NITROGEN TRANSFORMATIONS IN THE TOBACCO FLOAT TOBACCO SYSTEM[1]

R. C. Pearce, Y. Zhan, and M. S. Coyne[2]

Fertilizers containing urea may cause yellowing and stunted growth of tobacco transplants grown in the float system. The objective of this study was to determine the reason(s) for this poor growth. Float beds of a size to accommodate individual trays were prepared and filled with 40 L of water. Three commercial fertilizers containing 0%, 40%, or 80% of the total nitrogen (N) as urea-N were added at a rate of 100 mg L^{-1} total N. Two-hundred-cell polystyrene trays were filled with soilless media and 12 were seeded with TN90 burley primed tobacco seed; 12 additional trays were filled with soilless media and floated without seed. Float water and media solutions were collected weekly and analyzed for urea-N, ammoniacal-N (NH_4^+-N), nitrite-N (NCV-N), and nitrate-N (NO_3^--N). Media solutions were collected by vacuum filtration. Most probable number techniques were used to estimate the population of urease producing organisms and nitrifiers in the growing media. The untreated media had an estimated population of 3.0×10^5 urea decomposers g^{-1} dry media and 0.2×10^5 nitrifiers g^{-1} dry media. Urea-N in the float water slowly disappeared during the six-week growth period even in the absence of growing plants. No urea-N was recovered from the media solutions, suggesting rapid urea decomposition in the media. Ammoniacal-N decreased very slowly in the float water, but was rapidly nitrified in the media solution. Nitrate-N in the float water remained relatively stable until three weeks after seeding, when levels decreased in the seeded trays due to plant uptake. In the unseeded trays, NO_3^--N increased during this period. Nitrite-N accumulated to 60 to 80 mg L^{-1} at 2 to 3 wk after seeding, in float beds where 80% urea-N was used. Stunting of plants in these treatments was observed. The results of this study suggested that the reason for poor plant performance with urea fertilizers is due to nitrite toxicity.

Additional key words: nitrite toxicity, urea hydrolysis, nitrification, *Nicotiana tabacum* L.

INTRODUCTION

Over 75% of all burley tobacco *(Nicotiana tabacum* L.) transplants are produced in containerized systems, with most being grown in float systems. Water soluble fertilizers with high urea-N contents have caused stunting and death of transplants in the float system (8). In 1995, several tobacco producers in Kentucky were forced to discard numerous chlorotic, stunted plants because a water soluble 20-10-20 fertilizer was used, in which nearly all the nitrogen was supplied as urea-N. Flue-cured tobacco is also adversely affected by urea in the float water (12).

The soilless media used in the tobacco float systems typically does not contain any field soil. Such mixes have several advantages over soil based systems. Many of the components used in the media (peat, vermiculite, perlite) are nearly sterile, such that microbial populations are relatively low in the media as compared to field soils. This may reduce the potential for soil borne diseases, but it also means that populations of beneficial organisms, such as nitrifying bacteria, are low or nonexistent. The activity of some enzymes such as urease may also be different in the soilless media compared to field soils.

[1]Contribution (paper no 98-06-74) of the Kentucky Agric. Exp. Stn., published with the approval of the director.
[2]Assistant Extension Professor, Visiting Scientist, and Associate Professor, respectively. Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091.

Contribution received May 8, 1998. Tob. Sci. 42:82-88, 1998

The horticulture industry has been using soilless media for 15 to 20 yr. Studies with these media show that urea hydrolysis generally occurs to a much greater extent than nitrification (4). It has been suggested that this is due to the stability of urease under adverse conditions, and to the presence of urease in peat (4). Nitrifying bacteria, on the other hand, are known to be relatively scarce in peat (6). The hydrolysis of urea in the absence of nitrification, or the partial nitrification of ammonia to nitrite could lead to toxic conditions for plants fertilized with urea. Elliot (4) observed the accumulation of nitrite in soilless media that had been cropped for four weeks. He also reported that the inherent rates of urea hydrolysis and nitrification differed considerably from one type of potting media to the next.

Even though nitrifier populations are initially low in most soilless mixes, several studies show that the nitrification rate increases as a crop is grown in the media (4,7). In these studies, nitrification peaked at 4 to 6 wk of cropping, and then declined. The reason for a decline was not discussed. Such patterns could influence the availability of nitrogen to plants and the accumulation of toxic components. In most cases, tobacco transplants will only be in the float system for 7 to 8 wk, but may be held for longer periods. High fertilizer rates or a high proportion of NH_4^+-N also decrease nitrification activity in soilless media (7).

The aeration status of media in tobacco float systems may also influence the rates of nitrogen transformations. Nitrifying bacteria are obligate aerobes. In the float system, the media surface is often less than 7 cm from the water level. This results in nearly saturated conditions in the media, and may limit oxygen diffusion. The objective of this study was to investigate the transformation of urea-N in a typical tobacco float system, and to determine why fertilizer with urea-N results in stunting of tobacco transplants.

MATERIALS AND METHODS

A large float bed was constructed on the floor of a glass greenhouse at The University of Kentucky campus in Lexington, Kentucky. The bed was subdivided into units to accommodate single polystyrene float trays. Each individual unit was filled with 40 L of water, giving a water depth of approximately 15 cm. The fertilizer treatments consisted of three commercially available water soluble fertilizers (Table 1). One of the fertilizers did not contain any urea, one had 40% of the total N as urea-N, and the third had 80 % of the total N as urea-N. The fertilizer that contained 40% urea-N was 20-20-20 grade fertilizer, while the other two products were 20-10-20 grade. The additional phosphorous (P) in the 20-20-20 fertilizer was not expected to affect the results, since previous studies have shown that reducing the P rate in the float system did not affect the growth of tobacco seedlings until the solution N:P ratio was less than 20:1 (9). Just before tray seeding, the fertilizers were added to the individual float units. An appropriate amount of fertilizer was weighed and dissolved in the last 500 mL of water that was added to the bed to give an initial concentration of 100 mg L^{-1} total N in the float water. All units were thoroughly mixed after fertilizer addition.

Polystyrene Todd Cells (200 cells/ tray) were filled by hand with a soilless media manufactured by The Scotts Co. (Marysville, OH) composed of 55% to 65% vermiculite and 35% to 45% peat moss. A water saturation extract of samples of a different lot of the same brand of media was analyzed for phosphorous (3.3 mg P L^{-1}), potassium (193 mg K^{-1}), calcium (267 mg Ca L^{-1}), magnesium (194 mg L^{-1}), nitrate-N (115 mg NO_3^--N L^{-1}), and pH (6.25). Half of the filled trays were seeded with primed and pelleted TN90 burley tobacco seed and floated on the nutrient solution. The other half of the trays were floated without seed. Trays were arranged in a randomized complete block design with four replications.

Samples of the nutrient solutions were taken just after fertilizer addition and at weekly intervals thereafter. Before each sampling the water levels were adjusted to the original level, and the solutions were thoroughly mixed. Solution samples were refrigerated immediately after

Table 1. Form of nitrogen in three commercial fertilizers used in this study.

Fertilizer Grade	20-10-20	20-20-20	20-10-20
	----------% ----------		
NO_3^--N	12	6	—
NH_4^+-N	8	6	4
Urea-N	—	8	16
Urea-N/Total-N	0	40	80

collection, and analyzed within 48 h. Samples of the solution from the media matrix were collected at weekly intervals. The moist media was collected from five cells per tray. The solution was extracted by vacuum filtration through Whatman #42 filter paper. The collected solutions were refrigerated and analyzed within 48 h.

The plants were not clipped. Plant samples were collected at four, five, and six weeks after seeding. The plants were separated into roots and shoots, and oven dried at 70°C for 48 h. Plant tissues were ground and analyzed for total N, P, and K.

Ammoniacal-N in all samples was determined with *a* Technicon auto-analyzer utilizing the phenol hypochlorite (Berthelot) reaction. Nitrite-N was determined colorimetrically by the Griess reaction. Nitrate-N was determined by cadmium reduction to NO_2^--N which was then determined colorimetrically. Nitrate-N was corrected for the presence of NO_2^--N in the samples. Urea N was determined by the difference in NH_4^+-N after the addition of excess urease to the sample.

The Most Probable Number (MPN) technique outlined by Woomer (14) was used to estimate urease-producing and nitrifying bacteria populations. A medium or water sample was serially diluted 10-fold in sterile physiological saline (0.85% NaCl in distilled water) and 1 mL of each dilution was used to inoculate each of five replicate culture tubes. The culture tubes contained Christensen urea broth to support the growth of urease producers (11) or inorganic salts media with NH_4^+ to support the growth of autotrophic nitrifiers (10). The culture tubes were incubated 4 to 6 wk at 26°C until physiologically specific microbial activity was observed. For urease producers, this was sufficient alkalization of the urea broth to change a pH indicator (phenol red) from yellow to red. For nitrifiers, NO_2^- production was determined by a modified Griess-Ilosvay spot test (10) or NO_3~ production was determined by a spot test with acidic diphenylamine (13). The number of culture tubes showing specific microbial activity at each dilution was compared to published tables for MPN analysis (14) and used to estimate the MPN bacteria per gram of medium. The 95% confidence interval for this MPN design was ± 3.3 x calculated MPN.

RESULTS AND DISCUSSION

The MPN estimate of the population of urease producers in the medium prior to treatment was 3.0 x 10^5 organisms g^{-1} dry media. The estimated nitrifier population was 0.2 x 10^5 organisms g^{-1} dry media. These populations were lower than is typically found in natural mineral soils.

The disappearance of urea-N from the float water is shown in **Figure 1.** This suggested that urease or ureolytic organisms were present in the float water causing a slow, but steady

Figure 1. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of urea-N in float water fertilized initially at a rate of 100 mg L^{-1} total N. (Error bars represent standard error of the means.)

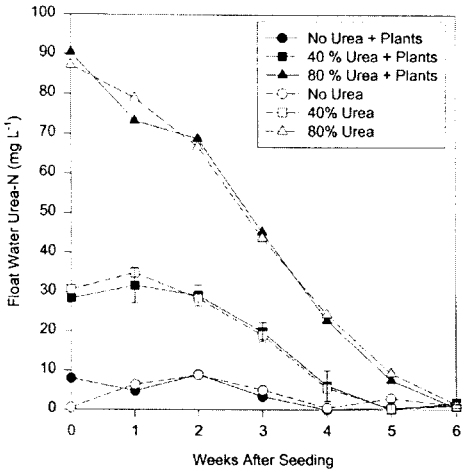
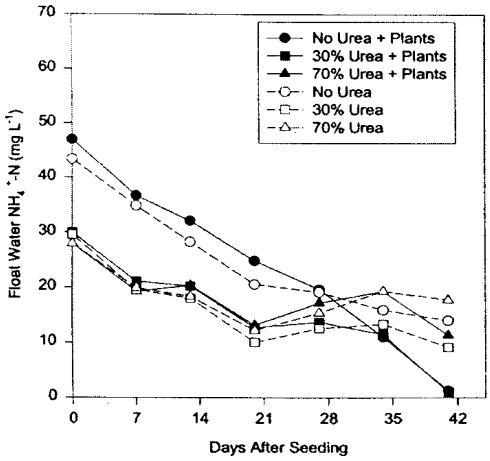


Figure 2. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of ammoniacal-N in float water fertilized initially at a rate of 100 mg L^{-1} total N. (Error bars represent standard error of the means.)



decomposition of urea. The decomposition rate was not affected by the presence of tobacco plants. No urea-N was detected in any of the solution samples collected from the media. Once urea entered the media it was rapidly converted to other forms of N. The MPN estimate of ureolytic organisms in the media four weeks after seeding averaged 2.0 x 10^7 organisms g^{-1} dry media across all six treatments. Population estimates between individual treatments were not significantly different at the 0.05 level of probability.

Relatively high rates of urea hydrolysis have been reported for commercial soilless media (4). Many organisms possess the urease enzyme, and extracellular urease has been extracted from soils. Extracellular urease may be absorbed onto organic colloids such as would be found in soilless media, thereby increasing its resistance to degradation. It can be concluded that appreciable amounts of urease may exist in soilless media that have been stored in a semi-desiccated condition.

The NH_4^+-N concentration in the float water decreased steadily over the six weeks of this study **(Figure 2).** In the media solution, a relatively high NH_4^+-N concentration was found 1 wk after seeding in all treatments **(Figure 3).** The NH_4^+-N source was most likely the starter fertilizer charge in the media. The NH_4^+-N concentration in the media solution steadily decreased during the study. After six weeks, very little NH_4^+-N was found in the media solution. Since NH_4^+-N disappearance was not affected by the absence of plants, it was postulated that the NH_4^+-N loss was due to nitrification, and not plant uptake. The MPN estimate for the population of nitrifiers in the media four weeks after seeding averaged 5.2 x 10^6 organisms g^{-1} dry media across all six treatments.

Nitrate-N in the float water decreased slightly during the first week after seeding and then leveled off until week four (Figure 4). In the presence of plants, NO_3^--N levels decreased rapidly after week four due to plant uptake. Media solution NO_3^--N levels as high as 350 mg L^{-1} were found one week after seeding. Approximately 140 mg L^{-1} NO_3^--N was present even in the fertilizer treatments that had no initial source of NO_3^--N. This NO_3^--N may have come from the starter charge in the media. Nitrate-N accumulated in the media of unseeded treatments during the course of the study **(Figure 5)**. This accumulation supported the assumption that the disappearance of urea-N and NH_4^+-N was at least partially due to urea hydrolysis and nitrification

Figure 3. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of ammoniacal-N in solution vacuum extracted from media samples. (Error bars represent standard error of the means.)

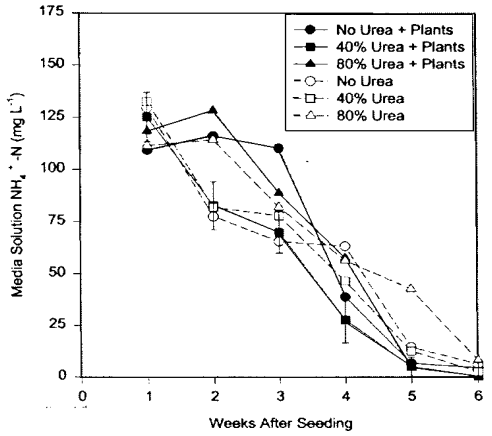


Figure 4. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of nitrate-N in float water fertilized initially at a rate of 100 mg L^{-1} total N. (Error bars represent standard error of the means.)

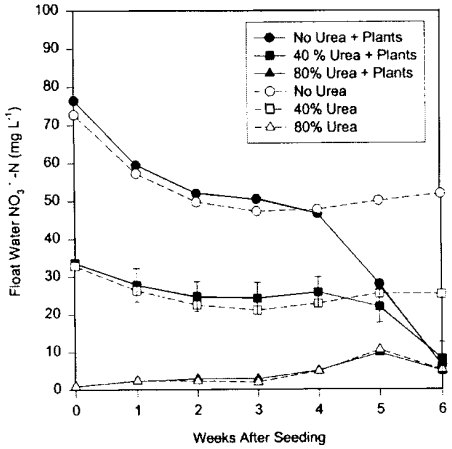


Figure 5. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of nitrate-N in solution vacuum extracted from media samples. (Error bars represent standard error of the means.)

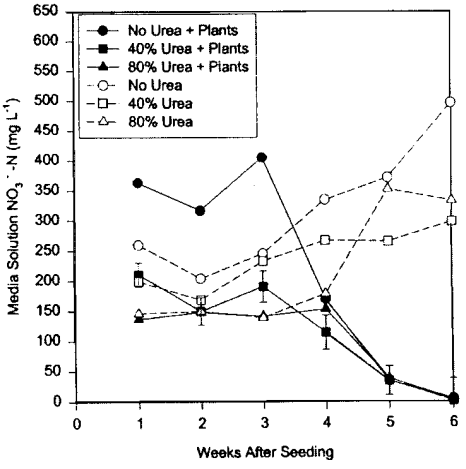
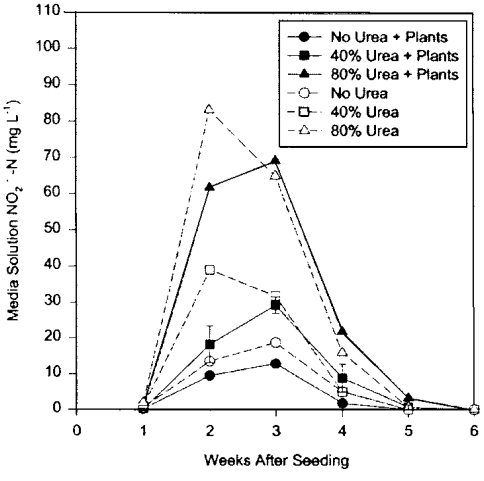


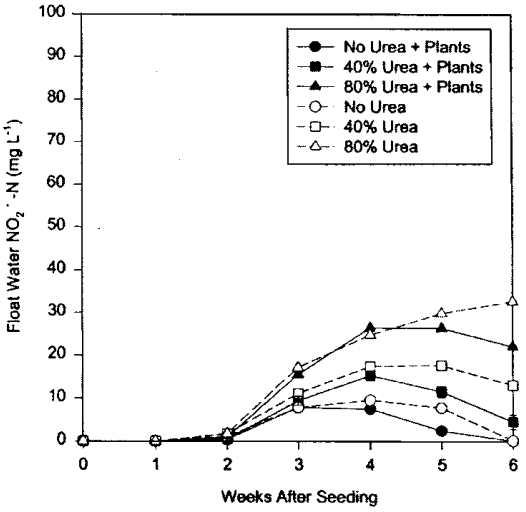
Figure 6. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of nitrite-N in solution vacuum extracted from media samples. (Error bars represent standard error of the means.)



in the media. The accumulation of nitrate in the high urea treatment was delayed by one week compared to the 0 urea treatment. In this study, the rate of urea decomposition did not appear to

limit N availability. Rather, urea hydrolysis contributed to NO_2^--N accumulation. Nitrite-N accumulated in the media solutions between weeks 1 to 3 after seeding (Figure 6). The NO_2^--N concentration was proportional to the amount of urea in the fertilizer treatment, although there was a slight accumulation of nitrite in the 0 urea treatments. Nitrite-N concentrations of more than 60 mg L^{-1} were observed in the media solution over a two week period for the high urea fertilizer. Nitrite-N levels in the media solutions dropped off sharply after three weeks, and were negligible by the fifth week after seeding. The appearance of NO_2^--N in the float water lagged behind the accumulation in the media solutions (Figure 7). The level of accumulation in the float water was proportional to the amount of urea in the fertilizer. The highest concentrations observed in the float water were 30 mg L^{-1} NO_2^--N.

Figure 7. Effect of the proportion of urea-N in water soluble fertilizer on the concentration of nitrite-N in float water fertilized initially at a rate of 100 mg L^{-1} total N. (Error bars represent standard error of the means.)



Nitrite, an intermediate in the oxidation of $NH4^+$-N to NO_3^--N, is known to be quite toxic to many plants and microorganisms (1). Normally, NO_2^--N is rapidly oxidized to NO_3^--N, so it rarely accumulates in mineral soils. Nitrite accumulation in soils is usually associated with two factors, alkalinity and high ammonium levels. Transitory NO_2^--N accumulations in acid soils have been reported as a result of the hydrolysis of urea, or the ammonification of organic matter (2,3,5). The hydrolysis of urea may produce locally high pHs and concentrations of NH_4^+-N that results in the suppression of the NO_2^- oxidizing bacteria, but not the NH_4^+ oxidizers. As the pH and NH_4^+ levels fall, due to NH_4^+ oxidation, the NO_2"oxidizers recover and the production of NO_3^- begins (1).

The pattern of NO_2^--N accumulation in the media solutions was consistent with the transitory events described above. The media pH, measured in a slurry consisting of one part media to two parts deionized water, one week after seeding was 6.0, 6.4, and 6.7 for the 0, 40%, and 80% urea fertilizer treatments, respectively. Even though the measured bulk pH was always less than 7.0, locally alkaline pHs could have occurred. The media slurry pH dropped 0.4 units in the high urea treatments between week 1 and week 2. By the fifth week the media slurry pH was 5.5 for all fertilizer treatments. As the pH and NH_4^+-N levels decreased in the high urea treatments, NO_2^--N also decreased (Figure 5), and NO_3^--N increased (Figure 4). The delayed

appearance of NO_2^--N in the float water may have been a result of NO_2^- diffusion from the media solution.

Visual top growth symptoms of stunting and yellowing were observed 17 d after seeding for plants growing in high urea fertilizer. This corresponded with the peak accumulation of NO_2^--N in the media solutions for these treatments. Five weeks after seeding, the urea fertilized plants had a normal color, but stunting was still evident at all growth stages (Table 2). Fertilizer

Table 2. Growth of burley tobacco seedlings in the float system as affected by the proportion of urea-N in the water soluble fertilizer.

Fertilizer Source (% Urea-N)	Week 4	Week 5	Week 6
	dry weight g/5 plants		
None	0.69 a[a]	2.04 a	5.16 a
40	0.48 a	1.70 ab	4.28 a
80	0.20 b	1.24 b	2.74 b

[a] Means followed by a different letter are significantly different at $P < 0.05$.

Table 3. Nutrient uptake by burley tobacco seedlings grown for six weeks in the float system with different proportions of urea-N.

Fertilizer Source (% Urea-N)	Nitrogen Uptake	Phosphorus Uptake	Potassium Uptake
	mg plant		
None	30.1 a[a]	2.6 a	26.8 a
40	22.7 ab	2.6 a	25.1 a
80	15.1 b	2.0 a	30.4 a

[a] Means followed by a different letter are significantly different at $P < 0.05$.

treatments had no significant effect on plant N concentration, but N uptake decreased significantly as the proportion of urea-N in the fertilizer increased (Table 3). The uptake of P and K were not significantly affected by fertilizer treatments. The reduced plant growth appeared to be the result of a toxic response, rather than a nutrient deficiency.

Solution culture studies have demonstrated that as little as 5 mg L^{-1} NO_2^--N damaged tobacco root tips, and few active root tips were found at 15 mg L^{-1} NO_2^--N (5). Even with significant root damage at 15 mg L^{-1} NO_2^--N, no visible symptoms were observed on the top growth. Nitrite has been reported to accumulate in tobacco fields as a result of organic matter decomposition, resulting in a disorder commonly referred to as organic matter toxicity (5). Organic matter toxicity occurs when a green manure crop is plowed under and tobacco is set soon after plowing. Hamilton and Lowe (5) observed that NO_2^--N reached 60 mg L^{-1} in the soil two weeks after organic matter addition, then decreased to zero during the next four weeks. Significant stunting occurred when tobacco was planted two weeks after the organic matter addition, but normal growth was obtained by delaying planting until six weeks after organic matter addition (5).

CONCLUSIONS

The soilless media used in this study had relatively low initial populations of N transforming organisms, but the populations increased when the media was hydrated. Nitrogen transforming activity was apparently sufficient to cause significant urea hydrolysis and $NH4^+$ oxidation. In the absence of urea, there was a slight increase in NO_2^--N. In the presence of urea, the NO_2^--N oxidizers were suppressed for two to three weeks by elevated pH and high $NH4^+$ concentrations, resulting in the accumulation of toxic levels of NO_2^--N. Plant growth was severely stunted from using fertilizer containing large amounts of urea. Water soluble or liquid fertilizers with little or no urea, are recommended for tobacco float systems.

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