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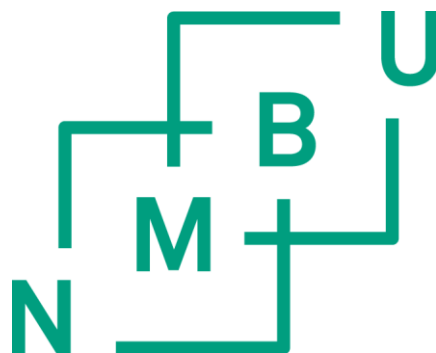
Potetkvalitet under lagring: Effekt av modningsgrad og ventilasjonsstrategier. Studier av lagringssykdommen *Fusarium* råte

Philosophiae Doctor (PhD) Thesis

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Abstract

Storage losses of Norwegian potatoes are estimated to approximately 10%. The losses are caused by respiration, transpiration, germination and diseases. These are processes that can be managed by controlling storage conditions and the quality of the potatoes that goes into storage. The main aim of this thesis is to gain knowledge on how to maintain tuber quality and reduce losses during storage, with special emphasis on tuber maturity, ventilation strategies and *Fusarium* dry rot.

During the growing seasons 2010, 2012 and 2013, three different maturity levels [M_{mat} (mature), M_{med} (medium mature) and M_{imm} (immature)] were obtained for the potato cultivars Asterix and Saturna by combining pre-sprouting strategy (pre-sprouted/not pre-sprouted), planting date (normal/late) and level of nitrogen fertilization (70/105/140 kg N ha^{-1}). To obtain $M_{mat,}$ a combination of pre-sprouting, planting at normal planting date and fertilizing with 70 kg N ha^{-1} was used. Maturity indicators measured 1, 2 and 3 weeks before harvest and at harvest were used to predict potato quality in potato tubers during and by the end of storage. The maturity indicators were haulm senescence (haulm maturity), skin set (physical maturity), dry matter content (physiological maturity) and contents of sucrose, glucose and fructose (chemical maturity). The quality parameters investigated during long-term storage were weight loss, respiration, dry matter content, fry colour and contents of sucrose, glucose and fructose.

Weight loss, respiration rate and dry matter contents were found to be influenced by maturity level, with immature potatoes having higher weight losses and respiration rates and lower contents of dry matter in average over three years. Sucrose, glucose and fructose were successfully predicted with linear regression models ($R^2 \geq 0.88$), and included dry matter, sucrose, glucose and fructose measured before harvest as significant continuous predictors. A weight loss model included skin set measured at harvest as a significant predictor and a fry colour model included sucrose measured before harvest as a significant predictor.

Moreover, the effect of maturity level on *Fusarium* dry rot development caused by four *Fusarium* species (*F. coeruleum, F. avenaceum, F. sambucinum* and *F. culmorum*) was studied on the same tuber material with different maturity levels. Maturity level significantly affected disease development in Asterix tubers when inoculated with *F. sambucinum*. There was no such effect of maturity on *Fusarium* dry rot development in the cultivar Saturna.

The effect of two different ventilation strategies on potato quality in Asterix and Saturna tubers with different maturity levels was examined both in small-scale experimental stores

and in large-scale commercial stores during the three storage seasons. One ventilation strategy included "natural ventilation", where the tubers were ventilated by continuous low air rates of 10-15 m^3 t^{-1} h^{-1}, resulting in an air volume of 240-360 m^3 t^{-1} day^{-1}. The other was "forced ventilation", with intermittent high air rates of 75-100 m^3 t^{-1} h^{-1}, resulting in an air volume of 150-200 m^3 t^{-1} day^{-1}. In average over three years, natural ventilation resulted in higher weight losses and lighter fry colours in Saturna in both small- and large-scale stores, lighter fry colours in Asterix in large-scale stores and lower contents of glucose and fructose in large-scale stores than did forced ventilation.

The prevalence of *Fusarium* dry rot in potatoes grown in Norway was investigated for three consecutive years in the period 2010 to 2012 with a total of 238 samples (comprising 23,800 tubers), representing different cultivars and production regions. Real-time PCR assays were tested for their suitability to detect Norwegian isolates of *Fusarium* species. Ten commonly grown potato cultivars in Norway were compared for their resistance to *F. coeruleum, F. avenaceum* and *F. sambucinum* in two trials during 2012 and 2013. To understand the impact of different inoculum sources on *Fusarium* dry rot development, seed and soil was inoculated with different concentrations of inoculum and dry rot development was quantified in progeny tubers.

A total of 718 isolates of *Fusarium* were recovered in the national survey. Seven *Fusarium* species were identified, and the four most prevalent species included *F. coeruleum*, *F. avenaceum*, *F. sambucinum* and *F. culmorum*. Less prevalent species were *F. cerealis, F. graminearum* and *F. equiseti*. The regions showed differences in prevalence of the *Fusarium* species. A previously developed *F. coeruleum* specific real-time PCR assay gave unexpectedly high Ct values. Hence, a new test for this species was developed which could successfully identify Norwegian isolates. There were differences in susceptibility to *Fusarium* spp. among the cultivars, of which the cultivars Berber, Rutt and Laila developed the most severe dry rot symptoms. In general, *F. sambucinum* was the most aggressive species and caused severe dry rot lesions. There were, however, significant differences between isolates. Soil infested with *F. sambucinum* (low and high levels) and *F. avenaceum* (only high levels) resulted in significantly more severe rots than did soil without inoculum. The inoculation of seeds did not result in any dry rot development in progeny tubers.

In conclusion, this study contributes with knowledge on how maturity and ventilation can be managed to improve potato quality during storage. It also provides knowledge that can support future control strategies of *Fusarium* dry rot.

Sammendrag

Lagring av poteter er nødvendig under norske forhold, men innebærer samtidig en stor fare for tap av verdier og ressurser. Lagringstapene, som i Norge estimeres til 10%, skyldes biologiske prosesser knyttet til respirasjon, transpirasjon, spiring og sykdomsangrep. For å lykkes med lagring er det viktig å ha fokus på råvarekvalitet, slik som potetens modningsgrad og sykdomssmitte, og på lagringsklima. Formålet med denne studien er å bidra med kunnskap som hjelper til med å opprettholde kvaliteten og redusere tapet under lagring. I arbeidet er det særlig fokus på ventilasjonsstrategier, på modning og på *Fusarium* råte.

Det meste av arbeidet er gjennomført i tre år (2010, 2012 og 2013) med sortene Asterix og Saturna med tre ulike modningsgrader [M_{mat} (moden), M_{med} (medium moden) og M_{imm} (umoden)]. De ulike modningsgradene ble oppnådd ved å kombinere lysgroing (lysgrodd/ikke lysgrodd), settetid (normal/sen) og gjødsling med forskjellige mengder nitrogen (7/10/14 kg N daa^{-1}). For eksempel var M_{mat} lysgrodd, hadde normal settetid og var gjødslet med 7 kg N daa^{-1}. Modningsindikatorer målt 3, 2 og 1 uker før høsting og ved høsting ble brukt til å forutsi potetkvaliteten gjennom lagringsperioden. Modningsindikatorene som ble brukt var grønnfarge i riset (risets modning), skallkvalitet (fysisk modning), tørrstoffinnhold (fysiologisk modning) og innhold av sukrose, glukose og fruktose (kjemisk modning). Modningsgrad, potetsort og år inngikk som faste prediktorer i alle modellene. Kvalitetsparametrene som ble målt under og etter langtidslagring var vekttap, respirasjon, tørrstoffinnhold, sukrose-, glukose- og fruktoseinnhold og friteringsfarge.

I gjennomsnitt hadde umodne poteter større vekttap, høyere respirasjon og lavere tørrstoffinnhold. Innholdet av sukrose, glukose og fruktose etter lagring kunne predikteres ved hjelp av lineære regresjonsmodeller ($R^2 \geq 0,88$). Signifikante faktorer i disse modellene var tørrstoff, sukrose, glukose og fruktose målt før innlagring. I en prediksjonsmodell for vekttap under lagring var skallkvalitet ved høsting en signifikant prediktor mens en modell for friteringsfarge inneholdt sukrose som en viktig prediktor.

Videre ble materialet med ulik modningsgrad benyttet til å studere betydningen av modning for smitte av *Fusarium,* med artene *F. coeruleum, F. avenaceum, F. sambucinum* og *F. culmorum.* Potetens modningsgrad påvirket utviklingen av *Fusarium* råte når denne var forårsaket av *F. sambucinum.* Det var ingen sikker effekt av modningsgrad på råteutvikling når knollene var smittet med de andre artene. For sorten Saturna hadde modningsgrad ingen betydning for utvikling av *Fusarium* råte i knollene.

Poteter med ulik modningsgrad av sortene Asterix og Saturna ble lagret på både småskala forsøkslagre og storskala kommersielle lagre. Målet var å se på effekten av to ulike

ventilasjonsstrategier på potetkvalitet. Med strategien "natural ventilation" ventileres potetene kontinuerlig med små luftmengder på 10-15 m^3 tonn^{-1} time^{-1}, og får dermed en total luftmengde 240-360 m^3 t^{-1} dag^{-1}. Med "forced ventilation" brukes store luftmengder i intervaller på 75-100 m^3 tonn^{-1} time^{-1}, noe som gir en total luftmengde på 150-200 m^3 t^{-1} dag^{-1}. I gjennomsnitt over tre år resulterte "natural ventilation" i større vekttap og lysere friteringsfarge i Saturna på både små- og storskala lagre, lavere innhold av glukose og fruktose og lysere friteringsfarge i Asterix på storskala lagre enn "forced ventilation".

For å kartlegge hvilke *Fusarium*-arter som er til stede i norske poteter ble det i tre vekstsesonger fra 2010 til 2012 samlet inn 238 potetprøver (23.800 knoller) med et representativt utvalg av potetsorter og geografiske regioner. Real-time PCR ble brukt til deteksjon av ulike *Fusarium* arter på norske isolater. Et forsøk med 10 av de mest vanlig brukte potetsortene i Norge ble gjennomført i 2012 og 2013 med hensyn til resistens mot tre *Fusarium* arter (*F. coeruleum, F. avenaceum* og *F. sambucinum*). For å kunne forstå mer om betydningen av forskjellige smittekilder for Fusarium ble den relative betydningen av jord- og knollsmitte undersøkt ved at jord og knoller ble tilført ulike sporekonsentrasjoner av inokulum.

Totalt ble det funnet 718 isolater, fordelt på syv forskjellige *Fusarium* arter. De fire vanligste artene var *F. coeruleum, F. avenaceum, F. sambucinum* og *F. culmorum*, mens mindre vanlige arter var *F. cerealis, F. graminearum* og *F. equiseti*. Ulike arter ble funnet i de forskjellige regionene. For *F.* coeruleum ble det funnet høye Ct verdier i en tidligere utviklet real-time PCR test og det ble derfor utviklet en ny test som er i stand til å identifisere norske isolater av arten. Det ble observert forskjellig mottakelighet mot *Fusarium* i ulike potetsorter. Berber, Rutt og Laila var de mest mottakelige sortene og utviklet mest råte. *F. sambucinum* var generelt den mest aggressive av artene, men det var forskjeller i aggressivitet mellom de undersøkte isolatene. Jordsmitte med *F. sambucinum* (lav og høy smittekonsentrasjon) og *F. avenaceum* (høy smittekonsentrasjon) resulterte i signifikant mer råte hos potet enn jord uten smitte. Ingen av behandlingene med knollsmitte resulterte i råteutvikling.

Samlet sett bidrar denne studien med kunnskap om hvordan råvarens modningsgrad og ulike ventilasjonsstrategier under lagring kan utnyttes til å bedre lagringskvalitet av potet. Kunnskapen om *Fusarium* kan brukes til å optimalisere tiltak for å unngå smitte og angrep av *Fusarium* råteorganismer.

List of papers

I. Heltoft P., Wold A-B., Molteberg E.L. (2016) Effect of ventilation strategy on storage quality indicators of processing potatoes with different maturity levels at harvest. Postharvest Biology and Technology 117: 21-29.

II. Heltoft P., Wold A-B., Molteberg E.L. (2016) Maturity indicators for prediction of potato quality during storage. *Submitted to Postharvest Biology and Technology*

III. Heltoft P., Brurberg M.B., Skogen M., Le V.H., Razzaghian J., Hermansen A. (2016) *Fusarium* spp. causing dry rot on potatoes in Norway and development of a real-time PCR method for detection of *Fusarium coeruleum*. Potato Research DOI: 10.1007/s11540-015-9313-5

IV. Heltoft P., Molteberg E.L., Nærstad R., Hermansen A. (2015) Effect of maturity level and potato cultivar on development of *Fusarium* dry rot in Norway. Potato Research 58: 205-219

V. Heltoft P., Brierley J.L., Lees A.K, Sullivan L., Lynott J., Hermansen A. (2016) The relative contribution of soil-borne inoculum to *Fusarium* dry rot in potato cultivars Asterix and Saturna. *Submitted to European Journal of Plant Pathology*

1. Introduction

1.1 General introduction

The Norwegian production of potatoes comprises approximately 350,000 tonnes, with a wholesale value of approximately NOK 500 million annually (Statistics, 2014). A major part of the potato production is stored for longer or shorter periods. Approximately 10 percent of the production is lost from Norwegian potato stores annually (Bengtsson et al., 1996). This represents both a major loss of resources in an environmental perspective and a high economic loss for the growers. The extent of the potato storage losses is basically determined by the conditions of the potato at harvest (maturity, mechanical damage and infection of disease and pests) and the conditions and duration of storage (Wustman and Struik, 2008).

In Norway, immature potatoes is a major concern, mostly due to the use of relatively late cultivars in combination with a short and cool growing season (100-110 days). Immature potatoes at harvest can lead to high losses caused by poor skin quality (dehydration, diseases), early sprouting and fry colour problems. The impact of maturity on the quality of potatoes after storage is confirmed in several studies (Herrman et al., 1995; Hogge et al., 1993; Kumar et al., 2004; Wiltshire et al., 2004). However, maturation is a complex process including both haulm maturity and physical, physiological and chemical maturity. They all have different effects on the potato quality and more studies are needed evaluating the effect of different aspects of maturity on the potato quality during storage including the effect of storage disease development. Chemical maturity is the most commonly used indicator in prediction models for potato quality (Hertog et al., 1997a; Sowokinos, 1978). Sowokinos (1978) found the level of sucrose at harvest to be a good indicator of subsequent processing quality and suggested to keep levels below 2.8 mg/g fresh weight. However, other studies investigating the relationship between processing quality and sucrose content did not succeed in using sucrose as a predictor (Briddon and Storey, 1996; Lærke and Christiansen, 2005; Wiltshire et al., 2004). More studies are thus needed, looking at the use of other maturity indicators in addition to chemical maturity. In practice, a model predicting tuber quality during and after storage, using measurements in the last part of the growing season and at harvest would be of great interest to the grower and to the processing industry. It could be useful in guiding decisions on length of storage and the order in which the crop should be processed.

Storage conditions, such as temperature, humidity, atmospheric conditions and ventilation are important elements in maintaining good quality and reducing loss of stored potatoes. The effect of ventilation strategy has been given little attention. There is thus a need for studies on the effect of different ventilation strategies on potato quality under Norwegian conditions during long-term storage.

Fusarium dry rot is an important storage disease, from which problems seem to have increased during the last decades. In order to support future control strategies, studies are needed exploring which *Fusarium* species are currently causing *Fusarium* dry rot in commercial potato production since the last survey was done in Norway (Bjor, 1978). In order to implement effective disease-management strategies for *Fusarium* dry rot, it is important to understand the impact of different inoculum sources on disease development. Studies of the relative importance of seed and soil-borne inoculum between different *Fusarium* spp. would be useful. Control strategies commonly include use of resistant cultivars. However, knowledge about resistance to *Fusarium* spp. among the currently most grown potato cultivars in Norway is limited. The Norwegian breeding company, Graminor, has previously done resistance testing in upcoming varieties, but in these tests, the inoculum was made as a mixture of *F. coeruleum* and *F. avenaceum* isolates and therefore no information was gained about resistance towards individual species and other species than *F. coeruleum* and *F. avenaceum*. Latent infections can occur in tubers pre-storage. Diagnostic tools can be used to detect these and validate the tuber storability. However, more knowledge is need to confirm the suitability of these tools on Norwegian *Fusarium* isolates.

1.2 Aim of the study

The main purpose of this thesis is to contribute with knowledge, which can support storage management strategies of potatoes that helps to maintain quality and reduce loss during storage. Special attention was given maturity of the crop, ventilation strategies and the storage disease *Fusarium* dry rot. The main objective was met by addressing the following research aims presented in five individual papers (I-V) (Figure 1):

- Study the effect of different maturity levels on storage quality of potatoes during long-term storage (Paper I).

- Investigate the potential of potato maturity indicators measured in the field prior to or at harvest to predict potato quality during and after storage (Paper II).

- Study the effect of maturity levels in potato tubers on *Fusarium* dry rot development caused by different *Fusarium* spp. (Paper IV).

- Examine the effect of ventilation strategies on storage quality of potatoes with different maturity during long-term storage (Paper I).

- Identify *Fusarium* species currently causing *Fusarium* dry rot in commercial potato production in Norway, including the extent of regional variation, and the effect of agronomic and storage factors (Paper III).

- Evaluate the resistance of commonly grown potato cultivars in Norway to different *Fusarium* species (Paper IV).

- Test the suitability of available real-time PCR assays for detection of *Fusarium* spp. common in Norway, development of new assays if needed (Paper III).

- Investigate the relative importance of seed- versus soil-borne inoculum of three species of *Fusarium* (*F. coeruleum, F. sambucinum* and *F. avenaceum*) in causing dry rot (Paper V).
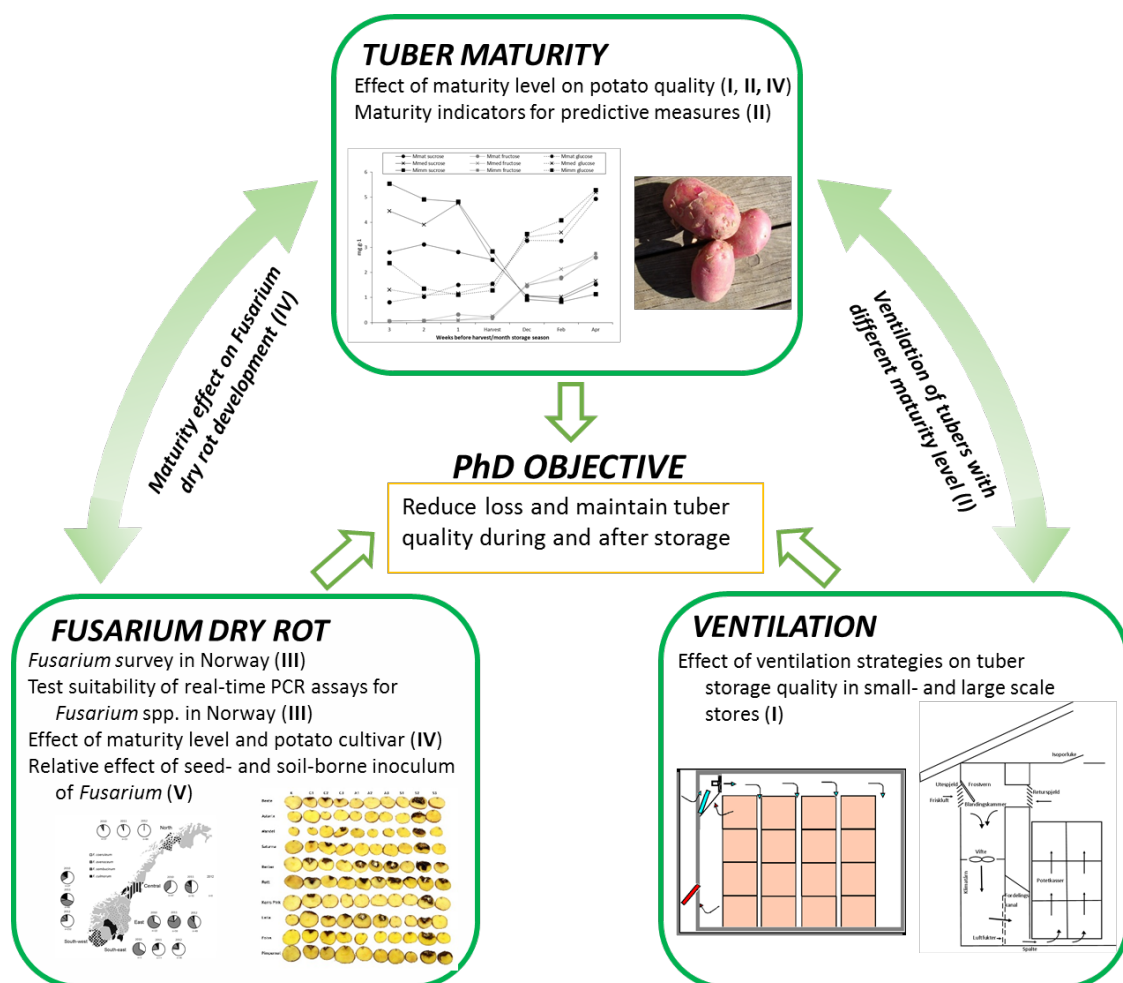

Figure 1 Graphical outline of the scientific papers (I-V) included in the thesis.

2. Background

2.1 Potato

Potato (*Solanum tuberosum* L.) belongs to the Solanaceae family. It originated from and was first domesticated in the Andes mountains of South America. Potato is now grown and consumed in temperate as well as in tropical countries and is ranked as the third most important food crop after wheat and rice with over 300 million t produced annually (CIP, International Potato Center).

2.1.1 Lifecycle

Potatoes are usually propagated using seed tubers. During the life cycle the potato tuber passes through several phases including dormancy, sprouting, tuberization, tuber bulking and maturity. Figure 2 show the traditional way of multiplying the potato (Struik, 2007). Just after harvest, the tubers undergo a period of dormancy for about 1-15 weeks, depending on cultivar, conditions before harvest and storage conditions (Sonnewald and Sonnewald, 2014; Struik et al., 2006; Suttle, 2004; Vreugdenhil, 2007; Wiltshire and Cobb, 1996). Once the dormancy is broken tubers can start growth. Sprouts are developed in the eyes of the seed tubers. Sprouting begin immediately if the conditions are right but cold temperatures in store or in the field may delay sprouting. Shoots are developed from the sprouts and the plant emerge. Stems, stolons, roots and inflorescences are developed on the shoots and photosynthesis begin. The tips of the stolons develops into new progeny tubers (tuberization) (Ewing and Struik, 1992; Jackson, 1999; Sonnewald and Sonnewald, 2014). In the next growth stage (tuber bulking) the tuber cell expands with the accumulation of nutrients, water and sugars and the tubers grow in size. Tubers become a storage organ for starch and storage proteins. In the final stage of the cycle (maturation), the plants growth slows and eventually ceases entirely. Photosynthesis in the leaves slow down, and the tubers stop growing.
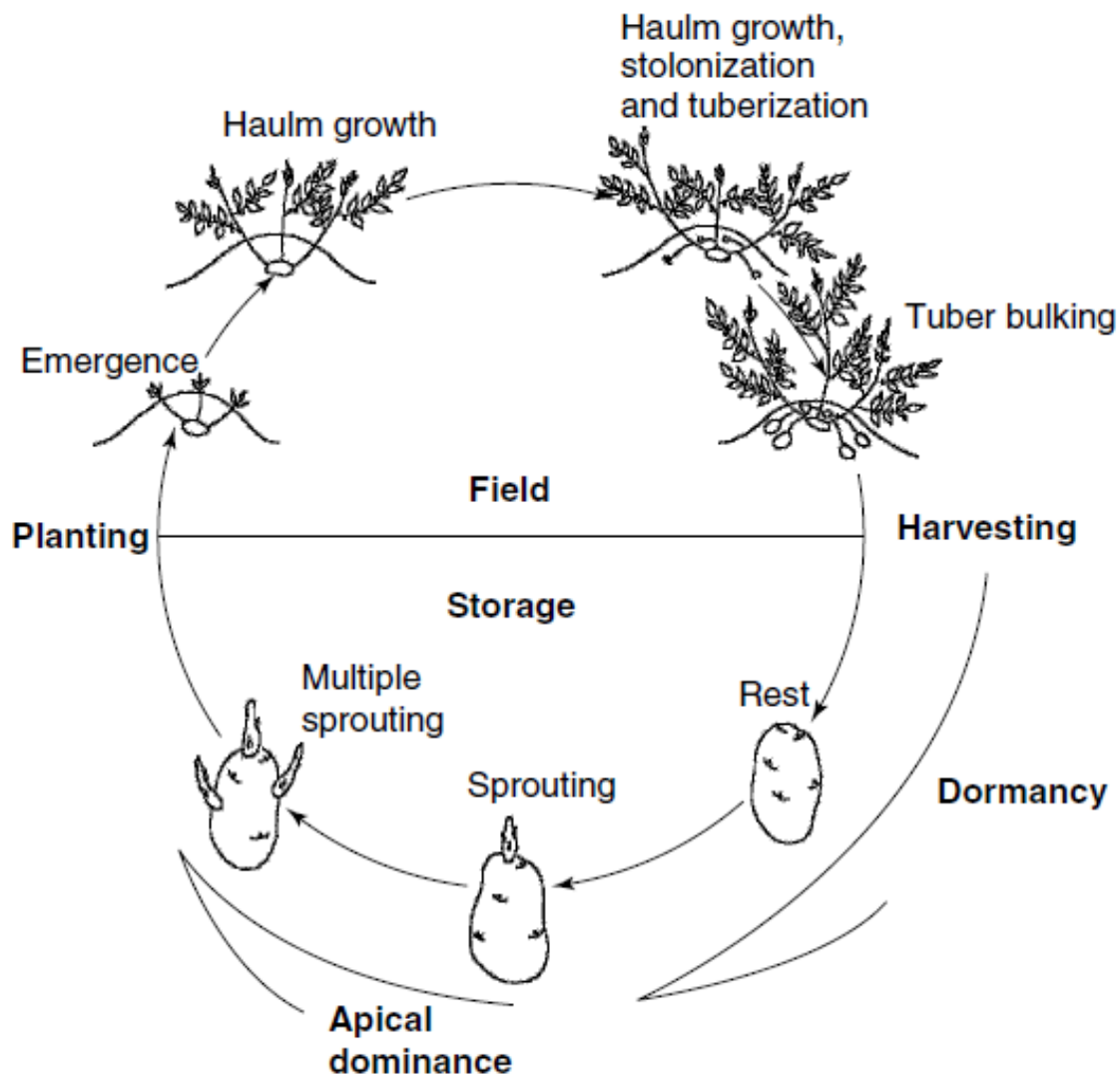
Figure 2 Life cycle of the potato. Seed tubers are harvested and stored under proper conditions to form sprouts. Seed tubers are planted (before or after sprouting) and will then produce new plants that produce progeny tubers (Struik, 2007)

2.2 Tuber maturity

Maturation is a continuous and complex process, and the result of individual and partly independent processes including senescence (haulm maturation), as well as skin set (physical maturation), accumulation of dry matter (physiological maturation) and lowering of sucrose content (chemical maturation) (Bussan et al., 2009; Kolbe and Stephan-beckmann, 1997; Sabba et al., 2007). The complexity of the maturation processes derives, as these processes does not necessarily peak at the same time (Kumar et al., 2004; Sabba et al., 2007; Sowokinos, 1978).

Maturity can be manipulated by various growth factors, including seed treatment, planting date, fertilization management and harvest strategy (Knowles and Plissey, 2008). Seed

treatments through manipulation of light and/or temperatures will affect the physiological age of the seed tuber and thus the growth of the crop. A process of presprouting tubers leads to earlier emergence and earlier tuber initiation and thus a more mature tuber than if not presprouting (Caldiz et al., 2001; Delaplace et al., 2008; Johansen and Molteberg, 2012). Presence of excess nitrogen can act as a growth regulator by stimulating haulm growth but delay tuber growth and maturing of the crop (Hope et al., 1960).

2.2.1 Haulm maturity

The maturation process of potatoes starts with maturation of the haulm. Haulm maturation occurs over the last 2 to 3 weeks of the potato plant growth. In the process of natural senescence the photosynthesis decreases, the allocation of carbohydrates to the tubers decline, bulking rates decrease and the tubers start to mature (Bussan et al., 2009). In practice, however, growers often stimulate the maturation by haulm desiccation. Through control of nutrient management, seed age and length of the growing season the haulm will senesce naturally.

2.2.2 Physical maturity

The tuber maturation process of potatoes includes the development of a mature and fully set periderm referred to as the physical maturation. The periderm consists of three layers of tissue (Figure 3): the phellem, phellogen and the phelloderm. The phellem is the outer tissue and is referred to as the skin. The middle layer is the phellogen, a thin region of immature meristematic tissue. The phelloderm is the inner tissue adjacent to the starch storing cortical tissue inside the tuber (Bussan et al., 2009; Lulai, 2002). When the tuber starts to mature, the periderm stop expanding and the skin starts to set and binds to the underlying tissues. Physically immature tubers are susceptible to skinning injury and have a skin that is permeable to water and can result in higher weight loss during storage due to higher transpiration rates (Sabba et al., 2007). In addition, physically immature potato tubers are more susceptible to wounding and important storage diseases can enter the tubers (Knowles and Plissey, 2008; Secor and Salas, 2001).
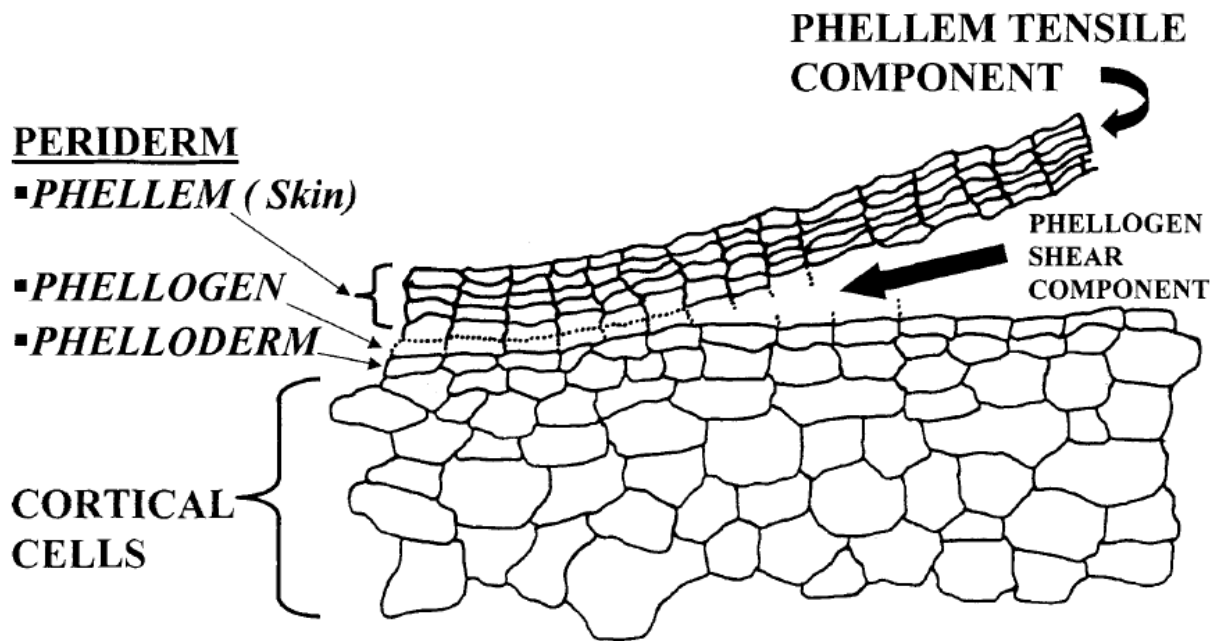
Figure 3 Outline of a micrograph of the cell walls of potato tuber periderm and neighbouring cortical cells (Lulai, 2002)

2.2.3 Physiological maturity

Physiological maturity is achieved when the dry matter content reach its maximum (Bussan et al., 2009; Sabba et al., 2007). The peak of the dry matter content usually coincides with a maximum starch accumulation. Specific gravity is closely related to dry matter content and indirectly also to starch content, since 80 to 85% of the dry matter content of a tuber is composed of starch.

2.2.4 Chemical maturity

Chemical maturity is related to minimum content of sucrose in the tuber. Immature potatoes have a high content of sucrose, as the rate of translocation to the tubers exceeds the rate of metabolism in the tuber (Sowokinos and Preston, 1988). Sucrose serve as a direct substrate for reducing sugar accumulation as sucrose is hydrolysed to glucose and fructose. A high content of reducing sugars is critical for the processing industry, as high reducing sugar levels might lead to dark fry colour and acryl amide production through the Maillard reaction (Amrein et al., 2003; Shallenberger et al., 1959; Sowokinos and Preston, 1988). Sowokinos (1978) suggested a maximum content of 2.8 mg g^{-1} sucrose in the tuber in order to obtain acceptable processing quality.

2.3 Storage conditions

Most potatoes are stored for longer or shorter periods and they are used fresh or after storage as table or processed potatoes. Some are also used as seed potatoes. To prevent post-harvest losses, proper storage is essential. In order to successfully store the potatoes, it is important to understand the factors affecting the storage of the crop. The potato tuber is a fresh product with a high moisture content and metabolic rate. Once the tubers are harvested they start utilizing their own stored resources for metabolic processes, which lead to both losses of mass and quality (Wustman and Struik, 2008).

Among quality defects caused by storage are diseased tubers, sprouting, weight loss, greening, black heart and dark fry colours. Important mechanisms involved in these processes are biochemical growth responses, respiration, transpiration and cold-induced sweetening. Important factors for development of quality defects are the physical, physiological and chemical state of the tubers (maturity and degree of skinning/mechanical stress), level of infection by diseases and levels and fluctuations in temperature, atmospheric composition and light (Pinhero et al., 2009; Pringle et al., 2009).

The storage conditions are of great importance for maintaining good quality and reducing losses of stored potatoes. Important parameters, such as temperature, relative humidity, atmospheric conditions and ventilation, must be controlled.

2.3.1 Temperature

Temperature affects various biological processes such as respiration, transpiration, cold induced sweetening and incidence of pests and diseases (Pinhero et al., 2009; Pringle et al., 2009; Wustman and Struik, 2008). Tubers have a higher respiration rate at higher temperatures than at low temperatures. Minimum respiration rates are seen at 5-6 °C and higher rates at elevated storage temperatures (Burton et al., 1955).

Wound healing is applied to the harvested crop in order to prevent infections from fungi and bacteria and to prevent moisture loss. During wound healing, suberin is formed between and below the damaged surface cells and provide an initial barrier to disease entry and new cells form soon after into a more impenetrable barrier. Suberin is described as a complex biopolyester comprised of a phenolic (aromatic or lignin-like) domain attached to the cell wall and an aliphatic (lipid, hydrophobic) domain which is probably attached to the phenolic

domain (Lulai and Orr, 1994; Lulai and Corsini, 1998). The rate of wound healing is primarily influenced by temperature. Wound healing is fastest at 20 °C and reduces to almost zero at 7 °C (Artschwager, 1927; Cunnington and Pringle, 2008; Pringle et al., 2009). Warm temperature speed wound healing but also favour development of disease. In general, infections with fungi and bacteria increase with increasing temperature (Pringle et al., 2009; Secor and Salas, 2001). However, since the skin is the main defence against disease, rapid wound healing at high temperature is usually a priority. Once wounds are healed, cooling can start. In this context it should however be mentioned that some species of *Fusarium* spp. and *Boeremia* spp. can continue disease development even at low temperatures (Kirk et al., 2013; Pringle et al., 2009; Secor and Salas, 2001).

Low temperature (3-4 °C) prolong the dormancy period of the potato tubers and prevent them from sprouting. Dormancy gives insight into how long the potato will store before it initiates sprout development (Suttle, 2004; Vreugdenhil, 2007; Wiltshire and Cobb, 1996). Sprouting initiates an increase in reducing sugar content, respiration rate and transpiration, which influences the tuber quality. The period of dormancy varies considerably between cultivars. Chemical treatments with sprout suppressors such as chloropham (CIPC), maleic hydrazide and ethylene can be used to control sprouting (Cunnington and Pringle, 2008; Kleinkopf et al., 2003; Wiltshire and Cobb, 1996).

Sprout suppressors are commonly used in stores with processing potatoes, which should be stored at temperatures above 6 °C. Lower temperatures result in high concentrations of the reducing sugars glucose and fructose, known as cold induced sweetening (Hertog et al., 1997b; Sowokinos and Preston, 1988). Elevated levels of fructose and glucose may result in dark fry colours as a result of the Maillard reaction where reducing sugars interact with free amino acids and produce acrylamide and a dark fry colour (Amrein et al., 2003; Shallenberger et al., 1959). Elevated levels of reducing sugars may be somewhat reduced through conditioning in the beginning of the storage period, or through re-conditioning at the end of the storage season prior to delivery. However, re-conditioning should take place before the beginning of senescent sweetening, where sugars are mobilised in the tubers for the benefit of development and growth of sprouts (Hertog et al., 1997b; Knowles et al., 2009; Pinhero et al., 2009; Sowokinos and Preston, 1988; Walsh, 1995).

2.3.2 Humidity

Potatoes have a high water content and during storage they lose moisture over time through transpiration and through the process of respiration (Cunnington and Pringle, 2008). Water loss caused by transpiration can be reduced by maintaining a high relative humidity (RH). RH is the amount of moisture in the air at a given temperature, relative to the maximum amount possible at that same temperature. RH is temperature dependent and warm air can hold more moisture than cold air. When the tubers are stored at 4 °C they reach equilibrium when the surrounding air is at 98% RH. Water loss from the tubers increase when RH of the air surrounding the tuber in store decrease (Oberg et al., 2013; Oberg and Kleinkopf, 2003). Condensation, which occurs as a result of the internal surface temperature falling below the dew point of the air next to the surface, should be prevented in order to avoid rotting and skin surface diseases of the tubers (Cunnington and Pringle, 2008; Pringle et al., 2009).

2.3.3 Atmospheric conditions

Potatoes are living organisms that respire and therefore needs oxygen. Lack of oxygen in a potato store can lead to blackheart (Cunnington and Pringle, 2008). During respiration, the potatoes produce CO_2, which will accumulate in the store atmosphere. High CO_2 levels can affect the fry colour in processing potatoes, resulting in darker fry colours (Copp et al., 2000; Daniels-Lake et al., 2005; Mazza and Siemens, 1990; Veerman and Wustman, 2005). Modern highly sealed stores are at risk of accumulating CO_2 unless precautions are taken to avoid this.

2.3.4 Ventilation

Ventilation is required during storage to maintain tuber quality (Bertolini and Guarnieri, 1990; Cunnington and Pringle, 2008; Sparks, 1980; Wustman and Struik, 2008). Ventilation remove moisture, field heat and respiratory heat from the potatoes and also prevent respiratory CO_2 accumulation in the storage facilities (Oberg and Kleinkopf, 2003). Recirculation of air is typically used in refrigerated stores. For ambient-air ventilation, outside air is usually combined with recirculation. Cooler ambient air replaces the warm inside air and cools the crop (Cunnington and Pringle, 2008; Pringle et al., 2009). Different air rates may be recommended during different phases of the storage period, depending primarily on tuber temperature and moisture on the tubers at harvest. High ventilation rate is required in the wound healing period, to remove moisture and heat. Newly harvested crops and especially

immature tubers, have a high rate of respiration, which can result in condensation. Furthermore, the moisture loss due to transpiration is high during the initial period of storage particularly in immature tubers with wounded tissue. (Pringle, 1996; Pringle and Robinson, 1996; Pringle et al., 2009).

Different ventilation strategies are in use, including forced ventilation and natural ventilation. Forced ventilation practices intermittent ventilation where the air is supplied from the top or bottom of the boxes and pressed through the boxes with a high air rate (typically 75-100 $m^3t^{-1}h^{-1}$) (Cunnington and Pringle, 2008; Pringle and Robinson, 1996). With natural ventilation, the air is supplied from channels in the floor using a low air rate (10-15 $m^3t^{-1}h^{-1}$). The air then rises naturally trough the boxes or pile as a result of natural convection (Forbord, 2013; Geyer and Gottschalk, 2008; Hylmö et al., 1975; Johansson, 1998; Pringle et al., 2009).

2.4 Storage diseases –*Fusarium* dry rot

Several storage diseases caused by both fungi and bacteria may cause significant yield losses during storage (Pringle et al., 2009). *Fusarium* dry rot is one of the most important storage diseases in potato tubers. The disease is caused by several fungal species in the genus *Fusarium* and can potentially cause significant yield losses with up to 60 percent of tubers affected. *Fusarium* spp. can infect almost all commonly grown cultivars (Leach and Webb, 1981; Secor and Salas, 2001). *Fusarium* species infect through wounds on tubers caused mainly by handling during planting, harvesting and grading (Secor and Salas, 2001).

2.4.1 Symptoms

The first symptoms of *Fusarium* dry rot are a sunken surface of the tubers with concentric circles (Kirk et al., 2013; Peters et al., 2008a). The colour of the rot is yellow-brown to dark-brown. Cavities can be seen inside the tuber. White, blue, pink or red coloured mycelium sometimes develops on the surface of the tubers or inside the cavity. In the beginning, the rot is V-formed towards the centre of the tuber and later the rot is spread to the whole tuber (Boyd, 1972; Kirk et al., 2013; Olofsson, 1976). Dry rot symptoms caused by *F. coeruleum* are shown in figure 4. Diagnosis of *Fusarium* dry rot can be complicated in the presence of soft rot bacteria, which often causes a secondary infection in the dry rot lesions. However, soft rot cause a wet rot that can very quickly encompass the entire tuber and mask the initial dry rot symptoms.

Figure 4 Potato tuber with *Fusarium* dry rot symptoms caused by *F. coeruleum* (photo: Pia Heltoft).

2.4.2 Causal organisms

Several species of *Fusarium* can cause *Fusarium* dry rot development in the tubers (Boyd, 1972; Secor and Salas, 2001)) and most of these species have a wide host range including *e.g.* cereals, legumes and beetroot (Peters et al., 2008b). In Great Britain and in the Nordic countries the most common species isolated from potato has been *F. coeruleum* (Bjor, 1978; Olofsson, 1976; Peters et al., 2008a; Seppänen, 1983). Macro- and chlamydospores of *F. coeruleum* are shown in figure 5. *F. sambucinum* is also an important species and is considered to be the most significant causal agent of *Fusarium* dry rot in other parts of Europe, in northern and western China and in North America (Du et al., 2012; Secor and Salas, 2001). Other important species includes *F. avenaceum* (Du et al., 2012; Peters et al., 2008a), *F. graminearum* (Estrada Jr et al., 2010) and *F. oxysporum* (Gachango et al., 2012)

Identification of *Fusarium* species can be done based on conidial morphology, production of chlamydospores, growth characteristics, and colony pigmentation (Gerlach and Nirenberg, 1982; Leslie and Summerell, 2006) or by using molecular methods. Real-time PCR assays providing fast identification and quantification of *Fusarium* spp. can be used to detect latent infections in tubers pre-storage, to validate their storability and/or suitability as seed potatoes (Cullen et al., 2005; Halstensen et al., 2006; Nicholson et al., 1998).

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Figure 5 Macro- and chlamydospores of *F. coeruleum* (photo: Pia Heltoft).

2.4.3 Disease cycle

Fusarium spp. are spread with contaminated seed tubers and soil (Adams and Lapwood, 1983; Jeger et al., 1996; Secor and Salas, 2001). Adams and Lapwood (1983) investigated the transmission of inoculum in the field, and found that *F. sambucinum* and *F. coeruleum* were transmitted from seed to progeny tubers. Another study (Leach, 1985) found that seed inoculated with *F. sambucinum* resulted in high levels of *Fusarium* dry rot in progeny tubers, whilst naturally occurring low levels of *F. coeruleum* in soil resulted in relatively less severe dry rot symptoms. Wounds caused during harvest and by other potato tuber handling operations serve as entry points for the *Fusarium* spores. Once the pathogen has penetrated the tuber skin, it begins to grow in the tubers tissue causing dry rot lesions at the point of entry (Kirk et al., 2013; Secor and Salas, 2001). *Fusarium* dry rot develops most rapidly at high relative humidity and temperatures of 15-20 °C. There is slower growth of *Fusarium* at lower temperatures but it can however continue its growth at the lowest temperature safe for storing potatoes (Secor and Salas, 2001).

2.4.4 Disease management

Present control strategies for *Fusarium* dry rot includes use of resistant cultivars and cultural practices such as crop rotation, use of disease free seed and wound healing prior to storage. As *Fusarium* spp. can only infect through wounds, avoiding injuries to tubers and providing conditions that promote wound healing are the most important management factors.

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Biological control agents and ultraviolet radiation are also used, as well as chemical control (Al-Mughrabi et al., 2013; Bojanowski et al., 2013; Bång, 1992; Gachango et al., 2012; Peters et al., 2008a; Ranganna et al., 1997; Secor and Salas, 2001). However, biological and chemical control methods are not commonly used targeted against *Fusarium* dry rot in Norway.

Integrated Pest Management (IPM) have received increased focus the last years. IPM is a sustainable approach to managing pests by combining biological, cultural and chemical tools in a way that minimises economic, environmental and health risks (Barzman et al., 2015). Cultivar resistance is a key element in the IPM strategies for control of *Fusarium* dry rot. Cultivars vary in their resistance to *Fusarium* spp. even though none of the potato cultivars have yet been found to be fully resistant to the whole *Fusarium* complex (Corsini and Pavek, 1986; Esfahani, 2005; Lees et al., 1998; Peters et al., 2008a; Wastie et al., 1989).

Controlling *Fusarium* dry rot can be challenging particularly with immature tubers, which often occurs at harvest after a short growing season. Immature tubers may be more susceptible to *Fusarium* dry rot. Boyd (1967) found higher infection rates in immature tubers and increased resistance with tuber maturation. He also concluded that susceptibility to *F. coeruleum* in immature tubers was closely related to the higher content of sucrose. Carnegie et al. (2001) reported that harvest date was an important factor affecting dry rot development of *F. coeruleum.*

3. Main materials and methods

3.1 Potato material

3.1.1 Potato material with different maturity level

The potato material used in paper I, II and IV were of the cultivars Asterix and Saturna grown on a loam soil (Cambisol, low erosion risk, moderate natural drainage)(WRB, 2006) in Østre Toten, Oppland, Norway (60.70°N, 10.87°E) in 2010, 2012 and 2013. The tubers were planted at 12 cm depth with a distance of 30 cm within rows and 80 cm between rows. In order to obtain experimental material with a maximum of variation in maturity at harvest, three different combinations of the factors pre-sprouting, planting date and level of nitrogen fertilization were used (Table 1). In all experiments the haulm was killed 8-10 days before

harvest. Tubers of all three maturity levels were harvested at the same date to avoid influence of different harvesting conditions within years.

Table 1 Different levels of maturity (M_{mat}, M_{med} and M_{imm}) in plant material of Saturna and Asterix obtained by a combination of presprouting, planting date and differentiated fertilization with nitrogen.

	M_{mat} ("mature")	M_{med} ("medium mature")	M_{imm} ("immature")
Presprouting	Yes	No	No
Planting date	Normal	Normal	2 weeks later than "Normal"
Fertilizing (kg N/Ha)	70	105	140

3.1.2 Potato material in the *Fusarium* experiments

The potato material (238 tuber samples, each 100 tubers) collected in the survey in paper III, came from main potato production areas in Norway. In total 26 different cultivars were collected. Different cultural practices are used in different regions; hence, some cultivars are only grown in specific regions of Norway. Agronomists, farmers, and store managers collected the samples. Information about geographical origin and potato cultivar was also collected.

Tuber material for the cultivar susceptibility test (paper IV) was grown at the same location as the material with different maturity levels (60.70° N, 10.87° E). The tubers were planted at 12 cm deep in 0.8-m row spacing and 20 cm within rows. The tubers were planted on 30 May in 2012 and 11 June in 2013, the haulm was killed between 10 and 20 August in both years based on visual inspection of the tuber size, to get equally sized tubers, and harvested 4–6 September in both 2012 and 2013. The tubers were stored in experimental facilities in 4 °C and 98% RH for 4 months prior to wounding and inoculation.

In paper V disease free seed tubers of the cultivars Saturna and Asterix were used. The seed material was obtained from a seed potato supplier in Scotland.

3.2 Maturity indicators

The study protocol for paper I and II are shown in figure 6. Paper I included maturity indicators measured at harvest. Paper II presents maturity indicators measured weekly from three weeks before the harvest until the date of harvest (1 to 22 September 2010, 23 August to 13 September 2012 and 22 August to 12 September 2013). Maturity indicators included haulm greenness (haulm maturity), skin set (physical maturity), dry matter content (physiological maturity) and content of sucrose, glucose and fructose (chemical maturity).
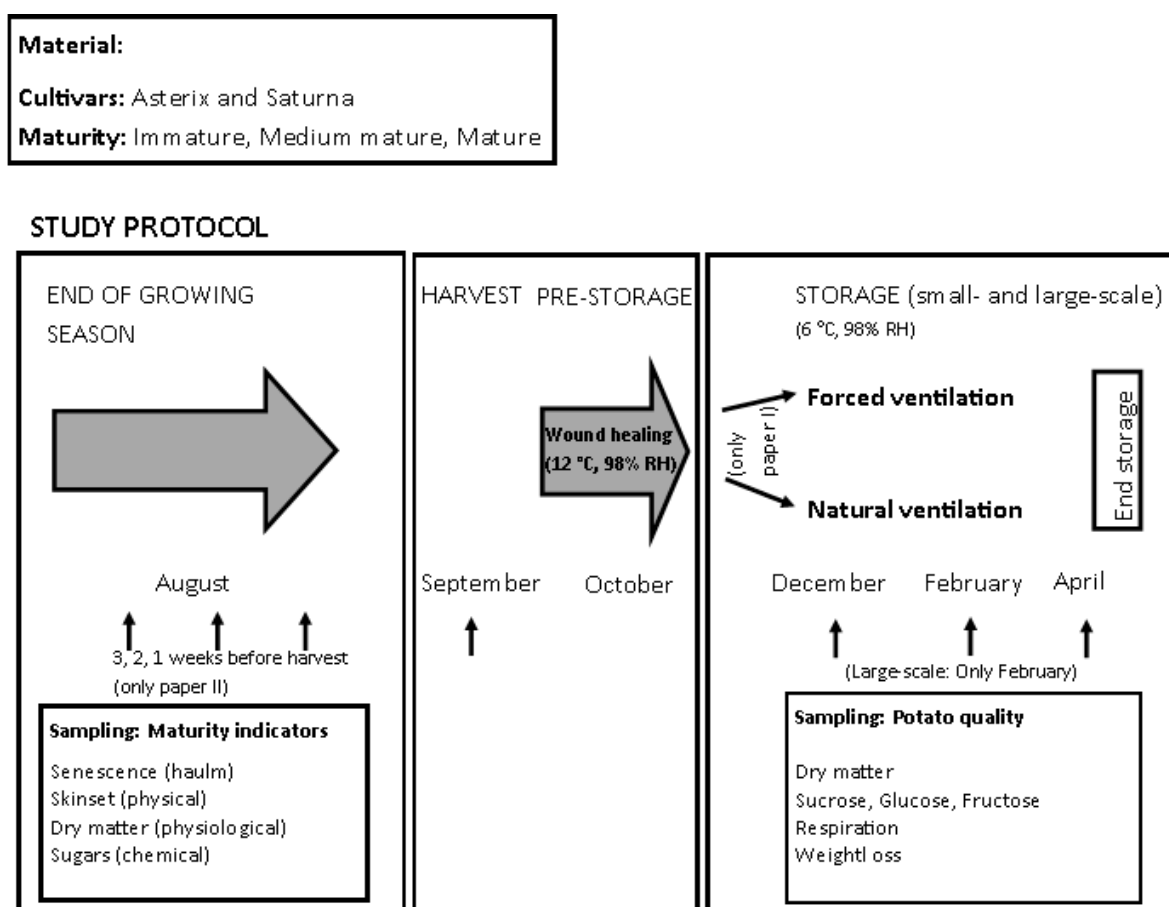
Figure 6 Study protocol, Paper I and II

3.3 Storage conditions

All tubers in paper I and II were wound healed at 12°C and 95% RH for two weeks just after harvest (Figure 6). Potato tubers of Asterix and Saturna with different maturity levels were stored in small-scale stores (paper I and II), and in large-scale commercial stores (paper I). The small-scale stores held 98% RH, and the temperature was stepwise down regulated over two months from October to December from 12 to 6°C. From December to April the

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temperature was held constant at 6°C. In the large-scale commercial stores, the temperature and humidity was regulated separately by the storage manager of the respective stores. The mean temperature during the main storage period (December to April) for all stores was 6.8°C (SD=1.3°C).

Two different ventilation regimes were investigated in both small and large-scale stores (paper I). One of the ventilation regime used was forced ventilation (figure 7), which is intermittent longitudinal flow ventilation, where air is supplied on top of the boxes and pressed down through them with a high air rate of 75-100 m^3 t^{-1} h^{-1} resulting in an air volume of 150-200 m^3 t^{-1} day^{-1}. This ventilation strategy is known in Norway as the "Agrovent" or "Hylleberg" method. The same method is also described as positive ventilation in Cunnington and Pringle (2008) and Pringle and Robinson (1996). The other ventilation regime was natural ventilation (figure 8) which is continuous ventilation with a low air rate of 10-15 m^3 t^{-1} h^{-1} resulting in an air volume of 240-360 m^3 t^{-1} day^{-1} from channels in the floor, where the air rises naturally through the boxes. This ventilation strategy is known as the "Findus" method (Forbord, 2013; Hylmö et al., 1975; Johansson, 1998). Both methods use outdoor air to control the temperature in store or may be equipped with a cooling system.
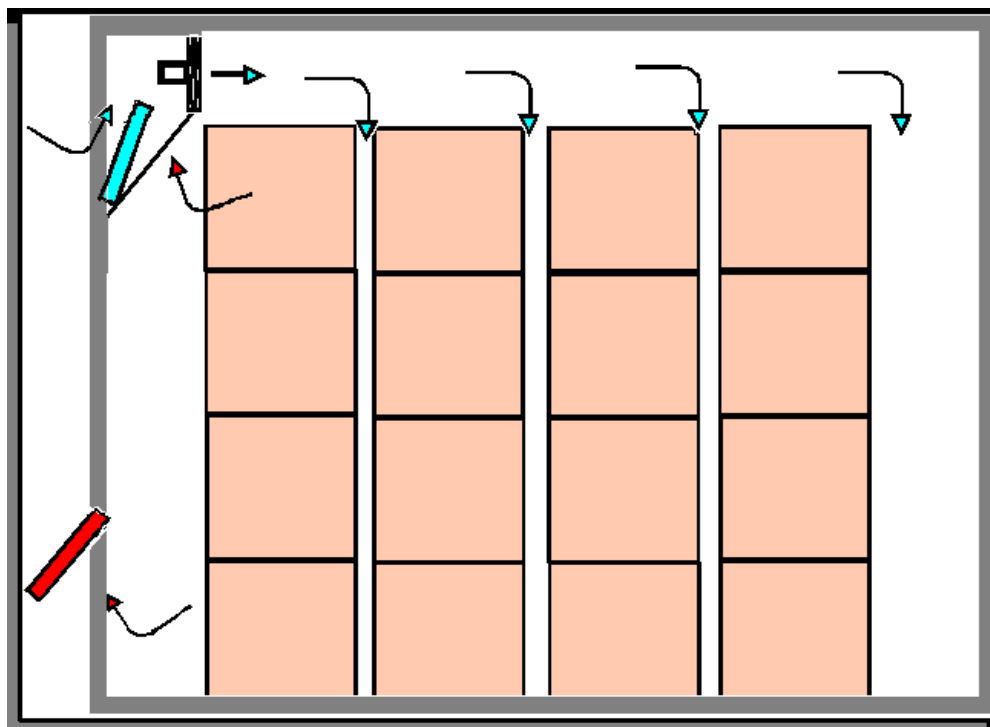
Figure 7 Air circulation with forced ventilation
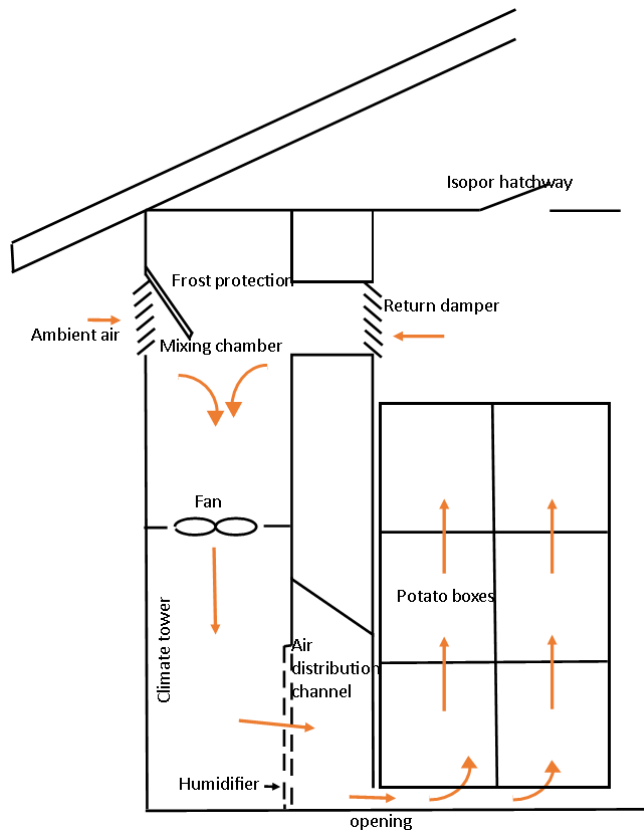
Figure 8 Air circulation with natural ventilation

3.4 Storage quality assessment

From the small-scale experimental store with 8 kg containers, samples of ten tubers were collected after 11, 20 and 27 weeks of storage (December, February and April) (paper I and II) (figure 4). Weight loss was calculated and the tubers were analysed for respiration rate, dry matter content, reducing sugars and fry colour. The same ten tubers were used for all quality measurements. In the large-scale commercial stores (paper I), potatoes (5 kg samples) were stored for five months and all samples were withdrawn for analyses in February, after 20 weeks of storage.

Respiration was measured as carbon dioxide concentrations using a Toray PG 100 (Toray Engineering Co., Ltd). Dry matter content was determined by over- and under-water weight to determine potato density and there after calculated using the equation of Lunden (1956). Content of sucrose, glucose and fructose were analysed by HPLC as described by Elmore et al. (2007) and fry colour was determined immediately after frying by visual inspection, using a scale from 1 (dark) to 9 (pale). From large-scale commercial stores colour was measured

by an Agtron reflectance spectrophotometer (Agtron Inc., Sparks, NV, USA) as described by Daniels-Lake et al. (2005).

3.5 Isolation and identification of *Fusarium* spp.

Fusarium spp. were isolated as described by Peters et al. (2008a) in the survey (paper III). *Fusarium* isolates from the survey were used in the experiments done in paper IV. In paper V, UK isolates originating from potato were used, as the experiment was performed in Scotland. Pure cultures of all isolates were grown on synthetic nutrient agar (SNA) and identified to species based on conidial morphology, production of chlamydospores, growth characteristics, and colony pigmentation as described by Leslie and Summerell (2006) and Gerlach and Nirenberg (1982). To confirm species identity, DNA extracts of the isolates were tested using PCR-based assays for *F. avenaceum* (Halstensen et al., 2006), *F. coeruleum, F. culmorum* and *F. sambucinum* (Cullen et al., 2005). Norwegian isolates of *F. coeruleum* were tested using the assay developed and described in Paper III.

3.6 Inoculation and incubation with *Fusarium* spp.

In the *Fusarium* experiments (paper IV and V), tubers were surface disinfested in 0.5% sodium hypochlorite and rinsed twice in sterile water before wounded with a nail board. Isolates were grown on Synthetic Nutrient Agar (SNA) in 9 cm^2 plates at approx. 18 °C for four weeks in alternating 12 h of light and 12 h of darkness. An inoculum slurry was made by mixing synthetic nutrient agar plates or scraping fungal colonies from the plates of four weeks old *Fusarium* cultures with sterile water of either *F. coeruleum, F. avenaceum, F. sambucinum* or *F. culmorum.* The inoculum was dispersed onto the wounds on each tuber. Two to three isolates of each species were used separately (paper IV) or together (paper V). Macroconidia was quantified using a haemocytometer and adjusted to the requested number (see paper IV and V). Control samples were wounded and sterile water or sterile water mixed with clean agar was dispersed onto the wounds. Inoculated tubers were placed in plastic-covered trays, and incubated at 10 °C and 95% relative humidity for eight weeks.

3.7 Disease assessment of *Fusarium* dry rot

After the incubation period (Paper III, IV and V), cuts exactly through each wounding point towards the centre of the tuber were made with a flame-sterilized knife. Disease development was described as rotted area around each wound. For the purpose of analysis, the rots were

assumed conical and width and depth of the rot was measured of each wounding point. Based on the method described by Peters et al. (Peters et al., 2008a), the volume of the rot in each wounded area was calculated using the equation: $Volume = \frac{1}{3} * \pi * h * r^2$ where r is half the width of the rot and h is the depth of the rot. To confirm the cause of symptoms for each isolate, random samples were taken for re-isolation. Tissue from the leading edge of tuber flesh showing dry rot symptoms was transferred to potato dextrose agar (PDA) and again to PDA for purification and then to synthetic nutrient agar (SNA) with a piece of filterpaper and identified morphologically.

3.8 Statistics

Statistical analyses were performed with Minitab® version 17.2.1 (paper I, II and V), R version 2.15.1 (www.r-project.org) (paper IV) or SAS 9.4 (paper III). General linear model (GLM) procedure were used in paper I, IV and V. The data were tested for significance of main effects and interactions. Differences between means were tested by Tukey's multiple comparison test.

Linear regression models were used to develop prediction models (paper II). All potential predictors were included initially in the models, and the models were subsequently reduced on the basis of the R^2-values obtained, p-values and where appropriate multicollinearity between the predictors.

Logistic regression was used to analyse the data in paper III. All data were converted from number of isolates found per sample of 100 tubers to 0 or 1 representing absence or presence of the *Fusarium* species, respectively. Prevalence of the individual *Fusarium* species in geographical region and potato cultivar is given in incidence and probability. Incidence is given as percentage of infected tubers per sample and probability, which indicate the likelihood of finding the given *Fusarium* species in a certain region or cultivar. Likelihood of finding is calculated from the formula: P(*Fusarium* spp.= 1)=1 − (e^estimate / (1+e^estimate)) where the estimate is given in the outcome of the logistic regression. Differences between means were tested by Tukey's multiple comparison test.

4. Main results and discussion

4.1 Effect of maturity on potato quality and *Fusarium* dry rot

Maturity significantly affected potato quality during storage (Paper I and II). Immature tubers had higher weight losses and respiration rates in the large-scale stores. This is in agreement with other studies that reported immature potatoes to be more susceptible to skinning injuries than mature tubers, in addition to have higher respiration rates at harvest (Bussan et al., 2009; Knowles and Plissey, 2008; Sabba et al., 2007). Increased weight loss in immature tubers may be related to increased transpiration in addition to increased respiration as immature potatoes with a poor skin set, have a skin that is permeable to water. This is in accordance to Lulai and Orr (1995), who reported skinned areas of tubers to have a transpiration rate that is 250 to 1000-fold higher than that of non-skinned areas.

Attempts were made to set up prediction models for potato quality after storage for tubers of different maturity levels (Paper II). Skin set (physical maturity) was found to contribute significantly to the models predicting weight loss. The weight loss models, however, was not further developed in the study as they showed low R^2-values (R^2<0.48). Skin set (physical maturity) should however be included in future prediction models for weight loss during storage. A poor skinset makes the surface permeable to water and susceptible to skinning and is thus expected to influence storage loss (Sabba et al., 2007) through a higher potential for weight loss due to transpiration.

Skin free areas in immature potatoes serve as an entry point for important storage diseases such as *Fusarium* dry rot, gangrene and bacterial diseases, which can potentially cause considerable losses during storage (Knowles and Plissey, 2008; Secor and Salas, 2001). Results confirmed that immature Asterix tubers, with poor skin set developed more severe *Fusarium* dry rot symptoms after inoculation with *F. sambucinum* than mature tubers (paper IV). This is in accordance with Carnegie (2001), who found less *Fusarium* dry rot development in more mature tubers with higher skin strength than in immature potatoes.

Dry matter contents of the tubers were, at all sampling dates, significantly influenced by maturity level for both Saturna and Asterix, always with the highest dry matter content in the most mature tubers (Paper I and II). This indicate the importance of reaching maturity at the time of harvest in order to maintain quality of the stored tubers i.e. for the purpose of frying.

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Herrman et al. (1995) and Wiltshire et al. (2004) found similar effects of maturity on tuber dry matter content.

The concentration of sucrose, glucose and fructose were monitored in the tubers during the last three weeks of the growing season and through the storage period (paper II). The results showed a decrease in sucrose content towards harvest and during the storage period until December when the concentration stabilized. Glucose and fructose contents increased from harvest and through the storage period. In Asterix sucrose contents increased significantly towards the last sampling date in April whereas in Saturna it remained at the same level. Similar trends, with decreasing sucrose content and an increase in reducing sugars, were observed in other studies (Hertog et al., 1997a; Knowles et al., 2009; Kolbe et al., 1995; Richardson et al., 1990) and provide support for the role of sucrose as a direct substrate for reducing sugar accumulation, where sucrose is hydrolysed to glucose and fructose. The increase of sugars in Asterix tubers towards the end of the storage, is probably a consequence of senescent sweetening, where sugars are mobilised for the benefit of development and growth of sprouts (Hertog et al., 1997b).

Maturity level was not found to influence tuber contents of sucrose, glucose and fructose during storage (Paper I and II). However, significant differences between maturity levels at sampling were observed before and at harvest (Paper II). The lack of differences between maturity levels during storage might be related to an effect of preconditioning at 12 °C for two weeks before lowering the temperature over six months to 6 °C (0.5 °C per week). During preconditioning some reducing sugars have respired and therefore the differences between maturity levels were offset (Pritchard and Adam, 1992; Sowokinos and Preston, 1988). Knowles et al. (2009) showed that the tubers were most sensitive to cold induced sweetening during the first months of storage and preconditioning of the tubers at a high temperature just after harvest reduced the sweetening response in the tubers. Lack of significant differences in reducing sugars among maturity levels may be the reason why there were no significant predictive effects of maturity on fry colour (Paper I and II). The correlation between reducing sugars and fry colour can be related to the Maillard reaction as reducing sugars interact directly with free amino acids and produce dark fry colours and acrylamide (Amrein et al., 2003; Shallenberger et al., 1959).

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Content of sucrose, glucose and fructose in the tubers measured before and at harvest served as successful continuous predictors, contributing significantly ($P \leq 0.01$) to the models predicting sugar content during storage. The sugar models showed high R^2-values ($R^2 > 0.89$). Previously, contents of sucrose, glucose and fructose have been used to predict sugar accumulation during storage (Hertog et al., 1997a; Richardson et al., 1990; Sowokinos, 1978). In addition, dry matter contributed significantly to all the sugar models, which can be explained by a close correlation between dry matter content and starch, and the role of starch in the synthesis of sucrose and thereafter the hydrolysis to glucose and fructose.

Prediction models for processing quality (fry colour) in Saturna and Asterix were explored and the best correlation ($R^2 = 0.51$) was found between contents of sucrose, glucose and fructose measured at harvest and fry colour in Saturna in April. For Asterix a correlation of 0.50 was found. It was concluded, that sugar contents at harvest was important when determining fry colour development during storage, but other measures should also be included in such a model. Lærke and Christiansen (2005) concluded that sucrose content in the tubers alone was not a reliable measure in predicting processing quality. Yet another study found that the relationship between sucrose at harvest and post-storage fry colour was only significant when very immature tubers with high sucrose content was used (Briddon and Storey, 1996). The content of free amino acids should be included in the prediction model as well as they contribute to the Maillard reaction, interacting with reducing sugars to produce a dark fry colour and acrylamide (Amrein et al., 2003; Shallenberger et al., 1959). Other measures, which should also be considered in future models for prediction of fry colour are temperature, humidity, CO_2 levels and ventilation within the stores. However, in this study these factors were standardized. Cultivar and maturity contributed significantly to all the models as categorical predictors, which indicate that maturity status of the crop should be considered in future models and that predictive models should take into account which cultivar is used. Richardson et al (1990) also observed differences between cultivars and concluded that predictions based on sugar contents at harvest should be determined on a cultivar specific basis.

4.2 Effect of ventilation strategy on potato quality

Ventilation strategy was found to affect weight loss in both small- and large-scale stores (Paper I). The tuber weight losses were higher with natural ventilation. These results

correspond with the results of Sparks (1973), who found intermittent ventilation to cause significantly less weight loss than continuous ventilation with the same air rate. Higher weight loss with natural ventilation can be related to the total higher air volume for natural compared to forced ventilation. Forced ventilation resulted in higher contents of glucose and fructose and a corresponding darker fry colour in large-scale commercial stores. Moreover, respiration rates were higher in tubers ventilated at forced ventilation. Darker fry colours have previously been associated with high respiration rates (Copp et al., 2000; Daniels-Lake et al., 2005; Mazza and Siemens, 1990). Copp et al. (2000) found similar results and suggested that monitoring respiration rates throughout the storage season could provide a continuous, non-destructive method for predicting the point at which tuber processing quality will decline. Another possible explanation for darker fry colours with forced ventilation in commercial large-scale storage may be elevated CO_2 levels. Periods of increased CO_2 levels may have occurred in the 2010-2011 storage season, which included a long period with cold outdoor temperatures (mean of -12.5°C in January and February) (www.yr.no). At low outdoor temperatures, ventilation may be switched to recycling of air, which may in time result in lower O_2 and higher CO_2 levels. The difference between the ventilation strategies may be a result of more airtight walls in newer stores, as were more common among the forced ventilation stores.

4.3 *Fusarium* species in Norway and cultivar susceptibility to *Fusarium* spp.

In the survey (Paper III), *Fusarium* species of various numbers were present in approximately half of the samples (each 100 tubers), indicating the potential risk of *Fusarium* dry rot in Norwegian potatoes, if the right conditions are present. The most prevalent species was *F. coeruleum*, which is consistent with previous findings (Bjor, 1978). *F. coeruleum* was also found to be the most common species isolated from potatoes in Great Britain (Peters et al., 2008a), Sweden (Olofsson, 1976) and Finland (Seppänen, 1983).

The prevalence of *F. coeruleum* was more frequent in northern Norway. This may be explained by a narrow crop rotation and widespread use of the susceptible cultivars Mandel and Gulløye. These cultivars are susceptible to *Fusarium* dry rot, with score 1 where 9 is most resistant (Møllerhagen, 2014). Other susceptible cultivars were observed in the survey (Paper III), e.g. Berber and Rutt, which were heavily infested with *F. coeruleum*. Heavy infestations

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in samples in the survey indicate the potential risk of *Fusarium* infections during storage. High susceptibility in the early cvs. Rutt and Berber to *F. coeruleum* as well as to *F. avenaceum* and *F. sambucinum* was confirmed in a susceptibility test of ten different potato cultivars (paper IV). Møllerhagen (2014) confirm the susceptibility of Rutt (score: 1). Laila which is also an early cultivar, was overall the third most susceptible of the ten cultivars tested. An earlier study conducted in Norway also found Laila to be of the most susceptible cultivars tested (Kirkerød, 1979). A large interaction effect of cultivar and *Fusarium* species in the cultivar test indicates that cultivar resistance to one *Fusarium* species does not imply resistance to all *Fusarium* spp.. Other studies have found the same (Esfahani, 2005; Peters et al., 2008a; Wastie et al., 1989). The variation in pathogenicity by different *Fusarium* spp. and isolates to different cultivars demonstrates the importance of using more than one species and isolate when screening the resistance of potato cultivars.

F. avenaceum was found to be a less aggressive species in potato than the other species (Paper III, IV and V) which was also observed by Peters et al. (2008a). However, *F. avenaceum* was the second most prevalent species in the survey. Similar findings were observed in previous surveys in Great Britain and China (Du et al., 2012; Peters et al., 2008a). A narrow crop rotation with cereals might affect the high prevalence of *F. avenaceum.* In Norway, *F. avenaceum* is the most commonly detected species of *Fusarium* in cereals*,* and *Fusarium* has been an increasing problem in cereals in Norway during the last ten years (Bernhoft et al., 2013). Gachango et al. (2012) discussed that crop rotation with cereals may have an implication on the prevalence of *F. avenaceum* in potatoes*.* However, when statistical analyses were applied in the present study, crop rotation did not have significant effect on the prevalence of the different *Fusarium* species (paper III). This could be a consequence of very few repetitions of the same crop rotation in the data or simply just the fact that *F. avenaceum* is commonly found in almost all crops grown in rotation with potatoes.

F. sambucinum was found to be a more aggressive species than the other *Fusarium* species investigated (Paper IV and V). A number of other studies (Esfahani, 2005; Gachango et al., 2012; Glorvigen, 1996; Peters et al., 2008a; Wastie et al., 1989) confirm this. However, differences in aggressiveness between isolates within *F. sambucinum* were found (paper IV). According to Desjardins (1995), the genetic diversity in *F. sambucinum* from potatoes is large in Europe, which can explain the differences in aggressiveness found in the present study. Despite high aggressiveness, *F. sambucinum* was the third most prevalent species in the

survey (Paper III). Relatively low prevalence of *F. sambucinum* was also reported from surveys in Michigan and in Great Britain, where it was the third and fourth most prevalent species, respectively (Gachango et al., 2012; Peters et al., 2008a). In the cultivar test, there was a significant interaction between isolates within *F. sambucinum* and cultivar, indicating race-specific resistance (paper IV).

4.4 Real-time PCR assays for *Fusarium* identification

The *Fusarium* species were identified by morphological characteristics. To confirm the species identity, DNA extracts were tested using real-time PCR assays specific to individual *Fusarium* species (Paper III, IV and V). However, unexpectedly high Ct-values were observed for the Norwegian isolates (Cullen et al., 2005) and a new real-time PCR assay was developed for *F. coeruleum* (Paper III). Furthermore, *F. culmorum* specific primers (Cullen et al., 2005) was not able to distinguish between *F. culmorum* and *F. cerealis*. This cross-reaction was also found by Nicolaisen et al. (2009). The *F. sambucinum* specific assay also had cross-reactivity with other species, even after testing different primer and probe concentrations to optimize the assay. However, this species was not considered to be important in Norway, because of the low prevalence and therefore no attempts was made to set up a new assay. Soil and potato peel were tested pre-storage with the *F. coeruleum* assay developed (Paper III). Only weak signals of *F. coeruleum* was detected, which indicate that the inoculum levels were low. Incubation for potential enrichment of Fusarium before the molecular teste might have enhanced the response. In two highly infested samples (cvs. Berber and Rutt) from the survey (paper III), *F. coeruleum* was not detected in potato peel. This indicates that the inoculum were present in the soil. However, it cannot be verified as no soil samples were taken together with the tuber samples.

4.5 Relative contribution of seed and soil-borne *Fusarium* inoculum

The presence of inoculum in soil or potato peel, can also lead to a discussion on the importance of soil- versus tuber-inoculum. The relative importance of seed- versus soil-borne inoculum of *F. coeruleum, F. sambucinum* and *F. avenaceum* was investigated for the two potato cultivars, Asterix and Saturna (paper V). Different levels of soil inoculum influenced the incidence and severity of *Fusarium* dry rot. Soil infested with *F. sambucinum* (low and high levels) resulted in significantly more severe rots than control treatments ($P<0.001$), whilst only high levels of *F. avenaceum* soil inoculum increased severity of tuber rot

compared with control treatments ($P<0.05$). Increases in disease severity observed as a result of addition of inoculum of *F. coeruleum* to soil were not significant. Seed inoculation did not significantly affect development of rots in progeny tubers for any of the *Fusarium* species in this study. The lack of effect of seed infection is in contrast to the findings of Adams and Lapwood (1983) who demonstrated that infected seed could result in infection and subsequent development of dry rot in progeny tubers. They found that whilst *F. coeruleum* was most readily transmitted from rotted seed tubers to progeny tubers, rather than from seed with symptomless infection, *F. sambucinum* was transmitted from highly contaminated seed (tubers inoculated with slurry containing 10^6 spores ml^{-1} just before planting) to progeny.

5. Main conclusions

Maturity level significantly influenced potato quality during storage, with the least mature tubers having higher weight losses, higher respiration rates and lower dry matter contents than the more mature tubers. Reaching maturity at the time of harvest is important in order to maintain good quality of the tubers during storage.

The maturity indicators dry matter content (physiological maturity) and sucrose, glucose and fructose content (chemical maturity) all contributed significantly as continuous predictors to the models predicting sucrose, glucose and fructose. Sucrose content contributed significantly to the fry colour model for Saturna and skin set to the weight loss model.

Immature potato tubers of Asterix had poor skin set and were more susceptible to *Fusarium* dry rot development caused by *F. sambucinum.* A general recommendation to the grower would be to ensure maturation of the skin to prevent wounding and thereby infection of the tubers.

Ventilation strategy influenced tuber quality during storage both in small- and large-scale stores. Natural ventilation resulted in higher weight loss than did forced ventilation, probably because of total higher air volumes passing between the potato tubers. In average over three years, forced ventilation strategies resulted in tubers with higher respiration rates and higher contents of reducing sugars in large-scale stores and darker fry colours in Saturna in both large- and small-scale stores than with natural ventilation. In the large-scale commercial stores, the dark fry colours might be a consequence of elevated CO_2 levels.

A *Fusarium* survey provided new information on the current occurrence of different *Fusarium* species causing *Fusarium* dry rot in potatoes in Norway. The four most important species were *F. coeruleum, F. avenaceum, F. sambucinum* and *F. culmorum*. Different regions showed differences in prevalence of the *Fusarium* species. The results indicated that crop rotation might influence the occurrence of *Fusarium*. There was also an indication of difference in resistance towards *Fusarium* dry rot between cultivars, which was confirmed by a cultivar susceptibility test.

Variation in pathogenicity by different *Fusarium* spp. and isolates to ten different cultivars demonstrated the importance of using more than one species and isolate when screening the resistance of potato cultivars.

A new *F. coeruleum* real-time PCR assay was developed, which can detect the species at an early stage. Detection of latent infections could validate recommendations on control strategies in order to avoid losses during storage and/or the suitability as seed potatoes.

Different levels of soil inoculum influenced the severity of *Fusarium* dry rot on progeny tubers. Understanding the impact of different inoculum sources on disease development is also important when aiming for reducing losses due to *Fusarium* dry rot. Different levels of seed inoculum did not influence disease development in progeny tubers.

6. Future perspectives

Maturity indicators measured before and at harvest may be used to predict potato quality in the cultivars Asterix and Saturna. Incorporating more factors in the models, such as temperature, humidity, CO_2 levels and ventilation would improve the models. The results indicated that cultivar specific models are needed in order to optimize prediction. Further studies on cultivar specific models in other potato cultivars than Asterix and Saturna would be of interest.

High CO_2 levels might influence fry colour in processing potatoes. Monitoring respiration rates of the tubers and CO_2 levels in the stores throughout the storage season may help in managing the stores and predicting sugar contents and fry colour. However, further studies would be useful to confirm this.

In the present study, the experiments were limited to investigate the ventilation strategies during the main storage period. It would be useful to study the effect of pre-storage ventilation strategies on the storage quality of potatoes. Moreover, it would be interesting to study the effect of ventilation strategies in combination with different temperature and humidity strategies both during the pre-storage and main storage period.

Results from the *Fusarium* survey showed that crop rotation might influence the occurrence of *Fusarium*. More studies are needed to investigate the influence of crop rotation on the prevalence of different *Fusarium* species in potato tubers.

It was suggested to use more than one species and isolate of *Fusarium* when screening the resistance of potato cultivars. However, before this can be recommended studies are needed to clarify potential antagonistic effects between the isolates. The study underline the importance of using resistant cultivars in an IPM strategy against *Fusarium* dry rot. Further studies are needed to explore resistance in different potato cultivars. Furthermore, it would also be interesting to study the resistance mechanisms in different cultivars.

There was no effect of seed inoculum on disease development in progeny tubers. Future experiments should include more susceptible cultivars for comparison and a re-evaluation of the contribution of seed inoculum to disease.

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Papers I-V

Paper I
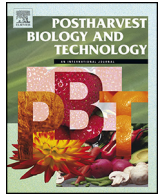
Effect of ventilation strategy on storage quality indicators of processing potatoes with different maturity levels at harvest

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ABSTRACT

Ventilation management and the tuber maturity at harvest are essential factors in maintaining potato quality during long-term storage. The aim of this study was to examine the effect of ventilation strategy on storage quality of potato tubers with three different maturity levels at harvest. Two potato cultivars, Saturna and Asterix, were stored in small-scale experimental stores and large-scale commercial stores. Both storage categories were ventilated by both low continuous air rates (natural ventilation) and intermittent high air rates (forced ventilation). The different maturity levels were obtained by a combination of pre-sprouting strategy, planting date and level of nitrogen fertilization of the seed tubers, where pre-sprouting, early planting date and low amount of nitrogen resulted in the most mature tubers. Storage quality parameters investigated during and after long-term storage (6 months in small-scale and 4 months in large-scale stores) included weight loss, respiration, dry matter, sucrose, glucose/fructose content and fry colour. In average over three years natural ventilation resulted in higher weight losses in small- and large-scale stores (1.36 and 3.93%), lower content of reducing sugars (glucose + fructose) in large-scale stores (2.35 mg g^{-1}) and lighter fry colour than did forced ventilation. Immature potatoes had higher weight losses (4.16%), higher respiration rates (1.68 mg CO$_2$ kg^{-1} h^{-1}) and lower dry matter content (22.3–22.5%) than more mature potatoes. This study show that both maturity and ventilation strategy affects storage quality of potatoes as measured by weight loss, sugar content and fry colour.

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1. Introduction

In order to ensure potatoes and potato product availability throughout the year, most potatoes must be stored for shorter or longer periods of time. In Norway, approximately 14,000 ha of potatoes are grown annually, resulting in a total yield of 350,000 tonnes with a wholesale value of approximately NOK 500 million (Statistics, 2014). The majority of harvested potatoes are put into storage for some length of time. An estimation of the annual loss during storage is approximately 10 percent (Bengtsson et al., 1996). Storage losses are due to various processes in the tuber, including transpiration, respiration, sprouting, changes in chemical composition, damage by extreme temperatures and diseases (Wustman and Struik, 2008). To minimize the loss of quality and quantity during storage, focus should be placed on quality of potatoes at harvest as well as the storage processes (Bussan et al., 2009; Knowles et al., 2009; Sparks, 1980).

In order to obtain good quality of stored potatoes, important storage parameters, such as temperature, relative humidity, atmospheric conditions and ventilation, must be controlled. Ventilation is required during storage to remove field heat and respiratory heat from the potatoes, as well as to prevent respiratory CO$_2$ accumulation in the storage facilities (Oberg and Kleinkopf, 2003), thus maintaining tuber quality (Bertolini and Guarnieri, 1990; Sparks, 1980; Wustman and Struik, 2008). Different ventilation rates are recommended during different phases of the storage period, depending primarily on tuber temperature and moisture on the tubers at harvest. In Norway, potatoes are mainly stored in 400 kg wooden boxes, and two different ventilation strategies are predominant. The first strategy (natural ventilation) includes continuous ventilation with a low air rate (10–15 m^3 t^{-1} h^{-1}) (t = tonne, h = hour) from channels in the floor, where the air rises naturally through the boxes, known as the "Findus" method (Forbord, 2013; Hylmö et al., 1975; Johansson, 1998). Natural ventilation is caused only by buoyancy forces as a result from both air density and temperature differences within the stack of boxes of respiring potatoes as described by Geyer and Gottschalk (2008). The other strategy (forced ventilation) uses

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intermittent longitudinal flow ventilation, where air is supplied on top of the boxes and pressed down through them with a high air rate (75–100 $m^3 t^{-1} h^{-1}$) (Pringle et al., 2009). In Norway, this ventilation strategy is known as the "Agrovent" or "Hylleberg" method. The same method is also described as positive ventilation in Cunnington and Pringle (2008) and Pringle and Robinson (1996). Both ventilation strategies use outdoor air to control the temperature in the store, and may be equipped with a cooling system. The effect of ventilation strategies during storage on end-use quality of potatoes for the industry has not been widely studied. One study conducted in USA with continuous and intermittent ventilation with the same air rate (Sparks, 1973), showed that intermittent ventilation, caused significantly less weight loss, flattening, shrivelling and sprouting than did continuous ventilation. Another study conducted in northern Italy recommended continuous ventilation with air rates above 0.0306 $m^3 t^{-1} s^{-1}$ (Bertolini and Guarnieri, 1990). Studies conducted in the UK recommended forced air ventilation to avoid condensation that may occur in naturally ventilated boxes especially on immature tubers that has a high metabolic activity (Pringle, 1996; Pringle and Robinson, 1996). However, this number of studies is limited and further information about the effect of ventilation strategies on potato quality is needed.

Maturity status of the tubers is another factor of importance for storability. Several studies have shown maturity to have an impact on the quality of potatoes after storage (Herrman et al., 1995; Hogge et al., 1993; Kumar et al., 2004; Wiltshire et al., 2004). The term "maturity" is, however, not easily defined or measured. Maturation may be considered as a continuous and complex process, and the result of individual and partly independent processes related to haulm maturation, as well as physical, physiological and chemical maturity (Bussan et al., 2009; Sabba et al., 2007). Haulm maturation refers to the natural senescence of the plant when photosynthesis decreases, the allocation of carbohydrates to the tubers declines and the tubers start to mature (Bussan et al., 2009). The physical maturity of tubers refers to skin set and the development of a mature periderm. Tubers that are physically immature have poor skin set, and a skin that is permeable to water and susceptible to skinning (Sabba et al., 2007). Physiological maturity refers to the dry matter content and is achieved when tuber dry matter reach a maximum. Chemical maturity is critical for the processing industry, and is obtained once sucrose concentrations reaches a minimum (Kolbe and Stephan-beckmann, 1997), preferably less than 2.8 mg g^{-1} fresh weight (Sowokinos, 1978). Maturity can be manipulated by various growth factors, such as seed treatment, planting date, nitrogen fertilizer management and harvest strategy.

In this study, the cultivars Asterix and Saturna were included, as they are among the main potato cultivars grown in Norway; Asterix for Table use and partly for chips and Saturna for crisps (Potetsorter, 2016 Fagforum Potet). Both are medium-late cultivars (ECPD, 2016 The European Cultivated Potato Database) that are at risk of not reaching maturity at the time of harvest under Norwegian growing conditions. Norway typically has short potato growing seasons (100–110 days), with low temperatures, particularly during spring and autumn (yr.no, NRK og Meteorologisk institutt).

The aim of the present study was to examine the effect of ventilation strategies during long-term storage on quality in potatoes with different maturity at harvest. Three different maturity levels were obtained by a combination of the factors planting date, pre-sprouting, and level of nitrogen fertilization where a combination of an early planting date, presprouting and fertilization with a small amount of nitrogen resulted in a mature crop. Two ventilation strategies were investigated in small-scale experimental stores as well as in large-scale commercial stores.

2. Materials and methods

2.1. Plant material

During the growing seasons of 2010, 2012 and 2013, Asterix and Saturna were grown on a loam soil (Cambisol, low erosion risk, moderate natural drainage) (WRB, 2006) in Østre Toten, Oppland, Norway (60.70°N, 10.87°E). The tubers were planted at 12 cm depth with a distance of 30 cm within rows and 80 cm between rows. In order to obtain experimental material with a maximum of variation in maturity at harvest, three different combinations of the factors pre-sprouting, planting date and level of nitrogen fertilization were used, as described by Heltoft et al. (2015). The most mature (M_{mat}), was presprouted for four weeks in 12 °C under full light (>100 lux), planted 16–23 May and fertilized with 70 kg N ha^{-1}. Medium mature (M_{med}), was not presprouted, planted the same dates as M_{mat} and fertilized with 105 kg N ha^{-1}. Immature (M_{imm}), was not presprouted, planted two weeks later than M_{mat} and M_{med} and fertilized with 140 kg N ha^{-1}. In all three treatments (M_{mat}, M_{med} and M_{imm}) the haulm was killed 8–10 days before harvest and tubers were harvested at the same date. This strategy was chosen to avoid influence of different harvesting conditions and to be able to start storage at the same dates within each year.

2.2. Tuber maturity indicators

To confirm different maturity levels of the tuber material at harvest, indicators of both physical, physiological and chemical maturity were used, in addition to haulm maturity.

2.2.1. Haulm maturity

Haulm maturity was determined before desiccation, both visually as relative greenness (0–100, 0 = dead and 100 = full greenness) and by measuring the chlorophyll content using a hand-held chlorophyll meter [model hydro-N-tester (HNT): Yara, Oslo, Norway], as described by Vos and Bom (1993). The chlorophyll meter measures the light transmittance of a leaf at 650 and 940 nm and measurements were made on the distal leaflet of the youngest fully expanded compound leaf (i.e. the fourth or fifth leaf from the apex). A total of 30 measurements as used to obtain each mean value of the chlorophyll content. The values obtained indicate the relative amount of chlorophyll present in the leaves.

2.2.2. Skin set

Skin set (physical maturity) was measured as described by Lulai and Orr (1993). The measuring device (Halderson Periderm shear tester) consisted of a measuring head attached to a torque meter, which measured the torsional force [mNm(milliNewton meters)] required to produce skinning injury. Skin set was measured on ten tubers selected from each maturity level of each variety. Tubers were left overnight at laboratory conditions (20 °C, 40–60% RH) before assessments of skin injury.

2.2.3. Dry matter

The dry matter content (physiological maturity) was determined by over- and under-water weight to determine potato density. The following equation was used to calculate the dry matter content (Lunden, 1956): *Dry matter* = 215.73 (*x* − 0.9825), where *x* is the specific weight calculated as weight in air/(weight in air − weight in water).

2.2.4. Sugar analysis

Glucose/fructose and sucrose contents in the tubers (chemical maturity) were analysed as described by Elmore et al. (2007).

Samples of 10 quarters (cut from apical to stolon end) of tubers from the same maturity level and cultivar were homogenized in a food processor. Samples of $2\,g \pm 0.005\,g$ were weighted into 50 mL screw-top bottles and 20 mL aqueous methanol (50%) containing 200 µL trehalose as internal standard was added. The sample was stirred for 15 min at room temperature. Aliquots (100 µL) were diluted in 900 µL aqueous methanol (50% (w/w)). The extracts were filtered through millipore filters PVCF 0.22 µm and analysed for sucrose, glucose and fructose using a HPAEC-PAD system (Dionex). Sugar values are given in $mg\,g^{-1}$ fresh weight (FW).

2.3. Ventilation strategies in small-scale experimental stores

At harvest, the potatoes were wound healed at 12 °C and 95% RH for approximately two weeks before transfer to tube-shaped PVC (polyvinyl chloride) storage containers (70 cm high, 16 cm diameter, 8 kg capacity). Each container had a netting bottom and was placed in a 16 cm diameter hole on top of an airtight box. The air entered the container through the bottom and left through the top, where the air flow was regulated by a screw tap. The air flow (m/s) was monitored using an anemometer (TSI INC, USA, model no.: 8350-1) and the volume of the air was calculated as m^3 per tonne potato per hour ($m^3\,t^{-1}\,h^{-1}$). Two different ventilation strategies were used: 1) Low air flow and continuous ventilation, with an air rate of $12.5\,m^3\,t^{-1}\,h^{-1}$ resulting in an air volume of $300\,m^3\,t^{-1}$ day^{-1} (natural ventilation) and 2) high air flow with air rates of $75\,m^3\,t^{-1}\,h^{-1}$ and ventilation in 15 min intervals eight times per day resulting in an air volume of $150\,m^3\,t^{-1}\,day^{-1}$ (forced ventilation). Each combination of ventilation, maturity and cultivar had three replicates. The storage containers, each holding a tuber sample of eight kg, were placed in controlled stores, with 96–98% RH, adjusted by humidifiers within the storage room providing moist to the air. The temperature was gradually lowered by approximately 0.5 °C per week over the first 11 weeks of storage, from 12 to 6 °C according to common practice in Norway. The tubers were stored for seven months from September to April.

2.4. Ventilation strategies in large-scale commercial stores

For large-scale storage, similar material of Asterix and Saturna with three maturity levels was stored in 5 kg bags in 13 commercial box-stores. One sample of each combination of cultivar and maturity level was stores in each store (six samples per store in total). The samples were located together at a height of 2–3 meters in one of the middle rows of 400 kg wooden boxes. All tuber samples were stored for four months from October to February. Details of stores are given in Table 1. All stores were in the south-eastern part of Norway (Hedmark, Oppland, Akershus and Vestfold counties) and had ventilation based on the principles of either high or low air flow systems. The five stores with continuous ventilation and low air rates (natural ventilation) were built in 1960–1999 with storage capacities from 200 to 800 tonnes, while the eight stores with high intermittent air rates (forced ventilation) were built from 2003 to 2011 and had storage capacities between 500 and 900 tonnes. The air temperature was in all stores regulated by mixing outdoor and indoor air (no refrigeration). Storage temperature was recorded every second hour using a temperature logger (Temprecord Multitrip™, Multi use temperature recorder) placed with the 5 kg tuber samples. The mean temperature during the main storage period for all stores was 6.8 °C (SD = 1.3 °C). In Table 1, the mean temperature of each store is given. Fig. 1 show temperature data from the storage season 2013–2014, during the storage period (October–February) in all stores. Similar curves were seen in the 2010–2011 and 2012–2013 seasons. Recorded temperature data from the large-scale commercial stores showed that there was some variation in temperature between the different stores. Statistical modelling of the data did however not find that this influenced the results of the comparisons between the two ventilation strategies.

2.5. Potato quality and storability

From the small-scale experimental store with 8 kg bags, samples of ten tubers were collected after 11, 20 and 27 weeks of storage (December, February and April). Weight loss was calculated and the tubers were analysed for respiration rate, dry matter content, reducing sugars and fry colour. The same ten tubers were used for all quality measurements. In the large-scale commercial stores, potatoes (5 kg samples) were stored for five months and all samples were withdrawn for analyses in February, after 20 weeks of storage.

Table 1
Details of large-scale commercial stores and mean temperature (average of three years 2010, 2012 and 2013).

Ventilation strategy	County	Build year	Tonnes in store	Ventilation	Temperature mean from November to February (°C)
Forced ventilation 75–100 $m^3\,t^{-1}\,h^{-1}$	Hedmark	2005	900	Internal indoor air 10–15 min every 12th hour. Intake of outdoor air when temperature regulation is needed.	5.7 (*0.7*)[a]
	Hedmark	2004	900	Internal indoor air 10–15 min every 4th hour. Intake of outdoor air when temperature regulation is needed.	8.0 (*1.2*)
	Hedmark	2004	900	Internal indoor air 18 min every 4th hour. Intake of outdoor air when temperature regulation is needed.	8.3 (*0.4*)
	Hedmark	2007	650	Internal indoor air 30 min every 6th hour, 15 min outdoor air every 12th hour followed by 5 min internal	6.6 (*1.3*)
	Oppland	2011	500	Internal indoor air 10–15 min every 4th hour. Intake of outdoor air when temperature regulation is needed.	5.9 (*0.3*)
	Akershus	2007	800	Internal indoor air 10–15 min every 4th hour. Intake of outdoor air when temperature regulation is needed.	6.0 (*0.5*)
	Akershus	2003	600	Internal indoor air 15 min every 4th hour. Intake of outdoor air when temperature regulation is needed.	7.7 (*1.2*)
	Vestfold	2003	500	Internal indoor air 30 min every 4th hour, 10 min outdoor air every 8th hour	7.9 (*0.6*)
Natural ventilation 10–15 $m^3\,t^{-1}\,h^{-1}$	Hedmark	1964	300	Continuous natural ventilation. Intake of outdoor air when temperature regulation is needed	5.8 (*0.9*)
	Hedmark	1999	330		7.5 (*0.5*)
	Hedmark	1985	450		6.3 (*0.3*)
	Vestfold	1980	200		6.7 (*0.6*)
	Hedmark	1990	800		5.6 (*1.1*)

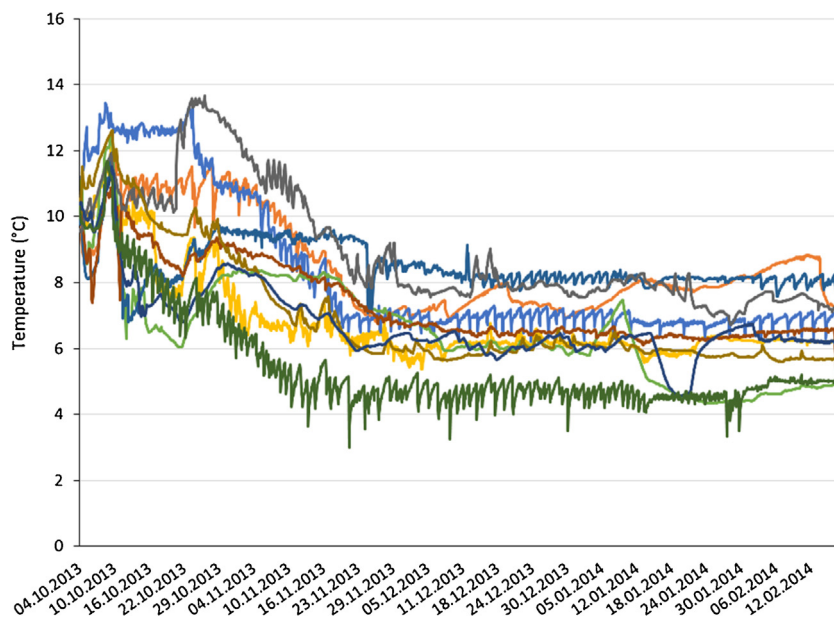[a] Between parentheses in italics: Standard deviation.

Fig. 1. Example of temperatures in large-scale commercial stores (natural and forced ventilation) in storage season 2013–2014.

2.5.1. Weight loss

The weight loss was calculated in percentage from the weight of the potato samples when put into storage, 8 kg and 5 kg, respectively, for small- and large-scale stores and the weight at each sampling date.

2.5.2. Respiration

We measured respiration as described by Kaaber et al. (2002). Ten tubers from each replicate were placed in sealed 3.0 L glass containers with a small silicone membrane. After 24 h, air samples (10 mL) were collected and carbon dioxide concentrations were measured using a Toray PG 100 (Toray Engineering Co., Ltd.). Respiration rates are given in mg CO_2 kg^{-1} h^{-1}.

2.5.3. Dry matter, carbohydrates and fry colour

Dry matter and carbohydrates were measured as previously described (2.2.3 and 2.2.4). Fry colour was determined for each replicate. For Saturna, ten tubers were peeled and three slices of 1.8 mm thickness were cut from the middle of each tuber. For Asterix tubers, strips (10-mm wide × 10-mm thick × length of tuber) were cut along the apical to basal axis, one from each of 10 tubers. The slices and strips were rinsed in tap water, gently wiped, and fried at 180 °C for 150 s in sunflower oil. From the small-scale stores, fry colour was determined immediately after frying by visual inspection, using a scale from 1 (dark) to 9 (pale). From commercial stores colour was measured by an Agtron reflectance spectrophotometer (Agtron Inc., Sparks, NV, USA) as described by Daniels-Lake et al. (2005). High Agtron scores represent lighter fry colours.

2.6. Statistics

Statistical analyses were performed with Minitab® version 17.2.1, using the general linear model (GLM) procedure. For small-scale stores weight loss, respiration rate, dry matter, sucrose, glucose/fructose content were analysed using ventilation strategy, maturity, cultivar and storage time as explanatory variables (fixed factors) and year as random replicate. Fry colour was modelled for each cultivar individually. For large-scale commercial stores, the same storability factors as mentioned above were analysed using ventilation strategy, maturity and cultivar as explanatory variables. Year was included as a random factor. The data were tested for significance of main effects and interactions. Differences between means were tested by Tukey's multiple comparison test.

Table 2

Indicators of maturity in Asterix and Saturna tubers with three levels of maturity (M_{mat}, M_{med}, M_{imm}) at harvest. Mean of three years (2010, 2012 and 2013).

Variety	Maturity level[a]	Haulm maturity (%)	Haulm maturity (relative units[b])	Skin set (mNm)	Dry matter (%)	Sucrose (mg g^{-1} FW)	Glucose + fructose (mg g^{-1} FW)
Asterix	M_{mat}	17.5b	279b	2.96a	23.5a	2.50	1.96
	M_{med}	22.5b	328b	2.66ab	22.0b	2.50	1.96
	M_{imm}	85a	470a	2.18b	21.1c	2.84	1.62
		**	***	**	**	n.s	n.s
Saturna	M_{mat}	20b	321b	3.47a	27.1a	1.07b	0.49
	M_{med}	25b	328b	2.96ab	25.6b	1.34ab	0.45
	M_{imm}	85a	455a	2.30b	25.1c	1.46a	0.40
		**	***	**	***	*	n.s

n.s = not significant ($P \geq 0.05$), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($P = 0.05$).
[a] Two cultivars(Asterix and Saturna) with three different maturity levels: Mature (M_{mat}), medium mature (M_{med}), immature (M_{imm}).
[b] The values obtained indicate the relative amount of chlorophyll present in the leaves,measured with a Hydro N-tester.

3. Results

3.1. Potato maturity at harvest

The maturity indicators used at the time of harvest confirmed that the intended variation in maturity was achieved. At the time of haulm desiccation the haulm in both Asterix and Saturna was significantly greener in the treatments which resulted in immature tubers (M_{imm}), while the haulm did not differ significantly in greenness between the other two treatments (Table 2). A significantly higher degree of skin set was found in M_{mat} than in M_{imm}, indicating differences in physical maturity. The dry matter content generally increased with increasing maturity. In Asterix there were significant differences between all maturity levels. Saturna had significantly higher dry matter contents than Asterix. Sucrose contents decreased with increasing maturity in Saturna, with the largest difference between M_{med} and M_{imm}. Glucose and fructose contents were not significantly influenced by maturity level in either of the cultivars.

3.2. Effect of maturity and ventilation during small-scale storage

Table 3–5 show the effects of maturity, storage time and ventilation system on weight loss, respiration, dry matter content, reducing sugar contents and fry colour in Asterix and Saturna tubers. No significant interactions were found.

3.2.1. Weight loss and respiration

The tubers ventilated with natural ventilation lost significantly more weight than tubers stored with forced ventilation (Table 3). There were no differences in weight loss among maturity levels, even though differences in skin set were observed at harvest. The respiration rates increased during storage and were for both cultivars significantly higher in April than in December.

3.2.2. Dry matter

Dry matter content did not change during storage (Table 3) and the differences found between cultivars and maturity levels at harvest, remained during storage. There were no differences between forced ventilation and natural ventilation strategies.

3.2.3. Carbohydrates and fry colour

The contents of sucrose and glucose/fructose were found to be significantly higher in Asterix than in Saturna. For the two cultivars together, both sucrose and glucose/fructose contents changed significantly from harvest (1.93 and 1.07 mg g^{-1} respectively) until the first sampling date during storage (0.90 and 3.34 mg g^{-1}) (results not shown). Sucrose contents in Asterix decreased during storage (Table 4), while the contents of glucose/fructose increased and were significantly higher in April than on previous sampling dates. The fry colour of Asterix also changed during the storage period and was significantly darker in February and April than in December. Sugar contents did not change significantly in Saturna (Table 5), but the fry colour became darker with time and was significantly darker in April than in December. Forced ventilation resulted in significantly darker fry colours in Saturna than natural ventilation. The contents of the measured sugars were not significantly influenced by the choice of storage strategy. There was a negative correlation between glucose/fructose content and fry colour in both Asterix and Saturna ($P < 0.01$).

3.3. Effect of maturity and ventilation during large-scale storage

3.3.1. Weight loss

Tubers from stores using natural ventilation lost significantly more weight than tubers stored with forced ventilation (Table 6). The immature tubers (M_{imm}) lost significantly more weight than both medium mature (M_{med}) and mature (M_{mat}) tubers. There was no difference between cultivars in weight loss.

3.3.2. Respiration rate

The respiration rate was higher in tubers stored with forced ventilation than with natural ventilation (Table 6). The immature tubers (M_{imm}) had higher respiration rates than the more mature tubers. In general, Asterix had higher respiration rates than Saturna.

3.3.3. Dry matter

The dry matter contents did not change significantly during large-scale commercial storage (Table 6) as was also seen in the small-scale storage experiment.

Table 3
Effect of ventilation strategy, maturity and storage time on weight loss, respiration and dry matter in potato cultivars Asterix and Saturna in small-scale stores.

	Weight loss (%)				Respiration (mg CO$_2$ kg^{-1} h^{-1})				Dry matter (%)			
	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean
Natural ventilation	0.71	1.60	1.56	1.36a	0.82	0.85	1.22	0.89	23.2	24.4	24.2	24.0
Forced ventilation	0.56	1.21	1.09	1.00b	0.71	0.91	1.06	0.97	23.4	24.4	24.2	23.9
				***				n.s				n.s
M_{mat}[a]	0.90	1.31	1.29	1.19	0.79	0.90	1.31	1.00	24.7	26.2	25.0	25.3a
M_{med}	0.51	1.44	1.22	1.12	0.73	0.94	0.83	0.84	23.6	24.7	24.8	24.4b
M_{imm}	0.49	1.49	1.48	1.23	0.78	0.80	1.28	0.95	21.6	22.3	22.9	22.3c
				n.s				n.s				***
Saturna	0.80	1.34	1.37	1.21	0.85	0.75	1.18	0.93	24.9	25.2	26.0	25.4a
Asterix	0.46	1.48	1.28	1.14	0.69	1.01	1.10	0.93	21.7	23.5	22.4	22.5b
				n.s				n.s				***
December	1.14	1.31	0.98	1.14ab	0.76	0.50	1.08	0.78b	22.6	24.6	24.1	23.8
February	0.95	1.42	1.57	1.31a	0.74	1.22	0.73	0.90b	23.5	25.0	23.7	24.1
April	0.81	1.01	1.44	1.09b	0.80	0.92	1.61	1.11a	23.7	23.6	24.8	24.0
				*				***				n.s

n.s = not significant ($P \geq 0.05$), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($P = 0.05$).
[a] Three different maturity levels: Mature (M_{mat}), medium mature (M_{med}), immature (M_{imm}).

Table 4
Effect of ventilation strategy, maturity and storage time on sucrose, glucose/fructose and fry colour in potato cultivars Asterix in small-scale stores.

	Sucrose (mg g^{-1} FW)				Glucose + fructose (mg g^{-1} FW)				Fry colour (scale 1–9)[b]			
	2010–2011	2012–2013	2013–2014	*mean*	2010–2011	2012–2013	2013–2014	*mean*	2010–2011	2012–2013	2013–2014	*mean*
Natural ventilation	0.97	1.05	1.40	*1.13*	6.11	6.02	5.53	*5.89*	6.8	6.4	6.5	*6.5*
Forced ventilation	1.00	0.99	1.22	*1.07*	6.58	7.32	5.01	*6.30*	6.8	6.1	6.6	*6.5*
				n.s				*n.s*				*n.s*
M_{mat}[a]	1.10	1.05	1.38	*1.18a*	6.76	5.08	5.51	*5.78*	6.9	6.2	6.5	*6.5*
M_{med}	0.98	1.10	1.33	*1.14ab*	6.76	7.02	5.17	*6.31*	6.4	6.3	6.7	*6.5*
M_{imm}	0.85	0.90	1.22	*0.99b*	5.54	7.90	5.13	*6.19*	6.7	6.2	6.5	*6.5*
				*				*n.s*				*n.s*
Saturna	–	–	–	–	–	–	–	–	–	–	–	–
Asterix	0.99	1.02	1.31	*1.10*	6.35	6.66	5.27	*6.09*	6.6	6.2	6.6	*6.5*
				–				–				–
December	1.36	1.17	1.49	*1.34b*	5.31	5.39	4.02	*4.91b*	7.7	7.2	7.1	*7.3a*
February	0.76	1.01	1.24	*1.00b*	5.97	5.42	5.31	*5.56b*	5.7	6.1	6.5	*6.1b*
April	0.84	0.89	1.19	*0.97a*	7.78	9.18	6.49	*7.82a*	6.5	5.5	6.1	*6.0b*
				**				***				***

n.s = not significant ($P \geq 0.05$), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($P = 0.05$).
[a] Three different maturity levels: Mature (M_{mat}), medium mature (M_{med}), immature (M_{imm}).
[b] 1 = dark and 9 = pale.

3.3.4. Carbohydrate and fry colour

After storage, the glucose/fructose contents were higher in tubers stored with forced ventilation while the content of sucrose was similar for the two ventilation strategies (Table 6). Average sucrose contents decreased from 1.93 mg g^{-1} at harvest to 1.26 mg g^{-1} in February, when samples were taken out of storage, while glucose/fructose contents increased from 1.07 mg g^{-1} at harvest to 2.60 mg g^{-1} in February. For fry colour, both Saturna and Asterix became significantly darker when tubers were stored with forced ventilation. There were negative correlations between glucose/fructose content and fry colour in both Saturna ($P < 0.001$) and Asterix ($P < 0.05$).

4. Discussion

4.1. Effect of maturity on tuber quality during and after storage

It is largely agreed that the storage potential of potatoes is influenced by their maturity at harvest (Burton, 1965; Bussan et al., 2009; Sabba et al., 2007). The term "maturity" is however not easily defined by a single parameter, but can be viewed as a combination of different processes which take place in the maturing plant. The processes involve haulm maturation, physical maturity (skin set), physiological maturity (maximum dry matter content) and chemical maturity (minimum content of sucrose). The processes

Table 5
Effect of ventilation strategy, maturity and storage time on sucrose, glucose/fructose and fry colour in potato cultivar Saturna in small-scale stores.

	Sucrose (mg g^{-1} FW)				Glucose + fructose (mg g^{-1} FW)				Fry colour (scale 1–9)[b]			
	2010–2011	2012–2013	2013–2014	*mean*	2010–2011	2012–2013	2013–2014	*mean*	2010–2011	2012–2013	2013–2014	*mean*
Natural ventilation	0.63	0.86	0.77	*0.74*	1.32	2.73	1.58	*1.88*	5.4	5.3	6.2	*5.7a*
Forced ventilation	0.64	0.91	0.73	*0.77*	1.4	2.81	1.79	*2*	5.4	4.9	5.4	*5.2b*
				n.s				*n.s*				*
M_{mat}[a]	0.60	1.10	0.71	*0.82*	1.01	2.84	1.91	*1.92*	5.5	5.3	5.8	*5.6*
M_{med}	0.65	0.78	0.78	*0.74*	1.30	2.72	1.79	*1.94*	5.8	5.4	5.7	*5.5*
M_{imm}	0.64	0.78	0.74	*0.71*	1.76	2.74	1.36	*1.96*	4.9	4.6	5.9	*5.1*
				n.s				*n.s*				*n.s*
Saturna	0.63	0.89	0.75	*0.76*	1.36	2.77	1.69	*1.94*	5.4	5.1	5.8	*5.4*
Asterix	–	–	–	–	–	–	–	–	–	–	–	–
				–				–				–
December	0.65	1.02	0.72	*0.79*	1.32	2.58	1.44	*1.78*	5.9	5.2	6.4	*5.8a*
February	0.55	0.77	0.75	*0.77*	1.35	2.98	1.71	*2.01*	5.5	5.8	4.9	*5.4ab*
April	0.69	0.86	0.77	*0.70*	1.41	2.75	1.92	*2.02*	4.8	4.3	6.0	*5.1b*
				n.s				*n.s*				**

n.s = not significant ($P \geq 0.05$), *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($P = 0.05$).
[a] Three different maturity levels: Mature (M_{mat}), medium mature (M_{med}), immature (M_{imm}).
[b] 1 = dark and 9 = pale.

Table 6

Main effect of ventilation strategy, maturity and cultivar on weight loss, respiration, dry matter, sucrose, glucose/fructose and fry colour in large-scale stores.

	Weight loss (%)				Respiration (mg CO_2 kg^{-1} h^{-1})				Dry matter (%)				Sucrose[a] (mg g^{-1} FW)				Glucose + fructose[b] (mg g^{-1} FW)				Fry colour Saturna (Agtron)[c]				Fry colour Asterix (scale 1–9)[d]			
	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean	2010–2011	2012–2013	2013–2014	mean
Natural ventilation	3.93	4.24	3.63	3.93a	1.48	1.50	1.21	1.45b	23.0	25.0	24.8	24.2	0.88	1.24	1.80	1.23	2.45	1.81	2.80	2.35b	45.8	38.4	38.0	40.7a	6.6	6.7	6.8	6.8a
Forced ventilation	2.91	3.65	2.27	2.91b	1.49	1.79	1.61	1.64a	22.9	25.0	24.3	24.0	0.96	1.09	1.72	1.26	2.63	2.24	3.48	2.78a	36.9	36.6	36.8	36.8b	5.4	6.3	6.6	6.2b
				***				***				n.s				n.s				**				*				***
M$_{mat}$	3.18	3.37	2.78	3.18b	1.40	1.54	1.42	1.46b	24.5	26.8	25.3	25.5a	0.97	1.15	1.75	1.26	2.38	1.98	3.31	2.53	45.5	38.6	36.9	40.3	6.4	6.7	6.6	6.6
M$_{med}$	2.94	3.42	2.29	2.94b	1.47	1.67	1.29	1.48b	23.3	25.0	24.9	24.4b	0.87	1.20	1.67	1.25	2.56	2.11	3.06	2.54	41.1	38.9	38.3	39.5	5.8	6.5	6.9	6.5
M$_{imm}$	4.16	4.87	3.23	4.16a	1.58	1.81	1.69	1.68a	21.0	23.2	23.3	22.5c	0.93	1.07	1.83	1.23	2.72	2.06	3.20	2.62	37.6	35.1	36.9	36.5	6.0	6.6	6.5	6.4
				***				***				***				n.s				n.s				n.s				n.s
Saturna	3.48	3.81	2.74	3.46	1.62	1.85	1.56	1.67	24.6	26.0	26.3	25.6a	0.42	0.98	1.60	0.98b	2.01	1.52	2.29	1.92a	41.4	37.5	37.4	38.8	–	–	–	–
Asterix	3.37	3.97	2.79	3.39	1.35	1.50	1.37	1.41	21.3	24.0	22.7	22.6b	1.39	1.30	1.90	1.51a	3.10	2.58	4.09	3.22a	–	–	–	–	5.9	6.5	6.7	6.7
				n.s				n.s				***				***				***				–				–

n.s = not significant ($P \geq 0.05$), $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($P = 0.05$).
[a] Mean value of content in Asterix and Saturna.
[b] Mean value of content in Asterix and Saturna.
[c] High Agtron scores represent lighter fry colours.
[d] 1 = dark and 9 = pale.

does not necessarily occur simultaneously, and tubers may be physically mature without being chemically and/or physiologically mature (Kumar et al., 2004; Sabba et al., 2007; Sowokinos, 1978). In the present study, potato tubers of the three maturity levels differed with respect to most maturity indicators at harvest. An exception was chemical maturity (sugar content). Even though not all indicators of maturity differed significantly between all maturity levels, there were significant differences for all maturity indicators between the most mature and the least mature tubers of both Asterix and Saturna.

After four months of storage in large-scale commercial stores, the immature tubers had significantly higher weight losses. Weight loss in healthy, not sprouted tubers is mainly caused by respiration and transpiration. Physically immature potatoes with a poor skin set, have a skin that is permeable to water and most of their weight loss is therefore probably due to higher respiration and transpiration (Bussan et al., 2009). Skinned areas of tubers have been found to have a transpiration rate that is 250 to 1000-fold higher than that of non-skinned areas (Lulai and Orr, 1995).

Respiration was highest in immature tubers in the present study. Previous studies have shown immature potatoes to be more susceptible to skinning injuries, and in addition to have higher respiration rates at harvest, than mature potatoes (Bussan et al., 2009; Sabba et al., 2007). Immature potatoes as a result of later planting dates have also been found to have higher respiration rates than mature tubers (Knowles et al., 2009).

The findings of the highest dry matter contents in the most mature tubers of both Asterix and Saturna are in accordance with Herrman et al. (1995) and Wiltshire et al. (2004), who found that more mature crops had higher dry matter contents than less mature crops.

The levels of contents of sucrose, glucose and fructose in Asterix and Saturna are similar to findings of others (Amrein et al., 2003; Hertog et al., 1997a; Skog and Alexander, 2006). Absence of differences in sucrose, glucose and fructose contents among maturity levels may be due to a slow decline in temperature from 12 to 6 °C (0.5 °C per week) because of conditioning at a high temperature during the first period of storage. Knowles et al. (2009) showed that the tubers were most sensitive to cold induced sweetening during the first months of storage and conditioning of the tubers at a high temperature just after harvest reduced the sweetening response in the tubers. Lack of significant differences in reducing sugars among maturity levels may be the reason why there were no significant effects of maturity on fry colour. The content of reducing sugars was negatively correlated with fry colour in the present study, as can be expected from the Maillard reaction where reducing sugars interact with free amino acids and produce acrylamide and a dark fry colour (Amrein et al., 2003; Shallenberger et al., 1959). In the small-scale storage experiments, sucrose content decreased during storage whilst the contents of glucose and fructose increased. Parallel to this, fry colour became darker. Similar results with decreasing sucrose content and an increase in reducing sugars during storage have also been observed in other studies (Hertog et al., 1997a; Knowles et al., 2009; Kolbe et al., 1995). The increase of sugars in Asterix tubers towards the end of the storage is probably a consequence of senescent sweetening, where sugars are mobilised for the benefit of development and growth of sprouts (Hertog et al., 1997b).

4.2. Effect of ventilation on tuber quality during and after storage

During storage, the tubers were exposed to two different ventilation strategies in both small- and large-scale stores, described as natural ventilation and forced ventilation. For both small- and large-scale storage, the tuber weight losses were higher with natural ventilation. These results correspond with the results

of Sparks (1973), who found intermittent ventilation to cause significantly less weight loss than continuous ventilation with the same air rate. In the small-scale storage, the total volume of air sent through the storage containers per day was larger for natural ventilation than forforced ventilation, amounting to 300 and 180 m^3 tonne^{-1} day^{-1} respectively. This means that the total air volume removed from the surface of the tubers was larger fornatural ventilation, explaining a higher weight loss in the tubers stored under these conditions. Similar results, with lower weight loss under reduced air flow, were found in a study of variable frequency drive as an alternative to manual on and off switching the ventilation fans run at full speed (Oberg and Kleinkopf, 2003). The present study found reducing sugar contents to be higher and fry colour darker in both Saturna and Asterix when ventilated with forced ventilation in large-scale commercial stores. In the small-scale store, forced ventilation only gave significantly darker fry colours in Saturna. Darker fry colours have previously been associated with high respiration rates (Copp et al., 2000; Daniels-Lake et al., 2005; Mazza and Siemens, 1990). This was confirmed by our study, as respiration rates were higher in tubers ventilated withforced ventilation when stored in the commercial large-scale stores. The same tendency was observed for small-scale stores. Forced ventilation also resulted in darker fry colour. In small-scale store, both tuber respiration rates and darkness of fry colour increased during storage with both ventilation methods and were significantly higher in April than earlier in the storage season. Copp et al. (2000) found similar results and suggested that monitoring respiration rates throughout the storage season could provide a continuous, non-destructive method for predicting the point at which tuber processing quality will decline. The difference in fry colour that was found between ventilation strategies in commercial large-scale storage may be explained by elevated CO_2 levels. Elevated CO_2 levels may enhance respiration and thus result in a rise in reducing sugars content. Periods of increased CO_2 levels may have occurred in the 2010–2011 storage season, which included a long period with cold outdoor temperatures (mean of $-12.5\,^\circ C$ in January and February) (www.yr.no). At low outdoor temperatures, ventilation may be switched to recycling of air, which may in time result in lower O_2 levels. The difference between the ventilation strategies may be a result of there being more airtight walls in newer stores, as were more common among the forced ventilation stores in the present study. A similar trend, with darker fry colour with forced ventilation, was found in small-scale storage of Saturna. This difference cannot be explained by elevated CO_2 levels, but seems to be reflected in a tendency towards more respiration and higher glucose/fructose contents in Saturna underforced ventilation.

In conclusion, the strategy used with various combinations of planting date, pre-sprouting and nitrogen fertilization, produced tubers with different maturity levels, as measured by haulm greenness, skin set, dry matter and partly by sucrose content at harvest. Maturity influenced storability significantly, with the least mature tubers having both a higher weight loss and a higher respiration rate than the more mature tubers. In both small- and large-scale stores, the ventilation strategy using low, continuous air flow resulted in higher weight losses than did intermittent ventilation with high air flow, probably as a result of total higher air volumes passing between the potatoes. Higher respiration rates, higher contents of reducing sugars and darker fry colour were observed for tubers stored with forced ventilation. In the forced ventilation large-scale stores, a possible explanation for darker fry colour may be elevated CO_2 levels due to closing of the outdoor air intake during cold periods. This study shows the importance of tuber maturity at harvest when aiming for good quality during and after storage. Monitoring respiration rates of the tubers and CO_2 levels in the stores throughout the storage season can help in managing the stores and predicting fry colour, especially in "forced ventilation stores.

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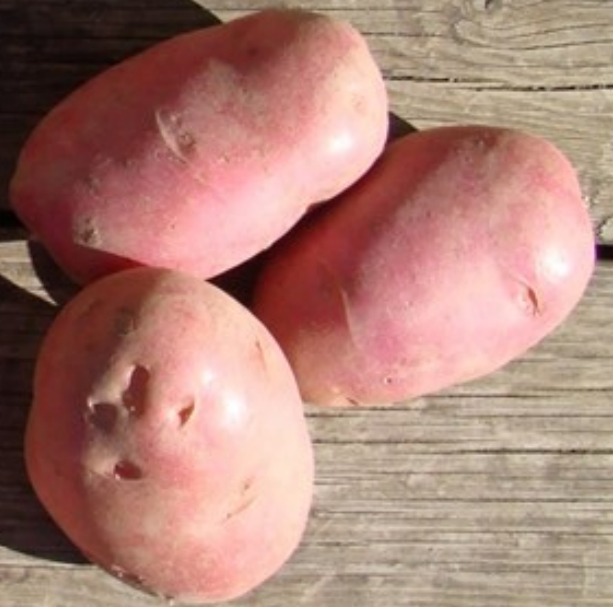
Maturity indicators for prediction of potato quality during storage

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Abstract

The use of maturity indicators as predictors of potato quality in potato tubers during and after storage was explored in cvs. Asterix and Saturna with three different maturity levels during three years (2010, 2012 and 2013). The maturity indicators measured 1-3 weeks before harvest and at harvest included haulm senescence (haulm maturity), skin set (physical maturity), dry matter content (physiological maturity) and contents of sucrose, glucose and fructose (chemical maturity). Potato quality parameters were measured three times during storage (December, February and April) and included dry matter content, sucrose, glucose and fructose contents, weight loss and fry colour. The data were modelled with linear regression. Cultivar and maturity level were included as categorical predictors and contributed significantly (P<0.001) to most of the prediction models. Dry matter, sucrose, glucose and fructose included as continuous predictors, contributed significantly (P<0.01) to the sucrose, glucose and fructose models and these models explained a high proportion of the variation ($R^2 \geq 0.88$). Skin set contributed significantly to the weight loss models (P<0.01) but the models showed low R^2-values ($R^2 < 0.48$). Sucrose contents contributed significantly (P=0.05) to the fry colour model for Asterix and the fry colour models for both Asterix and Saturna had R^2-values of 0.50 and 0.51 respectively. This study provides new information about the influence of maturity on potato quality during storage and the potential of using field measurements of maturity as predictors of storage potential for processing potatoes.

1. Introduction

Norwegian potato production has a value of approximately NOK 500 million annually and includes a total yield of 350,000 t grown on approximately 14,000 ha (Statistics, 2014). Potatoes are grown during a short summer season and consumed all year around, and thus need to be stored for longer or shorter periods of time. The estimated annual loss during storage is approximately 10 percent. Once the tubers are harvested, they utilize their own stored resources for metabolic processes. Mature potatoes store better than immature potatoes, as they are less susceptible to skinning injury, have lower respiration rates and weight loss and are less susceptible to diseases (Heltoft et al.,

2015; Knowles and Plissey, 2008; Lulai and Orr, 1995). Mature potatoes also have lower contents of reducing sugars than immature tubers (Driskill et al., 2007).

Immature potatoes at harvest are quite common in Norwegian potato production, due to the use of relatively late cultivars and a short growing season (100-110 days), with low temperatures, particularly during spring and autumn (www.yr.no). A prediction model for storage quality, based on measurements at harvest, would thus be useful in order to select the right potatoes for long-term storage. Potato maturity is a key factor in the management and prediction of processing quality after storage. Maturation of potatoes is complex and involves a variety of processes, including senescence (haulm maturation), skin set (physical maturation), accumulation of dry matter (physiological maturation) and lowering of sucrose content (chemical maturation) (Bussan et al., 2009; Sabba et al., 2007). However, these processes do not necessarily peak at the same time (Kumar et al., 2004; Sabba et al., 2007; Sowokinos, 1978). Chemical maturity is the most commonly used indicator in models which predict potato quality (Hertog et al., 1997a; Sowokinos, 1978). Sowokinos (1978) found the level of sucrose at harvest to be a good indicator of subsequent processing quality and suggested to keep levels below 2.8 mg g^{-1} fresh weight. However, other studies investigating the relationship between processing quality and sucrose content did not succeed in using sucrose as a predictor (Briddon and Storey, 1996; Lærke and Christiansen, 2005; Wiltshire et al., 2004). Maturity indicators other than chemical maturity, measured before and at harvest, are of interest to predict different quality measures during potato storage, either alone or in combination with chemical maturity. Herrman et al (1995) suggested that physical, physiological and chemical maturity measurements in combination, taken at or shortly before harvest, could be used for the purpose of selecting potato lots for long-term storage. Lærke and Christiansen (2005) proposed haulm maturity as a promising marker for fry colour during storage. A prediction model using measurements in the field in the last part of the growing season and at harvest, would be of great interest to the grower and to the processing industry, as it could guide decisions on length of storage and the order in which the crop should be processed.

The aim of this study was to investigate the potential of potato maturity indicators measured in the field or at harvest, to predict potato quality during and after storage in the cultivars Asterix and Saturna grown with different maturity levels.

2. Materials and methods

2.1. Plant material

During the growing seasons of 2010, 2012 and 2013, two potato cultivars, Asterix and Saturna, were grown on a loam soil (Cambisol, low erosion risk, moderate natural drainage)(WRB, 2006) in Østre Toten, Oppland, Norway (60.70°N, 10.87°E). The tubers were planted at 12 cm depth with a distance of 30 cm within rows and 80 cm between rows. In order to gain material with a maximum of variation in maturity at harvest, three different combinations of the factors pre-sprouting, planting date and level of nitrogen fertilization were used as described by Heltoft et al. (2015). The most mature (M_{mat}), was presprouted for four weeks in 12 °C under full light (>100 lux), planted 16 to 23 May and fertilized with 70 kg N ha^{-1}. Medium mature (M_{med}), was not presprouted, planted the same dates as M_{mat} and fertilized with 105 kg N ha^{-1}. Immature (M_{imm}), was not presprouted, planted two weeks later than M_{mat} and M_{med} and fertilized with 140 kg N ha^{-1}. In all three treatments (M_{mat}, M_{med} and M_{imm}) the haulm was killed 8-10 days before harvest and tubers were harvested at the same date. This strategy was chosen to avoid influence of different harvesting conditions and to be able to start storage at the same dates within each year.

2.2 Maturity indicators

Maturity indicators were measured once a week from three weeks before the set harvest until the date of harvest (1 to 22 September 2010, 23 August to 13 September 2012 and 22 August to 12 September 2013). Indicators included haulm greenness (haulm maturity), skin set (physical maturity), dry matter content (physiological maturity) and contents of sucrose, glucose and fructose (chemical maturity).

2.2.1 Haulm senescence (haulm maturity)

Haulm maturity was determined before haulm desiccation, both visually as relative greenness (0-100, 0 = dead and 100 = full greenness) and by measuring the chlorophyll content using a hand-held chlorophyll meter [model hydro-N-tester (HNT): Yara, Oslo, Norway] as described by Vos and Bom (1993). The chlorophyll meter measures the light transmittance of a leaf at 650 and 940 nm and measurements were done on the distal leaflet of the youngest fully expanded compound leaf (i.e. the fourth or fifth leaf from the apex). A mean of 30 measurements was used to obtain a value of the chlorophyll content. The values obtained indicate the relative amount of chlorophyll present in the leaves.

2.2.2 Skin set (physical maturity)
Skin set of potato tubers was measured as described by Lulai and Orr (1993), with a measuring device (Halderson Periderm shear tester) consisting of a measuring head attached to a torque meter, which measures the amount of torsional force [mN m (milliNewton meters)] required to produce skinning injury. After sampling, tubers were left overnight under laboratory conditions (20 °C, 40-60 % RH) before the measurements were taken.

2.2.3 Dry matter content (physiological maturity)
The content of dry matter was determined by weighing in air and in water to determine potato density as described by Lunden (1956). The following equation was used to calculate the content of dry matter, *Dry matter = 215.73 (x-0.9825),* where x is the specific weight calculated as weight in air / (weight in air – weight in water).

2.2.4 Sugar content (chemical maturity)
Analyses of sucrose, glucose and fructose contents in the tubers were done by ion chromatography as described by Elmore et al. (2007) with modifications. Samples of 10 quarters (cut from the heel to rose end) of tubers from the same batch were chopped in a food processor. Samples of 2 g ± 0.005 g were weighted into 50 mL screw-cap bottles and 20 mL aqueous methanol (50 %) containing 200 µL trehalose, as an internal standard, was added to each bottle. The samples were stirred for 15 min at room temperature. Aliqouts (100 µL) were diluted in 900 µL aqueous methanol (50 %). Then the extracts were filtered through a millipore filter PVCF 0.22 µm. The extracts were analysed for sucrose, glucose and fructose using a HPAC-PAD system.

2.3 During storage
All tubers were woundhealed at 12 °C and 95 % RH for two weeks and then transferred to controlled storage rooms. The storage rooms held 98 % RH, and the temperature was regulated down stepwise over two months from October to December, from 12 to 6 °C. Three times during the storage season (December, February and April), two samples of tubers from each combination of maturity level and cultivar were withdrawn for analyses of weight loss, dry matter content, contents of sucrose and reducing sugars (glucose and fructose) and fry colour. Fry colour was determined in each replicate. For Saturna, ten tubers were peeled and three slices of 1.8 mm thickness were cut from the middle of each tuber. For Asterix tubers, strips (10 mm wide x 10 mm thick x length of tuber) were cut along the apical to basal axis, one from each of 10 tubers. The slices and strips were rinsed in tapwater, gently wiped, and fried at 180 °C for 150 s in sunflower oil. Fry colour was determined by visual inspection immediately after frying, using a scale from 1 (dark) to 9 (pale).

2.5 Statistics

Statistical analyses were performed using Minitab® version 17.2.1. The data was modelled with linear regression using maturity, potato cultivar and year as categorical predictors and dry matter, sucrose, glucose, fructose, haulm senescence and skin set as continuous predictors. Response values included weight loss, dry matter, sucrose, glucose, fructose and fry colour. All potential predictors were included initially in the models, and the models were subsequently reduced on the basis of the R^2-values obtained, p-values and where appropriate multicollinearity between the predictors.

3. Results

3.1 Maturity indicators

3.1.1 Haulm senescence (haulm maturity)

The three different treatments to obtain the maturity levels (M_{mat}, M_{med} and M_{imm}) used for the cultivars Saturna and Asterix resulted in different haulm maturation. At all sampling dates, the least mature level (M_{imm}) was significantly greener than the other maturity levels for both cultivars (P<0.001) (fig. 1). Senescence measured by visual inspection and with the Yara-N tester correlated well for both Asterix and Saturna, R^2=0.89 and R^2=0.91 respectively.
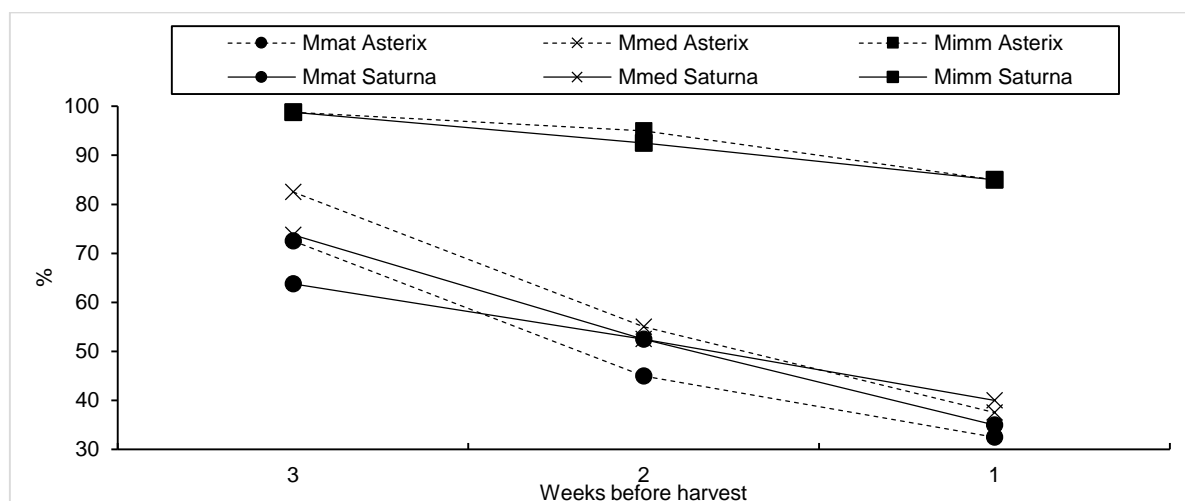

Fig. 1 Visual inspection of haulm maturity (0 -100, 0 = dead and 100 = full greenness) during the last three weeks before harvest for Asterix and Saturna with three maturity levels (M_{mat}, M_{med} and M_{imm}). Mean of three years.

3.1.2 Skin set (physical maturity)

There were significant differences in physical maturity for all three maturity levels on all sampling dates (P<0.001). Saturna had higher skin set values than Asterix (P<0.05). For both cultivars, the most mature level (M$_{mat}$) required the highest torsional force to cause skinning injury while the least mature level required the least force. The physical maturity increased for all maturity levels until harvest (fig. 2).
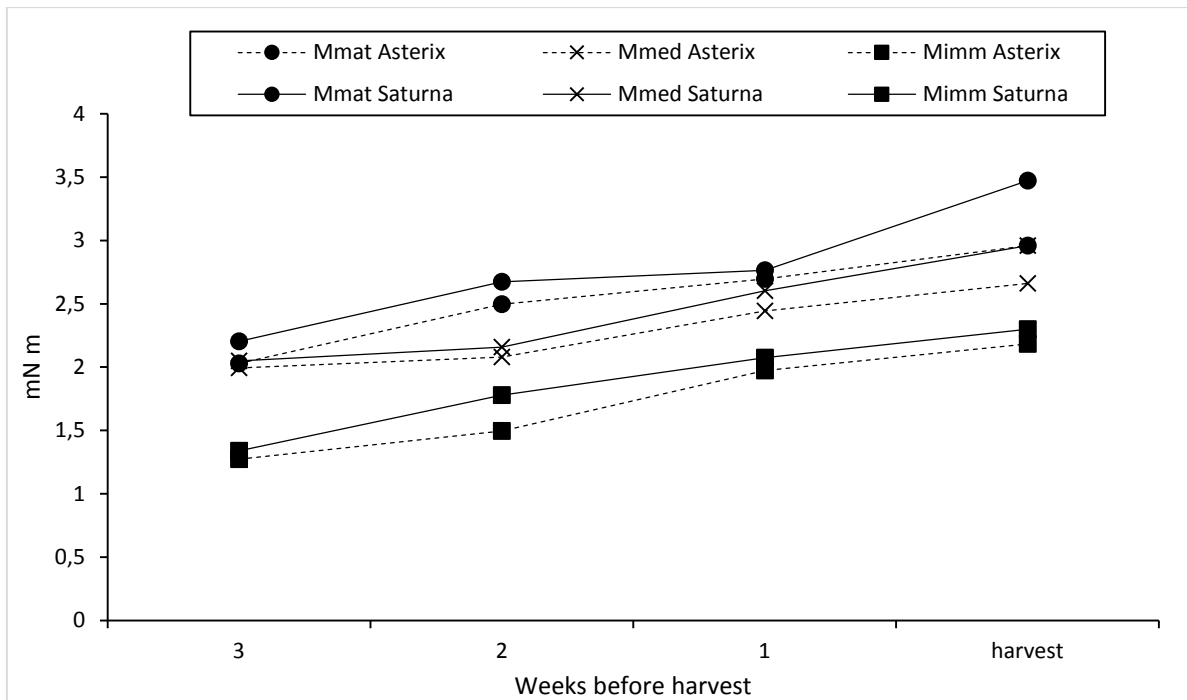

Fig. 2 Physical maturity measured with a torque meter in potato cultivars Saturna and Asterix with three maturity levels (M$_{mat}$, M$_{med}$ and M$_{imm}$) during the last three weeks prior to harvest. Mean of three years.

3.1.3 Dry matter content (physiological maturity)

The dry matter content showed significant differences (P<0.001) among the different maturity levels on all sampling dates from three weeks before harvest until the end of storage. The most mature tubers (M$_{mat}$) had the highest dry matter content followed by the medium mature ones (M$_{med}$) and the immature (M$_{imm}$) tubers. Saturna had significantly higher dry matter content than Asterix (P<0.001). The dry matter content increased until haulm desiccation and was stable after harvest and during storage (fig. 3).
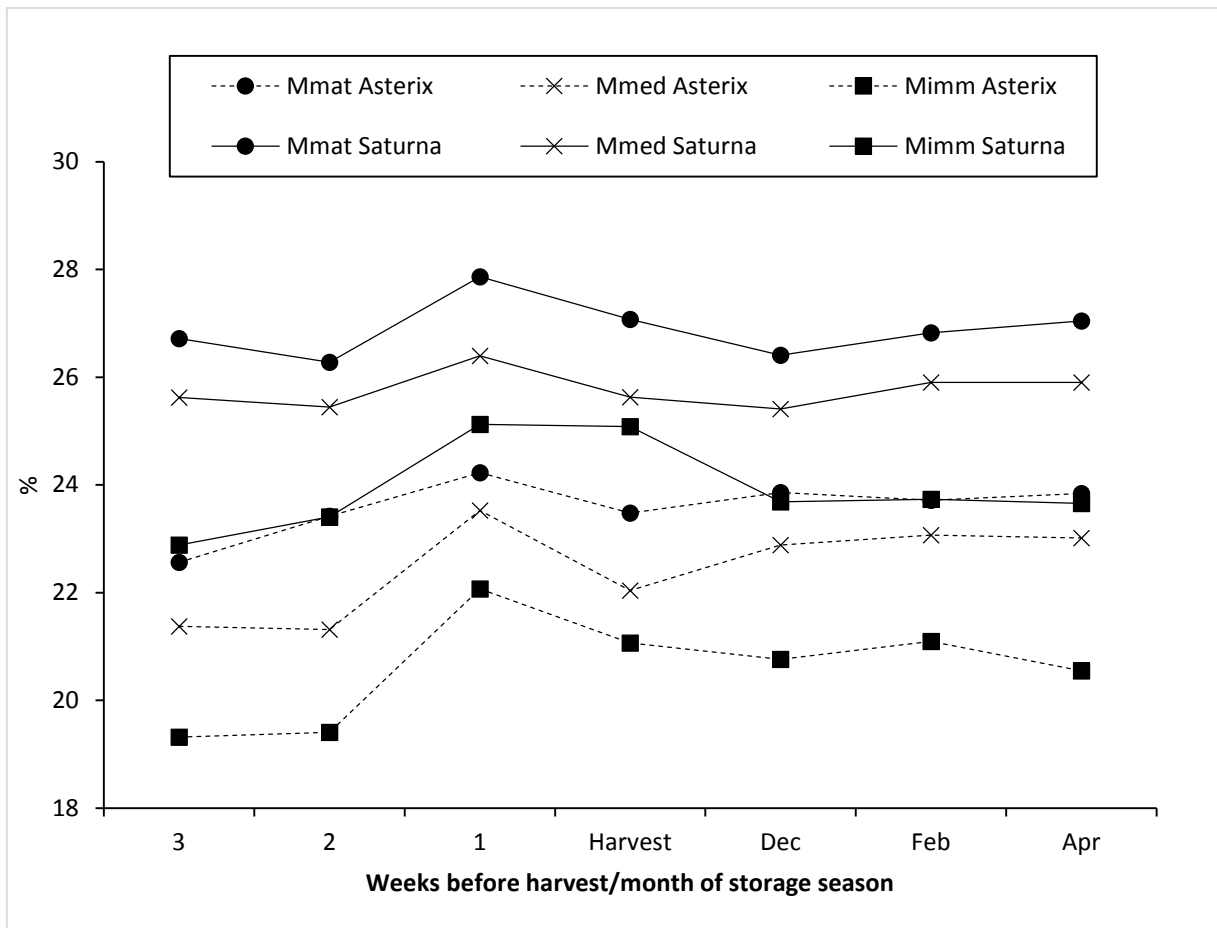
6

Fig. 3 Dry matter content in Asterix and Saturna tubers at three levels of maturity (M_{mat}, M_{med} and M_{imm}), from three weeks prior to harvest and during storage. Mean of three years.

3.1.4 Sugar content (chemical maturity)

The sucrose content decreased during the last part of the growing season at all maturity levels for both Asterix and Saturna (fig. 4 and 5) and continued to decrease during storage. There were significant differences in sucrose content among maturity levels prior to harvest, but not after harvest. At the time of harvest, Asterix had similar sucrose levels at the three maturity levels, while immature Saturna still had higher levels of sucrose at harvest.

Glucose and fructose contents increased and reached their highest levels in April. Immature tubers had significantly higher glucose and fructose contents than the more mature tubers at sampling two and three weeks before harvest ($P<0.001$), whereas after harvest no significant differences were observed among the different maturity levels. There were significant differences between the cultivars, and Asterix had higher contents of sugars than Saturna.
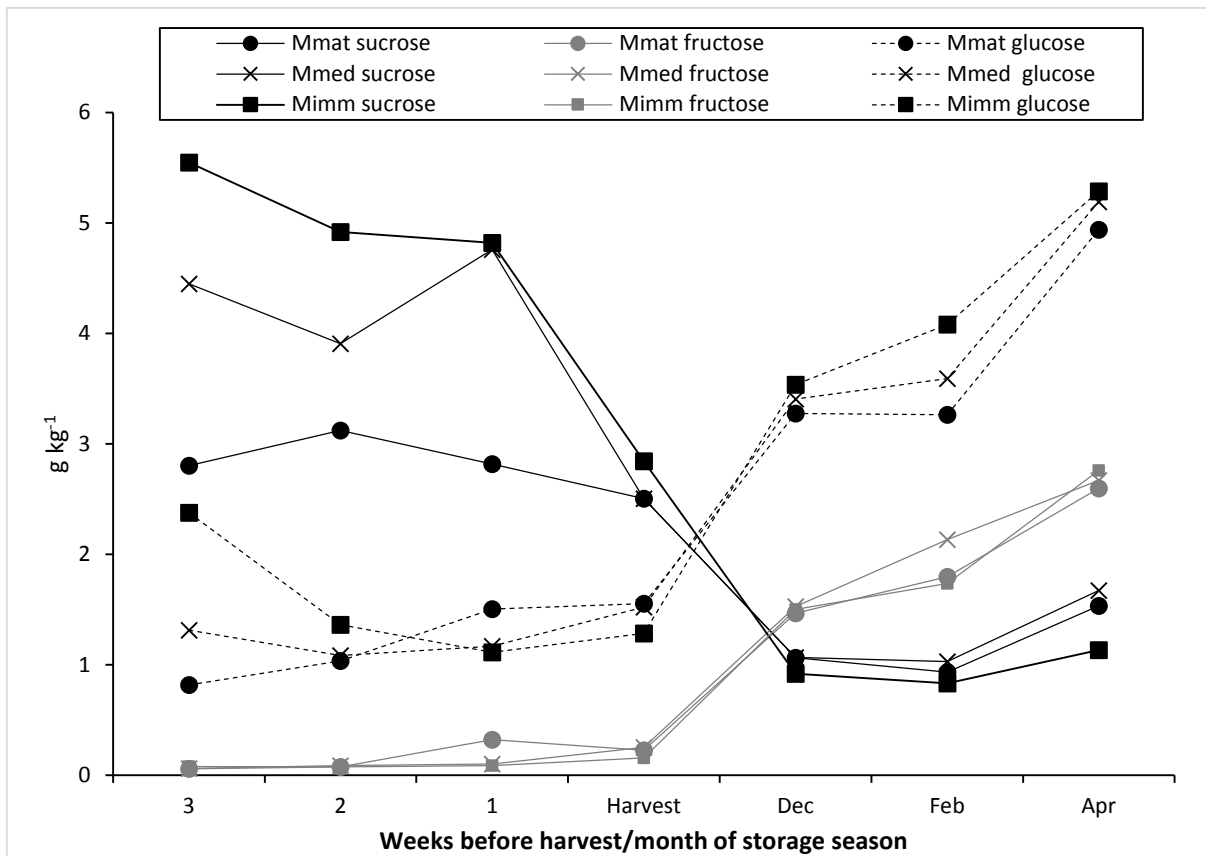
Fig. 4 Sucrose, glucose and fructose contents in Asterix tubers with three maturity levels, from three weeks prior to harvest and during storage. Mean of three years.
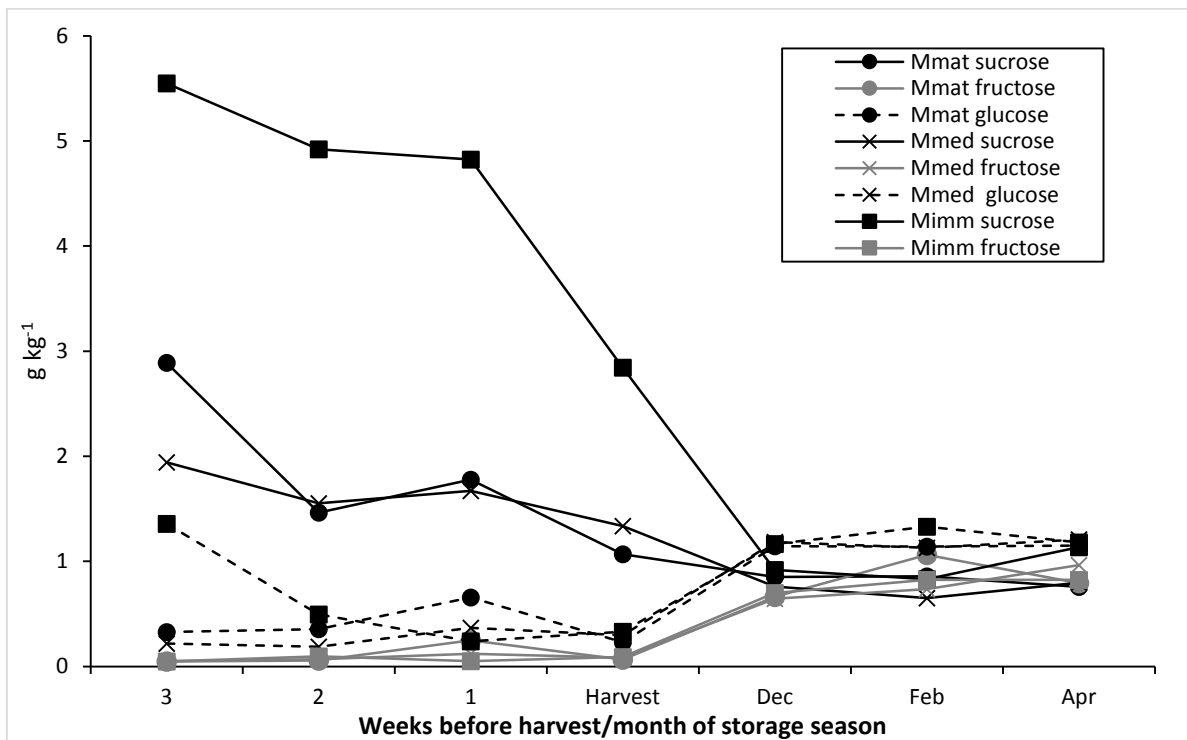

Fig. 5 Sucrose, glucose and fructose contents in Saturna tubers with three maturity levels, from three weeks prior to harvest and during the storage period. Mean of three years.

3.2 Predicting potato quality during storage

Linear regression models were developed to predict potato quality during storage. Different combinations of model data were explored. The models included both categorical predictors (maturity levels, potato cultivar and year) and continuous predictors (maturity indicators). The continuous predictors included dry matter, sucrose, glucose and fructose content, haulm senescence and skin set, measured 1-3 weeks before harvest and at harvest (Table 1). Mean values of selected predictors from the four measuring dates prior to or at harvest (mean) were also explored. The response values investigated included weight loss, dry matter, sucrose, glucose, fructose and fry colour, measured three times during storage (December, February and April), as well as an overall mean of the three sampling dates (mean). The models were reduced on the basis of their R^2-values, p-values and where appropriate multicollinearity between the predictors.

Table 1 shows that dry matter content was predicted well by all linear regression models that combined predictors from different sampling dates before/at harvest and dry matter measured at different times during storage ($R^2 > 0.84$) (Table 1). For response values sucrose, glucose and fructose, the December values and the mean value for the storage season were predicted best (highest R^2-values) (Table 1). Weight loss was not well predicted ($R^2 < 0.47$) but the weight loss model was the only one which contained a significant contribution ($P < 0.01$) from physical maturity (skin set), and thus the only one with skin set in the model. For both cultivars, the fry colour in April was generally best predicted from measurements around harvest. For Asterix the measurements at harvest resulted in the highest R^2-values ($R^2 = 0.50$), while Saturna had slightly higher values with measurements three weeks prior to harvest, $R^2 = 0.56$ versus 0.50 at harvest (Table 1).

Table 1 R^2-values for all combinations of sample dates after storage (December, February, April) and at harvest (0) or 1-3 weeks prior to harvest (separate and mean values), as mean for three years. The results are based on optimized regression models with all maturity indicators at or before harvest as predictors. Most models include both cultivars, while for fry colour separate models are used for each cultivar.

Weeks before harvest	Month sampling	Dry matter	Sucrose	Glucose	Fructose	Weight loss	Fry colour Asterix	Fry colour Saturna
3	December	0.87	0.87	0.84	0.80	0.36	0.29	0.30
3	February	0.85	0.62	0.71	0.54	0.32	0.39	0.44
3	April	0.87	0.60	0.57	0.68	0.43	0.47	0.56
3	mean	0.91	0.79	0.78	0.85	0.43	0.26	0.32
2	December	0.85	0.89	0.90	0.83	0.39	0.28	0.29
2	February	0.87	0.71	0.75	0.59	0.23	0.39	0.34
2	April	0.88	0.63	0.63	0.71	0.34	0.45	0.52
2	mean	0.92	0.84	0.84	0.86	0.37	0.26	0.32
1	December	0.87	0.91	0.89	0.81	0.38	0.14	0.30
1	February	0.86	0.61	0.71	0.58	0.25	0.41	0.46
1	April	0.90	0.64	0.64	0.71	0.33	0.36	0.48
1	mean	0.95	0.83	0.83	0.85	0.47	0.26	0.32
0	December	0.88	0.93	0.92	0.81	0.40	0.19	0.14
0	February	0.87	0.68	0.75	0.59	0.22	0.17	0.28
0	April	0.88	0.68	0.67	0.73	0.34	0.50	0.51
0	mean	0.91	0.84	0.83	0.79	0.38	0.28	0.30
mean	December	0.88	0.92	0.91	0.81	0.30	0.32	0.31
mean	February	0.88	0.70	0.76	0.59	0.27	0.06	0.36
mean	April	0.90	0.58	0.65	0.73	0.37	0.35	0.50
mean	mean	0.94	0.92	0.93	0.88	0.39	0.16	0.26

3.2.1 Optimized prediction models

Prediction models for sucrose, glucose and fructose were further developed into optimized prediction models, based on pooled sampling data as they showed high R^2-values (Table 1). Thus, the data for prediction included all samples prior to and at harvest (mean), while the response data used were the mean of all three sampling dates during storage. The continuous predictors of these models were dry matter, sucrose, glucose and fructose (Table 2).

In all optimized regression models for predicting sucrose, glucose and fructose, the categorical predictors included maturity level, cultivar and year, which contributed significantly to the models (P<0.001, not shown). The continuous predictors with significant influence on the model were dry matter, sucrose, glucose and fructose

(P<0.01), except foe´r fructose in the sucrose model (P=0.059). In the glucose and fructose models, four and three observations, respectively, were classified as outliers and therefore removed in order to optimize the models.

Table 2 Regression analysis of sugar variables, based on pooled data with predictors from all sampling dates before and at harvest and with response variables as mean of three dates during storage. Response variables in lines and continous predictors in colomn.

		Dry matter	Sucrose	Glucose	Fructose
Sucrose	Coeff	0.305	-0.052	0.359	-0.684
$R^2 = 0.92$	P-value	0.000	0.012	0.000	0.059
Glucose	Coeff	0.805	-0.267	1.334	-4.51
$R^2 = 0.93$	P-value	0.000	0.000	0.000	0.000
Fructose	Coeff	0.255	-0.102	0.339	-3.231
$R^2 = 0.88$	P-value	0.000	0.008	0.004	0.000

Separate prediction models for fry colour in Asterix and Saturna were also further developed. The combination of harvest sampling data and end of storage season data (April) were used for this. The regression models are presented in Table 3. Sucrose contributed significantly to the Asterix fry colour model. Sucrose, glucose and fructose were included in the model predicting fry colour in Saturna, but none of them contributed significantly to the model. Year and maturity level were included as categorical predictors in both models. Maturity was significant for Saturna fry colour only.

Table 3 Regression analysis of fry colour, with predictors from the date of harvest and frying colours in April as response variables (Asterix and Saturna). Continuous and categorical predictors in columns.

		Continuous predictors			Categorical predictors	
		Sucrose	Glucose	Fructose	Year	Maturity level
Fry colour (Asterix)	Coeff	-0.325				
$R^2 = 0.50$	P-value	0.05			0.462	0.344
Fry colour (Saturna)	Coeff	1.70	2.76	-10.5		
$R^2 = 0.51$	P-value	0.253	0.233	0.688	0.477	0.017

Discussion

Loss of greenness in potato haulm (senescence) is a visible sign of haulm maturation and the first indication that the physical, physiological and chemical processes of maturation of tubers have started. As expected, the least mature plants in the present experiment were more green on all sampling dates for both Asterix and Saturna. Yara-N sensor values correlated well ($r^2 \geq 0.89$) with visual inspection, indicating that both methods are suitable for evaluation of haulm maturity. Haulm senescence (haulm maturity) did not contribute significantly to any of the models in the present study. Two other studies identified a relationship between haulm maturity and processing quality (Lærke and Christiansen, 2005; Wiltshire et al., 2004). However, the relationships did not lead to a robust model for predicting processing quality.

Skin set (physical maturity) increased during the last weeks before harvest. There were significant differences among maturity levels in both Asterix and Saturna on all sampling dates. Saturna had higher skin set values than Asterix, which agrees well with the fact that Saturna is characterized as an early to intermediate cultivar in the European Cultivated Potato Database, while Asterix is characterized as intermediate to late. Skin set was the only predictor that contributed significantly to the models predicting weight loss. However, the weight loss models showed low R^2-values ($R^2 < 0.48$), and therefore these models were not developed further in the present study. Due to an important role of the skin in maturity development, skin set (physical maturity) should, however, be included together with other predictors in future models for predicting storage losses. Physical maturity is expected to influence storage loss because a poor skinset makes the surface permeable to water and susceptible to skinning (Sabba et al., 2007), thus giving potential for higher weight loss due to transpiration. Moreover, skin free areas in immature potatoes serve as an entry point for important storage diseases such as *Fusarium* dry rot, gangrene and bacterial diseases, which can potentially cause considerable losses during storage (Heltoft et al., 2015; Knowles and Plissey, 2008; Secor and Salas, 2001).

The dry matter content, an indicator of physiological maturity, increased during the growing season and peaked just after haulm desiccation, which stopped the transfer of photosynthesis assimilates from haulm to the developing tuber. Some of the photosynthesis assimilates are synthesized to starch, which is the main constituent of dry matter in potatoes and closely correlated to it. Haulm desiccation has previously been described by Knowles and Plissey (2008) to increase and initiate the stabilization of dry matter content in the tubers. The tuber dry matter content remained at the same level throughout the storage season. The stability of dry matter during storage shows how important the initial dry matter content is for the final quality of the tubers. In the present study, there were significant differences in dry matter among all maturity levels for both Saturna and Asterix, with the highest dry

matter content in the most mature tubers, from three weeks before harvest, at harvest and throughout the storage period. When aiming for high dry matter content, i.e. for the purpose of frying, reaching maturity at the time of harvest is important in order to maintain high quality in the stored tubers. Herrman et al. (1995) and Wiltshire et al. (2004) found similar relationships between maturity and tuber dry matter content.

The contents of sucrose and reducing sugars (glucose and fructose) were monitored during the last three weeks of the growing season, at harvest and three times during storage. The general trend was a decrease in sucrose content and increases in glucose and fructose contents until harvest and in storage until December, when sugar contents stabilized for Saturna. For Asterix, however, the content of glucose and fructose increased significantly ($P<0.01$) towards the last sampling date in April. Similar trends, with decreasing sucrose content and an increase in reducing sugars, have been observed in other studies (Hertog et al., 1997a; Knowles et al., 2009; Kolbe et al., 1995; Richardson et al., 1990) and provide support for the role of sucrose as a direct substrate for reducing sugar accumulation. However, the increase of sugars in Asterix tubers towards the end of the storage, could be a consequence of senescent sweetening, in which sugars are mobilised for the benefit of development and growth of sprouts (Hertog et al., 1997b). Reducing sugars increased more during storage in Asterix than in Saturna, probably due to a low susceptibility to cold induced sweetening in Saturna tubers, as was also observed by Hertog et al. (1997b). Cultivar contributed significantly to all the models as a categorical predictor, which indicates that the predictive models should take into account which cultivar is used. Richardson et al. (1990) also observed differences between cultivars and concluded that predictions based on sugar values at harvest should be determined on a cultivarspecific basis.

All sugar models developed in the present study were highly influenced by the sugar contents in the tubers at harvest or 1-3 weeks before harvest, as well as by the mean of the sampling dates. High R^2-values ($R^2>0.89$) were found for all sugar models. Significant influence for all sugar compounds in prediction of the various sugars after storage indicates strong interaction among the sugars. Contents of sucrose, glucose and fructose have previously been used to predict sugar accumulation during storage (Hertog et al., 1997a; Richardson et al., 1990; Sowokinos, 1978). Dry matter contributed significantly in all the sugar models. This can be explained by the close correlation between dry matter content and starch, and the role of starch, which is degraded first to sucrose and thereafter hydrolysed to glucose and fructose.

Attempts were made to develop prediction models for processing quality (fry colour) in Saturna and Asterix. The best R^2-value ($R^2=0.51$) was found for contents of sucrose, glucose and fructose measured at harvest and fry colour

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in Saturna in April. However, neither of these continuous predictors made a significant contribution to the model, with sucrose being the only continuous predictor that contributed significantly. These results indicate that sugar contents at harvest play a role in predicting fry colour. An improvement could have been achieved by including free amino acids in the prediction model for fry colour. These are included in the Maillard reaction, interacting with reducing sugars to produce a dark fry colour (Shallenberger et al., 1959). Storage conditions, such as temperature, humidity, CO_2 levels and ventilation, should also be considered in a future model, as they probably play a role for the fry colour. In our experiment these factors were standardized. Maturity level contributed significantly as a categorical factor to the Saturna fry colour model. This indicates that maturity status of the crop at harvest is important for product quality during storage, even though the selected maturity indicators in this study did not give a good prediction. Lærke and Christiansen (2005) also concluded that chemical maturity alone was not a reliable measure in predicting processing quality. Yet another study found that the relationship between sucrose at harvest and post-storage fry colour was only significant when very immature tubers with high sucrose content were used (Briddon and Storey, 1996).

Not all combinations of sampling dates at the end of the growing season and sampling during storage gave significant correlations between maturity indicators and response values for predicting tuber storage quality. This indicates a single sampling date may not be sufficient for the purpose of predicting tuber quality during storage. The same was concluded in another study where maturity indicators were used to predict processing quality during long-term storage (Herrman et al., 1995).

In conclusion, there is potential for using field measurements of maturity as indicators of storage potential. Skin set (physical maturity) contributed significantly to the weight loss model and should be included in future models for storage loss. Dry matter content (physiological maturity), sucrose, glucose and fructose contents (chemical maturity) all contributed significantly to the sucrose, glucose and fructose models. Sucrose gave significant contribution to the Saturna fry colour model. Maturity level contributed significantly to almost all the models and demonstrated the importance of allowing tubers to reach optimal maturity at the time of harvest. Cultivar was an important factor and cultivarspecific models should be considered. Further development of prediction models would be of great value to growers and the processing industry, as it would allow the processing quality to be predicted before or at harvesting of the potatoes.

Acknowledgement

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Paper III

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CrossMark

Fusarium spp. Causing Dry Rot on Potatoes in Norway and Development of a Real-Time PCR Method for Detection of *Fusarium coeruleum*

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Abstract The prevalence of *Fusarium* dry rot in potatoes produced in Norway was investigated in a survey for three consecutive years in the period 2010 to 2012. A total of 238 samples (comprising 23,800 tubers) were collected, representing different cultivars and production regions in Norway. *Fusarium* spp. were detected in 47% of the samples, with one to three species per sample. In total, 718 isolates of *Fusarium* spp. were recovered and identified to seven species. The most commonly isolated species was *Fusarium coeruleum*, comprising 59.6% of the total *Fusarium* isolates and found in 17.2% of the collected samples, followed by *Fusarium avenaceum* (27.2% of the isolates and found in 27.7% of the samples). *Fusarium sambucinum* was the third most prevalent species (6.4% in 8.8% of the samples) and *Fusarium culmorum* the fourth (5.2% in 6.3% of the samples). Less prevalent species included *Fusarium cerealis*, *Fusarium graminearum*, and *Fusarium equiseti* (<1% in 0.4 to 1.3% of the samples). *F. coeruleum* was the most prevalent species in northern and southwestern Norway, whereas *F. avenaceum* was dominating in eastern Norway. The potato cultivars Berber and Rutt were susceptible to all *Fusarium* spp. A new TaqMan real-time PCR assay specific for *F. coeruleum* was developed, which successfully identified Norwegian isolates. This and other previously developed real-time PCR assays targeting different *Fusarium* species were evaluated for their ability to detect latent infections in potatoes at harvest. This study provides new information on the current

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occurrence of different *Fusarium* species causing *Fusarium* dry rot in potatoes in Europe including areas far into the arctic in the north of Norway.

Keywords *Fusarium* · Molecular identification cultivars · Potato dry rot · Regions

Introduction

Fusarium dry rot is one of the most important storage diseases in potato tubers (*Solanum tuberosum* L.*)*, and affects almost all commonly grown potato cultivars (Leach and Webb 1981). Potentially, *Fusarium* dry rot can cause great yield losses with up to 60% of the tubers affected (Secor and Salas 2001). *Fusarium* dry rot pathogens infect through wounds on tubers caused mainly by handling during planting, harvesting, and grading.

The disease is caused by several species of *Fusarium* (Boyd 1972; Secor and Salas 2001) that in addition to potato have a wide host range including, e.g., cereals, legumes, and beetroot (Peters et al. 2008b). Thirteen *Fusarium* species are considered as causal agents of *Fusarium* dry rot in potatoes worldwide (Cullen et al. 2005). In Great Britain and in the Nordic countries, the most common species isolated from potato has been *Fusarium coeruleum* (Libert) Sacc. (Bjor 1978; Olofsson 1976; Peters et al. 2008a; Seppänen 1983). In other parts of Europe, in northern and western China and in North America *Fusarium sambucinum* (Fückel) sensu stricto is considered to be the most significant causal agent of *Fusarium* dry rot (Du et al. 2012; Secor and Salas 2001). In North Dakota, *Fusarium graminearum* (Schwabe) in addition to *F. sambucinum* was reported to be the most prevalent species causing *Fusarium* dry rot (Estrada et al. 2010). In a recent study on *Fusarium* dry rot in Michigan, *Fusarium oxysporum* Schlechtendal emend. Snyder & Hansen was the most prevalent species, but *F. sambucinum* was the most aggressive (Gachango et al. 2012).

Control strategies for *Fusarium* dry rot include use of resistant cultivars and cultural practices such as crop rotation, use of disease free seed, and wound healing prior to storage. Biological control agents and ultraviolet radiation are also used, as well as chemical control (Al-Mughrabi et al. 2013; Bojanowski et al. 2013; Bång 1992; Gachango et al. 2012; Peters et al. 2008a; Ranganna et al. 1997). However, chemical control is not common in use against *Fusarium* dry rot in Norway. Diagnostic tools providing fast identification and quantification of *Fusarium* dry rot pathogens could in principle validate the recommendations on the control strategy. Moreover, such tools can be used to detect latent infections in tubers pre-storage, to validate their storability and/or suitability as seed potatoes. Several molecular assays for detection of different *Fusarium* spp. found in potato and other crops have been developed (Cullen et al. 2005; Halstensen et al. 2006; Nicholson et al. 1998).

In Norway, the problems with *Fusarium* dry rot in potatoes seem to have increased the last decade. The objective of the current study was to identify which *Fusarium* species currently are causing *Fusarium* dry rot in commercial potato production in Norway, including the extent of regional variation and the effect of agronomic and storage factors. We also aimed to test the suitability of available real-time PCR assays for detection of *Fusarium* spp. common in Norway and, if needed, develop new assays.

Materials and Methods

Sample Collection

In total 238 potato tuber samples from fields in all major potato growing districts in Norway, covering a distance of more than 2000 km of the country from north to south, were collected in October 2010, 2011, and 2012. The main potato production areas in Norway are situated in the eastern and southeastern parts of the country, and hence, most of the samples came from these areas (Table 1). Different cultural practices are used in different regions; hence, some cultivars are only grown in specific regions of Norway. Cultivars sampled from different regions are listed in Table 1.

The samples were collected by agronomists, farmers, and store managers. Information about geographical origin and potato cultivar was also collected. The samples originated from 26 different cultivars. Only cultivars of which we received seven or more samples were included when analyzing the effect of cultivar (Table 4). In the other analyses, all 26 cultivars were included. The remaining cultivars, with less than seven representatives, included Arielle, Bruse, Gulløye, Juno, Kuras, Odin, Ostara, Pimpernel, Polaris, Sava, Solist, Tivoli, and Van Gogh. The majority of the samples were from potatoes intended for crisps and fries; hence, the cultivars Asterix, Lady Claire, and Saturna comprised more than 40% of the samples in this study. Each sample consisted of 100 tubers collected from box storages: 10 tubers just under the top layer from 10 different boxes from different heights in the storage. The samples were stored at 4 °C and 95% RH in experimental storage facilities for 6 months prior to wounding. A pre-study showed that tubers could be left in storage under these conditions without changes in *Fusarium* population and contaminations.

Isolation, Cultivation, and Identification of Fusarium

Fusarium spp. were isolated and identified as described by Peters et al. (2008a). Briefly, all tubers from each sample were wounded on both sides to a depth of 4 mm

Table 1 Number of potato samples from different regions and years and cultivars sampled in each region

Region[a]	2010	2011	2012	Total	Cultivars
North	4	4	4	12	Asterix, Gulløye, Mandel, Van Gogh
Central	16	8	4	28	Arielle, Asterix, Beate, Berber, Bruse, Folva, Lady Claire, Laila, Mandel, Oleva, Rutt, Saturna, Solist, Troll, Van Gogh
East	20	69	48	137	Arielle, Asterix, Beate, Berber, Bruse, Folva, Innovator, Juno, Kerrs Pink, Kuras, Lady Claire, Laila, Mandel, Odin, Oleva, Ostara, Peik, Pimpernel, Rutt, Saturna, Solist, Tivoli, Troll
Southeast	8	9	20	37	Asterix, Beate, Bruse, Fakse, Folva, Innovator, Kerrs Pink, Laila, Oleva, Saturna, Sava
Southwest	8	8	8	24	Arielle, Asterix, Beate, Berber, Fakse, Folva, Polaris, Rutt, Saturna
Total	56	98	84	238	

One sample consisted of 100 potato tubers collected from box storages

[a] Counties within regions: north: Troms; central: Nord-Trøndelag; east: Hedmark, Oppland, and Akershus; southeast: Vestfold, Østfold, and Buskerud; southwest: Rogaland, Aust-Agder, and Vest-Agder

using a sterile wounding device consisting of four spikes (each with a diameter of 1 mm) in a quadratic square (20 mm on each side). The tubers were placed in paper bags and incubated in experimental storage rooms holding a temperature of 10 °C and 95% relative humidity for 6 weeks.

After the incubation period, a cut exactly through each wounding point toward the center of the tuber was made with a flame-sterilized knife to detect potential *Fusarium* dry rot development. Tubers with typical *Fusarium* dry rot symptoms (shallow or sunken and wrinkled necrotic areas) in the wounding site or in natural infection sites on the tuber were registered. Rots were assumed to be conical for the purpose of analysis. Therefore, the volume of the rot was recorded and calculated using the formula: volume $= 1/3\pi hr^2$, where r is half the width of the rot and h is the depth of the rot. Four pieces of tissue from the edge of the rotten area were transferred to potato dextrose agar (PDA) with 200 mg l^{-1} streptomycin. The PDA plates were incubated at room temperature for 7–10 days in alternating 12 h of light and 12 h of darkness. Cultures resembling *Fusarium* were then transferred to fresh PDA and subsequently to synthetic nutrient agar (SNA) with a small piece of autoclaved filter paper. The pure cultures were identified to species based on conidial morphology, production of chlamydospores, growth characteristics, and colony pigmentation as described by Leslie and Summerell (2006) and Gerlach and Nirenberg (1982).

DNA Extraction and Molecular Identification of *Fusarium* spp.

Fusarium isolates were grown on PDA plates for 2–3 weeks at room temperature prior to DNA extraction. Mycelium was scraped off the agar plate using a scalpel. The mycelium was homogenized using a pestle and mortar and liquid nitrogen. DNA was extracted from the homogenized mycelium (100 mg) using DNeasy Plant Mini Kit (QIAGEN) according to the instructions provided by the manufacturer. DNA concentrations were estimated by comparing the intensity of genomic DNA bands in agarose gel to a DNA marker with known concentrations, or by using a NanoDrop spectrophotometer. To confirm species identity, DNA extracts of approximately 15% of the isolates were tested using PCR-based assays for *F. avenaceum* (Fries) Sacc. (Halstensen et al. 2006), *Fusarium culmorum* (W. G. Smith) Sacc., and *F. sambucinum* (Cullen et al. 2005). *F. coeruleum* was tested with the assay developed and described in this study (see below).

Statistics

All statistical analyses were performed using SAS 9.4. Logistic regression was used to analyze the data. The individual *Fusarium* species were modeled and analyzed one by one. Potato cultivar, geographical region, and year were used as fixed factors. All data was converted from number of isolates found per sample of 100 tubers to 0 or 1 representing absence or presence of the *Fusarium* species, respectively. Prevalence of the individual *Fusarium* species in geographical region and potato cultivar is given in incidence and probability. Incidence is given as percentage of infected tubers per sample and probability, which indicate the likelihood of finding the given *Fusarium* species in a certain region or cultivar, is calculated from the formula: P(*Fusarium* spp. $= 1$) $= 1 - (e^{estimate} / (1 + e^{estimate}))$ where the estimate is given in the outcome

of the logistic regression. Differences between potato cultivars and geographical regions were tested by Tukey's multiple comparison method.

Primer Design and Real-Time PCR for *F. coeruleum*

Five isolates of *F. coeruleum* originating from different parts of Norway were used for development of the assay. The internal transcribed spacer (ITS) regions (ITS1 and ITS2) of the rDNA genes of the isolates of *F. coeruleum* were amplified with the universal primers ITS1 and ITS4 (White et al. 1990). The PCR products were purified and sequenced at GATC Biotech AG. Sequence data were assembled and analyzed using the DNAStar Seqman II software. Five identical sequences were compared to other *Fusarium* sequences using ClustalW2, and primers and a TaqMan MGB probe were designed using Primer Express 2.0 and manual evaluation of the sequence alignment. A real-time PCR assay was selected because it is less labor intensive and less prone to contamination than standard PCR. Moreover, it is fast, sensitive, and amenable to quantification. Real-time PCR reactions were performed using TaqMan® Universal PCR Master Mix, 200 mM MGB probe (Fcoer1 P) (5′-FAM-CAGCGAGACCGCCAC-3′) and 300 mM of primer F (Fcoer1 F) (5′-TGTTAGCTACTACGCAATGGAAGCT-3′) and primer R (Fcoer1 R) (5′-GCCGGCCCCGAAATC-3′), with 2 μl template DNA in a total volume of 25 μl. PCR reactions were performed in duplicate in a 7900 HT Fast Real-Time PCR System (Applied Biosystem), and real-time data were analyzed using the Sequence Detection System version 2.2.1 (Applied Biosystems). The cycling protocol was as follows: 2 min at 50 °C, 10 min at 95 °C, 45 cycles of 95 °C for 15 s, and 60 °C for 1 min.

Sampling and DNA Extraction of Potato Peel and Soil

To evaluate the ability of the developed real-time PCR assay to detect *F. coeruleum* in tubers, we tested 40 potato peel samples from 2010 and 39 from 2012. These samples, consisting of 20 tubers each, came from the same fields as the tuber samples used for the survey. One peel strip (1–2 mm thick) including both rose and heel end was taken from each of 20 tubers using a hand held potato peeler. The peels were mixed with 50 ml SPCB buffer (120 mM sodium phosphate, 2% CTAB, 1.5 M NaCl, pH 8.0) (Lees et al. 2002) and homogenized using a kitchen blender for 2 min. A single 1.5 ml aliquot was taken from each sample for DNA extraction. DNA extraction was performed using the Molestrips DNA Plant kit (Mole Genetics, Lysaker, Norway) according to the manufacturer's protocol.

The plant internal control primers (COX F and COX R_W) and probe (COX) based on a previously described assay designed for the cytochrome oxidase (COX) gene (Weller et al. 2000) were used to detect host DNA, providing confirmation that DNA extraction was successful and thereby avoiding false-negative results for *F. coeruleum*.

The *F. coeruleum* assay was also tested with soil samples. In 2010, soil samples were taken after harvest from the 40 potato fields where the tuber samples were grown. Within these fields, soil was sampled from 50 to 60 points (0–10 cm deep) 10 m apart in a W-shape pattern using a soil corer. DNA was extracted from soil suspensions (60 g soil added 120 mL SPCB buffer) by physical disruption in a minibead beater using the method described by Cullen et al. (2001).

Results

Fusarium Species in Norway

In the years 2010–2012, a total number of 238 samples comprising 23,800 tubers were collected from commercial potato stores in Norway. *Fusarium* was isolated from 3% of the tubers divided over 47% of the samples; in total, 718 isolates were recovered and these were identified to seven species (Table 2). Most (98.4%) of these isolates were attributed to one of four *Fusarium* species: *F. coeruleum*, *F. avenaceum*, *F. sambucinum*, and *F. culmorum*. Other fungi isolated from rotted tissue of the sampled tubers included *Boeremia foveata* (Foister) Aveskamp, Gruyter & Verkley, *Colletotrichum coccodes* (Wallr.) Hughes, *Cylindrocarpon* spp. Wollenweber, *Helminthosporium solani* (Durieu & Montagne), *Polyscytalum pustulans* (M.N.Owen & Wakefield) M.B.Ellis, *Rhizoctonia solani* (A.B.Frank) Donk, and *Penicillium* sp., all potential plant pathogens.

Isolates were identified as *F. coeruleum* by their appearance of white-bluish mycelium after 2 weeks on PDA and production of weakly curved 4 septate macroconidia (25–50 × 5 μm) from slimy cream colored sporodochia around the filter paper on SNA. A previously developed *F. coeruleum* specific real-time PCR assay (Cullen et al. 2005) gave unexpectedly high Ct values (28.0–32.0), which lead us to develop a new test for this species (see below). The new test confirmed the identification based on morphology for 41 isolates (Ct values ranging from 17.7 to 22.9).

F. avenaceum produced white to rose mycelium after 2 weeks on PDA and long and slender 3–5 septate macroconidia from pale orange sporodochia around the filter paper on SNA. *F. avenaceum* specific real-time PCR assay (Halstensen et al. 2006) confirmed the identification based on morphology for 45 isolates (Ct values ranging from 23.1 to 29.1).

F. sambucinum was identified by production of white, yellow, or red to salmon pink mycelium on PDA and falcate, slender 3–5 septate macroconidia with a foot-shaped basal cell and pointed apical cell from orange sporodochia produced on SNA. *F.*

Table 2 Relative frequencies (%) of different *Fusarium* species isolated from commercial potato tubers sampled from Norwegian stores during 2010–2012

Fusarium spp.	Relative frequency (%) of isolated species (in % of samples)			
	2010	2011	2012	Total
Fusarium coeruleum	65.0 (*19.6*)	38.6 (*11.2*)	75.1 (*22.6*)	59.6 (*17.2*)
Fusarium avenaceum	28.5 (*26.8*)	40.4 (*32.7*)	12.8 (*22.6*)	27.2 (*27.7*)
Fusarium sambucinum	1.5 (*3.6*)	9.6 (*6.1*)	8.2 (*15.5*)	6.4 (8.8)
Fusarium culmorum	3.6 (*7.1*)	8.4 (*6.1*)	3.4 (*6.0*)	5.2 (6.3)
Fusarium cerealis	1.4 (*1.8*)	0.0 (*0*)	0.5 (*2.4*)	0.6 (1.3)
Fusarium graminearum	0.0 (*0*)	2.4 (*3.1*)	0.0 (*0*)	0.8 (1.3)
Fusarium equiseti	0.0 (*0*)	0.6 (*1.0*)	0.0 (*0*)	0.2 (0.4)
Total number of isolates	137	166	415	718
Total number of samples	56	98	84	238

Between parentheses in italics: percentage samples in which infection by the specific species occurred

sambucinum specific real-time PCR assay (Cullen et al. 2005) confirmed the identification for eight isolates (Ct values ranging from 19.2 to 25.0). Three isolates identified morphologically as *F. sambucinum* gave only weak signals in the real-time PCR test (Ct values ranging from 33.9 to 36.3). There was some cross-reactivity with other species giving Ct values from 32.6 to 44.1.

F. culmorum was identified by rapid production of white to orange-red mycelium and short, robust, and thick-walled 3–4 septate macroconidia from orange sporodochia produced on SNA. The species identification of five isolates was confirmed with *F. culmorum* specific real-time PCR assay (Cullen et al. 2005) (Ct values ranging from 18.6 to 20.2).

The most commonly isolated species was *F. coeruleum* comprising 59.6% of the total *Fusarium* isolates (Table 2). *F. coeruleum* was the most prevalent species in 2010 and 2012, whereas *F. avenaceum* was found slightly more than *F. coeruleum* in 2011. *F. coeruleum* was found in 17.2% of the samples and 1–88 isolates were recovered from each sample. Two samples from 2012, cultivars Berber and Rutt (each 100 tubers), were heavily infected with *F. coeruleum* and resulted in 81 and 88 isolates, respectively. These samples affected the total number of isolates greatly and resulted in a high total isolate number (415) this year. *F. avenaceum* was the second most prevalent species comprising 27.2% of the isolates. It was found in 27.7% of the samples (1–18 isolates per sample); hence, *F. avenaceum* was found in more samples than the other *Fusarium* species. None of these samples, however, was highly infected with *F. avenaceum*, indicating that *F. avenaceum* is a less aggressive species. *F. sambucinum* was the third most prevalent species, comprising 6.4% of the isolates, and was identified in 8.8% of the samples (1–10 isolates per sample). The relative frequency of *F. sambucinum* was considerably lower in 2010 than the two following years. *F. culmorum* was the fourth most prevalent species, comprising 5.2% of the isolates and was identified in 6.3% of the samples (1–9 isolates per sample). The species *F. cerealis* (Cooke) Sacc., *F. graminearum* and *F. equiseti* (Corda) Sacc. were less prevalent (0.2 to 0.6% of the isolates in 0.4 to 1.3% of the samples).

Volume of the dry rot was measured. The mean size of dry rot lesion for the four most prevalent *Fusarium* species varied from 3.6 to 10.1 cm^3. However, there was large variation in lesion development within the *Fusarium* species and there was no significant correlation between *Fusarium* species and lesion size.

Real-Time PCR Assay for *F. coeruleum*

Ten different isolates, identified morphologically as *F. coeruleum*, were analyzed with the real-time PCR assay developed by Cullen et al. (2005). The isolates gave Ct values ranging from 28.0 to 32.0, which is relatively high considering the fact that DNA templates were extracted from pure isolates. After several failed attempts to optimize the assay by Cullen et al. (2005), we developed a new TaqMan assay targeting the ITS1 region at a different site than the previously developed assay for *F. coeruleum*. The new real-time PCR assay was tested with various concentrations of primers (ranging from 50 to 900 mM) and probe (ranging from 50 to 250 mM). The optimal primer concentrations were 300 mM, and the optimal probe concentration was 200 mM. The TaqMan assay could reliably detect 10^{-15} g of *F. coeruleum* DNA. The standard curves for the real-time PCR test showed high correlation coefficient ($R^2 = 0.99$),

indicating a reproducible linear response in detection of increasing concentrations of *F. coeruleum* DNA (Fig. 1).

The assay was tested on 97 *Fusarium* isolates from Norway selected at random from the survey. DNA from isolates of *F. coeruleum* gave Ct values ranging from 17.7 to 22.9 (Fig. 2). The other *Fusarium* species, including *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, and *F. sambucinum* gave Ct values ranging from 34.6 to 44.4 or did not give any Ct value (shown as Ct value=0).

The *F. coeruleum* real-time PCR assay was tested for the ability to detect individual *Fusarium* species in potato peel samples and soil samples. In total, 79 potato peel samples were collected in 2010 and 2012 and 40 soil samples were collected in 2010. No *Fusarium* was detected with certainty in the samples from potato peel or from soil. However, there were weak signals of *F. coeruleum* in five potato peel samples giving Ct values ranging from 34.6 to 39.1 and in one soil sample giving a Ct value of 36.7. The COX internal control targeting the host plant DNA of potato yielded stable Ct values of around 25 for all the plant samples, indicating an overall high quality of the DNA extracts.

Regional Variation in *Fusarium* Occurrence

The incidence of and probability of finding the four most important *Fusarium* spp. varied between regions (Table 3). There were no significant differences between years. The probability of finding *F. coeruleum* was highest in northern Norway and significantly different from eastern Norway (Table 3). *F. coeruleum* was present in all sampled regions in 2010–2012 except in central Norway in 2012, where there was no incidence of *Fusarium* spp. at all (Fig. 3). There was a high incidence of *F. coeruleum* in northern Norway all three years compared to the other regions, and in 2012, no other species were detected. In southeastern and southwestern Norway, *F. coeruleum* was the most common species in 2010 and 2012; especially in 2012, the number of isolates collected in west Norway increased due to the, before mentioned, two heavily *F. coeruleum* infected samples (cultivars Berber and Rutt; each 100 tubers, 81 and 88 isolates per sample) (Fig. 3). *F. avenaceum* was found in all regions all years except in northern and central Norway in 2012. It was the most common *Fusarium* species found in eastern Norway all years (Fig. 3). *F. sambucinum* was isolated from the potato tuber samples in eastern and southwestern Norway in all years, as well as from central Norway in 2011
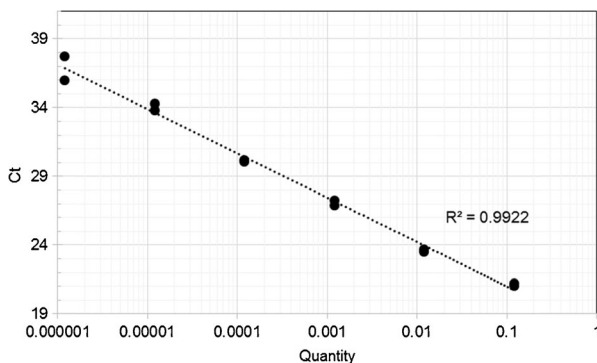

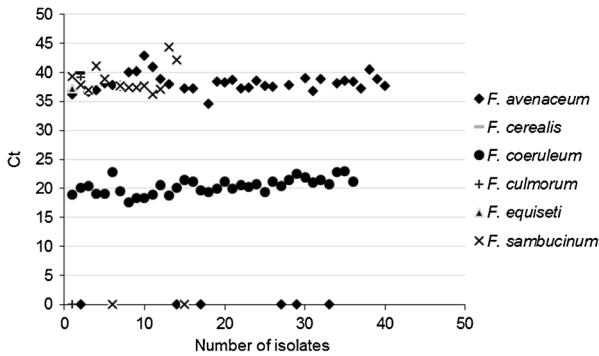**Fig. 1** Standard curve for *F. coeruleum* real-time PCR assay

Fig. 2 Ct values for 97 isolates belonging to six different species of *Fusarium* (*F. avenaceum*, *F. cerealis*, *F. coeruleum*, *F. culmorum*, *F. equiseti*, and *F. sambucinum*) when tested with *F. coeruleum* real-time PCR assay

and southeastern Norway in 2012 (Fig. 3). The probability of finding *F. sambucinum* was significantly higher in southwestern than in eastern and southeastern Norway (Table 3). *F. sambucinum* was not found in northern Norway (Table 3). *F. culmorum* had the total lowest incidence of the four *Fusarium* spp., but it was found in all Norwegian regions (Fig. 3). The probability of finding *F. culmorum* was significantly higher in southwestern than in eastern Norway (Table 3).

Effect of Cultivar on *Fusarium* Occurrence

The incidence of and probability of finding one of the four *Fusarium* species in 13 different potato cultivars are given in Table 4. The incidence of *F. coeruleum* was high (≥11.0) in Asterix, Berber, Laila, Mandel and Rutt. However, the probability of finding *F. coeruleum* was highest in Berber and Rutt. The incidence of *F. avenaceum* was high in Berber and so was the probability. The incidence of *F. sambucinum* was high in Berber and Rutt. However, the probability of finding *F. sambucinum* was low (≤0.002)

Table 3 Incidence (% infected tubers per sample) of different *Fusarium* spp. and probability (P(*Fusarium* spp. = 1)) of finding these *Fusarium* spp. on potato tubers sampled from different regions of Norway 2010–2012

	F. coeruleum		F. avenaceum		F. sambucinum		F. culmorum	
	%	P(F. coe = 1)	%	P(F. ave = 1)	%	P(F. sam = 1)	%	P(F. cul = 1)
North	12.58	0.500 a	0.33	0.331	0.00	<0.001 ab	0.08	0.083 ab
Central	1.21	0.184 ab	0.75	0.204	0.04	0.042 ab	0.04	0.035 ab
East	0.26	0.115 b	0.53	0.288	0.12	0.043 b	0.03	0.030 b
Southeast	1.10	0.186 ab	0.26	0.214	0.15	0.048 b	0.10	0.078 ab
Southwest	8.46	0.287 ab	2.21	0.331	1.17	0.312 a	0.96	0.250 a
		*		n.s		**		*

P(*Fusarium* spp. = 1) = 1 − (e^estimate / (1 + e^estimate)) (estimate given in the outcome of the logistic regression). Values within a column followed by different letters are significantly different according to Tukey's test

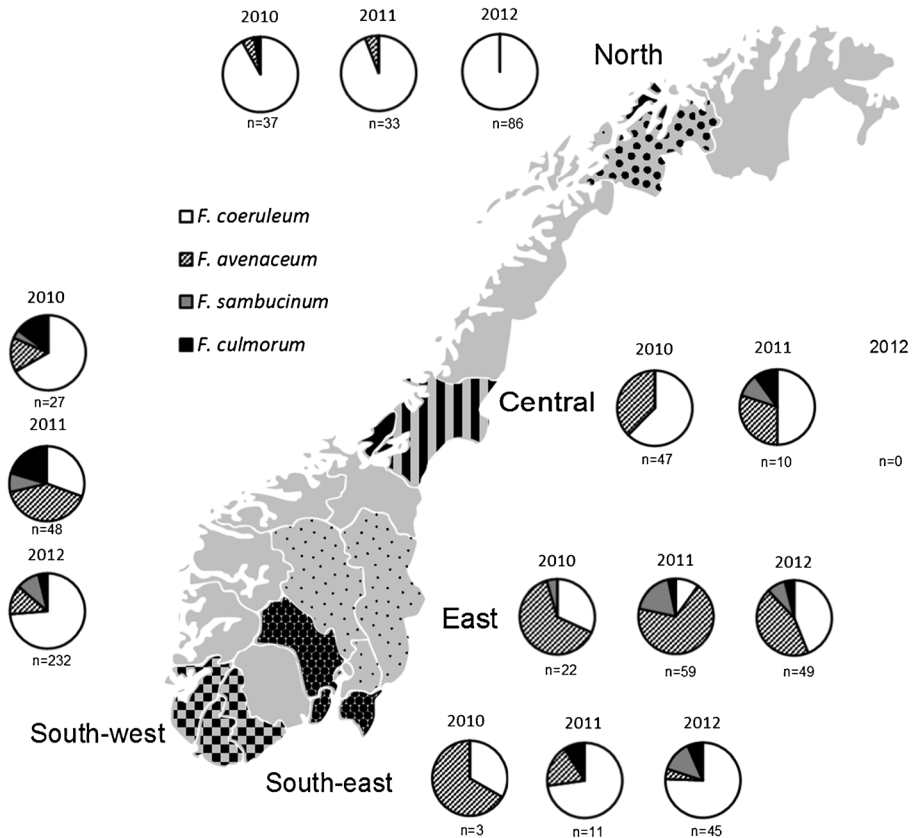n.s. = not significant ($p \geq 0.05$), *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$

Fig. 3 Incidence of *Fusarium* spp. on potato tubers in five regions in Norway in 2010–2012 (*n* = number of *Fusarium* isolates)

in all cultivars. *F. culmorum* was found more in Rutt than the other species. There were no significant differences between cultivars for any of the *Fusarium* species: *F. coeruleum* (*P* = 0.743), *F. avenaceum* (*P* = 0.927), *F. sambucinum* (*P* = 1.000), and *F. culmorum* (*P* = 0.939).

Discussion

During the period 2010–2012, *Fusarium* spp. were only found in 3% of the tubers, but *Fusarium* species—in varying number—were present in almost half of the samples (each 100 tubers), showing the potential risk of *Fusarium* dry rot development in Norwegian potatoes if the right conditions are present.

  F. coeruleum was the *Fusarium* species most frequently isolated from potato tubers in the samples, in total 428 isolates, which amounted to 59.6% of all the *Fusarium* isolates (Table 2). The high prevalence of *F. coeruleum* was consistent with previous findings in Norway (Bjor 1978). *F. coeruleum* was also found to be the most common species isolated from potato in Great Britain (Peters et al. 2008a), Sweden (Olofsson 1976), and Finland (Seppänen 1983). *F. coeruleum* was especially prevalent in northern

Table 4 Incidence (% infected tubers per sample) of *Fusarium* spp. and probability (P(*Fusarium* spp. = 1)) of finding *Fusarium* spp. on tubers of different potato cultivars sampled in 2010–2012 (number of samples in parentheses)

	F. coeruleum		F. avenaceum		F. sambucinum		F. culmorum	
	%	P(F. coe = 1)	%	P(F. ave = 1)	%	P(F. sam = 1)	%	P(F. cul = 1)
Asterix (25)	11.00	0.108	2.00	0.279	1.00	0.001	1.33	0.118
Beate (9)	1.00	0.092	4.00	0.336	1.00	0.002	0.00	<0.001
Berber (8)	28.33	0.371	6.75	0.501	10.00	0.001	0.00	<0.001
Fakse (7)	0.00	<0.001	2.50	0.272	0.00	<0.001	0.00	<0.001
Folva (12)	1.33	0.239	1.80	0.411	1.00	0.001	1.50	0.161
Innovator (8)	2.00	0.089	1.00	0.139	4.00	0.001	1.50	0.282
Lady Claire (20)	2.25	0.175	1.17	0.315	0.00	<0.001	1.00	0.054
Laila (9)	14.00	0.207	3.00	0.221	1.00	0.001	0.00	<0.001
Mandel (14)	22.00	0.293	1.25	0.280	0.00	<0.001	1.00	0.068
Oleva (7)	1.00	0.123	5.67	0.435	0.00	<0.001	0.00	<0.001
Rutt (7)	38.67	0.490	2.00	0.418	9.00	0.002	9.00	0.135
Saturna (52)	2.13	0.159	2.67	0.227	3.00	0.001	4.50	0.037
Troll (7)	1.00	0.123	0.00	<0.001	1.00	0.002	0.00	<0.001

Only cultivars of which we received seven or more samples (one sample = 100 tubers) are included in the table. P(*Fusarium* spp. = 1) = 1 − (e^estimate / (1 + e^estimate)) (estimate given in the outcome of the logistic regression)

Norway, which might be influenced by a crop rotation dominated by potato, the only described host for *F. coeruleum*. In 11 out of 12 samples, potato was also grown the year before on the same field and the most common cultivars grown were Mandel and Gulløye. These cultivars are known as susceptible to *Fusarium* dry rot, with score 1 of 9 where 9 is most resistant (Møllerhagen 2014). Two samples (from cultivars Berber and Rutt) heavily infected with *F. coeruleum* collected in southwestern Norway resulted in a high total number of isolates in 2012. These two samples show the potential risk of *Fusarium* infections in storage. The probability of finding *F. coeruleum* was high in Berber and Rutt in all three years of the survey. Møllerhagen (2014) confirms the susceptibility of Rutt (score 1), and we found Berber and Rutt to be very susceptible to *Fusarium* spp. in another study (Heltoft et al. 2015). We noticed that Berber and Rutt share one of their parents, Alcmaria, but no information regarding resistance to *Fusarium* spp. is registered for this cultivar (www.europotato.org).

F. avenaceum was the second most commonly isolated species, and it was even slightly more frequent than *F. coeruleum* in 2011 (Table 2). Furthermore, *F. avenaceum* was found in more samples than the other *Fusarium* species, but with a smaller number of isolates obtained per sample, indicating that *F. avenaceum* is a less aggressive species. *F. avenaceum* was the second most prevalent species in previous surveys in Great Britain and China (Du et al. 2012; Peters et al. 2008a), and this species was also considered as a relatively weak pathogen by Peters et al. (2008a). The prevalence of *F. avenaceum* in Norwegian potatoes may be affected by crop rotation, because potato is often grown in rotation with cereals. In Norwegian cereals, *F. avenaceum* is the most commonly detected

species of *Fusarium* and furthermore, *Fusarium* has been an increasing problem in cereals in Norway in the last 10 years (Bernhoft et al. 2013). In the present study, *F. avenaceum* was the most common species found in potatoes in eastern Norway, where a narrow crop rotation with cereals is normal practice. Gachango et al. (2012) also discussed that crop rotation with cereals may have an implication on the prevalence of *F. avenaceum* in potatoes. However, when statistical analyses were applied in our study, crop rotation did not have a significant effect on the prevalence of the different *Fusarium* species. This could be a consequence of very few repetitions of the same crop rotation in the data or simply just the fact that *F. avenaceum* is commonly found in almost all crops grown in rotation with potatoes. Further studies are needed to investigate crop rotation as a factor of increased *Fusarium* dry rot development in potato.

F. sambucinum was the third most prevalent *Fusarium* species in the present study. This species, however, was the most commonly isolated species in potato in other parts of Europe, in northern and western China, and in North America (Du et al. 2012; Estrada Jr et al. 2010; Secor and Salas 2001). In a number of studies, *F. sambucinum* was the most aggressive species in potato (Esfahani 2005; Gachango et al. 2012; Peters et al. 2008a; Wastie et al. 1989) and therefore, it cannot be readily explained why *F. sambucinum* is not more prevalent in the present study. However, relatively low prevalence of *F. sambucinum* was also observed in surveys in Michigan and in Great Britain, where it was the third and fourth most prevalent species, respectively (Gachango et al. 2012; Peters et al. 2008a). In particular, *F. sambucinum* was not found in northern Norway, where the climatic conditions normally are harsher than in the other regions. However, unfavorable climatic conditions cannot be used as an explanation for the absence of this species in northern Norway, because it is widely reported to be common in temperate and cool parts of the world (Leslie and Summerell 2006). Furthermore, *F. sambucinum* has previously been found in northern Norway (Abbas et al. 1987). A low number of samples per year in this region could also be the reason for the absence of *F. sambucinum*.

Even though the inoculum levels in the survey were unknown, the results indicate that they in general were very low. This is based on the results from the real-time PCR tests of soil and potato peel samples, where our assay was only able to detect weak signals of *F. coeruleum*. It can be discussed whether the potato peel should have been incubated for a period for potential enrichment of *Fusarium* before the molecular test. The high infection rate with *F. coeruleum* in 2012 and lack of *F. coeruleum* detected in potato peel that year indicates that the inoculum might have been present in the soil. However, this cannot be verified as no soil samples were analyzed in 2012.

The species identification based on morphology was confirmed by real-time PCR assays specific to each *Fusarium* species, and all isolates were tested with all assays. However, some problems occurred with the assays. It was found that the *F. culmorum* specific primers (Cullen et al. 2005) could not distinguish between *F. culmorum* and *F. cerealis*. The same cross-reaction was found by Nicolaisen et al. (2009), who developed another *F. culmorum* specific assay used for detection of the species in cereals. The *F. sambucinum* specific assay also had cross-reactivity with other species, even after testing different primer and probe concentrations to optimize the assay. However, this species was not considered an important species in Norway and therefore no attempts was made to set up a new assay. In contrast, a new real-time PCR assay was developed for the most prevalent species in the survey, *F. coeruleum*, because of unexpected high Ct values when using the assay previously developed (Cullen et al. 2005).

Seven species of *Fusarium* are currently causing *Fusarium* dry rot in commercial potato production in Norway, with *F. coeruleum* and *F. avenaceum* as the most important species. *Fusarium* spp. were present in almost half of the samples, and heavy infections may occur if the right conditions are present. The results of this study indicated differences between cultivars in resistance to *Fusarium* rot and can support future control strategies, for example by providing methods and targets for breeding programs However, further studies are needed looking at differences in cultivar resistance.

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	K	C1	C2	C3	A1	A2

Beate

Asterix

Mandel

Paper IV

Saturna

Berber

Rutt

Kerrs Pink

Laila

Folva

Pimpernel

Effect of Maturity Level and Potato Cultivar on Development of *Fusarium* Dry Rot in Norway

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Abstract The effect of maturity levels in potato tubers on *Fusarium* dry rot development caused by four *Fusarium* species (*Fusarium coeruleum*, *Fusarium avenaceum*, *Fusarium sambucinum* and *Fusarium culmorum*) was studied in two trials during 2012–2013. In addition, ten commonly grown potato cultivars in Norway were evaluated for resistance to *F. coeruleum*, *F. avenaceum* and *F. sambucinum*. *F. sambucinum* was the most aggressive species, while *F. avenaceum* and *F. culmorum* only caused minor dry rot symptoms in the tubers. Maturity, described as chemical, physical and physiological maturation as well as vine maturation, significantly affected dry rot development in tubers when inoculated with *F. sambucinum*. We found that immature tubers, having high sucrose content, low dry matter content and poor skin set were most susceptible to *Fusarium* spp. There were differences in susceptibility to *Fusarium* spp. among cultivars. The early maturing cultivars Berber, Rutt and Laila developed the most severe dry rot symptoms. In general, *F. sambucinum* caused more dry rot, but there were significant differences between isolates. The present study underlines that cropping the potatoes in order to reach a high level of maturity and the use of resistant cultivars are important elements in an integrated pest management (IPM) strategy against *Fusarium* dry rot in Norway.

Keywords Cultivar resistance · *F. coeruleum* · *F. sambucinum* · *Fusarium* dry rot · Maturity

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Introduction

Fusarium dry rot is a common storage disease in potatoes worldwide. In most years, the disease outbreaks are low, but severe losses can occur (Secor and Salas 2001). In a recent study in Norway, the main *Fusarium* species isolated from potatoes were *Fusarium coeruleum*, *Fusarium avenaceum*, *Fusarium sambucinum* and *Fusarium culmorum*, with *F. coeruleum* as the most prevalent species (Heltoft, unpublished data). This is in accordance with an earlier study by Bjor (1978), who also found *F. coeruleum* to be the most prevalent species in Norway. In a survey in Great Britain, they found the same species as in Norway (Peters et al. 2008). In the other Nordic countries, *F. coeruleum* is also the most widespread species isolated from potatoes (Olofsson 1976; Seppänen 1983). *F. sambucinum* is considered the most significant causal agent of *Fusarium* dry rot in parts of Europe, in Northern and Western China and in North America (Du et al. 2012; Secor and Salas 2001). However, in a recent survey on seed tubers in Michigan, *F. oxysporum* was the most prevalent species, whereas *F. sambucinum* was the most aggressive (Gachango et al. 2012).

Fusarium spp. enter the tubers via wounds. Immature potatoes are more susceptible to skinning and wounding during harvest and thus more susceptible to *Fusarium* dry rot (Boyd 1972; Knowles and Plissey 2008; Lulai and Orr 1995). Once the pathogen has passed the tuber skin through a wound, it begins to grow in the tuber tissue, causing dry rot lesions at the entry point.

Control strategies for *Fusarium* dry rot include the use of resistant cultivars and cultural practices such as crop rotation, use of disease free seed and wound healing prior to storage. Biological control agents may also be used as well as chemical control (Al-Mughrabi et al. 2013; Bojanowski et al. 2013; Bång 1992). However, biological and chemical control methods are not commonly used against *Fusarium* dry rot in Norway.

Control of *Fusarium* dry rot is a challenge because of the short growing season for potato production in Norway, with low temperatures, particularly in spring and autumn (www.yr.no). This often results in immature potatoes at harvest, which may be more susceptible to *Fusarium* dry rot. Maturation of potatoes involves a variety of processes, described as chemical, physical and physiological maturation, as well as haulm maturation (Bussan et al. 2009; Sabba et al. 2007), and this definition of maturity will be used in our study. Different maturity indicators such as tuber sucrose level, skin set, dry matter content and senescence of the plant measure the different processes. The maturity of the potato tubers may influence the susceptibility to *Fusarium*, and the different *Fusarium* spp. may also respond differently to maturity of the tubers.

It has been reported that infection rates of *F. coeruleum* was higher in immature tubers and that resistance increased with tuber maturation (Boyd 1967). Boyd (1967) also concluded that susceptibility to *F. coeruleum* in immature tubers was closely related to the higher content of sucrose. Carnegie et al. (2001) reported that harvest date was an important factor affecting dry rot development of *F. coeruleum*.

With an increased focus on integrated pest management (IPM), cultivar resistance is a key element in the strategies for control of *Fusarium* dry rot. None of the potato cultivars has yet been found to be fully resistant to the whole *Fusarium* complex, but they differ in susceptibility to the different *Fusarium* species (Esfahani 2005; Peters et al. 2008; Wastie et al. 1989). Thus, a certain species of *Fusarium* may be pathogenic

to one cultivar but not to another, even though the same methods of inoculation and incubation are used (Gachango et al. 2012; Leach and Webb 1981).

Knowledge about resistance to *Fusarium* spp. among the currently most grown potato cultivars in Norway is limited. Resistance testing has been done in the past, but in these tests, the inoculum was made as a mixture of *F. coeruleum* and *F. avenaceum* isolates (Anja Haneberg, Graminor, personal communication). Knowledge about resistance to individual *Fusarium* species is needed. Information about maturity and cultivar resistance is important element in the development of an IPM strategy against *Fusarium* dry rot in Norway.

The objectives of this study were (1) to study the effect of maturity levels in potato tubers on *Fusarium* dry rot development caused by different *Fusarium* spp. and (2) to evaluate the resistance of commonly grown potato cultivars in Norway to these species.

Materials and Methods

Effect of Maturity Level on Susceptibility to *Fusarium* Dry Rot

Plant Material with Different Maturity Levels

In the years 2012 and 2013, two cultivars of potato, Asterix and Saturna, were grown on loam soil (Cambisol, low erosion risk, moderate natural drainage) (WRB 2006) in Østre Toten, Oppland, Norway (60.70° N, 10.87° E). The tubers were planted 12 cm deep in 0.8-m row spacing and 30 cm apart within rows. Manipulation of the maturity status of the potato crop was done by multiple factors to gain experimental material with a maximum of variation in maturity at the time of harvest. Different maturity levels were obtained by a combination of *presprouting*, *planting dates* and *levels of nitrogen fertilization* of the seed tubers. The different levels of maturity were verified by indicators described below and are in accordance with definitions of maturity by Bussan et al. (2009) and Sabba et al. (2007). Seed tubers grown for maturity level 1 (M_{mat}), the most mature, were presprouted for 4 weeks in 12 °C under full light (>100 lx), planted on 23 May 2012 and 16 May 2013 and fertilized with 70 kg N ha^{-1}. Seeds for maturity level 2 (M_{med}), medium mature, were *not* presprouted, planted on the same dates as M_{mat} and fertilized with 105 kg N ha^{-1}. Seeds for maturity level 3 (M_{imm}), immature, were *not* presprouted, planted on 5 June 2012 and 7 June 2013 and fertilized with 140 kg N ha^{-1}. In all three plots (M_{mat}, M_{med} and M_{imm}), the haulm was killed 8–10 days before harvest and tubers were harvested at the same dates, 14 September 2012 and 5 September 2013, to avoid influence of different harvesting conditions within years.

Maturity Indicators

Skin set of potato tubers was measured as described by Lulai and Orr (1993). Measurements were performed before wounding and inoculation. The measuring device (Halderson periderm shear tester) consisted of a measuring head attached to a torque metre, measuring the amount of torsional force [mNm (milliNewton metres)] required to produce skinning injury. Tubers were left under laboratory conditions

(20 °C, 40–60% RH) overnight after sampling before the measurements were taken. The content of dry matter was determined by over- and underwater weight to determine potato density as described by Lunden (1956). The following equation was used to calculate the content of dry matter, Dry matter=215.73 (x−0.9825), where x is the specific weight calculated as weight in air/(weight in air−weight in water). Analyses of sucrose content in the tubers were done by ion chromatography as described by Elmore et al. (2007). Samples of 10 quarters (cut from the heel to rose end) of tubers from the same batch were chopped in a food processor. Samples of 2±0.005 g were weighted into 50-ml screw-top bottles and 20-ml aqueous methanol (50%) containing 200 µl trehalose as an internal standard was added to each bottle. The sample was stirred in 15 min at room temperature. Aliquots (100 µl) were diluted in 900 µl aqueous methanol (50%). Then, the extracts were filtered through a millipore filter PVCF 0.22 µm. The extracts were analysed for sucrose using HPAC-PAD system. Haulm greenness was determined visually as relative greenness (0–100, 0=dead and 100=full greenness) just before haulm desiccation.

Inoculation, Incubation and Disease Assessment

Tuber samples were taken 2 days after harvest in 2012 and 2013. Samples of 10 tubers were surface disinfested in 0.5% sodium hypochlorite and rinsed twice in sterile water before wounded to a depth of 4 mm with a nail board. The wounding device consisted of a wooden brick with four nails (each with a diameter of 1 mm) forming a quadratic square (20 mm sides). This device provided four equally sized wounds in each potato tuber. A thick inoculum slurry made by mixing synthetic nutrient agar plates of 4 weeks old *Fusarium* cultures with sterile water of either *F. coeruleum*, *F. avenaceum*, *F. sambucinum* or *F. culmorum* was dispersed equally with a spoon (0.75 ml with approx. 2× 10^5 macrospores ml^{-1}) onto the four wounds on each tuber. In each year, three isolates of each species collected from potatoes were used separately. The *Fusarium* isolates used in this study were all collected from potato in 2011 in different counties of Norway (Table 1). Pure cultures grown on synthetic nutrient agar (SNA) were identified to species based on conidial morphology, production of chlamydospores, growth characteristics and colony pigmentation as described by Leslie and Summerell (2006) and Gerlach and Nirenberg (1982). To confirm species identity, DNA extracts of the isolates were tested using PCR-based assays (Cullen et al. 2005; Halstensen et al. 2006). Control samples were wounded and not inoculated with *Fusarium*, but sterile water mixed with clean agar was dispersed onto the wounds.

Each sample was placed in a plastic box on a net of steel with a layer of moist paper towel in the bottom of the box. The boxes were sealed with a plastic lid and incubated in darkness for 7 weeks at 10 °C and 95% RH. Temperature and relative humidity was logged by Tinytag Plus 2.

After the incubation period, cuts exactly through each wounding point towards the centre of the tuber were made with a flame-sterilized knife. Disease development was described as a rotted area around each wound. For the purpose of analysis, the rots were assumed conical, so width and depth of the rot were measured of each wounding point. Based on the method described by Peters et al. (2008), we calculated the volume of the

Table 1 Origin of *Fusarium* species and isolates used in this study

Fusarium spp.	Isolate	Original code	Potato cultivar	Origin (county)[a]
F. coeruleum	C1	11-88 t6	Rutt	Aust-Agder
	C2	11-60 t19	Gulløye	Troms
	C3	11-60 t10	Gulløye	Troms
F. avenaceum	A1	11-93 t6	Saturna	Hedmark
	A2	11-48 t4	Saturna	Østfold
	A3	11-73 t18	Saturna	Oppland
F. sambucinum	S1	11-41 t1	Asterix	Rogaland
	S2	11-19 t4	Solist	Hedmark
	S3	11-91 t12	Saturna	Vest-Agder
F. culmorum	Cul1	11-91 t3	Saturna	Vest-Agder
	Cul2	11-91 t23	Saturna	Vest-Agder
	Cul3	11-43 t3	Folva	Rogaland

[a] Counties in Norway

rot in each wounded area using the equation Volume $= \frac{1}{3} * \pi * h * r^2$, where r is half the width of the rot and h is the depth of the rot. We calculated a mean rot volume for each tuber. To confirm the cause of symptoms for each isolate, random samples were taken for reisolation. Tissue from the leading edge of tuber flesh showing dry rot symptoms was transferred to potato dextrose agar and then to SNA and was identified morphologically as described above.

Susceptibility Test of Commonly Grown Potato Cultivars

In 2012 and 2013, ten potato cultivars were tested for their susceptibility to three *Fusarium* species: *F. coeruleum*, *F. sambucinum* and *F. avenaceum*. We used the isolates as in the maturity experiment (Table 1). Asymptomatic potato tubers of the most frequently grown cultivars in Norway were used: Asterix, Beate, Berber, Folva, Kerrs Pink, Laila, Mandel, Pimpernel, Rutt and Saturna. Tuber material used in the experiment was grown at the same location as the maturity trial (60.70° N, 10.87° E). The tubers were planted at 12 cm deep in 0.8-m row spacing and 20 cm within rows. The tubers were planted on 30 May in 2012 and 11 June in 2013, the haulm was killed between 10 and 20 August in both years based on visual inspection of the tuber size, to get equally sized tubers, and harvested 4–6 September in both 2012 and 2013. The tubers were stored in experimental facilities in 4 °C and 98% RH for 4 months. Samples of five tubers per *Fusarium* species were surface disinfested in 0.5% sodium hypochlorite and rinsed twice in sterile water before being wounded to a depth of 4 mm using a 4-mm steel pin. Based on experience from the maturity experiment, we used only one wound per tuber since *Fusarium* dry rot development was successful in almost all wounds. The wound however was made a bit larger in this experiment. Inoculum consisting of 20 µl of conidial suspension (approx. 5×10^4 macrospores ml^{-1}) made by synthetic nutrient agar with *Fusarium* spores mixed with sterile water was pipetted onto each wounded tuber. Sterile water was pipetted onto two wounded control

samples. The samples were placed in clean labelled paper bags and incubated in the dark at 10 °C and 95% RH for 6 weeks. After the incubation period, the tubers were cut across the wound, towards the centre of the tuber, and assessed for *Fusarium* dry rot development as described above.

Statistics

All statistical analyses were carried out using R version 2.15.1 (www.r-project.org). Both experiments were modelled by generalized linear models (GLM). In both experiments, disease development was measured as dry rot volume caused by the individual *Fusarium* species. In the maturity experiment, disease development was modelled with maturity, isolate and cultivar as explanatory variables and years as random replications. In the cultivar experiment, disease development was modelled with isolate and cultivar as explanatory variables and years as random replications. The data were tested for significance of the main effects and interactions. Differences of means were tested by Tukey's multiple comparison test.

Results

Effect of Maturity Level on Susceptibility to *Fusarium* Dry Rot

Different cultural practices resulted in different maturity levels of the tubers, as shown by the various maturity indicators (Table 2). Haulm greenness was higher in the immature tubers (M_{imm}) at the time of haulm desiccation, while M_{mat} and M_{med} were quite similar. For both cultivars, the highest levels of skin set were found for the most mature tubers (M_{mat}) while the least mature tubers (M_{imm}) had the lowest level of skin set (Table 2). The dry matter content generally increased with increasing maturity. Saturna had higher dry matter content than Asterix. Sucrose levels increased with decreasing maturity for Saturna, with the greatest difference between M_{med} and M_{imm}. Asterix only had higher sucrose levels in the least mature (M_{imm}) tubers.

Table 2 Maturity indicators measured just before haulm desiccation (haulm greenness) and just before inoculation (skin set, dry matter, sucrose levels) in Asterix and Saturna

Cultivar	Asterix			Saturna		
Maturity	M_{mat}	M_{med}	M_{imm}	M_{mat}	M_{med}	M_{imm}
Haulm greenness (%)	33 (±5.5)[a]	38 (±8.5)	85 (±5.0)	35 (±4.5)	40 (±8.0)	85 (±5.0)
Skin set (mNm)	3.2 (±0.5)	2.9 (±0.4)	2.2 (±0.2)	3.0 (±0.1)	2.7 (±0.2)	2.3 (±0.1)
Dry matter (%)	23.5 (±2.1)	22.0 (±1.3)	21.1 (±1.3)	27.3 (±1.5)	25.6 (±1.6)	25.1 (±0.2)
Sucrose (mg/g)	2.6 (±0.5)	2.4 (±0.6)	4.4 (±0.9)	2.5 (±0.4)	2.8 (±0.2)	3.2 (±0.4)

Mean of 2 years (2012 and 2013)

M_{mat} mature, *M_{med}* medium mature, *M_{imm}* immature

[a] Standard error of the mean

The *Fusarium* spp. caused typical dry rot lesions in the tubers. Controls showed no symptoms. Within the *F. sambucinum* isolates, isolate 2 caused significantly larger dry rot symptoms than isolates 1 and 3. Dry rot volumes for *F. sambucinum* isolates are shown in Fig. 1. Maturity level had effect on the dry rot development and the most mature (M_{mat}) were less affected.

The other *Fusarium* species did not differ in aggressiveness between isolates. The variation within *Fusarium* species was larger than the variation within isolates. Overall, the mean volume of the dry rot was significantly larger for *F. sambucinum* than for the other *Fusarium* species ($P<0.01$) (Figs. 2 and 3). *F. coeruleum* caused the second most rot, while *F. avenaceum* and *F. culmorum* caused minimal dry rot symptoms in the tubers.

When inoculated with *F. sambucinum*, maturity level had a significant effect on disease development in Asterix tubers, described as dry rot volume ($P<0.05$) (Fig. 2). The most mature tubers (M_{mat}) had the lowest dry rot volume while the least mature (M_{imm}) had a larger dry rot volume (Fig. 2). Such a difference was not found in Saturna tubers (Fig. 3). There were no significant differences in dry rot development between the maturity levels when inoculated with *F. coeruleum*. *F. avenaceum* and *F. culmorum* caused minor dry rot symptoms in both cultivars. For these three *Fusarium* species, there were no significant differences between years, and the general pattern was similar for both Asterix and Saturna.

Susceptibility Test of Commonly Grown Cultivars

An illustration of *Fusarium* dry rot development in tubers from the experiment is visualized by one representative tuber from each cultivar and isolate (Fig. 4). *Fusarium* dry rot development was similar in tubers with the same treatment, and the ranking of cultivars was similar in the 2 years of trials.

As for the previously described experiment, the mean rot volume caused by *F. sambucinum* was significantly higher than those of the other *Fusarium* spp. ($P<0.001$). However, there were also significant differences among isolates
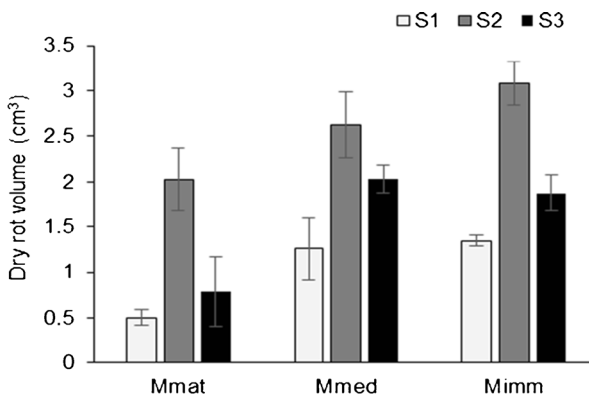

Fig. 1 Effect of maturity levels (M_{mat}, M_{med} and M_{imm}) on development of dry rot in Asterix potato tubers inoculated with three isolates of *F. sambucinum* (S1, S2 and S3). *Bars* denote the standard error of the means in both directions. Mean of 2 years (2012 and 2013)
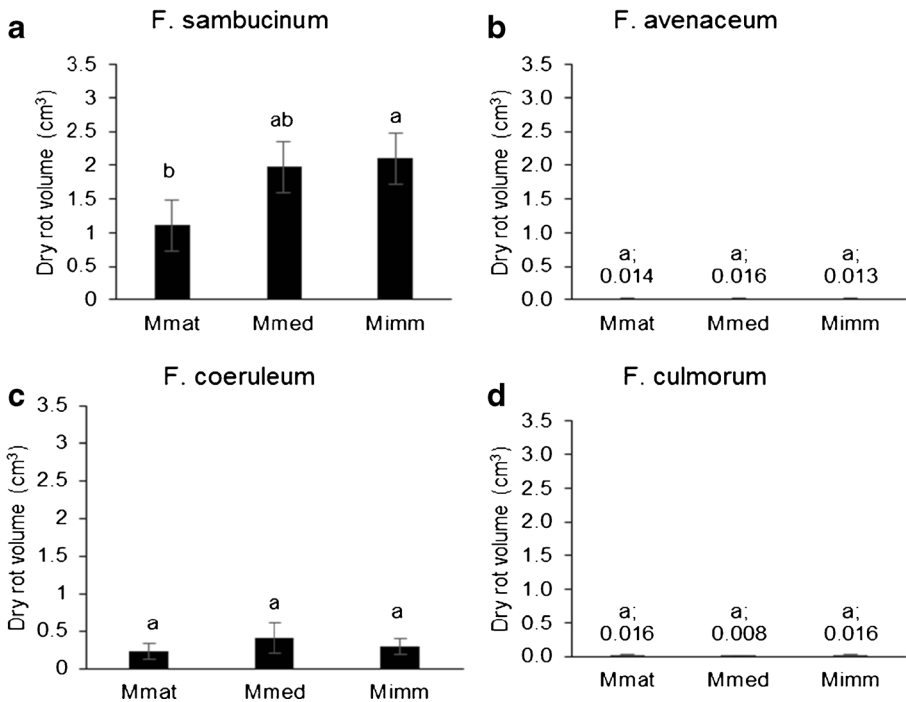
a   F. sambucinum

b   F. avenaceum

c   F. coeruleum

d   F. culmorum

Fig. 2 Dry rot volume as effect of maturity levels (M_{mat}, M_{med} and M_{imm}) in Asterix potato tubers inoculated with four Fusarium spp. (*F. sambucinum, F. avenaceum, F. coeruleum* and *F. culmorum*) (**a–d**). *Bars* denote the standard error of the means in both directions. *Different letters* indicate significance ($P<0.05$) among different maturity levels obtained by analysis of contrasts (Tukey) within the linear model. Mean of 2 years (2012 and 2013)

within *F. sambucinum*, with one isolate (S2) causing significantly larger rots than the other isolates ($P<0.001$) in both years (Fig. 5). There was a large interaction effect among isolates within *F. sambucinum* and cultivar indicating race-specific resistance. The *F. sambucinum* isolates ranked the cultivars differently and the pattern was similar in both years indicating race-specific resistance. There were no such differences among isolates for the other two *Fusarium* species.

The volume of rot varied among cultivars and the highest total dry rot volume for all *Fusarium* spp. was found in Berber, Rutt and Laila, whereas the lowest total rot volumes were found in Mandel, Saturna and Pimpernel (Fig. 6).

There was a large interaction effect between cultivar and *Fusarium* spp. ($P<0.001$), and the cultivar ranking order in susceptibility was different for each *Fusarium* spp. For *F. sambucinum*, the most susceptible cultivars were Berber, Asterix and Folva, with Berber having significantly highest volumes of dry rot. *F. avenaceum* caused significantly more rot in Berber and Rutt than in the other cultivars, while *F. coeruleum* was significantly most aggressive in Rutt, Laila and Berber. The levels were not significantly different from Folva and Saturna but higher than the five cultivars with the lowest disease level.
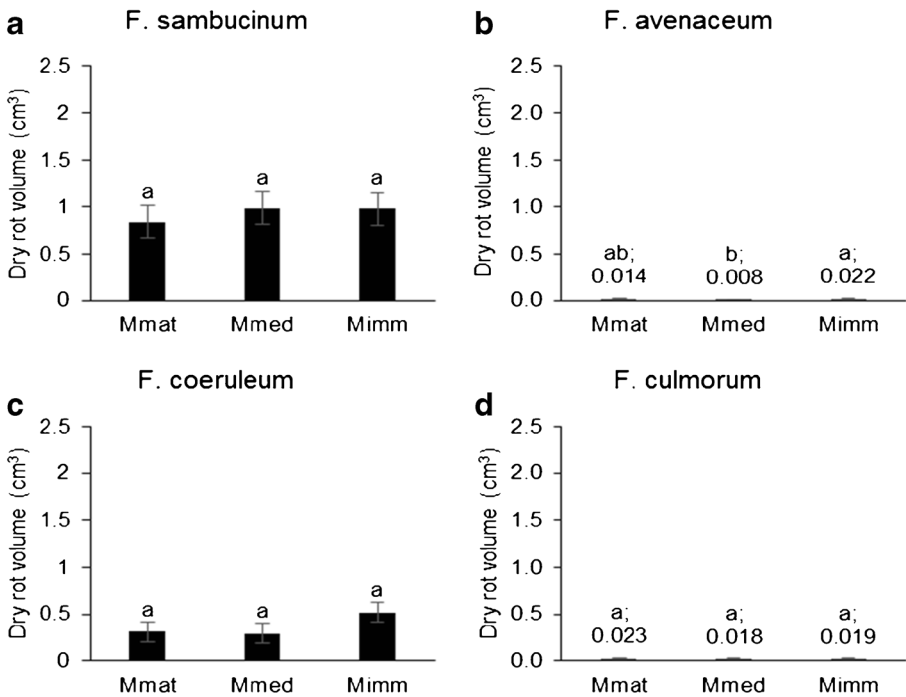
Fig. 3 Dry rot volume as affected by maturity levels (M_{mat}, M_{med} and M_{imm}) in Saturna potato tubers inoculated with four *Fusarium* spp. (*F. sambucinum*, *F. avenaceum*, *F. coeruleum* and *F. culmorum*) (**a–d**). *Bars* denote the standard error of the means in both directions. *Different letters* indicate significance ($P<0.05$) among different maturity levels obtained by analysis of contrasts (Tukey) within the linear model. Mean of 2 years (2012 and 2013)

Discussion

Differences in Aggressiveness of *Fusarium* spp.

In both the maturity level experiment and the cultivar resistance experiment, *F. sambucinum* was more aggressive, in causing dry rot than the other tested *Fusarium* species when looking at mean dry rot volume of the isolates. However, in our study, we found differences in aggressiveness between isolates within *F. sambucinum*. Isolate 2 was more aggressive and caused significantly larger rots than the two other *F. sambucinum* isolates. According to Desjardins (1995), the genetic diversity in *F. sambucinum* from potatoes is large in Europe which can explain the differences in aggressiveness found in our study.

In the cultivar experiment, eight out of ten cultivars got more severe dry rot symptoms after inoculation with *F. sambucinum* than with *F. coeruleum* or *F. avenaceum*. Similar results were also reported by Peters et al. (2008), who found that *F. sambucinum* gave larger rots than *F. coeruleum*, *F. avenaceum* and *F. culmorum* in most of the potato cultivars tested. Furthermore, Esfahani (2005) and Wastie et al. (1989) confirmed that *F. sambucinum* was a more aggressive species than *F. coeruleum*. Estrada et al. (2010) found *F. sambucinum* to be able to cause infection after a minor
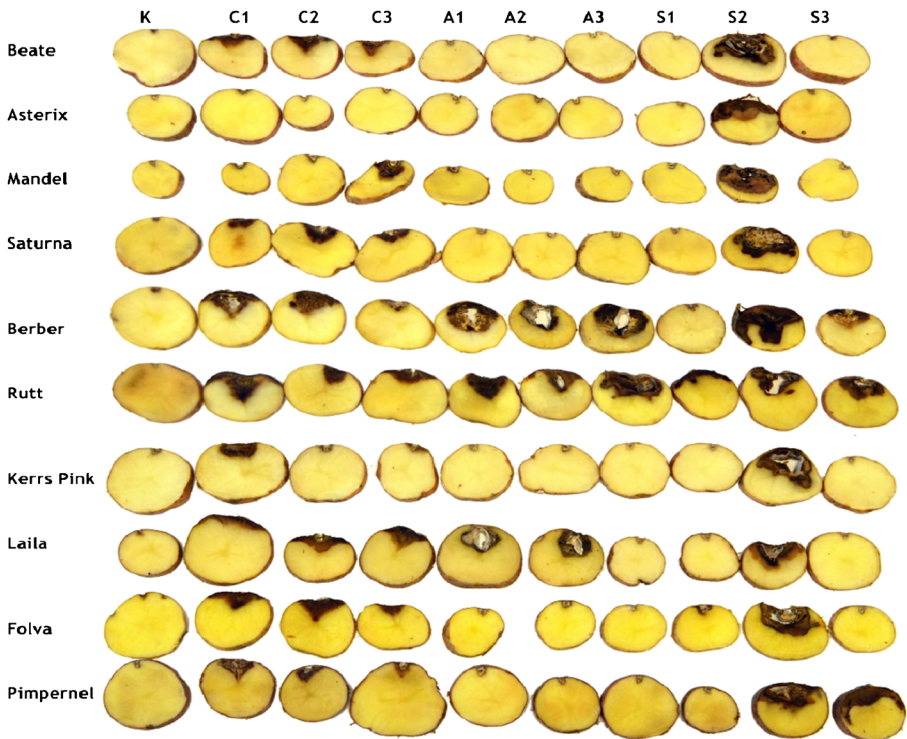
Fig. 4 *Fusarium* dry rot lesions in ten potato cultivars caused by *F. coeruleum* (C1, C2, C3), *F. avenaceum* (A1, A2, A3) and *F. sambucinum* (S1, S2, S3). K is uninoculated control

bruise or skinning of the periderm. Another study has shown *F. sambucinum* to be the only *Fusarium* spp. that could infect unwounded tubers (Tivoli and Jouan 1981).
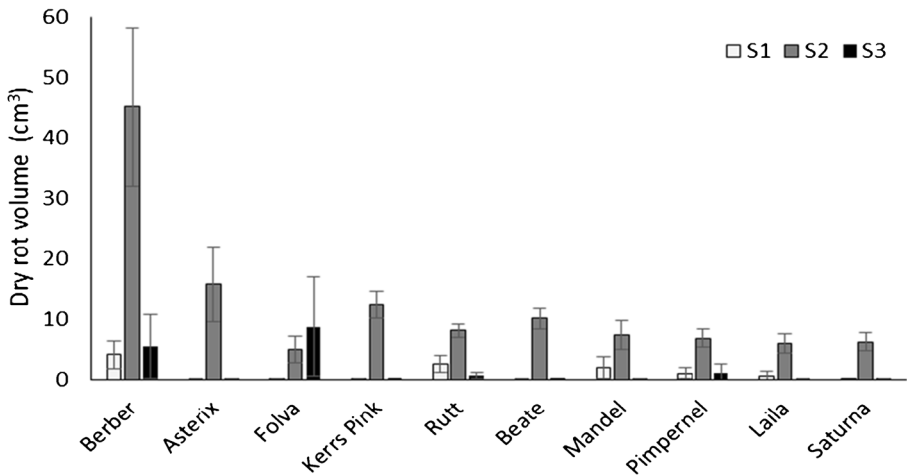

Fig. 5 Dry rot volume in potato cultivars caused by three isolates of *F. sambucinum* (S1, S2 and S3). *Bars* denote the standard error of the means in both directions. Mean of 2 years (2012 and 2013)
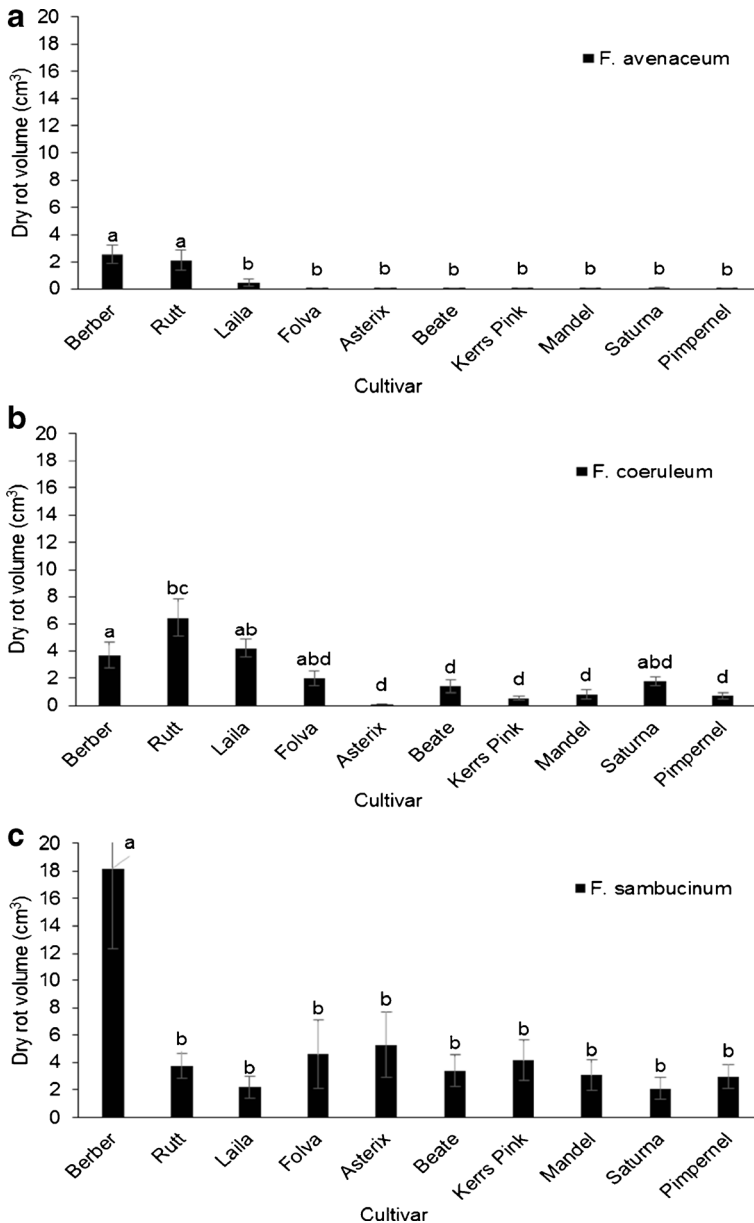
Fig. 6 *Fusarium* dry rot susceptibility of ten potato cultivars. Data shown are the mean of dry rot development (volume) caused by three *Fusarium* species calculated as mean of three isolates in two years (a–c). *Error bars* represent the standard errors of the mean. *Different letters* indicate significance ($P<0.01$) among cultivars obtained by analysis of contrasts (Tukey) within the linear model

Both *F. avenaceum* and *F. culmorum* caused only minor tuber symptoms in the maturity level experiment. In the cultivar susceptibility experiment, *F. avenaceum* showed less pathogenicity than *F. coeruleum* and *F. sambucinum* with minor dry rot symptoms in eight of ten cultivars. Peters et al. (2008) also concluded that

F. avenaceum and *F. culmorum* were relatively weak pathogens, at least in some cultivars. Saturna, which was also represented in both our experiments, was one of those cultivars.

Effect of Maturity on Dry Rot Development

Saturna and Asterix were selected in the maturity level experiment because they are among the most grown cultivars in Norway and belong to the same maturity group (medium–late).

We only got significant effects of maturity on rot development when using Asterix tubers and inoculation with *F. sambucinum*. There were larger rots in immature tubers than in mature tubers. Maturity level did not significantly affect the results after inoculation with *F. coeruleum*, *F. avenaceum* or *F. culmorum*, although there was a tendency to smaller dry rot symptoms in more mature tubers when inoculated with *F. coeruleum*. The general recommendation to potato growers is to ensure maturation of the skin to prevent wounding and thereby infection of the tubers (Knowles and Plissey 2008). In our study, we observed an increase in skin set with increasing maturity. Carnegie et al.(2001) also found an increase in skin strength with maturity and concluded that there was less dry rot development in the more mature tubers. Our results suggest that even other maturity indicators, representing the chemical and physiological maturity, are associated with less dry rot disease development in the tuber. Along with increasing skin set, there was also an increase in dry matter content and a decrease in sucrose levels. More mature tubers were more resistant towards *Fusarium* dry rot. Boyd (1967) concluded that sucrose content of immature tubers was closely related to the level of susceptibility. The same relationship was seen in the present study, where the least mature tubers had a higher content of sucrose. It can however be questioned whether this is a direct or an indirect effect.

Only a limited number of studies have reported the effect of maturity in potato tubers on the dry rot development caused by *Fusarium*. Boyd (1967) and Carnegie et al. (2001) studied the effect of harvest date on *F. coeruleum*. They concluded that harvest date had an effect on susceptibility. In these previous studies, maturity was obtained by different harvest dates, whereas maturity in our study was obtained by different planting dates, N-fertilization and presprouting. Several maturity indicators were measured in our study to document differences in tuber maturity. By harvesting potatoes of all maturity levels at the same date, we could prevent that differences caused by harvest dates (temperature and humidity) would influence development of disease.

Susceptibility of Potato Cultivars

In the cultivar susceptibility experiment, we found differences in *Fusarium* dry rot susceptibility among cultivars and differences in pathogenicity between the three *Fusarium* species commonly found in Norway and within *F. sambucinum* isolates.

Berber was susceptible to all *Fusarium* spp. This result supports findings in a survey of *Fusarium* spp. present in Norway, where the mean incidence of *Fusarium* spp. was the highest in Berber (Heltoft, unpublished data). In the same survey, the mean incidence of *F. coeruleum* was high in Rutt, which was also found in the present study. Berber and Rutt share one of their parents (Alcmaria), but data on this cultivar does not

include resistance towards *Fusarium* spp. (www.europotato.org). Furthermore, the other parent to Rutt is Laila, which also was found to be a susceptible cultivar to *Fusarium* dry rot. Surprisingly, Berber scores high on resistance to *Fusarium* in the Norwegian cultivar list (Møllerhagen 2014). However, in the resistance test used for creating this cultivar list, *F. sambucinum* has not been used as inoculum. Instead, a mixture of isolates of *F. coeruleum* and *F. avenaceum* has been used (Anja Haneberg, Graminor, personal communication). These two Fusarium species did however also cause large dry rot volume in Berber in the present study as well as in Rutt and Laila. These findings confirm the need of a new practice in the *Fusarium* resistance testing in Norway.

The early cultivars Berber, Rutt and Laila were more susceptible to *Fusarium* dry rot than the other seven cultivars in our study. These cultivars were all propagated in the same field and harvested at the same time, so they had different levels of maturity at harvest. In the maturity experiment, the resistance to *Fusarium* dry rot increased with maturity. In the cultivar resistance trial, the early cultivars developed more dry rot than the late cultivars despite being more mature. However, the cultivar experiment was done after 4 months of storage, whereas the maturity experiment was done shortly after harvest. Thus, the effect of cultivar resistance exceeds the effect of maturity in the cultivar experiment. The results indicate a negative correlation between earliness of the cultivar and resistance to *Fusarium* dry rot, as it has been shown for other potato diseases, e.g. late blight (Gebhardt et al. 2004).

The cultivar Saturna was relatively resistant to *Fusarium* spp., which correlates well with findings in Iran (Esfahani 2005) and Great Britain (Peters et al. 2008). In the British Potato Council Variety Database, Saturna is classified as medium resistant (6) (of 1–9 where 9 is resistant) to both *F. coeruleum* and *F. sambucinum*. In the same database, Asterix was resistant to *F. coeruleum* (8) and medium resistant (4) to *F. sambucinum* which correlates well with our results. In other databases (e.g. www.europotato.org) information about *Fusarium* dry rot resistance is either not present or presented on a general basis, not related to individual species. The large interaction effect of cultivar and *Fusarium* species indicates that cultivar resistance to one *Fusarium* spp. does not imply resistance to all *Fusarium* spp. This was also found in a study in Great Britain (Peters et al. 2008). Furthermore, Esfahani (2005) and Wastie et al. (1989), who studied the susceptibility to *F. coeruleum* and *F. sambucinum*, reported that resistance to each *Fusarium* species was independent. There was also a large interaction effect of isolates within *F. sambucinum* and cultivar indicating race-specific resistance. The variation in pathogenicity by different *Fusarium* spp. and isolates to different cultivars in Norway demonstrates the importance of using more than one species and isolate when screening the resistance of potato cultivars under Nordic conditions.

The present study underlines that both mature potatoes and resistant cultivars are important elements in an IPM strategy against *Fusarium* dry rot in Norway.

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Paper V

The relative contribution of soil-borne inoculum to *Fusarium* dry rot in potato cultivars Asterix and Saturna

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Abstract

A glasshouse experiment was carried out to quantify the relative contribution of seed- and soil-borne inoculum of three *Fusarium* spp. (*F. coeruleum, F. sambucinum* and *F. avenaceum*) in causing dry rot in two potato cultivars, Asterix and Saturna. Seed and soil were inoculated with different concentrations of inoculum; control (water only), low (10^2 conidia ml^{-1}) and high (10^5 conidia ml^{-1}) and disease severity on progeny tubers was subsequently assessed following an 8-week post-harvest storage period. Overall, *F. sambucinum* caused significantly ($P<0.05$) larger rots than *F. avenaceum*, with the severity of rots caused by *F. coeruleum* being intermediate, and disease severity was greater in cv. Asterix than cv. Saturna ($P<0.01$). None of the seed inoculation treatments resulted in dry rot development on progeny tubers. In contrast, soil infested with Fusarium species resulted in significantly more severe tuber rots on progeny tubers compared with controls ($P<0.01$). Soil infested with *F. sambucinum* (low and high levels) resulted in significantly more severe rots than control treatments ($P<0.001$), whilst only high levels of *F. avenaceum* soil inoculum increased severity of tuber rots compared with control treatments ($P<0.05$). Increases in disease severity observed as a result of the addition of inoculum of *F. coeruleum* to soil were not significant.

Keywords Potato dry rot; *F. sambucinum; F. coeruleum; F. avenaceum;* Inoculum potential

Fusarium dry rot is one of the most important storage diseases affecting potato (*Solanum tuberosum L.*), and affects many commonly grown potato cultivars (S.S. Leach and Webb 1981). However, cultivars vary in their resistance to different *Fusarium* spp. (Corsini and Pavek 1986; Lees et al. 1998; Peters et al. 2008; Heltoft et al. 2015).The disease can potentially cause significant yield losses, with up to 60 percent of tubers affected (Secor and Salas 2001). Control measures including crop rotation, wound healing prior to storage, fungicides and cultivar resistance are available. In northern Europe, *F. coeruleum* (Libert) Sacc is the most common *Fusarium* pathogen of potato (Olofsson 1976; Bjor 1978; Seppänen 1983; Peters et al. 2008; Heltoft et al. 2016). Other important species include

F. avenaceum and *F. sambucinum* (formerly *F. sulphureum* (Peters et al. 2008; Heltoft et al. 2016). *Fusarium* pathogens infect through wounds on tubers caused mainly during handling at planting, harvesting and grading. Adams and Lapwood (1983) investigated the transmission of inoculum in the field, and found that *F. sambucinum* and *F. coeruleum* were transmitted from seed to progeny tubers. Another study (Leach, 1985) found that seed inoculated with *F. sambucinum* resulted in high levels of *Fusarium* dry rot in progeny tubers, whilst naturally occurring low levels of *F. coeruleum* in soil resulted in relatively less severe dry rot symptoms. It is possible that the relative importance of seed and soil-borne inoculum varies between different *Fusarium* spp., and in order to implement effective disease-management strategies for *Fusarium* dry rot, it is important to understand the impact of different inoculum sources on disease development. The aim of the present study was to investigate the relative importance of seed- versus soil-borne inoculum of three species of *Fusarium* (*F. coeruleum, F. sambucinum* and *F. avenaceum*) in causing dry rot in two potato cultivars, Asterix and Saturna.

Seed tubers (24) of each of the two cultivars were assessed individually for *F. avenaceum, F. coeruleum* and *F. sambucinum* contamination using PCR-based assays (Cullen et al. 2005). The entire tuber was peeled (1-2 mm thick) using a hand held potato peeler and each tuber processed separately. The peels were mixed with 15 ml SPCB buffer (120 mM sodium phosphate, 2% CTAB, 1.5 M NaCl, pH 8.0) (Lees et al. 2002) and homogenised using a Homex grinder (Bioreba) and grinding bags (Bioreba). A single 1.5 ml aliquot was taken from each sample for DNA extraction. All tubers were shown to be free of the three *Fusarium* species used in the present study.

Inoculum of each *Fusarium* spp. was prepared using the following method. Two isolates of *F. avenaceum* and three isolates each of *F. sambucinum* and *F. coeruleum,* all UK isolates originating from potato, were grown on Synthetic Nutrient Agar (SNA) in 9 cm Petri dishes at 18 °C for four weeks in alternating 12 h of light and 12 h of darkness. To confirm species identity, DNA extracts of the isolates were tested using PCR-based assays (Cullen et al. 2005). An inoculum suspension consisting of a mixture of isolates of the same species was made by scraping fungal colonies from the Petri dishes into sterile distilled water (SDW). The concentration of macroconidia was quantified using a haemocytometer and adjusted to 10^5 (high) or 10^2 conidia ml^{-1} (low).

One day before planting the seed tubers were surface sterilized in 0.5% sodium hypochlorite and rinsed twice in sterile water before being wounded with a sterile device consisting of four spikes (each with a diameter of 1 mm) in a quadratic square (20 mm on each side) as described by Heltoft et al. (2016). Tubers were inoculated immediately after wounding with 20 µl of either a high (10^5 conidia ml^{-1}) or low (10^2 conidia ml^{-1}) conidial suspension or water only (control) in each of four wounds per tuber. The inoculated tubers were left overnight (12 h) in plastic-covered trays in room temperature before they were planted. The low and high conidial suspensions

were added (30 ml/6 l pot) to batches of James Hutton Institute compost (Invergowrie, Dundee), hereafter described as soil. The compost was assessed for the *Fusarium* species used in the present study using PCR-based assays. Infested soil was mixed thoroughly by hand before filling six litre pots. Control treatments had an equivalent volume of water added. The experiment was arranged in a randomized complete block design. For each *Fusarium* species, there were three replicates of each combination of soil and seed inoculum level (total of 162 pots with one tuber per pot). Plants were grown in a glasshouse at ambient temperature and watered by hand into saucers to minimize cross contamination between pots and to maintain conditions of constant dampness for five months prior to progeny tubers being harvested.

Harvested progeny tubers were wounded as described above (without the addition of inoculum) and placed in plastic-covered trays, and incubated under controlled environmental conditions (10°C and 95% relative humidity) for eight weeks. After storage, the incidence of tuber rots caused by the different *Fusarium* species was recorded and the rots which had developed at the wound sites were measured. Rots were assumed to be conical for the purpose of analysis. Therefore, the volume of the rot was recorded and calculated using the formula: Volume = $1/3\pi hr^2$, where r is half the width of the rot and h is the depth of the rot.

The statistical analysis was carried out using Minitab® version 17.2.1. The data were modelled by general linear model (GLM). Disease severity was measured as dry rot volume caused by the individual *Fusarium* species. Disease severity was modelled with cultivar, seed inoculum level and soil inoculum levels as explanatory variables with three random replicates. The data were tested for significance of the main effects and interactions. Differences of means were tested by Tukey's multiple comparison test.

Overall, the incidence of dry rotted tubers was significantly greater in progeny tubers grown in soil inoculated with *F. sambucinum* (68.3%) compared to either *F. coeruleum* (47.2%) or *F. avenaceum* (27.7%) (P<0.001). The results in Table 1 show the volume of rot in progeny tubers after eight weeks storage. Overall, *F. sambucinum* caused significantly larger rots than *F. avenaceum*, with the severity of rots caused by *F. coeruleum* being intermediate. In other studies, *F. sambucinum* was also found to be more aggressive than *F. coeruleum* and *F. avenaceum* (Wastie et al. 1989; Esfahani 2005; Peters et al. 2008; Heltoft et al. 2015).

Soil infested with *F. sambucinum* and *F. avenaceum* resulted in the development of significantly larger rots on tubers of both cultivars compared to control soil, whilst the increase in rot severity when soil was infested with *F. coeruleum* was not significant. Moreover, there was significantly higher incidence of dry rot overall of all *Fusarium* species in soils with high (67.4%) and low (57.4%) inoculum levels compared to the control soil (18.5%) (P<0.001), where all levels of seed inoculum are included in the mean. Leach (1985) found that naturally low soil

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levels of *F. coeruleum* resulted in no or little dry rot development in the progeny tubers and concluded that the absence of disease was due to low levels of soil inoculum or soil suppressiveness as pure cultures isolated from test soils gave good infection of the tubers.

There was no significant effect of seed inoculation on the development of rots in progeny tubers for any of the *Fusarium* species in this study. This indicates that the inoculation of tubers was not successful, but may also be caused by cultivar resistance. This is in contrast to the findings of Adams and Lapwood (1983) who demonstrated that infected seed could result in infection and subsequent development of dry rot in progeny tubers. They found that whilst *F. coeruleum* was most readily transmitted from rotted seed tubers to progeny tubers, rather than from seed with symptomless infection, *F. sambucinum* was transmitted from highly contaminated seed (tubers inoculated with slurry containing 10^6 spores ml^{-1} just before planting) to progeny.

Overall, there was a significantly higher incidence of *Fusarium* dry rot in cv. Asterix (54.9%) compared with cv. Saturna (40.5%) (P=0.036). Dry rot in treatments inoculated with *F. sambucinum* and *F. avenaceum* was more severe in cv. Asterix than cv. Saturna. These results are supported by ratings (on 1-9 scale of increasing resistance) where Saturna is moderately resistant to *F. sambucinum* (6) and Asterix is relatively susceptible (4) (British Potato Council Variety Database, http://varieties.ahdb.org.uk/varieties). No ratings are available for *F. avenaceum* in the database, but two studies have shown high resistance in both Asterix and Saturna (Peters et al. 2008; Heltoft et al. 2015). Considering that Asterix and Saturna are relatively resistant to *F. coeruleum* according to the variety data base with ratings of 8 and 6 respectively, the severity of rotting caused by *F. coeruleum* in this study was relatively high compared to rots caused by *F. avenaceum*. The results presented here support previous findings which demonstrated resistance to *Fusarium* spp. in cv. Saturna (Esfahani 2005; Peters et al. 2008; Heltoft et al. 2015).

This study showed that different levels of soil inoculum influence the incidence and severity of *Fusarium* dry rot on progeny tubers and that cultivar resistance should be considered an important component of disease control. Future experiments should include more susceptible cultivars for comparison and a re-evaluation of the contribution seed inoculum makes to disease.

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Table 1 Mean volume of rot (cm^3) in progeny tubers after storage

	All *Fusarium*	*F. sambucinum*	*F. coeruleum*	*F. avenaceum*
F. sambucinum	3.6a	-	-	-
F. coeruleum	2.1ab	-	-	-
F. avenaceum	0.6b	-	-	-
	*			
Asterix	3.2a	5.3a	2.9	1.2a
Saturna	1.0b	1.9b	1.1	0.1b
	**	**	n.s	**
Soil: high[a]	3.3a	6.5a	2.4	1.1a
Soil: low[a]	2.9a	3.4a	3.5	0.3ab
Soil: control[a]	0.1b	0.1b	0.2	0.1b
	**	***	n.s	*
Seed: high[b]	3.3	4.0	4.7	1.3
Seed: low[b]	1.6	3.5	0.8	0.4
Seed: control[b]	1.4	3.3	0.5	0.3
	n.s	n.s	n.s	n.s

n.s=not significant ($p \geq 0.05$), *$p<0.05$, ** $p<0.01$ and *** $p<0.001$. Values within a column followed by different letters are significantly different with Tukey's test ($p< 0.05$).

[a] Values include mean of seed: high, seed: low and seed: control

[b] Values include mean of soil: high, soil: low and soil: control