MANIPULATING THE PRODUCTION EFFICIENCY OF POTATO WITH PLANT GROWTH RETARDANTS AND CHARACTERIZATION OF DEVELOPMENTAL AND POSTHARVEST PHYSIOLOGICAL PHENOTYPES OF ENGINEERED CULTIVARS

By

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A dissertation submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
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DECEMBER 2019

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of GRAHAM DAY ELLIS find it satisfactory and recommend that it be accepted.

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ACKNOWLEDGEMENT

To begin, I would like to thank Dr. Richard Knowles, for whom provided the extraordinary opportunity for me to pursue my Doctor of Philosophy degree in Horticulture. Rick, you have been an exemplary mentor, whose candid guidance has opened my eyes to what it means to be a researcher with integrity, dedication, and to start projects always sooner rather than later. A many many thanks to Dr. Lisa Knowles for her numerous teachings and subtle guidance, and also for her imperturbable patience with me in the laboratory; I’ll strive to carry those traits into the future.

My other committee members, Dr. John K. Fellman and Dr. Duroy A. Navarre, thank you for your advice, I wish we would have had more opportunities at Senior Seminar to discuss science as it was and is today. I also would like to thank everyone else who helped with my research at Washington State University: MA Cody J. Dean, Dr. Derek J. Herman, Rudy Garza in Othello, and my other fellow graduate students and colleagues Paco Gonzalez, MA Alex Cruz, and Colton Thurgood, you all have helped make my research possible, answered my questions, and I will ever remember your camaraderie.

Finally, to my parents for having raised me to never shy away from a challenge, you both primed me for success not just in science, but for all difficult times in life. And to my bride-to-be, Cory, you supported me through trying times, helping keep me focused and smiling more than any other. Thank you to all my friends, and a special thanks to my dear friends Derek and Jess for the support you provided in the form of baked goods, games, and exciting hobbies; you’ve helped me succeed by keeping me sane through this entire process.

Thank you all again, for the opportunity, the guidance, the support, the love…thank you.
Abstract

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Management techniques that reduce agronomic inputs, control tuber size, and prolong the postharvest quality of potato and its products will enhance the economics and sustainability of production. The research had three objectives: investigate low O₂ storage for maintaining quality of fresh-cut (FC) potato, determine whether plant growth retardants can alter crop source/sink relationships to enhance production efficiency, and characterize the developmental and postharvest phenotypes of Innate®-engineered cultivars.

For objective one, we demonstrated that respiratory and cold-sweetening responses of FC and whole tubers are similar. The lower O₂ limit for aerobic metabolism in FC potato was ~2 kPa at 4°C, and lactate and EtOH accumulation were significant at ~1-1.5 kPa. Cold-sweetening and enzymatic browning of invertase-silenced (Innate®) and non-silenced FC tubers were reduced by hypoxia and abolished by anoxia. The results from this study inform development of modified atmosphere packaging for FC potato products.
For the second objective, we demonstrated that the GA biosynthesis inhibitor, paclobutrazol (PBZ), could reduce foliar growth and increase tuber set in ‘Bondi’, which inherently produces excessive canopy growth, low tuber-set, and over-sized tubers. The PBZ-induced changes in allometric relationships increased harvest index without decreasing tuber yield and depended on concentration and application timing. Application timing can be managed to control tuber size while preserving the increased source/sink production efficiency.

The third study demonstrated that developmental phenotypes and yields of the Innate®-engineered cultivars, Acclimate and Hibernate are equivalent to their respective parental cultivars, Ranger Russet and Atlantic. However, foliar growth and tuber-bulking rate of Glaciate was lower than Russet Burbank, resulting in lower yield. Changes in tuber respiration rates during sequential periods of cold-sweetening, reconditioning, and subsequent cold storage was comparable for the Innate® versus conventional cultivars, except for Glaciate where tuber respiration was elevated. Cold storage induced sucrose and reducing sugar buildup in Innate® and conventional tubers, respectively. Consequently, Innate® tubers maintained excellent fry and chip process quality regardless of storage temperature. Reconditioning permanently lowered the sucrose content of Innate® tubers. Heat stress exacerbated cold-induced sugar accumulation equally for all cultivars. The phenotyping trials provide information that will help optimize management practices.
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LIST OF ABBREVIATIONS

ACP, anaerobic compensation point
ANOVA, analysis of variance
Apogee®, Prohexadione-calcium
Asn, asparagine
Atl, Atlantic
AUFGC, area under the foliar growth curve
CS, cold-storage
CV, cultivar
DAP, days after planting
DD, degree days
DMSO, dimethyl sulfoxide
DW, dry weight
EB, enzymatic browning
EtOH, ethanol
FC, fresh-cut
FDA, Food and Drug Administration
Fru, fructose
FW, fresh weight
GA, gibberellin
Glc, glucose
Gln, glutamine
HI, harvest index
HS, heat-stress
LDPE, low density polyethylene
LOL, lower oxygen limit
LSD, least significant difference
LTS, low temperature sweetening
MAP, modified atmosphere packaging
PBZ, paclobutrazol
PHHS, postharvest heat stress
PM, physiological maturity
PPO, polyphenol oxidase
QSR, quick service restaurant
RAR, respiratory acclimation response
RB, Russet Burbank
RCBD, randomized complete block design
RH, relative humidity
RR, Ranger Russet
SFA, Snack Food Association
Suc, sucrose
UTC, untreated control
FOREWORD

This dissertation is a compilation of three independent studies that focus on different aspects of improving the production and postharvest handling of potato (*Solanum tuberosum* L.) and its products.

The first chapter investigates the respiratory and low-temperature sweetening (LTS) responses of fresh-cut potato (FC) to cold storage and low oxygen atmospheres. The findings are important to developing modified atmosphere packaging that can effectively preserve the quality of this highly perishable product as it moves through the supply chain to the consumer. Fresh-cut (FC) fruit and vegetables are peeled, sliced, shredded, or diced before packaging. The market for these products has grown and diversified during the past 25 years due to increased consumer demand for convenient meals (Ahvenainen, 1996; Rico et al., 2007). However, enzymatic browning has been the major impediment to the development and adoption of FC potato products. The recent approval and commercialization of potato cultivars with down-regulated polyphenol oxidase activity and associated enzymatic browning have opened the market for FC potato. Containing the wound-induced respiratory burst and high respiration rate of FC potato is also critical to slowing deterioration of this highly perishable product. While modified atmosphere packaging alone or in combination with cold storage, has been shown to decrease respiration and increase shelf-life of many commodities effectively (Cameron et al., 1995; Rico et al., 2007; Rojas-Graü et al., 2009), comparatively little work has been done with FC potato. Therefore, we characterized the physiological responses of FC potato to decreasing oxygen concentration and established the lower oxygen limit for maintaining aerobic respiration.

The second chapter investigates the efficacy of using gibberellin biosynthesis inhibitors to modulate tuber-set and size-distribution and improve source/sink efficiency of potato cultivars
with particular phenotypes. The cv. Bondi is a high-yielding, late-season cultivar with indeterminate growth habit developed by New Zealand Plant and Food Research for frozen (French fry) processing (Oliveira, 2015). Bondi has a distinctive phenotype when compared with industry-standard, late-season, frozen-processing cultivars such as Russet Burbank and Ranger Russet. The canopy growth of Bondi is excessively vigorous with elongated vines (Herman et al., 2016). Aboveground foliar fresh weight and dry matter are consistently 30-50% greater than Russet Burbank (Oliveira, 2015) and Ranger Russet. Additionally, Bondi inherently sets few tubers, leading to high percentage oversized tubers under late-season growing conditions. In short, the phenotype of Bondi is indicative of the effects of high endogenous GA concentrations on vine and tuber growth, making this cultivar ideal for developing/evaluating methods to modulate source/sink relationships in potato. We demonstrated that the GA biosynthesis inhibitor, paclobutrazol, effectively reduced foliar growth, increased tuber set, and reduced average tuber size, with minimum impact on overall yield. The result was an increase in harvest index and overall production efficiency, which may translate to a reduction in management inputs, increased sustainability, and improved profits for growers.

The last chapter characterizes the developmental and postharvest phenotypes of the Innate® cultivars, Glaciate, Acclimate and Hibernate, in comparison to their parental counterparts, Russet Burbank, Ranger Russet, and Atlantic in the Columbia Basin of Washington. The Innate®-engineered cultivars were developed by the J.R. Simplot Co. (Boise, ID) to have increased resistance to blackspot bruise, low acrylamide formation during frying, Late Blight (*Phytophthora infestans*) resistance, and resistance to LTS. These cultivars were approved for commercial production in 2017. Our studies involved (1) modeling foliar and tuber development over time and in relation to accumulated degree days from planting, (2) comparing the physiological
responses of tubers to changes in storage temperature during low temperature sweetening and reconditioning, and (3) evaluating how heat stress modulates subsequent cold-induced sweetening and process quality relative to the parental cultivars during storage. The growth profiling studies were designed to reveal cultivar-dependent differences in the timing of critical growth-stage ‘windows’ (emergence and plant establishment, tuberization, bulking, foliar senescence, tuber maturation), estimate the attainment of tuber physiological maturity (PM), and identify potential consequences of delayed harvest beyond PM for subsequent retention of process quality for each cultivar. Harvest indices and bulking rates were compared, along with developmental changes in tuber specific gravity, sugars, and free amino acid composition. In addition to PM, differences in the relative concentrations and distributions of Suc and reducing sugars in tubers during bulking and maturation were compared as indicators (Knowles et al., 2015) of propensity to develop physiological disorders, such as sugar or translucent (jelly) ends during storage (Thompson et al., 2008), which adversely affect process and nutritional qualities. We demonstrated that the developmental and yield phenotypes of Acclimate and Hibernate were equivalent to their parental cultivars. However, cv Glaciator exhibited reduced foliar growth and tuber bulking rate, which lead to considerable yield drag compared with Russet Burbank. Cold sweetening entailed the buildup of sucrose in Innate® tubers as opposed to reducing sugars in the parental cultivars, which subsequently lost process quality. Reconditioning was effective for moderating the cold-induced sucrose accumulation in Innate® tubers during long-term storage at 4°C. While heat stress exacerbated the cold-induced buildup of Suc in Innate® tubers and reducing sugars in the parental cultivars, the increases in total sugar (Suc + Glc + Fru) concentrations remained equal, indicating similarly elevated levels of starch catabolism during LTS of heat-stressed tubers. However, by virtue of silenced invertase, Innate® tubers were highly tolerant of temperature stress for retention
of process quality. The trials revealed those unique characteristics of the Innate® cultivars providing information that will be useful in optimizing management practices.
References


CHAPTER ONE

Respiratory and low-temperature sweetening responses of fresh-cut potato

\textit{(Solanum tuberosum L.)} tubers to low oxygen\textsuperscript{1}

Abstract

Enzymatic browning (EB) has impeded the commercialization of fresh-cut (FC) potato. However, recently introduced Innate\textsuperscript{®}-engineered cultivars with silenced polyphenol oxidase (PPO) have overcome this obstacle. As a supplement to refrigeration, low O\textsubscript{2} atmosphere may extend the shelf-life of FC potato by reducing wound-induced respiration, associated dry matter loss, EB, and low-temperature sweetening (LTS). Determining the O\textsubscript{2} concentration that minimizes these deteriorative physiological processes without invoking anaerobic metabolism is prerequisite to designing effective modified atmosphere packaging (MAP). Accordingly, FC tubers of cultivars Russet Burbank (RB), Ranger Russet (RR), and their Innate\textsuperscript{®} counterparts, Cultivate, Generate, and Glaciate, were stored (4\textdegree C) in ten O\textsubscript{2} atmospheres ranging from 0 to 21 kPa to determine the lower oxygen limit (LOL) for aerobic respiration and effects on LTS and EB. FC tissue stored at 21 kPa O\textsubscript{2} displayed a prominent cold-induced respiratory acclimation response (RAR), characterized by an initial decline in respiration rate followed by a steady increase through 48 h. The RAR decreased with O\textsubscript{2} and was extinguished at \( \leq 1.5 \text{ kPa} \). Lowering O\textsubscript{2} from 21-7 kPa had little effect on tissue respiration; however, rates fell from 4.23 to 3.41 \( \mu \text{g kg}^{-1} \text{s}^{-1} \) as O\textsubscript{2} decreased from 7 to 3.5 kPa, followed by a further 79\% reduction to 0.70 \( \mu \text{g kg}^{-1} \text{s}^{-1} \) at 0 kPa O\textsubscript{2}. Tissue lactate profiles revealed the onset of anaerobic metabolism at ca 1.5 kPa O\textsubscript{2} for all cultivars. Importantly, lactate and ethanol accumulation were negligible through 16 d at \( \geq 2 \text{ kPa O}_2 \) (= LOL) but increased considerably at \( \leq 1 \text{ kPa} \). Low O\textsubscript{2} attenuated the cold-induced synthesis of sucrose and reducing sugars in FC tissue from RB, and sucrose from Glaciate tubers in which acid invertase
is silenced. Enzymatic browning of FC RB and RR tubers was inhibited by anoxia, but extensive at ≥0.5 kPa O₂. FC Innate® tubers exhibited only minor EB at all O₂ concentrations. Collectively, these data inform the further development of MAP for FC potato.

Introduction

Minimal processing of fruit and vegetables involves peeling, slicing, shredding, or dicing before packaging. The market for these fresh-cut (FC) products has grown and diversified during the past 25 years due to increased consumer demand for convenient meals (Ahvenainen, 1996; Rico et al., 2007). Advances in the maintenance of cold-storage during distribution, modified atmosphere packaging, and use of inhibitors of enzymatic browning and microbial decay, have enabled this expansion (Watada and Qi, 1999; Artés et al., 2007; Rojas-Graü et al., 2009). These developments extend shelf-life by attenuating the effects of wounding on the subsequent deterioration of FC tissue (Watada et al., 1990; Brecht, 1995; Gunes and Lee, 1997).

Wounding of plant tissues induces a respiratory burst that must be minimized to preserve quality. Cold storage is commonly employed for this purpose (Watada et al., 1996); however, in the case of potato, the respiratory rate of FC French fries remained 5-fold higher than that of intact tubers even at 2 °C (Gunes and Lee, 1997). While modified atmosphere packaging (MAP) in low O₂, alone or in combination with cold storage, effectively decreases respiration and increases shelf-life (Cameron et al., 1995; Rico et al., 2007; Rojas-Graü et al., 2009), comparatively little work has been done with FC potato.

Physiological responses to wounding and the gaseous composition of MAP are commodity specific. Oxygen concentration, if too low, may invoke anaerobiosis, leading to the development of off-flavors and microbial decay (Artés et al., 2007). The concentration of O₂ at which respiratory metabolism switches from aerobic to anaerobic defines the lower oxygen limit (LOL) (Yearsley et al., 1996). Packaging systems that maintain O₂ concentration slightly above the LOL minimize aerobic respiration and preserve quality. Therefore, determination of LOL is a prerequisite to developing MAP for FC fruit and vegetables.
Although helpful in moderating respiration, low O₂ is not effective for eliminating enzymatic browning (EB) in FC products such as apple, pear, and potato (Gunes and Lee, 1997; Beltran et al., 2005; Rojas-Graü et al., 2009). EB results in surface discoloration from the action of polyphenol oxidase (PPO) on phenolic compounds in the presence of O₂ (Artes, 2007). EB reduces marketability, is difficult to control, and has impeded the development of FC potato (Laurila et al., 1998b; Limbo and Piergiovanni, 2006). The FC fruit and vegetable industry has addressed this problem through the use of antioxidant compounds such as ascorbate, citrate, sulfites, acidulants, and other reducing agents (Buta and Moline, 2001; Incelayi et al., 2014). Currently, however, consumers prefer to avoid chemical additives, particularly sulfites due to their allergenic properties (Ahvenainen, 1996; Laurila et al., 1998a), in favor of non-chemical treatments, such as hot water (Tsouvaltzis et al., 2011). Additionally, use of these inhibitors adds cost and in FC potato can potentially increase tissue metabolism (Rocculi et al., 2007) and darkening during cooking (O’beirne and Ballantyne, 1987).

Silencing PPO activity is an effective alternative to using anti-browning chemical additives for the prevention of EB. The Arctic® Golden, Arctic® Granny, and Arctic® Fuji apple cultivars can be FC and packaged without inhibitors of EB as a consequence of this technology (Carter, 2012; Waltz, 2015a). Similarly, the potato cultivars Cultivate, Generate, and Glaciate (J.R. Simplot Company, Boise, ID) can be FC with minimal subsequent EB due to silenced PPO activity (Waltz, 2015b).

Refrigeration remains essential to maintaining shelf-life following cutting of these engineered apple and potato cultivars and is legally required by the FDA to control microbial proliferation (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-guide-minimize-microbial-food-safety-hazards-fresh-cut-fruits-
and-vegetables#ch6, accessed June 11, 2019). However, unlike apple, potatoes are susceptible to low temperature sweetening (LTS) (Isherwood, 1973; Sowokinos, 2001), which can negatively affect processing and nutritional qualities. Storage of whole tubers of LTS-susceptible potato cultivars below ca 9 °C induces starch breakdown, resulting in the accumulation of reducing sugars (glc and fru) from sucrose via vacuolar acid invertase (Sowokinos, 2001; Bethke, 2014). Reducing sugars are substrates for the Maillard reaction (Maillard, 1916), which unacceptably darkens (Zommick et al., 2014; Herman et al., 2017) and increases the acrylamide content of fried potato products (Mottram et al., 2002; Becalski et al., 2003; Rosen et al., 2018). Acrylamide is classified as a probable human carcinogen (International Agency for Research on Cancer, 1994) and its formation is especially favored by elevated levels of glucose and asparagine (Becalski et al., 2003).

Although LTS is problematic for the processing industry, cold-storage has numerous benefits that include prolonged dormancy of tubers and reduced decay, respiration, and weight loss. Conventionally bred potato cultivars with resistance to LTS and the engineered Innate® varieties with silenced invertase and asparagine synthetase effectively alleviate the problems of reducing sugar buildup and acrylamide formation, allowing tubers for frozen processing to be stored cold (Clark et al., 2014; Herman et al., 2017; Novy et al., 2017). Therefore, in addition to reduced EB, LTS-resistant and low acrylamide-forming phenotypes are favorable attributes for quality retention of FC potato, particularly when coupled with reduced O₂ at low temperature to minimize respiration. The effects of temperature and O₂ on the sweetening responses of whole tubers have been studied extensively (Zhou and Solomos, 1998; Zommick et al., 2014; Herman et al., 2016); however, no information is available on the effects of low O₂ on the LTS responses of FC tubers of LTS-susceptible and resistant cultivars in relation to respiration during cold storage.
Development of potato cultivars with silenced PPO, invertase, and asparagine synthetase have paved the way for FC potato for retail and institutional markets. However, the LOL for storage or packaging of FC potato has not been reliably determined nor do we know the point at which anaerobic metabolites accumulate to a degree that might compromise quality. The studies reported herein used cultivars Ranger Russet, Russet Burbank, and their respective Innate® counterparts, Generate (silenced PPO and asparagine synthetase), Cultivate (silenced PPO and asparagine synthetase), and Glaciate (Russet Burbank with silenced PPO, asparagine synthetase and invertase) to determine how oxygen and cold storage affect respiration, LOL, EB, and LTS of FC potato. The results advance our understanding of the physiological responses of FC potato to low O₂ and cold storage and inform the selection of MAP films with appropriate permeability characteristics for minimizing aerobic respiration to preserve selected quality attributes of these cultivars.

**Materials and Methods**

*Plant Materials*

Potato tubers (*Solanum tuberosum* L.) were either obtained directly from the J.R. Simplot Co. (Boise, ID) (2014) or were grown at the Washington State University, Irrigated Agriculture Research and Extension Center (IAREC), Othello, WA (46°47.277’ N. LAT., 119°2.680’W. Long.) from certified seed potatoes provided by the J.R. Simplot Co. (2015 and 2017). Details of seed preparation, planting, crop maintenance, and harvesting of the Othello-grown tubers are as described in Herman et al. (2016). All tubers were subjected to an initial 10- to 14-d wound healing period at 10 °C (95 % RH) in October of each year before processing as described below. The cultivars, storage durations, and O₂ concentrations are summarized in Table 1 for each year of study.
Tuber processing and tissue respiration

All equipment and surfaces used in the preparation and storage of FC tissue were sanitized for 5 min with 10% (v/v) sodium hypochlorite prior to use. Tubers (171 - 284 g) from 10 °C were washed, surface sterilized with 10 % (v/v) sodium hypochlorite, peeled, and collectively diced using a Robot Coupe® CL50 vegetable processor (Robot Coupe USA, Inc., Jackson, MS) equipped with a 10 mm³ cutter at room temperature. The FC tissue was then apportioned to 125 mL Nalgene™ Unitary™ LDPE wash bottles (Thermo Fisher Scientific, Waltham, MA) on ice. The bottles were equipped with foam mesh mats to elevate the tissue (50 g tissue per bottle) ca 2 mm above the floor of each bottle. The bottles were placed in a walk-in cooler at 4 °C. The inlet ports were attached to cylinders of compressed gas that provided a continuous flow (0.5 mL s⁻¹) of the desired O₂ concentration (balance N₂) through each bottle for the duration of study (Table 1). Incoming air flowing through the inlet spout was directed to the floor of each bottle via the integrated tube and then flowed up through the tissue to the outlet port, which consisted of a 1 mL syringe barrel mounted in the lid of each bottle.

The outlet ports from the bottles were connected to an LI-7000 infrared CO₂/H₂O gas analyzer (LI-COR Inc., Lincoln, NB, USA) via computer-controlled 3-way solenoid valves and a manifold, and measurements began immediately. Carbon dioxide concentration in the outflow from each bottle was recorded every 2.5 – 4.5 h, depending on the study. All studies (Table 1) had three replicates (tissue from six tubers pooled per replicate) for each O₂ atmosphere and cultivar.

Tissue sampling

The 2014 and 2015 studies were designed to determine lower oxygen limit (LOL), which is defined as the minimum O₂ concentration needed to maintain predominately-aerobic respiration (Yearsley et al., 1996) in the FC tissue. These studies were terminated following approximately 3
incubation of the FC tubers in ten O₂ atmospheres (Table 1). The tissue from each chamber was immediately frozen in liquid N₂. Half the sample was then lyophilized for subsequent analysis of lactate and the other half stored at -80 °C for ethanol (EtOH) analysis.

The LTS-susceptible cultivar, Russet Burbank, and its Innate® LTS-resistant counterpart, Glaciate (silenced invertase), were selected for study in 2017 to assess the effects of low O₂ atmospheres on LTS in addition to the buildup of anaerobic metabolites over a longer, 16-d storage period at 4 °C. For this study, additional chambers containing 50 g of tissue (3 replicates, tissue from six tubers pooled per replicate) were maintained at five O₂ concentrations (Table 1) to provide samples for sweetening (glc, fru, suc) and anaerobic metabolite (lactate, EtOH) analyses at 4, 8, and 12 d of storage. The zero-time and final tissue samples were taken at tuber processing and from the respiration chambers at 16 d, respectively. All tissue samples were frozen and lyophilized as described above.

**Metabolite analyses**

Lyophilized tissue was ground (mortar and pestle) and sieved through a 60 mesh (246 µm) screen. L-lactate, glucose, fructose, and sucrose were extracted by vortexing the dried tissue for 4 min in 60 % (v/v) MeOH (80 g L⁻¹ DW) at room temperature. The crude extract was initially centrifuged at 1,470 g (4 min), further clarified at 17,000 g (15 min), and the supernatant stored at -20 °C for analysis. L-lactate was assayed in the methanolic extracts using a kit supplied by Megazyme International (Bray, Ireland) according to the instructions provided. The assay converts L-lactate to pyruvate coupled to the stoichiometric reduction of NAD (A₃₄₀, ε = 6,300 M⁻¹ cm⁻¹).

The spectrophotometric determination of glucose, fructose, and sucrose described by Bergmeyer (1974) and Bernt and Bergmeyer (1974) was modified for analysis using a microplate
reader (Knowles et al., 2009). The MeOH extracts (as above) were diluted to 20 g L$^{-1}$ DW for the assay. Quantitation was based on calibration curves of glucose, fructose, and sucrose (0.05 – 1.8 mM each).

Ethanol content of tissue was assessed by solid-phase microextraction (SPME)/gas chromatography. For the 2014 and 2015 studies, frozen tissue was ground to a fine powder in liquid N$_2$ and 2 g was transferred to a 10-mL reaction vial containing 1.8 g NaCl and 2.7 mL nanopure water. The vial was closed with a septum cap and 1 µL of 0.5 % (v/v) n-propanol was added through the septum to serve as an internal standard. The vial was stirred constantly at room temperature for 15 min, after which a SPME fiber of carboxen/polydimethylsiloxane (Sigma-Aldrich Corp, St. Louis, MO, USA) was inserted into the headspace for collection of EtOH for 45 min. The fiber was desorbed in the inlet of an Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA). Instrument conditions were as follows: 250 °C inlet; 5:1 split injection; oven program = 35 °C for 3 min, then 3 °C min$^{-1}$ to 50 °C, then 5 °C min$^{-1}$ to 225 °C; He flow rate = 1.1 mL min$^{-1}$, FID detector = 300 °C. Quantitation of EtOH was via a multi-point calibration curve of EtOH with constant level of n-propanol. For EtOH analysis in the 2017 study, sap from thawed tissue was extracted using a hydraulic press (Spectrum Technologies, Aurora, IL, USA). The sap was centrifuged (17,000 g, 12 °C, 15 min) and 1.8 mL of supernatant was transferred to a 4-mL vial containing 0.7 g NaCl. EtOH was analyzed as described for frozen tissue but collection from the headspace was reduced to 35 min. Assay calibration was based on aliquots of EtOH added to EtOH-free sap.

*Data analysis and presentation*

All studies (Table 1) were set up as randomized complete block designs with three replicates of treatments arranged factorially. Respiration rates (CO$_2$, µg kg$^{-1}$ s$^{-1}$ ±SE) are plotted
versus time and \( O_2 \) concentration. Sugar, lactate, and EtOH data were subjected to ANOVA with sums of squares partitioned into single degree-of-freedom contrasts for main effects (cultivar, oxygen, storage duration) and their interactions. Data (±SE) are plotted versus oxygen concentration and/or time with LSD values \( (P<0.05) \) provided for mean separation. Effects of cultivar, \( O_2 \) atmosphere, and storage duration on EB (tissue darkening) were not quantified but were documented in color photographs of the lyophilized tissue to facilitate qualitative comparison. Black and white negative images of tissue samples (processed with ImageJ, https://imagej.nih.gov/ij/, accessed June 11, 2019) are provided for increased resolution of the treatment effects.

Results

Short-term (64-h) storage studies

Respiration of FC tissue

The respiratory responses of FC tissue from cvs. Ranger Russet (Fig. 1A-C), Generate (Fig. 1D-F), Russet Burbank (Fig. 2A-C), and Cultivate (Fig. 2D-F) to decreasing \( O_2 \) concentration were similar over a 64-h storage period at 4 °C. At 21 kPa \( O_2 \), respiration rates fell from 3.39 to 2.60 \( \mu g \) kg\(^{-1}\) s\(^{-1}\) as tissue acclimated from approximately 10 to 4 °C over the initial 12-16 h of storage and then increased from this low point to 4.79 \( \mu g \) kg\(^{-1}\) s\(^{-1}\) through 64 h. This trend is similar to the respiratory acclimation response (RAR) that occurs in whole tubers when acclimating to 4 °C storage (Zommick et al., 2014). The magnitude of the initial decline in respiration rates from 0 to 12-16 h increased with decreasing \( O_2 \) concentration for all cultivars. The subsequent increase in respiration rates from 16-64 h, however, diminished as \( O_2 \) concentration fell and was completely absent below 1.5 kPa \( O_2 \) for Ranger Russet and Generate (Fig. 1A,D) and 1.0 kPa \( O_2 \) for Russet Burbank and Cultivate (Fig. 2A,D). This attenuation of the RAR with decreasing \( O_2 \) level is clear.
in three-dimensional surface plots of the normalized respiration rates versus time and O\textsubscript{2} concentration (Figs. 1B,E and 2B,E).

Reducing O\textsubscript{2} from 21-7 kPa had minimal effect on tissue respiration rates following 64 h of storage at 4 °C (Figs. 1C,F and 2C,F). However, averaged across cultivars, respiration rates fell from 4.23 to 3.41 µg kg\(^{-1}\) s\(^{-1}\) as O\textsubscript{2} decreased from 7 to 3.5 kPa, and to 0.70 µg kg\(^{-1}\) s\(^{-1}\) at 0 kPa O\textsubscript{2}. The lowest rates of CO\textsubscript{2} production by FC potato occurred under anoxia (0 kPa O\textsubscript{2}). Therefore, the concentration of O\textsubscript{2} at which respiratory metabolism transitions from aerobic to anaerobic could not be determined by changes in CO\textsubscript{2} production alone.

\textit{Anaerobic metabolites}

In contrast to CO\textsubscript{2} production, changes in lactate and EtOH content of FC tissue revealed the onset of anaerobic metabolism and thus the minimum concentration of O\textsubscript{2} needed to support aerobic respiration. Reducing O\textsubscript{2} from 21 - 2.5 kPa did not affect lactate content of FC tissue, which averaged 0.029 ±0.002 g kg\(^{-1}\) DW across cultivars (Figs. 1C,F and 2C,F). However, lactate increased to 0.126 g kg\(^{-1}\) DW at 1.5 kPa O\textsubscript{2} and continued to climb sharply, increasing to 0.962 g kg\(^{-1}\) DW in FC tissue from Ranger Russet and Generate as O\textsubscript{2} was further reduced to 0 kPa (Fig. 1C,F). Tissue EtOH concentrations for these two cultivars remained low and relatively constant at 5.4 µL kg\(^{-1}\) FW from 21–2 kPa O\textsubscript{2}, but then increased to 74 µL kg\(^{-1}\) FW on average as O\textsubscript{2} fell further to 0 kPa (Fig. 3A). Similar trends were observed for Russet Burbank and Cultivate where lactate increased from 0.026 to 0.036 g kg\(^{-1}\) DW from 2-1.5 kPa O\textsubscript{2}, followed by an increase to 1.69 g kg\(^{-1}\) DW (average) as O\textsubscript{2} was lowered to 0 kPa (Fig. 2C,F). Ethanol in FC Russet Burbank and Cultivate potatoes remained low and constant averaging 2.45 µL kg\(^{-1}\) FW from 21-1.5 kPa O\textsubscript{2}, then increased to 27.9 µL kg\(^{-1}\) FW on average as O\textsubscript{2} was lowered to 0 kPa (Fig. 3B). Averaged
across all cultivars, lactate concentrations indicated that the transition from aerobic to anaerobic metabolism for FC potato at 4 °C occurred between 1.5 to 2.0 kPa O₂.

_Browning of FC tissue_

Wound-induced changes in the color of FC tubers were not quantified but were clearly evident following 3 d storage at 4 °C (Fig. 4). Fresh-cut tissue from Ranger Russet tubers darkened considerably when stored at 21 kPa O₂, and much more so than tissue from Generate tubers. Anoxia (0 kPa O₂) inhibited browning the most; however, O₂ at ≤2 kPa mitigated the discoloration of FC tissue from both cultivars. The effect of PPO silencing on attenuation of O₂-dependent browning of tissue from Generate tubers was striking when compared with that from Ranger Russet tubers. Effects of O₂ on the EB of FC tubers of Russet Burbank and Cultivate were similar to Ranger Russet and Generate (data not shown).

_Long-term (16-d) storage study_

The effects of O₂ atmosphere on respiration, aerobic/anaerobic transition, and cold sweetening were also assessed over a 16-d storage interval, which approximates the desired shelf-life of FC potato from packaging to consumer (https://www.producebluebook.com/wp-content/uploads/KYC/Fresh-Cut-Produce.pdf, accessed June 10, 2019). Russet Burbank and its Innate®-engineered counterpart, Glaciate, were chosen for this study. Similar to Cultivate, PPO is silenced in Glaciate. However, unlike Cultivate, invertase has also been silenced in Glaciate (Clark et al., 2014), which renders this cultivar resistant to cold-induced sweetening and thus ideal to compare with Russet Burbank (cold sweetening susceptible) for effects of low O₂ on sugar buildup during the 16-d storage period. FC tissue from these cultivars was stored in 0, 1, 2, 3, and 21 kPa O₂ at 4 °C and changes in respiration rates, lactate, EtOH, sucrose, reducing sugars, and tissue browning were compared.
Respiration of FC tissue

The respiratory responses of FC Russet Burbank and Glaciate tubers to reduced O$_2$ concentration over the initial 3 d of storage at 4 °C were comparable to those described in the short-term studies (see 3.1.1 above). At 21 kPa O$_2$, tissue from both cultivars displayed a prominent cold-induced RAR from 0 to 48 h, which dissipated as O$_2$ was reduced to 2 kPa and was abolished at 0 kPa (Fig. 5). Respiration rates of tissue in 21 kPa O$_2$ then increased to a maximum at 9 d and subsequently decreased through day 12. Decreasing the O$_2$ concentration from 3–1 kPa attenuated and delayed this increase in respiration rate, effectively shifting the respiratory maxima later, and reducing maximum respiration rates in a concentration-dependent manner. Respiration rates of tissue held at 0 kPa O$_2$ decreased to 15 to 25 % of zero-time rates within 3 d and then remained low and relatively constant for the duration of study.

Anaerobic metabolites

The effects of O$_2$ concentration on lactate production by FC tissue over the 16-d storage period at 4 °C were similar for cvs. Russet Burbank and Glaciate (Fig. 6). Lactate concentrations remained low and constant throughout storage in tissue held at 2, 3, and 21 kPa O$_2$, reflecting aerobic metabolism. However, lactate increased linearly with time ($P<0.01$) in Glaciate and Russet Burbank tissues held at 1 kPa O$_2$, resulting in 1.48 and 0.60 g kg$^{-1}$ DW, respectively, by 16 d of storage, and signifying the induction of anaerobic metabolism. Lactate production was most rapid in tissues stored at 0 kPa O$_2$, where both cultivars had accumulated 3.4 g kg$^{-1}$ DW by 16 d. Results of ANOVA for lactate concentration appear in Table 2.

Tissue EtOH concentrations were compared at the end of the 16-d storage period. Cultivar had no effect on EtOH concentrations (Table 2). Averaged over cultivars, however, EtOH concentrations were relatively low and equivalent for the FC tissues stored at 21, 3, and 2 kPa O$_2$, 
averaging 0.77 µL kg\(^{-1}\) FW, but increased 2.7-fold to 2.08 µL kg\(^{-1}\) FW in tissue stored at 1 kPa O\(_2\) (Fig. 7; \(P<0.05\) for 1 versus 2, 3, and 21 kPa O\(_2\)). By contrast, tissue stored in 0 kPa O\(_2\) for 16 d averaged 195 µL kg\(^{-1}\) FW EtOH (Fig. 7).

**Sucrose and reducing sugars**

While the concentration of sucrose in Glaci\(\text{ate}\) tubers was nearly double that of Russet Burbank tubers \((P<0.01)\) at the beginning of storage (i.e. zero-time), the reducing sugar concentration was 5.7-fold lower \((P<0.01)\) in Glaci\(\text{ate}\) tubers (Fig. 8); a consequence of the effects of invertase silencing. Changes in sucrose and reducing sugars over time depended on cultivar and O\(_2\) concentration \((P<0.01, \text{Table 2})\). When stored at 21 kPa O\(_2\), sucrose concentrations increased linearly \((P<0.05)\) by 6.3 and 8.9 g kg\(^{-1}\) DW per day through the initial 8 d of storage at 4 °C for FC Russet Burbank and Glaci\(\text{ate}\) tubers, respectively (Fig. 8). Sucrose concentration then decreased in Russet Burbank tissue through 16 d, but remained significantly higher \((P<0.01)\) than the zero-time samples. By contrast, the concentration of sucrose in Glaci\(\text{ate}\) tissue held at 21 kPa O\(_2\) remained high and constant at approximately 28 g kg\(^{-1}\) DW from day 8 to 16.

Low O\(_2\) (1–3 kPa) significantly diminished the cold-induced buildup of sucrose in FC tissue from both cultivars over the 16-d storage interval (Fig. 8). However, at 0 kPa O\(_2\), sucrose concentration in Russet Burbank tissue fell linearly from 4.83 to 1.96 g kg\(^{-1}\) DW (2.5-fold) through 16 d. By contrast, the sucrose concentration in FC tissue from Glaci\(\text{ate}\) tubers stored at 0 kPa O\(_2\) remained relatively constant, averaging 10.4 g kg\(^{-1}\) DW over the 16-d storage period.

Reducing sugar concentrations in FC tissue from Russet Burbank increased only marginally over the initial 4 d of storage at 4 °C and were not affected by O\(_2\) (Fig. 8). However, further increases through the remaining 12 d of storage were significantly modulated by O\(_2\) concentration. Reducing sugars increased the most (2.7-fold) in FC Russet Burbank tubers stored
at 21 kPa O₂ during this period, which illustrates the low temperature-induced sweetening response of tissue from this cold sensitive cultivar. Lowering the O₂ concentration to 1 kPa attenuated LTS of Russet Burbank tissue and the response was abolished at 0 kPa O₂. In contrast to Russet Burbank, reducing sugar levels in FC Glaciate tubers remained low and constant over the 16-d storage period regardless of O₂ concentration, reflecting the LTS-resistant phenotype of this cultivar.

Unlike sucrose and reducing sugars, total sugar concentrations (suc + glc + fru) in Russet Burbank and Glaciate tubers were equivalent at zero-time (Fig. 8). Moreover, the effects of O₂ on the cold-induced changes in total sugars over time were similar for the two cultivars (Fig. 8; Table 2, cultivar x O₂ x time, ns). On average, total sugar concentrations increased 3-fold in FC tissue stored at 21 kPa O₂ over the 16-d storage period at 4 °C (Fig. 8). This cold-induced buildup in total sugars diminished with decreasing O₂ concentration and the trends were similar for both cultivars. Storage of FC tissue at 0 kPa O₂ prevented sugar accumulation at 4 °C, resulting in total sugar concentrations remaining relatively constant over the 16-d storage period. By 16 d at 0 kPa O₂, reducing sugars dominated the total sugar fraction of FC Russet Burbank tubers whereas sucrose dominated the total sugar fraction of FC Glaciate tubers.

**Browning of FC tissue**

Relative to the zero-time (fresh-cut) samples, enzymatic browning of FC Russet Burbank tubers was extensive during the initial 4 d of storage at 1, 2, 3, and 21 kPa O₂, but was significantly reduced by anoxia (Fig. 9A,B). Tissue darkening at 0 kPa O₂ remained relatively constant from 4 to 16 d but increased progressively with time when stored at 1, 2, 3, and 21 kPa O₂ (Fig. 9B). Therefore, the browning at 0 kPa O₂ occurred during the period from processing to full establishment of anoxia within the tissue (ca 3-4 h), reflecting the high affinity of polyphenol
oxidase for O₂. Relative to Russet Burbank, EB of FC Glaciate tubers was greatly reduced as a consequence of PPO silencing (Fig. 9A). However, all samples stored in the presence of O₂ developed an off-white color most closely resembling NN155A of the Royal Horticultural Society Colour Chart (http://rhscf.orgfree.com/, accessed June 11, 2019) within 4 d of processing. Tissue held at 0 kPa O₂ maintained the white coloration of FC tissue.

**Discussion**

Wound-induced respiration, enzymatic browning, and low-temperature sweetening (LTS) constitute physiological processes that can adversely affect the quality and thus shelf-life of FC potato. The Innate®-engineered cultivars (cvs) were approved for commercial production in 2015 and 2017. These cultivars are suitable for minimal processing and low-temperature storage by virtue of their silenced PPO, asparagine synthetase (cvs Cultivate, Generate, Glaciate) and invertase (cv Glaciate) activities (Table 1). Collectively, the traits controlled by these enzymes are important for maintaining the postharvest quality of whole and FC tubers. Low PPO activity greatly reduces the enzymatic browning that occurs in response to wounding (Fig. 4). Silenced invertase prevents the rapid buildup in reducing sugars that would otherwise occur in tubers of cold sweetening susceptible cultivars (e.g., Russet Burbank, Ranger Russet) stored at 4 °C (Fig. 8). Reducing sugars and asparagine can negatively affect quality through formation of brown pigments and acrylamide from the Maillard reaction during frying of potato (Novy et al., 2017). We characterized the effects of reduced O₂ atmosphere on the respiratory, enzymatic browning, cold-induced sweetening, and fry color phenotypes of FC tubers of these cultivars. Fresh-cut tubers were stored at 4 °C in O₂ atmospheres ranging from 0 to 21 kPa. The LOL for aerobic respiration was compared among the cultivars along with the effects of hypoxia and anoxia on LTS and enzymatic browning.
Regardless of cultivar, FC tubers at 21 kPa O₂ showed a prominent initial respiratory acclimation response (RAR) upon transfer from 10 to 4 °C (Figs 1, 2, and 5). A similar RAR has been well documented for whole tubers (Isherwood, 1973; Zhou and Solomos, 1998; Zommick et al., 2014; Herman et al., 2016) but the duration was longer at ca 5-6 d, presumably reflecting the greater time needed to cool and fully acclimate whole tubers compared with FC tissue. Respiration rates of the FC tissue at 21 kPa O₂ continued to increase from day 2 to 6 following the RAR but at a slower rate (Fig. 5). By contrast, the respiration rate of whole tubers declines steadily following the RAR through about 40 d at 4 °C and is thereafter maintained at a constant low rate through long-term (150 d) storage (Zommick et al., 2014; Herman et al., 2016). Importantly, the respiration rates of FC Russet Burbank and Ranger Russet tubers (Figs. 1 and 2) were approximately 3.5-fold higher than whole tubers (Zommick et al., 2014; Ellis and Knowles, unpublished) following acclimation to 4 °C for 48 h, and this difference increased to about 14-fold by 9 d of storage (Fig. 5), contributing to the increased perishability and higher potential for deterioration of FC tuber tissue.

Decreasing O₂ to approximately 1-1.5 kPa abolished the cold RAR and substantially reduced the respiration rate of FC tubers compared with 21 kPa O₂ (Figs. 1 and 2). Additionally, respiration remained relatively low and constant at 1-2 kPa O₂ through 7 d at 4 °C (Fig. 5). Isherwood (1973) demonstrated that cold-induced changes in respiration rates of whole tubers reflect the energetics (ATP equivalent cost) of starch catabolism to sugar during LTS. Given the increases in sucrose and reducing sugars in FC tubers at 4 °C (Fig. 8), it is reasonable to assume that much of the respiratory responses here were driven by temperature-dependent starch to sugar interconversions in the tissue. Moreover, FC Innate® tubers showed similar changes in respiration as their non-engineered counterparts, both in trend and magnitude during short and longer-term
(12 d) storage (Figs. 1, 2 and 5), despite major differences in cold-induced sucrose versus reducing sugar synthesis (Fig. 8). Hypoxia reduced cold-induced synthesis of sucrose and reducing sugars in FC Russet Burbank tubers, but only sucrose in FC Glaciate tubers where acid invertase was silent. This finding corroborates that of Herman et al. (2016) who reported that lowered reducing sugar accumulation in whole tubers stored at 2.5 kPa O$_2$ was the result of attenuated invertase activity. Therefore, consistent with Isherwood’s (1973; 1976) conclusions for the effects of cold-sweetening on respiratory responses of whole tubers, the O$_2$-induced effects on respiration impeded starch catabolism and sugar synthesis, since respiratory profiles (Fig. 5) and changes in total sugars (suc + glc + fru) (Fig. 8) were comparable for the conventionally bred and Innate®-engineered cultivars.

The respiratory CO$_2$ profiles under decreasing O$_2$ did not reveal the onset of anaerobic metabolism (Figs. 1 and 2). In many fruit and vegetables (e.g., carrot, pear, apple, peach), the induction of anaerobic metabolism initially involves decarboxylation of pyruvate to acetaldehyde, which is subsequently reduced to EtOH via alcohol dehydrogenase (Fig. 1C inset) (Boersig et al., 1988; Kubo et al., 1996; Yearsley et al., 1996; Kato-Noguchi and Watada, 1997). The LOL (Yearsley et al., 1996) for aerobic respiration in these EtOH-producing species can therefore be estimated by monitoring respiratory CO$_2$ output in response to decreasing partial pressure of O$_2$ in the surrounding atmosphere (Leshuk and Saltveit, 1990) and/or tissue (Yearsley et al., 1996). Respiration rate (CO$_2$ output) declines with O$_2$ partial pressure until the onset of anaerobic metabolism, which is ultimately detected as a rapid increase in CO$_2$ (Pasteur effect) from the increased pyruvate decarboxylase activity. The anaerobic compensation point (ACP) in EtOH-producing species is defined as the concentration of O$_2$ at which CO$_2$ production is minimum (Boersig et al., 1988).
In contrast, lactate is the first formed product of anaerobic metabolism in potato (Fig. 1 C inset) (Barker and El Saifi, 1952ab; Barker and Kahn, 1968). Anoxia first induces the reduction of pyruvate to lactate via lactate dehydrogenase (Shinozaki et al., 2015), which is favored by a slightly alkaline cytosol (Couldwell et al., 2009), with no attending increase in CO₂ to signal the onset of anaerobic metabolism in potato (Figs. 1 and 2; Barker and El Saifi, 1952ab). As shown in Figs. 1C,F and 2C,F, respiratory CO₂ continued to decrease in FC potato as O₂ fell to 0 kPa (anoxia). The ACP as defined by Boersig et al. (1988) is therefore non-existent in potato. However, as the first formed product of anaerobic metabolism in potato, the initiation of lactate buildup can be monitored to estimate the LOL for aerobic respiration. Accordingly, the LOL for aerobic respiration of FC potato in our studies was between 1.5 and 2 kPa O₂. Moreover, the changes in lactate with O₂ concentration and time were comparable for FC tubers of Russet Burbank, Ranger Russet, and their genetically engineered Innate® counterparts (Table 2, cultivar x O₂ x days, ns).

Under hypoxia, the initial increase in lactate of FC potato lowers cytosolic pH (Couldwell et al., 2009). According to the pH-stat hypothesis (Davies et al., 1974; Ratcliffe, 1995), this acidification induces pyruvate decarboxylase activity, which accounts for delayed buildup in EtOH relative to lactate in potato (Barker and El Saifi, 1952ab). EtOH buildup was indeed apparent in FC potato following 64 h as O₂ fell from ca 2 to 1 kPa (Ranger Russet and Generate), 1.5 to 0.5 kPa (Russet Burbank and Cultivate) (Fig. 3), and 2 to 1 kPa O₂ (Russet Burbank and Glaciator) at 16 d of storage. These changes in respiratory CO₂, lactate and EtOH production are consistent with previous work on whole tubers (Barker and Safi, 1952ab) and further demonstrate a notable Pasteur effect at ca 1-1.5 kPa O₂ for FC potato.
This report is the first to show that the respiratory and LTS responses of FC and whole tubers during cold acclimation are similar. While respiration rates of FC tubers are substantially higher than those of whole tubers, maintaining O$_2$ near the LOL (2 kPa) minimizes the cold RAR, subsequent increases in respiration rate, and LTS through 16 d. Process quality thus benefits in several ways. Lower reducing sugars decrease Maillard browning and acrylamide forming potential, while dry matter (starch) is preserved through reduced respiration. The Innate® cultivar, Glaciate, by virtue of its PPO and invertase silencing, has substantially mitigated EB and cold-induced reducing sugar buildup in FC tubers. Nevertheless, the starch/dry matter loss from FC Glaciate tubers would likely be equivalent to FC tubers of Cultivate (no invertase silencing) and Russet Burbank, as indicated by the comparable effects of low temperature and O$_2$ on total sugar accumulation (Fig. 8). The impact on sensory qualities associated with cold-induced sucrose (e.g., Glaciate) versus reducing sugar buildup (e.g., Cultivate) remains to be determined.

Acknowledgements

We gratefully acknowledge financial support from the USDA-ARS, Washington State Department of Agriculture (WSDA) Specialty Crop Block Grant program, and the J.R. Simplot Co. We thank Matthew McDonald, Simplot Food Group, for his insight and encouragement, and the J.R. Simplot Co. for providing Innate® seed potatoes.
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Table 1. Study parameters for comparing the respiratory responses and lower oxygen limits for aerobic metabolism of fresh-cut potatoes of Russet Burbank, Ranger Russet and their respective Innate® cultivars to low oxygen storage.

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Cultivars</th>
<th>Relevant Innate®-silenced genes</th>
<th>Storage Time (4 °C)</th>
<th>O₂ (kPa)</th>
<th>Physiological responses and metabolites</th>
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<tr>
<td>2014</td>
<td>Russet Burbank</td>
<td>Cultivate PPO Asn Synthetase</td>
<td>64 h</td>
<td>0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 7.0, 14, 21</td>
<td>Respiration, Lactate, EtOH</td>
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<tr>
<td>2015</td>
<td>Ranger Russet</td>
<td>Generate PPO Asn Synthetase</td>
<td>64 h</td>
<td>0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.5, 7.0, 14, 21</td>
<td>Respiration, Lactate, EtOH, tissue browning</td>
</tr>
<tr>
<td>2017</td>
<td>Russet Burbank</td>
<td>Glaciate PPO Asn Synthetase</td>
<td>16 d</td>
<td>0, 1, 2, 3, 21</td>
<td>Respiration, Lactate, EtOH, cold sweetening (Glc, Fru, Suc), tissue browning</td>
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<td></td>
<td></td>
<td>Acid Invertase</td>
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</table>


*a* Clark et al. (2014)

*b* balance N₂

*c* PPO, polyphenol oxidase; Asn, asparagine
Table 2. Sources of variation and levels of significance ($P$-values) for the effects of cultivar, oxygen atmosphere (0, 1, 2, 3, 21 kPa O$_2$) and storage duration (0, 4, 8, 12, 16 d) on sugar, lactate, and EtOH concentrations of fresh-cut tissue from Russet Burbank and Glaciate potato tubers.

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>Sucrose</th>
<th>Glc + Fru</th>
<th>Suc + Glc + Fru</th>
<th>Lactate</th>
<th>EtOH (16 d)</th>
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<td>Cultivar (C)</td>
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<td>0.01</td>
<td>0.04</td>
<td>ns$^1$</td>
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<tr>
<td>Oxygen (O)</td>
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<tr>
<td>Days (D)</td>
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<td>0.01</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>C x O</td>
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<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
<td>ns</td>
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<td>C x D</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
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</tr>
<tr>
<td>O x D</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
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$^1$ns, not significant.
Fig. 1. Changes in respiration rates and lactate concentrations (yellow symbols) of FC tubers of cvs Ranger Russet (A, B, C) and Generate (D, E, F) during cold acclimation as affected by O₂ atmosphere. Tubers were processed at 10 °C and the FC tissue was immediately placed at 4 °C with a continuous flow of O₂ (balance N₂) at the indicated concentrations for 64 h. (B, E) Respiration rate data were normalized to initial rates and plotted to model the three-dimensional profiles that define the time x [O₂] interactions (P<0.001) for each cultivar. Tissue lactate levels (C, F) were quantified at the end of the study and plotted with the final (64 h) tissue respiration rates versus O₂ concentration. Each point (C, F) represents the average of 3 replicates of pooled tissue from 6 tubers/replicate (±SE). Pathways leading to the synthesis of lactate and EtOH under anaerobic conditions are summarized in (C) (LDH, lactate dehydrogenase; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase). Effects of low O₂ on lactate buildup in FC tissues from Ranger Russet and Generate are directly compared in the inset histogram (F) (ANOVA results: cultivar, ns; O₂, P<0.001; cultivar x O₂, ns).
Fig. 2. Changes in respiration rates and lactate concentrations (yellow symbols) of FC tubers of cvs Russet Burbank (A, B, C) and Cultivate (D, E, F) during cold acclimation as affected by O$_2$ atmosphere. Tubers were processed at 10 °C and the tissue was immediately placed at 4 °C with a continuous flow of O$_2$ (balance N$_2$) at the indicated concentrations for 64 h. (B,E) Respiration rate data were normalized to initial rates and plotted to model the three-dimensional profiles that define the time x [O$_2$] interactions ($P<0.001$) for each cultivar. Tissue lactate levels (C, F) were quantified at the end of the study and plotted with the final (64 h) tissue respiration rates versus O$_2$ concentration. Each point (C, F) represents the average of 3 replicates of pooled tissue from 6 tubers/replicate (±SE). Effects of low O$_2$ on lactate buildup in the FC tissues from Russet Burbank and Cultivate are directly compared in the inset histogram (F) (ANOVA results: cultivar, ns; O$_2$, $P<0.001$; cultivar x O$_2$, ns).
Fig. 3. EtOH concentrations in FC tubers of cultivars (A) Ranger Russet and Generate, and (B) Russet Burbank and Cultivate following storage at 4 °C for 64 h in the indicated O₂ atmospheres. Each point represents the average of 3 replicates of pooled tissue from 6 tubers/replicate (±SE). ANOVA results for (A) and (B): cultivar, ns; O₂, $P<0.001$; cultivar x O₂, ns.
Fig. 4. Lyophilized samples of FC Ranger Russet and Generate tubers showing effects of O$_2$ atmospheres on the color of tissue (enzymatic browning) following 64 h storage at 4 °C. Each dish contains a pooled sample from six tubers.
Fig. 5. Respiration rates of FC tubers of cvs Russet Burbank (A) and Glaciate (B) in five O₂ atmospheres during 12 d at 4 °C. Rates have been normalized to initial zero-time rates (avg CO₂ production = 3.0±0.15 µg kg⁻¹ s⁻¹ Russet Burbank; 4.0±0.25 µg kg⁻¹ s⁻¹ Glaciate). Tubers were processed at 10 °C and the tissue was immediately placed at 4 °C under continuous flow of the designated O₂ concentrations (balance N₂). Each point represents the average of 3 replicates of pooled tissue from 6 tubers/replicate.
Fig. 6. Changes in lactate concentrations in FC Russet Burbank (A) and Glaciate (B) tubers stored in five O₂ atmospheres for 16 d at 4 °C. Each point represents the average of 3 replicates of pooled tissue from 6 tubers/replicate (±SE). See Table 2 for ANOVA results.
Fig. 7. EtOH concentrations in FC Russet Burbank and Glaciate tubers stored in five O₂ atmospheres for 16 d at 4 °C. Data are averaged over cultivar, which did not affect EtOH content (Table 2). Each bar represents the average of 3 replicates of pooled tissue from 6 tubers/replicate of each cultivar. Letters indicate mean separation by LSD ($P<0.05$). See Table 2 for ANOVA results.
Fig. 8. Changes in Sucrose (top row), reducing sugar (Glc + Fru; middle row), and total sugar (Suc + Glc + Fru; bottom row) concentrations in FC Russet Burbank (left column) and Glaciata (right column) tubers stored in five O2 atmospheres for 16 d at 4 °C. Each point represents the average of 3 replicates of pooled tissue from 6 tubers/replicate (±SE). See Table 2 for ANOVA results.
Fig. 9. (A) Lyophilized samples of FC Russet Burbank and Glaciate tubers showing the effects of O$_2$ atmosphere on changes in tissue color (enzymatic browning) over a 16-d storage period at 4 °C. Each dish contains a pooled sample from six tubers. (B) Black and white negative image with threshold adjusted to resolve the interaction effects of O$_2$ concentration x time x cultivar on darkening of the FC tissue.
CHAPTER TWO

Increasing the production efficiency of potato with plant growth retardants

Abstract

The vigorous growth phenotype of ‘Bondi’ is characteristic of high endogenous gibberellins, making it ideal for evaluating the efficacy of gibberellin biosynthesis inhibitors to improve allometric partitioning and production efficiency in potato. Foliar applications of prohexadione-calcium decreased foliar growth with no effects on tuber number, size, or yield. In contrast, when applied pre-tuberization, paclobutrazol decreased maximum foliar growth from 91 to 63 MT ha\(^{-1}\), increased tubers plant\(^{-1}\) from 6.4 to 8.9, and decreased average tuber fresh weight from 296 to 188 g tuber\(^{-1}\) with no reduction in tuber yield, which effectively increased the harvest index. However, when applied post-tuberization, PBZ reduced maximum foliar growth from 91 to 72 MT ha\(^{-1}\) with no effects on tuber number, size, or yield. Paclobutrazol can alter source/sink relationships in potato to increase production efficiency and this may translate to reduced agronomic inputs. Application timing and concentration are important for modulating tuber set and size.

Introduction

The potato cv. Bondi is a high-yielding, late-season cultivar with indeterminate growth habit developed by New Zealand Plant and Food Research for frozen (French fry) processing (Oliveira, 2015; https://www.plantandfood.co.nz/page/our-research/breeding-genomics/key-crops/potatoes/). Bondi has a rather unique phenotype when compared with industry-standard, late-season frozen-processing varieties such as Russet Burbank and Ranger Russet. The canopy growth of Bondi is vigorous with elongated vines (Herman et al., 2016). Aboveground foliar fresh weight and dry matter are consistently 30 to 50% greater than Russet Burbank (Oliveira, 2015) and Ranger Russet (Knowles, unpublished). Tuber set of Bondi is relatively low and this results in production of a high percentage (often >45%) of oversize (>397-g) tubers (Herman et al. 2016). Additionally, the tubers have an elongated phenotype as defined by high length-to-width ratio (Knowles, unpublished). Collectively, these phenotypic characteristics make this cultivar ideal for developing/evaluating methods to effectively modulate tuber set, size, and overall source/sink relationships in potato.

Our previous work (Herman et al. 2016) demonstrated that the low tuber set and excessive tuber size of cv. Bondi can be rectified by treating seed with GA prior to planting. GA seed treatments reduced apical dominance, resulting in increased stems, which in turn increased tuber set per plant and decreased average tuber size. However, these treatments did not appear to reduce the excessive foliar growth of this cultivar. Attenuating foliar growth may be advantageous if it leads to a decrease in the agronomic inputs needed to produce such a variety, without compromising yield.

While GA seed treatments increase tuber set indirectly through modulating apical dominance (Blauer et al. 2013a; Dean et al. 2018), foliar applications of GA can delay tuberization
and decrease tuber set (Caldiz et al. 1998; Sharma et al. 1998). This apparent contradiction is explained by the fact that stolon growth and tuberization are partly regulated by endogenous levels of GA. \( \text{GA}_{20} \) synthesized in leaves (Bömke and Tudzynski 2009) is translocated to stolons (Proebsting et al. 1992) where it is converted into bioactive \( \text{GA}_1 \). Continuous production of bioactive GA’s in stolons promotes continued elongation and prevents tuberization (Kumar and Wareing 1974; Xu et al. 1998). Environmental (e.g., short days, day/night temperatures) or other signals (e.g., low nitrogen, stress) that induce tuberization effectively decrease the production and increase the catabolism of bioactive GA in stolons, leading to the cessation of stolon elongation and promotion of tuber set (Hannapel 2007 and references therein; Kloosterman and Bachem 2014).

In addition to inhibiting tuberization (Kloosterman and Bachem 2014), high levels of endogenous GA stimulate internode elongation and can promote excessive foliar growth (Carrera et al. 2000; Davière and Achard 2013; Gonzalez et al. 2010; Huang et al. 1998). The vigorous-foliar-growth, low-tuber-set, and elongated-tuber phenotype of cv. Bondi suggests inherently high endogenous GA levels, which is further supported by the lower sensitivity of this cultivar to exogenous GA seed treatments relative to other cultivars (e.g., Ranger Russet, Bondi’s maternal parent) (Herman et al. 2016). Therefore, as suggested by Herman et al. (2016), GA biosynthesis inhibitors may constitute an even more effective approach than GA seed treatments to increase tuber set and decrease tuber size, with the added potential benefit of reducing the excessive foliar growth produced by cv. Bondi. This latter effect could translate to increased source/sink efficiency, which may warrant a reduction in management inputs (e.g., \( \text{H}_2\text{O} \), nitrogen fertility, etc.) and lead to a more sustainable and profitable production system for cv. Bondi and varieties with similar phenotype. To test this hypothesis, two growth retardants (prohexadione-calcium and
paclobutrazol) with different modes of action for inhibiting GA biosynthesis (see Discussion) were evaluated as an alternative to GA seed treatment. The overall objective was to assess the potential of these GA biosynthesis inhibitors to alter the allometric relationship between foliar and tuber growth and thereby improve production efficiency.

**Materials and Methods**

**Summary of trials**

The effects of gibberellin (GA) seed treatments and foliar applications of the GA-biosynthesis inhibitors, paclobutrazol (PBZ) and prohexadione calcium (Apogee®), on plant emergence, apical dominance, foliar growth, tuber set, yield, and tuber size distribution of cv. Bondi were evaluated in a series of field trials conducted at the Washington State University Irrigated Research Unit, Othello, WA (46° 47.277’ N. Lat., 119° 2.680’ W. Long.) during the 2015 to 2017 growing seasons. Planting and vine kill dates, timing of growth regulator applications, concentrations, and experimental designs for each trial are summarized in Table 1. The 2015 and 2016 studies focused on determining whether PBZ could modify tuber set and size distribution of plants developing from GA-treated and non-treated seed (described below). PBZ was initially tested over a relatively high concentration range in 2015 (0 to 250 mg L\(^{-1}\)), which was subsequently lowered to 0 to 120 mg L\(^{-1}\) in 2016. As an alternative to PBZ, the effects of prohexadione calcium on tuber set, size distribution, and yield were also evaluated in 2016. The 2017 trial focused on modeling foliar and tuber growth through the season in response to foliar applications of PBZ applied either pre- (51 DAP) or post-tuberization (61 DAP).

**Seed handling and GA treatment**

Certified seed tubers of cv Bondi (G3, generation three from nuclear) were acquired from a commercial seed grower in March 2015 and 2016 and held at 4 C (95% RH) for ca 30 days until
cutting, treating and planting. Since certified seed tubers of this cultivar were no longer available after 2016, non-certified seed tubers grown at Othello, WA in 2016 were used for the 2017 trial. Following harvest (Sept 26, 2016), the 113 to 283-g seed tubers were wound healed at 10 C (95% RH) for 3 weeks and then stored at 4 C (95% RH) until early April. Seed tubers (115-200 g) for all trials (Table 1) were hand cut into 50 to 64-g apical- and basal-end seed pieces 3 to 5 days prior to planting.

GA seed treatments were formulated and applied as described previously (Dean et al. 2018; Herman et al. 2016). Briefly, an 8 mg L⁻¹ GA treatment solution was prepared in water with 0.1 % (w/v) Tween 20 (polyoxyethylene (20) sorbitan monolaurate) from a 50 mg mL⁻¹ stock solution of gibberellin A₃ (≥90 %, Sigma-Aldrich, St. Louis, MO) in dimethyl sulfoxide (DMSO). The control solution (0 mg L⁻¹ GA) contained equivalent concentrations of Tween 20 and DMSO. Seed pieces were submerged in the control or GA solutions for 5 min, air dried at room temperature, and subsequently held at 10 C (95% RH) for 3 to 5 days prior to planting.

Field plot designs, planting and maintenance

Treatments in all trials were arranged in a randomized complete block design with four or five replicates (Table 1). Seed pieces were blocked for portion (apical versus basal ends) and planted 20-cm-deep (top of hill to bottom of furrow) in a Shano silt loam soil (Lenfesty 1967) with a two-row, assist-feed planter. The 2015 and 2016 trials consisted of 24 seed pieces (hills) per plot with seed spaced 25.4 cm apart (6.1-m plots). The plots were separated by 1.5-m alleys that included a single hill of cv Chieftain planted at the beginning and end of each plot to maintain competition for plants at the ends of plots and to facilitate plot separation during harvest. Treatment rows were flanked by guard rows of cv Bondi in all trials to provide uniform
competition and to protect plots from aerial drift during foliar applications of PBZ or Apogee® (see below). Rows were spaced 86-cm apart in 2015 and 81-cm apart in 2016 and 2017.

The effects of PBZ application timing on foliar and tuber growth were modeled over a 153-d growing season in 2017. This study entailed ten sequential harvests through the season to develop the foliar and tuber growth curves. At each harvest, four replicates of 8-hill plots for each of the 3 treatments (Table 1: 120 mg L⁻¹ PBZ applied at either 51 or 61 DAP vs control) were hand dug at ca 7-d intervals from 40 to 69 days after planting (DAP), followed by 15 to 18-d intervals through 153 DAP. Treatments were arranged in a randomized complete block design (10 harvest dates x 3 foliar treatments) with seed pieces blocked for portion (apical vs basal) and planted as described above. Each 8-hill treatment plot was planted with a single hill of cv Chieftain at either end. Treatment plots were separated by 1.3-m alleys (including the Chieftain hills).

All trials were watered with a center pivot, which also delivered fertilizer and pesticides based on best management practices for late season russet cultivars in the Columbia Basin (Hopkins et al. 2007; Lang et al. 1999; Schreiber et al. 2018). Irrigation scheduling was guided by data from neutron probes in conjunction with evapotranspiration models for potatoes grown at the WSU Othello Research Unit (WSU AgWeatherNet, http://weather.wsu.edu/awn.php). Soil moisture was maintained at a minimum of 65% field capacity. Fertility levels were initially adjusted based on pre-plant soil tests. Weekly petiole analyses helped guide subsequent in-season fertigation through the center pivot.

**Paclobutrazol and prohexadione-calcium foliar applications**

Concentrated solutions of analytical grade paclobutrazol (PBZ) (99.6 %, Sigma-Aldrich, St. Louis, MO) were prepared in 15 mL DMSO and subsequently added to 1.485 L deionized H₂O containing 0.1% (w/v) Tween 20 immediately prior to spraying. The final diluted spray solutions
thus contained 1% DMSO, Tween 20, and the PBZ concentrations indicated in Table 1. Spray solutions of prohexadione calcium were prepared by adding Apogee® (27.5% a.i.; BASF Corp., Park, NC) to 1.5 L deionized H₂O containing 0.1% (w/v) Tween 20 (immediately prior to spraying) to achieve 0, 150, 300, and 600 mg L⁻¹ prohexadione calcium. PBZ and Apogee® treatments were applied to foliage (187 L ha⁻¹) with a CO₂ backpack sprayer equipped with a TeeJet® flat fan nozzle (8004E, Bellspray Inc., R&D Sprayers, Opelousas, LA) at the DAP indicated in Table 1. The nozzle head was approximately 20 cm above the canopy during spraying.

**Plant growth and development measurements, harvesting and sorting**

Plant emergence counts for the GA x PBZ trials began 20 DAP and continued approximately every 2 days through 39 DAP in 2015 and 33 DAP in 2016. Additionally, the number of above ground stems per plant were counted at ca 48 DAP in both years for the 0 and 8 mg L⁻¹ GA-treated plots only, to quantify the effects of GA seed treatment on apical dominance. Foliar internode length, natural canopy height, and length of the longest vine were assessed at 58 DAP in 2015 and 62 DAP in 2016 (GA x PBZ and Apogee® trials) to document effects of the growth regulators on stem elongation and canopy development. Two (GA x PBZ trials) or three (Apogee® trial) plants from each of the 5 replicates (24-hill plots) for each treatment were randomly selected for these measurements. Internode length was measured between the fourth and fifth nodes distal to the apex of the longest vine on each plant. Natural canopy height was measured from the top of the hill to the top of the undisturbed canopy in each plot. Similarly, longest vine length was measured from the top of the hill to the apex of the longest fully extended vine. Longest vine length was not assessed in the Apogee® trial.

Emergence and stem count data were not collected for the 2016 Apogee study or the 2017 PBZ growth-profiling study since the foliar treatments in these trials were applied after the plants
had fully emerged. Foliar fresh weights, tuber number, tuber fresh weight, and specific gravities were assessed at each of the ten harvest dates in the 2017 growth profiling study (Table 1). For specific gravity measurements, five tubers of average weight were selected for each replicate, based on the average weight per tuber from the eight plants harvested per plot. Specific gravity was determined by the weight in air/weight in water method (Gould 1999). Additionally, total biomass (foliar + tuber biomass) and harvest indices (tuber fresh weight as percent of total biomass) were calculated at each harvest.

In preparation for machine harvest (trials 1 to 3, Table 1), vines were flail mowed at 152 to 155 DAP and harvested approximately two weeks later. Tubers were washed, counted, and individually weighed with an automated grader/sorter. Weight data were then sorted into the following seven categories to assess effects of the growth regulator treatments on final tuber size distribution: <113 g, 113 to 170 g, 170 to 284 g, 284 to 340 g, 340 to 397 g, >397 g, and culls. Total yield equals the sum of tuber weights from all size categories. U.S. No. 1 yield equals total yield minus culls and undersize (<113 g) tubers. Marketable yield equals U.S. No. 1 yield plus the yield of undersize tubers.

Data analysis and presentation

For all trials (Table 1), plant development and yield data were subjected to analysis of variance (ANOVA) with variation partitioned into single degree-of-freedom contrasts for main effects ([GA], [PBZ], [Apogee®], DAP, PBZ application timing) and interactions where appropriate. For the PBZ application timing trial (trial 4, Table 1), trends in foliar growth, tuber yield, and total biomass were best described by third- to fifth-degree polynomials (R² ≥0.96 to 0.99, P<0.001) and the data are plotted accordingly versus DAP, along with the associated cumulative growing degree-days (DD) from planting (7.2 C base temperature). The DD data were
obtained from the WSU AgWeatherNet (http://weather.wsu.edu/awn.php) portal for the WSU Othello, WA Research Unit during each year of study. Maximum foliar growth rates for each treatment were determined from plots of the first derivative of the polynomial models of foliar fresh weight (MT ha\(^{-1}\)) versus DAP. Area under the foliar growth curves (AUFGC) provided a measure of foliar biomass x duration and were determined using the area-below-curves macro function in SigmaPlot 11 (Systat Software Inc., www.systat.com). Harvest index, tuber number, average tuber fresh weight, and specific gravity data are plotted (±SE) versus DAP with significant differences between treatments at each harvest (DAP) indicated by LSD (\(P<0.05\)).

**Results**

**GA x PBZ dose responses**

*GA x PBZ\(_{0,125,250}\) (Trial 1)*

An initial study was conducted to determine how foliar applications of PBZ (125 and 250 mg L\(^{-1}\)) prior to tuberization (46 DAP; 319 DD from planting, Fig. 1) affect vine growth, tuber set, yield, and tuber size distribution from GA-treated and non-treated seed of cv Bondi. As expected and consistent with previous work (Herman et al., 2016), treatment of seed with 8 mg L\(^{-1}\) GA significantly (\(P<0.01\)) accelerated plant emergence relative to control (Table 2). Additionally, GA seed treatment decreased apical dominance, resulting in an additional 1.2 stems per plant compared with control. PBZ had no effect on plant emergence or stem number (Table 2) since it was applied post-emergence at 46 DAP (Table 1, Fig. 1).

Foliar internode length, canopy height, and length of the longest vine were compared at 58 DAP (12 days post application). Plants from GA-treated seed had longer internodes, which no doubt contributed to their significantly (\(P<0.01\)) taller canopies and lengthier vines than plants from non-GA-treated control seed (Table 3). Conversely, foliar applications of PBZ alone or in
combination with GA seed treatment reduced internode length and plant height linearly ($P < 0.01$) with increasing concentration, and these effects were visually apparent by 12 days after treatment (Fig. 2). PBZ at 125 mg L$^{-1}$ nullified the stimulatory effect of 8 mg L$^{-1}$ GA seed treatment on internode length and plant height relative to non-treated control (Table 3).

GA seed treatment increased tuber set from 6.4 to 9.5 tubers plant$^{-1}$, resulting in 50% (+144,700) more tubers ha$^{-1}$ than non-treated seed, and decreased average tuber weight by 110 g tuber$^{-1}$ (38%) (Table 2). The effects of PBZ on tuber set and average weight, however, depended on GA (GA x PBZ, $P < 0.01$). Absent GA seed treatment, PBZ increased tuber set from 6.4 to an average of 8.0 tubers plant$^{-1}$ over the two PBZ rates, resulting in 74,600 more tubers ha$^{-1}$ than control, but decreased average tuber weight by 119 g tuber$^{-1}$ (41%) at 250 mg L$^{-1}$. Conversely, PBZ treatment of plants from GA-treated seed decreased tuber set from 9.5 to an average of 7.7 tubers plant$^{-1}$ over the two PBZ rates, which was still 1.3 more tubers plant$^{-1}$ than the non-treated control. PBZ at 250 mg L$^{-1}$ decreased average tuber weight by 50 g tuber$^{-1}$ (28%) when combined with GA.

GA as a sole treatment did not affect total and marketable tuber yields but decreased ($P < 0.07$) the yield of U.S. No. 1 tubers by 12.8 MT ha$^{-1}$ (16%) (Table 2), partly reflecting the GA-induced shift in tuber size distribution to higher yield of undersize (<113-g, non U.S. No. 1) tubers. GA decreased the yields of >397-g and 340 to 397-g tubers by 25.2 (63%) and 2.6 MT ha$^{-1}$ (28%), respectively, and increased the yields of 170 to 384-g, 113 to 170-g, and <113-g tubers by 7.8 (47%), 8.0 (145%), and 7.5 MT ha$^{-1}$ (179%), respectively. As a percentage of marketable yield, the GA-induced shift in tuber size distribution from larger to smaller tubers was considerable (Fig. 3AB, blue polygons).
Regardless of GA seed treatment, total, U.S. No. 1, and marketable yields fell with increasing PBZ concentration and these effects were consistently greater in the GA/PBZ combination treatments despite the lack of a significant interaction (Table 2). Similarly, the effects of PBZ on tuber size distribution depended on GA. PBZ alone increased the yields of <113-g, 113 to 170-g, and 170 to 284-g tubers by 5.1 (121%), 5.2 (95%), and 5.3 MT ha\(^{-1}\) (32%), respectively, but decreased the yields of 340- to 397-g tubers by 3.6 MT ha\(^{-1}\) (39%) and >397-g tubers by 31.8 MT ha\(^{-1}\) (79%). When coupled with GA seed treatment, PBZ had comparably little effect on increasing the yield of undersize (<113-g) tubers, but decreased the yields of all other size classes by 37 to 75%, depending on class. Figure 3 summarizes the PBZ-induced shifts in tuber size distribution as percent marketable yield with and without GA seed treatment.

\(\text{GA x PBZ}_{0,40,80,120} \) (Trial 2)

The 2015 trial demonstrated that PBZ concentrations were likely too high when applied prior to tuberization at 187 L ha\(^{-1}\). PBZ concentrations were consequently lowered to 0, 40, 80, and 120 mg L\(^{-1}\) for the 2016 trial. Consistent with results in 2015, the 8 mg L\(^{-1}\) GA seed treatment in 2016 accelerated emergence, decreased apical dominance, increased plant height and tuber set, decreased average tuber weight, and shifted tuber size distribution toward higher yields of smaller (≤284-g) tubers at the expense of larger (>397-g) tubers (Tables 4 and 5). GA seed treatment alone had no effect on total, U.S. No. 1, and marketable yields in 2016 (Table 4). The resulting shift in tuber size distribution as percent marketable yield (Fig. 4AB, blue polygons) was thus comparable to that induced by GA in 2015 (Fig. 3AB, blue polygons).

In 2016, PBZ treatments were applied 44 DAP (349 DD from planting, Fig. 1) during the early stages of tuberization (stages III to IV) as defined by Weeda et al. (2009) (Fig. 5A). Consistent with the 2015 trial (Table 3), internode length decreased linearly with increasing PBZ
However, contrary to the results in 2015, GA seed treatment did not potentiate the PBZ-induced inhibition of internode elongation in 2016. PBZ at 40 and 80 mg L\(^{-1}\) had little effect on plant height. On the other hand, 120 mg L\(^{-1}\) PBZ decreased natural canopy height and vine length by 13% on average in plants from non-GA-treated seed, and by 30 and 24%, respectively, in plants from the GA-treated seed (GA x PBZ, \(P<0.05\)).

PBZ had no effect on tuber set of plants from the non-GA-treated seed, which averaged 6.6 tubers plant\(^{-1}\) (320,000 tubers ha\(^{-1}\)), but tuber set decreased from 10.2 (492,800 tubers ha\(^{-1}\)) to 8.9 tubers plant\(^{-1}\) (429,100 tubers ha\(^{-1}\)) with increasing PBZ concentration on plants from GA-treated seed (Table 4). Tuber fresh weight only dropped an average of 26 g tuber\(^{-1}\) (10%) as PBZ increased from 0 to 120 mg L\(^{-1}\) across GA treatments, reflecting the relatively modest (compared with 2015) decline in yields of larger tubers and increase in yields of smaller tubers. Therefore, the lower concentrations of PBZ in 2016 did not alter tuber size distribution meaningfully as a percentage of marketable yield (Fig. 4) when compared with the higher concentrations used in 2015 (Fig. 3).

Prohexadione-calcium dose responses (Trial 3)

Foliar applications of prohexadione-calcium (Apogee\(^\text{®}\)) were also evaluated for their potential to modulate foliar growth, tuber set, size, and yield in 2016. Like PBZ, Apogee\(^\text{®}\) was applied 44 DAP (422 DD); hence, emergence and stem numbers were not affected by treatments. Plant internode length and natural canopy height at 62 DAP (18 days post treatment) decreased linearly (\(P<0.005\)) by 32 and 26%, respectively, with increasing concentration. However, prohexadione-calcium had no effects on tuber number per plant (avg = 5.6 ±0.17), total, U.S. No. 1, and marketable tuber yields (avg = 84.7 ±2.6, 75.1 ±2.7, and 78.9 ±2.7 MT ha\(^{-1}\), respectively), tuber size distribution, and tuber fresh weight (avg = 290 ±5 g tuber\(^{-1}\)). Based on these results, prohexadione-calcium was not evaluated further.
PBZ application timing (Trial 4)

The 2015 and 2016 trials demonstrated that the effects of PBZ depended on concentration and GA seed treatment, and further suggested that PBZ application-timing relative to tuberization was important for effects on tuber set, average tuber size (Tables 2 and 4), tuber size distribution (Figs. 3 and 4), and possibly source/sink partitioning. To examine this directly, we compared the effects of PBZ applied before (51 DAP) and after (61 DAP) tuberization on the time course of foliar and tuber growth over a 153-d growing season in 2017.

While the first application (51 DAP) of PBZ in 2017 occurred 5 to 7 days later than in the 2015 and 2016 trials (Table 1), tuberization was significantly delayed by the comparatively cool spring in 2017 (Figs. 1 and 5B). The accumulated DD at early application in 2017 (311 DD) was thus comparable to that in 2015 (319 DD) when PBZ was applied pre-tuberization at 46 DAP (Fig. 1). Stolon development at 51 DAP in 2017 was exclusively stage I (Fig. 5B), “hooked…with no apparent swelling” (Weeda et al. 2009). Regardless of treatment, tuber initiation (stages II to III; swelling in terminal 5 mm of stolon with apical hook opening or fully open, Weeda et al. 2009) was first noted at 54 DAP in 2017, with plants averaging 0.36 tubers plant−1 and 156 mg tuber−1.

By 61 DAP (late application at 405 DD, Fig. 1), tuber development had progressed to stages IV (developing tuber is approximately twice the stolon diameter) to VII (2.5 to 5.0-g tubers; Weeda et al. 2009) and the 51-DAP-treated plants averaged 8.8 tubers (4.45 ±0.22 g tuber−1) compared with only 3.9 tubers plant−1 (4.42 ±0.46 g tuber−1) from the non-PBZ treated plants (P<0.05) (Fig. 6A). Tuber number per plant had reached maximum for all treatments by 69 DAP (Fig. 6A) where the early-treated plants averaged 11.1 tubers plant−1, compared with 7.9 and 7.3 tubers plant−1 from the check and late PBZ-treated plants (P<0.01), respectively. Average tuber fresh weights at 69 DAP remained unaffected by PBZ (avg = 18.7 ±0.91 g tuber−1) (Fig. 6B). Tuber numbers per plant
were somewhat variable with subsequent samplings through season end (153 DAP); however, from 61 to 153 DAP, the early-treated plants consistently produced more tubers plant\(^{-1}\) \((P<0.01)\) than the non- and late-treated plants, which did not differ (Table 6, Fig. 6A). From 69 to 153 DAP, the early-treated plants averaged 2.4 more tubers plant\(^{-1}\) \((P<0.05)\) than check and late-treated plants (Fig. 6A, inset).

In addition to increasing tuber set, the early application of PBZ significantly reduced tuber growth rate (g tuber\(^{-1}\) d\(^{-1}\)) from 61 to 153 DAP (Fig. 6B; Table 6, UTC vs PBZ\(_{51}\) x DAP, \(P<0.001\)). Tuber growth over this period was linear \((R^2= 0.99, P<0.001)\) regardless of treatment. However, tuber fresh weight only increased by 2.12 g tuber\(^{-1}\) d\(^{-1}\) for plants treated prior to tuberization (Fig. 6B; Table 6, UTC vs PBZ\(_{51}\) x DAP, \(P<0.001\)) compared with an average of 3.13 g tuber\(^{-1}\) d\(^{-1}\) from the non-treated and late-PBZ-treated plants (Fig. 6B; Table 6, UTC vs PBZ\(_{61}\) x DAP, ns). The plants treated prior to tuberization therefore produced smaller tubers (g tuber\(^{-1}\)) from 100 to 153 DAP than control and post-tuberization PBZ-treated plants (Fig. 6B). Importantly, the increased number of tubers resulting from application of PBZ prior to tuberization (Fig. 6A) offset the lower average fresh weight per tuber (Fig. 6B), and final tuber yields were equivalent for all three treatments \((avg = 98.0 \pm 10.2 \text{ MT ha}^{-1})\) (Fig. 7, Table 6).

Regardless of application timing, PBZ significantly reduced foliar growth rates when compared with the untreated control (UTC) (Fig. 7; Table 6, UTC vs PBZ\(_{51,61}\) x DAP, \(P<0.004\)). Foliar growth rates reached a maximum at ca 66 DAP for all treatments, averaging 2.55 (UTC) versus 1.42 and 1.48 MT fresh weight per hectare per day for the 51- and 61-DAP PBZ treatments, respectively. Similarly, foliar fresh weights (MT ha\(^{-1}\)) increased to a maximum at ca 104 DAP (1008 DD) for all treatments (Fig. 7), but maximum foliar yields were only 63 and 72 MT ha\(^{-1}\) for the pre- and post-tuberization PBZ treatments, respectively, compared with 95 MT ha\(^{-1}\) for the
UTC (Fig. 7). PBZ treatment prior to tuberization consequently reduced total biomass (foliage + tubers) the most over the 153-d growing season (Fig. 7, Table 6), resulting in higher ($P<0.05$) harvest indices (ratio of tuber yield to total biomass) at 69 and 153 DAP when compared with the non-treated plants (Fig. 8, Table 6). Plants treated with PBZ after tuberization had a higher harvest index (HI) than non-treated plants at 100, 135, and 153 DAP. (Fig. 8). The increased source/sink efficiency resulting from PBZ inhibition of foliar growth was also evident by comparing ratios of the areas under foliar growth curves (AUFGC) to final tuber yields. In this case, AUFGC is a measure of ‘source’ (foliar yield and duration over the 153-d growing season) with final tuber yield constituting the ‘sink’. Plants treated with PBZ prior to and after tuberization averaged 51.0 and 53.8 AUFGC per metric ton of tubers, respectively, compared with 72.6 for non-treated plants (Fig. 8 inset), underscoring the increased source/sink efficiency attributable to PBZ-inhibition of foliar growth in this cultivar. This increased efficiency was realized with no cost to tuber specific gravity (Fig. 9), an important indicator of tuber maturity that can affect frozen process quality (Wohleb et al. 2014).

Discussion

Gibberellins (GA) stimulate foliar growth and inhibit tuber initiation in potato (Carrera et al. 1999; Kloosterman and Bachem 2014; Sharma et al. 1998). GA$_1$ has been implicated since at least 1969 (Smith and Rapaport 1969) to be partly responsible for promoting stolon elongation and inhibiting tuber initiation (see also Carrera et al. 2000). Therefore, inhibiting GA biosynthesis by limiting the production of precursors of bioactive GA ($ent$-kaurene, $ent$-kaurenoic acid, GA$_{12, 53, 44, 19}$) and especially GA$_{20}$, a non-bioactive translocatable form (Binenbaum et al. 2018; Bömke and Tudzynski 2009), may provide a means to decrease the level of GA$_1$ in stolons and stimulate tuberization. This idea is supported by Kloosterman and Bachem (2014) who reported that the
expression of *StGA3ox2* decreases (codes for GA$_3$-oxidase, synthesizes GA$_1$) and *StGA2ox1* increases (codes for GA$_2$-oxidase which catabolizes GA$_1$) in stolons during tuber initiation (see also Kloosterman et al. 2007).

Paclobutrazol and prohexadione-calcium (Apogee®) are plant growth retardants that target different steps in the biosynthetic pathway of GAs. Paclobutrazol (PBZ), a triazole-based inhibitor of GA biosynthesis, blocks the action of *ent*-kaurene oxidase, decreasing the synthesis of *ent*-kaurenoic acid and thereby limits all subsequent steps leading to synthesis of bioactive GAs (Rademacher 2016). Foliar application of PBZ can thus decrease GA content throughout the plant (Rademacher 2000). On the other hand, prohexadione-calcium, an acylcyclohexanedione, specifically inhibits 3β-hydroxylation of GA$_{20}$ by GA 3-oxidase, preventing the synthesis of bioactive GA$_1$ (Brown et al. 1997). Both PBZ and prohexadione-calcium would therefore be expected to decrease foliar growth and thereby modify foliar/tuber allometric partitioning in potato. However, since prohexadione-calcium is mostly translocated acropetally (Evans et al. 1999), its inhibitory activity would be restricted to the foliage treated (Beam and Askew 2007). Consequently, in contrast to PBZ, prohexadione-calcium would likely not reduce GA$_1$ synthesis from GA$_{20}$ in stolons, resulting in continued elongation and no effect on tuberization. Indeed, prohexadione-calcium markedly reduced canopy height of cv Bondi by 16 days after treatment without affecting tuber number, average weight, or yield (see Results).

There are several possible explanations for the lack of significant effects of prohexadione-calcium on tuber set, size, and yield. First, prohexadione-calcium blocks the conversion of GA$_{20}$ to GA$_1$, resulting in an accumulation of GA$_{20}$. When the inhibitor effect dissipates after about two weeks (Ilias et al. 2007), the pool of GA$_{20}$ in foliage would be readily converted to GA$_1$, resulting in the resumption of normal foliar growth (Rademacher 2016). Moreover, while GA$_1$ synthesis
would be inhibited by prohexadione-calcium in foliage, translocation of GA$_{20}$ to stolons would likely continue unabated, providing substrate for the synthesis of GA$_1$ and continued inhibition of tuberization. Additionally, prohexadione-calcium was applied post tuberization (422 DD) in the 2016 trial. Further decrease in GA after tuber set (Fig. 5A) would likely not affect tuber number per plant, which also explains the lack of PBZ effect in the 2016 trial. Further work to quantify and compare GA levels as affected by timing of application is needed to fully explain the effects of PBZ and prohexadione-calcium on changes in the foliar/tuber allometric relationships characterized in our studies.

In 2015, PBZ was applied prior to tuberization at 319 DD (46 DAP) (Fig. 1). This treatment effectively increased tuber set (Table 2), decreased average tuber mass (Fig. 3), and reduced foliar growth (Fig. 2; Table 3). By contrast, PBZ applied post tuberization (44 DAP, 349 DD) as a sole treatment (i.e. without GA) at 120 mg L$^{-1}$ in 2016 decreased foliar growth (Table 5, PBZ$_{LT}$, $P<0.01$) without affecting tuber number and total and marketable yields compared with control (Table 4). Arpiwi (2003) successfully modulated tuber set and size of cvs Atlantic and Granola with foliar applications of PBZ (0–250 mg L$^{-1}$) applied 42 and 46 days after planting (DAP). Although tuber set was markedly increased by 74 DAP, this effect dissipated and by final harvest (104 DAP) the number of tubers per plant was unaffected by PBZ. The yield of smaller tubers, however, was significantly increased, demonstrating the efficacy of PBZ for modulating tuber size distribution when applied during early tuber initiation. Tekalign and Hammes (2005) showed that PBZ applied as a foliar spray (0–4 kg ha$^{-1}$) at 30 DAP, significantly reduced foliar growth while increasing tuber yields. The effects of PBZ on tuber number per plant and tuber size distribution were not documented in their study. To better resolve the effects of application timing on potato growth and development, Mabvongwe et al. (2016) applied foliar sprays of PBZ (250 g ha$^{-1}$) at 28,
35, and 42 DAP. All applications of PBZ shortened stems, decreased tuber set, and increased tuber yield. Collectively, these studies suggest the potential for using PBZ to optimize source/sink relationships in relation to management inputs. However, there were substantial dissimilarities in the quantity of product applied, timing of application, and yield-component data collected and analyzed among the studies. We therefore profiled crop growth and development through the season in 2017 to assess the relative effects of PBZ applied either prior to or after tuberization on foliar and tuber growth.

Foliar application of 120 mg L\(^{-1}\) PBZ prior to tuberization (311 DD, 51 DAP) increased tuber set per plant by an average of 2.4 tubers when compared with the non-treated and post-tuberization-treated plants (Fig. 6A). Furthermore, this increase in tuber number attenuated tuber growth rate (Fig. 6B), resulting in smaller average tuber size, without decreasing yield (Fig. 7, Table 6). These effects, along with the significant reduction in foliar growth are desirable for cv Bondi, which produces overly large tubers (Herman et al. 2016; Oliveira, 2015). By contrast, the post-tuberization application of PBZ at 405 DD (61 DAP) had no effect on tuber set (Fig. 6A; Table 6, UTC/PBZ\(_{61}\) x DAP, ns) or average tuber size (Fig. 6B; Table 6, UTC/PBZ\(_{61}\) x DAP, ns); however, the treated plants produced comparable tuber yield with less foliage, resulting in increased harvest index (Fig. 8 inset table; Table 6, UTC/PBZ\(_{51,61}\), \(P<0.01\)).

An improved efficiency of tuber biomass production per unit foliar growth has the potential to benefit potato production. We demonstrated that PBZ applied at 120 mg L\(^{-1}\) just prior to tuber set reduces foliar growth, increases tuber number per plant, and decreases average tuber size without reducing total yield. This shift in phenotype can be advantageous for many cultivars given the market-driven, financial incentives/penalties for delivery of particular tuber size classes (Bolotova and Patterson 2009). For example, Dean et al. (2018) demonstrated an increased seed
market value for cv Shepody by shifting tuber size distribution to favor higher yields of smaller tubers.

The PBZ-induced reduction in foliar growth without reduction in yield underscores the concept that source/sink relationships are not intrinsically optimized in potato varieties, as has been demonstrated for many crops (e.g., rice, wheat, tree fruits, etc.) (Davière and Achard 2013; Rademacher 2000). This creates opportunity for manipulating allometric relationships with plant growth regulators in potato to improve cropping efficiency and possibly profit potential. In the case of cv Bondi, reduction of foliar biomass by PBZ treatment did not necessarily equate to reduction in assimilate partitioning to support tuber (sink) growth. Tekalign and Hammes (2005) showed that applications of PBZ to potato plants in greenhouses increased chlorophyll $a$ and $b$ content by 72 and 121%, respectively, which contributed to increasing net photosynthesis by 61% compared with control. Other crops (e.g., wheat, rice, and apple) have also benefitted from diminished foliar growth through inhibition of GA, resulting in improvements in yield, water use efficiency, and abiotic stress resistance (Greene 1999; Lo et al. 2017; Rebetzke et al. 2012). Given the PBZ-induced reduction in plant biomass and increase in HI observed in our studies, further work should focus on determining the extent to which PBZ treatments enable reduction in agronomic inputs to enhance sustainability and profit potential in potato production.

**Acknowledgements**

Financial support was provided by the USDA Specialty Crop Block Grant program through the Washington State Department of Agriculture and the Northwest Potato Research Consortium.
References

Arpiwi, N.L.  2003.  The application of novel methods for increasing the yield of small round seed potatoes (*Solanum tuberosum* L.) varieties Atlantic and Granola.  MS Thesis, University of Western Australia: School of Plant Biology.


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Table 1. Plant growth regulator treatments and experimental designs for field trials on cv Bondi potatoes at Othello, WA.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Planting dates</th>
<th>Vine kill (DAP)</th>
<th>Randomized block designs and treatments$^a$</th>
<th>Replicates</th>
<th>Foliar applications$^b$ (DAP)</th>
<th>PBZ</th>
<th>Apogee$^c$</th>
</tr>
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<td>n/a</td>
</tr>
<tr>
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<td>4/15/16</td>
<td>153</td>
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<td>n/a</td>
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<tr>
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<tr>
<td>4</td>
<td>4/12/17</td>
<td>n/a$^d$</td>
<td>Factorial – UTC; PBZ$_120$ x 10 harvests</td>
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<td>51; 61</td>
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</tr>
</tbody>
</table>

$^a$GA, PBZ (paclobutrazol), and Apogee$^c$ (prohexadione calcium) subscripts indicate treatment concentrations in mg L$^{-1}$; UTC, untreated control; GA was applied as a seed treatment (2.5 L MT$^{-1}$) prior to planting.

$^b$187 L ha$^{-1}$

$^c$n/a, not applicable

$^d$Sequential hand harvest trial (10 harvest dates)
Table 2. Effects of gibberellin (GA) seed treatment and paclobutrazol (PBZ) foliar treatments on plant emergence, stem number per seed piece, yields, tuber size distributions, tuber number per plant, and average tuber weight of cv. Bondi potatoes at Othello, WA in 2015.

<table>
<thead>
<tr>
<th>GA&lt;sup&gt;a&lt;/sup&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>PBZ&lt;sup&gt;b&lt;/sup&gt; (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Emerg 32 DAP (%)</th>
<th>Stem No.</th>
<th>Bondi Tuber Yield (MT ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Marketable Tubers</th>
<th>Marketable Tubers</th>
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<td>10.7</td>
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<th>13.9</th>
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<th>10.8</th>
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<td>0.01</td>
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<sup>a</sup>Seed was treated with GA (2.5 L MT<sup>-1</sup>) 3 d prior to planting on April 13, 2015.
<sup>b</sup>PBZ was foliar applied (187 L ha<sup>-1</sup>) at 46 DAP.
<sup>c</sup>Marketable (Mkt) tubers = U.S. #1 tubers +<113-g tubers.
<sup>d</sup>LSD, least significant difference, P<0.05.
<sup>e</sup>Results of analysis of variance with sources of variation and associated P-values (LT, linear trend; Dev, deviations from linearity; ns, not significant).
Table 3. Effects of gibberellin (GA) seed treatment and paclobutrazol (PBZ) foliar treatments on foliar internode length, canopy height, and length of the longest vine of cv. Bondi potatoes at Othello, WA in 2015.

<table>
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<tr>
<th>GA (mg L⁻¹)</th>
<th>PBZ (mg L⁻¹)</th>
<th>Internode Length (cm)</th>
<th>Plant height (cm) at 58 DAP</th>
<th>Natural canopy</th>
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*Seed was treated with GA (2.5 L MT⁻¹) 3 d prior to planting on April 13, 2015.
*bPBZ was foliar applied (187 L ha⁻¹) at 46 DAP.
*cLength between fourth and fifth node distal to the apex of the longest vine.
*dMeasured from top of hill to top of undisturbed canopy.
*eTotal length from top of hill to apical meristem with longest vine fully extended
*fLSD, least significant difference, P<0.05.
*gResults of analysis of variance with sources of variation and associated P-values (LT, linear trend; Dev, deviations from linearity; ns, not significant)
Table 4. Effects of gibberellin (GA) seed treatment and paclobutrazol (PBZ) foliar treatments on plant emergence, stem number per seed piece, yields, tuber size distributions, tuber number per plant, and average tuber weight of cv. Bondi potatoes at Othello, WA in 2016.

<table>
<thead>
<tr>
<th>GA (mg L⁻¹)</th>
<th>PBZ (mg L⁻¹)</th>
<th>Emerg 24 DAP (%)</th>
<th>Stem No.</th>
<th>Bondi Tuber Yield (MT ha⁻¹)</th>
<th>Marketable Tubers</th>
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<tr>
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<td></td>
<td>Total</td>
<td>U.S. #1 &lt;113g</td>
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<td>90.0</td>
</tr>
<tr>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>86.3</td>
<td>76.7</td>
</tr>
<tr>
<td>80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84.9</td>
<td>73.5</td>
</tr>
<tr>
<td>120</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>80.4</td>
<td>70.3</td>
</tr>
</tbody>
</table>

LSD₉₅ₑₒᵦ 0.05 0.01 14.4 0.9 9.4 10.8 2.0 2.5 5.4 3.7 3.3 8.5 9.3 22.6 0.7 32.6

aSeed was treated with GA (2.5 L MT⁻¹) 4 d prior to planting on April 15, 2016.

bPBZ was foliar applied (187 L ha⁻¹) at 44 DAP.

cMarketable (Mkt) tubers = U.S. #1 tubers +<113-g tubers.

dLSD, least significant difference, P<0.05.

eResults of analysis of variance with sources of variation and associated P-values (LT, linear trend; QT, quadratic trend; Dev, deviations from linearity; ns, not significant).
Table 5. Effects of gibberellin (GA) seed treatment and paclobutrazol (PBZ) foliar treatments on foliar internode length, natural canopy height, and length of the longest vine of cv. Bondi potatoes at Othello, WA in 2016.

<table>
<thead>
<tr>
<th>GA(^a) (mg L(^{-1}))</th>
<th>PBZ(^b) (mg L(^{-1}))</th>
<th>Internode Length(^c) (cm)</th>
<th>Plant height (cm) at 62 DAP</th>
<th>Natural canopy(^d)</th>
<th>Longest vine(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>7.8</td>
<td>54.0</td>
<td>67.7</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>5.8</td>
<td>59.0</td>
<td>59.0</td>
<td>58.8</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>4.2</td>
<td>59.3</td>
<td>71.7</td>
<td>58.8</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>3.3</td>
<td>47.3</td>
<td>58.8</td>
<td>58.8</td>
<td></td>
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<tr>
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<td>8.5</td>
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<tr>
<td>40</td>
<td>6.5</td>
<td>61.3</td>
<td>74.7</td>
<td>76.3</td>
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</tr>
<tr>
<td>80</td>
<td>4.8</td>
<td>61.3</td>
<td>70.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>2.5</td>
<td>44.5</td>
<td>57.7</td>
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</table>

<table>
<thead>
<tr>
<th>LSD(_{0.05})</th>
<th>1.8</th>
<th>7.9</th>
<th>4.0</th>
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<tr>
<td>GA(^f)</td>
<td>ns</td>
<td>ns</td>
<td>0.1</td>
</tr>
<tr>
<td>PBZ(_{LT})</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>PBZ(_{QT})</td>
<td>ns</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>PBZ(_{Dev})</td>
<td>ns</td>
<td>ns</td>
<td>0.01</td>
</tr>
<tr>
<td>GA x PBZ(_{LT})</td>
<td>ns</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>GA x PBZ(_{QT})</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>GA x PBZ(_{Dev})</td>
<td>ns</td>
<td>ns</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\)Seed was treated with GA (2.5 L MT\(^{-1}\)) 4 d prior to planting on April 15, 2016.

\(^b\)PBZ was foliar applied (187 L ha\(^{-1}\)) at 46 DAP and canopy measurements were taken at 62 DAP.

\(^c\)Length between fourth and fifth node distal to the apex of the longest vine.

\(^d\)Measured from top of hill to top of undisturbed canopy.

\(^e\)Total length from top of hill to apical meristem with longest vine fully extended.

\(^f\)Results of analysis of variance with sources of variation and associated \(P\)-values (LT, linear trend; QT, quadratic trend; Dev, deviations from linearity; ns, not significant). LSD, least significant difference at \(P<0.05\).
Table 6. Sources of variation and levels of significance (P-values) for the effects of 120 mg L⁻¹ paclobutrazol (PBZ) applied as a foliar spray (187 L ha⁻¹) at 51 or 61 days after planting (DAP) on growth and yield components of cv Bondi (see Figs. 6, 7, 8, and 9 for growth profiles and related yield components).

<table>
<thead>
<tr>
<th>Sources of Variation¹</th>
<th>Crop Biomass (MT ha⁻¹)</th>
<th>Harvest Index</th>
<th>Tubers per plant</th>
<th>g/tuber</th>
<th>Specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foliage</td>
<td>Tubers</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UTC/PBZ₅₁,₆¹²</td>
<td>0.001</td>
<td>ns³</td>
<td>0.001</td>
<td>0.011</td>
<td>0.044</td>
</tr>
<tr>
<td>PBZ₅₁/PBZ₆¹</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.007</td>
</tr>
<tr>
<td>UTC/PBZ₅₁</td>
<td>0.001</td>
<td>ns</td>
<td>0.001</td>
<td>0.008</td>
<td>0.002</td>
</tr>
<tr>
<td>UTC/PBZ₆¹</td>
<td>0.001</td>
<td>ns</td>
<td>0.01</td>
<td>0.071</td>
<td>ns</td>
</tr>
<tr>
<td>DAP</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>UTC/PBZ₅₁,₆¹ x DAP</td>
<td>0.004</td>
<td>ns</td>
<td>0.029</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>PBZ₅₁/PBZ₆¹ x DAP</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>UTC/PBZ₅₁ x DAP</td>
<td>0.012</td>
<td>ns</td>
<td>0.026</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>UTC/PBZ₆¹ x DAP</td>
<td>0.012</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

¹Data for all yield components except specific gravity were analyzed from 54 to 153 DAP (3 treatments x 8 harvest dates). Specific gravity was analyzed from 61 to 153 DAP.
²UTC, untreated control; PBZ subscripts indicate the timing of foliar application (51 or 61 DAP).
³ns, not significant.
Fig. 1. Cumulative growing degree days (DD) from 0 to 70 DAP for paclobutrazol (PBZ) trials conducted at the WSU Research Unit, Othello, WA from 2015-17. The DAP and corresponding DD (7.2°C base) at PBZ application (circles) are shown in the inset table. See Fig. 5 for the stages of tuberization at PBZ application in the 2016 and 2017 trials.
Fig. 2. Photograph of adjacent PBZ-treated (125 and 250 mg L\(^{-1}\)) and untreated control (UTC) potato (cv Bondi) plots taken on June 10, 2015 (58 DAP) showing differences in natural canopy heights (yellow lines). PBZ was applied to foliage on May 29 (46 DAP) at 187 L ha\(^{-1}\). Tuber yield and canopy data are summarized in Tables 2 and 3, respectively.
Fig. 3. Polygonal plots showing the effects of foliar applications of 0, 125 and 250 mg L\(^{-1}\) PBZ (187 L ha\(^{-1}\)) on the tuber size distributions of cv Bondi grown from GA-treated (8 mg L\(^{-1}\)) and non-treated seed in 2015. Yields are plotted as percent marketable yield for each tuber size class. Tuber yields with ANOVA results appear in Table 2.
Fig. 4. Polygonal plots showing the effects of foliar applications of 0 to 120 mg L\textsuperscript{-1} PBZ on the tuber size distributions produced by cv Bondi grown from GA-treated (8 mg L\textsuperscript{-1}) and non-treated seed in 2016. Yields are plotted as percent marketable yield for each tuber size class. Tuber yields with ANOVA results appear in Table 4.
Fig. 5. Photographs depicting the degree of tuberization at the time of PBZ applications in the 2016 (44 DAP) and 2017 (51 and 61 DAP) trials (see Table 1 and Fig. 1). (A) PBZ was applied at tuberization stages III to IV as defined by Weeda et al. (2009); (B) PBZ was applied prior to tuberization; (C) PBZ was applied at post-tuberization stage VII (Weeda et al., 2009).
Fig. 6. (A) Changes in the number of tubers per plant with time in response to foliar treatments of cv Bondi with PBZ (187 L ha\(^{-1}\) of 120 mg L\(^{-1}\) PBZ) at 51 or 61 DAP in 2017 versus untreated control (UTC) (bars, ±SE). Tubers per plant are averaged from 69 to 153 DAP in the inset table. Letters indicate LSD \(P<0.05\) for comparison of treatments at each DAP and averaged over the 69 to 153-d interval (inset table) (see Table 6 for ANOVA results). Photo shows tubers harvested from a replicate of UTC and PBZ-treated (51 DAP) plots (8 plants per plot) at 69 DAP. (B) Changes in tuber fresh weight (g tuber\(^{-1}\)) with time. Letters indicate LSD \(P<0.05\) for comparison of treatments at each sampling (DAP) (bars, ±SE) (see Table 6 for complete ANOVA results).
Fig. 7. Effects of PBZ application timing on foliar growth, tuber yield, and total biomass (foliar + tuber) of cv Bondi in 2017 (see Table 6 for ANOVA results). PBZ was applied to foliage (187 L ha$^{-1}$ of 120 mg L$^{-1}$ PBZ) before (51 DAP) or after tuberization (61 DAP) versus untreated control (UTC) (see Fig. 5BC). The top axis of each graph shows cumulative degree-days (DD, 7.2°C base) from planting. The DAP, DD, and harvest indices (HI) at maximum foliar growth are shown for each treatment. Harvest index equals tuber fresh weight as percent of total plant (tubers + foliage) fresh weight calculated at maximum foliar growth. The DAP and yields at 50% HI (where foliar and tuber growth curves intersect) are circled.
Fig. 8. Changes in harvest index of cv Bondi as affected by PBZ applications at 51 DAP (pre-tuberization) or 61 DAP (post tuberization) in 2017 versus untreated control (UTC). Harvest index equals tuber yield as percent of total biomass (tubers + foliage, see Fig. 7) calculated at each sampling (DAP). Letters indicate LSD $P<0.05$ for comparison of treatments at each DAP (bars, ±SE) (see Table 6 for complete ANOVA results). Inset table summarizes the effects of PBZ on source/sink efficiency (AUFGC, area under the foliar growth curves shown in Fig. 7).
Fig. 9. Effects of PBZ application timing on tuber specific gravity of cv Bondi in 2017. PBZ was applied to foliage (187 L ha$^{-1}$ of 120 mg L$^{-1}$ PBZ) before (51 DAP) or after tuberization (61 DAP) (UTC, untreated control) (see Fig. 5BC). Letters indicate LSD $P<0.05$ at each DAP (bars, ±SE) (see Table 6 for complete ANOVA results).
CHAPTER THREE
Developmental and postharvest physiological phenotypes of engineered potatoes grown in the Columbia Basin

Abstract
The Innate® potato cultivars, Glaciate, Acclimate, and Hibernate are engineered for bruise and cold sweetening resistance, low acrylamide forming potential and increased Late Blight resistance. Phenotyping trials from 2015 to 2017 characterized the in-field performance, postharvest physiology, and process quality of these cultivars in the Columbia Basin of Washington. Plant growth, tuber yields and specific gravities of Acclimate and Hibernate were comparable to their respective parental cultivars, Ranger Russet and Atlantic. Glaciate exhibited lower yield and specific gravity compared with Russet Burbank. All Innate® cultivars had lower reducing sugars at harvest than their conventional counterparts resulting in superior process fry color. Asparagine (Asn) increased many-fold during development of Russet Burbank and Atlantic tubers but remained relatively low in Innate® tubers due to silenced Asn synthetase. Tuber respiration rates during sequential periods of low temperature sweetening (LTS, 4°C), reconditioning (16°C), and subsequent cold storage (4°C) were largely comparable for Acclimate versus Ranger and for Hibernate versus Atlantic. Glaciate tubers, however, maintained significantly higher respiration rates than Russet Burbank tubers. Silenced invertase conferred resistance to reducing sugar buildup at 4°C, resulting in superior process quality of the Innate® cultivars regardless of storage temperature. However, cold storage (4°C) induced substantial increases in Suc concentration of Innate® tubers, which was then lowered 43-73% by reconditioning at 16°C. Subsequent storage at 4°C had no further effect on Suc concentrations, demonstrating the efficacy of reconditioning as a
management technique to reduce and minimize Suc levels during prolonged cold storage of Innate® tubers. While heat stress exacerbated the cold-induced buildup of Suc in Innate® tubers and reducing sugars in the parental cultivars, the increases in total sugar (Suc + Glc + Fru) concentrations remained equal, indicating similar elevated levels of starch catabolism during LTS of heat-stressed tubers. However, by virtue of silenced invertase, Innate® tubers were highly tolerant of heat stress for retention of process quality.
1. Introduction

Russet Burbank, Ranger Russet, and Atlantic are important industry-standard cultivars for frozen French fry and chip production in North America (Belyea et al., 2010; NASS, 2018). These cultivars often dominate their respective markets because of superior processing and culinary attributes; however, they also have major weaknesses and are targets for replacement by breeding programs in the United States. One of the primary deficiencies in the three cultivars is their susceptibility to low temperature sweetening (LTS), an accumulation of sucrose and/or reducing sugars at storage temperatures below 8°C. As Maillard substrates (Maillard, 1916), reducing sugars are responsible for dark, undesirable process color and, in the presence of asparagine, elevated acrylamide in chips and fries (Mottram et al., 2002; Muttucumaru et al., 2014; Rosen et al., 2018). Russet Burbank (frozen processing) is susceptible to bruising, external and internal defects, LTS, stress-related physiological disorders, and various pathogens (Bethke et al., 2014; Love et al., 2003). Ranger Russet (frozen processing) is highly sensitive to blackspot bruise, LTS, and cannot be stored for long periods because of short dormancy (Love et al., 2003; Pavek et al., 1992; Woodell et al., 2004). Atlantic (chip processing) is also sensitive to environmental stressors, various defects (e.g., internal brown spot), many diseases, and sugar accumulation during storage, rendering it typically a straight-to-process cultivar (Cropwatch, 2019; Love et al., 2003; Pavlista and Ojala, 1997).

While breeding programs have successfully developed new cultivars with superior traits, these mainstay cultivars continue to dominate many production areas. This is partly due to the slow pace of adoption by processors and the quick service restaurant (QSR) industry. For example, Clearwater Russet (Novy et al., 2010), an LTS-resistant cultivar, took eight years of evaluation following its release in 2008 to be accepted by McDonald’s Corp. in 2016 as one of only seven
cultivars meeting its gold-standard French fry metric set by Russet Burbank. A cultivar may have many traits of value for late-season production, including disease resistance, low asparagine (Asn) and thus acrylamide-forming potential, and long dormancy but still be rejected by processors because of textural properties of fries not aligning with Russet Burbank.

The gradual pace of cultivar replacement is reflected by the change in acreage devoted to Russet Burbank over time. In 2013, 39.6% of the U.S. fall potato crop acreage (average of top seven potato producing states) was planted with Russet Burbank (NASS, 2013) and this cultivar still dominated fall production in 2018 at 37.8% (NASS, 2018). Similarly, Russet Burbank and Ranger Russet accounted for 42.0 and 13.8% of Pacific Northwest acreage in 2013, respectively, with only small decreases to 37.0 and 11.6% in 2018. The Pacific Northwest leads the nation in production of these late-season cultivars for the frozen French fry process industry (NASS, 2018).

As an alternative to conventional breeding, genetic engineering enables trait improvement for cultivars that already enjoy robust market share. Using genes from wild and domesticated potato, the J.R. Simplot Co. (Boise, ID) used Agrobacterium to transform cvs Russet Burbank, Ranger Russet, and Atlantic for improved traits. The first generation Innate® cultivars, approved for production in 2014, have increased resistance to blackspot bruise and acrylamide formation during frying by virtue of silenced polyphenol oxidase (PPO) and asparagine synthetase, respectively, and silencing of α-1,4 glucan phosphorylase-L and α-glucan water dikinase to preserve starch (Clark and Collinge, 2013). The second generation Innate® cultivars (Glaciate, Acclimate, Hibernate) added an R-gene for increased resistance to Late Blight (Phytophthora infestans), along with silenced vacuolar acid invertase to prevent cold-induced reducing sugar buildup (Clark et al., 2014; Pence et al., 2016). The U.S. Environmental Protection Agency approved these Innate® cultivars for commercial production in 2017.
Developing new cultivars through to adoption by industry entails extensive phenotyping, including evaluation of plant performance and yield potential in major production regions. As a part of this effort, we compared the developmental and postharvest physiological phenotypes of Acclimate and Hibernate to their respective parental cultivars, Ranger Russet and Atlantic. Acclimate and Hibernate were commercialized in 2017. We also compared Glaciate to its parental counterpart, Russet Burbank, but Glaciate has not been commercialized.

The project was conducted in the Columbia Basin of Washington, a major production region responsible for 65% of the U.S. fall crop of potatoes (total tonnage basis) (NASS, 2018). Environmental and edaphic conditions in this area are ideal for potato production, resulting in the highest average yield (ca 70 MT ha\(^{-1}\)) of potatoes worldwide. Yields of late-season frozen-processing russet cultivars are even higher in this area, with 75-90 MT ha\(^{-1}\) yields routinely produced. While these yields remain well below the estimated theoretical yield of 160 MT ha\(^{-1}\) for late season cultivars in this region (Haverkort and Struik, 2015; Kunkel and Campbell, 1987), in-season and postharvest management have been optimized for these late-season cultivars through many decades of production, making the Columbia Basin ideal for phenotyping the Innate\(^{®}\) cultivars. The trials revealed unique characteristics of the Innate\(^{®}\) cultivars that affect crop productivity and quality and provide information that may be useful in optimizing management practices.

2. Materials and Methods

2.1. Summary of trials

Differences in crop growth and development, yield, postharvest physiology, and process quality were evaluated for the potato (Solanum tuberosum L.) cultivars, Russet Burbank, Ranger Russet, and Atlantic, versus their respective Innate\(^{®}\)-engineered counterparts, Glaciate, Acclimate,
and Hibernate. Crop growth profiling and final yield phenotyping were accomplished in a series of field trials conducted during the 2015-17 growing seasons at the Washington State University (WSU) Irrigated Research Unit in the central Columbia Basin at Othello, WA (46° 47.277’ N. Lat., 119° 2.680’ W. Long.). The crop growth profiling studies focused on comparing plant emergence, foliar and tuber biomass production, and harvest indices (tuber biomass/foliar + tuber biomass) over time. These trials necessitated multiple in-season harvests to collect the foliar and tuber fresh weight data for modeling growth and development for each pair of cultivars (parental vs Innate®). Changes in tuber sucrose (Suc), reducing sugars (Glc + Fru), specific gravity, and free amino acids during tuber development were also compared. Separate trials conducted concurrently and using the same seed as the profiling studies characterized the effects of cultivar on final yields, tuber grades, and size distributions.

The postharvest phenotyping studies were conducted at the Postharvest Physiology and Biochemistry Laboratory, Department of Horticulture, WSU (Pullman, WA) using tubers grown full season from the field trials at Othello. These studies assessed the effects of cultivar on changes in tuber respiration, Suc, reducing sugars, and process quality (French fries and chips) before, during, and after sequential periods of low temperature sweetening (LTS, 4°C) and reconditioning (16°C). Effects of heat stress on the LTS phenotypes of parental versus Innate® tubers were also evaluated.

2.2. Crop growth and development profiling trials

The growth and development profiling trials were conducted during the 2015-17 growing seasons. These studies entailed hand harvesting aboveground foliage and tubers at approximately 16-d intervals from 44 to 184 days after planting (DAP) for Ranger Russet versus Acclimate in
2015 and Russet Burbank versus Glaciate in 2015 and 2016. The Atlantic versus Hibernate trials in 2016 and 2017 were harvested at 10-d intervals from 40 to 120 DAP.

2.2.1. Seed handling, field plot designs, and management

Seed tubers for all studies were provided by the J.R. Simplot Company (Boise, ID) each year in early April and held at 4°C (95% RH) until cutting and planting in mid-April. Seed preparation, planting, and field plot designs were as previously described (Ellis et al., 2020) with minor modifications. For the 2015 trials, single-drop seed tubers (70-100-g) were trimmed at their basal ends to produce 50–64-g apical seed pieces, which were then stored for five days at 9°C (95% RH) prior to planting. Seed pieces for the 2016 and 2017 trials were cut from 113–170-g seed tubers, trimmed into 50–64-g pieces, blocked for portion (apical or basal), and stored for 8 days at 9°C (95% RH) prior to planting.

Treatments for all profiling trials consisted of two cultivars (parental vs Innate®) x nine or ten harvest dates factorially arranged in randomized complete block designs with four replicates. The seed pieces were planted 20-cm deep (top of hill to bottom of furrow) in a Shano silt loam soil (Lenfesty, 1967) with a two-row, assist-feed planter. Treatment plots consisted of eight seed pieces (hills) spaced 25.4-cm apart within a row. An additional seed piece of cv All Blue was planted at either end of each treatment plot to facilitate plot identification and to maintain competition for plants at the ends of plots. Fifty-one centimeter alleys separated the treatment plots within each row and treatment rows were flanked by guard rows of the respective parental cultivar to provide uniform competition to plots. Rows were spaced 86-cm apart in 2015 and 81-cm apart in 2016 and 2017.

Each trial was managed using a center pivot system, which delivered water, fertilizer, and pesticides according to best management practices for production of mid and late-season potatoes.
in the Columbia Basin (Hopkins et al., 2007; Lang et al., 1999; Schreiber et al., 2018). Pre-plant fertility adjustments and in-season fertigation rates were determined based on soil tests and weekly petiole analyses, respectively. Neutron probe data in conjunction with evapotranspiration models for potato (WSU AgWeatherNet, https://weather.wsu.edu/) informed irrigation scheduling every season. Soil moisture was maintained at a minimum of 65% field capacity for all trials.

2.2.2. Data acquisition and sample processing

Plant emergence counts were taken ca every 2 days starting 20 DAP through 35-45 DAP depending on year and cultivar. Foliar fresh weights, tuber number, tuber fresh weight, and specific gravities were quantified at each harvest date. At each sampling, four replicates of eight plants per plot for each cultivar were hand harvested. The canopies were excised at hilltop level and total foliar fresh weights immediately recorded for each plot. The tubers were counted, collectively weighed, and five tubers, each approximating the average tuber weight (g tuber⁻¹) per plot, were selected for specific gravity determination. Specific gravity was determined by the weight in air and water method (Pavlista and Ojala, 1997). Each of the five tubers were then cut into apical and basal halves, which were subsequently split longitudinally along the apical to basal axis. A 1.5-mm-thick tissue slice was taken from the longitudinal face of the apical and basal halves of each tuber. The apical slices from the five tubers were pooled separately from the basal slices, resulting in tissue samples representing the apical and basal halves of the five-tuber sample from each plot. For smaller tubers from the initial harvests, the entire apical and basal half from each tuber was saved and combined with the corresponding portions from the other tubers. Tissue samples were frozen, lyophilized, ground (mortar and pestle), and sieved through a 60-mesh screen (246 μm) in preparation for analysis of Glc, Fru, Suc, and free amino acids.
2.2.3. Sucrose, reducing sugar, and amino acid analyses

Sugars were extracted from lyophilized tuber tissue with 60% (v/v) MeOH at room temperature as described in Ellis et al. (2019). Due to the large variation in tissue concentration of Suc and reducing sugars during tuber development, the initial extraction ratio of 80 mg mL\(^{-1}\) dry wt was diluted as necessary to bring the analytes within range of the Glc, Fru, and Suc calibration curves (0.05-1.8 mM each). Sugars in the apical and basal portions of tubers at each harvest were analyzed with the methods of Bergmeyer et al. (1974), Bergmeyer and Bernt (1974), and Bernt and Bergmeyer (1974) as modified by Knowles et al. (2009) for a microplate spectrophotometer.

For amino acid extraction and analysis, equal portions of apical and basal lyophilized tissue were pooled from the 68-, 84-, 113-, 145-, and 167-DAP Russet Burbank versus Glaciate and 50-, 60-, 78-, 99-, and 120-DAP Atlantic versus Hibernate trial samples in 2016. The Russet Burbank and Glaciate samples were extracted (30 mg mL\(^{-1}\)) with 60% (v/v) MeOH and a portion diluted to 5 mg mL\(^{-1}\). Atlantic and Hibernate samples were extracted similarly at 40 mg mL\(^{-1}\) with a portion diluted to 10 mg mL\(^{-1}\). The solutions of greater concentration were utilized for quantifying Gly, Leu, Ile, Thr, Pro, Met, Phe, Lys, His, Tyr, and Trp, whereas the lower concentration samples were used for Ala, Val, Ser, Asn, Asp, Glu, and Gln. Amino acids were derivatized using the EZ:faast\textsuperscript{TM} Amino Acid Analysis Kit for GC-FID (Phenomenex, Torrance, CA) at a volume of 100 μL and in the presence of norvaline (internal standard). The amino acids were analyzed on an Agilent (Santa Clara, CA) model 6890 gas chromatograph with operating conditions as per kit instructions. Calibration curves for individual amino acids were constructed using multiple levels of the kit-supplied authentic standard mixtures. Quantitation was achieved using peak heights relative to the internal standard (Zommick et al., 2013).
2.3. **Final yield and tuber size distribution trials**

Detailed assessments of the effects of cultivar on final yields, tuber grades, and size distributions were conducted during the 2015/16 growing seasons for cvs Russet Burbank versus Glaciate and Ranger Russet versus Acclimate. The Atlantic versus Hibernate trials were conducted in 2016/17. Since cultivar-dependent effects were similar in each year, data were averaged over the 2-year study periods for each pair of cultivars. These trials also provided tubers for the postharvest phenotyping studies (see 2.4 below).

Seed was acquired, prepared, and planted as described for the profiling studies in 2.2.1 above. The late-season frozen-processing cultivar trials (Russet Burbank, Ranger Russet and their Innate® counterparts) were planted in randomized complete block designs with four replicates on 4/15/15 and ten replicates on 4/14/16. The Atlantic versus Hibernate trials had five replicates planted 4/14 each year (2016/17). In contrast to the profiling studies, individual plots were 6.1 m long with 24 seed pieces per plot and were separated by 1.3-m alleys. A single hill of cv All Blue was planted at the ends of each plot and treatment rows were flanked by guard rows of the appropriate parental cultivar (Russet Burbank, Ranger Russet, or Atlantic). All trials were situated in close proximity to the profiling trials and were managed identically (fertility, irrigation, etc.). For the late-season frozen-processing cultivar trials, vines were mowed with a flail type mower 147 DAP in both years. Atlantic and Hibernate vines were mowed at 116 and 110 DAP, in 2016 and 2017, respectively.

Tubers in all trials were harvested with a single-row mechanical harvester approximately two weeks after mowing. Tubers from the Russet Burbank versus Glaciate and Ranger Russet versus Acclimate trials were washed, counted, and individually weighed. The data was then sorted to compare yields of the following tuber weight classes: undersize (<113 g), 113-170 g, 170-284...
Total yield was the combined weight of all tuber size classes. U.S. No. 1 yield equals total yield minus the yield of culls and undersize tubers. Marketable yield equals U.S. No. 1 yield plus the yield of undersize tubers. Atlantic and Hibernate tubers were also individually counted and weighed and the data converted to diameter grade categories relevant to round chipping potatoes using the weight-to-diameter conversion models of Blauer et al. (2013). The tuber diameter grades were: B (0-5.08 cm), A (5.08-8.26 cm), and oversize (>8.26 cm). Marketable yield for cvs Atlantic and Hibernate equals the sum of the yields within these categories.

### 2.4. Phenotyping postharvest physiology

#### 2.4.1. Tuber respiration, LTS, reconditioning, and process quality

Tubers (170-284 g tuber⁻¹) from the 2015 frozen processing russet and 2016 Atlantic versus Hibernate yield trials (section 2.3) were retained for postharvest phenotyping. All tubers were initially wound-healed at 9-10°C (95% RH) for 10-14 d directly following harvest. Storage studies for each pair of cultivars then compared the effects of sequential periods of LTS (4°C) followed by reconditioning (16°C) on changes in tuber respiration, sugar concentrations, and process quality as described by Zommick et al. (2014) with minor modifications. Tubers of each cultivar were blocked for size (190-230 g tuber⁻¹) and enclosed in 3.9-L glass chambers (six tubers per chamber) at 9°C. A continuous flow (ca 86 mL min⁻¹) of CO₂-free air from compressed gas cylinders was directed through the inlet ports to the bottom of the chambers. Outlet ports from the chambers were connected via 3-way solenoid valves and a manifold to an LI-7000 infrared CO₂/H₂O gas analyzer (LI-COR Inc., Lincoln, NB, USA). Respiration rate (CO₂, mg kg⁻¹ h⁻¹) measurements began immediately and continued at 3- to 4-h intervals for 56-77 days, depending on the cultivars.
under study. The studies were setup as randomized complete block designs with four (frozen processing cultivars) or six (Atlantic versus Hibernate) replicates of each pair of cultivars.

The tubers were initially acclimated in the chambers at 9°C for 3-5 days until respiration rates had stabilized. The storage temperature was then rapidly (15 min) lowered at zero-time to 4°C for a 31-d period of LTS and afterwards raised to 16°C for 21 days of reconditioning (29 days for Atlantic versus Hibernate). For the frozen processing cultivars, the temperature was again lowered to 4°C at day 52 for a further 73 days of cold storage. Additional tubers (170-284 g tuber⁻¹) of each cultivar were maintained in the storage for analysis of temperature-dependent changes in sugar and process quality over time. These destructive samplings occurred at 10-d intervals during the LTS period and thereafter weekly during the 21-d reconditioning period. Two additional samples were collected for the frozen processing cultivars during the final 4°C storage period on days 82 and 125.

At each sampling, twelve tubers were blocked for size into four replicates (three tubers per replicate). The tubers were halved longitudinally along the apical to basal axis and a complete ca 1.5-mm-thick slice was cut from the face of one of the halves representing each tuber. The slices from the three tubers comprising a replicate sample were combined, frozen, and lyophilized for subsequent determination of Suc and reducing sugar concentrations as described in section 2.2.3. To determine process quality, a single French fry plank (9.5 mm thick x 2.9 cm wide x length of tuber) was taken from the central portion of the remaining tuber halves. The twelve fry planks were collectively fried in soy oil at 191°C for 3.5 min. Color (i.e. lightness) of the apical and basal ends of each fry plank was quantified with a Photovolt reflectance meter (Model 577, Photovolt Instruments Inc., Indianapolis, IN) within 3 min of frying. A Photovolt reflectance value of ≤19
is considered unacceptably dark by industry standards (Pavlista and Ojala, 1997; Stark et al., 2018).

The Atlantic and Hibernate tubers were processed as chips using an Eagle chip slicer (Eagle Tool & Machine Co., Inc., Springfield, OH). Twelve tubers were halved (as above) from apical to basal end and two chips were shaved from the cut face of one of the halves. One chip was frozen and lyophilized while the second was combined with chips from the other tubers and collectively fried in soy oil at 191°C for 2 min. The chips were then individually pulverized to provide a homogeneous mix for a Photovolt reflectance reading, which represented the average color of each chip. Where pertinent, Photovolt reflectance data were converted to Snack Food Association (SFA) 1 (light) to 5 (dark) ratings based on a standard curve of SFA chip color ratings (subjectively acquired using the SFA chip color chart) versus Photovolt reflectance values ($R^2=0.95$, $P<0.001$, $n=344$). Photovolt reflectance values and SFA ratings are reported along with high-resolution images of representative fries and chips.

2.4.2. Effects of heat stress on LTS

Tuber samples from the 2015 frozen-processing and 2016 Atlantic versus Hibernate cultivar yield trials (section 2.3) were retained for evaluating the effects of heat stress on LTS and process quality phenotypes. Following a brief wound-healing period (see 2.4.1), the tubers (170-284 g tuber$^{-1}$) for each pair of cultivars were blocked for size into eight (frozen process cultivars) or four (chip cultivars) replicates (three tubers per replicate). The tubers were then subjected to heat priming and LTS temperature treatments using the postharvest heat stress (PHHS) protocol described by Herman et al. (2017). The four treatments included (1) control tubers stored at 9°C, (2) heat stressed (HS) tubers stored at 32°C for 21 d, (3) cold storage (CS) at 4°C for 32 days to induce LTS, and (4) HS followed by CS (HS+CS) to determine the extent to which HS modifies
the LTS phenotypes of the parental versus Innate® cultivars. Tubers were then analyzed for Suc, reducing sugars, and process quality as described in sections 2.2.3 and 2.4.1 above. Cultivar-dependent changes in sugar concentrations by treatment were equivalent for the frozen processing cultivars. Therefore, sugar data from cvs Russet Burbank and Ranger Russet were averaged and compared to the average sugar data from cvs Glaciate and Acclimate. French fry and chip sample images depicting the effects of PHHS treatments on process color are presented along with Photovolt reflectance data.

2.5. Data analysis and presentation

For all field trials (sections 2.2 and 2.3), crop development, carbohydrate, amino acid, yield and tuber size distribution data were subjected to analysis of variance (ANOVA) with variation partitioned into single degree-of-freedom contrasts for main effects (cultivar, DAP) and interactions (cultivar x DAP) where appropriate. Trends in foliar growth, tuber yield, total biomass, average tuber weight, and specific gravity with DAP were described by third- to fifth-degree polynomials ($R^2 \geq 0.97-0.99$, $P<0.001$) and the data are plotted accordingly. Tuber bulking rates were defined by the linear regression coefficients from 68-127 DAP for Russet Burbank versus Glaciate, 56-126 DAP for Ranger Russet versus Acclimate, and 71-110 DAP for Atlantic versus Hibernate. Tuber sucrose, reducing sugar and amino acid concentration data ($\pm$SE) are plotted versus DAP.

For the postharvest phenotyping studies, tuber respiration rates during the LTS and reconditioning phases of storage (section 2.4.1) were analyzed by factorial (cultivar x days) repeated measures ANOVA. Tuber sugar concentrations ($\pm$SE) are plotted versus days in storage. High-resolution images of sample French fries and chips are provided with Photovolt reflectance values (separated by LSD, $P<0.01$ or 0.05) to depict the effects of LTS and reconditioning on
process quality. Effects of the PHHS treatments (section 2.4.2) on tuber sugar, French fry, and chip colors were analyzed by factorial ANOVA (parental versus Innate® cultivars x four PHHS treatments) with means separated by LSD ($P<0.05$). Main effects and interactions are reported where pertinent.

3. Results

3.1. Russet Burbank versus Glaciate (late-season frozen processing cultivars)

3.1.1. Crop development, tuber carbohydrate, specific gravity, and amino acid profiles

Trends in plant emergence, foliar growth, and tuber growth of cvs Russet Burbank and Glaciate were similar over the 2015 and 2016 growing seasons. Data were thus averaged over years to characterize and compare the developmental phenotypes of each cultivar. Emergence of Glaciate plants was slightly (1-2 days) delayed ($P<0.05$) compared with Russet Burbank but both cultivars had reached 94 to 100% emergence by 32 days after planting (DAP) in both years (data not shown). Seasonal foliar growth (44 to 184 DAP) was best defined by fifth degree polynomials (avg $R^2=0.98$, $P<0.001$) with maximum foliar yields attained at 89 DAP (935 GDD from planting) for both cultivars (Fig. 1A). However, Glaciate produced 9.6% less foliar growth than Russet Burbank (63 vs 70 MT ha$^{-1}$) at maximum foliar development and 14.1% less area under the foliar growth curve (AUFGC, a measure of foliar yield x duration) from 44 to 184 DAP, characterizing the significant effect of cultivar ($P<0.006$) on foliar development (Table 1).

While not measured directly, tuber set was likely delayed in cv Glaciate compared with Russet Burbank, as evidenced by lower tuber yields ($P<0.05$) at 56 and 68 DAP (Fig. 1A). Increases in tuber yields from 44 to 184 DAP were best described by fourth degree polynomials ($R^2=0.99$, $P<0.001$). Regardless of cultivar, maximum tuber yield was reached at ca 145 DAP (1,696 GDD) when foliar growth had declined to about 35% of maximum. Bulking rates during
the linear phase (R²=0.99, P<0.001) of tuber growth from 68 to 127 DAP depended on cultivar. Over this period, tuber yields increased by 1.37 and 1.08 MT ha⁻¹ d⁻¹ for cv Russet Burbank and Glaciate, respectively, resulting in 15 MT ha⁻¹ lower final yield for Glaciate (80 vs 95 MT ha⁻¹) (CV x DAP, P<0.005, Table 1). Increases in average tuber fresh weights (g tuber⁻¹) with DAP also depended on cultivar (CV x DAP, P<0.002, Table 1), but final weights were equivalent (avg = 184 g tuber⁻¹) (Fig. 1B). The reduced foliar and tuber growth of cv Glaciate translated to lower total biomass (foliar + tuber yield) production than Russet Burbank over the 184-d growing season (Fig. 1A); however, harvest index (tuber yield/total biomass) was not affected by cultivar (Table 1).

On average, Suc concentrations in the stem and bud halves of Russet Burbank tubers fell from 74.1 to 6.1 mg g⁻¹ dry wt as tubers developed from 56 to 127 DAP, compared with a decline from 83.4 to 9.8 mg g⁻¹ dry wt in Glaciate tubers (Fig. 1B, Table 1). Glaciate tubers thus averaged 24% higher Suc concentration than Russet Burbank tubers during the linear phase of tuber growth. Following these initial declines, Suc remained relatively constant in Russet Burbank tubers, averaging 6.1 mg g⁻¹ dry wt from 127 DAP to season end (184 DAP), but increased from 9.8 to 12.4 mg g⁻¹ dry wt in Glaciate tubers over the same period.

In contrast to Suc, changes in tuber reducing sugars (Glc + Fru) over time depended on cultivar (CV x DAP, P<0.001, Table 1). Reducing sugar concentrations were highest in the bud versus stem halves of tubers at 56 DAP and declined rapidly through 84 DAP in both cultivars (Fig. 1C). Moreover, averaged over tuber portion (bud and stem ends), Glaciate® tubers maintained consistently lower reducing sugar concentrations (by 15 to 84% depending on DAP) than Russet Burbank tubers over the entire season. During tuber maturation (145 to 184 DAP), reducing sugars in Glaciate tubers remained low and constant, averaging 0.67 mg g⁻¹ dry wt. By
contrast, reducing sugars increased linearly ($R^2= 0.99, P<0.01$) from 0.68 to 5.12 mg g$^{-1}$ dry wt during maturation of Russet Burbank tubers, resulting in 21% darker stem end fry color than Glaciate tubers at harvest (Fig. 1C).

The specific gravity of Russet Burbank tubers increased from 1.045 (56 DAP) to a maximum of 1.086 at 150 DAP and then declined to 1.081 through 184 DAP (Fig. 1C). Despite similar trends over time, the specific gravity of Glaciate tubers was lower than Russet Burbank tubers throughout the season ($P<0.001$, Table 1), increasing from 1.040 at 56 DAP to a maximum of 1.079 at 156 DAP before declining to 1.076 at season end. Tuber physiological maturity (PM) was defined as the average DAP to reach maximum yield, maximum specific gravity, minimum Suc, and minimum reducing sugars in tubers (Knowles et al., 2015; Wohleb et al., 2014). PM occurred at 142 and 149 DAP for cvs Russet Burbank and Glaciate, respectively (Fig. 1C).

Cultivar-dependent changes in amino acid concentrations were also assessed during tuber development. Except for Asn and Gln, which together accounted for a relatively constant 49.7 ±1.3% (Russet Burbank) and 61.2 ±0.8% (Glaciate) of total free amino acids (mg g$^{-1}$ dry wt$^{-1}$) during tuber development (68-167 DAP), cultivar had little effect on changes in the 16 other amino acids. Therefore, only total free amino acids (sum of 18 amino acids), Asn, and Gln are reported. Total free amino acid, Asn, and Gln concentrations in Russet Burbank and Glaciate tubers at 68, 84, 113, 145, and 167 DAP were regressed against the average tuber fresh weights at each sampling to characterize cultivar-dependent differences with tuber development. Total free amino acid concentrations in Glaciate and Russet Burbank tubers averaged 54.8 and 40.2 mg g$^{-1}$ dry wt, respectively ($P<0.001$), and no clear trends with time (Table 1) or increasing tuber fresh weight (Fig. 2A) were apparent. By contrast, changes in Asn concentrations with tuber development depended on cultivar (CV x DAP, $P<0.007$; Fig. 2B). Asn increased from 2.4 to 7.0 mg g$^{-1}$ dry wt.
in Glaciate tubers as fresh weight increased from 12.9 to 133 g tuber\(^{-1}\) and then decreased to 5.1 mg g\(^{-1}\) dry wt with further growth to 210 g tuber\(^{-1}\). Asn concentration in Russet Burbank tubers, however, increased from 6.8 to 17.8 mg g\(^{-1}\) dry wt as tuber fresh weight increased from 23.3 to 225 g tuber\(^{-1}\) over the bulking period. While Gln concentration remained high and constant at 28.3 mg g\(^{-1}\) dry wt during growth of Glaciate tubers (Fig. 2C, Table 1), it decreased linearly (\(P<0.05\)) from 8.6 to 4.2 mg g\(^{-1}\) dry wt with increasing fresh weight of Russet Burbank tubers. The silenced Asn synthetase in Glaciate tubers was primarily responsible for these cultivar-dependent differences in concentrations of amide amino acids (Asn and Gln) during tuber development.

### 3.1.2. Yield and tuber size distribution trials

The effects of cultivar on final yields and tuber size-distribution phenotypes were evaluated in separate trials conducted alongside the profiling studies described in 3.1.1. Averaged over the 2-year study period, Glaciate produced 2.5 mainstems per seedpiece compared with 2.8 from Russet Burbank (\(P<0.01\)), which likely contributed to the cultivar-dependent differences in tuber set (Table 2). Glaciate averaged 8.8 tubers per plant compared with 10.8 for Russet Burbank (\(P<0.01\)), resulting in 94,000 fewer tubers per hectare for cv Glaciate. Consistent with results from the profiling studies (Fig. 1), total yield of cv Glaciate was lower (by 17.4 MT ha\(^{-1}\)) than that produced by Russet Burbank over the 147-d growing seasons (\(P<0.01\)) (Table 2). U.S. No. 1 and marketable (U.S. No. 1 + undersize) yields were also ca 20% (18.3 MT ha\(^{-1}\)) lower for cv Glaciate, representing significant yield drag for the this cultivar. The lower U.S. No. 1 yield of cv Glaciate was mainly attributable to reduced yields of 113-284-g tubers. Consistent with the profiling study (Fig. 1B), cultivar had no effect on final tuber fresh weight, which averaged 183 g tuber\(^{-1}\) (Table 2).
3.1.3. Phenotyping physiological responses of tubers to changes in storage temperature

Changes in respiration rates (CO$_2$ production), Suc, reducing sugar concentrations, and process quality of Russet Burbank and Glaciate tubers in response to low temperature sweetening (LTS, 4°C) and subsequent reconditioning at 16°C were distinctly cultivar-dependent. Tubers were initially acclimated to 9°C (98% RH) for 14 days following harvest before lowering the storage temperature to 4°C for a 31-d period of LTS. On average, the respiration rate of Glaciate tubers (4.09 ±0.22 mg kg$^{-1}$ h$^{-1}$) was 37% higher than Russet Burbank tubers (2.98 ±0.06 mg kg$^{-1}$ h$^{-1}$) during acclimation (Fig. 3A). Decreasing the storage temperature from 9 to 4°C at zero-time induced a notable respiratory acclimation response (RAR) in tubers of both cultivars that lasted ca 9 days. The cold-induced RARs were characterized by a precipitous 34% drop in respiration rates from 0 to 2 d, followed by a 103% increase from day 2 to 6, and subsequent rapid decline through day 9. Tuber respiration rates then gradually decreased from day 9 to 31. While the cold-induced RARs were similar for both cultivars, Glaciate tubers clearly respired at a significantly higher ($P<0.001$) rate than Russet Burbank tubers throughout the initial acclimation and LTS phases of storage.

Glaciate tubers contained higher Suc ($P<0.01$, Fig. 3B) and lower reducing sugar concentrations ($P<0.01$, Fig. 3C) than Russet Burbank tubers at the end of the 9°C acclimation period (i.e. zero-time). LTS (0-31 d at 4°C) of Glaciate tubers was characterized by comparatively little buildup in reducing sugars (Fig. 3C) but substantial accumulation of Suc (Fig. 3B) because of acid invertase silencing. By contrast, the LTS phenotype of Russet Burbank tubers was defined by rapid accumulation of reducing sugars to high levels (Fig. 3C) with only moderate increases in Suc concentration (Fig. 3B). The reducing sugar buildup in Russet Burbank tubers resulted in deterioration of French fry process color to unacceptable levels ($\leq$19 Photovolt reflectance units).
within the first 10 d of LTS (Fig. 4A). Glaciate tubers, however, maintained acceptable process color throughout the 31-d LTS period.

The storage temperature was increased to 16°C following the 31-d LTS period to compare the physiological responses of tubers to 21 days of reconditioning. Changes in tuber respiration during reconditioning depended on cultivar (CV x Days, \(P<0.001\)). As expected, the increase in temperature from 4 to 16°C prompted an immediate and rapid increase in tuber respiration rates to 15.2 ±1.14 and 11.5 ±0.82 mg g\(^{-1}\) dry wt at day 34 for Russet Burbank and Glaciate tubers, respectively (Fig. 3A). Respiration rates then declined precipitously to 3.19 ±0.11 (Russet Burbank) and 4.0 ±0.21 mg g\(^{-1}\) dry wt (Glaciate) at the end of the reconditioning period (at 52 d).

Reconditioning reduced the concentrations of Suc in Russet Burbank tubers from 19.9 ±4.3 to 11.3 ±2.73 mg g\(^{-1}\) dry wt (Fig. 3B) and reducing sugars from 46.3 ±0.60 to 24.2 ±3.56 mg g\(^{-1}\) dry wt (Fig. 3C). However, despite a 75% improvement in French fry color (Fig. 4A), process quality of Russet Burbank tubers remained unacceptable (≤19 Photovolt reflectance units) following 21 d of reconditioning. By contrast, reconditioning had little effect on the inherently low concentration of reducing sugars in Glaciate tubers (Fig. 3C), but decreased Suc concentration from 66.6 ±5.28 to 39.4 ±3.29 mg g\(^{-1}\) dry wt (Fig. 3B), and improved process color by 29% (Fig. 4A).

Following the 21-d reconditioning period (Fig. 3A), the storage temperature was again dropped to 4°C, resulting in rapid decline in tuber respiration rates from 52 to 57 days, and subsequent establishment of constant rates from 65 days through to the end of the monitoring period at 77 days. Similar to the initial 9°C acclimation period, LTS period, and last half of the reconditioning period, Glaciate tubers established a higher (\(P<0.001\)) rate of respiration (1.58
±0.004 mg g\(^{-1}\) dry wt) than Russet Burbank tubers (1.17 ±0.002 mg g\(^{-1}\) dry wt) during this final period of storage at 4°C.

Contrary to the initial LTS period (0-31 d), 4°C storage following the 21-d reconditioning treatment did not stimulate Suc accumulation in either cultivar. Sucrose concentrations remained constant at post-reconditioning levels, averaging 11.2 ±1.18 mg g\(^{-1}\) dry wt in Russet Burbank tubers and 40.8 ±1.16 mg g\(^{-1}\) dry wt in Glaciate tubers while stored at 4°C from 52 to 125 d (Fig. 3B). Increases in reducing sugar concentrations during this final storage phase were marginal when compared with the increases induced by storage at 4°C from 0-31 d (Fig. 3C). Importantly, reconditioning cv Glaciate tubers for 3 weeks at 16°C following initial storage at 4°C conditioned the tubers to withstand subsequent longer-term storage at 4°C with 39% less Suc (66.6 ±5.28 mg g\(^{-1}\) dry wt following LTS from 0 to 31 d versus 40.8 ±1.15 mg g\(^{-1}\) dry wt from 52 to 125 days). Consistent with the increases in reducing sugars during 4°C storage from 52 to 125 days (Fig. 3C), French fries from both cultivars darkened relative to the reconditioned tubers (Fig. 4A). However, in contrast to Russet Burbank, process quality of Glaciate tubers remained acceptable (Photovolt reflectance >19) over the entire 125-d storage period regardless of temperature, due to maintenance of low reducing sugar concentrations by virtue of silenced acid invertase.

3.2. Ranger Russet versus Acclimate (late-season frozen processing cultivars)

3.2.1. Crop development, tuber carbohydrate, and specific gravity profiles

Similar to Glaciate versus Russet Burbank, plant emergence from Acclimate was slightly delayed compared with Ranger Russet, however both cultivars reached full emergence by 35 DAP (data not shown). Foliar growth from 44 to 182 DAP was defined by 4\(^{th}\) degree polynomials (R\(^{2}\)= 0.97-0.98, \(P<0.001\) ) with maximum foliar biomass at 85 and 88 DAP (954 and 1004 GDD) for Acclimate and Ranger Russet, respectively (Fig. 5A). Changes in foliar growth with time were
similar for both cultivars (Table 1, CV x DAP, ns). Moreover, consistent with results from the Russet Burbank versus Glaciate trials (Fig. 1A), Ranger produced 14% higher maximum foliar biomass than Acclimate (Fig. 5A, 56.9 versus 50.0 MT ha\(^{-1}\)) and averaged 8.4% more foliage over the growing season (Table 1). Acclimate, however, averaged 12.3% higher foliar yield than Ranger toward season end (126-182 DAP), and the AUFGC (foliar yield x duration) for Acclimate was therefore only 7% less than Ranger, demonstrating nearly comparable seasonal canopy durations.

Tuber bulking rates during the linear phase (R\(^2\)= 0.99, P<0.001) of growth (56 to 114 DAP) were 1.23 MT ha\(^{-1}\) d\(^{-1}\) for Ranger versus 1.14 MT ha\(^{-1}\) d\(^{-1}\) for Acclimate (Fig. 5A), resulting in a relatively modest 5.9 MT ha\(^{-1}\) difference in maximum tuber yield when compared with that documented for Russet Burbank versus Glaciate (Fig. 1A). Maximum tuber yields were 97.8 MT ha\(^{-1}\) for Ranger at 158 DAP (1,915 GDD) versus 91.9 MT ha\(^{-1}\) for Acclimate at 168 DAP (1,997 GDD) (Fig. 5A) when foliar growth was 16% of maximum for both cultivars. However, a tenth and final machine harvest at 182 DAP showed no difference in total tuber yields of these cultivars. The maximum total biomass produced by Ranger at 121 DAP was 122 MT ha\(^{-1}\) compared with 110 MT ha\(^{-1}\) for Acclimate at 127 DAP, and Acclimate\(^{®}\) produced marginally lower total biomass than Ranger when averaged over the season (Table 1). Harvest indices at each sampling through the season were comparable for the two cultivars (data not shown). From 68 to 114 DAP, average tuber fresh weight increased by 2.87 and 2.29 g tuber\(^{-1}\) d\(^{-1}\) for Ranger and Acclimate, respectively, resulting in 218-g (Ranger) and 173-g (Acclimate) tubers by season end (Fig. 5B).

Tuber sucrose concentrations declined from an average of 65.4 mg g\(^{-1}\) dry wt at 56 DAP to 8.9 mg g\(^{-1}\) dry wt at 126 DAP and remained relatively constant thereafter as tubers developed to full maturity (Fig. 5B). Furthermore, in contrast to Russet Burbank versus Glaciate tubers (Fig.
sucrose concentrations in Ranger and Acclimate tubers were mostly equivalent during development (Table 1, CV x DAP, ns). Changes in tuber reducing sugars with time depended on cultivar (CV x DAP, \( P<0.001 \), Table 1) and the trends were similar to those described for Russet Burbank and Glaciate (section 3.1.1). From 56 to 100 DAP, reducing sugars in the bud and stem halves of Ranger tubers declined from 40.2 ±0.81 to 0.6 ±0.11 and 8.5 ±1.53 to 1.6 ±1.12 mg g\(^{-1}\) dry wt, respectively (Fig. 5C). By contrast, reducing sugar concentrations in Acclimate tubers were significantly lower because of invertase silencing, falling from 18.1 ±1.15 to 0.16 ±0.06 mg g\(^{-1}\) dry wt in the bud half and 3.2 ±0.55 to 0.15 ±0.05 mg g\(^{-1}\) dry wt in the stem half of tubers over the same period. Tuber physiological maturity (PM) was estimated at 140-143 DAP. Reducing sugars increased following PM in both the stem and bud ends of Ranger tubers, but only the stem ends of Acclimate tubers. Importantly, Acclimate\(^*\) tubers maintained substantially lower concentrations of reducing sugars than Ranger tubers during this post-PM maturation period, resulting in 50% lighter French fry color (Fig. 5C). Cultivar had no effect on changes in tuber specific gravity during development (Fig. 5C, Table 1).

### 3.2.2. Yield and tuber size distribution trials

Tuber set, yield, and tuber size distribution of Ranger was compared to Acclimate in 2015 and 2016 with 2-yr averages reported (Table 3). Cultivar had no effects on stem numbers per seed piece, tuber set (i.e. tubers plant\(^{-1}\)), or tubers ha\(^{-1}\) (Table 3). Total yields were also equivalent for Ranger and Acclimate, which is consistent with results from the final harvest in the 2015 profiling study (Fig. 5A). Acclimate produced a marginally lower (7\%, 6.2 MT ha\(^{-1}\)) yield of U.S. No. 1 tubers than Ranger, primarily due to lower yields of larger (>340-g) tubers. However, the yield of marketable (U.S. No. 1 + undersize) tubers was not affected by cultivar. The reduced yield of
>340-g tubers translated to lower average tuber fresh weights (g tuber\(^{-1}\)) for Acclimate, as was also revealed in the 2015 profiling study (Fig. 5B).

3.2.3. *Phenotyping physiological responses of tubers to changes in storage temperature*

The effects of LTS (4°C, 31 d) and reconditioning (16°C, 21 d) on tuber respiration, sucrose, reducing sugars, and process quality (French fry color) of cv Ranger Russet versus Acclimate were consistent with those described for Russet Burbank versus Glaciate (section 3.1.3, Fig. 3) with one notable exception; cultivar had little effect on tuber respiration rates (Fig. 6A). Respiration rates averaged 3.58 ±0.02 mg kg\(^{-1}\) h\(^{-1}\) during the initial acclimation period at 9°C (Fig. 6A). Rates then plummeted 36% to an average of 2.29 ±0.11 mg kg\(^{-1}\) h\(^{-1}\) during the first 24 h of LTS at 4°C, followed by a 97% increase to a maximum of ca 4.50 ±0.31 mg kg\(^{-1}\) h\(^{-1}\) for both cultivars at 6 days. Respiration rates then declined through the remainder of the 31-d LTS period to an average of 2.52 ±0.12 mg kg\(^{-1}\) h\(^{-1}\) just prior to reconditioning.

Similar to Glaciate versus Russet Burbank (Fig. 3B), Acclimate tubers contained higher Suc (\(P<0.01\), Fig. 6B) and lower reducing sugar concentrations (\(P<0.01\), Fig. 6C) than Ranger tubers just prior to LTS (i.e. zero-time). Thirty-one days of LTS increased Suc from 11.0 to 42.9 mg g\(^{-1}\) dry wt in Acclimate tubers compared with only 6.4 to 13.4 mg g\(^{-1}\) dry wt in Ranger tubers (Fig. 6B). On the other hand, reducing sugars increased the most in Ranger tubers during LTS, with comparatively little change in Acclimate tubers (Fig. 6C), reflecting the effects of invertase silencing. These LTS phenotypes had consequences for process quality. Like Russet Burbank, Ranger tubers produced unacceptably dark French fries (\(\leq 19\) Photovolt reflectance units) by 10 d of LTS and fry color continued to darken through 20 days (Fig. 4B). The low reducing sugar concentrations in Acclimate tubers, however, translated to acceptable process quality throughout the 31-d LTS period.
The change from 4 to 16°C for reconditioning triggered an immediate increase in the respiration rates of both cultivars to a maximum within 36-52 h, followed by a rapid but somewhat slower decline to 3.8 mg kg\(^{-1}\) h\(^{-1}\) through the remainder of the 21-d reconditioning period (Fig. 6A). At its maximum, the respiration rate of Acclimate tubers was lower than Ranger tubers, which is consistent with the response of Glaciate versus Russet Burbank tubers (Fig. 3A). Also consistent with Glaciate tubers, reconditioning had no effect on the inherently low concentration of reducing sugars in Acclimate tubers but greatly decreased Suc concentrations from 42.9 to 23.2 mg g\(^{-1}\) dry wt (Fig. 3B). The Suc concentration then remained constant during subsequent cold (4°C) storage of Acclimate tubers through 125 days. By contrast, reconditioning Ranger tubers dropped Suc levels from 13.4 to 7.3 mg g\(^{-1}\) dry wt and reducing sugars from 47.5 to 16.1 mg g\(^{-1}\) dry wt (Fig. 3C). Process fry color of Ranger thus improved 74% with reconditioning, from 11.8 (unacceptable) to an acceptable but relatively dark 20.5 fry Photovolt reflectance (Fig. 4B). While process quality of Acclimate tubers also improved (by 21%) with reconditioning, reconditioning was not necessary due to the low reducing sugar concentration and light fry color following LTS. Subsequent storage at 4°C from 52 to 125 days increased Suc and reducing sugar concentrations in Ranger tubers by 13 and 10.6 mg g\(^{-1}\) dry wt\(^{-1}\), respectively, resulting in further deterioration in fry color to unacceptable levels (Fig. 4B). By comparison, the reducing sugar concentration of Acclimate tubers only increased by 4.5 mg g\(^{-1}\) dry wt over this final period of cold storage and while process fry color darkened, it still remained relatively light and acceptable (USDA 1 or better).

3.2.4. Evaluating tolerance to heat stress

Our previous work demonstrated that heat stress could abolish the LTS-resistance of many conventionally bred cultivars (Herman et al. 2017; Zommick et al., 2014). We therefore
investigated the tolerance of Glaciate and Acclimate tubers to heat stress for maintaining low reducing sugar content during subsequent cold storage. Since the sweetening responses of Russet Burbank vs Glaciate and Ranger Russet vs Acclimate to heat stress (HS), cold storage (CS), and their sequential combination (HS + CS) were similar, sugar data were averaged for the parental cultivars and compared to the average of the Innate® cultivars.

All cultivars produced light, uniform French fry color when stored at 9°C for 7 (Fig. 7A) to 88 days (data not shown). However, consistent with their higher reducing sugar concentrations at 9°C (Fig. 7B), Russet Burbank and Ranger Russet produced significantly darker (but commercially acceptable) French fries than Glaciate and Acclimate at 9°C (Fig. 7A). On average, reducing sugar concentration in the parental cultivars fell in response to 21 d of HS (32°C) to levels equivalent to the Innate® cultivars (Fig. 7B). Sucrose concentrations, however, increased in response to HS regardless of cultivar. While HS resulted in slight darkening of French fries processed from Russet Burbank and Glaciate tubers (Fig. 7A), fry color remained well above the unacceptable limit of ≤19 Photovolt reflectance units. Heat stress improved process color of fries from Ranger Russet tubers but had no effect on fries from Acclimate tubers.

As expected, 32 d of CS at 4°C increased the reducing sugar concentrations in the parental cultivars from an average of 6.27 (9°C control) to 21.2 mg g\(^{-1}\) dry wt and Suc from 5.2 to 8.2 mg g\(^{-1}\) dry wt, reflecting their susceptibility to LTS. Conversely, CS had no effect on the intrinsically low reducing sugar content of Innate® tubers but increased the concentration of Suc from 11.1 (9°C control) to 26.4 mg g\(^{-1}\) dry wt as a consequence of invertase silencing. Cold stress thus rendered the process quality of the parental cultivars unacceptable while having no material effect on process quality of the Innate® cultivars (Fig. 7A).
Heat stress prior to CS (HS+CS) exacerbated subsequent CS-induced reducing sugar buildup in Russet Burbank and Ranger Russet tubers, compared with no effect of the combination treatment on reducing sugar concentrations in Innate® tubers (Fig. 7B). Consequently, process fry color of the heat-stressed parental cultivars darkened even more than induced by CS alone (Fig. 7A). By contrast, HS conditioned the Innate® tubers to accumulate even higher concentrations of Suc during subsequent CS than CS alone, with no effect on reducing sugar concentrations (Fig. 7B). Heat-stressed Innate® tubers thus maintained acceptable process fry color when subsequently stored at 4°C (Fig. 7A). Importantly, both the parental and Innate® cultivars were equally sensitive to prior heat stress for enhanced accumulation of total sugars (Suc + Glc + Fru) during subsequent cold storage (Fig. 7B), which implies elevated but similar levels of starch catabolism in the HS + CS tubers.

3.3. Atlantic versus Hibernate (mid-season chipping cultivars)

3.3.1. Crop development, tuber carbohydrate, specific gravity, and amino acid profiles

Foliar and tuber development of cvs Atlantic and Hibernate were profiled during the 2016 and 2017 growing seasons. Like the other Innate® cultivars, plant emergence from cv Hibernate was slightly delayed versus Atlantic, but both cultivars attained ≥90% emergence at the same time during both seasons (33 DAP on average). Moreover, trends in foliar growth from 40 to 120 DAP were identical and the cultivar main effect, while significant (P<0.04, Table 1), was minor and inconsequential (Fig. 8A). Maximum foliar fresh weight occurred 87 (806 GDD) and 92 DAP (874 GDD) for Atlantic and Hibernate, respectively. Foliar fresh weights at vine kill (120 DAP) were equivalent, averaging 36.1 MT ha\(^{-1}\) and the AUFGCs were also comparable. Tuber bulking rates from 60 to 101 DAP were similar, averaging 1.47 MT ha\(^{-1}\) d\(^{-1}\), and final yields averaged 88.9 MT ha\(^{-1}\) at 120 DAP. Cultivar had no effect on total plant biomass accumulation. The HI for
Hibernate was slightly higher than for Atlantic from ca 50 to 80 DAP (by 4% on average), but this difference dissipated, resulting in a final HI of 71% for both cultivars at 120 DAP (CV x DAP, \(P<0.02\), Table 1).

Sucrose concentrations were lowest in the stem halves of Hibernate versus Atlantic tubers early in tuber development (60 DAP), (Fig. 8B). However, concentrations fell rapidly from 60 to 80 DAP as average tuber fresh weight (g tuber\(^{-1}\)) increased from 20 to 82 g tuber\(^{-1}\), and by 101 DAP (147 g tuber\(^{-1}\)), Suc levels were equivalent at 2.76 mg g\(^{-1}\) dry wt regardless of tuber portion or cultivar. Suc then declined slowly, reaching 2.46 mg g\(^{-1}\) dry wt at 120 DAP.

Trends in reducing sugar concentrations with tuber development (Fig. 8C) were consistent with those described for the other late-season parental versus Innate\(^\circledast\) cultivars (Figs. 1 and 5, sections 3.1.1 and 3.2.1). Reducing sugars were highest in both cultivars at 60 DAP when tubers averaged only 20 g fresh weight (Fig. 8 C). Reducing sugar concentrations then fell rapidly with tuber development, reaching relatively constant and low levels from 80 to 120 DAP. Depending on DAP and tuber portion, Hibernate tubers contained anywhere from 43 to 915% lower reducing sugar concentrations than Atlantic tubers during development (CV x DAP, \(P<0.001\), Table 1). Reducing sugars averaged 0.29 and 0.13 mg g\(^{-1}\) dry wt in Atlantic and Hibernate tubers, respectively, at 120 DAP and this difference contributed to 49% lighter (\(P<0.05\)) chip color from the Hibernate versus Atlantic tubers (Fig. 8C). The average Photovolt reflectance values of chips translated to Snack Food Association values (1-5 scale) of 4.1 (darker) for Atlantic and 2.9 (lighter) for Hibernate (\(P<0.01\)). In addition to lighter process color, the specific gravity of Hibernate tubers was higher (\(P<0.001\), Table 1) than Atlantic tubers throughout development (Fig. 8C).

Similar to Russet Burbank and Glaciata, Asn and Gln collectively dominated the total free amino acid pools of Atlantic and Hibernate tubers during development, increasing from an average
of 50.4 to 67.4% on a mg g\(^{-1}\) dry wt basis as tuber fresh weight increased from 6.7 g tuber\(^{-1}\) (50 DAP) to 164.4 g tuber\(^{-1}\) (120 DAP) (Fig. 2 DEF). Moreover, aside from Asn and Gln, cultivar had no material effect on the remaining 16 amino acids (data not shown). Total free amino acid concentration in Atlantic tubers increased from 13.6 ±1.8 to 31.1 ±2.7 mg g\(^{-1}\) dry wt as tuber fresh weight increased from ca 5 to 70 g tuber\(^{-1}\) then decreased to 24.8 ±2.1 mg g\(^{-1}\) dry wt with further development to 168 g tuber\(^{-1}\) at 120 DAP (Fig. 2D). This increase was mainly attributable to Asn, which rose from 1.5 ±0.19 to 10.5 ±0.42 mg g\(^{-1}\) dry wt (Fig. 2E). Gln also increased as Atlantic tubers developed to ca 90 g tuber\(^{-1}\), then decreased (Fig. 2F). By contrast, total free amino acid concentration in Hibernate tubers remained constant at ca 18 mg g\(^{-1}\) dry wt with minimal increases in Asn and Gln during development (Fig. 2 DEF, Table 1). Consistent with Glaciate versus Russet Burbank, and because of silenced Asn synthetase, Gln dominated the amide amino acid profile of Hibernate tubers, whereas Asn concentration was only moderately higher than Gln in Atlantis tubers at harvest.

3.3.2. Yield and tuber size distribution trials

Yield and tuber size-distribution trials for cvs Atlantic versus Hibernate were conducted alongside the profiling studies described in 3.3.1 and the results were averaged over the 2-yr study period (2016-17). Cultivar had no effect on stem number per seed piece, tuber set (i.e. tubers plant\(^{-1}\)), average tuber size, market yield, or tuber size grade (Table 1). As a percentage of marketable yield, the cultivars averaged 74% A-size (premium) tubers for chip processing.

3.3.3. Phenotyping physiological responses of tubers to changes in storage temperature

The changes in respiration rates of Atlantic and Hibernate tubers in response to initial acclimation to 10°C storage, followed by LTS for 32 d at 4°C, and subsequent reconditioning for 29 d at 16°C were similar to those described for Ranger Russet versus Acclimate in 3.2.3. Tuber
respiration rates were comparable during the last ca 5 d of initial acclimation, decreasing to an average of 4.09 ±0.06 mg kg⁻¹ h⁻¹ at commencement of LTS (i.e. zero-time, Fig. 9A). While tuber Suc concentrations were also equivalent at this point (8.2 ±0.63 mg g⁻¹ dry wt) (Fig. 9B), Atlantic tubers contained a markedly higher concentration of reducing sugars than Hibernate tubers (3.18 ±0.45 vs 0.24 ±0.06 mg g⁻¹ dry wt, P<0.01, Fig. 9C), resulting in 41% lighter chips from the Hibernate tubers (Fig. 10). The chip Photovolt reflectance values at zero-time correspond to Snack Food Association (SFA) chip color ratings of 2.5 (marginally acceptable) for Atlantic and 1.4 (acceptable) for Hibernate.

Dropping the storage temperature from 10 to 4°C induced a prominent RAR characterized by respiration rates plummeting from 4.09 ±0.06 to 2.38 ±0.06 mg kg⁻¹ h⁻¹ within the initial 30 h (Fig. 9A) and then increasing rapidly to 3.76 ±0.10 (Atlantic) and 3.35 ±0.10 mg kg⁻¹ h⁻¹ (Hibernate) by 4 d of LTS. Rates then gradually declined to an average of 2.61 ±0.08 mg kg⁻¹ h⁻¹ at 21 d, just prior to reconditioning. Similar to the other Innate® versus parental cultivars (Figs. 3B and 6B), Suc concentration increased the most in Hibernate versus Atlantic tubers during LTS (Fig. 9B). LTS had little effect on the reducing sugar concentration of Hibernate tubers (Fig. 9C), a consequence of invertase silencing. By contrast, reducing sugars increased from 3.18 to 35.9 mg g⁻¹ dry weight in Atlantic tubers during LTS. LTS significantly decreased the process quality of both cultivars (Fig. 10). Atlantic chip lightness fell from 32.2 to 14.8 Photovolt reflectance units after only 10 d of LTS, resulting in a SFA rating of 4.6 (unacceptable). Atlantic chip color continued to deteriorate (darken) with further increases in reducing sugars from 10 to 32 d of LTS. Curiously, despite Hibernate tubers maintaining low reducing sugar concentration during LTS, chip Photovolt reflectance values fell from 45.5 to 18.1, which equates to SFA ratings of 1.4 (acceptable) and 4.2 (unacceptable), respectively.
The storage temperature was increased to 16°C to evaluate reconditioning following LTS. Respiration rates responded immediately to the change in temperature, rising from an average of 2.66 ±0.10 mg kg\(^{-1}\) h\(^{-1}\) to 10.1 ±0.40 mg kg\(^{-1}\) h\(^{-1}\) in Atlantic tubers at 34 d and to 8.88 ±0.24 mg kg\(^{-1}\) h\(^{-1}\) in Hibernate tubers at 36 d (Fig. 9A). Respiration rates then declined to comparable levels of ca 3.61 ±0.10 mg kg\(^{-1}\) h\(^{-1}\) at 56 d when measurements terminated. Reconditioning for 29 days decreased the Suc concentration in Hibernate tubers from 42.5 ±2.8 to 11.6 ±1.0 mg g\(^{-1}\) dry wt and in Atlantic tubers from 28.0 ±4.2 to 4.97 ±1.31 mg g\(^{-1}\) dry wt (Fig. 9B). By contrast, the concentration of reducing sugars in Hibernate tubers increased from 0.62 ±0.11 to 1.76 ±0.34 mg g\(^{-1}\) dry wt during reconditioning compared with a substantial decrease from 35.9 ±3.4 to 20.0 ±1.4 mg g\(^{-1}\) dry wt in Atlantic tubers (Fig. 9C). However, while Atlantic chip lightness nearly doubled in response to reconditioning, it remained unacceptably dark by industry standards (Fig. 10). Despite the relatively small absolute increase in reducing sugar concentration of Hibernate tubers, process chip color improved noticeably with reconditioning, increasing from 18.1 to 31.8 Photovolt reflectance units, which translated to 4.2 (unacceptable) to 2.6 (marginally acceptable) SFA values.

3.3.4. Evaluating tolerance to heat stress

Atlantic and Hibernate tubers were subjected to the PHHS treatments outlined in 2.4.2 to evaluate how heat stress affects subsequent LTS. Both cultivars produced relatively light and uniform chip color when stored at 9°C for 10 (Fig. 11A) to 63 days (data not shown). However, consistent with their higher reducing sugar concentrations (Fig. 11B), Atlantic tubers produced significantly darker (but marginally acceptable, SFA=2.5) chips than Hibernate tubers (SFA=1.4) at 9°C (Fig. 11A). The reducing sugar concentration in Atlantic tubers fell in response to 21 d of HS (32°C) to equal that of heat-stressed Hibernate tubers (Fig. 11B). Sucrose concentrations,
however, increased in response to HS regardless of cultivar. HS had no effect on the color of chips from Atlantic tubers (31.6 Photovolt, 2.6 SFA). Chip color from Hibernate tubers darkened by 21% in response to HS (Fig. 11A) but remained acceptable at an SFA rating of 2.2 (35.7 Photovolt reflectance).

As expected, 32 d of CS at 4°C increased the concentration of reducing sugars in Atlantic tubers from an average of 3.1 (9°C control) to 16.4 mg g⁻¹ dry wt and Suc from 9.1 to 16.8 mg g⁻¹ dry wt, reflecting the susceptibility of this cultivar to LTS. Conversely, CS had no effect on the intrinsically low reducing sugar content of Hibernate tubers, but increased the concentration of Suc from 10.7 (9°C control) to 25.7 mg g⁻¹ dry wt because of invertase silencing. Cold storage rendered the process quality of Atlantic and Hibernate tubers unacceptable, with SFA ratings of 4.5 and 3.3, respectively (Fig. 11A).

Heat stress prior to CS (HS + CS) exacerbated subsequent CS-induced reducing sugar buildup in Atlantic tubers, compared with no effect of the combination treatment on the reducing sugar concentration in Hibernate tubers (Fig. 11B). Consequently, process chip color of the heat-stressed Atlantic tubers darkened even more than induced by CS alone (Fig. 11A). By contrast, HS conditioned Hibernate tubers to accumulate even higher concentrations of Suc during subsequent CS than CS alone, with no effect on reducing sugar concentrations (Fig. 11B). Heat-stressed Hibernate tubers thus maintained equivalent chip color when subsequently stored at 4°C (Fig. 11A). Importantly, Atlantic and Hibernate tubers were sensitive to prior heat stress for enhanced accumulation of total sugars (Suc + Glc + Fru) during subsequent cold storage (Fig. 11B), which implies increased starch catabolism in the HS + CS tubers.
4. Discussion

We characterized the developmental and postharvest phenotypes of three Innate® cultivars in the Columbia Basin of Washington by: (1) modeling foliar and tuber development over time and in relation to accumulated degree days from planting, (2) comparing tuber physiological responses to low temperature sweetening and reconditioning during storage, and (3) evaluating how heat stress modulates subsequent cold-induced sweetening and process quality. The growth profiling studies were designed to reveal cultivar-dependent differences in the timing of critical growth-stage ‘windows’ (emergence and plant establishment, tuberization, bulking, foliar senescence, tuber maturation), estimate the attainment of tuber physiological maturity (PM), and identify potential consequences of delayed harvest beyond PM for subsequent retention of process quality (discussed below) for each cultivar. Harvest indices (a measure of source/sink relationships) and bulking rates were compared, along with developmental changes in tuber specific gravity, sugars, and free amino acid composition. In addition to PM, differences in the relative concentrations and distributions of Suc and reducing sugars in tubers during bulking and maturation were compared as indicators (Knowles et al., 2015) of propensity to develop physiological disorders such as sugar or translucent (jelly) ends during storage (Thompson et al., 2008), which adversely affect process and nutritional qualities.

Consistent with Pence et al. (2016), the growth and development phenotypes of Acclimate and Hibernate were comparable to their parental cultivars, resulting in equivalent total and marketable yields, with only minor differences noted in the U.S. No. 1 yield and tuber size-distribution profile of Acclimate (Table 3). Relative to Ranger Russet, which tends to produce a high percentage of oversize (>397-g) tubers (Knowles and Knowles, 2006; 2016), the reduced yield of >397-g Acclimate tubers is actually more desirable for frozen processing. There is
currently no financial disincentive for delivering oversize U.S. No. 1 potatoes to processors; however, larger tubers are more susceptible to internal defects (Jansky and Thompson, 1990; Nelson and Thoreson, 1986; Rex et al., 1987) and mechanical damage during harvest and handling (Baritelle and Hyde, 1999; Brook, 1996), which will trigger significant penalties in frozen process contracts. Furthermore, processors pre-cut oversize tubers to produce French fries of acceptable length (Gould, 1999), and a U.S. No. 1 tuber size profile with lower percentage oversize tubers reduces or negates this step. The robust resistance to blackspot bruise formation (Rommens et al., 2006), coupled with superior process qualities (conferred by silencing PPO and invertase activities), and comparable agronomic performance, makes cvs Acclimate and Hibernate vastly improved relative to Ranger Russet and Atlantic. Most significantly, resistance to reducing sugar accumulation at 4°C (Figs. 4, 6, 9, and 10), and tolerance of heat stress for retention of process quality when stored cold (Figs. 7 and 11), have rendered cvs Acclimate and Hibernate suitable for full-season storage, which is not the case for their parental cultivars.

In contrast to Acclimate and Hibernate versus their parental cultivars, Glaciate produced less foliar growth and bulked at a slower rate than Russet Burbank, resulting in lower tuber yields (Fig. 1, Table 2). While the Innate® cultivars underwent the same transformation events (Clark et al., 2014; Pence et al., 2016), differences in Agrobacterium cassette insertion can affect phenotype (Latham et al., 2006; Sinclair et al., 2004; Ziemienowicz, 2010). Clark et al. (2014) reported delayed emergence, lower plant vigor, and shorter canopy (plant) height of cv Glaciate, but no difference in yield versus Russet Burbank when averaged across multiple trial sites (ID, MN, ND, WA, and WI). These production regions vary in length of growing season, environmental conditions, and standard management practices, which in turn will affect the phenotype of each cultivar. Therefore, the reduced yield phenotype of Glaciate may only manifest in a long-season
environment like the Columbia Basin where environmental and crop management conditions are ideal for producing high yields of late-season potatoes.

For many late season cultivars (including Russet Burbank and Ranger Russet), prolonged maturation of tubers under dead vines following PM exposes them to fluctuating soil temperatures which hastens physiological aging, leading to earlier dormancy break, sweetening, development of sugar ends, and associated loss of process quality during storage (Knowles et al., 2015ab; Knowles et al., 2014; Wohleb et al., 2014). Knowles et al. (2019) refer to this stage of crop development as the ‘accelerated aging’ phase of tuber maturation. Timing the harvest in relation to tuber PM is thus important for storage longevity. As tuber age advances following PM, tuber specific gravity decreases and reducing sugars increase particularly in the basal (stem) ends of tubers (e.g., Figs. 1 and 5; Knowles et al., 2015a; Rosen et al., 2018; Wohleb et al., 2014). This can lead to sugar ends at harvest but more often manifests as sugar end development in storage, resulting in earlier loss of process quality (Knowles et al., 2015b). Here we show that by virtue of silenced invertase, Glaciate and Acclimate tubers were highly resistant to reducing sugar buildup during the ca 40-d post-PM accelerated-aging stage of development (Figs. 1 and 5). These cultivars are thus more ‘forgiving’ for harvest timing than their parental cultivars, which translates to greater flexibility for growers and processors in scheduling harvests without concern for compromising at-harvest or long-term process quality. Atlantic and Hibernate did not attain physiological maturity as their vines were mowed mid-season in preparation for harvest and in accordance with industry practice to avoid oversized tubers for chipping.

The respiratory and sweetening responses of tubers during cold acclimation, storage at 4oC, and subsequent reconditioning at 16oC defined the postharvest physiological phenotypes of Innate® and parental cultivars. Respiration rates are indicative of overall tuber metabolic rates.

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Changes in tuber respiration rates are driven by changes in “ATP-requiring activities of the cell that pull the process of ATP-yielding catabolism. ATP is generated only as fast as it is needed” through respiration to fuel metabolism (Lehninger, 1975). Temperature-dependent starch to sugar interconversion is a sink for metabolic energy (Isherwood, 1973).

All cultivars showed an initial prominent RAR during the first ca 9 days of acclimation to 4°C (Figs. 3A, 6A, and 9A). This response has been well defined in whole (Zommick et al., 2014) and fresh-cut tubers (Ellis et al., 2019) and reflects the direct effect of temperature (i.e. Q_{10}) on intermediary metabolism (Malone et al., 2006), and the increased demand for metabolic energy (ATP) to drive starch catabolism to sugars during LTS (Isherwood, 1973). The cold-induced (9→4°C) buildup of Suc (Innate® tubers) and reducing sugars (parental cultivars), and subsequent loss of sugar during reconditioning (4→16°C), did not translate to cultivar-dependent differences in tuber respiration rates, with all cultivars showing the same general trends in respiration (Figs. 3, 6, and 9). This is primarily because the temperature-induced increases in respiration rates are quantitatively related to the ATP-equivalent of the increased CO₂ output associated with Suc synthesis from starch and starch synthesis from sugars (Isherwood, 1973; Isherwood, 1976), and the LTS-induced increase in total sugar was equivalent for parental and Innate® cultivars.

Despite the overall respiratory trends being similar for Glaciate and Russet Burbank, the absolute respiration rate of Glaciate tubers was consistently higher (ca 35%) than Russet Burbank tubers throughout storage regardless of temperature (Fig. 3). Glaciate tubers from the 2014 growing season also had higher basal rates of respiration than Russet Burbank tubers during storage (data not shown). This elevated respiration phenotype is indicative of an additional sink for metabolic energy beyond that needed to fuel the temperature-dependent starch to sugar interconversions. While further work is required to identify the nature of this metabolism, the
practical implications include increased dry matter loss for Glaciate versus Russet Burbank tubers when both cultivars are stored at the same temperature. However, the ability to store Glaciate tubers at 4°C without buildup in reducing sugars and loss of process quality likely negates any potential negative impact of its elevated respiration rate on dry matter loss versus Russet Burbank tubers, since the parental cultivar must be stored at 9°C to minimize reducing sugar buildup and maintain process quality. Dry matter loss via respiration at 9°C would likely exceed the dry matter loss from Glaciate tubers stored at 4°C.

The cold-induced buildup in Suc concentration was greatest for Innate® tubers because of silenced invertase (Fig. 3B; Fig. 6B; Fig. 9B). Reducing sugars, however, increased the most during cold sweetening of the parental cultivars (Fig. 3C; Fig. 6C; Fig. 9C) and the time course was consistent with past reports (Herman et al., 2016; Isherwood, 1973; Zommick et al., 2014). Regardless of storage temperature, Innate® tubers maintained very low concentrations of reducing sugars, resulting in light French fry (Figs. 4, and 7) and chip colors (Fig. 10) relative to the parental cultivars. However, process color of the Innate® tubers darkened during cold sweetening (esp. Hibernate) relative to color at 9°C or following reconditioning at 16°C (Figs. 4 and 10). This darkening may be due to the high Suc levels, which are reported to contribute indirectly to the Maillard reaction via thermal hydrolysis to Glc and Fru (Leszkowiat et al., 1990; Townsend and Hope, 1959). Townsend and Hope (1959) explain that “the colour produced by hydrolyzed sucrose and amino acids would probably be masked in chips from tubers with an initially high content of reducing sugars but would become increasingly important in chips having a relatively low initial content.” This phenomenon underscores the importance of minimizing Suc buildup during cold storage of Innate® tubers.
Reconditioning cold-sweetened tubers at 16°C decreased both sucrose and reducing sugars in Russet Burbank, Ranger Russet, and Atlantic tubers, with associated improvements in process fry and chip colors. However, the drop in reducing sugar concentrations was insufficient to restore process quality of the parental cultivars to acceptable standards (Fig. 4; Fig. 10). Reconditioning cold-sweetened Hibernate tubers greatly reduced the Suc level (Fig. 9B) with virtually no impact on reducing sugar concentrations (Fig. 9C), which remained low throughout storage regardless of temperature due to invertase silencing. The reconditioning-induced drop in Suc correlated with improved chip color (Fig. 10), providing further evidence that the Suc buildup in Hibernate tubers stored at 4°C was contributing to deterioration of color. Reconditioning also dropped the Suc levels of Glaciate (Fig. 3B) and Acclimate tubers (Fig. 6B) with little impact on reducing sugar levels (Figs. 3C and 6C), resulting in marginal improvements in fry color (Fig. 4). Notably, Suc concentrations in these Innate® tubers did not increase during 73 d of storage at 4°C following reconditioning (Figs. 3B and 6B). Reconditioning may therefore be an effective management technique to moderate the cold-induced Suc accumulation in Innate® tubers during long-term storage.

In the broadest sense, LTS entails the breakdown of starch and buildup of Suc, Glc, and Fru. Since reducing sugars are the major concern for processing due to their participation in the Maillard reaction and acrylamide formation, much of the literature equates LTS as reducing sugar buildup only. However, there are at least several cultivar-dependent phenotypes defined by the relative changes in Suc versus reducing sugars during cold-sweetening. For example, Gemstar Russet accumulates Suc during LTS with minimal inversion to reducing sugars, thus maintaining process quality when stored cold (Zommick et al., 2014). This phenotype is synonymous with the Innate® cultivars where silenced invertase prevents hydrolysis of Suc during cold-sweetening. In
contrast, Premier Russet maintains low Suc and reducing sugars when stored cold (Zommick et al., 2014), which constitutes a true LTS-resistant phenotype. Russet Burbank, Ranger Russet, and Atlantic accumulate some Suc but mostly reducing sugars during LTS. The total sugar buildup (Suc + reducing sugars), however, is comparable to that of the respective Innate® cultivars, indicating similar levels of cold-induced starch catabolism and thus overall sweetening phenotype.

Heat stress exacerbated LTS, resulting in either enhanced Suc buildup (Innate® cultivars) or reducing sugar buildup (parental cultivars), with similar increases in total sugar, regardless of cultivar (Figs. 7 and 11). However, similar to Payette Russet (Herman et al., 2017), the LTS-induced Suc accumulating phenotype of heat-stressed Innate® tubers translates to retention of process quality due to minimal hydrolysis to reducing sugars. This phenotype is highly desirable for production in high-temperature environments like the southern Columbia Basin and ensures retention of process quality regardless of exposure to prior heat stress.

Acknowledgement

This work was supported by grants from the USDA ARS Federal-State Partnership Potato Program (2092-21220-001-00D), the USDA National Institute of Food and Agriculture Hatch project (Accession No. 1005995), “Physiological and agronomic studies to maximize the productivity and postharvest quality of new potato cultivars”, and through a sponsored research agreement with the J.R. Simplot Co.
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Table 1. Sources of variation and levels of significance (P-values) for the effects of cultivar and days after planting (DAP) on indices of crop development and tuber carbohydrate content during growth in the Columbia Basin (Othello, WA). Data are plotted in Figs. 1, 2, 5, and 8.

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<td>Foliage Tubers Total</td>
<td>Index g/tuber apical basal</td>
<td>apical basal</td>
<td>apical basal</td>
<td>Total</td>
<td>Asn</td>
</tr>
<tr>
<td>Russet Burbank vs Glaciote</td>
<td>Cultivar (CV)</td>
<td>0.006 0.001 0.001</td>
<td>ns</td>
<td>0.044 0.002 0.001</td>
<td>0.001 0.002 0.001</td>
<td>ns 0.001 0.001</td>
<td>ns 0.001 0.001</td>
</tr>
<tr>
<td></td>
<td>DAP</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.002 0.001</td>
<td>0.001 0.001 0.001</td>
<td>ns 0.001 0.001</td>
<td>ns 0.001 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV x DAP</td>
<td>ns 0.05</td>
<td>ns</td>
<td>0.002 ns</td>
<td>ns 0.002 ns</td>
<td>ns 0.007 ns</td>
<td></td>
</tr>
<tr>
<td>Ranger Russet vs Acclimate</td>
<td>CV</td>
<td>0.05 0.001 0.001</td>
<td>ns</td>
<td>0.001 ns</td>
<td>ns 0.001 ns</td>
<td>-    -    -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DAP</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.001</td>
<td>ns 0.001 0.001</td>
<td>-    -    -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV x DAP</td>
<td>ns 0.04</td>
<td>0.02</td>
<td>0.001 ns</td>
<td>ns 0.001 ns</td>
<td>ns 0.001 ns</td>
<td></td>
</tr>
<tr>
<td>Atlantic vs Hibernate</td>
<td>CV</td>
<td>0.04 ns</td>
<td>ns</td>
<td>0.001 ns</td>
<td>ns 0.001 ns</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.003</td>
</tr>
<tr>
<td></td>
<td>DAP</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.001</td>
<td>0.001 0.001 0.003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV x DAP</td>
<td>ns ns</td>
<td>0.02</td>
<td>0.02 ns</td>
<td>0.001 ns</td>
<td>0.002 0.001 ns</td>
<td></td>
</tr>
</tbody>
</table>

1Data were analyzed from 44 to 184 DAP (10 harvest dates) or from 56-184 DAP (9 harvest dates) depending on yield component for the late season frozen processing cultivar trials (see Figs. 1 and 5). For the early season chipping cultivars (Atlantic and Hibernate), data were analyzed from 40 to 120 DAP (9 harvest dates) or 60 to 120 DAP (7 harvest dates) depending on yield component (see Fig. 8).

2ns, not significant.
Table 2. Comparison of the yield components of cvs Russet Burbank and Glaciate averaged over the 2015 and 2016 growing seasons at Othello, WA. Cut seed was planted on 4/15/15 and 4/14/16 and vines were mowed 147 days after planting in both years.

<table>
<thead>
<tr>
<th>Yield (MT ha⁻¹) Components</th>
<th>Cultivar</th>
<th>R. Burbank</th>
<th>Glaciate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem No.</td>
<td></td>
<td>2.8</td>
<td>2.5**</td>
</tr>
<tr>
<td>Total Yld</td>
<td></td>
<td>99.6</td>
<td>82.2**</td>
</tr>
<tr>
<td>U.S. #1 Yld</td>
<td></td>
<td>86.3</td>
<td>68.7**</td>
</tr>
<tr>
<td>113-170 g</td>
<td></td>
<td>17.0</td>
<td>12.6**</td>
</tr>
<tr>
<td>170-284 g</td>
<td></td>
<td>34.3</td>
<td>26.9**</td>
</tr>
<tr>
<td>284-340 g</td>
<td></td>
<td>12.5</td>
<td>9.8ns</td>
</tr>
<tr>
<td>340-397 g</td>
<td></td>
<td>7.6</td>
<td>7.0ns</td>
</tr>
<tr>
<td>&gt;397 g</td>
<td></td>
<td>14.9</td>
<td>12.4ns</td>
</tr>
<tr>
<td>Undersize (&lt;113 g)</td>
<td></td>
<td>10.8</td>
<td>9.5ns</td>
</tr>
<tr>
<td>Culls</td>
<td></td>
<td>2.5</td>
<td>4.0*</td>
</tr>
<tr>
<td>Mkt Yld</td>
<td></td>
<td>97.1</td>
<td>78.2**</td>
</tr>
<tr>
<td>Tubers plant⁻¹</td>
<td></td>
<td>10.8</td>
<td>8.8**</td>
</tr>
<tr>
<td>Tubers ha⁻¹ (1000’s)</td>
<td></td>
<td>511.2</td>
<td>417.0**</td>
</tr>
<tr>
<td>g tuber⁻¹</td>
<td></td>
<td>184.7</td>
<td>181.7ns</td>
</tr>
</tbody>
</table>

*,**,**P<0.05 and 0.01, respectively. ns, not significant
Table 3. Comparison of the yield components of cvs Ranger Russet and Acclimate averaged over the 2015 and 2016 growing seasons at Othello, WA. Cut seed was planted on 4/15/15 and 4/14/16 and vines were mowed 147 days after planting in both years.

<table>
<thead>
<tr>
<th>Yield (MT ha(^{-1})) Components</th>
<th>Cultivar</th>
<th>Ranger</th>
<th>Acclimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem No.</td>
<td></td>
<td>2.5</td>
<td>2.5(^{\text{ns}})</td>
</tr>
<tr>
<td>Total Yld</td>
<td></td>
<td>99.0</td>
<td>94.1(^{\text{ns}})</td>
</tr>
<tr>
<td>U.S. #1 Yld</td>
<td></td>
<td>87.8</td>
<td>81.6(^{**})</td>
</tr>
<tr>
<td>113-170 g</td>
<td></td>
<td>10.7</td>
<td>11.1(^{\text{ns}})</td>
</tr>
<tr>
<td>170-284 g</td>
<td></td>
<td>28.2</td>
<td>31.4(^{\text{ns}})</td>
</tr>
<tr>
<td>284-340 g</td>
<td></td>
<td>14.0</td>
<td>13.5(^{\text{ns}})</td>
</tr>
<tr>
<td>340-397 g</td>
<td></td>
<td>10.6</td>
<td>8.8(^{*})</td>
</tr>
<tr>
<td>&gt;397 g</td>
<td></td>
<td>24.3</td>
<td>16.8(^{*})</td>
</tr>
<tr>
<td>Undersize (&lt;113 g)</td>
<td></td>
<td>7.5</td>
<td>9.3(^{\text{ns}})</td>
</tr>
<tr>
<td>Culls</td>
<td></td>
<td>3.2</td>
<td>2.9(^{\text{ns}})</td>
</tr>
<tr>
<td>Mkt Yld</td>
<td></td>
<td>95.3</td>
<td>90.9(^{\text{ns}})</td>
</tr>
<tr>
<td>Tubers plant(^{1})</td>
<td></td>
<td>9.1</td>
<td>9.5(^{\text{ns}})</td>
</tr>
<tr>
<td>Tubers ha(^{-1}) (1000's)</td>
<td></td>
<td>390.9</td>
<td>410.4(^{\text{ns}})</td>
</tr>
<tr>
<td>g tuber(^{1})</td>
<td></td>
<td>214.6</td>
<td>197.0(^{*})</td>
</tr>
</tbody>
</table>

\(\text{*,**P}<0.05\) and 0.01, respectively. \(\text{ns, not significant}\)
Table 4. Comparison of the yield components of cvs Atlantic and Hibernate grown at Othello, WA in 2016 and 17 (2-yr average). Cut seed was planted April 14 and vines were mowed 116 and 110 days after planting in 2016 and 2017, respectively.

<table>
<thead>
<tr>
<th>Yield (MT ha⁻¹) Components</th>
<th>Cultivar</th>
<th>Atlantic</th>
<th>Hibernate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem No.</td>
<td></td>
<td>2.3</td>
<td>2.5ns</td>
</tr>
<tr>
<td>Mkt Yld</td>
<td></td>
<td>85.1</td>
<td>83.2ns</td>
</tr>
<tr>
<td>B’s (0-5.08 cm)</td>
<td></td>
<td>1.8</td>
<td>1.9ns</td>
</tr>
<tr>
<td>A’s (5.08-8.26 cm)</td>
<td></td>
<td>60.0</td>
<td>64.5ns</td>
</tr>
<tr>
<td>Oversize (&gt;8.26 cm)</td>
<td></td>
<td>23.3</td>
<td>16.8ns</td>
</tr>
<tr>
<td>Tubers plant⁻¹</td>
<td></td>
<td>9.3</td>
<td>9.7ns</td>
</tr>
<tr>
<td>Tubers ha⁻¹ (1000’s)</td>
<td></td>
<td>448.0</td>
<td>467.4ns</td>
</tr>
<tr>
<td>g tuber⁻¹</td>
<td></td>
<td>199.2</td>
<td>185.9ns</td>
</tr>
</tbody>
</table>

*P<0.05 and 0.01, respectively. ns, not significant
Fig. 1. (A) Foliar growth, tuber growth, and total biomass (foliar + tuber) produced by potato cvs Russet Burbank and Glaciate grown at Othello, WA (average of 2015 and 2016 growing seasons). Planting dates were April 15, 2015 and April 14, 2016. Average cumulative degree-days (>7.2°C) from planting are indicated on the top axes. (B) Changes in tuber sucrose concentrations, average tuber weights, (C) reducing sugars (glucose + fructose) and specific gravity during development. Physiological maturity (PM) was estimated at 142 and 149 DAP for Russet Burbank and Glaciate tubers, respectively. Final-harvest (184 DAP) process quality is depicted by the French fry planks for each cultivar. Each fry plank represents a single tuber (numbers are average Photovolt reflectance values of the stem end of fry planks, *$P<0.05$). See Table 1 for ANOVA results and Table 2 for the effects of cultivar on tuber number, yields, and tuber size distributions.
Fig. 2. Changes in the concentrations of total free amino acids, asparagine, and glutamine in tubers of cvs Russet Burbank versus Glaciate (A,B,C) and Atlantic versus Hibernate (D,E,F) with increasing tuber fresh weight during the 2016 growing season at Othello, WA. *,**P<0.05 or 0.01, respectively (bars, ±SE for amino acids and tuber fresh weight). Each point represents an average of 12 tubers. See Figs. 1B and 5B for 2-year tuber growth profiles (g tuber$^{-1}$ vs days after planting).
**Fig. 3.** Effects of consecutive periods of low temperature sweetening (LTS) and reconditioning (Recond) on the respiration rates (A), sucrose (B) and reducing sugar (C) (Glc + Fru) concentrations of cvs Russet Burbank and Glaciata tubers. Tubers were initially stored for 15 days at 9°C directly following harvest to establish and compare basal respiration rates during wound healing. The storage temperature was then rapidly lowered at zero time to 4°C (arrow) for a 31-day period of LTS, followed by an increase to 16°C (arrow) for a subsequent 21-day period of reconditioning (from 31-52 days). At 52 days, the temperature was again dropped to 4°C for the remainder of the 125-d storage period. The reconditioning period (31-52 days) is shaded in yellow in B and C. For respiration rates (A), each point represents the average of 24 tubers (four replicates of six tubers). For sugars (B,C), each point represents the average of 12 tubers (four replicates of three tubers, ±SE). Samples of French fry planks are included at 0, 31, 52, 82 and 125 days (C) to show temperature-induced changes in process quality (each fry plank represents a single tuber). See Fig. 4 for a complete analysis of changes in fry color.
**Fig. 4.** French fry planks showing changes in process quality of (A) cvs Russet Burbank versus Glaciate and (B) Ranger Russet versus Acclimate tubers during consecutive periods of low temperature sweetening (LTS) and reconditioning. Tubers were initially wound-healed for 15 days at 9°C. The temperature was then rapidly lowered to 4°C for 31 days of LTS before increasing to 16°C for 6, 13, and 21 d of reconditioning. Following reconditioning, the temperature was again dropped to 4°C for an additional 30 and 73 days of LTS. Each fry plank is from a different tuber and the four planks shown for each treatment represent the average fry color from a 12-tuber sample. Fry planks are oriented apical (bud) end up and basal (stem) end (stolon attachment) down. Numbers are Photovolt reflectance values of the basal ends of fries (≤19 is unacceptable by industry standards). Letters indicate LSD *P*<0.01 within a cultivar pair (A or B) (n=12). See Figs. 3 and 6 for the effects of LTS and reconditioning on tuber respiration rates, sucrose and reducing sugar concentrations.
<table>
<thead>
<tr>
<th></th>
<th>LTS 4°C (days)</th>
<th>Reconditioning 16°C (days)</th>
<th>Storage 4°C (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RB</td>
<td>0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Glaciate</td>
<td>35.8b*</td>
<td>16.1b</td>
<td>9.9f</td>
</tr>
<tr>
<td>RR</td>
<td>30.5c*</td>
<td>15.4fgh</td>
<td>10.1i</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acclimate</td>
<td>45.4a</td>
<td>40.5b</td>
<td>25.6d</td>
</tr>
</tbody>
</table>

*Letters indicate significant differences among treatments.
Fig. 5. (A) Comparison of foliar and tuber growth, and total biomass (foliar + tuber) produced by potato cvs Ranger Russet and Acclimate grown at Othello, WA in 2015. Cumulative degree-days (>7.2°C) from planting (April 15) are indicated on the top axes. (B) Changes in tuber sucrose concentrations, average tuber weights, (C) reducing sugars (glucose + fructose) and specific gravity during development. Physiological maturity (PM) was estimated at 143 and 140 DAP for Ranger Russet and Acclimate tubers, respectively. Final-harvest (182 DAP) process quality is depicted by the French fry planks for each cultivar. Each fry plank represents a single tuber (numbers are average Photovolt reflectance values of the stem end of fry planks, *P<0.05). See Table 1 for ANOVA results and Table 3 for the effects of cultivar on tuber number, yields, and tuber size distributions.
Fig. 6. Effects of consecutive periods of low temperature sweetening (LTS) and reconditioning (Recond) on the respiration rates (A), sucrose (B) and reducing sugar (C) (Glc + Fru) concentrations of cvs Ranger Russet and Acclimate tubers. Tubers were initially stored for 15 days at 9°C directly following harvest to establish and compare basal respiration rates during wound healing. The storage temperature was then rapidly lowered at zero time to 4°C (arrow) for a 31-day period of LTS, followed by an increase to 16°C (arrow) for a subsequent 21-day period of reconditioning (from 31-52 days). At 52 days, the temperature was again dropped to 4°C for the remainder of the 125-d storage period. The reconditioning period (31-52 days) is shaded in yellow in B and C. For respiration rates (A), each point represents the average of 24 tubers (four replicates of six tubers). For sugars (B,C), each point represents the average of 12 tubers (four replicates of three tubers, ±SE). Samples of French fry planks are included at 0, 31, 52, 82 and 125 days (C) to show temperature-induced changes in process quality (each fry plank represents a single tuber). See Fig. 4 for a complete analysis of changes in fry color.
Tuber Respiration (mg CO$_2$ kg$^{-1}$ h$^{-1}$)

- Ranger Russet
- Acclimate

Reducing Sugars (mg g$^{-1}$ dry wt)

- Ranger Russet
- Acclimate

Sucrose (mg g$^{-1}$ dry wt)

- Ranger Russet
- Acclimate

Days in Storage

- Ranger Russet
- Acclimate
Fig. 7. Changes in sucrose, reducing sugars (glc + fru) and total sugar (suc + glc + fru) concentrations in Ranger Russet (RR) and Russet Burbank (RB) (cultivar average) versus Glaciate (W8) and Acclimate (X17) (cultivar average) tubers as affected by heat stress (HS, 21 d at 32°C), cold storage (CS, 32 d at 4°C) or the combination of HS followed by CS. Control tubers were stored at 9°C. Letters indicate LSD $P<0.05$ within a sugar (each bar represents 48 tubers; n=16, 3 tubers per rep). Samples of French fry planks from each cultivar are included to show the temperature-induced changes in process quality. Each fry plank is from a different tuber and the four planks shown for each treatment represent the average fry color from a 12-tuber sample. Fry planks are oriented apical (bud) end up and basal (stem) end (stolon attachment) down. Numbers are the mean Photovolt reflectance values of the basal ends of fries ($\leq 19$ is unacceptable by industry standards). Letters indicate LSD $P<0.01$ (n=12) within a cultivar pair (RB and Glaciate or RR and Acclimate).
**A**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sugars (mg g(^{-1}) dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB &amp; RR</td>
<td>15.1</td>
</tr>
<tr>
<td>W8 &amp; X17</td>
<td>8.1</td>
</tr>
<tr>
<td>Control 9°C</td>
<td>6.1</td>
</tr>
<tr>
<td>Ht Stress</td>
<td>10.7</td>
</tr>
<tr>
<td>CS</td>
<td>8.5</td>
</tr>
<tr>
<td>Ht Stress + CS</td>
<td>7.3</td>
</tr>
</tbody>
</table>

**B**

**Sucrose**

- **RB & RR**: 34.7 (bc)
- **W8 & X17**: 30.8 (cde)

**Glucose + Fructose**

- **RB & RR**: 32.5 (bcd)
- **W8 & X17**: 26.7 (ef)

**Total**

- **RB & RR**: 35.8 (b)
- **W8 & X17**: 45.2 (a)
Fig. 8. (A) Comparison of foliar and tuber growth, and total biomass (foliar + tuber) produced by potato cvs Atlantic and Hibernate grown at Othello, WA (average of 2016 and 2017 growing seasons). Average cumulative degree-days (>7.2°C) from planting (April 14) are indicated on the top axes. (B) Changes in tuber sucrose concentrations, average tuber weights, (C) reducing sugars (glucose + fructose) and specific gravity during development. See Table 1 for ANOVA results and Table 4 for the effects of cultivar on tuber number, yields, and tuber size distributions. Final-harvest (120 DAP) process quality is depicted by the potato chips for each cultivar (each chip represents a single tuber). Photovolt reflectance values and associated Snack Food Association (SFA) chip colors [1 (light) to 5 (dark)] are shown.
Fig. 9. Effects of consecutive periods of low temperature sweetening (LTS) and reconditioning on the respiration rates (A), sucrose (B) and reducing sugar (C) (Glc + Fru) concentrations of cvs Atlantic and Hibernate tubers. Tubers were initially stored for 10 days at 10°C directly following harvest to compare basal respiration rates during wound healing (yellow shading). The storage temperature was then rapidly lowered at zero time to 4°C (arrow) for a 32-day period of LTS, followed by an increase to 16°C (arrow) for a subsequent 29-day period of reconditioning (from 32-61 days). For respiration rates (A), each point represents the average of 36 tubers (six replicates of six tubers). For sugars (B, C), each point represents the average of 12 tubers (four replicates of three tubers, ±SE). Samples of chips are included at 0, 11, 32, 46 and 61 days (C) to show temperature-induced changes in process quality. See Fig. 10 for a complete analysis of changes in chip color.
A: Tuber Respiration (mg CO\textsubscript{2} kg\textsuperscript{-1} h\textsuperscript{-1})

B: Sucrose (mg g\textsuperscript{-1} dry wt)

C: Reducing Sugars (mg g\textsuperscript{-1} dry wt)

Days in Storage

Atlantic and Hibernate treatments at different temperatures.
Fig. 10. Changes in process quality of chips from cvs Atlantic and Hibernate tubers as affected by storage at 4°C for 32 days, followed by a 29-d period of reconditioning at 16°C. Tubers were initially wound-healed for 10 days at 10°C. The temperature was then rapidly lowered to 4°C for 32 d of low temperature sweetening (LTS) before increasing to 16°C for an additional 29 d of reconditioning. Each chip is from a different tuber and the four chips shown for each treatment represent the average chip color from a 12-tuber sample. Chips are oriented apical (bud) end up and basal (stem) end (stolon attachment) down. Numbers are Photovolt reflectance values. Letters indicate LSD $P<0.05$ (n=12). See Fig. 9 for the effects of LTS and reconditioning on tuber respiration rates, sucrose and reducing sugar concentrations.
Fig. 11. Changes in sucrose, reducing sugars (glc + fru) and total sugar (Suc + Glc + Fru) concentrations in Atlantic and Hibernate tubers as affected by heat stress (HS, 21 d at 32°C), cold storage (CS, 32 d at 4°C) or the combination of HS followed by CS. Control tubers were stored at 9°C. Letters indicate LSD $P<0.05$ within a sugar (each bar represents 12 tubers; $n=4$, 3 tubers per rep). Chip samples are included to show the temperature-induced changes in process quality. Each chip is from a different tuber and the four chips shown for each treatment represent the average color from a 12-chip (tuber) sample. Chips are oriented apical (bud) end up and basal (stem) end (stolon attachment) down. Numbers are mean Photovolt reflectance values. Letters indicate LSD $P<0.05$ ($n=12$).