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Development of leaf area and leaf number of micropropagated potato plants

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Abstract

Aboveground leaf area and leaf number development of *in vitro* produced potato plantlets was studied over three growth phases. *In vitro* plantlets were produced at 17 or 23°C (normalisation phase, 3 weeks), planted in soil at 18/12 or 26/20°C (transplant production phase, 2 weeks), and later transplanted at 18/12 or 26/20°C (tuber production phase, 6 weeks).

Boosts in leaf area increase and leaf appearance occurred in the first days after planting to soil. A shock in leaf area increase occurred after the later transplanting. Both for plant averages and most individual plants, leaf area increase in all growth phases was best described by logistic curves, indicating growth limitations occurred in all phases. These limitations were least severe during the relatively short transplant production phase. 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. Higher temperatures increased leaf number in all phases. After-effects of normalisation temperature occurred during transplant production but no longer during tuber production. After-effects of transplant production temperature occurred during tuber production. After-effects were direct (affecting plants at the beginning of the next phase) or appeared later.

Keywords: in vitro plantlet, leaf area, leaf number, leaf appearance, leaf expansion, logistic growth, *Solanum tuberosum* L., temperature, 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 in small pots with soil *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.* (2000) 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. 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 paper 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 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} thi-

amine HCl. Viable nodes were cut from plantlets (discarding tops) and cultured (one per tube) in sterilised 25 × 150 mm 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 trays with small cells (4.0 × 5.5 × 6.0 cm, w × l × d) filled with potting soil taking proper care to prevent damage of the delicate roots. The trays were placed in 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 a flux 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 nutrient solution (Lommen & Struik, 1992) three times a week. To avoid competition, only 8 plantlets were planted per tray of 32 cells (75 plants m^{-2}). Plantlets were grown 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-liter pots filled with potting soil to two glasshouses at a density of 16.0 plants m^{-2} to simulate the tuber production phase. To prevent damage of the roots the whole soil in the previous pots was transferred to the new 5-litre pots. 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 to study their growth and development during the early stages of the 'tuber production' phase. The tuber production phase of the experiment 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. The 128 plants reported on in the present paper were used for non-destructive leaf measurements and were part of a larger experiment with the same treatments, in total consisting of 992 plants, to allow for destructive measurements.

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 were taken every week. Green leaf area (including area of the explant leaf 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' and 'final leaf area', 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 to identify general patterns of growth and shocks or boosts upon transfer. Differences in initial and final leaf area were determined by ANOVA using Genstat 5 release 3.22 (1995). 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 describing the logistic increase between temperatures and pretreatments were determined using t-tests. Figure 1 illustrates the logistic type of growth 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. Initial and final leaf areas of plants following logistic growth and parameters describing this logistic growth were analysed by ANOVA. Differences between treatments were analysed by LSD tests at $P < 0.05$.

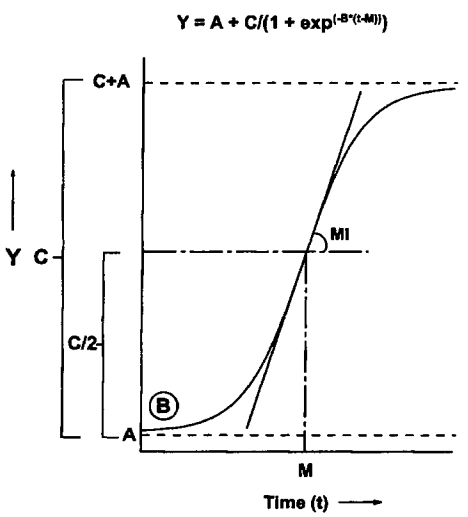

Figure 1. Logistic growth curve and the different parameters describing the curve: fitted minimum leaf area (A), fitted increment (C), and fitted midpoint (M). MI is the maximum rate of increase at M and is calculated as B×C/4. B represents the initial relative rate of increase.

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 three production phases are presented in Figure 2. Between phases, the planting of *in vitro* plantlets to soil and the later transplanting resulted in reductions in aboveground leaf area (Figure 2A, only showing the extreme treatments) and in number of aboveground leaves (Figure 2B), because only about half of the stem was left above the soil. 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, was observed in the increase of leaf area, but not in leaf number.

Beyond the phases of the shocks and boosts after (trans)planting and before strong interplant competition (LAI ≈ 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 (Figure 2). This will be treated further for the phases separately, with the emphasis on leaf area.

In vitro normalisation phase

When different types of curves were fitted through the average leaf area values of all plantlets, leaf area during *in vitro* growth increased logistically with time (Figure 3). At both temperatures, logistic fits described the increase in leaf area better than exponential or expolinear fits. Plantlets did not differ significantly in initial explant leaf area or in final leaf area between the two temperatures (Table 1), but the in-

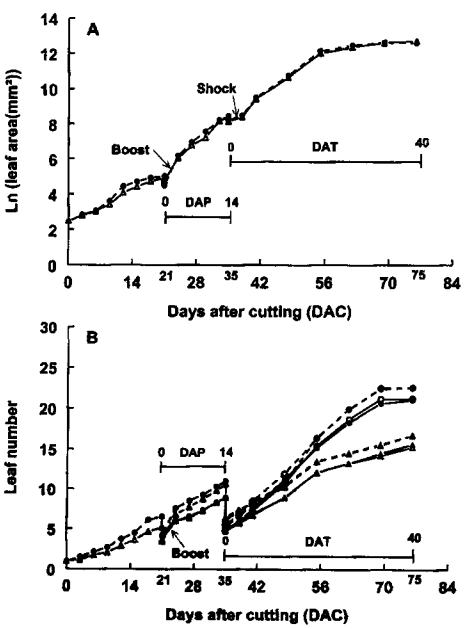

Figure 2. Aboveground leaf area (A) and main stem leaf number (B) development at different temperature treatments through the normalisation, transplant production and tuber production phases. The markers follow the pattern listed below in all graphs (except in those where the leaf area or leaf number 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).

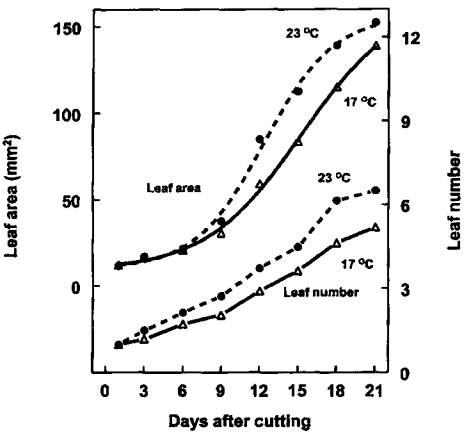

Figure 3. Logistic increase with time of the average leaf area and increase in average leaf number of *in vitro* propagated potato plantlets, grown at two temperatures *in vitro*. For curve fitting and curve parameters, see Table 1.

crease 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 (Figure 3, Table 1). Other parameters characterising the fitted curves or the maximum rate of increase (MI) were not significantly different between the two temperatures (Table 1). Leaf area of individual plants also generally increased logistically, although some plantlets showed exponential, expolinear or aberrant increases (Table 2; Figure 4). Temperature had no effect on the frequency at which the different types of increase occurred (Table 2). When the curves fitted through individual, logistically growing plantlets were compared, temperature effects on leaf area and leaf area increase were similar to those for plant averages, but leaf area increase of individual plants seemed to be characterised by a higher initial relative rate of increase (B), larger increments (C) and a higher maximum rate of increase (MI) than for plant averages. At the higher temperature plantlets reached the midpoint (M) of the fitted curve 2 days earlier than at the low temperature (Table 1).

Averaged over all plantlets, leaf appearance during the first 9 days was slower than thereafter (Figure 3). A higher temperature during normalisation resulted in a higher rate of leaf appearance during the major part of the phase. As a result, leaf number at the end of the phase was higher. 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 (Figure 4). In some plants classified as outliers the increase in leaf number started late or was very limited (Figure 4).

Transplant production phase

During transplant production, the increase in aboveground leaf area averaged over plants was best described by a logistic curve in all treatments (Figure 5, Table 3). At 26/20 °C, initial aboveground leaf area after planting was slightly lower than at 18/12 °C, but final leaf area was significantly larger (Table 3). Fitted curves at the higher 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 reached between day 10

Table 1. Effects of temperature during the normalisation (N) phase on initial and final leaf areas of *in vitro* propagated potato plantlets, and on parameters describing the logistic increase in average or individual leaf area (y) with time (x) during the normalisation phase.

Temperature in the respective phases (°C)			Initial leaf area (mm^2)	Final leaf area (mm^2)	n	Fitted parameters describing logistic growth $y = A + C/(1 + \exp^{(-B*(x-M))})$				Maximum rate of increase ($mm^2\ day^{-1}$) (MI)	r^2 of fit
N	TP	TB				Initial relative rate of increase (day^{-1}) (B)	Mid-point (days after cutting) (M)	Increment (mm^2) (C)	Minimum leaf area (mm^2) (A)		
Curves fitted through the average data of all plants per treatment											
17	–	–	12.8	148	64	0.27	15.3	157	9.2	10.6	0.99
23	–	–	11.3	156	64	0.37	12.4	146	10.0	13.5	0.99
Significance[a]			ns	ns		ns	*	ns	ns	ns	
Averages over curves fitted for individual, logistically growing plants											
17	–	–	12.0	148	49	0.37	15.0	163	10.4	13.9	0.99
23	–	–	11.7	158	58	0.40	13.0	156	9.9	15.2	0.99
Significance[b]			ns	ns		ns	***	ns	ns	ns	

[a] Initial and final leaf area tested by ANOVA, curve parameters tested by t-tests. ***: $P < 0.001$, **: $0.001 \leq P < 0.01$, *: $0.01 \leq P < 0.05$, ns: not significant, $P \geq 0.05$.

[b] Tested by ANOVA. For symbols, see above.

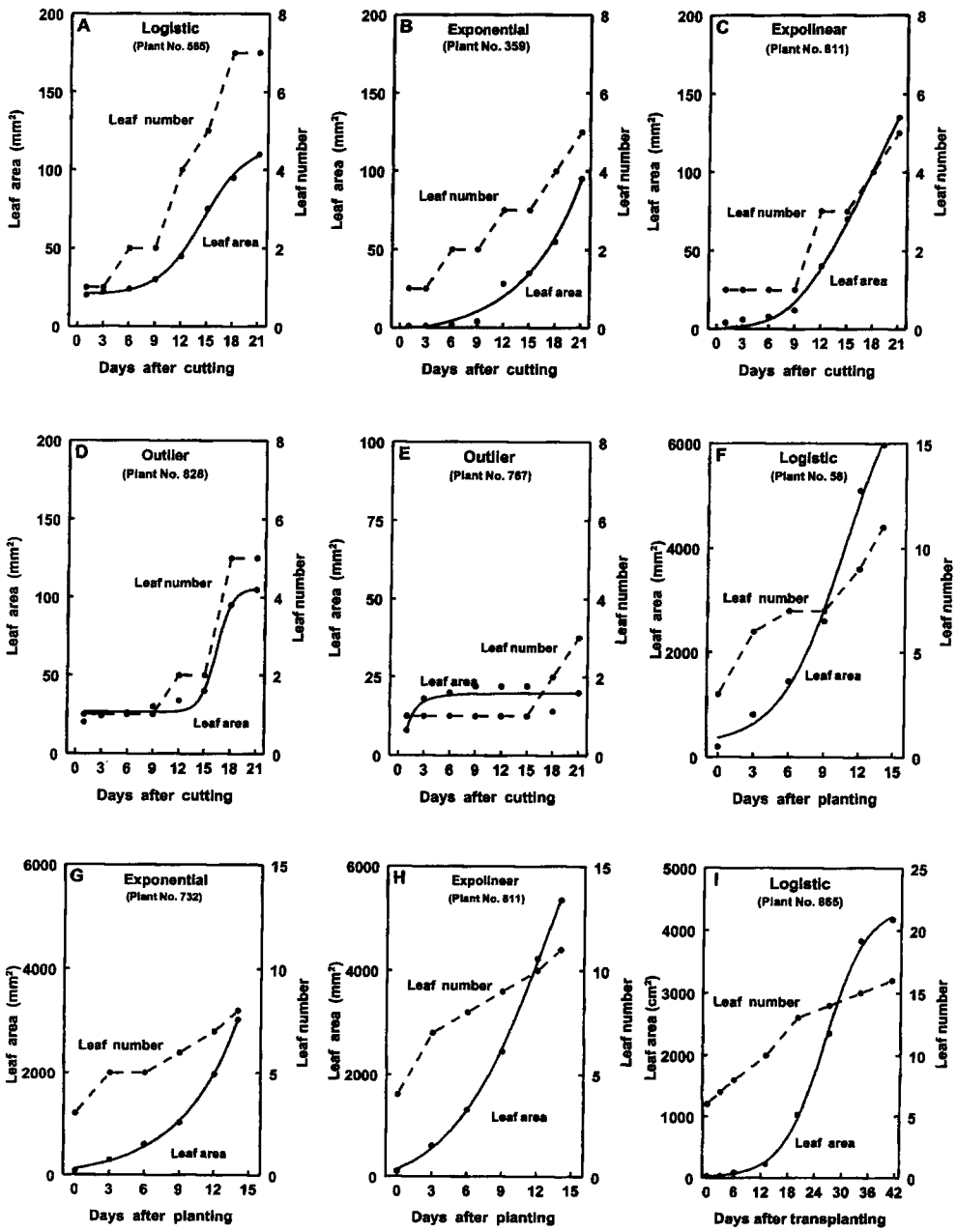

Figure 4. Examples of different curves describing the increase in aboveground leaf area and main stem leaf number with time, of individual plantlets during growth *in vitro* in the normalisation phase (A-E), transplant production phase (F-H) and tuber production phase (I). (Note that the scales along the axes are different).

Table 2. Percentage plants following different patterns of leaf area increase with time, as affected by temperature in three production phases.

Temperature (°C)	n	Logistic	Expolinear	Exponential	Outlier	Dead plants	Significance[a]
Normalisation phase (N)							
17	64	76.6	6.3	4.7	12.5	0.0	ns
23	64	90.6	4.7	4.7	0.0	0.0	
Transplant production phase (TP)							
18/12	64	59.4	21.9	9.4	0.0	9.4	ns
26/20	64	68.8	12.5	15.6	0.0	3.1	
Tuber production phase (TB)							
18/12	61	100.0	0.0	0.0	0.0	0.0	ns
26/20	59	100.0	0.0	0.0	0.0	0.0	

[a] Tested by X^2 – test on number of plants in the relevant categories.

and 13 of the 14-day period in both temperatures. In general, leaf area of individual plants also increased logistically, but some plants showed exponential or expolinear increases (Table 2, Figure 4). Temperature did not affect the frequency of the different types of curves (Table 2). For plants showing logistic increases in leaf area, no differences in initial aboveground leaf area were present between the two temperatures, whereas final leaf area again was greater at higher temperature (Table 3). As for plant averages, higher temperature resulted in plantlets having smaller B-values, smaller fitted minimum values (A) and larger fitted increases (C). Higher temperature also resulted in smaller MI-values. Temperature during the transplant production phase again had no effect on the time at which the mid-point M was reached, which was around 11 days after planting (Table 3). Leaf area increase of individual plants was characterised by higher initial relative rates of increase (B), higher minimum leaf areas (A), higher maximum rates of increase (MI), but lower increments (C) than leaf area increases over plant averages.

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 (Figure 5). A higher temperature resulted in a stronger boost and higher increase in leaf number in the next 3 days but not thereafter. The boost in leaf number was also clear in individual plants, but for some plants seemed to be followed by a check (Figure 4).

Pre-culturing plantlets at different temperatures in the preceding normalisation phase did not affect leaf areas shortly after planting or at the end of the phase (Table 3). Temperature during normalisation also did not affect fitted curve parameters in the transplant production phase (Table 3). The boost in leaf number after planting to soil seemed smaller for plantlets produced at high temperature during normalisation, but only when planted to the cool conditions (Figure 5).

Tuber production phase

Of the curve types tested, logistic curves again best described the increase in leaf

Table 3. Effects of temperature during transplant production (TP) and the preceding normalisation (N) phase on initial and final leaf areas of *in vitro* propagated potato plantlets, and on parameters describing the logistic increase in average or individual leaf area (y) with time (x) during the transplant production phase.

Temperature in the respective phases (°C)			Initial leaf area (mm^2)	Final leaf area (mm^2)	n	Fitted parameters describing logistic growth $y = A + C/(1 + \exp^{(-B*(x-M))})$				Maximum rate of increase ($mm^2\ day^{-1}$) (MI)	r^2 of fit
N	TP	TB				Initial relative rate of increase (day^{-1}) (B)	Mid-point (days after planting) (M)	Increment (mm^2) (C)	Minimum leaf area (mm^2) (A)		
Curves fitted through the average data of all plants per treatment											
17	18/12	–	116.8	4212	32	0.58	10.7	4538	323	658	0.97
23	18/12	–	104.1	3919	26	0.49	11.4	4749	239	582	0.97
17	26/20	–	93.5	5047	32	0.25	11.7	8337	–268	521	0.99
23	26/20	–	88.6	4688	30	0.28	12.8	8234	– 82	576	0.99
Significances[a]											
TP temperature			*	***		*	ns	**	*	ns	
N temperature			ns	ns		ns	ns	ns	ns	ns	
Averages over curves fitted for individual, logistically growing plants											
17	18/12	–	102.0	3970	21	0.73	10.7	4050	367	753	0.97
23	18/12	–	94.5	3875	15	0.63	11.4	4461	331	700	0.97
17	26/20	–	78.3	4899	24	0.33	10.6	7115	–218	562	0.99
23	26/20	–	96.6	4896	18	0.42	11.0	6589	15	679	0.99
Significances[b]											
TP temperature			ns	***		***	ns	***	***	*	
N temperature			ns	ns		ns	ns	ns	ns	ns	
TP*N interaction			ns	ns		**(0.10)	ns	*(689)	ns	ns	

[a] Initial and final leaf area tested by ANOVA, curve parameters tested by t-tests. For symbols see Table 1.
[b] Tested by ANOVA. For symbols, see Table 1. Between brackets: LSD 5%.

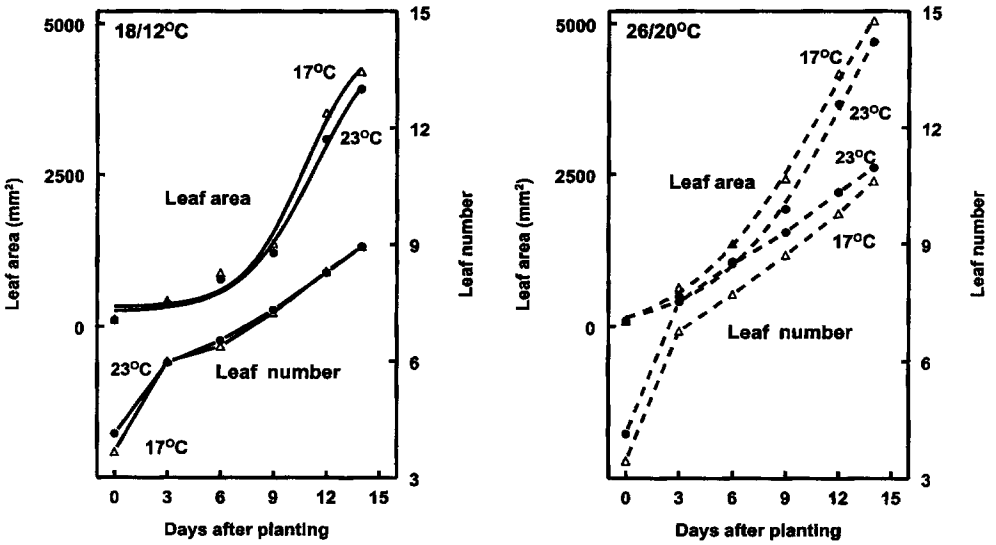

Figure 5. Logistic increase with time of the average aboveground leaf area and increase in average aboveground leaf number of *in vitro* propagated potato plantlets at two temperatures (18/12 or 26/20 °C) in the transplant production phase after they were grown at low (17 °C) or high (23 °C) temperatures during normalisation. For curve fitting and curve parameters, see Table 3.

area with time in the tuber production phase for plant averages (Figure 6). Transplants started with a slightly smaller aboveground 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 than at lower temperature (Table 4). 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 4). The increase in leaf area of all individual plants also was better described by a logistic curve than by exponential or expo-linear curves (Table 2, Figure 4). The effect of temperature on leaf area and leaf area increase of individual plants was comparable to that of plant averages (Table 4), but B-values and maximum rates of increase (MI) were higher in individual plants than for plant averages.

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 (Figure 6). A higher temperature always resulted in a higher rate of increase in leaf number. The leaf number increase of individual plants followed the pattern expressed by averages.

Transplants precultured at 26/20 °C had a higher initial aboveground leaf area at the start of the tuber production phase than transplants that were raised at 18/12 °C. There was, however, no effect anymore at the end (Table 4). For plant averages, the pre-treatment had no effect on any of the curve parameters of leaf area increase. For individual plants, those produced at 26/20 °C had larger fitted A-values and smaller

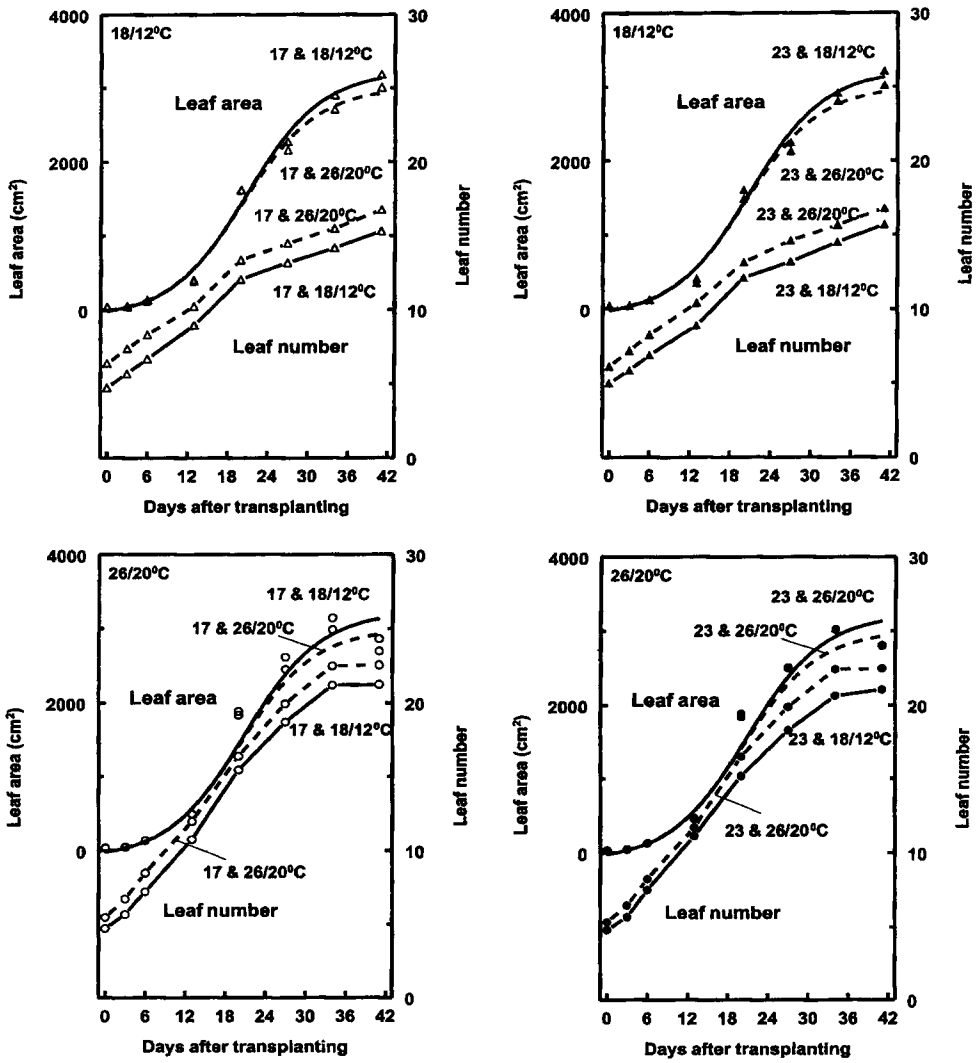

Figure 6. Logistic increase with time of the average aboveground leaf area and increase in average aboveground main stem leaf number of *in vitro* propagated potato plantlets at two temperatures (18/12 or 26/20°C) in the tuber production phase after they were grown at two temperatures (18/12 or 26/20°C)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

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.

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

Table 4. Effects of temperature during tuber production (TB) and the preceding transplant production (TP) and normalisation (N) phases on initial and final leaf areas of *in vitro* propagated potato plantlets, and on parameters describing the logistic increase in average or individual leaf area (y) with time (x) during the tuber production phase.

Temperature in the respective phases (°C)			Initial leaf area (cm^2)	Final leaf area (cm^2)	n	Fitted parameters describing logistic growth $y = A + C/(1 + \exp^{(-B*(x-M))})$				Maximum rate of increase ($cm^2\ day^{-1}$) (MI)	r^2 of fit
N	TP	TB				Initial relative rate of increase (day^{-1}) (B)	Mid-point (days after transplanting) (M)	Increment (cm^2) (C)	Minimum leaf area (cm^2) (A)		
Curves fitted through the average data of all plants per treatment											
17	18/12	18/12	34.1	3183	16	0.18	20.9	3321	−98.2	149	0.99
23	18/12	18/12	33.8	3213	14	0.17	21.2	3397	−114.4	144	0.99
17	26/20	18/12	44.3	3007	16	0.18	20.4	3087	−86.1	139	0.98
23	26/20	18/12	41.6	3017	15	0.18	21.4	3147	−67.0	142	0.99
17	18/12	26/20	33.6	2865	16	0.29	18.4	2958	19.3	215	0.99
23	18/12	26/20	32.4	2800	12	0.29	18.2	2854	13.3	207	0.99
17	26/20	26/20	40.1	2699	16	0.29	18.2	2784	22.8	202	0.99
23	26/20	26/20	36.5	2809	15	0.29	18.4	2864	18.8	208	0.99
Significances[a]											
TB temperature			*	***		***	***	**	***	***	
TP temperature			***	ns			ns	ns	ns	ns	
N temperature			ns	ns		...	ns	ns	ns	ns	
Averages over curves fitted for individual, logistically growing plants											
17	18/12	18/12	34.1	3183	16	0.20	20.6	3285	−79.6	162	0.99
23	18/12	18/12	33.7	3214	14	0.18	21.3	3425	−113.9	154	0.99
17	26/20	18/12	44.3	3007	16	0.20	20.2	3080	−79.4	149	0.98
23	26/20	18/12	41.6	3017	15	0.20	21.1	3114	−45.0	156	0.99
17	18/12	26/20	33.1	2886	16	0.30	18.6	3014	9.7	221	0.99
23	18/12	26/20	33.2	2792	12	0.33	18.1	2858	20.5	227	0.99
17	26/20	26/20	40.1	2699	16	0.30	18.3	2804	20.6	209	0.99
23	26/20	26/20	36.5	2809	15	0.30	18.4	2870	19.2	213	0.99
Significances[b]											
TB temperature			*	***		***	***	***	***	***	
TP temperature			***	ns		ns	ns	*	*	ns	
N temperature			ns	ns		ns	ns	ns	ns	ns	

[a] Initial and final leaf area tested by ANOVA, curve parameters tested by t-tests. For symbols see Table 1.
[b] Tested by ANOVA. For symbols, see Table 1. No interactions occurred.

Discussion

Logistic increase in leaf area

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, 3, 4). 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, total nutrient availability was not limiting growth. Insufficient rooting volume may have been the main limiting factor for leaf area increase, limiting availability of water and nutrient uptake. 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%, Table 2) was growing exponentially or expolinearly and the midpoint (M) of the logistic curve occurred only after about 11 days of the 14-d period.

During the tuber production phase, aboveground competition could not be avoided even though plants were spaced wider with time. Maximum leaf area indices approached 2 for the first time after 3 weeks in the tuber production phase. Moreover, high temperature caused early senescence of the plants. The decrease in leaf area caused by senescence at the end of the phase in the treatments growing at higher temperature will have forced the curve to take a logistic shape (Figure 4).

Boosts and shocks after transition to a new phase

After planting *in vitro* plantlets to soil, a boost in leaf area growth occurred (Figure 2A). Although this is consistent with a logistic growth pattern, the boost was very prominent compared to the previous phase and later part of the transplant production phase, and was also observed for leaf number (Figure 2B). Fig. 5 shows that logistic curves even underestimated the leaf area increase the first days after planting to soil. The increase in leaf number was likely associated with internode elongation allowing more leaves from the apex of the plant to be exposed and 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 elongation in potato (Rylski *et al.*, 1974). It is unknown if a different spectral quality of the light could have triggered elongation of the internodes. In the trans-

plant 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) was likely lower than in the normalisation phase. Leaf expansion could also have been increased. In addition, a small increase in aboveground 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

In the normalisation phase, the effect of a higher temperature (23 versus 17 °C) 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 (Table 1). 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 and Bodlaender, 1975; Steward *et al.*, 1981; Almekinders & Struik, 1994) but smaller (Bodlaender, 1963; Steward *et al.*, 1981) leaves. In our experiment, a higher temperature *in vitro* resulted in a higher rate of leaf appearance, but without a significant increase in leaf area (Figure 3), indicating that smaller leaves were produced.

During transplant production, leaf area and number increased more at higher than at lower temperature (Figure 5, Table 3). The effect appeared to be achieved mainly in the first part of the transplant production period (Figure 5). The faster increase in leaf area in the early part forced the fitted logistic curves to take a less clear S-shape at the higher temperature, with consequently lower fitted minimum leaf area (A) and B-values than at lower temperature (Table 3).

At the end of the tuber production phase, leaf area was less for plants growing at higher temperature (Table 4), whereas it was higher in the earlier stages (Figure 6). This growth pattern was reflected in earlier midpoints (M), lower fitted increments (C) and higher maximum rates of increase (MI) at the higher temperature (Table 4). In this phase, belowground 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 (Figure 6) 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 (Figure 6). Also more secondary stems were produced at the higher temperature (not shown), which is in agreement with Almekinders & Struik (1996). Final leaf area in our experiment was lower 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 (Figure 5). Temperature during normalisation also did not affect fitted curve parameters during transplant production (Table 3). 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.*, 2000). A higher normalisation temperature nevertheless resulted in greater leaf number at the end of the normalisation phase and the beginning of the transplant production phase. The boost in leaf number immediately after planting (Figures 2, 5) 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 3), and so was the initial aboveground leaf area in the subsequent tuber production phase (Table 4). This agrees with results from Tadesse *et al.* (2001). No significant effects of temperature during transplant production on leaf area were present anymore at the end of the tuber production phase (Table 4). However, individual plants pre-cultured at higher temperature (Table 4) 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 (Figure 6). 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.*, 2001). 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 (Figure 6). 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 was 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 cultivar used in this experiment, the very early cv. Gloria, was chosen for its ease of handling. Transplants derived from *in vitro* plants of this cultivar under field conditions may have restricted haulm development because of the strong partitioning of dry matter to tubers, limiting haulm development and ultimately yield (Lommen, 1999). Related research indicates that the performance of cv. Gloria is more difficult to manipulate by temperature than that of other (later) cultivars studied (Tadesse et al., 2000, 2001). We therefore surmise that after-effects found for cv. Gloria are also

relevant for other potato cultivars, and that after-effects in general even may more readily be found in other cultivars.

Leaf area increase of individual plants versus averages

In all phases, leaf area increase was more gradual with time for plant averages than for individual plants (Tables 1, 3, 4), as shown by lower B-values and lower maximum rates of increase (MI) for plant averages. The main reason is likely that 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 also increased less in leaf area than plantlets following logistic growth (not shown), likely because of a delayed shoot development or shock in development of new leaves (Figure 4). They thereby also reduced the rate of increase for plant averages, and most likely the B-, MI- and C-values (Table 1).

Also the smooth increase in leaf number found for plant averages (Figures 3 and 5) was not found in all individual plants (Figure 4), in which leaf appearance occurred more shock-wise. The results therefore show that large differences may exist between growth measured from a whole population of plants and growth of individual plants.

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