Nickel Supplements Improve Growth when Foliar Urea is the Sole Nitrogen Source for Tomato

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ABSTRACT. Concentrations of up to 1.0 μM NiCl_2 in a nutrient solution improved growth of tomato (*Lycopersicon esculentum* Mill. 'T-5') seedlings that received foliar urea as their sole nitrogen source. Nickel in the nutrient solution decreased the amount of urea present in the shoots and increased the amount in the roots, although it had no significant effect upon leaf urease activity. These results indicate that a) the presence of nickel in the nutrient solution improves growth of plants receiving foliar urea and b) the effect of nickel was related more to increased urea translocation from shoot to root than to enhanced leaf urease activity.

Foliar applications of urea may be phytotoxic even in moderate amounts (Krogmeier et al., 1989, 1991; Peltonen, 1993; Barel and Black, 1979). In a previous study (Nicoulaud and Bloom, 1996) plants treated with foliar applications of urea as the sole nitrogen source grew less than plants receiving NH_4NO_3 in the nutrient solution apparently because of urea toxicity. A possible cause of urea phytotoxicity is the NH_4^+ released during urea assimilation. High levels of NH_4^+ in plant tissues are toxic because they dissipate the pH gradients across biological membranes that are necessary for many metabolic processes including photosynthesis and respiration (Bloom, 1997). One approach that can be used to differentiate between the direct effects of urea and those of its breakdown products, such as NH_4^+ , is to apply urease inhibitors. Using phenylphosphorodiamidate to inhibit urease activity, Krogmeier et al. (1989) concluded that phytotoxicity from foliar urea applications derived from urea itself rather than NH_4^+ . This conclusion was supported by a subsequent experiment in which nickel deficient plants showed lower urease activity and more leaf-tip necrosis than nondeficient plants (Krogmeier et al., 1991). In wheat (*Triticum aestivum* L.), the necrotic leaf area was positively correlated to the total leaf urea content (Peltonen, 1993). The reasons for urea toxicity are not yet known.

One possible way to avoid urea toxicity is to increase urease activity. Urease is the first enzyme involved in urea assimilation by plant leaves (Hogan et al., 1983) and catalyzes the hydrolysis of urea. It is a nickel metalloenzyme of high molecular weight (≈ 590 kD) (Benchemsi-Bekkari and Pizelle, 1992). Leaf urease activities differ greatly among plant species (Hogan et al., 1983). For example, soybean (*Glycine max* L.) has a high leaf urease activity expressed as NH_3 evolved on a fresh mass basis ($20 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$), whereas tomato has low urease activity ($0.24 \mu\text{mol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$). Urease activity is strongly nickel dependent. *Robinia pseudoacacia* showed more than a 10-fold increase in urease activity when $10 \mu\text{M}$ of NiCl_2 was present in the nutrient solution (Benchemsi-Bekkari and Pizelle, 1992). Similar results were obtained for soybeans (Krogmeier et al., 1991).

In the following experiments, we tested whether or not the addition of nickel to a nutrient solution would increase leaf urease activity and improve growth of plants receiving foliar application of urea as the sole nitrogen source.

Material and Methods

Tomato seeds (*Lycopersicon esculentum* 'T-5') obtained from Dr. Richard Jones, Dept. of Vegetable Crops, Univ. of California, Davis, were germinated in the dark at 25°C in rolled germination paper standing in 2 mM CaSO_4 . After 7 d, seedlings were transplanted to 1.7 L opaque plastic containers at six plants per container. The containers were filled with a modified Hoagland's solution (Epstein, 1972) consisting of 2.0 mM CaSO_4 , 1.0 mM MgSO_4 , 0.6 mM K_2SO_4 , 1.0 mM K_2HPO_4 , micronutrients, iron, and $50 \mu\text{M}$ NH_4NO_3 . The nutrient solution was continuously bubbled with air. Plants were grown in the laboratory for 2 d at 20°C and with a PFD of 50 to $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at plant height during a 10-h day before they were transferred to a greenhouse. In the greenhouse, the containers were immersed in a water bath to maintain root temperature at $20 \pm 2.0^\circ\text{C}$. The plants were grown for 7 d before being transferred to a nutrient solution identical to that described above except it did not contain nitrogen. After 4 d, the plants were weighed and fitted with a 4 cm diameter, closed-cell foam plug that fit tightly around the stem. Plants then received $50 \mu\text{M}$ NH_4NO_3 in the nutrient solution or 0.2% (m/m) urea solution applied foliarly as the sole nitrogen source, and either 0.0, 0.1, 1.0, 10.0, or $100.0 \mu\text{M}$ NiCl_2 in the nutrient solution. Nutrient solutions were changed every day.

The urea treatment was applied with a handheld sprayer. The surfactant α -alkylaryl- Ω -hydroxypoly (oxyethylene) glycol ("Spray-Mate", National Chelating Corporation, Orange, Calif.) was added to the urea solutions in the proportion of 0.3% v/v. Plants were sprayed daily to drip point.

The plants were grown for 10 d and harvested. The plants were then dipped in liquid nitrogen, freeze-dried, weighed, ground, and analyzed for NH_4^+ and urea. Tomato plants at this developmental stage (four true leaves) show exponential growth (Smart and Bloom, 1993) so the relative growth rate was calculated by the formula $\text{RGR} = \ln(M_{\text{final}}/M_{\text{initial}})/t$, where RGR is the relative growth rate in $\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$, M_{final} is the plant fresh mass at the end of the experiment in g, M_{initial} is the plant fresh mass at the beginning of the experiment in g, and t is the duration of the experiment in days.

NH_4^+ and urea in the plant tissue were extracted with 1.0 mM CaSO_4 . NH_4^+ was determined with a custom automated system. In this system, 80 μL of 5.0 N KOH and 200 mM diethylene-

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triaminepentaacetic acid (DTPA) were added to 4 mL of sample, increasing the pH to ≈ 12 and chelating divalent cations. This mixture then flowed past a NH_3 -selective electrode (model 95-12; Orion Research, Boston), and the peak heights were recorded. Urea was analyzed by digestion of urea to NH_4^+ with an excess of urease (EC 3.5.1.5.; Sigma Chemical, St. Louis) and the NH_4^+ released determined as described above.

To determine the effects of nickel on in vivo leaf urease activity, tomato seedlings were grown using the above methods. After being transferred to a greenhouse, plants were grown for 7 d with $50 \mu\text{M}$ NH_4NO_3 in the nutrient solution and then placed in a nutrient solution without nitrogen for 4 d. Subsequently, the plants received one of four treatments for 10 d: a) $50 \mu\text{M}$ NH_4NO_3 and $1.0 \mu\text{M}$ NiCl_2 in the nutrient solution, b) $50 \mu\text{M}$ NH_4NO_3 and no nickel in the nutrient solution, c) 0.2 % (w/w) urea solutions applied to the leaves with $1.0 \mu\text{M}$ NiCl_2 in the nutrient solution, or d) 0.2 % (w/w) urea solutions applied to the leaves and no nickel in the nutrient solution. The method of Benchems-Bekkari and Pizelle (1992) was modified to estimate in vivo urease activity. Eighteen hours following the last urea application, one 10-mm leaf disk per sample was collected and vacuum infiltrated with a 10 mM phosphate buffer (pH 7.0) containing 0.1 % Triton X-100 and either 0.0 or 0.1 M urea. The disk was then incubated for 2 h at 30°C . After incubation, the amount of NH_4^+ released from the disk was determined and urease activity calculated from the difference between the amount of NH_4^+ released from the samples with and without urea.

Statistical analysis was performed with a one-way completely randomized ANOVA. We deemed probabilities of $<5\%$ as significant.

Results

Additions of 0.1 and $1.0 \mu\text{M}$ NiCl_2 to a nutrient solution increased the relative growth rate of plants receiving foliar applications of 0.2% (w/w) urea as the sole nitrogen source (Fig. 1). Concentrations of 0.1, 1.0, and $10.0 \mu\text{M}$ NiCl_2 did not affect the relative growth rate of plants growing with $50 \mu\text{M}$ NH_4NO_3 in the nutrient solution. Toxic effects were observed with the $100.0 \mu\text{M}$ nickel concentration for both the plants growing under foliar urea and those growing under NH_4NO_3 (Fig. 1).

The in vivo leaf urease activity was similar for all treatments ranging between 1.6 and $1.9 \mu\text{mol NH}_4^+$ released per gram fresh mass of leaf per hour (Fig. 2).

For plants receiving foliar urea or NH_4NO_3 in the nutrient solution, the presence of up to $10.0 \mu\text{M}$ NiCl_2 in the nutrient solution had little influence upon shoot NH_4^+ concentrations, whereas $100.0 \mu\text{M}$ NiCl_2 increased shoot NH_4^+ concentrations (Fig. 3). NiCl_2 concentrations of 0.1, 1.0, and $10.0 \mu\text{M}$ increased the root NH_4^+ concentrations of plants growing under 0.2 % urea solution as the sole nitrogen source and, with the exception of the $1.0 \mu\text{M}$ concentration, the same occurred with plants growing with $50 \mu\text{M}$ NH_4NO_3 in the nutrient solution. At $100.0 \mu\text{M}$ NiCl_2 , root NH_4^+ declined (Fig. 3).

Additions of up to $10.0 \mu\text{M}$ NiCl_2 decreased shoot urea concentrations in the plants receiving foliar urea; shoot urea concentrations of plants growing under NH_4NO_3 were relatively low and unaffected by nickel treatments (Fig. 4). For plants treated with foliar urea, root urea concentrations increased with increasing NiCl_2 concentrations up to $10.0 \mu\text{M}$, whereas for plants treated with NH_4NO_3 , the reverse trend was observed (Fig. 4).

Discussion

Moderate levels of nickel in a nutrient solution promoted the growth of tomato plants receiving foliar urea, but had little effect

Fig. 1. Relative growth rate of 3-week-old tomato plants receiving different concentrations of NiCl_2 in the nutrient solution. Plants were grown with $50 \pm 10 \mu\text{M}$ NH_4NO_3 in the nutrient solution, were deprived of nitrogen for 4 d, and then received for 10 d various concentrations of NiCl_2 and either foliar application of 0.2% (w/w) urea solution (closed bars) or $50 \pm 10 \mu\text{M}$ NH_4NO_3 (open bars) in the nutrient solution as the sole nitrogen source. Relative growth rate was calculated over the treatment period. Shown are means $\pm$ SE for six replicate plants.

upon control plants receiving NH_4NO_3 in the nutrient medium (Fig. 1). Neither the nickel supplement that was optimal for growth, nor foliar applications of urea influenced urease activity (Fig. 2). This contrasts with previous studies on barley (*Hordeum vulgare* L.; Chen and Ching, 1988) and wheat (Peltonen, 1993) that found a much higher urease activity in urea-treated plants and a

Fig. 2. In vivo leaf urease activity on a fresh mass basis for 3-week-old tomato plants treated with (open bars) or without (closed bars) NiCl_2 in the nutrient solution. Plants received either foliar application of 0.2% (w/w) urea solution or $50 \pm 10 \mu\text{M}$ NH_4NO_3 in the nutrient solution as the sole nitrogen source. Shown are mean $\pm$ SE for six replicate plants.

Fig. 3. Shoot and root NH_4^+ concentrations for 3-week-old tomato plants receiving different concentrations of NiCl_2 in the nutrient solution. Plants received either foliar application of 0.2% (w/w) urea solution (closed bars) or $50 \pm 10 \mu\text{M}$ NH_4NO_3 in the nutrient solution (open bars) as the sole nitrogen source. Shown are mean $\pm$ SE for six replicate plants.

correlation between higher urease activity and faster growth (Benchemsi-Bekkari and Pizelle, 1992). The urease activities found here were 10-fold higher than those previously reported for tomato (Hogan et al., 1983). The previous study examined mature tomato plants, whereas we examined seedlings. Urease activity can decline 10-fold with leaf age (Benchemsi-Bekkari and Pizelle, 1992), and this may explain the discrepancies between the two studies.

Fig. 4. Shoot and root urea concentrations for 3-week-old tomato plants receiving different concentrations of NiCl_2 in the nutrient solution. Plants received either foliar application of 0.2% (w/w) urea solution (closed bars) or $50 \pm 10 \mu\text{M}$ NH_4NO_3 in the nutrient solution (open bars) as the sole nitrogen source. Shown are mean $\pm$ SE for six replicate plants.

At the high nickel levels ($100 \mu\text{M}$), both NH_4^+ and urea accumulated in the shoot (Figs. 3 and 4) and growth was diminished (Fig. 1). In a nickel-sensitive species, *Silene italica*, root growth and cell division was inhibited at nickel concentrations as low as $7.5 \mu\text{M}$ (Gabbriellini et al. 1990).

Plants given foliar applications of urea as the sole nitrogen source grew slower than those grown receiving NH_4NO_3 in the nutrient solution (Fig. 1) confirming our previous observations (Nicoulaud and Bloom, 1996). Here, plants grown with foliar urea or NH_4NO_3 in the nutrient medium had similar concentrations of NH_4^+ in their shoots as well as in their roots (Fig. 3), indicating that the diminished growth under foliar urea was not the result of nitrogen deficiency. The diminished growth in the foliar-urea treatments seemed related to the larger concentration of urea present in the shoots (Fig. 4). Negative effects of foliar urea upon plant growth has been reported previously (Krogmeier et al., 1989; 1991; Peltonen, 1993). In the most drastic cases, foliar urea results in leaf necrosis (Peltonen, 1993). Whether phytotoxicity results from a direct effect of urea itself or from a product of its assimilation such as NH_3 has been addressed in two previous studies. In the first, inhibitors of urease activity indicated that urea itself was responsible for phytotoxicity in soybeans (Krogmeier et al., 1989). The second study used nickel deficient plants with low urease activities and came to similar conclusions (Krogmeier et al., 1991). Both studies support the contention that urea itself can be phytotoxic.

In this study, nickel in the nutrient solution may have decreased the amount of urea present in the shoot by increasing urea translocation from shoot to root. Differences in urea levels on a whole root basis corresponded to the total amount of urea disappearing from the shoots (Fig. 4). When urea was foliarly applied to soybeans, only a small percentage of the urea was found in the roots 18 h after the application (Vasilas et al., 1980; Morris and Weaver, 1983). A higher level of translocation was found in alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) in a field experiment where ^{15}N distribution in the plants was measured a month after the ^{15}N -urea application (Zebarth et al., 1991).

That nickel may play a role in urea translocation would indicate a new function for this element. Only recently has nickel been proposed to be an essential micronutrient for higher plants (Brown et al., 1987). The roles of nickel in plant metabolism remain mostly unknown; however, it was shown to have a role in transport of nutrients to the seed (Brown et al., 1987) in addition to its role as a cofactor for urease.

In summary, the results presented here indicate that 0.1 to $1.0 \mu\text{M}$ nickel in a nutrient solution enhanced growth in tomato seedlings receiving foliar urea applications. Diminished leaf urea content correlated with the growth enhancement. The diminished leaf urea content seemed to derive more from improved urea translocation from shoot to root than from any changes in urease activity.

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