AGRONOMIC AND STORAGE FACTORS AFFECTING ACRYLAMIDE FORMATION IN PROCESSED POTATOES

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Abstract

Acrylamide has been classified as a probable human carcinogen with neurological and reproductive effects. The compound is formed from reducing sugars and asparagine in the Maillard reaction during high-temperature processing, such as frying, baking and roasting. Among all foods containing acrylamide, fried potato products have been shown to be the highest contributors. Therefore, lowering acrylamide concentration in French fries and chips is a priority for the potato industry. The overall objective of this research is to determine effects of nitrogen (N) rate on tuber yield, tuber quality and acrylamide formation of recently developed potato cultivars, relative to the standard cultivars during the growing season and storage.

The first experiment determined the effect of N rate on tuber yield and tuber quality at harvest, and on tuber glucose concentrations and acrylamide concentrations in French fries and chips during storage at 7.2 ºC. New cultivars Alpine Russet, Dakota Trailblazer and Ivory Crisp were compared to conventional cultivars Russet Burbank and Snowden over two years. The new cultivars had similar or higher marketable yields than standard cultivars, which quadratically increased with greater N rate and optimized at 231 kg ha^{-1} in 2011 and 319 kg ha^{-1} in 2012. Critical petiole nitrate-N concentrations 50 and 70 days after planting for all cultivars were greater in 2012 than in 2011, suggesting that interpretation of critical values can be affected by growing conditions. Alpine Russet and Ivory Crisp had specific gravities suitable for commercial processing and low hollow heart incidence at all N rates. Dakota Trailblazer had high hollow heart incidence (greater

than 10 % at N rates above 125 kg ha^{-1}), and excessively high specific gravity, making it undesirable for processing but with potential to be a parent in a breeding program. In chip cultivars, glucose and acrylamide concentrations linearly decreased in 2011, and quadratically increased then decreased with greater N rates in 2012. The effect of N rate on French fry glucose concentrations varied with cultivar. Russet Burbank and Alpine Russet glucose concentrations decreased with increasing N rate, while they were not affected by N rate in Dakota Trailblazer. Glucose and acrylamide concentrations of chip cultivars generally increased during storage, while they were not changed, increased or decreased depending on year in the French fry cultivars. While N supply and storage time can affect acrylamide concentrations in fried potato products, the direction of response will depend upon cultivar and growing conditions, which often precludes the ability to predict effects on acrylamide formation.

The second experiment determined the effects of N rate on tuber yield, tuber quality, reducing sugars and asparagine concentrations during the growing season, glucose and acrylamide concentration during storage, and the relationships between acrylamide and its precursors for Dakota Russet and Easton compared to standard cultivar Russet Burbank over two years. Highest yield was produced by Easton, followed by Russet Burbank and then Dakota Russet in both years. New cultivars had less hollow heart tubers than Russet Burbank when environmental conditions were favorable for hollow heart formation. Dakota Russet and Easton had lower stem end reducing sugars than Russet Burbank at harvest under contrasting environmental conditions. Easton had lower asparagine concentrations at the stem and bud ends than Dakota Russet and Russet Burbank at harvest. Nitrogen rate effects were significant for yield, specific gravity and

asparagine concentration, but these parameters were sometimes influenced by sampling times and environmental conditions. The effect of storage time on glucose concentrations was significant, but differed by cultivar and year. When the potato crop experienced cold stress before harvest in 2014, higher stem end glucose concentration accumulated and then decreased during storage for all cultivars. However, in a growing season with minimal stress, stem end glucose concentrations either increased or did not significantly change after 32 weeks of storage depending on cultivar, while bud end glucose stayed at the same level for all cultivars. At 16 weeks of storage at 7.8 °C, acrylamide concentration linearly increased with increasing N rate. French fries produced from Dakota Russet and Easton contained significantly lower acrylamide concentrations than those produced from Russet Burbank both years. Glucose concentrations were positively correlated with acrylamide concentration with an $R^2 = 0.61$. Asparagine concentrations measured in tubers at harvest were also correlated with acrylamide concentration ($R^2 = 0.37$), when the ratio of asparagine/glucose was less than 1.06. These relationships suggest that in addition to tuber glucose and asparagine, other factors are involved with acrylamide formation in fried potato products.

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Introduction

Acrylamide has been classified as a probable carcinogen to humans by the International Agency for Research on Cancer (IARC 1994), with neurological and reproductive effects at high doses (Lea et al. 2007; Bethke and Bussan 2013). The detection of acrylamide existing in a wide range of high-temperature processed foods has greatly raised health concerns and prompted researchers to explore its formation and approaches to reduce its concentration in these products (Tareke et al. 2002; EFSA 2015). Among all food containing acrylamide, fried potato products have been shown to be the highest contributors (from 170 to 12000 ug kg^{-1}, Friedman 2003). Given the high consumption of processed potatoes in Europe and the U.S, lowering acrylamide concentration in French fries and chips remains a priority in the potato industry (Friedman and Levin 2008).

While the mechanisms of acrylamide synthesis have been proposed, the chemical steps are not yet fully understood (Knol et al. 2010; Parker et al. 2012; Bethke and Bussan 2013). Acrylamide is not present in raw tubers, but forms from reducing sugars (mainly glucose and fructose) and asparagine in the Maillard reaction during high-temperature processing, such as frying, baking and roasting (Michalak et al. 2011). Other compounds (such as melanoidin, pyrazine, pyrroles, furans, oxazoles, thiazoles, and thiophenes) that improve color, flavor and taste of the food products are also produced along with acrylamide in this reaction (Mottram 2007; Knol et al. 2010; Halford et al. 2012a).

Working to decrease the concentration of precursors in raw potatoes is one effective approach to reduce acrylamide in fried potato products (De Wilde et al. 2005; Lea et al. 2007). Between the two precursors, asparagine is often far in excess of the reducing sugars glucose and fructose. One study reported a 3.7 to 5.6 times higher concentration of asparagine than glucose or fructose from 17 potato cultivars (Amrein et al. 2003). Stark and Love (2003) reported reducing sugars from less than 0.04 to 4.8 mg g^{-1} dry weight, while Bethke and Bussan (2013) reported the asparagine concentration in six studies ranged from 4 to 25 mg g^{-1} dry weight. In view of the low concentration of reducing sugars in raw tubers, and the strong correlation between reducing sugars and acrylamide concentrations, reducing sugars are generally considered as the limiting factor in acrylamide formation (Amrein et al. 2003; Ohara-Takada et al. 2005; Elmore et al. 2015); although there are certain situations when asparagine can control the reaction as discussed below. Genetic modification has also demonstrated the importance of reducing sugars in acrylamide formation by silencing vacuolar invertase, an enzyme that converts starch to reducing sugars. This transformation resulted in significantly lower concentrations of reducing sugars in tubers and acrylamide in fried potato products (Ye et al. 2010; Bhaskar et al. 2010). However, genetically modified (GMO) potatoes are not currently accepted by the fast food industry (Philpott 2014).

To lower reducing sugar concentrations, evaluation of cultivar, agronomic factors (year to year variation in weather conditions, soil property, fertilizer type, timing and amount), storage temperature and storage duration have been investigated (Matsuura-Endo et al. 2004; Silva and Simon 2005; Friedman and Levin 2008). Potato tubers tend to have genetically determined sugar levels, which can consistently affect acrylamide

formation (Nelson and Sowokinos 1983; Amerin et al. 2003; Halford et al. 2012b). Nitrogen fertilizer rate is an additional variable that can greatly influence reducing sugar concentrations. Plants with adequate N supply generally produce tubers with low reducing sugar concentrations at harvest and through storage (Westermann et al. 1994; Kumar et al. 2004; De Wilde et al. 2006). However, this is not always the case, and this N rate effect might be type (French fry and chips), and cultivar-dependent (Muttucumaru et al. 2013).

During the growing season, sugar translocated from leaves to tubers is in the form of sucrose (Lalonde et al. 1999). The hydrolyzing reaction of sucrose to glucose and fructose by invertase is prevented by a protein inhibitor until harvest (Richardson et al. 1990). Low storage temperature (4℃) is ideal for preventing tubers from sprouting, but can greatly increase invertase activity and cause reducing sugar accumulation (Sowokinos 1990; Matsuura-Endo et al. 2006). Potato tubers need to be stored for a long time to assure a year-round supply for the fresh market and processing industry. Therefore, the post-harvest storage conditions can influence tuber sugar concentration and acrylamide-forming potential. An increasing amount of acrylamide from August to February in the year after harvest has been observed in commercial chip products over multiple years in both Europe and Japan (Powers et al. 2013; Tsukakoshi et al. 2013). However, the effect of storage time on reducing sugars differs with cultivar, and usually interacts with storage temperature (Matsuura-Endo et al. 2004). Therefore, it is important to investigate effects of post-harvest storage time on reducing sugars and acrylamide concentrations in recently released cultivars.

Besides N supply and storage conditions, in-season weather conditions and harvest time may also affect reducing sugar concentrations. Heat and cold stress can increase invertase activity and reducing sugar accumulation because of the disruption of normal biochemical processes (Krauss and Marschner 1984; Kumar et al. 2004; Thompson et al. 2008; Bethke and Bussan 2013). When a tuber is considered chemically mature, sucrose concentrations are at a minimum level. Therefore, to minimize tuber sugar concentrations, the recommended harvesting time is when the tubers are chemically mature (Sowokinos 1973; Sowokinos and Preston 1988). A harvest time that takes place before or after tuber chemical maturity is associated with high tuber sugar concentrations (Knowles et al. 2009; Knowles et al. 2015).

Asparagine is also associated with acrylamide formation and may be the limiting factor in tubers containing high amounts of reducing sugars (Amrein et al. 2003; Matsuura-Endo et al. 2006; Muttucumaru et al. 2017). This can occur when tubers are stored at low temperatures (2 to 4 ºC, Sowokinos 2001). Asparagine concentrations have been shown, in general, to increase with increasing N supply (De Wilde et al. 2006; Gerendás et al. 2007; Muttucumaru et al. 2013).

The overall objective of this research is to determine the effects of N rate on tuber yield, tuber quality and acrylamide formation of new cultivars, relative to the standard cultivars, during both the growing season and storage. In order for growers to adopt new cultivars, their agronomic performance and processing quality also needs to be determined. Therefore, in this research, N rate effects on agronomic performance were investigated for new French fry cultivars, Alpine Russet, Dakota Trailblazer and the new chipping cultivar, Ivory Crisp, relative to the standard cultivars Russet Burbank and

Snowden (Chapter 1). Then, the effects of N rate and storage time over a nine-month period on tuber reducing sugars and acrylamide formation were evaluated in Chapter 2. In Chapter 3, newly released cultivars Dakota Russet and Easton were monitored for continuous changes in reducing sugars and asparagine concentrations in response to N supply and contrasting environmental conditions, and compared to the standard cultivar Russet Burbank over two growing seasons. Tuber yield and quality characteristics of these new cultivars were also evaluated. In Chapter 4, glucose concentrations during storage were determined as affected by storage time, N rate and different growing conditions. The relationships among glucose, asparagine and acrylamide during storage were also explored and analyzed.

Chapter 1 - Nitrogen Response of French Fry and Chip Cultivars Selected for Low Tuber Reducing Sugars

Overview

New cultivars Alpine Russet, Dakota Trailblazer and Ivory Crisp have lower tuber reducing sugars and acrylamide-forming potential. Adoption of new cultivars by growers requires information about their responses to agronomic factors such as nitrogen (N) fertilizer. The objective of this study was to determine the effect of N rate on yield and quality of new cultivars relative to conventional cultivars Russet Burbank and Snowden. The experiment was conducted over two years as a randomized complete block design replicated four times with five N rates and five cultivars. The new cultivars had comparable or higher marketable yields, and a higher percentage of large tubers (greater than170 g) than the standard cultivars. Total and marketable yields responded quadratically to N and optimized at 231 kg ha^{-1} in 2011 and 319 kg ha^{-1} in 2012 for all cultivars. Dakota Trailblazer had high hollow heart incidence (greater than 10 % at N rates above 125 kg ha^{-1}), and excessively high specific gravity, making it undesirable for processing but with potential to be a parent in a breeding program. Alpine Russet and Ivory Crisp had specific gravity suitable for commercial processing, and low hollow heart incidence at all N rates. Critical petiole nitrate-N concentrations 50 and 70 days after planting for all cultivars were higher in 2012 than in 2011, suggesting that interpretation of critical values can be affected by growing conditions.

Introduction

Potato is a major food crop with high consumption around the world. Fried potato products (French fries and chips) represent more than 50 % of the market (National Potato Council 2014), and contributed 55 % of the dietary acrylamide in the United States (Katz et al. 2012). As in Europe, French fries contributed to most of the acrylamide intake, with the rest from chips and oven-cooked potatoes (Muttucumaru et al. 2015). Acrylamide is a suspected carcinogen and high amounts in fried potato products is a health concern (Mottram et al. 2002; European Food Safety Authority 2015). Previous studies have shown that reducing sugars and acrylamide forming potential depend on cultivar and in some cases nitrogen (N) rate, although results are not always consistent (Amrein et al. 2003; Long et al. 2004; Morales et al. 2008; Muttucumaru et al. 2013).

In addition to a high yield potential, new potato cultivars developed should have traits that result in improved processing quality such as low reducing sugars which reduce browning and acrylamide formation during high-temperature processing. New French fry cultivars Alpine Russet, Dakota Trailblazer, and a chip cultivar, Ivory Crisp were released in 2008, 2009, and 2002 respectively. A characteristic of these cultivars is their low concentrations of tuber reducing sugars, indicating a low potential for acrylamide formation during processing (de Wilde et al. 2005). In order for growers to adopt new cultivars, information about how they respond to management factors such as N fertilization is essential.

Nitrogen can significantly affect tuber quality and yield. Increased tuber yield and tuber size with increasing N rate have been reported by numerous investigators (White et al. 1974; Arsenault et al. 2001; Zebarth et al. 2004). Hollow heart is an internal disorder

in potato tubers which is generally associated with rapid tuber enlargement. The incidence of hollow heart is affected by cultivar, plant spacing, as well as rate and timing of fertilizer application, weather conditions, and soil moisture. The defect of hollow heart is usually prevalent in large tubers. Sufficient N is a prerequisite for hollow heart tubers, but not necessarily the cause. Nitrogen fertilizer application timing (shortly after tuber initiation) is crucial on hollow heart incidence (Rex and Mazza 1989). Variable effects of N supply on specific gravity have been reported, such as a linear decrease as N rate increases (Bélanger et al. 2002; Zebarth et al. 2004), and a decrease followed by an increase above the optimum N rate for yield (Long et al. 2004). Excessive N supply can cause other quality issues as well, such as tubers too large for the intended market, delayed tuber maturity, low dry matter concentration, and excessive stolons and vine growth (Belanger et al. 2002; Goffart et al. 2008). Excessive N fertilization will also lead to low N use efficiency, as well as potential groundwater contamination and greenhouse emissions because of N leaching and gaseous losses (Goffart et al. 2008; Fontes et al. 2010).

While yield depends on climatic conditions and N supply, it is also related to cultivar (Arsenault and Malone 1999; Zebarth and Rosen 2007). Differences in optimum N rate among cultivars exist because of the genetic variation in traits that contribute to total N uptake, N translocation and N assimilation (Xu et al. 2012). Fontes et al (2010) reported that the optimum N rates for yield ranged from 147 to 201 kg ha^{-1} depending on the four studied cultivars (Agata, Asterix, Atlantic and Monalisa). An N rate from 241 to 278 kg ha^{-1} was established to reach maximum yield for Russet Burbank on a Declo silt loam soil in Idaho (Love et al. 2005). In this three-year study, optimum N rates for

Summit Russet (230 to 293 kg ha^{-1}), Gem Russet (257 to 263 kg ha^{-1}), and Bannock Russet (211 to 213 kg ha^{-1}) were also presented. Another study (Atkinson et al. 2003) recommended an N rate of 280 to 325 kg ha^{-1} for Ranger Russet, and 246 to 291 kg ha^{-1} for Alturas, with two thirds applied pre-plant based on the relative total yield and relative U.S. No.1 yield in Idaho. Given the results of previous studies, it is, therefore, important to modify N fertilizer management based on the cultivar and the environment in which it is grown.

A few studies have been conducted on the agronomic performance of Alpine Russet and Ivory Crisp under growing conditions in the western U.S. (Love et al. 2003; Whitworth et al. 2011). Research on Dakota Trailblazer has been conducted in the Upper Midwest (Farnsworth et al. 2010). However, these cultivars have not been comparatively evaluated under a wide range of N fertilizer rates. In this study, the new French fry and chip cultivars and standard cultivars (Russet Burbank and Snowden) were evaluated for plant growth, tuber quality and yield under five N rates at Becker, Minnesota in 2011 and 2012. This research was initiated to investigate the interactive effects of cultivar and N management on potato yield and quality and was a part of a larger project to determine the effects of cultivar, N rate and storage time on acrylamide forming potential of fried potato products. The specific objectives of this study were to (1) determine the N fertilizer effects on yield potential and tuber quality of new and standard cultivars; (2) explore the possible interaction of cultivar by N rate on tuber yield and quality; 3) establish petiole nitrate-N sufficiency ranges for the new and standard cultivars.

Materials and Methods

This study was conducted at the Sand Plain Research Farm in Becker, Minnesota, on a Hubbard loamy sand (sandy, mixed, frigid Entic Hapludolls) soil in 2011 and 2012. The previous crop was rye (*Secale cereale* L.) for both years. Pre-planting soil chemical properties of each year are presented in Table 1-1. Prior to planting, 280 kg ha^{-1} 0-0-60 and 280 kg ha^{-1} 0-0-22 were broadcast and incorporated with a chisel plow in all plots.

Fertilizer Treatments

In this study, each cultivar was subjected to five N fertilizer treatments, 34, 135, 202, 269 and 336 kg N ha^{-1}. Because space was limited to five N treatments, 68 kg N ha^{-1} was omitted in order to obtain better precision at the higher N rates. At planting, all plots received 31 kg N ha^{-1} as monoammonium phosphate and 3 kg N ha^{-1} as ammonium sulfate applied in a band 8 cm to the side and 5 cm below the seed tuber. In addition to the N applied at planting, all plots received 146 kg P_2O_5 ha^{-1}, 203 kg K_2O ha^{-1}, 29 kg S ha^{-1}, 40 kg Mg ha^{-1}, 0.6 kg B ha^{-1} and 2.6 kg Zn ha^{-1}, applied as a blend of monoammonium phosphate, potassium chloride, potassium magnesium sulfate, ammonium sulfate, boric acid, and zinc sulfate. At emergence, N was side-dressed at 0, 101, 168, 235 and 302 kg N ha^{-1} as Environmentally Smart Nitrogen (Agrium, Inc., 44-0-0) and then hilled in.

Plot Design and Plant Management

A randomized complete block design was adopted with four replications using a factorial treatment arrangement of N rate and cultivar. Each plot consisted of four 6 m rows with the middle two as test rows. The spacing between rows was 0.9 m and seed tubers were spaced 0.3 m apart within each row. Test rows of each plot had two red potato plants at both ends as markers. Whole "B" seed (56 to 84 g) of Russet Burbank, and cut "A" seed (56 to 84 g) of Snowden, Alpine Russet, Dakota Trailblazer, and Ivory Crisp were hand planted in furrows. Growers in the region often use B seed for Russet Burbank (the main cultivar grown in Minnesota) to reduce the spread of seed borne diseases during the cutting process. Belay insecticide was applied in-furrow for Colorado potato beetle control, along with the systemic fungicide Quadris. Weeds, diseases, and other insects were controlled using standard practices (Midwest Vegetable Production Guide for Commercial Growers 2012). Rainfall was supplemented with sprinkler irrigation using the checkbook method of irrigation scheduling (Wright and Bergsrud 1991).

Sample Collection

Planting and harvesting dates were scheduled according to weather conditions, so the growing season length was different each year. French fry cultivars (Russet Burbank, Alpine Russet and Dakota Trailblazer) had 143 growing days (3949 growing degree days, GDD) in 2011, and 157 growing days (4365 GDD) in 2012. Chip cultivars (Snowden and Ivory Crisp) had 135 growing days (3835 GDD) in 2011, and 146 growing days (4122

GDD) in 2012. Growing degree days were calculated as (Maximum Daily Temperature + Minimum Daily Temperature)/2 – Base Temperature for potato plant 40 ^{o}F. Stem number data were collected based on 10 plants per plot in the test rows, on 14 June 2011 and 6 June 2012. Petiole samples (20 petioles per plot) from the fourth leaf from the terminal were collected 48, 56, 69 and 84 days after planting in 2011, and 55, 72, 84 and 98 days after planting in 2012. Petiole samples were oven-dried at 60 ^{o}C for at least 72 hours, then ground with a Wiley mill to pass through a 2-mm sieve. Petiole nitrate-N concentration was determined using a conductimetric procedure following the methods of Carlson (1986) and Carlson et al (1990).

Vines were killed on 15 September 2011 and 10 September 2012 for chip cultivars, 23 September 2011 and 21 September 2012 for French fry cultivars. All plots were machine harvested on 29 September 2011 and 2 October 2012. Tubers were then graded for yield based on the following weight categories: 85 g, 86 to 170 g, 171 to 283 g, 284 to 397 g and greater than 397 g. Tubers greater than 85 g were designated as marketable yield. Tuber subsamples (25 tubers per plot) were collected from different size categories, five tubers from the 86 to 170 g category, ten tubers from the 171 to 283 g category, seven tubers from the 284 to 397g category and four tubers from the greater than 397g category. These samples were used to determine specific gravity and the incidence of hollow heart. Tubers were weighed in air and water for the determination of specific gravity (Schippers 1976). The 25 tubers were cut apical to stem end to determine the incidence of hollow heart.

Statistical Analysis

Analysis of variance (ANOVA) was performed using PROC ANOVA of the SAS 9.4 statistical software package (SAS Institute Inc., Cary NC. USA) for each measured variable to assess the significance of main effects and interactions between cultivars nested within N rate (34, 135, 202, 269 and 336 kg ha^{-1}) and year (2011 and 2012). Means of interest were then compared using the least significant difference (LSD) at the 5 % probability level. A square root transformation was used when necessary to account for the heterogeneity of variance. Linear, quadratic or non-affected responses of yield, percentage of tubers greater than 170 g, hollow heart incidence and specific gravity as a function of N rate were determined using orthogonal contrasts. Linear or quadratic trends were analyzed within each cultivar and/or year when interactions with N rate were significant. A probability of 0.05 was considered significant for linear or quadratic trends, and the quadratic function was selected when both linear and quadratic trends were significant. Linear or quadratic regressions between N rates and petiole nitrate-N concentrations were determined at each sampling date for each cultivar and year. The critical petiole nitrate-N concentrations at the optimum N rates (231 kg ha^{-1} in 2011, and 319 kg ha^{-1} in 2012) were calculated from these linear or quadratic equations using Excel (Microsoft). A linear trend of critical petiole nitrate-N concentrations as a function of days after planting was then established based on calculated petiole nitrate-N concentrations from the regression equations. The critical concentrations established from the calculated equations were compared with the trends from the actual petiole nitrate-N concentrations for the 202, 269 and 336 kg N ha^{-1} treatments.

Results and Discussion

Weather Conditions

The monthly average temperature was higher in 2012 than in 2011 and the average for the past 30 years from April to July (Table 1-2). Seed tubers were planted on 3 May in 2011, 16 days later than in 2012, because of the cold early growing season in 2011.

Nitrogen fertilizer applications were completed by 25 May 2011 and 17 May 2012 using polymer-coated urea (ESN, Agrium INC.). The water holding capacity for the top 30 cm of Hubbard loamy sand soil in this study is 2.4 cm (Grimes, 1968). May 2011 and 2012, and July 2011 had more precipitation (16.8 cm, 28.6 cm, and 24.6 cm, respectively) than the average for the past 30 years (9.4 cm in May and 10.1 cm in July). Rainfall amounts greater than 2.4 cm would cause nitrate leaching because that amount would exceed the water holding capacity of the soil in the top 30 cm, where most of the potato roots are located. A total amount of 24.6 cm rainfall occurred in July 2011, with four rainfall events greater than 2.4 cm (ranging from 2.9 to 5.7 cm). In 2012, 28.6 cm rainfall occurred in May, with 15.6 cm occurring after emergence fertilizer applications (with three rainfall events greater than 2.4 cm).

Plant Growth Response

Stem number per plant was significantly affected by the interaction of cultivar by year (Table 1-3). Compared to the standards (Russet Burbank and Snowden), Alpine Russet, Dakota Trailblazer and Ivory Crisp had significantly fewer stems per plant both

years (Table 1-4). Dakota Trailblazer had the least stems per plant (2.7) in 2011 while Alpine Russet had the least stems per plant (1.9) in 2012. The cultivar effect on stem numbers has also been shown in a former study (Knowles and Knowles 2006). For Russet Burbank, whole "B" seed tubers were used at planting, resulting in more stems than for cut seed pieces. This may have resulted in Russet Burbank having the most stems (4.5 in 2011 and 2.5 in 2012) among the French fry cultivars. Fewer stem numbers per plant were correlated with the higher percentage of large tubers (Knowles and Knowles 2006), and yield of U.S. No. 1 tubers (Iritani et al. 1983). Fertilizer N rate had no effect on stem numbers, which is consistent with a previous study reported by De la Morena et al (1994).

All cultivars had fewer stems per plant in 2012 than in 2011.The year effect on stem numbers per plant has been reported before, and the reasons might be due to growing and storage conditions of seed tubers, germinating temperatures or time of planting (Iritani et al. 1972). In this study, fewer stem numbers per plant in 2012 may be attributed to the earlier planting and warmer temperatures during germination in 2012 than in 2011. Stem numbers of the new cultivars have not been reported in previous studies. The stem numbers per plant for Russet Burbank has been reported to range from 1.3 to 2.8 (Iritani et al. 1972), and from 2.3 to 4.5 (Iritani et al. 1983). The stem number of Russet Burbank in this study (4.5 in 2011 and 2.5 in 2012) is rational based on the results of previous studies.

Yield Response

The interaction of cultivar by year was significant for all three yield categories: total yield, marketable yield and percentage of tubers greater than 170 g (Table 1-3), with exception of marketable yield in 2012 for all cultivars (Table 1-5). Within chip cultivars, Ivory Crisp had significantly lower total yield, a higher percentage of tubers greater than170 g and the same level of marketable yield compared to Snowden. For French fry cultivars, Alpine Russet and Dakota Trailblazer had a significantly higher percentage of tubers greater than 170 g, and higher or comparable marketable yield than Russet Burbank. Alpine Russet had significantly lower total yield than Russet Burbank in 2011 possibly due to soft rot, which resulted in a poor plant stand (61.4 %). Inappropriate storage or shipping and handling problems of seed tubers might be the reason. Dakota Trailblazer had a comparable total yield in 2011, but a lower total yield in 2012 than Russet Burbank.

The N rate by year interaction was significant for total and marketable yields (Table 1-3). In both years, total and marketable yields increased quadratically with increasing N rate over all cultivars (Figure 1-1). Yields increased up to 231 kg N ha^{-1} and then started to decrease in 2011, but continued to increase up to 319 kg N ha^{-1} in 2012. Differences in yield response between the two years are likely due to the longer and warmer growing season in 2012.

In general, N supply increased the proportion of large sized tubers. The N rate by cultivar interaction was significant for the percentage of tubers greater than 170 g (Table 1-3). All cultivars responded to increasing N rate in a quadratic trend except for Dakota Trailblazer (Figure 1-2). Dakota Trailblazer had the highest percentage of large tubers at

an N rate lower than 152 kg ha^{-1}, and a slow linear increase of percentage large tubers with increasing N rates. New cultivars (Alpine Russet, Dakota Trailblazer and Ivory Crisp) had significantly higher percentages of tubers greater than 170 g than the standard cultivars under all N treatments. The proportions of tubers greater than 170 g at lower N rates (135 to 269 kg ha^{-1}) for new cultivars were even higher than for standards at N rates 269 kg ha^{-1} or higher.

The new cultivars had consistently higher or comparable marketable yields, significantly fewer stems, and higher percentages of tubers greater than 170 g than the standard cultivars both years. This is consistent with the conclusion of a previous study (Knowles and Knowles 2006) indicating stem numbers are correlated with the percentage of large tubers. The agronomic optimal N rate in 2011 was 231 kg ha^{-1}, and 319 kg ha^{-1} in 2012 for all cultivars. A previous study showed that the recommended N rate for Alpine Russet grown in southern Idaho ranged from 224 to 303 kg ha^{-1} to obtain 45 to 67 mt ha^{-1} yield potential (Whitworth et al. 2011). Love et al (2003) recommended 258 to 303 kg N ha^{-1} as optimum application rate in southeastern Idaho for Ivory Crisp. The optimum N rates for new cultivars in this study were in a reasonable range based on the results of the previous studies. The interaction of cultivar by N rate was significant for the percentage of tubers greater than 170 g, but not for total and marketable yield, suggesting different cultivar responses to N rate is primarily related to tuber size distribution. N fertilizer management affects tuber yield and size distribution, while cultivar selection is an additional effective approach for the management of tuber size distribution. Whole seed tubers have been reported to produce significantly higher total tuber yield and tuber

numbers per plant than cut seed (Strange and Blackmore 1990), which might contribute to the high yield of Russet Burbank in this study.

Tuber Quality Response

In this study, the three-way interaction of cultivar by N rate by year was significant for hollow heart incidence (Table 1-3 and Figure 1-3). The N rate effect on hollow heart incidence was significant for Dakota Trailblazer both years. Dakota Trailblazer had the highest percentage of tubers with hollow heart, linearly increasing with increasing N rate. The percentage of hollow heart tubers was greater than 10 % for N rates higher than 100 kg ha^{-1} in 2011 and 125 kg ha^{-1} in 2012. Hollow heart incidence in Russet Burbank linearly increased with increasing N rate in 2011, but was not affected by N rate in 2012. This suggests that hollow heart incidence in Russet Burbank was more related to weather and growing conditions than N supply, but when conditions are favorable for hollow heart formation, increasing N rate will increase hollow heart incidence. In 2011, Russet Burbank hollow heart incidence increased from 10 to 20 % as N rate increased from 205 to 348 kg N ha^{-1}. In all other cultivars (Alpine Russet, Ivory Crisp, and Snowden), hollow heart was not affected by N rate, and the incidence was less than 10% at all N rates both years.

Previous studies have reported an increase in the incidence of hollow heart in potatoes, with increased N supply (Rex and Mazza 1989). However, this is not always consistent and is often cultivar-dependent (Storey and Davies 1992; Zebarth and Rosen 2007). The results of this study suggest that hollow heart is affected by cultivar, growing

conditions and N rate. Under the growing conditions in this study, Dakota Trailblazer was prone to have a consistently higher percentage of hollow heart incidence than the other cultivars tested. Alpine Russet and Ivory Crisp were not susceptible to hollow heart incidence at any of the N rates tested. Low incidence of internal defects for these two cultivars has previously been reported (Love et al. 2003; Whitworth et al. 2011). No hollow heart or brown center tubers were found for Alpine Russet grown in Idaho, Oregon and Washington from 2002 to 2004. Ivory Crisp grown in these same states for 3 to 4 years, only had 3 % incidence of hollow heart.

A certain range of specific gravity is desired in the potato processing industry to produce high quality fried potato products. High specific gravity often results in high quality and yield of French fries and chips, and low oil content for chips (Smith 1951; Lulai and Orr 1979). A specific gravity between 1.080 and 1.089 is recommended for frozen French fries and chip processing. Chip cultivars with specific gravity greater than 1.089 is not suitable for processing due to the tendency for brittle chips (Rowe and Curwen 1993). In this study, the three-way interaction of year by N rate by cultivar was significant for specific gravity (Table 1-3). The response to N rate was affected by cultivar and year (Figure 1-4). In both years, the specific gravity of Alpine Russet and Snowden increased linearly with increasing N rate, while Ivory Crisp increased quadratically with increasing N rate. For Ivory Crisp, specific gravity increased as N rate increased from 34 to 269 kg N ha^{-1}, tending to level off in 2011, and decreasing in 2012. For Russet Burbank, specific gravity quadratically increased with increasing N rate in 2011, but was not affected by N rate in 2012. Specific gravity increased for Dakota

Trailblazer as N rate increased from 34 to 202 kg ha^{-1} and then tended to decrease in 2011, while linearly increasing with increasing N rate in 2012.

Russet Burbank had lower specific gravity in 2012 than in 2011 (Figure 1-4). In both years, Dakota Trailblazer had the highest level of specific gravity over two years (1.105 and 1.104 on average) which is above the range desired for commercial French fry processing. Alpine Russet had the same or lower level of specific gravity than Russet Burbank in two years.

The two chip cultivar had the same specific gravity both years at N rates above 135 kg ha^{-1} (from 1.084 to 1.093). Ivory Crisp had significantly lower specific gravity than Snowden at the low N rate. These results are consistent with previous studies. In a three-year study, Whitworth et al (2011) reported an average specific gravity of 1.072 and 1.084 for early and late harvested Alpine Russet, respectively. In North Dakota State University trials, Dakota Trailblazer was reported to have an average specific gravity of 1.091, lower than the values in this study (North Dakota State University Research Foundation). Love et al (2003) reported an average specific gravity of 1.085 for Ivory Crisp in a seven-year study conducted in three states.

These results suggest that cultivar selection is an effective way to provide tubers with specific gravity suitable for commercial processing. Environmental and cultural conditions including N management should also be taken into account, since specific gravity may be positively or negatively modified by cultivar.

Petiole Nitrate-N Concentrations

Determining petiole nitrate-N concentration from the most recently mature leaf during the growing season is a common approach for evaluating N sufficiency in the potato crop (Zebarth and Rosen 2007). A critical petiole nitrate-N value or range defines a situation where N is not limiting growth, but below which a yield reduction occurs (Westcott et al. 1991; Bélanger et al. 2003). A petiole nitrate-N within 5% range of the optimum N rate was considered sufficient in this study. Petiole nitrate-N concentration as a function of the number of days after planting for each cultivar was determined over the growing season (Figure 1-5). As reported in previous studies (Porter and Sission 1991; Bélanger et al. 2003), petiole nitrate-N decreased over the growing season for all cultivars in this study.

Porter and Sission (1991) reported that petiole nitrate-N could sensitively indicate N status of potato plants during the growing season, particularly 50 days after planting (DAP) when the tuber bulking stage often starts; this procedure was adopted for this study. The critical petiole nitrate-N concentrations for all cultivars were obtained at optimum N rates of 231 kg ha^{-1} in 2011, and 319 kg ha^{-1} in 2012. At 50 DAP in 2011, critical petiole nitrate-N concentrations of Russet Burbank, Alpine Russet and Dakota Trailblazer were 1.61 ± 0.08 %, 1.94 ± 0.10 %, and 1.78 ± 0.09 %, respectively. These French fry cultivars had higher critical petiole nitrate-N at 50 DAP in 2012, 1.83 ± 0.09 %, 2.19 ± 0.11 %, and 2.36 ± 0.12 % for Russet Burbank, Alpine Russet and Dakota Trailblazer respectively. Alpine Russet and Dakota Trailblazer had higher critical petiole nitrate-N levels than Russet Burbank both years. The critical petiole nitrate-N levels for chip cultivars were 1.70 ± 0.08 % and 1.83 ± 0.09 % for Ivory Crisp and Snowden at 50

DAP in 2011. Increased critical petiole nitrate-N levels were observed in 2012 at the same sampling time, 2.23 ± 0.11 % and 2.17 ± 0.11% for Ivory Crisp and Snowden. Nitrogen supply higher than 231 kg ha^{-1} in 2011, and 319 kg ha^{-1} in 2012 were rated as excessive for all French fry and chip cultivars.

At 70 DAP, the critical petiole nitrate-N ranges of Russet Burbank, Alpine Russet and Dakota Trailblazer were 0.94 ± 0.05 %, 0.85 ± 0.04 %, 0.89 ± 0.04 %, respectively in 2011, and 1.32 ± 0.07 %, 1.27 ± 0.06 %, 1.58 ± 0.08 %, respectively in 2012. For Ivory Crisp and Snowden, the ranges were 0.74 ± 0.04 %, 0.94 ± 0.05 %, respectively in 2011, and 1.36 ± 0.07 %, 1.53 ± 0.08 %, respectively in 2012. Ivory Crisp consistently had lower petiole nitrate-N concentrations than Snowden late in the growing season.

Porter and Sisson (1991) generalized petiole nitrate-N from a four-year study and reported a critical petiole nitrate-N concentration of 1.6 % for Russet Burbank at 50 DAP. A similar, but higher critical petiole nitrate-N value of 1.8 % at 55 DAP was reported for Russet Burbank by Waterer (1997). Nitrogen fertilizer rate and source, irrigation, growing site and climatic conditions have been shown to influence petiole nitrate-N levels (Bélanger et al. 2003). The critical petiole nitrate-N levels of Russet Burbank (1.61 ± 0.08 % in 2011 and 1.83 ± 0.09 % in 2012) in this study were within the range reported in the previous studies. Critical petiole nitrate-N levels of Alpine Russet, Dakota Trailblazer, Ivory Crisp and Snowden have not been previously reported.

Higher critical petiole nitrate-N concentrations in 2012 were associated with higher optimum N rates (319 kg ha^{-1}), and consequently higher tuber yields for all cultivars. It is speculated that the warm weather earlier in the growing season in 2012 was favorable for tuber bulking, and created high tuber sink strength, which demanded a high

level of nutrition (mainly nitrogen), and consequently resulted in higher tuber yield than the previous year (Oliveira et al. 2016).

Conclusions

Nitrogen supply significantly affected tuber yield and size distribution, but interacted with year and/or cultivar. The warm and longer growing season in 2012 resulted in higher tuber yield and a higher proportion of large tubers, requiring a higher N rate than the cooler, shorter growing season in 2011. The weather conditions were more favorable for tuber bulking and created higher tuber sink strength in 2012 than in 2011. The new cultivars, Alpine Russet, Dakota Trailblazer and Ivory Crisp, demonstrated higher or comparable marketable yield, and a higher proportion of large tubers (greater than 170 g) than standard cultivars Russet Burbank and Snowden.

Tuber quality (hollow heart incidence and specific gravity) responses to N supply were significant, but differed by year and cultivar. Alpine Russet and Ivory Crisp had low hollow heart incidence (less than 10 % and did not respond to N supply) and had a specific gravity suitable for commercial processing. Dakota Trailblazer had a consistent performance for tuber yield, tuber size distribution and quality, and was less affected by weather conditions compared to the other cultivars. Although Dakota Trailblazer had a higher incidence of hollow heart (greater than 10 % at N rates above 125 kg ha^{-1}) and specific gravity higher than desired for commercial processing, the consistent response in variable weather conditions suggests that this cultivar may be useful as a parent for developing superior cultivars in potato breeding programs.

Petiole nitrate-N concentration is a widely adopted indicator of N status for potato plants. However, the critical petiole nitrate-N value was affected by year for all cultivars in this study. The critical petiole nitrate-N concentration in 2012 would have been excessive in 2011. Weather conditions should be taken into account when establishing and interpreting critical petiole nitrate-N concentration for each cultivar.

Table 1-1. Pre-plant soil properties at the Sand Plain Research Farm in Becker, MN in 2011 and 2012

Year	pH	OM	Bray P	K	Ca	Mg	SO_4^{2-}-S	B	Zn	NO_3^--N	NH_4^+-N
		Top 15 cm Soil								Top 61 cm Soil	
		(%)					mg kg^{-1}				
2011	6.0	2.1	39.6	112	828	144	3.7	0.23	1.5	0.7	1.9
2012	5.7	1.5	35.8	101	547	106	5.0	0.20	1.6	3.9	1.1

Table 1-2. Monthly weather conditions during growing season at Becker, MN in 2011 and 2012

	Temperature (ºC)			Precipitation (cm)		
	2011	2012	Average (1985 to 2015)	2011	2012	Average (1985 to 2015)
April	6.50	8.70	7.60	6.90	4.50	7.50
May	13.6	16.3	14.4	16.8	28.6	9.40
June	19.0	20.9	19.1	7.80	11.0	11.2
July	24.3	24.5	21.5	24.6	10.2	10.1
August	21.2	20.2	20.0	8.30	3.20	10.7
September	15.4	15.6	15.7	1.50	0.40	7.60

The monthly average temperature and precipitation data from 1985 to 2015 were recorded in the Santiago, Minnesota weather station (approximately 19 km from the research site in Becker) and retrieved from the Minnesota Department of Natural Resources website (http://www.dnr.state.mn.us/climate/historical/acis_stn_meta.html)

Table 1-3. Analysis of variance for stem number, tuber quality and yields in 2011 and 2012

Sources of Variance	Degree of Freedom	Stems per Plant	Hollow Heart	Specific Gravity	Yield		
Main Effects					Total	Marketable	Tubers Greater than 170 g (%)
Cultivar (C)	4	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
N Rate (N)	4	0.8108	<.0001	<.0001	<.0001	<.0001	<.0001
Year (Y)	1	<.0001	0.0014	<.0001	<.0001	<.0001	<.0001
Interactions							
C * N	16	0.764	0.0032	0.0916	0.398	0.4047	<.0001
C * Y	4	<.0001	<.0001	0.0107	<.0001	<.0001	<.0001
N * Y	4	0.6427	0.0345	0.6689	<.0001	<.0001	0.1498
C * N * Y	16	0.6765	0.0427	0.0144	0.9513	0.8649	0.8654

NS = non-significant; * = significant at 0.05; ** = significant at 0.01

Table 1-4. Stem numbers of five cultivars in 2011 and 2012, across N treatments

Cultivar	Stems per Plant	
	2011	2012
Russet Burbank	4.5 b	2.5 c
Alpine Russet	3.5 c	1.9 d
Dakota Trailblazer	2.7 d	2.1 d
Ivory Crisp	3.6 c	3.0 b
Snowden	5.0 a	4.1 a
LSD ($\alpha = 0.05$)	0.4	0.3

Means within a year followed by the same letter are not significantly different at the 5% level

Table 1-5. Total and marketable (MKT) yields, and percentage of tubers greater than 170 g, in 2011 and 2012 across all N treatments

Cultivars	Total (t ha^{-1})		MKT (t ha^{-1})		Tubers greater than 170 g (%)	
	2011	2012	2011	2012	2011	2012
Russet Burbank	55.7 ab	61.1 ab	43.5 c	53.6	33.2 d	49.6 c
Alpine Russet	46.0 c	61.3 ab	40.7 c	58.4	55.1 b	75.7 a
Dakota Trailblazer	56.2 ab	57.3 c	53.3 a	55.7	67.5 a	71.0 a
Ivory Crisp	53.3 b	59.6 bc	49.8 b	56.1	65.9 a	59.9 b
Snowden	59.1 a	63.4 a	49.9 ab	55.2	37.9 c	37.1 d
LSD ($\alpha = 0.05$)	3.5	3.6	3.4	--	4.7	4.8

Means within a year followed by the same letter are not significantly different at the 5% level.

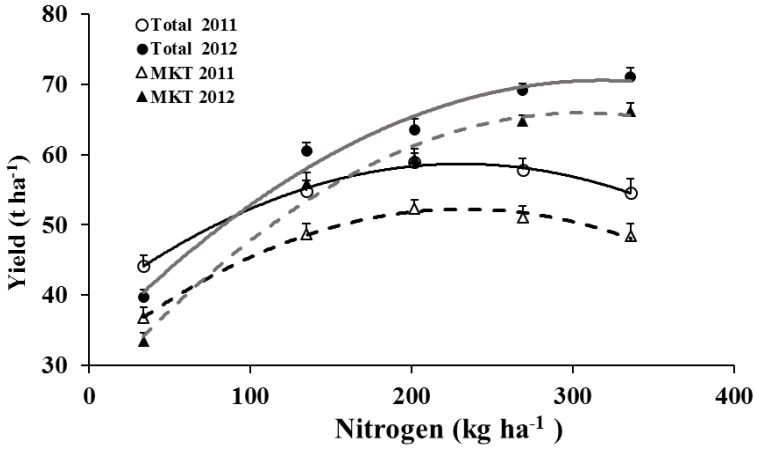

Figure 1-1. Nitrogen rate by year interaction effect on total and marketable (MKT) yield. Error bars indicate standard errors of the mean.
Equations
Total 2011: $y = -3.75\times10^{-4}\ x^2 + 0.17\ x + 38.68$, $R^2 = 1.00$
Total 2012: $y = -3.71\times10^{-4}\ x^2 + 0.24\ x + 32.83$, $R^2 = 0.98$
MKT 2011: $y = -3.89\times10^{-4}\ x^2 + 0.18\ x + 31.17$, $R^2 = 1.00$
MKT 2012: $y = -4.29\times10^{-4}\ x^2 + 0.26\ x + 25.77$, $R^2 = 0.98$

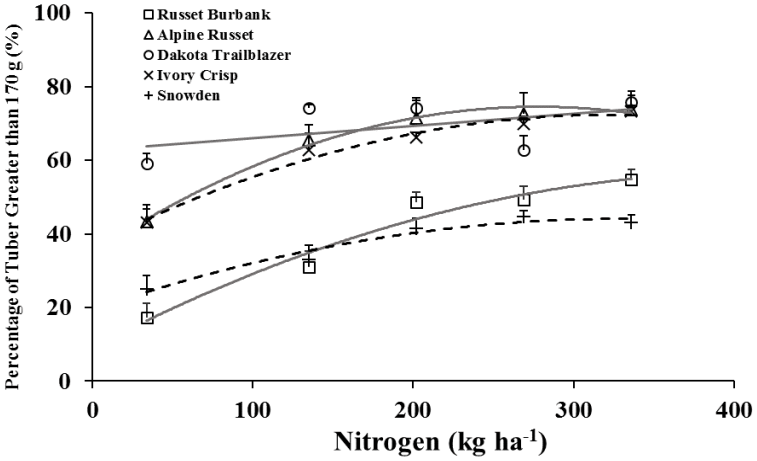

Figure 1-2. Cultivar by N rate interaction effect on the percentage of tubers greater than 170 g in 2011 and 2012. Error bars indicate standard errors of the mean.
Equations
Russet Burbank: $y = -2.74\times10^{-4}\ x^2 + 0.23\ x + 8.95$, $R^2 = 0.96$
Alpine Russet: $y = -5.13\times10^{-4}\ x^2 + 0.29\ x + 34.89$, $R^2 = 0.99$
Dakota Trailblazer: $y = 3.37\times10^{-2}\ x + 62.66$, $R^2 = 0.26$
Ivory Crisp: $y = -3.40\times10^{-4}\ x^2 + 0.22\ x + 36.85$, $R^2 = 0.98$
Snowden: $y = -2.25\times10^{-4}\ x^2 + 0.15\ x + 19.38$, $R^2 = 0.96$

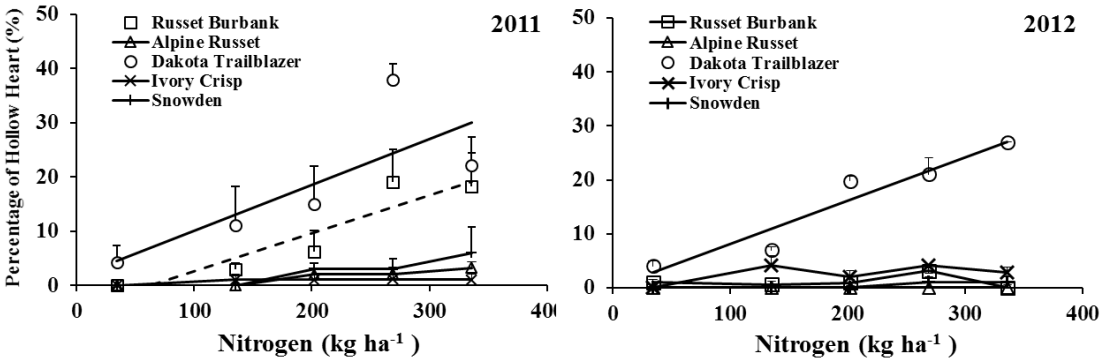

Figure 1-3. Interaction of the cultivar by N rate effect on hollow heart. Error bars indicate standard errors of the mean.

Equations

2011 Russet Burbank: $y = 6.99\times10^{-2}\ x - 4.35$, $R^2 = 0.86$

2011 Dakota Trailblazer: $y = 8.47\times10^{-2}\ x + 1.5$, $R^2 = 0.60$

2012 Dakota Trailblazer: $y = 8.05\times10^{-2}\ x + 0.05$, $R^2 = 0.92$

Hollow heart incidences for 2012 Russet Burbank, 2011 Alpine Russet, Ivory Crisp and Snowden, and 2012 Alpine Russet, Ivory Crisp and Snowden were not affected by N rate effect.

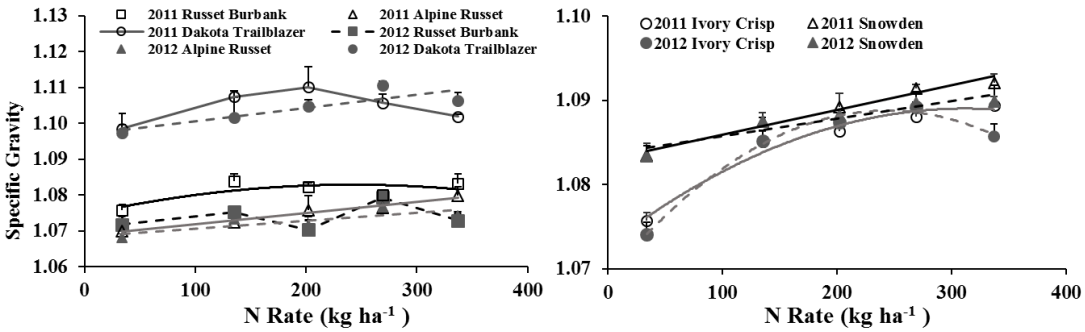

Figure 1-4. Three-way interaction of cultivar, N rate and year effect on specific gravity. Error bars indicate standard errors of the mean.

Equations

2011 Russet Burbank: $y = 1.42\times10^{-7}\ x^2 + 6.89\times10^{-5} + 1.07$, $R^2 = 0.57$

2011 Alpine Russet: $y = 3.14\times10^{-5}\ x + 1.07$, $R^2 = 0.98$

2011 Ivory Crisp: $y = -1.67\times10^{-7}\ x^2 + 1.04\times10^{-4}\ x + 1.07$, $R^2 = 0.97$

2011 Snowden: $y = 2.92\times10^{-5}\ x + 1.08$, $R^2 = 0.97$

2012 Alpine Russet: $y = 2.19\times10^{-5}\ x + 1.07$, $R^2 = 0.64$

2012 Dakota Trailblazer: $y = 3.72\times10^{-5}\ x + 1.10$, $R^2 = 0.76$

2012 Ivory Crisp: $y = -3.33\times10^{-7}\ x^2 + 1.63\times10^{-4}\ x + 1.07$, $R^2 = 0.99$

2011 Snowden: $y = 2.11\times10^{-5}\ x + 1.08$, $R^2 = 0.89$

Specific gravities for 2011 Dakota Trailblazer and 2012 Russet Burbank were not affected by N rate.

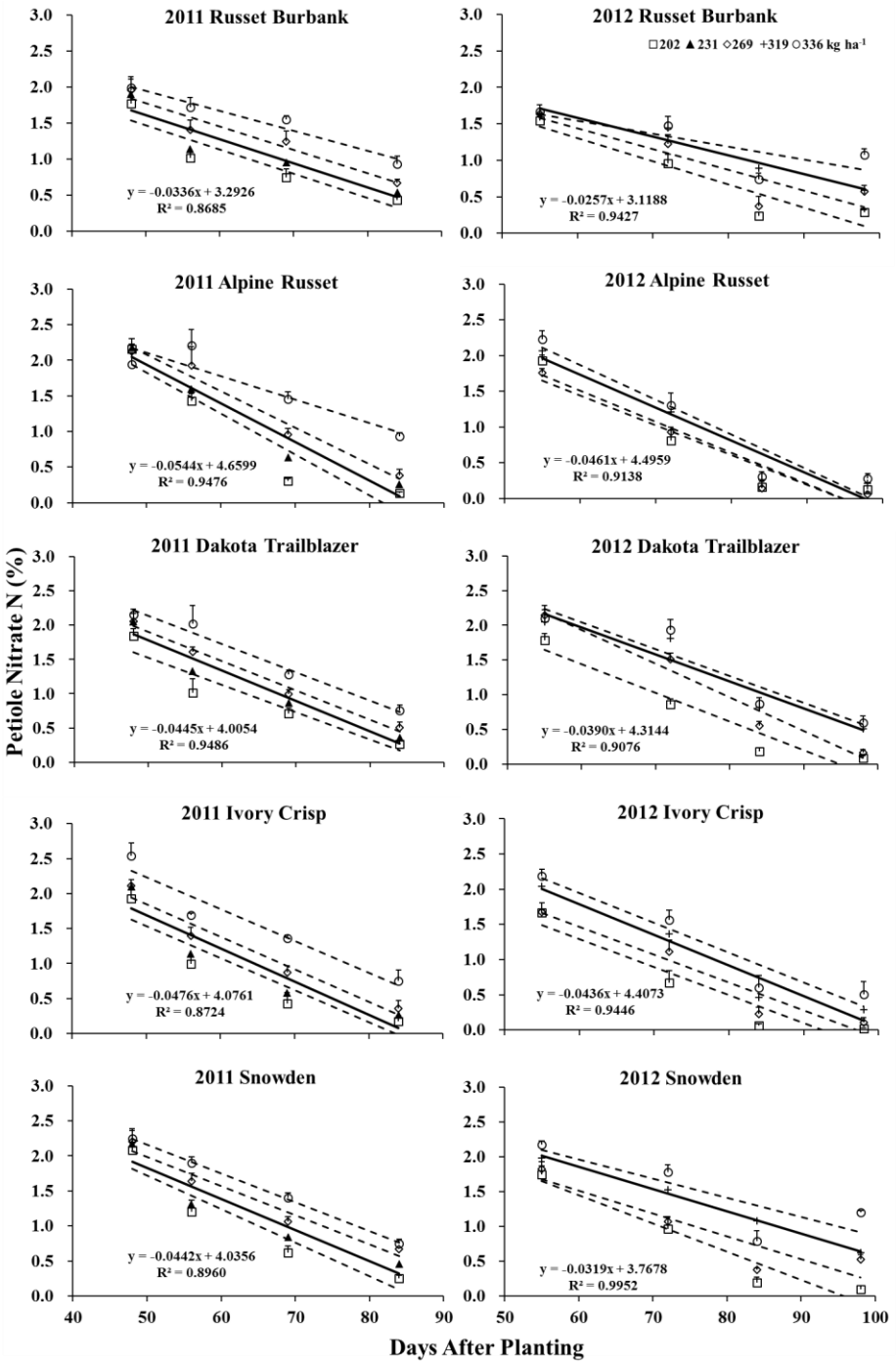

Figure 1-5. Petiole nitrate-N concentrations during the season in 2011 and 2012. Solid lines represent the linear trends of calculated critical petiole nitrate-N concentrations at the optimum N rates (231 kg ha^{-1} in 2011 and 319 kg ha^{-1} in 2012), and dotted lines represent the linear trends of actual petiole nitrate-N concentrations at N rates from 202 to 336 kg ha^{-1}

Chapter 2 - Acrylamide formation in processed potatoes as affected by cultivar, nitrogen fertilization and storage time

Overview

The affirmation of acrylamide as a probable carcinogen by the European Food Safety Authority has reinforced the need to lower acrylamide content in fried potato products. Selected for low reducing sugars and acrylamide-forming potential, recently released cultivars Alpine Russet, Dakota Trailblazer and Ivory Crisp were evaluated for their processing quality when grown with varying nitrogen fertilizer regimes. The objective of this study was to determine the effects of nitrogen fertilizer rate on tuber glucose and acrylamide concentration following processing of new cultivars relative to standard cultivars Russet Burbank and Snowden at harvest and after 3, 6 and 9 months of storage at 7.2 ºC. The study was conducted on an irrigated Hubbard loamy sand soil at the Sand Plain Research Farm in Becker, Minnesota in 2011 and 2012. All cultivars were subjected to five nitrogen rates (34, 135, 202, 269 and 336 kg ha^{-1}) in a randomized complete block design replicated four times. The glucose and acrylamide responses to nitrogen rate were similar for chip cultivars, which linearly decreased in 2011, and quadratically increased then decreased with increasing nitrogen rate in 2012. Nitrogen rate effect on French fry glucose concentration varied by cultivar. Glucose concentration of Russet Burbank and Alpine Russet decreased with increasing nitrogen rate, while Dakota Trailblazer did not respond to nitrogen rate. Glucose and acrylamide concentrations of chip cultivars generally increased during storage, with a dramatic increase in Snowden that resulted from senescence sweetening after 9 months of storage.

Glucose and acrylamide concentrations of all French fry cultivars generally increased during storage in 2011. In contrast, glucose concentrations of French fry cultivars were stable or increased, while acrylamide concentrations generally decreased during storage in 2012. Chip color of Snowden and Ivory Crisp was highly correlated to glucose concentration with $R^2 = 0.89$ in 2011 and 0.81 in 2012. Acrylamide concentrations of French fry and chip cultivars linearly increased with increasing glucose concentrations ($R^2 = 0.52$ and 0.66, respectively). Generally, potato tubers with low concentrations of acrylamide can be obtained from low reducing sugar cultivars that are provided with the appropriate N fertilizer rate during a growing season with minimal environmental stress.

Introduction

Acrylamide can have neurological and reproductive effects on humans and is considered a probable carcinogen by the World Health Organization and the International Agency for Research on Cancer (Friedman, 2003).The carcinogenic effect of acrylamide in higher doses has been shown for mice and rats (Johnson et al., 1986), but confirmed association between dietary acrylamide and human cancer occurrence is still lacking (Mucci et al. 2003, 2004, 2006 and 2008; Pelucchi et al. 2006; Hogervorst 2007; Weber et al. 2008). Because evidence in human studies is currently limited and inconclusive, the European Food Safety Authority (EFSA) reconfirmed the potential risk of dietary acrylamide consumption for developing cancer in humans of all ages (EFSA 2015). The large consumption of fried potato products contributes a substantial proportion of dietary acrylamide intake in Europe and the United States (EFSA 2011; Elmore et al. 2015). Therefore, reducing acrylamide concentrations in high acrylamide-containing food, such

as potato chips and fries (ranging from 170 to 12000 ug kg^{-1}, Friedman 2003) remains a high priority for the potato industry.

Acrylamide is not present in raw potato tubers, but can be formed from reducing sugars and asparagine in the Maillard reaction during high-temperature processing (Michalak et al. 2011). Reducing sugars are generally considered the limiting factor, because asparagine is often far in excess of reducing sugars in potato tubers, and a significant correlation exists between reducing sugars and acrylamide concentrations (Amrein et al. 2004; Ohara-Takada et al. 2005; Elmore et al. 2015). Raw potato tubers with low concentrations of precursors have been shown to reduce acrylamide-forming potential (De Wilde et al. 2005; Lea et al. 2007). For each cultivar, the propensity to contain a high or low concentration of tuber reducing sugars is heritable (Cunningham and Stevenson 1963). Cultivars with low reducing sugar concentrations usually generate less acrylamide in fried potato products, suggesting that cultivar selection would be an effective approach to decrease acrylamide formation (Halford et al. 2012).

Nitrogen (N) management is another practice that can influence sugar content and acrylamide-forming potential in fried potato products. Plants with adequate N supply have been shown to produce tubers with low reducing sugar content at harvest and through storage (Kumar et al. 2004). Increasing N fertilization has been shown to decrease tuber reducing sugars and acrylamide concentrations in French fries (De Wilde et al. 2006). Westermann et al. (1994) reported that increasing N application from 0 to 336 kg ha^{-1} increased reducing sugars in stem end, but decreased reducing sugars in the bud end for Russet Burbank. However, Amrein et al. (2003) reported that N fertilization did not significantly influence sugar content and acrylamide formation for 17 potato

cultivars in Switzerland. Muttucumaru et al. (2013) tested three N treatments (0, 100 and 200 kg ha^{-1}) applied to 13 cultivars (French fry, chip and boiling cultivars), and reported complex effects on glucose and acrylamide concentrations. The glucose concentrations of each type (French fry, chip and boiling cultivars) were not consistently increased or decreased with increasing N fertilization. The lowest glucose concentrations were reported with 100 kg N ha^{-1} for French fry and chip cultivars. All but one of the French fry cultivars and the boiling cultivar had acrylamide concentrations that increased with increasing N rate (0 kg ha^{-1} compared to 200 kg ha^{-1}). In contrast, chip cultivars had acrylamide concentrations that either decreased, slightly increased or remained unchanged with increasing N rate. The results of these previous studies suggest that the N fertilization effect on reducing sugars and acrylamide concentrations is not consistent, and is much likely type- (French fry and chips), and cultivar-dependent.

After harvest, potato tubers from some cultivars can be stored for up to one year to assure a year-round supply for fresh market and the processing industry. Low temperature (4 ºC) is ideal to prevent tubers from sprouting and weight loss during storage. However, it can greatly increase the activity of invertase that could hydrolyze sucrose to glucose and fructose, and cause reducing sugar accumulation (Sowokinos 1990; Matsuura-Endo et al. 2006). Consequently, a storage temperature of 8 to 9 ºC is typically recommended to ensure desirable sugar levels for French fry processing (Knowles et al. 2009), and 10-13 ºC for chip processing (Rowe and Curwen 1993) in the potato industry. Storage time has been reported to influence reducing sugar levels, but this effect usually depends on storage temperature and cultivar (Matsuura-Endo et al. 2004). Knowles et al. (2009) reported that reducing sugars in Ranger Russet increased during 230 days of

storage, while reducing sugars in Umatila Russet and Russet Burbank initially leveled off and then decreased following 111 days storage at 4.5 ºC. When storage temperature was raised to 9 ºC, reducing sugars levels of all three cultivars stopped increasing at 48 days in storage, and then started decreasing. Elmore et al. (2015) stored 20 potato cultivars at 8 ºC for 6 months, with reducing sugars and acrylamide concentrations determined after 2 and 6 months of storage. Five cultivars, Lady Rosetta, Daisy, Innovator, Markies and Ramos, showed a significant increase in reducing sugars, while reducing sugars of other cultivars did not change with storage. Acrylamide formation in response to storage time was cultivar dependent as well, with either an increase (Innovator), a decrease (Saturna) or no significant change (all other cultivars). Therefore, the post-harvest storage condition is a major management factor that can affect sugar content and acrylamide-forming potential, but also appears to be cultivar dependent.

Recently released French fry cultivars Alpine Russet, Dakota Trailblazer and a chip cultivar Ivory Crisp have a common characteristic of low tuber reducing sugars, and a low acrylamide-forming potential. The agronomic performance (tuber yield and quality) of these cultivars in response to N rate has been previously reported (Chapter 1). In the present study, the effects of N fertilization on tuber reducing sugars and acrylamide concentrations of French fries and chips were investigated. The specific objectives of this study were to (1) characterize the interactive effects of cultivar, N fertilization and storage on tuber glucose, acrylamide formation and fry color; and (2) determine the relationships between fry color ratings, glucose concentrations and acrylamide concentrations in the standard and new cultivars.

Materials and Methods

This study was conducted at the Sand Plain Research Farm in Becker, Minnesota, on a Hubbard loamy sand (sandy, mixed, frigid Entic Hapludolls) soil in 2011 and 2012. A randomized complete block design was adopted with four replications using a factorial treatment arrangement of N rate and cultivar. Three French fry cultivars Russet Burbank, Alpine Russet and Dakota Trailblazer, and two chip cultivars Snowden and Ivory Crisp were subjected to five N fertilizer treatments, 34, 135, 202, 269 and 336 kg ha^{-1}. All plots received 31 kg N ha^{-1} as monoammonium phosphate and 3 kg N ha^{-1} as ammonium sulfate applied in a band 8 cm to the side and 5 cm below the seed tuber at planting. The rest of the N fertilizer (0, 101, 168, 235 and 302 kg ha^{-1}) was side-dressed as Environmentally Smart Nitrogen (Agrium, Inc., 44-0-0) and then hilled in at emergence. Soil properties and further cultural practices used in this study can be found in Chapter 1.

Sample Collection and Analysis

Harvest date was scheduled according to weather conditions, and differed each year. All plots were planted on 3 May 2011, and 17 April 2012, and machine harvested on 29 September 2011 and 2 October 2012. After harvest, approximately 23 kg of tubers weighing between 170 and 283 g from each plot were shipped to the USDA-ARS Potato Research Worksite in East Grand Forks, Minnesota, preconditioned at 10 ºC for two weeks and then stored at 7.2 ºC for nine months. Five tubers from each plot were randomly taken out for glucose determination by a YSI-2700 Select Biochemistry Analyzer (Yellow Springs Instrument Co. Inc. Yellow Springs, Ohio) at harvest and after 3, 6 and 9 months of storage. A stem end sample (50 g) was collected from the 3.8 cm of

tuber tissues surrounding the stem scar from each tuber. A bud end sample (50 g) was collected from the remainder of the tuber. Before use, the juicerator was started and rinsed with three aliquots of 20 ml refrigerated 50 mM phosphate buffer (pH 7.2). The juicerator was then turned off and was allowed to spin down for one minute. The stem and bud end samples were ground separately, and brought up to a final volume of 100 ml with 50 mM phosphate buffer in a beaker. Samples were stored in a refrigerator (4 ºC) for 20 to 30 minutes and gently stirred without disturbing the precipitate (including starch) in the bottom of the beaker. For each sample, 15 ml of juice was transferred into a labeled scintillation vial and frozen for later analysis. The glucose concentration of the juice samples was determined by a YSI-2700 Select Biochemistry Analyzer.

To process the frying cultivars into French fries, tubers were steam-pealed for 30 seconds, washed at high pressure, cut on an Urschel cutter (9.5 mm by 9.5 mm), and blanched at 77 ºC for 7 to 10 minutes. After drying at 60 ºC until 9 to 11 % of their weight was lost, they were par-fried at 185 ºC for 90 seconds, and then at 191 ºC for 35 to 50 seconds, and sharp-frozen at -26 ºC. After one week, the frozen fries were fried at 177 ºC for 165 to 180 seconds.

For chips, tubers were steam-pealed for 30 seconds, followed by pressure washing and slicing (chip thickness 1.5 mm). Then the slices were rinsed in cold water, and fried at 185 ºC for 90 seconds. Chips were de-oiled, crushed and scanned with an Agtron (AGT) Analyzer (Agtron Inc., Reno, NV) to quantify chip color which was displayed as a number with a higher number indicating lighter color.

Fried samples were then shipped frozen to the University of Minnesota's St. Paul Campus for French fry color rating, acrylamide extraction and determination. French fry

color was estimated by visual comparison with the Munsell USDA Frozen French Fry Standard (Munsell Color) with a higher number indicating darker color.

Acrylamide was extracted using the following procedure. For each plot, three fries or 1.0 to 2.0 g of chips were ground for 30 seconds in a coffee grinder, and 0.8 to 1.0 g (for fries) or 0.20 to 0.25 g (for chips) of ground sample were placed in 15 ml Falcon ™ Conical Centrifuge tubes with ten parts distilled and deionized water, then vortexed for 30 seconds. After one hour of resting, the resulting suspension was centrifuged and the aqueous fraction was pipetted away from the fatty and solid fractions into 1.5 ml Eppendorf ™ Snap-Cap Microcentrifuge Safe-Lock ™ tubers. The centrifugation-isolation step was repeated twice, after which 1 ml of purified aqueous solution was pipetted into a 1.5 ml centrifuge tube for each sample, with addition of 100 pg of heavy acrylamide (Cambridge Isotope Laboratories, INC Andover MA; Acrylamide, 2, 3, 3-D3, 98%). Samples were then subjected to solid phase extraction with a Phenomenex Strata ™-X-C 33 µm Polymeric Strong Cation column.

To determine acrylamide concentrations: 20 µl of extracted samples were subjected to LC-MS/MS (SCIEX) using an Agilent autosampler with an analytical Thermo Hypercarb (100 L×1.0 mm I.D. Columns, 5 µm particle size) column connected to the Applied Biosystem 4000 ion trap fitted with a turbo V electrospray source. The samples were subjected to a linear gradient of 0 to 100 percent acetonitrile for 15 minutes at a column flow rate of 150 µl minute^{-1}. Transitions monitored were m/z 72 greater than m/z 44 and m/z 72 greater than 55 for the light acrylamide, and m/z 75 greater than 44 m/z and m/z 75 greater than m/z 58 for the heavy acrylamide. The data were analyzed using MultiQuant (ABI) providing the peak area ratio for the m/z 58// m/z 55 transition.

Standard curves for quantification were constructed using 100 pg heavy acrylamide/ml with light acrylamide ranging from 5 pg-1500 pg ml^{-1}. The amount of acrylamide was determined and expressed as ng acrylamide g^{-1} (ppb) on a fresh weight basis. All analysis was conducted at the Center for Mass Spectrometry and Proteomics at the University of Minnesota. Because of different frying methods, acrylamide concentrations were generally much higher in those tubers prepared for chips than for French fries.

Statistical Analysis

Analysis of variance (ANOVA) of fry color, sugars and acrylamide concentrations as functions of N rate, cultivar, storage time and year was conducted using PROC ANOVA in SAS 9.4 statistical software package (SAS Institute Inc., Cary NC. USA). Repeated measures were used for storage time. A square root transformation was used when necessary to account for the heterogeneity of variance. All data were presented separately for French fry and chip cultivars, due to the high acrylamide concentrations in chip cultivars that resulted from different frying methods. Means of fry color, glucose and acrylamide concentrations in 2011 and 2012 were compared among French fry and chip cultivars using PROC GLM and LSMEANS statement, or at each N rate using PROC GLM, MEANS statement and the least significant difference (LSD) at 5 % probability level in SAS. Linear or quadratic responses of glucose and acrylamide to N rate within each year and/or cultivar were determined using PROC GLM and CONTRAST statement in SAS. A probability of 0.05 was considered significant and the quadratic function prior to the linear function. AGT scores with glucose or acrylamide concentrations and within each treatment were plotted for Ivory Crisp and Snowden in

2011 and 2012, respectively, with linear regressions determined for each cultivar and each year in Excel (Microsoft). The regressions between fry color and glucose or acrylamide concentrations within each treatment, cultivar and year were determined using PROC REG, and then plotted in Excel for French fry cultivars. Acrylamide and glucose concentrations within each treatment were plotted for French fry and chip cultivars, respectively, with linear regressions determined for each type.

Results and Discussion

Interactive Effects on Glucose Concentrations

Tuber glucose concentrations were significantly affected by the interaction of N rate by cultivar by year for French fry cultivars (Table 2-1 and Figure 2-1). The effect of N rate was not influenced by storage time. Russet Burbank glucose concentrations decreased quadratically with increasing N rate in 2011 and 2012. For Alpine Russet, glucose concentrations decreased quadratically in 2011, but linearly in 2012 with increasing N rate. The glucose concentrations of Dakota Trailblazer were not affected by N supply in 2011, but minimally responded quadratically with increasing N rate in 2012. Compared to the standard cultivar Russet Burbank, Dakota Trailblazer had significantly lower glucose concentrations at all N rates both years. As for Alpine Russet, glucose concentrations were in the same range as Russet Burbank at the low (34 kg ha^{-1}) and high N ($\geq$ 269 kg ha^{-1}) rates in both years. At medium N rates, Alpine Russet had glucose concentrations higher than Russet Burbank in 2011, but lower than Russet Burbank in 2012, suggesting a significant effect of environmental conditions during the growing season on glucose concentrations for some cultivars.

The N rate by year, and N rate by cultivar interactions were significant for glucose concentrations in chip cultivars (Table 2-1 and Figures 2-2 and 2-3). Similar to French fry cultivars, the N rate effect on glucose concentration was not affected by storage time for chip cultivars. Glucose concentrations in Snowden were not affected by N supply. In contrast, glucose concentrations in Ivory Crisp linearly decreased with increasing N rate (Figure 2-2). When averaged over cultivar, glucose concentrations linearly decreased in 2011, but responded quadratically to N rate in 2012 with an increase at low N rates from 34 to 162 kg ha^{-1} followed by a decrease at rates higher than 162 kg ha^{-1} (Figure 2-3).

Glucose response to N rate was cultivar dependent in this study. Decreases in glucose concentrations with increasing N rate were observed for Russet Burbank, Alpine Russet and Ivory Crisp, but minimally affected by N rate for Snowden and Dakota Trailblazer. In addition, the N rate effect on glucose concentration was not affected by storage time for any of the cultivars (Table 2-1). Long et al. (2004) reported that increasing N fertilizer from 200 to 400 kg ha^{-1} had no effect on glucose concentrations for Snowden on an irrigated soil in central Michigan, which is consistent with results in this study. Knowles et al. (2015) reported decreased reducing sugar concentrations in the stem and bud end for Alpine Russet with increasing N rate with a delayed harvest at Othello, WA. Increasing N application from 0 to 336 kg ha^{-1} caused an increase or a decrease in reducing sugars in the bud and stem end for Russet Burbank, respectively, in Utah (Westermann et al. 1994). However, Zebarth et al. (2004) reported that increased N fertilizer from 0 to 160 kg ha^{-1} had little effect on reducing sugars concentration on Russet Burbank in New Brunswick, Canada. The contrasting results suggest that growing

conditions (environmental conditions and growing locations) also played a critical role in glucose concentrations of Russet Burbank. The effect of environmental conditions was confirmed in this study as well, such as the elevated glucose concentrations for French fry cultivars in 2012, and different glucose response to N rate for Alpine Russet in 2011 and 2012. The effect of N rate on glucose concentration has not been previously reported for Dakota Trailblazer and Ivory Crisp.

The three-way interaction of cultivar by storage time by year was significant for glucose concentration of both French fry and chip cultivars (Table 2-1). In 2011, glucose concentrations in tubers of French fry cultivars tended to increase over the 9-month storage period (Figure 2-4). For Dakota Trailblazer and Alpine Russet, glucose gradually increased over 9 months while glucose leveled off after 3 months for Russet Burbank. The glucose concentrations increased after 3-month storage for both chip cultivars, and leveled off after 6-month storage for Ivory Crisp (Figure 2-5), while glucose in Snowden dramatically increased (3.67 fold higher than the previous test after 6-month storage) after 9-month storage. This increase in tuber glucose concentrations for Snowden is due to senescent sweetening, which is irreversible, cultivar dependent, and occurs after an extended period of storage (Wiltshire and Cobb 1996).

In 2012, all French fry and chip cultivars had a higher level of glucose at harvest than in 2011 (Figures 2-4 and 2-5). The storage time effect on glucose concentrations differed by cultivar. Glucose concentrations in Alpine Russet tubers were not affected by storage time, while for Russet Burbank, they were not affected during the first 6 months of storage, but then significantly increased (Figure 2-4). At 3 months of storage, glucose concentrations decreased in Dakota Trailblazer (Figure2- 4), Snowden and Ivory Crisp

(Figure 2-5), compared to glucose concentrations at harvest. After 3 months, glucose concentrations of Dakota Trailblazer, Snowden and Ivory Crisp increased during storage, with the similar senescent sweetening for Snowden at 9 months as was observed in 2011 for this cultivar.

The higher glucose concentrations at harvest in all cultivars in 2012 compared with 2011 may have been induced by weather conditions during the growing season, mechanical stress at harvest, or chemical maturity (Kumar et al. 2004; Knowles et al. 2015). Because harvesting method, post-harvest handling and shipping was the same in 2011 and 2012, mechanical stress was not likely the reason. Chemical maturity is the state when tubers have the lowest sugar concentrations, and usually occurs before vine desiccation (Sowokinos and Preston 1988). Tubers harvested at chemical maturity often have lower reducing sugar concentrations during storage (Kumar et al. 2004). When harvested before or post chemical maturity, tubers often accumulate high reducing sugar concentrations (Sowokinos 1971; Knowles et al. 2009; Knowles et al. 2015). Knowles et al. (2009) reported a growing season length of 146 days and 154 days for Alpine Russet to reach chemical maturity under low N and high N supply, respectively, in a three-year study. Reducing sugar concentrations continued to increase in over-mature tubers, with higher concentrations at the stem end and low N treatment. In this study, tubers were harvested 149 and 168 days after planting in 2011 and 2012, respectively. Because a period of cold weather did not occur in either year, cold-sweetening was likely not the reason for the higher glucose concentrations at harvest in 2012. Over-maturity due to a longer growing season in 2012 was the likely reason for the high sugar concentrations that year. In-season temperature conditions might be another possible cause. Thompson

et al. (2008) reported that sugars tend to increase when air temperatures increase above 25 to 30 ºC during the growing season. In places like Minnesota, July and August are the times for tuber bulking and dry matter accumulation. The average maximum air temperature and numbers of days with maximum air temperature > 30 ºC in July 2011 and 2012 was generally the same (data not shown). However, in August, there were 14 days of maximum air temperature > 30 ºC in 2012, and 5 days of maximum air temperature > 30 ºC in 2011 (with a monthly average maximum air temperature 27.4 ºC in 2011 and 28.7 ºC in 2012). The high temperature in August might be another reason for the high sugar levels in 2012. Reconditioning is a phenomenon known in the potato industry that starch resynthesized from free sugars in potato tubers when storing under proper temperature (Elbashir and Saeed 2014). Among the five cultivars, the elevated glucose concentrations were re-conditioned to a lower level for Dakota Trailblazer, Snowden and Ivory Crisp, but not Russet Burbank and Alpine Russet (Figures 2-4 and 2-5). The results above suggested that growing season should be limited to a length when tubers are neither immature nor over matured, to prevent the accumulation of reducing sugars. In addition, proper use of irrigation should be considered to lower soil temperature during the maximum air temperature > 30 ºC days and the incidence of dark-ends French fries (Kincaid et al. 1993).

The effect of storage time on glucose concentration was significant and generally increased under the storage conditions in this study (7.2 ºC) for the tested cultivars (Figures 2-4 and 2-5). The exceptions were Alpine Russet in 2012, and the first 3 months of storage in 2012 for Dakota Trailblazer, Snowden and Ivory Crisp. Variable results due to storage temperature and cultivar have also been reported in previous studies

(Matsuura-Endo et al. 2006; Knowles et al. 2009; Muttucumaru et al. 2013). Generally, a storage temperature of 8 ºC or above is recommended to avoid a significant increase of reducing sugars (Blenkinsop et al. 2002; De Wilde et al. 2005; Vinci et al. 2012). However, this is not always the case. Elmore et al. (2015) stored 20 potato cultivars at 8 ºC for six months, with reducing sugars and acrylamide concentrations determined after 2-month and 6-month storage. Five cultivars Lady Rosetta, Daisy, Innovator, Markies and Ramos showed a significant increase in reducing sugars, while reducing sugars of other cultivars did not change with storage time. Muttucumaru et al. (2014) determined glucose in five chip cultivars and seven French fry cultivars at harvest and after storage of 5-6 months at 9 ºC. They found that glucose concentration in Lady Claire tubers decreased, while glucose concentrations increased in tubers of all other cultivars.

A few studies have reported on storage effects with the same cultivars used in this research. Knowles et al. (2009) reported that reducing sugars in Russet Burbank tubers stored at 6.7 ºC and 9 ºC, significantly increased during 17-48 days of storage, and then decreased for the next 182 days, but they never reached the initial glucose concentrations. Russet Burbank tubers stored at 9 ºC had lower reducing sugars than at 6.7 ºC after 111 days of storage. Whitworth et al. (2011) reported that glucose concentrations of Alpine Russet generally increased and then leveled off during the storage at 7.2 ºC. During the 250 days of storage, Alpine Russet glucose concentrations remained near or below 0.05 % on fresh weight basis, which was significantly lower than Russet Burbank. Glucose concentrations of Snowden tubers did not change significantly when stored at 6 and 8 ºC for 18 weeks, but the glucose concentration was higher at 6 ºC than at 8 ºC (Matsuura-Endo et al. 2006). The glucose concentrations of Alpine Russet in

this study were higher than those concentrations reported by Whitworth et al. (2011). Although the glucose concentrations were higher, the effects of storage on glucose concentrations were similar. In contrast, storage effects on Russet Burbank and Snowden in our study differed from those reported by Knowles et al. (2009) and Matsuura-Endo et al. (2006). These results indicate that the growing environment has a significant influence on tuber reducing sugar concentrations and changes in concentration that occur during storage.

Interactive Effects on Acrylamide Concentrations

The effect of N rate on acrylamide concentrations was influenced by year and by cultivar type (Table 2-1). Acrylamide concentrations of French fry cultivars responded quadratically to N rate (increased up to 212 kg N ha^{-1} and then decreased) in 2011, but did not respond to N supply in 2012 (Figure 2-6). However, for chip cultivars, acrylamide concentrations linearly decreased with increasing N rate in 2011, but responded quadratically to increasing N rate in 2012 (increased up to 212 kg N ha^{-1} and then decreased). Acrylamide concentrations in 2012 were higher than in 2011 for French fry cultivars at all N rates, and for chip cultivars above N rate 66.4 kg ha^{-1}.

Variable results (increase, decrease or quadratic responses) for N rate effects on acrylamide concentrations were observed for the cultivars evaluated in this study. Similar to glucose concentrations, the N rate effect on acrylamide was not affected by storage time (Table 2-1). For chip cultivars, glucose and acrylamide concentrations responded similarly to increasing N rate both years. However, glucose concentrations in French fry cultivars generally decreased in both years, while acrylamide concentrations increased

and then decreased in 2011, but were not affected by N rate in 2012. This inconsistent response has been previously reported by Muttucumaru et al. (2013). They reported increased acrylamide concentrations in six French fry cultivars (including Russet Burbank), and decreased acrylamide concentrations in cultivar Pentland Dell with increasing N supply from 0 to 200 kg ha^{-1}, while glucose concentrations changed minimally. Amrein et al. (2003) reported that N fertilization did not significantly influence acrylamide formation in 17 potato cultivars. In contrast, De Wilde et al. (2006) reported an increase of acrylamide-forming potential with increased N application in French fries produced from Bintje, Ramos and Saturna. Gerendás et al. (2007) reported an increase in acrylamide concentrations for the French fry cultivar Agria when grown with adequate N and inadequate K supply. In other studies, acrylamide concentrations either decreased, slightly increased or remained unchanged with increasing N rate for chip cultivars Hermes, Lady Clarie, Lady Rosetta, Saturna and Verdi (Muttucumaru et al. 2013). No studies have reported the N rate effect on acrylamide concentrations of the rest of test cultivars used in this study. Based on our results and those in the literature, glucose and acrylamide concentrations are not consistently affected by N supply and are cultivar dependent. Therefore, to reduce the acrylamide-forming potential, low reducing sugar cultivars should be selected and N should be managed to optimize yield.

Changes in acrylamide concentrations during storage depended on year for all cultivars (Figures 2-7 and 2-8). For French fry cultivars, acrylamide concentrations increased during storage in 2011, but decreased during storage in 2012 (Figure 2-7). Dakota Trailblazer had consistently lower acrylamide content than Russet Burbank and Alpine Russet. The changes in acrylamide concentrations in chip cultivars during storage

were different from those for French fry cultivars (Figure 2-8). Acrylamide concentrations in chip cultivars at harvest were generally higher than after 3 months of storage both years. For Ivory Crisp in 2011, acrylamide concentrations decreased until 6 months of storage, and then slightly increased after 9 months of storage. However, for Snowden in 2011 and 2012, and Ivory Crisp in 2012, acrylamide concentrations increased during the 3 to 9 month storage period. The large increase in acrylamide for Snowden after 9 months of storage corresponds with senescent sweetening (Figure 2-5). In general, the changes in acrylamide concentrations for all cultivars tended to be similar to changes in glucose concentrations, except for French fry cultivars in 2012 (Figures 2-4 and 2-5).

The effect of cultivar on glucose and acrylamide concentrations was consistent throughout this study. For example, Dakota Trailblazer had lower glucose and acrylamide concentrations than Russet Burbank and Alpine Russet. A similar trend was observed for Ivory Crisp and Snowden. In contrast, the effect of storage time on glucose and acrylamide concentrations depended on both year and cultivar, making it difficult to generalize about storage effects. Comparable results were observed in previous studies as well (Matsuura-Endo et al. 2006; Elmore et al. 2015). Matsuura-Endo et al. (2006) stored five cultivars at 6 ºC for 18 weeks, and reported that acrylamide concentrations decreased for Snowden and Hokkai, but increased for Toyoshiro, Inca-no-mezame, and Irish Cobbler. Snowden was stored for 9 months in this study, and acrylamide decreased after the first 12 weeks of storage, which is similar to the results reported in the Matsuura-Endo et al. (2006) study. Power et al. (2013) determined acrylamide concentrations in over 40000 fresh sliced potato chips from 2002 to 2011, and reported that the lowest

acrylamide concentration occurred in July, which corresponds to when new season potatoes were used. Acrylamide concentrations increased from October to January (about 3 to 6 months after harvest), and remained high until the next July. Similar results have also been observed in Japan between 2006 and 2010 (Tsukakoshi et al. 2012). The effect of storage time on acrylamide concentration in the Power et al. (2013) and Tsukakoshi et al. (2012) studies is similar to our results with Ivory Crisp in 2012, and Snowden before senescence sweetening occurred in both years of this study. Acrylamide concentrations in Russet Burbank were not significantly affected during storage at 8 ºC in the Elmore et al. (2015) study, while in our study, acrylamide increased in 2011 and decreased in 2012 during storage at 7.2 ºC. Differences between the two studies may have been due to differences in storage temperature or growing conditions. Selecting cultivars such as Dakota Trailblazer or Ivory Crisp that maintain low reducing sugars over long periods of storage is an effective approach to keeping acrylamide concentrations low.

Interactive Effects on Fry Color

The Maillard reaction not only produces acrylamide, but also generates melanoidin pigments that give a darker color to fried potato products (Bethke and Bussan 2013). The N fertilization effect was significant for color of French fries and chips, but the effect was minimal (Table 2-1, Supplementary Figures 2-12 and 2-13). Lighter color of French fries and chips was observed with increasing N supply. Knowles et al. (2015) also reported lighter fry color from tubers grown under high N fertilizer application.

French fry and chip color was significantly affected by the interaction of storage time by cultivar by year (Table 2-1, supplementary Figures 2-14 and 2-15). Fry color of

French fry cultivars generally increased during storage in 2011 and decreased in 2012, which was consistent with acrylamide response to storage time. Darker color chips during storage were observed for Ivory Crisp in 2012, and Snowden in 2011 and 2012, which generally corresponded with glucose and acrylamide responses to storage time.

Relationships between glucose, acrylamide and fry color

The relationship between fry color and glucose concentration was not significant individually for French fry cultivars within each year, except for Alpine Russet (R^2 = 0.44) and Dakota Trailblazer (R^2 = 0.29) in 2011. However, combined over all three French fry cultivars and both years, an increase in fry color was observed with increasing glucose concentration (R^2 = 0.40, Figure 2-9).

The relationship between glucose and AGT score was significant for chip cultivars (R^2 = 0.89 and 0.81 in 2011 and 2012, respectively, Figure 2-10). Chips with AGT color readings > 45 are considered acceptable (Santerre et al. 1986). Ivory Crisp was bred as a low reducing sugars cultivar and demonstrated lighter color (50 to 60 in 2011, and 43 to 60 in 2012) compared to Snowden during the 9-month storage. The equation coefficient for glucose concentration was lower in 2012 than in 2011. Produced from tubers with the same amount of glucose, chip fry color was darker in 2012 than in 2011. The results indicate that while glucose and chip color are correlated. The correlation can differ with growing season, suggesting that glucose is not the only component that determines chip color.

The relationships between acrylamide and glucose concentrations for chip and French fry cultivars had R^2 values of 0.66 and 0.52, respectively (Figure 2-11). This

suggests that even though the correlations were significant, there was still variability in acrylamide concentrations for the same glucose concentration. A similar result has been previously reported by Muttucumaru et al. (2014). They measured glucose concentrations in five chip cultivars and seven French fry cultivars at harvest and after 5 - 6 months of storage at 9 ºC, and found Lady Claire had a low glucose concentration with a high acrylamide concentration. In contrast, Hermes, Verdi and Markies cultivars had a high glucose concentration but low acrylamide concentration. Similar results were also reported by Elmore et al. (2015).

Significant correlations between acrylamide and reducing sugars, glucose or fructose have been reported before (Amrein et al. 2003; Chuda et al. 2003; De Wilde et al. 2005; Zhu et al. 2010; Parker et al. 2012; Muttucumaru et al. 2014). Tuber asparagine was not determined in this study. However, based on results reported by Amrein et al. (2003) and Matsuura-Endo et al. (2006), an improved correlation between acrylamide and glucose might be obtained when asparagine is taken into account. For example, a strong correlation of acrylamide-forming potential as a function of (0.5 * glucose + fructose) * asparagine with R^2 of 0.91 was reported for 17 cultivars (including both French fry and chip cultivars, Amrein et al. 2003). Matsuura-Endo et al. (2006) reported that the correlation coefficient for acrylamide and fructose could be significantly improved to 0.81 when only plotted for samples with high asparagine concentrations (fructose/asparagine molar ratios < 2). For those samples with high sugar concentrations, acrylamide was correlated to asparagine rather than fructose, with $R^2 = 0.68$.

The relationship between fry color and acrylamide was significant for all French fry cultivars over the two years, except for Russet Burbank in 2011 (supplementary

Figure 2-5). Darker French fries generally contained higher amounts of acrylamide. The AGT score was highly correlated with acrylamide concentration for Snowden and Ivory Crisp in 2011, with $R^2 = 0.88$ (supplementary Figure 2-17). However, the relationship between AGT score and acrylamide differed by cultivar and sampling time. In 2012, for the same chip color, Snowden and Ivory Crisp contained higher acrylamide concentrations at harvest than during storage. If harvest data is excluded, AGT score was negatively correlated with acrylamide concentrations for Snowden and Ivory Crisp, with $R^2 = 0.96$ and 0.81, respectively. Therefore, optimizing conditions to improve fry color will also result in lower acrylamide concentrations.

Conclusions

Darker French fries and chips were often produced from tubers containing higher glucose concentrations. Fry color was highly correlated to glucose concentration for chip cultivars and was moderately correlated for French fry cultivars, but the equation used for one year could not be used to predict the fry color for another year. The positive correlation between glucose and acrylamide was significant, but with great variability. Including asparagine data might be helpful for a better prediction of acrylamide formation.

The most consistent finding for glucose and acrylamide formation in this study was the effect of cultivar. Regardless of N rate or storage time, Dakota Trailblazer had lower glucose and acrylamide concentrations than Russet Burbank and Alpine Russet. For the chipping cultivars, storage time played an important role. Ivory Crisp and Snowden have similar glucose and acrylamide concentrations early in storage but

Snowden was more susceptible to senescent sweetening which caused a spike in glucose and acrylamide formation at 9 months of storage.

Nitrogen rate and storage time significantly affected glucose and acrylamide concentrations in the cultivars tested in this study. However, the effects were often influenced by cultivar and growing conditions. For example, increased N supply resulted in a decrease in glucose concentration in Russet Burbank, Alpine Russet and Ivory Crisp, but did not affect glucose concentrations in Dakota Trailblazer and Snowden. Acrylamide responses to N supply generally corresponded with responses of glucose to N rate for chip cultivars (lower acrylamide as N rate increased), but not for French fry cultivars.

The effect of time in storage on glucose and acrylamide concentrations appeared to be influenced by growing conditions. In a year with no environmental stress, both glucose and acrylamide concentrations increased with storage for all cultivars tested. However, when heat stress occurred during the bulking and maturation period, tubers contained higher glucose at harvest and had higher acrylamide concentrations compared with those grown in a season with minimal heat stress. In a year with heat stress, glucose response to storage time differed by cultivar. Storage time effects on acrylamide and glucose concentrations were similar for chip cultivars, but not for French fry cultivars.

Because of complex interactions, we conclude that while N supply and storage time can affect acrylamide concentration in fried potato products, the direction of response will depend upon cultivar and growing conditions, which often precludes the ability to generally predict their effects on acrylamide formation. To minimize acrylamide in fried potato products, cultivars should be selected with low reducing sugars and resistance to cold sweetening.

Table 2-1. Analysis of variance for glucose concentration, acrylamide concentration and fry color for French fry and chip cultivars in 2011 and 2012

Source of Variance	Degree of Freedom	Glucose		Acrylamide		Fry Color	
Main Effect		French Fry	Chip	French Fry	Chip	French Fry	Chip
Cultivar (C)	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
N Rate (N)	4	<.0001	<.0001	<.0001	<.0001	0.0142	0.0002
Year (Y)	1	<.0001	0.0014	<.0001	<.0001	<.0001	<.0001
Storage Time (S)	3	<.0001	<.0001	<.0001	<.0001	0.0003	<.0001
Interactions							
C * N	8	<.0001	0.0066	0.5611	0.0994	0.1661	0.9513
C * Y	2	<.0001	0.5032	0.0003	0.0683	0.7182	0.5525
N * Y	4	0.8612	<.0001	0.0466	0.018	0.7236	0.0393
T * Y	3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
C * S	6	0.0032	<.0001	0.2767	<.0001	0.4702	<.0001
N * S	12	0.5233	0.8063	0.4902	0.1670	0.9555	0.3245
N * S * Y	12	0.5943	0.1674	0.9273	0.6422	0.6686	0.4398
C * N * Y	8	0.0005	0.8025	0.5172	0.3594	0.446	0.396
C * S * Y	6	0.0047	<.0001	0.0007	0.0224	0.009	0.0012
C * N * S	24	0.2624	0.4502	0.0587	0.2974	0.8248	0.4756
C * N * S * Y	24	0.479	0.9188	0.3347	0.9711	0.9661	0.8641

NS = non-significant; * = significant at 0.05; ** = significant at 0.01

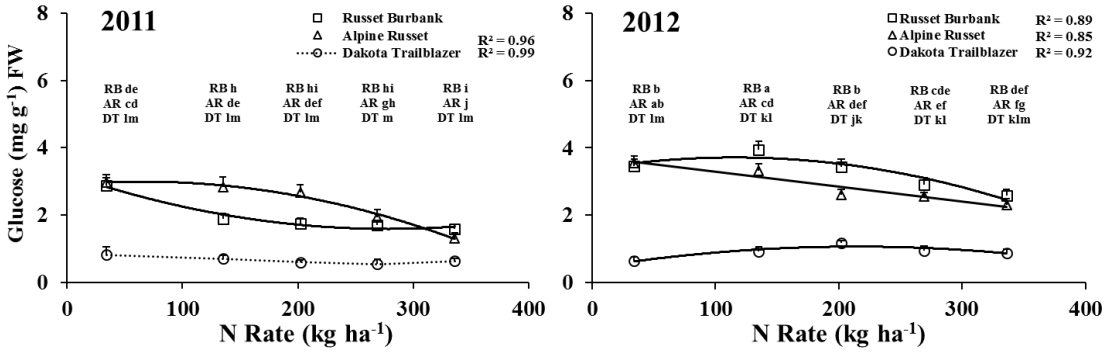

Figure 2-1. Three-way interaction of cultivar, N rate and year effect on glucose concentration for Russet Burbank (RB), Alpine Russet (AR) and Dakota Trailblazer (DT) (Mean glucose concentrations of each cultivar were separated with LSD at $\alpha = 0.05$ at each N rate. Mean values with the same letter indicate no significant differences between cultivars.)

Equations

Russet Burbank, 2011: $y = 2.66\times10^{-5}\ x^2 - 1.34\times10^{-2}\ x + 3.27$; 2012: $y = -2.58\times10^{-5}\ x^2 + 5.95\times10^{-3} x + 3.37$

Alpine Russet, 2011: $y = -1.82\times10^{-5}\ x^2 + 1.52\times10^{-3}\ x + 2.91$; 2012: $y = -4.38\times10^{-3}\ x + 3.72$

Dakota Trailblazer, 2012: $y = -1.15\times10^{-5}\ x^2 + 5.18\times10^{-3}\ x + 0.4$

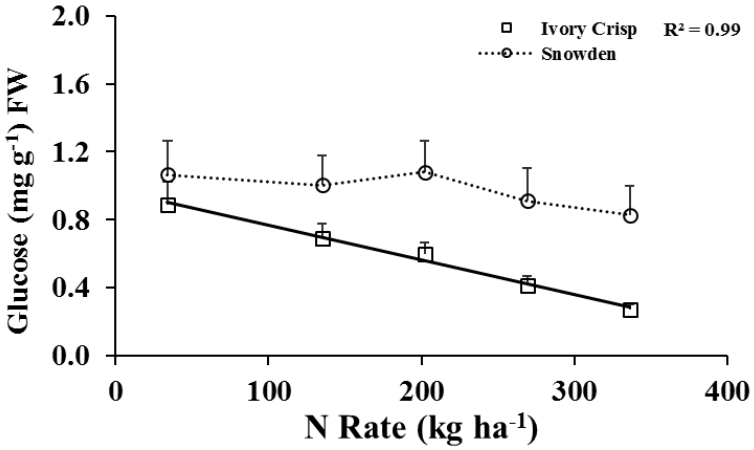

Figure 2-2. Nitrogen rate by cultivar interaction effect on glucose concentration for chip cultivars in 2011 and 2012
Equation
Snowden: $y = -2.0\times10^{-3}\ x + 0.97$

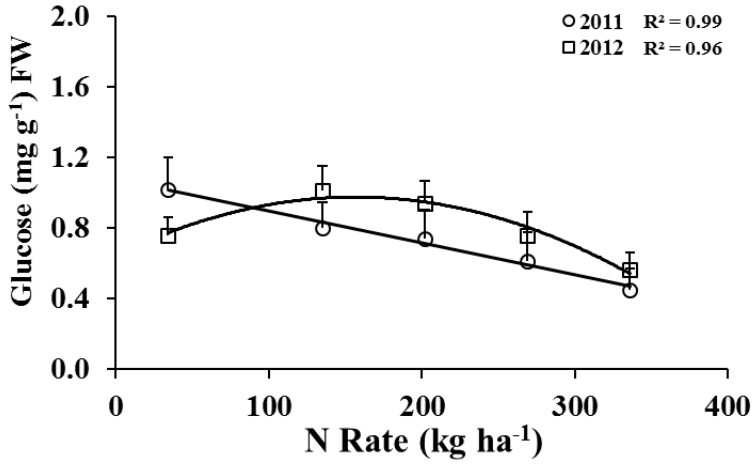

Figure 2-3. Nitrogen rate by year interaction effect on glucose concentration for chip cultivars
Equation
2011: $y = -1.35\times10^{-5}\ x^2 + 4.21\times10^{-3}\ x + 0.65$; 2012: $y = -1.80\times10^{-3}\ x + 1.08$

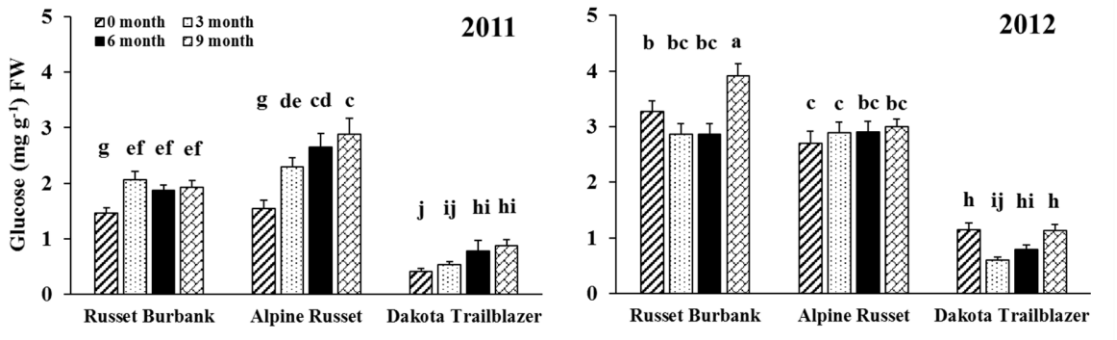

Figure 2-4. Three-way interaction of cultivar, storage time and year effect on glucose concentration for French fry cultivars (Means were separated with LSD at α = 0.05, with the same letter indicating no significant differences.)

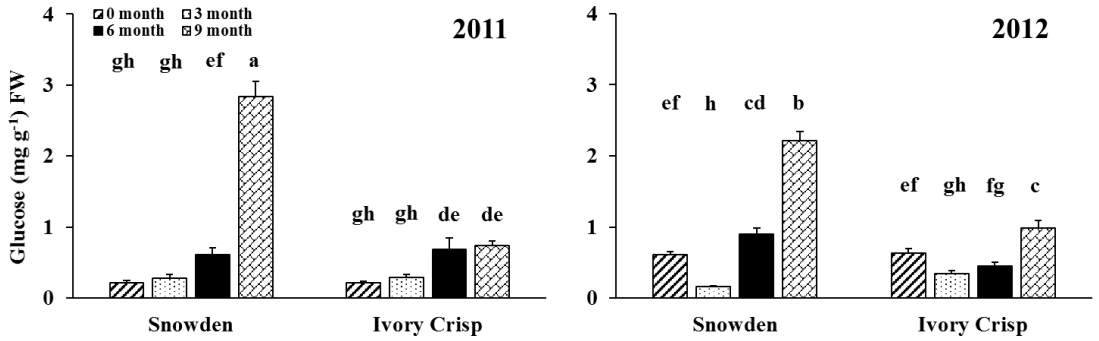

Figure 2-5. Three-way interaction of cultivar, storage time and year effect on glucose concentration for chip cultivars (Means were separated with LSD at α = 0.05, with the same letter indicating no significant differences.)

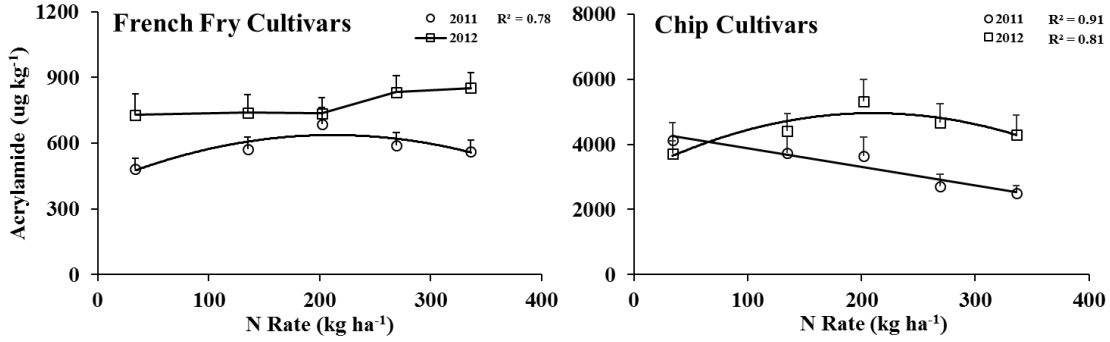

Figure 2-6. Nitrogen rate by year interaction effect on acrylamide concentration for French fry and chip cultivars

Equations

French fry cultivar 2011: $y = -5.11\times10^{-3}\ x^2 + 2.16\ x + 408.00$

Chip cultivar 2011: $y = -5.74\ x + 4462.80$; 2012: $y = -4.23\times10^{-2}\ x^2 + 17.79\ x + 3087.12$

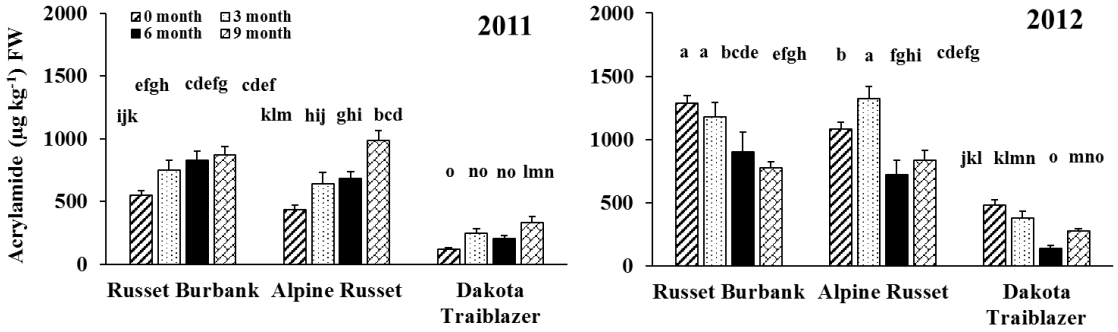

Figure 2-7. Three-way interaction of cultivar, storage time and year effect on acrylamide concentration for French fry cultivars (Means were separated with LSD at α = 0.05, with the same letter indicating no significant differences.)

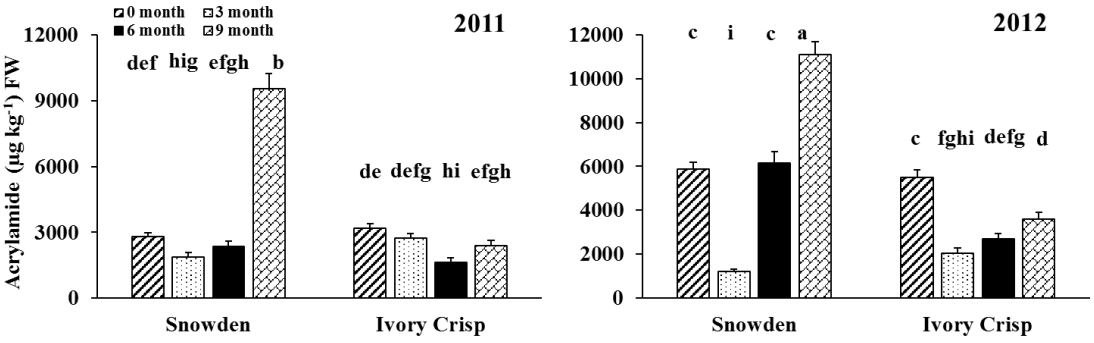

Figure 2-8. Three-way interaction of cultivar, storage time and year effect on acrylamide concentration for chip cultivars (Means were separated with LSD at α = 0.05, with the same letter indicating no significant differences.)

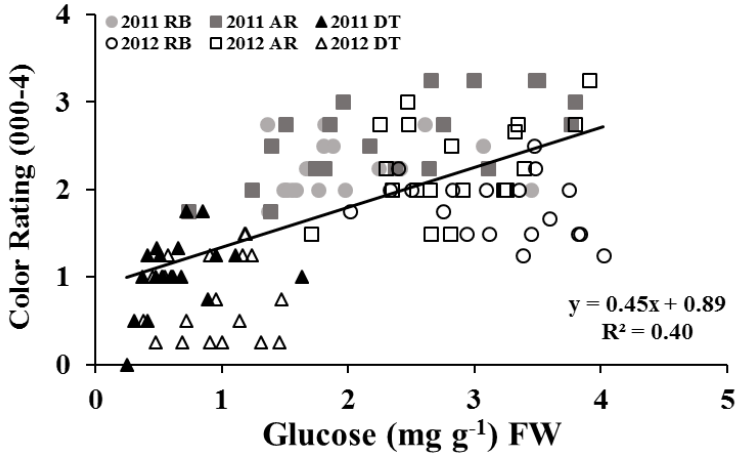

Figure 2-9. Relationships between glucose concentration and fry color for Russet Burbank (RB), Alpine Russet (AR) and Dakota Trailblazer (DT) in 2011 and 2012

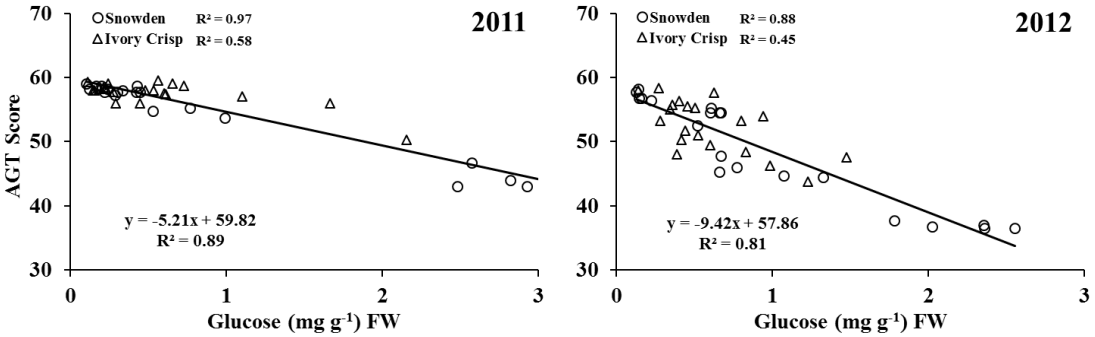

Figure 2-10. Relationships between glucose concentration and chip AGT scores for chip cultivars in 2011 and 2012

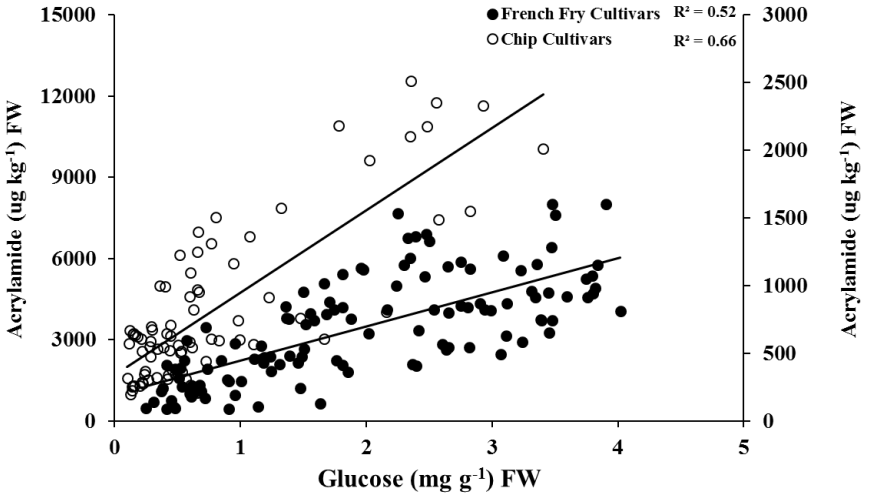

Figure 2-11. Relationships between acrylamide and glucose concentrations over cultivars and two years
Equations
French fry cultivars: y = 251.73 x + 191.15; Chip cultivars: y = 3045.49 x + 1694.20

Supplementary Figures

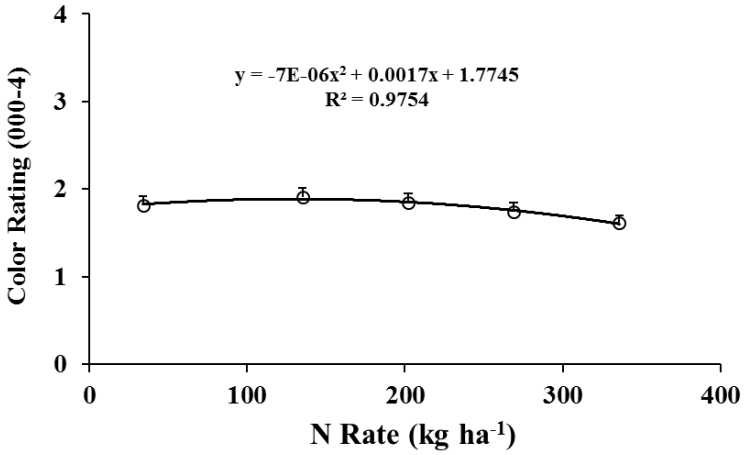

Figure 2-12. Nitrogen rate effect on color darkness for French fry cultivars

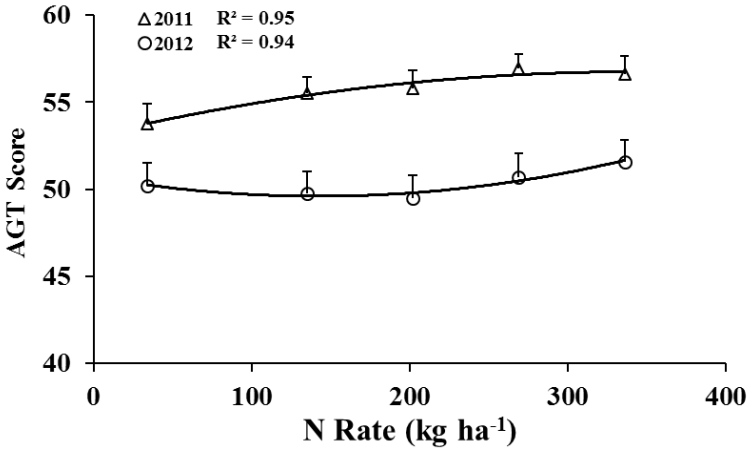

Figure 2-13. Nitrogen rate by year effect for chip AGT score
Equations 2011: $y = -3.08\times10^{-5}\ x^2 + 2.13\times10^{-2}\ x + 53.09$; 2012: $y = 5.49\times10^{-5}\ x^2 - 1.56\times10^{-2}\ x + 50.71$

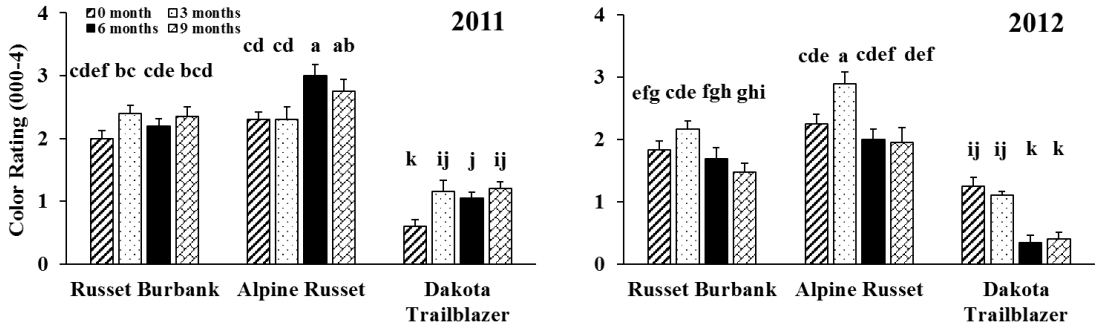

Figure 2-14. Three-way interaction of cultivar, storage time and year effect on fry color for French fry cultivars (Means were separated with LSD at α = 0.05, with the same letter indicating no significant differences.)

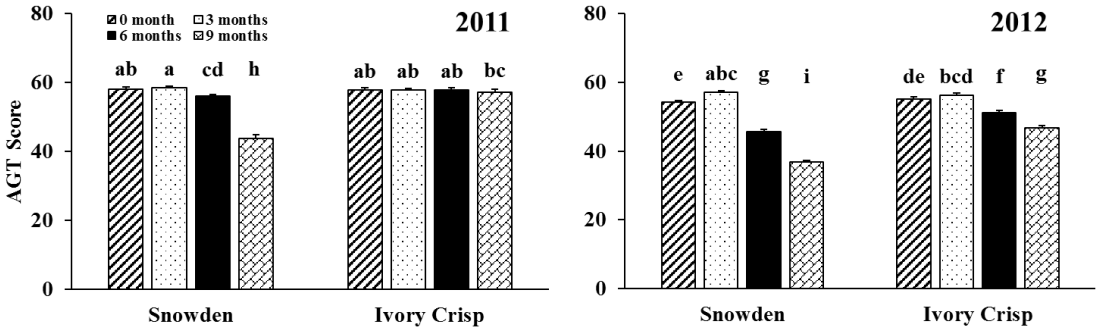

Figure 2-15. Three-way interaction of cultivar, storage time and year effect on AGT score for chip cultivars (Means were separated with LSD at α = 0.05, with the same letter indicating no significant differences.)

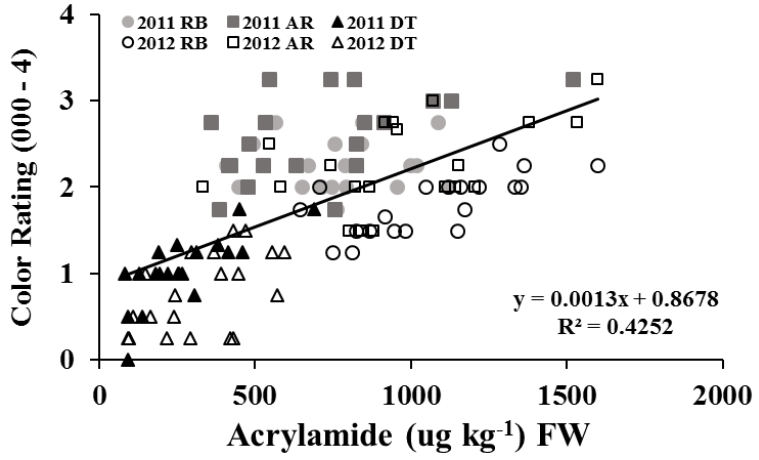

Figure 2-16. Relationships between acrylamide concentration and fry color for French fry cultivars

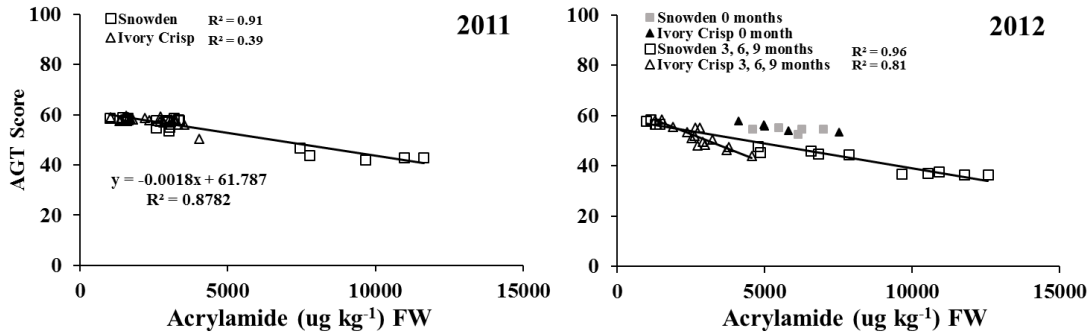

Figure 2-17. Relationships between acrylamide concentration and AGT score for chip cultivars
Equations
2012 Snowden: $y = -1.98\times10^{-3} x + 58.80$, Ivory Crisp: $y = -4.58\times10^{-3} x + 64.14$

Chapter 3 - Cultivar and Nitrogen Fertility Effects on Tuber Yield and In-Season Tuber Asparagine and Glucose concentrations

Overview

New potato cultivars Dakota Russet and Easton were selected for their low sugar accumulation during storage and low acrylamide concentration in fried products. Monitoring changes in reducing sugars and asparagine concentrations during the growing season may provide information on lowering acrylamide-forming potential in these two cultivars. The objectives of this study were to determine the effects of N rate (135 to 404 kg ha^{-1}) on tuber yield, tuber quality, reducing sugars and asparagine concentrations of Easton and Dakota Russet, relative to the standard cultivar Russet Burbank, over two years. The study was conducted on an irrigated loamy sand soil using a randomized complete block design. Total yield increased quadratically at harvest in 2014 and linearly in 2015. Highest yield was produced by Easton, followed by Russet Burbank and then Dakota Russet in both years. New cultivars had more large tubers (greater than 170 g), and less hollow heart tubers than Russet Burbank when environmental conditions were favorable for hollow heart formation. Dakota Russet and Easton had lower stem end reducing sugars than Russet Burbank at harvest under contrasting environmental conditions. Easton had lower asparagine concentrations at the stem and bud end than Dakota Russet and Russet Burbank at harvest. Nitrogen rate effects were significant for yield, specific gravity and asparagine concentration, but these parameters were sometimes influenced by sampling times and environmental conditions. In particular, cold weather before harvesting induced stem end reducing sugars accumulation in all

three cultivars, especially for Russet Burbank. The high ratio of reducing sugars/asparagine in Russet Burbank tubers at harvest may be a sign of asparagine as the limiting factor for acrylamide formation at that point in time.

Introduction

The potential harmful effect of dietary acrylamide on human health has been reconfirmed by the European Food Safety Authority (2015), but the chemical steps of its formation are complex (Bethke and Bussan 2013). Acrylamide is mainly formed from reducing sugars and asparagine. Tuber reducing sugars (glucose and fructose) are often considered to be the limiting factor for acrylamide formation in potato, because of their significantly lower concentration than asparagine, and the strong correlation between reducing sugars and acrylamide (Chuda et al. 2003; Amrein et al. 2004; De Wilde et al. 2005; Elmore et al. 2015). However, a few studies have reported that asparagine can also participate in predicting the acrylamide concentration in fried potato products. Amrein et al. (2003) reported acrylamide concentration as a function of (0.5*glucose + fructose)*asparagine for 17 potato cultivars with $R^2 = 0.91$. Muttucumaru et al. (2014a) reported that asparagine was also correlated with acrylamide for French fry cultivars that contained a high amount of reducing sugars. Matsuura-Endo et al. (2006) determined that the critical value of fructose/asparagine molar ratio is 2 for acrylamide formation, below which the limiting factor is reducing sugars and above which is asparagine. Therefore, asparagine concentration should be taken into account, as well as reducing sugars, when assessing the potential for acrylamide formation in fried potato products.

Tuber asparagine is synthesized in the tuber, rather than transported from leaf to tuber (Muttucumaru et al. 2014b). Asparagine accounts for 33 to 59 % of the total free amino acids and is used as a nitrogen (N) reservoir in potato tubers, when N supply is sufficient and protein synthesis is limited (Eppendorfer and Bille 1996; Gerendás et al. 2007; Lea et al. 2007). Zhu et al. (2010) reported asparagine concentrations ranged from 8.9 to 22.1 g kg^{-1} dry weight in nine potato cultivars obtained from supermarkets (approximately 1.8 to 4.4 g kg^{-1} fresh weight, assuming 80 % tuber fresh weight), while Vivanti et al. (2006) reported asparagine concentrations ranged from 0.15 to 7.6 g kg^{-1} fresh weight in nine cultivars sold in Italian markets and 22 cultivars sold in U.S. markets.

Nitrogen fertilizer application is an important and controllable agronomic practice during the growing season that affects tuber yield and quality (Bélanger et al. 2002; Zebarth et al. 2004). The N rate effect on tuber asparagine has attracted attention in the last decade, when researchers realized that reducing sugars might not be the only limiting factor for acrylamide formation during processing. Muttucumaru et al. (2013) grew 13 cultivars (5 chip cultivars, 7 French fry cultivars and 1 boiling cultivar) with 0, 100 and 200 kg ha^{-1} N, and reported increased asparagine at harvest with increasing N supply for all cultivars. Similar results of increased asparagine concentration with increasing N supply were observed at harvest for the French fry cultivar Agria (Gerendás et al. 2007). De Wilde et al. (2006) reported increased concentrations of asparagine and total free amino acid with increasing N rate in potato cultivars Bintje, Ramos and Saturna stored at 8 ºC. The effect of N fertilizer application on reducing sugars at harvest and during storage was cultivar dependent, and often interacted with growing and storage conditions (Kumar et al. 2004; Halford et al. 2010; Chapter 2).

The concentrations of acrylamide precursors have been investigated at harvest and during storage in separate studies. The effects of the growing season conditions (temperature, water availability, harvesting time and growing season length) on tuber asparagine and reducing sugars were significant, and likely to continue in storage (Kooman et al. 1996; Hijmans 2003; Bethke and Bussan 2013). Insufficient water availability increased reducing sugar concentrations and resulted in darker fry color at harvest and during storage (Eldredge et al. 1996; Kumar et al. 2004). However, irrigation has also been reported to increase acrylamide formation (Muttucumaru et al. 2015). The effect of drought on asparagine concentrations of potato plants was inconsistent, with an increase from 132 to 242 mmol kg^{-1} in the glasshouse, but was not affected in the field study (Muttucumaru et al. 2015). Increased asparagine concentrations under drought stress have also been reported in other crops, such as soybean, alfalfa and wheat (Lea et al. 2007).

To have good processing quality with low reducing sugars, the recommended harvesting time is at tuber chemical maturity when sucrose drops to a minimum level, which often occurs shortly before vine desiccation (Sowokinos 1973; Sowokinos and Preston 1988; Kumar et al. 2003). Air temperature is a factor that could affect the concentrations of reducing sugars. The optimum temperature for tuberization and growth ranges between 15 to 20 ºC. Temperatures below 8 to12 ºC, or above 25 to 30 ºC could cause an increase in tuber sugar concentration (Kumar et al. 2004). A period of pre-harvest cold weather is likely to increase the invertase activity and cause an accumulation of glucose and fructose from hydrolyzed sucrose (Bethke and Bussan 2013).

French fry cultivars Dakota Russet and Easton were recently released in 2012 and 2014, respectively. Dakota Russet was selected for low sugar accumulation during storage and resistance to cold-induced sweetening (North Dakota State University Research Foundation). Easton was rated as a high yielding cultivar that produced light colored French fries (Porter et al. 2014a). The effect of N fertility on reducing sugars and asparagine concentrations during the growing season for these two cultivars relative to the conventional cultivar Russet Burbank may provide insight into the factors that affect acrylamide forming potential in processed potatoes.

The objectives of this study were to: (1) determine the effects of N rate on tuber yield and tuber quality of Easton and Dakota Russet, relative to the standard cultivar Russet Burbank over two growing seasons; and (2) characterize in-season changes in tuber reducing sugars and asparagine concentrations as affected by cultivar and N rate.

Material and Methods

This study was conducted at the Sand Plain Research Farm in Becker, Minnesota, on a Hubbard loamy sand soil (sandy, mixed, frigid Entic Hapludolls) in 2014 and 2015. Pre-planting soil chemical properties of each year are presented in Table 1. Prior to planting, 224 kg ha^{-1} 0-0-60 and 224 kg ha^{-1} 0-0-22 were broadcast and incorporated with a disk plow in 2014, and a chisel plow in 2015 in all plots.

Fertilizer Treatments

Each cultivar was subjected to five N fertilizer treatments, 135, 202, 269, 336 and 404 kg ha^{-1}. All plots received 101 kg ha^{-1} as polymer-coated urea (ESN, Agrium, Inc.,

44-0-0) pre-planting, and 34 kg N ha^{-1} (31 kg ha^{-1} as monoammonium phosphate and 3 kg ha^{-1} as ammonium sulfate) at planting in a band 8 cm to the side and 5 cm below the seed tuber. In addition to the N application, all plots received 146 kg P_2O_5 ha^{-1}, 203 kg K_2O ha^{-1}, 49 kg S ha^{-1}, 22 kg Mg ha^{-1}, 0.6 kg B ha^{-1} and 1.1 kg Zn ha^{-1}, applied as a blend of monoammonium phosphate, potassium chloride, potassium magnesium sulfate, ammonium sulfate, boric acid, and zinc sulfate at planting. At emergence, N was side-dressed at 0, 67, 134, 201 and 269 kg N ha^{-1} as Environmentally Smart Nitrogen (Agrium, Inc., 44-0-0) and then hilled in on 5 June 2014 and 21 May 2015.

Plot Design and Plant Management

A randomized complete block design was adopted with four replications using a factorial treatment arrangement of N rate and cultivar. Each plot consisted of seven 7.6 m rows with 25 plants in each row. The spacing between rows was 0.9 m and seed tubers were spaced 0.3 m apart within each row. Tubers used for post-harvest detection were planted in row 4 and 5, which had two red potato plants at both ends as markers. Row 2 had two red potato plants at both ends, and every red potato plant between three plants of test cultivars, which were sampled six times during the growing season. Whole "B" seed (56 to 84 g) of Russet Burbank, and cut "A" seed (56 to 84 g) of Dakota Russet and Easton were hand planted in furrows. Belay insecticide was applied in-furrow for beetle control, along with the systemic fungicide Quadris. Weeds, diseases, and other insects were controlled using standard practices from the Midwest Vegetable Production Guide for Commercial Growers (2012). Rainfall was supplemented with sprinkler irrigation, which followed the checkbook method of scheduling (Wright and Bergsrud 1991).

Sample Collection

Planting and harvesting dates were scheduled according to weather conditions. There were 139 growing days in 2014 (planted on 6 May, vine-killing on 22 September, tuber-harvesting on 2 October), and 148 growing days in 2015 (planted on 21 April, vine-killing on 16 September, tuber-harvesting on 28 September). Emergence fertilizer was applied on 5 June 2014 and 21 May 2015. Three plants of the test cultivars were dug out in row 2 of each plot on 69, 79, 97, 112 and 125 days after planting (DAP) in 2014, and 63, 77, 105, 118 and 133 DAP in 2015. Total yield and specific gravity were determined at each sampling date, and on 149 and 160 DAP in 2014 and 2015, respectively. Reducing sugars and asparagine concentrations were determined at each sampling date, and on 149 and 148 DAP in 2014 and 2015, respectively. Hollow heart incidence and percentage of tubers greater than 170 g were determined at tuber harvest. 25 tubers of each plot were cut lengthwise for the determination of hollow heart incidence. Total yield was assumed not to change after vine kill, so vine kill dates (139 and 148 DAP) were considered as the last sampling day instead of tuber harvesting dates (149 and 160 DAP) for total yield.

On each sampling day, tubers were collected, washed and weighed from three plants in each plot. Ten tubers greater than 85 g from each plot were collected for specific gravity determination using the method from Schippers (1976), followed by determination of hollow heart incidence by cutting the tubers from apical to stem end. About 200 to 300 g of these chopped tuber samples were oven-dried at 60 ^{o}C for at least 72 hours, and ground with a Wiley mill to pass through a 2-mm sieve, then assessed for

tuber N concentration using a combustion analyzer (Elementar Vario EL III , Elementar Americas Inc., Mt. Laurel, NJ) following the methods of Horneck and Miller (1997). Six tubers greater than 85 g from each plot were collected for the determination of reducing sugars and asparagine concentrations. Total tuber numbers were less than 16 for some plots at the early sampling dates, and therefore tuber quality, reducing sugars and asparagine were determined from those tubers that were available. Petiole samples (20 petioles per plot) from the fourth leaf from the terminal were collected 51, 63, 77 and 86 days after planting in 2014, and 56, 65, 79 and 97 days after planting in 2015. Petiole samples were oven-dried at 60 ºC for at least 72 hours, then ground with a Wiley mill to pass through a 2-mm sieve. Petiole nitrate-N concentration was determined using a conductimetric procedure following the methods of Carlson (1986) and Carlson et al (1990).

Reducing Sugar and Asparagine Determination

Fresh tuber tissue was collected about 0.5 cm away from stem and bud end of tubers using a 7.8 mm Humboldt Brass Cork borer. Tissue samples were stored in Wheaton ™ 20 ml HDPE Liquid Scintillation vials at -20 ºC. The extraction of reducing sugars and asparagine was modified from a previous study (Knowles et al. 2008). An IKA Ultra-Turrax disperser (T-25 digital disperser, 18 mm) was used to grind 2.5 g samples for 1 min with 6 ml of triethanolamine HCl (TEA) buffer (30 mM, pH 7, with 1mM 1, 4-Dithiothreitol), 300 ul of 85 mM Carrez I solution, 300 ul of 250 mM Carrez II solution and 500 ul 0.1 mM NaOH until no visible chunk of tubers remained. The solution was transferred to a Falcon™ 15 ml centrifuge tube, vortexed 30 seconds for

better mixing and then centrifuged for 15 min at 1200 g. 2 ml supernatant was transferred to Eppendorf tubers and centrifuged (10000 g) for 10 min. Then 1.5 ml supernatant was transferred to a 2 ml Eppendorf ™ snap-cap microcentrifuge safe-lock tube and stored at -20 ºC for the determination of reducing sugars and asparagine. All the steps were conducted on ice to reduce the enzymic browning resulting from polyphenol oxidization.

The dinitrosalicylic colorimetric method as described by Lindsay (1973) was used to determine the concentration of reducing sugars. For this method, 100 µl Sumner's reagent (44 mM 3, 5-dinitrosalicylic acid (DNS), 2 M sodium hydroxide and 940 mM potassium sodium tartrate) were mixed with 100 µl supernatant in an Eppendorf ™ 96 well plate. Samples were heated at 94 ºC for 10 min and then chilled at 4 °C in the Eppendorf ™ 5331 MasterCycler Gradient Thermal Cycler. An aliquot of 150 µl from each sample (measuring reduction of DNS to 3-amino-5-nitrosalicylic acid by glucose) was determined at A550 using Bio-Tek Intruments EL800 Universal Microplate Reader. A standard curve for quantification of reducing sugars was constructed using 8 glucose concentrations ranging from 0 to 5 µM for each plate.

Asparagine quantification was determined by LC-MS/MS. For this method, deproteinization of 200 µl supernatant (described above) was performed by incubating the sample at 94 ºC for 3 min in an Eppendorf ™ 96-well PCR plate. Proteins were removed by centrifugation at 2000 g for 10 min. The supernatant was transferred to Eppendorf ™ tubes, and diluted 100 fold with the solution of two pairing ions, 0.1 % heptaflourobutyric acid (HFBA) and 0.1 % formic acid. The diluted solution was then transferred into the Agilent autosamplers and kept at 4 ºC. 10 µl diluted samples were subjected to LC-MS/MS using an Agilent Eclipse Plus C18 RP column on a Shimadzu

UFLC XR coupled to an AB SCIEX – Triple Quad 5500. The sample was subjected to a linear gradient of 0 to 100 percent acetonitrile for 5 minutes at a column flow rate of 0.4 ml / minute. The retention time for asparagine was 1 min. The source conditions were optimized for asparagine analysis. The specific selective reaction monitoring (SRM) transitions employed for asparagine were m/z 116 > 87 transition. The data were analyzed using Multi-Quant (ABI) providing the peak area for the m / z 116, 88 and 87 transition. The amount of asparagine was determined by comparison to sample peak area of a standard curve. The amount is expressed as ug asparagine g^{-1} potato fresh weight. All analysis was conducted by the Center for Mass Spectrometry and Proteomics at the University of Minnesota.

Statistical Analysis

Analysis of variance (ANOVA) was performed using PROC ANOVA with the SAS 9.4 statistical software package (SAS Institute Inc., Cary NC. USA) for each measured variable to assess the significance of main effects and interactions between cultivars interacted within N rate and year (for percentage of tubers greater than 170 g, hollow heart incidence and tuber N) or with sampling times (for total yield, specific gravity, reducing sugars and asparagine). Data for total yield, specific gravity, reducing sugars and asparagine were analyzed separately in 2014 and 2015 due to the different sampling dates in each year. Repeated measures were used for sampling time. Tuber size distribution at harvest (tubers greater than 170 g) and tuber N were combined over years and analyzed. A square root transformation was used when necessary to account for the heterogeneity of variance. Linear or quadratic regressions between N rate and total yield,

percentage of tubers greater than 170 g, specific gravity, asparagine and tuber N were determined using PROC GLM and CONTRAST statements in SAS. Probability levels less than or equal to 0.05 were considered significant for the linear or quadratic trends, and the quadratic trend was selected over the linear trend when both were significant. Means were separated using PROC GLM and the MEANS statement using the least significant difference (LSD) at the 5 % probability level in SAS for stem end asparagine and tuber N in 2014 and 2015. Means for total yield, specific gravity, hollow heart, reducing sugars at stem and bud end, and bud end asparagine in two years were separated using PROC GLM and LSMEANS statement in SAS. Reducing sugars and asparagine concentrations were converted to mM g^{-1}, and then the molar ratio of reducing sugars/asparagine at stem end bud ends was determined within each treatment during both growing seasons. Tuber N and average asparagine concentrations of stem and bud end were plotted and analyzed for significance of their relation within cultivar using PROC REG. The total yield of Russet Burbank on 125 DAP 2014 was an outlier and taken out of the quadratic trend. The cultivar effect was determined based on the estimated value from the quadratic equation for Russet Burbank at 125 DAP in 2014.

Results and Discussion

Weather Conditions

The monthly average temperature from May to August in 2014 and 2015 was compared with the average temperatures over the past 30 years (Table 3-2). A cold spring occurred with a monthly average temperature of 3.4 ºC in April 2014 compared to 7.8 and 7.6 ºC in 2015 and the average of past 30 years, respectively. The colder

temperatures in 2014 resulted in a late planting on 6 May. September 2015 with a monthly average temperature of 18.2 ºC was warmer than September 2014 and the average of past 30 years (15.6 and 15.7 ºC, respectively). Before tuber harvest on 2 October 2014, a period of cold weather occurred from 10 to 15 September and 17 to 18 September with the minimum temperature each day ranging from -1.1 to 6.7 ºC, while the temperatures during this same period in 2015 ranged from 3.3 to 17.2 ºC. Local growing degree days for potato plants also reflected a warm and long growing season in 2015 compared with 2014 (Supplementary figure 3-14). In 2014, precipitation above the 30-year average occurred in April, May, June and August. Precipitation was above the 30-year average during May, July and August in 2015. Assuming a water holding capacity of 2.4 cm in the top 39 cm of soil at this site (Gimes, 1968), there were three rainfall events following sidedress N application that exceeded the water holding capacity in 2014 and four rainfall events that exceeded the water holding capacity in 2015.

Yield and Size Distribution Response

For both years, tuber yield was determined during the growing season while tuber size distribution was determined only harvest. Total yield of Russet Burbank, Dakota Russet and Easton increased quadratically during the growing season (Figure 3-1). Small or no yield differences among cultivars were observed at early sampling dates (up to 97 DAP 2014 and 118 DAP 2015), suggesting similar initial tuber bulking rates for the three cultivars. After those time periods, tuber-bulking rates differed among the cultivars and yield differences became apparent. During the last two sampling dates, Easton was still bulking (6.3 % in 2014 and 11.8 % in 2015), while total yields of Russet Burbank and

Dakota Russet did not significantly increase during this time period. The high tuber-bulking rate of Easton late growing season resulted in higher total yields than the other two cultivars.

The interaction of N rate by sampling time was significant for total yield during the growing season in 2014 and 2015 (Table 3-3, Figure 3-2). Total yield did not respond to N supply at sampling dates up to 112 DAP in 2014 and 118 DAP in 2015, while yield increased with increasing N rate quadratically in 2014, and linearly in 2015 at the late sampling dates. Total yield responded to N rate up to 202 kg ha^{-1} in 2014, but linearly increased with increasing N rate from 135 to 404 kg ha^{-1} in 2015. Previous studies have reported the impact of environmental conditions on yield response to N rate (Chapter 1). A possible reason for the higher yield in 2015 was the favorable weather conditions for tuber bulking, which created a higher tuber sink resulting in a higher N demand and greater yield in this study. The high N demand exceeded the N supply for maximizing yield in 2015, while 202 kg N ha^{-1} was sufficient for yield in 2014. Similar yield responses to N rate with contrasting growing seasons were reported by Oliveira et al (2016) and Sun et al (Chapter 1).

The percentage of tubers greater than 170 g was affected by the interactions of cultivar by N rate, and year by N rate (Table 3-4, Figure 3-3). For Russet Burbank, the percentage of tubers greater than 170 g increased from 51 % to 73 % when the N rate increased from 135 to 339 kg ha^{-1}. Compared to Russet Burbank, tuber size distribution of the new cultivars Dakota Russet and Easton was only minimally affected by N supply. The percentage of large tubers in Easton increased quadratically from 81 % to 88 % when N supply increased from 135 to 320 kg ha^{-1}. The year effect also played an important role

in tuber size distribution response to N rate. The percentage of tubers greater than 170 g increased quadractically with increasing N rate both years, with a higher percentage of large tubers in 2014 than in 2015 for the same amount of N input.

The results from this study show that tuber size distribution was cultivar dependent. For cultivars like Russet Burbank, sufficient N supply increased the percentage of tubers greater than 170 g. However, Dakota Russet and Easton had a higher percentage of large tubers regardless of N rate, indicating a stronger effect of genotype than environmental conditions. Previous studies have reported that air temperature ranging from 15 to 24 ºC is optimum for foliar expansion, tuber initiation and tuber growth during the growing season (Kooman and Haverkort 1995). However, high temperatures (greater than 35 to 40 ºC) can significantly reduce tuberization, decrease the assimilate transport from leaves, and cause a high shoot/root ratio, reduced tuber dry matter, and consequently result in a low yield (Nowak and Colborne 1989; Basu and Minhas 1991; Gawronska et al. 1992). The results in this study are consistent with those reported in the previous studies. In this study, weather conditions in 2015 (warm weather early and late in the growing season) caused higher tuber numbers per plant (tuber initiation), and a lower average tuber weight (tuber bulking, data not shown) for all three cultivars, which resulted in higher total yield, but a lower percentage of large tubers in 2015 than in 2014.

Tuber Quality Response

Tuber specific gravity during the growing season was significantly affected by the interaction of cultivar by sampling time (Table 3-3, Figure 3-4). During the growing

season in 2014, specific gravity of three cultivars increased up to 112 DAP, then sharply decreased on 125 DAP, and then leveled off for all three cultivars. The cultivar effect on specific gravity was almost always (except 112 DAP) significant, with Dakota Russet and Easton significantly higher than Russet Burbank. The specific gravity of the three cultivars at tuber harvest in 2014 ranked as Easton = Dakota Russet > Russet Burbank, and ranged from 1.079 to 1.084. In 2015, the specific gravity of all three cultivars increased up to 105 DAP and then generally decreased (Dakota Russet) or leveled off (Russet Burbank and Easton) until tuber harvest. New cultivars Dakota Russet and Easton had a significantly higher or comparable level of specific gravity compared with Russet Burbank during the growing season. At tuber harvest in 2015, the specific gravity of three cultivars ranked as Easton > Dakota Russet = Russet Burbank and ranged from 1.076 to 1.079.

In 2014, specific gravity of all cultivars linearly decreased (from 1.087 to 1.077) with increasing N rate from 135 to 404 kg ha^{-1} (Figure 3-5). Decreasing specific gravity with increasing N rate was also observed in 2015. However, the decreasing rate of specific gravity differed among sampling times. A decreasing linear trend with increasing N rate occurred on 77 and 133 DAP, and a quadratic trend was observed on 105 and 118 DAP. By 160 DAP, specific gravity was not affected by N rate.

The effect of N rate on specific gravity has been reported in a number of previous studies with variable results. These include a linear decrease as N rate increased (Westermann et al. 1994; Bélanger et al. 2002; Zebarth et al. 2004), a linear or quadratic increase with increasing N rate (Chapter 1), a decrease followed by an increase above the optimum N rate for yield (Long et al. 2004), and no effect due to N supply (Chapter 1).

At tuber harvest, specific gravity of all three cultivars linearly decreased in 2014 with increasing N rate. In 2015, specific gravity was not affected by N supply. The N rate effect on new cultivars Dakota Russet and Easton has not been previously reported. As for Russet Burbank, specific gravity quadratically responded (increased from 34 to 242 kg N ha^{-1} and then decreased at N rate up to 336 kg ha^{-1}) in 2011, but was not affected by N rate in 2012 in a previous study conducted at the same location (Chapter 1), suggesting a significant impact of growing conditions. In North Dakota State University trials, Dakota Russet was reported to have an average specific gravity of 1.085, which is higher than the average values (1.082 in 2014 and 1.077 in 2015 at tuber harvest) in this study (North Dakota State University Research Foundation). Easton was reported to have a specific gravity of 1.081 in 17 trials at Maine state from 2007 to 2013 (Porter et al. 2014b). An average specific gravity of 1.084 in 2014 and 1.079 in 2015 at tuber harvest was found in this study. Rowe and Curwen (1993) recommended a specific gravity ranging from 1.080 to 1.089 for frozen French fry processing. Therefore, Easton had a specific gravity that was generally ideal for French fry processing, while Dakota Russet may have too low a tuber specific gravity under certain growing conditions (possibly a warmer and longer growing season that resulted in rapid tuber bulking for a long period). Sufficient water availability tends to have a negative effect on specific gravity. Adjusting water supply with irrigation has been suggested as a way to adjust specific gravity to a level that is proper for French fry processing under some conditions (Bélanger et al. 2002; Yuan et al. 2003).

Hollow heart incidence (data combined over two years) was determined and analyzed at tuber harvest (Table 3-4). Nitrogen supply did not significantly affect hollow

heart incidence either year. The cultivar effect was significant and differed by year (Table 3-4, Figure 3-6). In 2014, 27 % of Russet Burbank tubers had hollow heart, which was significantly higher than Dakota Russet (13.5 %) and Easton (3.5 %). However, the incidence of hollow heart in all three cultivars was lower than 5 %, with no significant differences in 2015. This indicates that environmental conditions in 2014 were favorable for the formation of hollow heart, especially in susceptible cultivars like Dakota Russet and Russet Burbank. Similar results have been reported in a previous study for Russet Burbank, which had up to 19 % hollow heart in one year and only 3.1 % in the following year (Chapter 1). Easton was highly resistant to hollow heart formation even under environmental conditions favorable for hollow heart development.

Reducing Sugars and Asparagine Response

The cultivar by sampling time interaction was significant for stem end reducing sugars in 2014 and 2015 (Table 3-3). In 2014, concentrations of stem end reducing sugars increased (69 to 79 DAP), decreased (79 to 112 DAP) and then increased again from 112 to 149 DAP for all three cultivars, although the increase was much higher for Russet Burbank than for Easton or Dakota Russet (Figure 3-7). The reason for higher reducing sugars in Russet Burbank may have been due to cold-induced sweetening. Tubers in 2014 were subjected to a period of cold weather (-1.1 to 6.7 ºC) from 127 to 135 DAP, which may have induced cold sweetening (Bethke and Bussan 2013). Reducing sugars concentrations in Russet Burbank (6.83 mg g^{-1}) were significantly higher than those in Dakota Russet (2.35 mg g^{-1}) and Easton (2.94 mg g^{-1}), suggesting it was more susceptible to weather conditions and more likely to cause sugar ends (browning of the stem end)

during French fry processing. In 2015, stem end reducing sugars concentration decreased from 63 to 105 DAP for all cultivars, and then increased for Russet Burbank or leveled off for Dakota Russet and Easton. Stem end reducing sugars in 2015 on 149 DAP were much lower with concentrations of 1.48, 0.50 and 0.67 mg g^{-1} for Russet Burbank, Dakota Russet and Easton, respectively.

The cultivar by N rate effect was significant for bud end reducing sugars in 2014 and the three-way interaction of cultivar by N rate by sampling time was significant for bud end reducing sugars in 2015 (Table 3-3). However, bud end reducing sugars in 2015 were only affected by N rate at the first two sampling times for all three cultivars, with no clear patterns. The cultivar by N rate interaction indicated that bud end reducing sugars of Dakota Russet and Easton were either not affected or minimally affected by N rate, while no consistent trends with N rate were observed for Russet Burbank bud end reducing sugars at the first two sampling times (data not shown).

The interaction of cultivar by sampling time is presented in Figure 3-7. In 2014, bud end reducing sugars of all cultivars increased from 69 to 79 DAP, decreased to low concentrations 112 DAP (ranging from 0.50 to 0.69 mg g^{-1}), then slightly increased at harvest (0.64 to 1.29 mg g^{-1}). At the later sampling times, bud end reducing sugars concentrations in Russet Burbank were not significantly different from those in the new cultivars, except for at harvest with Russet Burbank reducing sugars higher than Easton reducing sugars. In 2015, bud end reducing sugar concentrations of all three cultivars were the highest at 63 DAP, gradually decreased to low levels at 118 DAP (0.29 to 0.42 mg g^{-1}), and then leveled off for all three cultivars. In both years, the cultivar effect on

bud end reducing sugars was significant at the earlier sampling dates, but then gradually attenuated to a negligible level at harvest.

Stem end reducing sugars concentrations were lower than bud end concentrations at the early sampling dates, but higher than bud end at the late sampling dates for all three cultivars both years. Temperature stress can affect reducing sugars concentration at the stem end more than the bud end, as a result of disruption of normal biochemical processes (Krauss and Marschner 1984; Thompson et al. 2008). The high stem end reducing sugars concentration at tuber harvest in 2014 for Russet Burbank is consistent with previous reports that this is a sugar end susceptible cultivar (Shock et al. 1993). New cultivars Dakota Russet and Easton were also subjected to cold weather in 2014 with increased reducing sugars concentrations. However, the concentrations were lower than the concentrations in Russet Burbank, indicating that genotype plays an important role in resistance to sugar ends under stress conditions (Thompson et al. 2008).

Over both years, only main effects of cultivar, N rate and sampling time were significant for stem end asparagine, while cultivar by sampling time was significant for bud end asparagine in addition to the main effects (Table 3-3). To better monitor how reducing sugars and asparagine of each cultivar changed during the growing season, Figure 3-8 presents the interaction of cultivar by sampling time effect on stem end asparagine along with the average value of three cultivars at each sampling date. A consistent effect of cultivar on stem end asparagine concentrations was observed. Easton had significantly lower stem end asparagine concentrations than Russet Burbank and Dakota Russet during both growing seasons (Figure 3-8). Stem end asparagine concentrations generally increased and then decreased for all three cultivars. At 149 DAP

in 2014, stem end asparagine concentrations were 1.74, 1.49 and 1.08 mg g^{-1} for Russet Burbank, Dakota Russet and Easton, respectively. At 148 DAP in 2015, stem end asparagine concentrations for Russet Burbank, Dakota Russet and Easton (1.09, 1.06 and 0.62 mg g^{-1}, respectively) were lower than in 2014.

In both years, bud end asparagine generally increased and then decreased at 125 DAP in 2014, and 118 DAP in 2015 for all cultivars (Figure 3-8). Compared to Russet Burbank, Easton had the same concentrations of bud end asparagine at the early sampling dates, but lower concentrations later in the growing season both years (with exception of 63 DAP in 2015). Dakota Russet had comparable or higher concentrations of bud end asparagine than Russet Burbank at 69 to 97 DAP in 2014, and 63 to 105 DAP in 2015, then gradually decreased to the same (2015) or lower (2014) concentration. At vine kill, bud end asparagine concentrations were higher in 2014 compared to 2015.

The N rate effect on asparagine was consistent, showing an increase with increasing N rate at the stem and bud end for all cultivars both years (Figure 3-9). These results are similar to those reported in three previous studies (Lea et al. 2007; Gerendás et al. 2007; Muttucumaru et al. 2013) Stem end asparagine was higher than bud end, and showed an increase with increasing N supply. Asparagine concentrations in the stem and bud end in 2014 were significantly higher than in 2015, probably because of higher N demand for higher tuber yield in 2015, which resulted in a lower proportion of N contributed to asparagine as an N reservoir.

In contrast to the large differences between concentrations of stem end and bud reducing sugars at the end of the growing season, the concentrations of asparagine at stem and bud end were similar, and in a relatively narrow range (from 0.45 to 2.23 mg g^{-1}

in 2014, and 0.43 to 1.86 mg g^{-1} in 2015 including both ends). These results are similar to those reviewed and reported by Bethke and Bussan (2013) of 4 to 25 mg g^{-1} dry weight basis for asparagine, and 0.04 to 4.8 mg g^{-1} dry weight for reducing sugars. Asparagine concentrations changed in a similar manner with increasing N rate both years, indicating that they were not affected by the different environmental conditions in either year. While effects of environmental conditions on in-season asparagine concentrations were not apparent in this study for potato, asparagine accumulation under drought and salt stress has been reported in soybean, alfalfa and wheat (Lea et al. 2007). In contrast to significant effects of cultivar, storage conditions such as temperature and duration had minor effects on tuber asparagine concentrations (Olsson et al. 2004).

Reducing Sugar/Asparagine Molar Ratio

According to Matsuura-Endo et al. (2006), acrylamide concentrations are correlated to asparagine when the fructose/asparagine molar ratio is greater than 2. However, glucose was reported to be about half as effective as fructose in supporting acrylamide formation (Amrein et al. 2003). Therefore, the relationship between acrylamide and asparagine would occur when the reducing sugar/asparagine molar ratio is greater than 3. Figure 3-10 shows the reducing sugars/asparagine molar ratio in the stem and bud end of Russet Burbank, Dakota Russet and Easton in 2014 and 2015. Based on molar ratios, the limiting factor in the bud end for acrylamide formation was asparagine at the early sampling dates for Russet Burbank both years and for Dakota Russet and Easton in 2014. At later dates, the limiting factor switched to reducing sugars for all three cultivars both years. Reducing sugars in the stem end were the limiting factor

for all three cultivars during the whole season in 2015 when temperature stress did not occur. However, in 2014, when tubers were subjected to a period of cold weather before harvest, reducing sugars increased significantly for Russet Burbank, making asparagine the limiting factor. Therefore, for susceptible cultivars like Russet Burbank, asparagine might become the limiting factor for acrylamide formation in the stem end at harvest, but not in the bud end.

Tuber N Concentration and Its Relationship to Asparagine

The cultivar by N rate effect on tuber N concentration was significant and consistent both years (Table 3-4, Figure 3-11a). Tuber N concentration linearly increased with increasing N rate for all three cultivars, but increased at different rates within cultivars (Dakota Russet > Easton > Russet Burbank). Previous studies reported the same result of increased tuber N concentration with increasing N application in tuber and the whole plant (Joern and Vitosh 1995; Jamaati-e-somarin et al. 2009; Badr et al. 2012). When averaged over cultivar and N rate, tuber N concentration was higher in 2015 than in 2014, showing a significant year effect (Figure 3-11b).

The relationship between asparagine and tuber N concentration was analyzed because asparagine is often considered as an N reservoir with sufficient N supply (Eppendorfer and Bille 1996; Gerendás et al. 2007; Lea et al. 2007). While both asparagine and tuber N concentrations increased with increasing N supply, they were not correlated for three cultivars either year, except for Dakota Russet in 2015 with $R^2 = 0.29$ (Figure 3-12). A relationship between asparagine and tuber N in potato was not found in previous studies. Tuber N concentrations of the three cultivars were higher in 2015

(ranging from 0.80 to 1.88 %) than in 2014 (ranging from 0.69 to 1.65 %), while the average asparagine concentrations were higher in 2014 (ranging from 0.49 to 3.15 mg g^{-1} in 2014 and 0.40 to 1.64 mg g^{-1} in 2015). This indicates that even though total N uptake was greater in 2015, the N distribution was not proportionally distributed to asparagine in 2015 compared to 2014. Based on the higher yield and petiole nitrate concentrations in 2015 (Figure 3-2 and supplementary Figure 3-13), it is speculated that the N taken up was invested into yield rather than stored as asparagine under the favorable environmental conditions in 2015 for all three cultivars.

Conclusions

The new cultivars, Dakota Russet and Easton, produced a higher percentage of large tubers (greater than 170 g) than Russet Burbank. They also had higher specific gravity, less hollow heart incidence, and lower concentrations of asparagine and reducing sugars. Easton demonstrated continuous tuber bulking until vine kill, which resulted in higher total yield than Russet Burbank and Dakota Russet.

Increasing N supply greatly affected tuber yield and quality in this study. However, weather conditions (year) showed a strong influence on total yield response to N rate. In a warm and long growing season (2015), environmental conditions were favorable for tuber bulking, which created a high tuber sink strength and a high N demand. With increasing N supply (up to 404 kg ha^{-1}), a linear increase of total yield was observed. In contrast, in a shorter growing season like 2014, the lower N demand resulted in no significant yield increase above 202 kg N ha^{-1}. In addition, excessive N input in 2014 resulted in higher asparagine concentrations than in 2015.

Stem and bud end-reducing sugars concentrations changed over the growing season. Stem end-reducing sugars concentrations of Russet Burbank were significantly higher than Dakota Russet and Easton at harvest both years, which were also affected by different weather conditions. A period of cold weather before harvest in 2014 appeared to induce reducing sugars accumulation, especially for the susceptible cultivar Russet Burbank. Compared to Russet Burbank, the new cultivars Dakota Russet and Easton demonstrated better resistance to stress induced cold-sweetening. Because asparagine concentrations were not affected by weather conditions, high reducing sugar concentrations might lead to asparagine being the limiting factor for acrylamide formation. In that situation, tuber asparagine concentration should be taken into account for acrylamide mitigation in processed potatoes.

Table 3-1. Pre-plant soil properties at the Sand Plain Research Farm in Becker, MN in 2014 and 2015

	Top 15 cm Soil												Top 61 cm Soil
Year	pH	OM (%)	Bray P	K	Ca	Mg	SO_4^{2-}-S	B	Zn	Fe	Mn	Cu	NO_3^--N
								mg kg^{-1}					
2014	6.3	2.0	30.8	94	918	171	1.5	0.2	1.5	28.2	8.4	0.7	1.6
2015	6.0	2.2	35.5	119	911	153	2.7	0.3	1.9	40.5	11.3	0.6	3.8

Table 3-2. Monthly weather conditions during the growing season at Becker, MN in 2014 and 2015

	Temperature (ºC)			Precipitation (cm)		
	2014	2015	Average (1985 to 2015)	2014	2015	Average (1985 to 2015)
April	3.4	7.8	7.6	17.8	3.7	7.5
May	13.4	13.4	14.4	18.4	14.9	9.4
June	19.6	19.4	19.1	16.0	11.3	11.2
July	20.3	21.7	21.5	8.8	16.6	10.1
August	21.0	19.6	20	11.2	22.2	10.7
September	15.6	18.2	15.7	6.0	5.4	7.6

The monthly average temperature and precipitation data from 1985 to 2015 were recorded in the Santiago, Minnesota weather station (approximately 19 km from the research site in Becker) and retrieved from the Minnesota Department of Natural Resources website (http://www.dnr.state.mn.us/climate/historical/acis_stn_meta.html)

Table 3-3. Analysis of variance for total yield, tuber quality, reducing sugars and asparagine concentrations during the growing season in 2014 and 2015

2014 Source	Degree of Freedom	Total Yield	Specific Gravity	Reducing Sugars		Asparagine	
				Stem End	Bud End	Stem End	Bud End
Cultivar (c)	2	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
N Rate (N)	4	0.0899	<.0001	0.1692	0.6689	<.0001	<.0001
Sampling Time (S)	5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
C * N	8	0.9864	0.6135	0.8851	0.0507	0.6757	0.6334
C * S	10	<.0001	0.0034	<.0001	<.0001	0.1033	0.0016
N * S	20	<.0001	0.0947	0.7922	0.8788	0.0888	0.5791
C * N * S	40	0.5198	0.7907	0.4805	0.8559	0.0626	0.9999

2015 Source	Degree of Freedom	Total Yield	Specific Gravity	Reducing Sugars		Asparagine	
				Stem End	Bud End	Stem End	Bud End
Cultivar (C)	2	0.0034	<.0001	<.0001	<.0001	<.0001	<.0001
N Rate (N)	4	0.4768	<.0001	0.9174	0.644	<.0001	0.0014
Sampling Time (S)	5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
C * N	8	0.8386	0.5459	0.6825	0.1199	0.1736	0.1642
C * S	10	<.0001	<.0001	<.0001	<.0001	0.8539	0.0018
N * S	20	0.0073	0.0004	0.7501	0.4262	0.9695	0.2662
C * N * S	40	0.4921	0.0675	0.6343	<.0001	0.9939	0.3977

NS = non-significant; * = significant at 0.05; ** = significant at 0.01

Table 3-4. Analysis of variance for percentage of tubers greater than 170 g, hollow heart incidence and tuber N at harvest in 2014 and 2015

At Harvest 2014 and 2015 Source	Degree of Freedom	Tubers Greater than 170 g (%)	Hollow Heart Tubers (%)	Tuber Nitrogen (%)
Cultivar (C)	2	<.0001	<.0001	<.0001
N Rate (N)	4	<.0001	0.2675	<.0001
Year (Y)	1	<.0001	<.0001	<.0001
C * N	8	<.0001	0.7092	0.0209
C * Y	2	0.7508	0.0030	0.0921
N * Y	4	0.0486	0.2349	0.4967
C * N * Y	8	0.4449	0.8797	0.1774

NS = non-significant; * = significant at 0.05; ** = significant at 0.01

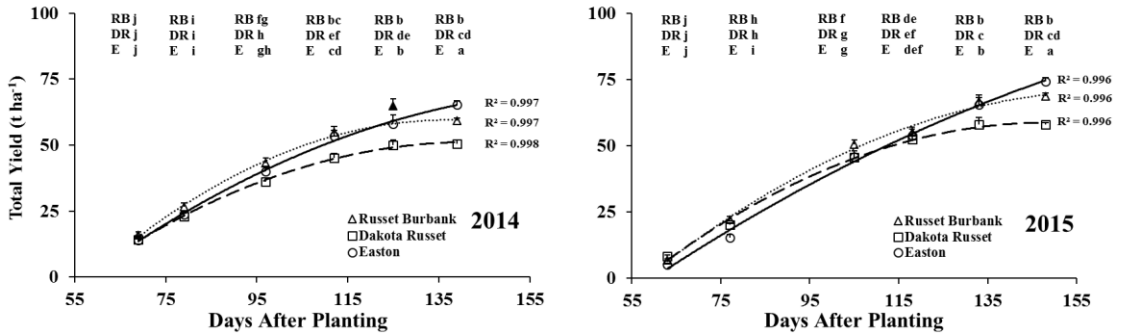

Figure 3-1. Total yield during growing season in 2014 and 2015 (Means were separated at the 5 % level, with the same letter following the cultivar indicating no significant difference.)

Equations

RB (Russet Burbank)

2014: $y = -0.95\times10^{-2}\ x^2 + 2.61\ x - 119.61$, 2015: $y = -0.63\times10^{-2}\ x^2 + 2.08\ x - 99.25$

DR (Dakota Russet)

2014: $y = -0.70\times10^{-2}\ x^2 + 1.99\ x - 90.36$, 2015: $y = -0.71\times10^{-2}\ x^2 + 2.10\ x - 97.36$

E (Easton)

2014: $y = -0.54\times10^{-2}\ x^2 + 1.87\ x - 89.46$, 2015: $y = -0.30\times10^{-2}\ x^2 + 1.46\ x - 76.66$

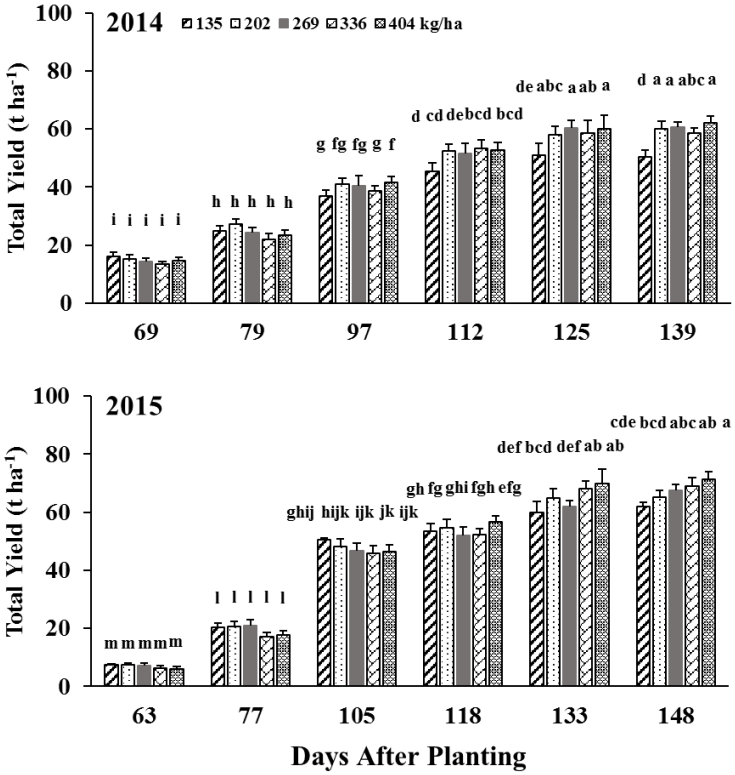

Figure 3-2. The N rate effect on tuber yield of three French fry cultivars during the growing season in 2014 and 2015 (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

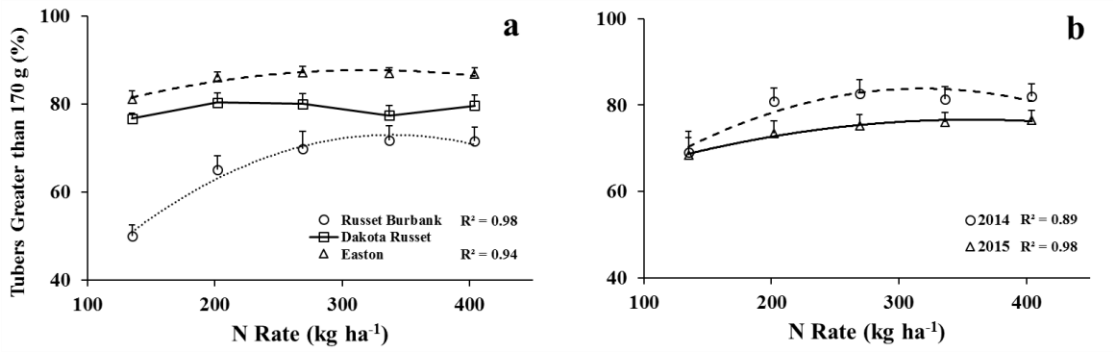

Figure 3-3. The interactions of cultivar by N rate (a), and year by N rate (b) effects on the percentage of tubers greater than 170 g in 2014 and 2015

Equations

3a. Russet Burbank: $y = -0.53\times10^{-3}\ x^2 + 0.36\ x + 12.24$, Easton: $y = -0.18\times10^{-3}\ x^2 + 0.12\ x + 69.00$

3b. 2014: $y = -0.40\times10^{-3}\ x^2 + 0.25\ x + 43.26$, 2015: $y = -0.16\times10^{-3}\ x^2 + 0.11\ x + 56.25$

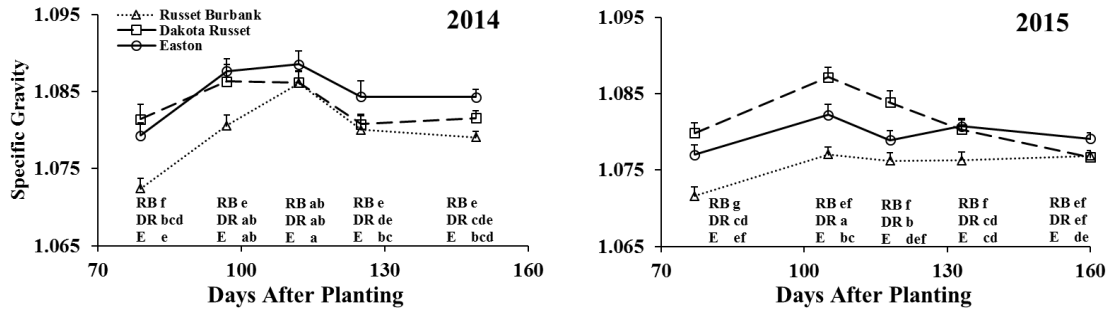

Figure 3-4. The specific gravity of three French fry cultivars during the growing season in 2014 and 2015 (Means were separated at the 5 % level, with the same letter following the cultivar indicating no significant difference.)

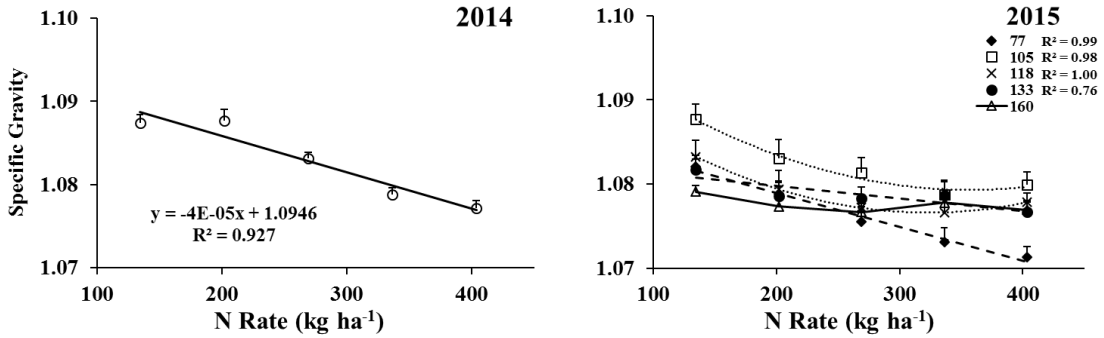

Figure 3-5. The N rate effect on specific gravity in 2014 and 2015 (interacted with sampling time)
Equations 2015
Linear 77 DAP: $y = -0.40\times10^{-4}\ x + 1.09$
133 DAP: $y = -0.15\times10^{-4}\ x + 1.08$
Quadratic 105 DAP: $y = -0.17\times10^{-6}\ x^2 + 0.12\times10^{-3}\ x + 1.10$
118 DAP: $y = -0.19\times10^{-6}\ x^2 + 0.12\times10^{-3}\ x + 1.10$

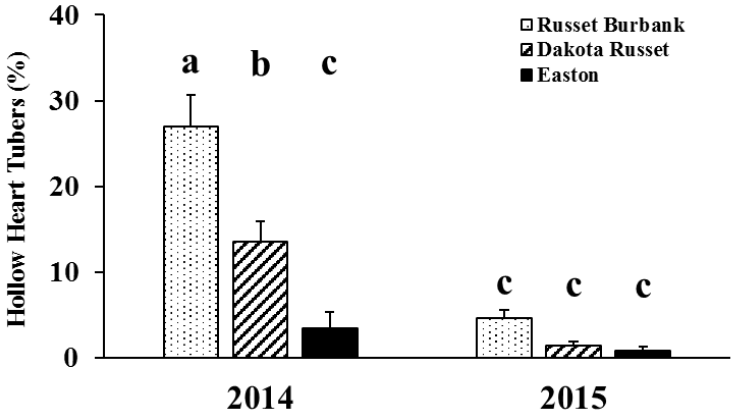

Figure 3-6. The interaction of year by cultivar effect on hollow heart at harvest in 2014 and 2015 (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

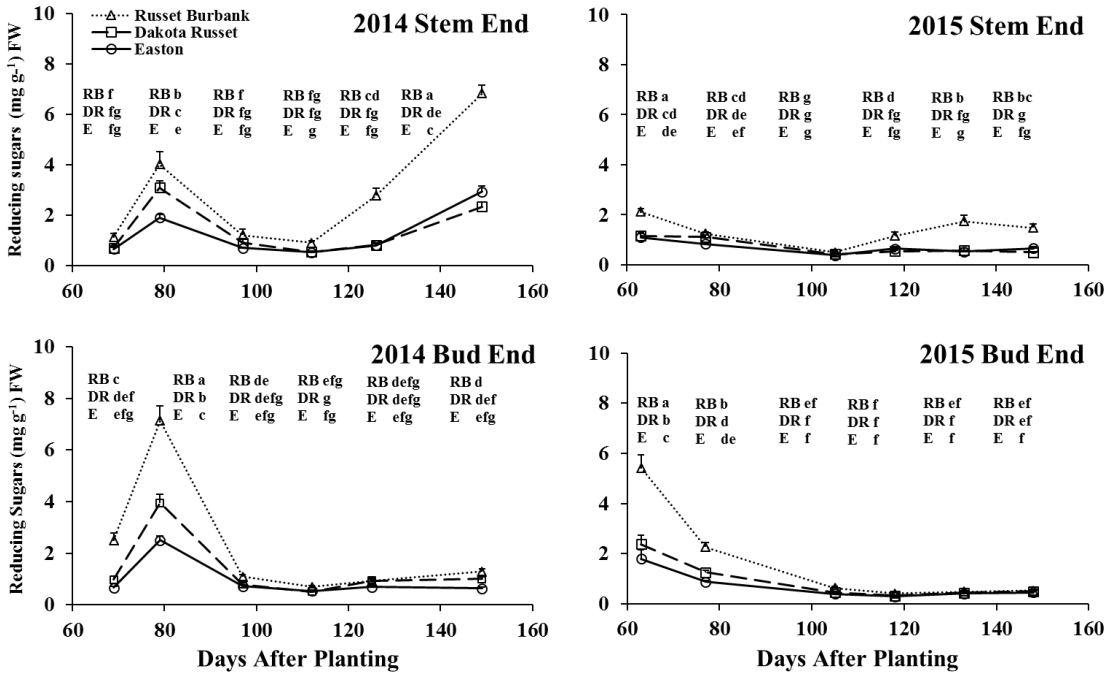

Figure 3-7. Reducing sugars concentrations at the stem and bud end during the 2014 and 2015 growing seasons (Means were separated at the 5 % level, with the same letter following the cultivar label indicating no significant difference.)

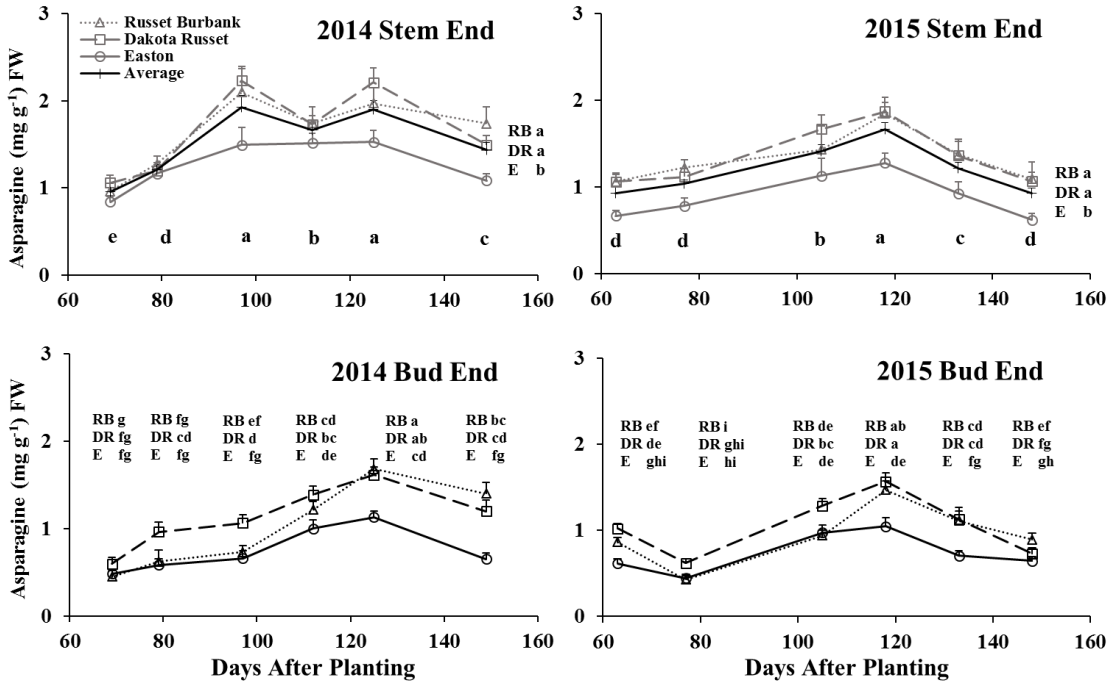

Figure 3-8. The asparagine concentrations in the stem and bud end during the 2014 and 2015 growing seasons. Asparagine concentrations in the stem end were compared to the average of all three cultivars by cultivar or sampling time (Means were separated at the 5 % level, with the same letter following the cultivar label indicating no significant difference.)

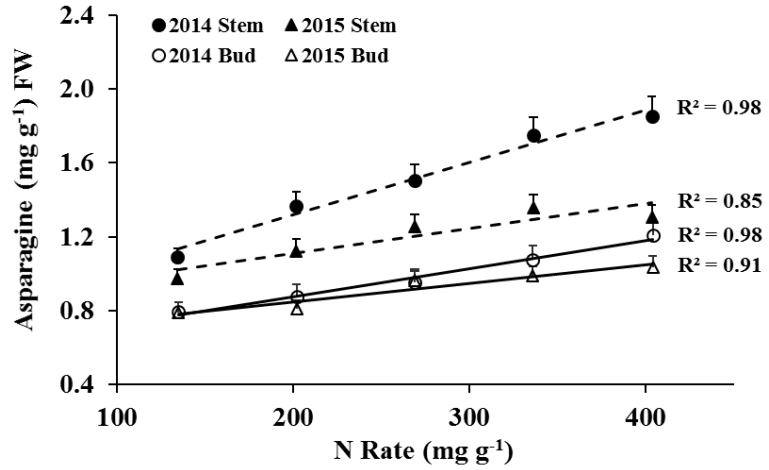

Figure 3-9. The N rate effect on asparagine concentration in the stem and bud end in 2014 and 2015
Equations:
2014 Stem: $y = 2.83\times10^{-3} x + 0.75$, 2015 Stem: $y = 1.35\times10^{-3} x + 0.84$
2014 Bud: $y = 1.52\times10^{-3} x + 0.57$, 2015 Bud: $y = 1.00\times10^{-3} x + 0.65$

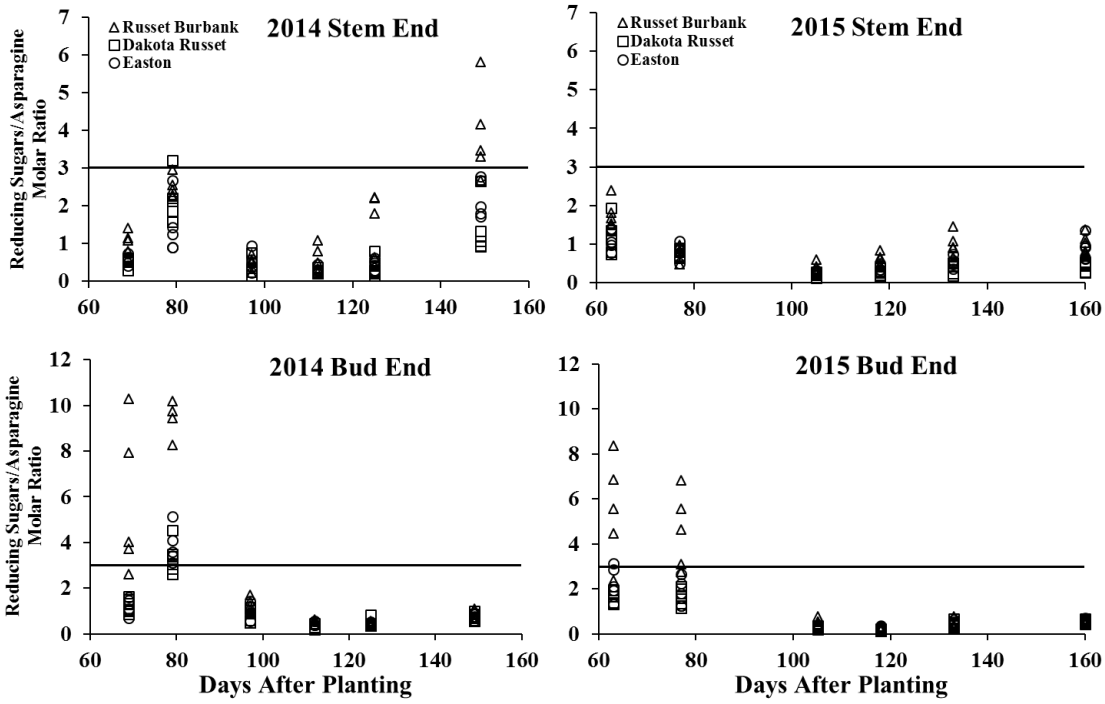

Figure 3-10. The molar ratio of reducing sugars/asparagine at the stem and bud end during the 2014 and 2015 growing seasons (straight line y = 3, data points greater than 3 indicate that asparagine is the limiting factor in acrylamide formation, and data points less than 3 suggest that reducing sugar is more essential.)

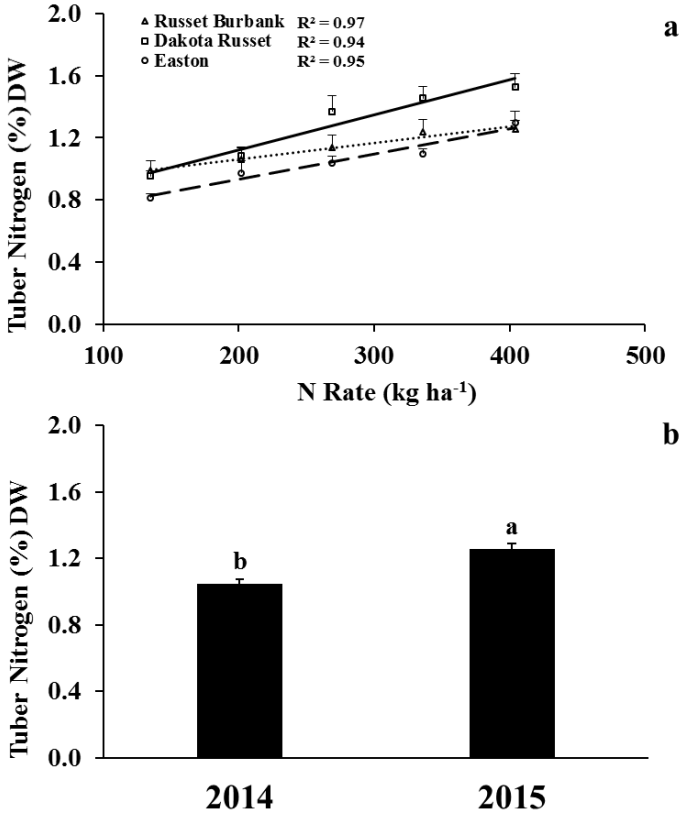

Figure 3-11. The cultivar by N rate effect (a), and main effect of year (b) on tuber N concentration at harvest in 2014 and 2015 (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)
Equations:
Russet Burbank: $y = 1.10\times10^{-3} x + 0.85$
Dakota Russet: $y = 2.30\times10^{-3} x + 0.67$
Easton: $y = 1.60\times10^{-3} x + 0.61$

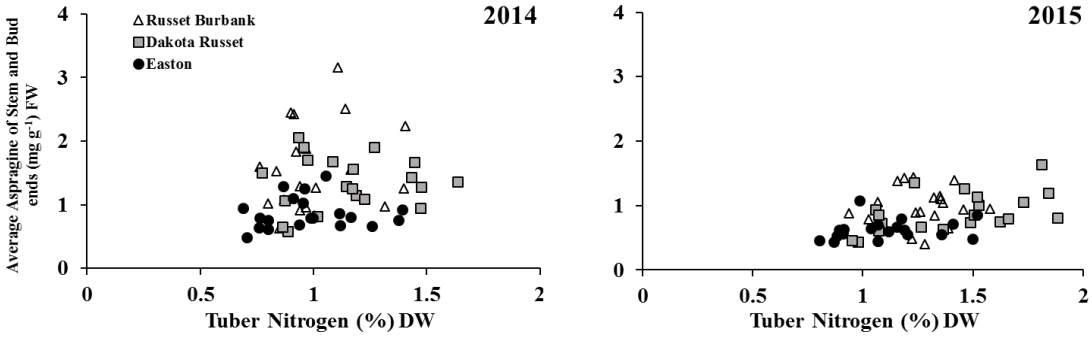

Figure 3-12. Relationship between tuber nitrogen and average asparagine concentrations of stem and bud end at harvest in 2014 and 2015

Supplementary Figures

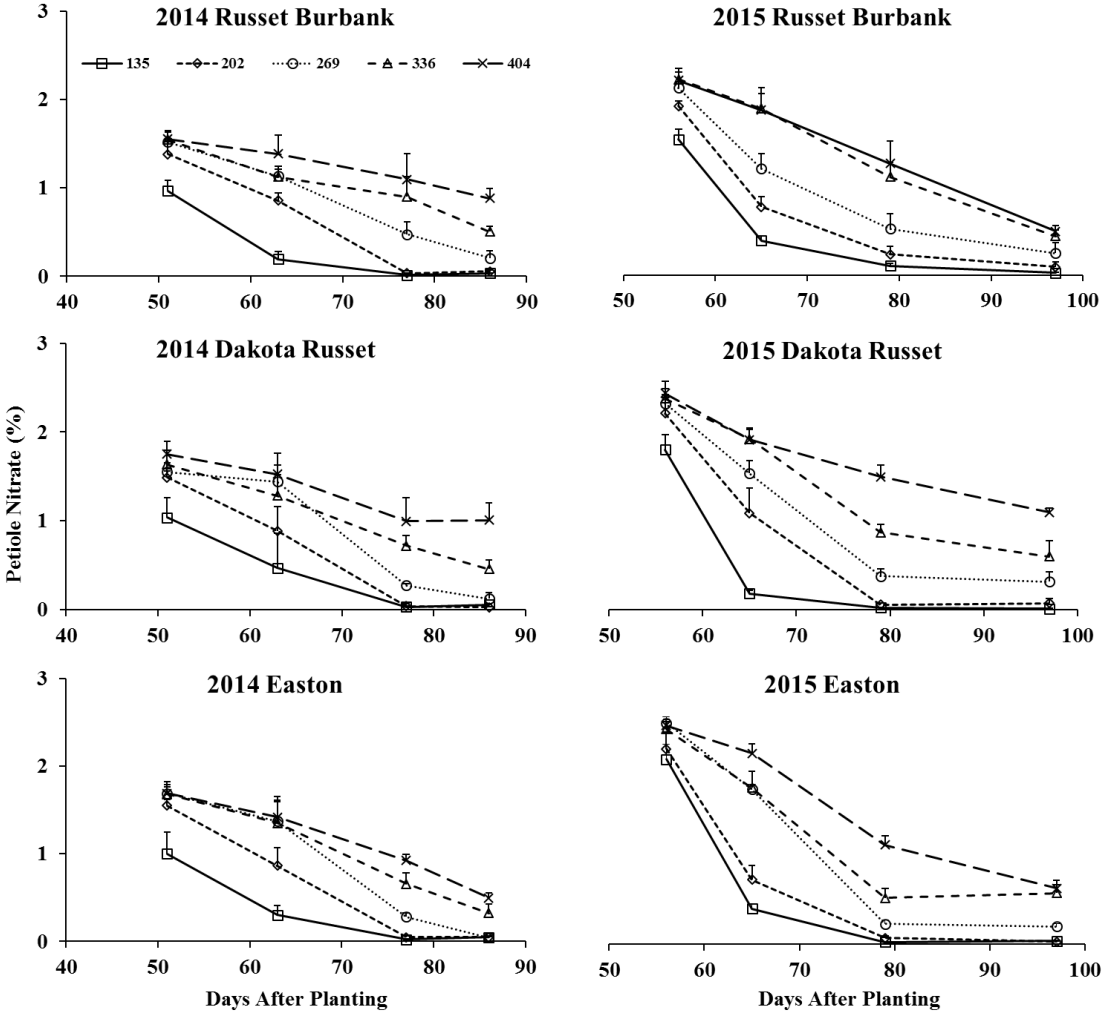

Figure 3-13. Petiole nitrate concentrations of Russet Burbank, Dakota Russet and Easton during the growing season in 2014 and 2015

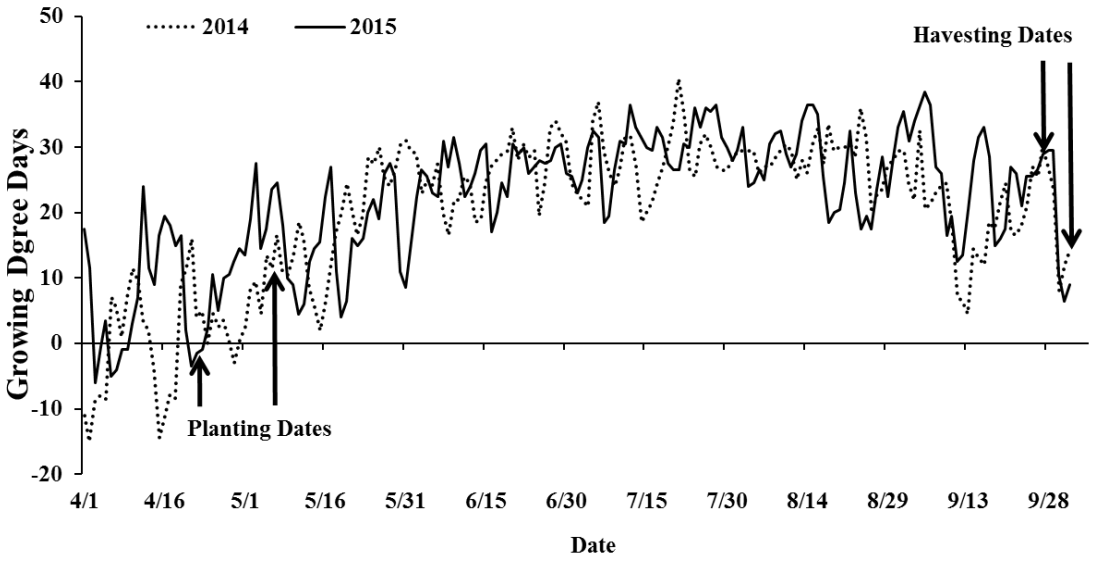

Figure 3-14. Accumulative growing degree days of potato during growing seasons in 2014 and 2015 at Santiago, MN. Growing degree days were calculated as (Maximum Daily Temperature + Minimum Daily Temperature)/2 – Base Temperature for potato plant 40 ^{o}F. Weather data recorded in the Santiago, Minnesota weather station (approximately 19 km from the research site in Becker) and retrieved from the Minnesota Department of Natural Resources website (http://www.dnr.state.mn.us/climate/historical/acis_stn_data_table.html?sid=217502&sname=SANTIAGO%203%20E&sdate=por&edate=por)

Chapter 4 - Changes in tuber glucose concentrations in storage as affected by cultivar and nitrogen rate: implications for acrylamide formation

Overview

Recently released cultivars Dakota Russet and Easton were bred with low acrylamide-forming potential. The objectives of this study were to determine the effects of N fertilizer rate, storage time and year on glucose concentration and the relationships between acrylamide and its precursors for Dakota Russet and Easton, relative to standard cultivar Russet Burbank. The study was conducted on an irrigated Hubbard loamy sand soil at the Sand Plain Research Farm in Becker, Minnesota in 2014 and 2015. All cultivars were subjected to five N rates from 135 to 404 kg ha^{-1} in a randomized complete block design with four replications. Tuber glucose concentration was determined at harvest, and after 16 and 32 weeks of storage at 7.8 ºC. New cultivars Dakota Russet and Easton had significantly lower glucose concentrations at the stem and bud end than Russet Burbank after 0, 16 and 32 weeks of storage both years. The storage time effect on glucose concentrations was significant, but differed by cultivar and year. When the potato crop experienced cold stress before harvest in 2014, higher stem end glucose concentration accumulated and then decreased during storage for all cultivars. However, in a growing season with minimal stress, stem end glucose either increased or did not significantly change after 32 weeks of storage depending on cultivar, while bud end glucose stayed at the same level for all cultivars. Variable results for stem and bud end

glucose concentration responses to N rate were observed. They either increased, decreased or did not significantly change with increasing N rate depending on cultivar and storage time. Acrylamide concentration was determined after 16 weeks of storage, with significantly lower concentration in Dakota Russet and Easton than in Russet Burbank both years. Acrylamide concentrations linearly increased with increasing N rate both years. Glucose concentrations were positively correlated with acrylamide concentration with $R^2 = 0.61$. Asparagine concentrations measured in tubers at harvest were also correlated with acrylamide concentration ($R^2 = 0.37$), when the ratio of asparagine/glucose was less than 1.06. Correlations between fry color and stem end glucose concentration were significant over three cultivars both years, but stronger in a growing season with minimal environmental stress.

Introduction

Acrylamide is a neurotoxin and probable carcinogen for humans and was first reported in fried potato products in 2002 (Mottram et al. 2002). Since then, numerous approaches to mitigate acrylamide in fried potato products have been evaluated, such as cultivar, soil nutrition, storage conditions, environmental conditions, genetic modification and processing parameters (Kooman et al. 1996; Amrein et al. 2003; Kumar et al. 2004; Long et al. 2004; Rommens et al. 2008; Ye et al. 2010; Bethke and Bussan 2013; Muttucumaru et al. 2013; Muttucumaru et al. 2015; Paul et al. 2016). As a result, considerable progress has been made in lowering the acrylamide concentration in potato chips with a mean value of 763 µg kg^{-1} in 2002 to 358 µg kg^{-1} in 2011 in 20 European countries (Powers et al. 2013). A significant decrease of acrylamide in potato chips was

also reported in Belgium in the time period 2008-2013 compared with 2002-2007 (Claeys et al. 2016). In the same study, however, acrylamide in French fries and total dietary acrylamide did not significantly change over the two time periods. Despite the success of some mitigation efforts, acrylamide remains a public concern (EFSA 2015).

Nitrogen (N) management is a common practice that can influence acrylamide precursors, reducing sugars and asparagine, and consequently, acrylamide-forming potential. Variable results have been reported for the effects of N rate on acrylamide precursors. For example in one study, inconsistent effects of N rate on reducing sugar concentrations were reported, while in another study N rate had no effect on reducing sugars (Amrein et al. 2003; Muttucumaru et al. 2013). Glucose concentration responded quadratically to N rate for chip cultivars, with an increase at low N rates and then a decrease at N rates greater than 162 kg ha^{-1} (Chapter 2). Another typical response is a decrease in reducing sugars at the stem end or in the whole tuber with increasing N rate (Westermann et al; 1994; Kumar et al. 2004; De Wilde et al. 2006; Argyropoulos et al. 2016). *SnRK1* is a gene that regulates the metabolism of reducing sugars. A reduced expression of the *SnRK1* gene was accompanied with a decrease in reducing sugars in the cultivars Spunta and Lady Rosetta when soil N supply increased from 300 to 600 mg kg^{-1} (Argyropoulos et al. 2016). In that study, increased amino acid concentrations were observed with increasing N rate, which then contributed to higher acrylamide concentrations even though reducing sugars were lower. A similar conclusion of low reducing sugars under high N supply was previously reported by Kolbe (1990) and Morales et al (2008). These authors found that biosynthesis of amino acids outcompeted

the accumulation of tuber reducing sugar under high N supply, resulting in low reducing sugars concentration and a high asparagine concentration.

Storage duration has a significant effect on glucose concentrations, but is often influenced by cultivar, location, environmental conditions and storage temperature (Knowles et al. 2009; Elmore et al. 2015; Muttucumaru et al. 2017; Chapter 2). A storage temperature of 8-13 ºC is recommended for French fry and chip cultivars, to avoid reducing sugar accumulation from cold-induced sweetening (Rowe and Curwen 1993; Knowles et al. 2009). Muttucumaru et al. (2017) reported the reducing sugars concentrations of 20 cultivars from two locations in the United Kingdom after 2 and 6 months of storage at 8 ºC. In that study, the reducing sugars of cultivar Pentland Dell and Umatilla Russet increased from 2 to 6 months of storage in Doncaster, but decreased in Woburn, suggesting a significant but opposite storage effect at two locations. At a storage temperature of 7.2 ˚C, an increase in glucose concentrations during the 9-month storage for Alpine Russet was reported in one year, but glucose concentrations were not affected by storage time in the following year, indicating a significant impact of growing season environment (Chapter 2).

Compared to the complex effect of storage time on reducing sugars, asparagine concentration is generally considered stable during storage (De Wilde et al. 2005; Ohara-Takada et al. 2005; Pavek and Knowles 2016). Olsson et al. (2004) investigated the asparagine concentrations of eight potato clones during long-term storage at 3 and 10 ˚C, and reported a minimal effect of storage conditions (both time and temperature) on asparagine, while genetic and year effects were substantial for some clones. Matsuura-

Endo et al. (2006) also reported little variation of asparagine concentration during the 18 weeks storage at 2 to 18 ˚C.

The relationship among reducing sugars, asparagine and acrylamide is complicated (Halford et al. 2012a). Reducing sugar concentration was considered the limiting factor for acrylamide formation due to its lower concentration than asparagine in potato tubers (De Wilde et al. 2005). A significant correlation between reducing sugar and acrylamide concentrations was reported in previous studies (Amrein et al. 2004; Ohara-Takada et al. 2005; Halford et al. 2012b; Elmore et al. 2015). However, a positive correlation between asparagine and acrylamide concentrations ($r = 0.84$) was also reported in another study (Zhu et al. 2010). Shepherd et al. (2010) stated that both reducing sugars and asparagine should be taken into account to explain most of the variation in acrylamide concentrations, while Amrein et al. (2003) reported acrylamide concentration as a function of (0.5*glucose + fructose)*asparagine, with $R^2 = 0.91$ for 17 potato cultivars. Higher amounts of reducing sugars relative to asparagine in some cultivars were suspected as the reason for the important role of asparagine in acrylamide formation (Muttucumaru et al. 2014). Asparagine was shown to affect acrylamide formation when its concentration was 2.26 times lower than that of reducing sugars for 20 cultivars grown in two locations after 2 and 6 months of storage (Mutturumaru et al. 2017). Matsuura-Endo et al. (2006) reported that asparagine was significantly correlated with acrylamide concentration ($R^2 = 0.68$) when the asparagine concentrations were less than half of the fructose concentration.

The effect of N rate on reducing sugars and asparagine was determined for recently released French fry cultivars Dakota Russet and Easton relative to Russet

Burbank during two growing seasons (Chapter 3). Weather conditions over the two years resulted in significant differences in stem end reducing sugar concentrations at harvest. This study investigates the effects of N rate and growing conditions on glucose concentrations during storage for these cultivars and implications for acrylamide formation in processed tubers.

The objectives of this study were to: (1) determine the effects of N rate and storage time on stem and bud end glucose concentrations of Easton and Dakota Russet cultivars, relative to the standard cultivar Russet Burbank over two contrasting growing seasons; and (2) evaluate the effects of N rate and growing season on acrylamide formation in these cultivars when processed after 16 weeks of storage.

Materials and Methods

This study was conducted at the Sand Plain Research Farm in Becker, Minnesota, on a Hubbard loamy sand soil (sandy, mixed, frigid Entic Hapludolls) in 2014 and 2015. A randomized complete block design was adopted with four replications using a factorial treatment arrangement of N rate and cultivar. Each plot consisted of seven 7.6 m rows with 25 plants in each row. The spacing between rows was 0.9 m and seed tubers were spaced 0.3 m apart within each row. Tubers used for post-harvest detection were planted in row 4 and 5, which had two red potato plants at both ends as markers. Three French fry cultivars, Russet Burbank, Dakota Russet and Easton, were subjected to five N fertilizer treatments, 135, 202, 269, 336 and 404 kg ha^{-1}. For each N treatment, 101 kg ha^{-1} N fertilizer was applied pre-planting, 34 kg N ha^{-1} at planting and the rest, 0, 67, 134, 201

and 269 kg N ha^{-1} at emergence. Soil properties and further cultural practices used in this study can be found in (Chapter 3).

Sample Collection and Analysis

Whole "B" seed (56 to 84 g) of Russet Burbank, and cut "A" seed (56 to 84 g) of Dakota Russet and Easton were hand planted in furrows on 6 May 2014, and 21 April 2015. Tuber harvest dates were scheduled according to weather conditions, on 2 October 2014 and 28 September 2015. After harvest, approximately 23 kg of tubers weighing between 170 to 283 g from each plot were shipped to the USDA-ARS Potato Research Worksite in East Grand Forks, Minnesota, preconditioned at 10 ºC for two weeks and then stored at 7.8 ºC for 32 weeks. Glucose concentrations in both stem and bud end were determined after 0, 16 and 32 weeks of storage at the worksite by an YSI-2700 Select Biochemistry Analyzer (Yellow Springs Instrument Co. Inc. Yellow Springs, Ohio). A stem end sample (50 g) was collected from the 3.8 cm of tuber tissues surrounding the stem scar from each tuber. A bud end sample (50 g) was collected from the remainder of the tuber. Before use, the juicerator was started and rinsed with three aliquots of 20 ml refrigerated 50 mM phosphate buffer (pH 7.2). The juicerator was then turned off and allowed to spin down for one minute. The stem and bud end samples were ground separately, and brought up to a final volume of 100 ml with 50 mM phosphate buffer in a beaker. Samples were stored in a refrigerator (4 ºC) for 20 to 30 minutes and gently stirred without disturbing the precipitate (including starch) in the bottom of the beaker. For each sample, 15 ml of juice was transferred into a labeled scintillation vial and frozen

for later analysis. The glucose concentration of the juice samples was determined by a YSI-2700 Select Biochemistry Analyzer.

Tuber samples were collected for asparagine analysis after vine kill both years. Fresh tuber tissue was obtained about 0.5 cm away from the stem and bud end using a 7.8 mm Humboldt Brass Cork borer. Tissue samples were stored in Wheaton ™ 20 ml HDPE Liquid Scintillation vials at -20 ºC. The extraction of asparagine was modified from a previous study (Knowles et al. 2008). An IKA Ultra-Turrax disperser (T-25 digital disperser, 18 mm) was used to grind 2.5 g samples for 1 min with 6 ml of triethanolamine HCl (TEA) buffer (30 mM, pH 7, with 1mM 1, 4-Dithiothreitol), 300 ul of 85 mM Carrez I solution, 300 ul of 250 mM Carrez II solution and 500 ul 0.1 mM NaOH until there were no visible chunks of tubers. The solution was transferred to a Falcon™ 15 ml centrifuge tube, vortexed 30 seconds for better mixing and then centrifuged for 15 min at 1200 g. Two ml of supernatant was transferred to Eppendorf tubers and centrifuged (10000 g) for 10 min. Then 1.5 ml supernatant was transferred into a 2 ml Eppendorf ™ snap-cap microcentrifuge safe-lock tube and stored at -20 ºC for the determination of reducing sugars and asparagine. All these steps were conducted on ice to reduce the enzymic browning resulting from polyphenol oxidization. Asparagine quantification was determined by LC-MS/MS. For this method, deproteinization of 200 µl supernatant (described above) was performed by incubating the sample at 94 ºC for 3 min in an Eppendorf ™ 96-well PCR plate. Proteins were removed by centrifugation at 2000 g for 10 min. The supernatant was transferred to Eppendorf ™ tubes, and diluted 100 fold with the solution of two pairing ions, 0.1 % heptaflourobutyric acid (HFBA) and 0.1 % formic acid. The diluted solution was then transferred into the Agilent autosamplers and kept at

4 ºC. Ten µl diluted samples were subjected to LC-MS/MS using an Agilent Eclipse Plus C18 RP column on a Shimadzu UFLC XR coupled to an AB SCIEX – Triple Quad 5500. The sample was subjected to a linear gradient of 0 to 100 percent acetonitrile for 5 minutes at a column flow rate of 0.4 ml/minute. The retention time for asparagine was 1 min. The source conditions were optimized for asparagine analysis. The specific selective reaction monitoring (SRM) transitions employed for asparagine were m/z 116 > 87 transition. The data were analyzed using Multi-Quant (ABI) providing the peak area for the m / z 116, 88 and 87 transition. The amount of asparagine was determined by comparison of the sample peak area to a standard curve. The amount is expressed as ug asparagine g^{-1} potato fresh weight. All analyses were conducted by the Center for Mass Spectrometry and Proteomics at the University of Minnesota.

After 16 weeks of storage, tubers were fried at the worksite in East Grand Forks. Five tubers from each plot were washed, cut (cross section dimension: 22 * 6.5 mm), blanched at 74 ºC for 6 minutes, and then fried in canola oil at 185 ºC for 2.75 minutes. After freezing at -26 ºC, all fries were refried for 1 minute at 191 ºC. Fry color was determined at East Grand Forks worksite with a Photovolt Reflectance Meter about 3 minutes after refrying (Photovolt Instruments Inc., Minneapolis, MN). The fried samples were then shipped to the University of Minnesota for acrylamide extraction using the following procedure. For each plot, three fries or 1.0 to 2.0 g of chips were ground for 30 seconds in a coffee grinder, and 0.8 to 1.0 g (for fries) or 0.20 to 0.25 g (for chips) of ground sample were placed in 15 ml Falcon ™ Conical Centrifuge tubes with 10 parts distilled and deionized water, then vortexed for 30 seconds. After one hour of resting, the resulting suspension was centrifuged and the aqueous fraction was pipetted away from

the fatty and solid fractions into 1.5 ml Eppendorf ™ Snap-Cap Microcentrifuge Safe-Lock ™ tubers. The centrifugation-isolation step was repeated twice, after which 1 ml of purified aqueous solution was pipetted into a 1.5 ml centrifuge tube for each sample, with addition of 100 pg of heavy acrylamide (Cambridge Isotope Laboratories, INC Andover MA; Acrylamide, 2, 3, 3-D3, 98%). Samples were then subjected to solid phase extraction with a Phenomenex Strata ™-X-C 33 µm Polymeric Strong Cation column. To determine acrylamide concentrations: 20 µl of extracted samples were subjected to LC-MS/MS (SCIEX) using an Agilent autosampler with an analytical Thermo Hypercarb (100 L×1.0 mm I.D. Columns, 5 µm particle size) column connected to the Applied Biosystem 4000 ion trap fitted with a turbo V electrospray source. The samples were subjected to a linear gradient of 0 to 100 percent acetonitrile for 15 minutes at a column flow rate of 150 µl minute^{-1}. Transitions monitored were m/z 72 greater than m/z 44 and m/z 72 greater than 55 for the light acrylamide and the m/z 75 greater than 44 m/z and m/z 75 greater than m/z 58 for the heavy acrylamide. The data were analyzed using MultiQuant (ABI) providing the peak area ratio for the m/z 58// m/z 55 transition. Standard curves for quantification were constructed using 100 pg heavy acrylamide/ml with light acrylamide ranging from 5 pg-1500 pg ml^{-1}. The amount of acrylamide was determined and expressed as ng acrylamide g^{-1} (ppb) on a fresh weight basis. All analysis was conducted at the Center for Mass Spectrometry and Proteomics at the University of Minnesota. Because of different frying methods, acrylamide concentrations were generally much higher in those tubers prepared for chips than for French fries.

Statistical Analysis

Analysis of variance (ANOVA) for glucose in stem and bud end as a function of N rate, cultivar, storage time and year was conducted using PROC ANOVA with repeated measures for storage time in SAS 9.4 statistical software package (SAS Institute Inc., Cary NC. USA). A square root transformation was used when necessary to account for the heterogeneity of variance. Acrylamide concentrations in fried potatoes at 16 weeks of storage were analyzed using PROC ANOVA as a function of N rate, cultivar and year. Means of interest was compared using the least significant difference (LSD) at the 5 % probability level. PROC GLM and CONTRAST statements were used to determine linear or quadratic effects of N rate on glucose concentration in stem and bud end at 0, 16 and 32 weeks of storage and acrylamide at 16 weeks of storage. Acrylamide and glucose or asparagine concentrations, acrylamide concentrations and the ratio of asparagine/glucose, fry color with glucose or acrylamide were plotted with linear regressions determined in Excel (Microsoft). A p value < 0.05 for the linear models was considered significant. The relationship between acrylamide and the molar ratio of asparagine/glucose was treated as a nonlinear model (Mutturumaru et al. 2017): acrylamide = (a + b * Asn/Glu) * (Asn/Glu < c) + (a + b * c +d * Asn/Glu) * (Asn/Glu > c). PROC NLIN was used to determine the constant c.

Results and Discussion

Glucose Concentrations

Stem end glucose concentrations in 2014 and 2015 were significantly influenced by the interaction of cultivar by storage time by year (Table 4-1). A decrease in glucose

concentration at stem end was observed for Russet Burbank, Dakota Russet and Easton during the 32-week storage in 2014 (Figure 4-1). For Easton, glucose concentrations decreased step wise over the 32 weeks storage, while a significant decrease occurred only after 32 weeks of storage for Russet Burbank and Dakota Russet. In 2015, changes in stem end glucose with storage time depended on cultivar (Figure 4-1). Glucose concentrations were not affected by storage for Russet Burbank and Easton, while they significantly increased after 16 weeks for Dakota Russet. Stem end glucose concentrations of Dakota Russet and Easton were significantly lower than for Russet Burbank during the 32-week storage both years.

The effect of N rate on stem end glucose concentrations depended on storage time and cultivar (Table 4-1 and Figure 4-2). Glucose concentrations in Russet Burbank tended to decrease with increasing N rate during the entire storage, but this decrease with N rate was only significant at 32 weeks. Glucose concentrations in the new cultivars only responded to increasing N rate at harvest. They decreased quadratically for Dakota Russet and increased linearly for Easton. Overall, the effect of N rate on stem end glucose of new cultivars was not as evident as for Russet Burbank.

Main effects of storage time, cultivar and N rate were significant for bud end glucose concentrations, but differed by year. Glucose concentrations decreased after 16 weeks of storage and then leveled off in 2014, while they were not affected by storage time in 2015 (Figure 4-3a). Dakota Russet and Easton had significantly lower concentrations of bud end glucose than Russet Burbank both years (Figure 4-3b). Bud end glucose concentrations of Dakota Russet were the same as Easton in 2014, but

significantly higher than Easton in 2015. Bud end glucose was not affected by N supply in 2014, but linearly decreased with increasing N rate in 2015 (Figure 4-3c).

In 2014, daily minimum temperature ranged from -1.1 to 6.7 ºC at 127 to 135 days after planting 2014 (one week before vine kill, Chapter 3). During this time, the cold-sweetening process was initiated and a high amount of reducing sugars accumulated, especially for the susceptible cultivar Russet Burbank. However, in 2015, no cold stress occurred during the month before tuber harvest (daily minimum temperature ranging from 3.3 to 20.6 ºC, with only one day below 4 ºC). Resulting glucose in the stem and bud end of all three cultivars either increased or stayed the same in storage. Even though all tubers were preconditioned at 10 ºC for two weeks after harvest, higher glucose concentrations were still observed in the stem end of Russet Burbank in 2014 (4.54 mg g^{-1}) than in 2015 (2.79 mg g^{-1}). Dakota Russet and Easton also had elevated stem end glucose at harvest in 2014 relative to 2015 (20 % higher for Dakota Russet and 9 % higher for Easton), but the difference was not as large as in Russet Burbank (63 %), suggesting a cold resistant characteristic of the new cultivars. Glucose concentrations decreased in the stem and bud end of all three cultivars during storage in 2014, which may be due to the proper storage temperature (7.8 ºC) for tuber reconditioning used in this study. These results are similar to those reported by Silva and Simon (2005). They found that glucose concentrations of seven potato genotypes increased from 0.32 to 2.64 % dry weight after they were stored at 2 ºC for 3 months. Then after the tubers were reconditioned at 15 ºC for 2 weeks, the average glucose concentration decreased to 0.58 % dry weight. Knowles et al. (2009) stored Ranger Russet, Umatilla Russet and Russet Burbank at low temperatures (4.5 and 6.7 ºC) for 31 days and induced reducing

sugar accumulation. Then after the storage temperature was increased to 6.7 and 9 ºC, they reported a decrease in reducing sugars over the next 220 days. The results above suggest that sugar accumulation from environmental stress is reversible with proper reconditioning temperatures.

The effect of N rate on glucose accumulation differed in stem and bud end, and often interacted with cultivar, storage time or year. Complex effects of N rate on glucose concentrations have been reported in previous studies (Westermann et al. 1994; Long et al. 2004; Zebarth et al. 2004; Gerendás et al. 2007; Muttucumaru et al. 2013; Knowles et al. 2015). Westermann et al. (1994) reported an increase in reducing sugars at bud end, and a decrease at stem end with increasing N supply from 0 to 336 kg ha^{-1} for Russet Burbank in Utah, which was consistent with the result in this study for the stem end. However, a minimal effect of N rate on reducing sugars was also reported for Russet Burbank (Zebarth et al. 2004), indicating an inconsistent N rate effect. Gerendás et al. (2007) reported an increased reducing sugar concentration with increasing N supply at harvest for cultivar Agria grown in Mitscherlich pots, which is consistent with the results for stem end glucose in Easton in this study. Knowles et al. (2015) reported a decreasing tendency of reducing sugars at stem and bud end with increasing N supply in a delayed harvest for Alpine Russet, while stem and bud end reducing sugars of the cultivar Sage barely responded to N supply at the same time. Gause (2014) reported that concentrations of total reducing sugars in Easton were not significantly affected by N rate, which is consistent with results of the present study for most of the storage dates. The effects of N rate on glucose concentrations in stem and bud end of Dakota Russet have not been previously reported.

Concentrations of Acrylamide and Acrylamide Precursors

The cultivar by year interaction was significant for whole tuber glucose at 16 weeks of storage (Table 4-2 and Figure 4-4). Due to higher stem end glucose concentrations than bud end concentrations in potato tuber, the whole tuber responses are the same as stem end glucose at 16 weeks of storage reported above.

Main effects of cultivar, year and N rate significantly affected whole tuber asparagine concentrations at harvest (Table 4-2, Figure 4-5). Because asparagine concentrations do not change significantly during storage (Olsson et al. 2004), we assumed the asparagine measured at harvest was similar to the asparagine at 16 weeks of storage. Asparagine concentrations in Dakota Russet and Easton were consistently lower than Russet Burbank. Averaged over cultivar and N rate treatment, whole tuber asparagine concentrations were lower in 2015 than in 2014, indicating a significant effect of environmental conditions. Weather conditions in 2015 (warm weather early and late in the growing season) were favorable for tuber bulking. The favorable growing conditions in 2015 relative to 2014 resulted in greater N uptake (data not shown) and greater yield (Chapter 3) and the N taken up was proportionally distributed to other N compounds related to yield rather than asparagine. Alternatively, higher tuber yield may have resulted in a dilution effect for tuber asparagine.

Acrylamide concentrations were affected by the cultivar by year interaction (Table 4-2). Russet Burbank had similar acrylamide concentrations both years (388 ug kg^{-1} in 2014 and 378 ug kg^{-1} in 2015), which were significantly higher than concentrations in the new cultivars Dakota Russet and Easton (Figure 4-4). Dakota

Russet (169 ug kg^{-1}) had a significantly higher concentration of acrylamide than Easton (127 ug kg^{-1}) in 2014, while concentrations of both cultivars were not significantly different in 2015 (209 and 203 ug kg^{-1}, respectively). The low acrylamide concentrations in French fries produced from Dakota Russet and Easton are consistent with reports from the North Dakota and Maine potato breeding programs (North Dakota State University Research Foundation; Porter et al. 2014a).

Averaged over cultivar and year, acrylamide concentrations increased linearly with increasing N rate (Figure 4-5). These results are consistent with those reported for Russet Burbank by Muttucumaru et al (2013) and Easton by Gause (2014). However, in another study, acrylamide concentrations in Russet Burbank were not affected by increasing N rate in one year, and quadratically changed in the following year (Chapter 2). These results suggest acrylamide response to N rate probably depends on growing location, environmental conditions and cultivar. For example, acrylamide concentrations decreased in Saturna and Hermes and increased in Lady Rosetta and Markies with increasing N supply in United Kingdom (Muttucumaru et al. 2013), but did not change with N rate in these four cultivars in Switzerland (Amrein et al. 2003). The effect of N rate on acrylamide concentration for Dakota Russet has not been previously reported.

Relationships between Acrylamide, Glucose and Asparagine

The relationship between glucose and acrylamide for three cultivars over two years was significant ($R^2 = 0.61$, Figure 4-6a), while the relationship between asparagine and acrylamide was not as strong ($R^2 = 0.15$, Figure 4-6b). These results are consistent with reports in previous studies showing a strong correlation between reducing sugars (or

glucose and fructose) and acrylamide with R^2 ranging from 0.73 to 0.95 (Amrein et al. 2004; Ohara-Takada et al. 2005; Muttucumaru et al. 2014; Elmore et al. 2015), and a weak relationship between asparagine and acrylamide with R^2 ranging from 0.03 to 0.27 (Ohara-Takada et al. 2005; Muttucumaru et al. 2014; Elmore et al. 2015).

The relationship between asparagine and acrylamide concentrations can be strong when tubers contain high concentrations of reducing sugars (Matsuura-Endo et al. 2006). In that study, asparagine was highly correlated with acrylamide with $R^2 = 0.68$, when the molar ratio of fructose/asparagine was greater than 2. Amrein et al (2003) reported that glucose is about half as effective as fructose in supporting acrylamide formation. Based on the results of these previous studies, we speculate that asparagine would become the limiting factor in acrylamide formation when tuber glucose concentration is higher than asparagine concentration (molar ratio of 1). The molar ratio of 1.06 derived from the PROC NLIN statement is supportive of this speculation, with R^2 that increased from 0.15 to 0.37 (Figure 4-9 and 4-10). Few studies have investigated the critical ratio of reducing sugars (or glucose and fructose) to asparagine when asparagine is the limiting factor. Mutturumaru et al. (2017) reported a significant correlation between asparagine and acrylamide when concentrations of reducing sugars were 2.257 fold higher than asparagine concentrations. More research results in acrylamide formation are needed to draw a conclusion based on the critical ratio of reducing sugars and asparagine.

Relationships between Fry Color, Glucose and Acrylamide at 16 weeks of storage

Glucose concentrations were significantly correlated with fry color at the stem end with $R^2 = 0.40$ and 0.75 in 2014 and 2015, respectively, while this relationship was

not significant or weak at bud end (R^2 = 0.20 in 2015, Figure 4-11). In general, tubers that contain high glucose concentrations produce dark color French fries at the stem end. Tubers containing the same amount of stem end glucose had lower photovolt readings in 2015 than in 2014. Thus, although this correlation was significant for the stem end both years, the equation in one year cannot be used to predict fry color or glucose concentration in another year. This is consistent with a previous study conducted with fry and chipping cultivars (Chapter 2). Fry color is considered acceptable when more than 80 % of French fries have photovolt readings greater than 23 (Shock et al. 1994; Driskill et al. 2007). Therefore, all tested cultivars produced French fries with acceptable color in this study. Due to the minimal contribution of bud end glucose to fry color, only the relationship between acrylamide and stem end fry color was analyzed. Over three cultivars and two years, acrylamide and stem end fry color was significantly correlated with R^2 = 0.57 (Figure 4-12).

Conclusion

New cultivars Dakota Russet and Easton contained significantly lower glucose concentrations at both tuber ends compared with Russet Burbank. Cold stress occurred during the growing season in 2014, which increased the stem end glucose concentrations at harvest in Russet Burbank. Weather conditions during the growing season appeared to affect stem and bud end glucose concentrations in storage time for all three cultivars. For example, glucose concentrations decreased during storage at both ends for all three cultivars in 2014, but increased or stay the same depending on cultivar in 2015 when no stress occurred.

At 16 weeks of storage, new cultivars had consistently lower glucose, asparagine and acrylamide concentrations than Russet Burbank. Asparagine and acrylamide concentrations increased with increasing N rate. Acrylamide was correlated with glucose, and asparagine when asparagine/glucose molar ratio was lower than 1.06. However, these results might not be valid for other cultivars under different frying parameters and further investigation into the mechanisms involved is needed.

Russet Burbank, Dakota Russet and Easton produced French fries with acceptable fry color under the treatments in this study. Fry color can be used as a straightforward indicator of acrylamide and glucose concentrations in stem end for test cultivars in this study, but the exact relationship between fry color and stem end glucose concentration was found to differ by year.

Table 4-1. The analysis of variance for glucose concentration during 32 weeks of storage in 2014 and 2015

Source of Variance	Degree of Freedom	Glucose during 32 Weeks of Storage	
		Stem End	Bud End
Main Effect			
Cultivar (C)	2	<.0001	<.0001
N Rate (N)	4	0.0718	0.4334
Year (Y)	1	0.1461	0.0018
Storage Time (S)	2	<.0001	0.0765
Interactions			
N * Y	4	0.3955	0.0142
N * S	8	0.3319	0.7266
S * Y	2	<.0001	0.0052
C * Y	2	<.0001	0.0049
C * N	8	0.0033	0.8609
C * S	4	<.0001	0.2078
N * S * Y	8	0.5359	0.2544
C * N * Y	8	0.4159	0.1309
C * N * S	16	0.0562	0.6043
C * S * Y	4	0.0295	0.2783
C * N * S * Y	16	0.4247	0.6849

NS = non-significant; * = significant at 0.05; ** = significant at 0.01

Table 4-2. The analysis of variance for the concentrations of glucose, asparagine and acrylamide in 2014 and 2015

Source	Degree of Freedom	Whole Tuber		
		Glucose (16 weeks)	Asparagine (after vine kill)	Acrylamide (16 weeks)
Main Effect				
Cultivar (C)	2	<.0001	<.0001	<.0001
N Rate (N)	4	0.3042	0.0002	<.0001
Year (Y)	1	0.3065	<.0001	0.0052
Interactions				
C * N	8	0.1617	0.7709	0.4667
C * Y	2	<.0001	0.3661	0.0049
N * Y	4	0.4420	0.4715	0.8615
C * N * Y	8	0.7170	0.9011	0.4493

NS = non-significant; * = significant at 0.05; ** = significant at 0.01

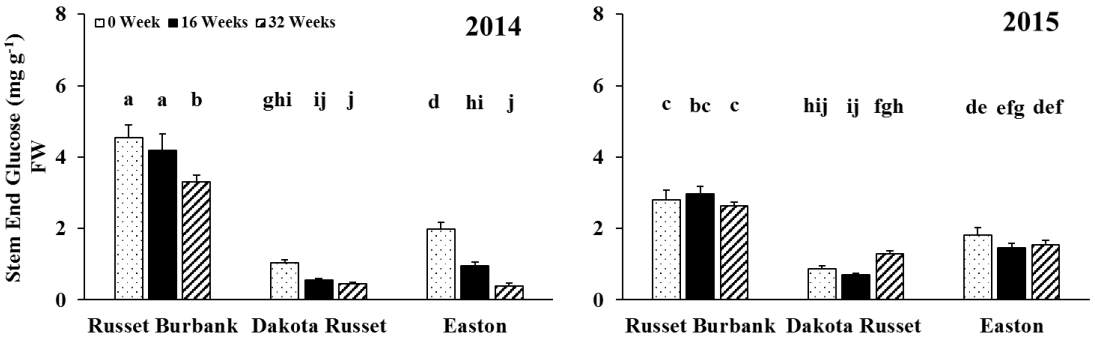

Figure 4-1. Three-way interaction of cultivar by storage time by year effect on stem end glucose concentrations in 2014 and 2015 (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

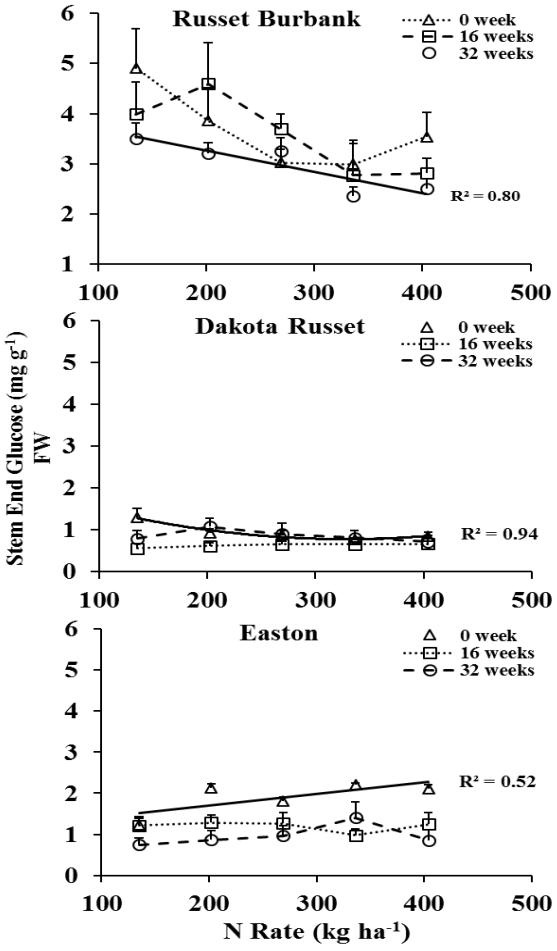

Figure 4-2. Three-way interaction of cultivar by N rate by storage time on stem end glucose in 2014 and 2015

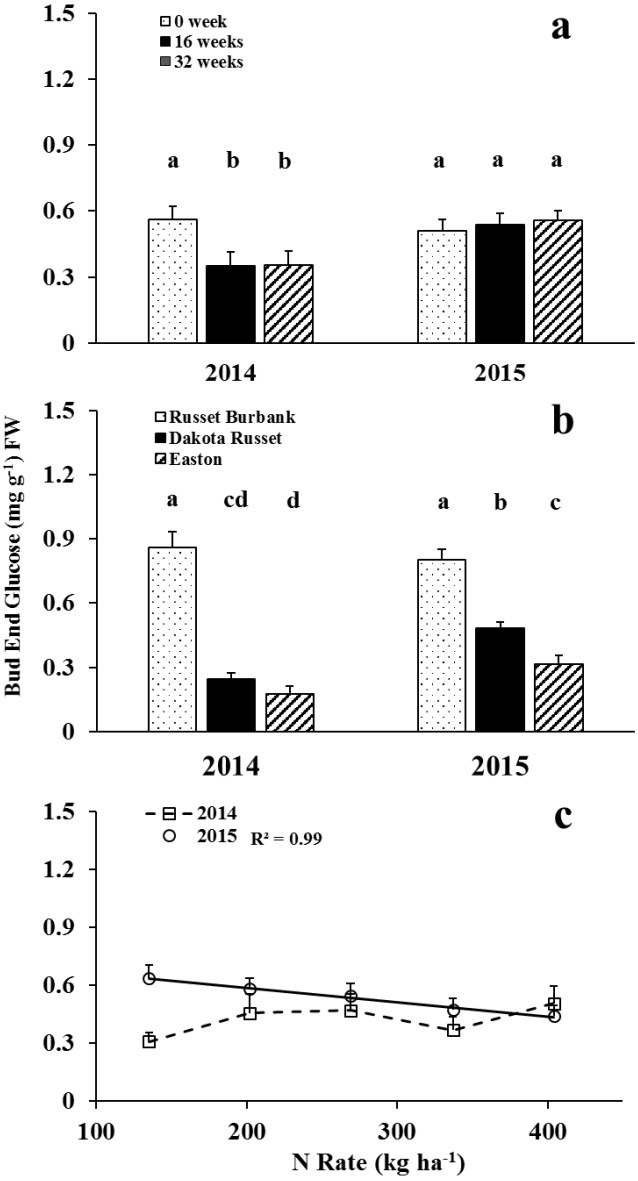

Figure 4-3. Interactions of storage by year, cultivar by year and N rate by year effects on bud end glucose (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

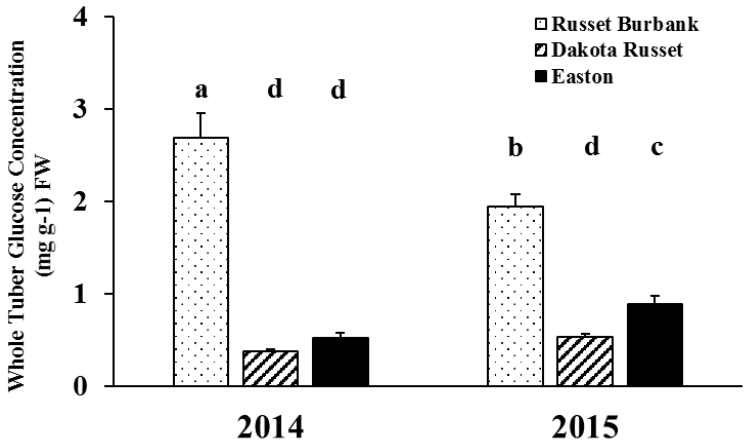

Figure 4-4. Cultivar by year interaction effect on whole tuber glucose concentrations at 16-week storage (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

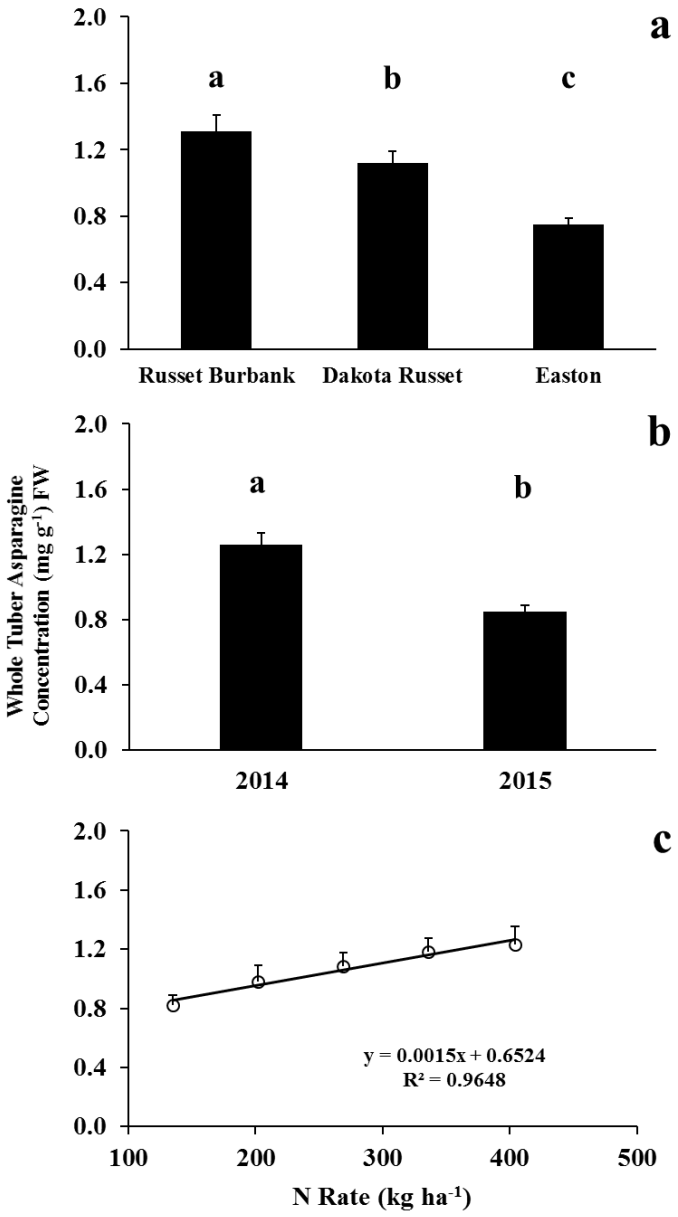

Figure 4-5. Maine effects of cultivar (a), year (b) and N rate (c) on whole tuber asparagine concentrations at 16-week storage (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

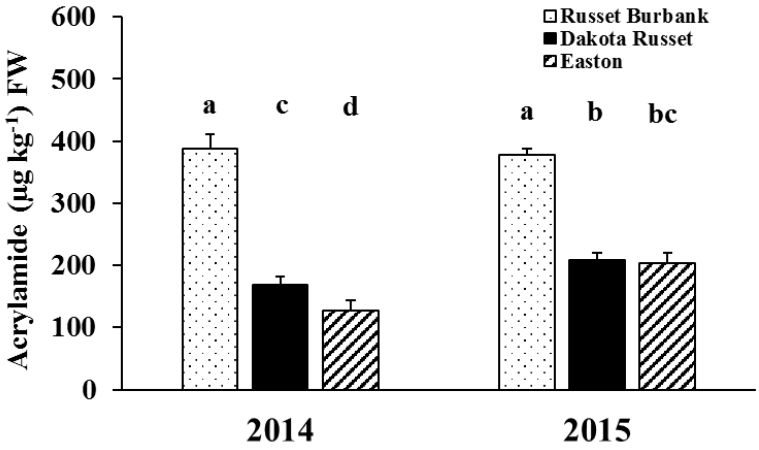

Figure 4-6. Cultivar by year interaction effect on acrylamide concentration at 16-week storage (Means were separated at the 5 % level, with the same letter above the bar indicating no significant difference.)

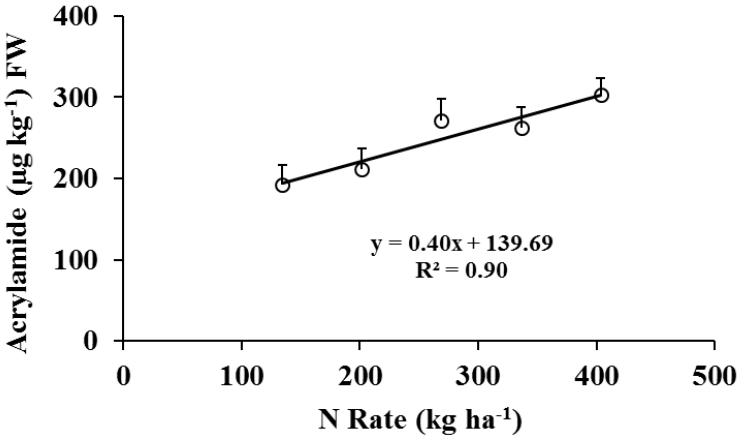

Figure 4-7. N rate effect on acrylamide concentration after 16 weeks storage

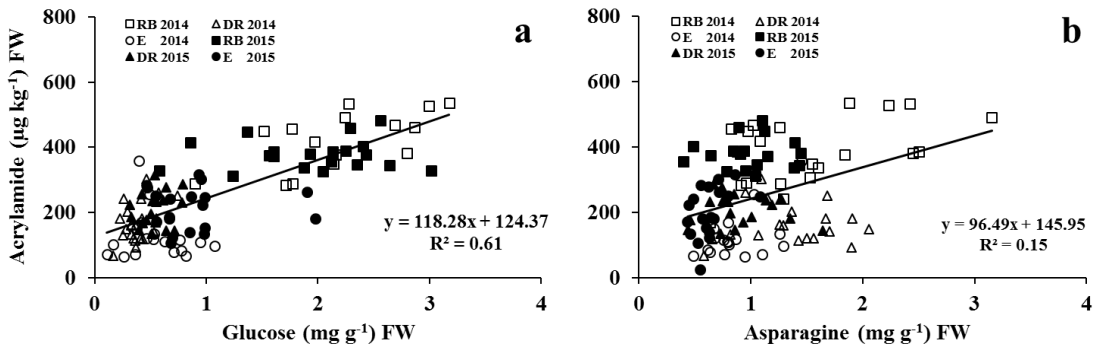

Figure 4-8. Relationships between acrylamide and glucose or asparagine concentrations of Russet Burbank, Dakota Russet and Easton after 16 weeks of storage in 2014 and 2015

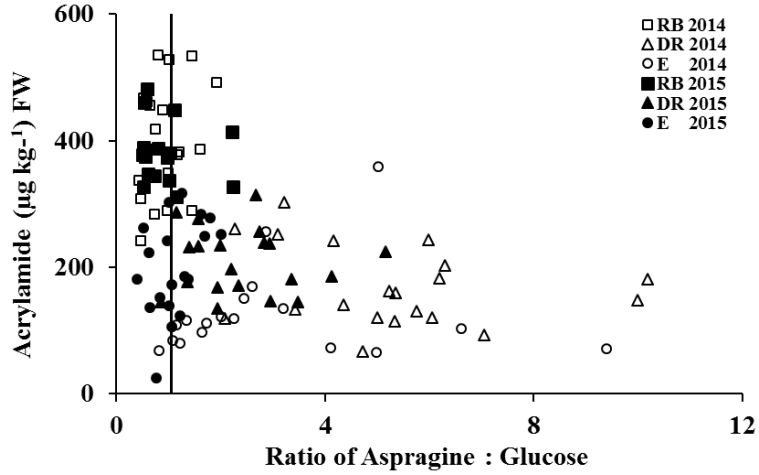

Figure 4-9. Relation of acrylamide and the molar ratio of asparagine to glucose (Solid line: y = 1.06)

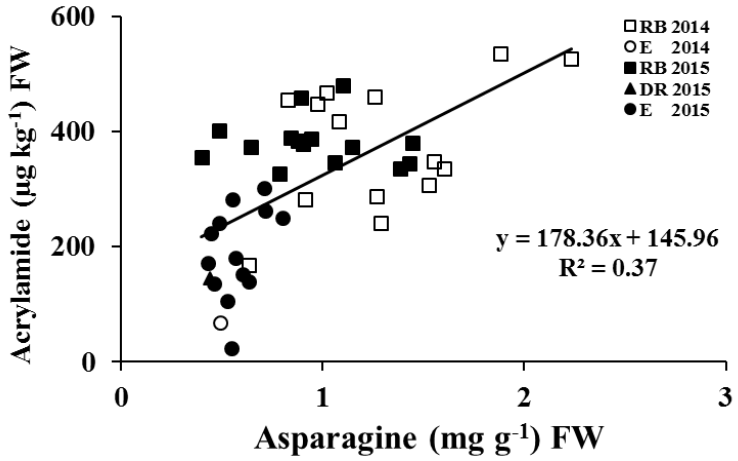

Figure 4-10. Relationship between acrylamide and asparagine: data presented when the asparagine/glucose molar ratio is lower than 1.06

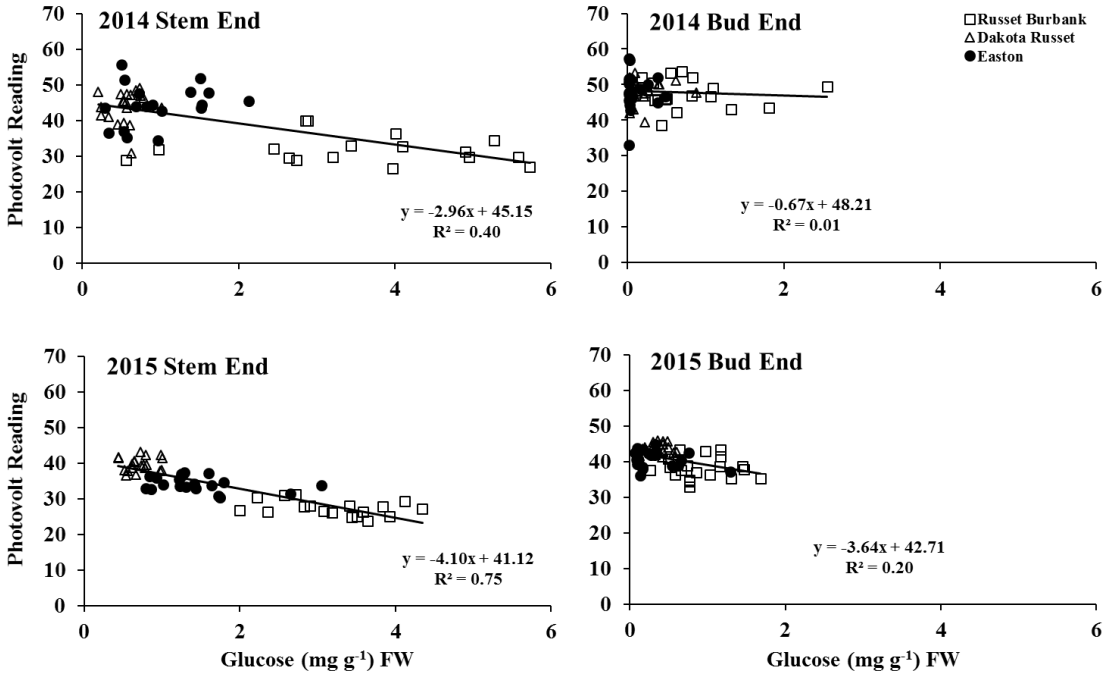

Figure 4-11. Relationships between tuber glucose and fry color at stem and bud end of French fries from tubers stored for 16 weeks at 7.8 ºC in 2014 and 2015

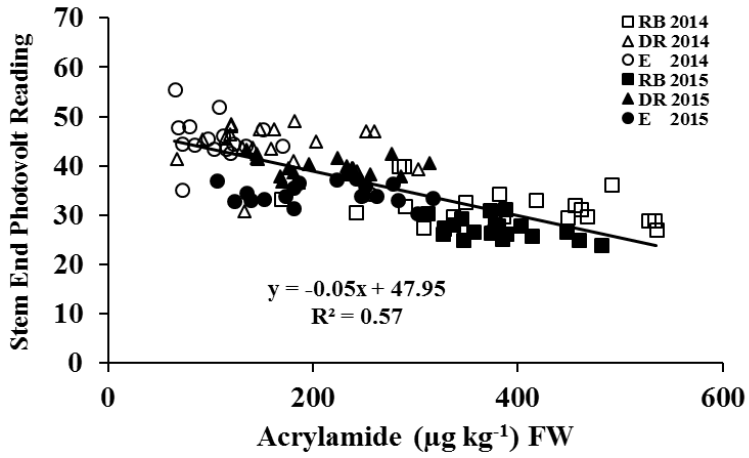

Figure 4-12. Relationship between stem end fry color and acrylamide concentration of French fries from tubers stored for 16 weeks at 7.8 ºC in 2014 and 2015

General Conclusions

Four new French fry cultivars (Alpine Russet, Dakota Trailblazer, Dakota Russet and Easton) and one new chip cultivar (Ivory Crisp) were evaluated for their agronomic characteristics and acrylamide forming potential as affected by nitrogen (N) fertilizer rate and in storage, relative to standard cultivars Russet Burbank and Snowden in multiple years. While cultivar, N rate and storage time had significant effects on tuber yield and acrylamide formation, the responses often depended on environmental conditions experienced during each growing season.

New cultivars generally had comparable or higher levels of desirable agronomic traits compared with the standard cultivars, with the exception of Dakota Trailblazer that had overly high hollow heart incidence and specific gravity, and Dakota Russet that had a lower yield potential than Russet Burbank. In this research, N sufficiency and environmental conditions affected tuber yield for each cultivar; although the cultivar by N rate interaction was never significant. Increasing N input increased tuber yield, but the maximum yield of each cultivar was limited by the environmental conditions. Two of the four growing seasons were warm and long which was favorable for tuber bulking. These conditions created a large tuber sink strength with high N demand, resulting in high yield. In contrast, the other two growing seasons were shorter with cooler temperatures. These conditions created a small tuber sink strength with low N demand, resulting in low yield. Therefore, when possible, N fertilizer should be applied according to environmental conditions to avoid nitrate leaching and optimize economic input. Another important tuber quality characteristic is hollow heart. In this study, hollow heart incidence of Russet

Burbank linearly increased to above 10 % with increasing N supply, but was not affected by N supply in 2012, 2014 and 2015 (lower than 10 % at any N rate). Nitrogen rate had inconsistent effects on specific gravity with more consistent effects associated with cultivar.

For French fry cultivars, reducing sugar concentrations were often higher at the stem end than bud end at harvest, which could easily result in sugar end defect (browning of the stem end during frying). During the growing season, stem end reducing sugar concentrations were not affected by N rate for the cultivars tested (Russet Burbank, Dakota Russet and Easton). Stem end reducing sugar concentrations increased early in the growing season, decreased, and then slightly or dramatically increased depending on the weather conditions before harvest. In two of the growing seasons, a period of cold weather occurred before harvest, which induced the cold-sweetening process and high amounts of reducing sugars at stem end, especially for sensitive cultivars like Russet Burbank. The new cultivars Dakota Russet and Easton had comparable or lower reducing sugar concentrations than Russet Burbank during the entire growing season under contrasting growing conditions. These results indicate that cultivar selection is an effective way to minimize accumulation of reducing sugars.

During storage, reducing sugars were affected by in-season N supply, cultivar, storage time and growing conditions, making it difficult to draw definitive conclusions. For example, glucose concentrations (whole tuber or stem end) decreased with increasing N rate for Russet Burbank, Alpine Russet and Ivory Crisp, but they were not affected or they were minimally affected for Dakota Trailblazer, Dakota Russet and Easton during storage. Although the effect of N rate on glucose (stem end or whole tuber) was cultivar

dependent, this N rate effect within each cultivar was often consistent over years (environmental conditions). For Russet Burbank, Alpine Russet and Ivory Crisp, adjusting N rate could be used to lower the reducing sugar concentration. Based on this research, the effect of N rate should be determined for each specific cultivar to minimize concentrations of reducing sugars and optimize yield.

Tuber glucose concentrations were affected by time in storage but the effects also depended on growing conditions and cultivar. In a year with minimal heat or cold stress, glucose concentrations (whole tuber or stem end) increased or were not affected during storage when stored at a proper temperature (7.2 and 7.8 ºC in this study). When tubers suffered a stressful growing season, proper storage conditions had a reconditioning effect on glucose concentrations, resulting in a decrease or a delayed increase in reducing sugars, depending on cultivar. While stress-induced increases in reducing sugars concentrations were reversible, reversal of senescence sweetening was not possible. The new chip cultivar Ivory Crisp had a comparable level of glucose to Snowden during the first six months storage, but a longer storability due to the senescence sweetening of Snowden.

Nitrogen rate affected acrylamide concentrations in fried tubers, but the effect was not consistent over year or cultivar. High concentrations of acrylamide were formed from tubers grown at low, medium and high N rates, depending on the cultivar and year. Therefore, N rate management is not a viable method to control acrylamide concentrations in fried potato products. Acrylamide concentrations were affected by storage time and environmental conditions during the growing season. In a year with minimal stress, acrylamide concentrations increased during storage. High acrylamide

concentrations were observed at harvest, and then decreased for up to six months during storage (at 7.2 and 7.8 ºC) when tubers experienced heat stress at tuber bulking and maturation. Therefore, storing at a proper temperature provides a second chance to lower the potential for acrylamide formation after harvest. However, the most consistent and straightforward approach is still cultivar selection. Cultivars selected for low concentrations of reducing sugars had low acrylamide concentrations in fried products under stressed and non-stressed conditions encountered in this study.

French fries and chips with high acrylamide concentrations generally have dark fry color. Glucose concentrations predicted fry color determined by Agtron and photovolt readings, but the regression equation and accuracy (R^2 value) differed each year. Compared to asparagine, tuber glucose concentrations correlated better with acrylamide concentrations for both French fry and chip cultivars. However, in a few situations, acrylamide was significantly correlated with asparagine when tubers contained high concentrations of reducing sugars relative to asparagine. The critical value for the molar ratio of asparagine to reducing sugars was 1.06 in this study, which is lower than other ratios reported in the literature.

Suggestions for Future Research

While the results of this study provide information on the effects of agronomic and storage factors on acrylamide precursors and acrylamide formation in fried potatoes, more systematic studies are needed to determine the effects of environmental stresses (e.g. temperature and moisture) on acrylamide formation in a broad range of cultivars. Determining reasons for the differences in molar ratios of asparagine to reducing sugars

reported in the literature also requires further investigation. Finally, continued breeding and cultivar selection for low reducing sugars and asparagine is needed to ensure that acrylamide formation in fried products remains low under a wide range of growing conditions.

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