for a total of 15 sites (Fig. 2). The recorded litter size for that rat was 11. This apparent transuterine migration by two blastocysts demonstrates that either the normal environment on the ULO side is not too hostile for the implantation process and some subsequent development, or that whatever ingredients are possibly needed can also migrate from the intact side and play a mitigating role, allowing nidation to occur. This cleared uterus provided no evidence of a common lower cervical canal as described by Young (1953).

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NITROGEN ION UTILIZATION BY TULIP POPLAR (Liriodendron tulipifera L.) SEEDLINGS

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ABSTRACT

Growth responses of one-year-old tulip poplar seedlings were determined for different nitrogen sources (NH_4NO_3, NH^+_4, NO^-_3, no nitrogen) at 336 ppm N in nutrient culture. At the end of three months, there were no significant differences in growth observed among treatments in terms of stem elongation, leaf area, and leaf size. After four months, however, seedlings of the NH_4NO_3 treatment exhibited significantly ($P < 0.05$) greater growth (final weight gain and stem elongation) than all other nitrogen sources. Growth was slightly less for the NO^-_3 treatment plants, but compared with NH^+_4 and no nitrogen treatments, both NH_4NO_3 and NO^-_3 treatments exhibited significantly greater growth responses. NO^-_3 is recommended as the sole nitrogen source, especially for small seedlings of tulip poplar.

INTRODUCTION

Species of plants differ in their ability to utilize ammonium and nitrate as sole nitrogen sources. Most investigations relating to such preferential utilization have shown different results, depending on age of the plant, pH, nutrient concentration, and other soil or nutrient media conditions (Street and Sheat 1958). Equal growth of loblolly pine (Addoms 1937), barley (Arnon 1937), and peach (Davidson and Shive 1934) has been shown to occur at a lower pH with NO^-_3 and a higher pH with NH^+_4. Although most studies have involved comparisons of NO^-_3 and NH^+_4 as soil nitrogen sources, Krajina et al. (1973) and Nelson and Selby (1974) have also found greater growth response to combined NO^-_3 and NH^+_4.

Several authors have investigated the effects of different levels of nitrogen on growth of tulip poplar (*Liriodendron tulipifera* L.); however, data are not available on the nitrogen form which is preferred by this species. Our primary interest in this study was in determining which form of nitrogen (NO^-_3, NH^+_4, NH_4NO_3 or no-nitrogen) would result in the greatest growth rate of tulip poplar. For that reason we chose to grow our seedlings at a pH closest to that at which tulip popular appears to make its best growth in natural stands in the Oak Ridge, Tennessee area; this occurs in limestone sinkhole areas where the pH is about six.

Methods

Treatments of NO^-_3, NH^+_4, NH_4NO_3, and no-nitrogen were distributed randomly in a greenhouse, which was heated when outside air temperatures were below 0°C and ventilated above outside temperatures of 24°C, with 14 trees (pots) per treatment. Day length was not controlled and supplemental lighting was not used. The basic nutrient solution used was a modification of Hoagland's solution, lacking nitrogen (Hoaglund and Arnon 1950). Different nitrogen sources (NH_4NO_3, NH_4Cl, $NaNO_3$) were added to all but the no-nitrogen treatment solution, and the pH was adjusted to 6.0 to approximate field pH levels. Three parts per million of emulsifiable N-serve active ingredient 2-chloro-6-(trichloromethyl) pyridine was added to all solutions to prevent nitrification of ammonium to nitrate (Goring 1962). A nitrogen concentration of 336 ppm was selected as the optimum nitrogen level based on Finn's (1966) results for tulip poplar growth in sand culture using ammonium nitrate as a nitrogen source.

Seedling roots were immersed for 2 h in full strength (336 ppm N) nutrient solutions three times per week, immediately after which the solution was drained from the pots to optimize root aeration. An automatic watering system supplied 600 ml demineralized water to each pot every other hour during the day and every fourth hour at night while the drains were open, keeping gravel and roots moist at all times. Five of the 14 trees in each treatment were selected at random from those seedlings with viable terminal buds for measurement of leaf area and terminal shoot length. Total increase in weight at the termination of the experiment was measured for all trees. Initial dry weights were estimated from initial fresh weights.

Leaf growth and shoot expansion were measured weekly. Leaf areas were computed using the equation derived by Harris et al. (1970) for tulip poplar. After four months, the experiment was terminated, roots were examined for general appearance and new root growth, and trees were divided into roots and shoots, dried, and weighed.

To evaluate the possible toxicity of NH_4 to tulip poplar, three-week-old seedlings were grown hydroponically with NH^+_4 concentrations ranging from 42 to 336 ppm.

Results

Seedlings with NH_4NO_3 and NO^-_3 grew much more rapidly than those with NH^+_4 or no-nitrogen (Fig. 1). Growth responses to treatments which were significantly different (according to ANOVA) always ranked in the same order: ammonium nitrate > nitrate > ammonium > no nitrogen (Table 1). A Duncan's multiple range test showed that mean values calculated for dry weight gain, leaf size, and total terminal shoot leaf area of the NH_4NO_3 and NO^-_3 treatments were significantly greater ($P < 0.05$) than NH^+_4 and no-nitrogen. Terminal shoot elongation ($N = 4$ or 5) and weight gain of all trees ($N = 14$) were significantly greater ($P < 0.05$) using NH_4NO_3 than using NO^-_3. No significant differences in growth responses were observed between NH^+_4 and no-nitrogen treatments.

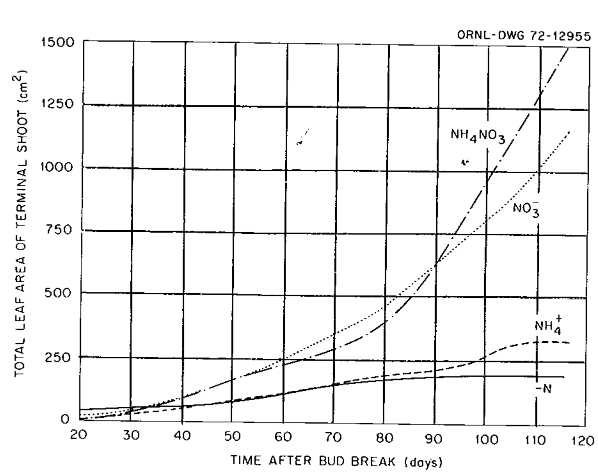

FIG. 1. *Cumulative increase in terminal shoot leaf area for tulip popular growing in different nitrogen solutions.*

TABLE 1. *Mean values of growth parameters for different nitrogen treatments. Values were determined at the termination of the experiment 120 d after bud break; means are calculated for five trees in the NO^-_3, NH^+_4, and no-nitrogen treatments and four trees in the NH_4NO_3 treatment. Means of change in weight are also calculated for all trees in each treatment ($N = 14$ for NO^-_3, NO^+_4, and no nitrogen; $N = 13$* for NH_4NO_3). Lines beneath means indicate no significant differences among treatments (Duncan's multiple range, $P < 0.05$).*

Growth parameter	NH_4NO_3	NO^-_3	NH^+_4	NO-N
Shoot elongation (cm)	41.7	25.8	7.32	4.62
Total leaf area of terminal shoot (cm2)	1506.1	1195.6	374.4	192.9
Largest leaf size (cm2)	196.9	149.4	67.0	39.3
Number of leaves per tree	14.0	16.4	14.2	10.2
Root/shoot ratio (g/g)	0.66	0.68	0.62	0.76
Change in wt (g) ($N = 4$* or 5)	18.9	18.1	1.1	1.3
Change in wt (g) ($N = 13$* or 14)	17.0	9.5	1.1	-0.4

* One of the trees from the NH_4NO_3 treatment died after 6 weeks, resulting in one less observation for this treatment.

Although there was no appreciable difference in appearance of shoots, leaves, or roots of seedlings receiving NH_4NO_3 versus those receiving NO^-_3, there was a striking difference in appearance of those receiving NH^+_4 versus no-nitrogen. Ammonium nitrate- and NO^-_3 supplied trees generally appeared healthy with bright green leaves, and roots also appeared quite healthy. Leaves of trees receiving no nitrogen became yellow and were leathery in texture. Few new roots were initiated by trees receiving no nitrogen, but root mortality was not evident. Leaves of ammonium-supplied trees that grew at all were dark blue-green and appeared wilted; roots grew in some cases but mortality and decay of branch roots was very evident.

In the ammonium toxicity experiment, 100% mortality occurred at concentrations > 84 ppm N (NH^+_4) within three weeks, but seedlings growing in all concentrations of NO^+_3 and NH_4NO_3 grew better than seedlings remaining in germination flats.

CONCLUSIONS

Although the presence of excess free NH^+_4 in plant cells is believed to be toxic, there is limited direct information on the character of this effect. While NH^+_4 toxicity was suspected in our three-week-old seedlings, the possible low concentrations of carbohydrate (organic acids) in very young seedlings may have been responsible for complete mortality at $\geqq$ 84 ppm NH^+_4, since there is some evidence for the influence of carbohydrates on NH^+_4 metabolism (McKee 1962) to nontoxic organic nitrogen compounds.

Several authors have investigated the effects of different levels of nitrogen on the growth of tulip poplar, although none compared types of nitrogen fertilizers. Ammonium has been used successfully as a nitrogen source in field fertilization experiments. Ike (1962) and Farmer et al. (1970) found that seedling growth approximately doubled in the first five years by using diammonium phosphate (36.7 kg N/ha) and ammonium nitrate (102 kg N/ha), respectively, and Finn and White (1966) found that 20-year-old slow growing trees doubled in growth in response to an undefined mixed fertilizer containing approximately one-half of the nitrogen as urea formaldehyde (61.7 kg N/ha). Nitrification rates were not taken into consideration in any of these studies, so the actual ionic form of nitrogen absorbed was not defined. However, Cummings (1941) found no response of one-year-old tulip poplar seedlings to NH_4SO_4 (55 kg N/ha) in acidic, sterile, strip mine spoil banks. Under such soil conditions, nitrification rates would be slow, and most nitrogen may have remained in the NH^+_4 form. In addition, seedlings were small (20-30 cm tall) and would not have had much carbohydrate reserve.

Within limits, the data from this study can be extrapolated to natural forest ecosystems. Seedling growth with NO^-_3 compared favorably to that observed with the balanced N-source (NH_4NO_3) at a pH found on optimum sites. In contrast, negligible tree growth occurred with NH^+_4. The favorable growth response to ammonium fertilizers in fertilizer trials in the past may have been due to nitrification of ammonium to nitrate. Our results provide evidence that either NH^+_4 or NO^-_3 can be absorbed by tulip poplar seedlings and that seedlings can utilize both NO^-_3 and mixed forms of nitrogen. Mature trees with greater carbohydrate reserves may be able to assimilate nitrogen in the NH^+_4 form alone. However, the apparent greater growth using a balanced nitrogen source should be taken into consideration in tulip poplar fertilization efforts, especially in establishing seedlings or in soils expected to be low in nitrifying capability.

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FIG. 1. *Cumulative increase in terminal shoot leaf area for tulip poplar growing in different nitrogen solutions.*

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