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Elina Virtanen

EFFECTS OF HAULM KILLING AND GIBBERELLIC ACID ON SEED POTATO *(SOLANUM TUBEROSUM L.)* AND TECHNIQUES FOR MICRO- AND MINITUBER PRODUCTION IN NORTHERN LATITUDES

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU,
FACULTY OF SCIENCE,
DEPARTMENT OF BIOLOGY;
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Abstract

Seed potato is the starting point in the potato (*Solanum tuberosum* L.) production chain. In order to secure potato production in a variety of production conditions, plant diseases must be controlled and the yield characteristics of the used cultivars ensured. In addition, production must be cost-effective. Characteristics particular to northern production conditions include long periods of daylight and a short growing season as well as a several months long seed potato storage period. The focus of the present study is on northern production conditions and methods, including haulm killing and sprout control, which are presumed to affect seed potato quality, as well as the initial stages of the seed potato production chain, i.e. micro- and minituber production, which could influence cost-effectiveness and propagation.

Haulm killing is one of the methods used in seed potato production to regulate tuber size. It is often carried out on unsenesced plants. The present results, however, indicate that cultivar properties have a greater effect on the sprouting and crop yield of seed potatoes than production-phase haulm killing or temperature sum accumulation. Nevertheless, haulm killing carried out three weeks after flowering (75 DAP) accelerated emergence. When the effect of haulm killing methods on seed potatoes was compared with natural haulm senescence, haulm killing was shown to increase disease pressure. Black scurf (*Rhizoctonia Solani*) was present in seed tubers whose haulm had been destroyed by mechanical or mechanical-chemical haulm killing. Naturally senesced haulm had less black scurf, and crop quantity and starch content developed to a level typical of the cultivar. Storage periods lasting several months make controlling seed potato sprouting more challenging. Therefore, use of the plant hormone gibberellic acid (GA) in sprout control was investigated. GA treatments at lower concentration (100 mM) increased the number of tubers in the cultivar Fambo. Thus, the timing of haulm killing and in the case of Fambo, GA treatment influenced the characteristics of seed potatoes.

Conventionally, the first tuber generation is produced using microplants to produce minitubers in greenhouses. This production method is, however, labour-intensive, and energy and investment costs are high. With the aim of increasing production efficiency in northern production conditions, the production of minitubers in the laboratory using a novel bioreactor technology and in growth rooms using the hydroponic technique was investigated. The Liquid Lab^{TM} Rocker bioreactor system was used *in vitro*, all the cultivars examined (Asterix, Timo, Van Gogh and Velox) produced microtubers. The quantity of tubers produced per dish varied between 30 (for the cultivar Asterix in eight weeks) and 75 (for the cultivar Velox in 11 weeks). The results showed hydroponic production of minitubers to be successful in indoor conditions: the cultivars Desiree and Van Gogh developed their first tuber three weeks faster than Asterix, and the minituber yield was 4.5 per plant for Desiree, 7.5 for Van Gogh and 4.0 for Asterix. When the results indicate that both the Liquid Lab^{TM} Rocker production method and the hydroponic production method are suitable for mass production of seed potatoes.

Keywords: gibberellic acid, haulm killing, microtuber, minituber, seed potato, Solanum tuberosum L., sprout, storage, technology

Virtanen, Elina, Varsistonhävityksen ja gibberelliinihapon vaikutuksia siemenperunaan (*Solanum tuberosum L.*) sekä tuotantotekniikoita mini- ja mikromukuloille pohjoisilla leveysasteilla.
Oulun yliopiston tutkijakoulu; Oulun yliopisto, Luonnontieteellinen tiedekunta, Biologian laitos; MTT Maa- ja elintarviketalouden tutkimuskeskus, Biotekniikka- ja elintarviketutkimus
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Tiivistelmä

Siemenperuna on lähtökohta perunan (*Solanum tuberosum* L.) tuotantoketjussa. Jotta perunantuotanto turvataan eri tuotanto-olosuhteissa, on hallittava siemenperunan kasvitaudit, taattava sadontuotto-ominaisuudet ja tuotannon on oltava lisäksi kustannustehokasta. Pohjoisissa tuotanto-olosuhteissa erityispiirteinä ovat valoisuudeltaan pitkät päivät ja kestoltaan lyhyet kasvukaudet. Lisäksi siemenperunoiden varastointijakso kestää useita kuukausia. Tässä tutkimuksessa selvitettiin vaikuttavatko pohjoiset tuotanto-olosuhteet tai tuotannossa käytetyt varsistonhävitys tai itämisen hallinta siemenperunoiden laatuominaisuuksiin. Lisäksi selvitettiin siemenperunoiden ensimmäisen mukulasukupolven (mikro- ja minimukula) tuotantoa eri tekniikoilla.

Siemenperunatuotannossa varsistonhävitystä käytetään mukulakoon säätelykeinona. Varsistonhävitys tehdään usein tuleentumattomaan kasvustoon. Saatujen tulosten perusteella lajikeominaisuudet vaikuttivat itämiseen ja sadontuotto-ominaisuuksiin enemmän kuin varsistonhävitys tai mukuloihin kerääntynyt lämpösumma. Varsistonhävitys kolme viikkoa kukinnasta (75 päivää istutuksen jälkeen) nopeutti kuitenkin siemenperunoiden taimettumista. Vertailtaessa varsistonhävitysmenetelmien vaikutusta siemenperunaan verranteena kasvuston luontainen tuleentuminen, varsistonhävitys lisäsi kasvitautipainetta. Mekaanis-kemiallisesti ja mekaanisesti varsistonhävityissä satomukuloissa tuli esiin seittirupea (*Rhizoctonia solani*). Luontaisesti tuleentuneen kasvuston sadoissa oli seittirupea vähemmän ja myös sadon määrä ja tärkkelyspitoisuus kehittyivät lajikkeelle luontaiselle tasolle. Useiden kuukausien varastointijakso vaikeuttaa siemenperunoiden itämisen hallintaa. Kun tutkittiin gibberelliinihapon (GA) käyttöä itämisen hallintaan, alhaisemman konsentraation (100mM) GA –käsittely lisäsi Fambo –lajikkeen mukulalukumäärää. Tulosten perusteella varsistonhävityksen ajoittamisella ja GA –käsittelyllä (Fambo –lajike) vaikutettiin siemenperunoiden ominaisuuksiin.

Siemenperunan ensimmäisen mukulasukupolven eli minimukuloiden tuottaminen tapahtuu perinteisesti kasvihuonekasvatuksena mikrokasveista. Tuotantotapa on työvoima-, energia- ja invetointikustannuksia vaativaa. Tuotannon tehostamiseksi pohjoisissa tuotanto-olosuhteissa tutkittiin mikro- ja minimukuloiden tuotantoa eri teknologioilla. Mikromukuloita tuotettiin bioreaktorimenetelmällä laboratoriossa ja minimukuloita hydroponisella menetelmällä kasvatushuoneissa. Tulokset osoittavat, että kaikki tutkitut lajikkeet (Asterix, Timo, Van Gogh ja Velox) tuottivat bioreaktorissa mikromukuloita. Mikromukuloiden määrä vaihteli 30:sta (Asterix, 8 viikon kasvatus) 75:een (Velox, 11 viikon kasvatus). Myös minimukuloiden hydroponinen tuotanto sisätiloissa on mahdollista; kaikki lajikkeet muodostivat mukuloita, Desiree ja Van Gogh 3 viikkoa nopeammin kuin Asterix. Desiree tuotti minimukuloita 4.5 kpl/kasvi, Van Gogh 7.5 ja Asterix 4.0. Tulokset osoittavat, että molemmat menetelmät (bioreaktori ja hydroponinen) soveltuvat mikro- ja minimukuloiden massatuotantoon.

Asiasanat: gibberelliinihappo, itäminen, mikromukula, minimukula, siemenperuna, Solanum tuberosum L., varastointi, varsistonhävitys

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The present study is based on research results obtained during 2003–2012 at MTT Agrifood Research Finland's Biotechnology and Food Research unit in Oulu. The results of the study address the most critical issues concerning seed potato (*Solanum tuberosum* L.) production in northern conditions.

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Oulu, March 2014 Elina Virtanen

Abbreviations

DAP	day after planting
DAH	day after haulm killing
L-L	Liquid Lab^{TM} Rocker system
HCS	hydroponic crop system
GA	gibberellic acid
PAI	physiological age index
RTI	relative thermal index
GDD	growing degree-day
PAR	photosynthetically active radiation
CIPC	chlorpropham
DMN	dimethylnaphthalene

List of original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Virtanen E, Häggman H, Degefu Y, Välimaa A-L & Seppänen M (2013) Effects of production history and gibberellic acid on seed potatoes. Journal of Agricultural Science 5: 145–153.

II Virtanen E & Seppänen M (2014) Effects of haulm killing on seed potato quality. Journal of Agricultural Science 6: 168–175.

III Kämäräinen-Karppinen T, Virtanen E, Rokka V-M & Pirttilä AM (2010) Novel bioreactor technology for mass propagation of potato microtubers. Plant Cell, Tissue and Organ Culture 101: 245–249.

IV Virtanen E & Tuomisto J (2013) Hydroponic minituber production in growth room conditions and carry-over effects of the technique on produced minitubers. (Manuscript).

Table of contents

1 Introduction

The potato (*Solanum tuberosum* L.) is one of the most important food plant in the world (Braun 2010). Potato can be cultivated in many regions of the world and used for many purposes. Potato cultivation contributes to meeting the increasing need for food created by world population growth. Potato tubers accumulate large amounts of starch and are low in fat, and their protein content is comparable to that of grains. In addition, potatoes contain vitamin C (Rodríguez-Falcón *et al.* 2006). In order to secure potato production in a variety of production conditions, plant diseases that affect seed potatoes must be controlled and yield characteristics ensured. In addition, production must be cost-effective (Corrêa *et al.* 2007). Potato production efficiency must be increased on a continent- and country-specific basis, as production conditions differ dramatically from one region to another.

The potato production chain starts with the seed potato (Fig. 1). The life cycle of the seed potato begins with tuber formation and ends with the growth of a new plant and the production of new tubers in the following year (Celis- Gamboa *et al.* 2003/4). Seed potatoes must be vigorous and free from plant diseases when producing new tubers. These yield characteristics are affected by many factors: the physiological state of the tuber (cultivar, age, size, growth, vigour), growing conditions (physical and chemical factors including light, temperature, precipitation, soil nutrients), harvesting techniques (haulm killing, harvest time) and crop storage (incl. temperature and humidity). Seed potato quality, i.e. its capacity to produce sprouts, shoots and daughter tubers, is measured only as it is producing a new crop (Struik & Wiersema 1999).

Finland's potato production area totals approximately 26,000 hectares and is among the northernmost in the world. Seed potato production covers 1,400 hectares of this area and is concentrated in Northern Ostrobothnia – a region is characterized by long days and a short growing season. Seed potato production in these northern conditions is subject to a number of special requirements. Expertise in and management of seed potato quality, the use of cultivars with different production characteristics, and the use of new technologies and methods aimed at improving production throughout the supply chain are among the key future challenges of the Finnish seed potato industry.

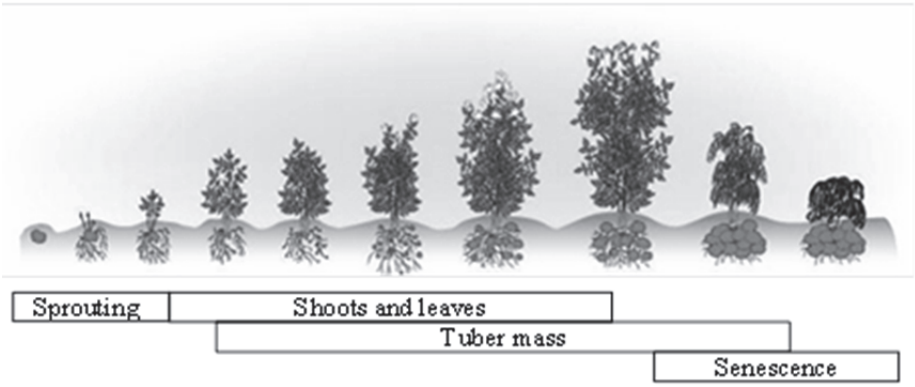

Fig. 1. The developmental stages of seed potato from yield production to natural senescence (based on Hack *et al.* 1993).

1.1 Northern production conditions

In Northern Ostrobothnia, most seed potato production takes place in the municipalities of Tyrnävä and Liminka, which form one of the five High Grade areas named by the European Union (64°46'N, 25°32'E) (Fig. 2). In addition to Finland, other EU-designated High Grade areas are located in Ireland, Great Britain, Portugal and Germany, and these vary considerably in area (ETYp 30.3.1993/231). Within the European Union, High Grade status is granted to a seed potato production area in which there are no dangerous potato plant pests or diseases or they have been successfully destroyed. Climate conditions affect the selection of seed potato production sites. In Finland the sites were selected based on the following key criteria: no potato plant disease, suitable soil and distinct summer and winter seasons. Although growing season conditions are the most important in terms of selecting seed potato production sites, year-round climate conditions also affect soil-borne plant disease control. Seed potatoes produced in the High Grade areas are an important part of European potato production. In Finland, a cluster of seed potato companies operate in the High Grade area and more than 20% of the seed potato production is exported.

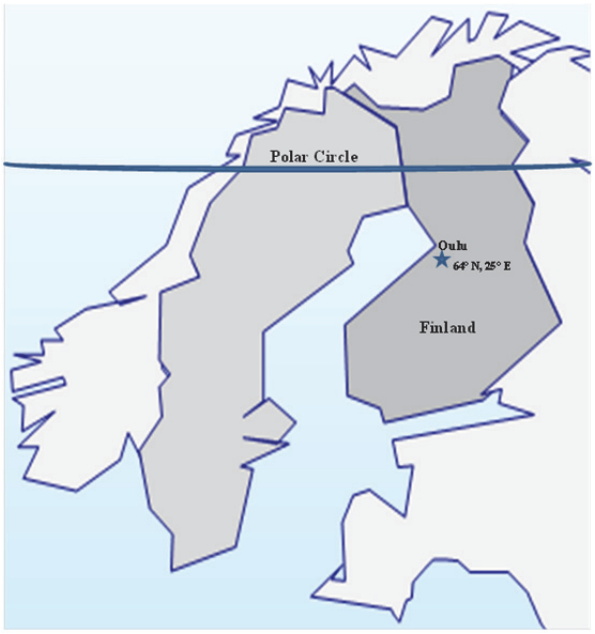

Fig. 2. The High Grade –seed potato area (Tyrnävä-Liminka municipalities) in Finland is shown as star.

1.1.1 Long days and short growing season

Finland's climate is strongly influenced by ocean currents, continental air masses, polar cyclones and the Gulf Stream. The climate is characterized by a clear seasonal rhythm: a snowy, cold winter, relatively short spring, warm but short summer and cloudy, chilly autumn. There are substantial variations in light conditions and temperatures along the parallels of latitude 64°–65°N. The winter is long and dark and the summer growing season is short (110–115 days) and bright. Annual precipitation is approximately 500–550 mm, 35–40% of which consists of snowfall (Finnish Meteorological Institute 2011).

Crop quality and quantity are substantially affected by the growing site climate, with the best potato yields achieved in cool and humid climatic conditions. The optimum temperature for potato growth is 15–20 ºC, with a lower limit of 5–10 ºC and an upper limit of 25 ºC (Reilly *et al.* 1996). At high temperatures, plant respiration is accelerated, resulting in loss of the energy created during photosynthesis and cessation of growth (Valkonen 2004). In

addition, potatoes are more sensitive to disturbances in water economy than other arable crops (Gregory & Simmonds 1992). Insufficient humidity reduces crop quantity, whereas excessive humidity may, for example, increase the occurrence of bacterial diseases in the foliage. Potatoes need approximately 350–500 mm of water during the growing season.

In Finland, dawn and dusk occur slowly, because as sunlight radiates to the polar regions it is refracted more steeply in the atmosphere than at the equator. As a result, the wavelength of light becomes longer and is experienced as twilight. At sunrise and sunset, sunlight travels longest through the atmosphere and the scattering of short wavelengths increases. As a result, the relative proportion of yellow and red light increases (Young & Freedman 2004) and, consequently, the daily quantity of far red light is higher in northern latitudes than in the further polar regions (Clapham *et al.* 1998). The long twilight periods in northern regions may substantially affect the photomorphogenetic (light-mediated development) properties of the plant. In some plants, long days increase the production of dry matter and flavour compounds (Hay 1990). According to Kozai *et al.* (1995), the fresh and dry weights of plants increase during long days, but no differences were found in dry-matter content in comparison to short days. The lengthening of shoots and internodes in the potato, caused by long periods of daylight and especially by far red light, has been observed in several different studies (Heyer *et al.* 1992, Yanovsky *et al.* 1998). In previous studies, long days and shading were found to delay tuber formation (Degamante & Vander Zaag 1988). However, sufficiently high quantities of illumination (a minimum of 400 μmol m^{-2} s^{-1}) have been observed to reverse the tuber-formation-inhibiting effect of long days (Wheeler & Tibbitts 1986). Based on the results of the studies of De Temmerman *et al.* (2002) on the response of potato to elevated O_3 and CO_2 concentrations, climatic conditions create changes relative to potato growth and yield. The results show tubers growing larger in size in Northern Europe (higher latitudes) as a consequence of lower temperatures, air humidity (vapour pressure deficit, VDP) and long-day conditions (De Temmerman *et al.* 2002).

In addition to environmental effects, plant development is also regulated by endogenous hormones, which serve as chemical messengers interacting with specific protein receptors linked to cellular signalling pathways (Taiz & Zeiger 2010). The plant hormone groups include auxins, gibberellins, cytokinins, ethylene, abscisic acid, brassinosteroids and strigolactones, which have specific roles in plant development. Moreover, jasmonic acid, salicylic acid and small polypeptides have roles in defence against herbivores or resistance to pathogens

(Taiz & Zeiger 2010). Auxin maintains a strong apical dominance and cytokinins and gibberellins are among the phytohormones that promote cell division and longitudinal growth of cells (Yang *et al.* 1996, Taiz & Zeiger 2006). Long days increase auxin, cytokinin and gibberellic acid levels and decrease the concentration of abscisic acid.

The potato stores glucose compounds formed during the growth season in the tubers as starch. Starch synthesis is an important factor in potato primary metabolism, as starch stored in the tubers functions as a carbohydrate reserve for new seed potato shoots and roots in the production of the next growth season's yield (Davies 1984, Geigenberger *et al.* 2004). The amount of starch in the tubers varies during the periods of growth and maturing as well as between cultivars (Liu *et al.* 2003, Fischer *et al.* 2013). Even though long days increase the length of time the potato uses for daily photosynthesis and the amount of absorbed carbon (De Temmerman *et al.* 2002), research data is required on how potato allocates carbon reserves to above-ground shoot growth and tuber yield production.

1.2 Special requirements for seed potato production

One of the reasons why seed potato production differs from that of other crops is that seed potatoes can be latently infected with plant diseases and their quality may deteriorate during storage (Struik & Wiersema 1999). After storage, seed potatoes should be disease-free and their physiological characteristics should also be suitable for yielding a crop in any production conditions. Seed potato quality is critically important in terms of yielding a crop and therefore, seed potato production, harvesting and storage should be carried out carefully (Corrêa *et al.* 2007).

After harvesting, the tubers undergo a period of dormancy and physiological rest lasts for a few months depending on the potato genotype and the physiological state. According to Lang *et al.* (1987), dormancy is divided into three categories: endo-, eco- and paradormancy. During deep dormancy (endodormancy), tubers cannot sprout even under favourable environmental conditions for sprouting. After deep dormancy (ecodormancy), tubers begin to sprout if temperature conditions are favourable, and further sprouting and natural sprouting begin as a result of physiological characteristics (paradormancy) (Suttle 2004, Chao *et al.* 2007). During dormancy, tubers undergo biochemical and physiological processes and, after dormancy, sprouting begins in phases: apical

dominance, normal sprouting, production of branched sprouts and incubation (tuber formation) (Struik & Wiersema 1999). In order to be able to control sprouting, particularly after a long storage period, methods or indicators are needed to indicate either dormancy or the seed potato physiological state (Caldiz *et al.* 2001).

1.2.1 Haulm killing

In northern production conditions, haulm killing is used in seed potato production primarily as a method of controlling tuber size, as tuber growth is relatively quick in long-day conditions (De Temmerman *et al.* 2002). Therefore, haulm killing is often carried out on highly immature plants that may still be flowering and the timing of haulm killing is not synchronized with foliage senescence or potato tuber maturation. According to Struik and Wiersema (1999), in conditions where the growing season is short, haulm killing can be used to advance harvesting, obtain a suitable tuber size, strengthen tuber skins before harvesting, and prevent plant pathogens from spreading among the foliage and crop. In particular, haulm killing aims to control soil-borne or seed-borne diseases including viral diseases, fungal or fungal-like diseases representing the genera *Rhizoctonia solani* (black scurf), *Phytophthora infestans* (late blight), *Phoma foveata* (gangrene) and *Verticillium dahlie* (Verticillium wilt) as well as bacterial diseases (Kempenaar & Struik 2007).

Besides cultivar-specific differences in tuber development, the developmental stage of different tubers in the same hill may vary at the time of haulm killing (Struik & Wiersema 1999). The effects of the timing of haulm killing on the seed potato physiological state have been studied in different production conditions and cultivars. Brown *et al.* (2003) reported that the timing of haulm killing affected seed potato physiological characteristics, whereas Wurr *et al.* (2001) found no clear effects. Bethke & Busse (2010) reported that haulm killing and tuber maturity at harvest have long term effects on tuber quality. According to Struik and Wiersema (1999), the differences in disease resistance between plants must be taken into consideration in determining the timing of haulm killing; if the cultivar has low disease resistance, it is recommended that the haulm is destroyed earlier than in cultivars with higher disease resistance. The presence of aphids must also be considered, along with the virus infection pressure in the production area. Early haulm killing reduces crop quantities; however late haulm killing creates a greater risk of increasing the presence of pathogens and potato diseases

(Struik & Wiersema 1999). Even though haulm killing can be used to reduce the occurrence of viral or other seed- or soil-transmitted plant diseases, some haulm killing methods may, however, contribute to the occurrence of plant diseases (Dijst 1988, Kempenaar *et al.* 2008, Tsor 2010).

There are several haulm killing methods; steaming, flaming, electrocuting, vine pulling and mechanical or chemical methods or combinations of both. In the studies of Misener & Everett (1981), disconnecting the haulm from the roots by pulling was found to be the most effective method: 98–100% of the haulm dried and re-growth appeared in only 2–3% of the tubers. Vine pulling is the quickest because it instantly inhibits haulm starch synthesis, prevents phloem transport of photosynthesis products to the tubers (Tiessen *et al.* 2002) and prevents the supply of growth-stimulating hormones (Weber & Bartel 1989). Regardless of the method used, haulm killing should be carried out when the haulm has already started to senesce naturally (van Evert *et al.* 2012). The use of haulm killing chemicals is recommended only when the haulm shows signs of natural senescence (Kempenaar *et al.* 2008, OEPP/EPPO 2010). The effects of the time between haulm killing and harvesting have become apparent primarily in the context of the physiological behaviour of seed potatoes (Wurr *et al.* 2001, Brown *et al.* 2003). The effects on external quality were not significant (Weber & Bartel 1989, Kumar *et al.* 2009, Bethke & Busse 2010). The recommended time between haulm killing and harvest is 10–14 days. Skin set typically takes 10–14 days, depending on the cultivar and soil conditions (Halderson & Henning 1993). If harvest is delayed longer than the recommended time, there is a real risk of increasing the likelihood of plant disease (Lootsma *et al.* 1996, Struik & Wiersema 1999). Tuber crops are known to be exposed to infection by black scurf if the haulm is destroyed while the root system is still in operation and the time between haulm killing and harvest is prolonged (Tsror 2010). Black scurf caused by *Rhizoctonia solani* (Kühn AG-3) leads to substantial economic losses (Lootsma & Scholte 1996) and in seed potato production it may be an obstacle to certification.

When determining the timing of haulm killing, methods and timing based on haulm senescence and production- as well as cultivar-specific requirements have to be taken into consideration (Ivany & Sanderson 2001, Pavlista 2001, Bethke & Busse 2010). More research is needed to optimize haulm killing to suppress the different soil- and seed-borne diseases and to create sensors or other methods to determine the timing or the amounts of chemicals used for haulm killing (van Evert *et al.* 2012).

1.2.2 Storage

Seed potato storage should be such that tuber transpiration and respiration and plant disease progress in the tubers are minimized. If the storage period is too short (0–3 months), the seed potato is in dormancy at the time of planting, or sprouting is controlled by apical dominance. If the storage period is too long, the seed potato may be physiologically too old. Well-implemented storage allows the seed potato to sprout while retaining its physiological vigour even during a long storage period (Oliveira *et al.* 2012).

In northern production conditions the seed potato storage period varies from 6 to 8 months. Frost periods of several months' duration place special structural and functional requirements on storage facilities, including optimization of temperature, relative humidity, ventilation and gas composition (Struik & Wiersema 1999). In order to manage respiration, the possible spread of bacterial diseases, and physiological decomposition, which reduce potato quality, the optimum potato storage temperature should be +4 °C, air humidity 90–95%, oxygen concentration 5–20% of storage air, and ventilation 15–20 m^3/t/h (Larsson & Bengtsson 1987). Even though seed potatoes can retain their viability for up to 3 years in the right storage conditions (+4 °C, 95% RH) (Weber 1990), in unfavourable conditions they may lose up to 4% of their moisture content in less than 6 months' storage (Zabrouskov *et al.* 2002).

The two developmental stages of seed potato occur in storage conditions – dormancy following tuber formation and sprouting following dormancy (Celis-Gamboa *et al.* 2003/4). During the storage period, dormancy duration and sprout control are also affected by seed potato production techniques, geographical location of production, tuber maturation at harvesting, and the interactions of various growth factors (Daniels-Lake & Prange 2007). Dormancy-controlling factors internal to the tuber include carbohydrate fluxes, tuber hormone levels and aging-related phenomena, such as the accumulation of free radicals and the resulting injury to cellular structures (Burton 1989). Celis-Gamboa *et al.* (2003/4) and Bethke & Busse (2010) reported that seed potato storage prolongs dormancy and that seed potatoes age in storage. However, aging occurs more slowly in lower than higher temperatures, although according to Shahba *et al.* (2007), the effects of storage temperature may be cultivar-specific.

Storage plays an important role in optimizing the agriculturally important characteristics of seed potatoes, because excessive sprouting of tubers occurring too early compromises seed potato production potential and results in reduced

yield (Veerman & Wustman 2005). Sprout control is important when seed potatoes are stored for long periods (Kleinkopf 2003). Research on tuber physiological aging and dormancy has led to the development of storage methods to delay sprouting and improve quality (Kerby *et al.* 2005). New sprout inhibitors have also been developed for use in storage conditions and have been introduced to the market (Daniels-Lake & Prange 2007). However, comprehensive sprout control still requires more in-depth knowledge of the potato's physiological state (Jin-Cheol *et al.* 2008, Salimi *et al.* 2010). Eltawil *et al.* (2006) concluded that storage conditions should be defined individually for different potato cultivars, taking their production history into consideration.

1.2.3 Sprout control

In areas with short growing seasons, seed potatoes are pre-sprouted before planting to accelerate foliage growth and to ensure senescence during the growing season (Hagman 2012). The challenge lies in enabling seed potato sprouting to occur at the preferred time, not too late and not too early. This challenge is complicated by the effect of the production history and storage conditions (Daniels-Lake & Prange 2007). Unwanted seed tuber sprouting during the storage period decreases tuber vigour leading to economic losses (Sonnewald 2001, Suttle 2004).

Sprouting mobilizes the starch in the tuber and consumes part of the tuber's biochemical reserves. This results in loss of tuber weight/biomass and withering. The loss in tuber quality is not desirable with respect to the production characteristics of seed potatoes, as sprout control after dormancy affects, in particular, the yielding capacity of seed potatoes (Daniels-Lake & Prange 2007). Sprouting should be controlled to occur at the preferred time and in the preferred manner (Daniels-Lake & Prange 2007, Teper-Bamnolker *et al.* 2010. 2012), as seed potato quality is measured by its ability to produce sprouts, shoots and daughter tubers.

The physiological state of seed potato is known to affect sprouting and yielding capacity (Knowles & Knowles 2006, Delaplace *et al.* 2009), but no definitive method exists for determining it. Physiological state has been assessed, for example, by means of accumulated temperature sum (Shahba *et al.* 2007), incubation period, or by combining chronological age and incubation period (Caldiz *et al.* 2001). However, these methods have inadequacies and cannot be used to evaluate physiological state in all circumstances (Johansen *et al.* 2008).

Delaplace *et al.* (2008a) consider the physiological age index (PAI) to be suitable for potato, although it only partially determines tuber aging. Yuan & Bland (2005) used in their studies the new thermally diffused relative thermal index (RTI), which is based on the modified beta distribution model, growing degree-day (GDD), physiological summation (Pday) and cumulative photosynthetically active radiation (PAR).

In northern production conditions, the temperature sum accumulated during the growing season is 1100–1200 degrees. This temperature sum is reduced by the haulm killing carried out in seed potato production. The physiological state of seed potato can be assessed by means of the temperature sum accumulated during the production history, i.e. in production during summer, in storage during winter, and during germination in the spring. The accumulated temperature sum (>4 °C) is registered per seed lot.

The differences between physiologically young and old seed potatoes have been extensively studied. According to Delaplace *et al.* (2008b), only one sprout develops in physiologically young seed potatoes, whereas several sprouts develop in older ones but they lack apical dominance. Physiologically older seed potatoes are also known to emerge faster than physiologically younger ones (O'Brien *et al.* 1986, Bodlaender & Marinus 1987, Knowles & Botar 1991, Jenkins *et al.* 1993, Essah & Honeycutt 2004, Eremeev *et al.* 2007).

Numerous methods have been developed for chemical control of dormancy and sprouting. For example, tuber treatment with ethylene chlorohydrin or ethanol favours dormancy breaking (Claassens *et al.* 2005, Daniels-Lake 2013). Maleic hydrazide, such as CIPC (chlorpropham), DMN (dimethylnaphthalene) (Campbell *et al.* 2010), and also volatile components of caraway and peppermint oils have been applied for tuber dormancy induction and prolongation (Kleinkopf *et al.* 2003, Eshel *et al.* 2009, Gomez-Castillo *et al.* 2013). However, the phytotoxicity of these sprout inhibitors must be tested before application (Sorce *et al.* 2005). Of the plant hormones, gibberellic acid (GA) has shown to be effective in interrupting the dormancy of potato minitubers (Salimi *et al.* 2010, Kulen *et al.* 2011). Other sprouting inhibitors (hydrogen peroxide, H_2O_2, bromoethane) have also been applied (Al-Mugharabi 2007, Akoumianakis *et al.* 2008), but the effects of the treatments on seed potatoes as carry-over effects have not been studied more widely (Jin-Cheol *et al.* 2008).

1.3 Technologies for micro- and minituber production

The requirements for seed potato production are the production efficiency of the first tuber generation (including both technologies and propagation), tuber vigour and absence of plant diseases. Cultivars are selected for production based on the purpose of use – for table, food industry or as starch potatoes. Cultivars have genetic differences in terms of length of dormancy, sprout growth rate, number of developing sprouts, growing season development rate (early, middle-early/late) and disease resistance.

In northern production conditions, lightness (long days) and relatively warm nights inhibit tuber formation (Degamante & Van de Zaag & van Loon 1987, Maladi & Burns 2007). Using conventional methods to produce the first tuber generation is not only labour-intensive but also causes extra costs in terms of furnishing greenhouses with the necessary equipment. Provision must be made for lighting and shading as well as for cooling and heating. Regardless of the investments, conventional greenhouse production enables only one additional production period when environmental conditions are utilized.

Conventionally, minitubers are produced from *in vitro* produced plantlets in greenhouses or screenhouses and minitubers are directly field-planted. Microtubers have been described as an alternative to plantlets and utilized for minituber production (Dhital & Lim 2012). New kinds of micro- and minituber production technologies have been implemented and commercialized. The implementation of micro- or minituber production systems has contributed to improved self-sufficiency and cost-effectiveness. Despite these different production systems, many interactions between growth parameters have appeared to be genotype-specific (Donnelly *et al.* 2003).

1.3.1 Cultivars

Potato is worldwide propagated vegetatively via seed tubers (Dhital & Lim 2012). Potato develops tubers from underground stems called stolons. Its equatorial origin makes potato essentially short-day dependent for tuberization (Simmonds 1997, Kloosterman *et al.* 2013). Cultivars processed in Europe are usually of Chilean origin, as tuber formation of the Peruvian potato – accustomed to short days – is relatively poor at European latitudes (Ames & Spooner 2005,). Many potato cultivars begin tuber formation more quickly under short-day production conditions compared to long-day conditions (Brown 2011). Cultivars have also

been observed to react in different ways to long-day conditions (Almekinders & Struik 1994). European potato breeders usually test their cultivars in the largest and southernmost production areas, and the yield potential of these cultivars has not yet been systematically assessed in long-day production conditions. Finland's official cultivar list includes over 50 cultivars, the majority of which have been developed by European potato breeders. The most widely produced, certified seed potato cultivars are Van Gogh, Nicola, Fambo and Asterix .

In short-day production conditions it is important that emergence, plant development and tuber formation occur undisturbed and as quickly as possible. Generally, long days, high temperatures and nitrogenous fertilizers delay tuber formation (Krauss 1985, Maladi & Burns 2007). Cultivar characteristics also have an effect and, therefore, sprouting rate can be classified by cultivar: 1) slow sprouting and quick senescence, 2) quick sprouting and slow senescence, and 3) quick sprouting and quick senescence (Reust 1986). Irrespective of the origin or properties of the cultivar, maximum tuber formation in production is the overriding priority, and attention must therefore be paid at the beginning of the production chain to the selection of cultivars suitable for northern production conditions (Ewing & Struik 1992, Brown 2011).

1.3.2 Propagation techniques

Starting seed potato field production with minitubers is the most viable option in terms of overall utility (Rolot & Seutin 1999). Viral, bacterial and fungal diseases easily infect tubers and are immediately passed on to the next generation, diminishing the production capacity of potato plants. In seed potato production, the source material must be disease-free.

Regardless of the production technology used, the source material for micro- or minitubers is produced from in vitro microplantlets and propagation is conventionally achieved by distributing the plantlets (Fletcher *et al.* 1998) on a substrate containing both micro- and macro-nutrients (Murashige & Skoog 1962). In northern conditions, *in vitro* microplantlets have been used to produce minitubers in conventional peat-greenhouse production. In commercial minituber production, the aim is always to produce a large number of tubers per plant (Milinkovic *et al.* 2012).

In northern production conditions, environmental factors restrict the utilization of light and temperature in conventional greenhouse production. Faster, more cost-effective technology solutions with a higher production capacity are

therefore sought for potato minituber production. Various soilless, hydroponic, aeroponic (Ritter *et al.* 2001, Chang *et al.* 2012) and bioreactor-type (Akita & Ohta 1998) production methods have already been developed and are in use. Hydroponic culture systems cover a range of techniques, including water culture (Wan *et al.* 1994), modified water culture, NFT (nutrient film technique) (Boersig & Wagner 1988; Wheeler *et al.* 1990, Molders *et al.* 2012), and aeroponic systems (Kang *et al.* 1996; Nichols & Christie 2002; Farran & Mingo-Castel 2006). Deep-water culture systems (Lommen 2007) have the same buffer capacities for pH, nutrients, and temperature as aerohydroponics (Soffer *et al.* 1991). These techniques differ in terms of water or nutrient input and how the plants utilize these liquids. Fermenters and bioreactors are used for microtuber production. Laboratories or different types of greenhouses can be equipped with new types of micro-minituber production technologies, enabling the elimination of disturbing conditions or plant-disease pressure caused by the production environment (Molders *et al.* 2012; Mateus-Rodriguez *et al.* 2013).

Soilless production provides an alternative for reducing the cost of minituber production and possibly increasing production volume (Rolot & Seutin 1999, Scherwinski-Pereira 2009). The yields of the new technique are typically comparable with the minituber yields of conventional *in vitro* propagated plants. Even if it would be possible to make conventional minituber production more effective, conventional minituber production from *in vitro* plantlets involves a contamination risk, in addition to high production costs and relatively low tuber yield (Rolot & Seutin 1999). The objective of the basic pre-seed potato production concept is not only to increase the effectiveness and speed of the production chain, but also to gain more information on cultivars during propagation.

1.4 Aims of the study

The general aim of the thesis was to investigate seed potato (*Solanum tuberosum* L.) cultivation in the northern latitudes, High Grade cultivation area defined by the European Union. The study focused on 1) seed potato production conditions and methods, especially haulm killing and sprout control, which are presumed to affect seed potato quality (Papers I and II) and 2) propagation methods used prior to seed potato production (i.e. micro-minituber production) to influence the propagation efficiency and general cost-effectiveness of potato production (Papers III and IV).

Paper I: We studied the sprouting management strategies required in seed potato production areas in northern Finland, where storage lasts several months and the growing season is short. The hypothesis was that management strategies might differ between cultivars with differing maturing properties. Therefore, the study focused on the effects of haulm killing as a response to temperature accumulation, and the role of GA in sprouting control on early (Fambo) and middle-late (Van Gogh) maturing cultivars of seed potatoes.

Paper II: We hypothesized that the haulm killing methods used in seed potato production along with the timing of haulm killing may affect the crop and quality characteristics of seed potatoes. The effects of the chemicals used in haulm killing, and the effect of haulm killing methods on seed potato quality as compared to naturally senesced seed potatoes were studied.

Papers III and IV: The aim was to investigate and compare different new propagation technologies to accelerate and enhance the growing phase of first-generation tubers as cost-effectively and productively as possible. Thus, we studied microtuber production using a novel Liquid-Lab bioreactor production method (III) and minituber production implemented using the hydroponic method (IV).

2 Materials and methods

The materials and methods used are described in brief below. Detailed descriptions are presented in the original papers (I-IV).

2.1 Seed potato history

The seed potatoes used in the studies (I, II) were produced in the Tyrnävä-Liminka High Grade Area (64°N, 25°E). The seed potato materials were produced in field experiments and factors related to soil conditions, the production method used and the production conditions were taken into consideration in the study. Cultivars were selected for field experiments based on how widely used the cultivars are and on differences in early-late senescence. In the study on the effects of production history (haulm killing, gibberellic acid) (paper I), the cultivars used were the early cultivar Fambo and the middle-late Van Gogh. In the comparative study of haulm killing methods (paper II), the cultivar used was the middle-late Matilda.

2.1.1 Timing of haulm killing

The haulm killing was carried out 50, 75 and 95 days after planting (DAP). The mechanical and mechanical-chemical haulm killing methods were used; natural haulm senescence was used as the control. Mechanical-chemical haulm killing was carried out by first crushing the haulm to a height of 20–30 cm, and by spraying it with a haulm-destroying chemical two days after crushing. In addition, the temperature sums accumulated in the seed potatoes were recorded at different haulm killing times. The temperature sums were collected from automatic observation stations located in the production areas.

Harvesting was conducted ten days after haulm killing. The tubers were stored temporarily at +15 °C until the last experimental plot was harvested, and the total harvest from all plots was transferred to cold storage (+4 °C, relative humidity 90%). The harvested tubers were analysed for nutrient content, weight, starch content, tuber size distribution, and external quality. Tubers 30–40 mm in size were numbered and placed in egg cartons in the storage facility for sprout control and carry-over testing. After 25 weeks of cold storage (+4 °C) and three weeks of pre-sprouting at +14 °C, the seed potatoes were transferred to a grow tunnel for carry-over testing (paper I). The emergence dates of each individual

plant were noted and the developmental stages observed. The number and fresh and dry weight of the stems and roots and the number, fresh weight, starch content and external quality of the tubers were assessed individually.

2.1.2 Method of haulm killing

The effects of the haulm killing methods on haulm killing effectiveness and the external quality of the seed potatoes were studied (paper II). Haulm killing was carried out based on the size of the developing tubers in the tuber nest, i.e. when no more than 5% of the crop tubers were over 50 mm in size, which follows the general seed potato production practice.

Both mechanical and mechanical-chemical haulm killing methods were used, and natural haulm senescence was the control. In mechanical-chemical haulm killing, the efficiency of two different chemicals (diquat dibromide and carfentrazone-ethyl) were compared. The efficiency of chemical haulm killing was observed as browning of the green parts of the foliage on a 0–100% scale at 3, 7, 14 and 21 days after the chemical was sprayed (Paper I) and 10 days after haulm killing (Paper II). Plant re-growth was assessed on a scale of 0–100 (0=no growth, 100=growth in each plant).

Harvesting was carried out 21–26 days after the haulm killing depending on the year (Paper II). Yields were weighed and graded (<35 mm, 35–55 mm, 55–70 mm and >70 mm). External quality was assessed visually with damages divided into the following categories: damage caused by disease (healthy 1, damage types 2–5; scab (*Streptomyces scabies*), black scurf (*Rhizoctonia solani*), soft rot (*Pectobacterium carotovora ssp. carotovora*), black leg (*Pectobacterium carotovora ssp. atroseptica*, *Dickeya solani*), other fungal (*Fusarium sulphureum F. solani*, *Phoma foveata*, *Phytophthora infestans*) or bacterial diseases (*Phytophthora erythroseptica*, *Pythium ultimum*). Damages due to physiological or other causes (damage categories 6–10); turgor or growth cracks, misshapes, greenings, black spots, hollow hearts, internal necrosis, other damage. As an additional definition of external quality, the quantity of black scurf was observed separately in each tuber as a percentage of tuber surface.

2.1.3 Gibberellic acid treatment

The effect of the plant hormone gibberellins (GA) on seed potato sprout development and yielding capacity was examined by immersing seed potatoes

taken from cold storage in two concentrations (100 mM and 400 mM) of gibberellic acid solution, water was as the control treatment. After these treatments, the seed potatoes were allowed to sprout and the number of sprouts was counted three times during the sprouting period. After cultivating seed potatoes in a greenhouse, at harvest, the number and weight of stems and tubers were determined individually plant by plant.

2.2 Micro- and minituber production technologies

The microtuber production-technology studies were carried out in a laboratory (paper III) and minituber production-technology in growth rooms (paper IV). The cultures in the Liquid Lab^{TM} (L-L) system (paper III) were illuminated with 22–70 lmol $m^{-2}s^{-1}$ at room (20 °C) temperature. The stem elongation phase was carried out over a 16h photoperiod, and for induction and growth of microtubers, short-day conditions (10h light/14h dark) were applied. In the hydroponic system (paper IV) the light illumination was scaled for 2383–2509 µmol $m^{-2}s^{-1}$, and short daytime light conditions were implemented with a 14h (07:00 a.m.–09:00 p.m.) /8h (10:00 p.m.–06:00 a.m.) day/night illumination. Growth room temperatures were adjusted to 20±2 °C for daytime and 14±2 °C for the night period, air humidity was adjusted to 60–80%.

2.2.1 Liquid Lab^{TM} Rocker system

The cultivars used in the Liquid Lab^{TM} (L-L) Rocker study included the early-maturing Timo, the middle Velox, the middle-late Van Gogh and the late Asterix. The stock plants of the cultivars were regularly subcultured to obtain an appropriate number of shoots for L-L cultivation. For shoot growing and microtuber induction, thin-film cultivations were performed in the L-L system. The L-L vessels were equipped with microporous patches to allow gas exchange. Folded cotton cloth was placed on the bottom of the vessels (Fig. 3). The L-L cultivations were performed using a liquid MS medium with a sucrose concentration of 2% (w/v) for the stem elongation phase, and with 8% (w/v) sucrose for the microtuber induction and growth phase.

The L-L rocker was adjusted to move liquid from one side to the other (regular pitch) once per hour in the stem elongation phase, and once every 2 min in the microtuber induction phase. The stem elongation phase in the L-L vessels varied between 4–5 weeks depending on the potato cultivar and its growth rate.

Microtuber induction periods of 8 weeks (short cultivation time) or 10–11 weeks (prolonged cultivation time) were tested. After the complete culture period in the L-L system, the microtubers were collected and divided into five classes primarily by weight. The five classes were >1.00 g, 0.99–0.50 g, 0.49–0.20 g, <0.20 g.

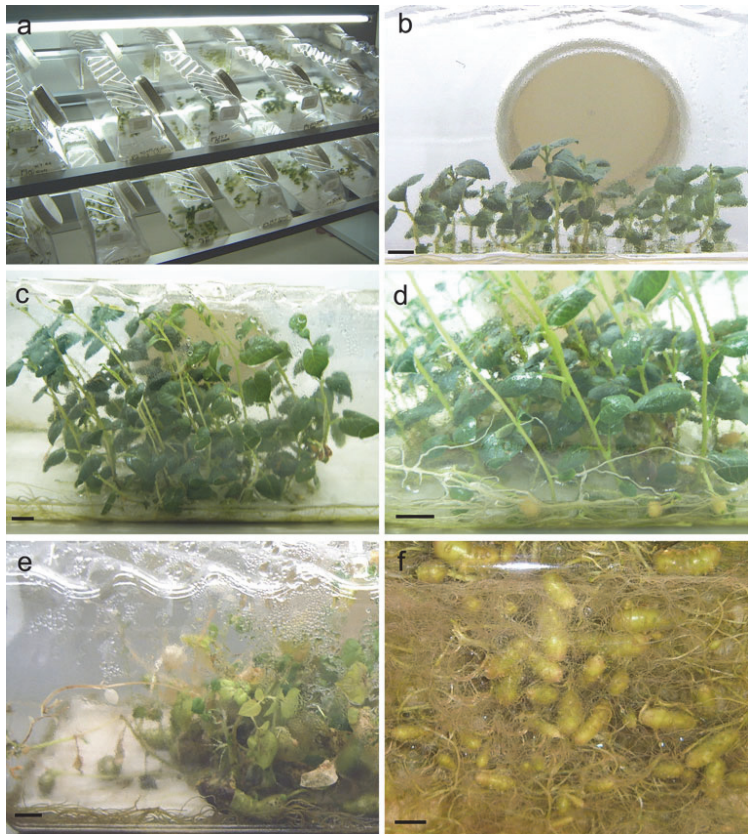

Fig. 3. Liquid Lab™ Rocker (L-L) system used for microtuber production of potato. The L-L system carrying vessels with potato explants (photo T. Kämäräinen-Karppinen).

2.2.2 Hydro Crop system

In the Hydro Crop system (HCS), the cultivars included the middle Desiree, the middle-late Van Gogh and the late Asterix. The roots of the microplantlets of cultivars grown on a regular semisolid MS medium were washed and the plants were transferred to hardening conditions. The hydroponic production system

consisted of a microplantlet hardening table and cultivation tables with growing trays. After 13 hardening days, the microplants were transferred to cultivation tables. In the hydroponic system, the plants are cultivated in growing trays; the roots are in contact with the cloth at the bottom of the tray and the cloth ensures uniform distribution of nutrient solution throughout the tray. The shoots of the microplantlets grow above a plastic sheet and the roots and the tubers develop between the cloth and the plastic sheet (Fig. 4.). The nutrient solutions recirculate via a filtersystem in the trays. Three different nutrient solutions were used in the circulation system depending on the plant developmental stage. During the microplantlet rooting phase and continuing until the initiation of tuber formation, nutrient solution 1 was used. At the initial phase of tuber formation, nutrient solution 2 was in the nutrient circulation. Solution 2 was then replaced by solution 3 during yielding. The nutrient solutions used were developed for hydroponic production. Hydroponic production was compared to a conventional minituber production method, and for this reason, microplantlets were also planted in a peat-based growing medium. Plant development and tuber formation were observed, and tubers were collected and divided into size classes of <20 and >20 mm. The dry matter and nutrient contents of leaf and tuber samples were analysed. After three months of cold storage (+4 °C) minitubers sized 10–-20 mm of the cultivars Van Gogh and Desiree were planted in a greenhouse for carry-over study. The emergence dates and the developmental stages were observed and at harvest, the number of stems, the number and weight of the tubers and the external quality of the tubers were assessed individually.

Fig. 4. Hydro Crop system (HCS) used for minituber production of potato. The HCS system carrying trays with potato plants (photo E. Virtanen).

2.3 Statistical analyses

The statistical analyses were conducted using the Mixed procedure of the SAS 9.2/SAS Enterprise Guide 4.3 (SAS Institute Inc., Cary, NC, USA) program, using a variance analysis model in compliance with the experimental model design (papers I, II, IV). In the study III analysis of variance (ANOVA, SPSS vers. 16.0 for Windows), two independent samples t-tests and a Bonferroni test were conducted.

3 Results and discussion

3.1 Seed potato history

Northern production conditions (temperature accumulation during the growing season, short growing season, long periods of daylight, and haulm killing), storage conditions (pre-storage 2 weeks +15 °C, cold storage 25 weeks +4 °C) and pre-sprouting (2 weeks +14 °C) factors affected the growth and yielding capacity of the seed potatoes. The sprouting efficiency of cultivars Fambo and Van Gogh differed after 20 and 25 weeks in the cold storage study. The early cultivar Fambo required only three weeks of sprouting to produce the maximum number of sprouts per potato eye (%), while Van Gogh required a longer period (paper I, figure 1.). According to Delaplace *et al.* (2008b), only one sprout develops in physiologically young seed potatoes, whereas several sprouts develop in older ones in which apical dominance no longer occurs.

3.1.1 Timing of haulm killing is important

Different haulm killing times (50, 75, 95 DAP) related to temperature accumulation sums did not result in any significant differences in the number of sprouts after three weeks of pre-sprouting. After production, 25 weeks of storage and three weeks of pre-sprouting, the seed potatoes accumulated total temperature sums of 3,198 °C (50 DAP), 3,284 °C (75 DAP) and 3,336 °C (95 DAP). Haulm killing affected the emergence of cultivars Fambo and Van Gogh as carry-over. Seed potatoes whose haulms were destroyed three weeks after flowering (75 DAP) emerged fastest, whereas those whose haulms senesced naturally until harvest (95 DAP) or were destroyed at the time of flowering (50 DAP) emerged more slowly. With regard to emergence rate, no significant difference was observed between the cultivars. The time of haulm killing in seed potato production has not previously been observed to have a carry-over effect on the emergence rate of seed potatoes as demonstrated by our study (paper I).

Haulm killing also affected the root and stem bulk produced. The physiologically older the seed potato, the larger the root and stem bulk at 95 days after planting. Root and stem bulk production was lowest in seed potatoes whose haulm had been destroyed at the time of flowering. The cultivars also differed significantly with regard to root and stem bulk, with Van Gogh being the most

productive. Naturally matured seed potatoes showed physiological behaviour similar to old seed potato. In most previous studies, older seed potatoes have produced several main stems (Iritani & Weller 1987; Knowles & Knowles 2006), whereas in the studies by Ezekiel (2004), seed potato age had no influence on stem number. The temperature sum accumulation increases the number of stems in seed potato (Bodlaender & Marinus 1987, Jenkins *et al.* 1993), whereas growing season temperature and daylength do not influence the number of seed potato stems or bulk stems (Johansen *et al.* 2002, Johansen & Nilsen 2004). Northern origin and daylength also do not influence yield capacity (Ezekiel 1997, Johansen *et al.* 2002, Knowles & Knowles 2006, Johansen *et al.* 2008). In the present study, haulm killing in seed potato production had no carry-over effects on yield, size or number of tubers. Thus the results of the present study are consistent also with Wurr *et al.* (2001).

3.1.2 Methods of haulm killing work well

In the study of haulm killing methods (paper II), haulm killing was conducted when no more than 5% of the tubers were over 50 mm in size, and the time between destruction and harvest (21–26 days) was similar to seed potato production practice. Mechanical-chemical haulm killing was effective, destroying the haulm completely in two weeks (paper II, Figure 1). Of the herbicides, diquat dibromide (200 g/l) acted faster than carfentrazone ethyl (60 g/l). The differences in efficiency of the chemicals used for haulm killing are generally minor. In earlier studies diquat was also found to have a slightly more rapid effect than other chemicals (Ivany 2003). Mechanical haulm killing also worked well.

However, when haulm killing was used to regulate tuber size in short growing-season production conditions, in many cases the haulm had to be destroyed while still completely green and capable of photosynthesis. Earlier studies have underlined that in order to reach adequate efficiency, more chemicals are needed to destroy vital foliage than to destroy more matured foliage (van Evert *et al.* 2012). To keep the use of haulm killing chemicals at a reasonable level, additional tuber growth and photosynthesis can be efficiently interrupted by means of mechanical-chemical haulm killing.

However, when haulm killing was applied to unsenesced foliage, our study showed re-growth. Re-growth and reformation of photosynthetic capacity manifested in one year as increased yield levels following mechanical haulm killing, as compared to mechanical-chemical haulm killing. The results were

contrary to the studies of Waterer (2007) in which the yield levels after mechanical haulm crushing were lower compared to yield levels after mechanical-chemical haulm crushing. Compared to other haulm killing methods, re-growth was least following haulm pulling (Misener & Everett 1981, Halderson *et al.* 1988) because disconnecting foliage from the roots leads to instant cessation of phloem transport and interruption of growth (Tiessen *et al.* 2002). After haulm killing, previously synthesized metabolites may increase in the still green stems and in the remaining bottom leaves (Halderson *et al.* 1988) and growth-stimulating hormones have an opportunity to exert their effect on re-growth (Tiessen *et al.* 2002). Regardless of the method used, haulm killing is most effective if carried out when the haulm has already started to senesce naturally (van Evert *et al.* 2012).

As the control, naturally senesced haulm produced the largest yield (40.3 t/ha) and the tuber starch content (17.2%) reached the level typical of the cultivar (paper II, Figure 2). On average, mechanical haulm killing affected yield quantities and starch contents in the same way as mechanical-chemical haulm killing. At the annual level, the variance was such that during the first year, both the yield quantity (31.4 t/ha) and starch content (14.8%) were higher in mechanical haulm killing than in mechanical-chemical haulm killing. During the other years, there were no significant differences. The average mechanical-chemical yield level was 29.2 t/ha and starch content varied between 12.3–15.8%. The smallest <35 mm part, 7% of the total yield, was produced by the control (natural senescence). The <35 mm size fraction was 17% in mechanical haulm killing and 20% in mechanical-chemical haulm killing. The 35–55 mm fraction was 71% in the control (natural senescence), 78% in mechanical haulm killing and 76% on average in mechanical-chemical haulm killing. Only naturally senesced haulm produced >70 mm tubers (6%).

The study showed that it is possible to regulate tuber size distribution by means of haulm killing. This is, however, achieved at the cost of yield quantity and starch content. As a method of controlling tuber size, haulm killing is easy to implement, and >70% of the tubers were distributed in the preferred size in our study. According to Struik & Wiersema (1999) and Kumar *et al.* 2009, the lower starch content and yield quantity produced by haulm killing, as compared to the yields produced by natural senescence, are the natural result of haulm killing carried out at an early stage. When haulm killing is used in seed potato production to achieve optimal tuber size distribution for commercial purposes, the physiological state of seed potatoes cannot be predicted. In addition to

physiological state, the relatively low starch content of seed potatoes to which haulm killing is applied, may affect their vitality (Sabba *et al.* 2007) to produce new sprouts and roots during the next growing season.

There was abundant black scurf in the potato tubers (paper II, figure 3). The quantity of black scurf varied between 0–60% by treatments and there was also variance between different years of the study. In the first two years mechanical-chemical haulm killing increased the occurrence of black scurf (30% on average) compared to other treatments. Mechanical haulm killing also increased the occurrence of black scurf (27% on average) compared to naturally senesced plants.

A 10–14-day interval between haulm killing and harvest is considered adequate in terms of periderm maturity, depending on the cultivar and the conditions (Waterer 2007). In northern seed potato production conditions, haulm killing is applied to immature plants and harvest is delayed by more than three weeks after haulm killing, weather conditions permitting. Tuber crops are known to be exposed to infection by black scurf if the haulm is destroyed while the root system is still active and if the time between haulm killing and harvest is prolonged (Tsror 2010). Black scurf caused by *Rhizoctonia solani* (Kühn AG-3) leads to substantial economic losses (Lootsma & Scholte 1996), and in seed potato production, it may be an obstacle to certification. In the present study, especially mechanical-chemical haulm killing increased black scurf. The results are similar to those of Dijst (1988), which showed that chemical haulm killing and stem cutting, along with a prolonged period between haulm killing and harvest, stimulated the formation of black scurf. According to Dijst (1988), water-insoluble components enable the formation of scleroses on the tuber surfaces, and the changes taking place in the relationship of inhibiting and stimulating factors after haulm killing could influence sclerosis formation. In addition, the colonization of *R. solani* on the ground before harvest is a factor that significantly influences the occurrence of black scurf in tubers. Weather conditions are also significant in terms of the occurrence of *R. solani*, and wet and low temperatures are in its favour (Lootsma & Scholte 1996). In the present study, black scurf occurrence in tubers was the lowest after a dry growing season and highest after cool and moist conditions.

3.1.3 Gibberellic acid is effective in interrupting dormancy

Gibberellic acid (GA) treatments did not result in any significant differences in the number of sprouts after three weeks of pre-sprouting. GA treatments increased the number of stems in both cultivars, with a greater effect in Fambo than in Van Gogh when compared to the control treatment, even though the treatments did not affect the number of sprouts. The GA treatments had a negative effect on crop yield (g/plant), in particular when the cultivar Fambo was treated with 400 mM GA. Correspondingly, a 100 mM GA concentration increased the number of tubers in Fambo, in comparison to water and 400 mM GA treatment (paper I, figure 2).

Salimi *et al.* (2010) have reported GA to be effective in interrupting the dormancy of minitubers. However, the sprouts that developed in the GA-treated tubers were easily broken during handling and planting. Other sprouting inhibitors (ethyle chlorothydrin, ethanol, CIPC, DMN) are also effective in interrupting dormancy or sprouting management (Beaver *et al.* 2003, Bajji *et al.* 2007, Teper-Bamnolker *et al.* 2010, 2012, Saraiva & Rodrigues 2011, Daniels-Lake 2013), but the effects of treatments on seed potatoes have not been studied. Alexopoulos *et al.* (2008) used GA at a concentration of 1–50 mg/l and found that treatment duration appears to be more important than GA concentration. In their sprouting management studies, Pruski *et al.* (2006) discovered that treatment of seed potatoes with ethylene during storage resulted in higher numbers of sprouts and tubers, but did not result in higher crop yield. In the present study, GA treatments were in line with the results of Pruski *et al.* (2006), i.e. 100 mM GA concentration increased the number of tubers in Fambo but did not affect crop yield.

3.2 Micro- and minituber production technologies

3.2.1 Liquid Lab^{TM} Rocker system for microtubers

All cultures of the Liquid Lab^{TM} Rocker system (L-L system) were carried out at room temperature and without contamination problems. Each cultivar (Asterix, Timo, Van Gogh, Velox) formed microtubers in the L-L -system. The mean number of microtubers per vessel (50 explants) varied between 30 (Asterix in 8-week tuber induction) and 75 microtubers (Velox in 11-week tuber induction). The majority (63%) of microtubers were of sufficient size and weight (above 200

mg) for further storage at dormancy. Velox yielded the highest number of microtubers with cultivation capacity. As a result of prolonged 2–3 week microtuber induction, more microtubers with potential for cultivation were obtained per cultivar, with the exception of Van Gogh. Nevertheless, the mean weight of the Van Gogh microtubers was significantly higher after prolonged microtuber induction (0.67g) compared to short induction (0.51 g). Thus, the microtuber yield was in accordance with that obtained by Nhut *et al.* (2006), but clearly less than reported by Jiménez *et al.* (1999) and Akita and Takayama (1994) (Paper III, Table 1). However, the amount of nutrient medium per explant in our experiment was lower in the tuber induction phase than that used by Jiménez *et al.* (1999) and Akita & Takayama (1994), which could explain the difference. The overall microtuber production time in the L-L system was reasonably short compared to other liquid cultivation techniques.

Several types of semisolid media in various cultivation vessels have been used for microtuber production of potato in the past (Donnelly *et al.* 2003). In addition, liquid cultures (Estrada *et al.* 1986) together with different temporary immersion techniques (Piao *et al.* 2003), such as ebb and flow in glass fermenters (Akita & Takayama 1994), the Rita^{TM} system (Teisson & Alvard 1999), the twin-flask system (Jiménez *et al.* 1999) and nutrient mist bioreactor (Hao *et al.* 1998), as well as plastic bag cultivations (Grigoriadou & Leventakis 2003) have been studied for production of potato microtubers. Most of these techniques, however, involve the use of relatively high-cost machinery and compressed air to aerate the cultures. In the L-L bioreactor, porous patches attached to each side of the vessel permit gas exchange, which at least partially may eliminate the effect of ethylene. One advantage of the L-L system over rotatory shakers is that the plants can be grown in large vessels, which reduces mechanical stresses that can cause growth abnormalities. Furthermore, mechanical stress may generate the production of ethylene, which has a negative influence on the growth of potato plants (Zobayed *et al.* 2001). Our experiments were carried out without any growth regulators or retardants to enhance microtuber formation (Harvey *et al.* 1991) or to increase microtuber number and weight (Piao *et al.* 2003).

3.2.2 Hydro Crop system for minitubers

The production of minitubers in the Hydro Crop system (HCS) succeeds in indoor conditions with the cultivars Desiree, Van Gogh and Asterix. Within 37 days from planting on the trays, Desiree and Van Gogh foliage covered the entire tray. Tuber

formation started after 41 days of cultivation in the Desiree and Van Gogh cultivars. Asterix foliage development was almost 3 weeks behind Van Gogh and Desiree. Asterix tuber formation started 62 days after the microplantlet phase. When plants were cultivated in growth rooms in a peat-based growing medium, foliage development occurred almost 3 weeks later and also tuber formation started approximately 18 days later in all cultivars compared to hydroponic production.

Hydroponically-grown tubers were harvested 96 days after planting on the hardening table. The minituber yield was 4.5 per plant for the cultivar Desiree, 7.5 for Van Gogh and 4.0 for Asterix. The minitubers were graded in the following size categories <20 mm and >20 mm; 56.1–58.4% of the Desiree and Van Gogh cultivars and 95.3% of the Asterix cultivar were in the <20 mm size category. The average weight of the minitubers was 11 g for the cultivar Desiree, 13 g for Van Gogh and 6 g for Asterix (paper IV, table 1). In peat-based production, Desiree produced 4.4 minitubers per plant, Van Gogh 4.6 and Asterix 3.3. The peat-produced minitubers were all <20 mm in size and weighed 5–9 g. The number of tubers per plant in hydroponics was higher than in peat-based production. Our results are similar to Corrêa *et al.* (2008).

Tuber formation of the Desiree and Van Gogh cultivars began similarly to that described by Chang *et al.* (2012) (within 30–65 days, depending on the cultivar and the production technique). Yields (4.5–7.5 per plant) are comparable to the hydroponic production levels obtained by Ritter *et al.* (2001) and Novella *et al.* (2008; also with regard to tuber weight (11–13 g). Tuber formation of the cultivar Asterix (63 days) was considerably slower than that of Desiree or Van Gogh, and also the average weight of the tubers was lower (6 g). According to Chang *et al.* (2008), hydroponic production techniques may not be favourable with late-maturing cultivars because the nutrient solutions may retard root and stolon growth.

There were no significant differences in the nutrient concentrations of leaf or tuber samples between hydroponic potato production and potatoes cultivated in a peat-based growing medium. Calcium concentrations were slightly (not significantly) higher in hydroponically-produced tubers compared to tubers produced using a peat-based growing medium. Correspondingly, manganese and iron concentrations were slightly (not significantly) higher in peat-produced tubers. In the carry-over study conventionally produced minitubers emerged faster, and performed better. The carry-over effects indicate that the cultivars used should be studied further in order to optimize minitubers yielding.

The heat produced by the high-pressure sodium lamps could not be controlled in the present study with the air conditioning equipment used. This was particularly evident in the variation in day and night temperatures from ca. 20–24 °C by day and 17–19 °C by night. However, this variation did not affect tuber formation, but but the total yields of cultivars could be higher. According to Vreugdenhil *et al.* (2007), long periods of daylight and high temperatures inhibit tuber formation and high night-time temperatures are more damaging than high daytime temperatures.

4 Conclusions

The results show that effective management strategies and methods for seed potato production are especially important in conditions where potato storage lasts several months and the growing season is short. Even though the metabolism of seed potatoes (dormancy, sprouting management) can be controlled, the effects of production history have to be predicted and managed. In this study, the effect of northern production conditions indicate that the tubers behaved as physiologically young seed potatoes regardless of cultivar properties or haulm killing. The physiological state of tubers designated as seed potatoes needs to be better recognized in order to optimize pre-sprouting and the use of sprouting inhibitors. In the case of Fambo, gibberellic acid treatment influenced the characteristics of seed potatoes produced in the present study, revealing a potential way to improve seed potato production in northern conditions.

The results of this study show that in seed potato production the timing of haulm killing should not be determined only by tuber size, even if the haulm killing methods used work effectively, as the most lucrative part of the potato yield in terms of tuber size may be lost due to plant disease. More research is needed to optimize the timing and methods of haulm killing to suppress the different soil- and seed-borne diseases. The results provide significant new insights regarding haulm killing in northern latitudes (65º40ºN), seed potato production of the cultivar Matilda, and potential haulm killing methods for use against plant diseases (e.g. black scurf).

Cost-effective and high-yield production of the first tuber generations (micro- or minitubers) offers several benefits for the seed potato industry. In this study, novel, efficient and rapid systems for seed potato production were compared. The Liquid Lab^{TM} Rocker is liquid culture technique for mass propagation. Liquid cultures offer several advantages over cultivation on semi-solid media by which microtubers are generally produced. In the present study, the replacement of liquid medium in 30 Liquid-Lab vessels, containing altogether 1 500 shoots, was carried out within one working day. The Liquid Lab^{TM} system saves time as, dozens of explants can be made at once. The tuber formation was induced by alteration of sucrose concentration combined with change in light regime for short-day conditions. Each cultivar (Asterix, Timo, Van Gogh, Velox) was able to form microtubers in the system. The mean number of microtubers per vessel (50 explants) varied between 30–75. In addition, the L-L machinery contains a lighting system, which together with the above-mentioned modifications enables

its use also in outside laboratory facilities. In conclusion, the L-L rocker system turned out to be very suitable for potato microtuber production.

In the northern conditions the environmental factors limit the utilization of illumination and temperature in conventional minituber production in greenhouses. Thus, soilless and indoor production techniques provide an alternative for minituber production. In the present study we proved that indoors hydroponic production system and artificial simulation (light, temperature, humidity) of the production environment enabled minituber production. Yields (4.5–7.5 per plant) were comparable to the hydroponic production levels obtained by greenhouses. Tuber formation of the cultivars Desiree, Van Gogh and Asterix differed, Asterix was considerably slower. As a comparison, minitubers from in vitro plantlets were grown in a peat-based growing medium and the yield of minitubers were higher in Hydro Crop System. However, in the carry-over study conventionally produced minitubers emerged faster, and performed better. The carry-over effects indicate that the cultivars used should be studied further in order to optimize the minituber production methods.

Our objective of seed potato studies was not only to use technology to increase the effectiveness and speed of the production chain, but also to gain more knowledge of the behaviour of the cultivars propagated by these techniques. This is, however, a good start for more in-depth studies at cultivar level to recognize the best cultivar / propagation technique combinations in order to lead cost effective high tuber yields.

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