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Nitrogen mobilization, nitrogen uptake and growth of cuttings obtained from poplar stock plants grown in different N regimes and sprayed with urea in autumn

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Summary Nitrogen mobilization, nitrogen uptake and growth of cuttings obtained from poplar stock plants fertigated with different nitrogen (N) treatments and sprayed with urea in autumn were studied. Stock plants propagated from poplar cuttings were trained to a single shoot and fertigated with 0, 5, 10, 15 or 20 mmol l^{-1} N during the first growing season. In October, a subset of stock plants from each N fertigation treatment was sprayed twice with either 3% urea or water, and overwintered outside. In March, total tree biomass and total N concentration and content of stems were estimated for stock plants in each treatment, and cuttings were taken from the middle of each stock plant and stored in plastic bags at 2 °C. In mid-April, cuttings were planted in 7.5-l pots containing N-free medium and grown outdoors with a weekly fertigation with nutrient solution containing 0 or 10 mmol l^{-1} $^{15}NH_4{}^{15}NO_3$. In mid-July, cuttings were harvested, and new shoot (new stems and leaves), shank (old cutting stem) and roots were analyzed for new biomass growth and total N and ^{15}N content. Growth of stock plants was positively related to N supply in the previous growing season. Foliar urea application in autumn had no effect on subsequent stock plant growth even though urea sprays increased both N concentration and content in stem tissues. Biomass growth of cuttings obtained from stock plants was closely related to their N content when the cuttings were grown in an N-free medium regardless of previous treatments applied to the stock plants. When N was supplied in the growth medium, the strength of the relationship between regrowth and N content of cuttings was significantly reduced. Cuttings from stock plants treated with foliar urea and grown in a N-free medium remobilized between 75 and 82% of their total N for new growth, whereas cuttings from plants receiving no urea spray remobilized only between 60 and 69% of their total N for new growth. Current N fertilization of the cuttings reduced the percentage of N remobilized. We conclude that new growth of poplar cuttings in spring was more dependent on currently applied N than on reserve N, and urea N applied as a spray in autumn was more easily remobilized than N taken up by roots during the previous season.

Keywords: biomass, fertigation, ^{15}N, Populus trichocarpa × P. deltoides.

Introduction

Poplar (*Populus* spp.), a fast-growing forest tree species, is widely used for timber, pulp and paper, and has potential as a source of biomass energy (Perry et al. 2001). Because stem cuttings of poplar readily initiate adventitious roots (Hartmann and Kester 1975, Heilman 1999), new poplar plantations are established by large-scale propagation of hardwood cuttings.

Deciduous trees store nutrients, and the early season growth of many species is supported by remobilization of stored nutrients before substantial root uptake occurs in the early spring, resulting in a close correlation between initial growth and quantity of nutrient reserves (Dong et al. 2001). The establishment and growth performance of nursery plants of several tree species is positively related to the amount of nitrogen (N) reserves at planting (Titus and Kang 1982, Millard 1996, Cheng et al. 2002). For this reason, increasing the amount of N reserves has become a goal of orchard and nursery management (Dong et al. 2002).

Foliar urea application is a widely accepted practice in fertilizer management for nursery production of fruit trees and certain ornamental plants. When applied in autumn, foliar application of urea can be used to build up N reserves (Shim et al. 1972, Cheng et al. 2002, Dong et al. 2002) and is often used to increase the quality of nursery plants. There is little information on the effects of foliar urea sprays on cutting propagation of woody plants and no information about the effects of foliar urea application on poplar nursery plants. Poplar cuttings readily initiate adventitious roots, and poplar trees are often propagated from cuttings (Hartmann and Kester 1975). Predictability and ease of rooting makes poplar cuttings a good model system to study N remobilization for new growth. In addition, use of cuttings ensures that all roots and shoots are the result of new growth. Furthermore, when cuttings are grown in a N-free medium or supplied with ^{15}N as the only N source, it is easy to calculate the percentage of N that has been remobilized.

Our study objectives were (1) to evaluate the effects of soil N application during the growing season and foliar urea application in autumn on biomass accumulation and N storage in poplar stock plants; and (2) to determine the effects of the previous N regimes on regrowth performance and N uptake by cuttings.

Materials and methods

Poplar stock plants (UCC-1, a hybrid of *Populus trichocarpa* × *P. deltoides*, Union Camp, Princeton, NJ) were propagated from hardwood cuttings (about 10 cm long) and planted in 7.5-l pots containing a 1:1:1 (v/v) mix of peat moss:perlite: loam soil in mid-April of their first growing season. The stock plants were grown outdoors in Corvallis, OR, and were trained to a single shoot and fertigated weekly until mid-June with 10 mmol l^{-1} N (about 200 mg N plant^{-1}) supplied as a commercial fertilizer (N,P,K, 20:10:20 with micronutrients; Plantex, ON, Canada). Uniform stock plants were selected based on shoot height and randomly assigned to one of five nitrogen fertigation treatments. The selected stock plants were fertigated weekly with 0, 5, 10, 15 or 20 mmol l^{-1} N as NH_4NO_3 from mid-June to mid-August, which supplied about 0–760 mg N plant^{-1}, respectively. All stock plants were well watered. In mid-October, half of the stock plants from each N fertigation treatment received two foliar applications of 3% urea 1 week apart (about 0.7–1.2 g N plant^{-1} depending on the stock plant size), and the remainder of the stock plants from each N fertigation treatment were sprayed twice with water. All stock plants were overwintered outside.

In March of the following year, four stock plants from each N fertigation + urea spray treatment were harvested, and tree biomass and total stem N concentration were determined. Three 10-cm cuttings were taken from the middle of the stem of each stock plant. Two of the cuttings were placed in plastic bags and stored at 2 °C, and the other cutting (stem section between other two cuttings) was freeze-dried and used to determine total N concentration and content.

In mid-April of the second season, the two cuttings from stock plants of each fertigation + urea treatment were removed from storage at 2 °C, soaked in tap water overnight, and planted in 7.5-l pots containing a N-free medium of vermiculite and perlite (1:1, v/v). Half of the cuttings were fertigated weekly with 500 ml of Hoagland's nutrient solution without N, and the remainder were fertigated weekly with 500 ml Hoagland's nutrient solution containing 10 mmol l^{-1} $^{15}NH_4{}^{15}NO_3$ (0.05% ^{15}N atom depleted, ISOTEC, Miamisburg, OH). The experiment thus comprised a factorial design of 5 (fertigation treatments) × 2 (urea treatments) × 2 (^{15}N treatments) with 4 replicates. All of the cuttings were grown outdoors in Corvallis, OR. In mid-July, new plants were harvested and separated into shoots (new stems and leaves), shank (old cutting stem) and roots. All samples were freeze-dried and ground with a Wiley mill (20 mesh) and reground with a cyclone mill (60 mesh) for determination of total N and ^{15}N.

Total N was determined by the Kjeldahl method (Schuman et al. 1973) at the Analysis Laboratory of Oregon State University. The $^{15}N/^{14}N$ ratio in samples was determined from the gas evolved from combustion of powdered tissue with an elemental analyzer coupled with a mass spectrometer at the Laboratory of Isotope Services (Los Alamos, NM) (Craswell and Eskew 1991). The percentage of N derived from labeled fertilizer in each tissue (NDFF%) was calculated as:

$$\mathrm{NDFF\%} = \frac{(\mathrm{atom\%}\,^{15}\mathrm{N})_{\text{natural abundance}} - (\mathrm{atom\%}\,^{15}\mathrm{N})_{\text{tissue}}}{(\mathrm{atom\%}\,^{15}\mathrm{N})_{\text{natural abundance}} - (\mathrm{atom\%}\,^{15}\mathrm{N})_{\text{fertilizer}}} 100$$

The mean natural abundance of ^{15}N in tissues of control samples was 0.3705%. The amount of N taken up by each cutting (new N) was calculated from NDFF% and total tissue N, and amount of new N in each tissue was calculated by multiplying the concentration of new N in tissue by the dry mass of the tissue. Total new N uptake per plant was calculated by summing the new N uptake in different tissues.

All data were subjected to analysis of variance (ANOVA). The correlations between new growth and initial N or current uptake N were calculated based on Pearson correlation (r). Differences between means were assessed by Duncan's multiple-comparison test. All statistical analyses were performed with NCSS 97 Statistical System Software (NCSS, Kaysville, UT).

Results

Stock plant growth

Total biomass of stock plants increased with increasing N concentration in the fertigation solution during their first growing season (Figure 1). A 5 mmol l^{-1} increase in N concentration of the fertigation solution increased total plant biomass by about 56 g. Increasing the concentration of N in the fertigation solution had little effect on root biomass (data not shown). Biomass was preferentially partitioned to shoot as the concentration of N in the fertigation solution increased. The shoot/root ratio increased from 1.1 ± 0.1 for stock plants in the 0 mmol l^{-1} N treatment to 2.3 ± 0.2 for stock plants in the 20 mmol l^{-1} N treatment. Foliar application of urea in autumn did not influence tree biomass or shoot/root ratio by the end of the first growing season, but leaf senescence and abscission was delayed in response to the urea spray (data not shown).

N concentration of stock plants and N content of new cuttings

Increasing the concentration of N in the fertigation solution

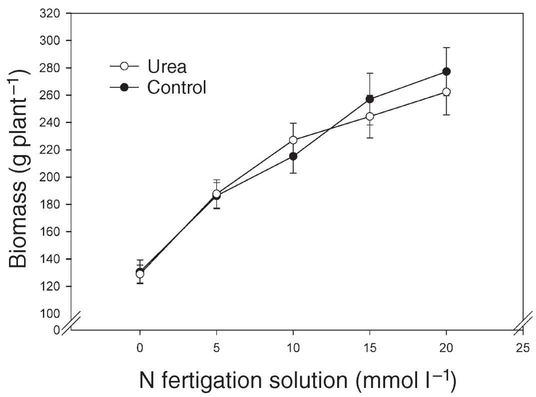

Figure 1. Total biomass of poplar stock plants in March of the second year after being grown at five different nitrogen (N) fertigation concentrations with (○) or without (●) 3% urea autumn spray in the first year. Bars represent standard errors of the mean of four replicates.

from 0 to 20 mmol l^{-1} during the first growing season increased stem N concentration of stock plants from 0.23 to 0.31% (Figure 2A), and increased the N content of new cuttings taken from these stock plants from 8.6 to 20.4 mg cutting^{-1} (Figure 2B). A 5 mmol l^{-1} increase in N concentration of the fertigation solution increased the N content of new cuttings by about 2.3 mg cutting^{-1}. Both the N concentration in stems of stock plants and the N content of new cuttings were significantly ($P < 0.0001$) increased by foliar application of urea to the stock plants in the previous autumn (Figures 2A and 2B). Foliar application of urea to stock plants increased N concentration in stems of stock plants by about 0.3% N and the N content of new cuttings by about 15 mg cutting^{-1}, and the responses were independent of N fertigation treatments applied to the stock plants during their first growing season. The fertigation treatments applied to the stock plants during the first growing season had a smaller effect on the N content of new cuttings than the foliar urea application in autumn.

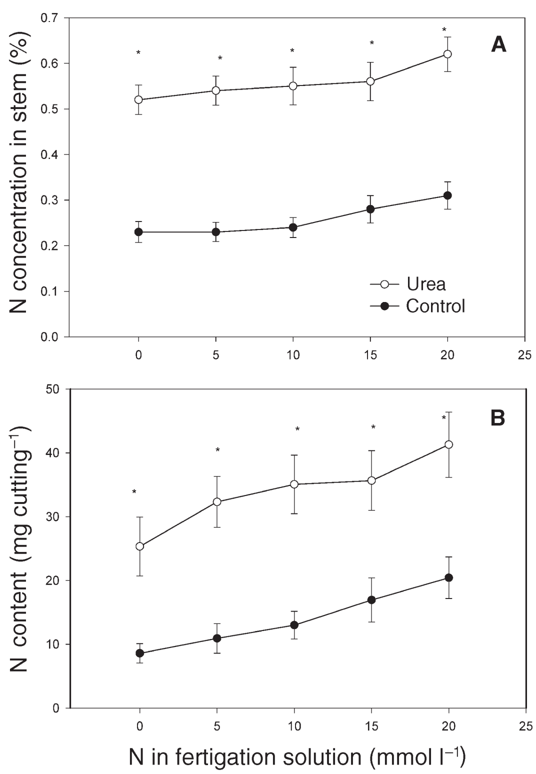

Figure 2. Nitrogen (N) concentration in stems of poplar stock plants (A) and N content of hardwood cuttings (B) taken from stock plants that had been grown at five different nitrogen (N) fertigation concentrations with (○) or without (●) urea (3%) spray in autumn of the first year. Measurements were made in March of the second year. Bars represent standard errors of the mean of four replicates. Asterisks indicate a significant difference between urea and control treatments within a N fertigation treatment ($P = 0.05$).

Biomass growth of new cuttings

Biomass growth of the new cuttings was assessed as the combined biomass of all new growth (stem, leaves and roots). Biomass growth of the new cuttings was influenced by N supply in the second growing season. When new cuttings were grown in N-free medium, there was a positive relationship between N content per cutting at the start of regrowth and total new biomass produced ($r^2 = 0.9005$) (Figure 3A). When N was supplied to the cuttings, total new biomass was significantly increased, but the strength of the relationship between the new growth and N content per cutting at the start of regrowth was

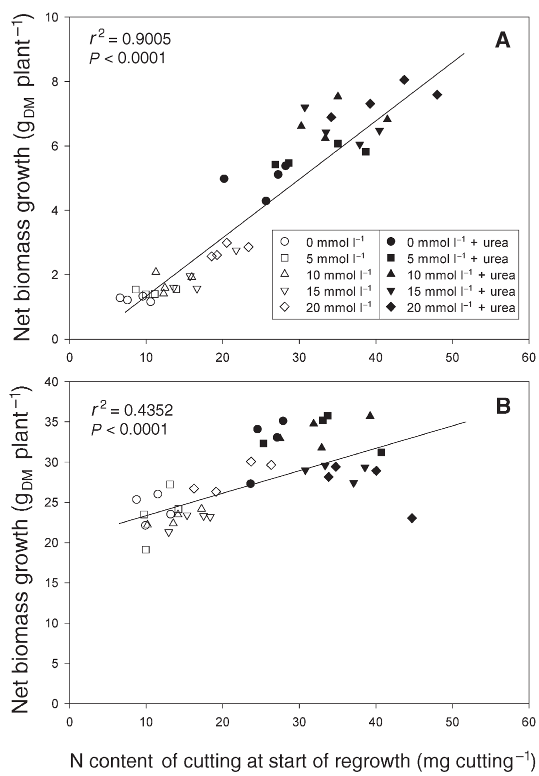

Figure 3. Relationship between new biomass growth (new stems, leaves and roots) and N content of poplar hardwood cuttings growing in a N-free medium (perlite:vermiculite = 1:1 by volume) without a current supply N supply (A) or with 10 mmol l^{-1} NH_4NO_3 (B).

significantly reduced ($r^2 = 0.4352$) (Figure 3B). There was no significant relationship between new growth and current uptake of N ($r^2 = 0.1226$). When grown in a N-free medium, cuttings from stock plants that received urea spray had significantly higher total new biomass than cuttings from stock plants that did not receive urea spray ($P < 0.0001$).

N remobilization from reserves

Cuttings from stock plants that received urea sprays remobilized between 70 and 82% of their total N for use in new growth, whereas cuttings from stock plants that did not receive urea sprays remobilized only between 56 and 69% of their total N (Table 1). The different N fertigation treatments applied to the stock plants in the previous year did not significantly change the percentage of total N remobilized in the new cuttings, but application of N to the new cuttings in the second season significantly reduced the percentage of total N remobilized (Table 1). The different N fertigation treatments applied to the stock plants in the previous year did not significantly change the distribution of remobilized N to new roots and shoots of the cuttings in the second season. In contrast, supplying the new cuttings with 10 mmol l^{-1} NH_4NO_3 generally reduced the amount of remobilized N partitioned to roots and increased the amount of remobilized N distributed to shoots compared with cuttings receiving 0 mmol l^{-1} NH_4NO_3 (Table 2).

Table 1. Percentage of total nitrogen (N) in hardwood poplar cuttings remobilized for new growth (stems, leaves and roots).

N Fertigation[1] (mmol l^{-1})	Control[2]		Urea	
	–N[3]	+N	–N	+N
0	65.7 ± 2.7[4]	60.1 ± 3.0	82.3 ± 1.8	75.9 ± 2.8
5	60.8 ± 4.2	57.3 ± 4.5	79.7 ± 3.5	73.8 ± 7.1
10	68.9 ± 4.8	56.5 ± 6.8	81.2 ± 1.6	76.6 ± 3.6
15	61.9 ± 3.3	55.6 ± 4.7	75.7 ± 4.4	72.2 ± 2.9
20	63.0 ± 4.4	57.5 ± 6.1	76.5 ± 5.1	70.1 ± 3.5

[1] Nitrogen concentrations of fertigation treatments applied to the stock plants in the previous year.
[2] Foliar spray treatments applied to the stock plants in the late season (October) of the previous year. Abbreviations: Control = water; and Urea = 3% urea.
[3] Nitrogen fertilization treatments applied during regrowth of the hardwood cuttings. Abbreviations: –N = no N in the nutrient solution; and +N = nutrient solution containing 10 mmol l^{-1} $^{15}NH_4{}^{15}NO_3$.
[4] Means of four replicates ± SE.

Uptake and distribution of ^{15}N

Nitrogen content of new cuttings and N concentration of stems of stock plants were significantly correlated ($P < 0.0001$ in both cases) with current uptake of N (new N) in cuttings. Cuttings with higher initial N content took up more new N than cuttings with lower initial N content. More than 80% of the new N taken up by the new cuttings was found in new shoot tissues (leaves and stems), about 11–14% in roots and less than 5% in shank (old cutting wood). The distribution of new N in the new cuttings was unaffected by the fertigation and urea treatments applied to the stock plants in the previous season.

Discussion

In many fruit trees, initial growth in spring is positively related to N storage from the previous year, irrespective of whether trees are supplied with N in the spring (Titus and Kang 1982, Millard 1995, Neilsen et al. 1997, Tagliavini et al. 1998, Cheng et al. 2001, Dong et al. 2001). Numerous experiments have shown that foliar urea application in autumn can increase N reserves and improve growth and fruiting of tree fruit crops in

Table 2. Distribution of remobilized nitrogen (N) in new roots and shoots (stems and leaves) of hardwood poplar cuttings.

Tissue	N Fertigation[1] (mmol l^{-1})	Control[2]		Urea	
		–N[3]	+N	–N	+N
Root	0	27.9 ± 2.5[4]	26.7 ± 2.0	36.7 ± 4.9	24.2 ± 2.9
	5	33.1 ± 3.6	26.2 ± 1.1	33.3 ± 2.5	26.7 ± 3.1
	10	32.9 ± 5.9	24.6 ± 4.6	34.7 ± 2.5	27.0 ± 4.6
	15	27.8 ± 3.4	26.2 ± 1.7	32.6 ± 2.3	24.5 ± 4.0
	20	34.9 ± 3.4	25.2 ± 2.1	28.2 ± 1.7	20.6 ± 2.7
Shoot	0	72.1 ± 2.0	73.3 ± 2.5	63.3 ± 4.9	75.8 ± 2.9
	5	66.9 ± 3.6	73.8 ± 1.1	66.7 ± 2.5	73.3 ± 3.1
	10	67.1 ± 5.9	75.4 ± 4.6	65.3 ± 2.5	73.0 ± 4.6
	15	72.2 ± 3.4	73.8 ± 1.7	67.4 ± 2.3	75.5 ± 4.0
	20	65.1 ± 3.4	74.8 ± 2.1	71.8 ± 1.7	79.4 ± 2.7

[1] Nitrogen concentrations of fertigation treatments applied to the stock plants in the previous year.
[2] Foliar spray treatments applied to the stock plants in the late season (October) of the previous year. Abbreviations: Control = water; and Urea = 3% urea.
[3] Nitrogen fertilization treatments applied during regrowth of the hardwood cuttings. Abbreviations: –N = no N in the nutrient solution; and +N = nutrient solution containing 10 mmol l^{-1} $^{15}NH_4{}^{15}NO_3$.
[4] Means of four replicates ± SE.

the following season (Oland 1963, Shim et al. 1972, Han et al. 1989, Sanchez et al. 1990, Khemira et al. 1998, Rosecrance et al. 1998, Cheng et al. 2002). Similarly, we found that foliar application of urea in autumn, when canopy growth had ceased, increased the N concentration and N content in poplar stock plants, independently of N supplied during the growing season. However, total new biomass growth of cuttings from these stock plants was significantly related to the N content of the cuttings only when the cuttings were grown in N-free medium, and the strength of this relationship was dramatically reduced when N was supplied during the regrowth period. The dependence of poplar cuttings on N reserves for new growth differed from that of tree fruit crops, which show little dependence on available soil N during the initial stages of spring growth. This difference may be a result of differences in growth rates between poplar and tree fruit crops. Poplar is a faster growing species than many tree fruit crops, and therefore may require more N to support initial spring growth than that available from storage. However, the difference is more likely associated with our use of cuttings. The root system is a storage organ in deciduous trees (Millard 1995, 1996), and poplar trees with a complete root system may show a different dependence on N reserves for new growth than poplar cuttings. Cuttings are only small segments of stems (10 cm in our experiment), and the amount of N in each segment is small and may not support new growth for a prolonged period. Our finding that N application to the cuttings increased new biomass growth by 5–10 times demonstrates that initial growth of the poplar cuttings depends more on current N application than on N reserves. However, we did not find a high correlation between new growth and uptake of new N, which may indicate that current N was so abundant that it was not the limiting factor for new growth.

Spray application of urea in autumn has been shown to increase N reserves in fruit trees (Shim et al. 1972, Han et al. 1989, Sanchez et al. 1990). We found that urea spray applied in the autumn to stock plants increased the N content of cuttings derived from these plants, and also increased the percentage of N in these cuttings that was remobilized for new growth in the following season (75–82% in cuttings grown in N-free medium and 70–76% in cuttings grown in medium containing N). We conclude that N from urea spray applied to leaves in the autumn is more easily remobilized for use in new growth than N taken up by the roots prior to foliar urea application. Other studies have also shown that N applied late in the season is more likely to be recycled than N applied early in the season (Sanchez et al. 1991, Millard 1995). In late season, active growth has ceased and most N taken up serves as storage N instead of structural N; consequently, an increased percentage of the N taken up late in the season is remobilized for new growth in the following season.

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