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

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Abstract

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

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

Introduction

Traditionally, the potato (*Solanum tuberosum* L.) is multiplied by producing seed tubers. Seed tuber production is carried out by highly specialized growers or institutions. A complete multiplication scheme can take more than 10 years. Main problems of a conventional seed programme are the low multiplication rate of field-grown potato plants and the susceptibility of potato to diseases, which may be transferred through the seed tubers. With each multiplication in the field, the risk of catching viral, bacterial or fungal diseases increases. The health status of the seed tubers may be improved by reducing the number of field multiplications

necessary to produce the desired seed lot. This requires a propagation material that can be produced in large numbers in protected environments. Only a few additionial years of conventional seed multiplication would then be necessary.

The last decades, alternative seed production programmes have been developed in which the first multiplication steps are speeded up by using in vitro plantlets (Jeffries, 1986), microtubers (Wang & Hu, 1982) or minitubers (van der Zaag, 1990). Microtubers (or in vitro tubers) are produced in vitro on in vitro propagated plantlets or shoots. They generally weigh 0.2 g per tuber or less (Hussey & Stacey, 1984; Estrada et al., 1986; Garner & Blake, 1989), though average weights of 0.4 g are reported when produced on liquid media containing growth regulators (Rossell et al., 1987; Lillo, 1989). Minitubers are produced on in vitro propagated plantlets, planted at high density in a soil medium in glasshouses and are larger than microtubers (Struik & Lommen, 1990). In vitro propagated plantlets and microtubers nowadays are commonly used (Jones, 1988) and perform well if raised under protected conditions, in beds (Wiersema et al., 1987) or as transplants in the field (Wattimena et al., 1983), provided the growing season is sufficiently long.

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

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

Materials and methods

In vitro multiplication

In vitro plantlets of *Solanum tuberosum* L. cv. Ostara (early) and cv. Bintje (mid-early) were multiplied routinely by subculturing single stem nodes every 4 weeks. Temperature in the growth room was 23 °C, photoperiod 16 hours and

light was supplied by fluorescent tubes (Philips 33) at an intensity of approximately 8 W m^{-2} (total radiation). The multiplication medium (pH 5.7) contained mineral salts and vitamins (Murashige & Skoog, 1962) plus 2.0 mg l^{-1} glycine, 8.0 g l^{-1} agar and 25.0 g l^{-1} sucrose. The normalization medium before transplanting had the same composition with in addition 0.01 g l^{-1} alar-85% (daminozide). The growing period from the last multiplication till transplanting was 17 days (cv. Ostara) or 18 days (cv. Bintje).

Culture in the glasshouse

In vitro plantlets were transplanted in a controlled glasshouse into a mixture of perlite and potting soil (50/50% v/v) in 13 × 13 × 13 cm pots. A plant density of 350 plants m^{-2} was obtained by planting 6 plants per pot in a row in the middle of the pot and joining all pots. Available N from the soil medium was approximately 230 mg per pot.

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

Treatments and experimental design

Growth and development were analysed after transplanting of the in vitro plantlets in the glasshouse. One series of treatments involved weekly, destructive harvests of undisturbed growing plants. At the moment the first tubers had a fresh weight of 0.3 g (3 weeks after transplanting), another series of treatments started: tubers ≥ 0.3 g were removed and plants were replanted. The removal of tubers was carried out, using a non-destructive harvesting procedure, suitable for practical use. Plants were lifted carefully from the soil mixture, tubers ≥ 0.3 g were removed and plants were replanted into the soil mixture. Whether the weight of the removed tubers was ≥ 0.3 g had to be estimated, using a diameter of approximately 8 mm as a criterium. Plants were always replanted deeper than before. Replanting depth was not recorded but depended on the harvest date, and increased as the length of the stem part without leaves increased. Care was taken not to damage stems and stolons. Damage of roots, however, could not be avoided. The non-destructive harvests were carried out 3, 4, 5, 6, 7 or 8 weeks after transplanting, and were each followed by a destructive harvest 3 weeks later, to establish growth and development. Treatments are schematically represented in Figure 1. Treatment codes represent the weeks after transplanting at which a harvest (non-destructive or destructive) took place.

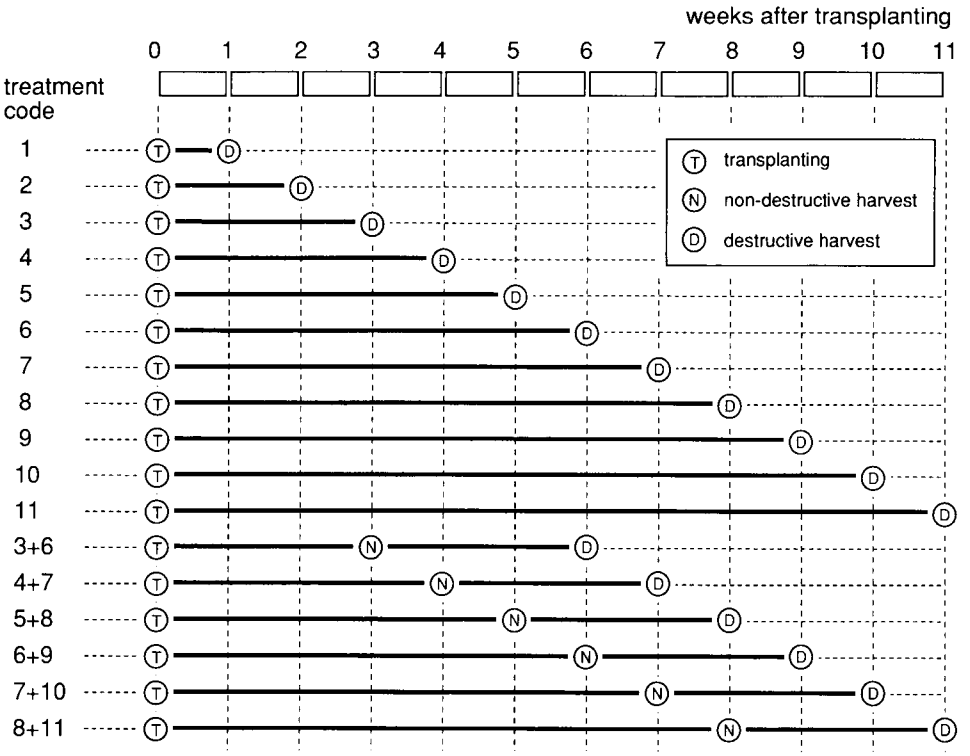

Fig. 1. Treatment codes and schematic explanation of treatments.

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

Observations

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

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

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

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

Analysis of data

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

Growth rates and relative growth rates

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

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

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

in which:

t = time of final harvest in weeks after transplanting
$W(t)$ = dry weight in g plant^{-1} at t
$W(t-21)$ = dry weight in g plant^{-1} 21 days before t
350 = number of plants per m^2
21 = number of days in 3 weeks period

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

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

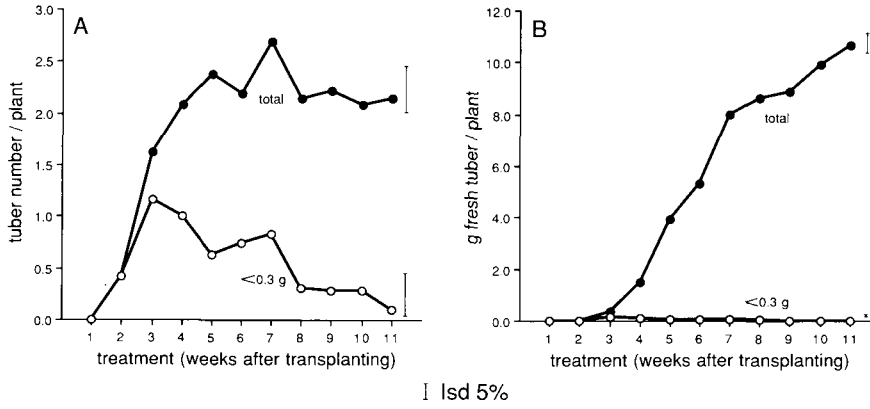

Fig. 2. Development over time of number (A) and fresh weight (B) of tubers in different grades, of undisturbed growing plants at a density of 350 plants m^{-2}. Average values of 2 cultivars.

Results

Tuber production during undisturbed growth

The in vitro propagated plantlets grew well after transplanting into the glasshouse at a plant density of 350 plants m^{-2}. First tubers were detected 2 weeks after transplanting (Figure 2). Total tuber number increased up to 7 weeks after transplanting to 2.69 tubers per plant and thereafter declined to approximately 2.15 tubers per plant, due to resorption (Figure 2A). Final total tuber number did not differ significantly from the number of tubers present 4 weeks after transplanting. The number of tubers < 0.3 g declined from 3 weeks after transplanting onwards, mainly due to passing into ≥ 0.3 g grading. The number of tubers ≥ 0.3 g gradually increased through the experiment, up to 2.04 tubers per plant. Tuber fresh weight increased up to 10.65 g per plant and more than 5 g per tuber. The contribution of tubers < 0.3 g to total fresh weight was negligible at the end of the experiment (Figure 2B).

Plant development and dry weight changes during undisturbed growth

The average overall growth rate during the experiment (week 1 to 11) was 11.8 g m^{-2} d^{-1}. Growth and development during undisturbed growth is shown in Figure

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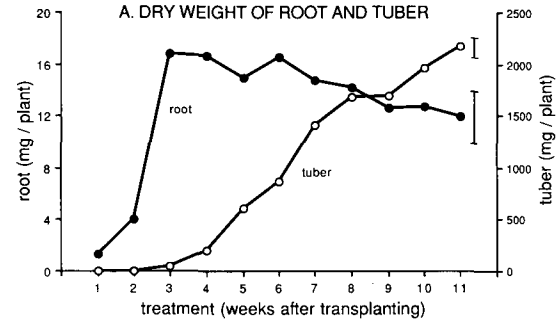
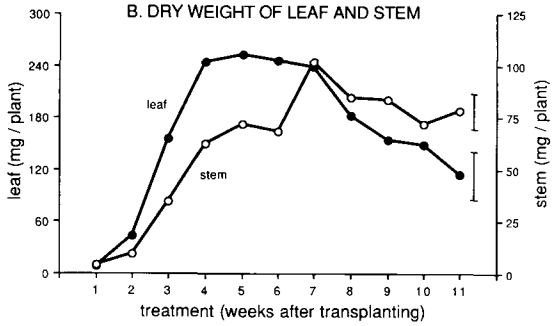
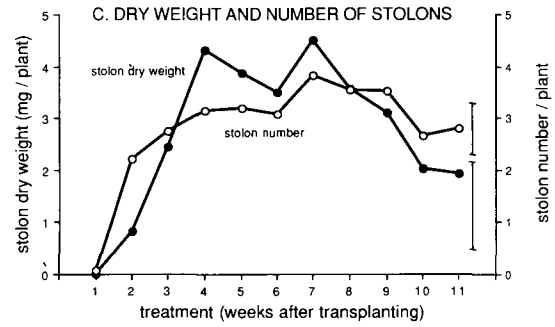
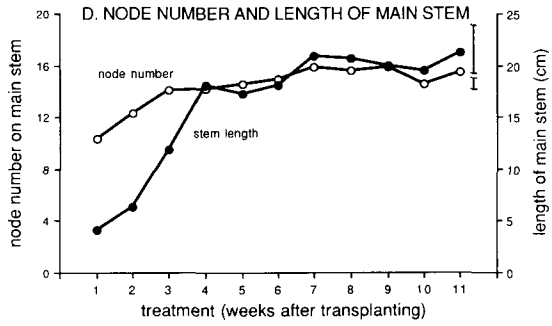
Fig. 3. Growth and development over time of undisturbed growing plants at a density of 350 plants m^{-2}. Dry weights per plant of root and tuber (A), dry weights per plant of leaf and stem (B), dry weight per plant and number of stolons (D) and node number and length of main stem (D). Average values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


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


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


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


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

I lsd 5%

3. During the 11 weeks of the experiment, the plants passed through 3 distinct growth phases: an early growth phase (0 - 4 weeks), a period of maximal growth (4 - 7 weeks), and a senescence period (7 - 11 weeks).

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

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

During the last growth phase, plants were clearly senescing: dry weights of root, stolons, leaf and stem decreased (Figures 3A, 3B and 3C). Only tuber dry weight still increased (Figure 3A). Stolon number declined (Figure 3C). Stem length (approximately 20 cm) and node number (approximately 16) ceased to increase (Figure 3D). Average overall growth rate during the last growth phase (week 7 to 11) was 7.6 g m^{-2} d^{-1}.

Influence of a non-destructive harvest at different time intervals after planting on tuber production

After removing tubers ≥ 0.3 g in a non-destructive harvest, many new tubers were initiated on existing stolons, newly formed stolons and directly on the below-ground part of the main stem. Number, fresh weight and size of tubers at the final harvest, 3 weeks after the non-destructive harvest, are shown in Table 1. Total

Table 1. Influence of timing of the non-destructive harvest on number, yield and size of tubers in different grades, recorded at the final harvest. Average values of 2 cultivars. See Figure 1 for treatment description.

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

tuber numbers at the last harvest were on average almost 4 times as high as the numbers observed in undisturbed growing plants (Figure 2A). The number of tubers ≥ 0.3 g increased on average by almost 30 %. Tuber number at the second harvest, however, depended strongly on the timing of the first harvest. Postponing the first harvest from 3 to 6 weeks after transplanting, increased the number of tubers in the second harvest. Further postponing decreased the total number of tubers in the second harvest, though it was still higher than in undisturbed plants. Highest tuber numbers in the second harvest were observed in Treatment 6+9, in which the plants were harvested both 6 and 9 weeks after planting: 12.77 tubers per plant. Highest numbers of tubers ≥ 0.3 g were also observed in this treatment: 3.44 tubers per plant. Tuber numbers in Treatment 5 + 8 were lower, but not significantly. The majority of the tubers in the second harvest, however, was smaller than 0.3 g. The later the first harvest, the higher the proportion of small tubers in the second harvest.

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

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

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

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

Influence of a non-destructive harvest at different time intervals after planting on plant development and dry matter production

Overall growth rate (GR) of harvested plants, calculated over the 3-weeks period between harvests, was on average 56 % of the overall GR of undisturbed growing plants (Table 3). The effect of a non-destructive harvest, however, depended on the timing of the first harvest. It was considerable at early harvests (Treatments 3+6 and 4+7), when GR of the harvested treatments was reduced to 43 % and

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

Treatment	Non-destructive harvest	Final harvest week	Tuber characteristics				Stolon characteristics		Haulm characteristics	
			number of sessile tubers per plant	number of tubers on stolons per plant	% of sessile tubers	number of tubers per stolon[1]	number of stolons per plant	length per stolon (cm)	main stem length (cm)	node number on main stem
6	no	6	0.23	1.96	9.6	0.66	3.1	2.1	18.1	14.9
7	no	7	0.19	2.50	5.4	0.66	3.8	2.0	20.9	15.9
8	no	8	0.10	2.04	5.1	0.69	3.5	2.3	20.6	15.6
9	no	9	0.10	2.12	4.6	0.94	3.5	1.8	20.0	15.9
10	no	10	0.15	1.94	7.2	0.81	2.7	1.1	19.5	14.6
11	no	11	0.10	2.04	4.1	0.89	2.8	1.4	21.2	15.5
mean			*0.15*	*2.10*	*6.0*	*0.77*	*3.2*	*1.8*	*20.0*	*15.4*
3+6	yes	6	0.58	3.06	14.2	0.84	3.7	1.8	17.5	15.4
4+7	yes	7	2.38	5.02	29.6	1.20	4.5	2.1	16.8	14.2
5+8	yes	8	2.68	7.04	29.8	1.20	6.7	1.8	21.6	16.2
6+9	yes	9	6.00	6.77	45.6	1.18	5.7	1.4	21.4	16.5
7+10	yes	10	5.00	4.35	57.3	1.36	3.2	1.4	19.5	14.0
8+11	yes	11	4.19	3.54	57.7	1.31	3.1	0.9	20.5	15.4
mean			*3.47*	*4.96*	*39.1*	*1.18*	*4.5*	*1.6*	*19.6*	*15.3*
Significance[2]										
– non-destructive harvest			**	**	**	***	***	ns	ns	ns
– final harvest week			ns	ns	ns	ns	***	***	ns	**
– interaction[3]			***	***	***	ns	ns	ns	ns	ns
LSD 5%			1.42	1.43	10.7					

[1] Tubers produced on stolons only.

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

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

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

Treatment	Non-destructive harvest	Final harvest week	Overall	Root	Stolon	Leaf	Stem	Tuber
6	no	6	15.6	–0.005	0.018	1.49	0.55	13.5
7	no	7	20.7	–0.031	0.003	–0.08	0.65	20.1
8	no	8	16.8	–0.012	–0.005	–1.19	0.22	17.8
9	no	9	12.4	–0.065	–0.007	–1.53	0.26	13.8
10	no	10	6.4	–0.035	–0.041	–1.50	–0.50	7.8
11	no	11	7.0	–0.039	–0.027	–1.11	–0.12	8.3
mean			13.2	–0.031	–0.010	–0.65	0.18	13.5
3+6	yes	6	6.7	–0.061	0.013	0.32	0.30	6.2
4+7	yes	7	7.1	–0.093	0.022	–1.23	0.02	8.4
5+8	yes	8	13.5	–0.018	0.141	–0.62	0.41	13.5
6+9	yes	9	10.7	–0.060	0.020	–1.37	0.22	11.9
7+10	yes	10	3.8	–0.046	–0.014	–1.60	–0.49	6.0
8+11	yes	11	1.3	–0.120	–0.027	–1.46	–0.31	3.2
mean			7.2	–0.067	0.025	–0.99	0.03	8.2
Significance[1]								
– non-destructive harvest			*	*	ns	ns	ns	*
– final harvest week			ns	ns	ns	*	***	ns
– interaction[2]			**	ns	*	*	ns	**
LSD 5%			4.4		0.067	0.79		3.7

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

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

34 % of the GR of undisturbed growing treatments, but most severe at a late harvest (Treatment 8+11), when GR was reduced to 19 %. Differences between undisturbed growing plants and harvested plants were not significant when plants were harvested for the first time after 5 or 6 weeks (Treatments 5+8 and 6+9).

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

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

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

A non-destructive harvest increased stolon numbers from 3.2 to 4.5 stolons per plant (Table 2). The timing of the non-destructive harvest did not significantly

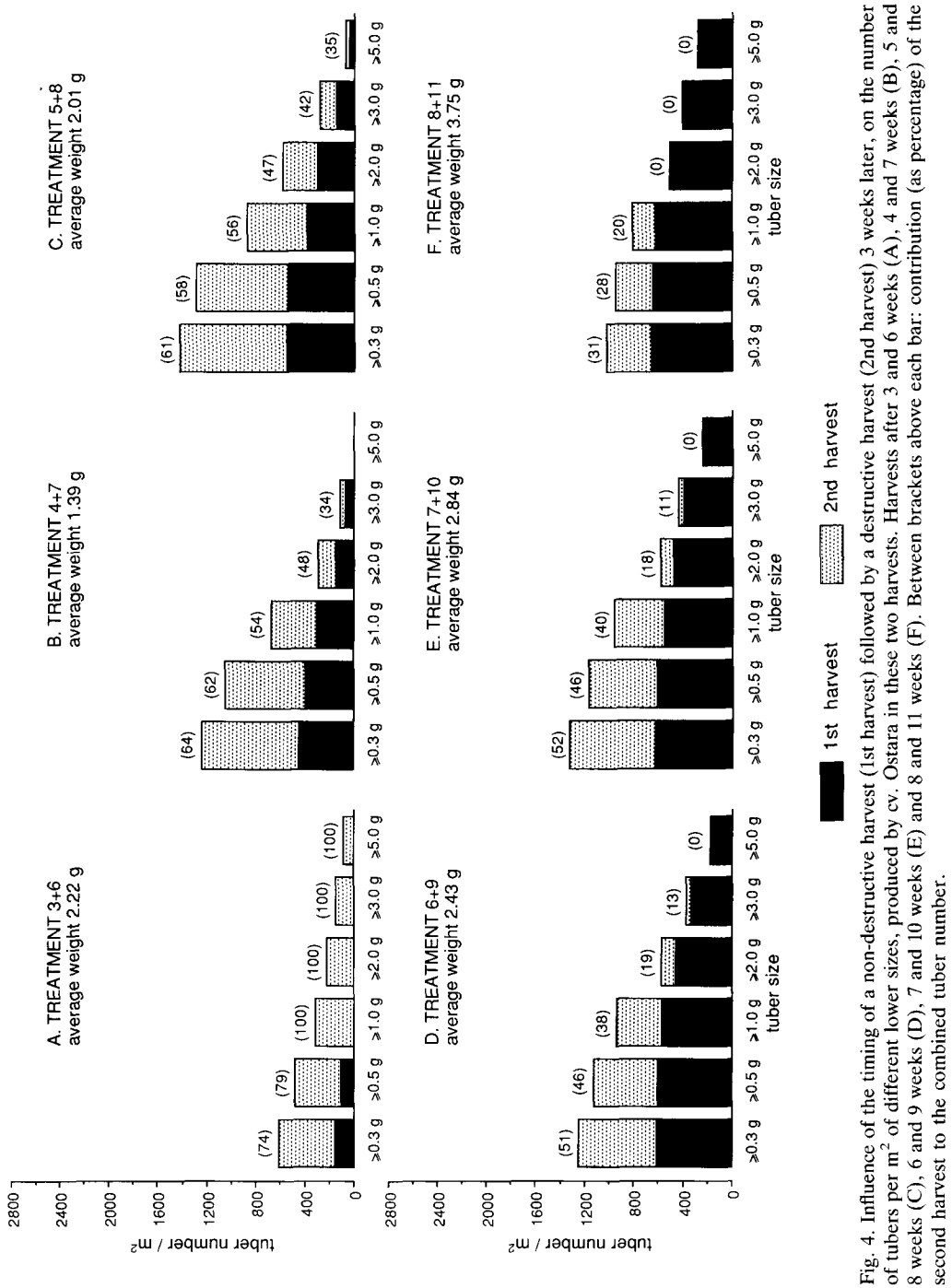


Fig. 4. Influence of the timing of a non-destructive harvest (1st harvest) followed by a destructive harvest (2nd harvest) 3 weeks later, on the number of tubers per m^2 of different lower sizes, produced by cv. Ostara in these two harvests. Harvests after 3 and 6 weeks (A), 4 and 7 weeks (B), 5 and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

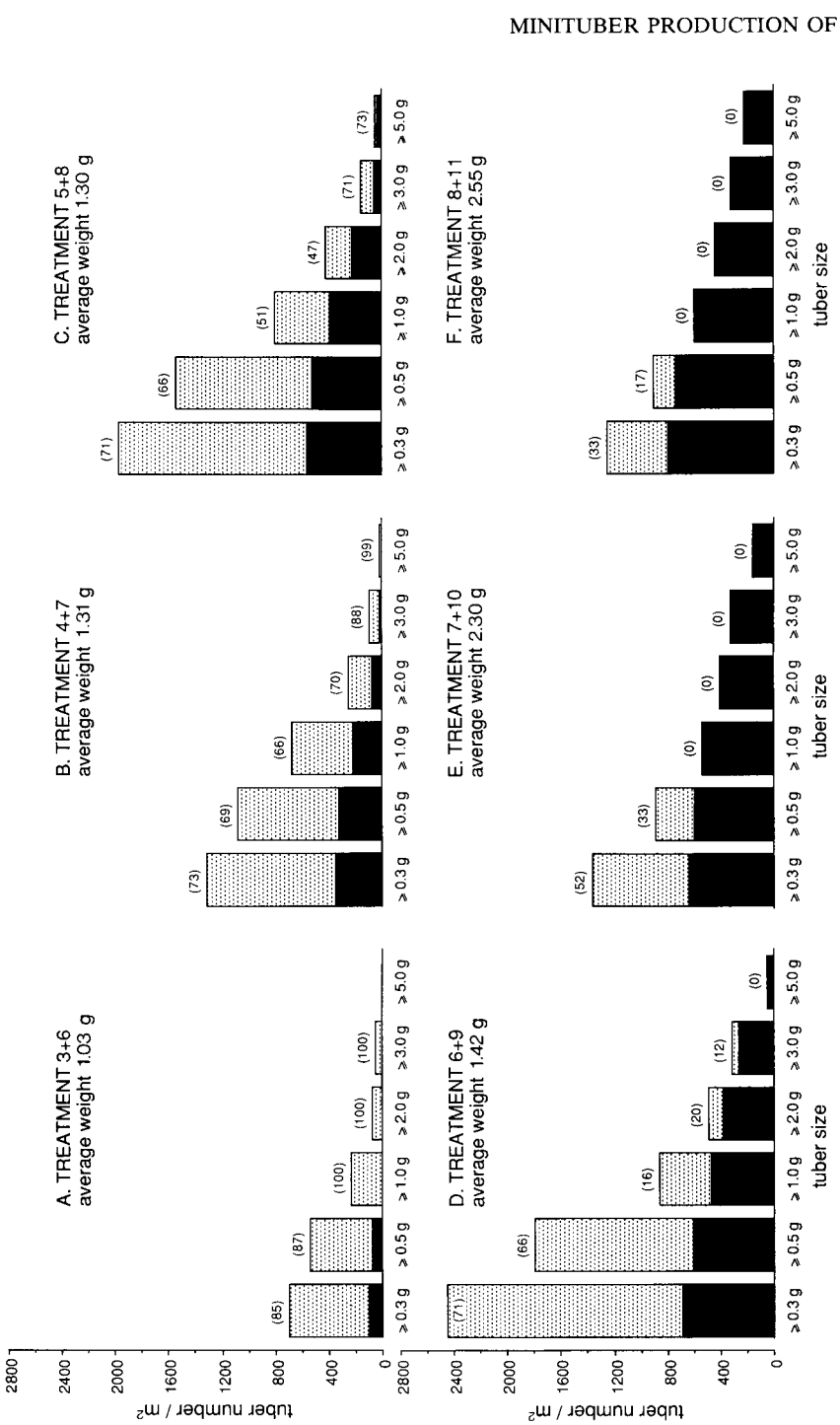


Fig. 5. Influence of the timing of a non-destructive harvest (1st harvest) followed by a destructive harvest (2nd harvest) 3 weeks later, on the number of tubers per m^2 of different lower sizes, produced by cv. Bintje in these two harvests. Harvests after 3 and 6 weeks (A), 4 and 7 weeks (B), 5 and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

Table 4. Relative growth rates (RGRs) of different parts, calculated over a 3-weeks period before the final harvest, in treatments with and without a non-destructive harvest 3 weeks before the final harvest. Average values of 2 cultivars and 6 final harvest weeks (d^{-1}).

Plant part	RGR control	RGR after non-destructive harvest	Significance[1]
Root	–0.007	–0.017	**
Stolon	–0.010	0.002	*
Leaf	–0.012	–0.018	ns
Stem	0.006	0.001	ns
Tuber	0.056	0.184	**
Overall	0.039	0.035	ns

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

affect this increase, but stolon GR was stimulated most when the first harvest took place after 5 weeks (Treatment 5+8, Table 3). Stolons did not branch.

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

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

Effect of cultivar

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

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

Both cultivars produced highest numbers of tubers in the second harvest in

Treatment 6+9. Cv. Ostara, however, reached its maximum tuber weight and its maximum number of tubers ≥ 0.3 g earlier than cv. Bintje.

Practical implications of a non-destructive harvest for a minituber production system

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

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

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

Discussion

Undisturbed growth at a high plant density

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

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

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

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

The final tuber number, however, was lower than the number of tubers initiated, because resorption occurred during plant senescence (Figure 2A). In our experiment, the dynamics of tuber number reflected the changes in growth and development during the different growth phases. The final number of tubers did not differ significantly from the number of tubers present at the end of the first growth phase (4 weeks after transplanting), i.e. the moment leaf dry weight ceased to increase (Figure 3B). During the second growth phase (4 - 7 weeks), leaf weight remained constant (Figure 3B). Deterioration of old leaves must have matched production and weight increase of new leaves, because node number still increased (Figure 3D). During this period of maximal leaf weight the number of tubers increased only slightly (Figure 2A), but overall and tuber dry weight increases were maximal (Table 3). The number of tubers initiated during this second growth phase, was similar to the number resorbed during senescence (7 - 11 weeks). This resorption was associated with a decay of stolons and a decrease in dry weight of all plant parts except tubers (Figure 3).

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

A non-destructive harvest of tubers ≥ 0.3 g involved three actions that could have caused the observed changes in plant growth and development:

1. removal of tubers, resulting in breaking of apical dominance of the dominant tuber at the stolon apex, and changes in the possibilities for assimilate partitioning;
2. damage of roots, resulting in a temporary drought stress, a change in root: shoot ratio, and possible changes in production of growth regulators;
3. replanting deeper than initially, resulting in more stem nodes being exposed to below-ground conditions.

The timing of the non-destructive harvest strongly influenced the effects of these actions.

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

The influence of drought stress on leaf expansion will be most important when young and expanding leaves are present, i.e. at early harvest moments. Significant reductions of leaf growth rates only occurred after early non-destructive harvests (Table 3). This explains why overall growth rate was reduced considerably after early harvests but less after intermediate harvests (Table 3). The reduction in total

growth rate, however, was most severe after the latest harvest date, since the senescing plants were not able to adapt anymore.

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

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

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

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

ting of our plants could have increased tuber number too, similar to stolon number.

The timing of the first harvest strongly influenced the tuber numbers in the second harvest (Table 1). After early non-destructive harvests, less tubers were produced than after intermediate non-destructive harvests. At early harvests, less tubers were removed since many tubers had not yet reached the desired size (Figure 2A, tubers < 0.3 g). Thus, the breaking of apical dominance was less important. Moreover, the later the non-destructive harvest, the deeper the plants were replanted, because of the longer stems (Figure 3D) or part of the stem that contained no green leaves. The number of tubers at the final harvest, however, was higher after intermediate harvests than after very late non-destructive harvests (Table 1). This difference was larger than the difference in tubers < 0.3 g remaining on the plants after the non-destructive harvests (Figure 2A). Possibly, at very late harvests, tuber initiation was limited by availability of mineral nutrients, which by then must have been very low, despite the replenishment of nutrients after 58 days. This agrees with the experiments of Nösberger & Humphries (1965), who concluded that after removal of tubers more meristems start to grow when the supply of N permits so. On the other hand, already some resorption of newly initiated tubers may have occurred, as was observed in the undisturbed senescing plants (Figure 2A). If so, at late harvest dates, the number of tubers initiated right after the non-destructive harvest, will be higher than the number of tubers observed after 3 weeks, at the final harvest.

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

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

Tuber growth rate and tuber size. The reduction of tuber growth rate caused by a non-destructive harvest, can be attributed to both tuber removal and the damage of roots. Burt (1964) observed lower dry weight gains of tubers, 13 days after removal of tubers. The first four days of the 21 days growth period in our experiment may not have been important for tuber growth. Burt (1964) found new tubers between three and six days after removal of tubers and Marschner et

al. (1984) observed a lag period of four days till normal total tuber growth rates were restored after removal of all fast growing tubers. Oparka (1987), however, showed that tuber removal also reduced final tuber yields under field conditions. Final tuber yields were also reduced after root damage (Oparka, 1987) or regular root pruning (Moore, 1937).

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

Practical consequences

Both undisturbed plants and plants that were harvested twice, produced more minitubers per in vitro propagated plantlet than commonly observed during the production of microtubers. The number of microtubers is only incidentally larger than one (Lillo, 1989), while the number of minitubers per plant produced by undisturbed plants was two and the total number of minitubers produced by plants harvested twice could be four to seven, depending on the cultivar. These minitubers were much larger than microtubers. All minitubers were $\geq$ 0.3 g and had average fresh weights of 5 g if plants grew undisturbed and 1 - 2 g if plants were harvested non-destructively at the optimal moment (Figures 4 and 5).

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

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

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

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