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

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Abstract

In vitro propagated potato plantlets, cultivars Gloria and Spunta, were pre-treated with 10 or 40 mg nitrogen per plant before transplanting to the field to determine after-effects of nitrogen on 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 field growth. 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 affect HI at the end of the season. Nitrogen pre-treatment can improve seasonal light interception of plantlets of transplant crops from early cultivars.

Keywords: 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 from early cultivars 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, based on the effects of such treatments on dry matter allocation in crops from seed tubers (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 paper 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, the latter 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 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 M & S 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 mmol 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 2.85 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 ha^{-1} N 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. The temperature sum (degree-days) is the product of the temperature above 0 °C and time in days. 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 or in the first two weeks of field growth (Fig. 1). Higher nitrogen pretreatment 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 (Fig. 1).

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	*	***	**	*	**	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

***: $P < 0.001$, **: $0.001 \leq P < 0.01$, *: $0.01 \leq P < 0.05$, ns: not significant, $P \geq 0.05$.
[a] DW: dry weight.

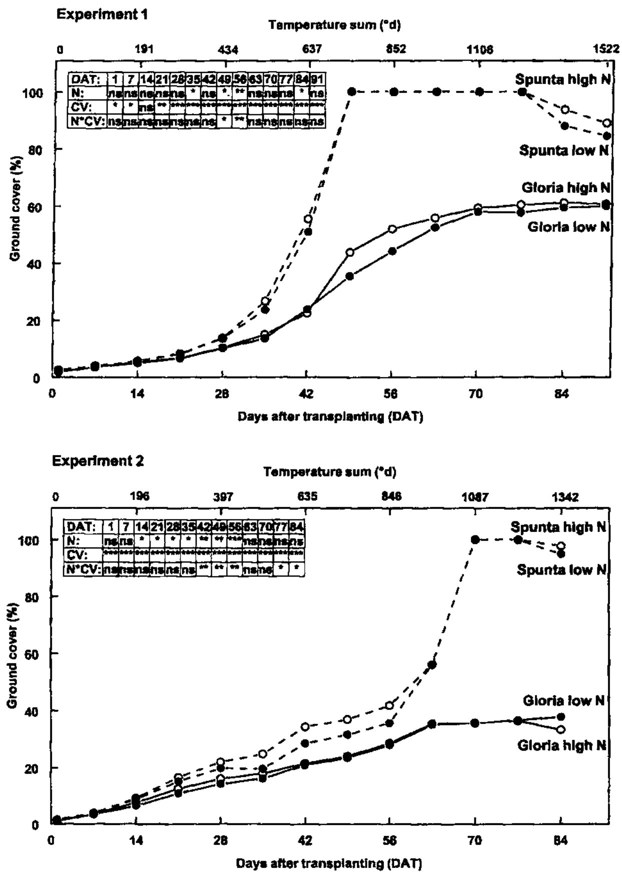

Fig. 1. Weekly ground cover of *in vitro* propagated potato plants of two cultivars pre-treated with 10 (low N) or 40 mg (high N) of nitrogen per plant before transplanting to the field, during the 1997 (Experiment 1) and 1998 (Experiment 2) field experiments, and the significances of the effects of the main factors nitrogen (N) and cultivar (CV) and their interaction. ***: $P < 0.001$, **: $0.001 \leq P < 0.01$, *: $0.01 \leq P < 0.05$, ns: not significant, $P \geq 0.05$.

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 in the first weeks after transplanting but slower thereafter (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.

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 2). 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 2). 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 2). There was no effect of nitrogen on total dry matter production in Experiment 2 (Table 2). 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 (Table 2), but had no effect on either of them in Experiment 2 (Table 2). Nitrogen had no effect on tuber dry matter concentration in both experiments (Table 2).

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.

	N	CV	DAT	N*CV	N*DAT	CV*DAT	N*CV*DAT
Tubers	ns	***	***	ns	**	***	ns
Leaves	*	***	***	ns	**	***	ns
Stems	ns	***	***	ns	ns	***	ns
Roots	ns	***	**	ns	ns	ns	ns

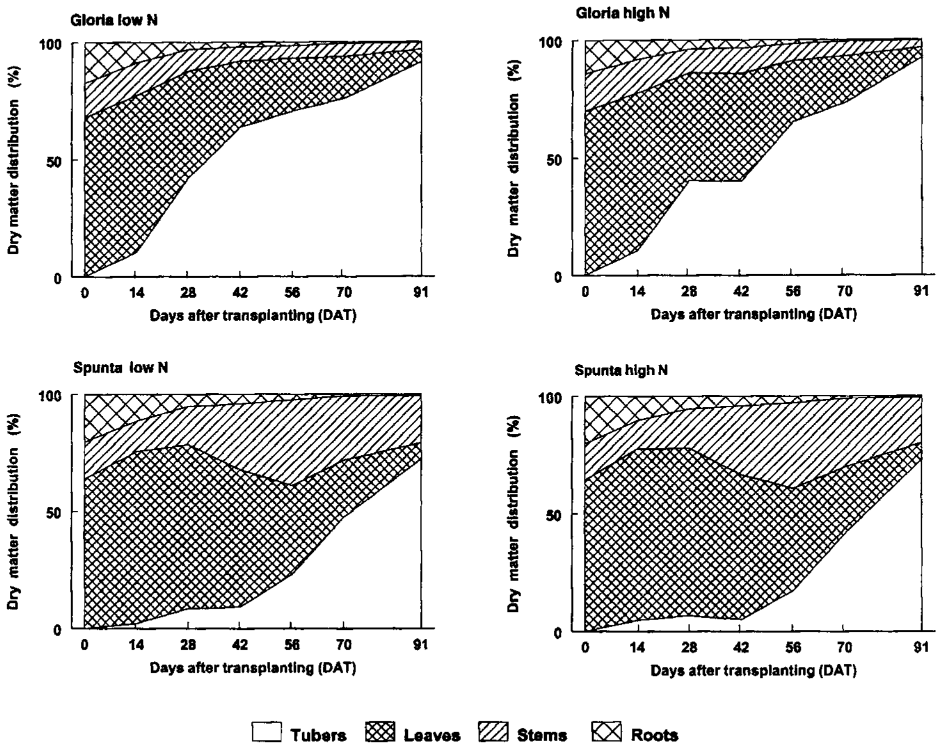

Fig. 2. Proportion (%) of leaves, stems, roots and tubers in the total plant dry matter during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars, pre-treated during transplant production with two nitrogen levels, in 1997 (Experiment 1). For explanation of the statistical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

Table 2. 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	**	***	***	ns	***	ns	***
CV	***	***	***	***	***	ns	***
N*CV	ns	ns	**(142)[c]	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)[c]	ns	ns	ns	ns	ns	ns

***: $P < 0.001$, **: $0.001 \le P < 0.01$, *: $0.01 \le P < 0.05$, ns: not significant, $P \ge 0.05$.
[a] AIR: accumulated intercepted radiation.
[b] DM: dry matter.
[c] LSD (5%) for comparisons of all means.

Tuber dry matter concentration was lower in cultivar Spunta than in cultivar Gloria only in Experiment 2 (Table 2).

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

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). 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). There was, however, no effect of nitrogen on the fraction of tubers in the total plant dry matter in Experiment 2 (Fig. 3).

	N	CV	DAT	N*CV	N*DAT	CV*DAT	N*CV*DAT
Tubers	ns	***	***	ns	ns	***	ns
Leaves	ns	***	***	ns	ns	***	ns
Stems	ns	***	***	ns	ns	***	ns
Roots	ns	***	***	ns	ns	***	ns

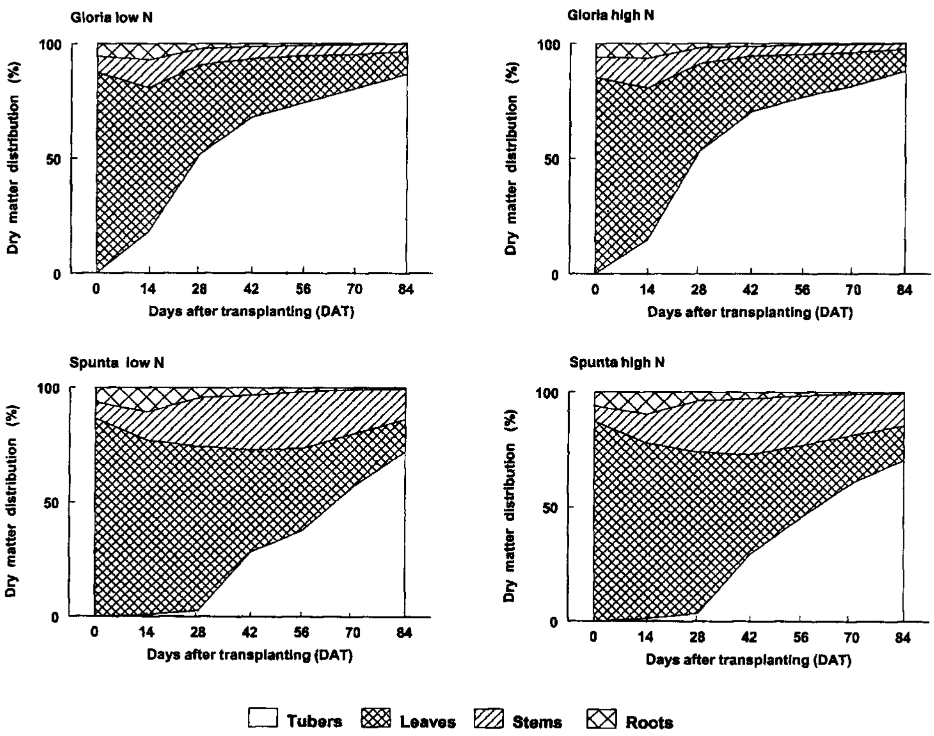

Fig. 3. Proportion (%) of leaves, stems, roots and tubers in the total plant dry matter, during field growth of transplants from *in vitro* propagated potato plantlets of two cultivars pre-treated during transplant production with two nitrogen levels, in 1998 (Experiment 2). For explanation of the statistical significances of the main experimental factors and their interactions, see Fig. 1.

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 first few weeks 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). The data, however, seem to suggest that a higher nitrogen pre-treatment increased tuber number between 28 and 56 DAT in cultivar Gloria and throughout the growing period in cultivar Spunta in Experiment 1 (Fig. 4). A nitrogen-cultivar interaction in Experiment 1 indicated that 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). 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).

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 an early phase of growth. However, even when there is a temporarily adequate supply, nitrogen may promote future haulm 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).

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

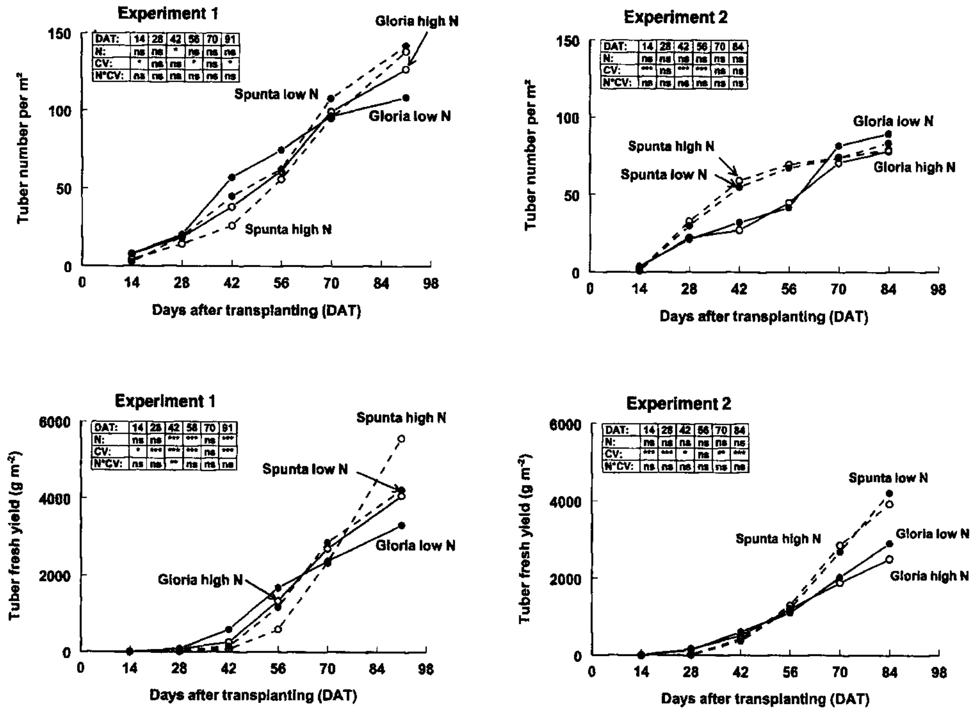

Fig. 4. Tuber number per m^2 and tuber fresh yield (g m^{-2}) of transplants from *in vitro* propagated potato plantlets of two cultivars pre-treated during transplant production with two levels of nitrogen, in 1997 (Experiment 1) and 1998 (Experiment 2). For explanation of the statistical significances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

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 slow 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)) 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.

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. Nevertheless, 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 2; 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 2).

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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