine I	—	Inhibition of feeding	0.01-1 mM	Hollister et al. 2001

1.6.5 Mineral elements

Mineral elements are a crucial part of plant nutrition and their presence plays an important role in plant growth. An excess or deficiency in potato tissues may disturb the metabolism or favor plant pathogens (Wang et al. 2013). Minerals also play an important role in protecting plants from pests. For example, potassium (KCl and KH_2PO_4) and sodium (NaCl) in potato leaves can act as co-factors of phagostimulants and enhance CPB feeding (Hsiao and Fraenkel 1968). Alyokhin et al. (2005) reported that boron has a strong negative effect on all beetle stages except for the overwintered adults, while zinc had a consistently positive effect. Mineral elements, such as phosphorus, iron, and manganese are also important in controlling the CPB and they can explain 40–57% of the variation in CPB populations because they are crucial factors in maintaining an optimal nutrient balance in plants, which can result in resistance to herbivory (Alyokhin et al. 2005). These minerals are inactive alone but act synergistically with other feeding stimulants. Furthermore, Ballan-Dufrançais (2002) mentioned that the insects possess mineral bioaccumulation structures in the cells of numerous organs. Currently, the mineral

accumulation mechanism in insects is still unclear. Further studies should focus on the roles of mineral elements in controlling the CPB feeding behavior.

1.7 Summary and future perspectives

This review investigates the response of CPB to potato foliar chemical composition. It showed that these chemical composition can stimulate or inhibit the feeding behaviors of the CPB. Volatiles and cuticular waxes on the leaf surface affect the recognition of the host plants by the CPB after their emergence from the ground. Sugars and protein amino acids can stimulate feeding behaviors and increase the survival rate of the CPB. Non-protein amino acids are used to biosynthesize toxic proteins and then damage the digestive functions of the CPB. High concentrations of glycoalkaloids can disorder the CPB's neural system and then reduce its feeding capability. Finally, mineral salts are co-factors in stimulating the feeding behaviors of the CPB while working with other stimulants.

Today, research is still being carried out to find effective alternatives to chemicals to control the CPB. Using nutrients in the host plants combined with naturally produced toxic chemicals to alter feeding behaviors and eventually reduce the beetles' population is a promising alternative to the use of chemical insecticides to manage CPB populations. Managing the chemical composition of potato leaves is important to increase the plant resistance to CPBs. When nutrients are deficient, such as amino acids and sugar, the CPB cannot biosynthesize enough protein and also lacks energy for its activity and diapause. Sufficient toxic chemicals (non-protein amino acids and glycoalkaloids) can damage the digestive function, which benefits CPB control. Therefore, CPB populations may be reduced significantly if a way to accumulate more toxic chemicals and fewer nutrients in host leaves is found. Many measures can be taken to alter the chemical composition of potato leaves. Using mineral fertilization, particularly N fertilizer, is a basic attempt because of the significant role of N in plant metabolism. Additionally, keep in mind that it is critical to balance the mineral fertilizers input which can dramatically enhance the host plant's resistance to pests. Another important measure is potato cultivar selection. Differences in the chemical composition of different host plants are basically dependent on genotype. In this literature, it is reported that some wild Solanum species have much higher levels of resistance sources, such as glycoalkaloids, against the CPB than potato cultivars (Friedman 2006). Therefore, intercrossing two potato cultivars may vary the levels of nutrients and toxic compounds and then improve the host plant's resistance. An innovative potato cultivar will probably accumulate a large fraction of beneficial nutrients in the tubers and toxic metabolites in the leaves. Many other factors, such as air temperatures and rainfall, also considerably influence the responses of CPB to host plants. In practice, the manipulation of potato foliar chemical composition is a complicated process, because the metabolism process in plants is really complex and is dependent on various climatic and cultivation conditions. More studies need to be carried out to investigate how the chemical composition of potato foliage can be varied without altering the quality and yield of tubers.

1.8 Acknowledgments

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Chapter 2 Nitrogen fertilization effects on the leaf chemical concentrations in Russet Burbank potato

Guoqi Wen, Athyna N. Cambouris, Annick Bertrand, Noura Ziadi, Haixiao Li, Mohamed Khelifi

This chapter contains pertinent results related to sugar and glycoalkaloid concentrations in Russet Burbank leaves when different N rates are applied under field conditions. This chapter was published in the journal of Field Crops Research.

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2.1 Résumé

Le doryphore de la pomme de terre (DPT), *Leptinotarsa decemlineata* (Say), est un ravageur destructif des cultures de pommes de terre (*Solanum tuberosum* L.). Les sucres foliaires sont considérés comme des sources énergétiques pour la croissance du doryphore, tandis que les glycoalcaloïdes sont utilisés pour la modification des comportements alimentaires du doryphore. La voie métabolique de ces composés foliaires est étroitement liée à la nutrition azotée. De plus, l'azote joue un important rôle dans l'obtention de rendements élevés en tubercules. L'objectif de cette étude était d'évaluer les effets de la fertilisation azotée sur les concentrations en sucres et en glycoalcaloïdes dans les feuilles de pommes de terre ainsi que sur le rendement en tubercules vendables dans la région de l'Est du Canada. Des expériences ont été réalisées sur trois sites et cinq doses d'azote (0, 60, 120, 180 et 240 kg N ha^{-1}) ont été appliquées selon un dispositif en blocs complètement aléatoires. Le cultivar de pomme de terre Russet Burbank a été utilisé. Vingt plants ont été sélectionnés au hasard dans chaque parcelle et la quatrième feuille au-dessus de chaque plant a été prélevée à 40, 54, 68 et 82 jours après la plantation afin d'analyser les concentrations en sucres (saccharose, glucose et fructose) et en glycoalcaloïdes (α-solanine et α-chaconine). Le rendement en tubercules vendables a été déterminé ainsi que l'accumulation en N de la plante entière (fanes + tubercule). Une diminution significative du contenu moyen en sucre total, incluant le saccharose et le glucose, a été observée avec des doses croissantes d'azote. La concentration moyenne en glycoalcaloïde total, dont l'α-chaconine, a augmenté significativement en réponse à l'augmentation des doses d'azote. D'après nos résultats, une dose de 205 kg N ha^{-1} a été identifiée pour l'obtention d'un rendement maximal en tubercules vendables ainsi que des concentrations élevées de glycoalcaloïdes et de faibles concentrations en sucres susceptibles d'inhiber l'alimentation du DPT.

Mots clés: *Solanum tuberosum* L., Ravageurs herbivores, Lutte antiparasitaire, Glycoalcaloïdes, Sucres.

2.2 Abstract

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) is a destructive pest of potato (*Solanum tuberosum* L.) crops. Leaf sugars are regarded as energy sources for the growth of the CPB, while glycoalkaloids are used to suppress the feeding behaviors of the CPB. The metabolic pathway of these leaf compounds is closely related to nitrogen (N) nutrition. Additionally, N plays an important role in determining high tuber yields. The objective of this study was to investigate the effects of N fertilization on the concentrations of sugars and glycoalkaloids in potato leaves and on marketable tuber yield under field conditions in eastern Canada. Experiments were conducted at three sites and five N rates (0, 60, 120, 180, and 240 kg N ha^{-1}) provided in a randomized complete block design. The potato cultivar "Russet Burbank" was used throughout the experiments. Twenty potato plants were randomly selected in each plot and the fourth leaf from the top of each plant was collected at 40, 54, 68, and 82 days after planting for analyzing the concentrations of sugars (sucrose, glucose, and fructose) and glycoalkaloids (α-solanine and α-chaconine). Marketable tuber yield was determined along with the whole plant (vine + tuber) N accumulation. A significant decline of the averaged total sugar, including sucrose and glucose, was observed with increasing N rates. The average concentration of total glycoalkaloid, including α-chaconine, increased significantly in response to the increasing N rates. Based on our results, a rate of 205 kg N ha^{-1} was identified to obtain a maximum marketable tuber yield along with high concentrations of glycoalkaloid and low sugar concentrations which may inhibit the CPB feeding.

Keywords: *Solanum tuberosum* L., Herbivorous pests, Pest management, Glycoalkaloids, Sugars.

2.3 Introduction

Potato (*Solanum tuberosum* L.) is the fourth most important crop in the world after rice (*Oriza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.), and has historically contributed to food and nutrition security (FAO, 2015). In 2016, potatoes were cultivated on more than 135,000 ha of arable land in Canada with a yield of 4.7 million tonnes (Statistics Canada, 2016). A large proportion of potato production in Canada is grown for French fries, with a smaller proportion for seed and the table market (Zebarth et al., 2012).

Colorado potato beetle (CPB) is a defoliating pest that can adversely affect potato yield and quality. The CPB is recognized as the most destructive pest of potato crops in Canada (Boiteau et al., 2008) and can completely destroy potato crops if left uncontrolled (Alyokhin, 2009). Approximately 40 cm^2 of potato leaves can be consumed by a single CPB during the larval stage, and 10 cm^2 of leaves are eaten per day during the adult stage (Ferro et al., 1985). In North America, potential yield losses due to the potato defoliation by the uncontrolled CPB populations have been estimated at around 30–50% (Boiteau, 2010).

Many efforts have been made to reduce the CPB population in potato crops. Chemical insecticides have historically served as the main method used to control the CPB, and they are likely to remain the predominant approach for the foreseeable future (Alyokhin, 2009). However, over the years, the CPB has developed resistance to most registered chemical insecticides, making it difficult to control (Alyokhin et al., 2008). The United States Department of Agriculture estimated that potato growers spent $75 to $100 million annually to control CPB (Perlak et al., 1993). Furthermore, the widespread use of toxic insecticides has already caused serious human health and environmental problems (Weisz et al., 1994). Thus, economical and environmentally friendly alternative methods of CPB control need to be developed.

Potato foliage is the major food source for CPB growth. High levels of chemicals such as sugars and glycoalkaloids in potato leaves can influence the behavioral responses and physiological processes of these beetles. Many studies reported that sucrose in potato foliage is a feeding stimulant which causes marked feeding responses in the CPB with a threshold value of 0.001 mol L^{-1} (Hsiao and Fraenkel, 1968; Mitchell and Harrison, 1984). The stimulant elicits a response from the galeal sensilla in the mouthparts of potato beetles, which contains nerve cells that are sensitive to sucrose in plant crops (Sen and Mitchell, 1987). Furthermore, the sugars (mainly glucose) in potato foliage provide the energy required for CPB flight (Weeda et al., 1979), which is the main way for the beetles to colonize new habitats and escape from hostile environments (Weber and Ferro, 1994). Glucose also provides energy for CPB metabolism, particularly during the starvation period (Weeda et al., 1979). Other important chemicals in potato foliage that are commonly regarded as toxic to herbivorous beetles are the secondary metabolites glycoalkaloids, which are synthesized when the alkaloid solanidine binds to carbohydrate moieties.

Glycoalkaloids are biosynthesized in various plant tissues, including fruit, leaves, stems, tubers, sprouts and damaged tissues (Percival and Dixon, 1996). The concentration of glycoalkaloid in plants is partially sensitive to stress, such as mechanical wounding, light exposure, and heat (Petersson et al., 2013). Jadhav et al. (1981) reported that approximately 95% of total glycoalkaloid in potato plants were found in tubers and vegetative parts depending on the cultivar, with leaves having a glycoalkaloid concentration 10 times greater than that in tubers (Friedman and Dao, 1992). Therefore, glycoalkaloids in potato foliage may influence the CPB behaviors. Chowński et al. (2016) observed that these chemicals can affect herbivorous insects at all levels of biological organization through the disruption of cellular and physiological processes, e.g., by altering redox balance, hormonal regulation, neuronal signalization, or reproduction. Glycoalkaloids also have an adverse effect on the central nervous system and the digestive system of insects (Hollister et al. 2001; Friedman and Levin 2009). So far, most commercial potato varieties are susceptible to the CPB infection, including the Russet Burbank frequently used in North America (Tai et al., 2015). New approaches to improve the resistance of Russet Burbank to the CPB include modification of gene expression, leading to an alteration of leaf metabolites through genetic engineering or intercross with some wild resistance varieties (Perlak et al., 1993; Tai et al., 2015).

Nitrogen (N) is an important macronutrient for potato production in affecting the tuber yield and quality. Sufficient N supply is required to achieve economically viable potato yields and to meet quality targets for processing potato production (Cambouris et al., 2016). However, excess N application increases the potential for N leaching, resulting in lower N use efficiency. Nitrogen fertilization may also have significant impacts on the dynamics of potato pests (Boiteau et al., 2008) because it can alter foliar chemical composition and distribution (Vos and van der Putten, 1998). Braun et al. (2016) reported that potato plants fertilized with a low N rate accumulated smaller amounts of soluble sugars in their leaves than adequately fertilized plants, probably due to the positive effect of N fertilization on photosynthetic capacity (Evans, 1989). Fragoyiannis et al. (2001) reported that a high N fertilization rate reduced the glycoalkaloid concentration in potato leaves. However, Jansson and Smilowitz (1985) observed that N application had a positive effect on the glycoalkaloid concentration due to the storage of excess foliar N as glycoalkaloids. In practice, there are two distinct ways where the foliar chemical concentrations are altered when different N rate is supplied. One is due to higher N fertilization promoting the CO2 assimilation by providing more proteins and enzymes and the other is due to the fact that N affects the catabolic activity, resulting in changes in the net accumulation of carbohydrates in leaves (Chen and Cheng, 2003).

Although many studies have examined the effects of different N fertilization strategies on tuber yield and N use efficiency, few studies have sought to quantify variations in leaf chemical concentrations in response to N application. The objective of our study was to investigate the impact of N fertilization on the concentrations of sugar and glycoalkaloid in potato foliage (cv. Russet Burbank) and on marketable tuber yield under field

conditions. We hypothesized that N fertilizer could modify the chemical composition of potato leaves which may influence the feeding behavior of the CPB and other insect pests.

2.4 Materials and methods

2.4.1 Site description, experimental design, and management

Experiments were conducted in commercial potato fields during two growing seasons (2015–2016) at three sites near Quebec City, QC, Canada. Two sites in Ste-Catherine (46°49′N, 71°39′W) were used in year 2015 (referred to as S_1) and 2016 (referred to as S_2) and a site in Beaumont was also used in year 2015 (46°49′N, 71°4′W) (referred to as S_3). The soil in the experimental fields was a sandy loam with an average pH value (in water) of 5.2 (Hendershot et al., 2008). The mineral N, total N, and total C of the soil were determined before potato planting as they could serve as an indicator of potential N release via mineralization and the residual N level in the soil could affect of sugar and glycoalkaloid concentrations. Average pre-plant soil mineral N (NO_3-N + NH_4-N) contents in the 0–30 cm depth were 101, 60, and 40 kg N ha^{-1} and the total N contents in the 0–20 cm depth were 2.0, 1.8, and 2.5 g kg^{-1} for S_1, S_2, and S_3, respectively. The total C concentrations (0–20 cm) were 25.6, 25.8, and 23.1 g kg^{-1} for each respective site. Total precipitation was 570, 545, and 478 mm for each respective site. The monthly mean temperatures of the three sites were 13.0, 16.0, 18.5, 19.0, and 15.4°C from May to September. The Russet Burbank, a widely grown potato cultivar in Canada for French fry processing, was used at all three sites (Bethke et al., 2014; Nassar et al., 2008). No irrigation was provided during the growing seasons.

Experiments were arranged in a randomized complete block design with five N rates (0, 60, 120, 180, and 240 kg N ha^{-1}) and four replications (blocks) for each treatment at each site. Nitrogen fertilizer was first band-applied at 60 kg N ha^{-1} as ammonium sulfate (22-0-0) at planting, except for the unfertilized treatment, and the remaining N treatment was applied as calcium ammonium nitrate (27-0-0) at the hilling stage just after the first leaves collection at 40 days after planting (DAP). Then, the calcium quantities for each treatment were 0, 0, 18, 36, and 53 kg ha^{-1}. Each experimental unit consisted of six rows measuring 8 m in length, with row spacing of 0.915 m and within-row spacing of 0.43 m (total area of 43.9 m^2).

The potato crop was planted on 20 May 2015 (S_1), 20 May 2016 (S_2), and 25 May 2015 (S_3). The insecticide-treated (Titan™) seed pieces were planted and covered with approximately 0.1 m of soil using a rake. Phosphorus and potassium fertilizers were applied at planting based on local recommendations combined with soil analyses (CRAAQ 2010). Thus, 150, 150, and 120 kg P_2O_5 ha^{-1} were banded as triple superphosphate (0-46-0) at S_1, S_2, and S_3, respectively. Potassium was applied as potassium chloride (0-0-60) at 300, 230, and 300 kg K_2O ha^{-1} to the three respective sites. To protect potato plants from the CPB damage, insecticide was applied once at S_1 and S_2. Delegate™ (Dow AgroSciences Canada Inc.) was applied on 15 July 2015 (56 DAP)

using 65 g in 150 L of water per hectare at S_1. Minecto Duo™ (Syngenta Canada Inc.) was applied on 1 August 2016 (73 DAP) using a concentration of 570 g ha^{-1} at S_2. No insecticide was used at S_3 during the growing season. The potato vines were desiccated using diquat as desiccant on 14 Sept. 2015, 10 Sept. 2016, and 15 Sept. 2015 for the three sites. Then, tubers were mechanically harvested on two 5-m length central rows in each plot on 2 Oct. 2015, 3 Oct. 2015, and 6 Oct. 2016 for S_1, S_2, and S_3, respectively to determine the marketable yield.

2.4.2 Sample collection and chemical analysis

In the spring preceding each potato growing season, one composite soil sample from 10 random soil cores was taken per block at the 0–20 cm depth to determine the initial soil physical and chemical characteristics, such as soil pH value. Soil samples were immediately air-dried. For mineral N analysis, 10 soil cores were collected randomly from each block at two incremental depths (0–15 and 15–30 cm) prior to planting and then combined to provide a composite sample for each depth. The soil samples were kept frozen until analysis. Then, soil NO_3-N was extracted with 1 mol L^{-1} KCl using a 1:10 soil/extractant ratio (Maynard et al. 2008). The NO_3-N and NH_4-N concentrations in the extract were determined by automated colorimetry (Lachat Instruments, QuickChem method 12-107-06-2-A and 12-107-04-1-B). The measurement results of mineral N content at the two depths were combined and are reported here for a 0–30 cm depth. Soil bulk density was measured using the core method (Hao et al., 2008). Before planting, three random cores (6.25 cm long by 5 cm in diameter) were sampled at depths between 5–12 and 20–27 cm in the blocks to represent the 0–15 and 15–30 cm depths, respectively. The NO_3-N and NH_4-N concentrations were multiplied by soil bulk density to obtain units of kg N ha^{-1}.

Whole potato plants were harvested from the two central rows, i.e., along a 5-m length per row in each plot (2 rows × 5 m × 0.915 m= 9.15 m^2). The plants were then partitioned into two components i.e., vines and tubers, for the determination of N accumulation in each component. A 500 g subsample of each component was ground to pass through a 2 mm sieve and the subsamples were analyzed using a CN analyzer (Elementar, model Varian Macro CN, Hanau, Germany) to determine total N and total C contents in each tissue. Total plant N accumulation was determined by adding together the N accumulation in vines and tubers calculated by multiplying the yield by their respective tissue N concentrations (Cambouris et al., 2016). Marketable tuber yield (tuber diameter > 47mm and length > 76 cm, without external defects such as greening, malformation, and soft rot), was also assessed in our study (Cambouris et al., 2016).

To study the effect of the N fertilization on sugar and glycoalkaloid concentrations in the foliage, 20 potato plants were randomly selected from each plot and the fourth leaf from the top of each plant was cut with scissors and collected at 40, 54, 68, and 82 DAP. The fourth leaf is well related to nutritional status of the whole plant and is sensitive to changes in the availability of nutrients (Westermann, 1993). Samples collected 40 DAP included N

rate-treatments of 0 and 60 kg N ha^{-1} and samples collected 54, 68, and 82 DAP included N rate-treatments of 0, 60, 120, 180, and 240 kg N ha^{-1}. Leaf samples were collected around the same time in the morning to minimize the influence of photosynthesis on chemical composition (Deahl et al., 1991b). All leaf samples were immediately placed in labeled plastic bags and stored in an ice-cooled incubator. Subsequently, samples were dried in a ventilated oven at 55°C for seven days. Samples were then ground to pass a 1 mm sieve using an IKA Werke grinder (MF10BS1, IKA, USA), and stored in the dark at room temperature. Before chemical analysis, the samples were dried at 105°C using a thermogravimetric analyzer (Model TGA701, Leco Corporation).

The soluble sugars measured in this study consisted of sucrose, glucose, and fructose, which were extracted from the foliage by adding 7 mL of methanol: chloroform: water (volume ratio of 12:5:3) to 200 mg of the dried and ground leaf samples. Extracts were incubated at 65°C for 20 min, cooled in an ice-water bath, and stored overnight at 4°C. A 1 mL subsample was collected and 250 µL of water was added. After centrifugation for 15 min at 4000 rpm, 750 µL of the methanol-water phase containing soluble sugars was sampled, evaporated to dryness using a Savant evaporator (SpeedVac Plus SC2104), and then re-suspended in water. Soluble carbohydrates in this extract were analyzed using a Waters High-Performance Liquid Chromatography (HPLC) analytical system controlled by Empower 2 software (Waters, Milford, MA). The HPLC was equipped with a model 1525 pump, a model 2707 autosampler and a model 2414 refractive index detector (Saïed et al., 2017). Distilled water was used as the mobile phrase and sugars were separated on a Waters Sugar-Pak column at 80°C.

In this study, α-solanine and α-chaconine were analyzed because they are two principal components of glycoalkaloid in potato plants (Friedman, 2004). Approximately 200 mg of dried and ground leaf sample was extracted by using an 80 mL mixture of 1% acetic acid and 0.4% heptanesulfonic acid and stored overnight at 4°C. The mixture was then centrifuged for 15 min at 4000 rpm. The supernatant was analyzed for glycoalkaloid concentrations by HPLC (Waters, Milford, MA) using a 3.9×200 mm Resolve C18 column eluted at a flow rate of 1 mL min^{-1} using acetonitrile, heptanesulfonic acid, and phosphoric acid with a ratio of 250 mL, 0.47 g, and 1 mL, respectively, as mobile phase and detection was set to a wavelength of 200 nm (Dual Absorbance Detector, Waters 2487) (Lafta and Lorenzen, 2000).

2.4.3 Statistical analysis

Because the experimental setup was different for observations taken at 40 DAP and those observed at 54, 68, and 82 DAP, the data analyses were made separately. In this study, we analyzed the effects of the N fertilization rate on the sugar and glycoalkaloid contents of potato foliage, marketable yield, and total plant N accumulation. The site and block were treated as random effects and the N rate as a fixed effect. For foliar sugars and glycoalkaloids, a generalized randomized block design was used for the data collected at 40 DAP, including the

N rates of 0 and 60 kg N ha^{-1}. The treatment 0 kg N ha^{-1} was repeated once in each block, while the 60 kg N ha^{-1} was repeated four times. A repeated randomized complete block design was used for the data collected at 54, 68, and 82 DAP, including the N rates of 0, 60, 120, 180, and 240 kg N ha^{-1} which was repeated once on specific plots per block by using sampling date as a repeated measurement. When the effects of N rate were significant, we used the protected LSD multiple comparison to investigate the difference among treatments. Since N rate is quantitative factor, we also used polynomial contrasts to study the link between the response variables and N rate. Based on the results, mixed regression models were fitted to the data in order to estimate the linear and quadratic terms for N-rates. The maximum marketable yield was estimated by setting the first partial derivative of the quadratic N fertilization rate response curve equal to zero (Cambouris et al., 2016).

We used the Shapiro-Wilk's statistic to test the normality assumption on the residuals of each model, while residual plots were used to verify the homogeneity of variances. When the normality assumption was not met, response variables were transformed using the Box-Cox. All analyses were performed using the Mixed procedure of SAS (SAS Institute 2010) at a significance level of $p<0.05$.

2.5 Results

2.5.1 Air temperature and precipitation

The air temperature varied sharply for each site during the entire growing season (Table 2-1). The average temperatures for each respective site were 16.2, 16.1, and 16.8°C which were 1.3, 1.2, and 1.1°C warmer than the 30-year average temperatures (Table 2-1). The average temperature increased from May to August and then reduced to September for each site. The total precipitations were 570, 478, and 545 mm for S_1, S_2, and S_3 from May to September (Table 2-1). Generally, the precipitation increased from May to July and then decreased until September. However, the precipitation in S_3 showed a different pattern resulting in a maximum value in September.

Table 2- 1 Mean air temperature and total precipitation measured during the entire potato growing season (May-September) on each site.

Year	Site	Mean air temperature (°C)						Total precipitation (mm)					
		May	June	July	August	Sept.	May-Sept.	May	June	July	August	Sept.	May-Sept.
2015	S_1	13.5	15.2	18.1	18.6	15.7	16.2	89.2	125.8	138.6	105.6	110.6	569.8
	S_3	13.3	15.8	18.8	19.3	16.6	16.8	105.4	124.6	156.6	79.2	78.8	544.6
2016	S_2	12.1	16.9	18.6	19.0	13.8	16.1	90.8	93.2	88.0	95.0	110.8	477.8
30-year average†	S_1 and S_2	10.9	15.6	18.4	17.4	12.3	14.9	128.5	127.8	133.3	126.6	133.2	649.4
	S_3	11.0	16.5	19.3	18.3	13.5	15.7	100.2	122.4	130.4	108.9	120.1	582.0

S_1, S_2, and S_3 indicated the potato field sites of Ste–Catherine in year 2015, Ste–Catherine in year 2016, and Beaumont in year 2015, respectively. †The 30-year average (1986-2015) of S_1 and S_2 was based on the Jean-Lesage weather station (46°48'N, 71°22'W) and the 30-year average of S_3 was based on the Lauzow weather station (71°16'N, 46°82'W).

2.5.2 Sugar composition and concentration

A significant decrease in total sugar from 24.2 mg g^{-1} dry matter (DM) at 0 kg N ha^{-1} to 18.5 mg g^{-1} DM at 60 kg N ha^{-1} were observed, mainly due to the decrease in sucrose and a slight decrease in glucose (Table 2-2). However, there was no significant variation in fructose concentration under different N rates at 40 DAP (Table 2-2).

Table 2- 2 Effects of N fertilizer rate on the concentrations of total sugar, sucrose, glucose, fructose, total glycoalkaloid, α-solanine, and α-chaconine in the leaves of Russet Burbank potatoes at 40 days after planting.

	Total sugar	Sucrose	Glucose	Fructose	Total glycoalkaloids	α-solanine	α-chaconine
Source of variance	---------- *p* values ----------						
N rate	0.0191	0.0194	0.0290	ns‡	ns	ns	ns
N rate (kg N ha^{-1})	---------- Concentrations (mg g^{-1} dry matter) ----------						
0	24.2a§	19.5a	2.5a	2.3	3.3	0.8	2.6
60	18.5b	13.8b	2.3b	2.4	3.5	0.9	2.6

The concentrations of each foliar chemical represented the average values of three sites. ‡ns, not significant at α=0.05. § Concentrations followed by the same letter are not significantly different at α=0.05.

Nitrogen fertilization significantly affected the total sugar concentration and the sucrose and glucose levels at 54, 68, and 82 DAP (Table 2-3). Increasing N application led to a reduction in total sugar from 53.7 and 54.6 mg g^{-1} DM under the rates of 0 and 60 kg N ha^{-1} to 50.0 and 48.9 mg g^{-1} DM under 120 and 180 kg N ha^{-1}, then to 44.0 mg g^{-1} DM under 240 kg N ha^{-1}. A similar pattern was observed for sucrose and glucose.

Sampling time, including 54, 68, and 82 DAP, significantly influenced the total sugar content and the sucrose, glucose and fructose levels (Table 2-3). The amounts of total sugar, sucrose, and fructose decreased from 54

to 68 DAP, and then increased at 82 DAP. For glucose, no noteworthy variation was found between 54 and 68 DAP, and subsequently the concentration increased to 5.8 mg g^{-1} DM at 82 DAP.

Table 2- 3 Effects of N fertilizer rate and sampling date on the concentrations of total sugar, sucrose, glucose, fructose, total glycoalkaloids, α-solanine, and α-chaconine in the leaves of Russet Burbank potatoes at 54, 68, and 82 days after planting (DAP).

	Total sugar	Sucrose	Glucose	Fructose	Total glycoalkaloids	α-solanine	α-chaconine
Source of variance	---------- *p* values ----------						
N rate	0.0001	0.0004	0.0020	ns‡	0.0256	ns	0.0100
Sampling date	<0.0001	<0.0001	<0.0001	0.0001	0.0011	0.0007	0.0013
N rate*sampling date	0.0232	0.0312	0.0420	ns	0.0137	ns	0.0154
Contrasts							
N rate linear	<0.0001	<0.0001	0.0001	0.0074	0.0015	ns	0.0005
N rate quadratic	ns	ns	ns	ns	ns	ns	ns
N rate (kg N ha^{-1})	---------- Concentrations (mg g^{-1} dry matter) ----------						
0	53.7a§	45.7a	3.7a	4.3	3.6b	1.0	2.6b
60	54.6a	46.0a	4.4a	4.3	3.3b	0.9	2.4b
120	50.0b	42.8a	3.5b	3.7	4.0a	1.0	2.9a
180	48.9b	41.7b	3.6b	3.7	4.2a	1.1	3.1a
240	44.0c	36.9c	3.4b	3.8	4.5a	1.1	3.4a
Sampling date (DAP)	---------- Concentrations (mg g^{-1} dry matter) ----------						
54	53.4b	46.5b	2.7b	4.3a	3.7b	1.1a	2.6b
68	34.0c	28.3c	2.7b	3.0b	4.8a	1.2a	3.6a
82	63.4a	53.0a	5.8a	4.6a	3.2b	0.8b	2.4b

‡ns, not significant at α=0.05. §Means followed by the same letter are not significantly different at α=0.05. The concentrations of each foliar chemical represented the average values of three sites.

Significant interactions between N fertilization and sampling date were observed for total sugar, sucrose, and glucose (Table 2-3). Total sugar concentration gradually decreased with increasing N rates at 54 and 68 DAP, and a similar pattern was observed for sucrose. In contrast, a gradual increase of total sugar and sucrose was observed up to 120 kg N ha^{-1} and then a slight decrease subsequently at 82 DAP (Figure 2-1A, B). For glucose, a progressive decrease occurred when the N rates increased from 0 to 240 kg N ha^{-1} at 54 and 68 DAP. However, the glucose concentration first increased and then decreased to a plateau value at 82 DAP (Figure 2-1C).

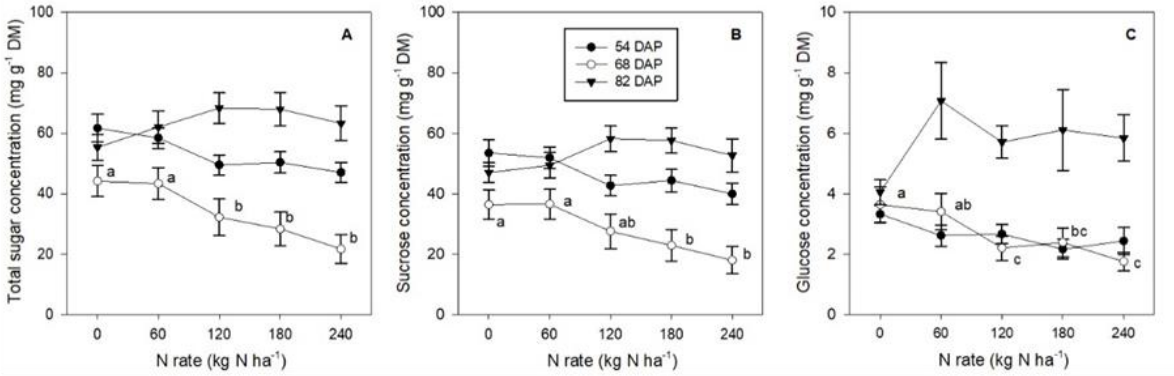

Figure 2- 1 Concentrations of (A) total sugar, (B) sucrose, and (C) glucose in the foliage of potato cv. Russet Burbank under different N fertilizer rates and sampling dates of 54, 68, and 82 days after planting

2.5.3 Glycoalkaloid composition and concentration

There was no significant effect of the N rates at 40 DAP (0 vs 60 kg N ha^{-1}) on total glycoalkaloids or on the α-solanine and α-chaconine concentrations (Table 2-2). However, N fertilization significantly influenced the concentrations of total glycoalkaloids and α-chaconine at 54, 68, and 82 DAP (Table 2-3). With increasing N fertilization, the total glycoalkaloid and α-chaconine contents increased linearly. However, no significant effect of N fertilization was observed on α-solanine.

Total glycoalkaloids, α-chaconine, and α-solanine responded significantly to sampling dates (Table 2-3). Total glycoalkaloids and α-chaconine followed an increasing trend from 54 to 68 DAP, and then decreased at 82 DAP. No significant difference was observed for α-solanine between 54 and 68 DAP. However, the α-solanine concentration showed a significant decrease, from 1.2 mg g^{-1} DM at 68 DAP to 0.8 mg g^{-1} DM at 82 DAP.

Significant interactions between N fertilization and sampling dates were observed for total glycoalkaloids and α-chaconine (Table 2-3). A slight increase in total glycoalkaloids was observed for the 60 kg N ha^{-1} application, and then a reduction until 180 kg N ha^{-1} followed by an increase for 240 kg N ha^{-1} at 54 DAP. However, a nearly opposite trend was observed at 68 DAP. At 82 DAP, a progressive increase in total glycoalkaloids was found with increasing N rate (Figure 2-2A). A similar pattern was observed for α-chaconine under different N rates at each sampling date (Figure 2-2B).

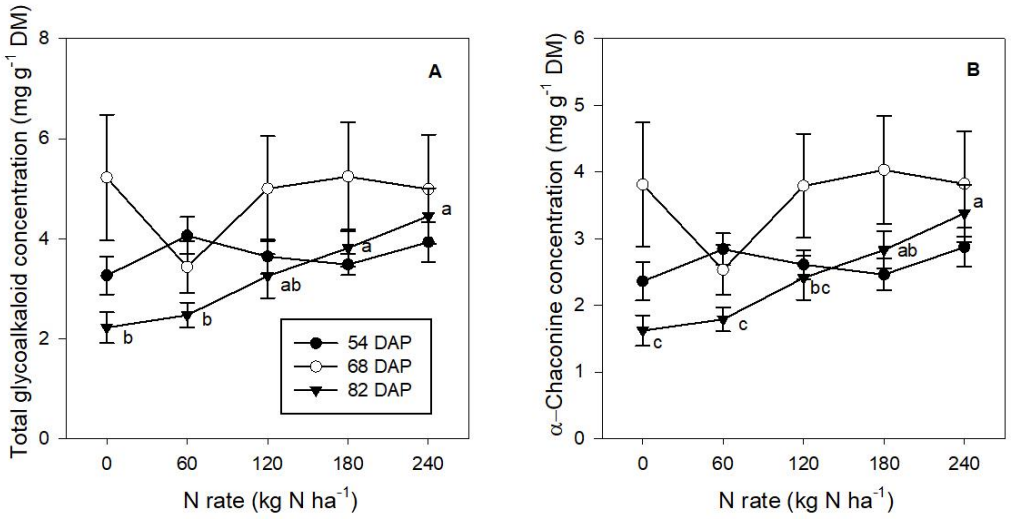

Figure 2- 2 Concentrations of (A) total glycoalkaloids and (B) α-chaconine in the foliage of potato cv. Russet Burbank under different N fertilizer rates (kg N ha^{-1}) at sampling dates of 54, 68, and 82 days after planting. Letters indicate the significant differences (p<0.05) in concentrations between different N rates on the corresponding sampling dates

2.5.4 Marketable yield

Marketable yield was significantly influenced by N fertilizer rate up to 120 kg N ha^{-1} (Table 2-4). Beyond this N rate, there was no significant increase in tuber yield with increasing N rate. In this study, marketable tuber yield ranged from 16.4 to 35.4 Mg ha^{-1} (Table 2-4). Based on the regression model of marketable tuber yield, we found that the optimal N rate (i.e. the N rate required to achieve maximum marketable tuber yield) was 205 kg N ha^{-1} with a maximum yield of 33.35 Mg ha^{-1} (Figure 2-3). Additionally, total plant N accumulation gradually increased from 75.7 to 186.8 kg N ha^{-1} with increasing N rates from 0 to 240 kg N ha^{-1} (Table 2-4).

Table 2- 4 Effects of N fertilizer rate on marketable tuber yield and total plant N accumulation.

	Marketable yield	Total plant N accumulation
Source of variance	---------- *p value* ----------	
N rate	<0.0001	<0.0001
Contrasts		
N rate linear	<0.0001	<0.0001
N rate quadratic	0.0010	0.0129
N rate (kg N ha^{-1})	----- Mg ha^{-1} -----	----- kg N ha^{-1} -----
0	16.4c[§]	75.7e
60	25.2b	119.3d
120	31.5a	147.1c
180	33.6a	165.0b
240	35.4a	186.8a

The marketable tuber yield and total plant N accumulation represented the average value of three sites. [§]Means followed by the same letter are not significantly different at α=0.05.

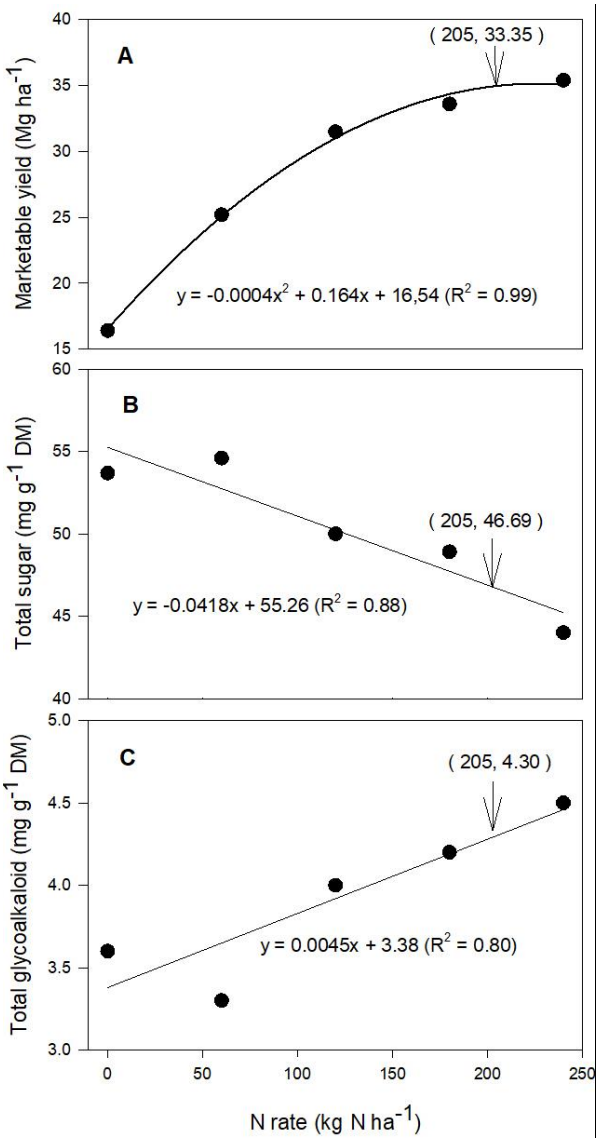

Figure 2- 3 Effects of N fertilizer rates on (A) marketable tuber yield, (B) total sugar, and (C) total glycoalkaloids. Arrows highlight marketable tuber yield, total sugar and total glycoalkaloid concentrations under the optimal N rate of 205 kg N ha^{-1}.

2.6 Discussion

2.6.1 Variation in sugar and glycoalkaloid concentrations in potato leaves

The growth of potato plants could be divided into five distinct life stages: sprout development, vegetative growth, tuber initiation, tuber bulking, and tuber maturation. At the first sampling (40 DAP), the growth stage of Russet Burbank was between vegetative growth and tuber initiation (Stark et al., 2004). During this stage, the average air temperature of three sites increased from 13°C in May to 18.5°C in July (Table 2-1). Timlin et al. (2006) reported that potato leaf appearance rate was positively and linearly related with temperature. Additionally,

moderate precipitation has a positive effect on the sugar biosynthesis (Farhad et al., 2011). This could explain the increase in sugars that we observed between 40 and 54 DAP (Table 2-2 and Table 2-3). When the plant enters the tuber bulking stage, up to 80% of photosynthetically fixed carbon is exported from leaves to tubers (Lemoine et al., 2013) in the form of sucrose which is converted into starch for tuber development (Nazarian-Firouzabadi and Visser, 2017). Liu et al. (2003) also observed that tuber starch content increased as the growth time increased during this period. Additionally, sucrose is used to synthesize starch in chloroplasts for leaf development (Lemoine, 2000). Thus, sucrose transport and conversion into starch explained the reduction of foliar sugar concentration that was observed between 54 and 68 DAP in our study. As a late-season potato cultivar, Russet Burbank had already acquired almost all the N, P, and K from the soil needed for its growth at about 80 DAP (Stark et al., 2004). Jackson and Haddock (1959) also reported that the potato growth rate reached its maximum around 95 DAP and the leaf area reached its highest value at 100 DAP for cv. Russet Burbank. Thus, there was a possibility that the sugar synthesis rate in leaves was higher than the sugar transportation rate to tubers as starch and then resulted in the foliar sugar accumulation. At that point, less sugar was required for starch synthesis in tubers when compared to the sugar photosynthesized in potato leaves (Sturm and Tang, 1999), which may explain why the sugar content in potato leaves once again increased between 68 and 82 DAP. Furthermore, the low air temperature observed after August in this study may promote the sugar accumulation since low temperature is an important abiotic stress of potato plant that could promote soluble sugars accumulation and the starch degradation (Sitnicka and Orzechowski, 2014).

Glycoalkaloids, which consist of a sugar moiety and an alkaloid, are toxic to humans and insects because of their anticholinesterase activity (Mondy and Munshi, 1990). In the potato, α-chaconine and α-solanine are the most abundant glycoalkaloids (Lachman et al., 2001). Glycoalkaloids are distributed in all plant organs but the concentrations are higher in the metabolically active parts, such as young leaves (Omayio et al., 2016). We observed that the total glycoalkaloid content in potato leaves (cv. Russet Burbank) was 3.8 mg g^{-1} DM during the entire growing season when averaged over the five N rates. This glycoalkaloid level is much lower than the concentration of 20.7 mg g^{-1} DM found in the leaves of the potato cultivar “new NDA 1725” (Friedman and Dao, 1992), but it falls within the range of 3.3 to 20.7 mg g^{-1} DM reported by Omayio et al. (2016) for a leaf moisture ratio of 93% (Liu et al., 2005). In general, α-chaconine content is higher than α-solanine in potato tissues (Omayio et al., 2016). In our study, the ratio of α-chaconine to α-solanine in the leaves of Russet Burbank was from 2.4 to 3.3, which agrees with the values ranging from 1.9 to 3.7 reported by Sinden and Webb (1974) in a study including six potato cultivars. The ratio of α-chaconine to α-solanine in Russet Burbank leaves was higher than the one reported in another study on tubers of the same cultivar (average ratio of 1.4) (Friedman and Levin, 2009). Therefore, the differences in glycoalkaloid concentrations seem to be both cultivar- and organ-dependent.

Our results showed that leaf glycoalkaloids first increased between 54 and 68 DAP and then decreased until 82 DAP (Table 2-3). Unlike sugars, glycoalkaloids do not translocate between organs within a potato plant (Hlywka et al., 1994). Thus, these variations in concentration are the result of glycoalkaloid metabolism, including anabolism and catabolism, within the leaves. Petersson et al. (2013) reported that concentration of glycoalkaloid in plants is partially sensitive to biotic and abiotic stress, such as heat, light intensity, and pest infection. Thus, the air temperature increase from May to July may have contributed to the increase in glycoalkaloid concentration between 54 and 68 DAP. Lafta and Lorenzen (2000) reported a similar 1.7 times increase in the potato foliar glycoalkaloid concentration when the air temperature increased from 27 to 32℃. On the other hand, we observed that the CPB infestation caused leaf damages around the second sampling date. This CPB attack likely stimulated the production of glycoalkaloids to protect the foliage. Pariera Dinkins et al. (2008) obtained similar results and concluded that glycoalkaloid production in potato plants was significantly greater following CPB defoliation.

2.6.2 Effect of N fertilization

2.6.2.1 Sugars and glycoalkaloids

Nitrogen fertilization is crucial for potato production because an increasing N rate can enhance tuber yield (Bélanger et al., 2000; Zebarth et al., 2012). Nitrogen fertilization can also stimulate potato leaf growth, including both leaf number and area (Millard and Marshall, 1986; Vos and Biemond, 1992). Furthermore, N is an essential element of chlorophyll and larger leaves have greater photosynthetic capacity than smaller leaves. Thus, N plays a critical role in the metabolism of sugars and N-containing glycoalkaloids in higher plants.

Vos and Biemond (1992) reported that N fertilization could significantly enlarge leaf size, suggesting that a biomass dilution effect on foliar sugar content may occur when the sugar synthesis rate is lower than the leaf growth rate. This could explain our finding that total sugar concentration decreased in leaves as N rate increased when measured at 40, 54, 68 DAP (Table 2-2 and Figure 2-1). On the other hand, we observed that N fertilization significantly increased tuber yield (Table 2-4). It is well known that starch is the major component of tubers (Bradshaw and Ramsay, 2009). At the tuber bulking stage (68 DAP), the tuber becomes the major sink for sucrose accumulation and starch synthesis (Kumar et al., 2004). Additionally, N fertilization can promote leaf and stem growth since it has a positive effect on the photosynthetic capacity of potato plants (Evans, 1989). The enlarged leaves are sinks also for sugar accumulation. Therefore, the reduction in leaf sugar associated with the increasing N rates was due in part to the translocation of sucrose from leaves to tubers to be stored as starch. In addition, high N fertilization may induce the synthesis of enzymes involved in the process of starch conversion (Koch, 2004).

As glycoalkaloids are N-containing compounds, their biosynthesis depends on N availability (Ginzberg et al., 2009; Najm et al., 2012; Friedman et al., 2017). Our results show that N fertilization increased the leaf glycoalkaloid concentrations, especially α-chaconine at the tuber bulking stage (Table 2-3), likely due to the increase in plant N accumulation. In our study, total plant N accumulation gradually increased from 75.7 to 186.8 kg N ha^{-1} with increasing N rates from 0 to 240 kg N ha^{-1} (Table 2-4). Nitrogen is an essential component of enzymes involved in the glycoalkaloid synthesis, such as glycosyltransferase enzymes (Friedman, 2006). However, Fragoyiannis et al. (2001) observed that the potato foliar glycoalkaloid concentration reduced with increasing N rates. This result is contradictory to our observations and the main reason may be due to the different cultivars which has different N requirements for optimal growth. The progressive increase of foliar total glycoalkaloid concentration at 82 DAP with increasing N rates (Figure 2-2) may be related to the previous applications of insecticide that we made in the fields at 56 DAP in S_1 and at 73 DAP in S_2. Zarzecka et al. (2013) reported that insecticides could either increase or decrease potato foliar glycoalkaloids depending on the potato cultivar and weather condition. However, the available literature lacks information on the impact of insecticides on changes in foliar glycoalkaloid content in cv. Russet Burbank. Additionally, pest infection could have played a role in modifying foliar glycoalkaloid concentrations. In our study, we observed a CPB emergence on plants at around 53 DAP in S_1. Pariera Dinkins et al. (2008) reported that glycoalkaloid concentration significantly increased while plants were undergoing defoliation by the CPB. Thus, the significant increase of foliar glycoalkaloids between 54 and 68 DAP may be linked to CPB infestation.

2.6.2.2 Marketable tuber yield

The marketable yield values found in our study, which ranged from 16.4 to 35.4 Mg ha^{-1} (Table 2-4), were slightly higher than those reported in a previous study in which similar N rates were applied as ammonium nitrate (Cambouris et al., 2016). These differences may be due in part to the higher mineral N concentration in the soil. The average soil mineral N level at the three sites was 67.2 kg N ha^{-1}, approximately twice the level found in the five-year field study conducted by Cambouris et al. (2016) with an average of 35.2 kg N ha^{-1}. Additionally, the different potato growth environments between the two studies, such as air temperatures and precipitation, could have played an important role in the tuber growth (Onder et al., 2005; Rykaczewska, 2015). In this study, we observed a quadratic increase in marketable yield with increasing N rates (Table 2-4), and the optimal N rate was 205 kg N ha^{-1} for a maximum yield of 33.35 Mg ha^{-1} (Figure 2-3). Potatoes are a high N demanding crop (Zebarth and Rosen, 2007) and N fertilization may enhance the photosynthetic capacity (Evans, 1989) and then increase the marketable tuber yield through starch biosynthesis. Starch biosynthesis is required as a mean of storing assimilated carbon (Bertoft and Blennow, 2009). This assumption is supported by our results showing that foliar sugar concentrations decreased with increasing N rates (Table 2-3), which suggests that photosynthetic-derived sugars are translocated and stored in tubers as well new stems and leaves as starch.

2.6.2.3 Role of nitrogen fertilization in CPB control

Although potato crops can tolerate a significant amount of defoliation (up to 20%) without a major impact on yields (Stieha and Poveda, 2015), the CPB can severely defoliate potato plants and thus highly reduce tuber yield. Generally, the control of CPB is mainly based on chemical insecticide applications (Alyokhin, 2009; Sablon et al., 2013). Although other approaches have also been used, such as crop rotation, straw mulch cover, biological control, transgenic plants, and RNA interference (Sablon et al., 2013), none of them has proven successful on a large scale for potato production. It is well established that the nutrients in potato leaves have noteworthy effects on CPB growth as well the overwintering survival and fecundity (Hsiao and Fraenkel, 1968; Szafranek et al., 2008) and the metabolism of these nutrients in plants is affected by N fertilization. Therefore, N fertilization may influence the CPB feeding through altering the nutrients composition of potato foliage.

As secondary metabolites, glycoalkaloids in potato foliage can protect plants against pests (Omayio et al., 2016). In this study, an increase in foliar glycoalkaloid concentration occurred with increasing N fertilization rates. Therefore, the N fertilization may play a role in preventing CPB feeding by changing the potato foliar glycoalkaloid concentrations, which were reported as well by previous researches results of Deahl et al. (1991a), Jonasson and Olsson (1994), and Sablon et al. (2013). It should be noted that N fertilization significantly increased the tuber glycoalkaloid concentration (Mondy and Mush, 1990), which could result in a bitter flavor and toxicity for humans (Friedman, 2006). We also evaluated the impact of N fertilization on marketable tuber yield and observed a quadratic response of marketable tuber yield to increasing N rates. The maximum yield of 33.35 Mg ha^{-1} was obtained when the N rate was 205 kg N ha^{-1}. This N rate is slightly higher than the recommended N rate (135 to 175 kg N ha^{-1}) in Quebec, Canada, which may be due to the fact that the sandy soils used in our study could have influenced the N uptake of potato plants (CRAAQ 2010). Since the roles of sucrose, glucose, and fructose in the CPB behaviors are similar based on our literature review (Wen et al., 2018), we only investigated the varied tendency of total sugar with increasing N rates. The same reason was considered for reporting the total glycoalkaloid. With the N rate of 205 kg N ha^{-1}, we obtained lower sugar (46.7 mg g^{-1} DM) and higher glycoalkaloid concentrations (4.3 mg g^{-1} DM) in the leaves based on our regression model, which may influence the CPB feeding behaviors to some extent.

2.7 Conclusion

Our results demonstrate that large variations in foliar chemicals as well as in tuber yield and N accumulation occur as a function of N fertilization. We observed that an increasing N rate significantly reduced sugar concentrations in potato foliage, most likely due to the high proportion of sugars synthesized in leaves that was translocated to tubers. Foliar glycoalkaloids gradually accumulated when the N rates increased from 0 to 240 kg N ha^{-1}. High N fertilizer rate also increased tuber yield and N accumulation. Based on our results, the N rate of 205 kg N ha^{-1} is recommended for the fertilization of potato plants in sandy soils for the highest marketable yield

with lower foliar sugar concentrations and higher glycoalkaloid levels which may influence the CPB feeding. Since this N rate is higher than that recommended in Quebec, Canada, N losing, particularly N leaching should be considered in the future studies. Additionally, the impacts of N fertilization with 205 kg N ha^{-1} on the actual CPB feeding and tuber quality should be addressed in future studies.

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Chapter 3 Nitrogen fertilization effects on the composition of foliar amino acids of Russet Burbank potato

Guoqi Wen, Athyna N. Cambouris, Noura Ziadi, Annick Bertrand, Mohamed Khelifi

This chapter presents important results related to amino acid concentrations variation in Russet Burbank leaves when different N rates are applied under field conditions. It was published as a scientific article in the American Journal of Potato Research.

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3.1 Résumé

Le doryphore de la pomme de terre (DPT), *Leptinotarsa decemlineata* (Say), est un ravageur important des cultures de pommes de terre. Les acides aminés foliaires de la pomme de terre jouent un rôle essentiel dans la croissance du DPT. Dans cette étude, les acides aminés ont été classés en quatre groupes en fonction de leurs différents rôles dans la promotion de la croissance du DPT. Ensuite, les effets de la dose d'azote sur les concentrations de groupes d'acides aminés ont été étudiés sous des conditions réelles au champ. Des expériences ont été effectuées avec cinq doses d'azote de 0, 60, 120, 180 et 240 kg de N ha^{-1} selon un dispositif en blocs complètement aléatoires. Vingt feuilles ont été recueillies 40, 54, 68 et 82 jours après la plantation (JAP) pour l'analyse des acides aminés. Les résultats ont démontré que la dose d'azote n'avait pas d'effet significatif sur les concentrations de chaque groupe d'acides aminés à 40 JAP. Cependant, leurs concentrations ont augmenté linéairement avec l'augmentation de la dose d'azote à 54, 68 et 82 JAP, ce qui suggère que des doses d'azote plus élevés pourraient potentiellement favoriser la croissance de DPT dans la phase d'initiation du tubercule.

Mots clés: Ravageur herbivore, engrais minéral, assimilation de l'azote, acides aminés.

3.2 Abstract

Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is an important pest of potato crops. Potato foliar amino acids play essential roles in CPB growth. In this study, amino acids were classified into four groups according to their different roles in promoting CPB growth. Then, nitrogen (N) rate effects on the concentrations of amino acid group were investigated under real field conditions. Experiments were carried out with five N rates of 0, 60, 120, 180, and 240 kg N ha^{-1} in a randomized complete block design. Twenty leaves were collected at 40, 54, 68, and 82 days after planting (DAP) for amino acids analysis. Results showed that N rate had no significant effect on the concentration of each amino acid group at 40 DAP. However, their concentrations linearly increased as N rate increased at 54, 68, and 82 DAP, suggesting that higher N rates could potentially favor CPB growth after potato enters tuber initiation stage.

Keywords: Herbivorous pest, mineral fertilizer, N assimilation, amino acids.

3.3 Introduction

Potato (*Solanum tuberosum* L.) is grown throughout the world and is now the fourth most important crop after rice *(Oriza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) (He et al. 2012). The extraordinary adaptive range of this crop and high nutritional content explain the steady increases in potato production around the world (DeFauw et al. 2012). In Canada, potato harvest reached 4.7 million tons in 2016 and potato consumption was approximately 80 kg per capita per year (Statistics Canada 2016). So far, the potato is still subjected to serious damage from the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), the most destructive foliar feeding insect pest of potato plants in North America (Boiteau et al. 2008). Both adults and larvae can defoliate the entire plant without discriminating among leaf tissues. Once potato foliage is consumed, beetles can continue to feed on stems and exposed tubers, though the latter two constitute a sub-optimal diet (Alyokhin 2009). Boiteau (2010) reported that potential potato yield losses due to the defoliating damage of CPBs could reach up to 30%–50% in North America.

For decades, chemical insecticides were the dominant approach to repress CPB populations and to maintain a high potato yield (Alyokhin 2009). However, high selection pressure, together with a natural propensity to adapt to toxic substances, eventually resulted in a large number of insecticide resistant CPB populations (Scott et al. 2015). In this context, a promising integrated pest management (IPM) was proposed to manage CPBs, with a major focus on the combined use of multiple control techniques, such as crop rotation and biological control, to repress the beetle populations to an economically tolerable level while reducing the use of insecticides (Alyokhin et al. 2015). However, IPM is not a silver bullet and it requires substantial extra effort to make it more effective to control the CPB by further supplementing appropriate techniques (Alyokhin et al. 2015).

During its life cycle, the CPB requires a huge quantity of nutrients from the host plants for its development from larva to adult. The quality and quantity of potato foliar nutrients could have a major effect on CPB growth and development and on some behaviors, such as feeding and flying. For instance, leaf carbohydrates could provide energy for CPB growth, particularly during overwintering, while glycoalkaloid could disturb the beetle's digestive system (Wen et al. 2019a). Foliar amino acids also play an important role in promoting CPB growth as they are basic units for protein synthesis (Brouwers and de Kort 1979). Domek et al. (1995) reported that some amino acids, such as glutamate (Glu) and proline (Pro), could provide energy for CPB flight, a critical activity for this beetle as it looks for food sources. Some amino acids, including γ-aminobutyric acid (GABA), alanine (Ala), and valine (Val), could promote CPB feeding even at a low concentration of 0.01 M (Hsiao and Fraenkel 1968). The metabolism of these chemical components in potato leaves is highly dependent on nitrogen (N) fertilization because chemical components are N-containing molecules and their metabolic process requires a considerable quantity of N-enzyme catalysts. We have already determined that N fertilization significantly increased glycoalkaloid concentrations and decreased sugar concentrations in potato leaves (Wen et al. 2019b).

Regarding potato foliar amino acids, few studies have been conducted to investigate the impacts of N fertilization on their concentrations while considering their different roles in promoting CPB growth, development, and behavior. The objectives of this study were (1) to classify the 20 amino acids identified from Russet Burbank leaves into four groups based on their different roles in promoting CPB growth and activity, and (2) to investigate the effects of N fertilization rates on the concentrations of these amino acid groups in potato leaves throughout the growing season using a multiple site field experiment.

3.4 Materials and methods

3.4.1 Site description, experimental design, and management

Field experiments used in this study were conducted as described in Wen et al. (2019b) at Ste-Catherine (2015 and 2016) and Beaumont (2015), near Quebec City, Canada. The soil characteristics and experimental design of each experimental field are presented in Table 3-1. The ground surface soil texture of the three sites varied from loamy sand to sandy loam. The average pH (in water) of the three sites was 4.7, 5.5, and 5.3, respectively. Average NO_3-N and NH_4-N concentrations in the 0–30 cm depth soil were 62 and 5.2 kg N ha^{-1}, respectively. Total N and C concentrations were 2.1 and 24.8 g kg^{-1} in the 0–20 cm depth soil. The weather conditions, including air temperature and total precipitation, are presented in Table 3-1. Average air temperature increased from May to August and then decreased until September at each site (Wen et al. 2019b). Crops of maize, canola (*Brassica napus* L.), and oat (*Avena sativa* L.) were previously planted for the three sites. The Russet Burbank potato cultivar was used for these field experiments since it was frequently grown in Canada for French fry processing (Bethke et al. 2014; Nassar et al. 2008). Insecticide-treated (Titan™, Active ingredient: Clothianidin) seed pieces were planted on 20 May 2015, 25 May 2015, and 20 May 2016 (Table 3-1).

Table 3- 1 Average values (n=4) of the pre-planting soil characteristics and experimental design and management of each potato field.

	Ste-Catherine (2015)	Beaumont (2015)	Ste-Catherine (2016)
Pre-planting soil characteristics			
Soil texture [a]	Loamy sand	Silty loam	Sandy loam
Soil pH (in water) [b]	4.8±0.1	5.5±0.4	5.3±0.1
Total N (g kg^{-1}) [c]	2.0±0.04	2.5±0.2	1.8±0.1
Total C (g kg^{-1}) [c]	25.0±0.7	23.5±1.0	25.8±1.4
NO_3-N (kg ha^{-1}) [d]	91.5±9.2	58.7±3.3	31.9±1.2
NH_4-N (kg ha^{-1})	18.4±3.6	1.0±1.5	5.4±2.2
Experimental design and management			
Previous crop	Corn	Canola	Oat
Planting date	20 May	25 May	20 May
Harvesting date	2 Oct.	6 Oct.	3 Oct.
Triple superphosphate (kg P_2O_5 ha^{-1})	150	120	150
Potassium chloride (kg K_2O ha^{-1})	300	300	230
Climatic conditions†			
Average air temperature (°C)	16.2	16.8	16.1
Total precipitation (mm)	569.8	544.6	477.8

[a] Soil texture was classified based on The Canadian System of Soil Classification (1998). [b] Soil pH (in water) was measured based on Hendershot et al. (2008). [c] Total N and total C were measured with an Elementar CN analyzer. [d] NO3-N was extracted with 1 M KCl using a 1:10 soil/extractant ratio. †The average air temperature and total precipitation in climatic conditions were monitored by Jean-Lesage Airport (46° 48′ N, 71° 22′ W) for Ste-Catherine and Lauzon station (71° 16′ N, 46° 82′ W) for Beaumont in the growing season from May to September.

Five N rates (0, 60, 120, 180, and 240 kg N ha^{-1}) were applied in a randomized complete block design (Wen et al. 2019b). A split N application was used in this study since this N supply strategy has been shown to increase tuber yield as compared with a single full application at planting or at hilling (Cambouris et al. 2007). Ammonium sulfate (22-0-0) was band-applied at planting at a rate of 60 kg N ha^{-1}, except for the unfertilized control. The remaining N fertilizer was applied as calcium ammonium nitrate (27-0-0) just after the first sampling at 40 days after planting (DAP). For this reason, the samples collected at 40 DAP included N rate-treatments of 0 and 60 kg N ha^{-1} and samples collected at 54, 68, and 82 DAP included N rate-treatments of 0, 60, 120, 180, and 240 kg N ha^{-1}. Triple superphosphate (0-46-0) and potassium chloride (0-0-60) were used at planting based on a local recommendation (CRAAQ 2010). Rates applied at each site are listed in Table 3-1.

To protect potato plants from pest damage, Delegate™ (65 g ha^{-1}, Active ingredient: Spinetoram, Dow AgroSciences Canada Inc., Montreal, Canada) was applied on 15 July 2015 in Ste-Catherine (2015) and Minecto Duo™ (570 g ha^{-1}, Active ingredient: Thiamethoxam and Cyantraniliprole, Syngenta Canada Inc., Ontario,

Canada) was applied on 1 August 2015 in Beaumont (2015). Potatoes were harvested in early October before winter came (Table 3-1).

3.4.2 Sample collection and chemical analysis

A composited soil sample of ten cores from 0–20 cm depth (0-30 cm depth for NO_3-N and NH_4-N) was collected from each block to determine the initial soil physical and chemical characteristics using the methodologies described by Wen et al. (2019b). The soil pH (1:2 in water) was determined based on the extract method of Hendershot et al. (2008). The NO_3-N was extracted with 1 M KCl using a 1:10 soil/extractant ratio, then NO_3-N and NH_4-N were determined by automated colorimetry (Maynard et al. 2008; Wen et al. 2019ba). Total C and N were measured with an Elementar CN analyzer (Elementar, model Varian Macro CN, Hanau, Germany).

Twenty potato plants were randomly selected in each plot and the fourth fully developed leaf from the top of each plant was collected at 40, 54, 68, and 82 DAP for amino acid analysis since the fourth leaf is well related to nutritional status of the whole plant and is sensitive to changes in the availability of nutrients (Westermann, 1993). Leaf samples were collected around the same time in the morning to minimize the influence of photosynthesis on chemical composition. All leaf samples were immediately placed in labeled plastic bags and stored in an ice-cooled incubator. Subsequently, samples were dried in a ventilated oven at 55°C for seven days and then were ground using an IKA Werke grinder (MF10BS1, IKA, USA) equipped with a 1-mm diameter sieve. Leaf samples were stored at room temperature in the dark for a duration of less than two weeks (Wen et al. 2019b). Before chemical analysis, ground samples were dried for two hours at 105°C using a Thermogravimetric Analyzer (TGA701, Leco) to determine dry mass.

The amino acids were determined based on the standard method provided by the Ultra Performance Liquid Chromatography (UPLC) Amino Acid Analysis Solution system guide (Waters Corporation). Amino acids were extracted by adding 7 mL of methanol, chloroform, and distilled water with a volume ratio of 12:5:3 to a 0.2 g dried and ground subsample. Extracts were incubated at 65°C for 20 min, cooled in an ice-water bath, and stored overnight at 4°C. A 1 mL subsample was collected and 250 µL of water was added. After centrifugation for 15 min at 4000 rpm, 750 µL of the methanol-water phase containing free amino acids was sampled, evaporated to dryness using a Savant evaporator (Speedvac plus SC2104) and resuspended in water. Free amino acids in this extract were analyzed by UPLC (Acquity, Waters, Milford, MA). Amino acids were separated on a high-efficiency 4-µm AccQ Tag Ultra C18 column (Dimension of 3.9 × 150 mm) and detected on a Tunable UV detector (Waters Acquity) according to the chromatographic conditions described in Cohen (2000).

3.4.3 Amino acid composition classification

In order to investigate the effects of N fertilization on the variation of CPB behaviors, the 20 amino acids were classified into four groups related to their different roles in affecting CPB behaviors: feeding stimulant (GABA, AABA, Gly, Asp, Ala, Glu, Pro, Ser, Asn, Gln, Arg, and Val), inheritable amino acid (Glu, Pro, Ser, Asn, Gln, His, Lys, Thr, and Tyr), flight-energy substrate (Ala, Glu, and Pro), and essential amino acid (Arg, Val, Ile, Leu, Met, Phe, His, Lys, and Thr) (Figure 3-1), as mentioned in the section 3.6.1.

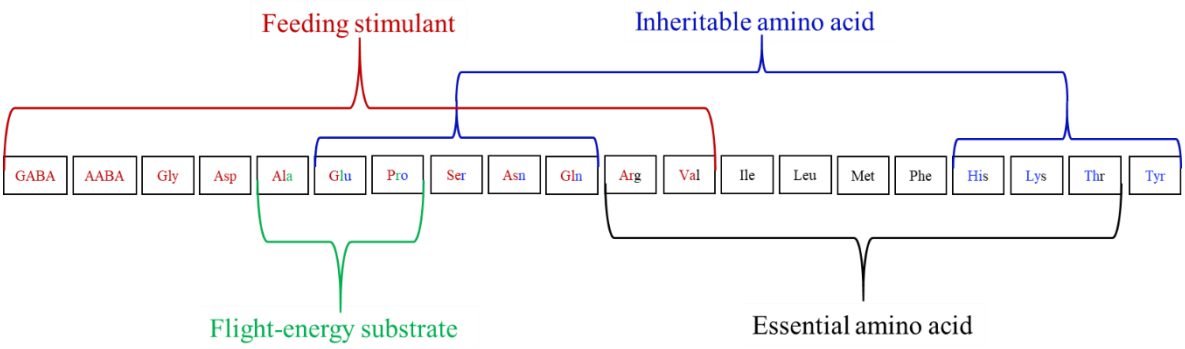

Figure 3- 1 Amino acid groups classified according to their different roles in promoting the growth of Colorado potato beetle (GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; Gly: glycine; Asp: aspartate; Ala: alanine; Glu: glutamate; Pro: proline; Ser: serine; Asn: asparagine; Gln: glutamine; Arg: arginine; Val: valine; Ile: isoleucine; Leu: leucine; Met: methionine; Phe: phenylalanine; His: histidine; Lys: lysine; Thr: threonine; Tyr: tyrosine). The different colors of amino acid abbreviations are included in different groups, i.e., red for Feeding stimulant, blue for Inheritable amino acid, green for Flight-energy substrate, and black for Essential amino acid.

3.4.4 Statistical analysis

Site and block were treated as random effects and the N rate as a fixed effect. Since the experimental design was different for samples collected at 40 DAP from those at 54, 68, and 82 DAP, the measured data were analyzed separately. For the data collected at 40 DAP, a generalized randomized block design was used. A repeated randomized complete block design was used for the data collected at 54, 68, and 82 DAP by considering the sampling date as a repeated measurement. Protected LSD multiple comparison was used to investigate the differences among N rates when a significant effect of N fertilization was obtained by ANOVA at $p < 0.05$. Polynomial priori contrasts were also used to investigate the relationship (linear or quadratic) between amino acid concentrations and N rates.

Shapiro-Wilk's statistic was used to test the normality assumption on the residuals of each model, while residual plots were used to verify the homogeneity of variances. When the normality assumption was not met, the

dependent variables were transformed by using the Box-Cox. All analyses were performed using the SAS Mixed procedure (SAS Institute, 2010) at a significance level of $p < 0.05$.

3.5 Results

No significant effect of N fertilization on the concentrations of the four group amino acids was observed at 40 DAP. The average concentrations of feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid were 65.86, 23.67, 28.80, and 23.76 μmol g^{-1} dry matter, respectively. The effect of N rates (0 vs 60 kg N ha^{-1}) at 40 DAP was not significant for any of the 20 selected amino acids (Table 3-2).

Table 3- 2 Effect of N rates on the concentration of each potato foliar amino acid at 40 days after planting (DAP).

	GABA[1]	AABA[1]	Gly[1]	Asp[1]	Ala[1,3]	Glu[1,2,3]	Pro[1,2,3]	Ser[1,2]	Asn[1,2]	Gln[1,2]
Source of variance	---------- *p* values ----------									
N rate	ns†	ns	ns	ns	ns	ns	ns	ns	ns	ns
N rate (kg N ha^{-1})	---------- Concentration (μmol g^{-1} dry matter) ----------									
0	19.45	0.12	1.53	3.28	19.66	1.95	4.68	2.30	2.87	1.84
60	19.16	0.10	1.47	2.84	19.16	1.97	4.57	2.42	3.06	1.79

	Arg[1,4]	Val[1,4]	Ile[4]	Leu[4]	Met[4]	Phe[4]	His[2,4]	Lys[2,4]	Thr[2,4]	Tyr[2]
Source of variance	---------- *p* values ----------									
N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N rate (kg N ha^{-1})	---------- Concentration (μmol g^{-1} dry matter) ----------									
0	1.33	6.16	3.89	4.12	0.03	2.79	0.40	1.52	1.69	3.63
60	1.42	5.68	3.94	4.32	0.03	2.73	0.40	1.57	1.75	3.62

†ns, not significant ($p > 0.05$). The amino acids marked with [1], [2], [3], and [4] indicate that they were classified into four groups: feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid, respectively. Leaf sample collected at 40 DAP included N rate-treatments of 0 and 60 kg N ha^{-1}. Amino acid abbreviations: GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; Gly: glycine; Asp: aspartate; Ala: alanine; Glu: glutamate; Pro: proline; Ser: serine; Asn: asparagine; Gln: glutamine; Arg: arginine; Val: valine; Ile: isoleucine; Leu: leucine; Met: methionine; Phe: phenylalanine; His: histidine; Lys: lysine; Thr: threonine; Tyr: tyrosine.

Concentrations of all the amino acids in the four groups linearly increased with increasing N rates from 0 to 240 kg N ha^{-1} at 54, 68, and 82 DAP (Table 3-3). The concentrations of amino acids in three groups (feeding stimulant, inheritable amino acid, and essential amino acid) were significantly influenced by the sampling date and their concentrations increased from 54 to 68 DAP, and then sharply decreased (Table 3-3). The concentration of flight-energy substrate amino acids was not affected by the sampling date ranging from 54 to 82 DAP. There were two significant interactions between N rate and sampling date in the concentrations of feeding stimulant and inheritable amino acid (Table 3-3). The N fertilization rate significantly increased the feed

stimulant amino acid concentrations at 68 and 82 DAP, but not at 54 DAP (Figure 3-2a). The inheritable amino acid concentration increased significantly with the increasing N rate only at 82 DAP (Figure 3-2b).

Table 3- 3 Effect of N rates and sampling dates on potato foliar amino acid concentrations in the four groups at 54, 68, and 82 days after planting (DAP).

	Feeding stimulant	Inheritable amino acid	Flight-energy substrate	Essential amino acid
Source of variance	---------- *p* values ----------			
N rate (N)	<0.0001	<0.0001	0.0026	<0.0001
Sampling date (D)	<0.0001	<0.0001	ns	<0.0001
N*D	0.0468	0.0012	ns	ns
Contrasts				
N linear	<0.0001	<0.0001	<0.0001	<0.0001
N quadratic	ns†	ns	ns	ns
N rates (kg N ha^{-1})	---------- Concentration (µmol g^{-1} dry matter) ----------			
0	59.35c‡	21.66c	26.60b	20.58b
60	58.40c	21.31c	27.00b	20.25b
120	68.35b	24.99b	30.27a	24.95a
180	71.00ab	26.23ab	31.43a	25.52a
240	75.61a	28.57a	33.76a	27.58a
Sampling dates (DAP)	---------- Concentration (µmol g^{-1} dry matter) ----------			
54	66.68b	20.92b	30.37a	23.31b
68	77.09a	35.36a	30.24a	28.22a
82	55.86c	17.38c	28.83a	19.80c

†ns, not significant ($p > 0.05$). ‡Concentrations followed by the same letter are not significantly different ($p > 0.05$). Leaf samples collected at 54, 68, and 82 DAP included N rate-treatments of 0, 60, 120, 180, and 240 kg N ha^{-1}.

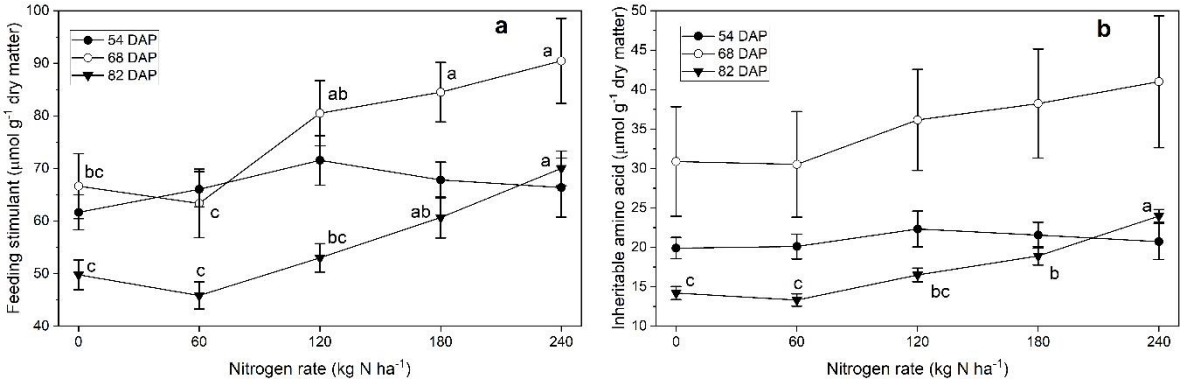

Figure 3- 2 Interaction effects of N rates and sampling dates on the concentrations of feeding stimulant (a) and inheritable amino acid (b). DAP: days after planting.

The concentrations of each amino acid (except for Met and Lys) measured in this study significantly increased with increasing N rates (Tables 3-4 and 3-5). All the amino acids, except for Met, were significantly affected by the sampling date (Table 3-4). Generally, the amino acid concentrations decreased over time; however, some amino acids, such as Pro and Asn, increased from 54 to 68 DAP and then decreased to 82 DAP (Table 3-6). There were significant interactions between the N rate and the sampling date in the concentrations of GABA, Pro, Gln, Arg, Ile, and Leu (Table 3-4). The concentrations of all these amino acids, except for Pro, significantly increased with increasing N rates at 68 and 82 DAP. However, the Pro concentration increased significantly only at 82 DAP (Figure 3-3).

Table 3- 4 ANOVA (*p* values) results on the effect of N rates and sampling dates on the concentration of each potato foliar amino acid at 54, 68, and 82 days after planting (DAP).

Source of variance	GABA[1]	AABA[1]	Gly[1]	Asp[1]	Ala[1,3]	Glu[1,2,3]	Pro[1,2,3]	Ser[1,2]	Asn[1,2]	Gln[1,2]
N rate (N)	<0.0001	0.0003	<0.0001	0.0053	0.0042	0.0046	0.0031	0.0084	0.0123	0.002
Sampling date (D)	<0.0001	<0.0001	<0.0001	<0.0001	0.0168	0.0005	<0.0001	<0.0001	<0.0001	<0.0001
N*D	0.0005	ns[†]	ns	ns	ns	ns	0.0052	ns	ns	0.0002

	Arg[1,4]	Val[1,4]	Ile[4]	Leu[4]	Met[4]	Phe[4]	His[2,4]	Lys[2,4]	Thr[2,4]	Tyr[2]
N rate (N)	<0.0001	<0.0001	<0.0001	<0.0001	ns	0.0005	0.0208	ns	0.0042	0.0005
Sampling date (D)	<0.0001	<0.0001	0.0155	0.0022	ns	<0.0001	0.0043	0.0002	0.0168	<0.0001
N*D	0.0443	ns	0.026	0.0145	ns	ns	ns	ns	ns	ns

[†]ns, not significant ($p > 0.05$). The amino acids marked with [1], [2], [3], and [4] indicate that they were classified into four groups: feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid, respectively. Leaf samples collected at 54, 68, and 82 DAP included N rate-treatments of 0, 60, 120, 180, and 240 kg N ha^{-1}. Amino acid abbreviations: GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; Gly: glycine; Asp: aspartate; Ala: alanine; Glu: glutamate; Pro: proline; Ser: serine; Asn: asparagine; Gln: glutamine; Arg: arginine; Val: valine; Ile: isoleucine; Leu: leucine; Met: methionine; Phe: phenylalanine; His: histidine; Lys: lysine; Thr: threonine; Tyr: tyrosine.

Table 3- 5 Effect of N rates on the average concentration of each potato foliar amino acid at 54, 68, and 82 days after planting (DAP).

N rate (kg N ha^{-1})	GABA[1]	AABA[1]	Gly[1]	Asp[1]	Ala[1,3]	Glu[1,2,3]	Pro[1,2,3]	Ser[1,2]	Asn[1,2]	Gln[1,2]
	---------- Concentration (μmol g^{-1} dry matter) ----------									
0	16.35b†	0.11c	0.88b	1.84ab	19.09c	1.85bc	5.66b	1.27c	4.50b	1.59bc
60	15.32b	0.13bc	0.87b	1.54b	19.77bc	1.71c	5.51b	1.34bc	4.68b	1.50c
120	17.82b	0.15ab	1.19a	2.30a	22.12ab	2.22ab	5.93a	1.58ab	5.66ab	1.98ab
180	18.49a	0.15ab	1.25a	2.34a	22.57a	2.37a	6.48a	1.56ab	6.21a	1.99a
240	18.80a	0.16a	1.29a	2.33a	24.38a	2.45a	6.93a	1.72a	7.13a	2.23a

	Arg[1,4]	Val[1,4]	Ile[4]	Leu[4]	Met[4]	Phe[4]	His[2,4]	Lys[2,4]	Thr[2,4]	Tyr[2]
	---------- Concentration (μmol g^{-1} dry matter) ----------									
0	1.10c	5.11b	3.59b	2.63c	0.08a	3.05b	0.48bc	1.35a	1.76ab	3.20b
60	1.07c	4.97b	3.50b	2.66c	0.08a	3.06b	0.47c	1.30a	1.65b	3.14b
120	1.38b	6.02a	4.24a	3.33b	0.08a	4.01a	0.51abc	1.49a	1.73b	3.89a
180	1.44ab	6.14a	4.29a	3.60b	0.07a	4.14a	0.54ab	1.47a	1.76ab	3.85a
240	1.61a	6.57a	4.61a	4.11a	0.06a	4.43a	0.57a	1.52a	1.91a	4.10a

†Concentrations in each column with the same letter are not significantly different ($p > 0.05$). The amino acids marked with [1], [2], [3], and [4] indicate that they were classified into four groups: feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid, respectively. Leaf samples collected at 54, 68, and 82 DAP included N rate-treatments of 0, 60, 120, 180, and 240 kg N ha^{-1}. Amino acid abbreviations: GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; Gly: glycine; Asp: aspartate; Ala: alanine; Glu: glutamate; Pro: proline; Ser: serine; Asn: asparagine; Gln: glutamine; Arg: arginine; Val: valine; Ile: isoleucine; Leu: leucine; Met: methionine; Phe: phenylalanine; His: histidine; Lys: lysine; Thr: threonine; Tyr: tyrosine.

Table 3- 6 Effect of sampling dates on the average concentration of each potato foliar amino acid at 40, 54, 68, and 82 days after planting (DAP).

Sampling date (DAP)	GABA[1]	AABA[1]	Gly[1]	Asp[1]	Ala[1,3]	Glu[1,2,3]	Pro[1,2,3]	Ser[1,2]	Asn[1,2]	Gln[1,2]
	---------- Concentration (μmol g^{-1} dry matter) ----------									
40	19.22	0.11	1.48	2.93	19.26	1.97	4.59	2.40	3.02	1.80
54	19.68a†	0.11b	1.38a	2.17a	22.88a	2.63a	4.86b	1.97a	2.30b	1.78ab
68	16.94b	0.18a	1.18a	2.92a	20.18b	1.74b	8.32a	1.70b	13.32a	2.27a
82	15.44c	0.14a	0.72b	1.11b	21.71ab	1.99b	5.13b	0.81c	1.29c	1.52b

	Arg[1,4]	Val[1,4]	Ile[4]	Leu[4]	Met[4]	Phe[4]	His[2,4]	Lys[2,4]	Thr[2,4]	Tyr[2]
	----------Concentration (μmol g^{-1} dry matter) ----------									
40	1.40	5.77	3.93	4.28	0.03	2.74	0.40	1.56	1.74	3.63
54	1.41a	5.51b	4.03ab	3.60a	0.07a	3.01b	0.50ab	1.57a	1.69b	3.61b
68	1.49a	6.85a	4.34a	3.29b	0.08a	5.62a	0.59a	1.29b	1.45c	4.67a
82	1.06b	4,92b	3.77b	2.90b	0.07a	2.58b	0.45b	1.41ab	2.14a	2.63c

†Concentrations in each column with the same letter are not significantly different ($p > 0.05$). The amino acids marked with [1], [2], [3], and [4] indicate that they were classified into four groups: feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid, respectively. The data collected at 40 days after planting were not used in the multiple comparisons in the statistical analysis since samples collected at 40 DAP included N rate-treatments of 0 and 60 kg N ha^{-1} and samples collected at 54, 68, and 82 DAP included N

rate-treatments of 0, 60, 120, 180, and 240 kg N ha^{-1}. Amino acid abbreviations: GABA: γ-aminobutyric acid; AABA: α-aminobutyric acid; Gly: glycine; Asp: aspartate; Ala: alanine; Glu: glutamate; Pro: proline; Ser: serine; Asn: asparagine; Gln: glutamine; Arg: arginine; Val: valine; Ile: isoleucine; Leu: leucine; Met: methionine; Phe: phenylalanine; His: histidine; Lys: lysine; Thr: threonine; Tyr: tyrosine.

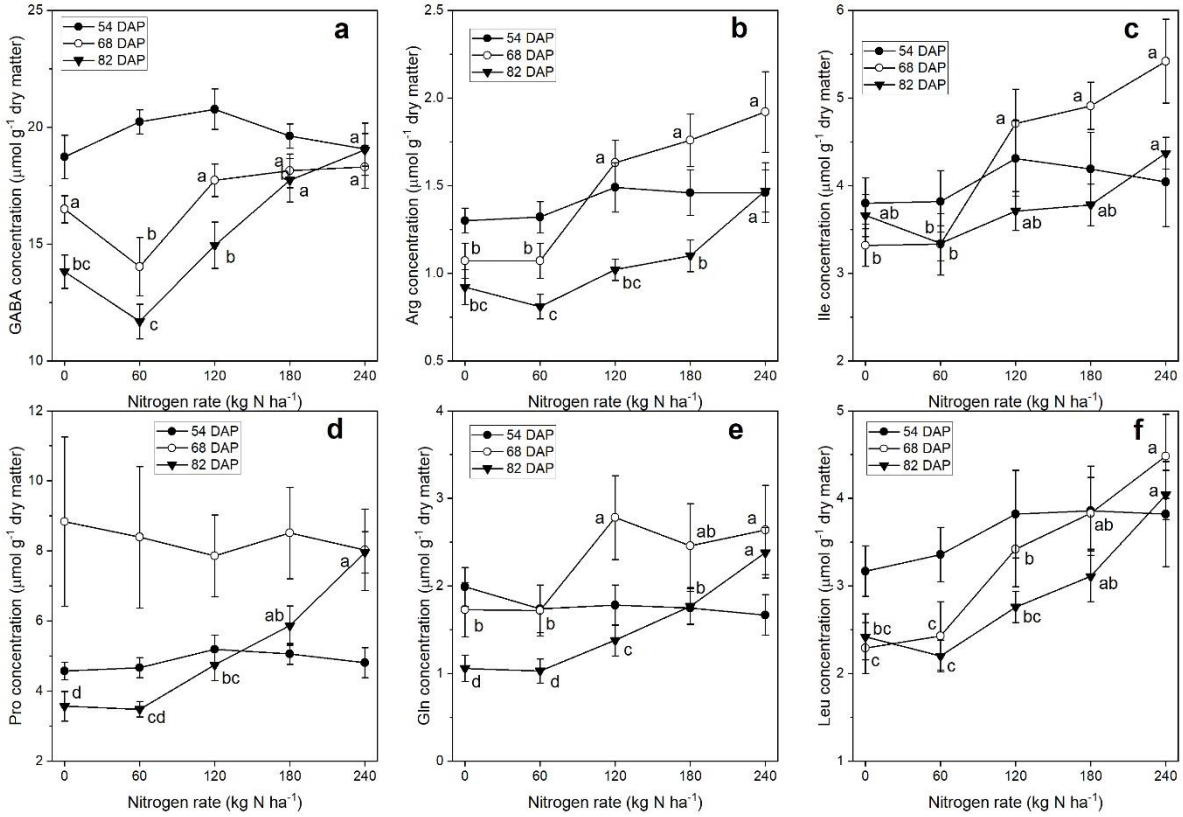

Figure 3- 3 Interaction effects of N rate and sampling date on the concentrations of GABA (a), Arg (b), Ile (c), Pro (d), Gln (e), and Leu (f). DAP: days after planting. GABA: γ-aminobutyric acid; Arg: arginine; Ile: isoleucine; Pro: proline; Gln: glutamine; Leu: leucine.

3.6 Discussion

3.6.1 Amino acid classification

According to Domek et al. (1995) and Hsiao and Fraenkel (1968), the amino acids of GABA, AABA, Gly, Asp, Ala, Glu, Pro, Ser, Asn, Gln, Arg, and Val could promote CPB feeding even at a low concentration of 0.01 M and were thus classified as feeding stimulant in this study. Some amino acids of CPB eggs, including His, Gln, Pro, Asn, Ser, Glu, Thr, Lys, and Tyr, could be transferred to CPB larvae and then influence the performance and survivability of the next CPB generation and, as such, have been classified as inheritable amino acid (Gelman et al. 2000). The amino acids Pro and Glu of potato foliage have been reported as energy sources for CPB flight during the host plant searching process while Ala is a precursor of Pro biosynthesis (Brouwers and de Kort 1979). Thus, Pro, Glu, and Ala have been classified as flight-energy substrate amino acids. Like mammals, CPBs require essential amino acids for their general activities and survivability. Cibula et al. (1967) reported that

the essential amino acids for CPB were Arg, Val, Ile, Leu, Met, Phe, His, Lys, Thr, and Tyr. In this study, we classified these amino acids as the essential amino acid group.

3.6.2 Effects of nitrogen supply and sampling date

3.6.2.1 Feeding stimulant

In the feeding stimulant group, Ala and GABA were the most abundant amino acids, accounting for 33% and 28%, respectively. The average concentrations of Ala and GABA were 21 and 17.8 μmol g^{-1} DM, which agreed with values ranging from 8.6 to 32 μmol g^{-1} DM for Ala and from 13.3 to 19.9 μmol g^{-1} DM for GABA, respectively, reported in potato leaves (cv. Desiree) by Urbanczyk-Wochniak et al. (2005) for a leaf moisture ratio of 93% (Liu et al. 2005). In a greenhouse experiment with potato cv. Atlantic, Yang et al. (2011) reported an Ala average concentration of 67.3 μmol mg^{-1} DM in potato leaves for a similar leaf moisture ratio. This larger value could be attributed to the differences in the potato cultivars under study or in cultivation conditions.

Amino acids are N-containing molecules that require N-rich enzymes for their assimilation and biosynthesis. Additionally, as an element of chlorophyll, N is essential for efficient photosynthesis (Fageria and Baligar 2005) that provides carbon skeletons for amino acid biosynthesis (Bouché and Fromm 2004). Through these pathways, N fertilization could increase amino acid biosynthesis in potato leaves. On the other hand, Vos and Biemond (1992) reported that N fertilization significantly enlarged potato leaf size, resulting in a biomass dilution effect on foliar amino acid concentrations. This dilution effect could explain the lack of variation in the concentration of feeding stimulant when different N rates were used at 40 and 54 DAP (Figure 3-2a). However, a significant increase in the concentrations of feeding stimulant and the respective amino acid of GABA, Arg, Pro, and Gln within this group was observed with increasing N rates at 68 and 82 DAP (Figure 3-2 and Figure 3-3). Harris (2012) demonstrated that the leaf expansion rate increases gradually after leaf emergence and reaches its maximum during the tuber bulking stage. Thus, the concentrations of amino acids may increase when their biosynthesis rate is larger than the potato leaf expansion rate, which was likely reduced between 68 and 82 DAP. We previously reported a significant decrease in foliar glucose concentration with increasing N rates (Wen et al. 2019b). Since glucose provides a carbon skeleton for amino acid formation (Bouché and Fromm 2004), we could suppose that a part of this glucose served as a substrate for the synthesis of feeding amino acids (Olsen and DeLorey 1999).

3.6.2.2 Inheritable amino acid

In this study, no significant variation was observed in the concentrations of inheritable amino acids and each individual amino acid in this group with increasing N rates at 40 and 54 DAP (Table 3-2 and Figure 3-2b). This result was mainly due to the high level of mineral N in the pre-planting soil with a concentration of 67 kg N ha^{-1}, which seems sufficient for plant growth (Stark et al. 2004).

Inheritable amino acid concentration increased with increasing N rates at 68 and 82 DAP (Figure 3-2b), which was partly due to the increase in Gln concentration (Figure 3-3e). At the late tuber bulking stage, Gln concentration could increase due to plant photorespiration. Also, N fertilization enhanced protein hydrolysis by increasing the biosynthesis of proteolytic enzymes (Muttucumaru et al. 2014).

The concentration of inheritable amino acid was significantly influenced by the sampling date (Table 3-3). This variation was partly due to the sharp increase of Asn at 68 DAP, which was approximately 6-fold of that at 54 DAP (Table 3-6). The Asn is a stress-sensitive amino acid that can accumulate to a considerable extent in response to wounding. Lea et al. (2007) reported that the Asn concentration reached a striking 800-fold increase when plant leaves were infected with a pathogen, which was induced by Gln-synthetase biosynthesis in the infected leaf mesophyll cells (Pérez-García et al. 1998). In our study, potato plants were infested by CPB at around 53 DAP and this infection lasted several days after insecticide application. Thus, CPB infection could explain the dramatic increase in Asn concentration from 54 to 68 DAP. Afterward, a significant reduction in Asn concentration was observed until 82 DAP. This observation was in accordance with the statement of Carillo et al. (2005) indicating that Asn accumulated in young but not old plants.

3.6.2.3 Flight-energy substrate

We observed a progressive increase in the concentration of flight-energy substrate with increasing N rates at 54, 68, and 82 DAP (Table 3-3), which was mainly due to the significant increase in Glu and Ala concentrations (Table 3-5). The Glu is a primary precursor of other amino acids, such as Ala, through transamination in plant leaves (Meza-Basso et al. 1986). On the other hand, the variation in Glu concentration in plants is considered a sensitive indicator of N fertilization because its synthesis needs N-enzymes, such as Glu-synthase (Geiger et al. 1999). Therefore, a considerable quantity of Glu is required to synthesize in potato leaves to meet the requirement for plant growth, especially at the tuber bulking stage. In this study, a significant increase in Pro concentration with an increasing N rate was only observed at 82 DAP (Figure 3-3d). Srivastava and Singh (2006) reported that Pro accumulation in plants follows exposure to an environmental stress to serve as a cryoprotectant. The application of the insecticide Minecto Duo™ at 73 DAP in Beaumont (2015) could be a possible reason for the dramatic increase in Pro concentration with increasing N rates at 82 DAP.

No significant response was observed in the flight-energy substrate concentration to the sampling date (Table 3-3). However, Glu and Pro concentrations changed remarkably over time (Table 3-4). An increase in Pro concentration and a decrease in Glu concentration were observed at 68 DAP when compared with those at 54 DAP (Table 3-6), which could be due to the transformation from Glu to Pro in plants (Meza-Basso et al. 1986). In addition, Tomlin and Sears (1992) observed a higher level of Pro when potato foliage was wounded by pests.

In our study, potato leaves were infected by CPB around 53 DAP and this condition lasted about two weeks, which could well explain the significant alteration in Pro concentration over time during potato cultivation.

3.6.2.4 Essential amino acid

A considerable quantity of essential amino acids could be biosynthesized in potato leaves to sustain plant growth. In this study, the concentration of essential amino acid progressively increased with increasing N rates (Table 3-3). We previously reported that N fertilization promotes N accumulation in potato plants (Wen et al., 2019b), which is mainly reflected in the increase in essential amino acid concentration (Millard, 1986). We also observed that the concentrations of Arg, Ile, and Leu increased only at 82 DAP with increasing N rates (Figure 3-3b, 3-3c, and 3-3f). When entering the maturation stage, potato tubers have already acquired all the necessary nutrients for their growth and proteins in plant leaves start to hydrolyze into free amino acids (Martin and Thimann, 1972). It seems that increasing N rates promoted the synthesis of the proteolytic enzyme (Muttucumaru et al., 2014), which could well result in the increase of these free amino acids at 82 DAP with increasing N rates.

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The concentration of essential amino acids was significantly dependent on sampling dates and this concentration reached a maximum at 68 DAP (Table 3-3). Also, the concentrations of Val, Phe, and Tyr in the essential amino acid group greatly increased from 54 to 68 DAP, and then decreased (Table 3-6). This variation partly resulted from the remarkable reduction in Glu concentration from 54 to 68 DAP which is basic unit of proteinous amino acids (Forde and Lea 2007).

3.7 Conclusion

In this study, amino acids were classified into four groups of feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid based on their different roles in CPB behavior and growth. We observed that N fertilization significantly increased the amino acid concentrations of all four groups after potato

plants entered the tuber initiation stage. This indicates that reducing N fertilization rates could decrease the concentrations of certain amino acids, potentially resulting in a repression of CPB growth and behaviors, including feeding and flighting. Based on our results, a low N rate of 60 kg N ha^{-1} could be a critical component of IPM by decreasing potato foliar amino acid composition. However, a maximum of tuber yield has recently been reported to be obtained with an N rate of 205 kg N ha^{-1} (Wen et al. 2019b). Thus, an N fertilization rate of around 180 kg N ha^{-1} could be a tradeoff to obtain an acceptable yield while minimizing CPB infestation for growing Russet Burbank potatoes. It should be worth noting that there is no strict boundary between amino acid groups because some amino acids have multiple functions. For example, Ala could promote CPB feeding and enhance the CPB flighting capability. Also, the amino acids in potato leaves can realize mutual transformation when potato plants are under stresses. On the other hand, CPB behaviors and growth are closely related to other chemicals in host plants, such as proteins and sugars. Therefore, further field studies are still needed to explore the effects of N rate on specific metabolites in potato leaves that could affect CPB behaviors. Additionally, the biological importance of inheritable amino acids and the key roles of foliar amino acids in building a protein in CPB body at the molecular level should be investigated in the next trials to better understand their impact on CPB growth.

3.8 Acknowledgments

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Chapter 4 Effects of nitrogen fertilization on the leaf chemical composition of two potato cultivars under controlled conditions

Guoqi Wen, Athyna N. Cambouris, Noura Ziadi, Annick Bertrand, Mohamed Khelifi

This chapter presents original results related to the variation in foliar chemical composition of potato cultivars Russet Burbank and Goldrush when different N rates were applied under controlled conditions. It was published in the American Journal of Potato Research.

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In this chapter, total sugar was defined as the sum of sucrose, glucose, and fructose. Similarly, total glycoalkaloid was regarded as the algebraic sum of α-solanine and α-chaconine and total amino acid is the total amount of γ-aminobutyric acid, α-aminobutyric acid, glycine, aspartate, alanine, glutamate, proline, serine, asparagine, glutamine, arginine, valine, isoleucine, leucine, methionine, phenylalanine, histidine, lysine, threonine, and tyrosine.

Abbreviations: GABA: γ-aminobutyric acid; Ala: alanine; Pro: proline; Ser: serine; Val: valine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Tyr: tyrosine.

4.1 Résumé

Le doryphore de la pomme de terre (DPT), *Leptinotarsa decemlineata* (Say), est le ravageur le plus destructeur de la culture de la pomme de terre et la composition chimique des feuilles de pomme de terre joue un rôle important dans la croissance et le développement du DPT. Les sucres et les acides aminés peuvent favoriser le développement du DPT en augmentant les comportements alimentaires et de vol tandis que les glycoalcaloïdes sont considérés comme des bio-insecticides pour réprimer les populations de DPT. Dans cette étude, les effets de la fertilisation azotée sur les concentrations en sucres, en acides aminés et en glycoalcaloïdes dans les feuilles des plants de deux cultivars de pommes de terre (Russet Burbank et Goldrush) ont été étudiés sous des conditions contrôlées. Une expérience en pots a été réalisée avec cinq doses d'azote (0, 60, 120, 180 et 240 kg N ha^{-1}) selon un dispositif en blocs complètement aléatoires. Les 3^{e}, 4^{e} et 5^{e} feuilles du sommet de trois plants de pomme de terre sélectionnés au hasard par traitement ont été cueillies à 61, 75, 89 et 103 jours après la plantation pour l'analyse des sucres, des glycoalcaloïdes et des acides aminés. La fertilisation azotée n'avait aucun effet significatif sur les concentrations en glycoalcaloïdes. Cependant, elle a augmenté quadratiquement les concentrations en sucres et en acides aminés dans les feuilles. Des concentrations relativement faibles de sucres et d'acides aminés ont été observées à 180 kg N ha^{-1}, ce qui suggère que cette dose d'azote pourrait être efficace pour le contrôle des populations de DPT. Des différences significatives dans la plupart des produits chimiques foliaires ont été observées entre les deux cultivars, ce qui nécessite des études supplémentaires pour examiner la composition chimique des feuilles de différents cultivars de pommes de terre au champ et leurs effets sur le comportement du DPT.

Mots-clés: *Solanum tuberosum* L.; Ravageur herbivore; Glycoalcaloïde; Sucre; Acide aminé

4.2 Abstract

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is the most destructive pest of potato crops and the chemical composition of potato leaves plays an important role in CPB growth and development. Sugars and amino acids can promote CPB development by increasing feeding and flight behaviors, whereas glycoalkaloids are considered as plant defensive compounds to suppress CPB populations. In this study, the effects of nitrogen (N) fertilization on foliar sugar, amino acid, and glycoalkaloid concentrations of two potato cultivars (Russet Burbank and Goldrush) were investigated under controlled conditions. A pot experiment was carried out with five N rates (0, 60, 120, 180, and 240 kg N ha^{-1}) in a randomized complete block design. The 3rd, 4th, and 5th leaves from the top of three randomly selected plants per treatment were collected at 61, 75, 89, and 103 days after planting for the analysis of sugars, glycoalkaloids, and amino acids. Nitrogen fertilization had no significant effect on glycoalkaloid concentrations; however, it quadratically increased foliar sugar and amino acids concentrations. Relatively low sugar and amino acid concentrations were observed at 180 kg N ha^{-1}, suggesting that this N rate may be effective for CPB management. Significant differences in most foliar chemicals were observed between both cultivars, warranting further studies to investigate the leaf composition of different potato cultivars under field conditions and their effects on CPB behaviors.

Keywords: *Solanum tuberosum* L.; Herbivorous pest; Glycoalkaloid; Sugar; Amino acid

4.3 Introduction

Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say), is a notorious defoliating pest of potato crops (Boiteau et al. 2008). For decades, many efforts have been made to control the CPB in order to maximize potato tuber yield. The CPB populations are generally suppressed by chemical insecticides (Alyokhin 2009). However, the CPB has the capability to rapidly develop resistance to registered chemicals, making it difficult to control (Alyokhin et al. 2008). Other approaches, such as crop rotation and physical control, have also been implemented (Wright 1984; Khelifi et al. 2007). Unfortunately, none of them are effective enough to suppress CPB populations below the economic injury level for large-scale potato crop production. With the aim of reducing chemical applications to control the CPB, integrated pest management (IPM), which combines multiple approaches and technologies, such as crop rotation and biological control, has been proposed (Alyokhin et al. 2015). However, supplemental techniques are nonetheless required to make the IPM strategy more effective in managing the CPB.

As an herbivorous pest, the CPB lives mainly on potato foliage. Thus, the chemical composition of potato leaves, such as sugars and glycoalkaloids, could play an important role in determining the behavioral responses and physiological processes of CPB. Mitchell and Harrison (1984) reported that potato foliar sugars, at a concentration as low as 0.001 M, could promote CPB feeding owing to the special sugar-sensitive sensilla in the CPB mouthparts (Sen and Mitchell 1987). Also, sugars provide essential energy for CPB flight and overwintering behaviors (Weeda et al. 1979; Weber and Ferro 1994). However, not all chemicals in potato leaves are beneficial to CPB growth, and some chemicals, such as glycoalkaloids, are widely regarded as inhibitors of CPB development because they adversely affect the CPB central nervous and digestive systems (Friedman and Levin 2009). Therefore, investigating the chemical composition of potato leaves could improve our knowledge about their nutrient content and their potential impact on CPB behaviors. This new knowledge could support the implementation of an IPM strategy to control CPB by identifying factors inducing changes in leaf chemical composition.

Nitrogen (N) is an essential macronutrient for potato crops. Sufficient N supply is required to achieve economically viable tuber yield and to meet quality targets for processing potato production (Cambouris et al. 2016). However, N fertilizer overuse could increase the potential for N leaching and result in lower N use efficiency. On the other hand, a suitable N fertilization strategy can protect potato plants against herbivore pests by altering potato foliar chemical composition (Vos and van der Putten 1998). Wen et al. (2019a) observed that N fertilization increased potato foliar glycoalkaloid concentration, which may inhibit CPB feeding behavior.

Besides N fertilization, the potato cultivar is another factor that could affect leaf chemical composition (Leonel et al. 2017) with a potential impact on CPB behavior (Wen et al. 2019b). For example, sugar concentration in

Russet Burbank leaves from field experiments varied between 18.5 and 63.4 mg g^{-1} dry matter (Wen et al. 2019a), which is lower than that reported for the cultivars Asterix and Atlantic (Braun et al. 2016) for a leaf moisture ratio of 93% (Liu et al. 2005). As such, the choice of a potato cultivar could be part of an IPM strategy for CPB infestation. Russet Burbank and Goldrush are two frequently used potato cultivars in Canada due to their substantial contents of essential dietary minerals (Nassar et al. 2012). The objective of this study was to investigate the effects of N fertilization on the concentrations of sugars, glycoalkaloids, and amino acids in leaves of potato cultivars 'Russet Burbank' and 'Goldrush'. This experiment was conducted in a greenhouse under controlled conditions to characterize the chemical composition of the leaves while minimizing the variability of environmental and soil conditions often encountered in the field. The ultimate goal is to increase knowledge of the effects of N fertilization on potato foliar chemical composition which may reduce CPB infestation.

4.4 Materials and methods

4.4.1 Experimental design and management

The pot experiment was conducted in a greenhouse of Progest Company (http://progest2001.com/) at Sainte-Croix near Quebec City, Canada. Each plastic pot was 21.3-cm in height and 20-cm in diameter with three drainage holes at the bottom. Each pot was filled with an Agromix substrate (Fafard Canada Ltd., Montreal, Canada) containing peat moss and silver sand at a volume ratio of 1:1, up to a level of 5-cm from the top. Potato cultivars 'Russet Burbank' and 'Goldrush' were used in this experiment. Intact potato tubers that were similar in size (length$\approx$5-cm and 2-cm < width < 3-cm) were selected for each cultivar and planted at a 5-cm depth in the substrate. Ammonium sulfate (22-0-0) was applied as N fertilizer at the following five rates: 0, 0.87, 1.73, 2.60, and 3.47 g N per pot, corresponding to 0, 60, 120, 180, and 240 kg N ha^{-1} based on soil density. With regard to potassium and phosphorus fertilizers, 1.02 g potassium chloride (0-0-60) and 1.33 g triple superphosphate (0-46-0) were applied to each pot, corresponding to 200 kg P_2O_5 ha^{-1} and 200 kg K_2O ha^{-1}. After seeding, the three fertilizers were well-mixed and broadcast on the substrate surface. During the growing period, all pots were routinely watered twice per week with a sprinkler. Potato plants were grown at 20 ± 1°C under natural daylight corresponding to photoperiods increasing from 10 h 24 min at planting date (February 16, 2017) to 15 h 51 min at the final sampling date, specifically 103 days after planting (DAP), which was monitored from Jean-Lesage Airport weather station (46°48' N, 71°22' W). This experiment was conducted in a randomized complete block design with four replications for each treatment. Three pots were pooled for each replicate and a total of 120 pots were used in this greenhouse experiment.

4.4.2 Leaf sample collection and chemical analysis

The 3rd, 4th, and 5th completely developed leaves from the top of three randomly selected plants from a pot were collected at 61, 75, 89, and 103 DAP, starting at 50% potato flowering and then once every two weeks. Nine

fresh leaves were collected from each experimental unit (a replication corresponding to leaves pooled from three pots) at each sampling date, and the concentration of foliar sugars, glycoalkaloids, and 20 amino acids were analyzed. Leaves were dried at 55°C for a week, then ground to pass through a 1-mm sieve using an IKA Werke grinder (MF10BS1, IKA, USA) and stored in the dark at room temperature before chemical analysis. The samples were dried again at 105°C, using a thermogravimetric analyzer (Model TGA701, Leco Corporation) to express results on a dry matter (DM) basis.

Sugars (sucrose, glucose, and fructose) and amino acids were extracted from 200-mg dried samples using 7 mL of methanol-chloroform-water solution at a volume ratio of 12:5:3. Soluble carbohydrates in the extracts were analyzed using a Waters High-Performance Liquid Chromatography (HPLC) system. Amino acids in the extracts were separated on an AccQ-Tag Ultra C18 column and analyzed by Ultra Performance Liquid Chromatography (Acquity, Waters, Milford, MA) with a TUV detector (Waters Acquity). For glycoalkaloids analysis, 200-mg dried samples were extracted by using an 80-mL mixture of 1% acetic acid and 0.4% hexanesulfonic acid. The supernatant was analyzed for glycoalkaloid concentrations by HPLC (Waters, Milford, MA) using a 3.9×200 mm Resolve C18 column.

4.4.3 Statistical analysis

The experiment in this study was a factorial design with two potato cultivars and five N rates in each block. Therefore, a repeated randomized complete block analysis of variance (ANOVA) was conducted using sampling date as a repeated measurement to investigate the effects of potato cultivar, N rate, and their interaction on potato foliar chemical composition, including sugars, glycoalkaloids, and amino acids. Potato cultivars and N rates were considered as fixed effects, whereas blocks (N rate × potato cultivar) were treated as a random effect. The normality assumption was investigated using the Shapiro-Wilk statistic, while the homogeneity of variance was verified using the residual plots. The transformation method recommended by the Box–Cox test was carried out when needed. The protected least significant difference (LSD) multiple comparison method was used after significant effects were found in the ANOVA analysis. Linear and quadratic effects of N rate and sampling date were determined using many *a priori* orthogonal contrasts. All effects were declared statistically significant at $p < 0.05$ using the Mixed procedure of SAS (SAS software, version 9.4, Cary, NC. USA).

4.5 Results

4.5.1 Sugars

Sugar concentrations were significantly influenced by potato cultivar, N fertilization, sampling date, and interactions of cultivar × N rate and cultivar × sampling date (Table 4-1). The concentrations of all sugar components in Goldrush leaves, including sucrose, fructose, and glucose, increased markedly in response to N

fertilization up to 120 kg N ha^{-1}, and then decreased at the rate 180 kg N ha^{-1}, and then increased again when 240 kg N ha^{-1} was used (Figure 4-1). However, N rate seems to have weaker impacts on sugar concentrations in Russet Burbank leaves (Figure 4-1).

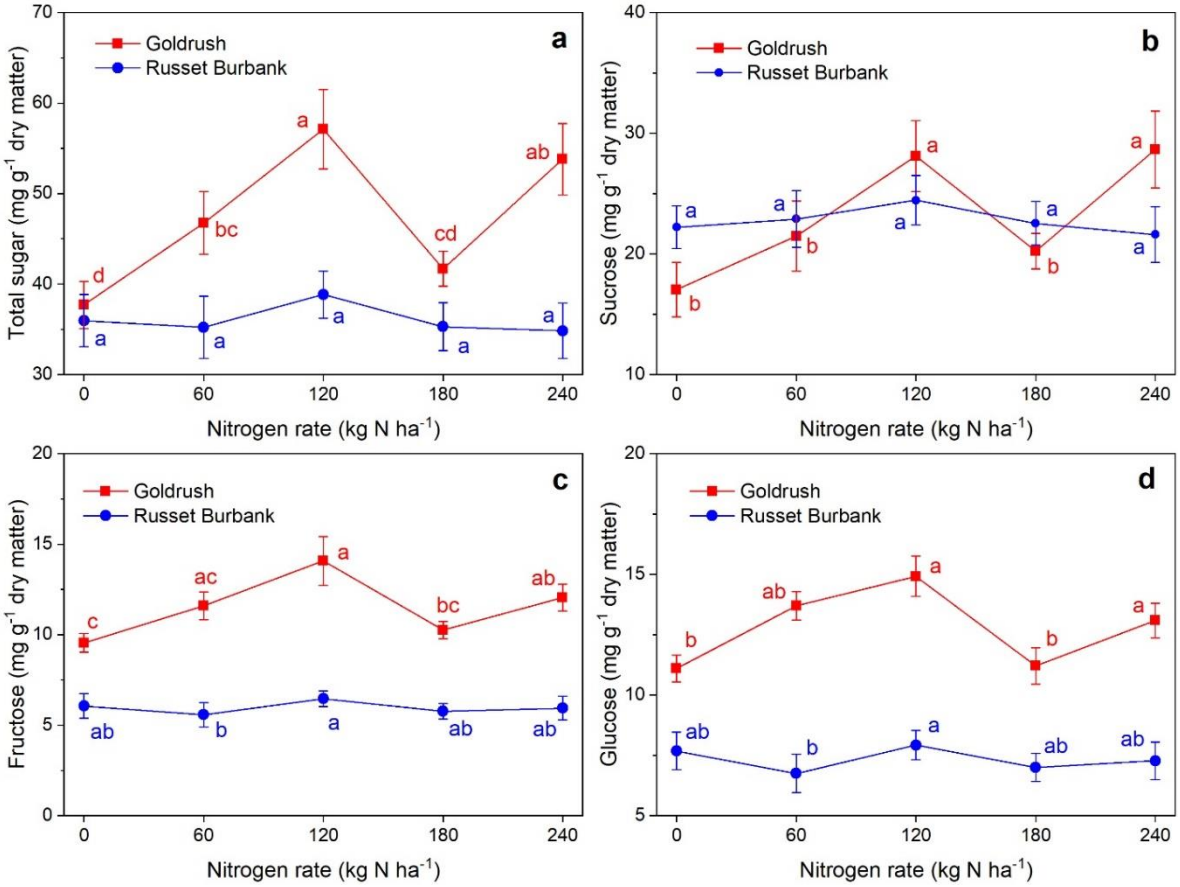

Figure 4- 1 Effects of N rate on foliar concentrations of total sugar (a), sucrose (b), fructose (c), and glucose (d) for each potato cultivar (Goldrush and Russet Burbank). Different letters indicate significant difference at $p < 0.05$ for each cultivar.

Table 4- 1 Analysis of variance (p values) and mean values on the effects of potato cultivar, N rate, and sampling date on concentrations of total sugar, sucrose, glucose, and fructose in potato leaves.

	Total sugar	Sucrose	Glucose	Fructose
Source of variance		---------- *p* values ----------		
Cultivar	<0.0001	ns†	<0.0001	<0.0001
N rate (N)	0.0002	0.0039	0.0039	0.0124
Cultivar*N	0.0030	0.0080	0.0106	ns
Sampling date (D)	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar*D	<0.0001	<0.0001	<0.0001	0.0081
N*D	ns	ns	ns	ns
Cultivar*N*D	ns	ns	ns	0.0232
Contrasts				
N linear	0.0196	0.0097	ns	ns
N quadratic	0.0169	ns	ns	ns
D linear	<0.0001	<0.0001	<0.0001	<0.0001
D quadratic	ns	ns	0.0236	ns
Cultivar		---------- Concentrations (mg g^{-1} Dry matter) ----------		
Goldrush	47.41a‡	23.1	12.80a	11.50a
Russet Burbank	36.02b	22.74	7.33b	5.96b
N rate (kg N ha^{-1})		---------- Concentrations (mg g^{-1} Dry matter) ----------		
0	36.82c	19.62c	9.39b	7.80b
60	40.99bc	22.18bc	10.22b	8.59b
120	47.97a	26.28a	11.42a	10.27a
180	38.49c	21.37bc	9.11b	8.01b
240	44.31ab	25.13ab	10.19b	8.99ab
Sampling date (DAP)		---------- Concentrations (mg g^{-1} Dry matter) ----------		
61	30.24d	15.54b	8.20c	6.51d
75	34.81c	16.61b	10.21b	7.98c
89	48.39b	28.71a	10.73ab	8.95b
103	53.42a	30.81a	11.11a	11.49a

†ns, not significant at $p > 0.05$. ‡Concentrations followed by different lower letters were significantly different at $p < 0.05$. DAP, days after planting.

Total sugar concentration increased progressively from 61 to 103 DAP in Russet Burbank leaves. But in Goldrush leaves, its concentration only increased significantly between 75 and 89 DAP (Figure 4-2a). A similar trend was observed for sucrose, which is the major type of sugar in potato leaves (Figure 4-2c). Glucose concentration in Goldrush leaves significantly increased from 61 to 75 DAP but decreased from 89 to 103 DAP. However, its concentration in Russet Burbank leaves progressively increased with increasing sampling dates from 61 to 103 DAP but remained lower than in Goldrush leaves (Figure 4-2b).

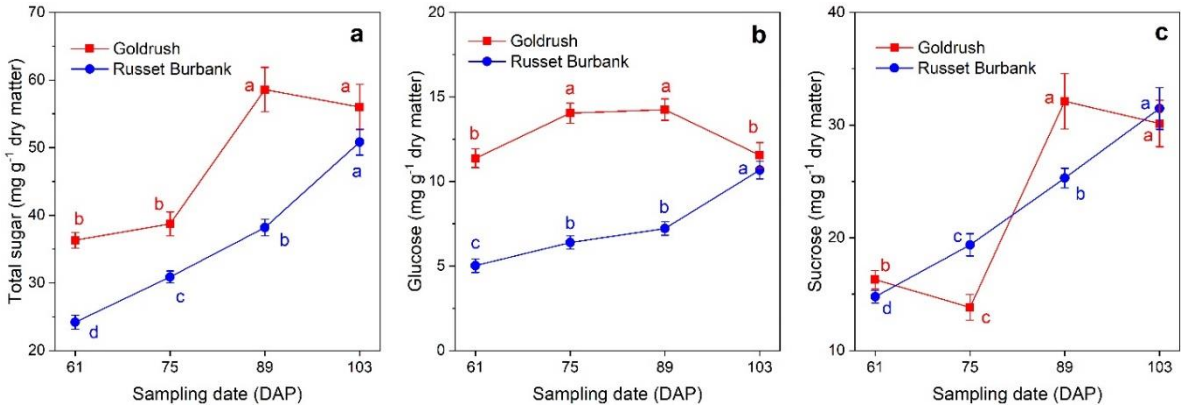

Figure 4- 2 Effects of sampling date on foliar concentrations of total sugar (a), glucose (b), and sucrose (c) for each potato cultivar (Goldrush and Russet Burbank). Different letters indicate significant difference at $p < 0.05$ for each cultivar.

4.5.2 Glycoalkaloids

The concentrations of total glycoalkaloids, α-solanine, and α-chaconine were significantly influenced by potato cultivar, sampling date, and the interactions of cultivar × sampling date and N rate × sampling date. Generally, potato cultivar Goldrush had significantly higher contents of glycoalkaloids, including α-solanine and α-chaconine, than Russet Burbank (Table 4-2). The foliar total glycoalkaloid and α-chaconine concentrations in Goldrush leaves remained stable, but α-solanine concentration significantly decreased from 61 to 75 DAP. After that, the concentrations of glycoalkaloids, including total glycoalkaloid, α-chaconine, and α-solanine, increased significantly from 75 to 103 DAP (Figure 4-3). Total glycoalkaloid and α-chaconine concentrations in Russet Burbank leaves gradually increased from 61 to 103 DAP. Foliar α-solanine concentration in Russet Burbank significantly increased from 61 to 89 DAP, then followed by a slight reduction at 103 DAP (Figure 4-3). There was no significant effect of N rate on the glycoalkaloid concentrations in either potato cultivar (Table 4-2).

Table 4- 2 Analysis of variance (*p* values) and mean values on the effects of potato cultivar, N rate, and sampling date on concentrations of of total glycoalkaloids, α-solanine, and α-chaconine in potato leaves.

	Total glycoalkaloids	α-Solanine	α-Chaconine
Source of variance	---------- *p* values ----------		
Cultivar	0.0017	0.0127	<0.0001
N rate (N)	ns†	ns	ns
Cultivar*N	ns	ns	ns
Sampling date (D)	<0.0001	<0.0001	<0.0001
Cultivar*D	0.0004	<0.0001	0.0344
N*D	0.0030	0.0003	0.0023
Cultivar*N*D	ns	ns	ns
Contrasts			
N linear	ns	ns	ns
N quadratic	ns	0.0275	ns
D linear	<0.0001	<0.0001	<0.0001
D quadratic	ns	ns	ns
Cultivar	---------- Concentrations (mg g^{-1} Dry matter) ----------		
Goldrush	10.37a‡	3.49a	6.88a
Russet Burbank	8.24b	2.94b	5.24b
N rate (kg N ha^{-1})	---------- Concentrations (mg g^{-1} Dry matter) ----------		
0	8.38	2.93	5.45
60	9.78	3.42	6.36
120	10.47	3.86	6.6
180	9	2.98	6.03
240	8.89	2.87	5.87
Sampling date (DAP)	---------- Concentrations (mg g^{-1} Dry matter) ----------		
61	7.02c	2.40b	4.62c
75	8.80b	2.94b	5.74b
89	10.32a	3.65a	6.66a
103	11.08a	3.86a	7.22a

†ns, not significant at $p > 0.05$. ‡Concentrations followed by different lower letters were significantly different at $p < 0.05$. DAP, days after planting.

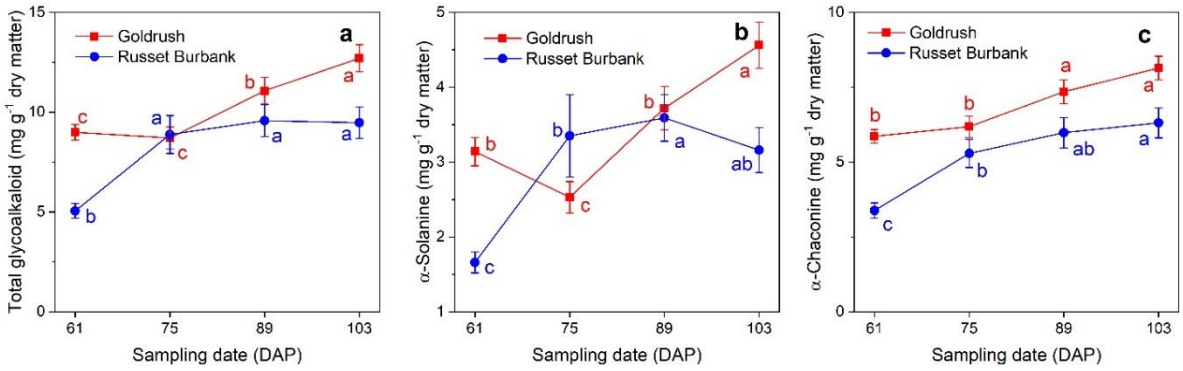

Figure 4- 3 Effects of sampling date on foliar concentrations of total glycoalkaloid (a), α-solanine (b), and α-chaconine (c) for each potato cultivar (Goldrush and Russet Burbank). Different letters indicate significant difference at $p < 0.05$ for each cultivar.

4.5.3 Amino acids

The total amino acid concentration was significantly influenced by N rate, sampling date, and the interaction of cultivar × sampling date (Table 4-3). Total amino acid concentration remained stable in response to N rates from 0 to 180 kg N ha^{-1} and then significantly increased in response to 240 kg N ha^{-1} (Table 4-3). A significant reduction in total amino acid concentration in Goldrush leaves occurred from 75 to 89 DAP. In contrast, its concentration in Russet Burbank leaves decreased significantly from 61 to 89 DAP and remained stable between 89 and 103 DAP (Figure 4-4a).

Table 4- 3 Analysis of variance (p values) and mean values on the effects of potato cultivar, N rate, and sampling date on concentrations of total amino acid, γ-aminobutyric acid (GABA), alanine (Ala), proline (Pro), serine (Ser), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and tyrosine (Tyr) in potato leaves.

	Total amino acid	GABA	Ala	Pro	Ser	Val	Ile	Leu	Phe	Tyr
Source of variance	---------- *p* values ----------									
Cultivar	ns†	<0.0001	ns	ns	ns	ns	ns	ns	<0.0001	ns
N rate (N)	0.0018	<0.0001	0.0114	ns	ns	ns	ns	ns	ns	ns
Cultivar*N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Sampling date (D)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar*D	0.0003	<0.0001	0.0002	ns	<0.0001	ns	ns	ns	0.0039	ns
N*D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cultivar*N*D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Contrasts										
N linear	0.0004	<0.0001	0.0169	0.0272	0.0170	ns	ns	ns	0.0099	0.0157
N quadratic	0.0338	0.0393	0.0264	ns	ns	ns	ns	ns	ns	ns
D linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.010	ns	<0.0001	<0.0001	<0.0001
D quadratic	ns	ns	ns	ns	<0.0001	0.003	<0.0001	ns	ns	0.0016
Cultivar	---------- Concentrations (mg g^{-1} Dry matter) ----------									
Goldrush	66.18	16.69b	16.17	3.60	2.20	4.09	2.96	3.71	3.09a	2.17
Russet Burbank	68.26	19.56a	17.00	3.41	2.09	3.82	2.87	3.80	2.50b	2.11
N rate (kg N ha^{-1})	---------- Concentrations (mg g^{-1} Dry matter) ----------									
0	63.49b‡	16.71c	15.96b	3.32	2.01	3.90	2.85	3.70	2.62	2.03
60	65.27b	17.19bc	16.50b	3.44	2.06	3.92	2.85	3.63	2.72	2.12
120	64.16b	17.09c	15.75b	3.33	2.09	3.84	2.82	3.57	2.79	2.09
180	67.36b	18.71b	15.93b	3.51	2.23	4.03	2.94	3.83	2.82	2.09
240	75.83a	20.92a	18.79a	3.92	2.33	4.09	3.11	4.03	3.04	2.39
Sampling date (DAP)	---------- Concentrations (mg g^{-1} Dry matter) ----------									
61	81.85a	21.00a	20.85a	3.87a	3.53a	3.93b	2.75b	4.16b	3.14a	2.13a
75	74.23b	18.79b	18.71b	4.14a	2.26b	4.50a	3.39a	4.80a	3.08a	2.61b
89	56.95c	16.16c	13.95c	3.12b	1.27d	3.80b	2.83b	2.99c	2.47b	1.93c
103	55.86c	16.54c	12.84c	2.87b	1.51c	3.59b	2.68b	3.06c	2.50b	1.91c

†ns, not significant at $p > 0.05$. ‡Concentrations followed by different letters were significantly different at $p < 0.05$. DAP, days after planting.

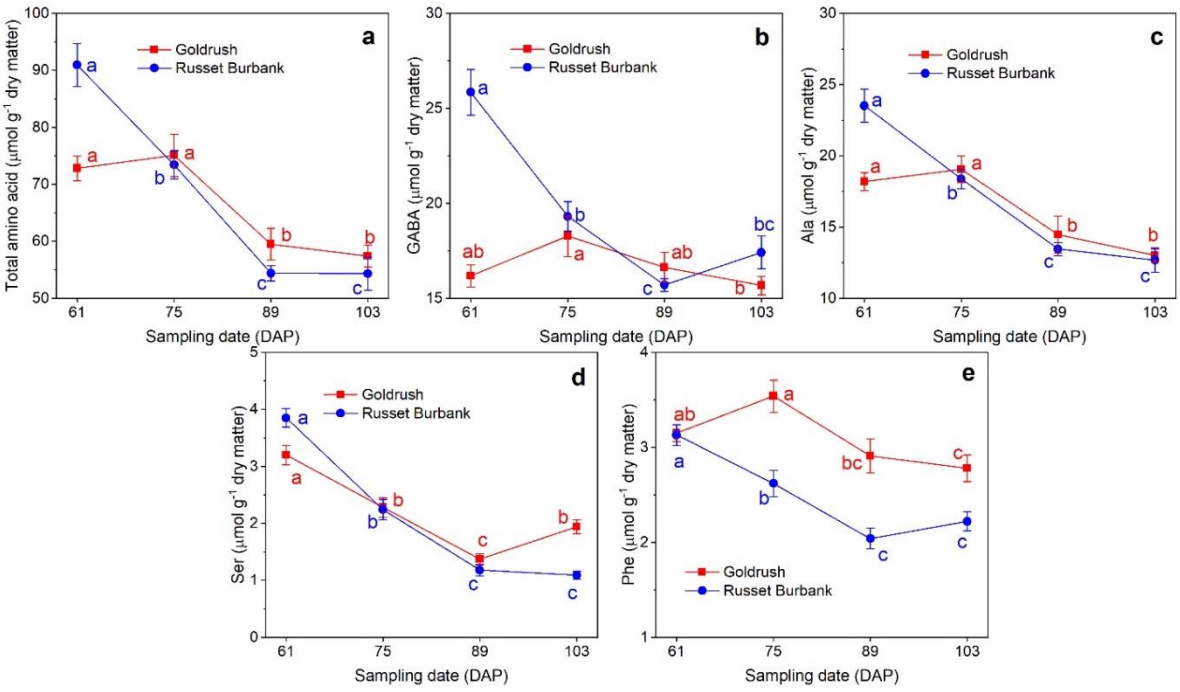

Figure 4- 4 Effects of sampling date on foliar concentrations of total amino acid (a), γ-aminobutyric acid (GABA, b), alanine (Ala, c), serine (Ser, d), and phenylalanine (Phe, e) for each potato cultivar. Different letters indicate significant difference at $p < 0.05$ for each cultivar (Goldrush and Russet Burbank).

Nitrogen rate, sampling date, and the interaction of cultivar × sampling date significantly influenced the concentrations of γ-aminobutyric acid (GABA) and alanine (Ala) (Table 4-3). The GABA concentration gradually increased from 17 to 21 μmol g^{-1} DM with increasing N rates from 0 to 240 kg N ha^{-1} (Table 4-3). Similar Ala concentration was observed with increasing N rates from 0 to 180 kg N ha^{-1} but its concentration increased significantly after 240 kg N ha^{-1} was supplied (Table 4-3). The GABA and Ala concentrations in Russet Burbank leaves significantly decreased from 61 to 89 DAP and then either increased or decreased slightly at 103 DAP. However, their concentrations in Goldrush leaves increased slightly from 61 to 75 DAP, then gradually decreased to 103 DAP (Figure 4-4b and 4-4c).

Concentrations of serine (Ser) and phenylalanine (Phe) were significantly influenced by sampling date and the interaction of cultivar × sampling date (Table 4-3). The Ser concentration in the leaves of both potato cultivars decreased significantly from 61 to 89 DAP. It subsequently increased greatly in Goldrush, but a similar level was observed in Russet Burbank leaves between 89 and 103 DAP (Figure 4-4d). The Phe concentration in Russet Burbank leaves significantly decreased from 61 to 89 DAP, with a similar concentration recorded between 89 and 103 DAP. However, in Goldrush leaves, Phe concentration remained stable from 61 to 75 DAP and from 89 to 103 DAP, with a significant reduction between 75 and 89 DAP (Figure 4-4e). Concentrations of proline (Pro), valine (Val), isoleucine (Ile), leucine (Leu), and tyrosine (Tyr) varied significantly during the course of the

experiment (Table 4-3). The Pro concentration decreased significantly over time, however, concentrations of Val, Ile, Tyr, and Leu increased from 61 to 75 DAP with a significant reduction at 89 DAP and remained stable up to 103 DAP (Table 4-3).

4.6 Discussion

4.6.1 Variation in sugar concentrations

The total sugar concentration was 36.02 mg g^{-1} DM on average in Russet Burbank leaves, which was slightly lower than in our previous study where the concentration ranged from 44 to 53.7 mg g^{-1} DM in Russet Burbank leaves in response to the same N fertilization rates under field conditions (Wen et al. 2019a). These differences were mainly due to changes in climatic conditions, such as diurnal light levels and water stress (Barnaby et al. 2015). Compared to Russet Burbank, a significantly higher total sugar concentration of 47.41 mg g^{-1} DM was observed in Goldrush leaves, which suggested that sugar metabolism is cultivar-specific (Braun et al. 2016).

Weeda et al. (1979) reported that potato foliar sugar can elicit marked feeding responses in CPB and its absence can inhibit CPB growth. Therefore, potato cultivar and N fertilization rate are two factors that can be used to strengthen the IPM strategy for controlling CPB because sugar concentrations were significantly influenced by these two factors (Table 4-1). We observed an increase in Goldrush foliar sugar concentrations in response to N rates from 0 to 120 kg N ha^{-1} (Figure 4-1). Evans and Clarke (2019) reported similar results, specifically that N fertilization could increase sugar accumulation in plants by promoting the biosynthesis of photosynthesis-related enzymes. Braun et al. (2016) also observed that potato plants fertilized with a high N quantity accumulated more sugars than those received low amounts of N. However, high rate of 180 kg N ha^{-1} caused a sharp decrease in Goldrush foliar sugar concentrations which could be due to sugar being transported to tubers and stored as starch. This explanation is supported by the findings of Lynch et al. (2012), who reported that a fertilizer rate of 190 kg N ha^{-1} maximized the tuber yield for cv. Goldrush. When the rate of 240 kg N ha^{-1} was used (Figure 4-1), sugars began accumulating in Goldrush leaves again, a pattern that could be linked with reports of reduced tuber yield in response to excess N fertilization (Lynch et al. 2012). Interestingly, the variations in sugar concentrations in response to N rates were moderate in Russet Burbank relative to Goldrush leaves. Vos and Biemond (1992) reported that N fertilization could significantly enlarge the plant leaf area. It indicated that sugar concentrations do not vary significantly, as the foliar sugar net accumulation rate (photosynthesis minus respiration) is equal to the leaf expansion rate. We also observed that sugar concentrations increased significantly with increasing sampling date (Table 4-1), which was mainly due to the progressive increase of light exposure (Simon et al. 2017). Khelifi et al. (2007) reported that the appeared larvae emerging in the summer can rapidly develop into adults within a three-week period. This result may be related to the progressive increase

in foliar sugar concentrations during potato plant development (Table 4-1). However, CPB growth in potato plants is also closely related to climatic conditions, such as air temperature (Ferro et al. 1985).

4.6.2 Variation in glycoalkaloid concentrations

Glycoalkaloids are frequently used as natural plant defensive compounds because their anticholinesterase activity can damage the digestion systems of insects (Mondy and Munshi 1990). Consequently, any factor that could modify potato foliar glycoalkaloid concentrations can be regarded as a potential component of IPM for CPB populations. Interestingly, we observed that foliar glycoalkaloid concentrations progressively increased during the course of potato growing for both cultivars (Figure 4-3), which could be due to the gradual increase in light exposure and duration (Mekapogu et al. 2016). This result is consistent with the finding of Lafta and Lorenzen (2000) that a reduction of irradiance resulted in a significant decrease of 40% in glycoalkaloid accumulation in potato plants. Besides, glycoalkaloid metabolism is highly genotype specific (Papathanasiou et al. 1999). In this study, Goldrush contained higher foliar glycoalkaloid concentrations than Russet Burbank (Table 4-2). This suggests that potato cultivars could be proposed as part of an IPM strategy for CPB and that the cultivar Goldrush seems to be a better choice than Russet Burbank for reducing CPB feeding.

4.6.3 Variation in amino acid composition

Amino acids in host plants are essential for CPB growth and influence their feeding and flight behaviors. Domek et al. (1995) reported that CPB preferred young potato foliage to old leaves because young potato leaf contained higher concentrations of Glu, Ser, Asp, Gln, Pro, His, and Arg. Thus, adjusting cultivation practices, such as N fertilization, to alter potato foliar amino acid composition could be included in an IPM strategy for CPB. In our study, N fertilization significantly increased total amino acid concentration in Russet Burbank leaves, mainly as a result of the increase in GABA and Ala concentrations with increasing N rates (Table 4-3). Wen et al. (2019a) reported that N fertilization promoted potato plant N accumulation and that this increasing N could be stored in the form of amino acids. In this study, total amino acid concentration remained stable in response to N rates from 0 to 180 kg N ha^{-1} and then increased from 180 to 240 kg N ha^{-1} (Table 4-3), suggesting that a rate of 180 kg N ha^{-1} could possibly be used for CPB management while maintaining a good tuber yield (Wen et al. 2019a). However, the potato cultivar grown seems to have a limited impact on CPB management in terms of amino acids because similar concentrations were found in Goldrush and Russet Burbank leaves. The variation in CPB populations between two different potato cultivars should be monitored in future field studies in order to confirm that potato foliar amino acid concentrations differ significantly between the cultivars under natural conditions and have an impact on CPB feeding.

4.6.4 Potential effects on herbivorous potato pests

Nitrogen fertilization significantly increased potato foliar sugar and amino acid levels but did not alter total glycoalkaloid concentration, which indicates that N fertilization could accelerate the CPB growth by the promotion effects of sugars and amino acids on CPB development. In this study, significantly different levels of foliar sugars and glycoalkaloids were observed between the two potato cultivars. Therefore, N fertilization and potato cultivar could be used as additional tools to develop an IPM for controlling CPB. This conclusion could also be applied to control other herbivorous pests because some chemicals, such as GABA, have different effects on the growth of other insect species. As a non-proteinogenic amino acid, GABA plays an adverse role in the growth of herbivorous pests in general through a detrimental effect on the peripheral nervous system of insects (Huang et al. 2011). However, Mitchell and Schoonhoven (1974) reported that GABA could promote CPB growth because this pest has a GABA-sensitive sensor in its mouth which allows it to effectively use the GABA as a nutriment (Mitchell and Harrison 1984).

4.7 Conclusion

In this study, potato cultivar and N rate were found to play an important role in altering potato foliar chemical composition. Generally, N fertilization significantly increased sugars and amino acids accumulation. Based on the findings in this study and tuber yield data reported in our previous article of Wen et al. (2019a), a rate of 180 kg N ha^{-1} was proposed for potato growers. This rate is near the recommended N rate (135 to 175 kg N ha^{-1}) for commercial farms in Quebec, Canada (CRAAQ 2010). Glycoalkaloid and sugar concentrations in Goldrush leaves were substantially higher than those in Russet Burbank leaves. Therefore, N fertilization and potato cultivar selection are possible approaches to supplement IPM strategies for CPB. But their effects on herbivorous pest growth, tuber yield, and environmental contamination should be further evaluated in field studies. Besides, it is noticeable that foliar chemical concentrations varied differently with increasing N rates when comparing potatoes grown in the field or under controlled conditions. Generally, potato crops can not reach their optimal growth status in containers because of the limited space that restricts tuber bulking and root extension. Differences could also be due to potato hilling management in our container study caused by the limited supply of substrate that could be added as compared as in a field study.

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General discussion

The CPB is the main insect pest that threatens the potato industry worldwide. In North America, potential yield losses due to the potato defoliation by the uncontrolled CPB populations have been estimated at around 30–50% (Boiteau, 2010). The CPB is widely regarded as one of the most difficult insect pests to control as it can thrive in a wide range of temperature conditions and successfully develop resistance towards various chemical insecticides (Dumas et al., 2019). Potato leaves are the major food source of the CPB and their chemical components play an important role in CPB growth and development. Interestingly, these foliar chemicals are closely related to N fertilization. Braun et al. (2016) reported that potato plants fertilized with a low N rate accumulated smaller amounts of soluble sugars in their leaves than adequately fertilized plants. Therefore, a good N fertilization strategy may be used to control the CPB populations by altering the potato foliar chemical concentrations. In this thesis, the effects of N fertilization on potato foliar sugar, glycoalkaloid, and amino acid concentrations have been investigated between two different potato cultivars under field and controlled conditions. Subsequently, the potential of using N fertilization for an eventual control of CPB populations was shortly discussed.

1. Effects of N rates on potato foliar sugar, glycoalkaloid, and amino acid concentrations

The potato crop is highly responsive to N fertilization, which is usually the most limiting essential nutrient for tuber growth. Sufficient N supply is required for potato plants to achieve high tuber yield and quality to meet the target for processing potato production (Cambouris et al., 2016). Nitrogen is also an essential element of chlorophyll and N-containing enzymes, which contribute to foliar chemical metabolic processes. Thus, N plays a critical role in the potato plant metabolism of sugars and N-containing chemicals, such as glycoalkaloids and amino acids.

Under field conditions, Russet Burbank foliar sugar concentrations decreased as N rates increased at tuber initiation and bulking stages (Table 2-2 and Figure 2-1). The major reason is the translocation of sucrose, the most important component of sugars, from leaves to tubers to be stored as starch (Kumar et al., 2004). Additionally, N fertilization could significantly increase the leaf size, suggesting that a biomass dilution effect on foliar sugar contents may occur when the sugar synthesis rate is lower than the leaf growth rate (Vos and Biemond, 1992). However, a significant increase in Russet Burbank foliar sugar concentrations with increasing N rates after tuber maturation was observed (Figure 2-1). Jackson and Haddock (1959) reported that the potato growth rate reached its maximum around 95 DAP for cv. Russet Burbank. Thus, it is possible that sugar synthesis rate in leaves was higher than the sugar transportation rate to tubers as starch and then resulted in the foliar sugar accumulation. At that point, less sugar was required for starch synthesis in tubers when

compared to the sugar photosynthesized in potato leaves (Sturm and Tang, 1999), which may explain the increase of sugar contents in potato leaves at 82 DAP. Interestingly, the variations in Russet Burbank foliar sugar concentrations in response to N rates were moderate under controlled conditions, which suggested that sugar metabolism in potato plants was closely related to climatic conditions. Different air temperature, precipitation (or irrigation), and light exposure duration between greenhouse and field experiments could significantly change the promoting effects of N fertilization on potato plant photosynthesis and then vary the foliar sugar accumulation patterns. An increase in Goldrush foliar sugar concentrations was also observed in response to N rates from 0 to 120 kg N ha^{-1}, followed by a decrease at 180 kg N ha^{-1}, and then an increase when 240 kg N ha^{-1} were applied (Figure 4-1). This indicated that potato cultivar had significant effects on foliar sugar concentrations with regard to applied N fertilizer rates.

As glycoalkaloids are N-containing compounds, their biosynthesis depends on N availability (Ginzberg et al., 2009; Najm et al., 2012; Friedman et al., 2017). Nitrogen fertilization progressively increased Russet Burbank foliar glycoalkaloid concentrations, especially α-chaconine (Table 2-3 and Figure 4-3) regardless of potato growing conditions (greenhouse or field) because N is an essential component of enzymes involved in the glycoalkaloid synthesis, such as glycosyltransferase enzymes (Friedman, 2006). Also, insecticide applications (Zarzecka et al., 2013) and CPB infestation (Pariera Dinkins et al., 2008) during potato growing stage under field conditions promoted foliar glycoalkaloid accumulation.

For foliar amino acids, they were classified into four groups in the field experiments according to their different roles in promoting the CPB growth and they were named feeding stimulant, inheritable amino acid, flight-energy substrate, and essential amino acid. Detailed information about this classification was presented in Chapter 3. Under controlled conditions, several major components of amino acids, such as GABA and Ala, were investigated to explore the N fertilization effects on their accumulation in potato leaves. Regardless of potato growing conditions, the concentrations of most amino acids progressively increased with increasing N rates (Figures 3-2 and 3-3, Tables 3-3 and 4-2). Amino acids are N-containing molecules that require N-rich enzymes for their assimilation and biosynthesis. As an element of chlorophyll, N is also essential for efficient photosynthesis (Fageria and Baligar, 2005) that provides carbon skeletons for amino acid biosynthesis (Bouché and Fromm, 2004), which in turn sustains a gradual reduction in foliar sugar concentrations with increasing N rates application. Through these pathways, N fertilization could increase amino acid biosynthesis in potato leaves.

In Chapter 2, the rate of 205 kg N ha^{-1} was recommended to Russet Burbank growers since the tuber yield reached the maximum at this N rate. Also, low sugar and relatively high glycoalkaloid concentrations were obtained at 205 kg N ha^{-1} when compared to other N rates. However, a rate of around 180 kg N ha^{-1} was

suggested in Chapter 3 for Russet Burbank potatoes. This was based on the foliar chemical concentrations, including total sugar, total glycoalkaloid, total amino acid, and marketable tuber yield (Tables 2-3, 2-4, and 3-3). Total sugar concentration did not vary between 120 and 180 kg N ha^{-1}, but significantly decreased with a rate of 240 kg N ha^{-1}. Total glycoalkaloid concentration significantly increased from 60 to 120 kg N ha^{-1} and did not change with more N fertilizer supply. Amino acid concentrations, particularly feeding stimulant and inheritable amino acid significantly increased with increasing N rates from 0 to 180 kg N ha^{-1} and did not change thereafter with more N fertilizer application. Tuber yield significantly increased with increasing N rates up to 120 kg N ha^{-1}. Thus, the rate of 180 kg N ha^{-1} is recommended to obtain an acceptable tuber yield while maintaining high glycoalkaloid and low sugar concentrations in Russet Burbank leaves. Regarding amino acids, their concentrations were much lower at 180 kg N ha^{-1} than at 240 kg N ha^{-1}, but the rate of 180 kg N ha^{-1} is close to the recommended N rate (135 to 175 kg N ha^{-1}) in Quebec, Canada (CRAAQ, 2010). However, the effects of N fertilization rates on CPB growth, tuber yield, and environment should be further investigated under field conditions.

This study indicated that foliar chemical concentrations varied with increasing N rates depending on the potato varieties. Overall, potato crops cannot reach their optimal growing status in the pots under controlled conditions because the narrow space significantly limits tuber bulking and root extension. Also, there may be not enough medium (peat moss in this study) to completely cover the tubers compared to field conditions, particularly after hilling.

2. Effects of potato cultivars on potato foliar sugar, glycoalkaloid, and amino acid concentrations

Under controlled conditions, total sugar concentration was 36.02 mg g^{-1} DM on average in Russet Burbank leaves and was significantly higher in Goldrush leaves with 47.41 mg g^{-1} DM, which suggests that sugar metabolism is cultivar-specific (Braun et al., 2016). Glycoalkaloid metabolism is highly genotype-specific as well, which was reported by Sinden and Webb (1974) after an investigation of six potato cultivars. In this study, Goldrush contained higher foliar glycoalkaloid concentrations than Russet Burbank under controlled conditions (Table 4-1). However, potato cultivar seems to have a limited impact on CPB management in terms of amino acids because similar concentrations were found in Goldrush and Russet Burbank leaves. As reported, Russet Burbank has a late maturation (Bethke et al., 2014) while Goldrush is a mid-season variety (Lynch et al., 2012). Therefore, the nutrient uptake and accumulation patterns in the leaves of these potato cultivars could greatly vary during their growing seasons. Since foliar chemicals (sugars and glycoalkaloids in this study) varied significantly between Russet Burbankd and Goldrush, potato varieties could be suggested as part of an IPM strategy for CPB management. However, the variation in CPB populations between different potato cultivars should be investigated in future under field studies.

3. Potential role of nitrogen fertilization in CPB control

Stieha and Poveda (2015) reported that potato crops can withstand a significant amount of defoliation up to 20% without a major impact on yields. However, the CPB can completely defoliate potato plants and highly reduce tuber yields if no effective control mean is used. For decades, chemical insecticides have been the predominant control method for CPB management (Alyokhin, 2009; Sablon et al., 2013). However, the CPB has the capacity to rapidly develop resistance to most commercial chemicals, making them ineffective after few year applications. Although some other approaches have been used, such as crop rotation, straw mulch cover, biological control, transgenic plants, and RNA interference, none of them has proven successfully on a large scale for potato production (Sablon et al., 2013). It is well known that nutrients in potato leaves have significant effects on the CPB growth as well as on the overwintering survival and fecundity (Hsiao and Fraenkel, 1968; Szafranek et al., 2008) and that the metabolism of these nutrients in plants is affected by N fertilization. Therefore, N fertilization may affect CPB feeding by altering the nutrient composition of potato foliage.

As secondary metabolites, glycoalkaloids are frequently used as natural bio-insecticides because their anticholinesterase activity can damage the digestion systems of insects (Mondy and Munshi, 1990). In this study, an increase in foliar glycoalkaloid concentrations occurred with increasing N fertilization rates. Therefore, N fertilization may play a significant role in preventing CPB feeding from potato leaves by changing the potato foliar glycoalkaloid concentrations as reported in previous research studies (Deahl et al., 1991; Jonasson and Olsson, 1994; and Sablon et al., 2013). On the other hand, concentrations of amino acids, promoting CPB growth and development, also significantly increased with increasing N rates, indicating that N fertilizer application is beneficial for CPB growth but unfavorable for CPB control. These results only provide a referential method to control the CPB. However, it has to be mentioned that previous publications simply provide general information about the promoting effects of sugars and amino acids and the inhibiting effects of glycoalkaloids on CPB growth. In our knowledge, the critical concentration of each foliar component that is required to stimulate or inhibit CPB development has never been discussed so far. Therefore, it is highly recommended to investigate the threshold values of each potato foliar chemical affecting the CPB behaviors. Afterward, the effects of N fertilization on the CPB growth and populations through varying potato foliar chemical composition should be studied at large scale in the field for different potato varieties. The obtained results could also be usefull to control other potato herbivorous pests.

General conclusion

As an above-ground pest, the CPB is the main insect pest of potato plants. Both adults and larvae feed on potato leaves and their damage can greatly reduce yields and even completely destroy potato plants. In North America, potential potato yield losses have been estimated at 30 to 50% due to uncontrolled CPB populations. Although many physical, biological, and chemical approaches have been implemented for decades, none of them is effective enough at large scales with a comparatively low economic invest. Thus, economical and environmentally friendly alternative methods need to be developed to control CPB populations.

Potato leaves are the main food source of the CPB and the nutrient components in their foliar juice play an important role in the CPB growth since they require a huge quantity of nutrients for their development. The investigation of the relationship between potato foliar chemicals and the CPB populations and feeding behaviors is therefore essential to better understand the role of the potato foliar nutrient composition in the behaviors of this beetle. In Chapter 1, chemical components in the potato leaf and their stimulating or inhibiting effects on the CPB feeding and growth were reviewed. Generally, carbohydrates, amino acids, and mineral elements are beneficial to the CPB development by providing energy for flight and overwinter activities. On the other hand, some chemicals, such as glycoalkaloids, are harmful to the CPB growth. It is important to mention that the volatile chemicals in potato leaf surface are also important in attracting the CPBs during their hosting stage.

Based on the relevant information summarised in Chapter 1, field experiments were carried out to investigate the responses of potato leaf chemicals (sugars and glycoalkaloids in Chapter 2 and amino acids in Chapter 3) to N fertilization. This original research confirmed that N fertilization plays an important role in altering the chemical concentrations of potato leaves. High N rate significantly increased glycoalkaloid and amino acid concentrations but decreased sugar contents under field conditions. A greenhouse experiment was also conducted to investigate the differences in foliar chemical components of two potato cultivars while different N rates were used. Results showed that N fertilization significantly increased potato foliar sugar concentrations with increasing N rates from 0 to 120 kg N ha^{-1}, then decreased at 180 kg N ha^{-1}, and re-increased after a rate of 240 kg N ha^{-1} was applied. The rate of 180 kg N ha^{-1} caused a sharp decrease in potato foliar sugar concentrations which could be due to sugar being conveyed to tubers and stored as starch. This explanation is supported by the findings of Lynch et al. (2012) and Wen et al. (2019), who respectively reported that fertilizer rates of 190 kg N ha^{-1} and 205 kg N ha^{-1} maximized the tuber yields of Goldrush and Russet Burbank cultivars. Regarding amino acids, a high N rate of 240 kg N ha^{-1} significantly increased their concentrations in potato leaves. However, no significant effect of N rate was observed on glycoalkaloid concentrations. Based on these results, a rate around 180 kg N ha^{-1} seems adequate to manage the CPB populations taking into consideration the effects of N fertilization on tuber yields and foliar chemical concentrations. Regarding the potato cultivars,

Goldrush leaves contained higher concentrations of sugars and glycoalkaloids than those of Russet Burbank and there was no obvious variation in total amino acid concentration between the two potato cultivars. Consequently, further investigation is required to determine the effects of potato cultivars on controlling the CPB by altering the foliar chemical composition of the leaves.

Perspectives

It is well known that the CPB mainly feeds on potato leaves. This indicates that the nutrient composition in potato leaves play an important role in the growth and development of CPBs. Decades ago, Hsiao and Fraenkel (1968) investigated the effects of the nutrients on CPB feeding and behaviors in a laboratory experiment and found that sugars and most of the amino acids could elicit marked feeding responses of CPBs and the absence of specific food elements could inhibit the CPB growth. Similar experiments were carried out subsequently by Weeda et al. (1979), Lefevere et al. (1989), Tomlin and Sears (1992), and Arrese and Soulages (2010). However, they did not point out the potential of altering potato foliar chemicals at large field scales to control the CPB. Researchers also focused on some second metabolites, particularly glycoalkaloids, and investigated their effects on CPB growth (Hollister et al., 2001). They also provided possible techniques to increase potato foliar glycoalkaloid concentrations, such as using high air temperature (Lafta and Lorenzen, 2000). Tai et al. (2015) attempted to increase the potato foliar glycoalkaloid concentrations using the hybridization technique to improve the potato plant resistance to CPB defoliation. The obtained results showed a potential resistance of potato plants to CPB defoliation under controlled conditions. However, large field-scale experiments have not been carried out so far. Interestingly, changing the potato foliar component concentrations seems a potential method to supplement the IPM to control the CPB under an economic level. In this context, we investigated the responses of foliar chemical concentrations in Russet Burbank and Goldrush to different N rates, an important macronutrient for potato crops to obtain high tuber yield and good tuber quality, under field and controlled conditions. Our results provided a basic referential guide for future studies if they also focus on the CPBs diet and nutrient composition in their food. This means that decreasing the CPB food nutrient as low as possible and largely increasing the poisonous compounds in their diets is a potential method to control the CPB as the nutrient composition in the CPB diet can be changed under different N fertilization rates. However, the real effects of N fertilization on CPB management has not been well demonstrated. Thus, several specific recommendations are suggested for future studies:

1. Effects of various chemical components in potato leaves on the CPB behaviors

Potato leaves contain various chemical components, including volatiles on the leaf surface, proteins, carbohydrates, and mineral elements. Most of these chemicals are partly related to CPB growth. For example, proteins are essential for CPB growth because they are hydrolyzed to amino acids after being digested by this beetle. These amino acids would provide energy to the CPB's overwinter and flight activities. On the other hand, some proteins called antibody in medical science are generally known to enhance the CPB resistance to environmental stresses. It is well established that the CPB can successfully develop resistance to chemical insecticides and this resistance capability should mainly be attributed to these proteins. Other potato foliar

chemicals, such as hexanoic acid, carboxylic acids, alcohols, and hormones should also be investigated to determine whether they stimulate or inhibit the CPB growth.

2. CPB behaviors after feeding on different potato cultivars

Potato foliar chemicals of the two potato cultivars Russet Burbank and Goldrush have already been investigated under greenhouse conditions. The results indicated that Goldrush leaves contained higher sugar and glycoalkaloid concentrations than those of Russet Burbank and that no significant variation in total amino acid content between the two cultivars was observed. Further studies are recommended to investigate the responses of the CPB populations to different potato cultivars under field conditions.

3. Effects of different nitrogen sources on the CPB behaviors

The ammonium nitrate was used to alter the chemical accumulation in potato leaves, including sugars, amino acids, and glycoalkaloids. Currently, some slow-release N fertilizers become more popular due to their high N use efficiency and low N losses. Since slow-release fertilizers have low solubility in the soil, their N release velocity might perfectly match the potato crops requirement for the tuber growth, which indicates that there may be no exceeded N to support the chemical biosynthesis in potato foliage. In this case, the CPB growth might slow down and its population could eventually highly decrease with time if an appropriate slow-release fertilizer is adopted. Based on these results, the slow-release N fertilizer, such as polymer-coated urea (PCU), is recommended to growers during the potato growing processes, particularly for the purpose of CPB management.

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