Manipulating the physiological quality of *in vitro* plantlets and transplants of potato

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Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr. ir. L. Speelman
in het openbaar te verdedigen
op dinsdag 5 december 2000,
des namiddags te vier uur in de Aula.

Financial support for the printing of this thesis was obtained from the Dr. Judith Zwartz Foundation, Wageningen, The Netherlands.

Tadesse Mehari (2000)

Manipulating the physiological quality of *in vitro* plantlets and transplants of potato.
Tadesse Mehari – [S.L.: s.n.]. Ill.
PhD Thesis, Wageningen University. – With ref.-
With summaries in English and Dutch.
ISBN: 90–5808–330–6
Subject headings: *Solanum tuberosum* L.; seed production/
in vitro propagation.

Propositions

1. Leaf area of *in vitro* propagated potato plantlets follows logistic growth in the normalisation, transplant production and tuber production phases, indicating that growth restrictions exist in all three phases. *(This thesis)*

2. The status of potato plants at the end of the transplant production phase influences performance of the plants in the tuber production phase. *(This thesis)*

3. Transition of potato plants from *in vitro* to *in vivo* conditions during transplant production promotes leaf growth. *(This thesis)*

4. Biotechnology in general and tissue culture in particular will enhance horticulture in Eritrea.

5. Long-term food aid is adverse to Africa.

6. Tissue culture techniques can promote reforestation programmes in Eritrea by rapid multiplication of indigenous tree species that are difficult to regenerate.

7. There is no future for agrification crops in The Netherlands; research on these crops, however, should be stimulated in The Netherlands.

8. The total is sometimes more, sometimes less than the sum of the components.

9. Spate irrigation is a unique technology in some low rainfall areas of Eritrea. It has allowed the nomads to become sedentary farmers in the marginal ecosystems of Eritrea.

10. Those who do not remember the past are condemned to repeat it.

Propositions belonging to the PhD thesis of Tadesse Mehari,
Manipulating the physiological quality of *in vitro* plantlets and transplants of potato.

Wageningen, 5 December 2000

ABSTRACT

Tadesse Mehari, 2000. Manipulating the physiological quality of *in vitro* plantlets and transplants of potato. Doctoral thesis, Wageningen University and Research Centre, Wageningen, The Netherlands, 230 pp., English and Dutch summaries.

In vitro techniques have been introduced in potato seed production systems in recent years. This research project aimed at studying the morphological and physiological changes in plants and crops in the last three phases of a seed production system that included an *in vitro* multiplication, an *in vitro* normalisation (growing cuttings to rooted plantlets), a transplant production, and a tuber production (field) phase.

Leaf area was identified as an important plant parameter for plant growth in the normalisation and transplant production phases. Explants and plantlets with larger initial leaf area performed better than those with smaller initial leaf area. *In vitro* treatments mainly affected leaf area of transplants through their effects on early above-ground leaf area. Leaf area increase was better described by logistic than by exponential or expolinear curves in all phases of growth, suggesting restriction of leaf area increase in all phases.

Low temperature decreased leaf and stem dry weights in all phases, and increased tuber fresh and dry yields, average tuber weight, leaf/stem ratio, specific leaf area and harvest index in the tuber production phase.

Growing *in vitro* plants at low normalisation temperatures increased leaf and total plant dry weights early in the transplant production and tuber production phases. It resulted in higher tuber yields, heavier individual tubers and higher harvest index.

Fertilising plants with higher nitrogen (40 versus 10 mg N per plant) during transplant production resulted in plants with higher groundcover in the field. This led to higher interception of solar radiation and higher tuber yield in one of the two experiments. Growing plants at higher temperature (26/20 versus 12/18 °C) during transplant production increased leaf area at the end of the transplant production phase. After transplanting to the field, it resulted in crops with higher groundcover, which intercepted more incoming solar radiation. Yield tended to be higher, but differences could not be assessed as statistically significant. A glasshouse experiment showed that high temperature during transplant production increased leaf and stem dry weights in the tuber production phase, but reduced tuber dry weights and harvest index when temperatures during tuber production were high. Thus, high temperature during transplant production may favour haulm growth and light interception in the field, but may also reduce dry matter partitioning to tubers.

Conditions in the tuber production phase were found to be of greater importance for final yield than conditions and treatments in earlier phases.

Strategies to optimise the production and use of propagules and transplants should focus on achieving leafy starting material, reducing stress during changes in environment and optimising conditions during tuber production. Production of transplants should be adjusted to the expected growth conditions in the tuber production phase.

Key words: *Solanum tuberosum* L., *in vitro* plantlet, seed production, normalisation, transplant production, tuber production, acclimatisation, leaf area, groundcover, logistic growth, temperature, nitrogen, dry matter production, specific leaf area, harvest index, radiation interception, radiation use efficiency.

LIST OF ABBREVIATIONS

AIR	Accumulated intercepted radiation (MJ m^{-2})
DAC	Days after cutting (d)
DAP	Days after planting (d)
DAT	Days after transplanting (d)
GC	Ground cover (mm^2, cm^2 or %)
HI	Harvest index (g g^{-1})
LA	Leaf area (mm^2 or cm^2)
LAI	Leaf area index (m^2 m^{-2})
N	Nitrogen
N	Normalisation phase
RH	Relative humidity (%)
RI	Relative increase in leaf area (increase/early leaf area)
RUE	Radiation use efficiency (g MJ^{-1})
SLA	Specific leaf area (cm^2 g^{-1})
TB	Tuber production (bulking) phase
TP	Transplant production phase

Logistic curve parameters

A	Fitted minimum leaf area (mm^2)
B	Initial relative rate of leaf area increase (day^{-1})
C	Fitted leaf area increment (mm^2)
M	Fitted curve mid-point (days after cutting or (trans) planting)
MI	Maximum rate of increase in leaf area at M (mm^2 day^{-1})

PREFACE

This thesis is a product of 4 years full-time PhD research work on potato tissue culture. The project was financed by MHO/NUFFIC as part of the linkage programme between the College of Agriculture and Aquatic Sciences (CAAS) of the University of Asmara, Eritrea and several institutions in The Netherlands including the Department of Plant Sciences, Wageningen University and Research (WUR), The Netherlands.

I sincerely hope that this thesis will, albeit modestly, contribute to the scientific knowledge of *in vitro* propagated transplant crops which are widely used as starting material in seed production systems in potatoes and many other crops. I would like to take this opportunity to express my sincere gratitude to the following individuals and institutions that directly or indirectly contributed to my work.

Special acknowledgement is due to the University of Asmara for granting me the scholarship to pursue my full time PhD study in The Netherlands, and special thanks go to WUR and especially to the Department of Plant Sciences for facilitating my research work and study.

First and foremost, I would like to express my heart-felt gratitude to my advisor Prof. Dr. Paul C. Struik for his day-to-day guidance, academic advice and for his continuous interest. I am very grateful for his unreserved support, concern and enthusiasm during the whole period of my work, which were encouraging, and for sparing much of his precious time editing and improving the manuscript. These simple words cannot, in any way, be adequate to explain the interest and enthusiasm of Paul.

I am deeply grateful to my co-advisor Dr. Willemien J.M. Lommen for her valuable guidance and continuous support both during my research work and in the preparation of the manuscript. Her door was always open for me to discuss whatever doubts I had in my mind.

I am greatly indebted to all the staff members of the Department of Plant Sciences (WUR) for their encouragement, support and expert advice. I really felt at home during my long stay in The Netherlands. Special thanks are due to Dr. E. Westphal for his continuous moral support and to Dr. K. Scholte and Dr. J. Vos for their academic advice.

I am also grateful to all the staff members of UNIFARM, without the continuous support and assistance of whom my research work would have been practically impossible. Mr. Herman Masselink and all the UNIFARM staff deserve special thanks. The TUPEA and Library staff at the Department of the Plant Sciences also deserve special thanks for their unreserved assistance and co-operation.

The liaison officer of the linkage programme, Drs. Jan van den Hoogen and his colleagues, Ms. Marianne Wijkniet, Ms. Barbara Rosemalen and Mr. Peter Holen

deserve special thanks for their continuous encouragement and for the administrative support they gave me to smoothly pursue my study.

My heart-felt thanks go to Ms. Gon Van Laar for her advice in the design of the lay out of my thesis and for her editorial suggestions. Special thanks are due to all my PhD colleagues in the department for their companionship and encouragement and especially to my office-mates, Maja Slingerland, Nick den Hollander and Harm Smit for their enthusiasm and support and, of course, also to Paula Westerman for her relentless weekend catering services. My colleagues from the University of Asmara, Woldeselassie Ogbazghi, Woldeamlak Araia and Mihreteab Tesfai also deserve special thanks for their companionship and encouragement.

The continuous encouragement and support I received from my Eritrean friends and their families in The Netherlands were actually my source of strength and helped me to feel at home during my stay in The Netherlands. I am, therefore, greatly indebted to the families of Negassi Sereke, Haile Habtagabir, Ali Salih, Samson Isack, Kahsai Tedros, Alamin Abdela, Semere Ghirmatsion, Asrat Kifle and Samuel Tekle and to my friends Solomon Ghebrekristos, Almaz Tesfai, Tekeste, Kibreab Woldeab, Kiflom Tesfamariam, Mehari Beyene and Angessom Habtenas.

I would also like to thank the Eritrean Consulate staff in The Hague for their unreserved encouragement and support.

Finally, I wish to express my everlasting sense of gratitude and love to my wife and kids, for their understanding and patience all the way throughout my stay in The Netherlands, I dedicate this thesis to them.

Tadesse Mehari

CONTENTS

CHAPTER 1

General introduction

General introduction

The potato plant

Potato is a Solanaceous plant which was given the Latin name *Solanum tuberosum* esculentum by Bauhin in 1596, a name essentially retained in the binomial *Solanum tuberosum* given by Linnaeus in his Species Plantarum in 1753. The potato originates from the highlands of South America where it has been an important food crop for a long time. It does not exist as a wild plant, other than as escapes and discards, and its wild origin is obscured by millennia of cultivation before it was introduced to Europe in the 16th century (Burton, 1989; Hawkes, 1990).

Potato is an annual plant about 30-100 cm tall and in general is vegetatively propagated through tubers. Like other Solanaceous genera, it shows considerable regenerative activity and produces three different types of stems – the leafy stems (sprouts), the stolons and the tubers. All of these possess apical and axillary buds which can potentially produce one of the above stems and it is this potential that can be exploited in the vegetative propagation of potato (Lommen, 1995).

Potato is grown in more than 70% of the countries of the world, it is the most important non-cereal world food crop and is next only to rice, wheat and corn as a major crop in terms of total production of fresh weight. In the past, most of the world potato production was limited to the temperate regions in the industrialised countries. However, during the last decades, potato production has been gradually shifting from the industrialised to the developing countries and from the temperate to the tropical and subtropical regions (Walker *et al.*, 1999). China has the largest area of potato, followed by Russia, Poland and India (Struik & Wiersema, 1999; Walker *et al.*, 1999).

Potato seed tuber production

Various methods are utilised for the production of potato seed tubers. These include seed tubers, plant regeneration from cell cultures such as protoplasts, callus etc. (Evans *et al.*, 1981), dissected meristems (Wang & Hu, 1980), stem and nodal cuttings (Goodwin *et al.*, 1980; Bryan *et al.*, 1981) and true potato seeds (TPS) (Umaerus, 1987). Next to the conventional seed production systems which utilise normal tubers, methods using nodal cuttings are preferred for seed tuber production purposes, because they conserve the genetic make-up of the parent plant since new plants are produced from existing buds (Lommen, 1995).

Conventional seed production

The conventional way of propagating potato involves the repeated multiplication of potato seed tubers. In many countries, healthy prebasic seed is produced by clonal selection, repeatedly propagating selected tubers which are pathogen-free and have the desired phenotype (i.e. 'true to type' plants). In areas where one crop of potato is grown in one year, the multiplication rate is only 12-20 per year (Beukema & Van der Zaag, 1990) as compared to 30 to 40 in wheat, 40 to 60 in barley and 150 to 240 in maize (Van der Zaag, 1990). Therefore, several years of field multiplication are required to produce the total quantity of potato seeds needed when starting from limited stock. In commercial varieties 10-15 % of the total area must be used for seed production (Struik & Wiersema, 1999).

From generation to generation the seed gradually degenerates mainly due to infection by viruses, the rate of degeneration varying from region to region and from cropping season to cropping season. In the high degeneration rate areas, seed that was initially clean (0 to 1% virus), after 1, 2 and 3 generations contains 10%, 45% and 100% virus, respectively (Beukema & Van der Zaag, 1990). Assuming that the seed should contain no more than 15-20% virus (Beukema & Van der Zaag, 1990), the clean seed introduced can only be multiplied once. This obviously limits potato seed production, especially in the high degeneration areas.

The main disadvantages of the conventional potato seed production system are: a) the slow and inflexible rate of multiplication of field-grown potato plants and b) the high risks of viral, bacterial and fungal infections with increasing numbers of field multiplications (Haverkort *et al.*, 1991). These disadvantages are impediments to a rapid introduction of new cultivars and to the production of high quality seed potatoes.

Production of in vitro plantlets

Several techniques have been developed over the past two to three decades to reduce the number of field generations and consequently the problems associated with the conventional seed production method. One of such techniques which is widely used in several seed production systems is the tissue culture technique or micropropagation (Hussey & Stacey, 1981). Tissue culture methods are nowadays widely employed to produce large quantities of healthy and genetically uniform *in vitro* plantlets for seed tuber production. As a tool for production of potato seeds, micropropagation has been expanding rapidly in the recent past and almost all seed producing areas in North America and Europe have either built one or modified the existing infrastructures to accommodate this type of propagation (Jones, 1988). The reasons for this are obvious:

a) large numbers of disease-free plants can be produced in a short period. Under laboratory conditions, multiplication rates of 10 to 25 fold per 8 weeks (Goodwin *et al.*, 1980) and 8 to 84 fold per 40 days (Marinus, 1983) are reported, b) losses due to pests, diseases etc. hardly occur, because production takes place under aseptic conditions, c) multiplication can take place whole year round in a small, controlled environment with easy storage of propagules (Struik & Lommen, 1990) and d) multiplication rate is constant within a variety – enabling good planning of the work (Marinus, 1985).

In vitro propagated plantlets are used as sources of starting material in several types of seed production systems. The fastest seed production system with the highest multiplication rate involves four phases (Fig. 1):

- Multiplication phase, where plantlets are propagated *in vitro* by producing single node cuttings;
- Normalisation phase, where single node cuttings develop into rooted *in vitro* plantlets;
- Transplant production (acclimatisation) phase, where rooted plantlets are acclimatised in a glasshouse; and
- Tuber production (field) phase, where transplants are grown in a field to produce seed tubers.

This system is also used in this thesis, albeit slightly modified for experimental purposes in some experiments.

This seed production system is very short, requiring only a few weeks between nodal cuttings and the transplant, for it does not require additional steps of *in vitro* tuber production, storage and/or pre-sprouting phases and it only involves one field cycle. This short production cycle is not only a great advantage for experimental purposes, but also when large quantities of large-sized seed tubers are needed for commercial purposes.

Much attention has also been given to the use of *in vitro* plantlets for the production of microtubers (*in vitro* tubers) or minitubers (tubers produced by *in vitro* plants in soil) (Hussey & Stacey, 1984; Lommen & Struik, 1995; Gopal *et al.*, 1998), but this remains out of the scope of the thesis.

The research project

Introduction

This research project was funded by MHO/NUFFIC as part of the linkage programme developed between the College of Agriculture and Aquatic Sciences (CAAS),

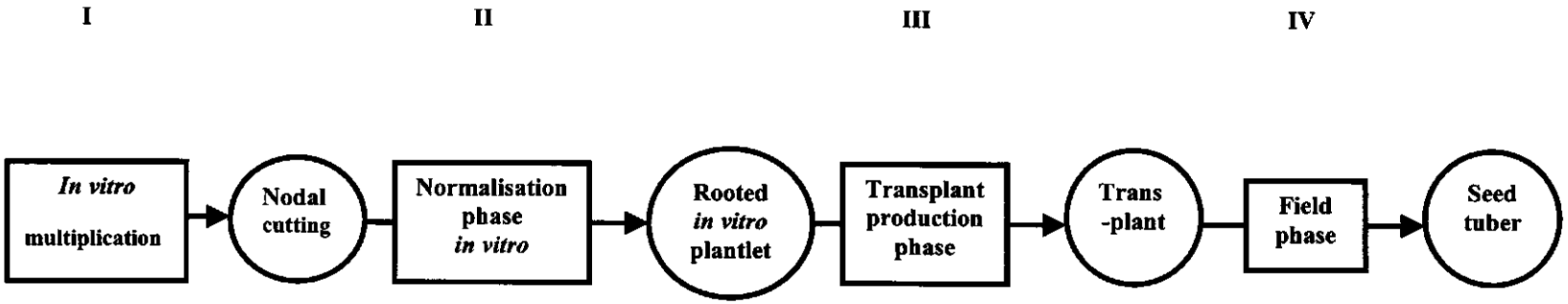

Fig. 1. A four-phase seed production system involving micropropagation (□ = Phase, ○ = End product of phase).

University of Asmara (UOA), Eritrea and several institutions in The Netherlands, including the Department of Plant Sciences, Wageningen University and Research Centre (WUR). One of the main objectives of the linkage programme is to equip Eritrea with the necessary scientific infrastructure, and to transfer the appropriate knowledge and technology, to enable it to develop and modernise its agricultural system and eventually attain food security and sustainability. Staff training was also one of the components of the linkage programme through which many CAAS staff members were trained at different levels to acquire the necessary scientific knowledge and expertise to enable them to tackle and solve problems related to agriculture independently so as to develop and modernise the agricultural sector of the country. The research project described in this thesis was partly the product of an analysis of research needs by a team of scholars from the WUR and the UOA, on the basis of these objectives (CAAS, 1996).

Although the majority of the farmers in Eritrea are subsistence farmers practising rain-fed agriculture, Eritrea has a long tradition of irrigated horticulture which is currently gaining much attention to produce fruits and vegetables using water that has been collected in newly built micro-dams throughout the country. Potato is selected as one of the priority crops to secure food sufficiency in Eritrea (World Bank, 1994). However, despite all the efforts made so far, potato production and yield remain low. One of the main reasons for this is the absence of a certified seed production system in the country. Poor quality, scarcity and high costs of potato seed tubers are some of the main constraints of potato production in Eritrea. Thus, developing a seed supply system that can ensure potato seed health and the availability of healthy seed tubers whenever farmers require them, is an important step forward. This can be achieved through micropropagation techniques where *in vitro* plantlets are multiplied rapidly to produce potato seed tubers (Hussey & Stacey, 1981). It is essential to identify and develop an appropriate seed production system that employs *in vitro* techniques and that also pays special attention to the vicissitudes of the *in vitro* plantlets after they leave the aseptic conditions.

Problem description

Plantlets are produced using various protocols in different laboratories (Goodwin & Brown, 1980; Miller *et al.*, 1985; Seabrook, 1987; Mastenbroek & Eising, 1987; Sipos *et al.*, 1988; Cournac *et al.*, 1991). Optimisation of conditions and techniques (Hussey & Stacey, 1981; Marinus, 1983) in the phase of *in vitro* multiplication has led to maximum numbers of nodal cuttings entering the normalisation phase. This part of the seed production system will therefore not receive further attention. Techniques and

conditions during normalisation may affect plant survival and field establishment when plants are directly planted to the field (Sipos *et al.*, 1988). Many authors believe that already at the end of the normalisation phase, the condition of the plantlets is essential for further growth and performance (e.g. Marinus, 1985; Mastenbroek & Eising, 1987; Kozai *et al.*, 1988). However, only a limited number of reports exist in which varying treatments in the normalisation phase (daminozide addition, Mastenbroek & Eising, 1987; Sipos *et al.*, 1988) or transplant production phase (changing the container volume, Thornton & Knutson, 1986) are shown to affect final tuber number or weight in the field. Unfortunately reported effects are generally not explained or further analysed, and results are not always consistent.

There is lack of information on how treatments in early phases affect (trans)plant growth, and on how the different end products lead to differences in growth in the next phase. There is also a gap of knowledge as to how treatments in previous phases influence growth of plantlets in subsequent phases and the final yield in the field. This gap of knowledge makes it difficult to design suitable protocols and to predict multiplication rates and final yield of seed tubers in the field.

Research objectives

The main objectives of this research project are to understand and analyse:

1. The important morphological and physiological changes that take place at the plant and crop level in the normalisation, transplant production and tuber production phases of the four-phase seed production system;
2. The way in which these changes are affected by different treatments during a phase; and
3. The way changes in a certain phase are affected by different treatments in earlier phases.

To realise these objectives, the following activities were conducted:

- Description of growth and development in different phases.
- Identification of relevant plant parameters that determine growth of the plantlets in the various phases and finally affect seed tuber yield.
- Analysis of the influence of these parameters on growth and yield.
- Manipulation of *in vitro* plantlets and transplants using different treatments to optimise plant growth in later stages and final yield.

The knowledge thus acquired will help to manipulate propagules at different propagation stages in order to direct growth and development, and affect yield in the last phase of the production system, the tuber production phase.

Thesis structure

Effects of *in vitro* treatments on leaf area growth of potato transplants during transplant production are examined in Chapter 2. This chapter further deals with the importance of leaf area of *in vitro* propagated plantlets for further growth during transplant production and the after-effects of *in vitro* treatments. It aims at establishing the relationship between leaf area of *in vitro* propagated plantlets at the beginning of the transplant production phase and leaf area of the plants at the end of this phase. It also deals with the way this relationship is affected by different cultivars and treatments *in vitro* and establishes the direction of the treatment effects in this relationship.

The following five chapters deal with characterisation of plant growth over the three phases – normalisation, transplant production and tuber production. In these chapters growth and development of *in vitro* propagated plantlets are assessed over the different growth phases in relation to time and temperature. The first chapter of these five, Chapter 3, focuses on leaf growth and development and analyses the changes in leaf area and leaf number that take place in the different phases of growth. Growth curves are plotted to describe leaf area and leaf number increase in all three phases of growth. Chapter 4 also deals with leaf area development in the three phases. It relates the parameters describing leaf area increase to the initial and final leaf area using linear correlations. Chapter 5 assesses the mutual relationships among the curve parameters themselves. Chapter 6 focuses on yield analysis and describes dry matter production under different temperatures, and explains how temperature conditions applied to the previous phases of growth affect dry matter production. The last chapter in this series, Chapter 7, explains how yield determining parameters such as tuber number, average tuber size, tuber fresh weight and harvest indices are affected by temperature in the current and previous phases.

Chapter 8 and 9 include investigations on the effects of pre-treatments during transplant production on the performance and yield of the transplants in the field. Chapter 8 deals with the effect of nitrogen pre-treatment and explains how pre-treatment of *in vitro* propagated plantlets with different levels of nitrogen during transplant production affects growth of the transplants and yield in the field. Chapter 9 assesses the effects of temperature pre–treatment in the transplant production phase on growth and performance of transplants and yield formation of the plants in the field.

The general discussion (Chapter 10) focuses on leaf number development over time in the different phases of growth, on leaf area as an indicator of quality of explants, plantlets and transplants and on the detailed analysis of leaf area growth. It also highlights how leaf area can be manipulated in the different production phases

and explains the side effects of leaf area manipulation. The importance of the different phases of growth for the seed production system is discussed. Finally, reflections are made on the original objectives.

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CHAPTER 2

Effects of *in vitro* treatments on leaf area growth of potato transplants during acclimatisation

Published (with minor adjustments of terminology) as:

Tadesse M., W.J.M. Lommen & P.C. Struik, 2000. Effects of *in vitro* treatments on leaf area growth of potato transplants during acclimatisation. *Plant, Cell, Tissue and Organ Culture* 61: 59-67.

2. Effects of *in vitro* treatments on leaf area growth of potato transplants during acclimatisation

Abstract

The importance of leaf area of *in vitro* propagated potato (*Solanum tuberosum* L.) plantlets for further growth during acclimatisation and the after-effects of *in vitro* treatments on growth were examined. The *in vitro* treatments included different levels of alar, nitrogen or mannitol or different temperatures during the last *in vitro* phase, the normalisation phase. Leaf area or ground cover was recorded one day after planting to soil and at the end of the first phase of *ex vitro* growth, the acclimatisation phase. Regression analysis showed that leaf area of a transplant at the end of acclimatisation phase was positively influenced by leaf area of the same plantlet at the beginning of the phase. The relative increase in leaf area during acclimatisation (increase/early leaf area) was linearly related to the inverse of the early leaf area, indicating almost comparable relative increases for plantlets having larger early leaf areas, but more variable responses for plantlets having smaller early leaf areas. *In vitro* treatments mainly affected leaf area of transplants through their effects on early leaf area. Adding alar, reducing nitrogen and reducing temperature increased leaf area. Reducing mannitol increased ground cover. A lower nitrogen concentration and higher temperature in some cultivars had slight negative effects on the relative increase in leaf area after acclimatisation. For nitrogen these negative effects were less significant than the positive effects through early leaf area. Results stress the importance of manipulation of leaf area *in vitro* to enhance plant performance in later stages of growth.

Key words: alar, *in vitro* plantlet, mannitol, nitrogen, normalisation, temperature.

Introduction

In vitro propagated potato (*Solanum tuberosum* L.) plantlets are used as starting material in various seed tuber production systems. Following the routine multiplication of *in vitro* plantlets, nodal cuttings, for example, can be used to produce rooted plantlets *in vitro* during the normalisation phase. These rooted plantlets are subsequently acclimatised *ex vitro* in a glasshouse to produce transplants in the acclimatisation phase before they are transplanted to the field to produce seed tubers (Marinus, 1983; Wattimena *et al.*, 1983).

The status of the plantlet at the end of the *in vitro* phase is important as it may affect further performance (Marinus, 1985; Mastenbroek & Eising, 1987; Kozai *et al.*,

1988; Hagman, 1990), but the mechanisms of such effects are still unknown. Leaves are the site of determinant physiological processes occurring in plantlets, including photosynthesis and transpiration. We therefore surmise that the leaf area at the onset of the acclimatisation phase is a good predictor of that status, whereas the rate of leaf initiation and growth reflects the vigour of young plantlets. As long as a young, vegetative plant is grown without inter-plant competition and free of stress, later leaf area is related to early leaf area (Goudriaan & Van Laar, 1994). Transferring an *in vitro* plantlet to *ex vitro* conditions, however, may be a traumatic event even when the *ex vitro* environment is stress-free, due to the morphology and anatomy of the plantlets (Grout, 1988; Sutter *et al.*, 1992).

Different experiments have shown that potato leaf area could be influenced by different treatments. Adding alar (daminozide) in a low concentration to the *in vitro* medium often leads to stronger plantlets with well-developed leaves (Marinus, 1985). Alar basically results in plantlets with shorter internodes, with darker green leaves and stems, and with shorter and more uniformly distributed roots (Sipos *et al.*, 1988). Reducing nitrogen content of a medium may increase leaf area and promote stem elongation (Charles *et al.*, 1992; Zarrabeitia *et al.*, 1997) and may increase chlorophyll content (Zarrabeitia *et al.*, 1997). This is in contrast to the general effect of nitrogen on normal potato crops. Higher temperatures *in vitro* basically increase the number of stem internodes (Hussey & Stacey, 1981; Caligari & Powell, 1989). However, in potato plantlets high temperature also develops a more normal habitus with leafy shoots instead of a stoloniferous habitus with scale leaves (Hussey & Stacey, 1981). High temperatures in normal potato plants reduce total leaf area (Ewing & Keller, 1982; Nagarajan & Bansal, 1990; Struik & Ewing, 1995). Mannitol addition to the *in vitro* medium results in a significant decrease in dry matter accumulation (Lipavska & Vreugdenhil, 1996), shoot growth (Siddiqui *et al.*, 1996) and root development (Harvey *et al.*, 1994). Along with leaf characteristics, all these treatments also may affect other plantlet characteristics relevant to further plant development.

More insight is, therefore, needed on how leaf area at the onset of the *ex vitro* phase may affect leaf area at the end of the acclimatisation phase and on additional effects of *in vitro* treatments on growth during acclimatisation. Such information would help to optimise techniques to manipulate plantlets in the normalisation phase to improve their performance in the phases of acclimatisation and field growth.

The aim of the study was to establish the relationship between leaf area of *in vitro* produced plantlets at the beginning of the acclimatisation phase and the leaf area of the transplants at the end of this phase, and the effects of different treatments *in vitro* on this relationship.

Materials and methods

Routine micropropagation

In vitro potato plantlets of cultivars Gloria, Spunta and Elkana were routinely multiplied every 4-5 weeks by single-node cuttings using virus-free stock plantlets cultured on a standard medium containing basic MS salts (Murashige & Skoog, 1962) with vitamins (2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 0.50 mg l^{-1} nicotinic acid, 0.50 mg l^{-1} pyridoxine HCl and 0.10 mg l^{-1} thiamine HCl), 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). Viable nodes were cut from the plantlets, discarding tops, and cultured in 25×150 mm culture tubes on 10 ml medium, one nodal cutting per tube. The tubes were closed with polycarbonate caps, sealed with household plastic foil, and placed at 23 °C and a photophase of 16 h supplied with Philips TL 84 fluorescent tubes with a photosynthetic photon flux density of 30 µmol m^{-2} s^{-1}.

Normalisation phase

Single-node cuttings were grown to rooted plantlets during 21 days under the same conditions as during routine multiplication, except for the variations due to the experimental treatments.

Experiment 1. Alar. Cuttings were grown in petridishes (8 per dish) on 20 ml of the standard medium containing alar or without alar.

Experiment 2. Nitrogen. Cuttings were grown on standard medium with one of the following three nitrogen levels: 0.84 (control), 1.42 and 2.00 g l^{-1} N. The control contained 0.84 g l^{-1} N available in the form of KNO_3 (1.90 g l^{-1}) and NH_4NO_3 (1.65 g l^{-1}) in the MS medium while an additional 0.58 or 1.16 g l^{-1} N in the form of NH_4NO_3 was added to the two highest nitrogen levels.

Experiment 3. Temperature. Cuttings were grown on standard medium in petridishes (8 per dish) at 17, 20, 23 or 26 °C in separate growth cabinets.

Experiment 4. Mannitol. Cuttings of cultivar Spunta were grown in petridishes on standard medium with either 0, 1 or 3% mannitol.

Acclimatisation phase

Rooted plantlets were planted into small round pots (8.5 × 9.0 cm, w × d; Experiment 1) or transplant trays with cells (4.0 × 5.5 × 6.0 cm, w × l × d; other experiments) containing potting soil. Approximately half of the stem was left above the soil. In Experiment 1, only the largest plants available for each treatment were planted. Plants were grown for 13 days each in a glasshouse (Experiments 1, 2, 3) or walk-in growth chamber (Experiment 4). Temperature and photoperiod conditions during acclimatisation were always within the range optimal for short-term vegetative shoot growth in potato. However, the environmental condition of the glasshouse/growth chamber depended on demands by other users and, thus, differed slightly among experiments. Day/night temperatures were 20/8, 18/12, 18/12 and 20/14 °C and photophases were 12 h, 16 h, 14 h and 16 h in Experiments 1, 2, 3 and 4, respectively. Relative humidity in all experiments ranged between 70 and 80%. Day light was supplemented by SON-T bulbs in the glasshouse, while light was supplied in the walk-in growth chamber by SON-T and HPI-T lamps and fluorescent tubes with a light intensity of 385 μmol m^{-2} s^{-1}. We are convinced that the range of conditions does not affect the relationships described in this paper, but absolute leaf growth rates during acclimatisation are not comparable over experiments. Each plant received 10 ml of a low-concentrated Steiner solution (Lommen & Struik, 1992) three times a week.

Experimental designs

In the acclimatisation phase, Experiment 1 was carried out in a completely randomised design with 16 replications and comprised of 2 (alar treatments) × 3 (cultivars) × 16 (replications) = 96 individual plants. Experiment 2 was carried out in a randomised block design in 16 blocks and each treatment was replicated three times within a block. Thus the experiment contained 3 (nitrogen treatments) × 3 (cultivars) × 16 (replications) × 3 (individuals per treatment-cultivar combination per replication) = 432 plants. Experiment 3 was carried out in a split-plot design with 16 blocks in which temperature treatments were randomised within cultivars in the main plots. Each treatment was replicated twice within a block. Thus, there were 4 (temperature treatments) × 3 (cultivars) × 16 (replications) × 2 (individuals per treatment-cultivar combination per replication) = 384 plants in the experiment. Mannitol treatments in Experiment 4 were randomised within 16 blocks and each treatment was replicated 4 times within a block. Thus, there were 3 (mannitol treatments) × 16 (replications) × 4 (individuals per treatment per replication) = 192 plants.

Measurements and statistical methods

During transplant production, leaf area (LA) was estimated by measuring the area of individual leaves of a plant using a transparent sheet with small (1×1 mm) and large (5×5 mm) grids (for bigger leaves) at 1 and 13 days after planting (DAP). In the mannitol experiment ground cover (GC), instead of LA, was estimated at 1 and 13 DAP using a miniature ground cover measuring device. The two methods are basically similar and equally accurate but they result in different variables. The measurement on Day 1 yielded the 'early leaf area', whereas the measurement on Day 13 gave the 'late leaf area'.

Data were subjected to analysis of variance (ANOVA) using Genstat 5 release 3.22 (1995) and differences between treatments were analysed by LSD tests at $P<0.05$. The relative increase in LA or GC (RI) was calculated as the increase in LA or GC between 1 and 13 DAP, divided by the LA or GC at 1 DAP. Simple regression analyses were carried out for data on individual plants to establish the relationship between early LA and late LA or RI, and to determine the contributions of early LA to the variance in late LA. Multiple regression analyses were used to assess the combined contributions of early LA or GC and experimental factors to the variance in late LA or GC and to assess whether *in vitro* treatments, cultivars and their interaction affected late LA at the end of transplant production only through their effects on early LA or whether other effects were involved as well. Intercepts and regression coefficients of fitted lines were compared using the t-test.

Results

Effects of in vitro *treatments on leaf area in the acclimatisation phase*

Adding alar to the normalisation medium led to a significantly higher LA of the plantlets in all three cultivars one day after plantlets were transferred to the glasshouse (1 DAP) and at the end of the acclimatisation phase (13 DAP) (Table 1). The cultivar effect was significant at 1 DAP but not at 13 DAP. There was no significant interaction between cultivars and treatments at either date.

Leaf area decreased with increasing nitrogen concentration in the normalisation medium for all three cultivars at the beginning (1 DAP) and at the end (13 DAP) of the acclimatisation phase. The largest contrast, however, existed between the lowest (0.84 g l^{-1}) and the two highest (1.42 and 2.00 g l^{-1}) nitrogen treatments (Table 1). A significant interaction at both 1 and 13 DAP indicated that cultivar Elkana suffered much more from increasing levels of nitrogen in the medium than Spunta and Gloria.

Table 1. Leaf area (Experiments 1, 2 and 3) or ground cover (GC) (Experiment 4) (mm^2) of *in vitro* derived potato plantlets of three cultivars at 1 and 13 DAP, as affected by various pre-treatments during normalisation.

	1 DAP			13 DAP		
	Gloria	Spunta	Elkana	Gloria	Spunta	Elkana
Experiment 1						
With Alar	79.4	84.7	95.3	2072	2400	2141
Without Alar	35.6	49.1	57.8	1528	1356	1572
LSD (5%)	10.5/12.8 [a]			272 [b]		
Experiment 2						
0.84 g l^{-1} N	30.3	51.2	79.3	371	526	998
1.42 g l^{-1} N	20.9	42.0	47.2	250	483	559
2.00 g l^{-1} N	21.4	39.6	39.4	217	362	514
LSD (5%)	9.3			131		
Experiment 3						
17 °C	40.6	73.3	56.7	867	1736	1595
20 °C	43.8	52.8	45.8	897	1073	968
23 °C	33.9	39.8	35.5	557	751	768
26 °C	22.2	31.3	32.5	338	598	661
LSD (5%)	10.5			270		
Experiment 4						
0% mannitol		37.3			2477	
1% mannitol		28.5			1965	
3% mannitol		10.0			1160	
LSD (5%)		5.3			263	

[a] first LSD for comparing treatment means, second one for comparing cultivar means

[b] LSD for comparing treatment means

A higher temperature during normalisation resulted in transplants with smaller LA in all cultivars at 1 and 13 DAP (Table 1). A significant interaction between cultivars and temperature treatments at 1 and 13 DAP indicated there was no difference in LA in Gloria between 17 °C and 20 °C at both dates, whereas for Spunta and Elkana differences between these two temperatures were large and LAs were lower with increasing temperature over the entire range investigated (Table 1).

Increasing mannitol concentration to the normalisation medium resulted in a significant decrease in GC of the transplants both at 1 and 13 DAP (Table 1).

Effects of early leaf area on growth during acclimatisation

When data on individual plants for all treatments and cultivars were combined for each experiment, those plants with higher LAs at the end of the acclimatisation phase already had higher LAs at the beginning of the phase, over the whole range tested (Fig. 1). Regression analysis indicated that a positive relationship existed between the early LA at 1 DAP and late LA at 13 DAP in the acclimatisation phase in all experiments. Linear, 2nd order polynomial, logistic or sigmoid and exponential fits all were highly significant (Table 2). Differences in coefficients of determination (R^2) were small. This suggests that the increase in LA or GC with time for individual plants was different for plants having different early LA or GCs. R^2-values were only slightly higher when early LA was combined with experimental factors in the regression model than when early LA alone is taken.

Fig. 2 shows the relation between the relative increase (RI) in LA or GC (i.e. the increase in LA or GC over the acclimatisation phase, divided by the early LA or GC) and the early LA or GC for different plants. Simple regression analysis over all data points, fitting the RI (y) to the inverse of the early LA or GC (1/x), showed higher RIs for plants with the smaller early LA or GCs in the experiments in which alar, nitrogen and mannitol were varied in the *in vitro* medium, and smaller RIs for plants with small early LAs when temperature was varied (Table 3).

After-effects of* in vitro *treatments on LA or GC increase during acclimatisation

If treatments in the normalisation phase have after-effects on the RI during acclimatisation, they affect the relation between early LA or GC and RI. Multiple regression analysis was carried out to check this. The proportion of the variance accounted for by adding the variables of pre-treatment with alar or mannitol to the regression model (data not shown) was not significantly improved. This suggests that alar and mannitol only affected late LA or GC through their effect on early LA or GC in these experiments. In the other experiments, adding cultivar, pre-treatment and their interaction to the model significantly improved the percentage variance accounted for (data not shown). This implies that the effects of these pre-treatments on the relation between early LA and RI existed, but depended on the cultivar.

Separate fits for individual nitrogen treatments within a cultivar (Table 4) showed no differences between fitted curves in cvs Gloria and Elkana. Only in cv. Spunta was there a lower constant for the lowest nitrogen level compared to the intermediate nitrogen level, implying a lower RI at the higher early LAs. Also a high regression coefficient was found in this cultivar for the lowest nitrogen pre-treatment

Table 2. Coefficient of determination (R^2) of the curves fitting LA or GC at the end of acclimatisation (y) to the early LA or GC after planting to soil (x) and after combining experimental factors (treatment × cultivar) with early LA or GC, for individual transplants produced under different conditions *in vitro*, in four experiments. Values for most suitable relations are underlined.

Experiment	n	R^2 of different relations[a]				Fitted line[b]	R^2 after adding experimental factors to the selected regression model[a]
		Linear	2nd order polynomial	Logistic/ sigmoid	Exponential		
1. Alar	96	<u>0.481</u>	0.486	0.480	0.487	$y = 770 + 16x$	0.512
2. Nitrogen	432	<u>0.633</u>	0.635	0.634	0.635	$y = 26 + 10.7x$	0.655
3. Temperature	384	<u>0.665</u>	0.665	0.664	0.665	$y = -96 + 23.3x$	0.700
4. Mannitol	192	0.258	<u>0.271</u>	0.268	0.273	$y = 800 + 56.5x - 0.384x^2$	0.297

[a] all R^2 values are significant at $P<0.001$

[b] fitted lines are linear for the alar, nitrogen and temperature experiments and polynomial for the mannitol experiment

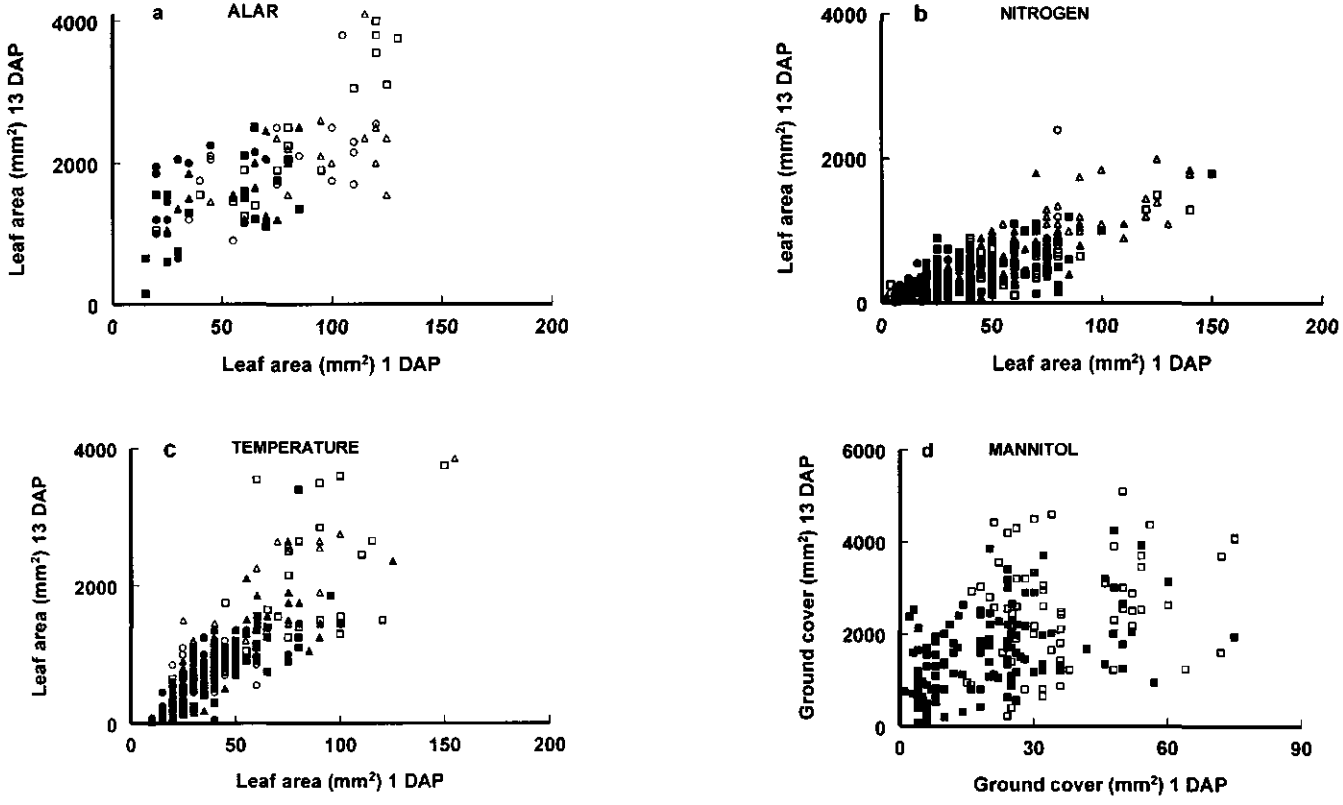

Fig. 1. Relationship between LA/GC at 1 and 13 DAP for *in vitro* treated plantlets of cultivars Gloria (○), Spunta (□) and Elkana (Δ) in the alar (a), nitrogen (b), temperature (c) and mannitol (d) experiments. (Alar: open = with alar, dark = without alar; Nitrogen: open = 0.84, grey = 1.42 and dark = 2.00 g l^{-1}; Temperature: open =17, grey = 20, dark = 23 and 26 °C; Mannitol: open = 0, grey = 1 and dark = 3%).

Table 3. Fitted line and coefficient of determination (R^2) of the relation between the relative increase (RI)[a] in LA during acclimatisation (y) and the early LA after planting to soil (x), for individual transplants, produced under different conditions *in vitro* in four experiments. All fitted constants and regression coefficients and all R^2 values are significant at $P<0.001$.

Experiment	n	Fitted line	R^2
1. Alar	96	$y = 15.75+744.2\times1/x$	0.405
2. Nitrogen	432	$y = 8.93+50.04\times1/x$	0.113
3. Temperature	384	$y = 24.73-187.0\times1/x$	0.105
4. Mannitol	192	$y = 34.62+952.2\times1/x$	0.543

[a] RI: increase in LA/GC between 1 and 13 DAP, divided by the early LA/GC at 1 DAP.

level (Table 4), implying a sharp decrease with increasing early LAs.

The pre-culture temperature treatments in the normalisation phase showed no differences in RI in cv. Elkana (Table 4). There was only a small effect of the temperature in the other cultivars. In those temperature treatments where there were differences (17 °C versus higher temperatures in cv. Gloria, and 20 °C versus 23 °C in cv. Spunta) they indicated a lower RI at higher early LAs when the temperatures were lower. Estimates for regression coefficients in this experiment often were negative, especially at higher temperatures, suggesting RI increased with increasing early LA (Table 4). However, estimates were generally not significantly different from zero in Spunta or Elkana. When differences between regression coefficients within one cultivar were significant (17 °C versus higher temperatures in cv. Gloria, 17 and 20 °C versus 23 °C, and 20 °C versus 26 °C in cv. Spunta and 17 °C versus 20 or 26 °C in cv. Elkana), coefficients were higher at the lower temperatures, indicating a sharper decrease or less strong increase with increasing early LA.

Discussion

Leaf area at the beginning of the acclimatisation phase was found to be an important characteristic for achieving a high leaf area at the end of this phase (Fig. 1), accounting for about 50% in the alar and for more than 60% in the nitrogen and temperature experiments to the percentage variance in late LA (Table 2). For the characteristic ground cover, assessed in the mannitol experiment, the percentage accounted for was lower, but still highly significant. The relationship between the early and late leaf area

Table 4. Regression lines fitting the relative increase in LA during acclimatisation (y) to the early LA after planting to soil (x), for individual transplants from three cultivars produced under different conditions *in vitro* in two experiments.

In vitro treatment	Curve parameters (y= a0 + a1 × 1/x)					
	Gloria		Spunta		Elkana	
	Intercept (a0)	Slope (a1)	Intercept (a0)	Slope (a1)	Intercept (a0)	Slope (a1)
Experiment 2						
0.84 g l^{-1} N	7.23 a *** [a,b]	73 a ***	4.65 a ***	212 b ***	9.84 a ***	137 a *
1.42 g l^{-1} N	9.92 a ***	19 a NS	9.82 b ***	29 a NS	9.14 a ***	88 a *
2.00 g l^{-1} N	7.59 a ***	9 a NS	6.56 ab ***	84 a NS	10.17 a ***	59 a ***
Experiment 3						
17 °C	7.33 a NS	543 b **	20.82 ab ***	144 bc NS	21.88 a ***	244 b NS
20 °C	25.65 b ***	–254 a *	13.67 a ***	281 c NS	24.24 a ***	–140 a NS
23 °C	26.49 b ***	–372 a ***	28.65 b ***	–424 a ***	24.30 a ***	–125 ab NS
26 °C	22.38 b ***	–181 a **	24.36 ab ***	–175 ab NS	24.92 a ***	–241 a NS

[a] different letters indicate that estimates differ from each other over nitrogen or temperature treatments within one cultivar (t-test: $P<0.05$).

[b] the second significance indicates how likely the constant or regression coefficient differs from 0 (***: $P<0.001$, **: $0.001 \leq P<0.01$, *: $0.01 \leq P<0.05$, NS: not significant $P \geq 0.05$).

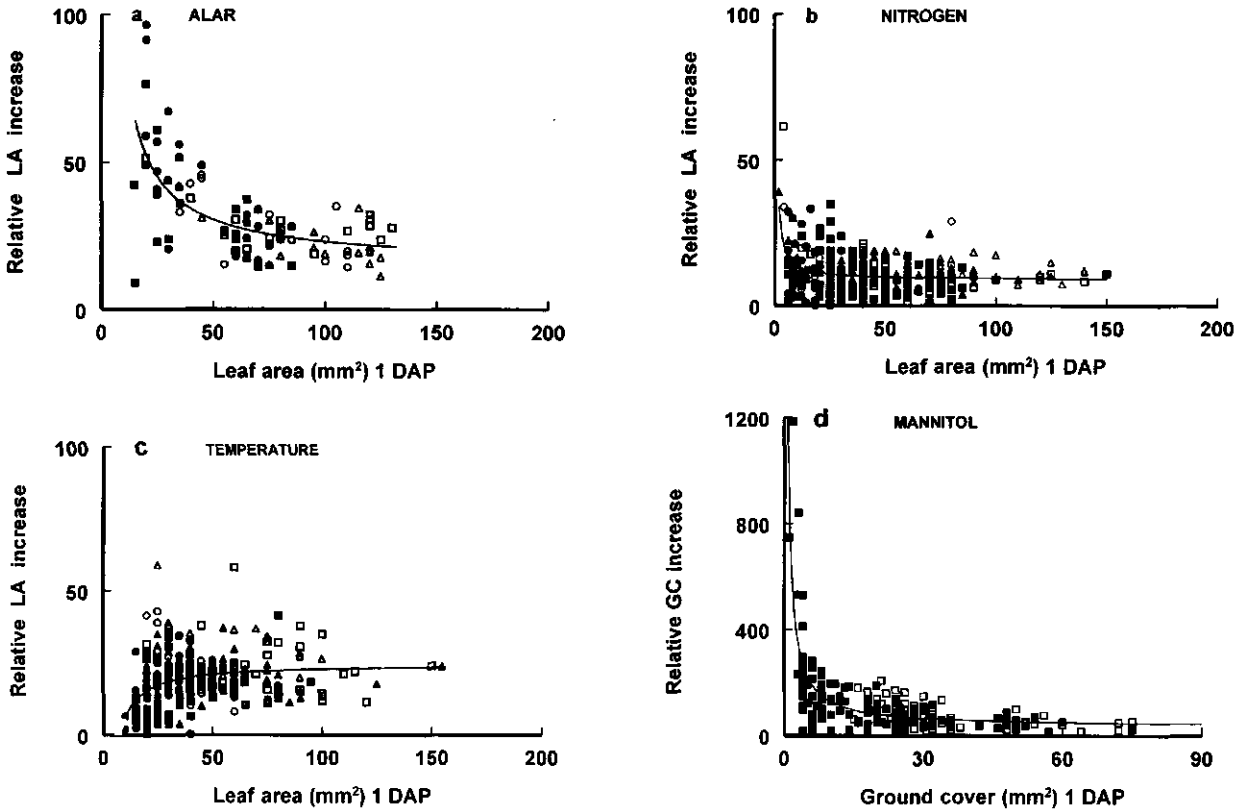

Fig. 2. Relationship between LA/GC at 1 DAP and the relative increase (RI: increase in LA/GC between 1 and 13 DAP, divided by the LA/GC at 1 DAP) for *in vitro* treated plantlets of cultivars Gloria (○), Spunta (□) and Elkana (Δ) in the alar (a), nitrogen (b), temperature (c) and mannitol (d) experiments. (Alar: open = with alar, dark = without alar; Nitrogen: open = 0.84, grey = 1.42 and dark = 2.00 g l^{-1}; Temperature: open =17, grey = 20, dark = 23 and 26 °C; Mannitol: open = 0, grey = 1 and dark = 3%). For fitted lines, see Table 3.

parameters was satisfactorily described by a linear curve in the alar, nitrogen and temperature experiments and by a 2^{nd} order polynomial curve in the mannitol experiment (Table 2).

The RI (relative increase in LA or GC) during acclimatisation also depended on the early LA or GC. The best fit was for a linear relation between RI and the inverse of the early LA or GC (Table 3). The relationships indicate almost comparable RIs for plantlets with intermediate to large early LAs in the range investigated (Fig. 2). Plantlets with smaller LA or GC had higher (Experiments 1, 2, 4) or lower (Experiment 3) RIs (Fig. 2, Table 3). Higher RIs under our experimental conditions are likely for plantlets suffering less from transplant shocks, or having more leaves in the expansion phase. Smaller plantlets will have fewer and less developed leaves and thus will show higher RIs than larger plantlets. However, the high variation among plantlets with small early LAs and the variable response over experiments (Fig. 2), even for control treatments (Table 3), suggest that these small LA plantlets also can be less vigorous. This may be because of more damage during handling, or of incomplete development or of high shoot:root ratios at transplanting.

In vitro treatments affected LA or GC at the end of the acclimatisation phase mainly through their effects on LA or GC at the beginning of the phase (Fig. 1, Table 2). Adding alar increased early LA of transplants, which is consistent with the findings of Marinus (1985). The higher early LA resulted in a concomitant increase in late LA at the end of acclimatisation phase (Table 1, Fig. 1). Alar affected late LA only through its effects on early LA and had no after-effects on LA increase during acclimatisation.

As was also reported by Charles *et al.* (1992) and Zarrabeitia *et al.* (1997), LA of plantlets increased with decreasing nitrogen concentration in the *in vitro* medium, leading to plantlets with higher above-ground LAs at the beginning of acclimatisation (Table 1), which also lead to higher LAs at the end of acclimatisation (Fig. 1, Table 2). In addition to the large positive effect of low nitrogen concentration on early and late LA, there was a small negative after-effect on the RI in cv. Spunta (Table 4). This may have been caused by the low nitrogen concentrations in the leaves of plantlets grown on the low nitrogen level, leading to lower relative leaf expansion rates.

The decrease in LA with increasing temperature during normalisation (Table 1) is consistent with literature on potato cultivation under controlled conditions (Struik & Ewing, 1995), but not with the observation from Hussey & Stacey (1981) on more leafy shoots being formed at higher temperatures. Increased temperature during normalisation reduced late leaf area at the end of acclimatisation through lowering early above ground leaf area (Fig. 1, Table 2). Also negative effects were observed on RI for some combinations of cultivar and temperature (Table 4). This is likely due to

the reduced leaf primordia size and subsequent leaf expansion. Also enhanced senescence of older leaves was observed at higher temperatures.

Mannitol addition *in vitro* decreased the early GC after planting to soil (Table 1), which is consistent with the negative effects of mannitol reported for dry matter accumulation and shoot growth (Lipavska & Vreugdenhil, 1996; Siddiqui *et al.*, 1996). There was no after-effect of mannitol on RI. Mannitol, therefore, reduced late LA of potato plantlets at the end of the acclimatisation period only through its effects on early LA.

Implications

The results stress the importance of a high early LA for achieving potato plantlets with high LA at the end of the acclimatisation phase. The early LA can be increased by specific treatments *in vitro,* but probably also by shallower planting or using older plants for transplanting. Still unknown is whether treatments *in vitro* aiming at high LA may have long-term after-effects in later growth stages. Results of experiments to test this will be reported in the near future.

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CHAPTER 3

Characterization of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Leaf area and leaf number

Submitted as:

Tadesse M., W.J.M. Lommen, P.E.L. Van der Putten & P.C. Struik, 2000. Characterization of plant growth of *in. vitro* propagated potato plantlets over three phases of culture under different temperatures: Leaf area and leaf number.

3. Characterization of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Leaf area and leaf number

Abstract

To assess leaf area and leaf number development of potato plantlets over three phases of growth, *in vitro* plantlets produced at 17 or 23 °C for 21 days (normalisation phase) were grown in soil in growth chambers at 18/12 or 26/20 °C for 14 days (transplant production phase), and after transplanting in glasshouses at 18/12 or 26/20 °C for 42 days (tuber production phase).

Boosts in leaf area and leaf appearance occurred in the first three days after planting to soil in the transplant production phase. A shock in leaf area increase occurred after transplanting to the tuber production phase. Leaf area increase was better described by logistic than by exponential or expolinear curves in all phases of growth and temperature treatments both for plant averages and for most individual plants, indicating growth limitation in all phases. The growth limitation was less severe during the relatively short transplant production phase as shown by later mid-points of the logistic curve and higher percentages of individual plants following exponential or expolinear growth than during other phases. Higher temperatures did not significantly increase leaf area during normalisation, increased leaf area during transplant production, and first increased but later reduced leaf area during tuber production. After-effects of normalisation temperature occurred during transplant production but not during tuber production. After-effects of transplant production temperature occurred during tuber production. These effects were direct – affecting leaf numbers and/or area at the beginning of the next phase – or appeared later.

Key words: potato, *Solanum tuberosum* L., *in vitro* plantlet, curve fitting, leaf area, leaf number, leaf appearance, leaf expansion, logistic growth, temperature, after-effects, transplant shock.

Introduction

Conventional potato seed production systems have low rates of multiplication and carry a high risk of disease infection with increasing number of field multiplication (Haverkort *et al.*, 1991). Micropropagation techniques have widely been introduced during recent decades to overcome these disadvantages (Jones, 1988; Struik & Wiersema, 1999). These techniques produce large numbers of disease-free plants

within a short period of time and losses due to infection hardly occur since production takes place under aseptic conditions.

In vitro produced plantlets are used as starting material in different seed production systems. The fastest seed production scheme with the highest multiplication rate per unit time, which was used in this study, involves four phases: **multiplication phase** (where plantlets are initiated *in vitro* from single-node cuttings), **normalisation phase** (where single-node cuttings develop into rooted *in vitro* plantlets), **transplant production** (or **acclimatisation**) **phase** (where rooted plantlets are acclimatised *ex vitro,* e.g. in a glasshouse, to produce transplants) and the **field** (or **tuber production**) **phase** (where transplants are grown in the field to produce seed tubers).

In practice, various conditions and methods are used to produce, grow and use *in vitro* plants in seed tuber production systems (Goodwin & Brown, 1980; Sipos *et al.*, 1988; Levy, 1988; Struik & Wiersema, 1999). It is not clear how these different protocols affect plant growth in the subsequent phase of a system or affect final yield in the field. Many of the protocols for earlier phases aim at maximum multiplication rates *in vitro* and maximum plant survival (e.g. Thornton & Knutson, 1986; Jones, 1988), but they often do not take into consideration what plant vigour might be in later stages of the scheme.

Tadesse *et al.* (2000a) recently showed that quantifying leaf area as affected by increases in leaf number and individual leaf area of *in vitro* plantlets is a meaningful way to express effects of conditions on vigour of plantlets and subsequent growth.

Vigour and growth during the normalisation, transplant production and tuber production phases mentioned above, as assessed in terms of leaf appearance, leaf expansion and dry matter accumulation in leaves need to be quantitatively described to understand the growth pattern of plants through the different phases. Such quantitative description also assists in understanding effects of conditions during various phases, to assess effects of transition from one phase to the other (shocks, boosts) and to quantify after-effects of conditions during one phase on growth during the next phase. Average trends and insights, into plant-to-plant variation developing during each phase are both relevant.

The aim of the current study is to assess growth and development of *in vitro* propagated plantlets over three phases of growth in relation to time. Temperature is varied in all three phases as the main environmental factor influencing growth and development. This chapter is the first of a series on this topic and focuses on development over time of leaf area and leaf number. Subsequent papers will relate initial leaf area at the beginning of each phase to subsequent performance and will analyse other aspects of growth and development.

Materials and methods

Plant culture and treatments

Potato (*Solanum tuberosum* L., cv. Gloria) plantlets were propagated *in vitro* by producing single-node cuttings using plantlets from virus-free stock. The plantlets were cultured on a standard medium containing MS salts (Murashige & Skoog, 1962), 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide), 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 0.50 mg l^{-1} nicotinic acid, 0.50 mg l^{-1} pyridoxine HCl and 0.10 mg l^{-1} thiamine HCl. Viable nodes were cut from plantlets (discarding tops) and cultured (one per tube) in sterilized 25 × 150 mm culture tubes containing 10 ml medium. The tubes, closed with polycarbonate caps, and sealed with household plastic foil, were placed at 17 or 23 °C and a photophase of 16 h supplied with Philips TL 84 fluorescent tubes with a photosynthetic photon flux density of 30 µmol m^{-2} s^{-1} for 21 days. This is the "normalisation" phase where single-node cuttings develop into rooted *in vitro* plantlets.

At the end of the normalisation phase, rooted *in vitro* plantlets were planted in transplanting trays with small cells (4.0 × 5.5 × 6.0 cm, w × l × d) filled with potting soil into growth chambers with day/night temperatures of 18/12 or 26/20 °C. A photophase of 14 h was supplied with a 1:1 ratio of SON-T and HPI-T lamps plus fluorescent tubes to improve light quality, providing an intensity above the plants of 420 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). Planting was done by leaving approximately half of the stem above the ground and each plant received 10 ml of a low-concentration Steiner solution (Lommen & Struik, 1992) three times a week. To avoid competition, only eight plantlets were planted per tray of 32 cells (75 plants m^{-2}). Plantlets were grown in the chambers for 14 days at a relative humidity of 80%. This is the phase where *in vitro* plantlets are acclimatised to *ex vitro* conditions to produce transplants and hence is referred to as the "acclimatisation" or "transplant production" phase.

The plants were then transplanted in 5-litre pots filled with potting soil to two glasshouses at a density of 16.0 plants m^{-2} to simulate the tuber production phase. Plants were spaced wider with time to 12.8, 9.6, and 6.4 plants m^{-2} at 7, 14, and 28 DAT, respectively. The glasshouses were kept at a day/night temperature of 18/12 or 26/20 °C and a relative humidity of 80%. The photophase was 16 h daylight supplemented with artificial light from SON-T light bulbs that provided a light flux density, above the plants, of 400 µmol m^{-2} s^{-1}. Transplants were grown under these conditions for 42 days in order to study their growth and development during the early stages of the 'tuber production' phase. The tuber production phase of the experiment

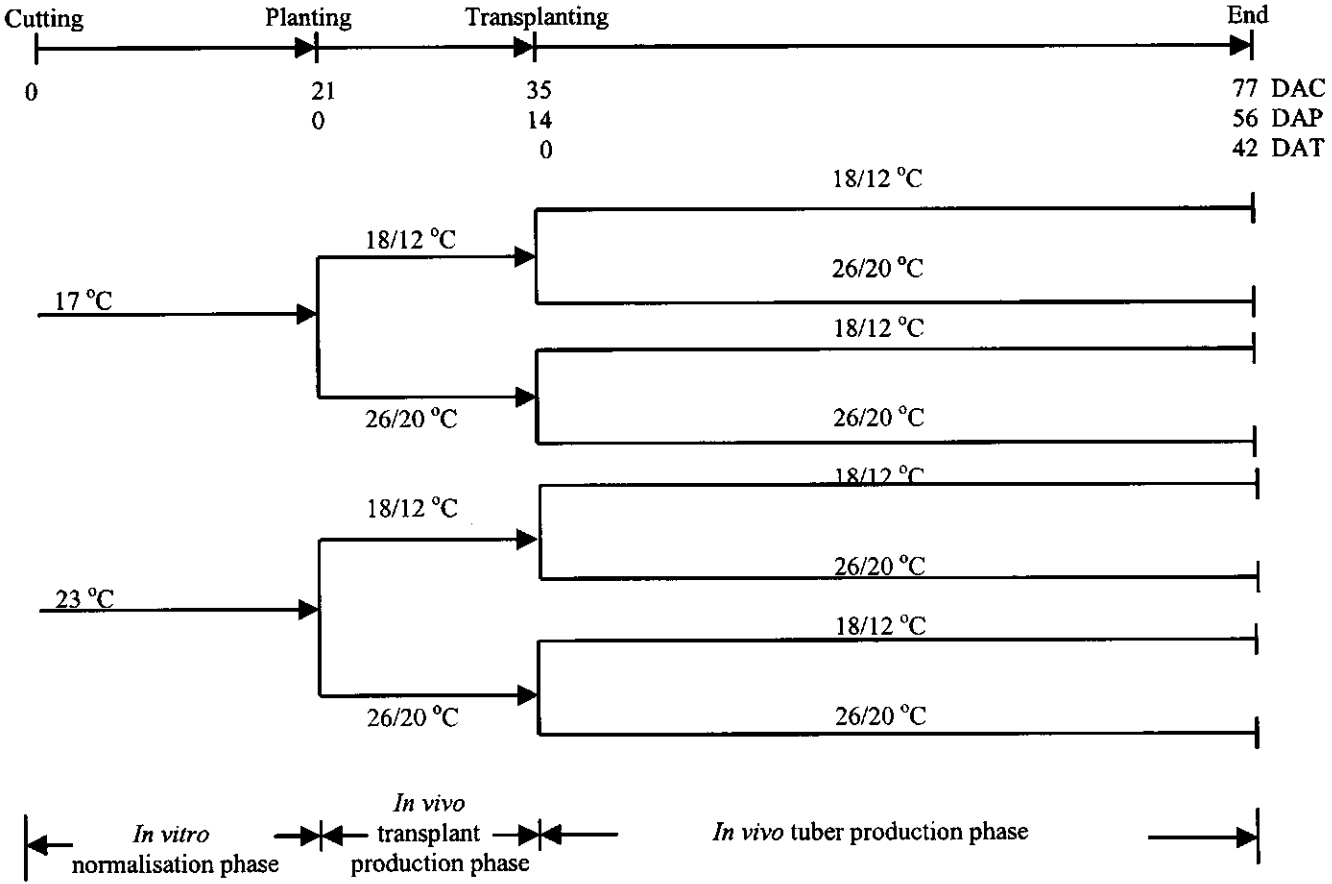

Fig. 1. Schematic representation of the different temperature treatments in the three growth phases. (DAC = days after cutting, DAP = days after planting, DAT = days after transplanting).

was carried out in Spring 1999.

Experimental design

The experiment was carried out in a split-split plot design in 16 blocks and comprised of 2 tuber production temperatures (TB) × 2 transplant production temperatures (TP) × 2 normalisation temperatures (N) × 16 replications. Temperature treatments of later phases were randomised within the temperature treatments of earlier phases of the experiment. The 128 plants reported in this study were used for frequent non-destructive measurements on leaf development and were part of a larger experiment with the same treatments, in total consisting of 992 plants, to allow for frequent destructive measurements. Fig. 1 illustrates the set-up of the experiment.

Measurements

Leaf area and leaf number of all individual plants were recorded before and after (trans)planting, every 3 days in the normalisation and transplant production phases and during the first week of the tuber production phase. From then on, measurements of all plants were taken every week. Green leaf area (including the explant leaf area in the normalisation phase) was non-destructively estimated using a transparent sheet with grids (1×1 mm or 5×5 mm) during the normalisation and transplant production phases, respectively. Leaf area during the tuber production phase was estimated by measuring the length and width of individual compound leaves and subsequently calculating leaf area using a shape factor (Biemond & Vos, 1992). Leaf number included the explant leaf in the normalisation phase. In later phases, only aboveground leaves of the main stem with a visible internode were counted. Measurements of leaf area on the first and last day of every phase gave the "initial leaf area" (ILA) and "final leaf area" (FLA), respectively, in all phases of growth.

Data processing and statistical analyses

Average leaf area (ln-transformed) and leaf number values were plotted against time over all three phases of growth in order to identify general patterns of growth, shocks or boosts upon transfer, after-effects of conditions during later phases and to quantify effects of plant competition in the final phase. Differences between ILA and FLA were determined using ANOVA. Average leaf area values for the different temperature treatments were plotted against time in the three phases and exponential, expolinear and logistic curves were fitted to determine the best fit. Differences in parameters

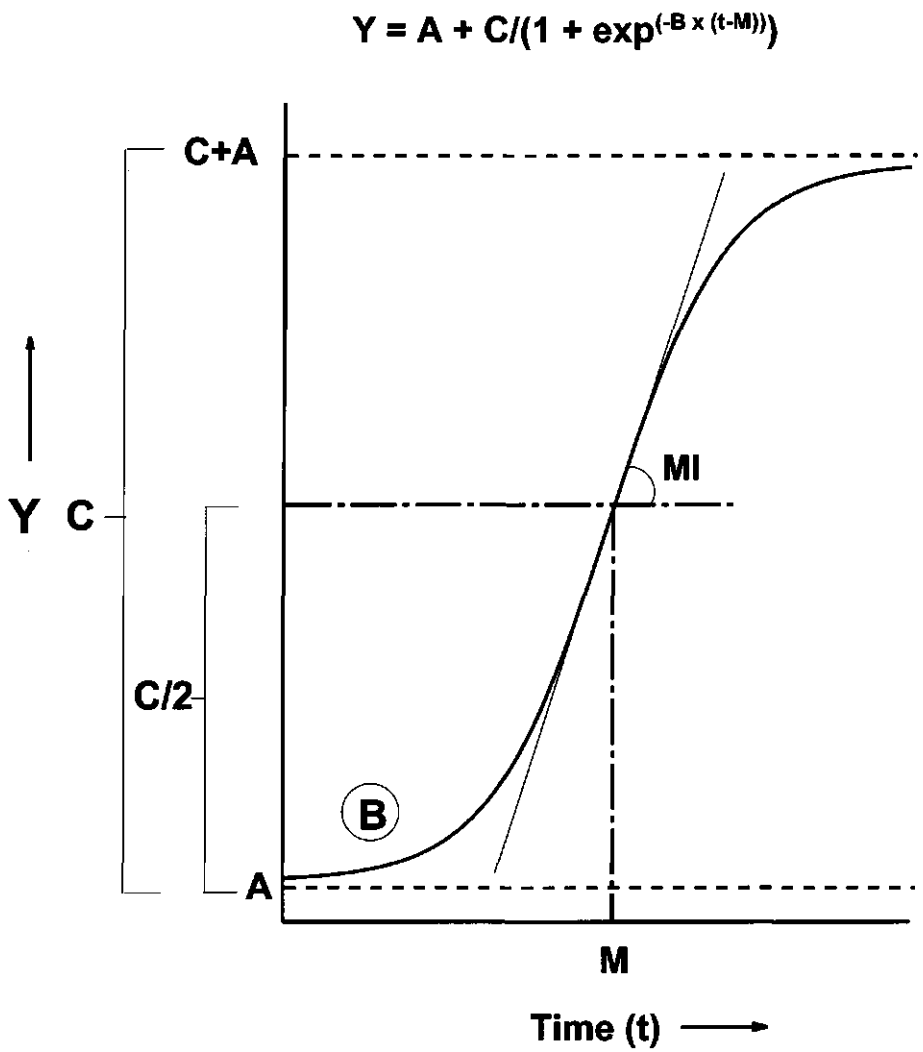

Fig. 2. Logistic growth curve and the different parameters describing the curve: fitted minimum growth (A), fitted increment (C), and fitted mid-point (M). MI is the maximum rate of increase at M and is calculated as B×C/4. B represents the initial relative rate of increase.

describing the logistic increase between temperatures and pre-treatments were determined using t-tests. Fig. 2 illustrates the logistic type of growth analysis and indicates parameters tested.

Exponential, expolinear and logistic curves were fitted to the leaf area points of all individual plants in the three phases and the best fits were determined on the basis of their r^2 values. Whether growth temperature affected the frequency of different growth patterns of individual plants was determined by Chi-square tests. Differences between ILA and FLA of logistically and non-logistically growing plants were tested by t-tests. Logistic fits had the highest r^2 in the majority of the plants in every phase of growth. Thus, initial and final leaf areas of plants following logistic growth and parameters describing this logistic growth were analysed separately by ANOVA and compared for the different temperature treatments in all three phases of growth using Genstat 5 release 3.22 (1995). Differences between treatments were analysed by LSD tests at $P<0.05$.

Results

Increase in leaf area and leaf number over three phases

The development with time of leaf area and main stem leaf numbers over all production phases: the *in vitro* normalisation phase, the transplant production phase and the tuber production phase are presented in Fig. 3. Between two phases, the planting of *in vitro* plantlets to soil and the later transplanting resulted in reductions in leaf area (Fig. 3A, only showing extreme treatments) and in number of aboveground leaves (Fig. 3B). During the first 3 days after planting to soil in the transplant production phase, boosts were observed in leaf area and leaf number increase. After transplanting to the tuber production phase a transplant shock reflected in a temporary fall back in the increase of leaf area, was observed, but not in leaf number.

Beyond the phases of the shocks and boosts after (trans)planting and before strong interplant competition (LAI $\approx$ 2) occurred, the increase in leaf area was not continuously exponential, as would be expected for vigorous unlimited growth, nor was the leaf number increase linear (Fig. 3). The development of leaf area and leaf number within a phase will be treated further for the phases separately, with the emphasis on leaf area.

In vitro normalisation phase

Averaged over all plantlets, leaf area during 3 weeks of *in vitro* growth increased

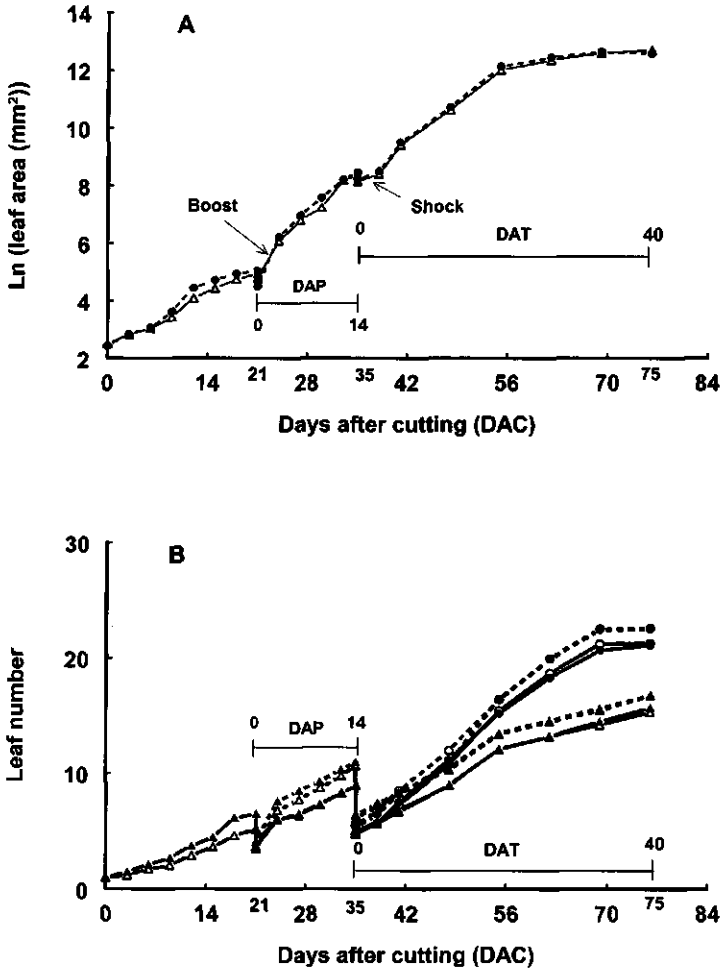

Fig. 3. Leaf area (A) and leaf number (B) increase at different temperature treatments through the normalisation, transplant production and tuber production phases. The markers follow the pattern listed below in all graphs (except in those where the leaf area or leaf number of individual plants is plotted): Normalisation phase: closed symbol = high, open symbol = low temperature; Transplant production phase: dotted line = high, solid line = low temperature; Tuber production phase: circle = high, triangle = low temperature. (Only the extreme treatments are shown in A).

logistically with time at both temperatures (Fig. 4). Logistic fits described the increase in leaf area better than exponential or expolinear fits. Plantlets did not differ significantly in initial explant leaf area (ILA) or in final leaf area (FLA) between the two temperatures after 21 days (Table 1), but the increase in leaf area was faster at 23°C than at 17 °C, as shown by the mid-point M of the fitted curve which was achieved almost 3 days earlier at the higher temperature (Fig. 4, Table 1). Other parameters characterizing the fitted curves (fitted initial leaf area (A), initial relative rate of increase (B) and fitted increment (C)) or the maximum rate of increase (MI) were not significantly different at the two *in vitro* temperatures (Table 1).

During normalisation, leaf appearance during the first 9 days was slower than thereafter (Fig. 4). A higher temperature during normalisation resulted in a higher rate of leaf appearance during the major part of the phase. Only in the middle part were differences between temperatures not obvious. As a result, leaf number at the end of the phase was higher at the high temperature.

Leaf area of individual plants also generally increased logistically, although some plantlets showed exponential, expolinear or aberrant increases (Table 2; Fig. 5). Temperature had no effect on the frequency at which the different types of increases occurred (Table 2). At 17 °C plantlets showing non-logistic increases in leaf area had a smaller explant leaf area than plantlets showing logistic increases, and had a smaller final leaf area at both temperatures (Table 2). Leaf number in individual plants often increased in steps of more than one leaf in the 3-days intervals between observations, whereas in other intervals there was no increase (Fig. 5). In some plants classified as outliers the increase in leaf number started late or was very limited (Fig. 5).

When only the logistically growing plantlets were compared after fitting curves to individual plantlets, temperature effects on leaf area increase were similar to those for plant averages. Explant leaf areas and final leaf areas did not differ significantly among temperatures, whereas at the higher temperature during normalisation plantlets reached the midpoint (M) of the fitted curve around 2 days earlier than at the low temperature (Table 3). Other parameters were not significantly different between the two temperatures.

Transplant production phase

During transplant production, the increase in above-ground leaf area averaged over plants was best described by a logistic curve, both at 26/20 and 18/12 °C during transplant production and after 17 or 23 °C during *in vitro* growth (Fig. 6, Table 4). At 26/20 °C, initial above-ground leaf area after planting was slightly lower than at 18/12 °C, but final leaf area was significantly higher (Table 4). Fitted curves at the higher

Table 1. Initial (ILA) and final (FLA) leaf areas of *in vitro* propagated potato plantlets grown at two temperatures in the normalisation phase *in vitro*, and parameters describing the logistic increase of average leaf area (y) with time (x) (n = 64).

			Parameters describing logistic growth $y = A + C/(1+\exp^{(-B \times (x-M))})$					
Temperature during normalisation	ILA (mm^2)	FLA (mm^2)	Fitted initial relative rate of increase (day^{-1}) (B)	Fitted mid-point (days after cutting) (M)	Fitted increment (mm^2) (C)	Fitted minimum leaf area (mm^2) (A)	Fitted maximum rate of increase ($mm^2\ day^{-1}$) MI	r^2
17 °C	12.8	148	0.27	15.3	157	9.2	10.6	0.99
23 °C	11.3	156	0.37	12.4	146	10.0	13.5	0.99
Significance [a]	NS	NS	NS	*	NS	NS	-	

[a] * $0.01 \leq P < 0.05$, NS not significant: $P \geq 0.05$

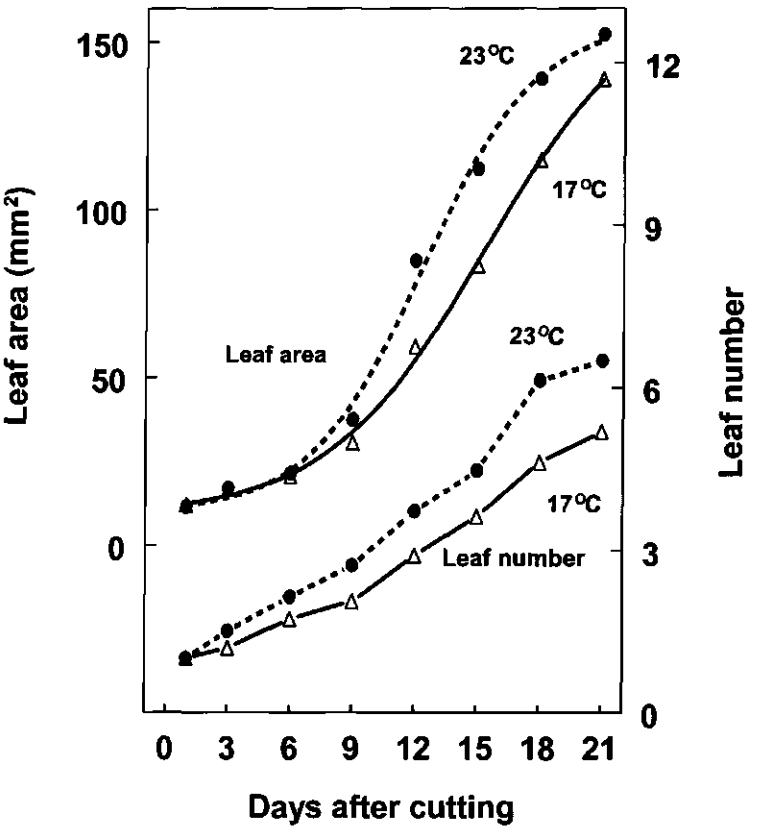

Fig. 4. Logistic increase with time of the average leaf area and increase in average leaf number of *in vitro* propagated potato plantlets, grown at two temperatures *in vitro*. For curve fitting and curve parameters, see Table 1.

temperature had lower B-values, lower fitted minimum values (A), higher increments (C), but did not differ in mid-point (M) or maximum rate of increase (MI) from those at the lower temperatures. Mid-points (M) were only reached between day 10 and 13 of the 14-day period in both temperatures.

The rate of increase in leaf number was fairly constant during transplant production after the boost in leaf numbers during the first 3 days after planting (Fig. 6). A higher temperature resulted in a stronger boost and higher increase in leaf number in the next 3 days but not thereafter.

Table 2. Number of best fits for different patterns of leaf area increase with time and the initial (ILA) and final (FLA) leaf areas of individual *in vitro* propagated plantlets showing logistic or non-logistic growth and grown at two temperatures *in vitro* in the normalisation phase.

Temperature during normalisation	Best fitting curve					ILA (mm^2)		FLA (mm^2)	
	Logistic	Expolinear	Exponential	Outlier	Total	Logistic	Non-logistic	Logistic	Non-logistic
17 °C	49	4	3	8	64	11.5 a [b]	3.2 b	147.8 a	36.0 b
23 °C	58	3	0	3	64	11.3 a	15.7 a	155.4 a	76.7 b
	$X^2 = 6.18$ (NS) [a]								

[a] X^2 - test on all categories

[b] different letters within a row and leaf area parameter denote a significant difference between logistic and non-logistic means ($P<0.05$).

Table 3. Initial (ILA) and final (FLA) leaf areas and growth parameters of *in vitro* propagated potato plantlets grown at two temperatures *in vitro* in the normalisation phase and that followed logistic growth (n = 49 at low and 58 at high temperature).

			Parameters describing logistic growth $y = A + C/(1 + \exp^{(-B \times (x - M))})$					
Temperature during normalisation	ILA (mm^2)	FLA (mm^2)	Fitted initial relative rate of increase (day^{-1}) (B)	Fitted mid-point (days after cutting) (M)	Fitted increment (mm^2) (C)	Fitted minimum leaf area (mm^2) (A)	Fitted maximum rate of increase ($mm^2\ day^{-1}$) MI	r^2
17 °C	12.0	148	0.37	15.0	163	10.4	13.9	0.99
23 °C	11.7	158	0.40	13.0	156	9.9	15.2	0.99
Significance [a]	NS	NS	NS	***	NS	NS	NS	

[a] *** $P < 0.001$, NS not significant: $P \geq 0.05$

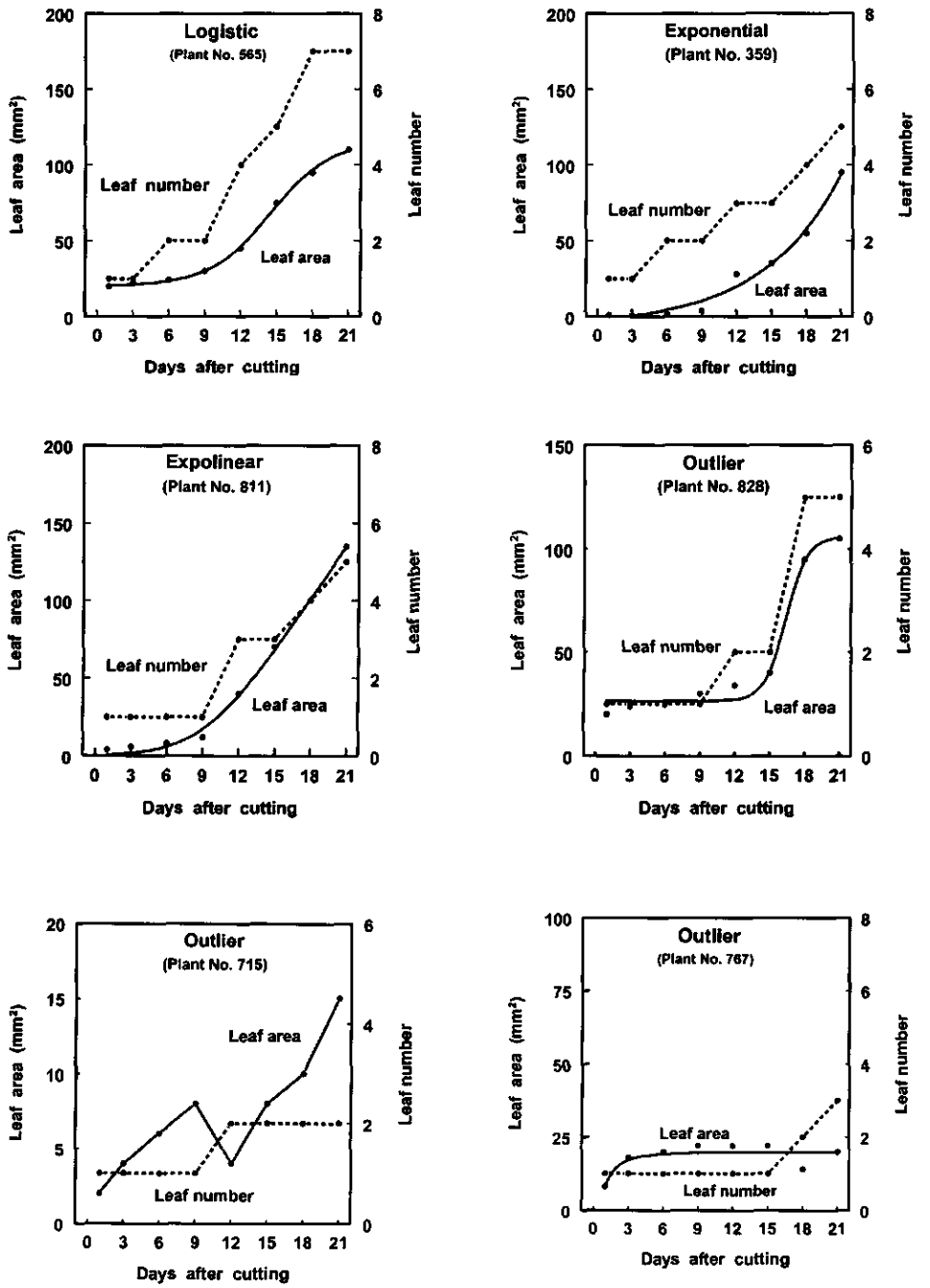

Fig. 5. Examples of different curves describing the increase in leaf area and leaf number with time, of individual plantlets during growth *in vitro* in the normalisation phase. (Note that the scale along the y-axis are different).

In general, leaf area of individual plants also increased logistically, but some plants showed exponential or expolinear increases (Fig. 7, Table 5). The frequency of the different types of curves did not depend on the temperature during transplant production (Table 5). Plants showing non-logistic increases did not differ significantly from plants showing logistic increases in initial above-ground leaf area or final leaf area after 14 days (Table 5). The boost in leaf number immediately after planting was also clear in individual plants (Fig. 7), but for some plants seemed to be followed by a check.

When only plants showing logistic increases in leaf area were compared, no differences in initial above-ground leaf area were present between the two transplant production temperatures, whereas final leaf area was higher at higher temperature (Table 6). A higher temperature also resulted in plantlets having lower B-values, lower fitted minimum values (A), higher fitted increases (C) and lower MI-values. The latter was especially clear when plants were pre-cultured at 17 °C during normalisation. Temperature during the transplant production phase had no effect on the time at which the mid-point M was reached, which was around 11 days after planting in all treatments (Table 6).

Pre-culturing plantlets at different normalisation temperatures did not result in plants with different leaf areas shortly after planting or at the end of the phase, for averages over all plants (Table 4) or for logistically growing plants (Table 6). Temperature during normalisation also did not affect fitted curve parameters in the next phase (Tables 4 and 6). The boost in leaf number seemed smaller for plantlets produced at high temperature during normalisation, but only when planted to the cool conditions during transplant production (Fig. 6).

Tuber production phase

Of the curve types tested, logistic curves again best described the increase in leaf area with time in the tuber production phase for plant averages, both at 26/20 and 18/12 °C during tuber production and for all four temperature pre-treatments (Fig. 8). Transplants started with a slightly smaller above ground leaf area at higher than at lower temperature. Leaf area at the higher temperature decreased at the end of the phase, resulting in lower final leaf areas at higher than at lower temperature (Table 7). Fitted curves at the higher temperature had higher B-values, earlier mid-points (M), smaller fitted increments (C), higher fitted start values (A) and higher rates of maximum increase (MI) than at the lower temperature (Table 7).

Table 4. Initial (ILA) and final (FLA) leaf area and parameters describing the logistic increase of average leaf area with time of *in vitro* propagated potato plantlets grown at two temperatures during transplant production (TP) after they were grown at two temperatures in the normalisation phase (N).

Temperature (°C) during:		n	ILA (mm^2)	FLA (mm^2)	Parameters describing logistic growth $y = A + C/(1 + \exp^{(-B \times (x-M))})$					r^2
TP	N				Fitted initial relative rate of increase (day^{-1}) (B)	Fitted mid-point (days after cutting) (M)	Fitted increment (mm^2) (C)	Fitted minimum leaf area (mm^2) (A)	Fitted maximum rate of increase ($mm^2\ day^{-1}$) MI	
18/12	17	32	116.8	4212	0.58	10.7	4538	323	658	0.97
18/12	23	26	104.1	3919	0.49	11.4	4749	239	582	0.97
26/20	17	32	93.5	5047	0.25	11.7	8337	-268	521	0.99
26/20	23	30	88.6	4688	0.28	12.8	8234	-82	576	0.99
Significance [a]										
N			NS	NS	NS [b]	NS	NS	NS	NS	
TP			*	***	*	NS	**	*	NS	

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$

[b] t - test for curve parameters carried out group-wise - low versus high temperature in the transplant production and normalisation phases.

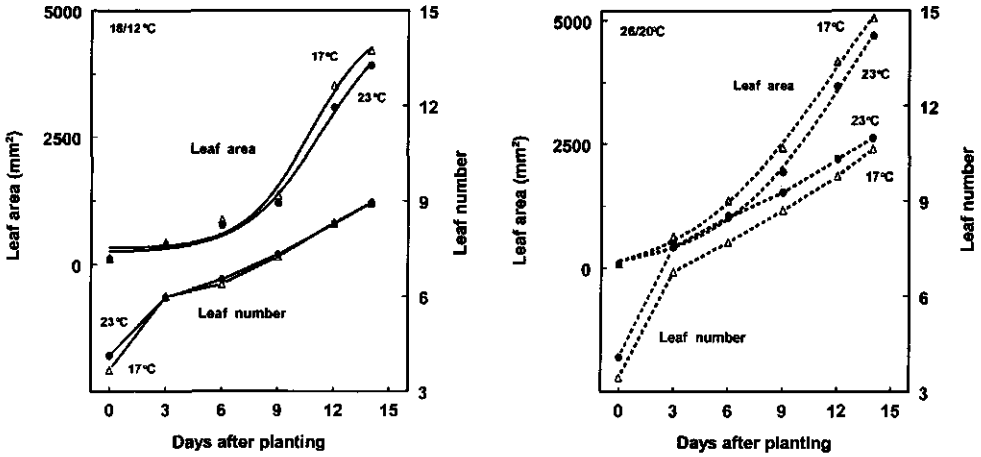

Fig. 6. Logistic increase with time of the average leaf area of *in vitro* propagated potato plantlets at two temperatures (18/12 or 26/20 °C) in the transplant production phase after they were grown at low (17 °C) or high (23 °C) temperatures during normalisation. For curve fitting and curve parameters, see Table 4.

The rate of increase in main stem leaf number in the tuber production phase was greater in the first three weeks than in the last three weeks for plants grown at 18/12 °C. By contrast, leaf appearance for plants grown at 26/20 °C was constant up to the last week, during which leaf number did not increase anymore (Fig. 8). A higher temperature during tuber production always resulted in a higher rate of increase.

The increase in leaf area of all individual plants was better described by a logistic curve than by an exponential or expolinear (Table 8, Fig. 9). The fitted midpoints (M) of the logistic curves were achieved earlier at higher than at lower temperature during tuber production. B-values, fitted minimum values (A) and maximum rates of increase (MI) were higher at higher temperature, and fitted increases (C) were lower (Table 9). The leaf number increase of individual plants also followed the pattern expressed by averages.

The initial aboveground leaf area at the start of the tuber production phase was higher for transplants that were raised at 26/20 °C than at 18/12 °C in the preceding transplant production phase, both for averages and for logistically growing plants. There was, however, no effect anymore at the end (Tables 7 and 9). For plant averages, the pre-treatment had no effect on any of the curve parameters of leaf area increase. For logistically growing plants, those raised at 26/20 °C had higher fitted A-values and smaller fitted increments in leaf area (C). Transplants produced at 26/20 °C increased faster in leaf number than transplants produced at 18/12 °C when grown at a high temperature during tuber production.

Table 5. Number of best fits for different patterns of leaf area increase with time and the initial (ILA) and final (FLA) leaf areas of individual *in vitro* propagated plantlets showing logistic and non-logistic growth and grown at two temperatures in the transplant production (TP) phase.

Temperature during TP	Best fitting curve			Dead plants	Total	ILA (mm^2)		FLA (mm^2)	
	Logistic	Expolinear	Exponential			Logistic	Non-logistic	Logistic	Non-logistic
18/12 °C	38	14	6	6	64	108.8 a [b]	139.2 a	4037 a	4776 a
26/20 °C	44	8	10	2	64	91.5 a	115.0 a	4938 a	5156 a
	$X^2 = 0.94$ (NS) [a]								

[a] X^2 - test on all categories

[b] same letters within a row and leaf area parameter denote no significant difference between logistic and non-logistic means of ILA or FLA (t-test: $P<0.05$).

Table 6. Initial (ILA) and final leaf area (FLA) and growth parameters of *in vitro* propagated potato plantlets grown at two normalisation (N) and two transplant production (TP) temperatures, that followed logistic growth in the transplant production.

					Parameters describing logistic growth $y = A + C/(1 + \exp^{(-B \times (x - M))})$					
Temperature (°C) during		n	ILA (mm^2)	FLA (mm^2)	Fitted initial relative rate of increase (day^{-1})	Fitted mid-point (days after planting)	Fitted increment (mm^2)	Fitted minimum leaf area (mm^2)	Fitted maximum rate of increase ($mm^2\ day^{-1}$)	r^2
TP	N				(B)	(M)	(C)	(A)	MI	
18/12	17	21	102.0	3970	0.73	10.7	4050	367	753	0.97
18/12	23	15	94.5	3875	0.63	11.4	4461	331	700	0.97
26/20	17	24	78.3	4899	0.33	10.6	7115	-218	562	0.99
26/20	23	18	96.6	4896	0.42	11.0	6589	15	679	0.99
Significance [a]										
N			NS	NS	NS	NS	NS	NS	NS	
TP			NS	***	***	NS	***	***	*	
N*TP			NS	NS	** (0.10) [b]	NS	* (689)	NS	NS	

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$

[b] numbers in brackets indicate LSDs for N*TP interaction

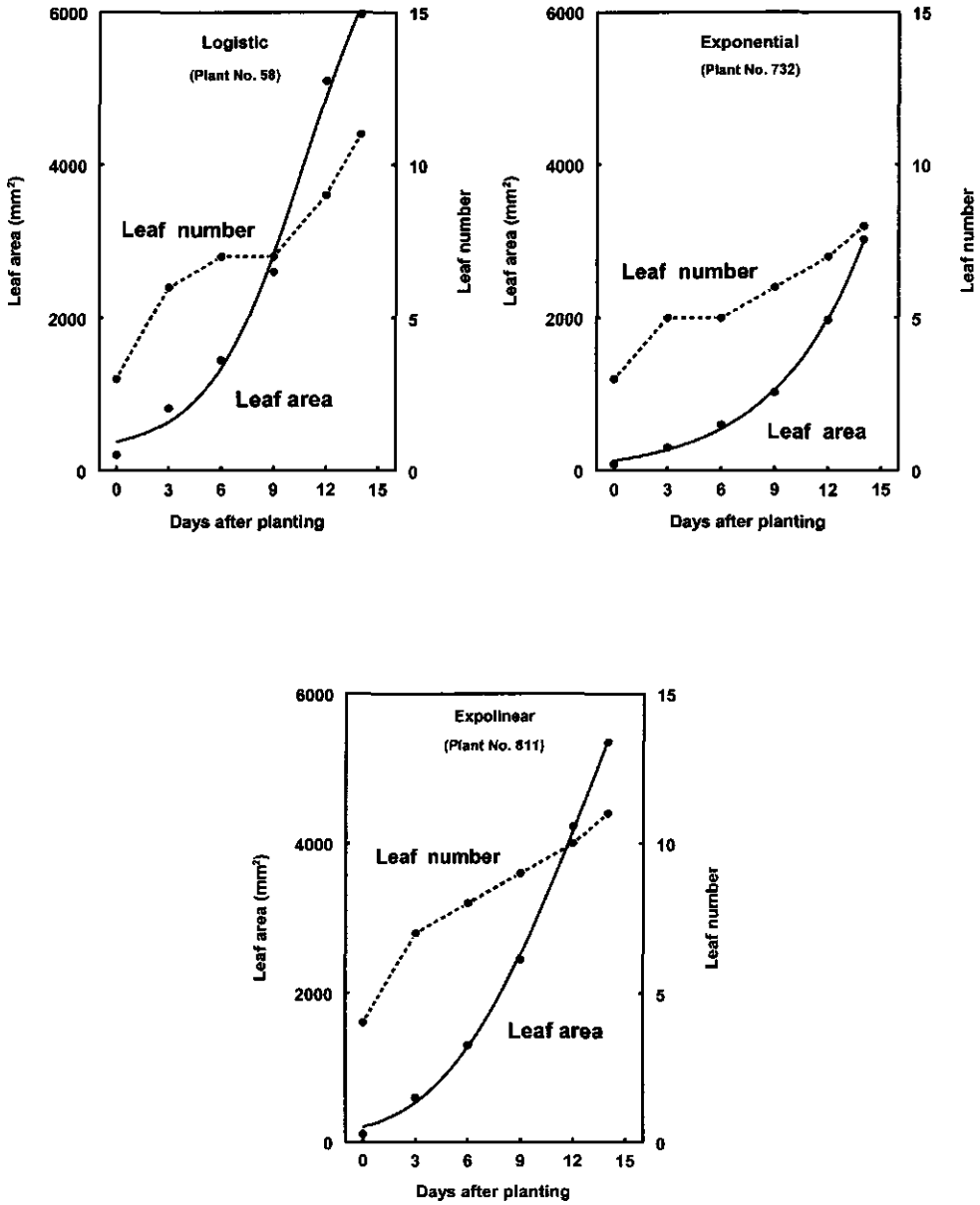

Fig. 7. Examples of different curves describing the increase in leaf area and leaf number with time, of individual plants during the transplant production phase.

Table 7. Initial (ILA) and final (FLA) leaf areas and parameters describing the logistic increase of average leaf area (y) with time (x) of *in vitro* propagated potato plantlets at two temperatures in the tuber production phase (TB) after they were grown at two temperatures in the normalisation (N) and transplant production (TP) phases.

Temperature (°C) During:			n	ILA (cm^2)	FLA (cm^2)	Parameters describing logistic growth $y = A + C/(1 + \exp^{(-B \times (x - M))})$					
						Fitted initial relative rate of increase (day^{-1})	Fitted mid-point (days after transplanting)	Fitted increment (cm^2)	Fitted minimum leaf area (cm^2)	Fitted maximum rate of increase ($cm^2\ day^{-1}$)	r^2
TB	TP	N				(B)	(M)	(C)	(A)	(MI)	
18/12	18/12	17	16	34.1	3183	0.18	20.9	3321	-98.2	149	0.99
18/12	18/12	23	14	33.8	3213	0.17	21.2	3397	-114.4	144	0.99
18/12	26/20	17	16	44.3	3007	0.18	20.4	3087	-86.1	139	0.98
18/12	26/20	23	15	41.6	3017	0.18	21.4	3147	-67.0	142	0.99
26/20	18/12	17	16	33.6	2865	0.29	18.4	2958	19.3	215	0.99
26/20	18/12	23	12	32.4	2800	0.29	18.2	2854	13.3	207	0.99
26/20	26/20	17	16	40.1	2699	0.29	18.2	2784	22.8	202	0.99
26/20	26/20	23	15	36.5	2809	0.29	18.4	2864	18.8	208	0.99
Significance [a]											
N				NS	NS	NS [b]	NS	NS	NS	NS	
TP				***	NS	NS	NS	NS	NS	NS	
TB				*	***	***	***	**	***	***	

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$; NS not significant $P \geq 0.05$ (all interactions were not significant: $P \geq 0.05$).

[b] t – test for curve parameters: low versus high temperature in the tuber production phase.

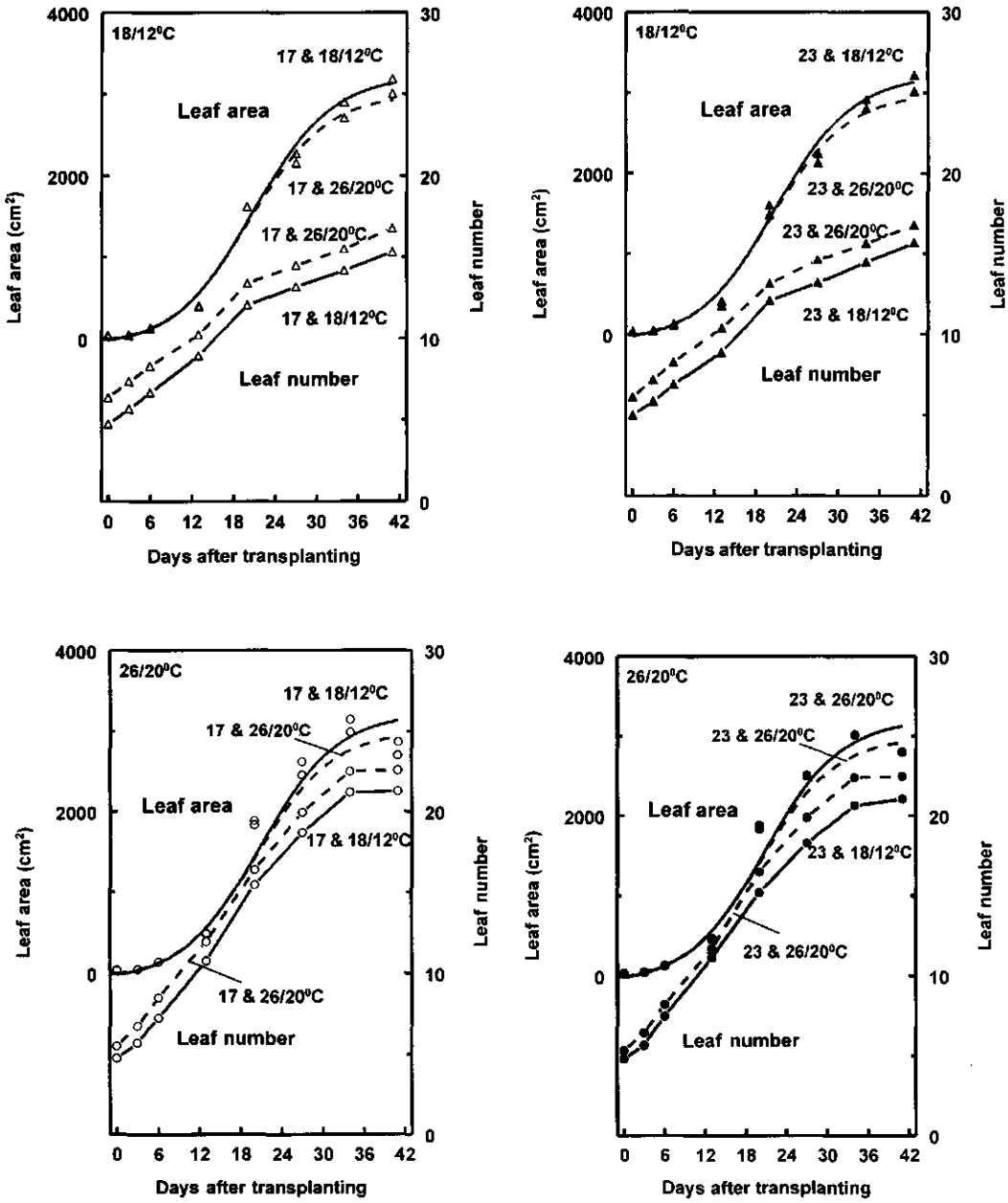

Fig. 8. Logistic increase with time of the average leaf area of *in vitro* propagated potato plantlets at two temperatures (18/12 or 26/20 °C) in the tuber production phase after they were grown at two temperatures (18/12 or 26/20 °C) in the transplant production and two temperatures in the normalisation (17 or 23 °C) phases. For curve fitting and curve parameters, see Table 7.

Table 8. Number of best fits for different patterns of leaf area increase with time and the initial (ILA) and final (FLA) leaf areas of individual *in vitro* propagated plantlets showing logistic or non-logistic growth and grown at two temperatures in the tuber production (TB) phase after they were grown at two temperatures in the transplant production and normalisation phases.

Temperature during TB	Best fitting curve				ILA (cm^2)		FLA(cm^2)	
	Logistic	Expolinear	Exponential	Total	Logistic	Non-logistic	Logistic	Non-logistic
18/12 °C	61	0	0	61	38.4	-	3105	-
26/20 °C	59	0	0	59	35.7	-	2797	-

There were no significant after-effects of normalisation pre-treatments in the tuber production phase.

Discussion

This discussion will first treat the best curve type describing the increase in leaf area in the different phases and the differences between fits for individual plants and averages. The boosts and shocks after transition to a new phase, the effects of temperature on development within phases and the after-effects of temperature on development in subsequent phases are discussed thereafter. Finally, the main conclusions are summarised.

Curve type

Increase in average leaf area with time was better described by logistic curves than by exponential or expolinear relations, with r^2-values being 0.97 or more in all phases (Tables 1, 4, 7). Exponential increase in leaf area would have been expected for plants growing in optimum conditions (e.g. Goudriaan & Van Laar, 1994). Logistic growth suggests that a maximum possible increment exists, and that the relative growth rate decreases linearly with the increase already achieved. Logistic curves therefore suggest that restrictions for leaf area increase occurred in all three phases. Insight in these restrictions may help to improve growth.

For the normalisation phase, total leaf area increase was probably limited by the shortage of O_2 for respiration, or CO_2 for assimilation, or of carbohydrates. The gas exchange in the sealed tubes could have been limited. This may reduce growth (cf. Cournac *et al.*, 1991), either through sub-optimal CO_2 or O_2 levels or by inhibitory compounds accumulating in the tubes. Probably also the supply of sucrose could be limited because of early enzymatic or chemical breakdown.

During transplant production, aboveground competition between plants was avoided by using only one out of each eight cells in the transplant tray. Because transplants received fertilisation in this stage total nutrient availability was not limiting growth. Insufficient rooting volume may have been the main limiting factor for leaf area increase. The growth restriction in this (relatively short) phase seemed less severe than in other phases, because a higher percentage of individual plants (32% versus 8 or 0%) was growing exponentially or expolinearly and the midpoint (M) of the logistic curve occurred only after about 11 days of the 14-day period.

During the tuber production phase, above-ground competition could not be avoided even though plants were spaced wider with time. Maximum leaf area indices

Table 9. Initial (ILA) and final (FLA) leaf areas and growth parameters of *in vitro* propagated potato plantlets grown at two normalisation (N), two transplant production (TP) and two tuber production (TB) temperatures and that followed logistic growth in the tuber production phase.

Temperature (°C) during:			n	ILA (cm^2)	FLA (cm^2)	Parameters describing logistic growth $y = A + C/(1 + \exp^{(-B \times (x - M))})$					r^2
						Fitted initial relative rate of increase (day^{-1})	Fitted mid-point (days after transplanting)	Fitted increment (cm^2)	Fitted minimum leaf area (cm^2)	Fitted maximum rate of increase ($cm^2\ day^{-1}$)	
TB	TP	N				(B)	(M)	(C)	(A)	(MI)	
18/12	18/12	17	16	34.1	3183	0.20	20.6	3285	-79.6	162	0.99
18/12	18/12	23	14	33.7	3214	0.18	21.3	3425	-113.9	154	0.99
18/12	26/20	17	16	44.3	3007	0.20	20.2	3080	-79.4	149	0.98
18/12	26/20	23	15	41.6	3017	0.20	21.1	3114	-45.0	156	0.99
26/20	18/12	17	16	33.1	2886	0.30	18.6	3014	9.7	221	0.99
26/20	18/12	23	12	33.2	2792	0.33	18.1	2858	20.5	227	0.99
26/20	26/20	17	16	40.1	2699	0.30	18.3	2804	20.6	209	0.99
26/20	26/20	23	15	36.5	2809	0.30	18.4	2870	19.2	213	0.99
Significance [a]											
N				NS	NS	NS	NS	NS	NS	NS	
TP				***	NS	NS	NS	*	*	NS	
TB				*	***	***	***	***	***	***	

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$ (all interactions were not significant: $P \geq 0.05$)

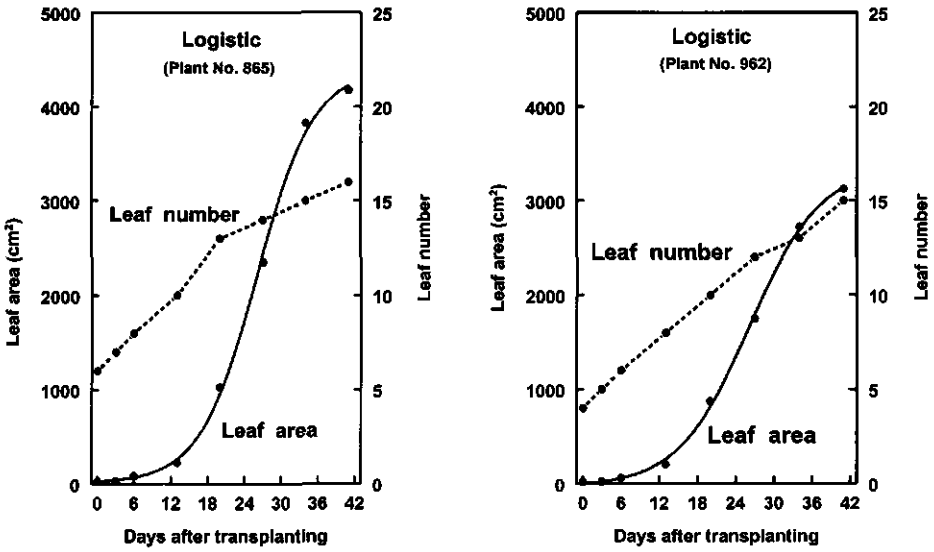

Fig. 9. Examples of logistic curves describing the increase in leaf area and leaf number with time, of individual *in vitro* propagated plants during the tuber production phase.

approached 2 for the first time after 3 weeks in the tuber production phase. Moreover, the decrease in leaf area caused by senescence at the end of the phase in the treatments growing at higher temperature (Fig. 7) will have forced the curve to take a logistic shape.

Leaf area increase of individual plants versus averages

In all phases, leaf area increase was more regular with time for plant averages (Tables 1, 4, 7) than for individual plants following logistic growth (Tables 2, 5, 8), as shown by lower B-values and lower maximum rates of increase (MI) for plant averages. This is likely as even small differences in onset of rapid growth of individual plants will level off the growth pattern found for all plants combined. Also outliers and plants following growth patterns other than logistic contributed to the growth patterns for plant averages in the first two phases. In the normalisation phase, these aberrant plants increased less in leaf area than plantlets following logistic growth (Table 2). They thereby also reduced the rate of increase for plant averages, and most likely the B- and MI-values. This also explains the lower increase (C) for plant averages (Table 1)

compared to the logistically growing plants (Table 2) in the normalization phase. Probable reasons for the lower FLA of non-logistically growing plants were a delayed shoot development or shock in development of new leaves (Fig. 5). In the transplant production phase, no difference in early or late leaf area existed between the logistically and non-logistically growing plants (Table 5). This difference therefore did not contribute to a more steady increase in leaf area for plant averages compared to individual plants. Some plants that were not developing properly died in this phase (Table 5) and were not taken into account for both individual plants and averages.

Boosts and shocks after transition to a new phase

After planting *in vitro* plantlets to soil, a boost in leaf area growth occurred. Althought this is consistent with a logistic growth pattern, the boost was very prominent and also observed for leaf number. The increase in leaf number likely was associated with internode elongation allowing more leaves to be counted. Elongation probably could have been triggered by the absence of ethylene, which is known to accumulate in sealed tubes (e.g. Roche & Cassells, 1996; Lê, 1996) and can reduce shoot (Rylski *et al.*, 1974) and stolon (Mingo Castel *et al.*, 1976) elongation in potato. It is unknown if a different spectral quality of the light could have triggered stem elongation. In the transplant production phase, the ratio between light in the blue-green range (380–520 nm) – known to inhibit stem elongation in potato *in vitro* (Seabrook & Douglass, 1998) – and the green to yellow range (about 520–650 nm) likely was lower than in the normalisation phase. Leaf expansion could also have been increased. In addition, a small increase in above-ground leaf area and number may have resulted from a very small drop in the soil level after watering the plants.

After transition of transplants to tuber production conditions, a shock was observed in leaf area growth, but not in leaf number. This was probably caused by some water stress, resulting from the unavoidable root damage taking place at transplanting.

Temperature effects

A higher temperature (23 versus 17 °C) did not increase leaf area at the end of the normalisation phase significantly (Tables 1, 3). The effect of a higher temperature on leaf area increase with time was mainly on advancement of growth, as expressed by the midpoint (M) of the logistic curves occurring 2-3 days earlier at higher temperature (Tables 1, 3). This is consistent with optimum temperatures of 20–25 °C reported for potato haulm growth in tuber-grown potato plants (Ingram & McCloud, 1984). Higher

temperature in potato generally leads to plants with more (Marinus & Bodlaender, 1975; Menzel, 1980; Steward *et al.*, 1981; Almekinders & Struik, 1994) but smaller (Bodlaender, 1963; Steward *et al.*, 1981; Wheeler *et al.*, 1986) leaves. The leaf appearance rate probably is maximum around 20 °C (Borah & Milthorpe, 1962). In our experiment, a higher temperature *in vitro* resulted in a higher rate of leaf appearance, but without a significant increase in leaf area (Fig. 4), indicating that smaller leaves were produced.

During transplant production, leaf area increased more at higher than at lower temperature (Fig. 6, Tables 4, 6). The effect appeared to be achieved mainly in the first part of the transplant production period (Fig. 6). It probably resulted from additional leaves appearing during the boost in leaf number after planting at the higher than at the lower temperature. After the boost, the leaf appearance on the main stem was not affected further by the temperature difference. The faster increase in leaf area in the early part of the transplant production period at the higher temperature forced the fitted logistic curves to take a less clear S-shape, with consequently lower fitted minimum leaf area (A) and B-values than at lower temperature (Tables 4, 6).

At the end of the tuber production phase, leaf area was lower for plants growing at higher temperature (Tables 7, 9), because it had decreased already, whereas it was higher in the earlier stages (Fig. 8). This growth pattern was reflected in earlier midpoints (M), lower fitted increments (C) and higher maximum rates of increase (MI) at the higher temperature (Tables 7, 9). In this phase below ground tuber production occurred, which was less strong at the higher temperature (not shown). The shift in assimilate partitioning to tubers is associated with slowing or cessation of shoot growth (Almekinders & Struik, 1996). The positive effect of high temperature on leaf area in the major part of the tuber production phase (Fig. 8) must have arisen from more leaves, because individual leaves generally are smaller at higher temperature (e.g. Steward *et al.*, 1981). The increase in main-stem leaf number indeed was greater at higher temperature, and lasted longer (Fig. 8). Also more secondary stems were produced at the higher temperature (not shown), which is in agreement with Almekinders & Struik (1996). Leaf area in our experiment decreased earlier at higher temperature because the life span of individual potato leaves is shorter at higher temperature (Struik & Ewing, 1995).

After-effects

After-effects due to different temperatures in earlier phases may result from direct effects on leaf area or number at the beginning of the next stage or from morphological or physiological changes exerting their effects later.

Growth at different temperatures during normalisation did not result in plants of cv. Gloria having significantly different leaf areas at the end of that phase or in the subsequent transplant production phase even though the increase in leaf area tended to be less for plantlets that experience the higher temperature during normalisation (Fig. 6). For other cultivars that responded more strongly, a negative effect of higher temperatures during normalisation on leaf area in the next phase was found (Tadesse *et al.*, 2000a). Temperature during normalisation also did not affect fitted curve parameters during transplant production (Tables 4, 6). A higher normalisation temperature nevertheless resulted in greater leaf number at the end of the normalisation phase and at the beginning of the transplant production phase. The boost in leaf number immediately after planting (Figs 3, 6) seemed slightly smaller for plantlets produced at a high temperature during normalisation, but only when grown at 18/12 °C during transplant production. After-effects on the subsequent rate of increase in leaf numbers did not occur during transplant production.

Leaf area at the end of the transplant production phase was larger for transplants raised at higher than at lower temperature (Table 6), and so was the initial above-ground leaf area in the subsequent tuber production phase (Tables 7, 9). This agrees with results from Tadesse *et al.* (2000b). No significant effects of temperature during transplant production on leaf area were present anymore at the end of the tuber production phase (Tables 7, 9). However, individual plants pre-cultured at higher temperature (Table 9) had higher fitted A-values and a smaller fitted increment in leaf area (C), which agrees with the tendency of a less strong increase in leaf area for transplants raised at higher temperature (Fig. 8). These results, however, were not consistent with a higher light interception found for field crops from transplants grown at a higher temperature (Tadesse *et al.*, 2000b). A higher temperature during transplant production also resulted in higher leaf numbers at the end of the transplant production and at the beginning of the tuber production phases. Differences in leaf number remained until the end. Transplants produced at 26/20 °C even tended to increase slightly faster in leaf number than transplants produced at 18/12 °C when grown at a high temperature during tuber production (Fig. 8). This probably was associated with a less strong partitioning of assimilates to tubers, resulting from a lower induction to tuberize (e.g. Struik & Ewing, 1995; Van Dam *et al.*, 1996), and causing later cessation of haulm growth (Almekinders & Struik, 1996). Also, the terminal inflorescence could have been initiated after more leaf primordia were initiated at the higher temperature during transplant production (cf. Almekinders & Struik, 1996).

Growth at different temperatures during the normalisation phase did not affect leaf area or leaf appearance rate in the tuber production phase.

The effects of leaf area at the beginning of a phase – resulting from either

different pre-treatments or variations in leaf area between plants - on leaf area development in that phase will be studied in a subsequent paper. After-effects exerting their influence through differences in tuber formation and dry matter partitioning between haulm and tuber growth are described in forthcoming papers.

Conclusions

- Logistic curves described leaf area increase of *in vitro* (derived) potato (trans)plants better than exponential and expolinear curves in three phases of growth: *in vitro* normalisation, transplant production and tuber production. This implies that restrictions for leaf area increase were present in all phases.
- Logistic curves described leaf area increase best for both plant averages and the majority of (or all) individual plants. The increase in leaf area was more regular for plant averages than for individual plants, which showed a clearer S-shaped pattern. This was reflected in higher B-values and higher maximum rates of increase (MI).
- Growth limitations were less severe in the relatively short transplant production phase than in other phases, because the midpoint (M) of the logistic curve occurred late during this phase and a larger percentage of individual plants followed an exponential or expolinear growth pattern than in other phases.
- Temperature did not significantly affect the type of curve best describing the increase in leaf area with time, or the proportion of plants following logistic or other types of increases.
- Temperature effects on leaf area increase between the beginning and end of a phase were different in the three phases: Higher temperatures did not significantly increase leaf area during normalisation, but did increase leaf area during transplant production. During tuber production, it first increased leaf area but later reduced it.
- Higher temperatures mainly advanced development, as shown by earlier midpoints (M) of the fitted logistic curves for leaf area in the normalization and tuber production phases, and by higher leaf appearance rates during parts of all phases.
- A boost in leaf area and leaf appearance occurred after planting *in vitro* plants to soil.
- A shock in leaf area increase took place after transplanting transplants to tuber production conditions, which was not found for leaf appearance.
- After-effects of different temperatures during normalisation on leaf area and number were only found during transplant production and not anymore during tuber production. After-effects of different temperatures during transplant production were found during tuber production.
- After-effects of a higher temperature on leaf number were both direct – a higher leaf

number at the beginning of the next phase – and indirect – a less strong boost in leaf appearance after planting to low temperature during transplant production, and a slightly higher leaf appearance rate during tuber production.

- After-effects of a higher temperature on leaf area were both direct – a higher leaf area after transplanting to the tuber production phase – and indirect – a reduction of the increase with time in the tuber production phase.

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CHAPTER 4

Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Effects of leaf area of individual plants on parameters describing individual leaf area increase

Submitted as:

Tadesse M., W.J.M. Lommen, P.E.L. Van der Putten & P.C. Struik, 2000. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Effects of leaf are of individual plants on parameters describing individual leaf area increase.

4. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Effects of leaf area of individual plants on parameters describing individual leaf area increase

Abstract

Leaf area increase of individual, *in vitro* produced plantlets (cultivar Gloria) was analysed over the last three phases of a seed production system: the *in vitro* normalisation, transplant production and tuber production phases. In each phase, initial leaf area (ILA) and final leaf area (FLA) of logistically growing plants were related to the values of the parameters describing the logistic curve (leaf area = $A+C/(1+\exp^{(-B \times (t-M))})$).

Higher ILA was associated with higher FLA at both temperatures during normalisation and transplant production, but no consistent association existed in the tuber production phase.

During normalisation, high ILA led to higher fitted minimum leaf area (A), higher fitted increment (C) and higher maximum rate of increase (MI). At higher normalisation temperature, higher ILA also resulted in higher initial relative rate of increase (B) and earlier mid-points (M). During transplant production, a higher ILA was associated with higher C. The positive association found between ILA and FLA mainly resulted from positive effects of a higher ILA on A, C and MI during normalisation, and on higher C during transplant production. No consistent association was found in the tuber production phase.

Leaf area increase of plantlets with a high FLA was characterised by higher A, C and MI-values and by advanced growth at higher temperature during normalisation, and by higher C and higher MI-values and by higher A at lower temperature during transplant production. Leaf area increase of plants having high FLA at the end of tuber production was characterised by higher C, M and MI-values.

Key words: potato, *Solanum tuberosum* L., *in vitro* plantlet, leaf area, logistic growth, growth analysis, regression coefficients, seed production, temperature.

Introduction

Conventional seed potato production systems have low rates of multiplication and high risks of infection with increasing numbers of field multiplication (Haverkort *et al.*, 1991). Micropropagation techniques, producing large numbers of disease free plants within a short period of time, may help to overcome these disadvantages (Jones, 1988;

Struik & Wiersema, 1999). These techniques have been incorporated into a seed production scheme which constitutes the multiplication, normalisation, transplant production (acclimatisation) and tuber production (field) phases.

Different methods and conditions are used in different laboratories to grow plants in the various phases of seed production systems (Sipos *et al.*, 1988; Levy, 1988; Struik & Wiersema, 1999). These protocols, however, do not limit plant-to-plant variation and do not take into account the consequences that this variation may have in subsequent phases. Plant-to-plant variation may be caused by differences in size or other physical and physiological characteristics of the explants, variation in conditions during their growth and by the interactions between these characteristics and later events. Especially the behaviour immediately after transition is relevant and may be very variable: plants are damaged during the transition, they are often re-planted deeper and they experience temporarily water loss. The same plants are also exposed to different growth conditions in the last three phases of the production scheme. All these factors contribute to transplanting shocks or boosts during transition of the plants from one phase to another, which will depend on their functional status (Sutter *et al.*, 1988; Grout, 1988; Sutter *et al.*, 1992; Tadesse *et al.*, 2000a). Transplant shocks or boosts will be reflected in leaf area growth and other developmental processes in the various phases.

Tadesse *et al.* (2000b) indicated that leaf area of transplants at the end of the acclimatisation phase was positively influenced by leaf area of the same plantlet at the beginning of the phase. They also stressed that the relative increase in leaf area during acclimatisation (increase/early leaf area) was linearly related to the inverse of the early leaf area, indicating almost comparable relative increase for plantlets having larger early leaf area but more variable responses for plantlets having smaller early leaf area. Tadesse *et al.* (2000b) also showed that temperature during the normalisation phase had a significant effect on leaf area growth during the transplant production phase that went beyond the effect of normalisation temperature on initial leaf area of the transplanted *in vitro* plantlet. The increase in leaf area with time usually follows a logistic growth in the normalisation, transplant production and tuber production phases (Tadesse *et al.*, 2000a). It is yet unknown how the leaf area of an individual plant at the beginning of a phase affects the values of the different curve parameters of the logistic growth function of leaf area, nor is it known which parameters are important for achieving a high leaf area at the end of a phase.

This study therefore aims at:

- Analysing plant-to-plant variation in leaf area growth in each of the three phases;
- Correlating initial leaf area to different growth parameters;
- Correlating final leaf areas to specific growth parameters in each phase;

- Describing the effects of temperature in each of the three phases on these correlations.

This study is the second of a series on leaf area development and growth in the normalisation, transplant production and tuber production phases of a seed tuber production system of potato.

Materials and methods

Details on the experiment described in this study have already been provided by Tadesse *et al.* (2000a). Below we briefly summarise the main aspects and provide further details relevant to this study.

Potato culture and treatments

Potato (*Solanum tuberosum* L., cv. Gloria) plantlets were propagated *in vitro* by producing single-node cuttings using virus-free stock plantlets and were cultured on a standard medium containing MS salts (Murashige & Skoog, 1962) with vitamins, 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). Viable nodes were cultured in sealed 25 ×150 mm culture tubes on 10 ml medium at 17 or 23 °C and a photophase of 16 h for 21 days in the "normalisation" phase. Rooted *in vitro* plantlets were then planted in transplanting trays with small cells filled with potting soil and grown in growth chambers with day/night temperatures of 18/12 or 26/20 °C, a photophase of 14 h and a relative humidity of 80% for 14 days. This is the "acclimatisation" or "transplant production" phase. After acclimatisation, the plants were transplanted into 5 liter pots (16 plants m^{-2}), filled with potting soil, and were grown in two glasshouses at a day/night temperature of 18/12 or 26/20 °C, a photophase of 16 h and a relative humidity of 80% for 42 days in the "tuber production" phase.

Experimental design

The experiment was carried out in a split-split plot design in 16 blocks where the tuber production temperature (TB) was randomised within the transplant production temperature (TP) and the latter was randomised within the normalisation temperature (N). Thus, a total of 128 plants (2 TB × 2 TP × 2 N × 16 replications = 128 plants) were used for frequent non-destructive measurements on leaf area. They were part of a larger experiment with the same treatments, in total consisting of 992 plants.

Measurements and statistical methods

Leaf area was measured before and after (trans)planting, and then every 3 days in the normalisation and transplant production phases and during the first week of the tuber production phase. From then on, measurements were taken every week in the tuber production phase. Leaf area (including the explant's) was non-destructively estimated using a transparent sheet with grids (1×1 mm or 5×5 mm) during the normalisation and transplant production phases, respectively. In the tuber production phase, leaf area was estimated by measuring the length (l) and width (w) of individual compound leaves of each of the 128 plants and subsequently calculating leaf area using the formula l × w × k. The shape factor, K, was derived from average measurements of typical leaves at a particular time of estimation. Measurements of leaf area on the first and last day of every phase gave the "initial leaf area" (ILA) and "final leaf area" (FLA), respectively in all phases of growth.

Only those plants that showed normal logistic growth were used in this study since logistic curve best explained leaf area increment in all phases of growth (Tadesse *et al.*, 2000a). The parameters of the logistic growth curve are: fitted minimum leaf area (A), initial relative rate of increase (B), fitted mid-point (M) and fitted increment (C). The maximum rate of increase in leaf area (MI) is calculated as B×C/4. To study the relationship between the ILA or FLA and the curve parameters three approaches were used: in the first, curve parameter values for individual plants were related to ILA and FLA by linear regression separately for high or low temperatures in all three phases of growth. In the transplant and tuber production phases, this was done for plants from all temperature pre-treatments combined. Equations are provided whenever the relationship was significant. In the second approach, correlation coefficients were assessed to identify correlations between ILA or FLA and the curve parameters for individual treatments. Finally regression analysis was carried out over individual treatments and regression coefficients were compared by t-tests.

Results

Leaf areas of individual plants at the beginning and at the end of a phase

In the normalisation phase, higher leaf areas of explants (ILA, initial leaf area) were associated with higher leaf areas of *in vitro* plantlets at the end of the period (FLA, final leaf area) under both temperatures (17 and 23 °C) of *in vitro* growth (Fig. 1a, Table 1).

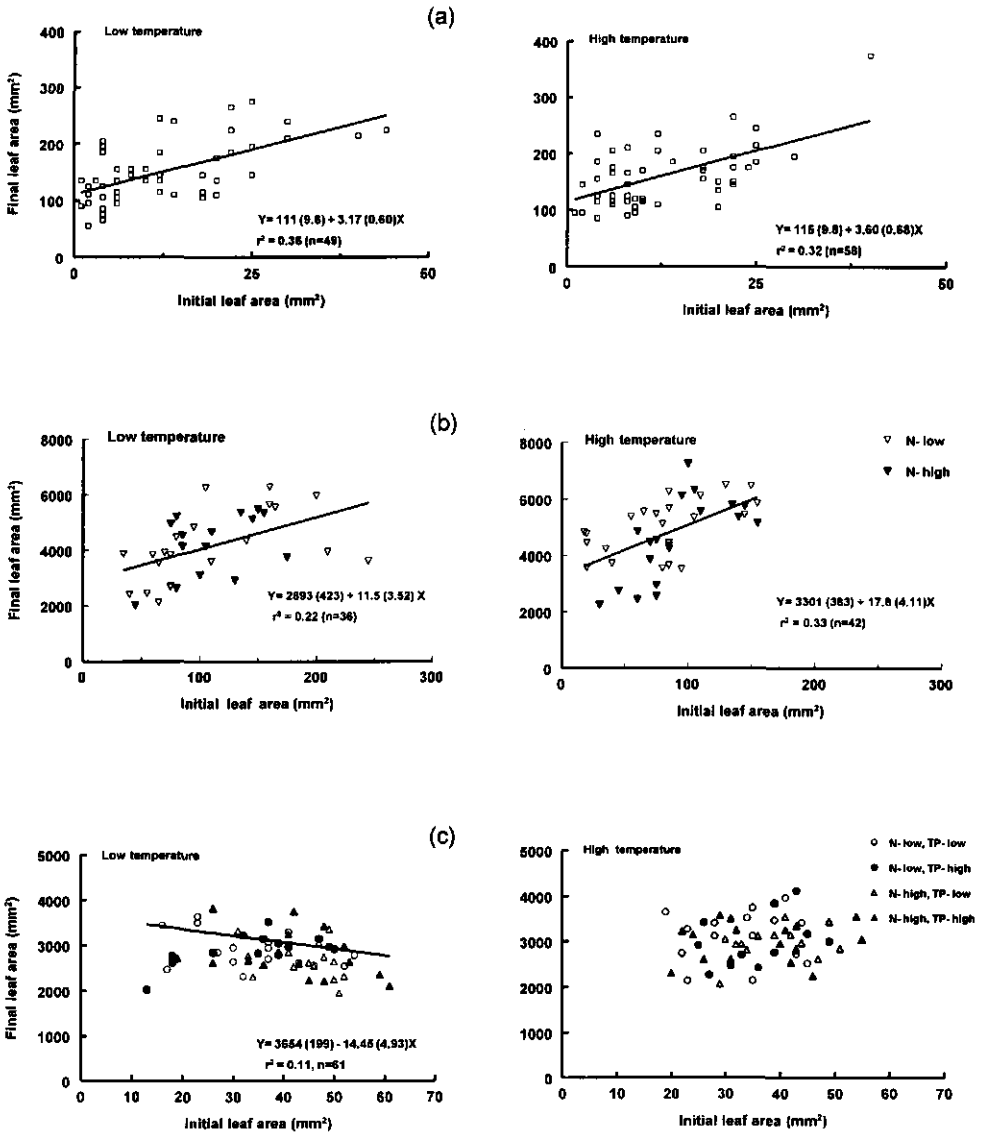


Fig. 1. Initial leaf area plotted against final leaf area at high and low temperatures in the normalisation (a), transplant production (b) and tuber production (c) phases.

Also after transplanting to soil, higher above-ground leaf areas were associated with higher leaf areas at the end of transplant production at both transplant production temperatures (18/12 and 26/20 °C), when data from both pre-treatments (17 and 23 °C during the normalisation period) were combined (Fig. 1b). A positive association was also found within most individual pre-treatments, but was not significant for *in vitro* plants produced at 23 °C and raised to transplants at 18/12 °C (see Table 2). High normalisation and low transplant production temperature-combinations do not promote leaf growth because previous studies by Tadesse *et al.* (2000a) have confirmed that high temperature during normalisation has a negative effect on leaf growth and similarly, low temperature during transplant production does not promote leaf growth.

After transplanting to the tuber production phase, higher initial leaf areas were associated with lower final leaf areas – for data from all pre-treatments combined – when temperature during tuber production was low (18/12 °C) (Fig. 1c). This negative association was only found within different pre-treatments for plants pre-cultured at both high normalisation and high transplant production temperatures (Table 3). Associations between ILA and FLA were not found at high (26/20 °C) temperature during tuber production, either for all data (Fig. 1, Table 3) or within different pretreatments.

Initial leaf area and parameters characterising its logistic increase

Normalisation phase

Under both temperatures during the normalisation phase, a higher leaf area of the explant leaf lead to higher values fitted for the lower asymptote (A) of the logistic curve describing leaf area increase of a plantlet during the phase, to higher fitted maximum increments in leaf area (C) and higher maximum rates of increase (MI) (Fig. 2, Table 1). At 17 °C *in vitro*, no associations between ILA and initial relative rate of increase (B) or fitted midpoint of the logistic curve (M) were found. At 23 °C, a higher ILA resulted in higher B-values and earlier midpoints (M) in addition to higher A, C and MI values (Fig. 2).

Regression coefficients for the relation between ILA and curve parameters were not different for the two temperatures (Fig. 3). This indicates that at a fixed difference in ILA between plantlets, the change in A, C or M was the same at the two temperatures.

Table 1. Matrix of linear correlations between initial (ILA) and final (FLA) leaf areas and parameters describing logistic growth at two temperatures in the normalisation phase (n = 49 at low and 58 at high temperatures).

	ILA (x)		FLA (y)	
	17 °C	23 °C	17 °C	23 °C
FLA	0.61 *** [1]	0.58 ***	–	–
A	0.90 ***	0.93 ***	0.41 **	0.42 ***
B	0.03 NS	0.29 *	–0.26 NS	–0.04 NS
M	–0.23 NS	–0.33 **	–0.22 NS	–0.39 **
C	0.29 *	0.36 **	0.81 ***	0.94 ***
MI	0.47 ***	0.60 ***	0.85 ***	0.84 ***

[1] ***: P<0.001, **: 0.001≤*P*<0.01, *: 0.01≤*P*<0.05, NS: not significant *P*≥0.05.

A - fitted minimum leaf area (mm^2)
B - initial relative rate of increase (day^{-1})
M - fitted mid-point (days after cutting/(trans)planting)
C - fitted increment (mm^2)
MI - maximum rate of increase in leaf area at M ($mm^2\ day^{-1}$)

Transplant production phase

During transplant production (and for data from both pre-treatments combined), similar associations between ILA and curve parameters were found at the lower temperature as were found at the lower temperature during normalisation. A higher ILA was associated with higher values for the lower asymptote (A), the maximum increment in leaf area (C) and the maximum rate of increase (MI), but not with the initial relative rate of increase (B) or midpoint (M) (Fig. 4). As at the higher temperature during normalisation, a higher ILA at the higher temperature during transplant production was associated with a higher increment (C) and an earlier midpoint (M). However, ILA at the higher temperature was not correlated with the A-value or maximum rate of increase (MI), and was negatively correlated with the B-value (Fig. 4).

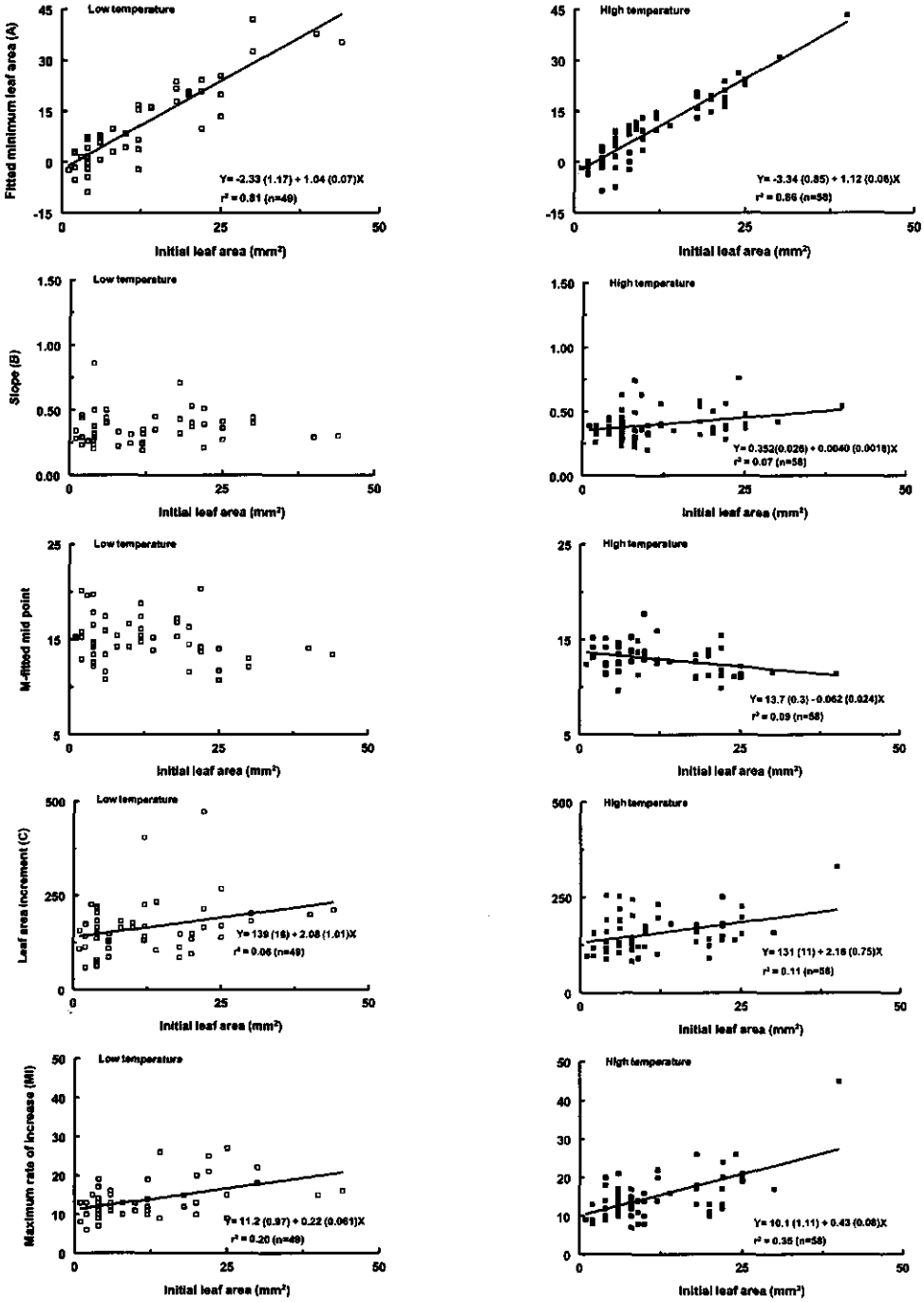


Fig. 2. Initial leaf area (ILA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low (17 °C) or high (23 °C) temperatures in the normalisation phase.

Table 2. Matrix of linear correlations between initial (ILA) and final (FLA) leaf areas and parameters describing logistic growth at two temperatures (18/12 or 26/20 °C) in the transplant production phase after growing at two temperatures (17 or 23 °C) in the normalisation phase (n = 36 at low and 42 at high temperature).

	ILA (x)				FLA (y)			
	18/12 °C		26/20 °C		18/12 °C		26/20 °C	
	17 °C	23 °C	17 °C	23 °C	17 °C	23 °C	17 °C	23 °C
FLA	0.52 * [a]	0.43 NS	0.56 **	0.69 **	–	–	–	–
A	0.63 **	0.28 NS	–0.30 NS	–0.42 NS	0.71 ***	0.86 ***	0.06 NS	–0.11 NS
B	–0.05 NS	–0.12 NS	–0.46 *	–0.42 NS	–0.04 NS	0.46 NS	–0.12 NS	–0.31 NS
M	–0.32 NS	0.37 NS	–0.17 NS	–0.79 ***	0.14 NS	–0.36 NS	0.07 NS	–0.75 ***
C	0.46 *	0.68 **	0.51 **	0.55 *	0.94 ***	0.55 *	0.69 ***	0.84 ***
MI	0.34 NS	0.36 NS	–0.05 NS	0.05 NS	0.86 ***	0.93 ***	0.56 **	0.49 *

[a] ***: P<0.001, **: 0.001≤*P*<0.01, *: 0.01≤*P*<0.05, NS: not significant *P*≥0.05.
For abbreviations see Table 1.

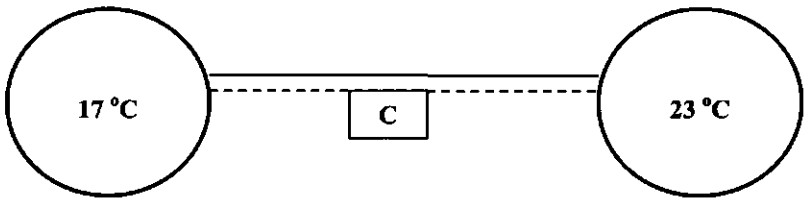


Fig. 3. Schematic representation of the statistical significance of differences between regression coefficients of curve parameters in relation to the initial (ILA) (——) or final leaf area (FLA) (------), at two temperatures in the normalisation phase. Only the curve parameter in which the treatment had a significant difference in regression coefficient is indicated. For abbreviation of curve parameter see Table 1.

Correlations between ILA and parameters of logistic growth during transplant production for individual pre-treatments were not significant when correlations over pre-treatments were not (Fig. 4, Table 2). Other correlations between ILA and parameters were not always significant for both pre-treatments. Only the positive associations between ILA and the fitted maximum increment (C) at both transplant production temperatures (Fig. 4) were consistently present for plants pre-cultured at both temperatures during normalisation (Table 2). The positive association between ILA and the A-values at 18/12 °C during transplant production and the negative association between ILA and the B-values at 26/20 °C (Fig. 4) were not significant for plants pre-cultured at 23 °C (Table 2). The positive association between ILA and the maximum rate of increase (MI) at 18/12 °C (Fig. 4) even was not significant within both pre-treatments (Table 2).

Regression coefficients between ILA and B, C or MI did not differ for different treatments (Fig. 5). Regression coefficients between ILA and A were different between plants grown at high normalisation and low transplant production temperatures and those grown continuously at high temperatures, between plants grown at low normalisation and high transplant production temperatures and those grown continuously at low temperatures, and between plants continuously grown at low and continuously at high temperatures. Regression coefficients between ILA and M were different for treatments continuously receiving higher temperature compared to all other treatments.

Tuber production phase

Over all pre-treatments, transplants with a higher ILA after transplanting to the tuber production phase, also had higher A-values at both temperatures during tuber

Table 3. Matrix of linear correlations between initial (ILA) and final (FLA) leaf areas and parameters describing logistic growth at two temperatures in the tuber production phase after growing at two temperatures (18/12 or 26/20 °C) in the transplant production and two temperatures (17 or 23 °C) in the normalisation phases (n ranges between 14 and 16 at the different temperature treatments).

	ILA (x)				FLA (y)			
Low temp. during tuber production	18/12 °C		26/20 °C		18/12 °C		26/20 °C	
	17 °C	23 °C	17 °C	23 °C	17 °C	23 °C	17 °C	23 °C
FLA	–0.46 NS [a]	0.40 NS	–0.36 NS	–0.51 *	–	–	–	–
A	–0.02 NS	0.55 *	–0.06 NS	0.42 NS	0.00 NS	0.33 NS	–0.16 NS	–0.45 NS
B	0.10 NS	0.58 *	0.06 NS	0.64 **	–0.28 NS	0.12 NS	–0.17 NS	–0.47 NS
M	–0.48 NS	–0.65 **	–0.45 NS	–0.90 ***	0.88 ***	0.18 NS	0.60 *	0.68 **
C	–0.42 NS	0.07 NS	–0.35 NS	–0.59 *	0.97 ***	0.89 ***	0.95 ***	0.99 ***
MI	–0.30 NS	0.63 *	–0.19 NS	–0.13 NS	0.61 *	0.59 *	0.53 *	0.73 **
High temp. during tuber production								
FLA	–0.10 NS	0.31 NS	0.14 NS	–0.04 NS	–	–	–	–
A	0.52 *	0.38 NS	0.28 NS	0.40 NS	–0.14 NS	–0.70 *	–0.03 NS	–0.43 NS
B	0.34 NS	–0.04 NS	0.23 NS	0.06 NS	–0.27 NS	–0.82 ***	–0.07 NS	–0.46 NS
M	–0.27 NS	–0.08 NS	–0.37 NS	–0.25 NS	0.80 ***	0.82 ***	0.34 NS	0.74 **
C	–0.04 NS	0.32 NS	0.18 NS	0.08 NS	0.99 ***	0.99 ***	0.98 ***	0.99 ***
MI	0.28 NS	0.45 NS	0.34 NS	0.04 NS	0.40 NS	–0.13 NS	0.42 NS	0.49 *

[a] ***: P<0.001, **: 0.001≤*P*<0.01, *: 0.01≤*P*<0.05, NS: not significant *P*≥0.05. For abbreviations see Table 1.

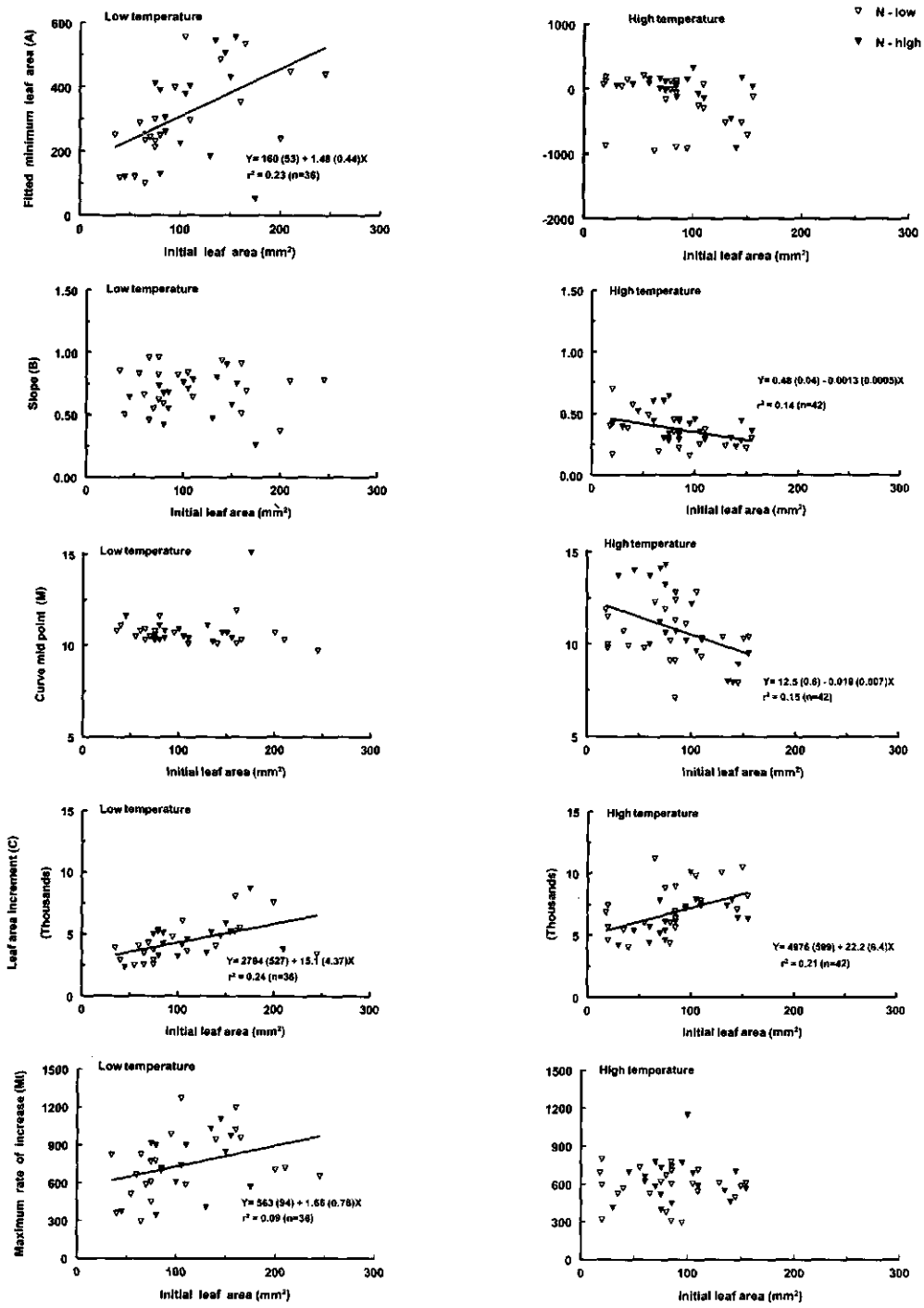
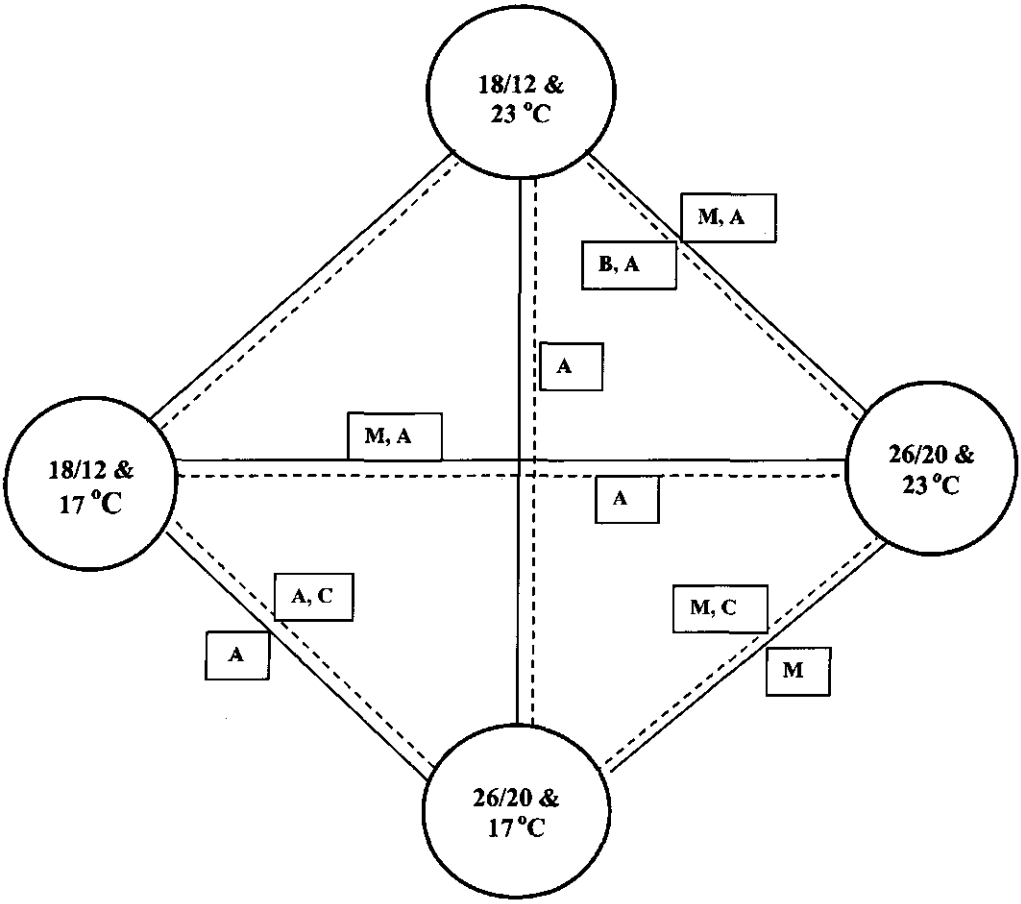


Fig. 4. Initial leaf area (ILA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low (18/12 °C) or high (26/20 °C) temperatures in the transplant production phase after they were pre-cultured at two temperatures in the normalisation phase.



Frequency of significance of difference between regression coefficients of ILA/FLA and the curve parameters for specific treatment comparisons in the transplant production phase. Maximum frequency is 6.

	A	B	M	C	MI
ILA	3	0	3	0	0
FLA	4	1	1	2	0

Fig. 5. Schematic representation of the statistical significance of differences between regression coefficients of initial (ILA) (———) or final leaf area (FLA) (------) and curve parameters at two temperatures in the transplant production phase after two different normalisation temperatures. (The first value represents the transplant production temperature and the second normalisation temperature). Only those curve parameters in which the treatments had a significant difference in regression coefficients are indicated. For abbreviations of curve parameters see Table 1.

production (Fig. 6). At the lower tuber production temperature, a higher ILA was also related to a higher B-value, lower increment (C) and later midpoint (M) (Fig. 6). These associations were not significant at the higher temperature during tuber production. ILA was not associated with the maximum rate of increase (MI) (Fig. 6).

When associations between ILA and fitted parameters were significant over all pre-treatments (Fig. 6), they not always showed for each pre-treatment (Table 3). The positive correlation between ILA and A (Fig. 6) at 18/12 °C only was found for plants pre-cultured at high normalisation plus low transplant production temperature, and at 26/20 °C only for plants pre-cultured at low normalisation plus low transplant production temperature (Table 3). The positive associations between ILA and the B-value or the negative association between ILA and M at the lower tuber production temperature (Fig. 6), were evident only for plants pre-cultured at high normalisation temperatures (Table 3), and the association between ILA and the C-value only for plants pre-cultured at high normalisation plus high transplant production temperature (Table 3).

Regression coefficients between ILA and A or B did not differ between treatments (Fig. 7). Regression coefficients between ILA and C differed between the treatment receiving low normalisation plus high transplant production plus high tuber production temperature and those receiving either high or low normalisation plus low transplant production and high tuber production temperatures, or receiving high normalisation plus high transplant production and low tuber production temperature. Regression coefficients between ILA and M differred between the treatment receiving low, high and high temperatures in the respective phases and the treatments receiving low, low and high, or continuously high or continuously low temperatures in the respective phases. The treatment receiving low, low and high temperatures in the respective phases also had different regression coefficient from the treatments receiving high, high and low temperature or continuously low temperature in the respective phases. Regression coefficient between ILA and the maximum rate of increase (MI) were only different between the treatment receiving continously low temperatures and the treatment receiving high, low and low temperatures in the respective phases.

Associations between logistic curve parameters and final leaf area

Normalisation phase

At both 17 and 23 °C in the normalisation phase *in vitro*, higher fitted values for the lower asymptote (A), higher values for fitted maximum increment in leaf area (C) and

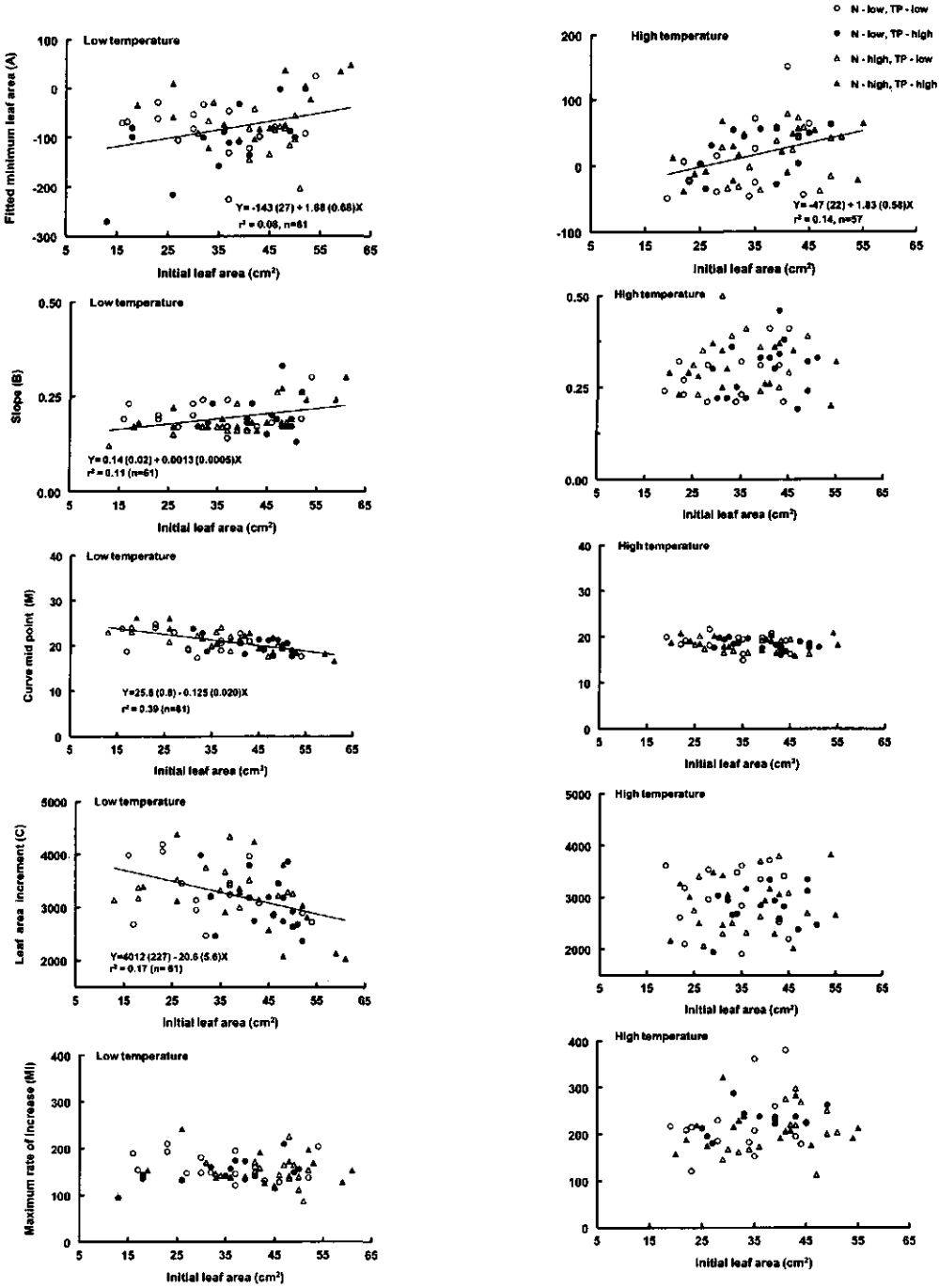
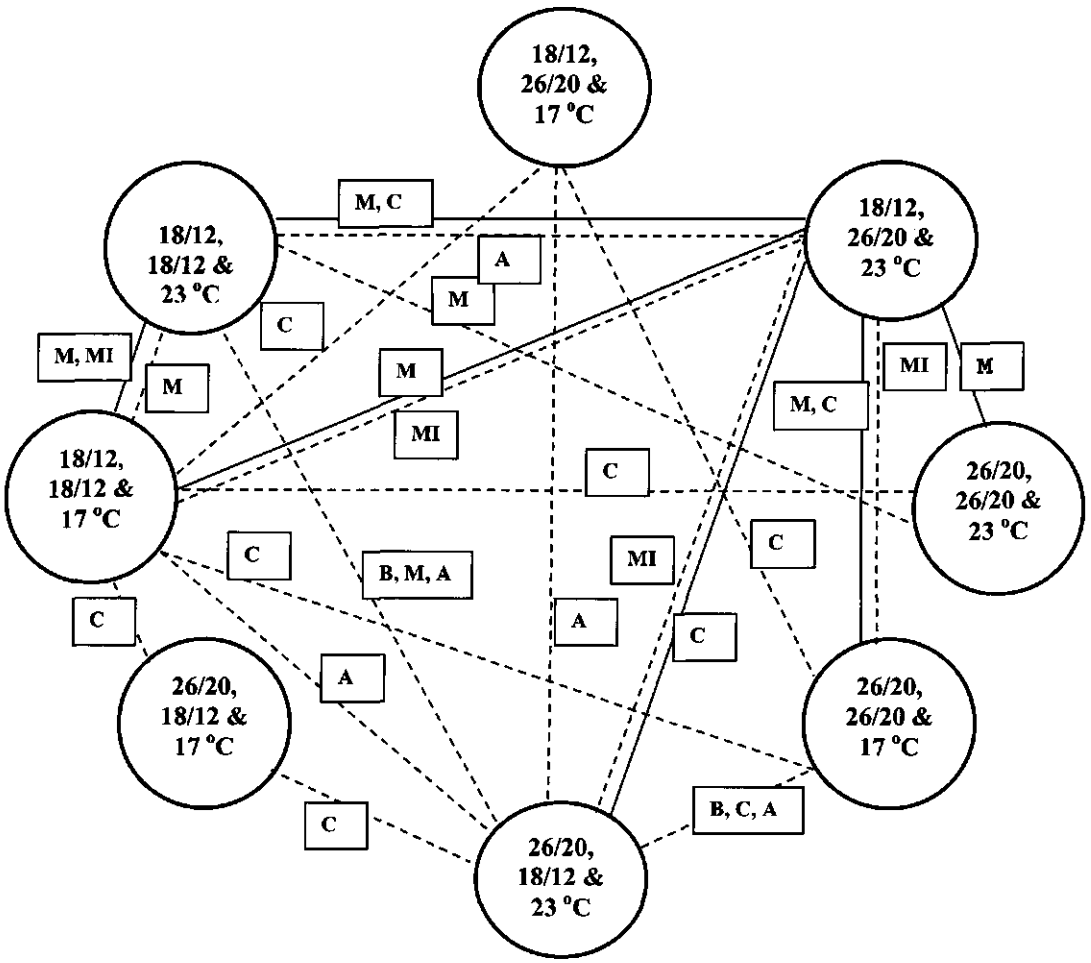


Fig. 6. Initial leaf area (ILA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low (18/12 °C) or high (26/20 °C) temperatures in the tuber production phase after they were pre-cultured at two temperatures in the normalisation and transplant production phases.



Frequency of significance of differences between regression coefficients of the ILA/FLA and the curve parameters for specific treatment comparisons in the tuber production phase. Maximum frequency is 28.

	A	B	M	C	MI
ILA	0	0	5	3	1
FLA	5	2	3	7	3

Fig. 7. Schematic representation of the statistical significance of differences between regression coefficients of the initial (ILA) (———) or final leaf area (FLA) (------) and curve parameters at two temperatures in the tuber production phase preceded by four different pre-treatments. (The 1st figure represents tuber production, the 2nd transplant production and the 3rd normalisation temperatures). Only those curve parameters in which the treatments had a significant difference in regression coefficients are indicated. For abbreviations of curve parameters see Table 1.

a higher maximum rate of increase (MI) were all associated with a higher final leaf area (FLA) at the end of the *in vitro* phase (Fig. 8, Table 1). No associations were found between the initial relative rate of increase (B) and the FLA. Only at 23°C an earlier midpoint of the fitted logistic curve (M) was related to higher final leaf areas.

Regression coefficients between FLA and the fitted increment (C) were different at the two temperatures (Fig. 3).

Transplant production phase

Associations – over both normalisation pre-treatments – between curve parameters and FLA in the transplant production phase were very similar to those in the normalisation phase. For both transplant production temperatures, higher fitted increments in leaf area (C) and higher maximum rates of increase (MI) were associated with higher FLAs. Initial relative rate of increase (B) and FLA were not associated, and only at the higher temperature an earlier M was related to a higher FLA (Fig. 9). A positive association between the A-value and the FLA was only found at the lower transplant production temperature and a negative association between A and M at higher transplant production temperature (Fig. 9).

Those associations being significant over pre-treatments (Fig. 9), were also generally significant for both pre-treatments separately (Table 2). The association between an earlier midpoint (M) and FLA at 26/20 °C was only significant for plants pre-cultured at 23 °C.

Regression coefficients between MI and FLA did not differ for different treatments (Fig. 5). Regression coefficients between A and FLA were usually different, except between plants grown continuously at low temperatures and those grown at high normalisation and low transplant production temperatures, and between those grown at low, followed by high and those grown continuously at high temperatures in the respective phases. The regression coefficients between B and FLA only differed between plants grown continuously at higher temperature and those grown at high normalisation and low transplant production temperatures. The regression coefficients between M and FLA only differed between plants grown continuously at higher temperature and those grown at low normalisation and high transplant production temperatures. Regression coefficients between C and FLA differed between plants grown at low normalisation and high transplant production temperatures and those grown continuously at high or at low temperatures.

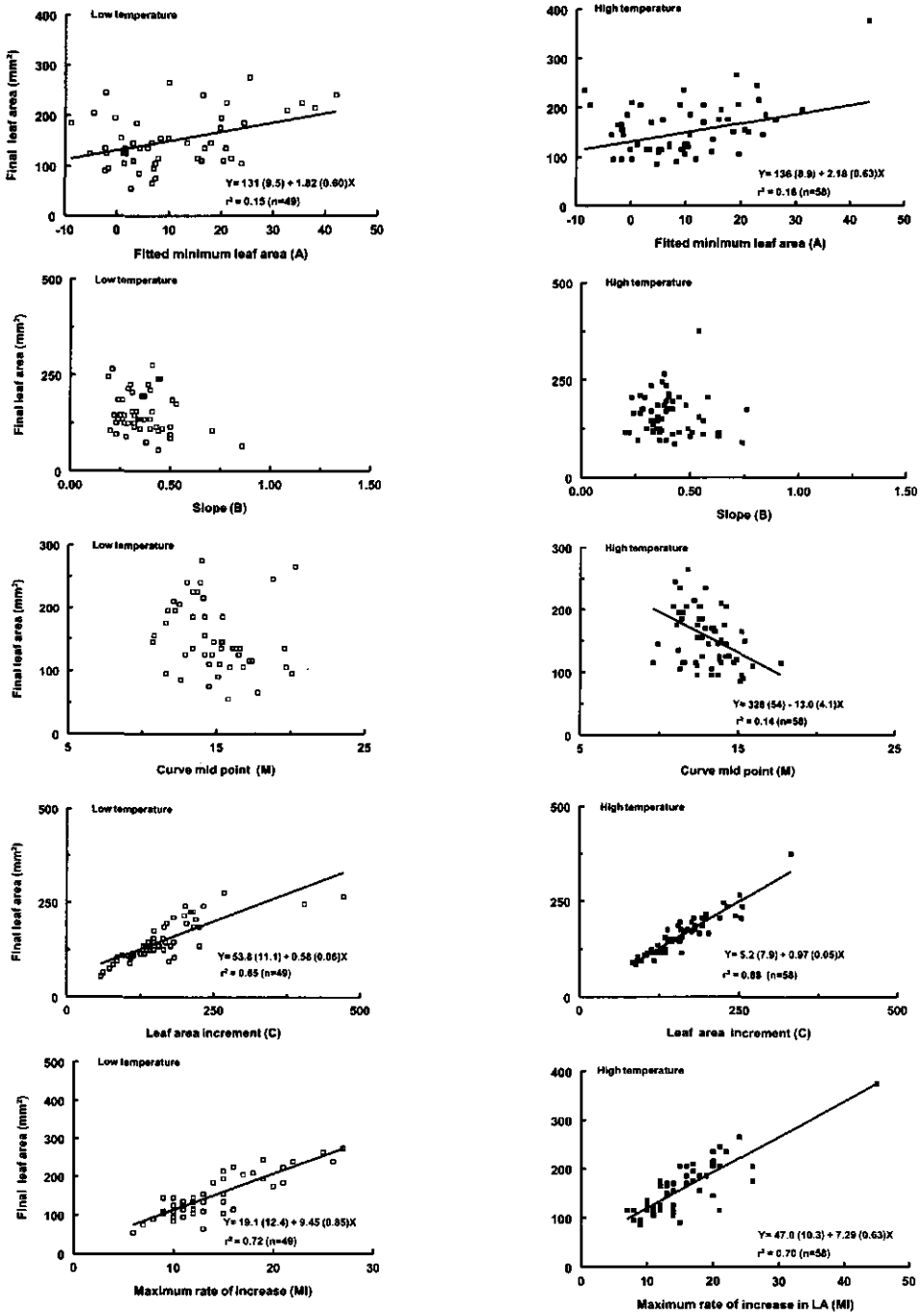


Fig. 8. Final leaf area (FLA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low (17 °C) or high (23 °C) temperatures in the normalisation phase.

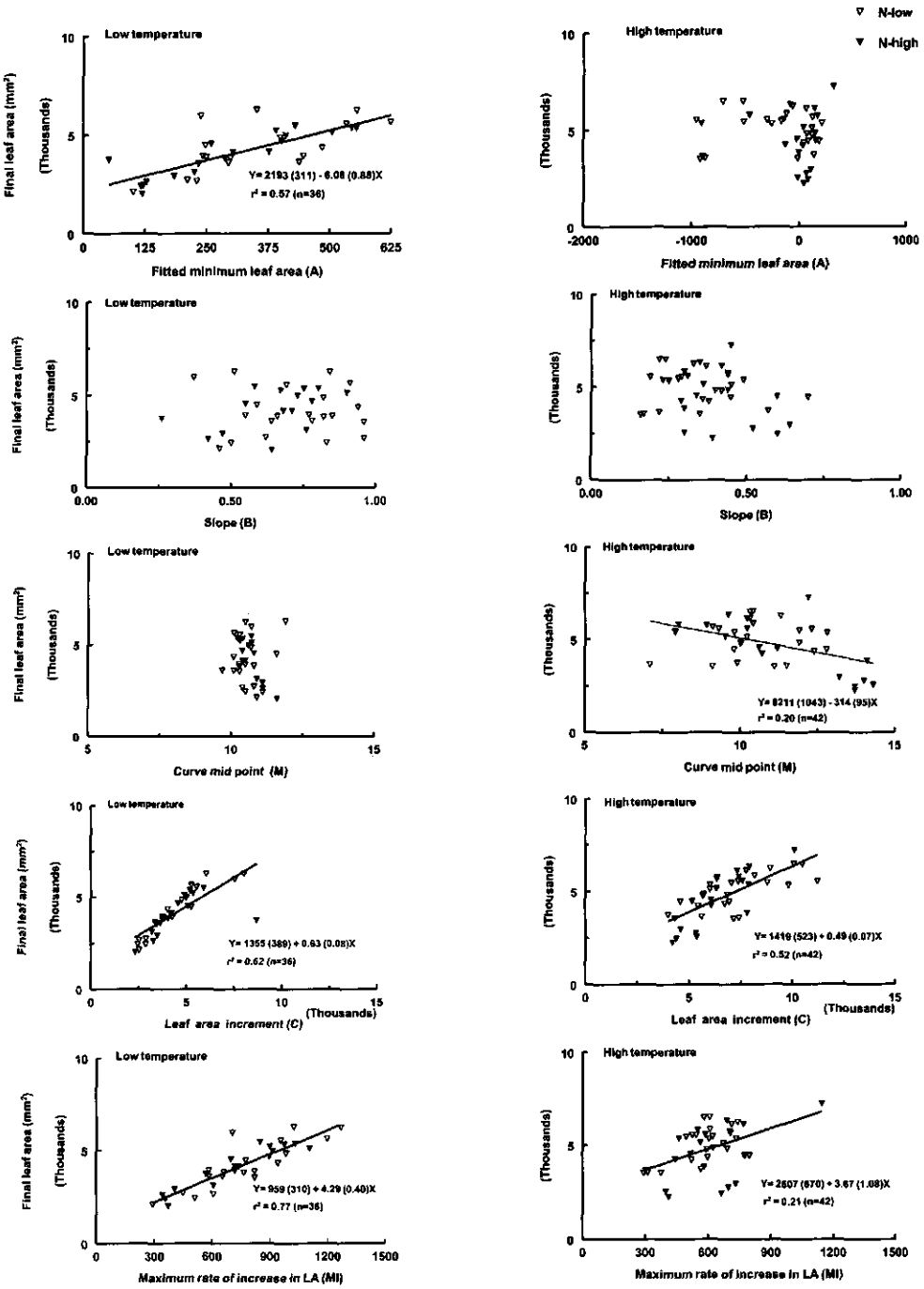


Fig. 9. Final leaf area (FLA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low (18/12 °C) or high (26/20 °C) temperatures in the transplant production phase after they were pre-cultured at two temperatures in the normalisation phase.

Tuber production phase

When values for all pre-treatments were combined, higher fitted increments (C), later midpoints (M) of the logistic curve and higher maximum rates of increase (MI), were all associated with higher FLAs at both temperatures during tuber production (Fig. 10). A higher initial relative rate of increase (B), however, was associated with lower FLAs at both temperatures (Fig. 10). Only at 26/20 °C during tuber production, higher values for the lower asymptote (A) were associated with lower FLAs (Fig. 10).

The positive association between the fitted increment (C) and the FLA (Fig. 10) was also found within all pre-treatments (Table 3). The associations between the midpoint (M) and FLA (Fig. 10) were significant for 3 out of 4 pre-treatments at each temperature in the tuber production phase. At 18/12 °C, only plants produced at low normalisation plus high transplant production temperature did not show a significant correlation, at 26/20 °C the plants produced at low normalisation plus high transplant production temperature did not show a significant correlation (Table 3). The positive association between MI and FLA (Fig. 10) was significant for all pre-treatments at the lower tuber production temperature, but only for plants produced at a high normalisation plus high transplant production temperature at the higher tuber production temperature (Table 3). The negative association between the B-value and FLA (Fig. 10) was not significant within all individual treatments at the low tuber production temperature, and only for plants produced at high normalisation plus low transplant production temperature at the high tuber production temperature (Table 3). The latter was also found for the negative association between the A-value and the FLA at the high tuber production temperature.

Regression coefficients between A and FLA differed between the treatment receiving high, low and high temperatures in the respective phases and those receiving low, high, low, or continuously low, or low, high, high, or high, low, low temperatures in the respective phases and between plants receiving high, high, low and high, low, low temperatures in the respective phases (Fig. 7). Regression coefficients between B and FLA differed between the treatment receiving high, low and high temperatures in the respective phases and those receiving high, low and low or low, high and high temperatures. Regression coefficients between C and FLA were different between the treatment receiving low, low and high temperatures in the respective phases and the treatments receiving high, low and high temperature. Regression coefficients between C and FLA were also different between the treatment receiving continuously low temperatures and all others growing at low temperature during tuber production, plus the one receiving continuously high temperatures. Regression coefficients were also different between the treatment receiving low, high

and high temperatures in the respective phases and the treatments receiving low, high and low, or high, low and high temperatures in the respective phases. Regression coefficients between M and FLA were different between treatments receiving high, low and low temperatures in the respective phases and the treatments receiving continuously low or continuously high or high, low and high temperatures in the respective phases. Regression coefficients between MI and FLA differed between the treatment receiving high, high and low temperatures in the respective phases and the treatments receiving continuously low temperatures, or high, low and high temperatures or low, high and high temperatures in the respective phases.

Discussion

Relationship between ILA and FLA

The relationships between the initial leaf area (ILA) and final leaf area (FLA) were more or less the same in the normalisation and transplant production phases: higher ILA was associated with higher FLA both at low and at high temperatures at the end of the normalisation and transplant production phases (Tables 1, 2). These associations prove that the status of a plantlet at the beginning of the normalisation and transplant production phases has an effect on the status of the plantlet at the end of the same phase. In line with this, Seabrook & Douglass (1994) reported that the removal of subtending leaf of explants resulted in plantlets with smaller leaf area. Tadesse *et al.* (2000b) also found that *in vitro* produced plants with higher leaf areas at the beginning of the transplant production phase end up with higher leaf areas at the end of the phase. The associations found are consistent with the general view for crop plants that, as long as a young, self supportive, vegetative plant is grown without inter-plant competition and free of stress, later leaf area is related to early leaf area (Goudriaan & Van Laar, 1994), and show that this also can occur under conditions where growth is limited as is the case under conditions where the leaf area increase is logistic. The positive correlations found during both the normalisation and transplant production phases, also imply that the use of nodal cuttings with larger explant leaves to start normalisation, may lead to transplants with larger leaf areas at the moment of transplanting into the field.

In the tuber production phase, associations between ILA and FLA were not found at high temperature (26/20 °C), either for all data or within different pre-treatments, probably because high temperature enhanced leaf senescence (cf. Struik & Ewing, 1995) and increased variation among plants. At the low temperature during tuber production, plants with higher ILA ended up with lower FLA at the end of the

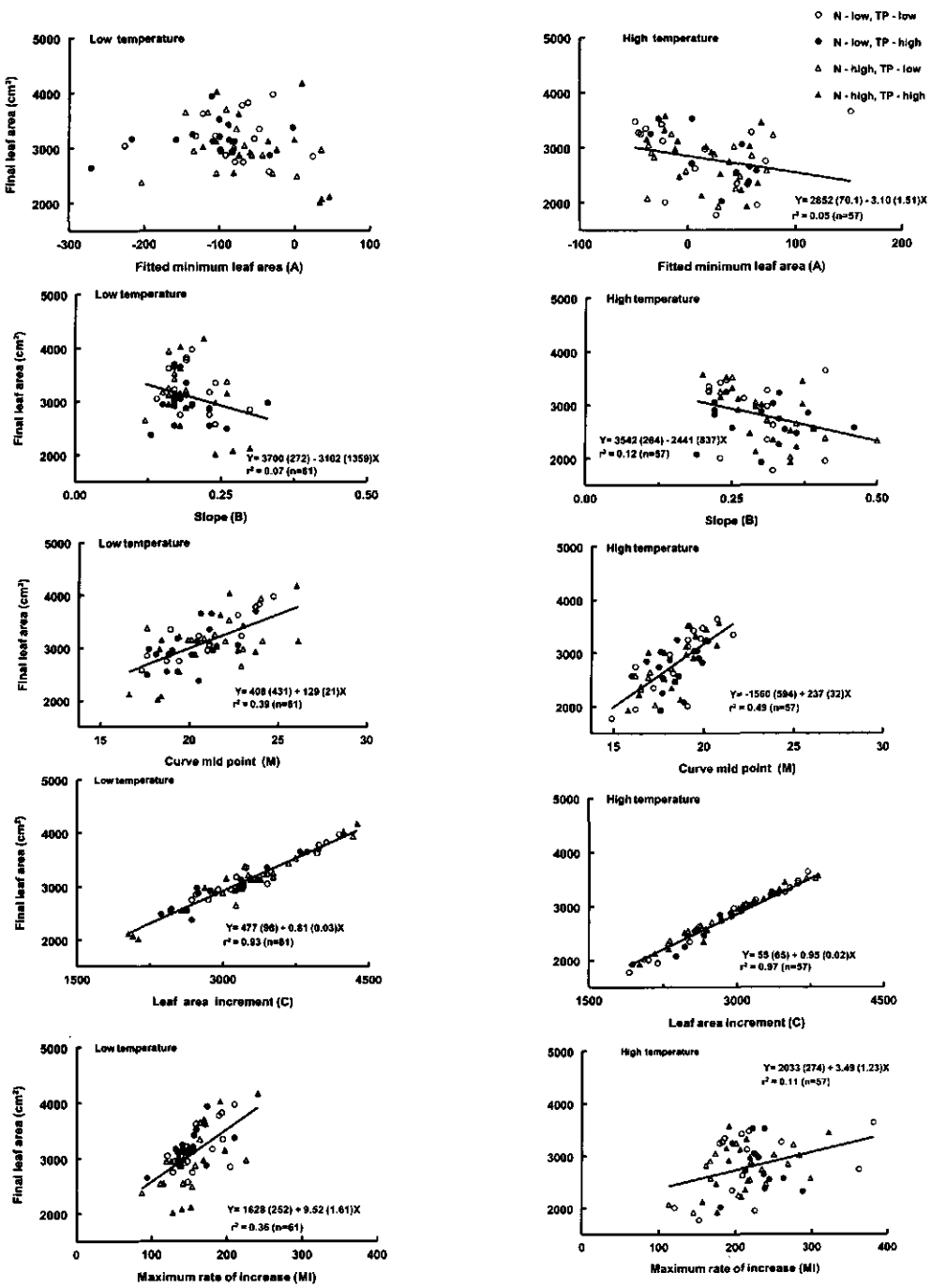


Fig. 10. Final leaf area (FLA) plotted against curve parameters defining logistic growth of *in vitro* propagated potato plantlets grown at low (18/12 °C) or high (26/20 °C) temperatures in the tuber production phase after they were pre-cultured at two temperatures in the normalisation and transplant production phases.

phase when all data were combined, probably because the most advanced plants also had the highest ILA at the beginning of the phase and were the first to senesce or were earlier to partition a relatively large part of their dry matter to tubers, thus limiting haulm development. However, the negative correlation at low temperature during tuber production was only found to be significant within one pre-treatment (i.e. plants pre-treated at high temperatures during normalisation and transplant production phases) (Table 3), indicating that also the pre-treatments themselves affected the overall relationship (Fig. 1c).

Relationship between ILA and logistic curve parameters

Normalisation phase

Associations between the ILA of individual plants at the onset of the normalisation phase and the parameters characterising their logistic increase in leaf area indicated that a higher ILA led to higher fitted minimum leaf area (A) and a higher fitted increment (C) at both temperatures. The latter likely resulted from a more steep increase of the logistic curve, and was associated with a higher maximum rate of increase (MI). A higher ILA, therefore, leads to faster growth. This, plus the higher fitted minimum leaf area, explains the positive association found between ILA and FLA in the normalisation phase. The positive associations between ILA and the initial relative rate of increase (B) and the negative ones between ILA and the curve mid-point (M) at 23 °C indicate that at the high temperature during normalisation larger explants also had higher initial relative increase rates and a more advanced plant development.

Transplant production phase

At low temperature during transplant production, associations between ILA and curve parameters (over all data) were similar to those in the normalisation phase: a higher ILA was associated with higher fitted minimum leaf area (A), the fitted increment in leaf area (C) and the maximum rate of leaf area increase (MI). These findings are consistent with the idea that plants with a higher ILA started at a higher level and increased faster. However, only the correlations between ILA and C were significant for all pre-treatments. Also at high temperature during transplant production, only the positive correlations between ILA and C were significant for all pre-treatments. The positive association between ILA and FLA in the transplant production phase, therefore, is caused by a stronger leaf area increase in plants with a higher ILA.

Tuber production phase

In the tuber production phase, none of the associations found between ILA and curve parameters (Fig. 6), also was found consistently within all pre-treatments. Different responses for plants from different pre-treatments may therefore have directed the regression over all data (Fig. 6). The lower number of plants available in each pre-treatment also made it more difficult to establish the significance of a correlation, but often correlations within pre-treatments also were extremely weak (Table 3). Clearest were the negative associations between ILA and M at both temperatures, suggesting a more advanced growth for the transplants with the largest leaf areas. The association between ILA and the curve parameters usually not being significant at high temperature in the tuber production phase – and for many pre-treatments at the low temperature – however, is consistent with the absence of a clear association between ILA and the FLA in this phase.

Relationship between logistic curve parameters and FLA

Normalisation phase

At the end of the *in vitro* phase, higher fitted minimum leaf area (A), higher fitted increment (C) and a higher maximum rate of leaf area increase (MI) were associated with a higher FLA. One of the probable reasons for a higher fitted minimum leaf area (A) is a higher ILA and usually a higher ILA most likely results in a higher FLA before plants senesce. Besides, a higher C value indicates that the logistic curve is more steep with a higher maximum rate of leaf area increase (MI) and, thus, will give rise to a higher FLA. An earlier mid-point was associated with a higher FLA at 23 °C in the normalisation phase, indicating that also growth of plants achieving a higher final leaf area was more advanced at that temperature.

Transplant production phase

Associations between curve parameters and FLA in the transplant production phase were very similar to those in the normalisation phase. Higher FLAs were correlated with higher fitted increments in leaf area (C) and higher maximum rates of increase (MI), and at low temperature also with higher A-values.

Tuber production phase

At both temperatures during the tuber production phase, higher fitted increments (C), later mid-points (M) and higher maximum rates of increase (MI) were all associated with higher FLAs. Later mid-points suggest that leaf area development over time was slower and thus plant senescence was probably delayed. The latter relationship between the logistic curve parameters and FLA did not exist in the earlier phases.

Conclusions

- Explants with larger leaf areas gave rise to plantlets with larger leaf areas at the end of the normalisation phase.
- Explants with larger leaf areas showed higher fitted increments in leaf area during normalisation and higher fitted start values. At 23 °C, they also showed higher initial relative rates of increase and a more advanced growth.
- For *in vitro* plantlets with a higher leaf area at the end of the normalisation period, the increase in leaf area was characterised by higher fitted increments, higher fitted minimum leaf area and higher maximum rate of increase, and at 23 °C also a more advanced growth.
- Plants with a larger above ground leaf area after planting generally had larger leaf areas at the end of the transplant production phase.
- Plants with a larger above ground leaf area after planting showed a higher fitted increment in leaf area during transplant production.
- For transplants with a larger leaf area at the end of the transplant production period, the increase in leaf area was characterised by higher fitted increments, higher maximum rates of increase and at the lower temperature also higher fitted minimum leaf area.
- No clear associations were found between the leaf area of individual plants after transplanting and their leaf area at the end of tuber production.
- No clear associations were found between the leaf area of individual plants after transplanting and parameters characterising their logistic increase in leaf area.
- For plants with a larger leaf area at the end of the tuber production period, the increase in leaf area was characterised by higher fitted increments and higher maximum rates of increase, and by a later midpoint of the logistic curve.

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CHAPTER 5

Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Correlations between parameters describing individual leaf area increase

Submitted as:

Tadesse, M., W.J.M. Lommen, P.E.L. Van der Putten & P.C. Struik, 2000. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Correlations between parameters describing individual leaf area increase.

5. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Correlations between parameters describing individual leaf area increase

Abstract

Leaf area of *in vitro* propagated potato plantlets increases logistically in the normalisation, transplant production and tuber production phases of a seed production system. Logistic curves were fitted to the leaf area of individual plants in these three growth phases to identify associations between growth parameters. Curve parameters for each phase (fitted minimum leaf area (A), initial relative rate of increase (B), fitted increment (C), fitted mid-point (M) and maximum rate of increase (MI)) were related to each other using linear correlations. Plants received two treatments in each of the three phases. Consequently 2, 4 and 8 sets of plants were analysed in the respective phases.

A and B were significantly and positively correlated in all 14 cases. A higher A-value was also significantly associated with a lower M. Similarly, B was significantly correlated with M in 11 out of 14 cases, out of which 8 showed a negative relation. Lower M-values indicate that the curve mid-point is reached earlier. Both A and B were positively, significantly correlated with MI in 12 out of 14 cases each. This likely results from MI being a function of B (MI = B × C/4), whereas A was correlated well to B. Correlations between A and C and between B and C were only significant in a few cases. There was hardly any relationship between M and MI and between C and MI.

Physiologically relevant relations were those between M and C in the tuber production phase.

Key words: potato, *Solanum tuberosum* L., *in vitro* plantlet, logistic growth, growth analysis, leaf area, temperature, seed production.

Introduction

In vitro techniques have widely been introduced in potato seed production systems during the past few decades to overcome the problems associated with the conventional seed production system based on clonal selection (Struik & Wiersema, 1999). These techniques produce large numbers of disease-free plants within a short period of time all year round (Jones, 1988) and losses due to re-infection hardly occur since production takes place under aseptic conditions. The fastest *in vitro* seed

production system constitutes four phases: *in vitro* multiplication (through nodal cuttings), normalisation (growing single-node cuttings into rooted *in vitro* plantlets), transplant production (acclimatising *in vitro* plantlets in a glasshouse), and tuber production (in the field or greenhouses).

Different practices are employed in different laboratories to produce, grow and use *in vitro* plants in seed production systems (Goodwin & Brown, 1980; Sipos *et al.*, 1988; Levy, 1988; Struik & Wiersema, 1999). However, these protocols do not limit the variations that might exist between plants and do not take into account the consequences of these variations in subsequent phases. Differences in size or other physiological characteristics of the explant and differences in growth conditions may cause plant-to-plant variations. In the *in vitro* technique of seed tuber production, the same plants are exposed to different growth conditions in the last three phases of the production scheme. Transplant shocks or boosts may, therefore, occur during transition of the plants from one phase to another (Sutter *et al.*, 1988; Grout, 1988; Sutter *et al.*, 1992; Tadesse *et al.*, 2000a) and these will be reflected in leaf area growth and other developmental processes in the various phases.

Tadesse *et al.* (2000b) found that leaf area is an important growth parameter for *in vitro* plantlets and indicated that leaf area of transplants at the end of the transplant production phase was positively influenced by leaf area of the same plantlet at the beginning of the phase. Tadesse *et al.* (2000a) showed that the increase in leaf area with time usually follows a logistic growth in the normalisation, transplant production and tuber production phases. The effect of leaf area of an individual plant at the beginning of a phase on the values of the different curve parameters of the logistic growth function of leaf area of the same plant was described by Tadesse *et al.* (2000c). Results indicated that a higher initial leaf area (ILA) led to higher fitted minimum leaf area (A), higher increment (C) and higher maximum rate of increase (MI) in the normalisation phase and to a higher increment (C) in the transplant production phase. Although the relationship between the leaf area of a plant and the different curve parameters of the logistic growth curve has been determined, the correlations of the various curve parameters with each other are not known.

The aim of the current study is, therefore, to assess the relationship of the curve parameters among themselves using a matrix of linear correlation. This chapter is the third of a series on leaf area development and growth in the normalisation, transplant production and tuber production phases of an *in vitro* seed tuber production system.

Materials and methods

Details on the experiment described in this study have already been provided by Tadesse *et al.* (2000a). Below we briefly summarise the main aspects and provide further details relevant to this study.

Potato culture and treatments

Potato (*Solanum tuberosum* L., cv. Gloria) plantlets were propagated *in vitro* by producing single-node cuttings using virus-free stock plantlets and were cultured on a standard medium containing MS salts (Murashige & Skoog, 1962) with vitamins, 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). Viable nodes were cultured in sealed 25 × 150 mm culture tubes on a 10 ml medium at 17 or 23 °C and a photophase of 16 h for 21 days in the "normalisation" phase. Rooted *in vitro* plantlets were then planted in transplanting trays with small cells filled with potting soil and grown in growth chambers with day/night temperatures of 18/12 or 26/20 °C, a photophase of 14 h and a relative humidity of 80% for 14 days. This is the "acclimatisation" or "transplant production" phase. After acclimatisation, the plants were transplanted into 5 litre pots at an initial planting density of 16 plants m^{-2} declining with time to 12.8, 9.6, and 6.4 plants m^{-2} at 7, 14, and 28 DAT, respectively. The pots filled with potting soil, were grown in two glasshouses at a day/night temperature of 18/12 or 26/20 °C, a photophase of 16 h and a relative humidity of 80% for 42 days in the "tuber production" phase.

Experimental design

The experiment was carried out in a split-split plot design in 16 blocks where tuber production temperature (TB) was randomised within the transplant production temperature (TP) and the latter was randomised within the normalisation temperature (N). Thus, a total of 128 plants (2 TB × 2 TP × 2 N × 16 replications = 128 plants) was used for frequent non-destructive measurements on leaf development. They were part of a larger experiment with the same treatments, in total consisting of 992 plants, to allow for frequent destructive measurements.

Measurements and statistical methods

Leaf area was measured before and after (trans)planting, and then every 3 days in the normalisation and transplant production phases and during the first week of the tuber

production phase. From then on, measurements were taken every week in the tuber production phase. Leaf area (including the explant's) was non-destructively estimated using a transparent sheet with grids (1 × 1 mm or 5 × 5 mm) during the normalisation and transplant production phases, respectively. In the tuber production phase, leaf area was estimated by measuring the length (l) and width (w) of individual compound leaves of each of the 128 plants and subsequently calculating leaf area using the formula l × w × k. A shape factor, K, was derived from average measurements of typical leaves at a particular time of estimation. Measurements of leaf area on the first and last day of every phase gave the "initial leaf area" (ILA) and "final leaf area" (FLA), respectively in all phases of growth.

Only those plants that showed normal logistic growth were used in this study since logistic curves best explained leaf area increment in all phases of growth (Tadesse *et al.*, 2000a). The relevant curve parameters in this type of growth curve were described by Tadesse *et al.* (2000a). To study which parameters account for the greatest part of the variations in leaf area at the end of a phase, logistic growth curve parameters were related to each other using a matrix of linear correlation. Fitted minimum leaf area (A), initial relative rate of increase (B), fitted mid-point (M), fitted increment (C) and maximum rate of increase (MI) were correlated with each other at the different temperature treatments in the normalisation, transplant production and tuber production phases. Maximum numbers of data pairs per treatment were 64 for the normalisation phase, 32 for the transplant production phase and 16 for the tuber production phase. The total number of cases per combination of parameters was 2 (for normalisation phase), plus 4 (for transplant production phase) plus 8 (for tuber production phase) = 14.

Results

Matrices of linear correlations between parameters describing logistic growth in the normalisation, transplant production and tuber production phases showed that the relationship between some parameters were consistently affected in a similar way in all phases of growth.

The fitted minimum leaf area (A) and the initial relative rate of leaf area increase (B) were significantly, positively correlated in the three phases of growth in all 14 possible cases (Tables 1, 2, 3).

A higher fitted minimum leaf area (A) was significantly associated with an earlier fitted mid-point (M) in the three phases of growth in 7 out of 14 cases (Tables 1, 2, 3). Six out of the seven significant correlations and six out of the remaining seven non-significant correlations between A and M showed a negative relation. The

Table 1. Matrix of linear correlations between parameters describing logistic growth at 17 °C (upper half of matrix) or at high 23 °C temperatures (lower half of matrix) in the normalisation phase.

17 °C (n = 49)	A	B	M	C	MI
23 °C (n = 58)					
A	–	0.29*[1]	–0.20 NS	0.01 NS	0.41**
B	0.48***	–	–0.17 NS	–0.50***	0.17 NS
M	–0.32*	–0.44**	–	0.25 NS	–0.10 NS
C	0.15 NS	–0.28 NS	–0.20 NS	–	0.66***
MI	0.58***	0.46***	–0.54***	0.69***	–

[1] ***: $P<0.001$; **: $0.001 \leq P<0.01$; *: $0.01 \leq P<0.05$; NS: not significant $P \geq 0.05$.

A - fitted minimum leaf area (mm^2)

B - initial relative rate of increase (day^{-1})

M - fitted mid-point (days after cutting or after (trans) planting in later tables)

C - fitted increment (mm^2)

MI - maximum rate of increase in leaf area at M ($mm^2\ day^{-1}$).

significant correlations were mainly found under high temperature.

The fitted minimum leaf area (A) was usually not significantly correlated with the fitted increment (C) (Tables 1, 2, 3). There were, however, a significant positive and a significant negative correlations at low and at high temperatures, respectively, in the transplant production phase (Table 2), and a single significant negative correlation at high temperature in the tuber production phase (Table 3). All non-significant correlations in the transplant production and tuber production phases were negative.

The fitted minimum leaf area (A) was well and positively correlated with the maximum rate of increase in leaf area (MI) in 12 out of 14 cases (Tables 1, 2, 3).

The initial relative rate of increase in leaf area (B) was significantly correlated with the fitted mid-point (M) in the three phases of growth, in 11 out of 14 cases. Of these, 8 were negative (Tables 1, 2, 3). A higher initial relative rate of increase in leaf area (B) resulted in a lower fitted mid-point (M) at higher temperature (23 °C) in the normalisation phase (Table 1) and at lower temperature (18/12 °C) in the transplant production phase (Table 2). In the tuber production phase plants pre-cultured at high transplant production temperature showed a negative correlation, while those pre-

Table 2. Matrix of linear correlations between parameters describing logistic growth at 18/12 or 26/20 °C in the transplant production phase after growing at 17 or 23 °C in the normalisation phase. Matrices for temperature combinations at the top of the table are listed in the upper part and those for temperature combinations below them are presented in the lower part of the correlation matrix.

17 & 18/12 °C (n = 21)	A	B	M	C	MI
17 & 26/20 °C (n = 24)					
A	–	0.43*	–0.40 NS	0.45*	0.83***
B	0.84***	–	–0.55**	–0.32 NS	0.45*
M	0.05 NS	–0.13 NS	–	0.40 NS	–0.06 NS
C	–0.52*	–0.66***	0.47*	–	0.66**
MI	0.72***	0.66***	0.27 NS	0.06 NS	–
23 & 18/12 °C (n = 14)	A	B	M	C	MI
23 & 26/20 °C (n = 18)					
A	–	0.77**	–0.66*	0.12 NS	0.92***
B	0.62**	–	–0.72**	–0.33 NS	0.71**
M	0.50*	0.41 NS	–	0.57*	–0.42 NS
C	–0.21 NS	–0.49*	–0.42 NS	–	0.39 NS
MI	0.54*	0.55*	0.05 NS	0.44 NS	–

[1] ***: P<0.001, **: 0.001≤*P*<0.01; *: 0.01≤*P*<0.05; NS: not significant *P*≥0.05.
For abbreviations see Table 1.

cultured at low transplant production temperature had a positive correlation in three out of four cases.

The correlation of the initial relative rate of increase (B) with the fitted increment (C) was only significant in 5 out of the 14 cases and was consistently negative in all cases including the non-significant correlations (Tables 1, 2, 3).

The initial relative rate of leaf area increase (B) was consistently significantly correlated with the maximum rate of increase in leaf area (MI), in 12 out of the 14 cases. All correlations (including the two non-significant ones) were positive (Tables 1, 3).

The fitted mid-point (M) of the logistic curve had a significant, positive correlation with the fitted increment (C) in the transplant production and tuber production phases in 8 out of the 12 possible cases (Tables 2, 3). M and C were not significantly correlated in the normalisation phase (Table 1).

The fitted mid-point (M) was significantly negatively correlated with the maximum rate of increase in leaf area (MI) at high temperature in the normalisation phase (Table 1). There was no significant correlation in the transplant production phase. Only one (positive) correlation was significant in the tuber production phase (Table 3).

The fitted increment (C) was only positively correlated to the maximum rate of increase in leaf area (MI) at both temperatures in the normalisation phase (Table 1), at low temperature in the transplant production phase after a low normalisation temperature (Table 2) and at one temperature combination (high normalisation-high transplant production-low tuber production temperature) in the tuber production phase (Table 3).

Discussion

Relationships among parameters characterising the logistic leaf area increase of individual plants in different phases may exist for different reasons. Firstly, there can be mathematical relations between parameters. For instance, the maximum rate of increase (MI) is a function of B and C ($MI = B \times C/4$). Secondly, effects on different parameters could have the same physiological basis. For instance, larger explant leaves may lead to higher fitted minimum leaf areas (A) in the normalisation phase (Tadesse *et al.*, 2000c) and also to faster growth, thus increasing C or MI (cf. Tadesse *et al.*, 2000c; Seabrook & Douglass, 1994). Finally, relationships among parameters may result from the actual way the logistic curve is best fitting through the data values. Slight changes in data may cause effects on curve fitting that could result in simultaneous changes in values of different parameters. For instance, when the S-

Table 3. Matrix of linear correlations between parameters describing logistic growth at 18/12 or 26/20 °C in the tuber production phase after growing at 18/12 or 26/20 °C in the transplant production and at 17 or 23 °C in the normalisation phases. Matrices for temperature combinations at the top of the table are listed in the upper part and those for temperature combinations below them are presented in the lower part of the correlation matrix.

17, 18/12 & 18/12 °C (n = 16)	A	B	M	C	MI
17, 18/12 & 26/20 °C (n = 15)					
A	–	0.86***[1]	–0.25 NS	–0.22 NS	0.71**
B	0.84***	–	0.58 **	–0.49 NS	0.59*
M	–0.28 NS	0.55*	–	0.94***	0.27 NS
C	–0.16 NS	–0.30 NS	0.80***	–	0.42 NS
MI	0.75**	0.76***	0.05 NS	0.37 NS	–
17, 26/20 & 18/12 °C (n = 16)	**A**	**B**	**M**	**C**	**MI**
17, 26/20 & 26/20 °C (n = 16)					
A	–	0.90***	–0.54*	–0.41 NS	0.65**
B	0.92***	–	–0.71**	–0.44 NS	0.74**
M	–0.59*	–0.75***	–	0.78***	0.18 NS
C	–0.12 NS	–0.19 NS	0.45 NS	–	0.28 NS
MI	0.84***	0.86***	–0.48 NS	0.34 NS	–

[1] ***: P<0.001; **: 0.001≤*P*<0.01; *: 0.01≤*P*<0.05; NS: not significant *P*≥0.05.
For abbreviations see Table 1.

Table 3. Cont'd ...

23, 18/12 & 18/12 °C (n = 14)	A	B	M	C	MI
23, 18/12 & 26/20 °C (n = 12)					
A	–	0.85***[1]	–0.36 NS	–0.06 NS	0.85***
B	0.80**	–	–0.73 **	–0.31 NS	0.87***
M	–0.80**	0.90***	–	0.52 NS	0.48 NS
C	–0.71**	–0.83***	0.81**	–	0.21 NS
MI	0.56*	0.67**	0.56*	0.15 NS	–
23, 26/20 & 18/12 °C (n = 15)	A	B	M	C	MI
23, 26/20 & 26/20 °C (n = 15)					
A	–	0.89***	–0.38 NS	–0.50 NS	0.24 NS
B	0.88***	–	–0.66**	–0.56*	0.25 NS
M	–0.62*	–0.72**	–	0.77***	0.30 NS
C	–0.42 NS	–0.51 NS	0.76**	–	0.65**
MI	0.49 NS	0.53*	0.04 NS	0.45 NS	–

[1] ***: P<0.001; **: 0.001≤*P*<0.01; *: 0.01≤*P*<0.05; NS: not significant *P*≥0.05.
For abbreviations see Table 1.

shape becomes a bit less sharp not only the B-value, but also the fitted A value will decrease.

Out of the relations tested, three groups seem to be of interest: (1) relations among A, B, and MI, (2) relations between C and MI and (3) relations among C and M and between M and A or B.

Relationships among A, B and MI

The fitted minimum leaf area (A) and the initial relative rate of leaf area increase (B) were so well and positively correlated in all possible cases that it is tempting to suggest that a higher fitted minimum leaf area is likely to occur when initial leaf area (ILA) is high and that this is associated with more substrate for growth to realise a higher initial relative rate of leaf area increase (B). However, Tadesse *et al.* (2000c) reported that high ILA in the transplant production and tuber production phases were not consistently related to a higher A or higher B. It is, therefore, likely that in these phases the very clear positive associations between A and B mainly resulted from the simultaneous effect that a change in the course of the logistic curve has on both parameters, and much less from a higher initial leaf area as a shared physiological cause.

Both A and B were very well correlated with the maximum rate of increase (MI). Since MI is a function of B (MI = B×C/4), it is logical that B and MI are correlated. The correlation between A and MI is likely to result indirectly from the very clear correlation between A and B. It is less likely that the correlation between A and MI resulted from larger initial leaf areas (ILAs) and consequently higher growth rates, because both the correlations between ILA and A were not consistently found (Tadesse *et al.*, 2000c) and the correlations between A and C were weak.

Relation between C and MI

Since MI is also a function of C (MI = B×C/4), it is also logical that C and MI are correlated. However, correlations between C and MI in the transplant and tuber production phase were usually not significant (although positive). Only those between B and MI were. Correlations between B and C tended to be negative. All this suggests that the relations between C and MI were mainly of a non-physiological nature.

Relations between M and C, M and A and M and B

The positive correlation between M and C often found in the tuber production phase suggests that plants that were later in development achieved a higher leaf area increase. This is likely because of less senescence of leaves that was already very prominent at high temperature during tuber production (Tadesse *et al.*, 2000a) or a delayed restriction of haulm growth because of later tuber bulking (Van Dam *et al.*, 1996).

Especially at the higher temperature during tuber production, M and A were clearly negatively correlated, suggesting that plants with larger fitted minimum leaf areas were more advanced. Alternatively, because of the clear association between A and B, it might also suggest that plants with a clearer S-shaped curve were more advanced. The latter, however, seems less likely because the association between B and M within some treatments was positive but negative in others. Direct associations between A or B and C in the tuber production phase, however, were usually not significant, though negative.

Temperature effects

When significant, correlations between parameters in the normalisation and tuber production phases never had opposite signs at the two temperatures during a phase, but often were clearer at one of the temperatures. In the transplant production phase, however, a significant positive correlation between A and C was found at low temperature, but a negative at high temperature. Also a negative correlation was found between A and M at the low temperature and a positive at the high temperature. It is unknown where these differences arise from.

Also pre-treatments never had significant opposite effects on correlation coefficients, except for those between B and M in the tuber production phase. These were negative when plants were pre-grown at high transplant production temperature and positive in three out of the four treatments in which plants were pre-grown at low transplant production temperature.

Conclusions

- There was a consistent significant positive correlation between the fitted minimum leaf area (A) and the initial relative rate of increase (B).
- Both the fitted minimum leaf area (A) and the initial relative rate of increase (B) correlated well with the maximum rate of increase (MI), which is a function of B.

- There was hardly any relationship between the fitted increment (C) and the maximum rate of increase (MI), although MI is a function C.
- In the tuber production phase, the fitted increment in leaf area (C) was higher for plants being less advanced, as shown by a later midpoint (M). A later midpoint (M) was - especially under high temperature - associated with a higher fitted minimum leaf area (A).
- Most likely, only the latter conclusion has a physiological basis.

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CHAPTER 6

Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Dry matter production

Submitted as:

Tadesse M., W.J.M. Lommen & P.C. Struik, 2000. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Dry matter production.

6. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Dry matter production

Abstract

To assess effects and after-effects of temperature treatments on dry matter production, *in vitro* potato plantlets produced at 17 or 23 °C (normalisation phase) were grown in soil in growth chambers with day/night temperatures of 18/12 or 26/20 °C for 14 days (transplant production phase). They were then transplanted in 5-liter pots to two glasshouses with day/night temperatures of 18/12 or 26/20 °C (tuber production phase). Some plants were left to grow for one more week in the normalisation and transplant production phases.

Transition of plants from one phase to another, especially from *in vitro* to *in vivo* conditions, greatly increased leaf growth and to a smaller extent stem growth. In all phases, higher temperature increased leaf and stem dry weights, reduced tuber dry weights in the tuber production phase and decreased leaf/stem ratio and SLA in the phases for which they were assessed. Total plant dry weight was higher at high than at low temperature at the beginning of the tuber production phase but lower at the end.

Normalisation and transplant production temperatures showed significant after-effects in later phases. Growing plants at lower normalisation temperature significantly increased leaf and total plant dry weights early in the transplant production phase and in the first part of the tuber production phase. Growing plants at lower transplant production temperature reduced stem weight during tuber production, but increased tuber weight early in the tuber production phase and later when temperatures during tuber production were high.

Key words: potato, *Solanum tuberosum* L., after-effects, normalisation, transplant production, tuber production, transplanting, acclimatisation, dry matter production, *in vitro* plantlet, temperature, specific leaf area.

Introduction

The conventional way of propagating potato (*Solanum tuberosum* L.) involves the repeated multiplication of potato seed tubers. The multiplication rate is very low, only 12-20 per year (Beukema & Van der Zaag, 1990) in areas where one crop is grown per year. A large proportion of the potato hectarage must be used for seed potato production; world-wide this propagation takes about 12% of the potato acreage (Struik & Wiersema, 1999). Potato is susceptible to many viral, bacterial and fungal diseases

and the seed stock gradually degenerates with increasing numbers of field multiplication (Haverkort *et al.*, 1991). Healthy basic seed, therefore, needs to be maintained and used as a renewed starting point of the production scheme over and over again. It needs to be multiplied several times in the field to produce commercial seed in the amounts required. This obviously limits potato seed production in many regions of the world, especially in those where degeneration is rapid. Routine production of disease-free seed tubers is costly and difficult but necessary to maintain adequate yields of ware potatoes (Hussey & Stacey, 1981).

Several techniques have been developed during the past few decades to overcome the problems associated with the conventional seed production system (Struik & Wiersema, 1999). Micropropagation (tissue culture) is one of such techniques which is nowadays widely used to produce large quantities of healthy and genetically uniform *in vitro* plantlets for seed tuber production purposes (Goodwin *et al.*, 1980; Marinus, 1983). The fastest seed production system with the highest multiplication rate involves four phases: *in vitro* multiplication of nodal cuttings, normalisation (where single-node cuttings develop into rooted *in vitro* plantlets), transplant production (acclimatisation) and tuber production.

The physiology of the plantlets throughout all phases is complicated. Firstly, in the last three phases of this seed production system plantlets are subsequently exposed to different conditions which affect growth during the current and later phases. Secondly, plantlets experience sudden changes in growth conditions as a result of planting or transplanting. The performance of plantlets during later phases may be manipulated by treatments and/or cultural conditions in earlier phases (Hussey & Stacey, 1981; Tadesse *et al.*, 2000a, b, c). There still is no clear view on how *in vitro* produced potato plantlets develop through all these phases and on how pre-treatments affect further growth. In this paper we will focus on temperature effects during different phases on production of *in vitro* propagated potato plants.

Growth and development of potatoes are profoundly affected by high soil and air temperatures (Ewing, 1981). Potato is well adapted to a mean temperature of 17 °C. Haulm growth is enhanced by an increase in temperature until an optimum of 20-25 °C (Ingram & McCloud, 1984). High temperature delays stolon and tuber initiation (Gregory, 1956; Slater, 1963) and onset of early tuber growth, the optimum being 15-19 °C (Van Dam *et al.*, 1996). High temperature also delays and reduces partitioning of dry matter to the tubers resulting in low harvest indices (Struik & Ewing, 1995). Van Dam *et al.* (1996) stressed that already a slight increase in temperature above the optimal range of 15-19 °C strongly reduces dry matter partitioning to tubers. Low temperature, on the other hand, restricts haulm growth and promotes the accumulation of dry matter in the tubers (Menzel, 1985). A delay in tuber formation, however, may

stimulate final yield when the growing season is sufficiently long to profit from the increased duration of ground cover (Struik & Ewing, 1995).

These general effects of temperature on potato physiology may also be relevant to seed production systems starting from *in vitro* plantlets and may determine the efficiency of the system. Tadesse *et al.* (2000a) reported that leaf area of *in vitro* propagated potato plantlets increased logistically through time in all three phases of the production system and that temperature had an effect on leaf area and leaf number in the various phases. Tadesse *et al.* (2000b) found that growing potato plantlets at higher temperature during the transplant production phase promoted leaf area of plantlets during the tuber production phase in the field. Higher leaf area may increase yield directly by enabling the plants to intercept more solar radiation. The effect of pre-culture conditions on dry matter partitioning during later phases may also be important. According to Lommen (1999), the lower accumulated intercepted radiation and hence lower yields in transplant crops from early cultivars may be associated with their dry matter partitioning – limiting haulm growth and favouring tuber growth – in the early phase of field growth.

Information on the pattern of dry matter production of *in vitro* produced plantlets and the effect of pre-treatments on this pattern can help to understand yield formation processes within a crop. This study, therefore, assesses dry matter production of *in vitro* propagated potato plantlets over three phases of growth as influenced by temperature during various phases.

Materials and methods

Details on the experiment described in this chapter have already been provided by Tadesse *et al.* (2000a). In the following sections we briefly summarise the main aspects of culture, treatments and design, and provide further details on data collection and analysis relevant to the results presented.

Potato culture and treatments

Potato (*Solanum tuberosum* L., cv. Gloria) plantlets were propagated *in vitro* by producing single-node cuttings using virus-free stock plantlets. The plantlets were cultured on a standard medium containing MS salts (Murashige & Skoog, 1962) with vitamins, 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). Viable nodes were cut from plantlets discarding tops and cultured in 25×150 mm culture tubes on a 10 ml medium (one per tube) at 17 or 23 °C and a photophase of 16 h for 21 days in the "normalisation" phase.

Rooted *in vitro* plantlets were then planted in transplanting trays with small cells filled with potting soil and grown in chambers with day/night temperatures of 18/12 (optimal for tuber growth) or 26/20 °C (optimal for haulm growth), a photophase of 14 h and a RH of 80% for 14 days in the "transplant production" phase.

At the end of the transplant production phase, plants were transplanted in 5 liter pots filled with potting soil to two glasshouses at an initially density of 16.0 plants m^{-2} subsequently declining, by repetitive sampling, to 12.8, 9.6 and 6.4 plants m^{-2}. The pots were placed in two glasshouses at a day/night temperature of 18/12 or 26/20 °C, a photophase of 16 h and a relative humidity of 80% for 42 days in the "tuber production" phase.

Experimental design

The experiment was carried out in a split-split plot design in 16 blocks where the tuber production temperature (TB) was randomised within the transplant production temperature (TP) and the latter was randomised within the normalisation temperature (N). The tuber production phase comprised of 2 TB × 2 TP x 2 N × 5 harvests (H) × 16 blocks = 640 plants. For harvesting during the transplant production phase an additional set of plants was available: 16 blocks × 2 TP × 2 N × 3 H = 192 plants, and for harvesting in the normalisation phase 16 blocks × 2 N × 5 H = 160 plants were present. The total number of plants in the experiment was 992.

Harvests were carried out at 0, 7, 14, 21 and 28 days after cutting (DAC) in the normalisation phase, at 7, 14 and 21 days after planting (DAP) in the transplant production phase, and at 7, 14, 28 and two groups of plants at 42 days after transplanting (DAT) in the tuber production phase (Fig. 1). Of those plants, thirty two (16 × 2 temperature treatments, harvest 28 DAC) in the normalisation and 64 plants (16 × 4 temperature combinations, harvest 21 DAP) in the transplant production phase were allowed to grow one extra week in their respective phases compared to other plants, in order to follow up their pattern of undisturbed growth (Fig. 1). Two groups of 128 (16 × 8) plants – one group selected for continuous measurement of plant growth characteristics throughout the three phases of growth and another group that was not exposed to measurements – were harvested at the end of the experiment (42 DAT) and were also compared.

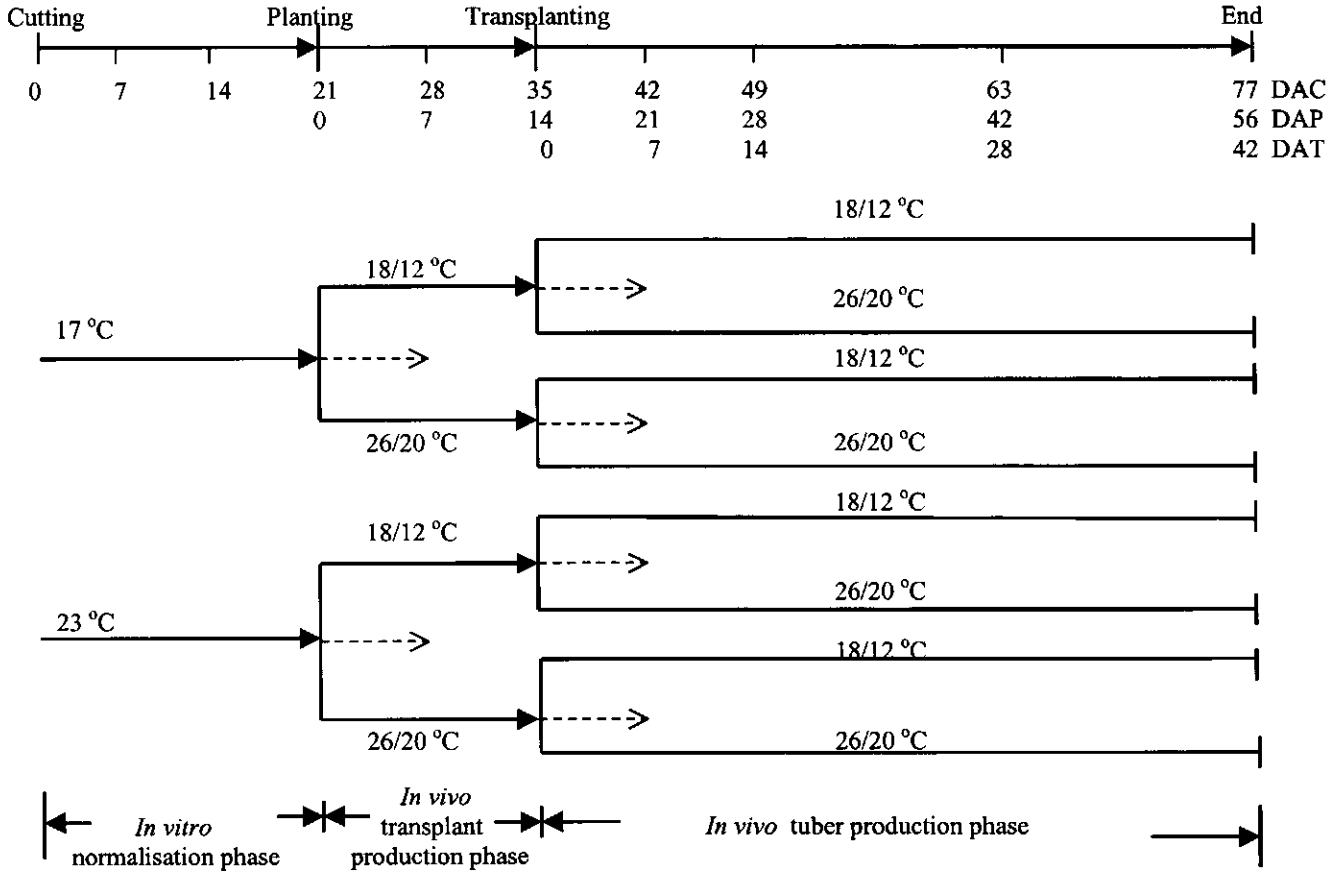

Fig. 1. Schematic representation of the different temperature treatments in the three growth phases. DAC = days after cutting, DAP = days after planting and DAT = days after transplanting.

Measurements and statistical methods

Dry matter increase through time was analysed by destructively harvesting plants, separating the different plant parts and drying them in an oven at 105 °C for 16 hours. Leaf dry weight, above and below ground stem dry weight and total plant dry weight were measured in all three phases of growth. Stolons were included in the stem fraction. In addition, tuber dry weight was measured and leaf/stem ratio and SLA (leaf area per unit weight) were calculated in the tuber production phase. Roots were not included in any data collection or processing. Leaf, stem, tuber and total dry weights of the two groups of 128 plants were also compared at final harvest to establish possible thigmomorphogenetic effects caused by frequent measurements. Data were subjected to analysis of variance (ANOVA) using Genstat 5 release 3.22 (1995) and differences between treatments were analysed by LSD tests at $P<0.05$.

Results

Normalisation phase

Leaf dry weight was not significantly different at the two temperatures in the normalisation phase although there was a gradual increase in the difference between the two temperature treatments. For stem dry weight, the temperature effect also increased over time and stem weight was significantly higher at higher than at lower temperature at the end of the normalisation phase (21 DAC) (Table 1). Total plant dry weight was also not significantly different at the two temperatures in the normalisation phase. Leaf, stem and total dry weights were not significantly different for the two temperatures after the extended growth at 28 DAC (Table 1).

Transplant production phase

There was no significant effect of temperature on leaf dry weight in the normalisation phase, but some effect was carried over to the next phase: plants pre-treated with lower normalisation temperature had higher leaf dry weight at 7 DAP in the transplant production phase, which is in contrast with the trend during normalisation. The effect, more or less, disappeared later (Table 2). Leaf dry weight was significantly higher at higher than at lower transplant production temperature at the end of the transplant production phase (14 DAP) but there was no difference in leaf dry weight at the two temperatures after the one week extended growth (21 DAP) (Table 2). For stem dry weight no after-effects of normalisation conditions were observed. Stem dry weight

Table 1. Leaf, stem and total dry weights at various harvests, of *in vitro* propagated potato plantlets grown at low (17 °C) or high (23 °C) temperatures during the normalisation (N) phase. DAC = days after cutting.

	Normalisation harvests (DAC)				
	0	7	14	21	28[a]
Leaf dry weight (mg/plant)					
17 °C	0.10	0.54	1.15	2.78	4.50
23 °C	0.17	0.66	1.55	3.14	4.30
Significance [b]					
N	NS	NS	NS	NS	NS
Stem dry weight (mg/plant)					
17 °C	0.37	0.79	1.09	1.10	1.86
23 °C	0.39	0.98	1.33	1.64	2.32
Significance [b]					
N	NS	NS	NS	**	NS
Total dry weight (mg/plant)					
17 °C	0.47	1.32	2.24	3.88	6.36
23 °C	0.56	1.64	2.88	4.78	6.62
Significance [b]					
N	NS	NS	NS	NS	NS

[a] results of the one week extended growth

[b] ** $0.001 \leq P < 0.01$; NS: not significant $P \geq 0.05$

was significantly higher at higher than at lower transplant production temperature throughout the transplant production phase and at the end of the extended growth period (Table 2). A tremendous increase in stem dry weight was observed during the one-week extended growth (14-21 DAP) at both temperatures in the transplant production phase (Table 2).

An after-effect of the normalisation temperature was present for total plant dry weight at the early stages of the transplant production phase. Plants pre-grown at lower normalisation temperature produced higher total plant dry weight in the transplant production phase than those grown at higher normalisation temperature (Table 2).

Table 2. Leaf, stem and total plant dry weights at various harvests during the transplant production phase (TP), of *in vitro* propagated potato plantlets pre-grown at two normalisation (N) temperatures. For data at planting, see the last harvest (21 DAC) of the previous phase (Table 1). DAP = days after planting.

		Transplant production harvests (DAP)		
		7	14	21[a]
Leaf dry weight (mg/plant)				
Temp. (°C) during				
TP	N			
18/12	17	19.4	115	345
18/12	23	14.7	104	343
26/20	17	24.5	172	433
26/20	23	19.5	142	365
Significance[b]				
N		*	NS	NS
TP		NS	**	NS
Stem dry weight (mg/plant)				
Temp. (°C) during				
TP	N			
18/12	17	2.9	7.9	133
18/12	23	2.6	7.7	128
26/20	17	4.2	21.7	189
26/20	23	3.8	17.7	168
Significance[b]				
N		NS	NS	NS
TP		**	***	**
Total dry weight (mg/plant)				
Temp. (°C) during				
TP	N			
18/12	17	22.3	123.1	504
18/12	23	17.3	111.7	489
26/20	17	28.6	194.0	657
26/20	23	23.2	159.7	539
Significance[b]				
N		*	NS	NS
TP		*	***	*

[a] results of the one week extended growth

[b] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$ (all interactions were not significant).

Total plant dry weight was significantly higher at higher than at lower transplant production temperature throughout the phase including the one week extended growth (Table 2).

Tuber production phase

Leaf dry weight

On some dates, after-effects of the normalisation temperature on leaf dry weight were significant but small. Plantlets pre-treated at lower normalisation temperature had higher leaf dry weight than those pre-treated at higher temperature at 7 and 28 DAT in the tuber production phase (Table 3). An interaction at 28 DAT indicated that the positive effect of low normalisation temperature on leaf dry weight was only significant for plants grown at low transplant production temperature, followed by high temperature during tuber production.

The transplant production temperature also showed significant after-effects during the tuber production phase but the effect was opposite: plants pre-treated with high transplant production temperature had significantly higher leaf dry weight at 14 and 28 DAT in the tuber production phase than those pre-treated with low transplant production temperature (Table 3). At 28 DAT the positive effect of a high transplant production temperature on leaf dry weight was only significant for plants grown at the highest normalisation and tuber production temperatures.

Higher tuber production temperature also enhanced leaf dry weight throughout the tuber production phase. A three-way interaction between temperatures of the three phases at 28 DAT indicated that the effect of high temperature on leaf dry weight during the tuber production phase was relatively small when temperatures had been low during transplant production but high during normalisation, whereas the effect was relatively large when temperatures had been low during both earlier phases.

Stem dry weight

An after-effect of the normalisation temperature on stem dry weight was significant during the tuber production phase: plants pre-treated with lower normalisation temperature had a significantly higher stem dry weight at 7 DAT in the tuber production phase (Table 3). This positive effect also showed at 14 DAT for plants growing at higher tuber production temperature, and at 28 DAT for plants growing at high tuber production temperature after low temperature during transplant production.

Table 3. Leaf, stem and total plant dry weights at various harvests during the tuber production phase (TB), of *in vitro* propagated potato plantlets pre-grown at two normalisation (N) and two transplant production (TP) temperatures. For data at planting, see the last harvest (14 DAP) of the previous phase (Table 2). DAT = days after trasplanting.

			Harvest dates during tuber production (DAT)			
			7	14	28	42
Leaf dry weight (g/plant)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.61	2.60	8.71	11.53
18/12	18/12	23	0.54	2.30	7.99	12.39
18/12	26/20	17	0.69	2.87	9.17	10.95
18/12	26/20	23	0.61	2.99	8.71	11.99
26/20	18/12	17	0.83	3.02	12.00	14.80
26/20	18/12	23	0.63	2.59	9.29	15.30
26/20	26/20	17	0.86	3.31	11.64	15.76
26/20	26/20	23	0.75	2.83	11.32	16.11
Significance[a]						
N			*	NS	**	NS
TP			NS	*	*	NS
TB			*	***	***	***
N*TP*TB			NS	NS	* (1.15)[b]	NS
Stem dry weight (g/plant)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.11	0.55	2.58	2.75
18/12	18/12	23	0.10	0.52	2.37	2.80
18/12	26/20	17	0.16	0.67	3.31	3.33
18/12	26/20	23	0.13	0.71	2.83	3.45
26/20	18/12	17	0.17	0.84	7.51	7.95
26/20	18/12	23	0.13	0.72	5.60	8.07
26/20	26/20	17	0.22	1.07	7.83	9.24
26/20	26/20	23	0.20	0.87	7.81	10.56
Significance[a]						
N			*	NS	**	NS
TP			***	***	***	***
TB			***	***	***	***
N*TB			NS	* (0.13)[b]	NS	NS
N*TP*TB			NS	NS	** (0.86)	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS: not significant $P \geq 0.05$

[b] numbers in brackets show LSD for interactions (all other interactions were not significant).

The after-effect of the transplant production temperature was persistent throughout the tuber production period: stem dry weight usually was significantly higher for plants pre-grown at high transplant production temperature than those grown at low transplant production temperature (Table 3).

A high tuber production temperature enhanced stem dry weight compared to a low temperature during this phase (Table 3). The positive effect was larger after a lower than after a higher normalisation temperature at 14 DAT. The significant three-way interaction between the temperatures of the three phases at 28 DAT was consistent with the same interaction for leaf dry weight.

Specific leaf area (SLA)

After-effects of the normalisation temperature indicated that plantlets grown at lower temperature during the normalisation phase had a lower SLA at 7 DAT in the tuber production phase (Table 4). At 28 DAT, this effect of low normalisation temperature was only found for plants grown at high temperature during transplant production (Table 4).

A lower transplant production temperature resulted in a higher SLA than a higher transplant production temperature at 14 DAT in the tuber production phase, and at 28 DAT for plants grown at higher normalisation temperature (Table 4).

A lower tuber production temperature resulted in plants with higher SLA throughout the tuber production phase (Table 4).

Leaf/stem ratio

Normalisation temperature sometimes had after-effects on leaf/stem ratio in the subsequent phases. Significant interactions between normalisation and tuber production temperatures at 14 and 42 DAT indicated that a lower normalisation temperature resulted in plants with higher leaf/stem ratios for plants grown for 14 DAT at a low tuber production temperature and for 42 DAT at a high tuber production temperature.

The effect of the temperature during the transplant production phase was also, carried over to the tuber production phase: lower transplant production temperature resulted in plants with a higher leaf/stem ratio throughout the tuber production phase (Table 4). The positive effects of transplant production temperature (28 DAT) on leaf/stem ratio were higher at lower than at higher tuber production temperatures.

Leaf/stem ratio was higher at lower than at higher tuber production temperature and the difference became larger at later stages of growth in the tuber production phase

Table 4. Specific leaf area (SLA) and leaf/stem ratios at various harvests during the tuber production phase (TB), of *in vitro* propagated potato plantlets grown at two normalisation (N) and two transplant production (TP) temperatures.

			Harvest dates during tuber production (DAT)			
			7	14	28	42
SLA ($cm^2\ g^{-1}$)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	242	266	209	155
18/12	18/12	23	299	289	234	154
18/12	26/20	17	230	259	196	153
18/12	26/20	23	276	255	200	154
26/20	18/12	17	210	281	160	121
26/20	18/12	23	253	292	177	120
26/20	26/20	17	204	272	154	120
26/20	26/20	23	234	278	151	120
Significance[a]						
N			*	NS	*	NS
TP			NS	*	***	NS
TB			**	**	***	***
N*TP			NS	NS	* (12)[b]	NS
Leaf/stem ratio						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	5.70	4.93	3.45	4.31
18/12	18/12	23	5.73	4.47	3.65	4.78
18/12	26/20	17	4.43	4.36	2.81	3.55
18/12	26/20	23	5.07	4.15	3.04	3.99
26/20	18/12	17	5.01	3.68	1.65	1.93
26/20	18/12	23	4.94	3.67	1.82	1.81
26/20	26/20	17	4.00	3.14	1.50	1.78
26/20	26/20	23	4.18	3.30	1.40	1.46
Significance[a]						
N			NS	NS	NS	NS
TP			***	***	***	**
TB			***	***	***	***
N*TB			NS	* (0.27)[b]	NS	* (0.48)
TP*TB			NS	NS	* (0.21)	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS: not significant $P \geq 0.05$

[b] numbers in brackets show LSD for interactions (all other interactions were not significant).

(Table 4). A significant interaction between transplant production and tuber production temperatures at 28 DAT indicated that when plants had been exposed to a lower temperature during the transplant production phase, the effects of the tuber production temperature on leaf/stem ratio was larger than when the plants had been exposed to a higher transplant production temperature (Table 4). At 42 DAT, the positive effect of low tuber production temperature was higher for plants grown at high normalisation temperature.

Tuber dry weight

There was no after-effect of normalisation temperature on tuber dry weight in the tuber production phase. A lower transplant production temperature pre-treatment, however, resulted in a higher tuber dry weight for plants grown at the lower tuber production temperature at 14 DAT, and for plants grown at the higher tuber production temperature at 42 DAT in the tuber production phase (Table 5).

Tuber dry weight was significantly higher at lower than at higher tuber production temperature throughout the tuber production phase except at the beginning when tubers were barely present (Table 5). At 14 DAT, the positive effect of a lower tuber production temperature was larger after a lower than after a higher transplant production temperature, whereas at 28 and 42 DAT it was smaller after a lower than after a higher transplant production temperature.

Total plant dry weight

After-effects of the normalisation and transplant production temperatures on total plant dry weight were prominent in the tuber production phase (Table 5). Plantlets pre-treated with lower normalisation temperature had higher total plant dry weight at both temperatures in the tuber production phase at 7 DAT and for plants grown at a high tuber production temperature at 28 DAT. Plants pre-treated with higher transplant production temperature also had higher total plant dry weight at 7 DAT but this effect disappeared soon in the tuber production phase (Table 5).

Total plant dry weight was significantly higher at higher than at lower temperature at the beginning of the tuber production phase (7 DAT), but this effect was reversed at later stages (28 and 42 DAT) in the tuber production phase (Table 5). A significant interaction between the normalisation and tuber production temperatures at 28 DAT indicated that the negative effect of a higher tuber production temperature on total plant dry weight was larger after a high than after a low normalisation temperature.

Table 5. Tuber dry weight and total plant dry weight at various harvests during the tuber production phase (TB), of *in vitro* propagated potato plantlets pre-grown at two normalisation (N) and two transplant production (TP) temperatures. For data on total dry weight at planting, see the last harvest (14 DAP) of the previous phase (Table 2).

			Harvest dates during tuber production (DAT)			
			7	14	28	42
Tuber dry weight (g/plant)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.02	0.49	18.17	57.14
18/12	18/12	23	0.01	0.34	16.88	53.54
18/12	26/20	17	0.01	0.12	18.16	56.59
18/12	26/20	23	0.01	0.04	18.05	57.82
26/20	18/12	17	0.02	0.04	6.74	34.39
26/20	18/12	23	0.01	0.05	4.62	30.78
26/20	26/20	17	0.001	0.000	5.52	30.53
26/20	26/20	23	0.004	0.004	3.10	26.33
Significance[a]						
N			NS	NS	NS	NS
TP			NS	***	NS	NS
TB			NS	***	***	***
TP*TB			NS	*** (0.11)[b]	* (1.88)	* (3.76)
Total dry weight (g/plant)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.74	3.64	29.46	71.42
18/12	18/12	23	0.65	3.17	27.18	68.73
18/12	26/20	17	0.86	3.65	30.64	70.87
18/12	26/20	23	0.75	3.75	29.59	73.27
26/20	18/12	17	1.02	3.90	26.26	57.14
26/20	18/12	23	0.77	3.37	19.92	54.14
26/20	26/20	17	1.08	4.38	24.99	55.54
26/20	26/20	23	0.96	3.70	22.23	53.00
Significance[a]						
N			*	NS	*	NS
TP			*	NS	NS	NS
TB			***	NS	***	***
N*TB			NS	NS	* (2.60)[b]	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS: not significant $P \geq 0.05$

[b] numbers in brackets show LSD for interactions (all other interactions were not significant).

Comparison of two data sets on 42 DAT

Growth analysis of the 128 plants that were subjected to continuous measurement throughout the experiment showed no significant difference in any of the growth parameters studied when compared to plants harvested with them at the end of the field phase (Table 6). In all parameters, the difference between the two groups averaged over all treatments was about 1% in favour of the plants that were not continuously measured.

Discussion

The assumption that frequent measurements of some of the plants throughout the experiment might interfere with their growth was not supported by data obtained from the two groups of plants. Thus, conclusions on the growth of the plants and their final yield were derived safely from the plants that have been measured continuously during the experiment.

Effects of transition

Transition of the plants from one phase to another as compared to plants that were left to grow in the earlier phases had a strong stimulating effect on leaf growth and a smaller on stem growth. This was manifested by the 4 fold increase in leaf dry weight at 7 DAP in the transplant production phase (Table 2) when compared to the leaf dry weight of similar plants that were allowed to grow for one more week in the normalisation phase (28 DAC, Table 1). Both groups of plants were 28 days old. The first group of plants has been transferred to a glasshouse (*in vivo)* while the other group continued to grow *in vitro* for one more week (28 DAC) in the normalisation phase. Thus, transition of plantlets from *in vitro* to *in vivo* conditions stimulated leaf growth tremendously. Differences in temperature, light intensity and quality, relative humidity and water availability in the two phases of growth must have contributed to the difference in leaf growth. Leaf growth was best described by a logistic curve in the three phases of growth suggesting that there was a limitation of growth in all phases Tadesse *et al.* (2000a). They also reported a boost in leaf growth of *in vitro* plantlets after they were transferred to soil in the glasshouse in the transplant production phase. This indicates that the limitation of growth at the end of the normalisation phase has been overcome and plants started to grow vigorously. Similarly, leaf dry weight of the one week old transplants in the tuber production phase (7 DAT) was twice that of the plants that were allowed to grow for one more week (21 DAP) in the transplant

Table 6. Growth analysis results, at final harvest (42 DAT) at two temperatures in the tuber production (TB) phase, of the normal plants (Group 1) and the 128 plants that were continuously measured (Group 2), after growing at two normalisation (N) and two transplant production (TP) temperatures.

Temperature (°C) during			Leaf dry weight (g)		Stem dry weight (g)		Tuber dry weight (g)		Total dry weight (g)	
			Group 1	Group 2	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
TB	TP	N								
18/12	18/12	17	11.53	10.97	2.75	2.71	57.14	57.66	71.42	71.34
18/12	18/12	23	12.39	10.90	2.80	1.99	53.54	53.64	68.73	66.52
18/12	26/20	17	10.95	12.24	3.33	3.74	56.59	58.00	70.87	73.98
18/12	26/20	23	11.99	11.90	3.45	3.07	57.82	55.88	73.27	70.85
26/20	18/12	17	14.80	15.44	7.95	8.54	34.39	33.51	57.14	57.49
26/20	18/12	23	15.30	14.34	8.07	7.89	30.78	28.46	54.14	50.87
26/20	26/20	17	15.76	17.08	9.24	10.52	30.53	29.30	55.54	56.89
26/20	26/20	23	16.11	14.62	10.56	9.12	26.33	27.45	53.00	51.19
Average			**13.60**	**13.44**	**6.02**	**5.95**	**43.39**	**42.99**	**63.01**	**62.39**

The effect of temperature in the tuber production phase on the dry weights of the different plant parts was highly significant ($P<0.05$) in the two groups of plants, but all interactions were not. Differences between the two groups were not significant for any parameter.

production phase (Tables 2 and 3). Also transplanting, therefore, stimulated leaf growth, despite the transplant shock reported for leaf area increment by Tadesse *et al.* (2000a) when plants were transplanted to the tuber production phase. The transplanting effect must have been smaller than the planting effect because of the transplanting shock and because growth limitation was also reported to be less severe in the relatively short transplant production phase than in the other phases (Tadesse *et al.*, 2000a)

Main effects of temperature

The positive effect of high temperature on leaf dry weight (Tables 1, 2 and 3) was not always significant but was large at the end of the transplant production phase and throughout the tuber production phase. The positive effect of high temperature on stem dry weight was consistent over the three phases of growth (Tables 1, 2 and 3). These results agree with reports for tuber-derived plants (e.g. Ben Khedher & Ewing, 1985; Basu & Minhas, 1991), in the range of temperatures used in our experiment. When effects could not be proven statistically the observed trends were still consistent (e.g. Table 1).

High temperature had a negative effect on leaf/stem ratio throughout the tuber production phase (Table 4), as commonly found in potato (Gregory, 1956; Bodlaender, 1963). Stem dry matter production has a higher optimum temperature than leaf dry matter production, resulting in a lower leaf/stem ratio at higher temperatures (Bodlaender, 1963; Wheeler *et al.*, 1986). The effect of temperature was greater at later stages of growth in the tuber production phase.

SLA was higher at lower than at higher tuber production temperature (Table 4). Some reports (e.g. Struik & Ewing, 1995) indicate that higher temperatures result in higher SLA in tuber-derived plants. Burton (1989), however, has shown that haulm growth increased with temperature over the range 15-25 °C but after the first few weeks, the absolute leaf area, and the leaf area per unit weight (SLA) were all maximal at 20 °C. Hotsonyame & Hunt (1998) also indicated that, as temperatures increased from 8 to 26 °C, SLA of field grown wheat increased to a maximum value achieved at 18-20 °C and then decreased. Dry weight of individual leaves increases with an increase in temperature as a result of increased assimilate supply in potato (Basu & Minhas, 1991) and in tomato (Heuvelink & Marcelis, 1996). Leaf expansion (Ben Khedher & Ewing, 1985; Wheeler *et al.*, 1986) and the size of individual leaves (Struik & Ewing, 1995) are optimum at cooler temperatures. Optimum temperature for the SLA may, therefore, be around 18-20 °C.

Tuber dry weight was significantly higher at lower than at higher tuber production temperature throughout the tuber production phase except at the beginning of the phase when tubers were barely present. Van Dam *et al.* (1996) also confirmed that low temperature (15-19 °C) is optimal for tuber initiation and initial growth. High temperature delays tuber initiation (Struik *et al.*, 1989b) and strongly reduces the proportion of total dry matter partitioned to tubers (Ewing, 1981; Ben Khedher & Ewing, 1985; Struik *et al.*, 1989a; Bennett *et al.*, 1991; Wheeler *et al.*, 1986).

Total plant dry weight was higher at higher temperature at the beginning of the tuber production phase (7 DAT) but this effect was reversed at later stages (28 and 42 DAT) in the phase (Table 4). At that time leaf area had reached its maximum and high temperature therefore did not result in better light interception. Maintenance respiration may also have been large in the leafy crop at higher temperature, and may have lowered total plant dry weight. Total biomass generally is also reduced at high temperature (Nagarajan & Bansal, 1990), because haulm growth is more expensive in terms of energy requirements than starch production in tubers (Penning de Vries *et al.*, 1974). In addition, lack of strong tuber sinks may have reduced total dry weight increase as illustrated by a lower rate of assimilate transport from the leaves (Basu & Minhas, 1991; Lorenzen & Ewing, 1992) and the subsequent reduction in photosynthesis.

After-effects of temperature pre-treatments

After-effects of the normalisation temperature were significant for leaf and total dry weights at the beginning of the transplant production phase. A lower normalisation temperature pre-treatment resulted in higher dry weights at 7 DAP (Table 2). This is consistent with the effect of temperature on above ground leaf area after planting (Tadesse *et al.*, 2000a, c). After-effects of the normalisation temperature were also frequently significant during the tuber production phase. A lower normalisation temperature pre-treatment resulted in higher leaf, stem and total dry weights but lower SLA at 7 and 28 DAT in the tuber production phase (Tables 3, 4, 5). The higher dry weights could be the results of higher weights during transplant production phase and the lower SLA could be associated with this because normally SLA drops when plants increase in dry weight (Table4).

Although after-effects of the transplant production temperature were often present in the tuber production phase they were variable in their duration. Plants pre-treated with higher transplant production temperature had higher leaf dry weight (14 and 28 DAT), stem dry weight (7-42 DAT) and total plant dry weight (7 DAT) in the tuber production phase. On the other hand, plants pre-treated with a lower transplant

production temperature resulted in higher tuber dry weight (14 DAT), SLA (14 and 28 DAT) and leaf/stem ratios (7-42 DAT) in the tuber production phase. Wheeler *et al.* (1986) showed that as temperature increased, tubers contributed proportionally less to the total dry matter yield while stems and leaves contributed more. Low temperature has a stimulating effect on tuber formation and partitioning of dry matter to tubers (Menzel, 1985; Van Dam *et al.*, 1996). Tadesse *et al.* (2000b) reported that higher temperature pre-treatment promoted leaf area at the end of the transplant production phase and resulted in plants with higher ground cover at the end of the growing season. Leaf/stem ratio and SLA are, thus expected to drop at high temperature in the transplant production phase because SLA decreases as plants increase in weight.

Optimum temperature combinations

Significant two and three-way temperature interactions throughout the experiment suggest that some combinations of temperature treatments may give better results than other combinations.

A combination of low normalisation and high transplant production temperatures increased leaf and/or stem dry weights in the tuber production phase (Table 3). Thus, lower normalisation and higher transplant production temperature combinations may promote vegetative growth and could have yield advantages under conditions where a higher radiation interception is limiting yield and partitioning of dry matter to tubers is still sufficient to ensure higher tuber yields. This was not the case in this experiment, because there was no effect on final tuber yield (Table 5). In fact, producing transplants at lower temperature at some dates in the tuber production period resulted in higher tuber dry weight, than producing transplants at a higher temperature (Table 5), especially when the temperature during tuber production was high. This probably resulted from a stimulating effect of low temperature during transplant production on tuber formation and partitioning of dry matter to tubers.

Conclusions

Effects of different temperatures on *in vitro* propagated potato plants were similar to the effects on tuber-derived plants in the same temperature range and were also more or less consistent throughout the phases. Leaf and stem dry weights were higher at higher transplant production and tuber production temperatures, leaf/stem ratio, SLA and tuber dry weight were lower. Total plant dry weight was higher at higher temperature at the beginning of the tuber production phase but was reversed at later stages of the same phase.

Transition of plants from one phase to another, especially from *in vitro* to *in vivo* conditions, greatly increased leaf growth and to some extent stem growth.

After-effects of previous phase temperatures occurred for some plant growth characteristics in subsequent phases. Growing *in vitro* plantlets at lower temperature during normalisation significantly increased leaf and total plant dry weight early in the transplant production and tuber production phases, and later when the plants were grown at high temperature during tuber production. Growing transplants at lower temperature during transplant production increased stem dry weight in the tuber production phase. It also significantly increased tuber dry weight early in the tuber production phase and later when the plants were grown at high tuber production temperature. This suggests that acclimatising transplants at low temperature could have yield advantages when partitioning of dry matter to tubers is limiting tuber yield.

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CHAPTER 7

Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Tuber number, tuber fresh yield and harvest index

Submitted as:

Tadesse M., W.J.M. Lommen & P.C. Struik, 2000. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Tuber number, tuber fresh yield and harvest index.

7. Characterisation of plant growth of *in vitro* propagated potato plantlets over three phases of culture under different temperatures: Tuber number, tuber fresh yield and harvest index

Abstract

To assess main and after-effects of temperature (pre-)treatments on tuber number, tuber size, tuber fresh weight, tuber dry matter concentration and harvest index (HI), *in vitro* plantlets produced at 17 or 23 °C (normalisation phase) for 21 days were grown in soil in growth chambers at 18/12 or 26/20 °C for 14 days (transplant production phase). They were then transplanted to glasshouses at 18/12 or 26/20 °C and grown for 42 days (tuber production phase).

Stolons were initiated at the end of the transplant production phase and tubers during the first week of the tuber production phase.

Effects of normalisation temperature were not significant in the transplant production phase but were carried over to the second half of the tuber production phase. A lower normalisation temperature pre-treatment resulted finally in higher tuber yield and higher HI at the end of the tuber production phase at both tuber production temperatures. In the first 14 days after transplanting, a lower transplant production temperature resulted in more tubers and higher tuber fresh yield. Finally it resulted in higher dry matter concentration and higher HI for plants grown at high tuber production temperature. Tuber production temperature was especially important for final yields and tuber numbers. A lower tuber production temperature resulted in higher tuber fresh yield, average tuber weight, tuber dry matter concentration and HI, and in higher tuber number when normalisation temperature had been high. Low temperature pre-treatment in the normalisation phase promoted tuber yield in the tuber production phase and hence could have yield advantages.

Key words: potato, *Solanum tuberosum* L., harvest index, *in vitro*, multiplication, tuber number, temperature.

Introduction

Micropropagation techniques have widely been introduced in potato seed production systems during the past few decades to alleviate problems associated with the conventional seed production system based on clonal selection (Jones, 1988; Struik & Wiersema, 1999). These techniques have been developed because large numbers of disease-free plants can be produced within a short period of time all year round (Jones,

1988). The common production system constitutes four phases: *in vitro* multiplication (through nodal cuttings), normalisation (where single-node cuttings develop into rooted *in vitro* plantlets), transplant production (acclimatisation) and tuber production.

A possible advantage of this system is that plantlets can be manipulated in the normalisation and transplant production phases with different treatments (e.g. temperature sequences) to influence plant growth and development (Hussey & Stacey, 1981; Marinus, 1985; Charles *et al.*, 1992; Tadesse *et al.*, 2000a, b, c), for example towards a higher multiplication rate. In the last phase of the seed production system high tuber numbers and tuber yields are required to have higher multiplication rates and temperature could possibly play an important role in this repect.

Tubers in potato plants are formed on stolons when conditions for the formation of both stolons and tubers are favourable. This process is strongly influenced by climatic factors such as photoperiod and temperature (Ewing & Struik, 1992). Van Dam *et al.* (1996) reported that temperatures in the range 15-19 °C are optimal for tuber initiation and observed a delay in the onset of tuber growth with an increase in temperatures from 15-27 °C, confirming results of Borah & Milthorpe (1962), Bodlaender (1963), Midmore (1984) and Menzel (1985). Low temperature, therefore, may increase tuber yield. However, at higher temperature, primary stolons are able to form branches and can have numerous potential tuber sites (Struik *et al.*, 1989). Higher temperature thus may increase tuber number. Slater (1963) identified 29 °C as the temperature at which no tubers are formed anymore.

Temperature in the four-phase seed production system mentioned above not only exerts effects through its direct influence on tuber formation but also through its effects on leaf area growth. A boost in leaf area growth and a rapid increase in leaf number were reported by Tadesse *et al.* (2000a) when *in vitro* plantlets were transferred to soil. Higher temperatures did not significantly increase leaf area during normalisation, increased leaf area during transplant production and first increased but later decreased leaf area during tuber production (Tadesse *et al.*, 2000a). Tadesse *et al.* (2000b) also found that higher temperature pre-treatment during the transplant production phase promoted leaf growth enabling the plants to intercept more solar radiation after transplanting to the field. This may increase yield. Tadesse *et al.* (2000d) found that, the positive effect of a lower tuber production temperature was larger after a lower than after a higher transplant production temperature at 14 DAT, whereas it was smaller after a lower than after a higher transplant production temperature at 28 and 42 DAT. Growing *in vitro* plantlets at lower temperature during normalisation significantly increased leaf area and total plant dry weight early in the transplant production and tuber production phases (Tadesse *et al.*, 2000d). Temperature may influence the vegetative growth and final yield of a potato crop,

affecting tuber yield, tuber number and average tuber weight.

This study assesses the yield components – tuber number, tuber weight, tuber fresh yield and harvest index (HI) – of *in vitro* propagated potato plantlets over the normalisation, transplant production and tuber production phases as influenced by different temperature treatments.

Materials and methods

Details on the experiment described in this chapter have already been provided by Tadesse *et al.* (2000a). In the following sections we briefly summarise the main aspects and provide further details relevant to the results presented in this study.

Potato culture and treatments

Potato (*Solanum tuberosum* L., cv. Gloria) plantlets were propagated *in vitro* by producing single-node cuttings using virus-free stock plantlets. The plantlets were cultured on a standard medium containing MS salts (Murashige & Skoog, 1962) with vitamins, 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). Viable nodes were cut from plantlets discarding tops and cultured in 25 × 150 mm culture tubes (one per tube) on 10 ml medium at 17 or 23 °C and a photophase of 16 h for 21 days in the "normalisation" phase.

Rooted *in vitro* plantlets were then planted in transplanting trays with small cells filled with potting soil and grown in growth chambers with day/night temperatures of 18/12 or 26/20 °C, a photophase of 14 h and a RH of 80% for 14 days in the "transplant production" phase.

At the end of the transplant production phase, plants were transplanted in 5 litre pots filled with potting soil to two glasshouses at a density of 16.0 plants m^{-2} decreasing with time as plants were regularly harvested to 12.8, 9.6, and 6.4 plants m^{-2} at 7, 14, and 28 DAT, respectively. The glasshouses were kept at a day/night temperature of 18/12 or 26/20 °C, a photophase of 16 h and a relative humidity of 80% for 42 days in the "tuber production" phase.

Experimental design

The experiment was carried out in a split-split plot design and comprised of 2 tuber production (TB) temperatures × 2 transplant production (TP) temperatures × 2 normalisation (N) temperatures × 4 harvests (H) × 16 replications (R) in the tuber production phase and 16 (R) × 2 (TP) × 2 (N) × 3 (H) during the transplant production

phase. The tuber production temperature was randomised within the transplant production temperature and the latter was randomised within the normalisation temperature.

Harvests were carried out at 7, 14 and 21 days after planting (DAP) in the transplant production phase, and at 7, 14, 28 and 42 days after transplanting (DAT) in the tuber production phase. The plants from the harvest 21 DAP in the transplant production phase were grown one week longer in the phase than the actual duration of the phase for other plants (extended growth) in order to compare undisturbed plants to plants transferred from one phase to the next. These plants were part of a large experiment with the same treatments, in total consisting of 992 plants.

Measurements and statistical methods

Stolon numbers from main stems were counted in the transplant production phase. Tuber numbers (tubers with a diameter of 0.5 mm and above) were counted in the extended growth of the transplant production phase and in the tuber production phase. Tuber fresh yield was determined and average weight per tuber calculated. Dry matter was determined in the tuber production phase by separating the different plant parts and drying them in an oven at 105 °C for 16 hours. Total dry matter did not include roots. From this harvest index (HI, the proportion of tubers in the total dry matter) was calculated, in the tuber production phase. Tuber dry matter concentration was calculated as the percentage of tuber dry weight in the tuber fresh weight. Data were subjected to analysis of variance (ANOVA) using Genstat 5 release 3.22 (1995) and differences between treatments were analysed by LSD tests at $P<0.05$.

Results

Transplant production phase

Stolons appeared at the end of the transplant production phase (14 DAP). There were no after-effects of the normalisation temperature on stolon number in the transplant production phase. Stolon number was not significantly different at the two temperature treatments in the transplant production phase, but was higher at lower than at higher temperature after the one week extended growth at 21 DAP (Table 1).

Tubers were not present during the 14-day transplant production period, but were produced after the one week extended growth at 21 DAP. The temperature effect was not significant, although the trend showed that relatively more tubers were produced at lower than at higher temperature during transplant production (Table 1)

Table 1. Stolon and tuber numbers at two harvests during the transplant production phase, of *in vitro* propagated potato plantlets grown at two normalisation (N) × two transplant production (TP) temperatures. Stolons and tubers were not yet present at 7 days after planting (DAP).

		Harvest dates during transplant production (DAP)	
		14	21 [a]
Stolon number (per plant)			
Temp. (°C) during			
TP	N		
18/12	17	1.0	4.4
18/12	23	1.1	3.7
26/20	17	1.3	2.3
26/20	23	1.3	2.4
Significance [b]			
N		NS	NS
TP		NS	***
Tuber number (per plant)			
Temp. (°C) during			
TP	N		
18/12	17	0	0.44
18/12	23	0	0.35
26/20	17	0	0.19
26/20	23	0	0.04
Significance [b]			
N		-	NS
TP		-	NS

[a] Results of the one week extended growth

[b] *** $P < 0.001$, NS: not significant $P \geq 0.05$ (interactions were not significant).

Tuber production phase

Tuber number

After-effects of normalisation temperature were present at 28 DAT in the tuber production phase (Table 2). Plants pre-grown at lower normalisation temperature had

more tubers than those pre-grown at higher normalisation temperature at 28 DAT, but only at a higher temperature during tuber production.

After-effects of the transplant production temperature were present only in the first two weeks after transplanting (7-14 DAT). All plants pre-grown at lower transplant production temperature had more tubers than those pre-grown at higher temperature early in the tuber production phase. After 14 DAT the difference was significant only for plants growing at low temperature during tuber production (Table 2).

Tuber number was higher at lower than at higher tuber production temperature throughout the tuber production phase except at 7 DAT when tuber production had barely started (Table 2). An interaction between the normalisation and tuber production temperatures at 28 DAT indicated that the positive effect of a low tuber production temperature on tuber number was larger after higher than after lower normalisation temperature.

Tuber fresh yield

After-effects of the normalisation temperature expressed themselves in the second half of the tuber production phase. Tuber fresh yield was significantly higher for those plants pre-cultured at low than at high normalisation temperature only for plants growing at high temperature in the tuber production phase at 28 DAT but for all plants at 42 DAT (Table 2).

An after-effect of the transplant production temperature was only present at the harvest of 14 DAT: plants pre-grown at low transplant production temperature had higher tuber fresh yield than those grown at a high transplant production temperature (Table 2), but only at low tuber production temperature.

Tuber fresh yield was significantly higher at lower than at higher tuber production temperature throughout the tuber production phase except at 7 DAT when tuber bulking had just started and at 14 DAT for plants that received a high transplant production temperature and were least advanced in tuber formation (Table 2).

Average tuber weight

There were no significant after-effects of the normalisation or transplant production temperatures (Table 3). Average tuber weight was significantly higher at lower than at higher tuber production temperature throughout the tuber production phase except at 7 DAT (Table 3).

Table 2. Tuber number and tuber fresh yield at various tuber production harvests, of *in vitro* propagated potato plantlets grown at two normalisation (N) × two transplant production (TP) × two tuber production (TB) temperatures.

			Harvest dates during tuber production (DAT)			
			7	14	28	42
Tuber number (per plant)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.38	5.81	15.62	15.69
18/12	18/12	23	0.45	3.76	13.43	16.16
18/12	26/20	17	0.06	1.37	15.00	16.31
18/12	26/20	23	0.26	1.02	16.83	16.86
26/20	18/12	17	0.75	1.19	12.75	14.19
26/20	18/12	23	0.37	1.33	8.51	12.66
26/20	26/20	17	0.06	0.00	11.25	13.44
26/20	26/20	23	0.17	0.19	9.38	13.14
Significance [a]						
N			NS	NS	*	NS
TP			*	**	NS	NS
TB			NS	***	***	***
N*TB			NS	NS	* (1.90) [b]	NS
TP *TB			NS	** (1.06)	NS	NS
Tuber fresh yield (g/plant)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.16	4.39	127.8	293.4
18/12	18/12	23	0.12	3.11	118.5	279.4
18/12	26/20	17	0.06	1.32	124.0	297.0
18/12	26/20	23	0.12	0.36	124.5	277.5
26/20	18/12	17	0.23	0.36	48.9	196.1
26/20	18/12	23	0.13	0.51	33.7	169.3
26/20	26/20	17	0.01	0.00	42.0	183.2
26/20	26/20	23	0.04	0.04	23.9	164.3
Significance [a]						
N			NS	NS	NS	**
TP			NS	***	NS	NS
TB			NS	***	***	***
N*TB			NS	NS	* (13.5) [b]	NS
TP*TB			NS	** (1.05)	NS	NS

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$ (interactions which are not listed were not significant at any date)

[b] numbers in brackets show LSD for interactions.

Table 3. Average tuber fresh weight and tuber dry matter concentration at various tuber production harvests, of *in vitro* propagated potato plantlets grown at two normalisation (N) × two transplant production (TP) × two tuber production (TB) temperatures.

			Harvest dates during tuber production (DAT)			
			7	14	28	42
Average tuber weight (g/tuber)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.47	0.76	9.63	20.70
18/12	18/12	23	0.28	0.83	9.52	17.55
18/12	26/20	17	0.50	0.96	8.95	18.72
18/12	26/20	23	0.36	0.35	7.70	17.44
26/20	18/12	17	0.43	0.30	3.91	14.10
26/20	18/12	23	0.34	0.38	4.64	13.29
26/20	26/20	17	0.40	0.00	4.45	14.29
26/20	26/20	23	0.26	0.21	2.86	13.11
Significance [a]						
N			NS	NS	NS	NS
TP			NS	NS	NS	NS
TB			NS	**	***	***
Tuber dry matter (%)						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	10.71	11.19	14.06	19.29
18/12	18/12	23	11.03	11.35	14.00	19.31
18/12	26/20	17	10.73	12.88	14.62	20.05
18/12	26/20	23	10.56	16.47	14.31	19.74
26/20	18/12	17	10.48	14.05	13.27	16.69
26/20	18/12	23	10.05	11.59	13.18	17.85
26/20	26/20	17	10.46	8.30	13.08	16.56
26/20	26/20	23	10.51	9.14	13.23	15.68
Significance [b]						
N			NS	NS	NS	NS
TP			NS	NS	NS	NS
TB			NS	**	***	***
TP*TB			NS	***(3.58) [b]	NS	*** (1.22)

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$ (interactions not listed were not significant at any date)

[b] numbers in brackets show LSD for interactions.

Tuber dry matter concentration

Normalisation temperature had no effect on tuber dry matter concentration in the tuber production phase. Plants pre-cultured at lower than at higher transplant production temperature had higher tuber dry matter concentration at 14 DAT when grown at lower tuber production temperature (Table 3).

A lower temperature during tuber production resulted in a higher tuber dry matter concentration except at 7 DAT when tubers were barely present. At 14 DAT the effect was only significant for plants pre-cultured at high transplant production temperature. At 42 DAT, however, the positive effect of transplant production temperature on tuber dry matter concentration was larger at lower than at higher tuber production temperature.

Harvest index

An after-effect of the normalisation temperature was significant only at the end of the tuber production phase: plants pre-cultured at lower normalisation temperature had a higher harvest index (HI) than those pre-cultured at higher normalisation temperature at 42 DAT (Table 4).

After-effects of the transplant production temperature were now and then present throughout the tuber production phase. Plants grown at lower transplant production temperature had higher HI than those grown at higher transplant production temperature (Table 4). However, this effect was only significant at lower tuber production temperature at 14 DAT and at higher tuber production temperature at 28 and 42 DAT.

HI was significantly higher at lower than at higher tuber production temperature throughout the tuber production phase except at 7 DAT (Table 4).

Discussion

Main effects of temperature

The experiment indicated that stolons were produced at the end of the transplant production phase (14 DAP). Transplant production temperature had no effect on stolon number during the transplant production phase at 14 DAP but a high temperature during this phase resulted in fewer stolons when the transplant production phase was prolonged. Thus, low temperature promotes stolon initiation in the transplant production phase.

Table 4. Harvest index at various tuber production harvests, of *in vitro* propagated potato plantlets grown at two normalisation (N) × two transplant production (TP) × two tuber production (TB) temperatures.

			Harvest dates during tuber production (DAT)			
			7	14	28	42
Harvest index (g g^{-1})						
Temp. (°C) during						
TB	TP	N				
18/12	18/12	17	0.018	0.13	0.61	0.80
18/12	18/12	23	0.017	0.08	0.56	0.76
18/12	26/20	17	0.007	0.03	0.59	0.80
18/12	26/20	23	0.017	0.01	0.60	0.79
26/20	18/12	17	0.018	0.01	0.25	0.60
26/20	18/12	23	0.016	0.01	0.21	0.57
26/20	26/20	17	0.001	0.00	0.22	0.56
26/20	26/20	23	0.008	0.00	0.15	0.50
Significance [a]						
N			NS	NS	NS	*
TP			NS	***	NS	NS
TB			NS	***	***	***
TP*TB			NS	*** (0.03) [b]	* (0.05)	* (0.04)

[a] *** $P < 0.001$, ** $0.001 \leq P < 0.01$, * $0.01 \leq P < 0.05$, NS: not significant $P \geq 0.05$ (interactions not listed were not significant at any date).

[b] numbers in brackets show LSD for interactions.

Tuber fresh yield, average tuber weight, tuber dry matter concentration and HI were all affected in a similar manner: they were all significantly higher at lower than at higher temperature in the tuber production phase except at the beginning (7 DAT), during which time tuber production was barely starting (Tables 2, 3 and 4). This agrees with the results reported for tuber-derived potato plants by Borah & Milthorpe (1962), Bodlaender (1963), Midmore (1984) and Van Dam *et al.* (1996) and is mainly because a higher proportion of the total dry matter is allocated to tubers than to the haulm at low temperature. Tuber number was also significantly higher at lower than at higher temperature in the tuber production phase except at 42 DAT, when it was only still higher when normalisation temperature had been high. The higher tuber dry matter concentration at lower than at higher temperature throughout the tuber production

phase is associated with the positive effect of relatively low temperature on starch synthesis (Wolf *et al.*, 1991; Lafta & Lorenzen, 1995).

After-effects of temperature pre-treatment

There were no significant after-effects of normalisation temperature on stolon or tuber numbers at 7 and 14 DAP in the transplant production period or at the end of the extended growth (21 DAP) in the transplant production phase (Table 1), but a few after-effects of the normalisation temperature were carried over to the later part of the tuber production phase. A lower normalisation temperature pre-treatment resulted in a higher tuber fresh yield and a higher HI at the end of the tuber production phase (42 DAT). Pre-culturing plants at low normalisation temperature resulted in more tubers at higher tuber production temperature at 28 DAT.

Effects of the transplant production temperature were also carried over to the tuber production phase but contrary to those of the normalisation temperature, most of them were present during the early stages (7-14 DAT) of the tuber production phase. After-effects of both phases were more or less similar in their action. A lower transplant production temperature pre-treatment resulted in more tubers, higher tuber fresh yield and higher HI during the first two weeks of the tuber production phase indicating that low temperature favours tuberization and advances tuber formation.

Previous research showed that higher transplant production temperature increased leaf area (Tadesse *et al.*, 2000a) and leaf dry weights (Tadesse *et al.*, 2000d), and resulted in plants with higher ground cover at the end of a short growing season (Tadesse *et al.*, 2000b) indicating that high temperature promotes or prolongs haulm growth. Associated with these partitioning effects, tuber initiation and formation were delayed. Lower transplant production temperatures, on the other hand, increased the proportion of the total dry matter partitioned to tubers (see also Menzel, 1985; Van Dam *et al.*, 1996). Also Tadesse *et al.* (2000d) found that a low transplant production temperature resulted in higher tuber dry matter yield when the growth cycle was relatively short.

Optimum temperature combinations

Tuberization and initial exponential growth of tubers were late at high temperature during normalisation, transplant production and tuber production periods. The expression of the effects induced during the different phases depended on the temperature during tuber production thus causing some interactions between treatments for tuber number or tuber fresh weight. During the final two weeks of the

tuber production phase, tuberization generally levelled off for treatments at a low tuber production temperature but still increased at high tuber production temperature after a high normalisation temperature. At the same time, average individual tuber fresh weight increased by 8-11 g for all eight temperature-combinations during the last two weeks indicating that once tuber bulking started, its rate was not much affected by temperature.

Interactions between the different phase temperatures indicate that certain combinations of temperature treatments are optimum to obtain higher yield and tuber numbers within a given short growth period. Maximum tuber numbers were obtained at low tuber production temperature with no significant after-effects of temperature during previous phases. This temperature effect confirms results reported by Menzel (1985) and Van Dam *et al.* (1996) on tuberization and initial growth, and are also in agreement with results from Struik (1987, 1989) who indicated that temperature effects on tuber number are only existing when temperature is varied shortly before or during tuber formation.

At the end of the tuber production phase, tuber fresh yield, average tuber weight and HI were significantly higher for plants pre-cultured at low normalisation temperature. Low temperature during normalisation advances crop development (Tadesse *et al.*, 2000c) and may thus result in a higher yield at the end of a short growing season.

Only the low transplant production and low tuber production temperature-combination resulted in some tuber yield at 14 DAT in the tuber production phase. Lower temperature during transplant production increased tuber dry weight and tuber dry matter concentration, but only significantly at higher temperature during tuber production.

To optimise the production of potato seed tubers using the four-phase scheme of seed production, an appropriate combination of temperature treatments must be selected. The results of this experiment indicate that the temperature of the tuber production phase is most important for final yield. However, the production of large-sized seed tubers (15-20 g) requires low tuber production temperature preferably in combination with a low normalisation temperature.

Conclusions

- Stolons were produced at the end of the transplant production phase while tubers were produced after transplanting plants to the tuber production phase (7 DAT).
- After-effects of the normalisation temperature were not present at the transplant production phase but some were carried over to the tuber production phase and

most of them were manifested at the end of the phase (42 DAT). Low temperature pre-treatment during normalisation resulted in higher tuber yield and higher HI at the end of the tuber production phase.

- After-effects of the transplant production temperature were, in most cases, only present during the early stages of the tuber production phase (7-14 DAT). A lower transplant production temperature resulted in more tubers, and higher tuber fresh yield at 14 DAT and finally resulted in higher dry matter concentration and higher HI for plants growing at high tuber production temperature.
- Although previous phase temperatures also had some influence on growth of the plants and final yield, conditions during tuber production were most important.
- Once tuber bulking started, its rate was not much affected by temperature.
- Pre-culturing plants at low temperature during normalisation followed by transplanting to low tuber production temperature could have yield advantages.

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CHAPTER 8

Effects of nitrogen pre-treatment of transplants from *in vitro* produced potato plantlets on transplant growth and yield in the field

Submitted as:

Tadesse M., W.J.M. Lommen & P.C. Struik, 2000. Effects of nitrogen pre-treatment of transplants from *in vitro* produced potato plantlets on transplant growth and yield in the field.

8. Effects of nitrogen pre-treatment of transplants from *in vitro* produced potato plantlets on transplant growth and yield in the field

Abstract

In vitro propagated potato plantlets, cultivars Gloria and Spunta, were pre-treated with 10 or 40 mg of nitrogen per plant before transplanting to the field to determine after-effects of nitrogen on the field performance of these transplants in two experiments. Yield, ground cover (GC), accumulated intercepted radiation (AIR), radiation use efficiency (RUE) and harvest index (HI) were assessed. Spunta had higher GC, AIR, total dry matter and tuber fresh and dry weights but lower RUE and HI than Gloria at final harvest. Nitrogen pre-treatment had no clear effect on plant growth at the end of the transplant production phase or during early growth in the field. Later, higher nitrogen pre-treatment resulted in a slightly higher GC and AIR. In one experiment, this resulted in a significantly higher yield. Pre-treatment did not consistently affect RUE or HI. Nitrogen pre-treatment can improve seasonal light interception of plantlets, especially of early potato cultivars.

Key words: harvest index, dry matter distribution, ground cover, leaf area, radiation use efficiency, radiation interception, seed potato, *Solanum tuberosum* L.

Introduction

In vitro propagated potato (*Solanum tuberosum* L.) plantlets are commonly used in seed tuber production systems (Marinus, 1983; Jones, 1988). Field crops grown from transplants have lower accumulated intercepted radiation (AIR) and hence have lower yields than crops from seed tubers, especially in early cultivars (Lommen, 1999). The relatively poor performance of transplants may be associated with their dry matter partitioning in the early phase of field growth. Lommen (1999) suggested that high nitrogen, high temperature or long day treatments, either during transplant production or in the field may improve the field performance of *in vitro* propagated potato plantlets. The general effects of such treatments on dry matter allocation in crops from seed tubers in potato are described by Van Heemst (1986), Wolf *et al.* (1990), Biemond & Vos (1992) and Van Dam *et al.* (1996). However, it is still largely unknown how treatments and growing conditions during the production of transplants in the phase immediately preceding transfer to the field affect the growth of these transplants and their subsequent field performance. In this chapter we will focus on

nitrogen supply.

Nitrogen fertilisation during production of transplants of cauliflower (Booij, 1992) and leek (Gray & Steckel, 1990) was shown to improve the field performance of transplants, mainly because of enhanced canopy development. Whether such mechanism would also apply to potato remains to be seen.

The growth of normal potato plants is extremely responsive to nitrogen fertilisation. Nitrogen increases leaf number and leaf size, especially through its effect on rate of leaf expansion (Vos & Biemond, 1992). It enhances sympodial growth and delays senescence, both of the individual leaf and (independent of that) of the entire plant (Burton, 1989; Harris, 1992; Vos & Biemond, 1992).

Total dry matter yield is directly related to the solar radiation intercepted by the crop. It is thus enhanced by nitrogen effects on leaf area and canopy duration (Gunasena & Harris, 1968). The efficiency with which intercepted solar radiation is converted into dry matter also plays a role (MacKerron & Waister, 1983; Harris, 1992). This radiation conversion efficiency is generally higher in crops well endowed with nitrogen (Dyson & Watson, 1971; Van der Zaag, 1984).

However, high levels of nitrogen also decrease the induction to tuberize and delay tuber initiation (Oparka *et al.*, 1987). The more nitrogen available to the plant, the lower will be the percentage of plant dry matter that will be partitioned to the tubers early in the season (Harris, 1992). This will favour haulm growth but postpone tuber bulking. This difference in partitioning usually diminishes with time, and there may be no difference by the end of the growing season (Harris, 1992). Effects on yield may, therefore, be negative in short cycle crops but positive in long cycle crops (Van Heemst, 1986). Moreover, nitrogen may increase the number of tubers (Harris, 1992; Roy & Jaiswal, 1998; Gabr & Sarg, 1998), by enhancing individual stem vigour, although effects are not always consistent.

In transplant potato crops, treating plantlets with more nitrogen during transplant production could promote haulm development of the subsequent field crops, either directly by enhancing its haulm growth or indirectly by delaying tuber bulking, thus prolonging haulm growth. This may ultimately increase the field performance of such transplants, especially in early (short cycle) cultivars in which haulm growth may be particularly limiting. However, a yield increase can only be achieved if an adequately long tuber bulking period is guaranteed and if a pre-treatment with extra nitrogen is not associated with negative side effects such as through an increase in the transplant shock.

The aim of the current study is therefore to understand how nitrogen pre-treatment in the very early cultivar Gloria and the mid-early cultivar Spunta affects growth of plantlets in the transplant production phase, and growth and yield of

transplant crops in the field. We will analyse these effects of pre-treatments of nitrogen on shoot and root growth of transplants, the severity of the transplant shock, the development of haulm and tubers after transplanting, the efficiency of the use of radiation and the dry matter partitioning.

Materials and methods

In two experiments during 1997 and 1998, potato (*Solanum tuberosum* L.) plantlets, cultivars Gloria (very early) and Spunta (mid early), were propagated *in vitro* by producing single-node cuttings using virus-free stock plantlets. The plantlets were cultured on a standard medium containing MS salts (Murashige and Skoog, 1962) with vitamins, 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). The vitamins added were 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 0.50 mg l^{-1} nicotinic acid, 0.50 mg l^{-1} pyridoxine HCl and 0.10 mg l^{-1} thiamine HCl and the medium was adjusted to a pH of 5.7. Viable nodes were cut from plantlets discarding tops and cultured in petri-dishes on 20 ml medium (8 per dish). The petri-dishes were sealed with household plastic foil, and placed at 23 °C and a photophase of 16 hours supplied with Philips TL 84 fluorescent tubes with a photosynthetic photon flux density of 30 μmol m^{-2} s^{-1} for 21 days. This is the *normalisation phase* in which single-node cuttings develop into rooted *in vitro* plantlets.

After 21 days, rooted *in vitro* plantlets were planted in transplanting trays with small cells (4.0 × 5.5 × 6.0 cm, w × l × d) filled with ca 100 ml potting soil, leaving approximately half of the stem above the soil. The potting soil contained 17.4 mg of nitrogen per 100 ml soil in the form of NH_4NO_3. The plantlets were then transferred to a glasshouse in Experiment 1 (1997) and to a growth room in Experiment 2 (1998) with day/night temperatures set at 18/12 °C and a photophase of 14 h. The day light in Experiment 1 was supplemented with SON-T lamps providing an intensity above the plants of 570 μmol m^{-2} s^{-1}, light was provided by SON-T, HPI-T and TL 84 lamps with an intensity of 420 μmol m^{-2} s^{-1} in Experiment 2. Relative humidity was 70-80% in both experiments. Plants were grown for 14 days after planting (DAP) and the temperature was lowered to 12/8 °C two days before transplanting to the field. During this phase, plantlets were fertilised with 15 ml (per plant per time) of complete Steiner solution (Lommen & Struik, 1992) with a low or a high nitrogen level two times a week for two weeks, receiving a total of 10 (low N) or 40 (high N) mg of nitrogen per plant, respectively. The high nitrogen treatment was prepared by adding 0.5 g l^{-1} NH_4NO_3 to the Steiner solution. This is the *acclimatisation* or *transplant production phase* where *in vitro* plantlets were acclimatised to *ex vitro* conditions to produce transplants.

The transplants were transplanted in low ridges in the field on May 14, 1997 (Experiment 1) and on May 20, 1998 (Experiment 2), leaving approximately half of the stem above the soil at a distance of 75 cm between the rows and 20 cm between the plants. The experiment was carried out in a sandy soil in Achterberg, near Wageningen (The Netherlands), in a randomised block design with 5 blocks. The two cultivars and two nitrogen treatments were randomised within each block and the six harvests (at 14, 28, 42, 56, 70 and 91/84 days after transplanting (DAT)) were randomised within the main plots. The first harvest was 0 DAT (at transplanting) and the last field harvest 91 DAT in Experiment 1 and 84 DAT in Experiment 2.

The experimental unit consisted of 8 net plants per sub-plot (2 rows × 4 plants) and these were surrounded by 16 guard plants. The field was fertilised with 121.5 kg N ha^{-1} in the form of NH_4NO_3 before planting. Other nutrients were also applied as recommended. Hilling (to ca 20 cm high) was done 35 DAT in both experiments and the field was irrigated as required.

The first growing season (1997) was dry and sunny with a daily average temperature of 16.6 °C while the second season (1998) was relatively wet and cloudy and had a daily average temperature of 16.2 °C.

Measurements and statistical methods

Ground cover was estimated every week using a grid of 100 squares (90 × 75 mm each) and growth was analysed by harvesting plants every 14 days throughout the field phase. Daily ground cover was intrapolated from the weekly measurements and was multiplied by the total daily global radiation to calculate the daily radiation intercepted and the total accumulated intercepted radiation (AIR) during the growing season. Radiation use efficiency (RUE) was calculated by dividing the total dry matter production by the AIR. Leaf, stem and root dry weights were recorded during the early stages of the field phase and in addition, tuber numbers, tuber fresh and dry weights were recorded at later stages. Shoot/root ratios and harvest index (HI; the proportion of tubers in the total dry matter) were calculated. Data were subjected to analysis of variance (ANOVA) using Genstat 5 release 3.22 (1995) and differences between treatments were analysed by LSD tests at $P<0.05$.

Results

Initial plant dimensions

There were no effects of nitrogen treatment on plant growth characteristics at the end of the transplant production phase in both experiments, except for a positive effect of high nitrogen on stem dry weight in cultivar Gloria in Experiment 2 (Table 1). Plantlets of cultivar Spunta generally had higher leaf, stem, root and total dry weights at transplanting than those of cultivar Gloria in each experiment (Table 1).

Plantlets of the two experiments differed in characteristics at planting. Leaf, shoot and total dry weights were higher in the second experiment than in the first (Table 1). However, transplants in the second experiment had lower root weights resulting in shoot/root ratios of about three times higher than those in the first experiment (Table 1).

Ground cover

Nitrogen pre-treatment had no effect on ground cover immediately after transplanting to the field (Table 1) or in the first two weeks of field growth (Table 2). Higher nitrogen pre-treatment resulted in a significantly higher ground cover at 35 DAT for both cultivars in Experiment 1, and at 49 and 56 DAT in cultivar Gloria when Spunta already had maximum ground cover. At 84 DAT, ground cover was again higher at higher nitrogen in both cultivars indicating a slower decrease in ground cover in cultivar Spunta at higher nitrogen. Both cultivars had a higher ground cover at higher than at lower nitrogen between 14 and 35 DAT in Experiment 2 and cultivar Spunta also between 42 and 56 DAT (Table 2; Fig. 1).

Cultivar Spunta had a higher ground cover than cultivar Gloria throughout the two experiments (Fig. 1). Cultivar Spunta reached full ground cover at 49 DAT in Experiment 1, while cultivar Gloria had then only about 40% cover (Fig. 1). In Experiment 2, cultivar Spunta reached full cover at 70 DAT, at which time cultivar Gloria had about 35% cover (Fig. 1). The average ground cover in cultivar Gloria at the end of the growing season was about 60% in the first and only 35% in the second experiment (Fig. 1).

Ground cover increased relatively faster in the second than in the first experiment in both cultivars (Fig. 1). Differences among years in patterns of canopy development were not associated with differences in temperature sum (Fig. 1) but were consistent with other potato experiments using *in vitro* plantlets on the same site.

Table 1. Plant characteristics at transplanting (0 DAT), of *in vitro* propagated potato plantlets pre-treated with 10 mg (low N) or 40 mg (high N) of nitrogen per plant, during the 1997 (Experiment 1) and 1998 (Experiment 2) field experiments.

	Leaf DW[a] (mg/plant)	Stem DW (mg/plant)	Shoot DW (mg/plant)	Root DW (mg/plant)	Total DW (mg/plant)	Shoot/root ratio	Leaf area (cm^2)
Experiment 1							
Gloria low N	18.1	3.8	21.9	5.0	26.9	5.3	12.3
Gloria high N	17.2	3.8	20.9	3.8	24.7	6.5	12.4
Spunta low N	29.1	7.2	36.2	9.1	45.3	4.0	19.5
Spunta high N	34.4	7.8	42.2	11.3	53.4	4.3	19.9
Significance							
N	NS	NS	NS	NS	NS	NS	NS
CV	* [b]	***	**	*	**	NS	**
N*CV	NS	NS	NS	NS	NS	NS	NS
Experiment 2							
Gloria low N	36.4	3.1	39.5	2.2	41.7	17.6	13.7
Gloria high N	47.1	4.8	51.9	3.1	55.0	16.6	14.6
Spunta low N	55.9	4.7	60.6	4.2	64.8	14.4	23.5
Spunta high N	59.0	4.3	63.3	3.9	67.2	16.1	23.8
Significance							
N	NS	NS	NS	NS	NS	NS	NS
CV	**	NS	**	**	**	NS	**
N*CV	NS	*	NS	NS	NS	NS	NS

[a] DW dry weight

[b] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

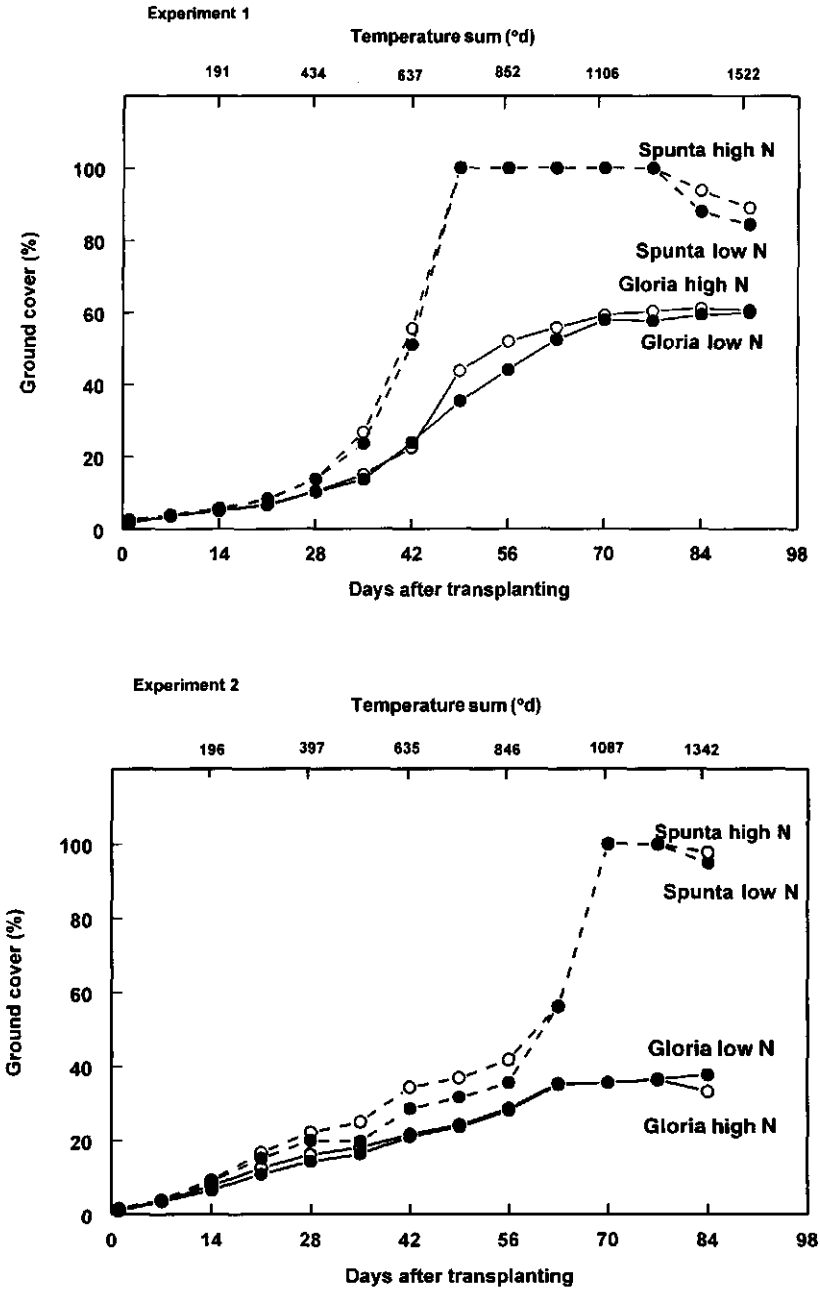

Fig. 1. Weekly ground cover of *in vitro* propagated potato plants of two cultivars pre-treated with 10 (low N) or 40 mg (high N) of nitrogen per plant before transplanting to the field, during the 1997 (Experiment 1) and 1998 (Experiment 2) field experiments. For statistical significances, see Table 2.

Table 2. Statistical significances of the effects of the main factors nitrogen (N) and cultivar (CV) and their interaction (N*CV) on the weekly ground cover of transplants from *in vitro* propagated potato plantlets during field growth in 1997 (Exp. 1) and 1998 (Exp. 2) presented in Fig. 1.

	Days after transplanting													
	1	7	14	21	28	35	42	49	56	63	70	77	84	91
Experiment 1														
N	NS	NS	NS	NS	NS	*	NS	*	**	NS	NS	NS	*	NS
CV	* [a]	*	NS	**	***	***	***	***	***	***	***	***	***	***
N*CV	NS	NS	NS	NS	NS	NS	NS	*	**	NS	NS	NS	NS	NS
Experiment 2														
N	NS	NS	*	*	*	*	**	**	***	NS	NS	*	NS	
CV	***	***	***	***	***	***	***	***	***	***	***	***	***	
N*CV	NS	NS	NS	NS	NS	NS	**	**	**	NS	NS	*	*	

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

Crop yield analysis

At the end of the field phase, AIR was significantly higher in the crop from transplants receiving the high nitrogen fertilisation before field planting than that receiving lower nitrogen, for both cultivars in Experiment 1 and for cultivar Spunta in Experiment 2 (Table 3). Radiation Use Efficiency (RUE) was significantly higher at higher than at lower nitrogen pre-treatment in Experiment 1 but there was no significant effect in Experiment 2 (Table 3). More total dry matter was produced at higher than at lower nitrogen in cultivar Spunta in Experiment 1, but not significantly in cultivar Gloria (Table 3). There was no effect of nitrogen on total dry matter production in Experiment 2 (Table 3). Higher nitrogen pre-treatment had no effect on the final harvest index (HI) in both experiments. Higher nitrogen pre-treatment increased tuber fresh yield and tuber dry matter production at the end of the growing season in Experiment 1, but had no effect on either of them in Experiment 2 (Table 3). Nitrogen had no effect on tuber dry matter concentration in both experiments (Table 3).

At final harvest, cultivar Spunta had a higher AIR, total dry matter and tuber fresh and dry weights than cultivar Gloria but a lower RUE and HI in both experiments. Tuber dry matter concentration was lower in cultivar Spunta than in cultivar Gloria only in Experiment 2 (Table 3).

AIR, harvest indices and tuber fresh yield were relatively higher in Experiment 1 than in Experiment 2, in both cultivars (Table 3).

Dry matter distribution

In Experiment 1, higher nitrogen pre-treatment resulted in a significantly higher fraction of leaves in the total plant dry matter at 42 and 56 DAT (Fig. 2; Table 4). There was, however, no effect of nitrogen in Experiment 2. Nitrogen had no effect on the fraction of stems or on the fraction of roots in the total plant dry matter during the two seasons of growth. The fraction of tubers in the total plant dry matter was lower at higher than at lower nitrogen at 42 DAT in Experiment 1 (Fig. 2; Table 4). There was, however, no effect of nitrogen on the fraction of tubers in the total plant dry matter in Experiment 2 (Fig. 3; Table 4).

The fraction of leaves, stems and roots in the total plant dry matter was significantly higher in cultivar Spunta than in cultivar Gloria over much of the growing season, in both experiments. The fraction of tubers was, however, lower except at the beginning before tubers were initiated.

In Experiment 1 compared to Experiment 2, the fraction of leaves in the total plant dry matter was lower but the fraction of roots higher in both cultivars during the

Table 3. Crop yield analysis after 91 (Exp. 1) or 84 (Exp. 2) days of field growth, of transplants from *in vitro* propagated potato plantlets of two cultivars pre-treated with 10 mg (low N) or 40 mg (high N) of nitrogen per plant before transplanting to the field.

	AIR[a] ($MJ\ m^{-2}$)	Radiation use efficiency ($g\ MJ^{-1}$)	Total DM[b] production ($g\ m^{-2}$)	Harvest index ($g\ g^{-1}$)	Tuber DM production ($g\ m^{-2}$)	Tuber DM concentration ($g\ g^{-1}$)	Tuber fresh yield ($g\ m^{-2}$)
Experiment 1							
Gloria low N	352	2.08	731	0.91	669	0.20	3315
Gloria high N	380	2.28	864	0.92	797	0.20	4073
Spunta low N	853	1.36	1161	0.72	843	0.20	4229
Spunta high N	877	1.77	1545	0.73	1127	0.20	5566
Significance							
N	**[c]	***	***	NS	***	NS	***
CV	***	***	***	***	***	NS	***
N*CV	NS	NS	**(142)[d]	NS	NS	NS	NS
Experiment 2							
Gloria low N	292	2.52	736	0.87	635	0.22	2907
Gloria high N	293	2.17	632	0.88	556	0.22	2503
Spunta low N	621	1.81	1122	0.72	804	0.19	4204
Spunta high N	647	1.66	1072	0.71	756	0.19	3929
Significance							
N	**	NS	NS	NS	NS	NS	NS
CV	***	**	***	***	**	***	***
N*CV	**(12)[d]	NS	NS	NS	NS	NS	NS

[a] AIR accumulated intercepted radiation

[b] DM dry matter

[c] *** $P<0.001$; ** $0.001 \leq P < 0.01$; NS not significant: $P \geq 0.05$

[d] LSD (5%) for comparisons of all means.

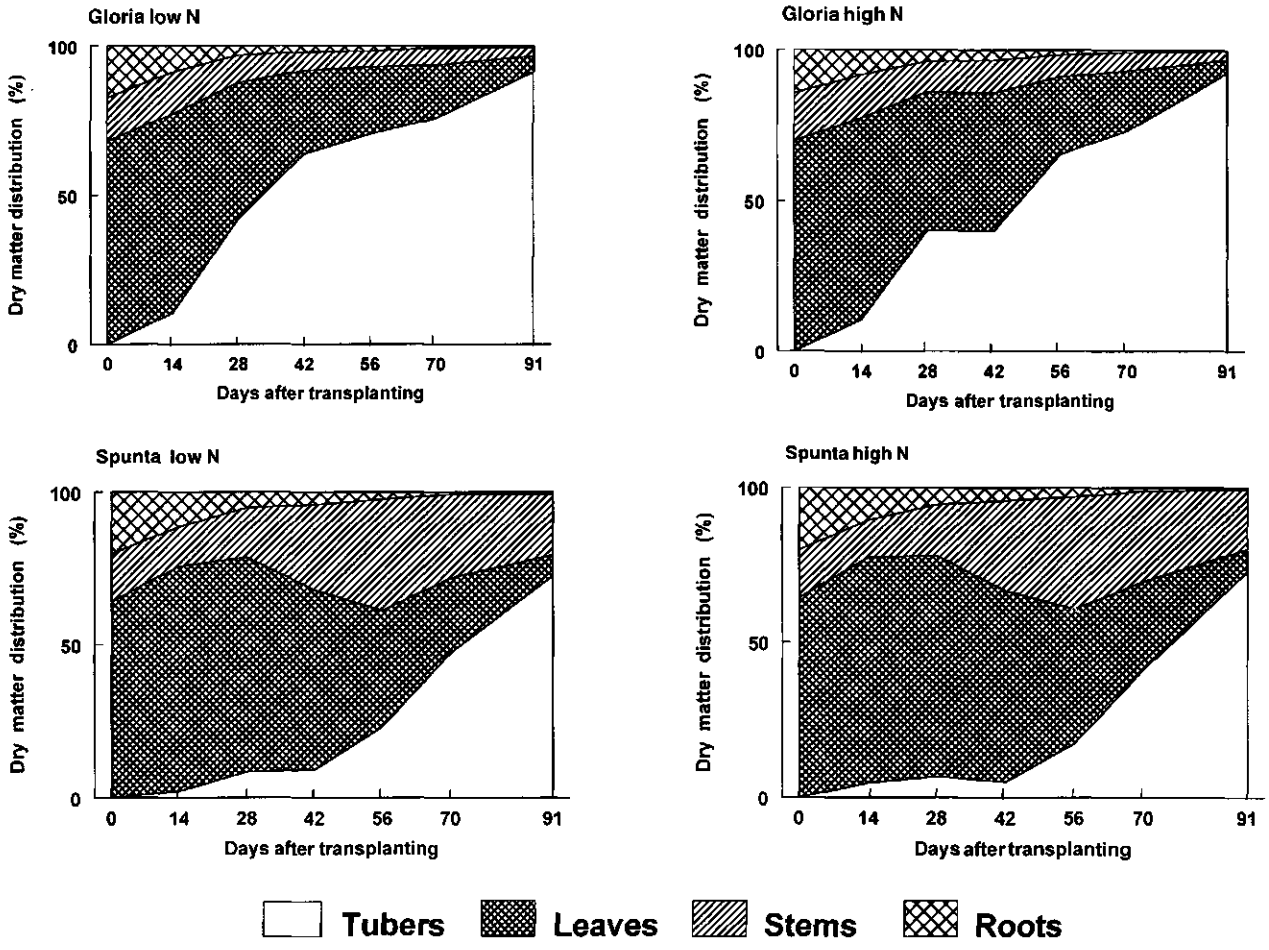

Fig. 2. Proportion (%) of leaves, stems, roots and tubers in the total plant dry matter during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars, pre-treated during transplant production with two nitrogen levels, in 1997 (Experiment 1). For statistical significances, see Table 4.

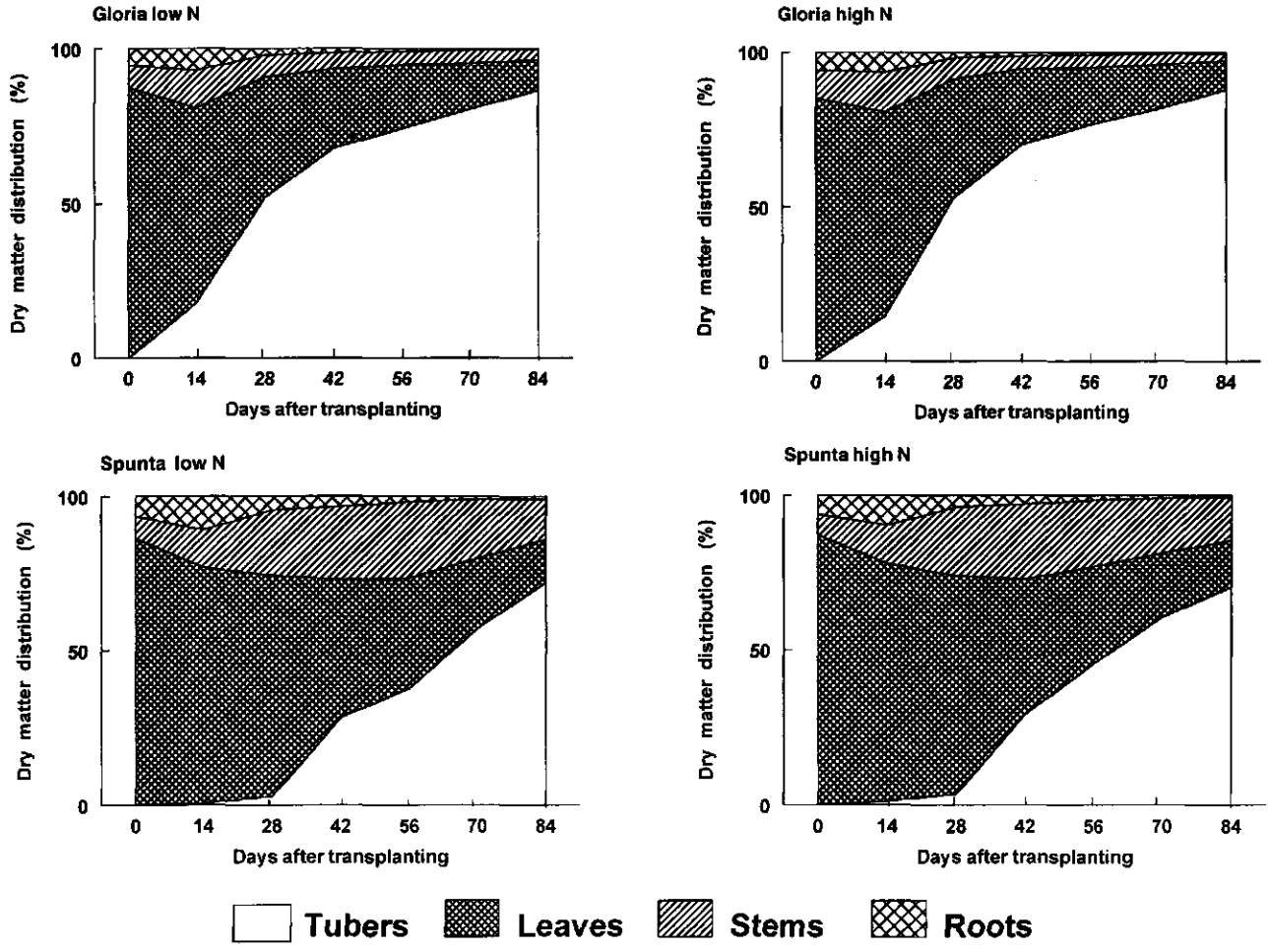

Fig. 3. Proportion (%) of leaves, stems, roots and tubers in the total plant dry matter, during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars pre-treated during transplant production with two nitrogen levels, in 1998 (Experiment 2). For statistical significances, see Table 4.

Table 4. Statistical significances of the effects of the experimental factors nitrogen, cultivar, harvest and their interactions on the proportion of different plant parts in the total plant dry matter of transplants from *in vitro* propagated potato plantlets during field growth in 1997 and 1998, presented in Figs. 2 and 3.

Plant part	Nitrogen (N)	Cultivar (CV)	Harvest (H)	N*CV	N*H	CV*H	N*CV*H
Experiment 1							
Leaves	* [a]	***	***	NS	**	***	NS
Stems	NS	***	***	NS	NS	***	NS
Roots	NS	***	**	NS	NS	NS	NS
Tubers	NS	***	***	NS	**	***	NS
Experiment 2							
Leaves	NS	***	***	NS	NS	***	NS
Stems	NS	***	***	NS	NS	***	NS
Roots	NS	***	***	NS	NS	***	NS
Tubers	NS	***	***	NS	NS	***	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

first few days of growth (Figs. 2 and 3). The fraction of stems in the total plant dry matter was higher in cultivar Spunta during the last stages of growth in Experiment 1 than in Experiment 2.

Tuber numbers and tuber fresh yield

Nitrogen pre-treatment usually had no effect on tuber numbers in both experiments (Fig. 4; Table 5). Based on the data, however, it may be suggested that a higher nitrogen pre-treatment reduced tuber number between 28 and 56 DAT in cultivar Gloria and throughout the growing period in cultivar Spunta in Experiment 1 (Fig. 4). In Experiment 1, a higher nitrogen pre-treatment resulted in significantly lower tuber fresh yield in cultivar Gloria at 42 DAT and in both cultivars at 56 DAT and this was reversed at the end of the season (Fig. 4; Table 5). Nitrogen had no effect on tuber fresh yield in Experiment 2.

In Experiment 1 cultivar Gloria had a higher tuber number than cultivar Spunta at 56 DAT but lower at final harvest. By contrast cultivar Spunta had a higher tuber number than cultivar Gloria at 42 and 56 DAT in Experiment 2. In both experiments, cultivar Gloria had higher tuber fresh yield during the early stages of growth and cultivar Spunta towards the end of the growing season (Fig. 4; Table 5).

Comparing the two experiments, tuber numbers and tuber fresh yield were generally higher in Experiment 1 than in Experiment 2 in both cultivars (Fig. 4).

Discussion

Nitrogen had almost no effect on plant growth characteristics measured at the end of the transplant production phase in both experiments (Table 1), indicating that a potential effect of nitrogen was not visible immediately in the transplant production phase but was carried over to the next phase, the field phase. The lack of a direct response may be due to the ample supply compared to the need in early phase of growth. However, even when there is a temporarily adequate supply, nitrogen may promote future haulm growth by increasing leaf nitrogen concentration, initial leaf primordia size (cf. discussion in Vos *et al.*, 1996), by affecting future dry matter allocation, postponing tuber bulking, or by influencing future radiation use efficiency (cf. discussion in Vos & Biemond, 1992; Biemond & Vos, 1992).

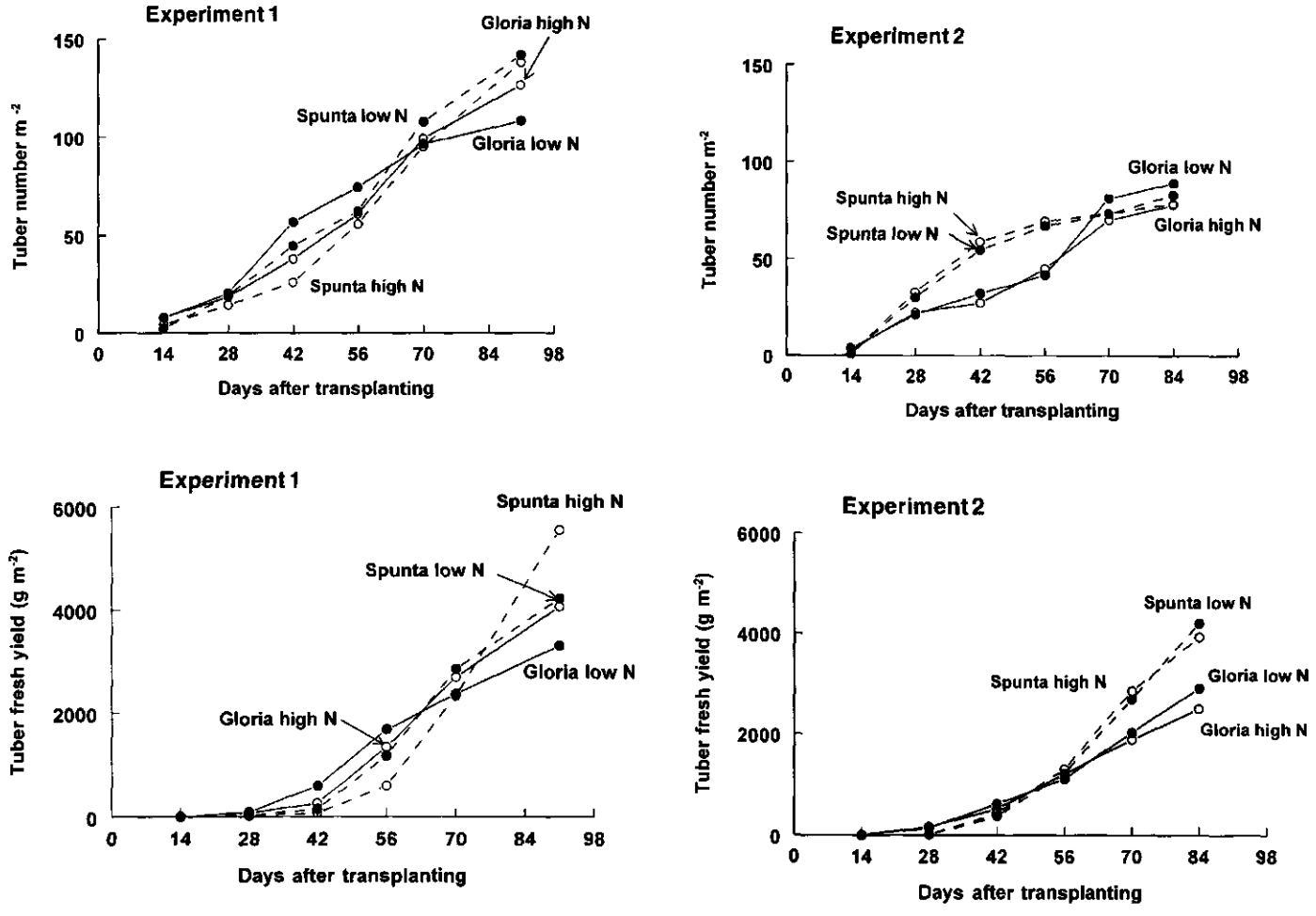

Fig. 4. Tuber number (m^{-2}) and tuber fresh yield (g m^{-2}) of transplants from *in vitro* propagated potato plantlets of two cultivars pre-treated during transplant production with two levels of nitrogen, in 1997 (Experiment 1) and 1998 (Experiment 2). For statistical significances, see Table 5.

Table 5. Statistical significances of the effects of the main factors nitrogen (N) and cultivar (CV) and their interaction on tuber number and tuber fresh weight of transplants from *in vitro* propagated potato plantlets during field growth in 1997 (Experiment 1) and 1998 (Experiment 2), presented in Fig. 4.

	Days after transplanting					
	14	28	42	56	70	91/84
Tuber number						
Experiment 1						
N	NS	NS	*[a]	NS	NS	NS
CV	*	NS	NS	*	NS	*
N*CV	NS	NS	NS	NS	NS	NS
Experiment 2						
N	NS	NS	NS	NS	NS	NS
CV	***	NS	***	***	NS	NS
N*CV	NS	NS	NS	NS	NS	NS
Tuber fresh weight						
Experiment 1						
N	NS	NS	***	***	NS	***
CV	*	***	***	***	NS	***
N*CV	NS	NS	**	NS	NS	NS
Experiment 2						
N	NS	NS	NS	NS	NS	NS
CV	***	***	*	NS	**	***
N*CV	NS	NS	NS	NS	NS	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

Nitrogen pre-treatment in the transplant production phase had no immediate effect on ground cover after transplanting or in the first weeks of field growth (Fig. 1; Table 1). However, higher nitrogen pre-treatment resulted in plants with higher ground cover in both cultivars starting at 14 DAT in Experiment 2 and 35 DAT in Experiment 1 (Fig. 1). This is in contrast with results of nitrogen pre-treatment in other transplant crops (Gray & Steckel, 1990; Booij, 1992). These authors showed a large effect of nitrogen on transplant size but a reduction in effect after transplanting. Vos *et al.* (1996), however, indicated that for Brussels sprouts the first effect of additional nitrogen is observed in plant nitrogen concentration and net changes in leaf growth started about 15 days after a switch in nitrogen regime. The main effect was through a change in specific leaf area (SLA). They concluded that there is a slowly operating mechanism of control of leaf size, which is consistent with our results. The higher ground cover (possibly partly through a higher SLA and partly through an effect on dry matter distribution (Fig. 2; Table 4)) led to a higher interception of solar radiation. In one experiment there was even a simultaneous increase in radiation use efficiency. These effects resulted in a higher dry matter production and also (by absence of clear effects on harvest index and tuber dry matter content) in higher tuber yields at the end of the growing season. Similar effects were also reported for crops from seed tubers by MacKerron & Waister (1983), Van der Zaag (1984) and Haverkort *et al.* (1991).

The very-early cultivar Gloria had lower ground cover than the mid-early cultivar Spunta in both experiments (Fig. 1). The slower increase in ground cover in cultivar Gloria after transplanting, which is a typical behaviour of transplants of early cultivars (Lommen, 1999), is due to the allocation of a higher proportion of the plant dry matter to tubers and little to the haulm early in the growing season, thereby reducing the haulm growth rate. The end of leaf growth, defined by Kooman & Haverkort (1995) and Kooman *et al.* (1996) as the moment when 90% of the dry matter produced was allocated to tubers (Lommen, 1999), must have occurred in cultivar Gloria when the ground cover was still far below full cover in both experiments but especially in Experiment 2. This effect came on top of the fact that Gloria had already shown a much slower increase in ground cover during the first part of the season. As a result, cultivar Gloria had a much lower AIR than cultivar Spunta in the two experiments.

The amount of nitrogen pre-treatment applied to the plantlet in the transplant production phase was very little compared to the nitrogen fertiliser applied to the field and which will be readily available to the plants later. Still higher nitrogen pre-treatment resulted in higher ground cover and more AIR in both seasons and higher tuber yield at the end of the growing season in Experiment 1 (Table 3; Fig. 4). Effects

of nitrogen on other plant growth parameters were, however, not significant in Experiment 2 probably because other environmental factors such as the abundant rainfall and dull weather were dominant. Such conditions increase the leaf nitrogen concentration and may thus have after-effects similar to the effect triggered by a temporary over-supply of nitrogen. Thus, the effect of nitrogen pre-treatment could have been overwhelmed (Table 3).

In conclusion, nitrogen pre-treatment can improve the performance of early potato cultivars and thus, increase yield by affecting the seasonal light interception of the plants.

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CHAPTER 9

Effects of temperature pre-treatment of transplants from *in vitro* produced potato plantlets on transplant growth and yield in the field

Submitted as:

Tadesse M., W.J.M. Lommen & P.C. Struik, 2000. Effects of nitrogen pre-treatment of transplants from *in vitro* produced potato plantlets on transplant growth and yield in the field.

9. Effects of temperature pre-treatment of transplants from *in vitro* produced potato plantlets on transplant growth and yield in the field

Abstract

In vitro produced potato plantlets of cultivars Gloria and Spunta were exposed to low and high temperatures for 14 days. They were then transplanted to the field to assess the after-effect of temperature on the performance of short cycle crops in two field experiments. Accumulated intercepted radiation (AIR) and radiation use efficiency (RUE) were calculated from ground cover and incoming radiation, and dry matter production and fresh tuber yield were analysed. High temperature pre-treatment caused higher leaf area at the end of the transplant production phase and resulted in a higher AIR at the end of the field phase than low temperature. Higher AIR, however, was not associated with higher tuber yield. The proportion of dry matter partitioned to tubers was lower at higher temperature. Cultivar Spunta had higher ground cover and AIR than cultivar Gloria. High temperature pre-treatment may improve the performance of plantlets, especially of early potato cultivars.

Key words: acclimatisation, dry matter distribution, ground cover, harvest index, radiation interception, radiation use efficiency, *Solanum tuberosum* L.

Introduction

Field crops grown from transplants produced from *in vitro* plantlets, especially those of early cultivars, have lower accumulated intercepted radiation (AIR) and lower yields than crops from seed tubers (Lommen, 1999). The probable cause is a slower increase in ground cover after transplanting and consequently a lower AIR over the whole growing season for some cultivars. The slow increase in ground cover was attributed to a relatively high proportion of dry matter being allocated to tubers before and immediately after transplanting, resulting in reduced haulm growth rates and subsequently total growth rates (Lommen, 1999). If indeed this is the main cause for the lower yield of transplant crops (especially for the early cultivars), exposing plantlets to non-inducing conditions during transplant production could improve their performance if the effects of such conditions persist for a while in the field phase. However, there is no conclusive information on how treatments and growth conditions during transplant production affect growth of the transplants and their subsequent performance in the field. This chapter will focus on temperature pre-treatments.

Temperature is one of the major environmental factors influencing potato growth and yield (Bennett *et al.*, 1991). Potato is well adapted to mean temperature of 17 °C (Bushnell, 1925), but also responds positively to diurnal temperature fluctuations; night temperature is especially crucial (Struik & Ewing, 1995).

Tuber initiation and growth are temperature dependent (Gregory, 1956; Slater, 1963). Van Dam *et al.* (1996) confirmed that temperatures of 15-19 °C are optimal for tuber initiation and initial growth. High temperature delays or even inhibits tuber initiation (Struik *et al.*, 1989b) and strongly reduces the proportion of total dry matter partitioned to tubers (Ewing, 1981; Ben Khedher & Ewing, 1985; Struik *et al.*, 1989a; Bennett *et al.*, 1991) and increases partitioning to the haulm (Basu & Minhas, 1991). A slight increase in temperature already strongly reduces partitioning to tubers (Van Dam *et al.*, 1996).

Treating plants with relatively high temperature during transplant production could promote haulm growth of the subsequent field crops either directly by enhancing haulm growth or indirectly by delaying tuber initiation. This may improve the field performance of the transplants, especially in early cultivars in which haulm growth is limiting. Yield increase can only be achieved if the tuber bulking period will be long enough and if the temperature pre-treatment of transplants is not associated with yield limiting side effects.

This paper, therefore, summarises the results of two experiments in which *in vitro* propagated potato plantlets were grown at two different temperatures during transplant production before they were transplanted to the field. The aim of the current study is to quantify temperature effects on *in vitro* propagated plantlets and to understand how temperature pre-treatment in the very early cultivar Gloria and mid-early cultivar Spunta affects growth, development and yield formation in the subsequent field phase.

Materials and methods

Potato (*Solanum tuberosum* L.) plantlets cvs. Gloria and Spunta were propagated *in vitro* by producing single-node cuttings using virus-free stock plantlets on a standard medium containing MS salts (Murashige & Skoog, 1962) with vitamins, 25 g l^{-1} sucrose, 8 g l^{-1} agar and 0.0133 g l^{-1} alar-64% (daminozide). The vitamins in the MS salts were 2 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 0.50 mg l^{-1} nicotinic acid, 0.50 mg l^{-1} pyridoxine HCl and 0.10 mg l^{-1} thiamine HCl. The pH of the medium was adjusted to 5.7. Viable nodes were cut from plantlets discarding tops and cultured in petri-dishes containing 20 ml medium (8 per dish). The petri-dishes, sealed with household plastic foil, were placed in a growth chamber at 23 °C and a photophase of

16 hours supplied by Philips TL 84 fluorescent tubes with a photosynthetic photon flux density of 30 μmol m^{-2} s^{-1} for 21 days. This is the "normalisation" phase during which single-node cuttings develop into rooted *in vitro* plantlets.

At the end of the normalisation phase, rooted *in vitro* plantlets were planted in transplanting trays with small cells (4.0 × 5.5 × 6.0 cm, w × l × d) filled with (ca. 100 ml) potting soil into walk-in chambers with day/night temperatures of 18/12 or 26/20 °C. A photophase of 14 h was supplied with SON-T and TL 84 lamps providing an average intensity above the plants of 465 μmol m^{-2} s^{-1}. Planting was done by leaving approximately half of the stem above the ground at a density of 300 plants m^{-2}. Each plant received 10 ml of a low-concentrated Steiner nutrient solution (Lommen & Struik, 1992) three times a week. The relative humidity (RH) in the growth chambers was kept at 70-80% and plantlets were cultured in these chambers for 14 days. The temperature in the growth chambers was reduced to 12/8 in the low and 20/16 °C in the high temperature chambers two days before transplanting. This 14-days phase in which *in vitro* plantlets are acclimatised to *ex vitro* conditions to produce transplants is referred to as the "acclimatisation" or "transplant production" phase.

The transplants were then transplanted to a sandy soil in Achterberg, near Wageningen (The Netherlands) on the 13th of May 1998 (Experiment 1) or on the 12th of May 1999 (Experiment 2). Planting was done in low ridges at a density of 6.67 plants m^{-2} by leaving approximately half of the stem above the soil at a row distance of 75 cm and a plant distance within the row of 20 cm. The ridges were hilled to a height of about 20 cm, 35 days after transplanting (DAT) in both experiments.

The field phase of the experiment was carried out in a split-split plot design in 5 blocks. The two cultivars were randomised within each block, the two temperature treatments within each cultivar and six harvests (at 14, 28, 42, 56, 70 and 84 DAT) within each temperature treatment. A first harvest was done at transplanting (0 DAT). The experimental unit consisted of 6 net plants (2 rows × 3 plants) and these plants were surrounded by 14 guard plants. The field was fertilised beforehand with 122 kg N ha^{-1} in the form of NH_4NO_3 and 60 kg K ha^{-1} in the form of K_2O. No phosphorus fertiliser was added to the field since the phosphorus status was adequate for potato cultivation.

The first growing season (1998) was wet and cloudy with a daily average temperature of 16.2 °C while the second season (1999) was less wet and cloudy, with a daily average temperature of 16.3 °C.

Measurements and statistical methods

Ground cover was assessed every week using a grid of 100 squares (90 × 75 mm

each). Daily ground cover was intrapolated from the weekly measurements and was multiplied by the total daily incoming global radiation to calculate the daily radiation intercepted and the total accumulated intercepted radiation (AIR) during the growing season. Yield formation was analysed by harvesting plants every 14 days throughout the field phase. Radiation use efficiency (RUE) was calculated as the total dry matter production per AIR. Leaf, stem and root dry weights and (if present) tuber fresh and dry weights were measured and tuber numbers counted. Shoot/root ratio and harvest index (HI) (the proportion of tubers in the total dry matter) were calculated from the yield analysis. Data were subjected to analysis of variance (ANOVA) using Genstat 5 release 3.22 (1995) and differences between treatments were analysed by LSD tests at $P<0.05$.

Results

Initial plant dimensions

At the end of the transplant production phase, leaf, stem, shoot and total dry weights were significantly higher at higher than at lower temperature in Experiment 2 only (Table 1). Leaf area at the end of the transplant production phase was higher at higher than at lower temperature in cultivar Spunta in Experiment 1 and in both cultivars in Experiment 2. Temperature pre-treatment, however, had no effect on root dry weight or shoot/root ratios in both experiments (Table 1).

In general, plantlets of cultivar Spunta had higher dry weights and larger leaf areas than those of cultivar Gloria in both experiments (Table 1). Plantlets in Experiment 2 had comparatively larger leaf areas and higher leaf, stem, shoot and total dry weights than those in Experiment 1. Root growth was, however, comparatively lower in Experiment 2 resulting in a much higher shoot/root ratio in plantlets in Experiment 2 than in Experiment 1 (Table 1).

Ground cover

In Experiment 1, ground cover was significantly higher at higher than at lower temperature during transplant growth in both cultivars throughout the growing season, except at 84 DAT when the positive effect was only significant in cultivar Gloria (Fig. 1a; Table 2). In Experiment 2, ground cover was significantly higher at higher than at lower temperature pre-treatment during most of the growing period (when ground cover was not yet 100%) (Fig. 1b; Table 2).

Table 1. Plant growth characteristics at transplanting (0 DAT) of *in vitro* propagated potato plantlets of two cultivars (CV) grown at two temperatures (T) during transplant production in the 1998 (Experiment 1) and 1999 (Experiment 2) field experiments.

	Leaf dry weight (mg/plant)	Stem dry weight (mg/plant)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)	Total dry weight (mg/plant)	Shoot/root Ratio	Leaf area (cm^2)
Experiment 1							
Gloria low T	18.0	3.4	21.4	4.8	26.2	5.2	14.8
Gloria high T	15.7	3.6	19.3	3.5	22.9	5.9	16.8
Spunta low T	32.1	7.1	39.2	10.6	49.8	3.9	20.1
Spunta high T	38.6	8.2	46.7	13.6	60.3	4.0	28.8
Significance							
T	NS	NS	NS	NS	NS	NS	***
CV	*[a]	**	*	*	*	NS	**
T*CV	NS	NS	NS	NS	NS	NS	*** (3.01)[b]
Experiment 2							
Gloria low T	39.7	7.0	46.7	2.5	49.1	19.5	20.4
Gloria high T	64.3	12.3	76.7	4.1	80.8	25.8	39.0
Spunta low T	61.0	9.3	70.3	4.0	74.3	17.9	34.6
Spunta high T	97.7	20.3	118.0	6.5	124.5	20.7	51.9
Significance							
T	**	**	**	NS[c]	**	NS	***
CV	***	**	***	NS[d]	***	NS	***
T*CV	NS	NS	NS	NS	NS	NS	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$

[b] LSD (0.05) for comparisons of all means

[c] P = 0.053

[d] P = 0.059

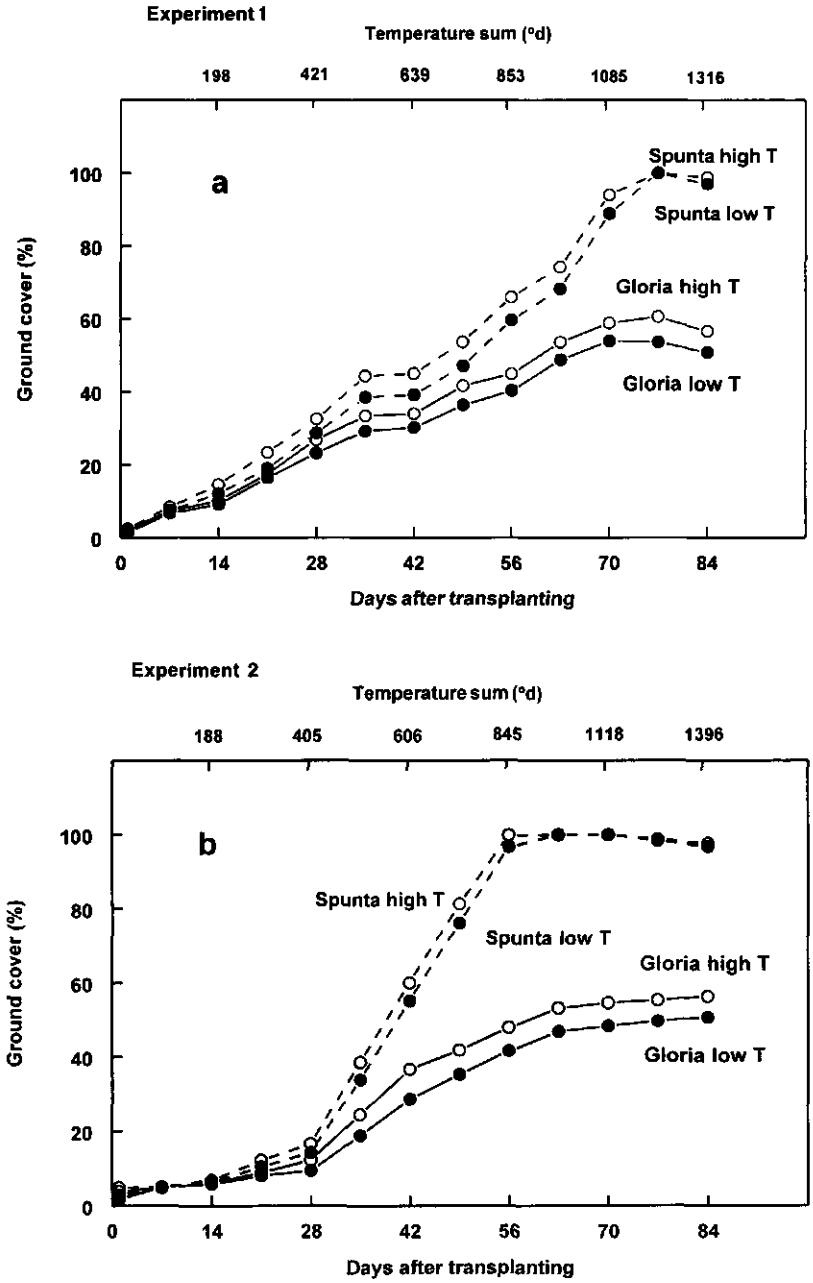

Fig. 1. Ground cover during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars grown at 18/12 (low) or 26/20 (high) °C before transplanting to the field in two experiments, (a) during 1998 (Experiment 1) and (b) during 1999 (Experiment 2). For statistical significances, see Table 2.

Table 2. Statistical significances of the effects of the main factors temperature (T) and cultivar (CV), and their interaction (T*CV) for the weekly ground cover of transplants from *in vitro* propagated potato plantlets during field growth in 1998 (Experiment 1) and 1999 (Experiment 2) presented in Fig. 1.

	Days after transplanting												
	1	7	14	21	28	35	42	49	56	63	70	77	84
Experiment 1													
T	*** [a]	**	***	***	***	***	***	***	***	***	***	***	**
CV	**	**	***	***	*	***	***	***	***	***	***	***	***
T*CV	NS	NS	**	**	NS	NS	NS	NS	*	NS	NS	NS	*
Experiment 2													
T	***	NS	***	***	***	***	***	***	***	***	**	**	**
CV	*	NS	*	**	***	***	***	***	***	***	***	***	***
T*CV	NS	NS	*	**	NS	NS	*	NS	*	***	**	*	*

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

Cultivar Spunta reached full ground cover at 77 DAT in Experiment 1 and at 56 DAT in Experiment 2 (Fig. 1a and b). For Gloria the maximum ground cover was about 55% in both experiments (Fig. 1a and b).

Ground cover increased faster in Experiment 1 than in Experiment 2, during the early stages of growth (Fig. 1b). At later stages, however, ground cover had a higher rate of increase in Experiment 2 than in Experiment 1, especially in cultivar Spunta. The characteristic behaviour of ground cover development in Experiment 1 was also observed in another experiment in the same year and site using transplants as propagules.

Crop yield analysis

At the end of the growing season, the crop from transplants that received high temperature pre-treatment had a significantly higher AIR than that which received low temperature pre-treatment, in both cultivars and both experiments (Table 3). The higher AIR, however, did not result in a significantly higher total dry matter yield in the field. In both experiments, temperature pre-treatment also had no significant effect on RUE, harvest index, tuber dry matter production, tuber dry matter concentration, or tuber fresh yield, at final harvest (Table 3). There was, however, a trend towards higher values for all yield parameters (total dry matter, tuber dry matter and tuber fresh yield) at higher than at lower temperature in both cultivars and in both experiments (Table 3). The effect of temperature on RUE was small and inconsistent over the two cultivars and in the two experiments.

There was a significant cultivar effect on most of the plant growth characteristics at final harvest in both experiments (Table 3). AIR, total dry matter production, tuber fresh yield and tuber dry matter production were higher in cultivar Spunta than in cultivar Gloria in both experiments. Tuber dry matter concentration and harvest indices were higher in the earlier cultivar Gloria (Table 3). There was, however, no cultivar effect on RUE at final harvest in both experiments (Table 3).

AIR, total dry matter production and tuber fresh yield were comparatively higher but harvest indices and tuber dry matter concentration were comparatively lower in Experiment 2 than in Experiment 1 (Table 3).

Dry matter distribution

During field growth, there was generally no effect of temperature pre-treatment on the fraction of leaves in the total plant dry matter except in cultivar Gloria in Experiment 2 where a higher temperature during pre-treatment resulted in a higher fraction of leaves

Table 3. Yield analysis at final harvest (84 DAT), of crops from transplants from *in vitro* propagated potato plantlets of two cultivars (CV) grown at 18/12 (low T) or 26/20 °C (high T) in the transplant production phase, before transplanting to the field.

	AIR[a] ($MJ\ m^{-2}$)	Radiation use efficiency ($g\ MJ^{-1}$)	Total DM[b] production ($g\ m^{-2}$)	Harvest index ($g\ g^{-1}$)	Tuber DM production ($g\ m^{-2}$)	Tuber DM concentration ($g\ g^{-1}$)	Tuber fresh yield ($g\ m^{-2}$)
Experiment 1							
Gloria low T	309	1.40	433	0.90	391	0.23	1697
Gloria high T	345	1.65	570	0.89	507	0.23	2222
Spunta low T	466	1.77	822	0.77	634	0.20	3142
Spunta high T	536	1.56	841	0.76	645	0.20	3298
Significance							
T	*** [c]	NS	NS	NS	NS	NS	NS
CV	***	NS	***	***	*	*	*
T*CV	*** (18)[d]	NS	NS	NS	NS	NS	NS
Experiment 2							
Gloria low T	348	1.73	601	0.76	455	0.18	2452
Gloria high T	399	1.58	628	0.75	469	0.19	2505
Spunta low T	689	1.69	1162	0.53	608	0.17	3556
Spunta high T	713	1.72	1228	0.56	685	0.18	3904
Significance							
T	***	NS	NS	NS	NS	NS	NS
CV	***	NS	***	***	**	**	**
T*CV	***(13)[d]	NS	NS	NS	NS	NS	NS

[a] AIR accumulated intercepted radiation

[b] DM dry matter

[c] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$

[d] LSD (0.05) for comparisons of all means.

(Fig. 3; Table 4). The fraction of stems was higher at higher than at lower temperature in Experiment 1. It was also higher at 14 and 28 DAT in Experiment 2 but lower at 70 DAT (Fig. 3; Table 4). There was no effect of temperature pre-treatment on the fraction of roots in the total plant dry matter except at 14 DAT, in Experiment 2 which was higher at high temperature (Fig. 3). The fraction of tubers in the total plant dry matter was significantly higher at lower than at higher temperature during pre-treatment in Experiment 1 (Fig. 2). This was only found at 14 DAT in Experiment 2 (Fig. 3).

In both experiments, the fractions of leaves and stems in the total plant dry matter decreased consistently through time in cultivar Gloria but increased in between in cultivar Spunta, before they decreased continuously thereafter (Figs 2 and 3). In both experiments, the fraction of roots decreased continuously with time in both cultivars. The fraction of tubers increased consistently over time in both cultivars in Experiment 1 and in cultivar Gloria in Experiment 2, but declined first at 28 DAT and then increased throughout the growth period in cultivar Spunta, in Experiment 2 (Fig. 3).

In both experiments, there was a higher fraction of leaves and stems in the total plant dry matter in cultivar Spunta than in cultivar Gloria for the greatest part of the growing season except for few days at the beginning (Figs 2 and 3). The fraction of roots in the total plant dry matter was higher in cultivar Spunta than in cultivar Gloria between 0 and 28 DAT in Experiment 1 and between 14 and 42 DAT in Experiment 2. The fraction of tubers in the total plant dry matter was, however, higher in cultivar Gloria than in cultivar Spunta throughout the growing period in both experiments.

A higher proportion of dry matter was allocated to the leaves and lower to the tubers in both cultivars in Experiment 2 than in Experiment 1 (Figs 2 and 3). The proportion of dry matter allocated to stems was also relatively higher in Experiment 2 than in Experiment 1 at later stages of growth in both cultivars. A higher proportion of dry matter was allocated to the roots in cultivar Gloria during the first two weeks of growth in the first than in the second experiment (Figs 2 and 3).

Tuber number, tuber fresh yield and tuber growth rate

There was no after-effect of temperature on tuber number in Experiment 1 (Fig. 4). In Experiment 2, however, tuber number was higher at higher than at lower temperature during pre-treatment in cultivar Gloria but not in cultivar Spunta (Fig. 4).

In Experiment 1, tuber number was higher in cultivar Gloria at 28 DAT but in cultivar Spunta during the rest of the season. In Experiment 2, tuber number was significantly higher in cultivar Spunta than in cultivar Gloria at 42 DAT (Fig. 4).

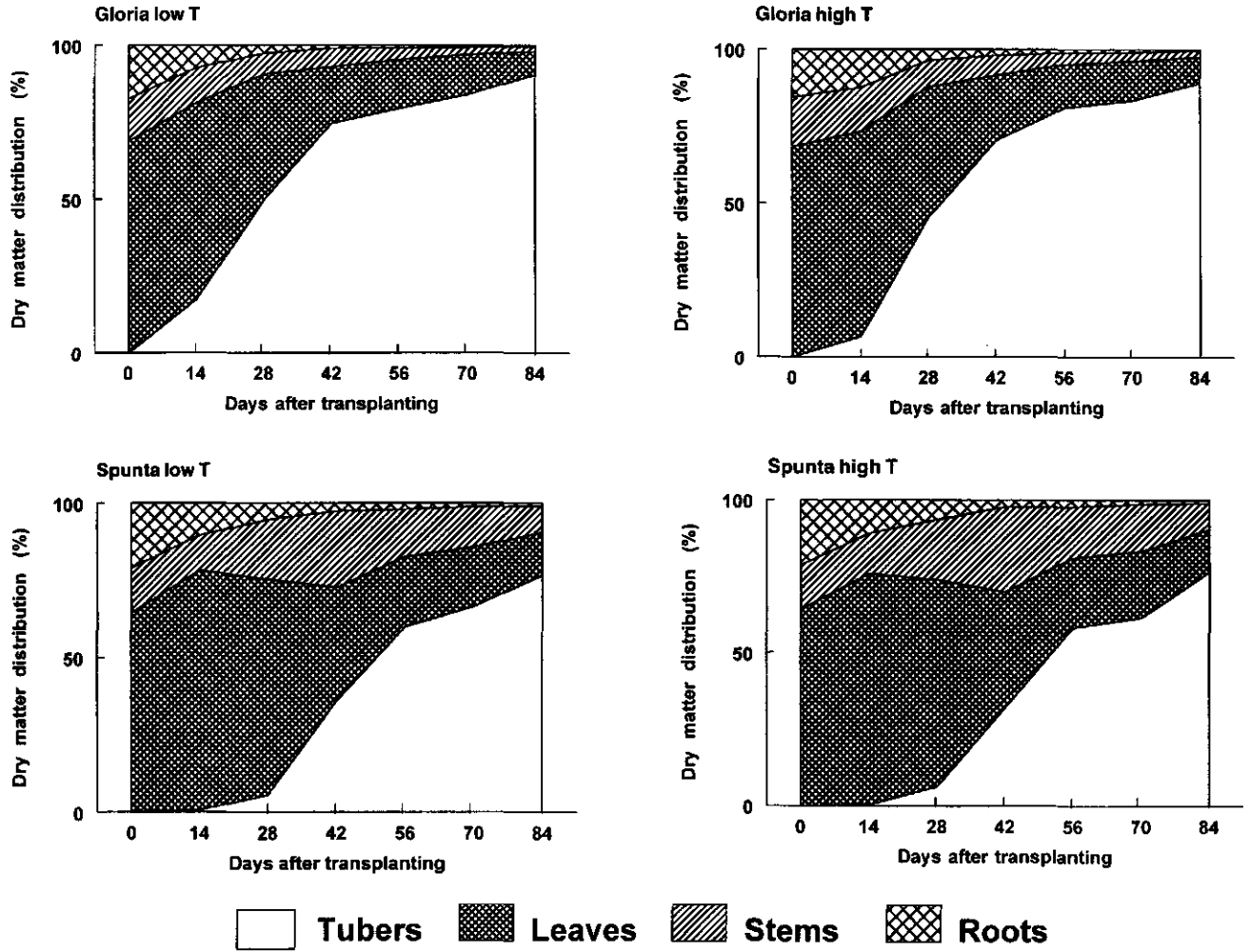

Fig. 2. Proportion (%) of leaves, stems, roots and tubers in the total plant dry matter during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars grown at 18/12 (low) or 26/20 (high) °C before transplanting to the field, in 1998 (Experiment 1). For significances, see Table 4.

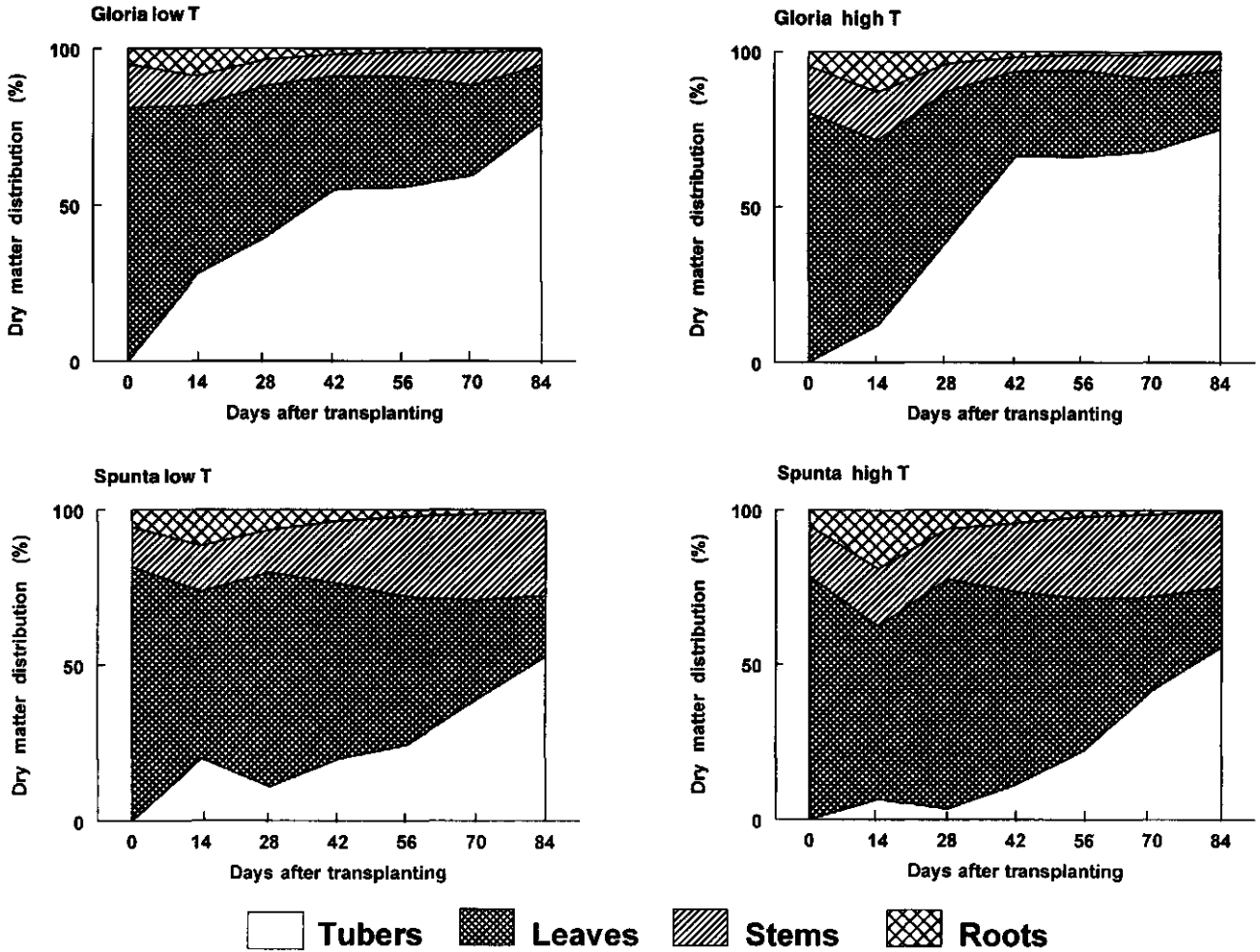

Fig. 3. Proportion (%) of leaves, stems, roots and tubers in the total plant dry matter during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars grown at 18/12 (low) or 26/20 (high) °C before transplanting to the field, in 1999 (Experiment 2). For significances, see Table 4.

Table 4. Statistical significance of the proportion of different plant parts in the total plant dry matter of transplants from *in vitro* propagated potato plantlets during field growth in 1998 and 1999, presented in Figs 2 and 3.

Plant part	Temperature (T)	Cultivar (CV)	Harvest (H)	T*CV	T*H	CV*H	T*CV* H
Experiment 1							
Leaves	NS	***	***	NS	NS	***	NS
Stems	** [a]	***	***	NS	NS	***	NS
Roots	NS	**	***	NS	NS	*	NS
Tubers	**	***	***	NS	NS	***	NS
Experiment 2							
Leaves	***	***	***	*	NS	***	NS
Stems	NS	***	***	NS	**	***	NS
Roots	**	**	***	NS	***	**	NS
Tubers	NS	***	***	*	***	***	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

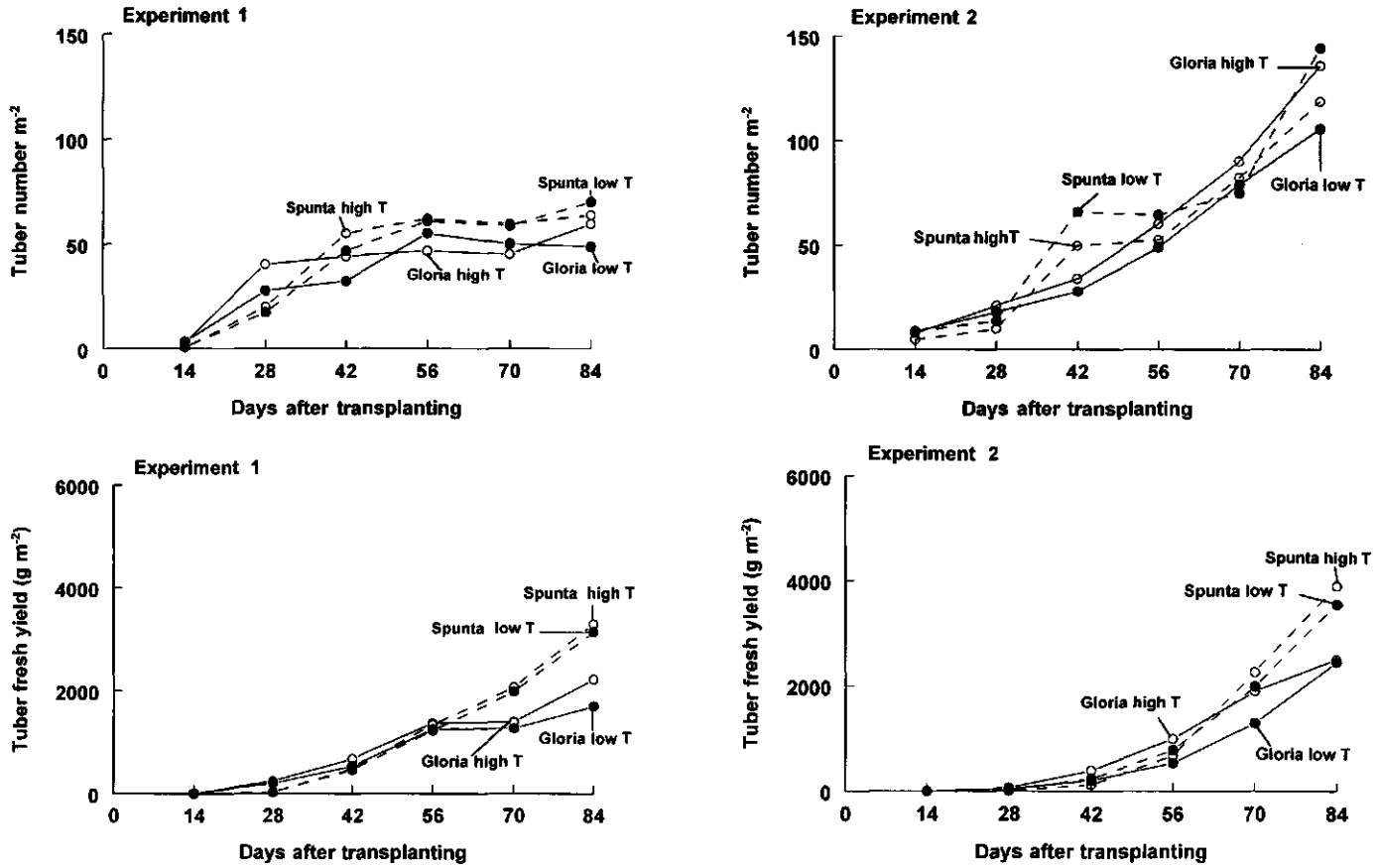

Fig. 4. Tuber number and fresh yield during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars grown at 18/12 (low) or 26/20 (high) °C before transplanting to the field, in 1998 (Experiment 1) and 1999 (Experiment 2). For significances, see Table 5.

Table 5. Statistical significances of the effects of the main factors temperature (T) and cultivar (CV) and their interaction (T*CV) for tuber number and tuber fresh weight of transplants from *in vitro* propagated potato plantlets during field growth in 1997 (Experiment 1) and 1998 (Experiment 2), presented in Fig. 4.

	Days after transplanting					
	14	28	42	56	70	84
Tuber number						
Experiment 1						
T	NS	NS	NS	NS	NS	NS
CV	*[a]	**	*	NS	NS	NS
T*CV	NS	NS	NS	NS	NS	NS
Experiment 2						
T	NS	NS	NS	NS	NS	NS
CV	*	**	***	***	NS	NS
T*CV	NS	NS	*	*	NS	NS
Tuber fresh weight						
Experiment 1						
T	NS	NS	NS	NS	NS	NS
CV	*	**	NS	NS	**	*
T*CV	NS	NS	NS	NS	NS	NS
Experiment 2						
T	*	NS	NS	**	NS	NS
CV	NS	*	*	NS	NS	**
T*CV	NS	NS	***	***	NS	NS

[a] *** $P < 0.001$; ** $0.001 \leq P < 0.01$; * $0.01 \leq P < 0.05$; NS not significant: $P \geq 0.05$.

Tuber number increased with time in both experiments except at 70 DAT in Experiment 1 (Fig. 4) where a slight decrease took place followed by an increase. Over the whole season, tuber number was generally higher in Experiment 2 than in Experiment 1 in both cultivars (Fig. 4).

In both experiments, cultivar Spunta had higher tuber fresh yield than cultivar Gloria at the end of the growing season (70 and 84 DAT).

Discussion

Effects of temperature during pre-treatment

High temperature pre-treatment resulted in transplants with higher dry weights and higher leaf areas in both cultivars (Table 1). This is because potato plants show (within the relevant temperature range) considerable increase in internode length (Marinus & Bodlaender, 1975), in the number of leaves (or internodes) per stem (Marinus & Bodlaender, 1975; Struik & Ewing, 1995) and perhaps also in Specific leaf area (SLA) (Midmore & Prange, 1992; Struik & Ewing, 1995) at higher temperature. The negative effects of temperature on leaf size and leaf/stem ratio, however, do not manifest themselves within the temperature range applied. These effects of temperature pre-treatment were evident at the end of the transplant production phase but partly faded out in the subsequent phase of growth, the field phase.

After-effects of temperature pre-treatment

Pre-treatment with higher temperature resulted in plants with higher ground cover in both cultivars in the two experiments. This effect persisted until full ground cover was reached (Fig. 1). As a result, plantlets of both cultivars intercepted more incoming radiation in both experiments until the end of the growing season resulting in a higher AIR (Table 3). Higher AIR, however, was not associated with a significant increase in yield in either of the two experiments (Table 3), because effects on AIR were either too small or were associated with a small reduction in RUE.

Given the temperature effects during transplant production on leaf and stem dry weights (in Experiment 2) and leaf area (in both experiments), it is likely that the functional condition of transplants differed for the two temperature pre-treatments (see e.g. Wheeler *et al.*, 1986). Other plant characteristics not measured in the experiments, such as number and size of leaf primordia (cf: Vos, 1995), SLA (cf: Vos, 1995; Struik & Ewing, 1995), induction to tuberise (Struik & Ewing, 1995) and dry matter partitioning (Ewing & Struik, 1992) might have been different as well. These likely

effects may be responsible for possible after-effects during the field phase, but expression of these after-effects depends on conditions during field production. After-effects may be expressed or not, or disappear rapidly.

Struik *et al.* (1988) investigated after-effects of short periods with long days in normal tuber-derived plants and found effects on stolon development, onset of tuberization and tuber size distribution (shift towards smaller sizes) but found no effect on yield. Struik (1987) also investigated after-effects of short periods of high temperature and found that short periods of high temperature well before tuberization boost stolon development, that similar treatments during tuberzation had little and variable effect and that such treatments after tuberization affected tuber size distribution. Struik (1989) showed that short periods of heat enhanced shoot growth and reduced harvest index. Effects on tuber number and growth were only considerable when heat was applied just before, during or after tuber set and not when applied well before tuber set.

Based on this literature on normal tuber-derived plants, it is unlikely to have long term after-effects on tuber number and tuber yield unless there is an after-effect on plant vigour, leaf area or dry matter partitioning. Plant vigour was affected (see early leaf area, Table 1) and effects on leaf area persisted probably also partly through effects on number and size of leaf primordia. Effects of pre-treatments on partitioning were present but not very persistent. The following effects however were relevant.

Stems contributed a higher proportion to the total plant dry matter at higher than at lower temperature pre-treatment in Experiment 1, because high temperature promotes stem growth (Marinus & Bodlaender, 1975; Wheeler *et al.*, 1986). The allocation of dry matter to the stems and roots was higher in the early stages but lower at later stages of growth in Experiment 2 (Fig. 3). Analogous to reports by Ewing (1981) and Ben Kheder & Ewing (1985) on normal plants, the proportion of total dry matter partitioned to tubers was reduced at high temperature in both experiments (Figs 2 and 3). This is because high temperature increases the proportion of dry matter allocated to the haulm (Basu & Minhas, 1991), prolonging the vegetative growth of the plants and thereby delaying tuber initiation and bulking.

Differences among cultivars

The proportion of leaves in the total plant dry matter was higher in cultivar Gloria at the early stages of growth and in cultivar Spunta during the rest of the growth period (Fig. 2). Gloria had a higher proportion of leaves in the total plant dry matter before tuber initiation, but as it started allocating part of its dry matter to the tubers early in the season, the proportion allocated to the leaves declined and thus Spunta had a

higher proportion at later stages.

The fraction of tubers in the total plant dry matter was higher in cultivar Gloria than in cultivar Spunta throughout the growing season in both experiments. This is mainly because the growing season (12 weeks) was not sufficiently long for the mid-early cultivar Spunta to fully develop and attain its maximum harvest index. It was, however, sufficiently long for the very-early cultivar Gloria to allocate the maximum possible dry matter to the tubers before the end of the season (Table 3).

Total dry matter production, AIR, tuber fresh yield and tuber dry matter production were higher but harvest indices lower in cultivar Spunta than in cultivar Gloria, in both experiments (Table 3). Cultivar Spunta had invested a higher proportion of its dry matter in the haulm early in the growing season and intercepted more solar radiation to synthesise more dry matter. Harvest index data also indicated that cultivar Spunta was still allocating dry matter to the tubers while cultivar Gloria had allocated about 75% of its dry matter to the tubers by the end of the growing season (Table 3).

The very-early cultivar Gloria never reached full cover in either of the two experiments and the low maximum ground cover is in accordance with the behaviour of this cultivar in other research (Lommen, 1999; Tadesse *et al.*, unpublished). Cultivar Gloria allocated a higher proportion of its dry matter to tubers early in the growing season before the haulm was fully developed. This substantially reduced the haulm growth rate, as was also reported by Lommen (1999), and prevented it from achieving full cover. The end of leaf growth, defined by Kooman & Haverkort (1995) and Kooman *et al.* (1996), as the moment when 90% of the dry matter produced was allocated to tubers, occurred when the ground cover was about 55% in cultivar Gloria in Experiment 1 (Fig. 1). The end of leaf growth was not reached in Experiment 2 because cultivar Gloria had allocated only about 77% of its total dry matter to the tubers by the end of the growing season (Table 3).

Differences among experiments

During the early stages of growth, ground cover increased faster in Experiment 1 than in Experiment 2 (Fig. 1), but there was a higher rate of increase in Experiment 2 at later stages, especially in cultivar Spunta. As a result of their larger size and higher shoot/root ratios, transplants in Experiment 2 may have suffered a larger transplant shock immediately after transplanting (Fig. 1). After overcoming this shock, however, there was a boost in growth at 28 DAT and ground cover increased faster in both cultivars (Fig. 1). Cultivar Spunta attained full cover earlier and maintained full cover longer in Experiment 2 than in Experiment 1 (Fig. 1).

Conclusion

Studies on the effect of *in vitro* treatment on leaf area growth (e.g. Marinus, 1985; Mastenbroek & Eising, 1987; Hagman, 1990) have shown the importance of the status of the propagules at the end of the *in vitro* phase for further performance of the plantlets in the following phases. Our data for two field experiments support the importance of that status.

Higher temperature pre-treatment promoted leaf area at the end of the transplant production phase and resulted in plants with higher ground cover at the end of the growing season. As a result, plants pre-treated at high temperature were able to intercept more solar radiation in the field. Higher AIR, however, did not lead to significant differences in yield.

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CHAPTER 10

General discussion

General discussion

Introduction

The seed tuber production system selected for this research project has four phases:

a) The 'multiplication' phase, where *in vitro* plantlets are propagated *in vitro* using single-node cuttings in a culture medium;
b) The 'normalisation' phase, where nodal cuttings are cultured *in vitro* to produce rooted plantlets;
c) The 'transplant production' phase, where rooted *in vitro* plantlets are acclimatised *in vivo* in a glasshouse to produce transplants, and
d) The 'tuber production' phase, where transplants are grown in the field to produce seed tubers.

For research purposes this system was slightly changed. For example, the fourth phase took place in a greenhouse when environmental conditions needed to be controlled.

This research project focuses on the last three phases and its main objectives are to analyse and understand:

1. The important morphological and physiological changes that take place at the plant and crop levels in the normalisation, transplant production and tuber production phases of the four-phase seed production system;
2. The way in which these changes are affected by different treatments during a phase, and
3. The way changes in a certain phase are affected by different treatments in earlier phases.

Knowledge on these aspects will help to develop protocols to manipulate the propagules at different propagation stages in order to direct growth and development, and affect yield in the last phase, the tuber production phase in the field.

This general discussion will focus on leaf number development over time in the different phases of growth, on leaf area as an indicator of quality of explants, plantlets and transplants, and on the description and detailed analysis of leaf area growth. It will also highlight how leaf area can be manipulated in the different production phases and will explain the side effects of this leaf area manipulation. The importance of the different phases of growth in the seed production system will be discussed. Finally, reflections will be made on the original objectives.

Leaf number development over time

Under constant environmental conditions the increase in leaf number during the vegetative phase of a plant is usually linear (Dale, 1982; Dale & Milthorpe, 1983; Vos & Biemond, 1992). In this research a sudden switch in the environment of the plant affected leaf appearance: planting *in vitro* plantlets to soil in the transplant production phase temporarily hastened leaf appearance. Also the increase in leaf number in the different phases of growth proved not always to be linear. These aspects are discussed in detail for the different phases below.

Normalisation phase

In the *in vitro* normalisation phase, leaf appearance was faster in the last 12 days than in the first 9 days of growth (Fig. 4, Chapter 3). This was likely to be caused by the variation among plantlets in the moment at which the first leaf appeared.

The interval between two observations of leaf number was almost similar to the expected plastochron (Vos, 1995), so that approximately one leaf was produced in the 3-day interval between successive observations. The rate of leaf appearance was, however, not consistent among individual plants. Some plants produced more than one new leaf in the 3-day interval between observations, while others showed no increase at all during an interval (or even several successive intervals in extreme cases) (Fig. 5, Chapter 3).

Higher temperature during normalisation promoted leaf appearance during the major part of the phase and leaf number was significantly higher at higher than at lower temperature at the end of the phase.

Transplant production phase

A boost in leaf appearance was observed during the first 3 days after planting to soil in the transplant production phase (Fig. 3, Chapter 3). The boost was smaller for plantlets produced at high temperature during normalisation, when planted to low transplant production temperature. Higher temperature during transplant production resulted in a larger boost in leaf number.

The boost in leaf number was most probably associated with etiolation of the internodes resulting in more leaves becoming visible. This sudden etiolation could have been triggered by the absence of ethylene in the transplant production phase, whereas ethylene is known to accumulate in sealed tubes (Roche & Cassells, 1996; Lê, 1996). It could also have been caused by the switch in light spectral quality during the

change from the normalisation to the transplant production phases. The ratio between the blue-green light range (380–520 nm) which inhibits stem elongation in potato *in vitro* (Seabrook & Douglass, 1998) and green-yellow range (520–650 nm) was lower in the transplant production than in the normalisation phase.

After the initial boost, the rate of increase was fairly constant (Fig. 6, Chapter 3). The rate of increase after the boost was not affected by temperature during the transplant production phase.

The optimal temperature for leaf appearance is between the lower and higher temperature imposed in this experiment (Borah & Milthorpe, 1962).

Tuber production phase

In the tuber production phase, the rate of leaf appearance on the main stem was higher during the first half of the phase than during the second half, for plants growing at low temperature during tuber production. For plants growing at high temperature, the rate of leaf appearance was constant up to the last week of field growth, after which leaf number did not increase anymore (Fig. 8, Chapter 3). Higher temperature during tuber production promoted leaf appearance of the main stem.

The observed effects are likely to be caused by the interaction between haulm growth and tuber growth. Tuber production was less at higher temperature (Table 5, Chapter 6), whereas stem and leaf production were higher (Table 3, Chapter 6). The shift in assimilate partitioning to tubers is associated with cessation of haulm growth (Almekinders & Struik, 1996). The interaction between haulm growth and tuber growth is also illustrated by the positive effect of high temperature on leaf area in the first part of the tuber production phase (Fig. 8, Chapter 3): this growth can only result from continued leaf production, supported by sympodial growth. For a given stem and level of growth, high temperature generally leads to potato plants with more (Marinus & Bodlaender, 1975; Menzel, 1980; Steward *et al.*, 1981; Almekinders & Struik, 1994) but smaller (Bodlaender, 1963; Steward *et al.*, 1981; Wheeler *et al.*, 1986) leaves. During tuber production, transplants produced at 26/20 °C showed a slightly faster increase in leaf number than transplants produced at 18/12 °C provided temperature during tuber production was high. Also this was probably associated with less strong partitioning of assimilates to tubers, resulting from a lower induction to tuberize (Struik & Ewing, 1995; Van Dam *et al.*, 1996) and a late cessation of haulm growth (Almekinders & Struik, 1996). Also the terminal inflorescence could have been initiated after more leaf primordia were initiated at the higher temperature during transplant production (cf. Almekinders & Struik, 1996).

Leaf area as an indicator of quality of explants, plantlets and transplants

Leaves are the sites where the most important physiological processes, including photosynthesis and transpiration, occur. It is, therefore, logical to assume that leaf area of an explant or a plantlet at the beginning of a particular phase of growth reflects the plant vigour and that early leaf area is a good predictor of the status of that explant or plantlet at the end of the same phase.

The initial leaf area (ILA) and final leaf area (FLA) at both temperatures during the normalisation phase were correlated (Table 1, Chapter 4), indicating that using a nodal cutting with a larger leaf area results in an *in vitro* plantlet with a larger leaf area at the end of the normalisation phase. The fact that Seabrook & Douglass (1994) found that removing the leaf of the explant resulted in *in vitro* plantlets with lower leaf area, supports the idea that a positive effect of a high ILA was indeed related to the leaf and not merely to the bud from which the new shoot developed. They also suggested that the subtending leaf supplied metabolites to the growing axillary bud thus enhancing the growth of the bud.

Also in the transplant production phase, a high ILA, in this case the above ground leaf area after planting an *in vitro* plant to soil, was positively associated with a high FLA at the end of the transplant production period (Table 4, Chapter 3; Fig. 4, and Table 2, Chapter 4).

In the tuber production phase, no clear correlations between above-ground leaf area of individual transplants and the leaf area at the end of the tuber production period could be established (Table 3, Chapter 4), probably because high temperature enhanced leaf senescence (cf. Struik & Ewing, 1995) and increased variation among plants. Nevertheless, high temperature treatments during transplant production resulted in higher leaf areas of transplants (Table 1, Chapter 9) or above-ground leaf area of transplants after transplanting to tuber production conditions (Table 9, Chapter 3). Thereafter it also resulted in higher leaf or stem dry weights during tuber production (Table 3, Chapter 6) or in a higher accumulated intercepted radiation (AIR) by the crop at the end of tuber production phase in the field (Table 3, Chapter 9). Positive effects on tuber production, however, could not be assessed (Table 3, Chapter 9; Table 5, Chapter 6; Table 2, Chapter 7).

Leaf area growth

Leaf area increase was better described by logistic than by exponential or expolinear curves in all phases of growth (with R^2 values of 0.97 and above). This was found at all temperature treatments and both for plant averages and most individual plants

(Chapter 3). As was reported by Goudriaan & Van Laar (1994) exponential increase in leaf area would have been expected for plants growing in optimum conditions. Logistic growth suggests that a maximum possible increment exists, and that the relative growth rate decreases linearly with the increase already achieved. This implies that restriction for leaf area increase was present in all phases of growth.

Leaf area increase was probably limited by the very low gas exchange in the sealed tubes during the *in vitro* normalisation phase. Shortage of O_2 for respiration or CO_2 for assimilation or inadequate supply of carbohydrates may reduce leaf growth (Cournac *et al.*, 1991). Accumulation of inhibitory compounds in the tubes may also limit leaf area increase of *in vitro* plantlets.

Transplants were fertilised during the transplant production phase and thus total nutrient availability will not have limited growth. Above ground competition of the plants for light was also avoided by planting very sparsely in the transplant tray (only one out of eight cells was used for planting). Insufficient rooting volume is thus suspected to have been the main limiting factor for leaf area increase. The growth restriction was less severe in the transplant production phase than in other phases as indicated by a higher percentage of individual plants that was growing exponentially or expolinearly as compared to other phases (Tables 2, 5, 8; Chapter 3). Also, during the transplant production phase the curve mid-point was reached relatively late, after about 11 days of the 14-day period.

The restriction of leaf area increase during the tuber production phase may have been associated with plant senescence and the competition between tubers and haulm for assimilates.

Detailed analysis of leaf area growth, as affected by the initial leaf area

The relationships between ILA and FLA were analysed in more detail by correlating ILA to different parameters describing logistic leaf area increase with time in all phases, by relating these curve parameters to FLA, and by establishing the relation between ILA and the relative increase in leaf area during the transplant production period.

During normalisation, high ILA led to higher fitted minimum leaf area (A), higher fitted increment (C) and higher maximum rate of increase (MI) (Table 1, Chapter 4). At higher normalisation temperature, higher ILA also resulted in higher initial relative rate of increase (B) and earlier mid-points (M). Leaf area increase of plantlets with a higher FLA was characterised by higher A, C and MI-values and by advanced growth (earlier mid-points) at higher temperature during normalisation (Table 1, Chapter 4). No association was found between the initial relative rate of

increase (B) and FLA. These results indicate that the faster rate of growth and the higher initial leaf area of the explant itself explain why higher ILAs are associated with higher FLAs at both temperatures during normalisation. At 23 °C, also a more advanced growth explains the positive association.

During transplant production, a higher ILA was associated with higher A and C values (Table 2, Chapter 4). Leaf area increase of plantlets with a higher FLA was characterised by a higher C (Table 2, Chapter 4). The positive association found between ILA and FLA during transplant production therefore will have mainly resulted from a faster leaf area increase in plants with higher ILA. The relative increase in leaf area ((FLA–ILA)/ILA) between day 1 and 13 of the transplant production period was linearly related to the inverse of the ILA at day 1 (Table 3, Chapter 2). The relation was in such a way that the relative increases in leaf area for plants with ILAs in the larger ranges were almost comparable, but that relative increases were more variable for plants with ILAs in the smaller ranges.

There were no consistent associations between ILA and FLA in the tuber production phase. The low number of plants per treatment also made it more difficult to establish a correlation between ILA and parameters describing logistic growth, but often correlations within pre-treatments were also extremely weak. Leaf area increase of plants having a high FLA at the end of tuber production was characterised by higher C, M and MI-values (Table 3, Chapter 4).

Manipulation of leaf area

In different experiments described in this thesis, the leaf area growth during a certain phase was manipulated by different factors.

Manipulation of leaf area growth during normalisation

During normalisation, the first factor that affected leaf area of *in vitro* plants was the size of the explant leaf, as originated by the natural variation expressed during the *in vitro* production of rooted cuttings. A larger explant leaf at the beginning of the normalisation phase was correlated with a larger leaf area at the end of the phase (Table 1, Chapter 4). This is consistent with reports by Seabrook & Douglass (1994) who observed that excising leaves from single-node cuttings was clearly deleterious to the growth and vigour of the resultant plants.

In Chapter 2, it was shown that the addition of daminozide (0.00133 %) to the medium resulted in *in vitro* plantlets with a larger above ground leaf area after planting. This is consistent with reports by Marinus (1985) and Sipos *et al.* (1988).

Growing plantlets at low temperature during normalisation produced plantlets with larger leaf areas after planting to soil than at high temperatures in the range 17-20-23-26 °C in Chapter 2. In Chapter 3 no significant positive effect of a lower temperature (17 versus 23 °C) on the leaf area at the end of the normalisation phase was found. The response of cultivar Gloria to the different *in vitro* treatments was very low compared to the other cultivars in Chapter 2. This relatively low response of cultivar Gloria (the only cultivar used in Chapter 3) and perhaps also variation among experiments in the proportion of leaf area left above ground at transplanting may have contributed to the discrepancy that existed between experiments described in Chapters 2 and 3.

Different levels of nitrogen added to the *in vitro* medium (in the form of NH_4NO_3) above the standard level present in the MS medium decreased the leaf area of *in vitro* plantlets at planting to soil (Chapter 2). This may be consistent with the effects of nitrogen on leaf area of *in vitro* plants described by Charles *et al.* (1992) and Zarrabeitia *et al.* (1997), who observed that reducing nitrogen content of a medium may increase leaf area.

Lowering the level of mannitol (from 3 to 1 to 0 %) in Chapter 2 in the *in vitro* medium also increased ground cover.

Manipulation of leaf area growth during transplant production

During transplant production, the first factor that positively affected leaf area growth of transplants was a high initial leaf area of the plantlet after planting. This may result both from natural variation among plantlets or from *in vitro* treatments applied. The effects of *in vitro* treatments on the leaf area of transplants were mainly through their effects on early leaf area. Adding daminozide, reducing nitrogen and reducing temperature *in vitro* increased early leaf area during transplant production. The positive association between initial leaf area (ILA) and final leaf area (FLA) in the normalisation phase also implies that using explants with a high leaf area *in vitro* resulted in transplants with a larger leaf area at the end of the transplant production period.

Transplant growth at higher temperature (26/20 versus 18/12 °C, Table 4, Chapter 3 and Table 1, Chapter 9) during transplant production resulted in a higher leaf area at the end of the transplant production period.

Higher nitrogen fertilisation (40 versus 10 mg nitrogen per plant) during transplant production (Table 1, Chapter 8) did not have any effect on leaf area of the transplant at the end of the transplant production phase, but an effect was carried-over to the tuber production phase. The lack of a direct response may be due to the short

period of transplant production (14 days) and the ample supply from the soil compared to the need in early phase of growth.

Manipulation of leaf area growth during tuber production

Initial leaf area growth during the tuber production phase was affected by the leaf area of the transplants at the end of the transplant production phase.

Higher temperature during tuber production increased leaf area (Table 7, Fig. 8, Chapter 3) during the early stages of growth in the tuber production phase but later it was reduced probably because the life span of individual potato leaves is shorter at higher temperature (Struik & Ewing, 1995).

Leaf area during tuber production could also be influenced by treatments in earlier phases. Pre-treatments during the normalisation phase had no effect on leaf area during the tuber production phase. Pre-treatments during the transplant production phase affected leaf area in the tuber production phase in some of the experiments. Higher nitrogen pre-treatments during transplant production had no effect on ground cover immediately after transplanting to the field (Fig. 1, Chapter 8). At later stages, however, higher nitrogen pre-treatment resulted in a significantly higher ground cover. Ground cover in the field was also significantly higher almost throughout the tuber production phase when transplants had been produced at higher temperature during transplant production (Fig. 1, Chapter 9). However, in the greenhouse experiment described in Chapter 3, no significant effect of transplant production temperature on leaf area was present in the tuber production phase.

Side effects of leaf area manipulation

After-effects of different treatments applied in earlier phases may result from direct effects on either leaf area or leaf number in subsequent phases or from morphological and/or physiological changes exerting their effects later.

Pre-treating plants with higher temperature in the transplant production phase resulted in transplants with high leaf area at the end of the transplant production (Table 1, Chapter 9; Table 4, Chapter 3), but also in plants with higher ground cover during the field phase. This effect persisted until full ground cover was achieved. As a result, plantlets intercepted more incoming radiation resulting in higher accumulated intercepted radiation. Higher accumulated intercepted radiation was, however, not associated with a significant increase in yield probably because effects on accumulated intercepted radiation were either too small or were associated with a small reduction in the radiation use efficiency (RUE). Experiments described in Chapters 3-7 show that

transplants grown at higher temperature during transplant production not only increased leaf area during transplant production, but also resulted in higher stem dry weights in the tuber production phase (Table 3, Chapter 6). High temperature during transplant production also significantly decreased tuber dry weight early in the tuber production phase and also later if the plants were grown at high tuber production temperature (Table 3, Chapter 7). This suggests that acclimatising transplants at low temperature could have yield advantages when partitioning of dry matter to tubers is limiting tuber yield. Thus, whether one should acclimatise transplants at high or low temperatures depends on the temperature conditions during the tuber production phase. Acclimatising transplants at low temperature could be advantageous if the tuber production temperature is high. On the other hand, acclimatising transplants at high temperature may promote leaf growth and enhance plant growth and development and could be beneficial if field conditions favour tuberization.

Pre-treating plants with different levels of nitrogen in the transplant production phase had almost no effect on the plant growth parameters measured at the end of the transplant production phase (Table 1, Chapter 8). Yet, a nitrogen effect on leaf area was carried over to the tuber production phase, perhaps through the increase in leaf primordia size or through an increase in nitrogen content of the plantlet when transplanted. Higher nitrogen pre-treatment therefore resulted in higher ground cover and higher accumulated intercepted radiation (AIR) in the tuber production phase. It also resulted in higher yield in one out of two field experiments (Table 3, Chapter 8).

Importance of different phases for seed production

The research in this thesis showed that the status of plants at the beginning of a particular phase was very crucial to the performance of those plants in that phase and that manipulation of growth was possible in all three phases studied.

The physiology of a plantlet going through the successive phases of the seed production system is highly complicated. Yet, the effects of temperature were surprisingly similar in the different phases. This is illustrated by Table 1, in which the effects of high temperature in the experiment described in Chapters 3-7 on the different plant characteristics are shown. High temperature positively affected leaf number, stem length, stem weight, internode number, internode length and haulm dry weight in the three phases of growth. But it reduced specific leaf area (SLA) and leaf/stem ratio throughout the phases. Tuber number and tuber dry weights were also reduced, but this effect was only expressed in the tuber production phase.

Although the sequence of events in the different phases of growth in the four-phase seed production system is essential, conditions in the last phase, the tuber

Table 1. Summary of the possible effects of high temperature on various plant growth characteristics of *in vitro* propagated plantlets grown at two different temperatures in the three phases of growth. Partly based on unpublished data from research described in Chapters 3, 6 and 7.

	Growth phase		
	Normalisation	**Transplant production**	**Tuber production**
Leaf			
Total leaf area	±	+	±
Individual leaf size	–	±	–
Leaf number	+	±	+
SLA	–	–	–
Senescence	+	±	+
Stem			
Total length	+	+	+
Total weight	+	+	+
Internode number	+	+	+
Indiv. node length	+	+	+
Leaf/Stem ratio	–	–	–
Haulm			
Dry weight	+	+	+
Tuber			
Time	×	×	✓
Number	×	×	–
Dry weight	×	×	–

+ Positive effect, – negative effect, ± indifferent, ✓ tuber present, × not present

production (field) phase, were overriding. For instance, seasonal differences had a larger impact on ground cover development with time (Fig. 1; Chapters 8 and 9) and final yield (Tables 3, Chapters 8 and 9) than pre-treatment with nitrogen or temperature during transplant production. Similarly, temperature during tuber production had a larger influence on total dry weight and tuber yield than temperature pre-treatments in the normalisation or transplant production phases (Table 5, Chapter 6). This is likely to result from the following:

a) The tuber production phase is the last and the longest of the phases.
b) Even though earlier phases may have an impact on tuber induction, tuber initiation and tuber bulking take place in the tuber production phase and many processes associated with these physiological events are strongly influenced by momentous factors. Thus conditions during this phase are crucial for tuber fresh yield and tuber numbers.

Reflections on original objectives

The research has greatly contributed to the understanding of the important morphological and physiological changes that take place at the individual plant and crop level in the four-phase seed production system used in this study. Leaf area was identified as an important plant parameter reflecting plant growth in the normalisation and transplant production phases of the seed production system. Explants and plantlets with larger initial leaf area performed better than those with smaller initial leaf area. When trying to increase vigour of propagules, one may focus on protocols that would enhance leaf area growth, without increasing the trauma during the transfer from one phase to the next.

The morphological and physiological changes could be influenced by manipulating *in vitro* plantlets in the different production phases. Manipulating plantlets improved performance of the plant in the different phases of growth and in some cases increased final yield at the end of the growth period. Yield was improved directly by promoting leaf area of the plantlets or indirectly by changing assimilate partitioning between the above ground parts of the plant and tubers. This promoted assimilate partitioning to the haulm during the early stages of field growth and may prevent early tuber initiation or strong partitioning of dry matter to tubers. In other cases, e.g. at high temperature during tuber production, dry matter partitioning to the tubers may increase yield. The pattern of dry matter distribution could be influenced by the temperature conditions during transplant production.

Manipulating plantlets at the different phases of the seed production system has also made it possible to assess the relative importance of conditions or treatments

during the last three phases of the seed production system for final yield. Although previous phase temperatures had some influence on growth of the plants and final yield, conditions during tuber production were overriding.

The importance of initial leaf area and the overriding impact of conditions during the tuber production phase together suggest that strategies to optimise the production and use of propagules and transplants and reduce the costs of production should be focused on achieving leafy starting material, reducing the stress during changes in environment and optimising conditions during tuber production. Production of transplants should be adjusted to the expected effects of growth conditions in the tuber production phase.

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Summary

The conventional way of propagating potato involves the repeated multiplication of seed tubers. Seed tubers currently occupy about 12% of the potato acreage. Major disadvantages of this seed production system are the low rate of multiplication and the susceptibility of the crop to many diseases that can be transmitted from one generation to the next.

Micropropagation techniques have widely been introduced in potato seed production systems during the past few decades to solve problems associated with the conventional seed production system. These techniques received wide acceptance in seed production systems because they can produce large quantities of disease-free and genetically uniform plantlets *in vitro* for seed tuber production purposes within a short period of time and all year round.

The fastest propagation system for the production of seed tubers constitutes of four phases: *in vitro* 'multiplication' phase in which nodal cuttings are produced, *in vitro* 'normalisation' phase in which rooted *in vitro* plantlets are produced from single node cuttings, 'transplant production' or 'acclimatisation' phase in which plantlets are raised to transplants in glasshouses and 'tuber production' phase in which large-sized tubers are produced in the field. Plants have a complicated physiology throughout these phases because they are exposed to different conditions that may affect growth immediately in the current phase or in later phases. The plants may also experience sudden changes in growth conditions as a result of planting to the glasshouse or transplanting to the field.

Although many authors suggest that the status of a plant at the end of the normalisation or transplant production phase can influence further performance, there is no insight in the way treatments in earlier phases affect final yield in the last phase.

The main objectives of this research project are to understand and analyse:

1. The important morphological and physiological changes that take place at the plant and crop level in the normalisation, transplant production and tuber production phases of the four-phase seed production system;
2. The way changes are affected by different treatments during a phase, and
3. The way changes in a certain phase are affected by different treatments in earlier phases.

Leaf area as an indicator of quality of explants, plantlets and transplants

A series of experiments with cultivars Gloria, Spunta and/or Elkana is described in Chapter 2, in which the importance of leaf area of *in vitro* propagated plantlets after

planting for further leaf area growth during acclimatisation and the after-effects of *in vitro* treatments on growth, were examined. *In vitro* plantlets were pre-treated with different levels of alar, nitrogen, or mannitol or different temperatures during the normalisation phase and were then grown in small pots with soil in a glasshouse in the transplant production phase for 13 days. Leaf area or ground cover was recorded one day after planting (1 DAP; early leaf area) and at the end of the acclimatisation phase (13 DAP; late leaf area).

Regression analysis results indicated that leaf area at the onset of the transplant production phase was found to be an important parameter for achieving a high leaf area at the end of the same phase, accounting for about 50-60% of the variance in late leaf area. The relative increase (RI) in leaf area during acclimatisation, i.e. the increase divided by the early leaf area, was linearly related to the inverse of the early leaf area. This indicates that the relative increases for plantlets having larger early leaf areas were more or less comparable, but that of those plantlets having smaller early leaf areas were variable.

In vitro treatments mainly affected leaf area of transplants through their effects on early leaf area. Low nitrogen, low temperature and adding alar increased leaf area, while low mannitol increased ground cover.

Leaf area and leaf number development

Plant vigour and growth during the three phases of growth after the *in vitro* multiplication can be assessed in terms of leaf appearance, leaf expansion and dry matter accumulation in leaves and need to be quantitatively described in order to understand the growth pattern of plants through the different phases. To study this in detail, an experiment was designed to assess leaf area and leaf number development (Chapters 3-5) of *in vitro* propagated plantlets of cultivar Gloria over three phases of growth in relation to time. Temperature as the main environmental factor influencing growth and development, was varied in all phases. During the normalisation phase temperature was 17 or 23 °C, during the later two phases 18 (day)/12 (night) °C or 26/20 °C.

Results of the experiment indicated that leaf area (LA) increase of *in vitro* derived plants was better described by logistic than by exponential or expolinear curves. This was found both for plant averages and for most individual plants and for all phases of growth and temperature pre-treatments (Chapter 3). Logistic curves imply that there were restrictions of LA increase in all three phases of growth. The restriction was less severe in the relatively short (14-day) transplant production phase. This was shown by the higher percentages of individual plants that followed exponential or

expolinear growth in the transplant production phase compared to other phases, and a relatively late mid-point of the logistic curve.

A boost in LA and in leaf appearance occurred during the first three days after planting the *in vitro* plantlets to soil in the transplant production phase. A shock in leaf area increase, but not in leaf appearance, was observed after transplanting transplants to the tuber production phase.

Temperature effects on LA increase were not consistent in the three phases: higher temperature did not significantly increase LA during normalisation, increased LA during transplant production and first increased LA but later reduced it during tuber production. After-effects of different temperatures during normalisation on LA and leaf number were only found during transplant production and not any more during tuber production; after-effects of temperature during transplant production were found during tuber production.

Effects of leaf area of individual plants on parameters describing individual logistic leaf area increase were assessed in Chapter 4. Explants with larger leaf areas and transplants with higher above ground leaf areas after planting gave rise to plants with larger leaf areas at the end of the normalisation and transplant production phases, respectively. In the normalisation phase, a higher initial leaf area was associated with a higher fitted minimum leaf area and a higher fitted total increment. At 23 °C, a higher initial leaf area was also associated with a higher initial relative rate of increase and an earlier mid-point, indicating more advanced growth. In the transplant production, a higher initial leaf area resulted in a higher total increment. This last parameter contributed to a high final leaf area in all phases of growth.

Logistic growth curve parameters were related to each other using matrices of linear correlations in Chapter 5. Results indicated that the fitted minimum leaf area (A) and the initial relative rate of increase (B) were significantly (positively) correlated in all phases of growth in all treatments. Higher A and B values were significantly correlated with a higher maximum rate of increase.

Effects on yield and tuber number

Having studied how leaves of *in vitro* produced plants of cultivar Gloria develop in the three phases of growth, dry matter production, tuber yield and tuber numbers were analysed. In Chapters 6 and 7 the main and after-effects of temperature on dry matter production and partitioning and on tuber number, size, tuber fresh yield, tuber dry matter concentration and harvest index (HI) of *in vitro* produced plantlets were assessed.

Results indicated that effects of different temperatures on *in vitro* propagated

potato plants were consistent with the effects commonly found for tuber-derived plants in the same temperature range and were also more or less consistent throughout the phases. Higher temperature increased leaf and stem dry weights in all phases, but reduced tuber dry weights, leaf/stem ratio and specific leaf area (SLA) in the tuber production phase. Total plant dry weight was higher at high than at low temperature at the beginning of the tuber production phase but this was reversed at later stages of the same phase.

Transition of plants from one phase to another, especially from *in vitro* to *in vivo* conditions, greatly increased leaf growth and to a small extent stem growth. This was manifested by the four-fold increase in leaf dry weight at 7 DAP in the transplant production phase when compared to the leaf dry weight of similar plants that were left to grow for one more week in the normalisation phase.

After-effects of previous phase temperatures occurred for some plant growth characteristics in subsequent phases. Growing *in vitro* plantlets at lower temperature during normalisation significantly increased leaf and total plant dry weight early in the transplant production and tuber production phases. Growing transplants at low temperature during transplant production significantly reduced stem weight in the tuber production phase and increased tuber weight when tuber production took place at high temperature. This suggests that acclimatising transplants at low temperature could have yield advantages in conditions where a strong partitioning of dry matter to the haulm is limiting tuber yield.

Chapter 7 shows that stolons were initiated at the end of the transplant production phase (14 DAP), while tubers were produced soon after transplanting plants to the tuber production phase (7 DAT).

After-effects of the normalisation temperature were not significant in the transplant production phase but were carried over to the tuber production phase and most of them were manifested at the end of the phase (42 DAT). Plantlets pre-cultured at low normalisation temperature resulted in higher tuber fresh yield, higher tuber sizes and higher harvest index (HI) at both temperatures at the end of the tuber production phase.

After-effects of the transplant production temperature were, in most cases, present during the early stages of the tuber production phase (7-14 DAT). A lower transplant production temperature resulted in more tubers and higher tuber fresh yields at 14 DAT and finally resulted in higher dry matter concentration and higher HI for plants growing at high tuber production temperature.

Although previous phase temperatures influenced growth and final yield of the plants, conditions during tuber production were more important. Low temperature during tuber production resulted in higher tuber fresh yield, average tuber weight,

tuber dry matter concentration and HI. Pre-culturing plants at low temperature during normalisation and transplanting them to low tuber production temperature could have yield advantages.

Manipulating plantlets to affect growth and yield

In vitro plantlets of the very-early cultivar Gloria and mid-early cultivar Spunta were pre-treated with different levels of nitrogen or different temperatures during the transplant production phase (of 14 days). The effects of nitrogen and temperature on the transplants and on their performance after transplanting to the field were assessed. The length of the field period was 12-13 weeks.

Results of the nitrogen experiment (Chapter 8) proved that Spunta had higher ground cover (GC), accumulated intercepted radiation (AIR), total dry matter and tuber fresh and dry weights but lower radiation use efficiency (RUE) and harvest index (HI) than Gloria at final harvest. Nitrogen pre-treatment had no clear effect on plant growth at the end of the transplant production phase or during early growth in the field. Later in the field phase, higher nitrogen pre-treatment resulted in plants with higher GC in both cultivars, which in turn led to higher interception of solar radiation, with even simultaneous increase in RUE in one of the experiments. These effects resulted in a higher dry matter production and also in higher tuber yields at the end of the growing season in one of the experiments. Thus, optimum nitrogen pre-treatment can increase yield of early potato cultivars by affecting the seasonal light interception of the plants.

Results of the temperature experiment (Chapter 9) indicated that the effects of temperature pre-treatment were evident at the end of the transplant production phase but partly faded out in the field phase. This suggests that a longer period of pre-treatment might be necessary to achieve a persistent effect. High temperature pre-treatment promoted leaf area in both cultivars at the end of the transplant production phase and resulted in plants with higher GC at the end of the growing season. As a result, plants pre-treated at high temperature were able to intercept more solar radiation in the field. This, however, did not lead to higher tuber yields because effects on AIR were either too small or were associated with delayed or reduced partitioning of dry matter to tubers.

Data for these two cases studied showed that the status of the plants at the end of the transplant production phase is important for further field performance.

The general discussion (Chapter 10) focuses on leaf number development over time in the different phases of growth, on leaf area as an indicator of quality of

explants, plantlets and transplants and on the detailed analysis of leaf area growth. It also highlights how leaf area can be manipulated in different production phases and explains the side effects of leaf area manipulation. Also the importance of the different phases of growth for the seed production system is discussed. Finally, reflections are made on the original objectives.

The study described in this thesis has contributed to the understanding of the morphological and physiological changes that take place at the plant and crop level in the four-phase seed production system and to our insight in how they are affected by different factors. The information gained in this study will help to manipulate the propagules at different propagation stages to optimise seed tuber production of potato.

Samenvatting

Samenvatting

Aardappel wordt normaliter vegetatief vermeerderd via de productie van verschillende opeenvolgende generaties pootgoedknollen. Het areaal pootgoed neemt wereldwijd ongeveer 12% van de totale aardappelteelt in beslag. Het conventionele pootgoed-productiesysteem heeft grote nadelen. De vermeerderingsfactor is laag. Bovendien is het gewas zeer gevoelig voor ziekten en plagen die met het pootgoed van de ene generatie naar de andere kunnen overgaan.

In de laatste decennia zijn er echter snelle vermeerderingstechnieken ontwikkeld en geïntroduceerd die deze nadelen niet of in mindere mate hebben. Met behulp van deze technieken kunnen grote aantallen ziektevrije en genetisch uniforme planten worden geproduceerd in korte tijd. Dat gebeurt dan onder *in vitro* (dat wil zeggen aseptische) omstandigheden en de productie kan het gehele jaar plaatsvinden. In het snelste vermeerderingssysteem wordt pootgoed in vier fasen geproduceerd: in de eerste fase vindt *in vitro* vermeerdering plaats (de vermeerderingsfase); in de tweede fase worden *in vitro* stekjes opgekweekt tot bewortelde plantjes (de normalisatiefase); in de derde fase worden de plantjes in kassen tot een zekere grootte opgekweekt en afgehard (de transplantproductiefase), en tenslotte worden deze zogenaamde transplants in het veld uitgeplant om grote knollen te vormen (de knol-productiefase). Plantjes worden derhalve aan uiteenlopende omstandigheden blootgesteld en in elke fase wordt hun fysiologie beïnvloed door de groeiomstandigheden. Sommige van deze effecten kunnen ook nawerken in een volgende fase. Bovendien worden de plantjes blootgesteld aan plotselinge overgangen in omstandigheden, als gevolg van het planten (van *in vitro* naar *in vivo*) of het overplanten (van kas naar veld).

Hoewel algemeen gesuggereerd wordt dat de hoedanigheid van een plant aan het eind van de normalisatiefase of de transplantproductiefase de verdere groei kan beïnvloeden, bestaat er geen inzicht in de wijze waarop behandelingen tijdens vroege fasen de uiteindelijke opbrengst in de laatste fase beïnvloeden.

Het onderzoeksproject dat in dit proefschrift wordt beschreven, had tot doel de veranderingen in morfologische en fysiologische kenmerken te beschrijven en te begrijpen, zoals die optreden op het niveau van de individuele plant en het gewas in de laatste drie fasen van dit vier-fasensysteem en te analyseren hoe deze veranderingen door verschillende factoren (kunnen) worden beïnvloed. Meer begrip van de morfologie en de fysiologie is nodig bij het manipuleren van het uitgangsmateriaal in de verschillende stadia teneinde uiteindelijk de knolopbrengst en het knolaantal in de laatste fase, de knolproductiefase, te sturen.

Bladoppervlakte als kwaliteitskenmerk

In Hoofdstuk 2 wordt een reeks proeven met de rassen Gloria, Spunta en/of Elkana beschreven die werd uitgevoerd om te onderzoeken wat het belang was van bladoppervlakte van *in vitro* plantjes voor hun verdere groei gedurende de transplantproductiefase. Ook werd nagegaan in hoeverre er sprake was van na-effecten van behandelingen tijdens de normalisatiefase op de groei in de transplantproductiefase. *In vitro* plantjes werden voorbehandeld met verschillende concentraties alar, stikstof of mannitol of werden blootgesteld aan verschillende temperaturen gedurende de normalisatiefase. Vervolgens werden ze in kleine potjes uitgeplant en voor de transplantproductiefase gedurende 13 dagen in een kas opgekweekt. Het bladoppervlak of de bodembedekking werd waargenomen op dag 1 na planten en aan het eind van de transplantproductiefase (13 dagen na planten).

Met behulp van regressie-analyse werd aangetoond dat het bladoppervlakte van een plant aan het begin van de transplantproductiefase van groot belang was voor het bereiken van een grote bladoppervlakte aan het eind van die fase. Van de variantie in bladoppervlakte van individuele planten op dag 13 na planten werd 50-60% verklaard door de bladoppervlakte op dag 1 na planten. De relatieve toename in bladoppervlakte gedurende de transplantproductie (de toename per eenheid aanwezige bladoppervlakte), bleek lineair gecorreleerd met de inverse van de bladoppervlakte op dag 1 na planten. Dit geeft aan dat voor planten met grote bladoppervlaktes op dag 1 na planten een toename in bladoppervlakte nauwelijks invloed had op de relatieve toename van de bladoppervlakte tussen dag 1 en dag 13 na planten, terwijl kleine individuele plantjes wel degelijk variatie vertoonden in de relatieve toename in bladoppervlakte tussen dag 1 en dag 13 na planten.

De behandelingen tijdens de *in vitro* fase hadden vooral een effect op de bladoppervlakte van de transplant aan het einde van de transplantproductiefase via hun effect op de bladoppervlakte op dag 1 na planten. Een lage concentratie N in het medium, een lage temperatuur, toevoeging van alar en een lage concentratie mannitol verhoogden de bladoppervlakte of de bodembedekking.

Ontwikkeling van bladoppervlakte en bladaantal

Groeivermogen en actuele groei (gedurende de drie fasen van groei na de *in vitro* vermeerdering) kunnen worden gekarakteriseerd op basis van bladverschijning, bladstrekking en drogestofophoping in de bladfractie. Deze kenmerken dienen kwantitatief te worden beschreven om het groeipatroon van de planten over de verschillende fasen goed te kunnen doorgronden. Om dit in detail te kunnen

bestuderen werd een proef uitgevoerd met het ras Gloria waarin de ontwikkeling van bladoppervlakte en bladaantal in de tijd werd gemeten gedurende de normalisatiefase, de transplantproductiefase en de knolproductiefase (Hoofdstukken 3-5). De temperatuur (als belangrijkste omgevingsfactor voor groei en ontwikkeling) werd in elk van deze fasen gevarieerd. Gedurende de normalisatiefase was de temperatuur 17 of 23 °C, in de latere fasen was de temperatuur 18 (dag)/12 (nacht) °C of 26/20°C.

Uit de resultaten van dit experiment bleek dat de toename in bladoppervlakte van uit *in vitro* vermeerdering verkregen plantjes voor elk van de fasen het best kon worden beschreven met een logistische functie. Een dergelijke fit leverde in elk geval betere resultaten dan fits door exponentiële of expolineaire curves. Dit gold zowel voor de individuele planten als voor het gemiddelde van alle planten en voor alle acht temperatuurbehandelingen (Hoofdstuk 3). De logistische groei geeft aan dat er in elk van de drie fasen sprake was van groeibelemmerende factoren. Deze belemmering was het minst duidelijk voor de relatief kortstondige transplantproductiefase van 14 dagen, zoals bleek uit het relatief hogere percentage van de individuele planten die in deze fase exponentiële of expolineaire groei vertoonden. Bovendien was het buigpunt van de logistische groeicurve relatief laat.

Gedurende de eerste drie dagen na het planten van de *in vitro* plantjes (dus aan het begin van de transplantproductiefase) bleek er een duidelijke versnelling in de toename van de bladoppervlakte en de bladverschijning op te treden. Omgekeerd was er sprake van een duidelijke groeiremming bij het overplanten van de transplant-productiefase naar de knolproductiefase. Die laatste remming trad overigens alleen op voor de toename in bladoppervlakte en niet voor die in bladaantal.

De effecten van temperatuur waren niet consistent over de drie fasen van groei: een hogere temperatuur gaf geen significante toename in bladoppervlakte gedurende de normalisatiefase, resulteerde in een grotere bladoppervlakte gedurende de trans-plantproductiefase en had aanvankelijk een positief maar later een negatief effect op de bladoppervlakte gedurende de knolproductiefase. Na-effecten van de temperatuur-behandeling gedurende de normalisatiefase werden slechts waargenomen gedurende de transplantproductiefase en niet meer gedurende de knolproductiefase. Na-effecten van temperatuurbehandelingen tijdens de transplantproductiefase werden wel gevonden tijdens de knolproductiefase.

Effecten van de bladoppervlakte van een individuele plant aan het begin van een groeifase op de groeiparameters die de toename in individuele bladoppervlakte tijdens die fase kwantificeren, worden beschreven in Hoofdstuk 4. Explantaten en plantjes met een grotere (bovengrondse) bladoppervlakte na het planten resulteerden in planten met een grotere bladoppervlakte aan het eind van respectievelijk de normalisatiefase en de transplantproductiefase. Een grotere bladoppervlakte van het

explantaat in de normalisatiefase resulteerde in hogere waardes voor de geschatte minimale bladoppervlakte en de geschatte totale toename in bladoppervlakte. Bij de hoge temperatuur tijdens de normalisatiefase (23 °C) waren de plantjes ook verder in groei, zoals blijkt uit een eerder moment waarop de logistische curve zijn buigpunt bereikt en was de initiële relatieve bladgroeisnelheid hoger. Een hogere initiële bladoppervlakte in de transplantproductiefase resulteerde in een hogere geschatte toename in bladoppervlakte. Deze parameter droeg bij aan een hoge uiteindelijke bladoppervlakte aan het eind van alle fasen.

In Hoofdstuk 5 werden lineaire correlatiecoëfficiënten berekend voor de verschillende parameters van een logistische groeicurve onderling. Uit de resultaten bleek dat de gefitte minimum bladoppervlakte (A) en de initiële relatieve groeisnelheid (B) in alle fasen en in alle behandelingen significant en positief gecorreleerd waren. Hogere waardes voor A en B waren significant gecorreleerd met een hogere maximale groeisnelheid.

Effecten op opbrengst en knolaantal

Na het in detail analyseren van de groei van de bladeren in de opeenvolgende groeifasen, werden de drogestofproductie, de knolopbrengst en het knolaantal geanalyseerd. De Hoofdstukken 6 en 7 beschrijven een proef met het ras Gloria waarin de hoofd- en na-effecten van temperatuurbehandelingen op drogestofproductie, drogestofverdeling, knolaantal, knolgrootte, knolversgewicht, drogestofgehalte van de knollen en oogstindex werden vastgesteld.

De waargenomen effecten van de temperatuurbehandelingen op het gedrag van *in vitro* vermeerderde planten bleken niet af te wijken van wat gebruikelijk wordt waargenomen bij planten of gewassen uit pootgoed in hetzelfde temperatuurtraject. Bovendien waren de waargenomen effecten min of meer consistent over alle drie de groeiperioden. Een hogere temperatuur leidde tot een hoger drooggewicht van blad en stengel, maar tot een lager drooggewicht aan knollen, een lagere blad:stengel verhouding en een lagere specifieke bladoppervlakte gedurende de knolproductiefase. Aan het begin van de knolproductiefase was het totale drooggewicht per plant hoger bij een hoge dan bij een lage temperatuur, maar dit effect was tegengesteld aan het einde van dezelfde fase.

De overgang van de ene fase naar de andere (en dan vooral de overgang van *in vitro* naar *in vivo* omstandigheden) had een groot positief effect op de bladgroei, en een kleiner op de stengelgroei. Dit bleek uit de viervoudige toename in het bladdrooggewicht 7 dagen na het planten in de transplantproductiefase ten opzichte van de toename van vergelijkbare planten die een week langer onder normalisatie-

omstandigheden werden voortgekweekt.

Voor sommige plantkenmerken werden na-effecten van temperatuurbehandelingen gedurende de vorige fase waargenomen. Indien *in vitro* plantjes waren opgekweekt bij een lagere temperatuur gedurende de normalisatiefase, dan hadden ze een significant hoger bladgewicht en totaalgewicht dan bij een opkweek bij hogere temperatuur. Dit werd waargenomen aan het begin van de transplantproductiefase en aan het begin van de knolproductiefase. Indien transplants bij een lagere temperatuur werden opgekweekt dan leidde dat tot lagere stengelgewichten in de knolproductiefase, en tot een significant hoger knoldrooggewicht wanneer knolproductie plaatsvond onder hoge temperatuur. Dit suggereert dat acclimatiseren van transplants bij een lage temperatuur tot een hogere opbrengst kan leiden onder niet-inducerende omstandigheden waarbij een al te eenzijdig verdeling van de droge stof in de richting van het loof leidt tot geringe knolopbrengsten.

Hoofdstuk 7 maakt duidelijk dat de eerste stolonen verschenen aan het eind van de transplantproductiefase (14 dagen na planten) en de eerste knollen kort na het overplanten tijdens de knolproductiefase (7 dagen na overplanten).

De temperatuurbehandelingen tijdens de normalisatie leidden niet tot significante verschillen in stoloon- of knolparameters tijdens de transplantproductiefase, maar bleken wel een na-effect te hebben tijdens de knolproductiefase, met name aan het eind van deze periode (42 dagen na overplanten). Plantjes die tijdens de normalisatie aan een lage temperatuur waren blootgesteld gaven uiteindelijk een hogere knolopbrengst, een grotere maat knollen en een hogere oogstindex bij beide temperaturen tijdens de knolproductiefase.

Na-effecten van de temperatuurbehandelingen tijdens de transplantproductiefase waren in de meeste gevallen juist aanwezig aan het begin van de knolproductiefase (7-14 dagen na overplanten). Een lagere transplantproductietemperatuur leidde tot meer knollen en hogere knolversopbrengsten op 14 dagen na overplanten en resulteerde uiteindelijk in een hoger drogestofgehalte en een hogere oogstindex, wanneer de temperatuur tijdens de knolproductiefase hoog was.

Hoewel er sprake was van verschillende na-effecten van omstandigheden tijdens de normalisatie en de transplantproductiefase waren de condities gedurende de knolproductie toch overheersend. Een lagere temperatuur tijdens die fase resulteerde in een hogere verse knolopbrengst, een hoger gemiddeld knolgewicht, een hoger drogestofgehalte van de knollen en een hogere oogstindex. Een voorbehandeling met lage temperaturen tijdens de normalisatie gevolgd door de teelt onder lage knolproductietemperaturen kan evenwel leiden tot hogere opbrengsten.

Het manipuleren van plantjes ter verbetering van groei en opbrengst

In vitro plantjes van het zeer vroege ras Gloria en het middelvroege ras Spunta werden voorbehandeld met verschillende stikstofniveaus of temperaturen gedurende de transplantproductiefase van 14 dagen. Vervolgens werden de effecten op de transplants en op het gewas na overplanten in het veld geanalyseerd. De lengte van de veldperiode was 12-13 weken.

Bij voorbehandelingen met verschillende stikstofniveaus (Hoofdstuk 8) bleek allereerst dat Spunta bij de eindoogst een betere grondbedekking, een grotere totale hoeveelheid onderschepte straling, een hogere knoldrogestofopbrengst, een hogere knolversopbrengst, maar een lagere lichtbenuttingsefficiëntie en een lagere oogstindex vertoonde dan Gloria. De voorbehandeling met stikstof had geen duidelijk effect op de omvang van de planten aan het eind van de transplantproductiefase of gedurende de eerste groei in het veld. Later in het veldseizoen echter, bleek een hogere stikstofgift tijdens de transplantproductie bij beide rassen te leiden tot een gewas met een betere grondbedekking, hetgeen resulteerde in meer onderschepte straling, met zelfs een gelijktijdige toename van de lichtbenuttingsefficiëntie in één van de proeven. Deze effecten resulteerden in een hogere totale drogestofproductie en in hogere knolopbrengsten aan het eind van het groeiseizoen, althans in één van de proeven. Een juiste stikstofdosering tijdens de transplantproductiefase kan derhalve leiden tot een betere lichtonderschepping en derhalve een hogere opbrengst aan het eind van de veldperiode.

Ook in de proeven met verschillende temperaturen tijdens de transplantproductiefase (Hoofdstuk 9) werden verschillen gevonden. Aan het eind van de transplantproductiefase waren verschillen aanwezig, maar deze vervaagden tijdens de veldperiode. Daaruit zou kunnen worden geconcludeerd dat voor een groot effect de behandelperiode langer zou moeten duren. Een hogere temperatuur tijdens de transplantproductiefase leidde in beide rassen tot een grotere bladoppervlakte aan het eind van de transplantproductiefase en tot een betere grondbedekking aan het eind van het (korte) groeiseizoen. De planten van onder hogere temperatuur gekweekte transplants onderschepten dan ook meer straling. Dit effect op de hoeveelheid onderschepte straling resulteerde echter niet in een significant hogere opbrengst, waarschijnlijk omdat de effecten te klein waren of gekoppeld waren aan een uitgestelde of verminderde toedeling van drogestof naar de knollen.

Uit deze proeven onder veldomstandigheden blijkt in elk geval dat de status van de plantjes aan het eind van de transplantproductiefase wel degelijk van belang kan zijn voor hun prestaties in het veld.

De algemene discussie (Hoofdstuk 10) beschrijft de ontwikkeling van het

bladaantal in de tijd gedurende de verschillende groeifasen en het belang van de bladoppervlakte als kwaliteitskenmerk voor explantaten, plantjes en transplants. Vervolgens wordt de toename van de bladoppervlakte in elk van de fasen in detail bediscussieerd. Tevens wordt besproken hoe de bladoppervlakte kan worden gemanipuleerd in de verschillende fasen en wat de bij-effecten van deze manipulatie zijn. Tenslotte worden de oorspronkelijke doelstellingen aan een nadere beschouwing onderworpen.

De studie die in dit proefschrift wordt beschreven heeft bijgedragen aan het begrijpen van de morfologische en fysiologische veranderingen die plaatsvinden op het niveau van de plant en het gewas in het vierfasen-pootgoedproductiesysteem. Daarnaast heeft de studie geleid tot meer inzicht in de effecten van verschillende factoren daarop. Dit inzicht kan leiden tot het verstandig manipuleren van uitgangsmateriaal in de verschillende fasen van vermeerdering teneinde de aardappelpootgoedproductie te optimaliseren.

CURRICULUM VITAE

Tadesse Mehari was born on the 21st of July 1954 in Asmara, Eritrea. After obtaining his BSc degree in Biology from the University of Asmara, Asmara, Eritrea, he started teaching at different senior high schools in Eritrea during which time he also served as a coordinator of the Biology Section of the *"Pedagogical Animation Programme"* launched by the Ministry of Education in association with the World Bank. After obtaining his MSc degree in Microbiology in 1988 from Addis Ababa University, Addis Ababa, Ethiopia, he joined the Biology Department of the University of Asmara. The College of Agriculture and Aquatic Sciences (CAAS) was founded within the Biology Department of the University of Asmara in 1989 and he was one of the staff members who worked for the realisation of the college and also served as head of one of its departments, the Department of Plant Sciences and later as Dean of the College. He joined Wageningen University and Research Centre (WUR) in November 1996 to pursue a full time PhD study as part of the linkage programme started between WUR and the University of Asmara.