Basic studies on the production and performance of potato minitubers

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Stellingen

1.
Verschillen in veldgedrag tussen miniknollen en conventionele pootaardappelknollen worden behalve door verschillen in gewicht ook veroorzaakt door verschillen in fysiologische ontwikkeling van de knollen.
Dit proefschrift.

2.
Naarmate aardappelknollen lichter zijn, zijn de effecten van absolute en relatieve verschillen in gewicht op het gedrag van de knollen en planten uit die knollen duidelijker. Het gebruik van klein pootgoed vraagt dan ook een andere benadering dan het gebruik van conventionele knollen.
Dit proefschrift

3.
De lagere gewichtsopbrengsten aan knollen die door gewassen uit kleinere miniknollen op een in Noordwest-Europa voor pootaardappelen gebruikelijk oogsttijdstip worden gerealiseerd, zijn zowel een gevolg van een lagere lichtonderschepping door het loof als van een lagere oogstindex.
Dit proefschrift.
Marshall, B. & H. Taylor, 1990. Radiation interception and growth of minitubers as affected by seed size. *Abstracts 11th Triennial Conference of the European Association for Potato Research, Edinburgh, UK*, pp. 380-381.

4.
Beschrijvend en verklarend onderzoek naar de gezondheid, de genetische kwaliteit en de groeikracht van latere generaties 'normale' knollen geproduceerd uit micro- en miniknollen, blijft hard nodig.

5.
Gebrek aan consistentie in de definiëring van begrippen of in de analyse van problemen of processen kan duiden op wetenschappelijke vooruitgang.

6.
Bij het ontwerpen van nieuwe produktieprogramma's voor uitgangsmateriaal ontbreken methoden waarmee ongelijksoortige kwaliteitseigenschappen (zoals gebruiksgemak, betrouwbaarheid en gezondheid) bij elkaar kunnen worden opgeteld.

7.
De invloed van de grootte of het gewicht van het gebruikte uitgangsmateriaal op de ontwikkeling en opbrengstvorming van een gewas is nog grotendeels onbegrepen.

8.
Het is verwarrend om het moment waarop een aardappelknol wordt geïnitieerd, aan te merken als het moment waarop de kiemrust begint, omdat tijdens de groei van de knol de apex doorgaat met het afsplitsen van bladeren.
Burton, W.G., 1963. Concepts and mechanisms of dormancy. In: J.D. Ivins and F.L. Milthorpe (eds), *The Growth of the Potato*. Butterworths, London, pp. 17-41.

9.
Het heeft weinig zin de invloed van toevoeging van groeiregulatoren op de opbrengst aan hoogwaardige aardappelknollen *in vitro* te bestuderen in batchcultures, als de samenstelling of de hoeveelheid van het basismedium al limiterend is voor deze opbrengst.
Leclerc, Y., D.J. Donelly & J.E.A. Seabrook, 1994. Microtuberization of layered shoots and nodal cuttings of potato: The influence of growth regulators and incubation periods. *Plant Cell, Tissue and Organ Culture* 37: 113-120.

10.
Het is onwaarschijnlijk dat onder veldomstandigheden een aardappelknol met een diameter van 5 mm uit eigen reserves een stevige, groeikrachtige plant met een bladoppervlakte van meer dan 2 cm^2 kan produceren.

11.
Bij een strakke studieplanning staan procedures die vragen om vroegtijdige goedkeuring en planning van (vooral) veldonderzoek het realiseren van de leerdoelen van afstudeervakken in de weg.

12.
In de agronomie zou het een enorme vooruitgang zijn wanneer ook oudere literatuur in geautomatiseerde literatuurbestanden werd opgenomen.

Stellingen behorend bij het proefschrift 'Basic studies on the production and performance of potato minitubers', door W.J.M. Lommen.
Wageningen, 7 april 1995.

W.J.M. Lommen

Basic studies on the production and performance of potato minitubers

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 7 april 1995,
des namiddags om vier uur in de Aula
van de Landbouwuniversiteit te Wageningen.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Lommen, W.J.M.

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Subject headings: Solanum tuberosum L. ; seed production / Solanum tuberosum L. ; rapid multiplication.

ABSTRACT

Lommen, W.J.M., 1995. Basic studies on the production and performance of potato minitubers. Doctoral thesis, Wageningen Agricultural University, Wageningen, the Netherlands, 181 pp., English and Dutch summaries.

Minitubers are small seed potato tubers that can be produced year-round in glasshouses on *in vitro* propagated plantlets planted at high density. The research reported in this thesis studied the agronomical and physiological principles of the production of minitubers and their performance under Dutch field conditions. The minitubers had fresh weights between 0.125 and 4.000 g.

More than 3000 minitubers per m^2 were produced in 10 weeks (average fresh weights 1 - 2 g), when tubers were harvested 4, 7 and 10 weeks after planting, using a non-destructive harvesting technique in the first two harvests. Removing tubers in the first harvest resulted in initiation of new tubers because more potential tuber sites became available that were not subjected to the dominance of rapidly growing tubers. Part of the newly initiated tubers grew to a harvestable size within three weeks, but the number of tubers in harvestable sizes did not increase thereafter, whereas part of the undersized tubers was resorbed. The second harvest stimulated growth of tubers that otherwise would have been resorbed or would have remained too small.

Almost all minitubers ≥ 0.5 g survived storage at 2 °C for 1.5 years. After 6 months of storage, growth of plants from minitubers was still poor. Largest leaf areas were achieved after 10 - 11 months of storage, highest stem numbers, progeny tuber weights and harvest indices after 14 - 15 months of storage for cv. Agria and after 18 - 19 months for cv. Liseta.

The performance of minitubers was affected considerably by their weight. Lighter tubers had a longer dormant period, partly because of a slower sprout growth up to 2 mm (used to assess the end of dormancy). Plants from lighter tubers took longer to emerge and at emergence had thinner stems, lower root weights, and higher shoot:root ratios. Crops from lighter minitubers produced lower yields because of less radiation intercepted (slower ground cover) and a lower harvest index. Multiplication factors per planted tuber were lower in crops from lighter minitubers because fewer plants emerged or survived, and fewer progeny tubers and lower weights were produced per plant. Yield variation within a crop was higher in crops from lighter minitubers, but - when properly nursed - variation in yield over years was not affected by the weight. Effects of minituber weight generally became less clear in the higher weight ranges. Differences in performance between minitubers and conventional tubers were attributed to weight and age of seed tubers, presprouting method and crop husbandry.

Minitubers can be used in the first year of potato seed production programmes to speed up multiplication and to increase the quantity of seed from new cultivars.

Keywords: *Solanum tuberosum* L., seed production, minitubers, rapid multiplication, *in vitro*, tuberization, tuber pruning, non-destructive harvest, seed weight, nutrient supply, plant density, cold treatment, sprouting, water loss, physiological age, presprouting, planting depth, shoot:root ratio, emergence, ground cover, radiation interception, radiation conversion, harvest index, variation.

Reference to chapters 2 - 10 should be made citing the original publications.

CONTENTS

WOORD VOORAF

Ruim acht jaar geleden kwamen de eerste berichten uit het buitenland over miniknollen die ongekende mogelijkheden leken te bezitten voor een snelle, goedkope produktie van gezonde pootaardappelen, ook in Nederland. Hoewel verschillende firma's produktiemethoden of miniknollen te koop aanboden, was er nauwelijks betrouwbare, open informatie over de produktie en het gedrag van de knollen beschikbaar en ervaring met hun produktie en gedrag ontbrak. Om dit te verbeteren werd dankzij de inzet van met name dr ir D.A. van der Zaag en dr ir I. Mastenbroek een onderzoeksproject opgezet dat gedeeltelijk werd gefinancierd door het aardappelbedrijfsleven (via het Produktschap voor Aardappelen en de Nederlandse Aardappel Associatie) en gedeeltelijk door de Landbouwuniversiteit. Het project werd door mij van september 1987 tot en met december 1990 uitgevoerd op de toenmalige vakgroep Landbouwplantenteelt en Graslandkunde van de Landbouwuniversiteit Wageningen, nu de vakgroep Agronomie en het proefcentrum Unifarm. Een enthousiaste begeleidingscommissie, die bestond uit dr ir D.E. van der Zaag (voorzitter tot 1 januari 1990), ir C.D. van Loon (voorzitter vanaf januari 1990), prof. dr ir P.C. Struik (secretaris), drs K.J. Hartmans, dr ir I. Mastenbroek en dr D. Vreugdenhil, bewaakte namens de Nederlandse Aardappel Associatie de voortgang van het onderzoek, bediscussieerde de resultaten en de ontwikkelingen elders, gaf advies over de te volgen onderzoekslijnen en zorgde ervoor dat naast het wetenschappelijke belang ook het praktische, maatschappelijke belang voldoende aandacht kreeg. Bij het onderzoek werd ik de eerste jaren geassisteerd door Evelien van Heusden. Ze heeft me wegwijs gemaakt op de vakgroep en in het gewas aardappel. Mede dankzij tal van medewerkers van de vakgroep, studenten, stagiairs en gastmedewerkers, werd in korte tijd een schat van informatie verzameld over de produktie en het gedrag van miniknollen. De duizenden *in vitro* planten die in deze periode voor proeven werden gebruikt, werden geproduceerd door Theo Meulendijks en zijn team van de Stichting Begeleiding Snelle Vermeerdering van Aardappelen. Van hem heb ik niet alleen veel geleerd over de produktie en het gebruik van *in vitro* aardappelplanten in de praktijk, maar ook over de teelt en keuring van pootaardappelen.

Het idee om een proefschrift te schrijven over miniknollen ontstond pas toen ik een baan kreeg als universitair docent bij de Landbouwuniversiteit en was afkomstig van mijn promotor Paul Struik. De wetenschappelijke publikaties die in dit proefschrift zijn opgenomen, zijn grotendeels geschreven als onderdeel van mijn huidige taak. Ze zijn gebaseerd op de resultaten van de proeven die in het kader van het bovengenoemde onderzoeksproject waren gedaan en enkele proeven die daarna zijn uitgevoerd. Het Engels uit de reeds gepubliceerde hoofdstukken werd gecorrigeerd door mijn vroegere buurvrouw - tevens vertaalster - Miep Schilte, en door de language-editors van het tijdschrift Potato Research, de heren Fox, Hide en Wastie. Wampie van Schouwenburg zorgde voor druk op de ketel nadat de artikelen waren geaccepteerd.

Aan de proeven die zijn verwerkt in dit proefschrift werd meegewerkt door de studenten Ruilof van Putten, Harm Kuipers, Lukas Wolters, Roelof Kramer, Jan Broos, Fokko Prins en Bert Waterink, de stagiair(e)s Ben Glas, Sigrid Wiersum en Ankie Bos en gastmedewerkster Jadwiga Płodowska. Vanuit de vakgroep en het proefbedrijf zorgden vooral Elco van Doorn, Jan van der Pal, Lammert

Haalstra en Ton Blokzijl er voor dat alles groeide zoals het hoorde, maar ook John van der Lippe, Teus Bleyenberg, Wim van der Slikke, André Maassen en vele anderen hebben regelmatig aan de voorbereiding, de verzorging of het oogsten van proeven meegewerkt. Voor praktisch advies of om uit te blazen kon ik altijd terecht bij mijn collega's. Vooral bij mijn lotgenoten Conny Almekinders en Leon Mol liep ik regelmatig binnen.

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Willemien Lommen

NOTE

Chapters 2 - 9 of this thesis have been published in Netherlands Journal of Agricultural Science (chapters 2 and 3) or Potato Research (chapters 4 - 9). Chapter 10 has been submitted for publication by Potato Research.

As presented in this thesis, the published chapters differ in the following ways:

(1) The original running title of chapter 2 is: Minituber production of potato plantlets;

(2) The original running title of chapter 3 is: Potato minituber production;

(3) The listing of references has been standardized and updated;

(4) Minor alterations have been made to guarantee a more consistent spelling throughout the thesis;

(5) Minor alterations have been made in the presentation of Tables.

The structure of the papers has not been changed.

CHAPTER 1

General introduction

1 GENERAL INTRODUCTION

1.1 Reproduction of potato

Potato (*Solanum tuberosum* L.) is one of the world's major food crops, grown for its edible tubers. Worldwide, it is planted on approximately 18 million hectares in 128 countries (FAO, 1994). Seed tubers are by far the most important planting material used and around 10 % of the area under potato cultivation is necessary for the production of successive generations of seed tubers. Because of the problems (see later) occurring when seed tubers successively are produced from other seed tubers, new production systems for seed tubers are being developed.

Except from seed tubers, potato plants can be produced from several types of propagation material including protoplasts, callus and explants from different tissues (all reviewed by Evans et al., 1981), dissected meristems (e.g. Wang & Hu, 1980), cuttings made from sprouts or stems segments with at least one bud (shoot tips, nodal or apical cuttings, e.g. Goodwin et al., 1980; Bryan et al., 1981a, b, c) and true potato seeds (e.g. Umaerus, 1987). For seed production purposes, multiplication methods using tubers or nodal and apical cuttings are preferred because they are genetically conservative as new plants are produced from existing buds. This thesis studies the production and performance of minitubers, one of the new types of propagules that can be used for the production of conventionally sized seed tubers.

1.2 Morphology of the potato plant

Details on the morphology of the potato plants are recently described by Beukema & van der Zaag (1990) and Cutter (1992). Under normal conditions and crop husbandry, the potato plant possesses one or more negatively geotropic (upright) growing stems, the main stems, which carry leaves above ground. Below ground buds of the main stems may produce orthotropically (more or less upright) growing leafy stems, called secondary stems. The leafy stems often branch and may end in inflorescences, which produce berries that contain the true potato seeds. Below ground, also stolons develop from axillary buds of the stems. Stolons are thin and elongated diageotropically (horizontally) growing stems with scale leaves and a hooked tip. Roots develop adventitiously, usually on leafy stems and stolons. Potato tubers actually are also drastically shortened and thickened stems. They normally form at the tip of the stolons or their branches.

From the above description it is clear that a potato plant can produce three main types of stems: the normal stems (either sprouts or leafy stems), the stolons and the tubers. All these possess apical and axillary buds that potentially can produce one of the three types of stems, depending on the internal and external conditions. This potential can be exploited in vegetative propagation of potato.

1.3 Multiplication of potato by means of seed tubers: the conventional seed production system

When potato is multiplied by seed tubers, the multiplication factor (here the ratio between the weight

of tubers produced and planted) is 12 - 20 (Beukema & Van der Zaag, 1990). This is low compared to other important food crops like wheat, rice and maize with multiplication factors of 50, 100 and 250 respectively (Van der Zaag, 1987) and soya bean with a multiplication factor of 30 - 100 (area basis; Fehr, 1978), but higher than of cassava of which around 10 cuttings per plant can be taken (Silvestre, 1989). If one crop of potatoes is grown in one year, as in most north-west European countries, the multiplication rate is only 12 - 20 per year. Therefore, several years of field multiplication are necessary to produce the total quantity of seed needed. Special efforts are needed to maintain a high health standard in subsequent generations, because potato is susceptible to diseases which may be transferred through the seed tubers.

In many countries, healthy seed is produced by clonal selection, repeatedly propagating a sample of tubers that often originates from one plant having the desired phenotype ('true to type') and being free of diseases. A complete seed production programme consists of the production of three categories of seed: (1) clonal selection or pre-basic seed in the first 1 - 4 years, (2) basic seed in the next 1 - 3 years, and (3) certified seed production in the final 1 - 3 years. Within a category, there are different quality classes. During the first years of multiplication, seed tubers are produced by specialised growers, growers' cooperatives, companies or institutes, depending on the country (Oosterveld, 1987). In later years, seed tubers are produced by growers. The quality of the seed is usually checked by the inspection services, which certify the seed when it meets the quality standards. Seed tubers in the Netherlands are automatically declassified one class after each step of multiplication.

Seed tuber production is characterised by crop husbandry techniques aiming at reducing the risk of obtaining diseases or multiplying off-types. They include using healthy planting material, roguing diseased and deviant plants, and controlling pathogens or the vectors that transmit them. An apparent feature of seed potato production in the Netherlands is the short growing season: the haulm of the seed crop is killed before the number and the activity of aphids that transmit viruses from diseased to healthy plants become unacceptably high.

1.4 Recent developments in seed production: new production systems

1.4.1 Why new systems?

The main disadvantages of a conventional seed programme are the low multiplication rate of field-grown potato plants, resulting in a slow and inflexible system, and the increasing risk of catching viral, bacterial or fungal diseases with an increasing number of field multiplications. In north-west Europe, especially *Erwinia* species are threatening because they may remain latent (e.g. Weber, 1990) and are difficult to control. Seed programmes in which the desired amount of seeds is produced in fewer years would alleviate both disadvantages and may improve the health status of the seed ultimately produced.

A reduction in the number of multiplication years requires a propagule that can be produced in large numbers in protected environments in a short period. Multiplication of plantlets *in vitro* meets

these prerequisites. Two types of propagules can be considered: plantlets produced *in vitro* or tubers produced on these plantlets or on plant parts.

1.4.2 Production of *in vitro* plantlets

Many techniques have been developed during the last decades for producing potato plantlets on nutrient media in aseptic environments, being referred to as '*in vitro*'. Many of these techniques are useful in breeding of new varieties, but there are two valuable in seed tuber production: meristem culture and the multiplication of plantlets by nodal cuttings.

Meristem culture is the culture of a dissected portion of the meristematic region of a shoot tip, often after heat treatment of the plants, on a nutrient medium for plant regeneration (e.g. Wang & Hu, 1980). It is used for rendering diseased cultivars free of (mainly) virus diseases. Although the use of pre-organised meristems is generally regarded to have a low risk of obtaining aberrant plants (e.g. Evans & Bravo, 1986), Wright (1983) observed altered characteristics in 1 out of 30 clones regenerated by meristem culture. This indicates that meristem culture should not be used without good reason for initiation of *in vitro* cultures at the start of seed production programmes.

When large numbers of *in vitro* plantlets with a high genetic and health status are needed in a short period, the plantlets are commonly multiplied by nodal cuttings (Nozeran et al., 1977; Hussey & Stacey, 1981; Marinus, 1985) or other techniques that use existing buds for shoot formation (Goodwin et al., 1980).

1.4.3 Microtubers and minitubers

Throughout the year, two types of small tubers can be produced on *in vitro* plantlets: microtubers and minitubers.

Microtubers or *in vitro* tubers are produced *in vitro* on complete plantlets or on plant organs by changing the nutrient medium and/or the external conditions. *In vitro* produced tubers generally weigh 0.2 g per tuber or less (Hussey & Stacey, 1984; Estrada et al., 1986; Garner & Blake, 1989), though average weights of 0.4 g are reported when produced on liquid media containing growth regulators (Rossell et al., 1987; Lillo, 1989) and even higher weights are claimed by commercial companies. If produced on whole plantlets, the number of microtubers usually is limited to one per plant or explant.

Minitubers are small tubers that can be produced year round in glasshouses on *in vitro* propagated plantlets, planted at high density. Their size is 5 - 20 mm (Struik & Lommen, 1990) or slightly larger. In existing literature the term minitubers sometimes is used for *in vitro* tubers (Hussey & Stacey, 1984; Rosell et al., 1987; Ortiz-Montiel & Lozoya-Saldaña, 1987) or for larger tubers produced in containers from *in vitro* plantlets (Jones, 1988; Melching et al., 1993). The number of minitubers produced can be more than ten per *in vitro* propagated plant.

1.4.4 The potential of the new types of propagation material

In vitro plantlets (Jeffries, 1986; Mastenbroek & Eising, 1987; Jones, 1988) and microtubers (Jones, 1988) now are commonly used for speeding up multiplication at the start of seed programmes. *In vitro* plantlets and microtubers perform well when they are raised under protected conditions, in beds (Wiersema et al., 1987) or as transplants in the field (Wattimena et al., 1983) and the growing season is sufficiently long.

Only minitubers appear suitable for use in the first year of a seed programme in which the number of conventional field multiplications is to be reduced drastically, because this requires a propagule that can be planted on a large area by seed growers, directly in the field. For this, the propagule not only has to be vigorous and of an excellent health and genetic status, but also has to be available in large numbers at planting time, implying that it needs to be stored and distributed relatively easily. In addition, it has to produce more common sized seed potatoes of a high quality under less protected conditions in a growing season that in many countries (e.g. the Netherlands) is short because of early haulm killing. *In vitro* plantlets and microtubers do not meet these prerequisites. *In vitro* propagated plantlets are not suitable for large-scale use because they require careful handling, cannot be stored without loss of early growth vigour and are bulky (especially after transplanting), which makes transport laborious. Microtubers mainly appear less suitable because all published production methods yield very small tubers which have a low early growth vigour if planted directly in the field (e.g. Haverkort et al., 1991). Only minitubers seem promising propagules for direct field planting (Horváth & Föglein, 1987) on a large scale.

1.5 The research project

At the start of the research project leading to this thesis, the knowledge on minitubers was limited. Although *in vitro* plantlets were successfully used for tuber production (Marinus, 1985; Mastenbroek & Eising, 1987), no special production techniques for glasshouse production of minitubers existed in the Netherlands by which large numbers of tubers could be produced per *in vitro* plantlet and per unit area of glasshouse in a short time period throughout the year. This was in contrast to some other countries (e.g. Leth Pedersen & Föglein, 1987).

The research project aimed at studying the possibilities of producing large numbers of minitubers per *in vitro* plantlet and per unit area of glasshouse space throughout the year, and their performance under Dutch conditions. The results also might contribute to developing new production methods and new production systems for potato seed tubers. The project covered the following three phases of a production method of potato seed using minitubers: (1) the tuber production phase in which minitubers were produced on *in vitro* propagated plantlets in the glasshouse, (2) the storage phase comprising the period from the harvest of minitubers until planting, and (3) the field phase, in which minitubers were planted in the field to produce more normal sized seed tubers. A phase preceding these three, the production of *in vitro* plantlets, remained out of the scope of the research because techniques were already available and more or less optimized (Marinus, 1985).

Practical results of the project, experiences, and guidelines for the production, storage and use of minitubers were published in a report (Lommen, 1990) and a paper (Lommen, 1991), both in Dutch. This thesis concentrates on underlying processes and the mechanisms by which these processes are affected. It describes, quantifies and analyses effects of techniques employed during production of minitubers on processes like stolon formation, tuber formation and plant growth and development, and effects of seed weight and techniques employed during storage on processes like water loss, sprout growth and plant and crop growth.

The quality of the progeny tubers (health, trueness to type, physiological aberrations, performance) and their subsequent progenies was not studied here because of practical limitations and the not yet optimized methods for producing and using minitubers.

1.6 The structure of the thesis

The first part of the thesis deals with the production of minitubers. In chapters 2 and 3 a basic technique for the production of minitubers is developed and analysed, which yields large numbers of minitubers per *in vitro* plantlet and per unit area of glasshouse space throughout the year. In chapter 4, effects of crop husbandry techniques are described on yield characteristics of minitubers produced by the technique developed. After this, sufficiently large numbers of minitubers could be produced to study their performance during storage and in the field. The dry-matter concentration and dormancy of minitubers are described and quantified in chapter 5, the losses occurring during storage in chapter 6. Effects of the storage duration on the performance after planting under controlled conditions are assessed in chapter 7. In chapter 8, detailed studies are described on sprout growth during storage and on effects of sprout length and planting depth on emergence and plant characteristics at emergence. The field performance of minitubers is studied in chapters 9 and 10. Chapter 9 analyses crop establishment and yield formation, chapter 10 yield variation and multiplication factors. Chapters 5, 6, 8, 9 and 10 all include investigations on the effects of the initial weight of tubers on their performance. Understanding these effects will facilitate applying the results to other types of small tubers, e.g. microtubers and seedling tubers. The general discussion, chapter 11, first analyses how plant growth and tuber formation in the tuber production phase are affected by (a) the preceding *in vitro* phase, determining the physical and physiological status of the plantlets used for minituber production, (b) the environmental conditions during the glasshouse phase, and (c) the repeated harvesting technique employed for the production of minitubers. Subsequently, effects of the storage period and the weight of the minitubers on their performance are analysed, and possibilities to improve the field performance of the minitubers are explored. Finally, the possibilities to incorporate of minitubers in a seed production programme in the Netherlands are discussed.

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CHAPTER 2

Influence of a single non-destructive harvest on potato plantlets grown for minituber production

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2 INFLUENCE OF A SINGLE NON-DESTRUCTIVE HARVEST ON POTATO PLANTLETS GROWN FOR MINITUBER PRODUCTION

Abstract

Incorporating a step of minituber production in seed production programmes of potato, may speed up multiplication and improve seed tuber quality. Therefore, growth, development and minituber production of *in vitro* propagated potato plantlets were studied, after transplanting in the glasshouse at 350 plants per m^2 under tuber inducing conditions. Plants growing undisturbed were compared to plants from which tubers ≥ 0.3 g were removed in a single non-destructive harvest, 3 to 8 weeks after transplanting. In undisturbed plants, tuber initiation slowed down 4 weeks after transplanting, and only 2 tubers per plantlet were harvested in 11 weeks (average weight 5 g). After a non-destructive harvest, new stolons and tubers were initiated. However, overall and tuber growth rates were reduced. Effects of a non-destructive harvest were probably caused by the combined influences of tuber removal, root damage and deep replanting of the plantlets. The effects of the non-destructive harvest depended on the growth phase of the plants at the moment the non-destructive harvest took place: highest tuber numbers and lowest growth rate reductions were observed when growth was at its maximum. Using this non-destructive harvesting procedure, over 1400 and 2400 minitubers ≥ 0.3 g could be produced per m^2 within 8 and 9 weeks after transplanting for cultivars Ostara and Bintje, respectively. These minitubers (average weight 1 - 2 g) seem suitable for large scale use in a seed production programme.

Key words: Solanum tuberosum L., minitubers, rapid multiplication, seed production, tuber pruning, tuber initiation, tuber growth, stolon initiation.

Introduction

Traditionally, the potato (*Solanum tuberosum* L.) is multiplied by producing seed tubers. Seed tuber production is carried out by highly specialized growers or institutions. A complete multiplication scheme can take more than 10 years. Main problems of a conventional seed programme are the low multiplication rate of field-grown potato plants and the susceptibility of potato to diseases, which may be transferred through the seed tubers. With each multiplication in the field, the risk of catching viral, bacterial or fungal diseases increases. The health status of the seed tubers may be improved by reducing the number of field multiplications necessary to produce the desired seed lot. This requires a propagation material that can be produced in large numbers in protected environments. Only a few additional years of conventional seed multiplication would then be necessary.

The last decades alternative seed production programmes have been developed in which the first multiplication steps are speeded up by using *in vitro* plantlets (Jeffries, 1986), microtubers (Wang & Hu, 1982) or minitubers (Van der Zaag, 1990). Microtubers (or *in vitro* tubers) are produced *in vitro* on *in vitro* propagated plantlets or shoots. They generally weigh 0.2 g per tuber or less (Hussey

& Stacey, 1984; Estrada et al., 1986; Garner & Blake, 1989), though average weights of 0.4 g are reported when produced on liquid media containing growth regulators (Rossell et al., 1987; Lillo, 1989). Minitubers are produced on *in vitro* propagated plantlets, planted at high density in a soil medium in glasshouses and are larger than microtubers (Struik & Lommen, 1990). *In vitro* propagated plantlets and microtubers nowadays are commonly used (Jones, 1988) and perform well if raised under protected conditions, in beds (Wiersema et al., 1987) or as transplants in the field (Wattimena et al., 1983), provided the growing season is sufficiently long.

For a more drastic reduction of the number of conventional field multiplications, however, these alternative propagules need to be used on a very large scale, directly for field production. *In vitro* propagated plantlets are not suitable for large-scale use because they require careful handling, cannot be stored without loss of early growth vigour and are bulky (especially after transplanting), which makes transport laborious. Microtubers seem less suitable for direct field planting because they are very small. Thus, minitubers appear to be promising propagules for large-scale use (Struik & Lommen, 1990). Introduction of minitubers in a seed production programme, however, will only be successful if they are superior (economically and/or in quality) to both conventional seed and microtubers.

Therefore, a research programme was started in which the production, storage and field performance of minitubers were investigated. This paper deals with their production and concentrates on increasing the number of minitubers produced per *in vitro* propagated plantlet. Tuber numbers could possibly be increased by removal of existing tubers (cf. Nösberger & Humphries, 1965), although this reduces total yield (Burt, 1964; Nösberger & Humphries, 1965). Preliminary experiments have shown that removal of tubers could indeed increase tuber number, also using a practical non-destructive harvesting procedure. Tuber number per plantlet, however, depended on the timing of tuber removal (W.J.M. Lommen, unpublished data). A comprehensive experiment is described in this paper.

Materials and methods

In vitro multiplication. *In vitro* plantlets of *Solanum tuberosum* L. cv. Ostara (early) and cv. Bintje (mid-early) were multiplied routinely by subculturing single stem nodes every 4 weeks. Temperature in the growth room was 23 °C, photoperiod 16 hours and light was supplied by fluorescent tubes (Philips 33) at an intensity of approximately 8 W m^{-2} (total radiation). The multiplication medium (pH 5.7) contained mineral salts and vitamins (Murashige & Skoog, 1962) plus 2.0 mg l^{-1} glycine, 8.0 g l^{-1} agar and 25.0 g l^{-1} sucrose. The normalization medium before transplanting had the same composition with in addition 0.01 g l^{-1} alar-85 % (daminozide). The growing period from the last multiplication till transplanting was 17 days (cv. Ostara) or 18 days (cv. Bintje).

Culture in the glasshouse. *In vitro* plantlets were transplanted in a controlled glasshouse into a mixture of perlite and potting soil (50/50 % v/v) in 13 x 13 x 13 cm pots. A plant density of 350 plants per m^2 was obtained by planting 6 plants per pot in a row in the middle of the pot and joining

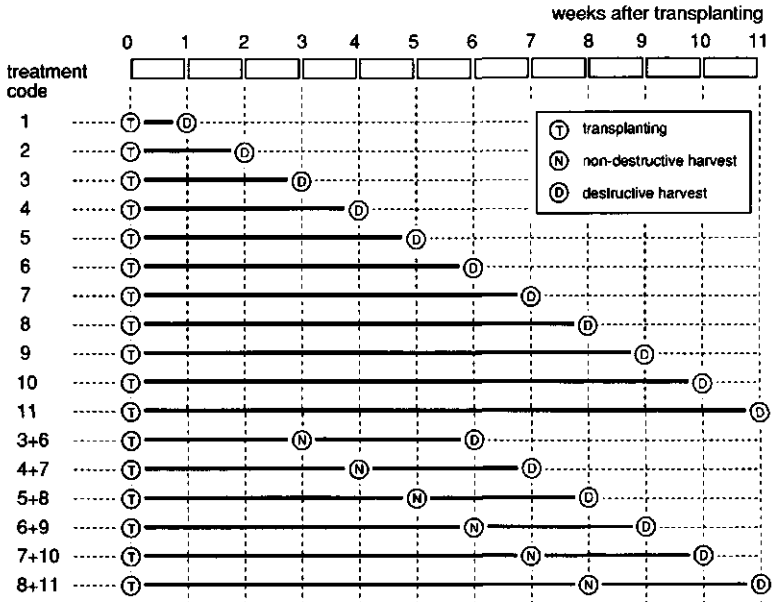


Fig. 1. Treatment codes and schematic explanation of treatments.

all pots. Available N from the soil medium was approximately 230 mg per pot.

The experiment was carried out during winter (December 15 - March 1). Photoperiod in the glasshouse was 12 hours. Natural light was supplemented to at least 80 W m^{-2} (total radiation) using high-pressure sodium lamps (Philips SON-T). Day temperature was set at 18 °C, night temperature at 12 °C. After 58 days, every pot received 200 ml of a low-concentrated nutrient solution ($Ca(NO_3)_2.4H_2O$ 0.890 g l^{-1}, KNO_3 0.446 g l^{-1}, KH_2PO_4 0.135 g l^{-1}, K_2SO_4 0.140 g l^{-1}, $MgSO_4.7H_2O$ 0.472 g l^{-1}, H_2SO_4 0.034 g l^{-1}, FeEDTA 0.035 g l^{-1}, $MnSO_4.1H_2O$ 2.0 mg l^{-1}, H_3BO_3 3.0 mg l^{-1}, $ZnSO_4.7H_2O$ 0.5 mg l^{-1}, $Na_2MoO_4.2H_2O$ 0.1 mg l^{-1} and $CuSO_4.5H_2O$ 0.1 mg l^{-1}, pH 6.0).

Treatments and experimental design. Growth and development were analysed after transplanting of the *in vitro* plantlets in the glasshouse. One series of treatments involved weekly, destructive harvests of undisturbed growing plants. At the moment the first tubers had a fresh weight of 0.3 g (3 weeks after transplanting), another series of treatments started: tubers ≥ 0.3 g were removed and plants were replanted. The removal of tubers was carried out, using a non-destructive harvesting procedure, suitable for practical use. Plants were lifted carefully from the soil mixture, tubers ≥ 0.3 g were removed and plants were replanted into the soil mixture. Whether the weight of the removed tubers was ≥ 0.3 g had to be estimated, using a diameter of approximately 8 mm as a criterium. Plants were

always replanted deeper than before. Replanting depth was not recorded but depended on the harvest date, and increased as the length of the stem part without leaves increased. Care was taken not to damage stems and stolons. Damage of roots, however, could not be avoided. The non-destructive harvests were carried out 3, 4, 5, 6, 7 or 8 weeks after transplanting, and were each followed by a destructive harvest 3 weeks later, to establish growth and development. Treatments are schematically represented in Fig. 1. Treatment codes represent the weeks after transplanting at which a harvest (non-destructive or destructive) took place.

The experimental unit was a pot containing 6 plants. Pots were arranged in a complete randomized design with 4 replications, 2 cultivars and 17 treatments. Plant density was maintained at 350 plants per m^2 throughout the experiment. One row of guard pots surrounded the experiment.

Observations. At a destructive harvest, plants were separated into the following fractions: leaf (petiole, rachis + leaflets), stem, stolon, root and tuber. Included in the root fraction of plants harvested non-destructively, were only the roots that were still attached to the plant, and not the roots that were disrupted at the non-destructive harvest.

Total numbers of sessile tubers (tubers produced at the nodes of the main stem, with no visible stolon part) and tubers on stolons were separately recorded. Tubers on the stolon apex had a diameter of at least twice the stolon diameter. Classification into stolons or sessile tubers and tubers directly on stolon nodes was based on shape. Tubers were graded into different fresh weight classes.

Stem length of the main stem was measured from the original cutting to the point were new leaves appeared. Number of nodes was counted on the main stem, including the visible leaves in the top part.

At a non-destructive harvest, only tubers $\geq$ 0.3 g were harvested and graded into fresh weight classes.

Analysis of data. Treatment effects were compared after analysis of variance. Depending on the kind of comparison, different subsets of data were analysed. For growth analyses of undisturbed growing plants, only the undisturbed growing plants were analysed (11 treatments x 2 cultivars x 4 replications). For studying tuber production in a second harvest, only the treatments with non-destructive harvests were compared (6 treatments x 2 cultivars x 4 replications). For determining the effect of a non-destructive harvest and the timing of this harvest, only the treatments with a final harvest from week 6 onwards were analysed, using harvest number and final harvest time as factors (2 harvest numbers x 6 final harvest times x 2 cultivars x 4 replications).

Growth rates and relative growth rates. Growth rates (GRs) and relative growth rates (RGRs) that were analysed statistically, were calculated over a period of 3 weeks prior to the final harvest, using the following formulas:

$$\overline{GR(t)} = \frac{\overline{W(t) - W(t\text{-}21)}}{21} \times 350 \qquad (g\ m^{-2}\ d^{-1})$$

$$RGR(t) = \frac{\ln(0.001+W(t)) - \ln(0.001+W(t-21))}{21} \quad (d^{-1})$$

In which:

t = time of final harvest in weeks after transplanting,

$W(t)$ = dry weight in g per plant at t,

$W(t\text{-}21)$ = dry weight in g per plant 21 days before t,

350 = number of plants per m^2,

21 = number of days in 3-weeks period.

Growth rates were calculated for the different plant fractions. All fractions were combined to produce overall growth rate.

Average overall growth rates over the whole experiment or part of the experiment, were calculated from the average dry weight values.

Results

Tuber production during undisturbed growth. The *in vitro* propagated plantlets grew well after transplanting into the glasshouse at a plant density of 350 plants per m^2. First tubers were detected 2 weeks after transplanting (Fig. 2). Total tuber number increased up to 7 weeks after transplanting

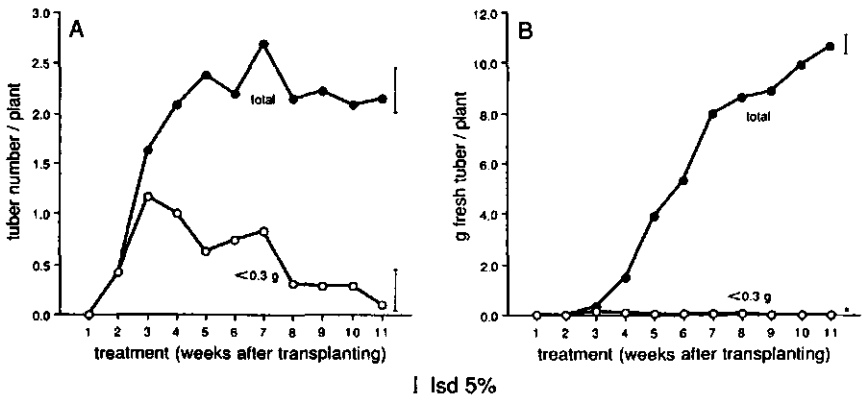
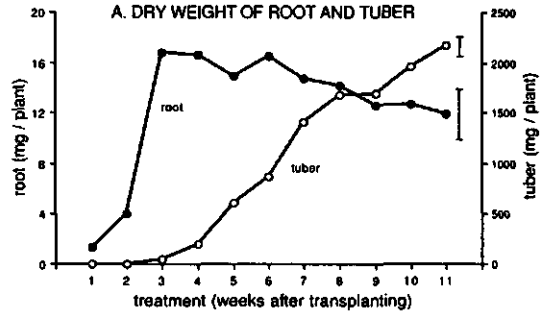
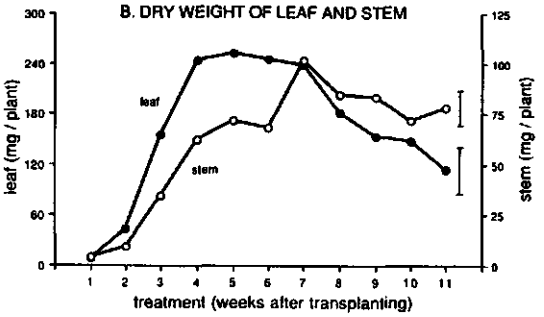
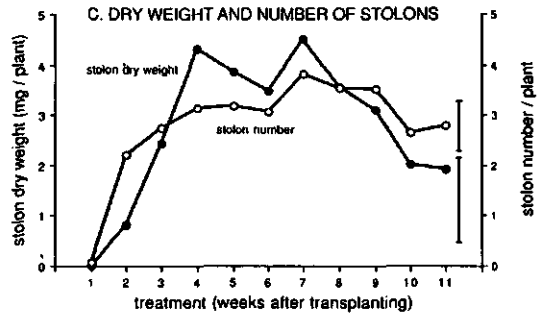


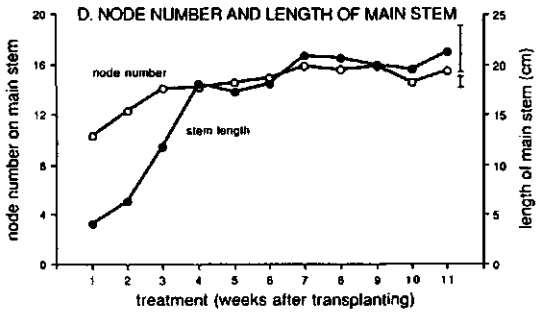
Fig. 2. Development over time of number (A) and fresh weight (B) of tubers in different grades, of undisturbed growing plants at a density of 350 plants per m^2. Average values of 2 cultivars.

Fig. 3. Growth and development over time of undisturbed growing plants at a density of 350 plants per m^2. Dry weights per plant of root and tuber (A), dry weights per plant of leaf and stem (B), dry weight per plant and number of stolons (D) and node number and length of main stem (D). Average values of two cultivars. →


A. DRY WEIGHT OF ROOT AND TUBER
root (mg / plant)
tuber (mg / plant)
root
tuber
treatment (weeks after transplanting)


B. DRY WEIGHT OF LEAF AND STEM
leaf (mg / plant)
stem (mg / plant)
leaf
stem
treatment (weeks after transplanting)


C. DRY WEIGHT AND NUMBER OF STOLONS
stolon dry weight (mg / plant)
stolon number / plant
stolon dry weight
stolon number
treatment (weeks after transplanting)


D. NODE NUMBER AND LENGTH OF MAIN STEM
node number on main stem
length of main stem (cm)
node number
stem length
treatment (weeks after transplanting)

I lsd 5%

to 2.69 tubers per plant and thereafter declined to approximately 2.15 tubers per plant, due to resorption (Fig. 2A). Final total tuber number did not differ significantly from the number of tubers present 4 weeks after transplanting. The number of tubers < 0.3 g declined from 3 weeks after transplanting onwards, mainly due to passing into ≥ 0.3 g grading. The number of tubers ≥ 0.3 g gradually increased through the experiment, up to 2.04 tubers per plant. Tuber fresh weight increased up to 10.65 g per plant and more than 5 g per tuber. The contribution of tubers < 0.3 g to total fresh weight was negligible at the end of the experiment (Fig. 2B).

Plant development and dry weight changes during undisturbed growth. The average overall growth rate during the experiment (week 1 to 11) was 11.8 g m^{-2} d^{-1}. Growth and development during undisturbed growth is shown in Fig. 3. During the 11 weeks of the experiment, the plants passed through 3 distinct growth phases: an early growth phase (0 - 4 weeks), a period of maximal growth (4 - 7 weeks), and a senescence period (7 - 11 weeks).

The first growth phase was characterized by increases in dry weight of all plant parts; root dry weight, however, only till 3 weeks after transplanting (Figs 3A, 3B and 3C). First stolons were detected 1 week after transplanting. Stem length, node number of the main stem and stolon number, all increased during this first growth phase (Figs 3C and 3D). The average overall growth rate, calculated between week 1 and 4 was 8.6 g m^{-2} d^{-1}.

During the second growth phase, leaf, root and stolon dry weights remained at more or less constant levels (Figs 3A, 3B and 3C). Stem and tuber dry weights still increased (Figs 3A and 3B). Stolon number, stem length and node number also continued to increase (Figs 3C and 3D). Stolons did not branch and reached an average length of 2 cm, while 3.8 stolons per plant were formed. Average overall growth rate between week 4 and 7, was 20.7 g m^{-2} d^{-1}.

During the last growth phase, plants were clearly senescing: dry weights of root, stolons, leaf and stem decreased (Figs 3A, 3B and 3C). Only tuber dry weight still increased (Fig. 3A). Stolon number declined (Fig. 3C). Stem length (approximately 20 cm) and node number (approximately 16) ceased to increase (Fig. 3D). Average overall growth rate during the last growth phase (week 7 to 11) was

Table 1. Influence of timing of the non-destructive harvest on number, yield and size of tubers in different grades, recorded at the final harvest. Average values of two cultivars. See Fig. 1 for treatment description.

Treatment	Tuber number/plant			Fresh tuber weight (g)/plant			Fresh weight (g)/tuber		
	total	≥ 0.3 g	< 0.3 g	total	≥ 0.3 g	< 0.3 g	total	≥ 0.3 g	< 0.3 g
3+6	3.65	1.50	2.15	2.86	2.78	0.08	0.81	1.97	0.04
4+7	7.40	2.50	4.90	3.42	3.19	0.24	0.48	1.26	0.05
5+8	9.72	3.23	6.49	4.58	4.21	0.37	0.50	1.38	0.06
6+9	12.77	3.44	9.33	3.78	3.31	0.48	0.29	1.08	0.05
7+10	8.75	2.02	6.71	2.07	1.75	0.32	0.24	0.86	0.06
8+11	7.71	1.17	6.54	1.12	0.84	0.28	0.15	0.77	0.05
LSD 5 %	3.28	0.68	2.94	0.95	0.88	0.14	0.15	0.35	0.02

7.6 g m^{-2} d^{-1}.

Influence of a non-destructive harvest at different time intervals after planting on tuber production. After removing tubers ≥ 0.3 g in a non-destructive harvest, many new tubers were initiated on existing stolons, newly formed stolons and directly on the below-ground part of the main stem. Number, fresh weight and size of tubers at the final harvest, 3 weeks after the non-destructive harvest, are shown in Table 1. Total tuber numbers at the last harvest were on average almost 4 times as high as the numbers observed in undisturbed growing plants (Fig. 2A). The number of tubers ≥ 0.3 g increased on average by almost 30 %. Tuber number at the second harvest, however, depended strongly on the timing of the first harvest. Postponing the first harvest from 3 to 6 weeks after transplanting, increased the number of tubers in the second harvest. Further postponing decreased the total number of tubers in the second harvest, though it was still higher than in undisturbed plants. Highest tuber numbers in the second harvest were observed in treatment 6+9, in which the plants were harvested both 6 and 9 weeks after planting: 12.77 tubers per plant. Highest numbers of tubers ≥ 0.3 g were also observed in this treatment: 3.44 tubers per plant. Tuber numbers in treatment 5+8 were lower, but not significantly. The majority of the tubers in the second harvest, however, was smaller than 0.3 g. The later the first harvest, the higher the proportion of small tubers in the second harvest.

Tuber fresh weight in the second harvest (Table 1) was reduced, compared to undisturbed growing treatments (Fig. 2B). Like tuber number, tuber fresh weight in the second harvest also depended strongly on the timing of the first harvest. Postponing the first harvest first increased and later decreased tuber yield. The increase in yield, however, was not as strong as the increase in tuber number. Maximum tuber yield was attained by treatment 5+8, with treatment 6+9 not differing significantly. The decrease in tuber yield by further postponing the first harvest was much stronger than the decrease in tuber number. The contribution of tubers < 0.3 g in total tuber yield was smaller than the contribution of tubers ≥ 0.3 g.

The later the first harvest, the lower the average weight per tuber in the second harvest (Table 1). The average weight per tuber remained below 1 g when all tubers were taken into account, and below 2 g when only tubers ≥ 0.3 g were taken into account.

Both higher numbers of sessile tubers and of tubers on stolons were produced in the second harvest (Table 2). While in undisturbed growing plants only 6.0 % of the tubers were sessile, in a second harvest on average 39.1 % of the tubers were sessile. However, the later the first harvest, the higher the percentage of sessile tubers. Postponing the first harvest from 3 to 8 weeks, increased the proportion of sessile tubers from 14.2 % to 57.7 %.

A non-destructive harvest increased the number of tubers per stolon without increasing the average stolon length (Table 2).

Influence of a non-destructive harvest at different time intervals after planting on plant development and dry matter production. Overall growth rate (GR) of harvested plants, calculated over the 3-weeks period between harvests, was on average 56 % of the overall GR of undisturbed growing plants

Table 2. Influence of a non-destructive harvest, 3 weeks before the final harvest, and final harvest week on tuber, stolon and haulm characteristics, recorded at the final harvest. Average values of two cultivars. See Fig. 1 for treatment description.

Treatment	Non-destructive harvest	Final harvest week	Tuber characteristics				Stolon characteristics		Haulm characteristics	
			number of sessile tubers	number of tubers on stolons	% of sessile tubers	number of tubers per stolon[a]	number of stolons per plant	length per stolon (cm)	main stem length (cm)	main node number on main stem
6	no	6	0.23	1.96	9.6	0.66	3.1	2.1	18.1	14.9
7	no	7	0.19	2.50	5.4	0.66	3.8	2.0	20.9	15.9
8	no	8	0.10	2.04	5.1	0.69	3.5	2.3	20.6	15.6
9	no	9	0.10	2.12	4.6	0.94	3.5	1.8	20.0	15.9
10	no	10	0.15	1.94	7.2	0.81	2.7	1.1	19.5	14.6
11	no	11	0.10	2.04	4.1	0.89	2.8	1.4	21.2	15.5
mean			*0.15*	*2.10*	*6.0*	*0.77*	*3.2*	*1.8*	*20.0*	*15.4*
3+6	yes	6	0.58	3.06	14.2	0.84	3.7	1.8	17.5	15.4
4+7	yes	7	2.38	5.02	29.6	1.20	4.5	2.1	16.8	14.2
5+8	yes	8	2.68	7.04	29.8	1.20	6.7	1.8	21.6	16.2
6+9	yes	9	6.00	6.77	45.6	1.18	5.7	1.4	21.4	16.5
7+10	yes	10	5.00	4.35	57.3	1.36	3.2	1.4	19.5	14.0
8+11	yes	11	4.19	3.54	57.7	1.31	3.1	0.9	20.5	15.4
mean			*3.47*	*4.96*	*39.1*	*1.18*	*4.5*	*1.6*	*19.6*	*15.3*
Significance[b]										
- non-destructive harvest			**	**	**	***	***	ns	ns	ns
- final harvest week			ns	ns	ns	ns	***	***	ns	**
- interaction[c]			***	***	***	ns	ns	ns	ns	ns
- LSD 5 %			1.42	1.43	10.7					

[a] Tubers produced on stolons only.

[b] Mean squares of main effects were tested against error mean squares if no interaction occurred. Otherwise, mean squares of main effects were tested against interaction mean squares. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

[c] Influence of the timing of harvest on the effect of the non-destructive harvest.

(Table 3). The effect of a non-destructive harvest, however, depended on the timing of the first harvest. It was considerable at early harvests (treatments 3+6 and 4+7), when GR of the harvested treatments was reduced to 43 % and 34 % of the GR of undisturbed growing treatments, but most severe at a late harvest (treatment 8+11), when GR was reduced to 19 %. Differences between undisturbed growing plants and harvested plants were not significant when plants were harvested for the first time after 5 or 6 weeks (treatments 5+8 and 6+9).

The negative GRs of the root fraction were reduced even more by a non-destructive harvest (Table 3).

The influence of a non-destructive harvest on leaf GRs depended on the timing of the harvest (Table 3). Leaf GR was reduced when the non-destructive harvest took place early (treatments 3+6 and 4+7).

Table 3. Influence of a non-destructive harvest, 3 weeks before the final harvest, and final harvest week on growth rates of different plant parts and overall, calculated over a 3-weeks period before the final harvest, in g m^{-2} d^{-1}. Average values of two cultivars. See Fig. 1 for treatment description.

Treatment	Non-destructive harvest	Final harvest week	Growth rates					
			overall	root	stolon	leaf	stem	tuber
6	no	6	15.6	-0.005	0.018	1.49	0.55	13.5
7	no	7	20.7	-0.031	0.003	-0.08	0.65	20.1
8	no	8	16.8	-0.012	-0.005	-1.19	0.22	17.8
9	no	9	12.4	-0.065	-0.007	-1.53	0.26	13.8
10	no	10	6.4	-0.035	-0.041	-1.50	-0.50	7.8
11	no	11	7.0	-0.039	-0.027	-1.11	-0.12	8.3
mean			*13.2*	*-0.031*	*-0.010*	*-0.65*	*0.18*	*13.5*
3+6	yes	6	6.7	-0.061	0.013	0.32	0.30	6.2
4+7	yes	7	7.1	-0.093	0.022	-1.23	0.02	8.4
5+8	yes	8	13.5	-0.018	0.141	-0.62	0.41	13.5
6+9	yes	9	10.7	-0.060	0.020	-1.37	0.22	11.9
7+10	yes	10	3.8	-0.046	-0.014	-1.60	-0.49	6.0
8+11	yes	11	1.3	-0.120	-0.027	-1.46	-0.31	3.2
mean			*7.2*	*-0.067*	*0.025*	*-0.99*	*0.03*	*8.2*
Significance[a]								
- non-destructive harvest			*	*	ns	ns	ns	*
- final harvest week			ns	ns	ns	*	***	ns
- interaction[b]			**	ns	*	*	ns	**
LSD 5 %			4.4		0.067	0.79		3.7

[a] Mean squares of main effects were tested against error mean squares if no interaction occurred. Otherwise, mean squares of main effects were tested against interaction mean squares. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

[b] Influence of the timing of harvest on the effect of the non-destructive harvest.

No significant influence of a non-destructive harvest was observed on GRs of stems (Table 3), stem length (Table 2) or node number (Table 2).

A non-destructive harvest increased stolon numbers from 3.2 to 4.5 stolons per plant (Table 2). The timing of the non-destructive harvest did not significantly affect this increase, but stolon GR was stimulated most when the first harvest took place after 5 weeks (treatment 5+8, Table 3). Stolons did not branch.

A non-destructive harvest also reduced tuber GRs (Table 3). Similar to overall growth rate, the effect was most severe when the first harvest took place early (treatments 3+6 and 4+7) or late (treatment 8+11).

The influence of a non-destructive harvest on average relative growth rates (RGR) is shown in Table 4. RGRs of roots were lower in treatments which were harvested non-destructively. Differences in stem, leaf and overall RGRs were not significant at a 5 % level. RGRs of stolons and tubers were higher in treatments which were harvested non-destructively. Tubers had higher RGRs than other plant fractions. Tubers were followed by stems when plants were growing undisturbed. In treatments in which plants were harvested twice, however, stolons had higher RGRs than stems.

Effect of cultivar. Generally, treatment effects were highly significant, even if mean squares were tested against mean squares of a cultivar x treatment interaction, in case such an interaction existed. Therefore, only average values of the two cultivars were presented.

Cv. Ostara, however, showed a slightly faster development than cv. Bintje. Leaf and total dry weights of cv. Ostara increased faster, but cv. Ostara also showed an earlier decline in growth rate. Cv. Bintje usually produced more tubers than cv. Ostara, but the individual tuber weight was lower.

Both cultivars produced highest numbers of tubers in the second harvest in treatment 6+9. Cultivar Ostara, however, reached its maximum tuber weight and its maximum number of tubers ≥ 0.3 g earlier than cv. Bintje.

Table 4. Relative growth rates (RGRs) of different plant parts, calculated over a 3-weeks period before the final harvest, in treatments with and without a non-destructive harvest 3 weeks before the final harvest. Average values of two cultivars and six final harvest weeks (d^{-1}).

Plant part	RGR control	RGR after non-destructive harvest	Significance [a]
Root	-0.007	-0.017	**
Stolon	-0.010	0.002	*
Leaf	-0.012	-0.018	ns
Stem	0.006	0.001	ns
Tuber	0.056	0.184	**
Overall	0.039	0.035	ns

[a] Mean squares of main effects were tested against error mean squares if no interaction with final harvest week occurred. Otherwise (leaf), mean squares of main effects were tested against interaction mean squares. ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

Practical implications of a non-destructive harvest for a minituber production system. For practical purposes, tubers ≥ 0.3 g of both the non-destructive harvest (1st harvest) and the final harvest (2nd harvest) are of interest. In Figs 4 and 5, tuber numbers of both harvests are combined and presented on a square meter basis, separately for both cultivars and different grades. Cultivar Ostara (Fig. 4) produced over 1400 tubers ≥ 0.3 g per m^2, when harvested 5 and 8 weeks after transplanting (treatment 5+8). The number of tubers produced by this cultivar in treatment 6+9 was lower, but not significantly. Cultivar Bintje (Fig. 5) produced over 2400 tubers ≥ 0.3 g per m^2, when harvested 6 and 9 weeks after transplanting (treatment 6+9). Further postponement of the first harvest caused a severe drop in the number of tubers ≥ 0.3 g produced by cv. Bintje.

In general, the contribution of the second harvest to the combined tuber number decreased, when the first harvest was later (Figs 4 and 5). The decrease was stronger for the larger tuber sizes. While in treatment 3+6 all tubers ≥ 2 g were produced in the second harvest, all tubers ≥ 2 g in treatment 8+11 were produced in the first harvest.

Combining tubers of both harvests, the average fresh weights of tubers ≥ 0.3 g were always larger than 1.0 g (Figs 4 and 5).

Discussion

Undisturbed growth at a high plant density

Undisturbed growing plants completed their growth cycle very rapidly (Fig. 3). This will have been caused by the experimental conditions, known to hasten plant senescence:

1. the conditions in the glasshouse, stimulating tuber initiation;
2. the high plant density of 350 plants per m^2;
3. the choice of early and mid-early cultivars;
4. the low fertilization.

Tuber formation was very early: first stolons were observed 1 week after transplanting (Fig. 3C), first tubers 2 weeks after transplanting (Fig. 2A). The short photoperiod, an intermediate temperature and the additional illumination all accelerated tuber initiation (Bodlaender, 1963) and therefore may have reduced the number of tubers.

Undisturbed plants produced only 2.14 tubers $plant^{-1}$ (749 tubers per m^2) in 11 weeks (Fig. 2A). This apparently low tuber number was not merely caused by a lack of stolons or possible tuber sites, because under undisturbed conditions, the number of stolons (Fig. 3C) was always larger than the number of tubers (Fig. 2A).

The final tuber number, however, was lower than the number of tubers initiated, because resorption occurred during plant senescence (Fig. 2A). In our experiment, the dynamics of tuber number reflected the changes in growth and development during the different growth phases. The final number of tubers did not differ significantly from the number of tubers present at the end of the first growth phase (4 weeks after transplanting), i.e. the moment leaf dry weight ceased to increase (Fig. 3B). During the second growth phase (4 - 7 weeks), leaf weight remained constant

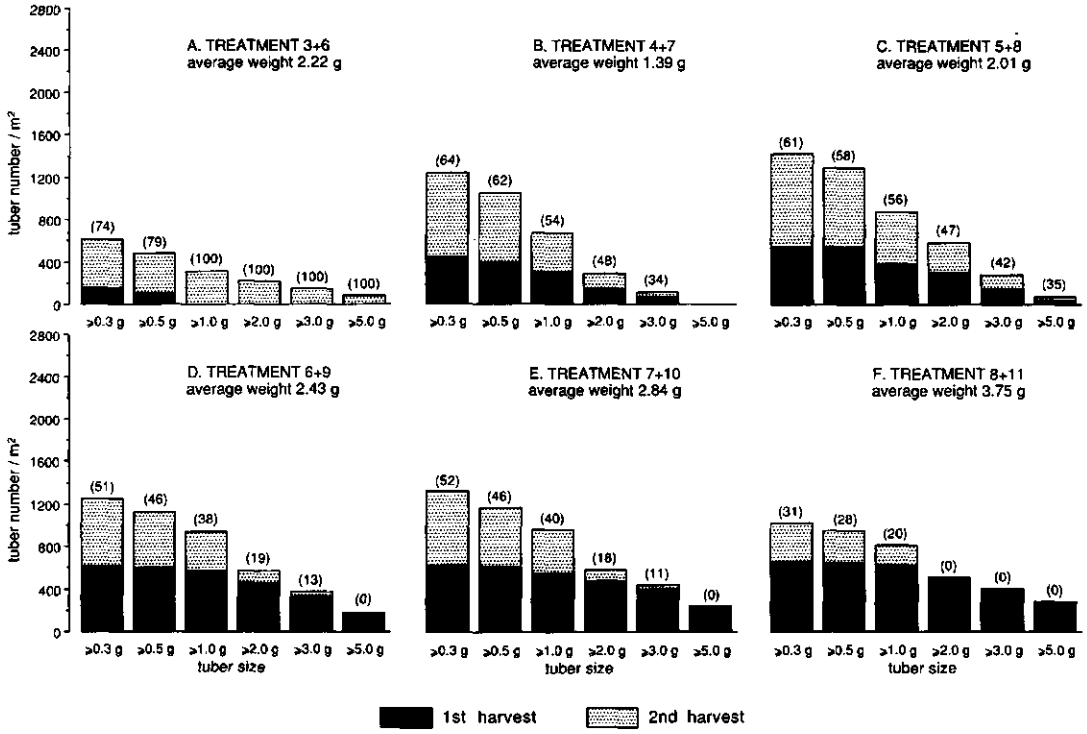


Fig. 4. Influence of the timing of a non-destructive harvest (1st harvest) followed by a destructive harvest (2nd harvest) 3 weeks later, on the number of tubers per m^2 of different lower sizes, produced by cv. Ostara in these two harvests. Harvests after 3 and 6 weeks (A), 4 and 7 weeks (B), 5 and 8 weeks (C), 6 and 9 weeks (D), 7 and 10 weeks (E) and 8 and 11 weeks (F). Between brackets above each bar: contribution (as percentage) of the second harvest to the combined tuber number.

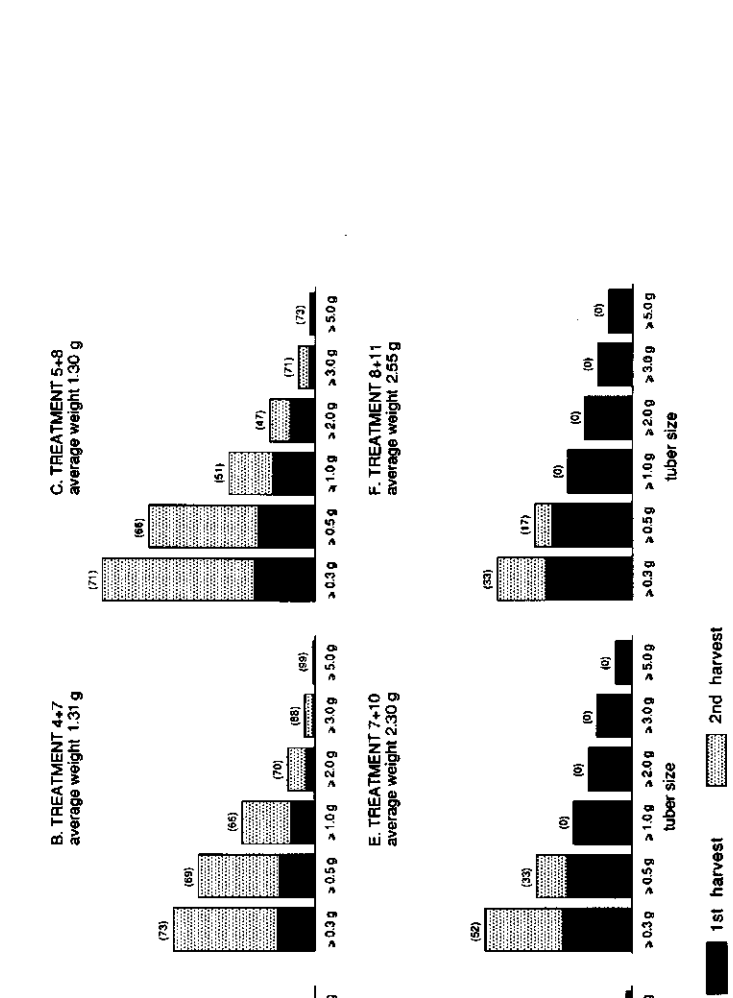


Fig. 5. Influence of the timing of a non-destructive harvest (1st harvest) followed by a destructive harvest (2nd harvest) 3 weeks later, on the number of tubers per m^2 of different lower sizes, produced by cv. Bintje in these two harvests. Harvests after 3 and 6 weeks (A), 4 and 7 weeks (B), 5 and 8 weeks (C), 6 and 9 weeks (D), 7 and 10 weeks (E) and 8 and 11 weeks (F). Between brackets above each bar: contribution (as percentage) of the second harvest to the combined tuber number.

(Fig. 3B). Deterioration of old leaves must have matched production and weight increase of new leaves, because node number still increased (Fig. 3D). During this period of maximal leaf weight the number of tubers increased only slightly (Fig. 2A), but overall- and tuber dry weight increases were maximal (Table 3). The number of tubers initiated during this second growth phase, was similar to the number resorbed during senescence (7 - 11 weeks). This resorption was associated with a decay of stolons and a decrease in dry weight of all plant parts except tubers (Fig. 3).

Influence of a non-destructive harvest on plant growth and development

A non-destructive harvest of tubers ≥ 0.3 g involved three actions that could have caused the observed changes in plant growth and development:
1. removal of tubers, resulting in breaking of apical dominance of the dominant tuber at the stolon apex, and changes in the possibilities for assimilate partitioning;
2. damage of roots, resulting in a temporary drought stress, a change in root:shoot ratio, and possible changes in production of growth regulators;
3. replanting deeper than initially, resulting in more stem nodes being exposed to below-ground conditions.
The timing of the non-destructive harvest strongly influenced the effects of these actions.

Overall growth rate. The reduction of overall growth rate observed in our experiment (Table 3) can be attributed to both the removal of tubers and the damage of roots. Removal of tubers (Burt, 1964; Moll, 1986) or tubers plus stolons (Nösberger & Humphries, 1965) reduces overall growth rates and net assimilation rates, by lowering the rate of photosynthesis. In our experiment, root damage will also have contributed to the reduction of the overall growth rate. The plants showed visible wilting, but always recovered within 2 days. This drought stress may have reduced production by reducing the photosynthesis per cm^2 of leaf (cf. Moorby et al., 1975; Vos & Oyarzún, 1987) and by reducing the leaf area as a result of a reduced leaf expansion (cf. Munns & Pearson, 1974).

The influence of drought stress on leaf expansion will be most important when young and expanding leaves are present, i.e. at early harvest moments. Significant reductions of leaf growth rates only occurred after early non-destructive harvests (Table 3). This explains why overall growth rate was reduced considerably after early harvests but less after intermediate harvests (Table 3). The reduction in total growth rate, however, was most severe after the latest harvest date, since the senescing plants were not able to adapt anymore.

Haulm characteristics. No significant differences were found in stem growth rates (Table 3), stem length and node number (Table 2) between undisturbed growing plants and harvested plants. The same applies to leaf growth rates at later harvest dates (Table 3). This contrasts with Burt (1964) and Nösberger & Humphries (1965), who found higher stem and leaf dry weights after removal of tubers. In our experiment, however, the damage of roots will have counteracted this effect. Root damage generally reduces the weight of the upper plant parts, as found by Moore (1937) after root pruning.

Root growth rate. In our experiment, root growth rate was calculated by subtracting the root dry weight of harvested plants from that of undisturbed plants. Consequently, lower growth rates of roots (Table 3) in harvested plants only show that the plants were not able to compensate completely for the root damage within a 3-weeks period. The root:shoot ratio of undisturbed growing plants generally was higher than that of plants which had been harvested non-destructively. This difference, however, was not significant ($P = 0.11$, results not shown).

Stolon characteristics and tuber number. The non-destructive harvest increased stolon number (Table 2). As the harvested plants were replanted deeper than initially, more nodes were exposed to stolon inducing conditions (see also: Kumar & Wareing, 1972). Our results agree with those of Svensson (1962) who found higher stolon numbers when emerged potato plants were hilled up early. In addition, the removal of tubers probably stimulated the development of buds into stolons, similarly to the increase in number of lateral branches of stems, observed by Nösberger & Humphries (1965) after removal of tubers plus stolons. No obvious lateral branching of stems or stolons was observed in our experiment.

The breaking of apical dominance by removing the dominant tuber on the stolon apex and the deeper replanting most probably explain the overall increase in tuber number caused by a non-destructive harvest. An increase in tuber number compared to undisturbed growing plants was also observed by Nösberger & Humphries (1965) in one of their experiments after removal of tubers and stolons. Oparka (1987) observed high numbers of small tubers two weeks after he had removed the apices of the primary stolons. However, he found no influence on the final tuber number, which he attributed to one tuber on every node becoming the dominant sink, while the other tubers were resorbed or shed before harvest. Similarly, he found no influence of removing tuber initials on the number of tubers present at the final harvest. In our experiment the time period between the non-destructive harvest and the final harvest was only three weeks. This time period was chosen arbitrarily, but a preliminary experiment (not published) had shown that this regrowth period was long enough to enable growth of some newly initiated tubers to a size of ≥ 0.3 g. If finally only one tuber on each node would become dominant, this probably would not have shown yet. The deeper replanting of our plants could have increased tuber number too, similar to stolon number.

The timing of the first harvest strongly influenced the tuber numbers in the second harvest (Table 1). After early non-destructive harvests, less tubers were produced than after intermediate non-destructive harvests. At early harvests, less tubers were removed since many tubers had not yet reached the desired size (Fig. 2A, tubers < 0.3 g). Thus, the breaking of apical dominance was less important. Moreover, the later the non-destructive harvest, the deeper the plants were replanted, because of the longer stems (Fig. 3D) or part of the stem that contained no green leaves. The number of tubers at the final harvest, however, was higher after intermediate harvests than after very late non-destructive harvests (Table 1). This difference was larger than the difference in tubers < 0.3 g remaining on the plants after the non-destructive harvests (Fig 2A). Possibly, at very late harvests, tuber initiation was limited by availability of mineral nutrients, which by then must have been very low, despite the replenishment of nutrients after 58 days. This agrees with the experiments of

Nösberger and Humphries (1965), who concluded that after removal of tubers more meristems start to grow when the supply of N permits so. On the other hand, already some resorption of newly initiated tubers may have occurred, as was observed in the undisturbed senescing plants (Fig. 2A). If so, at late harvest dates, the number of tubers initiated right after the non-destructive harvest, will be higher than the number of tubers observed after 3 weeks, at the final harvest.

Tuber position. After a non-destructive harvest, the percentage of sessile tubers considerably increased (Table 2), most probably because of a lack of possible tuber sites on the stolons. Due to tuber inducing conditions in the glasshouse, stolons in both undisturbed growing plants and harvested plants remained very short (Table 2). After a non-destructive harvest, the average length of the stolons was 1.6 cm. These short and unbranched stolons had only a few potential tuber sites, especially because some of them had already one tuber removed from the stolon apex in the first harvest. In the final harvest, 1.2 tubers per stolon were produced.

The higher percentages of sessile tubers observed after late non-destructive harvests compared to early harvests (Table 2) are in accordance with this view. Stolon numbers were lower at later harvests (Table 2). Thus, the total number of tuber sites on stolons was more limited at late harvests. Presumably, the number of tubers was less reduced by the limited nutrient supply at later harvests than the number of stolons, possibly because of a higher sink activity of the tubers.

Tuber growth rate and tuber size. The reduction of tuber growth rate caused by a non-destructive harvest, can be attributed to both tuber removal and the damage of roots. Burt (1964) observed lower dry weight gains of tubers, 13 days after removal of tubers. The first four days of the 21 days growth period in our experiment may not have been important for tuber growth. Burt (1964) found new tubers between three and six days after removal of tubers and Marschner et al. (1984) observed a lag period of four days till normal total tuber growth rates were restored after removal of all fast growing tubers. Oparka (1987), however, showed that tuber removal also reduced final tuber yields under field conditions. Final tuber yields were also reduced after root damage (Oparka, 1987) or regular root pruning (Moore, 1937).

The reduction in average tuber size after a non-destructive harvest (Table 1) compared to undisturbed growing plants, may fully be explained by the higher tuber number combined with the lower tuber yield after a non-destructive harvest. Postponing the first harvest resulted in a clear decrease of the average weight per tuber in the final harvest (Table 1), because tuber numbers were not affected in the same way by postponing of the first harvest as tuber fresh weights. Initially tuber numbers increased more than tuber fresh weights and later tuber numbers decreased less than tuber fresh weights (Table 1).

Practical consequences

Both undisturbed plants and plants that were harvested twice, produced more minitubers per *in vitro* propagated plantlet than commonly observed during the production of microtubers. The number of

microtubers is only incidentally larger than one (Lillo, 1989), while the number of minitubers per plant produced by undisturbed plants was two and the total number of minitubers produced by plants harvested twice could be four to seven, depending on the cultivar. These minitubers were much larger than microtubers. All minitubers were ≥ 0.3 g and had average fresh weights of 5 g if plants grew undisturbed and of 1 - 2 g if plants were harvested non-destructively at the optimal moment (Figs 4 and 5).

Because of the high plant density, glasshouse space was used efficiently. Tuber number of undisturbed growing plants was 714 tubers per m^2 ≥ 0.3 g, but by repeated harvesting 1400 - 2400 tubers per m^2 could be obtained within 8 - 9 weeks. This number is comparable with the number of microtubers obtained by Wang & Hu (1982), who produced 36 000 microtubers per 10 m^2 in 4 months in a growth chamber. Our minitubers, however, had average weights of more than six times the weight of these microtubers.

It was quite possible to produce minitubers without using growth regulators. The short photoperiod and the additional illumination in the glasshouse stimulate tuber initiation but also reduce stem length (cf. Bodlaender, 1963). Short stems make the plantlets more suitable for a non-destructive harvest, because they are less susceptible to damage. Because no growth regulators were used during the production of minitubers, they are more suitable for the production of seed potatoes than microtubers, during the production of which cytokinins and CCC (chlormequat) are commonly used (Estrada et al, 1986; Rosell et al, 1987; Lillo, 1989). Cytokinins can increase the risk of obtaining adventive meristematic structures, the development of which should be avoided producing seed potatoes (Hussey & Stacey, 1981) and CCC can retard sprouting of the tubers (Goburdhun, 1978), reduce tuber yield of the progeny (Dekhuijzen & Bodlaender, 1973) or hinder roguing of undesired genotypes and diseased plants.

While producing minitubers in practice, it may be difficult to fix the harvest dates at which highest minituber numbers are produced. The optimal date for the first harvest could not be judged from the plant habitus. It may vary as the climatic conditions will slightly vary with each culture of minitubers. An early harvest may be better than a late harvest, because in the latter case both tuber number and tuber size decrease (Table 1, Figs 4 and 5) and glasshouse space is used less efficiently. An early harvest may even offer the opportunity of a third harvest, because plants are not senesced at the moment of the second harvest. In addition, the date of the second harvest may be altered, because the interval between harvests may affect tuber numbers in the second harvest. Thus, more research should clarify the influence on tuber number and size of (1) increasing the interval between harvests and (2) a second non-destructive harvest, followed by a third harvest. We will report on that in a forthcoming paper.

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CHAPTER 3

Production of potato minitubers by repeated harvesting: Plant productivity and initiation, growth and resorption of tubers

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3 PRODUCTION OF POTATO MINITUBERS BY REPEATED HARVESTING: PLANT PRODUCTIVITY AND INITIATION, GROWTH AND RESORPTION OF TUBERS

Abstract

Plant growth and tuber initiation, growth and resorption were studied in two potato cultivars, grown for minituber production under tuber inducing conditions. Plants were harvested up to three times within 11 weeks, using one or two non-destructive harvests at which minitubers (≥ 0.3 g) were removed and plants were replanted. The first non-destructive harvest stimulated the initiation of new tubers. A part of these tubers grew to a size of ≥ 0.3 g within three weeks. The other tubers remained < 0.3 g and many of them were resorbed during plant senescence. A second non-destructive harvest, three to four weeks after the first harvest, stimulated initiation of new tubers only in young plants of one cultivar, but always stimulated growth of tubers that otherwise would have been resorbed or would have remained < 0.3 g. Again, a part of the tubers grew to a size of ≥ 0.3 g within three weeks. Thus, the number of minitubers increased after both non-destructive harvests. Tuber and overall growth rates, however, were reduced.

A production scheme for practical minituber production is suggested, consisting of 3 harvests and yielding over 1800 minitubers per m^2, all ≥ 0.3 g and weighing on average 1 - 2 g.

Keywords: Solanum tuberosum L., minitubers, rapid multiplication, growth rate, tuberization, tuber pruning, non-destructive harvest

Introduction

Minitubers are small seed potato tubers, produced in the glasshouse on *in vitro* propagated plantlets, planted in a high density. They are considered to be the most suitable progagule to reduce the number of field multiplications in a seed programme (Lommen & Struik, 1992). The production of minitubers consists of two phases (1) the multiplication of plantlets *in vitro* and (2) the production of minitubers on these plantlets in the glasshouse. This paper deals with the second phase.

Previous research showed that the number of minitubers could be increased by 100 % - 250 % if plants were harvested twice instead of once, using a non-destructive harvesting procedure in the first harvest (Lommen & Struik, 1992) at which tubers ≥ 0.3 g were removed and plants were replanted. Three weeks later, the plants were harvested a second time. A weight of 0.3 g was used as a lower limit for being counted as a minituber.

The non-destructive harvest stimulated the initiation of new stolons and tubers, but the majority of the newly initiated tubers was smaller than 0.3 g at the second harvest. Number of minitubers in the second harvest, and overall and tuber growth rates between two harvests depended on the age of the plants at the first harvest. Highest number of minitubers in the second harvest was produced when the first harvest took place in the period of maximum plant growth. The timing of the first harvest was very critical, but it was impossible to asses it on the basis of plant habitus.

From a practical point of view, two questions remained: (1) how are minituber numbers at the second harvest affected by extending the growing period between two harvests and (2) how can high numbers of minitubers be produced reliably? For answering these questions one needs a better understanding of tuber formation after a non-destructive harvest.

The number of minitubers in a second harvest will depend on (a) the total number of tubers remaining on the plant after the first harvest and initiated thereafter and (b) the proportion of these tubers that is able to grow to the desired weight before the second harvest. The length of the growing period between two harvests might be crucial for the tuber numbers in different grades at the second harvest. Therefore, the effects of extending the growing period between two harvests, on tuber numbers in different grades and growth rates of different plant parts are described in this paper. In addition, the effects of a second non-destructive harvest were investigated. The cultivar choice, the non-destructive harvesting procedure and the experimental conditions were similar to the ones described previously (Lommen & Struik, 1992). A production scheme for practical production of minitubers will be suggested, aiming at high numbers of minitubers per m^2 and suitable for different cultivars.

Materials and methods

In vitro multiplication. In vitro plantlets of *Solanum tuberosum* L. cv. Ostara (early) and cv. Bintje (mid-early) were produced by subculturing single-node stem cuttings approximately every 4 weeks. Temperature in the growth room was 23 °C, photoperiod 16 hours and light was supplied by fluorescent tubes (Philips 33) at an intensity of 8 W m^{-2}. The multiplication medium, pH 5.7, contained mineral salts and vitamins according to Murashige and Skoog (1962), 2.0 mg l^{-1} glycine, 8.0 g l^{-1} agar and 25.0 g l^{-1} sucrose. To the normalization medium before transplanting an additional 0.01 g l^{-1} alar-85 % (daminozide) was added.

In vitro plantlets were produced using the same procedure as before (Lommen & Struik, 1992), but the length of the growing period on the normalization medium from the last subculturing until transplanting was 8 to 11 days, and therefore shorter than in earlier research.

Culture in the glasshouse. In vitro plantlets were transplanted in a controlled glasshouse into 13 x 13 x 13 cm pots with a mixture of perlite and potting soil (50/50 % v/v). Available N from the soil medium was approximately 230 mg per pot. A plant density of 350 plants per m^2 was obtained by planting 6 plants per pot in a row in the middle of the pot, spaced approximately 2.2 cm from each other and joining all pots. This plant density was maintained throughout the experiment. One row of guard pots surrounded the experiment.

The experiment was carried out in a glasshouse in Wageningen, the Netherlands, during summer (June 14 - August 30, 1988; in contrast with previous research, which was carried out in winter; Lommen & Struik, 1992). Photoperiod was reduced to 12 hours and natural light was supplemented to at least 80 W m^{-2} (total radiation) by high-pressure sodium lamps (Philips SON-T). Day temperature was set at 18 °C, night temperature at 12 °C. After 63 days, every pot received 100 ml

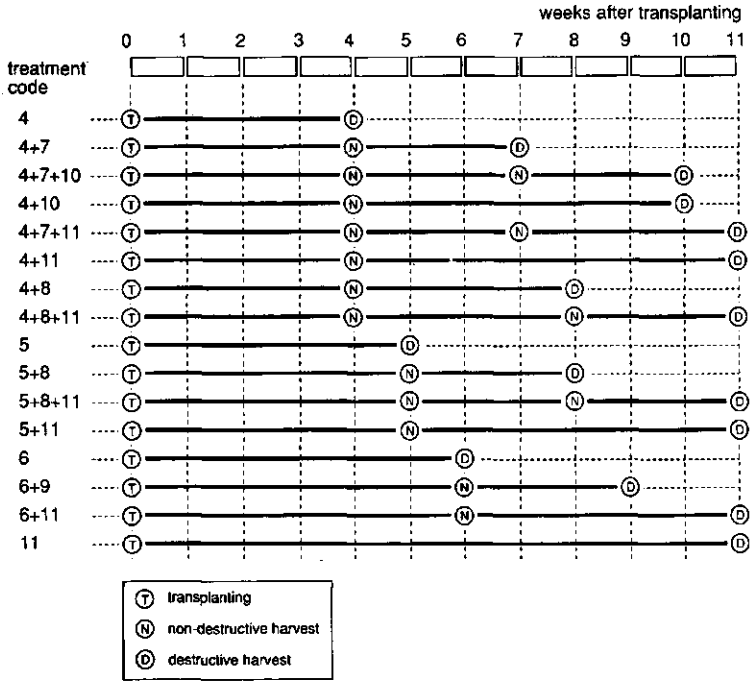

Fig. 1. Treatment codes and schematic explanation of treatments.

of a low concentrated nutrient solution ($Ca(NO_3)_2.4H_2O$ 0.890 g l^{-1}, KNO_3 0.446 g l^{-1}, KH_2PO_4 0.135 g l^{-1}, K_2SO_4 0.140 g l^{-1}, $MgSO_4.7H_2O$ 0.472 g l^{-1}, H_2SO_4 0.034 g l^{-1}, FeEDTA 0.035 g l^{-1}, $MnSO_4.1H_2O$ 2.0 mg l^{-1}, H_3BO_3 3.0 mg l^{-1}, $ZnSO_4.7H_2O$ 0.5 mg l^{-1}, $Na_2MoO_4.2H_2O$ 0.1 mg l^{-1} and $CuSO_4.5H_2O$ 0.1 mg l^{-1}, pH 6.0). Thus, nutrients were supplied 5 days later in a lower dose than in previously described research (Lommen & Struik, 1992).

Treatments and experimental design. Plants grew undisturbed, or were harvested non-destructively and replanted one or two times. At a non-destructive harvest, all tubers ≥ 0.3 g were removed. The removal of tubers was carried out, using a procedure suitable for practical use (Lommen & Struik, 1992). Root damage could not be avoided, but care was taken not to damage stems and stolons. Plants were replanted in the same soil, deeper than initially. At the final harvest of each treatment, plants were analysed completely (destructive harvest). The number and timing of the harvests varied. All treatments are schematically presented in Fig. 1. Treatment codes represent the weeks after transplanting at which the (non-destructive and final/destructive) harvests in a treatment took place.

The experimental unit was a pot containing 6 plants. Results, however, will generally be expressed on a per plant basis. Pots were arranged in a complete randomized design with 6 replications, 2 cultivars and 16 treatments.

Plant analysis. At a non-destructive harvest, only tubers ≥ 0.3 g were harvested. At the final harvest, tubers were graded into 2 fresh weight classes: ≥ 0.3 g and < 0.3 g. Plants were divided into leaves (petioles, rachides + leaflets), stems, stolons, roots and tubers. Stem length and node number of the main stem were recorded. Branching hardly occurred. Node number included the nodes of all visible leaves in the top. Growth rates (GRs) were calculated from the dry weight data at the final (destructive) harvests, for different plant fractions; leaves, stems and stolons at the final harvests were combined into a non-tuber fraction and tubers from all harvests were combined into a tuber fraction. Growth rates presented are mean growth rates. They were calculated over variable periods, comprising different growth phases, but are always expressed in g m^{-2} d^{-1}. More details on methods were described by Lommen & Struik (1992).

Definitions.

Plant age: time passed since the transplanting of the *in vitro* plantlets to the glasshouse.

Minituber number: number of tubers ≥ 0.3 g.

Total tuber number: number of tubers > 0.0 g.

Combined tuber number: the sum of the number of tubers from non-destructive harvest(s) (always ≥ 0.3 g) and final harvest (respectively ≥ 0.3 g and > 0.0 g, for combined number of minitubers and combined total tuber number).

Actual tuber number: number of tubers present at the plant.

Results

Stolon and tuber characteristics after a first non-destructive harvest. Comparisons of treatments 4, 5 and 6 (showing the situation at the first harvests), with treatments 4+7, 5+8 and 6+9 (in which the growing period between the first and final harvest was 3 weeks), showed that the number of stolons only increased after a first non-destructive harvest of 5 and 6 weeks old plants of cv. Bintje (Table 1). Stolons were short (on average 1.6 cm), both at the first non-destructive harvest and 3 weeks later (Table 1).

In the 3-weeks growing period between the first and final harvest, many new tubers were initiated (Figs 2A and 2B). Most tubers were initiated in the 3-weeks growing period after a non-destructive harvest of 6 weeks old plants in cv. Ostara and 5 weeks old plants in cv. Bintje. However, the influence of plant age on the initiated number of tubers was smaller in cv. Ostara (Fig. 2A) than in cv. Bintje (Fig. 2B). Three weeks after a first non-destructive harvest, a much larger proportion of the tubers was < 0.3 g than before (Table 1). This proportion was much larger in cv. Ostara than in cv. Bintje.

If the final harvest was postponed from 3 weeks after the first harvest (treatments 4+7, 5+8 and 6+9) till 11 weeks after transplanting (treatments 4+11, 5+11 and 6+11) total number of tubers decreased: many of the newly initiated small tubers were resorbed (Figs 2A and 2B). The later the first harvest, the more tubers were resorbed in the period from 3 weeks after the first harvest till 11

Table 1. Stolon and tuber characteristics of two cultivars at a first non-destructive harvest and a second/final harvest at different moments. Data of the destructive harvest of each treatment only. See Fig. 1 for treatment codes.

Treatment	First non-destructive harvest	Stolon number per plant[a]		Length (cm) per stolon[a]		Percentage of tubers < 0.3 g		g fresh per tuber > 0 g		g fresh per tuber ≥ 0.3 g	
		Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje
at first non-destructive harvest											
4	no	2.33	2.00	1.71	1.39	36.6	21.3	0.81	1.05	1.24	1.28
5	no	2.93	1.89	1.54	1.63	42.9	33.3	1.38	1.79	2.54	2.82
6	no	2.36	1.90	2.28	1.70	34.9	13.1	2.53	2.87	3.77	3.42
at second/final harvest, 3 weeks after first non-destructive harvest											
4+7	4 w[b]	1.92	2.28	1.51	1.54	61.8	46.3	1.05	0.87	2.62	1.37
5+8	5 w	2.28	3.33	1.46	1.66	67.5	53.1	0.84	0.53	2.62	1.05
6+9	6 w	3.23	4.28	1.51	1.22	70.8	47.9	0.61	0.58	1.91	1.05
at second/final harvest, 11 weeks after transplanting											
4+11	4 w	-[a]	-[a]	-[a]	-[a]	35.2	23.1	2.97	1.74	4.78	2.20
5+11	5 w	-[a]	-[a]	-[a]	-[a]	19.3	18.7	2.42	1.07	3.10	1.29
6+11	6 w	-[a]	-[a]	-[a]	-[a]	16.8	33.3	2.03	0.97	2.39	1.31
LSD 5 % (all means)		0.96		ns[c]		17.5		0.57		0.77	

[a] In older plants, no accurate measurements could be made of number and length of stolons, due to stolon and plant senescence.
[b] w: weeks after transplanting.
[c] ns: not significant, no LSD 5 % calculated.

weeks after transplanting.

Although extending the growing period from 3 weeks after the non-destructive harvest till 11 weeks after transplanting decreased total tuber numbers, it hardly affected the number of tubers ≥ 0.3 g (Figs 2C and 2D). No significant increase was observed. When cv. Bintje was harvested late for the first time, extending the growing period even significantly reduced the number of tubers ≥ 0.3 g (Fig. 2D). The average weight per tuber, however, increased by extending the growing period (Table 1).

Growth rates after a first non-destructive harvest. Table 2 shows that from 4 to 6 weeks after transplanting (the moments at which the first harvests took place) no new leaf appearance occurred in undisturbed plants, but stems were still elongating. Leaf dry weight reached its maximum 5 weeks after transplanting, but dry weights of most other plant parts increased until at least 6 weeks after transplanting. Total dry weight of the non-tuber fraction (excluding roots) was highest 6 and 5 weeks after transplanting for cvs. Ostara and Bintje respectively (not shown). On average, tubers made up 30 % of the dry matter when young (4 weeks) plants were harvested for the first time and 62 % when older (6 weeks) plants were harvested for the first time.

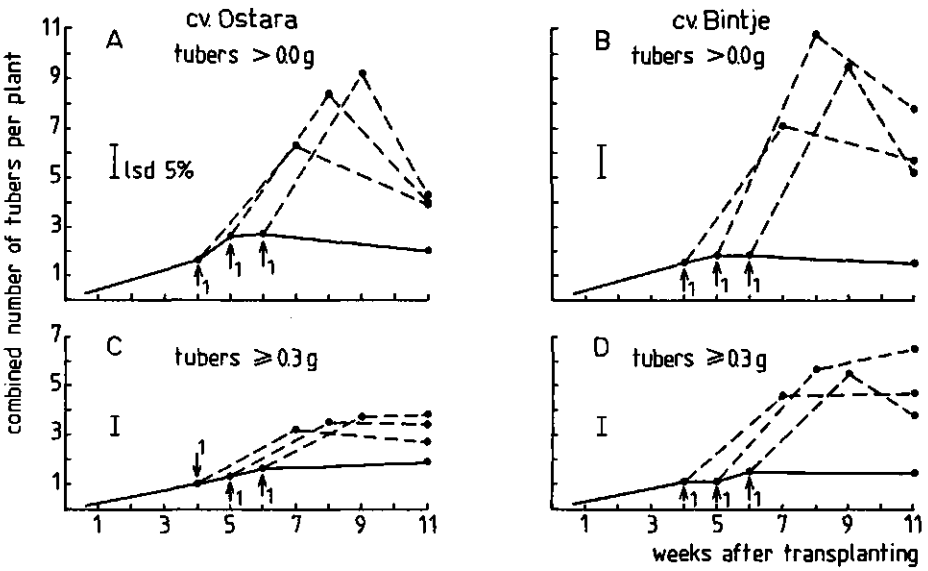

Fig. 2. Development over time of the combined number of tubers from all harvests of plants growing undisturbed and after a first non-destructive harvest that took place 4, 5 or 6 weeks after transplanting. Total number of tubers (> 0 g) of cvs. Ostara (A) and Bintje (B); Number of minitubers (≥ 0.3 g) of cvs. Ostara (C) and Bintje (D). ——— undisturbed growth, – – – – after first non-destructive harvest. Arrows indicate first non-destructive harvest.

During the 3 weeks after a non-destructive harvest of 4 weeks old plants, the dry weight of the non-tuber fraction hardly changed: growth rates (GRs) were around zero (Table 3). However, during the 3 weeks after a non-destructive harvest of older plants, GRs of the non-tuber fraction were negative. In contrast, tuber GRs were positive and not affected by the age of the plants at the first harvest (Table 3). Overall GRs during the first 3 weeks after a non-destructive harvest were positive, but slightly lower (though not significantly so) when plants were older at the first harvest.

When the second harvest was postponed till 11 weeks after transplanting, non-tuber GRs in the period from 3 weeks after the non-destructive harvest till 11 weeks after transplanting, were clearly negative (Table 3). The later the first non-destructive harvest, the more negative these GRs. Tuber and overall GRs during this time interval were lower, the later the first harvest. Due to severe plant senescence, all three GRs were negative when the growing period after a harvest of 6 weeks old plants was extended from 9 to 11 weeks after transplanting (Table 3). This could not be explained merely by the period over which GRs were calculated: when the non-destructive harvest took place 4 weeks after transplanting, overall and tuber GRs were even positive in the last week (not shown).

Compared to undisturbed growing plants, a first non-destructive harvest did not affect GRs of the non-tuber fraction over the time interval from the non-destructive harvest moment till 11 weeks after

Table 2. Characteristics of undisturbed growing plants of two cultivars at first non-destructive harvests and 11 weeks after transplanting. See Fig. 1 for treatment codes.

Treatment	Node number on main stem		Stem length (cm)		Dry weights (mg/plant)										Harvest index[a]	
					leaf		stem		stolon		root		tuber			
	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje	Ostara	Bintje
4	12.6	13.9	21.3	25.9	308	290	77	96	3.15	2.45	16.0	17.8	159	192	27.2	31.9
5	12.6	13.6	23.8	26.0	379	366	116	113	4.34	2.42	20.2	19.8	438	478	45.1	47.5
6	12.5	13.6	24.2	30.2	363	329	147	123	7.44	1.82	26.9	23.0	892	805	62.6	61.8
11	12.5	14.3	27.3	34.0	36	57	73	72	0.86	1.24	10.4	8.0	2154	2120	94.8	94.0
LSD 5 % (all means)	0.8		2.9		62		29		3.11		8.7		173		6.9	

[a] Roots included.

Table 3. Growth rates (GRs) in g m^{-2} d^{-1} of different plant fractions of two cultivars from a first non-destructive harvest at different moments till 3 weeks after the non-destructive harvest and from 3 weeks after the non-destructive harvest till 11 weeks after transplanting.

Timing of first non-destructive harvest	Period during which GRs were calculated	Non-tuber GR		Tuber GR		Overall GR	
		Ostara	Bintje	Ostara	Bintje	Ostara	Bintje
from non-destructive harvest till 3 weeks after non-destructive harvest							
4 w[a]	4 w - 7 w	0.2	-0.5	14.0	13.9	14.2	13.4
5 w	5 w - 8 w	-1.9	-2.3	14.8	13.2	12.8	10.9
6 w	6 w - 9 w	-3.2	-2.1	13.0	13.0	9.0	10.9
LSD 5 %		1.5		ns[b]		ns	
from 3 weeks after non-destructive harvest till 11 weeks after transplanting							
4 w	7 w - 11 w	-3.4	-3.3	11.8	7.5	8.4	4.2
5 w	8 w - 11 w	-4.6	-4.1	5.1	8.4	0.5	4.3
6 w	9 w - 11 w	-5.9	-5.5	-0.3	-5.3	-6.2	-10.8
LSD 5 %		1.3		9.9		10.4	

[a] w: weeks after transplanting.
[b] ns: not significant, no LSD 5 % calculated.

transplanting (Table 4). Tuber and overall GRs, however, were reduced strongly by a non-destructive harvest. The later the first non-destructive harvest, the more severe the reduction.

Tuber characteristics after a second non-destructive harvest. In the 3-weeks growing period between a second and third harvest, no initiation of new tubers was observed (Fig. 3A, 3B, 3E, 3F), except in cv. Ostara when the second harvest took place 7 weeks after transplanting (Fig. 3A, comparison of treatments 4+7 and 4+7+10). After a second harvest of 8 week old plants, total tuber number remained constant when the first harvest had taken place 4 weeks after transplanting (Figs 3A and 3B, comparison of treatments 4+8 and 4+8+11), but decreased when the first harvest had taken place 5 weeks after transplanting (Figs 3E and 3F, comparison of treatments 5+8 and 5+8+11).

In all cases tubers ≥ 0.3 g were still formed after all tubers ≥ 0.3 g were removed in a second non-destructive harvest (Figs 3C, 3D, 3G and 3H). Approximately half of the tubers had not reached a size of ≥ 0.3 g within 3 - 4 weeks after a second harvest (Table 5). The average fresh weight of all tubers was higher than 0.3 g, while the average fresh weight of the tubers ≥ 0.3 g in general was higher than 1 g (Table 5).

Growth rates after a second non-destructive harvest. After a second non-destructive harvest, non-tuber GRs were always negative (Table 6). The decreases in non-tuber dry weight were similar to the decreases in plants left undisturbed after a first non-destructive harvest. Tuber GRs generally were positive, but were less than half of tuber GRs of plants left undisturbed after the first non-destructive harvest (Table 6). Compared to plants left undisturbed after the first harvest, a second

Table 4. Growth rates (GRs) in g m^{-2} d^{-1} of different plant fractions of two cultivars after a first non-destructive harvest at different moments and during undisturbed growth from the non-destructive harvest moments until 11 weeks after transplanting.

Timing of first non-destructive harvest	Period during which GRs were calculated	Non-tuber GR		Tuber GR		Overall GR	
		Ostara	Bintje	Ostara	Bintje	Ostara	Bintje
after a first non-destructive harvest							
4 w[a]	4 w - 11 w	-1.9	-2.1	12.8	10.2	10.9	8.1
5 w	5 w - 11 w	-3.3	-3.2	9.9	10.8	6.7	7.6
6 w	6 w - 11 w	-4.3	-3.5	7.7	5.7	3.0	2.3
		mean:	*-3.0*	*mean:*	*9.5*	*mean:*	*6.4*
without first non-destructive harvest							
no	4 w - 11 w	-2.0	-1.8	14.2	13.8	12.3	11.9
no	5 w - 11 w	-3.2	-2.9	14.3	13.7	11.1	10.8
no	6 w - 11 w	-4.2	-3.2	12.6	13.2	8.0	9.9
		mean:	*-2.9*	*mean:*	*13.6*	*mean:*	*10.7*
LSD 5 % (all means)		0.7		3.0		3.2	
Significance of effect of non-destructive harvest[b]		ns		***[c]		***	

[a] w: weeks after transplanting.
[b] *** $P < 0.001$, ns not significant: $P \geq 0.05$.
[c] Mean squares tested against error mean squares. Interaction of effect of non-destructive harvest on tuber GRs with timing of harvest, however, was significant ($0.05 > P > 0.01$).

harvest reduced overall GRs severely. Overall GRs were slightly positive or negative after the second harvest, except during the 3 weeks after the earliest harvest (Table 6).

Combined tuber numbers. For practical purposes, tubers from all harvests are of interest. Fig. 4 shows the combined tuber numbers. In cv. Ostara, highest tuber numbers were observed when plants were harvested three times, with treatment 4+7+10 performing best (Figs 4A and 4C). In cv. Bintje, however, the combined number of tubers was increased less by a second non-destructive harvest (Figs 4B, 4D and 4F), especially when the timing of the first harvest was right (5 weeks after transplanting, Fig. 4H). In this cultivar, highest number of tubers > 0.0 g was produced in treatment 5+8 (Fig. 4F), highest number of minitubers (≥ 0.3 g) in plants left undisturbed after this first non-destructive harvest (treatment 5+11, Fig. 4H, while treatment 5+8 did not differ significantly from treatment 5+11). Averaged over both cultivars, however, treatment 4+7+10 produced most minitubers. Prolonging the interval between the second and the third harvest (treatment 4+7+11) did not increase the number of minitubers, averaged over two cultivars. Plants were senescing seriously at that time. When instead of 4 weeks after transplanting (treatment 4+7+10) the first harvest was carried out 5 weeks after transplanting (treatment 5+8+11), combined tuber numbers were lower, but not significantly.

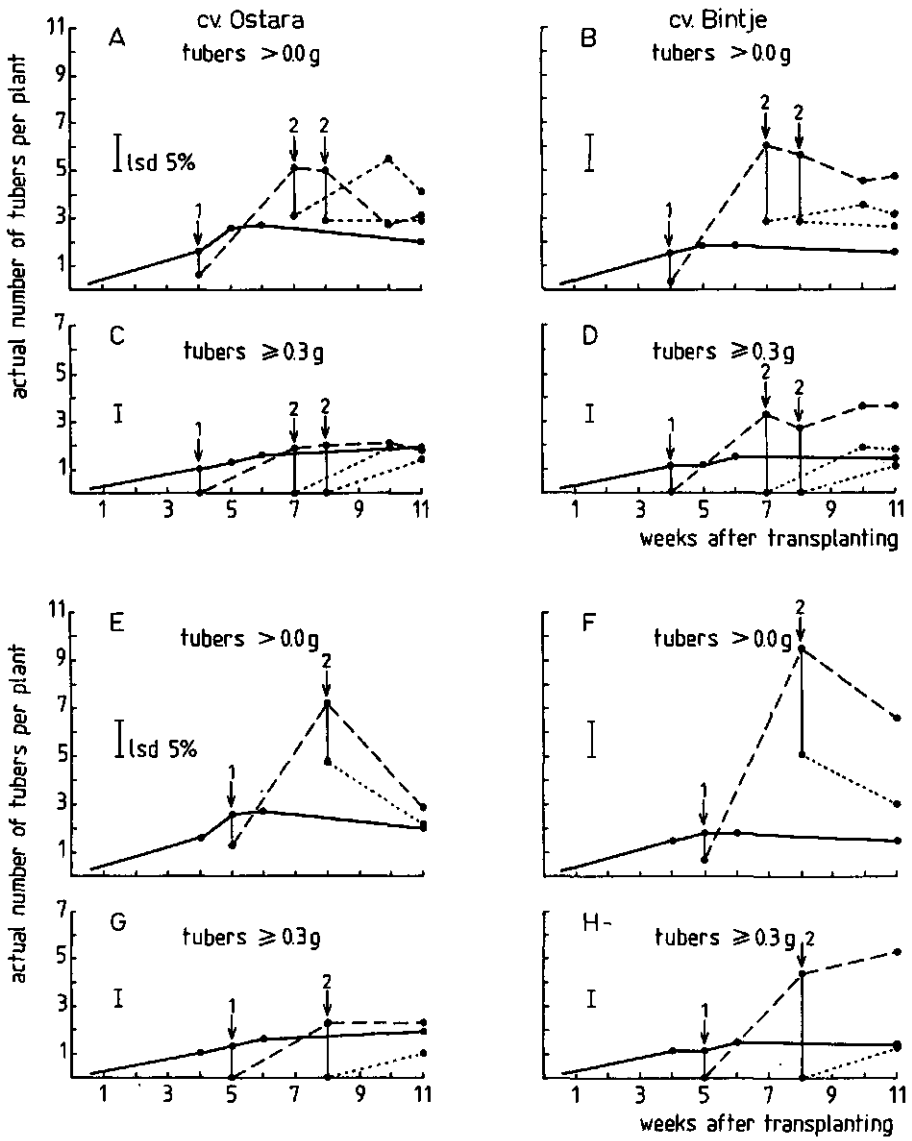

Fig. 3. Development over time of the actual number of tubers, present on plants growing undisturbed and after one and two non-destructive harvests. Total number of tubers (> 0.0 g) of cvs. Ostara (A) and Bintje (B), and number of minitubers (≥ 0.3 g) of cvs. Ostara (C) and Bintje (D) when the first non-destructive harvest took place 4 weeks after transplanting; Total number of tubers of cvs. Ostara (E) and Bintje (F), and number of minitubers of cvs. Ostara (G) and Bintje (H) when the first non-destructive harvest took place 5 weeks after transplanting. ——— undisturbed growth, – – – – after first non-destructive harvest, ····· after second non-destructive harvest. Arrows indicate first or second non-destructive harvest.

Table 5. Tuber characteristics of two cultivars, at a second non-destructive harvest and a third/final harvest at different moments. Data of the destructive harvest of each treatment only. See Fig. 1 for treatment codes.

Treatment	First non-destructive harvest	Second non-destructive harvest	Percentage of tubers < 0.3 g		g fresh per tuber ≥ 0 g		g fresh per tuber ≥ 0.3 g	
			Ostara	Bintje	Ostara	Bintje	Ostara	Bintje
at second non-destructive harvest								
4+7[a]	yes	no	61.8	46.4	1.05	0.87	2.62	1.37
4+8	yes	no	53.1	47.7	1.76	1.22	3.40	2.14
5+8[a]	yes	no	67.5	53.1	0.84	0.53	2.62	1.05
at third/final harvest								
4+7+10	yes	yes	61.6	43.6	0.66	0.68	1.63	1.13
4+7+11	yes	yes	47.1	43.7	1.13	0.82	2.16	1.31
4+8+11	yes	yes	45.9	55.8	0.62	0.45	1.14	0.83
5+8+11	yes	yes	55.9	55.1	0.50	0.57	1.05	1.13
LSD 5 % (all means)			17.4		0.58		0.72	

[a] Same values as shown in Table 1.

Table 6. Growth rates (GRs) in g m^{-2} d^{-1} of different plant fractions of two cultivars after a second non-destructive harvest and during undisturbed growth after a first non-destructive harvest.

Timing of first non-destructive harvest	Timing of second non-destructive harvest	Period during which GRs were calculated	Non-tuber GR		Tuber GR		Overall GR	
			Ostara	Bintje	Ostara	Bintje	Ostara	Bintje
after a second non-destructive harvest								
4 w[a]	7 w	7 w - 10 w	-3.2	-3.3	8.9	5.4	5.6	2.1
4 w	7 w	7 w - 11 w	-2.8	-3.4	7.8	2.1	5.0	-1.4
4 w	8 w	8 w - 11 w	-3.8	-3.5	4.1	0.6	1.1	-2.9
5 w	8 w	8 w - 11 w	-5.4	-4.5	-0.7	5.0	-6.1	0.4
			mean:	*-3.8*	*mean:*	*4.2*	*mean:*	*0.5*
without second non-destructive harvest								
4 w	no	7 w - 10 w	-4.3	-3.7	12.8	8.4	8.5	4.7
4 w	no	7 w - 11 w[b]	-3.4	-3.3	11.8	7.5	8.4	4.2
4 w	no	8 w - 11 w	-3.2	-3.1	10.5	7.2	7.4	4.0
5 w	no	8 w - 11 w[b]	-4.6	-4.1	5.1	8.4	0.5	4.3
			mean:	*-3.7*	*mean:*	*9.0*	*mean:*	*5.3*
LSD 5 % (all means)			1.2		5.9		6.2	
Significance[c] of effect of second non-destructive harvest			ns		***		***	

[a] w: weeks after transplanting.
[b] Same values as shown in Table 3.
[c] *** $P < 0.001$; ns not significant: $P \geq 0.05$.

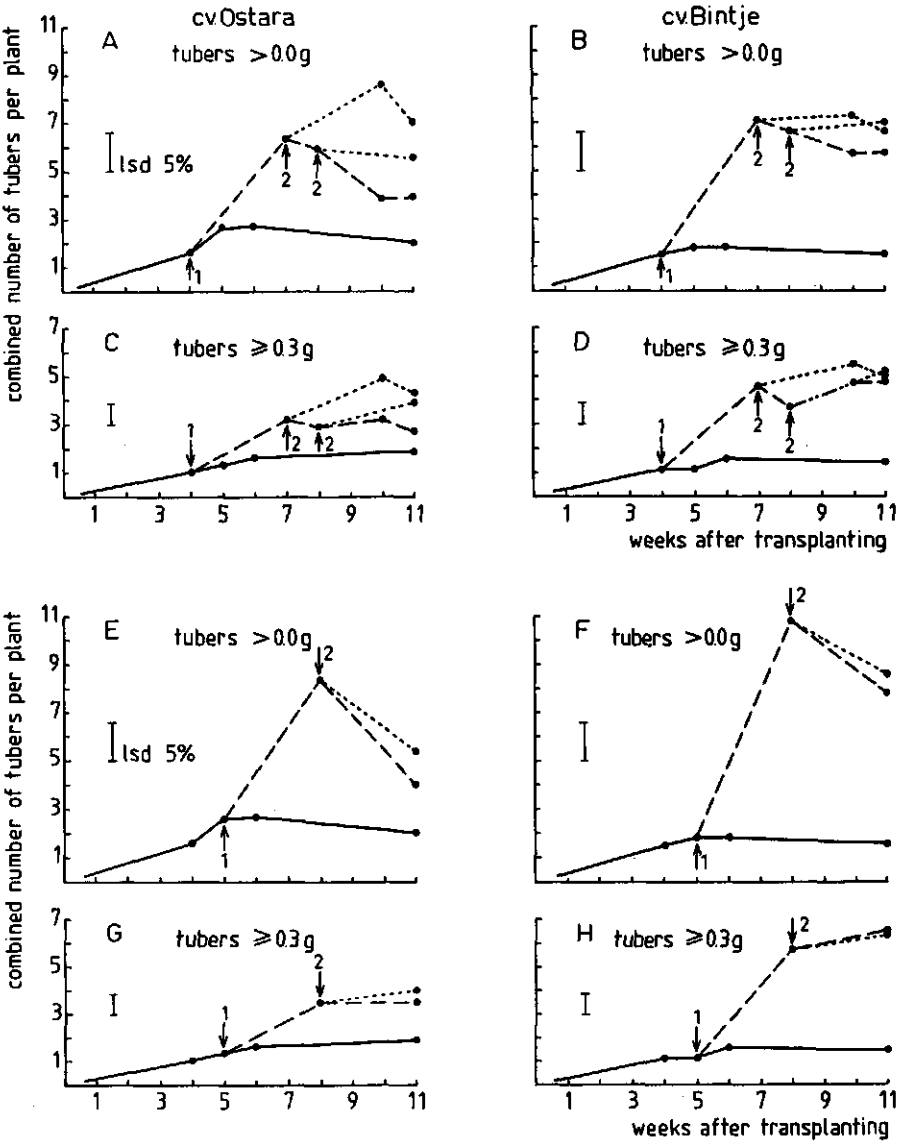

Fig. 4. Development over time of the combined number of tubers from all harvests of plants, growing undisturbed and after one and two non-destructive harvests. A to H and legend as in Fig. 3.

Discussion

Tuber initiation and resorption after a first non-destructive harvest. The extensive initiation of new tubers that occurred within 3 weeks after removal of tubers in a first non-destructive harvest (Figs 2A and 2B), confirms previous results (Lommen & Struik, 1992) and results obtained by Oparka (1987) after removal of stolon apices and by Nösberger & Humphries (1965) after removal of tubers plus stolons. This initiation of new tubers was attributed to the breaking of the dominance of the removed tuber and the deeper replanting of the plantlets after a non-destructive harvest, exposing more nodes to below ground conditions (Lommen & Struik, 1992).

In the experiment described in this paper, the number of tubers initiated within 3 weeks after a first non-destructive harvest proved to be much higher than the number of tubers that finally set (Figs 2A and 2B). Many tubers were resorbed. Similar results are reported with plants under field conditions, where considerable resorption of tubers is often observed after treatments causing the initiation of many tubers, like a high moisture level (Krug and Wiese, 1972), temperatures favouring early stem and haulm development (Cho & Iritani, 1983) or the removal of stolon apices (Oparka 1987), if these treatments are followed by normal plant senescence. Ewing & Struik (1992) suggested that after treatments causing extensive tuber initiation, the number of surviving tubers would not be increased, unless productivity also was increased by these treatments. In our experiment, however, no significant effect of a non-destructive harvest was observed on non-tuber GRs, and overall and tuber GRs even were reduced (Table 4). Still, 11 weeks after transplanting, tuber numbers (> 0.0 g) in plants after a first non-destructive harvest, always were higher than in treatments in which plants grew undisturbed (Figs 3A, 3B, 3E and 3F). In our experiment, resorption of tubers always occurred during plant senescence (i.e. when dry weights of the non-tuber fraction decreased). Due to the tuber inducing conditions and the high plant density of 350 plants per m^2, the plants completed their growth cycle very rapidly. Larger decreases in tuber number (Figs 2A and 2B), were associated with lower (more negative) GRs of the non-tuber fraction (Table 3).

Tuber growth after a first non-destructive harvest. In general, the number of tubers ≥ 0.3 g did not decrease during tuber resorption (Figs 2C and 2D). Thus, it seems plausible that in our experiment an individual tuber weight of 0.3 g was large enough to become a competitive tuber. Only in strongly senescing, deteriorating plants, when even tuber GRs became negative (Table 3), a decrease in number of tubers ≥ 0.3 g was observed. However, no significant increase in number of tubers ≥ 0.3 g was observed either, later than 3 weeks after a non-destructive harvest (Figs 2C and 2D). This shows that prolonging the growing period, and thus increasing the total amount of assimilates, was not effective in increasing the number of tubers ≥ 0.3 g.

High numbers of tubers initiated, may increase the number of tubers that can grow to a size of ≥ 0.3 g, provided tuber GRs are sufficiently high. Under field conditions, MacKerron et al. (1988) observed more uniform tuber sizes of tubers > 15 mm at higher tuber numbers. In our experiment, the timing of the first non-destructive harvest strongly affected the number of tubers initiated within

3 weeks after a first harvest in cv. Bintje (Fig. 2B), while tuber GRs were similar (Table 3). Higher numbers of tubers initiated indeed were associated with higher numbers of tubers ≥ 0.3 g, 3 weeks after a non-destructive harvest. Thus, the timing of the first harvest also affected the number of tubers ≥ 0.3 g that was produced finally. Differences between cultivars were remarkable. In cv. Ostara, less tubers were initiated after a first non-destructive harvest than in cv. Bintje (Fig. 2), while tuber GRs were similar (Table 3). Moreover, in cv. Ostara, a smaller proportion of the initiated tubers did grow to a size of ≥ 0.3 g within 3 weeks (Table 1), thus becoming unlikely to be resorbed.

Low tuber GRs during the 3 weeks interval after a non-destructive harvest, however, may reduce the proportion of the initiated tubers that can grow to a size of 0.3 g within 3 weeks under almost similar conditions: Lommen & Struik (1992) showed that postponing a non-destructive harvest reduced tuber GRs more than tuber initiation. This was reflected in much lower numbers of tubers ≥ 0.3 g, 3 weeks after a non-destructive harvest. In our experiment, however, averaged weights of all tubers were much higher than 0.3 g (Table 1). Thus, differences in initial weight and/or growth rates between individual tubers must have influenced the proportion of tubers that could grow to a size of ≥ 0.3 g.

Tuber initiation and resorption after a second non-destructive harvest. Three weeks after a second non-destructive harvest, no initiation of new tubers was observed, except in cv. Ostara harvested non-destructively after 4 and 7 weeks (Fig. 3A). In general, tuber numbers remained constant or declined (Figs 3A, 3B, 3E and 3F). There could be several possible explanations for the difference in response after a first and a second non-destructive harvest: (a) the number of possible tuber sites was limited, (b) the tubers that remained on the plant after a harvest because they had not reached the desired size became dominant before new tubers were initiated, (c) the newly initiated tubers were already resorbed before the plants were analysed and (d) plants approached senescence and tuber initiation period had ended. The age of the plants itself (possibility d), most probably did not prevent initiation of tubers. Under similar conditions, Lommen & Struik (1992) observed extensive initiation of tubers even when senescing plants were harvested non-destructively for the first time. The first three possible explanations, however, can not be ruled out.

The number of tuber sites is not accurately known, but might have been limiting. Many sites had already been occupied by removed tubers, and plants, including stolons, were seriously senescing at the time of the second harvest. The different response of cv. Ostara, harvested non-destructively after 4 and 7 weeks (treatment 4+7+10), supports the view that the number of tuber sites may have limited tuber initiation. In this treatment, plants were less senescent than older plants and less tuber sites had been occupied thus far compared to cv. Bintje (Figs 2A and 2B). On the other hand, formation of above-ground tubers is often observed when plants are induced to tuberize and tuber sites below ground are lacking, for instance after removing stolons plus tubers (Abdel-Waheb & Miller, 1963; Paiva et al, 1983). In our experiment, above ground tubers were formed only incidentally and not significantly more after a second non-destructive harvest than without this non-destructive harvest (results not shown).

A second explanation is a rapid restoration of dominance by tubers remaining on the plant. More

tubers remained on the plant after a second non-destructive harvest than after a first non-destructive harvest (Figs 3A, 3B, 3E and 3F), and often more tubers remained on the plant than were likely to set and start bulking. Marschner et al. (1984) observed a restoration of normal tuber growth rates, 4 days after removal of all fast growing tubers, thus dominance in our experiment, could have been restored very rapidly. In favour of this second explanation is the different change in tuber number after the second harvest of plants harvested non-destructively after 4 plus 8 weeks (Figs 3A and 3B) and after 5 plus 8 weeks (Figs 3E and 3F). In the latter treatment, more tubers remained on the plant after the second harvest, resulting in a decrease in tuber number after the second harvest. In the first treatment less tubers remained on the plant and tuber numbers did not change.

Finally, already some resorption may have occurred within the 3 weeks after the second non-destructive harvest, because plants were seriously senescing after the second harvest. This view is supported by the fact that extending the growing period from 3 to 4 weeks after the second non-destructive harvest, tended to decrease the number of newly initiated tubers in cv. Ostara (Fig. 3A).

Tuber growth after a second non-destructive harvest. The number of tubers ≥ 0.3 g always increased after a second non-destructive harvest, regardless of how total tuber numbers changed (Figs 3C, 3D, 3G and 3H), and their average weight in general was higher than 1 g (Table 5). The increase in number of tubers ≥ 0.3 g indicates that a second harvest stimulated the growth of tubers that would have been resorbed or had not reached the final phase in tuber formation without a second non-destructive harvest. Only around half of all tubers was ≥ 0.3 g (Table 5), but the average fresh weight of all tubers was higher than 0.3 g. Therefore, again, differences in initial weight or growth rates between individual tubers must have influenced the proportion of tubers growing to a size of ≥ 0.3 g after a second non-destructive harvest.

Fitting in the effect of a non-destructive harvest into the general concept of tuber formation. Based on papers by Struik et al. (1988) and Vreugdenhil & Struik (1989), eight phases can be distinguished in the process of tuber formation: (1) stolon induction, (2) stolon initiation, (3) stolon growth, (4) cessation of stolon growth, (5) tuber induction, (6) tuber initiation, (7) tuber set and (8) tuber growth. Tuber resorption, however, can replace tuber set and tuber growth. Before the first non-destructive harvest, at least part of the tubers was already growing rather rapidly. Initiation of more tubers was limited, most probably by the lack of tuber sites that were not subjected to the dominance of the rapidly growing tubers. At a non-destructive harvest, the tubers that were most advanced in the process of tuber formation, were removed. Thereafter, many new tubers were initiated. Only a part of them was able to grow through all phases of tuber formation. Plants were already senescing by then and many tubers were resorbed if plants were left undisturbed after the first harvest. If, instead, plants were harvested non-destructively a second time, the tubers removed again included the dominant tubers that could prevent the initiation or growth of other tubers. After removal of these, tubers that would have been resorbed or had not reached the final phase in tuber formation yet, were able to develop further.

Growth reductions after a non-destructive harvest. The long-term (5-7 weeks) reduction in tuber and overall growth caused by a first non-destructive harvest, was most severe when older plants were harvested non-destructively (Table 4). This is in accordance with previous results (Lommen & Struik, 1992) in which it was shown that 3 weeks after a non-destructive harvest, growth was reduced most in senescent plants. This was attributed to the senescent plants not being able to adapt after the non-destructive harvesting procedure, in which not only tubers were removed, but which also caused root damage.

Like the first non-destructive harvest, a second harvest again caused a severe reduction in overall and tuber GRs, compared to plants left undisturbed after a first non-destructive harvest (Table 6). This reduction in growth will have similar causes as the reduction observed in rather old plants after a first non-destructive harvest (Lommen & Struik, 1992). If old plants (8 weeks) were harvested non-destructively a second time, however, even overall GRs were only slightly positive or negative. We surmise that in those cases most of the gain in tuber dry weight was caused by redistribution from the non-tuber plant fraction.

The reduction in tuber GRs after a second non-destructive harvest, compared to undisturbed growth after a first non-destructive harvest, implies that under circumstances where no significant gain in tuber numbers can be expected (Fig. 4H), a second harvest will only reduce tuber yield.

Practical implications. For commercial production of high numbers of minitubers, all larger tubers from non-destructive and final harvests can be used. The procedure described in this paper, growing plants under tuber inducing conditions at a plant density of 350 plants per m^2 and using two non-destructive harvests of tubers $\geq$ 0.3 g and a third and final harvest, proved to be a suitable method for producing high numbers of minitubers, both per m^2 and per plantlet. The first non-destructive harvest stimulated the initiation of new tubers and the second non-destructive harvest in general stimulated the set and growth of tubers which otherwise would have remained small or would have been resorbed.

With a production scheme using intervals of 3 weeks between harvests, high numbers of tubers were obtained in both cultivars; the first harvest could be carried out around 4 weeks after transplanting (harvest scheme 4+7+10). Using this method 1740 minitubers per m^2 were produced in cv. Ostara, and 1946 in cv. Bintje in 10 weeks. These minitubers had average weights higher than 1 g (Tables 1 and 5). For some cultivars, however, a harvest scheme consisting of only 2 harvests may be used. In cv. Bintje, a second non-destructive harvest was less effective in increasing the combined number of minitubers; higher yields and tuber numbers could therefore be obtained in a production scheme with only 2 harvests (harvest scheme 5+11). However, in this scheme the timing of the first harvest was very critical. The optimal moment could not be assessed on the basis of the plant habitus. Using a 3-harvests scheme would therefore be more safe. Optimizing the production technique for minitubers and establishing their storage behaviour and field performance, will be reported in forthcoming papers.

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CHAPTER 4

Production of potato minitubers by repeated harvesting: Effects of crop husbandry on yield parameters

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4 PRODUCTION OF POTATO MINITUBERS BY REPEATED HARVESTING: EFFECTS OF CROP HUSBANDRY ON YIELD PARAMETERS

Additional keywords: Solanum tuberosum L., seed production, rapid multiplication, tuber pruning, non-destructive harvest, nutrient supply, plant density, plant arrangement

Summary

Minitubers can be produced in large quantities by repeated harvesting of tubers from *in vitro* propagated plantlets at 4, 7 and 10 weeks after transplanting to the glasshouse at high plant densities. Yield parameters of minitubers can be manipulated by crop husbandry.

By supplying nutrients or using a square plant arrangement, minituber yield increased. Effects on numbers of tubers were cultivar-dependent.

Changing plant density from 50 to 800 plants per m^2 or the minimal diameter of harvested tubers from 5 to 12 mm, did not significantly affect tuber yield per m^2. Higher plant densities resulted in more tubers per m^2 but fewer tubers per plant. Removing smaller tubers greatly increased the number of small tubers, but did not affect yield and number of tubers in larger grades.

Crop husbandry techniques affected minituber yield mainly through their effects on leaf area duration, and the number of minitubers through their effects on growth of tubers to a harvestable size.

Introduction

Minitubers are small seed potato tubers, produced on *in vitro* propagated plantlets after transplanting to the glasshouse at a high plant density. By using minitubers in a seed potato programme, the number of field multiplications can be reduced.

In a previous paper (Lommen & Struik, 1992b), a production method for minitubers was suggested by which over 1800 minitubers could be produced per m^2 within 10 weeks. This method was suitable for several cultivars and it consisted of growing plants at a high plant density and removing tubers ≥ 0.3 g in three harvests, of which two were non-destructive. The first non-destructive harvest stimulated the initiation of new tubers (Lommen & Struik, 1992a, b), the second non-destructive harvest stimulated the growth of tubers which otherwise would have been resorbed or would have remained too small (Lommen & Struik, 1992b). Compared to plants left undisturbed, the number of minitubers was greatly increased, but the weight of the tubers was reduced, probably because of root damage and the removal of tuber sinks (Lommen & Struik, 1992a).

When producing minitubers, five mutually dependent yield parameters may be manipulated; (1) the number of minitubers per *in vitro* plantlet, (2) the number of minitubers per unit area, (3) the average weight per minituber, (4) the minituber yield per plantlet, and (5) the minituber yield per unit area. Which parameters are favoured, will depend on the costs and availability of facilities and labour and the intended use of the minitubers. Yield parameters may be manipulated by crop

husbandry during minituber production.

In undisturbed plants of normal crops, **number of tubers per plant** increases when plants are fertilized before tuber initiation (cf. Gunasena & Harris, 1968). Number of tubers per stem may increase (cf. Wurr, 1974) or remain constant (cf. Vander Zaag et al., 1990) at lower plant densities. **Number of tubers per unit area** increases at higher plant densities (Ifenkwe & Allen, 1978). **Average weights per tuber** are reported to be higher at lower densities (cf. Vander Zaag et al., 1990; Bremner & Taha, 1966) and in fertilized compared to non-fertilized plants (cf. Simpson, 1962). **Tuber yields per plant** are higher at lower plant densities (cf. Bremner & Taha, 1966). **Tuber yield per unit area** may be increased by fertilization (cf. Ryan, 1961) or higher plant densities (cf. Bremner & Taha, 1966); it may (Svensson, 1972) or may not (Bleasdale & Thompson, 1963) increase by less rectangular plant arrangements. If these cultivation techniques are also effective on *in vitro* plantlets, they could be used to manipulate minituber production. Therefore, the effects of nutrient supply, plant density and plant arrangement on yield parameters of minitubers were studied. In addition, the effects were studied of changing the diameter of the tubers, removed at the three harvests. Decreasing this diameter may increase the number of minitubers harvested, because many tubers initiated do not grow to the desired size (Lommen & Struik, 1992b).

Materials and methods

Production of in vitro plantlets. In vitro plantlets of *Solanum tuberosum* L. cv. Ostara (early), cv. Bintje (mid-early) and cv. Elkana (late) were produced by subculturing single-node stem cuttings about every 4 weeks. Details are described by Lommen & Struik (1992a, b). The growing periods from the last subculturing until transplanting were 17 days (Expt 1), 8 days (Expt 2), 13 days (Expt 3, cvs. Ostara and Bintje) or 9 days (Expt 3, cv. Elkana).

Culture in the glasshouse. All experiments were conducted in Wageningen, the Netherlands. *In vitro* plantlets were transplanted to a controlled environment glasshouse into a mixture of perlite and potting soil (50/50 % v/v, containing 131.4 mg N l^{-1}). Photoperiod was fixed at 12 hours. Natural light was supplemented to at least 80 W m^{-2} (total radiation) by high-pressure sodium lamps (Philips SON-T). Day temperature was set at 18 °C, night temperature at 12 °C. For fertilization, a low concentration nutrient solution was used ($Ca(NO_3)_2.4H_2O$ 0.890 g l^{-1}, KNO_3 0.446 g l^{-1}, KH_2PO_4 0.135 g l^{-1}, K_2SO_4 0.140 g l^{-1}, $MgSO_4.7H_2O$ 0.472 g l^{-1}, H_2SO_4 0.034 g l^{-1}, FeEDTA 0.035 g l^{-1}, $MnSO_4.H_2O$ 2.0 mg l^{-1}, H_3BO_3 3.0 mg l^{-1}, $ZnSO_4.7H_2O$ 0.5 mg l^{-1}, $Na_2MoO_4.2H_2O$ 0.1 mg l^{-1} and $CuSO_4.5H_2O$ 0.1 mg l^{-1}, pH 6.0).

Tubers were removed in 3 harvests at 4, 7 and 10 weeks after transplanting. The minimum diameter of the tubers removed differed between experiments. A non-destructive harvesting procedure was used at the first two harvests (Lommen & Struik, 1992a). At these harvests, root damage could not be avoided, but care was taken not to damage stems and stolons. After a non-destructive harvest, plants were replanted deeper than initially.

Experiment 1. Influence of start of nutrient supply. Cvs Ostara and Bintje were fertilized with the low concentration nutrient solution, starting at different times:
(1) 11 days after transplanting, just before tuber initiation;
(2) 28 days after transplanting, right after the first non-destructive harvest;
(3) 47 days after transplanting, right after the second non-destructive harvest.
In a fourth treatment no nutrient solution was applied.

The experimental unit was a square pot (13 x 13 cm) containing 1.75 l of soil mixture. Pots were arranged contiguously in a block design with three blocks. At least one row of guard pots surrounded the experiment. Plants were grown at a density of 350 plants per m^2, by planting 6 plants in a row in the middle of each pot. Pots received nutrient solution in doses of 100 ml, if possible twice a week, but only if the plants needed water. Total volumes of nutrient solution supplied were 1500 ml, 1000 ml and 600 ml per pot for treatments starting 11, 28 and 47 days after transplanting respectively. Unfertilized pots and pots in which the fertilization had not yet started, received the same quantities of tap water. At the three harvests, tubers ≥ 0.3 g were removed. The weight of these tubers in the non-destructive harvests was estimated, using a diameter of 8 mm as a criterion. Experiment 1 was carried out from January 8 - March 18.

Experiment 2. Influence of plant density and plant arrangement. Cvs Ostara and Bintje were planted at densities of 50, 200, 400 and 800 plants per m^2, using a distance of 10 cm between rows, and at 2 additional plant arrangements of 400 plants per m^2: 5 cm x 5 cm and 1.25 cm x 20 cm. All plants were single-stemmed and were grown on glasshouse benches covered with a sheet of plastic film without perforation, in 18 cm deep soil mixture. They were fertilized twice a week from the first harvest onwards, with 100 ml of nutrient solution per 6 plants. Additional watering was necessary. In all three harvests, only tubers ≥ 0.3 g (i.e. diameter ≥ 8 mm) were removed. Experiment 2 was carried out from May 10 - July 19. Treatments were replicated in four blocks, which were harvested by different persons. Within each block, plots with increasing plant densities were contiguous. Cultivars were assigned at random to one or other half of a block. Each net plot consisted of one row of six plants. A group of three net plots, used for determination of leaf area at the three harvest dates, was surrounded by guard plants. The number of guard plants increased at increasing densities, to ensure a uniform plant size in the net plots. At each harvest date all plants (including guard plants) were harvested, but the tuber data presented were collected from the plants from which leaf area was determined at the final harvest. Below ground between guard plants and net plants, a 5 cm wide strip of plastic prevented entanglement of stolons and roots. After a harvest, the guard plants were replanted at different positions to guard the remaining plots. Thus, plant densities and plant arrangements were maintained throughout the experiment.

Experiment 3. Influence of the diameter of the removed tubers. Tubers with diameters ≥ 5 mm, ≥ 8 mm and ≥ 12 mm were removed at each of three harvests from cvs Ostara, Bintje and Elkana. Plant density was 350 plants per m^2, achieved by planting 6 plants in a row in the middle of a pot measuring 13 x 13 x 13 cm and joining all pots. Each pot contained 1.75 l of soil medium. Nutrient

supply started after the first harvest, using 100 ml of nutrient solution per pot, twice a week. Additional watering was necessary. Expt 3 was carried out from March 30 - June 8. The experimental unit was a pot with 6 plants. Pots were arranged in a completely randomized design with 4 replications. One row of guard pots surrounded the experiment.

Results

Start of nutrient supply (Expt 1). Fertilization increased minituber yields in both cultivars (Table 1). However, the optimal moment for starting nutrient supply differed. In cv. Ostara tuber yield was highest when fertilization started after the first harvest. In cv. Bintje, the sooner the fertilization started, the higher the tuber yield. Late applications only had small effects.

Cv. Bintje produced more tubers than cv. Ostara (Table 1). In cv. Ostara the number of minitubers $\geq$ 8 mm was not influenced by the fertilization treatments. In cv. Bintje, more minitubers were produced after the first harvest when nutrient supply started earlier.

Average tuber weight in cv. Ostara was higher than in cv. Bintje (Table 1). Only in the third harvest was the average weight of the minitubers significantly higher in the fertilized treatments (Table 1). Fertilization did not affect the average tuber weight significantly if results of all harvests were combined (Table 1).

At the end of the experiment the haulm of unfertilized plants had deteriorated more than the haulm of fertilized plants.

Plant density (Expt 2). By increasing plant density from 50 to 800 plants per m^2, minituber yield per plant and number of minitubers per plant decreased in both cultivars (Table 2; data and analysis of treatments with standard row distance only). These effects were clear from the second harvest onwards. Differences between 400 and 800 plants per m^2 were small. The average weight per minituber decreased at increasing plant densities in all harvests of both cultivars (Table 2).

Tuber yield per m^2 was not significantly influenced by plant densities from 50 to 800 plants per m^2 (Table 3; data and analysis of treatments with standard row distance only), except in the first harvest. More tubers were produced per m^2 at higher plant densities, in all harvests and both cultivars (Table 3). This effect was clear between 50 and 200 plants per m^2, but not significant between 200 and 400 plants m^{-2}. At still higher densities, numbers of tubers per m^2 again clearly increased. Coefficients of variation, however, were high (Table 3), because data per plant were converted to data per m^2. These high coefficients of variation could be reduced by ln (1+x) or square root transformations (not shown), but differences in tuber yield per m^2 and differences in number of minitubers per m^2 at 200 and 400 plants per m^2 remained statistically non-significant from the second harvest onwards.

In the first two harvests and both cultivars, Leaf Area Index (LAI) was higher at higher plant densities (Table 3). In the final harvest, LAI tended to be higher at lower densities. At all but the lowest plant density, LAI was maximal by the first harvest. Thereafter, leaf area declined at variable rates, leading to large differences between replicates and high coefficients of variation (Table 3). In

Table 1. Influence of the start of nutrient supply on yield, number and size of tubers ≥ 8 mm of two cultivars, expressed per plant. Plant density: 350 plants per m^2; distance between rows: 13 cm (Expt 1). Treatments in braces were similar until that harvest.

Start of nutrient supply	Tuber yield (g fresh per plant)				Number of tubers (number per plant)				Tuber size (g fresh per tuber)			
	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined
Cultivar Ostara												
Tuber initiation	1.0	5.6	2.9	9.4	1.1	2.2	1.1	4.3	1.0	2.6	2.6	2.2
1st harvest	0.6	7.0	3.8	11.4	0.6	2.6	1.8	4.9	0.9	2.8	2.2	2.3
2nd harvest	1.2	5.5	2.5	9.2	1.1	2.6	1.1	4.8	1.1	2.2	2.3	2.0
No fertilization	1.0	5.1	2.2	8.4	0.8	2.3	1.7	4.8	1.3	2.2	1.4	1.8
Cultivar Bintje												
Tuber initiation	1.4	6.4	3.4	11.2	1.3	5.8	3.0	10.1	1.1	1.1	1.1	1.1
1st harvest	0.9	5.7	2.8	9.5	0.9	4.5	2.4	7.8	1.0	1.3	1.1	1.2
2nd harvest	0.4	5.3	3.2	8.9	0.6	3.7	2.6	6.9	0.8	1.6	1.4	1.3
No fertilization	0.9	4.6	1.7	7.2	0.8	3.7	2.1	6.6	0.9	1.3	0.8	1.1
Statistical analysis[a]												
Nutrient supply (NS)	ns	*	ns	***/ns	ns	ns	ns	*/ns	ns	ns	**	ns
Cultivar (CV)	ns	ns	ns	ns	ns	***/*	**	***/*	ns	***	***	***
Interaction (NS x CV)	*	ns	ns	*	ns	*	ns	**	ns	ns	ns	ns
CV%	42.9	13.6	40.7	9.1	36.3	17.7	41.9	14.5	30.7	22.0	22.8	13.5
SE	0.4	0.8	1.1	0.9	0.3	0.6	0.8	0.9	0.3	0.4	0.4	0.2

[a] The first indications of statistical significance presented and the CV% and SE are the results of a standard analysis of variance. Only if both main effects and interaction were significant, an F-test was performed on mean squares of main effects and interactions, to determine the relative importance of the main effect. The results of this test are presented as the second indications of statistical significance. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

Table 2. Influence of plant density on yield, number and size of tubers ≥ 8 mm of two cultivars, expressed per plant. Distance between rows: 10 cm; start of fertilization: after first harvest (Expt 2).

Plant density (plants per m^2)	Tuber yield (g fresh per plant)				Number of tubers (number per plant)				Tuber size (g fresh per tuber)			
	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined
Cultivar Ostara												
50	2.2	24.8	30.2	57.2	0.7	7.0	6.7	14.4	3.4	3.7	4.5	4.0
200	1.1	12.4	9.5	23.0	0.6	4.4	3.0	8.0	1.9	2.9	3.1	2.9
400	1.3	5.5	2.4	9.2	0.8	2.1	1.3	4.2	1.6	2.5	1.7	2.1
800	0.6	3.3	2.2	6.1	0.6	1.7	1.1	3.4	1.0	1.6	1.7	1.6
Cultivar Bintje												
50	0.8	34.9	35.7	71.4	0.3	10.6	9.8	20.7	2.0	3.3	3.7	3.5
200	0.8	9.8	6.3	16.9	0.5	4.8	3.5	8.8	1.6	2.1	1.7	1.9
400	0.6	4.0	2.0	6.6	0.4	2.3	1.0	3.7	1.4	1.6	2.0	1.7
800	0.5	4.4	1.1	6.0	0.3	2.9	1.0	4.3	1.4	1.5	1.2	1.4
Statistical analysis[a]												
Plant density (PD)	ns	***/*	***	***/**	ns	***/*	***/*	***/*	**	***	***	***
Cultivar (CV)	*	ns	ns	ns	*	**/ns	**/ns	**/ns	ns	ns	ns	*
Interaction (PD x CV)	ns	**	ns	*	ns	*	***	***	ns	ns	ns	ns
CV%	66.8	27.2	27.7	21.6	54.4	23.3	22.4	17.3	43.9	35.3	34.8	24.4
SE	0.7	3.4	3.1	5.3	0.3	1.0	0.8	1.5	0.8	0.8	0.9	0.6

[a]) The first indications of statistical significance presented and the CV% and SE are the results of a standard analysis of variance. Only if both main effects and interaction were significant, an F-test was performed on mean squares of main effects and interactions, to determine the relative importance of the main effect. The results of this test are presented as the second indications of statistical significance. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$. The analysis was conducted only on the set of plots planted at 10 cm spacing.

Table 3. Influence of plant density on yield and number of tubers ≥ 8 mm per m^2 and LAI of two cultivars. Distance between rows: 10 cm; start of fertilization: after first harvest (Expt 2).

Plant density (plants per m^2)	Tuber yield (g fresh per plant)				Number of tubers (number per plant)				Leaf Area Index		
	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest
Cultivar Ostara											
50	109	1239	1509	2858	33	352	335	721	2.0	3.7	2.0
200	215	2478	1904	4597	117	883	600	1600	5.1	4.9	2.3
400	522	2178	969	3669	300	850	533	1683	8.4	4.0	0.7
800	480	2613	1767	4860	467	1333	900	2700	7.5	7.5	2.1
Cultivar Bintje											
50	42	1744	1784	3570	17	529	490	1035	2.5	3.8	2.7
200	164	1967	1257	3387	108	950	708	1767	6.6	5.2	1.8
400	242	1593	815	2655	167	917	383	1467	8.0	6.0	0.8
800	411	3534	853	4799	267	2333	833	3433	11.0	10.6	0.4
Statistical analysis[a]											
Plant density (PD)	**	ns	ns	ns	***	***	*	***	***/*	*	*
Cultivar (CV)	ns	ns	ns	ns	ns	ns	ns	ns	***/ns	ns	ns
Interaction (PD x CV)	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns
CV%	71.1	61.5	53.4	51.3	68.4	46.3	45.1	39.3	14.7	58.9	70.7
SE	194.7	1332.7	724.4	1948.0	126.2	471.2	269.1	708.2	0.9	3.4	1.1

[a] The first indications of statistical significance presented and the CV% and SE are the results of a standard analysis of variance. Only if both main effects and interaction were significant, an F-test was performed on mean squares of main effects and interactions, to determine the relative importance of the main effect. The results of this test are presented as the second indications of statistical significance. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$. The analysis was conducted only on the set of plots planted at 400 plants per m^2.

some plots, especially at 400 plants per m^2, haulm had senesced completely by the third harvest.

Plant arrangement (Expt 2). Differences between plant arrangements were significant in the third harvest and when yields from all harvests were combined. In both cultivars, tuber yield was highest at a square plant arrangement (Table 4).

The effect of the different plant arrangements at 400 plants per m^2 on number of minitubers per plant, again depended on the cultivar (Table 4). Number of tubers in cv. Ostara was hardly affected by the different plant arrangements. Only in the third harvest did the number of tubers slightly increase when the within-row spacing increased, but when results of all harvests were combined, no effect of plant arrangement on number of minitubers was observed in cv. Ostara. In contrast, cv. Bintje clearly produced most tubers in a square arrangement; this showed from the second harvest onwards.

Average tuber weight tended to be higher at a square spacing (Table 4), but this effect was not significant at the 5 % level.

Diameter of tubers removed (Expt 3). The size of the tubers removed did not affect tuber yield (Table 5), except in the first harvest when many tubers had not yet grown to a size of 12 mm. Regardless of the diameter of the tubers removed, the yield of tubers $\geq$ 12 mm was similar (results not shown). In addition, yield of tubers $\geq$ 8 mm was similar in the treatments in which tubers from 5 or 8 mm upwards were removed (not shown).

The smaller the diameter of the tubers removed, the more tubers were harvested in all cultivars (Table 5). Regardless of the diameter of the tubers removed, similar numbers of tubers $\geq$ 12 mm were produced (results not shown) and similar numbers of tubers $\geq$ 8 mm were produced by the treatments in which tubers from 5 and 8 mm upwards were removed (not shown).

From the second harvest onwards, the average tuber weight was higher, when the diameter of the tubers removed was larger (Table 5). Even if tubers $\geq$ 5 mm were removed, the average weight of the tubers was still over 1 g in all cultivars.

Discussion

Effects of crop husbandry on tuber yield. Effects of the different treatments on minituber yield, will have been exerted through their effects on the canopy. Under field conditions in normal crops, Leaf Area Duration or Intercepted Radiation correlate well with tuber yield when fertilizer application (Gunasena & Harris, 1969) or plant density (Vander Zaag et al., 1990) are varied. In our experiments with *in vitro* plantlets, effects of plant density on LAI showed trends similar to those of density effects on yield per m^2, if treatments were compared within a single harvest date (Table 3). A higher maximum LAI (cf. Gunasena & Harris, 1971) may have contributed to the higher minituber yields per plant after fertilization (Table 1). Because a complete nutrient solution was supplied, the availability of all essential minerals increased. However, when the supply of nutrients in cv. Ostara was begun at tuber initiation, the increase in tuber yield was less than when it was begun after the

Table 4. Influence of plant arrangement on yield, number and size of tubers ≥ 8 mm of two cultivars, expressed per plant. Plant density: 400 plants per m^2; start of fertilization: after first harvest (Expt 2).

Plant arrangement	Tuber yield (g fresh per plant)				Number of tubers (number per plant)				Tuber size (g fresh per tuber)			
	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined
Cultivar Ostara												
5.00 x 5.00 cm	0.7	4.6	5.0	10.3	0.5	1.5	1.8	3.8	1.8	3.7	2.9	2.8
10.00 x 2.50 cm[a]	1.3	5.5	2.4	9.2	0.8	2.1	1.3	4.2	1.6	2.5	1.7	2.1
20.00 x 1.25 cm	0.7	5.8	2.2	8.7	0.5	2.3	0.9	3.7	1.3	2.7	2.1	2.2
Cultivar Bintje												
5.00 x 5.00 cm	0.6	10.5	7.7	18.8	0.3	5.0	3.0	8.4	1.8	2.2	2.5	2.2
10.00 x 2.50 cm[a]	0.6	4.0	2.0	6.6	0.4	2.3	1.0	3.7	1.4	1.6	2.0	1.7
20.00 x 1.25 cm	0.7	5.8	2.1	8.6	0.5	3.2	1.6	5.3	1.2	1.8	1.3	1.6
Statistical analysis[b]												
Plant spacing (PS)	ns	ns	***	*	ns	ns	***/ns	*/ns	ns	ns	ns	ns
Cultivar (CV)	ns	ns	ns	ns	ns	*/ns	*/ns	*/ns	ns	ns	ns	ns
Interaction (PS x CV)	ns	ns	ns	ns	ns	*	*	*	ns	ns	ns	ns
CV%	65.3	49.8	63.5	50.7	45.2	39.3	34.2	32.4	57.0	55.5	56.6	37.5
SE	0.5	3.0	2.3	5.3	0.2	1.1	0.5	1.6	0.9	1.4	1.2	0.8

[a] Same values as shown in Table 2.

[b] The first indications of statistical significance presented and the CV% and SE are the results of a standard analysis of variance. Only if both main effects and interaction were significant, an F-test was performed on mean squares of main effects and interactions, to determine the relative importance of the main effect. The results of this test are presented as the second indications of statistical significance. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

Table 5. Influence of diameter of removed tubers on yield, number and size of tubers of three cultivars, expressed per plant. Plant density: 350 plants per m^2; start of fertilization: after first harvest (Expt 3).

Diameter of removed tubers	Tuber yield (g fresh per plant)				Number of tubers (number per plant)				Tuber size (g fresh per tuber)			
	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined	1st harvest	2nd harvest	3rd harvest	all harvests combined
Cultivar Ostara												
≥ 5 mm	3.1	7.1	4.0	14.2	1.6	3.5	3.5	8.6	1.9	2.0	1.1	1.6
≥ 8 mm	4.3	6.6	4.3	15.2	1.3	2.1	2.1	5.5	3.7	3.1	2.0	2.8
≥ 12 mm	2.0	8.1	5.2	15.3	0.8	2.2	1.4	4.4	2.4	3.8	3.7	3.5
Cultivar Bintje												
≥ 5 mm	3.5	7.3	3.6	14.5	1.5	7.3	3.5	12.3	2.4	1.0	1.0	1.2
≥ 8 mm	3.0	8.1	3.1	14.2	1.3	5.2	3.2	9.7	2.4	1.5	1.0	1.5
≥ 12 mm	2.8	5.1	2.3	10.2	1.1	2.0	1.2	4.3	2.7	2.6	1.9	2.4
Cultivar Elkana												
≥ 5 mm	1.4	8.5	3.6	13.5	1.0	4.7	3.5	9.2	1.1	1.9	1.0	1.5
≥ 8 mm	1.6	7.1	4.2	12.9	0.9	2.8	2.6	6.3	1.8	2.6	1.7	2.1
≥ 12 mm	0.6	7.6	4.4	12.7	0.3	1.9	1.5	3.7	2.5	4.0	2.9	3.4
Statistical analysis[a]												
Tuber diameter (TD)	*	ns	ns	ns	***	***/ns	***	***/**	ns	***	***/*	***
Cultivar (CV)	***	ns	*	ns	***	***/ns	ns	***/ns	ns	***	***/ns	***
Interaction (TD x CV)	ns	*	ns	ns	ns	***	ns	***	ns	ns	*	ns
CV%	38.2	19.9	29.6	15.7	29.8	17.8	19.2	10.8	40.1	20.6	26.2	16.0
SE	1.0	1.5	1.1	2.1	0.3	0.6	0.5	0.8	0.9	0.5	0.5	0.4

[a] The first indications of statistical significance presented and the CV% and SE are the results of a standard analysis of variance. Only if both main effects and interaction were significant, an F-test was performed on mean squares of main effects and interactions, to determine the relative importance of the main effect. The results of this test are presented as the second indications of statistical significance. *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

first harvest (Table 1). Although the nutrient solution was low in concentration, the total dose of N added at this early stage may have reduced the partitioning of assimilate to the tubers (cf. Simpson, 1962; Gunasena & Harris, 1968).

In addition, minituber yield was higher because haulm senescence was reduced or delayed in fertilized plants, at a square plant arrangement, and at lower densities (Table 3). This reduced senescence is often observed in the field after fertilization (Simpson, 1962; Van Burg, 1967) but is in contrast with the general view expressed by Proctor (1969) that higher yields at more uniform plant arrangements are caused by a delay in competition for light, and thus a higher production early in the growing season. In our experiment, tuber yields were higher at the square spacing because plants suffered less from the second non-destructive harvest as they had shorter stems (cf. Proctor, 1969) and were more compact. Stem damage also contributed to the haulm senescence of the more etiolated plants at high densities. A higher rate of senescence at higher densities may also be observed under field conditions (Bodlaender & Reestman, 1968; Van Burg, 1967), but then the availability of nutrients (Bremner & El Saeed, 1963; Van Burg, 1967) or water is often thought to limit yield at high plant or stem densities. In our experiment each plant received the same volume of nutrient solution, thus fertilizer application per m^2 was higher at high plant densities.

Yield of minitubers may also be affected by the photosynthetic efficiency of the canopy: Lommen & Struik (1992b) showed that tuber growth rates after a non-destructive harvest were lower than those of plants left undisturbed, but non-tuber growth rates were similar. After a non-destructive harvest, photosynthesis may be reduced by a temporary drought stress or the removal of tuber sinks. The reduction caused by removal of tuber sinks may be less severe in better fertilized plants, as observed by Nösberger & Humphries (1965), though not by Burt (1964). Under the well fertilized conditions of Expt 3, where tubers with different diameters were removed and as a consequence a varying number and size of tuber sinks remained on the plants, no effect on minituber yield in the second harvest was observed (Table 5).

Effects of crop husbandry on number of tubers. Lommen & Struik (1992b) showed that the number of minitubers ≥ 8 mm after non-destructive harvests was not limited by the total number of tubers and tuber initials present, but by the growth of these tubers to a harvestable size. Thus, if crop husbandry techniques increase number of minitubers harvested per plant, it may be through their effects on tuber growth. Our research shows that their effects on number of minitubers harvested per plant strongly depended on the cultivar.

Combined over all harvests, in cv. Ostara, fertilization and a square plant arrangement did not affect number of minitubers, although they increased minituber yield. Only when plant density was lowered and tuber yield per plant increased substantially, did the number of tubers per plant growing to ≥ 8 mm increase (Table 2). Similar effects of density on tuber number per stem are generally observed in the field (cf. Wurr, 1974) or in beds (Wiersema, 1986), although not always (Vander Zaag et al., 1990).

Combined over all harvests, in cv. Bintje all crop husbandry techniques that increased minituber yield per plant (fertilization, lowering plant density and a square plant arrangement) also increased

number of minitubers per plant (Tables 1, 2, 4). Also, under field conditions numbers of tubers are higher where plants are fertilized with N before tuber initiation (Gunasena & Harris, 1971).

At very high densities (400 to 800 plants per m^2) and in both cultivars, the number of minitubers per plant appeared to level off at a minimal number when density increased (Table 2). This lead to a discontinuous increase in the number of tubers per m^2 with an increase in density. Because of the relatively low number of tubers produced by cv. Bintje at 400 plants per m^2 in this experiment, the discontinuity occurring in this experiment was extremely strong. A discontinuous increase, however, was also observed in a preliminary experiment (W.J.M. Lommen, unpublished), and similar discontinuous effects of plant density are reported in other crops, for instance on ear number in winter wheat (Darwinkel, 1978).

In Expts 1, 2 and 3, 1731, 1683 and 1925 tubers per m^2 respectively were produced by cv. Ostara and 2722, 1467 and 3383 by cv. Bintje in those treatments with 350, 400 (10 cm rows) and 350 plants per m^2, in which tubers $\geq$ 8 mm were removed and plants were fertilized from the first harvest onwards. Numbers of tubers per m^2 in Expt 2 were lower than in Expts 1 and 3 due to the low number produced by cv. Bintje. In cv. Ostara, differences between experiments were statistically not significant, while in cv. Bintje differences between Expts 1 and 3 were not significant. Part of the differences between experiments may have been caused by the smaller plantlets used for transplanting in Expt 2 (9 days old, compared to 17 and 13 days in Expts 1 and 3) and brighter weather during Expt 3. In addition, slightly more damage may have occurred in Expt 2 during harvesting, because of the smaller distance between rows (10 cm) and the difficulties of harvesting and replanting plants on fixed positions in large plots, while in Expts 1 and 3 pots could be harvested one by one.

Possibilities of manipulating yield parameters by crop husbandry and the practical implications. Adjusting plant density (Tables 2 and 3) and the diameter of the tubers removed (Table 5) proved to be perfect tools in all cultivars for manipulating minituber number and size. These practices had no significant effect on tuber yield per m^2; thus increases in number of tubers per m^2 were directly reflected in decreases in average tuber weight.

Number and size of minitubers could not be manipulated in all cultivars by adjusting the time at which the supply of nutrient solution started (Table 1) or the plant arrangement (Table 4). When results of all harvests were combined, numbers of tubers in cv. Ostara were not affected. Supplying nutrients and using a square plant arrangement, however, increased minituber yield in both cultivars and did not reduce average tuber weight. No significant interactions between cultivars and treatments in average tuber weight were observed, although the cultivars responded similarly in tuber yield and differently in tuber number.

For practical production of minitubers, a continuous supply of a low-dose nutrient solution starting at the first harvest may be adopted as a means of increasing tuber yield. Square plant arrangements, however, were less convenient than row arrangements for carrying out the non-destructive harvests.

When controlled glasshouse space is limited and high numbers of tubers per unit area are desirable, plant density may be increased. At 800 plants per m^2, 2700 and 3400 minitubers per m^2

were produced by cvs Ostara and Bintje respectively (Table 3). The average weights were 1.4 and 1.6 g and all tubers were ≥ 8 mm. Smaller tubers may also be removed. When tubers ≥ 5 mm were removed at 350 plants per m^2 in Expt 3, 3000, 4300 and 3200 minitubers per m^2 were produced by cvs Ostara, Bintje and Elkana (calculated from Table 5), still with average weights between 1.2 and 1.6 g.

When *in vitro* plantlets are expensive and a high number of minitubers per plantlet is preferred over a high number of minitubers per m^2, plant density can be lowered. Lowering plant density was the only treatment investigated which increased the number of minitubers ≥ 8 mm per plantlet in all cultivars, except at very high plant densities. Simultaneously, the average tuber weight increased. For further studies, we adopted a plant density of 175 plants per m^2, favouring high numbers of tubers per plantlet to high numbers of tubers per m^2. To increase the number of tubers per plantlet even further, the diameter of the tubers removed may be lowered from 8 to 5 mm. From a practical point of view, this seems logical: it increases number of tubers considerably, does not affect the number and weight of tubers in the larger (≥ 8 or ≥ 12 mm) grades, and will hardly affect the time necessary to carry out the harvest. Tubers between 5 and 8 mm, however, may prove less suitable for direct field planting (cf. Struik & Lommen, 1990).

The effects of climatic factors on yield parameters of minitubers and the behaviour and performance of minitubers of different sizes during storage and in the field, will be reported in forthcoming papers.

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CHAPTER 5

Post-harvest characteristics of potato minitubers with different fresh weights and from different harvests. I. Dry-matter concentration and dormancy

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5 POST-HARVEST CHARACTERISTICS OF POTATO MINITUBERS WITH DIFFERENT FRESH WEIGHTS AND FROM DIFFERENT HARVESTS. I. DRY-MATTER CONCENTRATION AND DORMANCY

Additional keywords: Solanum tuberosum L., seed size, rapid multiplication, seed production, non-destructive harvest, cold treatment, sprouting

Summary

Dry-matter concentration and dormancy were studied in minitubers of cvs Agria and Liseta, using five fresh weight classes (< 0.50 g, 0.50 - 0.99 g, 1.00 - 1.99 g, 2.00 - 2.99 g and ≥ 3.00 g) and three successive harvests of the same plantlets. The average dry-matter concentration of the minitubers, assessed one day after harvest, was 17.8 %. Dry-matter concentration increased with tuber weight for tubers from the second and third harvests. In minitubers ≥ 0.5 g, dry-matter concentration was higher in tubers from later harvests. The dormant period (days from harvest to 50 % sprouting) was longer in minitubers with lower weights than with higher weights, and longer in tubers from the first harvest than from later harvests. A cold-storage period of 6 weeks, starting 14 days after harvest, reduced the dormant period by an average of 11 days.

Introduction

Minitubers are small seed potato tubers, produced in the glasshouse on plantlets propagated *in vitro* and planted at high density. Large numbers of small tubers can be produced by removing tubers from the same plantlets three times in 10 weeks (Lommen & Struik, 1992a). Minitubers can be produced throughout the year and are principally used for the production of pre-basic or basic seed by direct field planting.

The feasibility of incorporating a minituber production step into a seed multiplication programme will depend on the behaviour of minitubers during and after storage. If field planting is only possible once a year, year-round production of minitubers implies that many of them must be stored until planting. Moreover, as minitubers are likely to be dormant for some time after harvest, some tubers may have to be stored for more than one year, until the next planting season. Information on the dormancy and storability of **minitubers** is lacking, although small **'normal' tubers** are known to possess a longer dormant period than larger tubers (cf. Emilsson, 1949; Van Ittersum & Struik, 1992). Information on dormancy of **microtubers** (*in vitro* tubers) is scarce and often not supported by clear data. They have been reported to sprout immediately (Ortiz-Montiel & Lozoya-Saldaña, 1987) or after a dormant period varying from 60 to 210 days when stored at 4 °C, depending on the conditions (Tovar et al., 1985).

In this paper data are presented on the dry-matter concentration and the duration of dormancy of minitubers differing in fresh weight and from three harvests of one planting. In a subsequent paper, the behaviour of minitubers during storage for up to 1.5 years will be reported (Lommen, 1993).

Table 1. Procedure, timing and growing conditions during production of minitubers from two cvs in 1988.

Procedure	Cultivar		Conditions
	Agria	Liseta	
Final multiplication *in vitro*	March 30	April 15	Lommen & Struik (1992a,b)
First transplanting of *in vitro* plantlets	April 15	April 25	Glasshouse; natural day length; potting soil (70 % organic matter, pH 5.5)
Second transplanting	May 4	May 4	Glasshouse; photoperiod 12 h; day/night temperature set at 18/12 °C; 350 plants per m^2; perlite-potting soil 50/50 % (v/v)
First harvest: removal of tubers ≥ 8 mm; plants replanted	May 25	May 25	Start of fertilization (Lommen & Struik, 1992c)
Second harvest: removal of tubers ≥ 8 mm; plants replanted	June 15	June 15	
Third harvest: removal of tubers ≥ 8 mm	July 6	July 6	

Materials and methods

Minituber production. Minitubers from cvs Agria and Liseta were produced on *in vitro* propagated plantlets in a glasshouse in Wageningen, the Netherlands, in 1988. The tubers were removed from the plantlets in three successive harvests at intervals of 3 weeks. The first two harvests were non-destructive (Table 1). Plantlets were transplanted twice because they were not meant to serve for production of minitubers. After harvest, minitubers were dried overnight at room temperature in a shallow layer in metal boxes. Adhering soil was then removed and the tubers were sorted by fresh weight into five classes: 1: < 0.50 g; 2: 0.50 - 0.99 g; 3: 1.00 - 1.99 g; 4: 2.00 - 2.99 g; 5: ≥ 3.00 g.

Tuber categories. There were 30 tuber categories planned, representing all combinations of five fresh weight classes, three harvests and two cultivars. No tubers were obtained in class 5 of the first harvest of cv. Liseta, and tubers from other classes of the first harvest of cv. Liseta were in short supply. Average tuber weights are presented in Table 2.

Dry-matter concentration. The dry-matter concentration of tubers was determined one day after each harvest, after sorting the tubers into fresh weight classes, by drying the sliced tubers at 105 °C for

Table 2. Average weight of minitubers from various categories one day after each harvest.

Cultivar	Harvest	Fresh weight class				
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g
Agria	first	0.26	0.71	1.48	2.47	5.11
	second	0.27	0.72	1.43	2.36	4.78
	third	0.23	0.71	1.39	2.35	3.92
Liseta	first	0.23	0.67	1.20	2.22	-
	second	0.25	0.69	1.39	2.32	4.77
	third	0.22	0.76	1.41	2.35	4.85

40 h. Two samples were taken from each tuber category. The number of tubers per sample depended on the class and the availability of tubers, and varied from 30 - 60 in class 1, 5 - 18 in class 2 and 5 - 10 in classes 3, 4 and 5, except for the first harvest of cv. Liseta, from which no tubers were available in classes 3 and 5 and only two in class 4.

Length of dormancy. After sorting by fresh weight, the tubers were cured at 18 °C until 2 weeks after harvest and then cold stored at 2 °C for up to 12 weeks. During curing and cold storage the tubers were kept in a shallow layer in crates lined with cheese-cloth in complete darkness at 80 % r.h. Tubers which deteriorated were removed.

To assess the length of dormancy tubers were sampled before and after curing (0 weeks of cold storage) and after 2, 4, 6, 8, 10 and 12 weeks of cold storage. Tubers were placed with the apical eye upwards on a thin layer of air-dry sand under the same conditions as for curing: complete darkness, 18 °C and 80 % r.h. Sprouting was checked weekly. The dormant period was regarded as the period from harvest until 50 % of the tubers bore a sprout of at least 2 mm, and was determined by linear intrapolation. Three samples of 10 tubers were analysed from each available tuber category, but due to a lack of tubers it was not possible to analyse the desired number of tubers from all categories of cv. Liseta at each date. Tubers which deteriorated (shown first by loss of rigidity and followed by excessive shrinkage, accompanied by a brownish discoloration) before sprouting were excluded.

Statistical analysis. Analysis of variance was used and four-way interactions were always included in the residual variance. If both main effects and their interactions were significant when tested against the residual mean square, their relative importance was tested by F-tests on mean squares. If all interactions were of minor importance, LSD's are presented for the main factor only. If one or more two-way interactions could not be ignored, their mean squares were tested against all appropriate significant three-way interactions. LSD's for two-way interactions are presented if three-way interactions proved to be of minor importance, otherwise LSD's for three-way interactions are presented.

Table 3. Dry-matter concentration of minitubers from two cultivars, three successive harvests and five fresh weight classes, assessed one day after harvest.

Cultivar (CV)	Harvest (H)	Fresh weight class (CL)					Mean over classes
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g	
Agria	first	16.9	16.6	16.6	16.6	16.6	16.6
	second	16.3	17.9	20.4	18.1	20.6	18.7
	third	17.7	17.9	18.6	19.4	20.3	18.8
Liseta	first	19.5	17.1	-	15.8	-	17.0
	second	16.0	16.9	17.0	17.8	17.2	17.0
	third	16.2	19.2	19.0	19.6	19.2	18.6
Mean over cultivars	first	18.2	16.9	16.6	16.2	16.3	16.8
	second	16.1	17.4	18.7	18.0	18.9	17.8
	third	17.0	18.5	18.8	19.5	19.7	18.7
SE	0.9						

Significances [a]:
CV: significant, but CV*H*CL interaction (LSD 5 % = 1.8) could not be ignored
H : significant, but H*CV (LSD 5 % = 0.8) and H*CL interaction (LSD 5 % = 1.0) could not be ignored
CL: significant, but CV*H*CL interaction (LSD 5 % = 1.8) could not be ignored

[a] See Materials and methods.

Estimates of missing values and of mean values for missing categories (e.g. cv. Liseta, first harvest) were derived using Genstat.

Results

Dry-matter concentration. One day after harvest, the average dry-matter concentration of the minitubers was 17.8 %. Dry-matter concentration was higher in cv. Agria than in cv. Liseta, but differences between cultivars were not consistent and were affected by tuber weight and the harvest (Table 3).

Dry-matter concentration increased with tuber weight in tubers from the second and third harvests. In cv. Agria, the dry-matter concentration of minitubers of the first harvest did not depend on the fresh weight, and in cv. Liseta the highest dry-matter concentration in the first harvest was observed in tubers from the lowest fresh weight class (Table 3).

The dry-matter concentration was higher in tubers from later harvests for tubers ≥ 0.5 g. In cv. Agria, the difference in dry-matter concentration between tubers from the first and second harvest was significant, while in cv. Liseta the difference between tubers from the second and third harvest was significant (Table 3).

Table 4. Duration of dormancy (days after harvest) of minitubers from two cultivars, three successive harvests and five fresh weight classes, when kept at 18 °C, 80 % RH and darkness from one day after harvest onwards. Means of samples taken before and after curing.

Cultivar (CV)	Harvest (H)	Fresh weight class (CL)					Mean over classes
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g	
Agria	first	157	154	148	140	124	144
	second	152	142	137	134	132	139
	third	156	147	140	135	126	141
Liseta	first	155	152	-	-	-	144
	second	140	135	133	126	127	132
	third	144	132	126	121	120	129
Mean within classes		150	144	139	132	126	138

SE 1.0 (means of harvests); 3.2 (all other means)

Significances [a]:
CV: significant, but CV*H interaction (LSD 5 % = 1.6/2.2 for comparisons within/between harvests) could not be ignored
H : significant, but CV*H interaction (LSD 5 % = 1.6/2.2 for comparisons within/between harvests) could not be ignored
CL: significant, LSD 5 % = 1.5

[a] See Materials and methods.

Dormancy of minitubers at 18 °C. When tubers were kept at 18 °C after sorting (no cold storage), the duration of dormancy was 138 days, averaged over all tuber classes, harvests and cultivars. There was no statistically significant difference between the samples taken before and after curing, and therefore only means are presented (Table 4). Minitubers from cv. Agria had a longer dormancy than those from cv. Liseta, except for the tubers from the first harvest, which were similar.

In both cultivars, minitubers of lower weight had a longer dormancy. Differences in dormancy between the lowest and highest fresh weight classes were approximately 27 days in cv. Agria and 23 days in cv. Liseta.

The effect of the harvest on the length of the dormant period depended on the cultivar. In both cultivars, tubers from the first harvest had a longer dormancy than those from later harvests (Table 4), but in cv. Agria tubers from the second and third harvests did not differ significantly, while in cv. Liseta there was a clear decrease in dormancy in tubers from later harvests. The maximum difference between harvests was much smaller in cv. Agria (5 days) than in cv. Liseta (15 days).

Effects of a cold-storage period on the length of the dormant period. The time from harvest until 50 % of the tubers had produced a sprout of 2 mm could be reduced by inserting a cold-storage period of 14 - 70 days at 2 °C after the curing period (Fig. 1). A cold period of 42 days gave a significantly

higher reduction than other cold-storage periods: compared to sprouting without a cold-storage period, the reduction was on average 11.2 days. Longer periods of cold storage increased the total time from harvest to 50 % sprouting, but still reduced the period between removal from cold storage and 50 % sprouting (Fig. 1). Although two-way and three-way interactions occurred in the total number of days to 50 % sprouting, they were of minor importance compared to the main effect of the cold treatment ($P < 0.001$).

Discussion

Dry-matter concentration of minitubers. In 'normal' field grown potato crops the relationship between tuber size and dry-matter concentration is of a negatively quadratic nature (Wurr & Allen, 1974): dry-matter concentration increases with tuber size, and after a maximum generally decreases. In my experiment, only minitubers from the second and third harvests showed a higher dry-matter concentration at higher fresh weights, and no decrease was observed (Table 3). In cv. Agria, the dry-matter concentration of tubers from the first harvest was not affected by their fresh weight, and in cv. Liseta the dry-matter concentration of tubers from the first harvest was highest in the lowest fresh

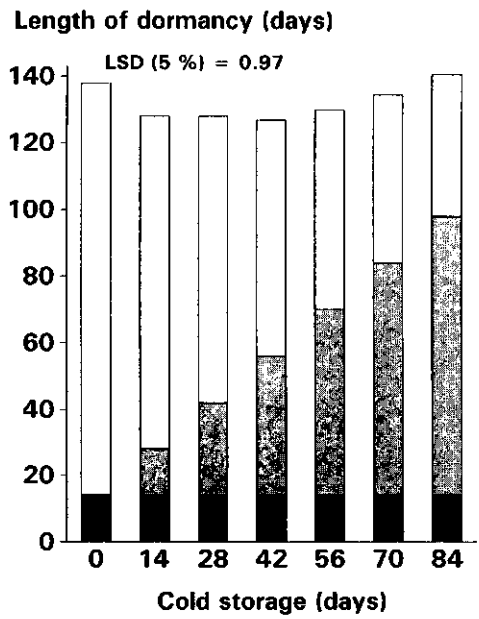

Fig. 1. Effect of the length of a cold-storage period on the duration of dormancy (total number of days from harvest until 50 % of the minitubers had produced a sprout of at least 2 mm). Drying + curing period: ■, cold-storage period: ▩, sprouting period: □. Average values of two cultivars, five fresh weight classes and three harvests.

weight class. The aberrant response of tubers from the first harvest may be meaningful. Ifenkwe & Allen (1978) showed that in younger plants of cv. Maris Piper the tuber size giving maximum dry-matter concentrations was lower than in older plants. In their second experiment, the decrease in dry-matter concentration at the earliest harvest date started at a very small, or the smallest size studied. However, in my research, part of the higher dry-matter concentration of tubers from the lowest fresh weight class of the first harvest, especially of cv. Liseta, may have been caused by high water losses between harvest and the assessment of dry-matter concentration one day later. Tubers with lower fresh weights, tubers from the first harvest and tubers from cv. Liseta are especially prone to weight losses (Lommen, 1993).

In field-grown tubers, the dry-matter concentration of a certain tuber size initially increases as the plant ages (Ifenkwe & Allen, 1978), but later declines. However, in minitubers produced by repeated harvesting, only an increase was observed (Table 3). The dry-matter concentration in minitubers from later harvests may have been higher because more tubers were growing on the plants in later harvests than in the first harvest (cf. Lommen & Struik 1992c). Wurr et al. (1978) showed that higher tuber numbers were positively correlated with higher dry-matter concentrations later in the growing season. Secondly, the availability of water may have been lower in later harvests due to root damage during the non-destructive minituber harvests (cf. Lommen & Struik, 1992b). Unfortunately, the variable response of the two cultivars cannot be explained from the data available.

Dormancy of minitubers. Minitubers showed a dormant period after they were harvested (Table 4) and therefore cannot be planted immediately. As is common in 'normal' tubers, the length of this dormant period depended on the cultivar and decreased at increasing tuber weights (cf. Emilsson, 1949; Van Ittersum & Struik, 1992; Table 4 of this paper). Because the period of sprout growth up to 2 mm is included in the dormant period, part of the differences between fresh weight classes may have been caused by differences in the rate at which the sprout increases in length, which was shown to be a positive function of tuber weight in sprouts from lateral buds of tubers weighing 53 - 200 g and growing in diffuse light (Morris, 1966).

The length of dormancy of minitubers was also affected by the harvest: minitubers from the first harvest had a longer dormancy (calculated from each harvest onwards) than minitubers from later harvests (Table 4). The reasons are not known, but theoretically, differences could have been caused by (a) the age of the tubers at harvest, (b) an influence of the physiological status of the plant, and/or (c) differences in availability of nutrients for sprout growth up to 2 mm. The age of the tubers at harvest is not known, but from the results of Lommen & Struik (1992a, b) it may be concluded that tubers from the first harvest had been initiated 0 - 3 weeks before harvest, whereas those from the last harvest were most probably initiated 0 - 6 weeks before harvest. This could partly explain the longer dormancy (measured from harvest onwards) of tubers from the first harvest. No data are available to support or refute the other possibilities.

The shortening of the dormancy of minitubers by cold-storage periods after curing (Fig. 1) is consistent with the positive effects of cold storage in 'normal' tubers on the number of sprouted tubers (cf. Tedin, 1938) and the sprout length (Wurr & Allen, 1976), and the reduction of the time

to 80 % sprouting in cultivars with an intermediate and long dormancy (Van Ittersum & Scholte, 1992).

Practical implications. It took 5 months for 50 % of the lightest tubers of cv. Agria to produce a sprout of 2 mm (Table 4) and cold-storage periods only enhanced sprouting by 1 - 2 weeks (Fig. 1). Consequently, minitubers must be produced more than 5 months before they are planted, or dormancy has to be broken by methods not studied in this paper. When still dormant at planting time, minitubers have to be stored for an additional year in climates with only one growing season. How minitubers perform during storage will be reported in a subsequent paper (Lommen, 1993).

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CHAPTER 6

Post-harvest characteristics of potato minitubers with different fresh weights and from different harvests. II. Losses during storage

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6 POST-HARVEST CHARACTERISTICS OF POTATO MINITUBERS WITH DIFFERENT FRESH WEIGHTS AND FROM DIFFERENT HARVESTS. II. LOSSES DURING STORAGE

Additional keywords: Solanum tuberosum L., seed size, rapid multiplication, seed production, non-destructive harvest, water loss

Summary

Storage losses were studied in minitubers of cvs Agria and Liseta, using five fresh weight classes (< 0.50 g, 0.5 - 0.99 g, 1.00 - 1.99 g, 2.00 - 2.99 g, ≥ 3.00 g), and three successive harvests of the same plantlets. After each harvest, tubers were dried at room temperature (1 day), cured at 18 °C (13 days) and stored at 2 °C (540 days). Two kinds of storage losses were considered: (a) losses of entire tubers because of deterioration, and (b) fresh weight losses of the other tubers. Both kinds of losses were higher in cv. Liseta, in tubers with lower fresh weights and in tubers from the first harvest. Almost all minitubers ≥ 0.5 g from later harvests and from both cultivars survived storage for 1.5 years. Deterioration occurred mainly from 6 to 12 months of storage. Tubers which deteriorated during cold storage had already shown high weight losses during curing.

Introduction

Minitubers are small potato tubers intended for producing basic and pre-basic seed by direct field planting. They can be produced throughout the year by planting *in vitro* propagated plantlets at high density in a glasshouse, and harvesting tubers from the same plantlets in three successive harvests (Lommen & Struik, 1992a).

Because they are meant for direct field planting and can be produced all year round, many of the tubers must be stored until the next planting season. As minitubers are dormant immediately after harvest (Lommen, 1993), they must be stored at least until dormancy is over. In climates with only one growing season each year, minitubers that are still dormant at the start of a growing season may need to be stored for a further year until the following planting season.

Burton (1973) showed that small, immature 'normal' tubers could suffer extremely high weight losses during the first few hours after harvesting. Furthermore, during storage they may show higher weight losses than larger tubers, because of their higher surface area:volume ratio. Minitubers may therefore prove to be very susceptible to weight losses. Because there is no published information on the storability of minitubers, I studied the behaviour during storage for up to 1.5 years of minitubers of different weight, originating from three harvests of one planting. The performance of these minitubers after different storage periods will be reported upon in a subsequent paper (Lommen & Struik, 1993).

Table 1. Contribution (%) of minitubers from five fresh weight classes and three harvests to the total number of tubers produced by cvs Agria and Liseta.

Cultivar (CV)	Harvest (H)	Fresh weight class (CL)					Mean over classes
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g	
Agria	first	4.0	4.1	5.8	4.2	9.2	27.3
	second	5.6	5.9	9.7	6.2	10.4	37.8
	third	12.0	8.0	8.9	3.6	2.5	35.0
	Sum	21.6	18.0	24.4	14.0	22.1	
Liseta	first	16.0	4.7	2.2	0.1	0.0	23.0
	second	6.8	5.5	7.7	5.0	9.9	34.9
	third	14.1	6.9	8.4	5.2	7.3	41.9
	Sum	36.9	17.1	18.3	10.3	17.2	

Materials and methods

Minituber production. Minitubers were produced on *in vitro* propagated plantlets in a glasshouse in Wageningen in summer 1988. The tubers were removed from the plantlets in three successive harvests at intervals of 3 weeks. The first two harvests were non-destructive (Lommen & Struik 1992b). Details of the growing conditions and harvest procedure have been reported (Lommen, 1993).

Minituber storage. After every harvest, the storage period consisted of (1) a drying period of one day at room temperature, (2) a curing period of 13 days at 18 °C in darkness and 80 % r.h., and (3) a period of cold storage of 540 days at 2 °C in darkness and 80 % r.h. After drying, soil adhering to the tubers was removed and tubers were sorted according to their weight.

Tuber categories. There were 30 tuber categories planned, representing all combinations of: (a) five fresh weight classes: class 1: < 0.50 g, class 2: 0.50 - 0.99 g, class 3: 1.00 - 1.99 g, class 4: 2.00 - 2.99 g and class 5: ≥ 3.00 g; (b) three harvests: first, second and third; (c) two cultivars: Agria and Liseta. No tubers were available in classes 4 and 5 from the first harvest of cv. Liseta. Table 1 shows the relative contribution of the fresh weight classes and harvests to the total number of tubers produced by each cultivar.

Unless stated otherwise, the values presented were not corrected for relative contributions of every tuber category to the total tuber number. If necessary, missing values were estimated by the statistical programme Genstat, for the two missing tuber categories of cv. Liseta. Means derived from these estimated values were omitted from the time series (tuber losses during storage, Figs 2, 3 and 4) if they were higher than at a later assessment.

Losses during storage. Weight losses were determined by individually weighing 20 tubers from each category. To assess weight losses during curing, the individual tuber weights were determined 2 and 15 days after harvest, i.e. one day after the start of the curing period and one day after its end. The weight loss (%) during this period was related to the weight of the tubers 2 days after harvest. To assess weight losses during cold storage, the individual weights of all tubers were determined every week from 15 until 50 days after harvest, every 2 weeks from 50 until 106 days after harvest, every 3 weeks from 106 until 358 days after harvest and finally at 554 days after harvest. The weight loss (%) during cold storage was related to the weight of the tubers 15 days after a harvest, i.e. one day after the cold-storage period started. Tuber weights were always determined at room temperature after acclimatization for 1.5 hours. The individual weights of 60 tubers were determined immediately after harvest. Tubers that deteriorated considerably were removed and regarded as tuber losses (by

Table 2. Fresh weight loss (%) during curing of minitubers which did not subsequently deteriorate during storage for 554 days. Weight losses were determined from 1 day after the start of the curing period (i.e. 2 days after harvest) until 1 day after the end of the curing period (i.e. 15 days after harvest), on minitubers from two cultivars, five fresh weight classes and three harvests. SE = 2.0 [a].

Cultivar	Harvest	Fresh weight class				
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g
Agria	first	-	-	5.6	-	-
	second	7.0	4.4	3.9	4.1	3.0
	third	6.7	4.6	4.0	3.5	3.4
Liseta	first	-	-	-	-	-
	second	7.1	5.2	5.9	5.1	4.7
	third	7.9	5.7	5.1	4.5	4.2

[a] SE of all means. The number of surviving tubers can be calculated from Table 4.

Table 3. Fresh weight loss (%) during cold storage of minitubers which did not subsequently deteriorate during storage for 554 days. Weight losses were determined from 1 day after the start of the cold-storage period (i.e. 15 days after harvest) until 554 days after harvest, on minitubers from two cultivars, five fresh weight classes and three harvests. SE = 6.3 [a].

Cultivar	Harvest	Fresh weight class				
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g
Agria	first	20.5	16.8	14.9	11.2	9.7
	second	18.5	11.2	10.4	10.5	7.0
	third	19.3	14.8	10.7	9.1	8.5
Liseta	first	40.0	29.4	23.3	-	-
	second	29.2	17.9	15.5	12.8	10.9
	third	33.3	20.7	16.8	13.7	12.5

[a] SE of all means. The number of surviving tubers can be calculated from Table 4.

number). These tubers were omitted from the analysis of weight losses.

Results

Weight losses during curing. From 2 to 15 days after a harvest, weight losses were lower in cv. Agria than in cv. Liseta (Table 2). Data refer only to tubers that did not deteriorate later. During curing, minitubers from the lowest weight class lost a higher percentage of their fresh weight (7 - 8 %) than those from the highest weight class (3 - 5 %, Table 2). No clear differences existed between tubers from the second and third harvests. The importance of weight loss during the curing period was only realized after the weight losses of one tuber category from the first harvest were determined. Therefore, weight losses of all but one tuber category from the first harvest are missing in Table 2. An additional weight loss test on 60 tubers (0.5 - 3 g) from the second harvest of cv. Agria, however, revealed that the first two days immediately after a harvest were even more important: one day after the curing period started, these tubers had already lost on average 5.7 % of the weight recorded immediately after harvest. During curing, the tubers from these categories lost 4.1 % of their weight one day after the start of the curing period (calculated from Table 2).

Weight losses during cold storage. During storage at 2 °C from 15 to 554 days after harvest, the minitubers that did not deteriorate lost only 15 % of their fresh weight recorded at 15 days after harvest. Weight losses were again consistently lower in cv. Agria than in cv. Liseta, and considerably higher in minitubers from the lowest weight class (19 - 40 %) than from the highest weight class (7 - 13 %) (Table 3). In addition, weight losses were higher in tubers from the first harvest than in those from later harvests (Table 3).

Because evaporative weight losses are generally higher in smaller than larger tubers, due to their higher surface area:volume ratio (Burton, 1973), curves were fitted in which the weight losses of minitubers during the cold-storage period were related to their weight at the start of this period. These curves had to allow comparisons between minitubers from different cultivars and different harvests, irrespective of their initial fresh weight. Because it was uncertain how weight would be lost with time, different curves were fitted for different dates. The relation $y = b \times x^{2/3}$ proved to be most suitable (both accurate and simple), in which y = fresh weight loss from 15 to a certain number of days of storage (22 - 358 days, intervals of 6 weeks), and x = the tuber weight 15 days after a harvest. The $x^{2/3}$ is a measure of the tuber surface, regarding the tuber as being globular and having a specific gravity independent of the fresh weight. Estimates of the regression coefficient b, all significant at $P < 0.001$, showed that after storage periods of 148 days or more, weight losses per tuber in cv. Agria were lower than in cv. Liseta, and that weight losses of tubers from the first harvest were higher than from later harvests (Fig. 1). The estimated value of the coefficient b is also the predicted weight loss (g) of a tuber with an initial weight of 1.00 g. If the weight losses were fitted to x instead of to $x^{2/3}$, the curves explained a smaller part of the variance on all but one occasion (51 out of 52 curves). Tubers of cv. Liseta were more elongated than those of cv. Agria, and therefore the surface area:volume ratio may have been higher in cv. Liseta. There were no

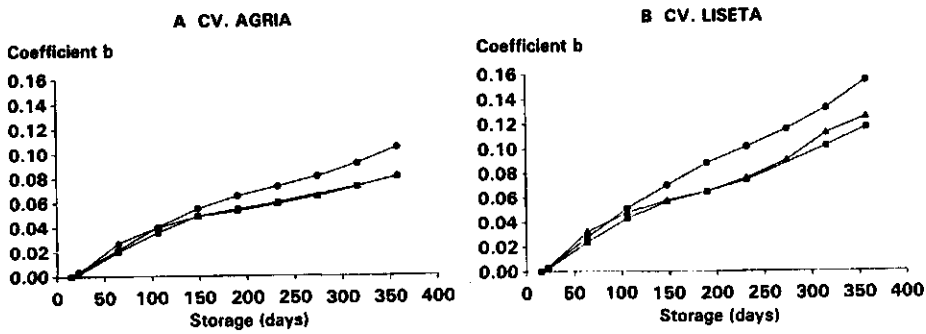

Fig. 1. Progressive fresh weight loss during cold storage of minitubers from the first (●), second (■) and third (▲) harvests, as characterized by the regression coefficient b from the relation $y = b \times x^{2/3}$ in which y = fresh weight loss from 15 days of storage onwards, and $x^{2/3}$ is a measure of the tuber surface of a tuber with a weight x, 15 days after a harvest, regarding the tuber as being globular and having a specific gravity that is independent of the fresh weight. Different curves were fitted for each date. The estimated value of the coefficient b is also the predicted weight loss of a tuber with an initial fresh weight of 1.00 g. A: cv. Agria, B: cv. Liseta.

obvious differences in tuber shape between tubers from different harvests.

Tuber losses (by number). Fewer tubers of cv. Agria deteriorated during storage than of cv. Liseta (Fig. 2). Tubers which were regarded as being lost initially lost turgor and later shrivelled excessively. These changes were accompanied by a brownish discolouration of the surface. There were no indications that the losses were caused by pathogens, but if tubers were not removed from storage some of them became infected by fungi. No tubers were regarded as lost at the end of the curing period, for most losses occurred between 232 and 358 days of storage in cv. Agria and

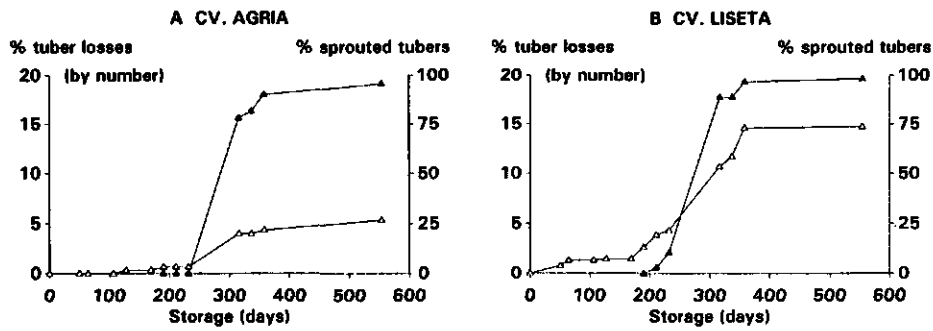

Fig. 2. Percentage (by number) of minitubers deteriorating during storage (▵) and percentage of non-deteriorating tubers showing visible sprouting (▲). A: cv. Agria, B: cv. Liseta. Mean values of five fresh weight classes and three harvests.

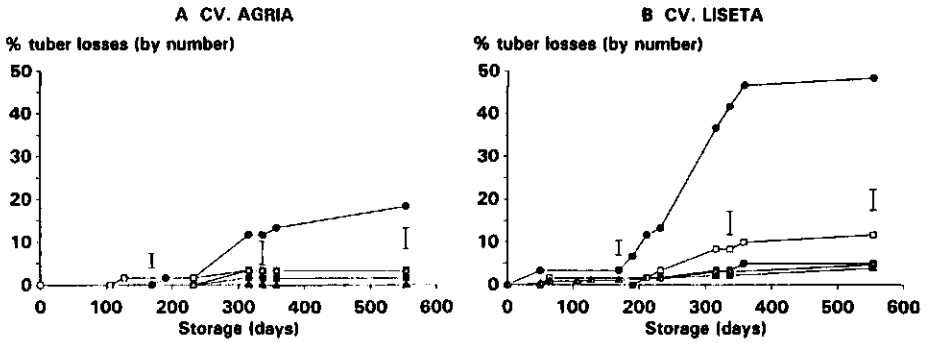

Fig. 3. Percentage (by number) of minitubers deteriorating during storage, originating from fresh weight classes < 0.50 g (●), 0.50 - 0.99 g (□), 1.00 - 1.99 g (■), 2.00 - 2.99 (△) and ≥ 3.00 g (▲). A: cv. Agria, B: cv. Liseta. Mean values of three harvests. Bar: LSD 5 %.

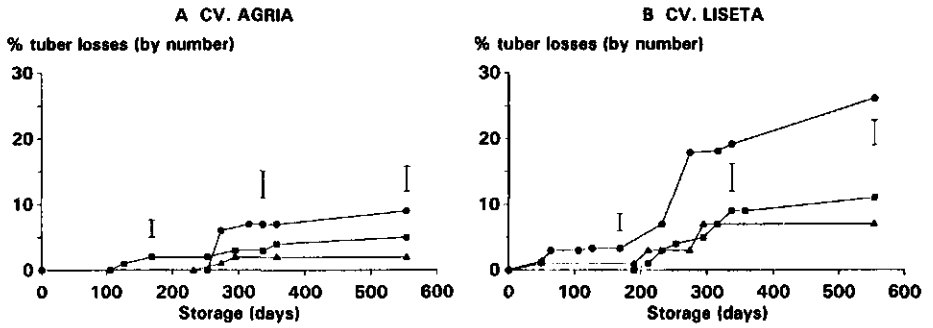

Fig. 4. Percentage (by number) of minitubers deteriorating during storage, originating from the first (●), second (■) and third (▲) harvests. A: cv. Agria, B: cv. Liseta. Mean values of five fresh weight classes. Bar: LSD 5 %.

between 169 and 358 days in cv. Liseta. Thereafter, losses were small. The periods during which most losses occurred coincided with the period during which the first sprouts became visible (Fig. 2).

In both cultivars, most tubers were lost from the lowest weight class (Fig. 3). In cv. Agria, hardly any tubers ≥ 0.5 g (classes 2 - 5) were lost, and differences between these fresh weight classes were negligible (Fig. 3A). In cv. Liseta, losses of tubers of 0.5 - 1 g were still higher than losses of tubers with higher fresh weights (Fig. 3B).

From later harvests, fewer tubers deteriorated during storage than from earlier harvests (Fig. 4). The magnitude of the differences depended on the cultivar. In cv. Agria, differences between harvests were small: the percentage of deteriorating tubers from the second harvest did not differ

Table 4. Percentage of minitubers from three successive harvests, two cultivars and five fresh weight classes which had not deteriorated 554 days after a harvest. Sample size = 20 tubers.

Cultivar	Harvest	Fresh weight class				
		< 0.50 g	0.50 - 0.99 g	1.00 - 1.99 g	2.00 - 2.99 g	≥ 3.00 g
Agria	first	70	95	95	95	100
	second	80	95	100	100	100
	third	95	100	100	95	100
Liseta	first	20	75	85	-	-
	second	55	90	100	100	100
	third	65	100	100	100	100

significantly from that at the other harvests (Fig. 4A). In cv. Liseta, the percentage of deteriorating tubers from the first harvest was considerably higher than that from the second harvest (Fig. 4B).

Table 4 shows the percentage of tubers surviving storage up to 554 days for every tuber category. Only 20 % of the tubers < 0.5 g from the first harvest of cv. Liseta survived storage during this period, while only about 60 % of the tubers < 0.5 g from the other harvests of this cultivar could be stored for this period. The storability of both cultivars was good in tubers ≥ 0.5 g (classes 2 to 5), especially if produced in the second or third harvest. From Table 4 it appears that the minimum fresh weight of tubers showing good storability was lower the later the harvest took place.

Tubers were regarded as being lost on the basis of visual inspection. Their individual weights, however, had been recorded up until rejection. It appeared that the tubers which deteriorated during cold storage showed much higher weight losses during curing than the tubers which survived storage for 554 days (Fig. 5). At the time that they were regarded as being lost, tubers appeared to have lost 9 - 85 %, but in general 40 %, of the weight they had 15 days after harvest.

Discussion

Storage losses. Fresh weight losses may have occurred through evaporation (water loss) and respiration (dry-matter loss). No data were collected on the relative contribution of each, but evaporative weight losses can be assumed to have been more important than respiratory weight losses (cf. Appleman et al., 1928; Wilcockson et al., 1985). Evaporation of unsprouted tubers is proportional to their surface area, and is inversely related to the resistance of the tuber periderm (including lenticels). Higher relative weight losses therefore may be expected in smaller tubers, which have a higher surface area:volume ratio. The better fit of the calculated regression lines with $x^{2/3}$ than with x itself supports the view that the surface area was an important characteristic in determining weight losses.

Because evaporation is inversely related to the resistance of the periderm, it will be higher in tubers with an incomplete or less suberized periderm, or in tubers with more or more permeable

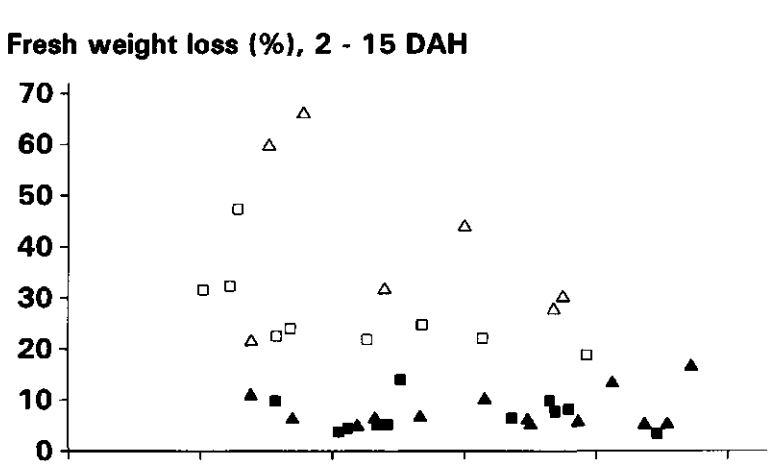

Fig. 5. Relation between the initial fresh weight and the fresh weight loss (%) during curing, of minitubers that survived storage for 554 days (closed symbols) or deteriorated during this period (open symbols). Weight losses were determined from one day after the start of the curing period (i.e. 2 days after harvest) until one day after the end of the curing period (i.e. 15 days after harvest), on individual tubers (cv. Liseta) from the second (▵ , ▲) and third (□ , ■) harvests. DAH: days after harvest.

lenticels. In minitubers, a lower periderm resistance may explain the higher proportional weight losses in tubers from the first harvest (Tables 2 and 3; Fig. 1), part of the differences between cultivars (Tables 2 and 3, Fig. 1), part of the differences between individual tubers of similar weight (Fig. 5), and probably also part of the higher proportional weight losses of smaller tubers, which are likely to have a higher proportion of their surface area wounded at harvest. No data concerning periderm thickness, periderm suberization, wounding or lenticels were collected, but observation of the tubers at harvest revealed that skin set of tubers from the first harvest was not complete, especially at the bud end of the tuber, and that its skin could be easily removed during handling.

Because the highest losses by weight and number occurred in the same tuber categories, the deteriorating tubers may have been those individuals which - due to normal variability - showed the highest weight losses within each category (cf. Fig. 5). However, depletion of substrate available for respiration may also have been important, because (a) the losses occurred especially in immature and small tubers, which are likely to have both the highest respiration rates immediately after harvest (Burton, 1964) and the lowest carbohydrate reserves; (b) most losses occurred in the period after the onset of sprouting (cf. Fig. 2), when respiration normally increases (Burton, 1974), and (c) the deteriorating tubers initially seemed to resemble tubers which were being resorbed (e.g. loss of turgor and shrivelling).

Improvement of storability. Because tubers which deteriorated during the cold-storage period showed

high weight losses during the curing period (Fig. 5), the treatment of the minitubers immediately after harvest appears extremely important. The air drying for one day, used to remove soil from the minitubers was probably harmful, especially to the smallest and immature tubers. Burton (1973) showed that immediately after harvesting the weight losses of small, immature tubers could be more than 1 % per hour. The dry conditions may have inhibited periderm formation and suberization (cf. Wigginton, 1974). In later experiments, minitubers were cleaned after harvest by washing them with tap water.

A curing period is extremely important to reduce weight losses during storage (cf. Wilcockson et al., 1985). Conditions during curing were chosen to be suitable for wound healing (cf. Wigginton, 1974). However, weight losses during the first 15 days after harvest were only slightly lower than weight losses during the next 540 days of cold storage. Curing should be carried out at a higher r.h. or with reduced air movement from ventilation in order to reduce weight losses. In later experiments, the trays in which minitubers were cured were covered loosely with a sheet of plastic, still allowing abundant exchange of air. If respiration also contributes to the observed losses in tuber number, curing should be carried out at a lower temperature or for a shorter period.

The storability of tubers from the first harvest might be improved simply by delaying this harvest for a few days, thus enabling the smallest tubers to grow to a larger and therefore more storable size.

From a practical point of view, losses in tuber number are more important than weight losses. Even without optimizing the storage conditions, minitubers $\geq$ 0.5 g from the second and third harvests can be stored without large losses for a period of 1.5 years. Fortunately, most of the minitubers produced in three harvests are produced in the last two harvests (Table 1; Lommen & Struik, 1992a). By multiplying the relative contribution of each tuber category (Table 1) by the proportion of tubers surviving storage for 554 days (Table 4), it can be calculated that 96 % of all minitubers of cv. Agria and 77 % of those of cv. Liseta survived storage for 1.5 years.

Acknowledgements

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CHAPTER 7

Performance of potato minitubers in a controlled environment after different storage periods

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7 PERFORMANCE OF POTATO MINITUBERS IN A CONTROLLED ENVIRONMENT AFTER DIFFERENT STORAGE PERIODS

Additional keywords: Solanum tuberosum L., rapid multiplication, seed production, non-destructive harvest, physiological age, cultivar, stem number

Summary

Minitubers of cultivars Agria and Liseta were harvested from the same plantlets on three dates. After each harvest, tubers were dried (1 day), cured (13 days) and cold stored at 2 °C in darkness and 80 % r.h. Their performance was studied 65, 128, 191, 254, 317, 380, 443, 506 and 569 days after harvest. Minitubers (1 - 2 g) were planted in pots and grown for 8 weeks in a controlled environment. After 191 days of storage their growth was still extremely poor. In both cultivars, tallest plants and largest leaf areas per plant were observed in plants from tubers from the second and third harvests that had been stored for 317 days. Highest stem numbers, yields (total dry matter, tuber fresh weight) and harvest indices were achieved with 443 days storage with cv. Agria and 569 days storage with cv. Liseta. Tubers from the first harvest behaved slightly differently.

Introduction

After transplanting *in vitro* propagated plantlets in a glasshouse at high plant density, minitubers can be harvested at intervals of three weeks from the same plantlets (Lommen & Struik, 1992). The period from planting to the last harvest may extend to 10 weeks, and so with several plantings minitubers can be produced throughout the year. They can be used to produce prebasic or basic seed in the field.

After planting, the performance of minitubers may be affected by physiological age, as observed with 'normal' seed tubers (Madec & Perennec, 1955). The physiological age of tubers increases with increasing storage duration (Krijthe, 1962; Bodlaender & Marinus, 1987) and is affected by conditions and treatments during tuber growth (cf. Krijthe, 1962; Van Ittersum et al., 1993) and during storage and presprouting (O'Brien et al., 1983; Van Ittersum et al., 1993). Also patterns of physiological ageing differ between cultivars.

When minitubers are required for planting in the field, the physiological status of different batches can vary considerably. Firstly, because they have been stored for different periods; the tubers originate from several glasshouse plantings and various harvests of one planting. In countries with only one planting season for field production, the storage duration may vary from a few months (for tubers in which dormancy has just ended) to more than one year (for tubers that were dormant at the start of the planting season in the preceding year). Secondly, minitubers originating from subsequent harvests of one glasshouse planting differ, even if the time elapsed after harvest is similar. This is illustrated by differences in dry matter concentration (Lommen, 1993a), length of the dormant period (Lommen, 1993a) and weight losses during storage (Lommen, 1993b). Finally, the

physiological status of minitubers from different plantings may vary because the external conditions during the course of a year change, even in a controlled glasshouse. As a result of a varying physiological nature, the performance of the minitubers may vary too.

Because there are no reports on physiological ageing of minitubers or other types of small tubers, the performance of minitubers under controlled conditions was studied after storage up to 1.5 year.

Materials and methods

Minituber production. Details on production of the minitubers in this experiment were reported by Lommen (1993a); minitubers of cvs Agria and Liseta were produced on *in vitro* propagated plantlets grown in a soil-perlite mixture at 350 plants per m^2 in a glasshouse under tuber inducing conditions. Tubers were harvested on three dates at three-week intervals (May 25, June 16 and July 6) after removing plants from the growing medium. After the first two harvests, plants were replanted deeper than originally.

Minituber storage. After harvest, minitubers were left to dry at room temperature for one day in a thin layer in open metal boxes, to allow removal of soil. They were then sorted by fresh weight and cured at 18 °C in complete darkness at 80 % r.h. for 13 days. Long-term storage was at 2 °C in complete darkness and 80 % r.h. Maximum length of the storage period of tubers from each harvest was 569 days, consisting of (a) the drying period of one day, (b) the curing period of 13 days and (c) the cold-storage period up to 555 days. During curing and cold storage, tubers were kept in a thin layer in crates lined with cheese-cloth; those that deteriorated were discarded.

Performance tests. Performance tests were carried out after 65, 128, 191, 254, 317, 380, 443, 506 and 569 days of storage using minitubers (1.00 - 1.99 g) of both cultivars from all harvests. Tests were started with tubers from different harvests at three-week intervals. At the start of the tests, tubers had accumulated respectively 358, 484, 610, 736, 862, 988, 1114, 1240 and 1366 day degrees > 0 °C after harvest. Room temperature during the first day was assumed to be 22 °C. With cv. Agria, 18 tubers were used in each test. With cv. Liseta, fewer tubers were produced and the numbers used in each test are shown in Table 1. Tubers were not presprouted or conditioned and were planted singly 3.1 cm deep in 20 cm diameter pots containing 5 liter of potting soil. No fertilizer was applied. Pots were arranged in two contiguous rows in a growth room at 18/12 °C day/night and 12 hours photoperiod. Light (90 W m^{-2}, total radiation at plant level) was supplied by Philips SON-T and HPL-T lamps, supplemented by fluorescent tubes (Philips-33). Missing tubers of cv. Liseta were replaced by other minitubers (not analysed) to give 36 pots in each test. The position of the pots in the growth room was changed every 3 weeks to enable similar positions of pots in all tests. Plants were harvested 8 weeks after planting. Tubers used in the last test of cv. Liseta were weighed at room temperature at regular intervals during storage to determine weight loss during storage (Lommen, 1993b).

Table 1. Number of tubers from three harvests of cv. Liseta used in performance tests after storage periods of 65 to 569 days.

Harvest	Duration of storage (days)								
	65	128	191	254	317	380	443	506	569
First	10	10	18	18	10	-	-	-	18
Second	18	10	9	18	14	15	-	-	18
Third	18	18	18	18	18	18	9	-	18

Observations. Shoot emergence was recorded daily, starting with minitubers from the third harvest in the third performance test. In later tests, emergence date during the weekend was estimated. All stems originating from one tuber were regarded as one plant. At 8 weeks after planting, the weights of stems, leaves (petiole, rachis and leaflets), stolons and tubers were recorded. Leaf area was determined and numbers of main stems (above-ground stems arising from the mother tuber) were recorded. Stem length of the longest main stem was measured from the mother tuber to the point where new leaves appeared. Harvest index was the dry weight of tubers as a percentage of total dry weight, excluding roots.

Results

Emergence. After storage for 65 days, most minitubers were dormant and did not emerge within the 8 weeks period (Figs 1A and B). After tubers had been stored for 128 days most plants emerged, and with further storage the proportion of emerged plants rapidly increased up to 100 % whereas the time taken to emergence decreased (Figs 1C and D). Emergence was slightly slower with cv. Agria than with cv. Liseta. However, when tubers of cv. Agria were stored for more than 443 days, the time taken to emerge increased and at the end not all tubers produced a plant. By contrast, emergence of cv. Liseta at that time still was rapid and complete.

As all plants had 8 weeks available for emergence and post-emergence growth, the time to emergence directly affected the length of the post-emergence period; the average number of days with above-ground shoots can be derived from Figs 1C and D. With cv. Agria, this was maximum from 317 to 506 days of storage, with cv. Liseta from 317 days of storage onwards.

Stem number. Minitubers usually produced one main stem following storage periods up to 254 days (Fig. 2). After longer storage, numbers of main stems increased. In cv. Agria, maximum numbers were produced after 443 days storage (2 - 3 main stems per plant), whereas numbers of main stems decreased after prolonged storage. In cv. Liseta, highest numbers of main stems were recorded after the longest storage period tested (569 days, 4 - 6 main stems per plant). At these high stem numbers, however, stems were much thinner and weaker than at the lower stem numbers observed earlier.

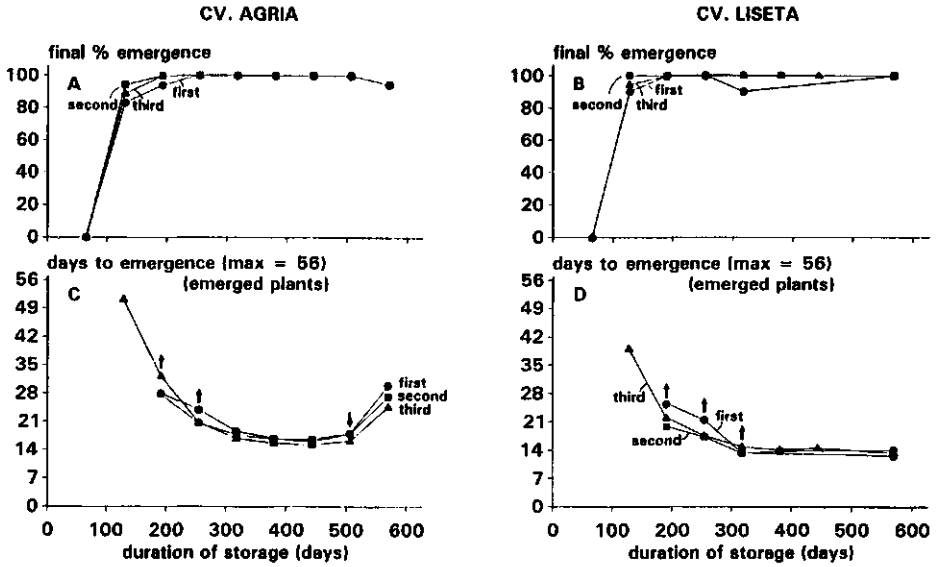

Fig. 1. Influence of length of storage period on the percentage emerged plants (A, B) and the number of days to emergence of emerged plants only (C, D) from minitubers originating from three repeated harvests of the same plantlets. Duration of test: 56 days. A and C: cv. Agria. B and D: cv. Liseta. Significant differences ($P < 5$ %) between harvests are indicated in the graphs as: ↑ = highest value differs from the others, ↓ = lowest value differs from the others, ↕ = only highest and lowest values differ, a = all values differ from each other.

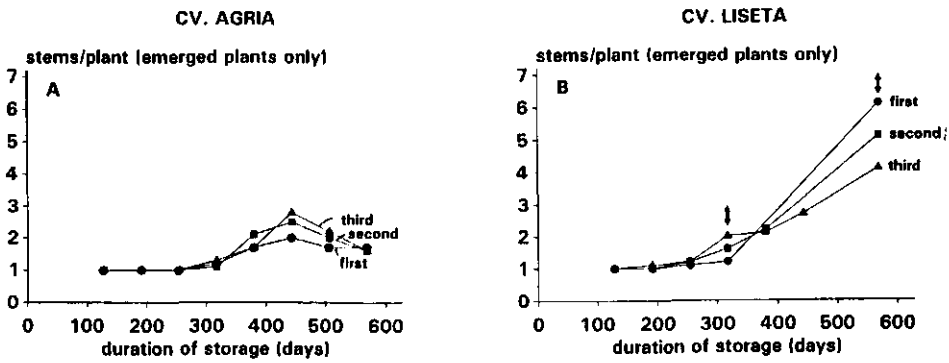

Fig. 2. Influence of length of storage period on numbers of main stems produced by minitubers (emerged plants only) originating from three repeated harvests of the same plantlets. A: cv. Agria. B: cv. Liseta. Untransformed data, assessed 8 weeks after planting. Significant differences between harvests (see Fig. 1) were assessed after square root transformation.

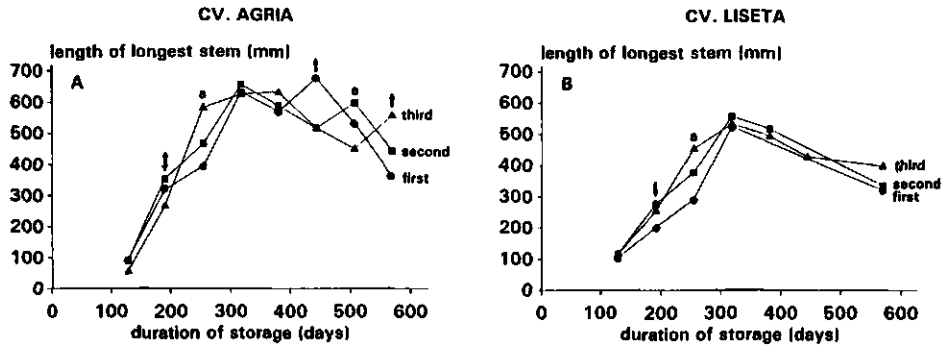

Fig. 3. Influence of length of storage period on the length of the longest main stem of emerged plants from minitubers of three harvests, 8 weeks after planting. A: cv. Agria. B: cv. Liseta. For significant differences see Fig. 1.

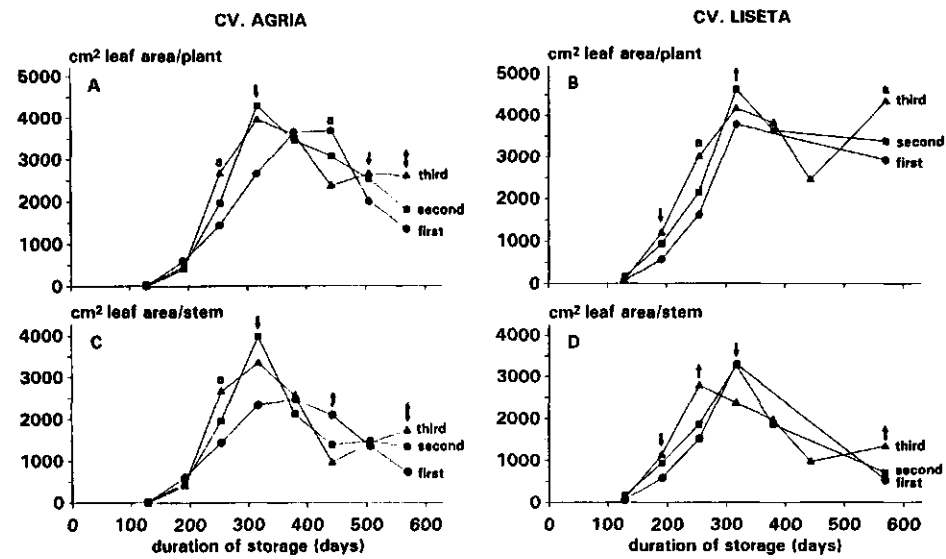

Fig. 4. Influence of length of storage period on leaf area per plant (A, B) and per main stem (C, D) produced by emerged plants of minitubers from three harvests, 8 weeks after planting. A and C: cv. Agria. B. Cv. Liseta. For significant differences see Fig. 1.

Differences in stem numbers produced by tubers from the three minituber harvests never were significant with cv. Agria. With cv. Liseta, tubers from the first harvest tended to produce fewer stems than those from the third harvest when given intermediate storage periods (difference significant after 317 days of storage), but more stems following very long storage (569 days).

Stem length. In both cultivars, length of the longest main stem per mother tuber increased with increasing storage duration up to approximately 317 days (Fig. 3). Thereafter, stem length was more variable, and tended to decrease.

Before maximum stem length was achieved, tubers from the first harvest often produced shorter stems than those from later harvests. In cv. Agria, the decrease in stem length following prolonged storage was initially less with tubers from the first harvest.

Leaf area. In plants of both cultivars originating from tubers of the second and third harvests, leaf area per plant increased to approximately 4000 - 4500 cm^2 with increasing storage duration up to 317 days (Figs 4A and B). In cv. Agria this was mainly due to a larger leaf area per stem (Figs 2A, 4C). In cv. Liseta, the leaf area per stem increased only with storage up to 254 days for tubers from the third harvest (Fig. 4D), but leaf area per plant was larger after 317 days of storage because of high stem numbers. With storage periods longer than 317 days, leaf areas per stem decreased in both cultivars (Figs 4C and 4D). As stem numbers did not compensate for the smaller leaf areas per stem, leaf area per plant decreased after maximum values were achieved. This was most clear with cv. Agria.

Plants originating from tubers of the first harvest usually produced smaller leaf areas per plant than those of later harvests after storage for up to 317 days. With cv. Agria, they apparently needed longer storage periods to achieve maximum leaf areas. Nevertheless, with both cultivars, plants originating from tubers of the first harvest again had smaller leaf areas than those of later harvests, when stored for 569 days.

Total dry matter production, yield of progeny tubers and harvest index. Total dry matter production (excluding roots, Figs 5A and B), fresh weight of progeny tubers (Figs 5C and D) and harvest index (Figs 5E and F), showed similar responses to increasing storage duration. In cv. Agria, yields increased with increasing storage up to 443 days to about 30 g dry matter and 110 g fresh tuber weight per plant. The harvest index had by that time increased to about 60 %. After 569 days of storage, both yields and the harvest index had clearly decreased. With cv. Liseta, highest yields and highest harvest indices were achieved with the longest storage period tested. Maximum yields were higher than those obtained from cv. Agria, although maximum harvest indices were almost similar.

When differences in yield between the 3 tuber sources were significant, plants originating from tubers of the first harvest generally yielded less. These differences were most obvious with cv. Liseta (Figs 5B and D). By contrast, harvest indices of plants originating from tubers of the first harvest did not consistently differ from those of later harvests.

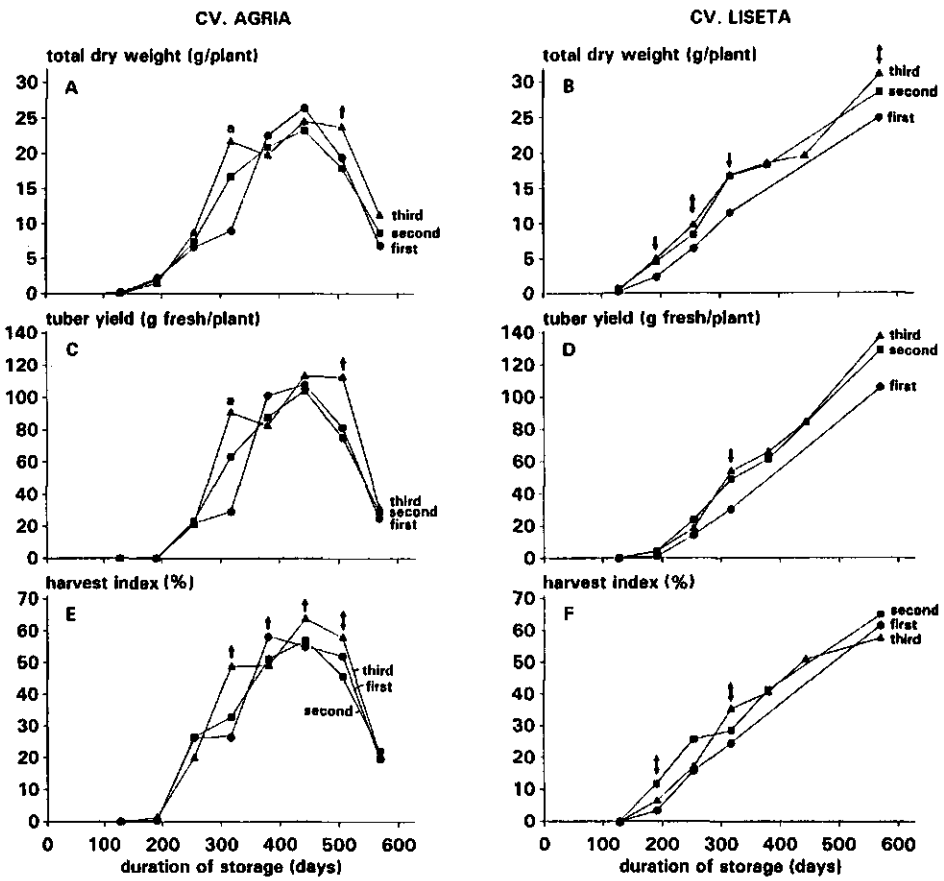

Fig. 5. Influence of length of storage period on total dry matter (A, B), tuber fresh weight (C, D) and harvest index (E, F) produced by emerged plants of minitubers from three harvests, 8 weeks after planting. A, C and E: cv. Agria. B, D and F: cv. Liseta. For significant differences see Fig. 1.

Discussion

Performance of minitubers after storage. Minitubers (1 - 2 g) showed patterns of increasing and (eventually) decreasing performance with increasing storage duration similar to those known to occur with normal seed tubers (Bodlaender & Marinus, 1987).

As in normal tubers, patterns were cultivar dependent. Cv. Agria performed best after storage periods of approximately 443 days when rate of emergence, number of main stems, total dry weight and tuber fresh weight were highest (Figs 1, 2 and 5); with longer storage, performance declined considerably. By contrast, cv. Liseta produced highest stem numbers and highest yields after the longest storage period in this experiment (569 days). However, not all plant characteristics were

maximal after the indicated storage periods. For example, maximum leaf areas and stem lengths were observed following shorter storage (Figs 3 and 4).

Patterns of physiological ageing of tubers slightly differed between harvests. With cv. Agria, the rate of increase in stem length, leaf area per plant and yield with increasing storage duration appeared to be lower when tubers originated from the first than from later harvests, whereas the decrease after maximum values were attained appeared to be greater with tubers from the first harvest (Figs 3A, 4A, 5A and 5C). With cv. Liseta, the performance of tubers from the first harvest was much poorer than that of tubers from later harvests (cf. Figs 5B and D), which may have resulted from slower physiological ageing or from a smaller amount of carbohydrate reserves in minitubers from the first harvest. Average tuber weights of cv. Liseta from the first harvest were slightly lower than from later harvests (respectively 1.2, 1.4 g; Lommen, 1993a). Minitubers from the three harvests were compared after similar storage durations. At a certain calendar date, however, minitubers from the three harvests differ in storage duration by 21 days each. Simultaneous planting, approximately 10 months after the last harvest, will reduce differences in performance between tubers from the first and second harvests, but will increase differences between those from the second and third harvests.

Extrapolation to field performance. Because conditions during the tests differed from those prevailing in the field, we cannot predict precisely the effect of storage duration on field performance of minitubers. Effects of physiological age are most clear under adverse conditions (Allen et al., 1979), whilst post-emergence conditions in the field also may level out the effects of physiological age (Fischnich & Krug, 1963). However, results (Figs 1 - 5) show that differences in performance are to be expected between cultivars and between tubers stored for different periods.

It is likely that the optimum storage duration of minitubers for field planting will be shorter than those giving the highest tuber yields in our tests (Fig. 5). Minitubers will usually be presprouted and hardened, and this increases their physiological age (cf. Allen et al., 1992). Also plants from physiologically younger tubers may develop more haulm before tubers are initiated but may, when the growing period is long enough, achieve higher tuber yields because of greater light interception (O'Brien et al., 1983). Therefore, the larger leaf areas of our plants after storage periods shorter than those giving highest yields in performance tests may result in higher yields of progeny tubers under field conditions when the growing period for seed production is long enough. However, leaf areas achieved by longer-stored minitubers may be high enough to give complete ground cover under field conditions, whilst the rate of tuber bulking may be higher. This will depend on plant density and arrangement used in the field. It seems unlikely that the optimum storage duration for field planting will be much shorter than 317 days, because emergence is likely to be delayed (Fig. 1) and therefore the length of the post-emergence period reduced.

Practical implications and manipulating physiological ageing of minitubers. As minitubers have a dormant period (Lommen, 1993a), it is necessary to produce them some time before they are planted in the field or to break their dormancy artificially. Our results suggest that their performance may

be poor if they are produced 6 or 7 months or less before planting. Therefore, they should be produced earlier and stored until their performance is better (10 - 11 months under our conditions) or optimal.

By increasing temperature during the whole or part of the storage period, the ageing of tubers possibly could be accelerated. Heat treatments soon after curing may increase vigour shortly after the end of dormancy (Van Ittersum et al., 1993) and presprouting at high temperatures in light followed by cold storage (O'Brien et al., 1983) could increase their physiological age. By adjusting the timing of this high-temperature presprouting, it is possible that sprout numbers also could be manipulated. However, desprouting following high temperature storage (Bodlaender & Marinus, 1987) would seem to be undesirable for practical use as a significant amount of the minituber reserves already may have been spent in producing the first sprout.

The timing of production of minitubers with optimal physiological age may pose problems when minitubers are produced throughout the year in countries with one planting season. Very young tubers will need to be stored until the next planting season. Although most minitubers ≥ 0.5 g can be stored for more than 1.5 year (Lommen, 1993b), our results indicate that not all cultivars perform well after storage for that period (Figs 5A and C). Also, the timing of production of minitubers and storage conditions will require correct adjustment to fit the patterns of physiological ageing of different cultivars, because age may have a large effect on their performance.

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CHAPTER 8

Effect of weight of potato minitubers on sprout growth, emergence and plant characteristics at emergence

published as:

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8 EFFECT OF WEIGHT OF POTATO MINITUBERS ON SPROUT GROWTH, EMERGENCE AND PLANT CHARACTERISTICS AT EMERGENCE

Additional keywords: Solanum tuberosum L., seed production, tuber size, presprouting, sprout length, planting depth, shoot:root ratio

Summary

The behaviour of minitubers in five weight classes, having mid-point values between 0.19 and 3.00 g, was studied during sprouting and emergence under controlled conditions. Lighter tubers took longer to produce sprouts of 2 mm, and their sprouts grew more slowly between 2 and 4 mm and 4 and 6 mm. As sprouts lengthened their rate of growth increased. The influence of tuber weight was less for heavier tubers and also decreased as the sprouts grew longer. When tubers with sprouts of the same length were planted in pots, sprouts from lighter tubers took longer to emerge. Emergence was later and differences between weight classes were larger when tubers were planted deeper (6 or 9 cm) or when they had shorter sprouts at planting (2 or 4 mm). At emergence, plants from lighter tubers had thinner stems and lower stem and root weights, but higher stem weights proportional to tuber weights and higher shoot:root ratios.

Introduction

Potato minitubers can be used for seed tuber production under field conditions. However, early attempts to grow a crop from minitubers were often unsuccessful because of reduced or delayed emergence, probably mainly due to an insufficiently long sprouting period, associated with the small tuber size. Insufficient sprouting could have resulted from the long dormant period of minitubers, which is longer with lighter tubers (Emilsson, 1949; Van Ittersum & Struik, 1992; Lommen, 1993), and therefore makes a relatively short period available for sprouting. Even after the same period for sprout growth, sprouts from tubers with lower weights could be smaller at planting, because the rate of sprout elongation during the sprouting period is positively correlated with the amount of substrate available for growth (Morris, 1966). If sprouts are smaller at planting, this will result in a later emergence (Sadler, 1961). In addition, other problems resulting from biotic or abiotic stresses may occur between planting and emergence.

This paper describes the results of three related experiments, carried out to obtain information on the effects of the weight of minitubers on sprout elongation during the sprouting period and on growth between planting and emergence.

Materials and methods

Three experiments were carried out in growth chambers, using minitubers from cvs Ostara, Bintje and Elkana in five weight classes: I 0.13 - 0.24 g; II 0.25 - 0.49 g; III 0.50 - 0.99 g; IV 1.00 - 1.99

g; V 2.00 - 3.99 g. The range of weights doubled with each consecutive class, and results are presented by plotting the middle value of each class on a log scale.

Expt 1. Minitubers were cured after harvest for 14 days and stored at 2 °C in darkness until 105 days after harvest. They were sprouted in darkness (to promote sprout elongation) at 18 °C and 80 % r.h. Tubers were placed with the apical eye upwards in a thin layer of dry sand to keep them in an upright position. The length of the sprouts was measured every 2 days until they were 8 mm long. Measurements took place under low intensity green light. Ten tubers, each producing one sprout, were used for each combination of cultivar and fresh weight class.

Expts 2 and 3. Time to emergence and plant growth until emergence were studied in two partly overlapping experiments. Individual tubers producing one (apical) sprout were sprouted as in Expt 1 until the sprout was 2, 4 or 8 mm long. The tubers were then stored at 4 °C to prevent further increase in length until 205 days after harvest when all tubers had sprouts of the desired length. Thereafter, sprouted tubers were exposed to light for 11 days and planted in individual 12.5 cm high pots containing 350 ml of coarse quartz sand supplemented with a complete nutrient solution.

Treatments applied in Expt 2 were all combinations of:

(a) Planting depth: 3, 6 and 9 cm;

(b) Tuber fresh weight class: I to V;

(c) Cultivar: Ostara, Bintje and Elkana.

All tubers had sprouts of 2 mm at planting.

Treatments applied in Expt 3 were all combinations of:

(a) Sprout length at planting: 2, 4 and 8 mm;

(b) Tuber fresh weight class: I to V;

(c) Cultivar: Ostara, Bintje and Elkana.

In Expt 3, all tubers were planted 6 cm deep.

In both experiments, all treatments were replicated ten times, using a completely randomized design. The temperature after planting was 18 °C during the day (16 h) and 12 °C during the night (8 h). Emergence was checked daily and plants were analysed the day they emerged for fresh weights of root, stem (= shoot) and mother tuber, as well as the length of the stem and its diameter in the middle region.

Results

As the main purpose of this paper was to study the effects of the weight of the mother tuber, all results are presented as average values for the three cultivars. Interactions with cultivar sometimes occurred ($P < 0.05$) but they were always less significant than the effects presented in the Table and Figures, and merely reflect differences in the clearness of the effects.

Increase in sprout length during sprouting (Expt 1). Heavy minitubers produced a 2 mm apical sprout sooner than light ones (Table 1). Sprouts from heavy minitubers also grew more rapidly between 2 and 4 mm and between 4 and 6 mm than those from lighter tubers. However, differences

Table 1. Expt 1. Influence of the fresh weight class of minitubers on the time required for the apical sprout to grow to 2 mm, from 2 to 4 mm, from 4 to 6 mm and from 6 to 8 mm. Mean of three cultivars.

	Mid-point of minituber weight class (mg)					LSD 5 %
	187	375	750	1500	3000	
Days from harvest to produce a sprout 2 mm long	153.9	146.7	142.1	136.7	134.7	1.7
Additional days taken to grow from: 2 - 4 mm	16.6	14.1	10.9	8.2	7.5	1.8
4 - 6 mm	7.1	5.4	3.9	5.3	4.9	1.6
6 - 8 mm	3.5	3.1	2.6	2.9	3.3	0.9

between weight classes were smaller in the upper ranges of tuber weight and when sprouts became larger (Table 1). The time to grow from 6 to 8 mm did not differ significantly between the weight classes. In all classes, the increase in length was faster as the sprouts became longer ($P < 0.001$).

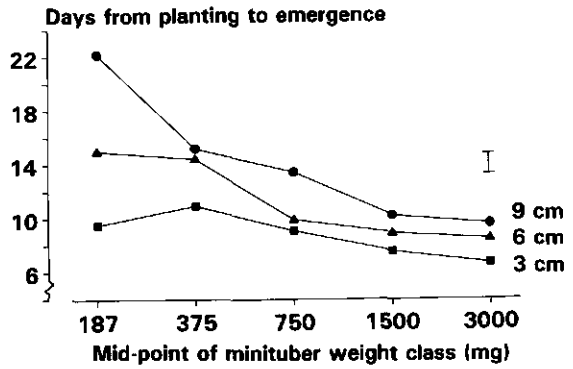

Fig. 1. Expt 2. Influence of the minituber weight class on the time to sprout emergence from three depths of planting in pots. Average values of three cultivars; sprout length at planting 2 mm. Bar: LSD 5 %.

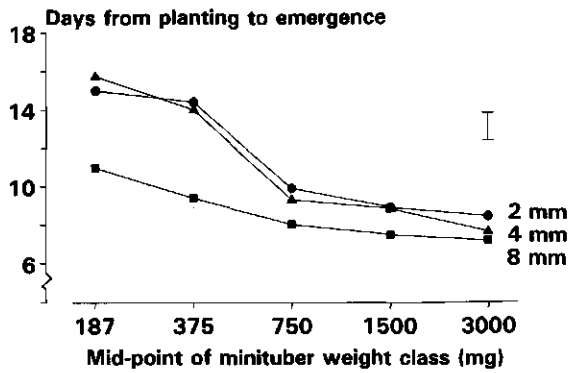

Fig. 2. Expt 3. Influence of the minituber weight class on the time to sprout emergence for three sprout lengths at planting. Average values of three cultivars; planting depth 6 cm. Bar: LSD 5 %.

Emergence and pre-emergence growth (Expts 2 and 3). When tubers, with 2 mm sprouts, were planted 3, 6 or 9 cm deep (Expt 2), the sprouts from on average 5.7 % of the tubers from the lowest weight class did not emerge within 30 days. Sprouts from all other tubers emerged. Non-emerging plants were excluded from further analysis and so were plants that had produced more than one main stem (6.3 %) or a branched main stem before emergence (5.2 %; they were mainly in the two lowest weight classes). Of the remaining tubers, the mean time to emergence decreased for heavier tubers and when minitubers were planted less deep (Fig. 1). Differences in time to emergence between weight classes were much larger with deeper planting (Fig. 1).

When tubers with sprouts of 2, 4 and 8 mm were planted 6 cm deep (Expt 3), sprouts from on average 4.2 % of the lightest tubers did not emerge. All heavier tubers produced an emerging sprout. Again, the non-emerging plants and the plants that had produced more than one main stem (4.5 %) or a branched main stem (4.3 %) were excluded from further analysis. The mean time to emergence again decreased with increasing minituber weight (Fig. 2). The time to emergence was shorter when tubers had longer sprouts at planting (Fig. 2). In addition, differences in time to emergence between low and high weight classes were larger when minitubers had small (2 or 4 mm) compared to larger (8 mm) sprouts at planting (Fig. 2).

The results of Expt 3, averaged over the three initial sprout lengths, showed that stems from lighter tubers were thinner than those from heavier tubers (Fig. 3) and that they grew further before emerging (Fig. 3). In addition to possible slight differences in planting depth (probably of no more

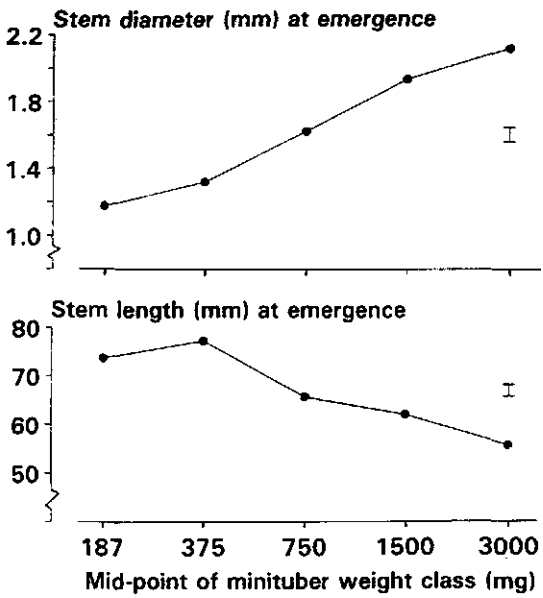

Fig. 3. Expt 3. Influence of the minituber weight class on stem diameter and stem length at emergence. Average values of three cultivars and three sprout lengths at planting; planting depth 6 cm. Bar: LSD 5 %.

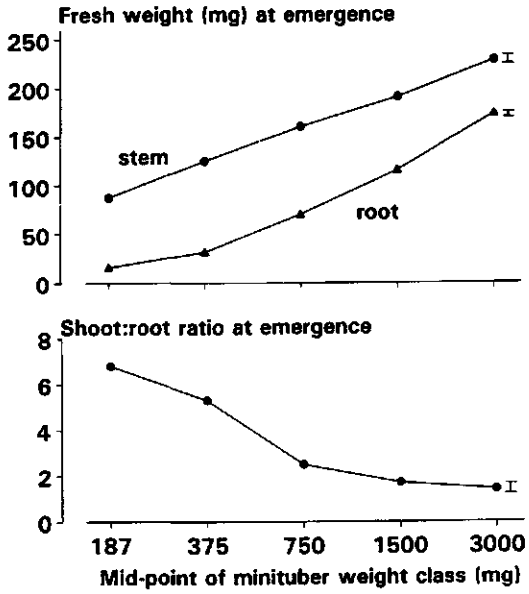

Fig. 4. Expt 3. Influence of the minituber weight class on stem and root weights and shoot:root ratio (fresh weight basis) at emergence. Average values of three cultivars and three sprout lengths at planting; planting depth 6 cm. Bar: LSD 5 %.

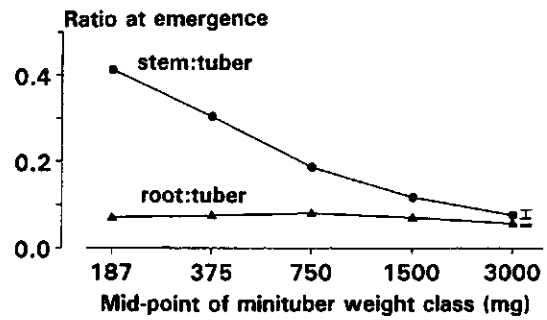

Fig. 5. Expt 3. Influence of the minituber weight class on the ratios between fresh weights of stem and mother tuber, and between root and mother tuber, at emergence. Average values of three cultivars and three sprout lengths at planting; planting depth 6 cm. Bar: LSD 5 %.

than 6 - 7 mm) due to different tuber diameters, the greater length was because most of these stems did not grow straight to the surface.

At emergence, both stem and root fresh weights increased with increasing weight of the minitubers (Fig. 4), but the shoot:root ratio was much higher in plants from lighter tubers (Fig. 4). This higher shoot:root ratio was mainly due to a much higher stem weight compared to the weight of the mother tuber at emergence in plants from smaller tubers (Fig. 5). The ratio between root fresh weight and fresh weight of the mother tuber differed only slightly between tubers of different weights (Fig. 5).

Discussion

Increase in sprout length during sprouting. The dormant period (defined here as the period from harvest until the apical sprout of a tuber is 2 mm long) was longer for lighter minitubers (Table 1). This is in accordance with other studies on minitubers (Lommen, 1993) and conventional tubers (Emilsson, 1949; Van Ittersum & Struik, 1992) although slightly different characteristics were used to define the dormant period. A slower rate of initial sprout growth up to 2 mm almost certainly contributed to this effect, because sprouts of tubers from lower weight classes were still growing more slowly between 2 and 4 mm and 4 and 6 mm (Table 1). No data are available on the onset of sprout growth. The results confirm the observation of Krijthe (1962), that initial sprout growth up to 3 mm was slower in smaller tubers. Van Ittersum et al. (1992) observed no consistent effects of tuber weight on initial sprout elongation. This was probably because they used heavier tubers (25 g and 80 g), for differences in sprout elongation rate between fresh weight classes diminished when tuber weights became higher (Table 1).

Emergence and pre-emergence growth. Table 1 suggests that if all tubers are sprouted for the same period, those from the lowest weight classes will have the smallest sprouts. Consequently, differences in time to emergence between tuber classes will be extremely large because (a) stems from lighter tubers emerge later even if the sprouts are the same length at planting (Figs 3, 4); and (b) stems from tubers with smaller sprouts emerge later (Fig. 2; Sadler, 1961; Headford, 1962; Firman et al., 1992). By planting minitubers with longer sprouts (up to 8 mm, the maximum studied), both the time to emergence can be shortened and differences between tubers with different weights can be reduced. However, this will require a much longer sprouting period for tubers with lower weights, and consequently an adjustment of the sprouting period depending on the tuber weight. In practice, this could probably be achieved by narrow grading of the tubers after curing, and sprouting batches separately for the appropriate period.

The later emergence of stems from smaller tubers was mainly due to a slower increase in length after planting (Figs 3, 4). In addition, however, stems from lighter tubers grew farther before they emerged (Fig. 3), probably partly because their growth had not been completely negatively geotropic. Some stems, however, especially the thinner stems from light tubers, seemed to have been impeded by the soil, as indicated by the fact that they were curved or coiled. The coarse sand used may have enhanced this effect.

Time to emergence is probably negatively related to the rate at which reserves from the mother tuber become available for stem growth (cf. Moorby, 1967) and positively to the total weight of the stem produced at emergence. Sprouts emerge earlier if they were larger when the tubers were planted (Fig. 2; Sadler, 1961; Headford, 1962) probably because in these tubers the substrate becomes more quickly available for growth. Although during sprouting the availability of calcium is thought to limit sprout growth (Davies, 1984), between planting and emergence the availability of carbohydrates is more likely to restrict stem growth, as mineral nutrients could be absorbed during emergence (confirmed by unpublished data). Although the stems of lighter tubers were thinner (Fig. 3) and had

lower weights at emergence (Fig. 4), the growth of these stems will have required a higher proportion of mother tuber reserves, because the stem:tuber ratio at emergence was considerably higher in plants from lighter tubers (Fig. 5; only fresh weight data available). Thus, if a similar proportion of tuber reserves becomes available each day for stem growth in all weight classes, stems from tubers with lower weights will emerge later because in total a higher proportion of the tuber reserves is necessary for emergence (cf. Fig. 5). This also implies that if plants from smaller tubers are damaged before or soon after emergence (e.g. by night frost) fewer reserves from the mother tuber (both absolutely and relatively) are available to resume growth.

The weight of the root system at emergence was lower in plants from lighter tubers (Fig. 4), but much more proportional to the weight of the mother tuber than that of the stem (Fig. 5). Consequently, in plants from lighter tubers the root system has to provide water and nutrients to a much larger shoot in relation to its weight (higher shoot:root ratio; Fig. 4). This may partly explain the slower foliar ground cover of plants from smaller tubers (Wiersema & Cabello, 1986; Struik & Lommen, 1990; Allen et al., 1992) and - if this situation persists during further growth - it may render plants from small tubers more susceptible to drought and to second growth of progeny tubers, as reported by Struik & Lommen (1990). Because of the limited root system at emergence the use of small tubers may require special cultural techniques, such as fertilizer placement, irrigation, etc. to make full use of their potential.

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CHAPTER 9

Field performance of potato minitubers with different fresh weights and conventional seed tubers: Crop establishment and yield formation

published as:

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9 FIELD PERFORMANCE OF POTATO MINITUBERS WITH DIFFERENT FRESH WEIGHTS AND CONVENTIONAL SEED TUBERS: CROP ESTABLISHMENT AND YIELD FORMATION

Additional keywords: Solanum tuberosum L., seed production, emergence, ground cover, radiation interception, radiation conversion, harvest index

Summary

Field performance of five fresh weight classes of minitubers ranging from 0.13 - 0.25 g to 2.00 - 3.99 g and conventional seed tubers was studied in a short growing season (79 or 82 days) in two years. The heavier minitubers gave a more regular emergence, faster ground cover soon after emergence, higher dry-matter yields, and higher fresh tuber yields. Radiation conversion coefficient (RCC) did not differ. Higher tuber yields resulted from more radiation intercepted due to a faster ground cover, and a higher harvest index. All minitubers produced plants with one primary stem. In one experiment when heavier minitubers had long sprouts, time to 50 % emergence decreased with tuber weight, whereas dry-matter concentration of progeny tubers increased. Conventional tubers appeared superior to minitubers in all characteristics mentioned except RCC, which was similar. Differences in performance between minitubers and conventional tubers were attributed to weight and age of seed tubers, presprouting method and crop husbandry.

Introduction

Potato minitubers and microtubers may be used in seed production programmes to reduce the number of field multiplications. This may increase the flexibility of seed production, improve the health status of the ultimate commercial seed produced and reduce the time for adequate volumes of seed from new cultivars to become available. Mini- and microtubers, however, will only be used on a large scale if they reliably produce acceptable yields before haulm has to be killed, and produce high quality progeny tubers both in the first and later generations.

Because of their larger weight, minitubers appear more suitable for direct field planting than microtubers (Struik & Lommen, 1990). They can be produced in a glasshouse (Lommen & Struik, 1992), but little accurate information is published on their field performance. Crops from minitubers intercept less radiation during the growing period than those from conventional seed tubers and produce lower tuber yields (Marshall & Taylor, 1990), although the difference may be small in some years (Ogilvy et al., 1990). More is known about microtubers. Plants from them have fewer main stems than those from conventional tubers (Wattimena et al., 1983; Haverkort et al., 1991) and crops cover the ground less rapidly (Haverkort et al., 1991). Yield of progeny tubers may be lower (Haverkort et al., 1991) or equal (Wattimena et al., 1983) to that of conventional tubers.

Is it not clear how seed weight affects the field performance of minitubers and whether it is the

only cause of differences between new and conventional seed types. Therefore, the effects of seed weight on the field performance of minitubers were studied and their field performance was compared with that of conventional tubers. The present paper analyses crop establishment and yield formation.

Materials and methods

General. The field performance of minitubers from five fresh weight classes (Table 1) and conventionally produced seed tubers (grade 25/28 mm or 28/35 mm) was studied in 2 years. The relative range of minituber weights was equal in each class and cv. Bintje (mid-early) was planted in Expt 1 (1989) and cvs Bintje, Ostara (early) and Elkana (late) were planted in Expt 2 (1990). The growing period for field production was short (79 or 82 days) because minitubers must produce progeny tubers before haulm is normally killed to prevent infection by viruses.

Production, storage and presprouting of mother tubers. Minitubers were produced according to Lommen & Struik (1992) and were harvested on November 29, 1988 (Expt 1) and November 27, 1989 (Expt 2). In Expt 1 they were cured for 15 days (18 °C), cold-treated for 14 days (2 °C) and stored/presprouted in a layer of a few tubers thick in metal boxes for 84 days (18 °C), all in darkness at 80 % r.h. Tubers were sorted by fresh weight before sprouting. They generally produced one (apical) sprout, which was longer in tubers from the higher weight classes (Table 1). Sprouted tubers were hardened for 41 days in a glasshouse before planting. In Expt 2, minitubers were cured for 14 days, cold-treated for 42 days, sorted by fresh weight and presprouted at the same conditions as in Expt 1. Individual tubers were presprouted with the apical eye upwards until the apical sprout was 3 mm and then stored at 4 °C in darkness until 87 days after the start of presprouting. Before planting they were hardened for 7 days in a glasshouse. Sprout length (Table 1) was more uniform than in Expt 1, both within and between fresh weight classes.

Table 1. Fresh weight range of minitubers from five weight classes assessed before sprouting, and sprout length assessed at the start of the hardening period (Expt 1) or at planting (Expt 2).

Minitubers	Fresh weight range (g)	Sprout length (mm)			
		Expt 1	Expt 2		
		Bintje	Bintje	Ostara	Elkana
class I	0.13 - 0.24	0.5	2.3	2.1	1.7
class II	0.25 - 0.49	1.3	2.5	2.4	1.8
class III	0.50 - 0.99	3.6	3.1	2.4	2.3
class IV	1.00 - 1.99	4.8	2.7	2.6	2.3
class V	2.00 - 3.99	5.9	2.5	2.5	2.5

Conventional seed tubers were produced commercially and the precise treatment between harvest and presprouting was unknown. They were presprouted for 7 (Expt 1) or 6 (Expt 2) days, and hardened for 7 days until planting under similar conditions as minitubers. Sprouts were longer and more numerous than those of minitubers.

Experimental design. The experiments were laid out in blocks with four blocks and six treatments (five minituber classes + conventional tubers) in Expt 1, and a split-plot design of four blocks with three cultivars assigned to main plots and six treatments randomized within a cultivar in Expt 2. Plots comprised 56 planting positions (4 rows x 14 tubers) and observations were made on the inner 16 positions (2 rows x 8 tubers) in Expt 1 and 20 positions (2 rows x 10 tubers) in Expt 2.

Field practice. Tubers were planted by hand on May 2 1989 (Expt 1), and April 26 1990 (Expt 2) into a light sandy soil in Achterberg, the Netherlands. They were spaced 20 cm apart in ridges 75 cm wide (66.667 tubers per ha), with their upper surface 6 cm below the soil surface. Plants were harvested 79 (Expt 1) or 82 (Expt 2) DAP (days after planting). Mean soil temperatures at 5 cm during 2 weeks after planting were respectively 16.0 and 18.3 °C and mean air temperatures at 10 cm during the rest of the growing period 16.8 and 15.6 °C.

Fertilizer was broadcast at 80 kg N/ha, 50 kg P_2O_5/ha and 100 kg K_2O/ha 7 DAP in Expt 1, and at 70 kg N/ha (immediately after planting), 48 kg P_2O_5/ha and 104 kg K_2O/ha (14 days before planting) in Expt 2. Weeds were controlled chemically before emergence and later by hand. Plots were earthened up by hand with 4 cm soil 51 DAP in Expt 1, and after individual plots had a ground cover of 25 % in Expt 2. The experimental field was irrigated.

Observations and calculations. Emergence was recorded two (Expt 1) or three (Expt 2) times a week and the number of days to 25 %, 50 % and 75 % emergence was estimated by linear interpolation. Ground cover of the first 90 cm of the inner plants of the centre two rows of each plot was estimated on 51 and 76 DAP in Expt 1 and weekly from 18 to 74 DAP in Expt 2 using a 90 x 75 cm grid with 100 compartments. The number of days to 10 % and 30 % ground cover was estimated by linear interpolation in Expt 2.

Records were made of fresh weights of progeny tubers and in Expt 1 of above-ground haulm. Fresh and dry weights of different plant parts and stem and branch numbers were assessed on plants at four fixed positions.

The accumulated intercepted global radiation was calculated from the daily global radiation and the proportion of soil covered by the haulm as recorded in the weekly measurements. Radiation Conversion Coefficient (RCC) was the total dry-matter production (excluding roots) divided by the accumulated intercepted global radiation.

Statistical analysis. To determine whether the minituber weight affected performance, only data of the five minituber classes were subjected to analyses of variance. When interactions between class

Table 2. Emergence and survival of plants produced by minitubers of five weight classes and conventional seed tubers.

	Percentage emerging plants				Percentage plants at harvest[a]			
	Expt 1	Expt 2			Expt 1	Expt 2		
	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Ostara	Elkana
Minitubers								
class I	89	98	99	96	86	93	91	95
class II	97	99	100	100	92	99	96	99
class III	98	100	100	100	95	100	97	99
class IV	91	100	100	100	89	100	100	99
class V	84	100	100	100	80	100	99	100
Difference between minituber classes[b]	ns	---------- * ----------			ns	---------- ** ---------		
Conventional seed tubers	100	100	100	100	100	100	99	100
Difference between conventional seed and all minituber classes[b]	ns	--------- ns ---------			ns	---------- ns ---------		

[a] Number of plants as percentage of number of tubers planted, assessed 59 DAP in Expt 1 and 82 DAP in Expt 2.
[b] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, ns not significant: $P \geq 0.05$.

and cultivar could not be disregarded compared to the main effect (tested by an F-test on mean squares), differences between treatments were analysed for each cultivar by an LSD test and significances are presented for each cultivar separately.

To determine whether conventional tubers differed from all minituber classes, all data were subjected to analyses of variance. When there were treatment effects, the significance of the smallest difference between the relevant minituber class and conventional tubers was tested by a LSD test, and when there were interactions between treatments and cultivars that could not be disregarded, this was done for cultivars separately.

Results

Plant emergence and establishment. In Expt 1, 84 - 97 % of the minitubers emerged. This percentage was variable, both within and between classes, and not significantly affected by the weight of the minitubers (Table 2). In Expt 2, 96 - 100 % of the minitubers emerged (Table 2) and the percentage increased with increase in weight of minitubers. Emergence of conventional seed tubers was complete in both experiments.

Of the plants that emerged in Expt 1, the period from planting to 25 % or 50 % emergence was longest for minitubers from the lowest weight class, but no differences were found in the time

between 25 % and 50 % emergence (Fig. 1A). In Expt 2, when sprout length at planting was similar for all minituber classes (Table 1), no significant differences between minituber classes could be detected in the number of days to 25 % or 50 % emergence or between 25 % and 50 % emergence (Figs 1B, 1C, 1D). However, in both experiments it took longer for the remaining tubers to emerge: the time period from 50 % to 75 % emergence was longer for the lightest minitubers (Fig. 1).

In both experiments the time to 25 % or 50 % emergence was shorter for conventional tubers than for minitubers (Fig. 1). They also emerged more uniformly (Fig. 1), but the differences compared with the most uniformly emerging minituber classes were not significant.

Not all the plants that emerged survived until harvest and plant numbers at harvest in Expt 2 even were more affected by the fresh weight of the minitubers than the percentage emerged plants (Table 2).

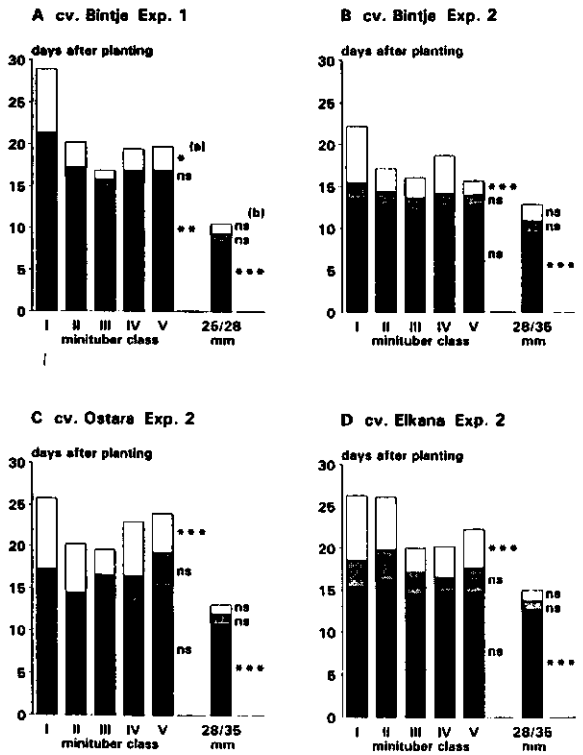

Fig. 1. Emergence parameters (days from planting to 25 % emergence ■, from 25 % to 50 % emergence ■ and from 50 % to 75 % emergence □) of plants that emerged from minitubers of five weight classes (see Table 1) and conventional seed tubers (25/28 mm or 28/35 mm). Significant differences in the length of each period are indicated of (a) comparisons within minituber classes, (b) comparisons between all minituber classes and conventional seed tubers. *** = $P < 0.001$, ** = $0.001 \leq P < 0.01$, * = $0.01 \leq P < 0.05$, ns = not significant. No interaction occurred between cultivar and treatment in Expt 2.

Table 3. Number of stems and branches on plants produced by minitubers of five weight classes and conventional seed tubers, 82 DAP Expt 2.

	Number of primary stems per plant			Total number of branches per primary stem			Percentage of branches originating below-ground		
	Bintje	Ostara	Elkana	Bintje	Ostara	Elkana	Bintje	Ostara	Elkana
Minitubers									
class I	1.0	1.1	1.1	6.6	3.5	6.7	76	67	65
class II	1.1	1.0	1.0	5.7	6.2	5.9	72	68	61
class III	1.1	1.0	1.0	6.2	5.3	6.6	72	60	62
class IV	1.0	1.0	1.0	6.6	5.5	7.1	58	64	46
class V	1.3	1.0	1.0	4.7	7.1	6.1	56	46	56
Difference between minituber classes[a]	--------- ns ---------			--------- ns ---------			--------- ** --------		
Conventional seed tubers	3.3	2.3	1.6	1.2	2.1	2.1	84	80	63
Difference between conventional seed and all minituber classes[a]	***	***	*	-------- *** --------			--------- ns --------		

[a] Significant differences were analysed after square root transformation of the data. For symbols see Table 2.

Canopy structure. Minitubers usually produced one primary stem originating from the mother tuber with an average of six branches (Table 3). Some branches were secondary stems originating from below-ground nodes and others were above-ground branches. The total anumber of branches per primary stem was not significantly affected by the weight of the minitubers (Table 3), but the proportion produced on below-ground nodes was lower in the plants from minitubers with the higher fresh weights (Table 3).

Plants from conventional tubers had more primary stems than those from minitubers and fewer secondary stems and above-ground branches per primary stem (Table 3).

Ground cover. Data from Expt 2 show that in all cultivars foliar ground cover soon after emergence increased faster in crops from minitubers with the higher weights (Fig. 2). The period from 50 % emergence to 10 % ground cover was longer for crops from the lighter minitubers. After 10 % ground cover had been reached, the increase with time up to 30 % ground cover appeared similar for all weight classes, except in the early cv. Ostara (Fig. 2). Differences between weight classes in the time between 10 % and 30 % ground cover were not statistically different, but in some of the lowest weight classes half of the plots did not reach 30 % ground cover (dotted upper lines in right part of Fig. 2). For conventional tubers from all cultivars, both the period from 50 % emergence to 10 % ground cover and the period from 10 % to 30 % ground cover were shorter than for minitubers (Fig. 2).

In Expt 1, ground cover at harvest was complete except for the crop from the lightest minitubers (0.13 - 0.25 g), which did not advance beyond 86 %. In Expt 2, plots with conventional tubers also

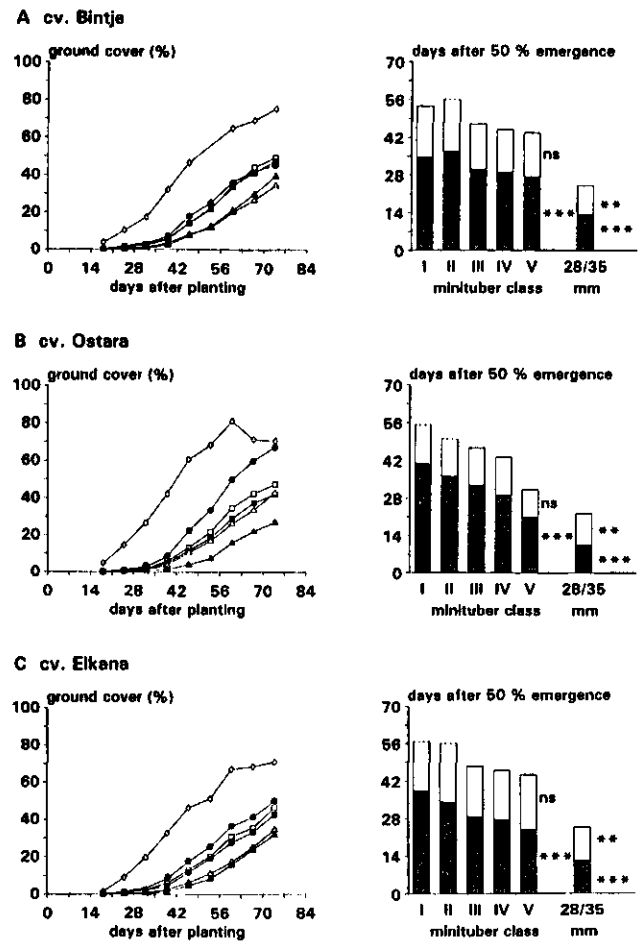

Fig. 2. Development of ground cover with time and the time from 50 % emergence to 10 % ground cover and from 10 % to 30 % ground cover, of minitubers from five weight classes (see Table 1) and conventional seed tubers (28/35 mm), Expt 2. For indication of significant differences see Fig. 1. Minituber class I: ▲, class II: ▲, class III: ■, class IV: □, class V: ●. Conventional tubers: ◊. Days from 50 % emergence to 10 % ground cover: ▭, and from 10 % to 30 % ground cover: ▭.

did not achieve complete ground cover, although the maximum value was higher than for minitubers (Fig. 2).

In both experiments, the haulm of plants from minitubers was green at harvest whereas plants from conventional tubers had turned slightly yellow.

Yield parameters. Plots with plants from the heavier minitubers intercepted more radiation during the growing period and had a higher total dry-matter production at harvest (Table 4). Differences

Table 4. Yield characteristics of plants from minitubers of five weight classes and conventional seed tubers, 79 DAP Expt 1 (cv. Bintje) and 82 DAP Expt 2 (mean of cvs Bintje, Ostara and Elkana).

	Accumulated intercepted global radiation (MJ/m^2)		Dry-matter production (g/m^2)		Radiation conversion coefficient (RCC) (g/MJ)		Harvest index (g/g)		Tuber dry-matter concentration (g/g)		Fresh tuber yield (g/m^2)	
	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Minitubers												
class I	-	136.6	294	231	-	1.70	0.33	0.62	0.16	0.20	617	710
class II	-	163.6	462	270	-	1.60	0.48	0.64	0.18	0.20	1266	862
class III	-	213.5	629	361	-	1.69	0.55	0.65	0.18	0.20	1752	1221
class IV	-	232.5	600	378	-	1.64	0.56	0.65	0.19	0.20	1830	1275
class V	-	279.6	642	453	-	1.68	0.54	0.70	0.18	0.20	1968	1592
Difference between minituber classes[a]	-	***	***	***	-	ns	***	b	**	ns	***	***
Conventional seed tubers	-	514.6	1076	801	-	1.58	0.78	0.79	0.22	0.22	3776	2911
Difference between conventional seed and all minituber classes[a]	-	***	***	***	-	ns	***	***	***	*	***	***

[a] For symbols see Table 2.
[b] Effect of minituber class was highly significant (***), but interaction between minituber class and cultivar could not be disregarded.

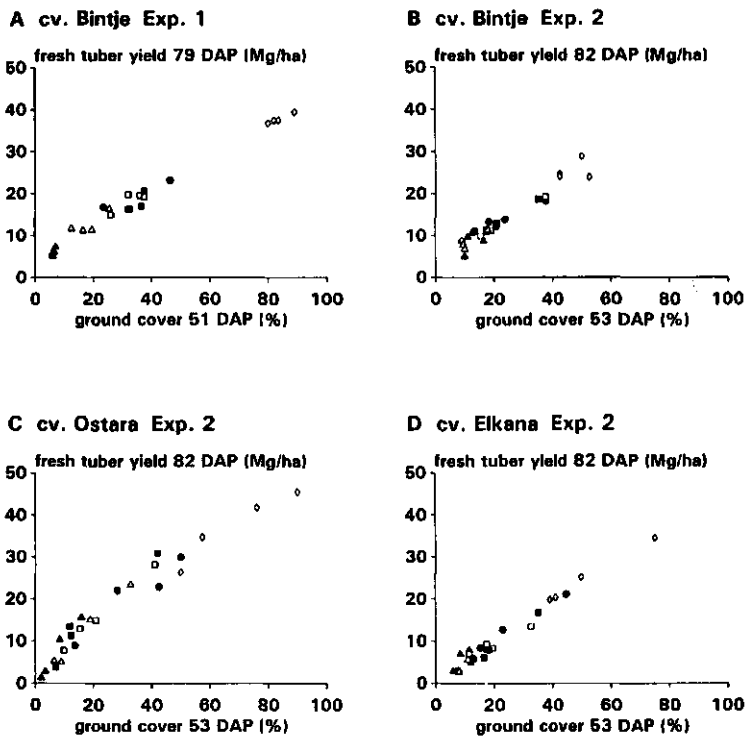

Fig. 3. Relationship between mid-season ground cover (51 or 54 DAP) and final fresh tuber yield (79 or 82 DAP) in individual plots planted with minitubers from five weight classes and conventional seed tubers. For symbols see Fig. 2.

in RCC were not significant between minituber classes, but the harvest index was higher in plots from the heavier minitubers (Table 4). In Expt 2, effects of minituber class on harvest indices were more variable within a cultivar, but in all cultivars minituber class I significantly had a lower harvest index than class V. Dry-matter concentration of the progeny tubers was variable, and only in Expt 1 was it significantly higher in plants from minitubers with the higher fresh weights (Table 4). Fresh yield of progeny tubers increased with the weight of the mother(mini)tubers (Table 4).

All yield parameters were higher for conventional tubers than for minitubers (Table 4) except the RCC which did not differ significantly.

Relation between mid-season ground cover and fresh tuber yield. In both experiments and in all cultivars, fresh yields of progeny tubers were closely related to the mid-season ground cover and Fig. 3 shows the fresh tuber yields at harvest plotted against mid-season ground cover for individual experimental plots. The relations appeared to be almost linear or slightly negatively quadratic.

Discussion

Effects of fresh weight on the performance of minitubers. The long period between planting and 50 % emergence for the lighter minitubers in Expt 1 (Fig. 1) could have resulted from the shorter sprouts after presprouting (Table 1; Sadler, 1961; Headford, 1962; Lommen, 1994). Minituber weight did not affect this period in Expt 2 when sprout length at planting hardly differed between fresh weight classes. Both the less uniform emergence after 50 % of the plants had emerged (Fig. 1) and the lower final emergence of tubers with lower weights in Expt 2 (Table 2) may be related to the more delicate stems from these tubers and the high proportion of tuber reserves necessary to cause emergence (cf. Lommen, 1994).

The heavy branching of the stems from minitubers was comparable to that of single-stemmed plants derived from *in vitro* plantlets (e.g. Leclerc & Donnelly, 1990). However, the observation that plants from tubers with lower weights had proportionally more secondary stems compared with above-ground branches, whereas the total number of secondary stems + above-ground branches was not affected (Table 3), probably resulted from the crop husbandry technique employed. At hilling, all plots received 4 cm additional soil. Plants from the lighter minitubers could have had shorter internodes and so more branches would have been covered with soil.

The much slower ground cover of crops from the lighter minitubers soon after emergence was probably caused by a slower haulm development per plant due to the relatively small root system of plants from the light tubers at emergence and the smaller amount of tuber reserves available for growth (cf. Lommen, 1994). Fig. 2 shows two weeks difference between the lowest and highest weight class in days from 50 % emergence to 10 % ground cover. In addition the longer time for all plants to emerge (4 days difference between the lowest and highest class in time from 50 % to 75 % emergence), the lower final percentage emergence (2 % between the lowest and highest class) and the higher plant death after emergence (7 % between the lowest and highest class) may also have contributed. As presprouting of the seed tubers at 18 °C started 4 to 8 weeks after harvest, the growth of sprouts other than the apical one was suppressed (cf. Krijthe, 1962) resulting in plants with one primary stem regardless of the fresh weight. Thus the slower ground cover development in crops from the lighter tubers was not caused by fewer main stems per plant but by a less ground cover per stem.

The lower dry-matter production of crops from the lighter minitubers (Table 4) can be explained mainly through the smaller quantity of radiation intercepted (Table 4) due to slower ground cover development soon after emergence (Fig. 2). As observed by Marshall & Taylor (1990), RCC did not differ between fresh weight classes (Table 4). In addition, harvest index increased with seed weight, which indicates that plants from tubers with different weights may differ in time of tuber initiation and/or in the allocation of dry matter to the tubers. Tuber dry-matter concentration increased with minituber weight only in Expt 1. Thus, fresh yield of progeny tubers increased with the fresh weight class of the mother tubers mainly because of a higher quantity of radiation intercepted and a higher harvest index.

Comparison of performance of minitubers and conventional seed tubers. Although conventional tubers were about 5 - 9 times heavier than the heaviest minituber class, these differences are likely to explain only part of the difference in crop establishment and yield formation between conventional seed tubers and minitubers. Also tuber age, conditions between harvest and planting and crop husbandry techniques employed in the field will have affected the differences.

The consistently shorter time to 25 % or 50 % emergence (Fig. 1) of conventional tubers probably resulted from more rapid stem growth after planting because of their age (cf. Firman et al., 1992) and the size of sprouts at planting (cf. Sadler, 1961; Firman et al., 1992; Lommen, 1994). In addition, the total distance the stems had to grow was smaller because planting depth was not adjusted for sprout length. The tendency to a more uniform emergence of conventional seed tubers compared to minitubers (Fig. 1) will have resulted from the larger number of sprouts following longer and colder storage in association with a higher seed weight. Damage to a single sprout at planting would have left other sprouts intact to emerge whereas with minitubers new sprouts or branches had to be produced.

Both faster emergence of conventional tubers compared to minitubers (Fig. 1) and faster ground cover after emergence (Fig. 2) led to a higher amount of radiation being intercepted during the growing season. The faster increase in ground cover in conventional tubers could have been caused partly by the higher stem numbers (Table 3). Because of the increased quantity of radiation intercepted, dry-matter production from conventional tubers was also higher. RCC was slightly lower but not significantly less than with minitubers. Harvest index was higher in conventional tubers than in minitubers, a difference that can be explained partly by mother tuber weight (Table 4), but mainly by the age of conventional tubers. Tuber dry-matter concentration was much higher in conventional tubers than in minitubers, possibly because of the more advanced developmental stage of the plants. Although dry-matter concentration of the progeny tubers was higher, fresh tuber yield always was higher in plants from conventional tubers, due to the much higher dry-matter production and higher harvest index.

Practical implications. Because yield of progeny tubers in a short growing season was affected mainly by the radiation intercepted and the harvest index, methods to increase tuber yield from small mother tubers could concentrate on (a) reducing the time to emergence, (b) increasing the proportion emerging and surviving plants, (c) enhancing haulm growth after emergence, and (d) increasing the harvest index. Emergence will be earlier with sufficiently presprouted tubers (Headford, 1962; Lommen, 1994) and/or tubers of a greater physiological age (Firman et al., 1992; Lommen & Struik, 1993). Using older tubers may also increase the percentage emerging minitubers (Lommen & Struik, 1993), but methods to improve the survival after emergence are not yet clear. Crop husbandry techniques such as floating plastic film may accelerate both emergence and early haulm growth after emergence. Haulm growth probably also could be stimulated by crop husbandry techniques including irrigation or fertilizer placement which reduce negative effects of the smaller root system of plants from the lighter tubers (Lommen, 1994). However, the precise causes of slower haulm development are not yet understood. The amounts of radiation intercepted per hectare may also be increased by

planting more of the lighter tubers and Fig. 3 suggests that this may result in higher progeny tuber yields per hectare without appreciably reducing yields per plant. Harvest index may be increased by using slightly older minitubers (Lommen & Struik, 1993).

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CHAPTER 10

Field performance of potato minitubers with different fresh weights and conventional seed tubers: Multiplication factors and progeny yield variation

submitted as:

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10 FIELD PERFORMANCE OF POTATO MINITUBERS WITH DIFFERENT FRESH WEIGHTS AND CONVENTIONAL SEED TUBERS: MULTIPLICATION FACTORS AND PROGENY YIELD VARIATION

Additional keywords: Solanum tuberosum L., seed production, variability

Summary

Multiplication factors and progeny yield variation in crops from minitubers of five weight classes (ranging from 0.13 - 0.25 g to 2.00 - 3.99 g) and conventional seed tubers were studied in field experiments in three years. Multiplication factors were calculated as the number and weight of progeny tubers produced per planted tuber or per unit planted tuber weight. They were lower for the lighter minitubers when calculated per tuber and higher when calculated per weight. Yield variation was described by coefficients of variation for the number and weight of progeny tubers produced. Variation over individual plants of a crop was higher in stands from the lighter minitubers. Variation over plots within a field was sometimes higher for the lighter minitubers, but variation over years was similar for all minituber classes. Variation over plots in progeny tuber weight was higher for minitubers than for conventional tubers.

Introduction

Potato minitubers can be used as a propagation material for the production of high quality seed in a seed programme comprising only a few field multiplications. Minitubers may have weights from 250 mg upwards. The average weight of the minitubers, however, depends on the technique used for their production, as do the numbers of minitubers produced (e.g. Lommen & Struik, 1992a, b). Consequently, the choice for a certain way of producing minitubers may affect multiplication factors when minitubers are planted in the field and also the reliability with which a crop can be grown from them.

Multiplication factors can be expressed in terms of the number or weight of progeny tubers produced per planted tuber or per unit planted weight. For multiplication purposes, the number of progeny tubers above a certain minimum weight produced per seed tuber may be more important than the weight produced per seed tuber. When small mother tubers (minitubers, small conventional tubers or seedling tubers) with different weights are compared at equal planting densities, the number of progeny tubers produced is sometimes higher for the heavier mother tubers (De Vries, 1990; Allen et al., 1992), but not always (Wiersema & Cabello, 1986; Horváth & Föglein, 1987). The weight of progeny tubers produced at equal planting densities is generally higher for heavier minitubers (De Vries, 1990; Marshall & Taylor, 1990; Ogilvy et al., 1990; Lommen & Struik, 1994), especially in a confined growing period. Multiplication factors for small mother tubers based on planted weight are rarely published, but are probably higher for lighter mother tubers (cf. De Vries, 1990).

Introduction of light minitubers in a seed production programme, however, will only have

perspective if crops can be grown reliably from them. In previous experiments (Lommen & Struik, 1994), crops from lighter minitubers seemed more variable than those from heavier minitubers or conventional tubers. Variation may show at various levels, e.g. over individual plants within a crop, over plots within a field, and over years. A potentially higher plant-to-plant variation and plot-to-plot variation reduce the possibilities of growing a uniform crop. A higher year-to-year variation reduces the yield stability.

This paper reports the multiplication factors obtained in field experiments in three years in crops from minitubers with regularly increasing weights and compares them with those of conventional seed tubers in two years. The paper also quantifies the yield variation over plants, plots and years of plants and crops produced from these tubers.

Materials and methods

General. Minitubers from five weight classes were planted in the field in three years (1989, 1990, 1994) and were compared with larger, conventionally produced seed tubers in two of the years (1989, 1990). Cv. Bintje (mid-early) was planted in all years and cvs Ostara (early) and Elkana (late) only in 1990. The growing period for field production was short because minitubers must produce progeny tubers before haulm is normally killed to prevent infection by viruses.

Production, storage and presprouting of tubers. In all experiments (Expts 1 - 3), minitubers were produced according to Lommen & Struik (1992b) and harvested in the last week of November 1988 (Expt 1), 1989 (Expt 2) and 1993 (Expt 3), cured for about 2 weeks and stored cold. Tubers were sorted into 5 weight classes: 0.13 - 0.25 g, 0.26 - 0.49 g, 0.50 - 0.99 g, 1.00 - 1.99 g and 2.00 - 3.99 g. The relative range of weights was equal in each class in all expts, but in Expt 3 it was ensured that also the coefficient of variation for weight of the minitubers to be planted in every row of every plot was equal (21 %). The tubers were presprouted before planting. In Expt 1, sprout length increased with the weight class from 0.5 to 5.9 mm. In Expts 2 and 3, sprout length at planting was constant at about 3 mm.

Conventional seed tubers of grade 25/28 mm, class A in Expt 1 and grade 28/35 mm, class SE in Expt 2 had been produced commercially and the precise treatment after harvest was unknown. They were presprouted for about one week.

Experimental design. The experiments were laid out in blocks with four replications and six treatments (five minituber classes plus conventional tubers) in Expt 1, a split-plot design of four blocks with three cultivars assigned to main plots and six mother tuber sizes (five minituber classes plus conventional tubers) in Expt 2, and five blocks and five minituber classes in Expt 3. Plots comprised 56 planting positions (4 rows x 14 tubers) in Expts 1 and 2, and 39 planting positions (3 rows x 13 tubers) in Expt 3. Weights and numbers of tubers were assessed from the inner 16 planting positions (2 rows x 8 tubers) in Expt 1, 20 positions (2 rows x 10 tubers) in Expt 2, and 9 positions (1 row x 9 tubers) in Expt 3.

Field practice and temperatures. Tubers were planted by hand on May 2 1989 (Expt 1), April 26 1990 (Expt 2) and May 10 1994 (Expt 3) in a light sandy soil in Achterberg, the Netherlands. They were spaced 20 cm apart in ridges of 75 cm (66,667 tubers per ha). Progeny tubers were harvested 79, 82 or 84 DAP (days after planting) in Expts 1, 2 and 3 respectively. Mean soil temperatures at 5 cm during 2 weeks after planting were 16.0, 18.3 and 15.6 °C respectively, mean air temperatures at 10 cm during the rest of the growing period were 16.8, 15.6 and 17.5 °C (data from a nearby station). Fertilizer was broadcast before or immediately after planting at 80, 70 and 125 kg N/ha, 50, 48 and 150 kg P_2O_5/ha and 100, 104 and 230 kg K_2O/ha respectively. Details on field practice of Expts 1 and 2 were described by Lommen & Struik (1994).

Yield assessment. Progeny tubers were harvested by hand without prior haulm killing. In Expt 3, every plant was harvested and processed separately. Fresh yield and number of tubers > 20 mm were determined after passing the tubers over a square mesh hand grader.

Multiplication factors. To calculate multiplication factors per planted tuber, the number and weight of progeny tubers per plant were corrected for the proportion of the planted tubers that actually had resulted in a plant at harvest. To calculate multiplication factors per planted tuber weight, these values were divided by the mid-point of the appropriate minituber weight class. Because the weight of the conventional tubers was unknown, no accurate multiplication factors based on fresh weight could be calculated, but to allow rough comparisons between minitubers and conventional seed tubers, estimated values were calculated from the mean number and weight of progeny tubers per planted seed tuber and an estimated weight per conventional seed tuber of 16 g in Expt 1 and 25 g in Expt 2.

Estimating the variation in progeny tuber yield. As a measure of variation in progeny tuber yield, the coefficients of variation for number and fresh weights of progeny tubers were estimated for the different minituber weight classes and conventional tubers.

Statistical analysis. Analysis of variance was carried out on the data of the minituber weight classes and significances are presented in Tables 1 and 2. If in Expt 2 interaction between class and cultivar was significant ($P < 0.05$), mean squares of the weight class effects were tested against interaction mean squares by an F-test and the results of the F-test are presented as a second indication of significance.

To determine whether conventional tubers differed from all minituber classes, all data were subjected to analyses of variance. An LSD-test was performed to determine whether the smallest difference between a minituber class and conventional tubers was still significant. The significances presented in Tables 1 and 2 refer to the probability level at which the LSD value indicated significance. In case of interaction, a similar procedure was performed as described above.

Coefficients of variation were calculated for minituber classes from plot or year means. Whether these coefficients decreased with increasing weight class of the minitubers was tested by linear

regression against the logarithm of mid-points of classes. In Expt 3 coefficients of variation were also calculated over plants and planting positions within plots. These coefficients were subjected to analysis of variance, and their means were tested as described above.

Results

Although results are presented for progeny tubers larger than 20 mm, similar trends were observed if also smaller tubers were taken into account (> 0 mm in Expts 1 and 3 and > 10 mm in Expt 2).

Yield characteristics per plant. Not all of the tubers which were planted, especially not those of the lighter minitubers, produced emerged leafy shoots or plants that survived until harvest (Table 1). Of the plants actually present at harvest, the number of progeny tubers per plant increased with the weight of the minitubers planted, although differences between the higher minituber classes were negligible (Table 1). The weight of progeny tubers produced per plant increased more clearly and consistently with the mother tuber weight (Table 1) and consequently the average weight per progeny tuber increased when plants originated from heavier minitubers (Table 1). Effects were consistent over cultivars in 1990 and over years for cv. Bintje, although the magnitude of the effects could differ.

Plants from conventional seed tubers produced more progeny tubers and a higher tuber weight than plants from minitubers (Table 1), partly because they had higher numbers of stems than plants from minitubers, which on average produced only one main stem (cf. Lommen & Struik, 1994).

Multiplication factors. The lighter minitubers produced fewer progeny tubers and a lower tuber yield per planted tuber (Table 2), but differences in number of progeny tubers among the higher weight classes were small. When calculated per fresh weight planted, the lighter minitubers produced more progeny tubers and a higher progeny tuber weight than heavier tubers (Table 2).

As expected, conventional tubers produced more progeny tubers and a higher progeny tuber yield than minitubers if multiplication factors were calculated per planted tuber (Table 2), but the estimated multiplication factors based on fresh weight planted were much lower.

Effects of minituber weight on the variation in yield over plants within a plot. In Expt 3, coefficients of variation in progeny tuber yield (number and weight) were calculated over individuals plants. Both for tuber number and for tuber weight, the coefficients of variation were much higher in crops originating from the lighter minitubers (Table 3). Because not all tubers planted in weight classes I and II had resulted in a plant surviving until harvest (Table 1), coefficients of variation were also calculated per planting position. These coefficients of variation were slightly higher.

Effect of minituber weight and tuber type on the variation in progeny tuber yield over plots. Within one year, differences in weight of progeny tubers between plots of one tuber class could be large. Averaged over all minituber treatments, the highest yielding plots produced 2.85 times higher

Table 1. Percentage of surviving plants and yield characteristics of progeny tubers > 20 mm from minitubers in five weight classes and conventional seed tubers of three cultivars.

	Percentage plants at harvest[a]					Number of progeny tubers per plant					Progeny tuber weight per plant (g fresh)					Average weight per progeny tuber (g fresh)[b]				
	Expt 1	Expt 2			Expt 3	Expt 1	Expt 2			Expt 3	Expt 1	Expt 2			Expt 3	Expt 1	Expt 2			Expt 3
	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Bintje	Ostara	Elkana	Bintje
Minituber classes																				
0.13 - 0.24 g	86	93	91	95	93	4.6	5.0	2.3	2.6	6.4	100	134	123	79	125	21.2	26.3	39.5	27.0	19.3
0.25 - 0.49 g	92	99	91	99	98	7.3	4.4	2.8	2.3	9.5	202	130	189	70	171	27.6	27.1	56.2	27.1	18.0
0.50 - 0.99 g	95	100	98	99	100	8.6	6.4	3.3	3.4	9.6	269	197	226	126	211	31.4	29.2	56.7	32.3	22.2
1.00 - 1.99 g	89	100	100	99	100	8.9	6.1	3.4	3.5	10.8	302	189	238	143	268	34.4	28.7	61.2	40.1	25.5
2.00 - 3.99 g	80	100	99	100	100	9.3	6.1	3.8	3.5	10.1	371	212	319	185	313	43.6	32.0	77.5	47.3	31.1
Significance[c]	ns	---	***	---	*	**	---	***	---	**	***	---	***	---	***	***	---	***/*	---	***
Conventional																				
seed tubers	100	100	99	100		13.3	9.2	7.3	5.8		560	374	559	372		31.2	38.3	74.0	62.1	
Significance[d]	ns	---	ns	---		***	---	***	---		***	---	*	---		ns	---	ns	---	

[a] Percentage of the tubers planted that actually produced a plant that survived till harvest; results of Expts 1 and 2 from Lommen & Struik (1994).
[b] Derived from the total weight and number per plot.
[c] Differences between minituber classes: *** = $P < 0.001$, ** = $0.001 \leq P < 0.01$, * = $0.01 \leq P < 0.05$, ns = not significant: $P \geq 0.05$.
[d] Differences between conventional seed and all minituber classes; for symbols see above.

Table 2. Multiplication factors based on number and weight of planted minitubers in crops from minitubers of five weight classes and some additional data for conventional tubers of three cultivars. Only progeny tubers > 20 mm were considered.

	Number of progeny tubers per planted tuber (nr/nr)					Progeny tuber weight per planted tuber (g fresh/nr)					Number of progeny tubers per weight planted (nr/g fresh)					Progeny tuber weight per weight planted (g fresh/g fresh)				
	Expt 1	Expt 2			Expt 3	Expt 1	Expt 2			Expt 3	Expt 1	Expt 2			Expt 3	Expt 1	Expt 2			Expt 3
	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Bintje	Ostara	Elkana	Bintje	Bintje	Bintje	Ostara	Elkana	Bintje
Minituber classes																				
0.13 - 0.24 g	3.9	4.6	2.1	2.5	6.0	86	124	112	75	118	16.7	24.5	11.3	13.1	32.0	456	662	598	401	628
0.25 - 0.49 g	6.7	4.4	2.7	2.3	9.2	185	128	183	70	166	14.4	11.7	7.2	6.0	24.6	493	341	489	186	442
0.50 - 0.99 g	8.2	6.4	3.3	3.4	9.6	256	196	223	125	211	8.8	8.5	4.3	4.5	12.8	341	262	297	167	281
1.00 - 1.99 g	7.8	6.1	3.4	3.5	10.8	268	189	238	141	268	4.2	4.1	2.3	2.3	7.2	179	126	159	94	179
2.00 - 3.99 g	7.4	6.1	3.8	3.5	10.1	295	212	315	185	313	2.2	2.0	1.3	1.2	3.4	97	71	105	62	104
Significance[a]	**	-------	***	---------	**	***	--------	***	--------	***	***	-------	***/***	------	***	***	---------	***	--------	***
Conventional seed tubers	13.3	9.2	7.2	5.8		560	374	553	372		0.8[b]	0.4[b]	0.3[b]	0.2[b]		35[b]	15[b]	22[b]	15[b]	
Significance[c]	***	-------	***	--------		***	---------	***	--------											

[a] Differences between minituber classes, for symbols see Table 1.
[b] Estimated values, see Materials and methods section.
[c] Differences between conventional seed and all minituber classes, for symbols, see Table 1.

Table 3. Coefficients of variation (%) for number and weight of progeny tubers > 20 mm of cv. Bintje at different area and time scales, in five minituber weight classes and conventional tubers.

	Over plants	Over plots within a year			Over years
	Expt 3	Expt 1	Expt 2	Expt 3	Expts 1-3
Coefficients of variation on number of progeny tubers					
Minituber classes					
0.13 - 0.24 g	75 (82)[a]	17	23	34	22
0.25 - 0.49 g	45 (48)	6	16	23	35
0.50 - 0.99 g	44	19	33	17	20
1.00 - 1.99 g	36	7	38	14	28
2.00 - 3.99 g	33	11	25	11	26
Decrease with class[b]	* (*)	ns	ns	**	ns
Conventional tubers		8	20		
Coefficients of variation on weight of progeny tubers					
Minitubers					
0.13 - 0.24 g	87 (94)[a]	14	28	40	19
0.25 - 0.49 g	58 (61)	21	23	22	18
0.50 - 0.99 g	62	12	30	14	15
1.00 - 1.99 g	43	14	33	20	19
2.00 - 3.99 g	32	21	19	11	20
Decrease with class[b]	** (**)	ns	ns	*	ns
Conventional tubers		3	10		

[a] Between brackets: coefficient of variation over planting positions.
[b] Significance tested by linear regression of means against the logarithm of mid-points of classes; for symbols see Table 1. Analysis of variance carried out on coefficients of variation over plants and planting positions showed a highly significant effect of minituber class ($P < 0.001$).

weights of progeny tubers than the lowest yielding plots. However, consistently in all cultivars and all seasons, yield in both the highest and lowest yielding plots of one weight class were higher, when the planted tubers were heavier. The highest yielding plots from lighter minitubers usually produced a higher progeny tuber weight than the lowest yielding plots from next heavier minitubers.

The coefficients of variation over plots for number and weight of progeny tubers decreased significantly with increasing weight class in cv. Bintje in Expt 3 (Table 3) and in the early cultivar Ostara in Expt 2 (not presented). In the other cases, the coefficient of variation was very variable and no decrease with increasing weight class was significant. For conventional tubers, the coefficients of variation over plots for progeny tuber number were comparable with those of minitubers (Table 3), but the coefficients of variation for tuber weight were much lower than for minitubers (Table 3).

Effects of minituber weight on the variation in progeny tuber yield over years. For cv. Bintje, yield data from the same tuber classes were available from three years (Table 2) and coefficients of

variation for progeny tuber yield over years were calculated from the yearly tuber yields per weight class. Coefficients of variation for tuber number varied between weight classes, and no decrease with increasing minituber weight could be detected (Table 3). For progeny tuber weight, coefficients of variation varied little between weight classes (Table 3), indicating that relative differences in tuber yield over years were not higher for crops from lighter minitubers.

Discussion

Multiplication factors. The relative differences in multiplication factors between classes were calculated from Table 2, assuming class I and V to be 100 % for multiplication factors based respectively on the tuber weight an tuber number planted. Means of these multiplication factors over cultivars and years are presented in Fig. 1. One minituber from the lowest weight class had around half the multiplication factor of one minituber from the highest weight class, but when equal weights were compared multiplication factors of the lowest class were seven times higher (Fig. 1). Multiplication factors per planted tuber were lower for the lighter minitubers because fewer plants survived until harvest (Table 1, Expts 2 and 3 only) and a lower number and weight of progeny tubers were produced per plant (Table 1) although there were no differences in number among the higher weight classes (III - V). The lower progeny weight from lighter tubers was caused by a lower

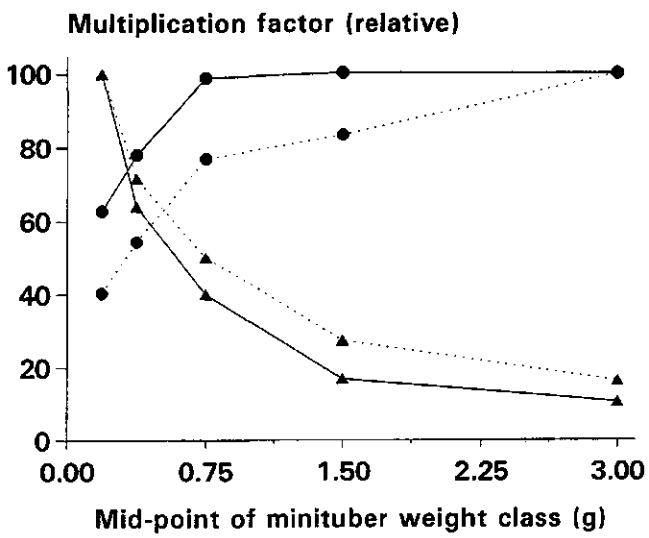

Fig. 1. Relationships between the weight per mother tuber and relative multiplication factors of minitubers from five weight classes. Mean percentages of all cultivars and experiments. ●——● : number of progeny tubers > 20 mm produced per number of mother tubers planted (class V = 100%); ●---● : weight of progeny tubers > 20 mm per number planted (class V = 100 %); ▲——▲ number of progeny tubers > 20 mm per weight planted (class I = 100 %); ▲---▲ weight of progeny tubers > 20 mm per weight planted (class I = 100 %).

light interception by their canopy (cf. Lommen & Struik, 1994), but the lower tuber number is difficult to explain. Partly, the number of progeny tubers was lower because relatively more progeny tubers had not grown to the minimum size of 20 mm. However, also the total number of tubers per plant was lower for the lighter minitubers (not shown). The number of main stems produced by plants from minitubers is unlikely to explain the difference because usually one stem was produced per plant in all weight classes (cf. Lommen & Struik, 1994).

Because progeny tubers from lighter minitubers also had lower average weights (Table 1), they are likely to produce fewer stems per progeny tuber (e.g. Reestman & De Wit, 1959; Allen et al., 1992). Consequently, the potential number of stems to be produced by all progeny tubers originating from one minituber will increase with increasing weight of the mother tubers, even for the higher weight classes (III - V) where the number of tubers produced per planted tuber was similar. Consequently, the replantable area will be larger.

Conventional tubers had higher multiplication factors per planted tuber than all minitubers because they produced higher progeny tuber weight and more tubers per plant. The higher weight will be related to the higher radiation interception of plants from conventional tubers (cf. Marshal & Taylor, 1990; Lommen & Struik, 1994), and the higher number of tubers mainly to their higher number of stems.

Variation in tuber yield. Coefficients of variation are lower when absolute yield differences are smaller or when the absolute variation (standard deviation) is smaller or when the average yield is higher. Lower average yields per plant or unit area (Tables 1 and 2) contributed to the higher coefficients of variation over individual plants or planting positions in stands from lighter minitubers (Table 3), but for number of progeny tubers also the absolute variation over plants and planting positions tended to be higher for lighter minitubers (not shown).

Coefficients of variation over plots were high in Expt 2 because the experiment was carried out in a field with large differences in soil conditions and yields generally were low. The coefficients of variation over plots were higher for plots planted with the lighter tubers in Expt 3 (Table 3), due to lower average numbers and weights and for numbers also higher absolute differences. In Expts 1 and 2, the low number of replicated plots (4) may have hindered detection of an effect of the weight class, whereas in Expt 3 also variation over plants or planting positions still may have contributed to the high variation over plots from the lighter tubers. The high coefficients of variation over plots suggest that differences in growing conditions or crop husbandry may have a large effect on the uniformity of a crop from minitubers.

No effects of the weight class on the variation in yield over years were obvious for cultivar Bintje (Table 3), suggesting that the yield of crops from lighter tubers is as stable as that of crops from heavier minitubers. The mother tubers in the experiments, however, were handled carefully and even for the lightest tubers plant survival was fairly good in all years (Table 1). It cannot be excluded that if tubers are handled less carefully a greater proportion of the lightest tubers may fail to produce plants in some years.

The lower coefficients of variation for tuber yield over plots with conventional tubers compared

to minitubers (Table 3) were mainly caused by higher tuber yields for conventional tubers.

Practical implications. If the choice for a production method for minitubers is based on multiplication factors only, a method producing high numbers of tubers appears preferable to a method producing low numbers of larger tubers even though the minitubers may be smaller. This is justified by the higher multiplication factors for the lighter minitubers when based on tuber weight planted (Fig. 1). If also progeny tubers below 20 mm would have been taken into account, multiplication factors for number produced per weight planted would have been even much more favourable for the lighter minitubers (not shown). Because the variation in progeny yield over years was not affected by the minituber weight (Table 3) there was no reason to expect a less stable yield from crops of lighter tubers provided the light tubers and their plants were handled properly.

In this paper all weight classes of minitubers were compared at the same planting density. Consequently, when a similar weight of lighter and heavier tubers was planted, the planting area needed would be larger for the lighter tubers. Multiplication factors of lighter minitubers would have been relatively lower if a similar seed tuber weight would have been planted per unit area for all tuber classes. Still, doubling the planting density in a crop from the lightest minitubers from 66,667 to 133,333 tubers per ha was not thought to affect the progeny tuber weight per plant greatly (Lommen & Struik, 1994). Thus, two of the lightest tubers (total weight of seed tubers 0.375 g) may produce almost the same progeny tuber weight as one of the heaviest minitubers (total weight 8 times higher) when planted on the same area, whereas more progeny tubers might be produced because of more stems. Consequently, a production strategy for minitubers may accept a reduction in total weight of the tubers produced if more minitubers are produced. Very light minitubers nevertheless still have two major drawbacks. Firstly, minitubers with weights below 0.50 g were more difficult to store than larger minitubers (Lommen, 1993) and some losses of tubers may occur during storage. Secondly, the variation in progeny tuber yield over plants in a crop from light tubers was higher (Table 3) and most probably also the variation in haulm development. This may lower the quality of seed tubers because an irregular crop is more difficult to rogue and may attract more aphids.

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CHAPTER 11

General discussion

11 GENERAL DISCUSSION

Before conventionally sized seed tubers can be harvested from minitubers, four main phases have to be completed: (1) the *in vitro* phase in which *in vitro* potato plantlets are produced and multiplied from explants, (2) the minituber production phase in which minitubers are produced in the glasshouse on these *in vitro* produced plantlets, (3) the storage phase comprising the period from harvest in the glasshouse until planting of the minitubers into the field, and (4) the field phase in which crops are grown from minitubers yielding conventionally sized seed tubers. Experimental results considering phases 2 - 4 and their practical implications have been reported and discussed in chapters 2 - 10, which have been published by Lommen & Struik (1992a, b, c), Lommen (1993a, b), Lommen & Struik (1993a), Lommen (1994) and Lommen & Struik (1994, 199x), respectively. This general discussion first concentrates on how the processes of plant growth and tuber formation in the minituber production phase could be affected by the *in vitro* plantlets used, and by the climatic conditions and repeated harvesting technique employed during this phase. The second part of the discussion explores how the processes determining the performance of the tubers are affected by the storage period and the weight of the mother tubers, and which possibilities there are to improve the field performance of the minitubers. In the last part, the possibilities of incorporating a step of minituber production into a seed production programme are assessed. For the reader's convenience, some brief repetitions of methodology and results are included.

11.1 The production of potato minitubers

The production method for minitubers developed and studied by Lommen & Struik (1992a, b, c) basically involved planting of *in vitro* propagated plantlets in a glasshouse under tuber inducing conditions and removing tubers by repeated harvesting. With this method it was possible to produce around 3500 minitubers $\geq$ 5 mm per m^2 when 350 plants per m^2 were planted and tubers were harvested after 4, 7 and 10 weeks (Lommen & Struik, 1992c).

The success of this method may have been determined by the following three factors: (1) the state of the *in vitro* propagated plantlets when they were planted in the glasshouse, (2) the climatic conditions during minituber production in the glasshouse and (3) the repeated harvesting and the timing of the consecutive harvests. How these three factors may have affected the growth of the plants and the formation of tubers is discussed below.

11.1.1 Possible effects of the status of the *in vitro* propagated plantlets

The *in vitro* plantlets used for the production of minitubers were routinely multiplied *in vitro* by single-node stem cuttings every few weeks, and grew at 23 °C and a 16 h photoperiod (fluorescent light, Philips TL-33, approximately 8 W m^{-2} total irradiance) on a standard solid nutrient medium containing M&S mineral salts and vitamins (Murashige & Skoog, 1962) supplemented with 2.0 mg l^{-1} glycine, 8.0 g l^{-1} agar and 25.0 g l^{-1} sucrose. To the last medium before transfer to the

glasshouse also 0.01 g l^{-1} alar-85 % (active ingredient: daminozide, N-dimethylaminosuccinamic acid) was added. The *in vitro* phase was not studied here because procedures had already been optimized (Marinus, 1985), the plantlets produced had been successfully used for tuber production (Mastenbroek & Eising, 1987) and the status of the plantlets proved not to be critical for the production of minitubers. However, the environmental conditions imposed and the composition of the growing medium in the *in vitro* phase may have had a marked effect on the plant growth and tuber formation after planting into the glasshouse.

Effects on plant growth. The procedure resulted in approximately 90 % rooted plantlets (not reported) which were planted between 8 and 18 days after the last subculturing (Lommen & Struik, 1992a, b, c). Survival after planting into the glasshouse was virtually 100 % (not shown), possibly because daminozide was added to the normalization medium before planting. Daminozide addition results in darker, shorter and more vigorous plants (Marinus, 1985; Sipos et al., 1988; Lommen, 1990) with shorter roots but equal root mass, and increases the percentage survival (Sipos et al., 1988). Growth after planting was satisfactory: Leaf Area Indices of more than 5 m^2 m^{-2} were attained within 4 weeks at 200 plants per m^2 (Lommen & Struik, 1992c), whereas growth rates by that time could be 20 g m^{-2} d^{-1} (Lommen & Struik, 1992a; 350 plants per m^2). Comparisons over experiments suggest that the performance of older plantlets (13 or 17 days after last subculture, Lommen & Struik, 1992c) is slightly better than of younger plantlets (9 days after last subculture). This can probably be attributed to a larger leaf area at planting and consequently a higher initial growth rate and larger leaf area at tuber initiation and/or to a higher root:shoot ratio.

Effects on tuber formation. The *in vitro* plantlets depend solely on external conditions and the nutrient medium to produce or receive the stimulus (or stimuli) which trigger(s) tuber initiation. In plants from tubers, also the mother tuber may contribute (e.g. Madec & Perennec, 1959) - or counteract, depending on the conditions. It is unlikely that during routine multiplication *in vitro* the plantlets become strongly induced to tuberize, because the external conditions and the composition of the medium *in vitro* are not likely to stimulate the formation of tubers. The fairly high temperature (23 °C) and relatively long day length (16 h) are not enhancing tuber initiation in vivo (cf. Bodlaender, 1963) and also *in vitro* favour the development of a leafy shoot. At shorter day lengths *in vitro* (8 h compared to 16 or 24 h) or lower temperatures (15 °C compared to 20 or 25 °C) the plantlets may have a more stoloniferous habit (Hussey & Stacey, 1981). The medium used for routine multiplication of nodal cuttings did not contain additions that generally stimulate the formation of tubers *in vitro*, such as an elevated sucrose level (Garner & Blake, 1989), cytokinins (Wang & Hu, 1982; Hussey & Stacey, 1984), or the growth retardants CCC (e.g. Hussey & Stacey, 1984) or coumarin (e.g. Stallknecht & Farnsworth, 1982). Probably the addition of the growth retardant daminozide to the normalization medium before planting could have stimulated tuber formation slightly, because in the field, alar is known to increase the proportion of dry matter allocated to the tubers (Bodlaender & Algra, 1966) and the number of tubers (Humphries & Dyson, 1967).

In vitro tubers were not observed except when plants were left to grow undisturbed for several months under the same conditions. The buds in the leaf axils of the nodal cuttings also consistently developed as leafy shoots. If single node cuttings are taken from glasshouse or field grown plants, shoot development from the buds of the cuttings indicates that the mother plants were not or barely induced to tuberize, otherwise the buds would develop into stolons of tubers, depending on the degree of induction of the mother plants (Ewing, 1985). Exposure to light, however, may have prevented the buds of *in vitro* cuttings to develop into tubers, because light delays the initiation of tubers *in vitro*, especially if the medium contains additions that stimulate the formation of tubers (cf. Slimmon et al., 1989; Pelacho & Mingo-Castel, 1991; Nowak & Asiedu, 1992).

11.1.2 Possible effects of the conditions in the glasshouse

Minitubers were produced throughout the year in glasshouses at day temperatures set at 18 °C, night temperatures at 12 °C, a fixed photoperiod of 12 h, and with natural light supplemented to at least 80 W m^{-2} (total irradiance) with high-pressure sodium lamps (Philips SON-T). These conditions were chosen because cool temperatures, long nights and a high light intensity would stimulate the induction of tubers (e.g. Bodlaender, 1963), whereas sufficient growth would still be possible. Tuber inducing conditions not only shorten the time until tuber initiation, they also affect overall plant development. Ewing and Struik (1992) recently reviewed possible effects: larger and thinner leaves, shorter stem internodes, a higher ratio of leaf to stem dry weight, a wider angle between leaves and stem, a higher rate of photosynthesis per unit of leaf dry weight, suppression of axillary and sympodial branching, lower root dry weights, replacement of stolon growth by tuber growth, a higher proportion of dry matter partitioned to the tubers and an earlier plant senescence. If plants become induced too strongly, their yield may remain low (Ewing, 1985), because they may initiate tubers before sufficient haulm has developed to allow high growth rates, whereas further haulm growth is limited. Partitioning of dry matter to the tubers may cause early haulm senescence. The conditions chosen for the production of minitubers, however, allowed sufficient growth (see 11.1.1) even though tubers were initiated already 2 weeks after planting the *in vitro* plantlets to the glasshouse (Lommen & Struik, 1992a). Regular fertilization with a complete, low dosed nutrient solution delayed haulm senescence and probably thereby increased minituber yield (Lommen & Struik, 1992c). Because tuber inducing conditions also result in short stem internodes, the plants were robust and easy to handle. The additional illumination allowed production of minitubers in periods with low natural light intensity (winter time). In summer, the short photoperiod may have reduced negative effects of an unavoidable increase in temperature (cf. Wolf et al., 1990), whereas the high light intensity allowed high growth rates.

11.1.3 Effects of the non-destructive harvests

The non-destructive harvests involve three actions that may explain the higher number of tubers and the lower total tuber weight compared to plants left undisturbed (Lommen & Struik, 1992a, b). These

actions are (1) the root damage that occurred when the plantlets were lifted from the soil mixture, (2) the removal of all tubers larger than a minimum size, and (3) the deeper replanting of the plants. An attempt will now be made to explain the effects of the non-destructive harvests on plant growth and on the process of tuber formation.

Effects on plant growth. The non-destructive harvests reduced overall growth rates compared to plants left undisturbed (Lommen & Struik, 1992a, b). This effect should be attributed mainly to the damage of roots. Root pruning is long known to reduce the weight of the upper plant parts and tubers (Moore, 1937) and simulating the non-destructive harvest by damaging roots and replanting deeper without removing tubers reduces the final tuber weight (Lommen & Struik, 1993b). Root damage sometimes caused wilting in plants for minitubers production, but they always recovered within two days. This drought stress can reduce total photosynthesis by lowering the leaf expansion (cf. Munns & Pearson, 1974) or the photosynthesis per unit area of leaf (cf. Moorby et al., 1975; Vos & Oyarzún, 1987). A significant reduction of leaf growth rates occurred when young and expanding leaves were present, i.e. when the non-destructive harvests took place after 3 or 4 weeks (Lommen & Struik, 1992a). Removal of tubers (Burt, 1964; Moll, 1986) and removal of tubers plus stolons (Nösberger & Humphries, 1965) are also reported to reduce overall growth rates, net assimilation rates or tuber yields, most probably because of removal of sinks. However, the removal of tubers of different sizes in non-destructive harvests (leaving different-sized sinks on the plant) had no effect on the total weight of minitubers produced (Lommen & Struik, 1993b). In addition, Tibbits et al. (1994) observed only a minor reduction in tuber yield when tubers were removed regularly in a nutrient film technique system in which root damage was limited. Also the replanting depth after a non-destructive harvest had no significant effect on the total weight of tubers (Lommen & Struik, 1993b). Consequently, under the conditions studied, the non-destructive harvests may have reduced overall plant growth mainly because of the root damage occurring at the non-destructive harvests.

Effects on tuber formation. Tuber initiation occurred within two weeks after planting and slowed down 4 weeks after planting, when part of the tubers was growing rapidly and leaf weight had about reached its maximum level (Lommen & Struik, 1992a). This slow-down in tuber initiation should be attributed to a lack of tuber sites that are not subjected to the dominance of the rapidly growing tubers.

If the one or two most advanced tubers were removed in a non-destructive harvest around 4 weeks after planting, many new tubers were initiated, because new possible tuber sites became available below ground that were not subjected to the dominance of rapidly growing tubers. Both the removal of the dominant tubers and the deeper replanting contributed to this, as shown by Lommen & Struik (1993b). Extensive initiation of new tubers was also observed by Oparka (1987) after removal of stolon apices and by Nösberger & Humphries (1965) after removal of tubers plus stolons. The new tubers were initiated on new stolons, existing stolons and directly on the stem (Lommen & Struik, 1992a). The non-destructive harvest increased the number of stolons (Lommen & Struik, 1992a) probably mainly because of the deeper replanting, and thus the increase in number of nodes being

exposed to stolon inducing conditions (cf. Svensson, 1962; Kumar & Wareing, 1972). The proportion of sessile tubers (produced on the stem) was probably high (increasing from 30 % to 57 %, 3 weeks after non-destructive harvests that took place 4 to 7 weeks after planting; Lommen & Struik, 1992a) because of a limited number of possible tuber sites on the stolons, which generally remained short due to tuber inducing conditions in the glasshouse (Lommen & Struik, 1992a).

Not all of the newly initiated tubers were able to pass all phases of tuber formation. Only a part of them grew to a harvestable size in the three weeks after a non-destructive harvest (Lommen & Struik, 1992b) and delaying the next harvest generally did not increase this number (Lommen & Struik, 1992b), whereas many of the undersized tubers were resorbed. Similar results are reported for plants under field conditions, where considerable resorption of tubers is often observed after treatments causing initiation of many tubers, like a high moisture level (Krug and Wiese, 1972), temperatures favouring early stem and haulm development (Cho & Iritani, 1983) or the removal of stolon apices (Oparka 1987), if they are followed by normal plant senescence. Generally, the number of tubers $\geq$ 0.3 g did not decrease during tuber resorption (Lommen & Struik, 1992b) and it seems plausible that a weight of 0.3 g was large enough to become a competitive minituber.

The most advanced tubers again could be removed in a second non-destructive harvest 3 weeks after the first non-destructive harvest. Now, the smaller tubers that otherwise would have been resorbed or had not yet reached the final phase in tuber formation, were able to grow further. After this second non-destructive harvest, the initiation of new tubers probably could be restricted by the limited number of possible tuber sites or be suppressed by dominant tubers. Many sites had already been occupied by removed tubers, and plants were often senescing when no additional fertilization was supplied. Without proper fertilization, tuber initiation may have been limited by the availability of mineral nutrients. Also Nösberger and Humphries (1965) concluded that after removal of tubers plus stolons more meristems start to grow when the supply of N permits so. In addition, tubers that remained on the plant after the second harvest may have become dominant (probably within 4 days, cf. Marschner et al., 1984) before new tubers were initiated.

The timing of the harvests strongly influenced the number of tubers initiated (Lommen & Struik, 1992a, b). At very early non-destructive harvests (3 weeks after planting), the number and weight of tubers that can be removed is low, the replanting cannot be deep and probably the plants are less strongly induced to tuberize. The number of new tubers was highest after intermediate first harvests (4 - 6 weeks) and again decreased thereafter (Lommen & Struik, 1992a). Postponing the first harvest in order to yield larger tubers does not increase the number of tubers in this harvest, and reduces the numbers of tubers in later harvests, because plants become senescent. Postponing the second harvest also does not increase the total number of larger tubers, whereas smaller tubers are likely to become resorbed.

11.2 The performance of potato minitubers

The performance of the minitubers during storage and after planting in the field was affected by external factors after harvest, such as temperature during storage (Lommen, 1993a), the total length

of the storage period (Lommen, 1993b; Lommen & Struik, 1993a), the sprout length to which tubers were presprouted (Lommen, 1994), the planting depth (Lommen, 1994) and the experimental year (Lommen & Struik, 1994, 199x), and by intrinsic factors, such as the harvest from which the tubers originated (Lommen, 1993a, b; Lommen & Struik, 1993a), the cultivar (Lommen, 1993a, b; Lommen & Struik, 1993a, 1994, 199x) and the weight of the tubers (Lommen, 1993a, b, 1994; Lommen & Struik, 1994, 199x). Especially the length of the storage period and the weight of the minitubers had an enormous influence on their performance. In this section I will analyse how these two factors affect the performance of the minitubers, and how the field performance of minitubers can be improved.

11.2.1 Effects of the length of the storage period

The period between harvest and planting of minitubers can be subdivided into:
(1) the curing period in which freshly harvested minitubers are left undisturbed at a high temperature and high humidity (around 2 weeks);
(2) the main or cold storage period in which minitubers are stored for a longer period (several months up to more than one year);
(3) the presprouting period in which minitubers are placed at a higher temperature to enhance the growth or elongation of the sprouts (a few weeks or months).
The conditions during these periods and the length of the periods may affect (1) the processes of periderm formation and suberization, water loss and respiration, associated with the storability of the tubers and the amount of reserves available for growth, and (2) the processes of dormancy, physiological ageing and sprout growth, which affect the growth and development of plants from the tubers.

Processes taking place during storage. Immediately after harvest, minitubers are sensitive to weight losses as are other small, immature tubers. Lommen (1993b) reported fresh weight losses of 5.7 % in 2 days for minitubers, Burton (1973) of more than 1 % per hour for small immature tubers. Weight losses may occur through evaporation (water loss) and respiration (dry-matter loss) which both are likely to be higher in minitubers than in conventional seed tubers. Evaporation of unsprouted tubers is proportional to the surface area and inversely related to the resistance of the tuber periderm. Therefore, evaporative losses in minitubers could be high because of a high surface area:volume ratio and low resistance of the periderm (low degree of suberization, more or more permeable lenticels, and more wounding at harvest). Respirative losses in minitubers may be high because immature small tubers have relatively high respiration rates after harvest (Burton, 1964).

The high temperature and high relative humidity during the curing period (18 °C, 80 % r.h.) enhance periderm formation and suberization (cf. Wigginton, 1974), and consequently increase the resistance to water losses (cf. Wilcockson et al., 1985). On the other hand, the relatively high temperature may result in high respirative weight losses and consequently will also reduce the quantity of substrate available for maintenance and growth. The rate of weight loss during the

following cold storage tended to decrease slightly with time, but tended to increase when sprouts became visible. This could be caused by increased evaporation through the sprout or by a higher respiration (Burton, 1974). Losses in tuber number (i.e. deterioration of tubers) also occurred mainly in the period after the onset of sprouting (Lommen, 1993b).

When minitubers were maintained at a higher temperature after the curing period (no cold storage between the curing and sprouting period), 50 % of the tubers had produced an apical sprout of 2 mm after four to five months (Lommen, 1993a). This period from harvest until 50 % of the tubers had a sprout of 2 mm, is referred to as the dormant period, but in fact it consists of the period after harvest until the actual date of onset of sprout growth and the period of sprout growth up to 2 mm. Inserting a period of cold storage (0 - 84 days) between the curing period and the presprouting period reduced the length of this presprouting period at a higher temperature, necessary to produce an apical sprout of 2 mm on 50 % of the tubers (Lommen, 1993a). This has to be attributed to an earlier end of the actual dormant period and/or to an increased rate of sprout growth (cf. Wurr & Allen, 1976). Therefore, a cold-storage period of 6 weeks shortened the total period necessary after harvest to produce sprouts of 2 mm on 50 % of the tubers most (Lommen, 1993a).

A longer cold storage period in the range of 0 - 555 days considerably affected the performance of plants from minitubers (1 - 2 g, Lommen & Struik, 1993a) under controlled conditions in a similar way as an increasing physiological age (brought about by longer storage or storage at higher temperatures) affects the performance of plants from conventionally sized tubers (e.g. Bodlaender & Marinus, 1987). Depending on the cultivar, rate of emergence, number of main stems, total dry weight and tuber fresh weight were highest after 443 days of cold storage and declined thereafter, or were highest after 569 days, the longest period tested (Lommen & Struik, 1993a). Maximum leaf areas and stem lengths were observed following shorter storage (Lommen & Struik, 1993a). Also under field conditions, Struik & Lommen (1990) reported higher stem and tuber numbers in plants from minitubers (1 - 2 g) with a higher physiological age.

A longer presprouting period resulted in longer sprouts, and accelerated the emergence of the tubers after planting (Lommen, 1994).

Modifications by the status of the minitubers at harvest. The status of the minitubers at harvest may modify the processes taking place during storage. Tubers from the first of the three sequential harvests of minitubers had the longest dormant period (Lommen, 1993a), showed higher weight losses during storage (Lommen, 1993b), and their performance appeared to increase less with increasing physiological age or started to increase at a higher physiological age, and probably decreased faster (Lommen & Struik, 1993a). This may be related to a different physiological pattern with age and/or be associated with the amount of carbohydrate reserves in minitubers, which may have been lower in some tubers from the first harvest (Lommen, 1993a). It is not known whether the changing performance with age is affected by the tuber size.

Contribution of the storage period to the differential performance of minitubers and conventional tubers in the field. The storage conditions and duration may have contributed to the differences in

the field performance between minitubers and conventional seed tubers (Lommen & Struik, 1994, 199x). They generally resulted in a lower physiological age and smaller sprouts for minitubers than for conventional tubers. Consequently, they may partly account for the longer time to 25 % or 50 % emergence of minitubers (cf. Sadler, 1961; Firman et al., 1992; Lommen, 1994). The tendency to a less uniform emergence of minitubers (Lommen & Struik, 1994) may have resulted partly from their low number of sprouts (generally one) because of an early start of presprouting. Damage to the sprout at planting or before emergence would require new sprouts or branches to be produced, whereas in conventional seed other sprouts may emerge. A lower physiological age also may have contributed to the slower ground cover after emergence (both directly or through the number of main stems) and a lower harvest index in minitubers.

11.2.2 Effects of weight of the minitubers

Performance before planting. During curing and subsequent cold storage, the lighter tubers showed higher weight losses (Lommen, 1993b) and during long term storage they were more likely to deteriorate (Lommen, 1993b). This must have been caused by higher evaporative weight losses (water losses) and respirative weight losses (dry weight losses). Evaporation in lighter tubers will be higher because of a higher ratio between the surface area and the weight, and probably also because of a lower periderm resistance due to an incomplete (wounding or fewer periderm layers) or less suberized periderm, or relatively more or more permeable lenticels. Also respiration immediately after harvest is highest in small, immature tubers (Burton, 1964).

As commonly observed in conventional seed tubers (cf. Emilsson, 1949; Van Ittersum & Struik, 1992), minitubers showed a dormant period after harvest which was longer for lighter tubers if a sprout length of 2 mm was used as a criterion for establishing the end of dormancy (Lommen, 1993a). For minitubers, a slower rate of initial sprout growth up to 2 mm almost certainly contributed to this effect, because sprouts of tubers from lower weight classes were still growing more slowly between 2 and 4 mm and 4 and 6 mm after removal from cold storage (Lommen, 1994). The slower sprout growth at least partly may have been caused by differences in tuber reserves, because Morris (1966) showed that the rate at which the sprout increases in length was a positive function of tuber weight and could be lower when more sprouts were growing.

Performance after planting. As observed in conventional tubers (Sadler, 1961; Headford, 1962), stems from lighter minitubers emerged later (Lommen, 1994), even if they had sprouts of similar length at planting. This was mainly due to a slower increase in length after planting (Lommen, 1994) and partly to the fact that stems were longer when they emerged (Lommen, 1994). The slower increase in length may be related to the rate at which reserves from the mother tuber become available for stem growth (Morris, 1966). The longer stems at emergence may be caused partly by differences in planting depth but mainly by the fact that stems from lighter tubers often were not straight due to not completely negatively geotropic growth or impedance by the soil.

Because of the thinner stems (Lommen, 1994) plants from lighter tubers may be more prone to

attack by diseases and pests during emergence, which partly may account for the lower final percentage emergence for lighter tubers observed in some field experiments (Lommen & Struik, 1994, 199x). In addition, plants from lighter tubers need a larger proportion of tuber reserves for emergence (Lommen, 1994) and consequently they have fewer reserves from the mother tuber (both absolutely and relatively) available to resume growth after damage by pathogens, pests or night frosts before or soon after emergence. The slower foliar ground cover of crops from lighter tubers (Wiersema & Cabello, 1986; Allen et al., 1992; Lommen & Struik, 1994) can be partly explained by the longer time for all plants to emerge (Lommen, 1994; Lommen & Struik, 1994), the lower final percentage emergence (Lommen & Struik, 1994, 199x) and the higher plant death after emergence (Lommen & Struik, 1994). Also a slower haulm development per plant must have contributed, probably mainly by a slower rate of appearance and shorter final size of leaves on the main stem, as suggested by Lommen (1993c). This may be associated with the lower weight of the root system at emergence in plants from lighter tubers (Lommen, 1994), that had to provide water and nutrients to a much larger shoot in relation to its weight (higher shoot:root ratio; Lommen, 1994). This and the smaller quantity of tuber reserves may lead to lower initial growth rates after emergence. In the physiologically young tubers used in the field experiments, the number of main stems and the number of secondary stems + branches per main stem were not higher in plants from heavier tubers (Lommen & Struik, 1994) and consequently the number or size of individual leaves per main stem may account for the major part of the differences in ground cover observed.

The slower ground cover development soon after emergence in crops from lighter tubers (Lommen & Struik, 1994) lead to a smaller quantity of radiation intercepted during the growing period (Lommen & Struik, 1994) and this directly accounted for the lower dry-matter production of crops from the lighter minitubers (Lommen & Struik, 1994). The radiation conversion coefficient did not differ between fresh weight classes (Marshall & Taylor, 1990; Lommen & Struik, 1994). Also harvest index was lower when the crop originated from lighter minitubers, which may be associated with the date of tuber initiation or the allocation of dry matter to the tubers. Consequently, the total dry matter of tubers in crops from lighter minitubers was lower and - because tuber dry-matter concentration decreased with minituber weight only in one experiment (Lommen & Struik, 1994) - also the fresh yield of progeny tubers.

The number of progeny tubers produced in crops from lighter tubers - all with single sprouts - in the range 0.125 - 4 g was lower because fewer plants survived until harvest (true for some experiments, Lommen & Struik, 199x) and the number of tubers produced per plant was lower (Lommen & Struik, 199x), although there were hardly differences among plants from minitubers of 0.5 g - 4 g. Because progeny tubers from lighter minitubers also have lower average weights (Lommen & Struik, 199x), they will produce fewer stems per tuber (e.g. Reestman & De Wit, 1959; Allen et al., 1992). Consequently, the potential number of stems from the progeny of one minituber will increase with increasing weight of the mother tubers, even in those ranges where the number of progeny tubers produced per planted tuber was similar.

Planting lighter tubers lead to less regular crops: The coefficients of variation in progeny tuber yield and number over individual plants and planting positions were higher in stands from lighter

minitubers (Lommen & Struik, 199x). Partly this must have been caused by the fact that at the moment of emergence the variation in the amount of reserves from the mother tuber is relatively large for lighter mother tubers, as a larger proportion of the reserves is needed for emergence (cf. Lommen, 1994). In addition, all factors contributing to an incomplete or irregular emergence may contribute to the higher variation.

General effects. Over experiments, absolute differences between the highest and lowest weight classes studied were generally larger than those between the most extreme levels of other experimental factors studied simultaneously, e.g. the harvest from which the minitubers originated (Lommen, 1993a, b), the cultivar (Lommen, 1993a, b; Lommen & Struik, 1994, 199x), the length of the sprouts at planting (Lommen, 1994), the planting depth (Lommen, 1994) or the experimental year (Lommen & Struik, 1994, 199x). Effects of tuber weight studied can be classified into 3 categories: (1) effects on the performance of the tubers or plants or crops from these tubers after a fixed time period (losses during storage, progeny tuber number and weight), (2) effects on the time required to reach a certain stage (end of the dormant period, fixed sprout length, emergence, fixed ground cover), and (3) effects on the characteristics of the tubers or plants from the tubers at a certain growth or developmental state (dry-matter concentration of the minitubers at harvest, shoot:root ratios at emergence). The existence of the differences of type 3 implies that the effects of weight on plant growth and development were not merely caused by a delay in growth or development, but also by a differential development of the plants at important stages, e.g. harvest or emergence. Differences in crop development in addition are determined by differences between plants (e.g. uniformity of emergence, percentage emergence).

For almost all characteristics studied, the influence of the weight on the performance generally became smaller when the weight classes became larger (even though they increased logarithmical in most experiments), suggesting that other factors than those related to weight may have become limiting factors for the processes studied.

11.2.3 Improvement of the field performance of light tubers

Some strategies could be employed to increase weight and number of progeny tubers and the uniformity of crops from light tubers grown in a short growing season.

Increasing the weight of progeny tubers. Because the weight of progeny tubers was mainly determined by the radiation intercepted and the harvest index (Lommen & Struik, 1994), methods to increase tuber weight from light mother tubers could concentrate on increasing (a) the period during which radiation is intercepted, (b) the daily amount of radiation intercepted, and (c) the harvest index.

The period during which radiation can be intercepted will be longer when emergence is earlier or harvest is later. Emergence is early when tubers are sufficiently presprouted (Headford, 1962; Lommen, 1994) and/or have a greater (up to a maximum) physiological age (Firman et al., 1992;

Lommen & Struik, 1993a). Also crop husbandry techniques such as floating plastic film, irrigation, adequate fertilization may accelerate emergence, whereas early planting may accelerate the emergence date. The harvest date will generally depend on the number and activity of aphids that may transmit virus (beyond direct control) and the maturity of the crop. Using minitubers of a higher physiological age may give rise to crops that around the date of haulm killing have acquired a higher degree of mature plant resistance (virus is translocated to the tubers less rapidly after infection of the leaf).

The daily amount of radiation intercepted can be increased by increasing the number of emerging and surviving plants and enhancing haulm growth soon after emergence. The number of emerging plants may be increased by using sufficiently old tubers (Lommen & Struik, 1993a) and by crop husbandry techniques that accelerate emergence (see above) and consequently reduce the time that the growing sprouts are prone to attack by diseases and pests, or by crop husbandry techniques that control the attack by diseases or pests during emergence. Curing the tubers after harvest in light or hardening the sprouted tubers in light before planting combined with shallow planting probably may reduce attack by *Rhizoctonia solani* or by wireworms, which preferably do not feed on tissues containing glycoalcaloids (Jonasson & Olsson, 1994). These methods may also improve the survival after emergence. Early haulm growth after emergence may be enhanced by floating plastic film due to higher soil temperatures or retention of water. Haulm growth probably also could be stimulated by crop husbandry techniques which reduce negative effects of the smaller root system of plants from lighter tubers (Lommen, 1994), e.g. irrigation when necessary, adequate fertilization or fertilizer placement. Also encapsulation of tubers (Melching et al., 1993) falls into this category. The daily amount of radiation intercepted per unit area in addition could be increased by a higher planting density. For the lighter tubers, planting 133,333 instead of 66,667 tubers per hectare may not reduce progeny tuber weights per plant appreciably at an early harvest date (Lommen & Struik, 1994).

Harvest index will generally be higher when tubers are initiated earlier or a higher proportion of the dry matter produced is allocated to the tubers. Up to an optimum, it will be higher when using older minitubers (Lommen & Struik, 1993a).

Some of the methods mentioned, however, may have side-effects. Emergence early in the growing season (when soil and air temperatures and photoperiod are still relatively low) leaves a longer period between emergence and harvest (positive), but also may result in relatively early initiation of tubers, which limits haulm development (negative), but increases the harvest index (positive). Floating plastic film and a high nitrogen fertilization, however, may accelerate emergence (positive) and enhance early haulm development (positive) but may lower the harvest index (negative). The actual outcome of the methods to increase progeny tuber weight therefore will depend on the differential effects on the periods between planting and emergence, between emergence and between tuber initiation and harvest, and the haulm growth until tuber initiation, the radiation intercepted thereafter, the partitioning of dry matter to the tubers and the dry-matter concentration of the tubers. The planting density and cultivar will affect the outcome considerably.

Increasing the number of progeny tubers. The number of progeny tubers produced by crops from

minitubers depends on the number of emerging and surviving plants and the number of progeny tubers per plant, and both were lowest for the lighters minitubers (Lommen & Struik, 199x). Ways to increase the number of progeny tubers may aim at one of these options. Possibilities to increase the number of emerging and surviving plants have been discussed above. Methods to increase the number of tubers per plant could concentrate on increasing the number of stems per plant or the number of tubers per stem. Using physiologically older tubers may increase the number of stems (Bodlaender & Marinus, 1987; Struik & Lommen, 1990; Lommen & Struik, 1993) and the number of tubers per plant (Struik & Lommen, 1990). Also early N fertilization (Gunasena and Harris, 1968) or encapsulation of the tubers (Melching et al., 1993) may safeguard a high number of tubers per plant. It is uncertain whether the number of progeny tubers per stem could be increased by hilling, planting depth treatments or presprouting treatments. Increasing the number of tubers per plant, however, inevitably will reduce their size when the total progeny weight produced per plant remains at the same level.

Decreasing the variation over plants within a crop. The variation over plants or over planting positions in tuber number or yield per plant was higher in crops from lighter minitubers, even though the variation in seed tuber weight at planting was equal for all weight classes of minitubers (Lommen & Struik, 199x). All factors contributing to a more complete or regular emergence or to less variation in the amount of mother tuber reserves at the moment of emergence may improve the regularity of the crop. Consequently, again all methods for increasing the percentage emergence or accelerating emergence (see above) and closer grading could contribute to reducing the variation over plants within a crop of light tubers.

From all the possibilities mentioned to improve the field performance of light tubers the most promising ones are the use of tubers of a suitable physiological age and properly presprouted, the optimization of the application of fertilizer and the use of floating plastic film.

11.3 Incorporating a step of minituber production into a seed production programme: an assessment

To reduce the number of field multiplications for the production of basic seed in the Netherlands, minitubers may have to be field planted on approximately 1000 ha (Van der Zaag, 1987). This may improve the quality of the seed produced and shorten the time until adequate volumes of seed become available. It requires around 60 - 100 million minitubers to be available at planting time. For this type of application of minitubers in the Netherlands, the following prerequisites may be defined:

(1) The production technique has to be reliable and to yield large numbers of minitubers;
(2) The minitubers should be storable;
(3) The minitubers should have an excellent genetic quality;
(4) The minitubers should have an excellent health quality;
(5) The minitubers should have an optimum growth vigour at planting time;

(6) The minitubers must be distributed for planting by seed growers;
(7) The minitubers should reliably produce acceptable yields after field planting, before haulm has to be killed;
(8) The progeny tubers must have a high quality, both in the first and later generations;
(9) The production costs of the minitubers and their progeny should be low enough to be economically feasible.

Without aiming at being complete, it will be discussed in the following paragraphs to what extent these demands are already met or could be met in the future, and which gaps in knowledge or technology still need to be bridged.

(1) The production technique has to be reliable and to yield large numbers of minitubers

If large numbers of tubers are needed, these tubers have to be produced throughout the year because seasonal production will result in an undesirable and unmanageable peak in labour and equipment. The time required for the production of one batch should be short to reduce production costs. Techniques for production of *in vitro* plantlets are already optimized (Marinus, 1985) and plantlets can be produced throughout the year. With 3 - 6 new cuttings produced every 4 weeks (Miller et al., 1985), multiplication rates are very high. Also minitubers can be reliably produced throughout the year on these plantlets in controlled glasshouses, (cf. Lommen & Struik, 1992a, b, c; Lommen, 1993a; Lommen & Struik, 1994). Depending on the production technique employed, the number of minitubers produced differed. At a planting density of 350 plants per m^2, more than 3500 minitubers ($\geq$ 5 mm) per m^2 were produced when minitubers were harvested non-destructively at four, seven and ten weeks after planting (Lommen & Struik, 1992c) and more than 700 tubers ($\geq$ 0.3 g) if plants were left to grow undisturbed.

Consequently, the production method for minitubers (Lommen & Struik, 1992b, c) met the prerequisites of reliably producing large numbers of minitubers in a short period and throughout the year, and therefore seems suitable for further optimization.

(2) The minitubers should be storable

Losses of minitubers in the storage period occurred mostly in tubers from the first harvest and in tubers < 0.5 g. However, 96 % of all minitubers of cv. Agria and 77 % of those of cv. Liseta survived storage for 1.5 years, even without trying to optimize the storage conditions, i.e. when minitubers were left to dry after harvest for one day, cured for 13 days (18 °C, 80 % r.h.) and stored cold thereafter (2 °C, 80 % r.h., Lommen, 1993b). Most losses occurred in that part of the cold-storage period in which the sprouts became visible (around 7 - 11 months after harvest). The storability of the minitubers might be improved by delaying the first harvest for a few days until the tubers are $\geq$ 0.5 g and by proper treatment of the minitubers starting immediately after harvest. Optimizing the treatment the first hours and days after harvest appears extremely important, because

most minitubers that deteriorated during the cold-storage period showed already high fresh weight losses during the curing period (Lommen, 1993b). Drying after harvest to remove soil should be omitted or minitubers could be cleaned by washing with water. To reduce evaporative weight losses during curing, curing might be carried out at a higher r.h. and with reduced air movement from ventilation, still allowing abundant exchange of air. It probably should be carried out at a lower temperature or for a shorter period if research shows that respiration also contributes to the observed losses in tuber number.

Consequently, there are no bottlenecks in the storage of minitubers and there are possibilities for optimization.

(3) The minitubers should have an excellent genetic quality

In vitro cultures were initiated from plants that were true to type. The risk of obtaining genetically altered plants was minimized by initiating *in vitro* cultures from axillary buds of single node stem segments and not from dissected small meristems. This last technique often is employed to eradicate possible (virus) diseases (Wang & Hu, 1980), but may incidentally produce a clone with changed characteristics (e.g. Wright, 1983; one deviant clone out of 30 tested). Also routine multiplication *in vitro* was carried out using single node stem cuttings, which technique produces no albino's, leaf variegation, abnormal anthocyan production or gross distortions, as observed in plantlets derived from adventitious sprouts from callus or stem explants (Cassells et al., 1983). In addition, the genetic quality was safeguarded by refraining from NAA, IAA and kinetin in the medium. These occasionally are used in media for propagation by nodal segments (Jones, 1988), but may increase the risk of obtaining callus (cf. Okazawa et al., 1967; Dhingra et al., 1987), or differentiation or growth of adventitious sprouts, especially in stem explants (Wang & Huang, 1975). Also during the production of minitubers on these plantlets in the glasshouse, no growth regulators were used. Multiplying several clones of one cultivar separately may reduce the risk of a complete stock becoming genetically changed.

Consequently, all procedures used aimed at reducing the risk of obtaining mutations and although data on large numbers of plantlets fail, there is no reason to expect a high genetic variation.

(4) The minitubers should have an excellent health quality

All *in vitro* plantlets originated from plants that were verified to be free from diseases. Because of the aseptic conditions it was unlikely that plants became infected with diseases during culture initiation and routine multiplication *in vitro*. The conditions employed in the glasshouse resulted in robust plants. These appear less susceptible to damage at the non-destructive harvests, and consequently to infection. Also lowering the planting density or using techniques in which the repeated harvesting is circumvented, may reduce this risk. For commercial production of minitubers precautions should be taken to reduce risk of infection with diseases in the glasshouse, but these were not studied here. There also are no published reports on the susceptibility of the minitubers for

attack by micro-organisms during storage and also this part still needs attention. The losses in tuber number during storage (Lommen, 1993b) were not thought to have been caused by micro-organisms.

Consequently, the plantlets entering the tuber production phase are likely to have a good health status. This should be maintained in the glasshouse and during storage, but the health status of the minitubers produced still needs to be established and studied.

(5) The minitubers should have an optimum growth vigour at planting time

Minitubers are dormant after harvest (Lommen, 1993a) and their performance under controlled conditions is still poor when they are produced 6 or 7 months or less before planting and have been stored cold (Lommen & Struik, 1993a). Consequently, they should be produced earlier and stored until their performance is better (10 - 11 months) or optimal, or treatments may have to be applied to break dormancy or accelerate the physiological ageing of the tubers. It is possible to store minitubers that are dormant at planting time for an additional year (Lommen, 1993b), but not all cultivars still performed well 1.5 year after harvest, when tested under controlled conditions (Lommen & Struik, 1993a). Also under field conditions the physiological age of the tubers affected their performance (Struik & Lommen, 1990) and it is likely that after field planting the performance will first increase and later decrease with increasing physiological age of the tubers, as observed under controlled conditions (Lommen & Struik, 1993a). Differences in physiological age may show especially if field conditions are adverse (cf. Allen et al., 1979).

By breaking their dormancy and/or temperature treatments during the storage period, the end of the dormant period and the ageing of tubers could be accelerated. Cold treatments accelerated the end of the dormant period in minitubers (Lommen, 1993a), heat treatments soon after curing may increase vigour shortly after the end of dormancy (Van Ittersum et al., 1993) and presprouting at high temperatures in light followed by cold storage (O'Brien et al., 1983) or higher temperatures during (part of) the storage period (e.g. Bodlaender & Marinus, 1987) could enhance their physiological age. By adjusting the timing of a high-temperature treatment, sprout numbers possibly could be manipulated. An adequate understanding of the effects of the conditions and treatments during this period is of the utmost importance when minitubers are going to be produced year-round whereas their performance has to be optimum at planting time. This is especially needed since minitubers with different weights, from different harvests or grown under different climatic conditions throughout the year, may respond differently to the treatments.

The length of the presprouting period after removal from long-term storage, should depend on the weight of the tubers, because the lighter tubers will have the smallest sprouts if all tubers are presprouted for the same period (Lommen, 1994). Then, differences in time to emergence will be extremely large because stems from lighter tubers and stems from tubers with smaller sprouts emerge later (Sadler, 1961; Headford, 1962; Firman et al., 1992; Lommen, 1994). By planting minitubers with longer sprouts also differences in time to emergence between tubers with different weights can be reduced (Lommen, 1994). In practice, a uniform sprout length could probably be achieved by narrow grading of the tubers before sprouting, and sprouting batches separately for the appropriate

period.

Consequently, with the present status of knowledge it is not possible to produce minitubers throughout the year having an optimum age at planting in countries with one planting season, but there are possibilities of manipulating the physiological age of the tubers at planting and improving their performance. How this age can and should be modified, and in what way and to what extent the physiological age affects the field performance need further study.

(6) The minitubers must be distributed for planting by seed growers

The moment at which minitubers should be transported to the seed growers is not yet clear. The least vulnerable period appears the period between curing and the end of dormancy. Before properly cured, the tubers may dry out or become wounded easily, or suffer from a lack of oxygen when packed. After dormancy has ended, unwanted sprout growth during transport should be avoided, either by rapid or cooled transport. As bulk transport of presprouted tubers is precarious, minitubers preferably have to be transported to growers before presprouting. However, clean and controlled storage probably could be carried out most suitably by the company or institution producing the minitubers. Also when minitubers need treatments during storage, e.g. to speed up their physiological ageing, these treatments can be given most competently by the producer. Even presprouted minitubers may have to be distributed, especially if stem numbers have to be controlled by early presprouting treatments, followed by long-term storage. Thus, the least vulnerable period for transport seems not to be the most obvious period.

Consequently, the later the minitubers are distributed to growers, the higher the demands for a rapid, cool and careful transport. The tubers then can be pretreated for planting by the producer. The sooner after curing minitubers are transported, the less vulnerable the tubers are, but the higher the demands on the facilities, skills and treatments to be met by the growers.

(7) The minitubers should reliably produce acceptable yields after field planting, before haulm has to be killed

Whether yields are acceptable will depend on the cost/benefit ratios of the crop from minitubers, subsequent crops and those of competing technologies. In all years and cultivars tested, the weight and/or number of progeny tubers produced by crops from minitubers within 76 - 82 days after planting, increased when the minitubers originated from heavier weight classes (Lommen & Struik, 1994, 199x). With proper handling of the tubers and considerable care after planting, however, the variation in yield over years was not affected by the weight class of the minitubers planted (Lommen & Struik, 199x; data of one cultivar over three years). It is questionable whether this is also true for adverse conditions. Possibilities for improving the field performance of minitubers have been elaborated earlier in this chapter (11.2.3).

Consequently, yields in crops from minitubers depend on the weight class of the minitubers used. There are considerable possibilities for yield improvement.

(8) The progeny tubers must have a high quality, both in the first and later generations

Crops from the lighter minitubers compared to the heavier minitubers and crops from minitubers compared to conventional seed tubers show a higher variation in progeny tuber yield over plants within a crop (Lommen & Struik, 199x) and most probably also in haulm size. This irregularity may hinder roguing, and consequently reduce the genetic or health quality of the progeny tubers. On the other hand, no CCC or other persistent growth regulators were used during the production of minitubers, which may interfere with visual assessment of the plants. An irregular crop, however, not only hinders roguing, but probably also may attract more aphids and consequently have a higher risk of becoming infected with virus diseases. Also crops from the progeny tubers of - especially the lighter - minitubers may show a relatively high variation over plants, because a higher proportion of progeny tubers shows second growth symptoms (Struik & Lommen, 1990) and secondary tubers may behave like physiologically younger tubers when compared to normal or primary tubers (Jefferies & MacKerron, 1987). Also the high coefficients of variation over plots in crops from minitubers (Lommen & Struik, 199x) indicate that the progeny tubers from minitubers may vary considerably. It is uncertain, however, whether crops from minitubers and their second generation crops need considerable roguing. Van der Zaag (1990) estimated the roguing costs in crops from the progeny tubers from minitubers lower than in those from conventional seed, because of their high health status.

Apart from their effects on crop uniformity, more second growth symptoms in progeny tubers from lighter minitubers, also hinder size grading and planting, increase damage during handling or decrease the storability.

Whether progeny tubers from minitubers are less or more infected by virus than conventional tubers remains to be established. Plants from conventional tubers probably have a more advanced developmental stage and consequently possess a higher degree of mature plant resistance to virus infection of the tubers (cf. Beemster, 1972). In addition, the more closed canopy of conventional tubers may attract fewer aphids. Indirect evidence for this last assumption is the observation of Birecki & Roztropowicz (1963) that at a low spacing a higher percentage of plants was infected with leafroll and virus Y. If crops from minitubers indeed appear to be more susceptible to virus diseases, the use of physiologically older minitubers and a slightly higher planting density, combined with effective control of aphids and of virus transmission will increase the performance of minitubers in this respect.

Unpublished results from the author and results from Hide et al. (1992) suggest that plants from lighter tubers are more susceptible to *Rhizoctonia solani* than those from heavier tubers. Thus far, however, there is no evidence that the higher susceptibility of plants from lighter tubers also will lead to progeny tubers with more black scurf (cf. Hide et al., 1992). Coombs (1992) suggested that minitubers (as compared to conventional tubers) could contribute to the control of black scurf, but he had to base this suggestion on comparisons of progenies of one minituber stock and one conventional tuber stock for three years at different sites. However, clean seed of whatever source may contribute to the control of *Rhizoctonia solani*, because there is a positive relationship between

the disease incidence on seed tubers and that of the stored progeny tubers (Adams & Hide, 1980).

Coombs (1992) observed more common scab in progeny tubers from minitubers and attributed this to their later emergence and dryer soil conditions during tuber initiation. Unpublished results from the author confirm that the percentage of surface of the progeny tubers covered with *Streptomyces scabies* was higher for minitubers than for conventional tubers, irrespective of the weight of the minitubers. A higher incidence of *Streptomyces scabies* may result in higher weight losses during storage, but the consequences for the field performance of later generations remain to be established.

Drawing sound conclusions on the health status of further generations of seed tubers derived from minitubers is not yet allowed. Both Copeland (1990) and Coombs (1992) compared minitubers to tubers derived from virus-tested stem cuttings and suggested that minitubers had potential to increase the health standard of potato crops. Copeland (1990) observed that their first and second generation crops had a lower incidence of bacterial (*Erwinia carotovora* subsp. *atroseptica*) diseases, although they were possibly more susceptible to weakly pathogenic soil-inhabiting fungi developing during storage. Coombs (1992), by contrast, observed more *Erwinia carotovora* subsp. *atroseptica*, but less subsp. *carotovora*. A sound assessment of the differences in the health status in seed derived originally from minitubers and conventional seed tubers will be difficult because of the low incidence of certain diseases (e.g. blackleg) and the huge effects of differences in crop husbandry on health.

Consequently, at present it still is not known how the quality of the progeny tubers and that of their subsequent progenies are affected.

(9) The production costs of the minitubers and their progeny should be low enough to be economically feasible

The production costs of the progeny tubers from minitubers will be lower when the seed costs (e.g. the production costs or price of minitubers) and the non-seed production costs are lower, or when the yield obtained is higher.

If prices for minitubers are relatively high (e.g. 5 times that of conventional seed) and yields after field planting are relatively (e.g. half that of conventional seed tubers; Lommen & Struik, 1994, 199x), they appear important constraints for large-scale use of minitubers. Van der Zaag (1990) concluded after extensive calculations that most seed growers in the Netherlands then will not be prepared to pay the extra costs for seed tubers produced directly from minitubers compared with conventional seed from S tubers (the progeny of 3rd or 4th year clones).

Methods to reduce the production cost per weight of progeny tubers in the Netherlands may concentrate on reducing the seed or non-seed production costs or increasing the yield. Methods by which the seed or non-seed production costs can be reduced have not been published in detail. Possibilities for increasing yield have been elaborated earlier in this chapter. Partly they also increase the production costs per unit area (e.g. floating plastic film will increase the non-seed production costs, whereas increasing the planting density will increase the seed costs), but they nevertheless may reduce production costs.

At present, however, minitubers have more prospect if the seed they have to replace is more expensive (e.g. the basic plants in a clonal selection system or higher grade pre-basic seed) or in countries which depend on expensive, imported seed. Even some growers in countries like the Netherlands might pay the extra costs for tubers produced in two generations from minitubers when the quality of the seed produced proves to be higher and the price of minitubers is low (Van der Zaag, 1990). Also in countries which allow higher yields from light tubers, e.g. where the growing season is longer, the use of minitubers may more readily become economically feasible.

Consequently, the production costs of minitubers and their progeny tubers at present do not allow large-scale use in the Netherlands for the production of basic seed, but other applications may have more prospective.

General assessment. Potato minitubers of good quality can be produced reliably from *in vitro* propagated plantlets. Application of minitubers in seed production programmes, however, will only be successful if they yield progeny tubers that are economically and/or in quality superior to tubers produced by existing technologies. At present minitubers are successfully used, but mainly to replace the basic plants in the clonal selection system, to speed up seed production to a limited extent, and to increase the quantity of seed from new cultivars. Technically it is possible to produce large numbers of minitubers throughout the year, which is required for large-scale use of minitubers. However, before large-scale application could be considered, further studies are needed on the processes which affect the growth vigour and the genetic and health quality of the minitubers and their progeny, and the techniques by which these processes can be manipulated. Both these uncertainties and economical reasons are at present the main obstacles for large-scale application of minitubers in countries like the Netherlands. Nevertheless, as knowledge and the reliability concerning quality increases, an increasing part of the world's pre-basic seed production may be derived from minitubers or other tubers produced from *in vitro* plantlets, e.g. microtubers. This will be enhanced when the price ratio between minitubers and conventionally produced seed tubers declines, and when the demand for seed from new cultivars increases.

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Summary

SUMMARY

Minitubers are small potato tubers (5 - 20 mm or slightly larger) that can be produced year-round in glasshouses on plantlets that are propagated *in vitro* (i.e. on nutrient medium in aseptic environments) and are planted at high density. Minitubers are thought to combine the advantages of tubers and *in vitro* propagated plantlets: tubers can be stored and transported relatively easily; *in vitro* propagated plantlets can be produced in large quantities throughout the year by means of single node cuttings and - when properly produced - are likely to have a high quality with respect to the health status. Minitubers are meant to serve as potato seed tubers in the first year of seed production programmes. At the start of the research project leading to this thesis, the knowledge on minitubers was scarce. Incorporating a step of minituber production in seed production programmes of potato was thought to speed up multiplication and consequently improve the quality of the seed produced in later generations. Conventional seed tuber production takes many years because of the low multiplication rate of potato, whereas the high number of field multiplications increases the risk of (re-)infection with diseases.

The research project aimed at studying the possibilities of producing large numbers of minitubers per *in vitro* plantlet and per unit area of glasshouse space throughout the year, and at studying their performance under Dutch conditions. It covered (a) the phase in which minitubers were produced in glasshouses, (b) the phase in which the produced minitubers were stored en (c) the field phase in which conventional sized seed tubers were grown from minitubers. Practical results, experiences, and guidelines for the production, storage and use of minitubers were published in Dutch. This thesis concentrates on the underlying agronomical and physiological principles.

The production of minitubers. The research on the production of minitubers (chapters 2 - 4) resulted in a production method for potato minitubers by which in all cultivars studied (Ostara, Bintje and Elkana) more than 3000 minitubers per m^2 could be produced in 10 weeks, with average fresh weights of 1 - 2 g per tuber. The production method involved planting of *in vitro* propagated plantlets at 350 plants per m^2 into a mixture of perlite and potting soil (1/1 v/v) in glasshouses under tuber inducing conditions (18/12 °C day/night temperature, 12 h photoperiod, and a minimum light intensity of 80 W m^{-2} total irradiance), and repeatedly removing tubers $\geq$ 5 mm 4, 7 and 10 weeks after planting, using a non-destructive harvesting procedure in the first two harvests. Plants were fertilized from the first harvest onwards. At the non-destructive harvests, plants were lifted carefully from the soil mixture, tubers were removed and plants were replanted deeper than initially.

The effects of non-destructive harvests on plant growth and tuber formation were analysed in cvs Ostara and Bintje (chapters 2 and 3). In plants growing without interference, the initiation of new tubers slowed down 4 weeks after planting while the existing tubers continued to increase in weight. When, instead, the largest tubers were removed in a non-destructive harvest, new stolons and tubers were initiated. Part of these newly initiated tubers grew to a harvestable size within three weeks and at that moment tubers could be harvested a second time. Postponing the second harvest until more than three weeks after the first harvest only increased the weight but not the number of tubers in the

harvestable sizes, whereas part of the undersized tubers was resorbed. A second non-destructive harvest, three to four weeks after the first harvest, stimulated growth of tubers that otherwise would have been resorbed or would have remained too small. Again, part of these tubers grew to a harvestable size within three weeks after the second non-destructive harvest. After a non-destructive harvest, tuber and overall growth rates were reduced compared to plants left to grow without interference.

Effects of crop husbandry on the yield parameters of minitubers produced by repeated harvesting were analysed in chapter 4. Supplying nutrients and using a square plant arrangement increased the fresh weight of the minitubers produced, but the effects on the number of minitubers produced were cultivar-dependent. Increasing the plant density from 50 to 800 plants per m^2 or the minimal diameter of harvested tubers from 5 to 12 mm did not affect the weight of minitubers per m^2. Increasing the plant density resulted in more tubers per m^2 but fewer tubers per plant. Removing smaller tubers ($\geq$ 5 mm) greatly increased the number of small tubers, but did not affect the weight and number of tubers in larger grades ($\geq$ 8 mm or $\geq$ 12 mm). Crop husbandry techniques in the glasshouse phase affected the weight of minitubers produced mainly through their effects on leaf area duration, and the number of minitubers through their effects on growth of tubers to a harvestable size.

In chapter 11 it is discussed that the success of the production method for minitubers may have depended on (1) the state of the *in vitro* plantlets when they were tranferred to the glasshouse, resulting in a high plant survival rate and rapidly growing plants which were unlikely to be already strongly induced to tuberize at planting, (2) the conditions chosen in the glasshouse, resulting in early tuber initiation, although still allowing sufficient growth, and (3) the non-destructive harvesting procedure. The effects of the non-destructive harvests were probably caused by the combined influences of (1) the root damage occurring when lifting the plants from the soil mixture, (2) the removal of the most dominant tubers, resulting in more potential tuber sites that were not subjected to the dominance of rapidly growing tubers, and (3) the deeper replanting of the plantlets, resulting in more nodes below ground, and consequently more stolon positions. The root damage explained the reduction in growth rates and in fresh tuber yield after non-destructive harvests, the removal of dominant tubers and the deep replanting explained the extra tubers initiated.

The performance of the minitubers between harvest and planting. After a harvest, tubers were dried at room temperature for one day to allow removal of adhering soil, cured at 18 °C until 14 days after harvest to enhance periderm formation and suberization, and stored cold at 2 °C for varying periods. The drying for one day was omitted in later studies. Research on the performance of minitubers during storage (chapters 5 and 6) was carried out using minitubers from two cultivars (Agria and Liseta), five fresh weight classes ($<$ 0.50 g, 0.50 - 0.99 g, 1.00 - 1.99 g, 2.00 - 2.99 g and $\geq$ 3.00 g) and three successive harvests of the same plantlets. On average, the dry-matter concentration of the minitubers assessed one day after harvest was 17.8 % (chapter 5). Dry-matter concentration increased with tuber weight for tubers from the second and third harvests. In minitubers $\geq$ 0.5 g, dry-matter concentration was higher in tubers from later harvests.

Storage losses were (a) losses of entire tubers because of deterioration, and (b) fresh weight losses of tubers surviving storage for 1.5 year. Both kinds of losses were higher in lighter than in heavier tubers, in tubers from the first harvest than in those from later harvests and in cv. Liseta than in cv. Agria (chapter 6). It is argued that weight losses were caused by evaporation (water losses) and respiration (dry-matter losses). Both losses were probably higher in young, lighter tubers. Especially the high surface area:volume ratio in lighter tubers and a less resistant periderm may contribute to high water losses. It was assumed that high evaporation and/or a high respiration contributed to the deterioration, but the real causes are unknown. Tubers which deteriorated during cold storage had already shown large weight losses during curing, whereas deterioration occurred mainly between 6 and 12 months of storage, which coincided with the period the first sprouts became visible and respiration is likely to increase. However, almost all minitubers $\geq$ 0.5 g from later harvests and from both cultivars survived storage for 1.5 years.

The dormant period (i.e. the period from harvest until 50 % of the tubers had produced a sprout of 2 mm when kept in darkness, at 18 °C and 80 % r.h.) was on average 138 days. The dormant period was longer for lighter than for heavier minitubers, for tubers from the first harvest than from later harvests and cultivar-dependent (chapter 5). A cold-storage period of 6 weeks, starting 14 days after harvest, reduced the period until 50 % of the tubers had a sprout of 2 mm by an average of 11 days. The longer dormant period for lighter tubers must partly be attributed to a slower rate of sprout growth up to 2 mm, because in research concerning sprout growth (cvs Ostara, Bintje, Elkana, chapter 8) after removal from cold storage, sprouts from lighter tubers took longer to grow to 2 mm, but also grew more slowly between 2 and 4 mm and 4 and 6 mm (chapter 8). The influence of tuber weight on sprout growth was less clear for tubers in the larger weight ranges and when sprouts grew longer. In both cases the rate of sprout growth was relatively high.

The performance of minitubers after planting. The performance of plants and crops from minitubers were principally influenced by the storage period and the weight of the minitubers.

The length of the storage period affected the performance of plants from the minitubers mainly through its effects on the physiological ageing of minitubers (chapter 7). Minitubers of 1 - 2 g of cvs Agria and Liseta were harvested, dried for 1 day, cured for 13 days and thereafter stored cold at 2 °C in darkness and 80 % r.h. Their performance was studied after taking them out of storage at regular intervals up to more than 1.5 years of storage. Tubers were planted individually in pots in a growth chamber, and plants were harvested exactly 8 weeks after planting. After 6 months of storage the growth was still extremely poor. In both cultivars, tallest plants and largest leaf areas per plant were observed in plants from tubers of the second and third harvests that had been stored for 10 - 11 months. Highest stem numbers, dry weights, tuber fresh weights and harvest indices were achieved after 14 - 15 months of storage with cv. Agria and after 18 - 19 months of storage with cv. Liseta. Under field conditions a higher physiological age (up to a maximum) may lead to earlier emergence, earlier haulm development, higher stem and tuber numbers, and a higher harvest index.

When tubers with sprouts of the same length were planted in pots (chapter 8), sprouts from lighter tubers took longer to emerge because they increased slower in length and did not grow straight to

the surface. Emergence was later and differences between weight classes were larger when tubers were planted deeper (6 or 9 cm versus 3 cm) or when they had shorter sprouts at planting (2 or 4 mm versus 8 mm). At emergence, plants from lighter tubers had thinner stems and lower stem and root weights, but higher stem weights proportional to tuber weights and higher shoot:root ratios.

The field performance of five fresh weight classes of minitubers ranging from 0.13 - 0.25 g to 2.00 - 3.99 g was studied in a short growing seasons of 79 - 84 days in 1989, 1990 and 1994, and was compared to that of conventional seed tubers in the first two years (chapters 9 and 10). Plants from heavier minitubers emerged more regular, showed a faster ground cover by the haulm soon after emergence, had higher dry-matter yields and higher fresh tuber yields. Radiation conversion coefficients (RCC) did not differ between weight classes. Higher tuber yields resulted from more radiation intercepted due to a faster ground cover, and a higher harvest index. All minitubers produced plants with one primary stem due to their young physiological age. In one experiment when heavier minitubers had long sprouts, time to 50 % emergence decreased with tuber weight, whereas dry-matter concentration of progeny tubers increased. Conventional tubers were superior to minitubers in all characteristics mentioned except RCC, which was similar. Differences in performance between minitubers and conventional tubers were attributed to weight and age of seed tubers, presprouting method and crop husbandry.

Multiplication factors were calculated as the number and weight of progeny tubers > 20 mm, produced per planted tuber or per unit planted tuber weight. Multiplication factors per planted tuber of the lightest (0.13 - 0.25 g) minitubers were half of those of the heaviest (2 - 4 g) minitubers. Multiplication factors were lower for the lighter minitubers because fewer plants in the crop survived until harvest (lower % emergence and a higher plant death after emergence), and because fewer progeny tubers and a lower progeny tuber weight were produced per plant. Differences in the number of progeny tubers produced by plants from minitubers of the highest weight classes were only small, but because the average weight of the progeny tubers increased with increasing weight of the minitubers, the potential number of stems to be produced by the progeny tubers from heavier minitubers was higher. When calculated per unit of planted minituber weight, multiplication factors of the lightest minitubers were about 7 times higher than those of the heaviest minitubers.

Yield variation was described by coefficients of variation for the number and weight of progeny tubers > 20 mm. Variation over individual plants in crops from lighter minitubers was higher than in stands from heavier minitubers. Variation over plots within a field was sometimes higher for the lighter minitubers, but variation over years (calculated for cv. Bintje in three years) was similar for all minituber classes. This suggests that yield stability does not depend on the weight of the minitubers used, provided the tubers and plants are properly nursed. Variation over plots in progeny tuber weight was higher for minitubers than for conventional tubers.

Strategies to improve the field performance of light tubers (increasing the weight and number of progeny tubers and reducing the variation) are discussed (paragraph 11.2.3). The most promising crop husbandry techniques for this appear to be (a) using tubers of a suitable physiological age and properly presprouted, (b) optimizing the application of fertilizer, and (c) using floating plastic film.

Incorporating minitubers in seed production systems. Minitubers can be reliably produced from *in vitro* plantlets, and are successfully used to replace the basic plants in the clonal selection system, to speed up seed production slightly, and to increase the quantity of seed from new cultivars. Technically it is possible to produce large numbers of minitubers throughout the year, which is required for use of minitubers on a large scale. In countries like the Netherlands, economical reasons and the gaps in knowledge on processes affecting the growth vigour, the genetic quality and the health quality of the minitubers and their progeny, and on techniques by which these processes can be manipulated, are the main obstacles for large-scale application of minitubers. On a world's scale the use of minitubers or similar propagules in seed production programmes will probably increase.

Samenvatting

SAMENVATTING

Miniknollen zijn kleine aardappelknollen met een diameter van 5 tot 20 mm of iets groter. Ze worden in kassen geproduceerd aan planten die *in vitro* (d.w.z. op een voedingsmedium onder aseptische omstandigheden) zijn vermeerderd en die in een hoge plantdichtheid zijn geplant. Miniknollen zijn bedoeld als uitgangsmateriaal in de eerste fase van een produktieprogramma voor aardappelpootgoed. Ze lijken de voordelen van knollen en *in vitro* planten te combineren: knollen zijn goed te bewaren en gemakkelijk te vervoeren en af te leveren; *in vitro* planten kunnen door middel van stekken gedurende het hele jaar in hoge aantallen worden geproduceerd en zijn vrij van ziekten - mits correct geproduceerd. Voor aanvang van het onderzoeksproject dat heeft geleid tot dit proefschrift was de kennis over miniknollen gering. Inpassen van miniknollen in de teelt van aardappelpootgoed leek echter een mogelijkheid om het aantal jaren veldvermeerdering in vergelijking met op de conventionele manier geproduceerd aardappelpootgoed aanzienlijk te verminderen en daarmee tevens de kwaliteit van de volgende generaties pootgoed te verhogen. Het vermeerderen van pootaardappelen op de conventionele manier is langzaam door het geringe aantal nakomelingen per pootaardappel, terwijl het hoge aantal veldvermeerderingen een groot risico op infectie van pootgoed met ziekten met zich mee brengt.

In het onderzoeksproject werden de mogelijkheden onderzocht om jaarrond hoge aantallen miniknollen te produceren, zowel per *in vitro* plant als per vierkante meter kas. Daarnaast werden hun gebruiksmogelijkheden onderzocht onder Nederlandse omstandigheden als uitgangsmateriaal voor pootgoedproduktie. Dit gebruiksdoel houdt in dat er na poten op het veld slechts een kort groeiseizoen beschikbaar is om dochterknollen te produceren. Het onderzoeksproject bestreek de volgende fasen: (a) de produktie van miniknollen aan *in vitro* planten in de kas, (b) de bewaarfase en (c) de veldfase. Praktische resultaten, ervaringen en protocollen voor de teelt en het gebruik van miniknollen zijn reeds gepubliceerd in Nederlandstalige publikaties. Dit proefschrift analyseert de basisprincipes van de produktie, bewaring en het gebruik van miniknollen.

De produktie van miniknollen. Het onderzoek naar de produktie van miniknollen leidde tot een produktiemethode die bruikbaar was om jaarrond hoge aantallen miniknollen te produceren. Hierbij werden *in vitro* vermeerderde planten in kassen geplant bij een plantdichtheid van 350 planten per m^2 in een mengsel van potgrond en perliet (elk 50 % op volumebasis). De omstandigheden in de kassen waren gunstig voor de aanleg van knollen: een daglengte van 12 uur, een dag/nacht temperatuur van 18/12 °C en een lichtintensiteit van minimaal 80 W m^{-2} totale straling. Van de planten werden 4, 7 en 10 weken na planten alle op dat moment gevormde knollen met een diameter van 5 mm of meer verwijderd. De oogsten na 4 en 7 weken werden niet-destructief uitgevoerd: de planten werden voorzichtig uit de grond gelicht, de knollen werden verwijderd en de planten werden dieper dan oorspronkelijk teruggeplant. Bij de rassen Ostara, Bintje en Elkana konden op deze manier meer dan 3000 miniknollen per m^2 worden geproduceerd in 10 weken, met een gemiddeld versgewicht tussen 1 en 2 g.

De invloed van niet-destructieve oogsten op de plantegroei en de vorming van knollen werd

bestudeerd bij de rassen Ostara en Bintje (hoofdstukken 2 en 3). Wanneer planten ongestoord groeiden, stopte de aanleg van knollen ongeveer 4 weken na planten nagenoeg, terwijl vervolgens de aanwezige knollen doorgroeiden. Wanneer in plaats daarvan de grootste knollen werden verwijderd in een niet-destructieve oogst, werden nieuwe stolonen en nieuwe knollen aangelegd. Een deel van deze knollen nam binnen drie weken na de eerste oogst voldoende toe in gewicht om te worden verwijderd in een tweede oogst. Door deze tweede oogst uit te stellen van drie weken na de eerste oogst naar een later tijdstip, nam het aantal knollen in de oogstbare maat (in deze proeven nog ≥ 8 mm) niet toe - wel het gewicht - en werd een deel van de te klein gebleven knollen geresorbeerd. Een tweede niet-destructieve oogst, drie tot vier weken na de eerste oogst, stimuleerde echter het doorgroeien naar een oogstbare maat van een deel van de knollen die anders te klein zouden zijn gebleven of zouden zijn geresorbeerd. Deze konden drie weken na de tweede oogst worden geoogst. Hoewel door het niet-destructief oogsten het aantal knollen steeg, daalde de groeisnelheid van de planten en van de knollen in vergelijking met planten die niet werden geoogst.

De invloeden van teeltmaatregelen in de kas op opbrengstparameters van miniknollen werden geanalyseerd in hoofdstuk 4. Regelmatige bemesting met een laag gedoseerde voedingsoplossing en het planten van de *in vitro* planten in vierkantsverband verhoogden de gewichtsopbrengst aan miniknollen per m^2, maar verhoogden slechts bij één van de twee onderzochte cultivars (Bintje) ook het aantal knollen. Verhogen van de plantdichtheid van 50 naar 800 planten per m^2 of van minimum diameter van de geoogste knollen van 5 naar 12 mm hadden geen invloed op het gewicht aan miniknollen per m^2. Verhoging van de plantdichtheid leidde tot meer knollen per m^2, maar tot minder knollen per plant. Door kleinere knollen te oogsten (≥ 5 mm), werd het totaal aantal miniknollen aanzienlijk verhoogd zonder dat het aantal en het gewicht aan knollen in de grotere fracties (≥ 8 mm of ≥ 12 mm) significant veranderde. Teeltmaatregelen leken het gewicht aan miniknollen vooral te beïnvloeden via de bebladeringsduur, en het aantal miniknollen door uitgroei van knollen naar een oogstbare maat.

Het succes van de beschreven produktiemethode werd waarschijnlijk vooral beïnvloed door drie factoren (hoofdstuk 11): (1) de toestand van de *in vitro* plantjes op het moment van planten, die borg stond voor een hoog percentage overleving en goed groeiende planten die waarschijnlijk nog niet sterk tot knolvorming waren geïnduceerd; (2) de klimaatsomstandigheden in de kas, die borg stonden voor vroege knolaanleg waarbij toch voldoende groei mogelijk was; (3) de niet-destructieve oogsten. De effecten van de niet-destructieve oogsten werden veroorzaakt door (1) wortelbeschadiging bij het losmaken van de planten uit het grondmedium, (2) verwijdering van de grootste knollen, waardoor meer mogelijke knolplaatsen niet meer beïnvloed werden door dominante knollen, en (3) dieper terugplanten na een oogst, waardoor er meer knoppen ondergronds kwamen, d.w.z. onder condities die gunstig zijn voor de aanleg van stolonen. De wortelbeschadiging veroorzaakte met name de groeireducties, het verwijderen van knollen en het dieper terugplanten de initiatie van nieuwe knollen.

Het gedrag van miniknollen tussen oogst en planten. Na een oogst werden miniknollen gedurende 1 dag gedroogd om aanhangende grond te kunnen verwijderen, vervolgens werd tijdens een

helingsperiode bij 18 °C tot 14 dagen na oogst de vorming en de verkurking van de schil gestimuleerd, en tenslotte werden de knollen koud bewaard bij 2 °C gedurende kortere of langere tijd. Het drogen gedurende 1 dag werd in latere studies achterwege gelaten om uitdroging van de knollen tegen te gaan. Het onderzoek aan het gedrag van miniknollen tijdens de bewaarperiode (hoofdstukken 5 en 6) werd in het algemeen uitgevoerd met miniknollen afkomstig van twee cultivars (Agria en Liseta), vijf gewichtsklassen (< 0,50 g, 0,50 - 0,99 g, 1,00 - 1,99 g, 2,00 - 2,99 g en ≥ 3,00 g) en de drie opeenvolgende oogsten van dezelfde planten.

Het drogestofgehalte van de miniknollen 1 dag na de oogst was gemiddeld 17,8 %. Bij miniknollen uit de tweede en derde oogst was het drogestofgehalte hoger naarmate de knollen zwaarder waren. In knollen ≥ 0,50 g was het drogestofgehalte bovendien hoger wanneer de miniknollen afkomstig waren van latere oogsten.

Tijdens bewaring traden de volgende verliezen op: (1) uitval van knollen en (2) gewichtsverliezen van knollen die bewaring gedurende 1,5 jaar schijnbaar goed doorstonden. Beide typen verliezen waren hoger in miniknollen met lagere gewichten, in knollen van de eerste oogst in vergelijking met latere oogsten en in knollen van cv. Liseta in vergelijking met cv. Agria (hoofdstuk 6). De gewichtsverliezen werden vermoedelijk veroorzaakt door evaporatie (waterverlies) en respiratie (drogestofverlies). Het is aannemelijk dat beide hoger zijn in jonge, lichte knollen. Vooral de hoge oppervlakte:inhoud verhouding van lichte miniknollen en een lage weerstand van de schil voor verdamping leken hoge gewichtsverliezen te bevorderen. Een hoge evaporatie en een hoge respiratie droegen waarschijnlijk ook bij aan de uitval van knollen tijdens de bewaring, maar de exacte oorzaken hiervan bleven onduidelijk. Knollen die uitvielen, vertoonden al relatief hoge gewichtsverliezen tijdens de helingsperiode, terwijl de meeste knollen uitvielen in de periode tussen 6 en 12 maanden bewaring, waarin ook de eerste kiemen zichtbaar werden en de respiratie waarschijnlijk hoger werd. Uiteindelijk bleken echter 96 % van alle knollen van cv. Agria en 77 % van alle knollen van cv. Liseta een bewaarperiode van 1,5 jaar te overleven.

De lengte van de kiemrustperiode (hier gedefinieerd als de periode van oogst tot het moment waarop 50 % van de knollen een kiem van 2 mm had gevormd wanneer de knollen werden bewaard in het donker, bij 18 °C en een relatieve luchtvochtigheid van 80 %) was gemiddeld 138 dagen. De kiemrustperiode was langer voor lichte dan voor zwaardere miniknollen, langer voor knollen uit de eerste oogst dan uit latere oogsten en afhankelijk van de cultivar (hoofdstuk 5). Door een koudeperiode van 6 weken bij 2 °C die 14 dagen na de oogst begon, kon de lengte van de periode tot 50 % van de knollen een kiem van 2 mm had gevormd met 11 dagen worden bekort. De langere kiemrustperiode van lichte knollen werd hoogstwaarschijnlijk deels veroorzaakt door een trage groei van de spruiten tot 2 mm. Onderzoek met de rassen Ostara, Bintje en Elkana waarbij de kiemgroei werd gemeten van knollen die na een periode van koude bewaring bij hogere temperatuur werden geïncubeerd (hoofdstuk 8), toonde namelijk aan dat kiemen van lichtere knollen niet alleen gemiddeld meer dagen nodig hadden om een lengte van 2 mm te bereiken, maar dat ze ook nog duidelijk trager groeiden tussen 2 en 4 mm en tussen 4 en 6 mm. De invloed van het knolgewicht op de kiemgroei werd minder duidelijk bij hogere knolgewichten en wanneer de kiemen langer werden (tot 8 mm, de maximaal gemeten lengte), terwijl kiemen dan tevens sneller groeiden.

Het gedrag van miniknollen na planten. Met name de bewaarperiode en het gewicht van de miniknollen hadden invloed op de manier waarop planten en gewassen uit miniknollen zich ontwikkelden.

De duur van de bewaarperiode had met name invloed op het gedrag van de knollen na planten door beïnvloeding van de fysiologische leeftijd van de miniknollen (hoofdstuk 7). Met miniknollen van 1 - 2 g van de rassen Agria en Liseta die werden bewaard bij 2 °C, werden groeikrachttesten uitgevoerd na verschillende bewaarperiodes. Hierbij werden knollen individueel in potten in klimaatcellen geplant en exact 8 weken na poten geoogst. Na een bewaarperiode van 6 maanden was de groeikracht nog extreem laag. Bij beide cultivars werden de langste planten en hoogste bladoppervlakten gemeten aan planten uit knollen die 10 - 11 maanden waren bewaard. De hoogste stengelaantallen, drooggewichten, versgewichtopbrengsten aan dochterknollen en oogstindices werden bij cv. Agria bereikt na 14 - 15 maanden bewaring en bij cv. Liseta na 18 - 19 maanden, de langste periode die werd getest. Het lijkt aannemelijk dat ook onder veldomstandigheden een hogere fysiologische leeftijd (tot een maximum) zal leiden tot een snellere opkomst, hogere stengel- en knolaantallen per plant en een hogere oogstindex.

Wanneer knollen met kiemen van gelijke lengte individueel in potten werden geplant in klimaatcellen (rassen Ostara, Bintje, Elkana, hoofdstuk 8), duurde het langer voor de planten uit lichtere knollen opkwamen. Dit werd veroorzaakt door een tragere stengelgroei, maar ook doordat de stengels van kleine knollen niet volledig recht naar de grondoppervlakte groeiden. Wanneer de knollen dieper werden gepoot (6 of 9 cm in vergelijking met 3 cm) of kleinere kiemen hadden (2 of 4 mm in vergelijking met 8 mm) was de opkomst later en namen de verschillen tussen de gewichtsklassen toe. Bij opkomst hadden planten uit kleinere knollen dunnere stengels, lagere stengelgewichten, lagere wortelgewichten, maar een hoger stengelgewicht ten opzicht van het gewicht van de moederknol en een hogere spruit:wortel verhouding.

Het veldgedrag van miniknollen die varieerden in gewicht tussen 0,13 - 0,24 g en 2,00 - 3,99 g werd bestudeerd in 1989, 1990 en 1994 in groeiperioden van 79 - 84 dagen, en vergeleken met dat van conventionele pootgoedknollen in 1989 en 1990 (hoofdstukken 9 en 10). Planten uit zwaardere miniknollen kwamen regelmatiger boven, bedekten na opkomst de grond sneller met loof, hadden hogere droge-stofopbrengsten en hogere knolopbrengsten. De efficiëntie waarmee de door het loof opgevangen straling werd omgezet in drogestof, verschilde niet tussen gewassen uit miniknollen van verschillende gewichtsklassen. Hogere knolopbrengsten bij gewassen uit zwaardere miniknollen werden veroorzaakt door meer opgevangen straling als gevolg van een snellere grondbedekking en door een hogere oogstindex. Alle miniknollen produceerden planten met gemiddeld één hoofdstengel. Alle genoemde parameters waren hoger of beter voor gewassen uit conventionele pootgoedknollen, behalve de efficiëntie waarmee opgevangen straling werd omgezet, die niet verschilde. De verschillen in veldgedrag tussen gewassen uit miniknollen en conventionele pootgoedknollen werden toegeschreven aan verschillen in gewicht en fysiologische leeftijd van de moederknollen, de manier van voorkiemen en de teeltmaatregelen tijdens de veldfase.

Vermeerderingsfactoren werden berekend als het aantal en het gewicht aan dochterknollen > 20 mm per geplante knol en per eenheid geplant gewicht. De vermeerderingsfactoren per geplante

miniknol waren voor de laagste gewichtsklasse miniknollen (0,13 - 0,24 g) ongeveer de helft van de vermeerderingsfactoren voor de hoogste gewichtsklasse miniknollen (2,00 - 3,99 g). Dit werd veroorzaakt doordat minder miniknollen uiteindelijk resulteerden in een plant (lager opkomstpercentage en meer uitval na opkomst) en doordat per gevestigde plant minder knollen werden gevormd en een lager knolgewicht werd geproduceerd. Planten uit de hogere gewichtsklassen miniknollen verschilden onderling niet significant in aantal dochterknollen per plant. Omdat het gemiddeld gewicht per dochterknol echter hoger was bij planten uit zwaardere miniknollen, is het waarschijnlijk dat het aantal stengels dat uiteindelijk geproduceerd kan worden uit de opbrengst van een miniknol, wel hoger is bij zwaardere miniknollen. Wanneer vermeerderingsfactoren werden berekend per eenheid geplant gewicht, waren ze ongeveer 7 keer hoger voor de lichtste miniknollen dan voor de zwaarste.

De variatie in opbrengst werd beschreven door variatiecoëfficiënten in aantal en gewicht aan dochterknollen > 20 mm. In gewassen uit lichtere miniknollen was de variatie over individuele planten hoger dan in gewassen uit zwaardere miniknollen. De variatie over veldjes binnen een proefveld was soms hoger voor lichtere miniknollen, maar de variatie over jaren (berekend voor cv. Bintje over drie jaar) was vergelijkbaar voor alle gewichtsklassen. Dit suggereert dat de opbrengststabiliteit niet afhangt van het gewicht van de gebruikte miniknollen, mits de knollen en de gewassen uit deze knollen zorgvuldig worden behandeld. De variatie over veldjes binnen een proefveld was hoger voor miniknollen dan voor conventionele pootgoedknollen.

Mogelijkheden om het veldgedrag van miniknollen te verbeteren (verhogen van het aantal en gewicht aan dochterknollen en verlagen van de variatie) werden aangegeven in paragraaf 11.2.3. De meest veelbelovende teeltmaatregelen hiervoor zijn: (a) gebruik van miniknollen van een geschikte fysiologische leeftijd, (b) optimaliseren van de toediening van meststoffen en (c) gebruik van folie.

Inpassen van miniknollen in een produktiesysteem voor pootaardappelen. Miniknollen kunnen betrouwbaar worden geproduceerd aan *in vitro* planten en worden met succes gebruikt ter vervanging van basisplanten in een stamselectiesysteem, om het aantal jaren veldvermeerdering op beperkte schaal te reduceren en om de beschikbare hoeveelheid pootgoed van nieuwe cultivars te verhogen. Technisch is het mogelijk om hoge aantallen miniknollen jaarrond te produceren, hetgeen nodig is voor grootschalige toepassing van miniknollen. De belangrijkste obstakels voor grootschalige toepassing van miniknollen in landen als Nederland zijn de hoge kosten en de nog te geringe kennis over de processen die de groeikracht, gezondheid en genetische kwaliteit van miniknollen en hun dochterknollen bepalen, en over de mogelijkheden deze processen te beïnvloeden. Waarschijnlijk zal op wereldschaal het gebruik van miniknollen of vergelijkbaar vermeerderingsmateriaal toenemen.

CURRICULUM VITAE

Willemien Josepha Maria Lommen werd geboren op 1 juli 1956 in Tegelen. Na het behalen van het Gymnasium-ß diploma aan het St.-Thomascollege te Venlo, begon ze in september 1974 aan een studie Biologie aan de toenmalige Landbouwhogeschool in Wageningen. Later werd deze studierichting verruild voor de studierichting Landbouwplantenteelt, waarvan in juni 1981 het doctoraaldiploma werd gehaald. Doctoraalvakken waren de leer van het grasland, de microbiologie en de bodemvruchtbaarheid. Van juli 1981 tot en met oktober 1985 werkte ze voor de Nederlandse Organisatie voor Zuiver-Wetenschappelijk Onderzoek (Z.W.O.) als wetenschappelijk assistent bij de vakgroep Microbiologie van de toenmalige Landbouwhogeschool aan een onderzoek naar de genetisch-fysiologische aspecten van de erwt-*Rhizobium*-symbiose. Van augustus 1986 tot januari 1989 deed ze onder meer journalistiek werk. Van september 1987 tot en met december 1990 verrichtte ze als toegevoegd onderzoeker bij de vakgroep Landbouwplantenteelt en graslandkunde van de Landbouwuniversiteit onderzoek naar de produktie en gebruiksmogelijkheden van miniknollen bij de teelt van pootaardappelen. Dit onderzoek werd via het Produktschap voor Aardappelen gedeeltelijk gefinancierd door het aardappelbedrijfsleven. Vanaf januari 1991 werkt ze bij de vakgroep Agronomie van de Landbouwuniversiteit als universitair docent, in het bijzonder op het gebied van de produktie en kwaliteit van uitgangsmateriaal.