Fertilizer Research 29: 309–316, 1991.

Absorption of three slow-release nitrogen fertilizers by perennial ryegrass turf

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Received 23 January 1991; accepted in revised form 28 May 1961

Key words: Lolium perenne, Coron, N-Sure, Nitrazine, melamine, urea, nitrate, N metabolism

Abstract

The absorption of three new slow-release fertilizers (Coron, N-Sure and Nitrazine) by perennial ryegrass turf was compared to uptake of NO_3^- and urea using a nutrient solution culture system. Each source of nitrogen was supplied to turf cultures at a rate equivalent to 21 kg N ha^{-1} every five days during a twenty day experimental period. Nitrate and urea produced the most growth, while growth on Coron and N-Sure was reduced 30%. Growth on Nitrazine-N was further reduced to only 40% of that on NO_3^- and urea. Coron and N-Sure were absorbed relatively rapidly during the first 24 hour period, with cumulative absorption over the five day period amounting to approximately 80% of the total supplied. Nitrazine-N was absorbed more slowly, with only 40% of the N absorbed after five days. Perennial ryegrass was apparently capable of metabolizing both Coron and N-Sure. The slow-release N component of Nitrazine (melamine) was inhibitory to photosynthesis, and at higher solution concentrations, was toxic to the turf.

Introduction

Although roots are capable of absorbing nitrogen as ammonium, it is widely considered that nitrogen is absorbed mainly as nitrate under field conditions, with ammonium fertilizers being nitrified prior to absorption [10]. However, turfgrasses may represent an exception to this rule. Bowman et al. [5] reported that ammonium applied to a field-grown turfgrass was biologically immobilized by turfgrass and associated microorganisms within 48 hours of application, with little evidence of significant nitrification during that period. Further, absorption of both nitrate and ammonium by perennial ryegrass was enhanced to a similar extent following a period of nitrogen deficiency [4].

Turfgrasses are typically fertilized with either highly soluble fertilizers, such as potassium nitrate, ammonium sulfate and urea, or slow-release fertilizers, such as UF and IBDU. These latter sources are formulated to provide nitrogen gradually to the root system, the rate of supply being controlled, at least partially, by the low solubility and mineralization rate of the fertilizer [1]. Several slow-release N fertilizers have recently been introduced for use on turf. Both Coron (Coron Corp., Souderton, PA) and N-Sure (Arcadian Corp., Parsipany, NJ) are novel slow release fertilizers in that they are formulated as a liquid containing 28% soluble N with a low potential for foliar injury. As such, they are particularly well-suited for foliar application by the home lawn care industry. The principle sources of slow release N in Coron are amine-modified polymethylene ureas and in N-Sure it is triazone (Fig. 1).

Nitrazine (Pursell Industries, Sylacauga, AL) is another new slow release fertilizer on the turfgrass market. This granular fertilizer has a

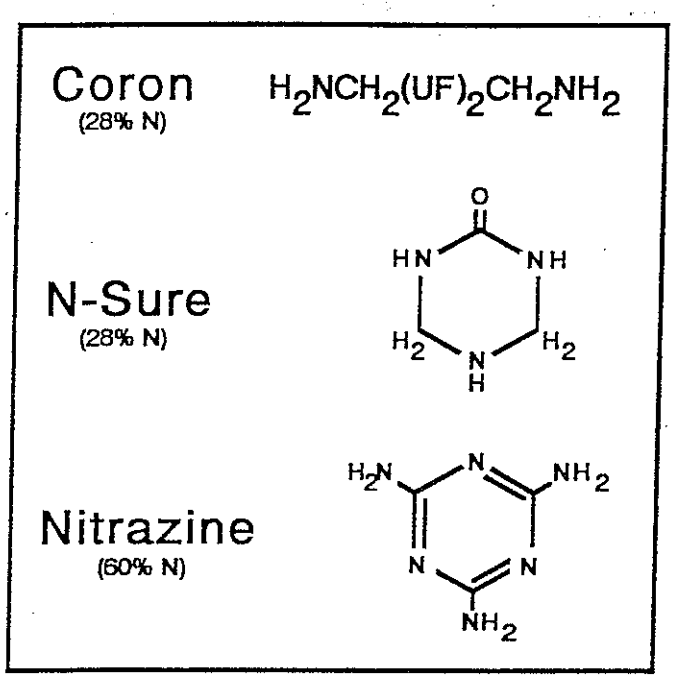

Fig. 1. Molecular structure of the primary source of slow-release nitrogen in Coron, N-Sure and Nitrazine. (UF) in the Coron chain represents ureaformaldehyde.

much higher analysis at 60% N, but its slow-release nitrogen source, melamine (triamino-triazine) is relatively insoluble. Urea comprises approximately 25–28% of the total N in all three fertilizers.

The slow release characteristics of each of these fertilizers is presumably based on breakdown by soil microorganisms, although to date few studies have examined the nitrogen dynamics of these fertilizers. In one study comparing NH_3 volatilization from urea and N-Sure, Kissel and Cabrera [11] reported that approximately 40% of the triazone-N had mineralized 24 days after application to a bare soil. Mosdell et al. [12] applied melamine to Kentucky bluegrass at a rate of 10 g N m^{-2} and found that the mineralization rate was too slow over a four month period to supply adequate N to maintain turf quality.

A second factor, in addition to mineralization, that could conceivably determine the rate of nitrogen availability from these fertilizers is direct absorption of the slow release molecule by plant roots and subsequent metabolism by the plant. Rapid and direct absorption of these fertilizers would reduce their predicted rate of nitrogen release and, thus, modify turf response. This might be particularly important for Coron and N-Sure, since they are supplied in solution, and barring strong retention on soil colloids, would be present at high concentrations in the soil solution. The objective of this study was to evaluate the potential for direct absorption and metabolism of the three fertilizers by a perennial ryegrass turf grown in a solution culture system. Absorption was determined as depletion from solution, while metabolism was measured indirectly by turf growth response.

Materials and methods

Plant culture

Nutrient solution culture was used to measure N uptake by turf grown in the greenhouse. The culture unit consisted of a 19 cm deep round plastic container having a surface area of 167 cm^2 and holding 2.3 liter of nutrient solution. For each container, seed of perennial ryegrass (*Lolium perenne* L. 'Manhattan II') was sown at a rate of 400 kg ha^{-1} on a sheet of glass wool supported by a rigid plastic mesh. The container was filled with tap water (moistening the glass wool and seeds by capillarity) and covered with a shaded plastic cap. Germination and subsequent establishment took place in a greenhouse operated at 23°C day and 15°C night under natural light. Seeds germinated in 7 days, after which the seedlings were uncovered and grown for an additional 7 days on tap water. Subsequently, the turf was established on a 0.25 strength Hoagland's solution [9], with the following composition: 3.75 mM NO_3-N, 1.25 mM K^+, 1.25 mM Ca^{2+}, 0.5 mM Mg^{2+}, 0.25 mM $H_2PO_4^-$, 0.5 mM SO_4^{2-}, 46 μM B, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, 0.1 μM Mo, and 1 mg Fe $liter^{-1}$ as Fe-EDDHA. The initial pH of the solution was 6.0. Supplemental Fe as $FeSO_4 \cdot 7H_2O$ was periodically added at a rate of 0.4 mg Fe $liter^{-1}$ to prevent chlorosis. Solutions were aerated and were changed with each mowing (every 4–7 days, depending on growth rate). The turf cultures were mowed regularly to a height of 4 cm starting 3 weeks after seeding and continuing during the preculture period. Cultures were used for experimentation twelve weeks after seeding. The turf was dense by this time, with 4 to 5 tillers per plant, and had developed a healthy root system.

Experiment 1: Growth on slow-release sources of nitrogen

On May 14, the turf cultures were removed from the preculture solutions, mowed, and then transferred to 0.25 strength minus-N Hoagland's solution (NO_3^- salts replaced by SO_4^{2-} salts) to induce moderate nitrogen deficiency. After seven days growth, the cultures were mowed and transferred to one of seven treatment solutions consisting of 2.3 liters of minus-N Hoagland's solution supplemented with 35 mg N as either KNO_3, urea, Coron, N-Sure or Nitrazine, or with 10 mg N as KNO_3 or urea. These latter two treatments were included because each of the three commercial slow release fertilizers supplied approximately 10 mg N of the total 35 mg N as urea. On a land area basis, 35 mg N culture^{-1} is equivalent to 21 kg N ha^{-1}. The pH of all solutions was adjusted to 6.0 with either H_2SO_4 or KOH.

The turf cultures were grown in these treatment solutions for a total of twenty days, during which time the solutions were replaced and the turf mowed every five days. Clippings from each mowing were dried, weighed, and ground to pass a 40 mesh screen. The nitrogen content of the clippings was determined by Kjeldahl digestion.

Nitrogen uptake

Net uptake was determined for a five day period between days 15 and 20 by measuring the depletion of N from solution, corrected for volume loss due to evapotranspiration and sampling. A 10 ml sample from each container was obtained daily at noon, starting on day 16. An exception to this was that a sample of 20 ml was obtained from the three slow-release nitrogen treatments on day 16 to provide adequate volume for separate urea-N and total-N analyses. During this period, the pH of the solutions was adjusted to 6.0 ± 0.5 daily.

Nitrate in the KNO_3 solutions was measured directly by the Carlson method [6, 7]. Urea was determined in a 5 ml aliquot of those solutions containing urea by first hydrolyzing the urea to NH_4^+ using jackbean urease (Sigma) and then measuring the NH_4-N by the Carlson method. Total nitrogen in the solutions containing the slow-release forms of N was determined by Kjeldahl digestion. Samples of the solutions were passed through a serum filter to remove particulates, and then a 10 ml aliquot was transferred to 75 ml digestion tubes. To this was added, in sequence, 4.5 ml concentrated H_2SO_4, 3 ml H_2O_2, 1.5 g K_2SO_4, and 0.33 ml of 8% $CuSO_4 \cdot 5H_2O$. Tubes were placed on a cold digestion block and the temperature was gradually raised to 360°C to safely evaporate the liquid. Final digestion was at 360° for 60 min. Reduced N in the digests was determined directly by the Carlson method. Preliminary data indicated that this digestion procedure was adequately sensitive, being able to resolve absorption of 0.2–0.3 mg N culture^{-1}. The method was also quite accurate, recovering 96–99% of the nitrogen contained in dilute standard solutions of Coron, N-Sure and Nitrazine comparable in concentration to those used in this experiment.

Photosynthesis

As a general indicator of plant health, net photosynthesis of the turf cultures was measured at the termination of the experiment prior to mowing (day 20). Individual cultures were placed in an enclosed Plexiglas chamber totaling 11.0 liters in volume and equipped with several fans to ensure rapid mixing of the air. Gas exchange measurements were obtained at saturating light intensity (1200–1500 μE m^{-2} s^{-1}), ambient temperature and CO_2 concentrations, using a portable gas exchange system (Licor model 6000).

Experiment 2: Effect of melamine concentration on nitrogen uptake

Because of the relatively small amount of melamine-N depleted from solution in the first experiment, the results were inconclusive with regard to the potential for absorption of the slow-release nitrogen source in Nitrazine. Consequently, a second short-term experiment was conducted to determine specifically if perennial ryegrass absorbs melamine-N directly. Turf cultures were produced as detailed above and transferred on June 11 to a minus-N Hoagland's solution. Seven days later, the cultures were transferred to one of five treatment solutions containing melamine at rates of 0, 100, 200, 400 and 800 mg N liter^{-1}. The turf cultures were grown on these melamine solutions for a single

five day period. On day 5, samples of the solutions were passed through serum filters and the nitrogen content determined on 10 ml aliquots of the filtrate, as above. Net photosynthesis was also measured on day 5, prior to harvesting the clippings for nitrogen analysis. The experimental design was a completely randomized block with four replicates of each treatment in both experiments.

Results

Experiment 1

Based on growth measurements, the seven treatments segregated by day 5 into three groups. The 35 mg N level of NO_3^- and urea (group 1) consistently ranked the highest (Fig. 2), with clipping production on NO_3^- being slightly greater on days 15 and 20. The Coron and N-Sure treatments supplying 35 mg N (group 2) were very similar with an intermediate growth rate averaging approximately 70% of the 35 mg $NO_3 - N$ treatment. The third group, which exhibited the slowest growth rate, consisted of the 10 mg N level of NO_3^- and urea, and Nitrazine supplied at 35 mg N. For all three groups, growth declined between the first and second harvest. Thereafter, growth was relatively stable for groups 1 and 2 but continued to slowly decline in group 3.

Percent N in the clippings (Fig. 3) ranged from approximately 3.1% to 3.8%, with the treatments again segregating as described above. Total nitrogen removed in the clippings per harvest, averaged over the final three harvests, was approximately 29, 17 and 10 mg N culture^{-1} for groups 1, 2 and 3, respectively.

Nitrogen uptake was measured during the final 5 days of the experiment. After 1 day, all of the nitrogen supplied as either NO_3^- or urea, at both the 35 and 10 mg N level, had been absorbed (data not shown). This was also the case for the urea component of the Coron, N-Sure and Nitrazine treatments. Absorption of N from Coron and N-Sure (Fig. 4) was identical, with 23 mg N absorbed during the first day. This initial rapid absorption was followed by a much slower rate of approximately 1.2 mg N d^{-1} over the next four days, as determined by linear regression. By contrast, absorption of Nitrazine during the first

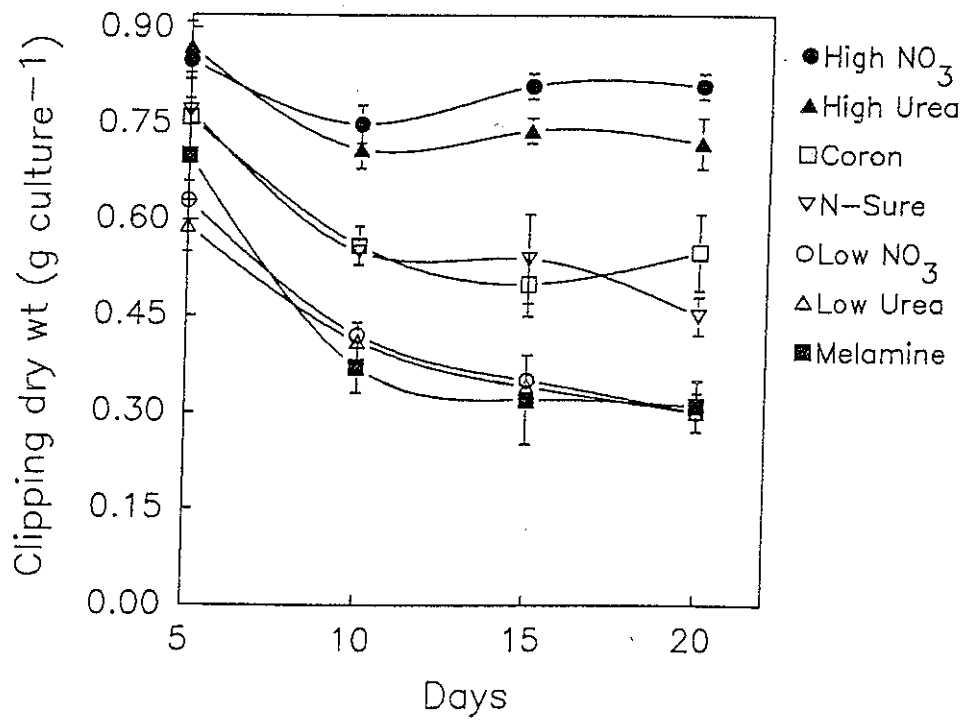

Fig. 2. Dry weight of perennial ryegrass clippings over the twenty day experimental period for the seven fertilizer treatments. Values are means of four samples ±standard deviation.

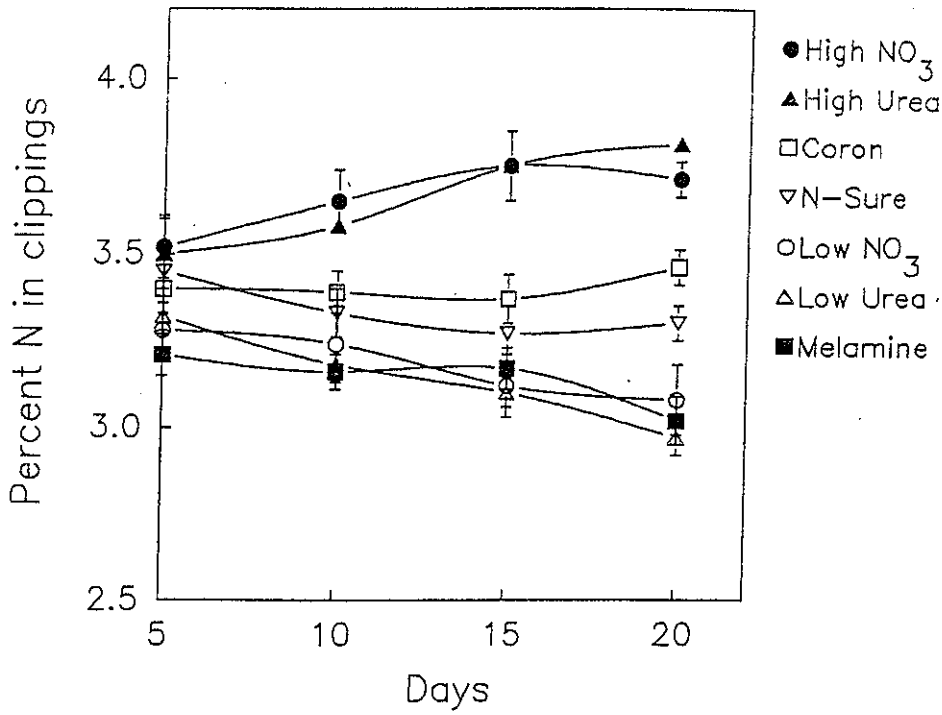

Fig. 3. Percent N in the clippings of perennial ryegrass over the twenty day experimental period for the seven fertilizer treatments. Values are means of four samples ±standard deviation.

24 hours amounted to only 10 mg N, due to absorption of the urea component. Again, a much slower uptake rate of 0.94 mg N d^{-1} was observed between days 2 and 5.

There were significant differences on day 20 in photosynthetic gas exchange between the treatments (Table 1). Urea at 35 mg N had the highest rate of photosynthesis at 21.0 μMol CO_2 m^{-2} s^{-1}, whereas Nitrazine had the lowest at 12.6 μMol CO_2 m^{-2} s^{-1}. Nitrazine was significantly reduced compared to both the 10 mg urea-N and NO_3-N treatments. Surprisingly, photosynthesis in the 35 mg NO_3-N treatment was depressed 20% relative to the 35 mg urea-N treatment.

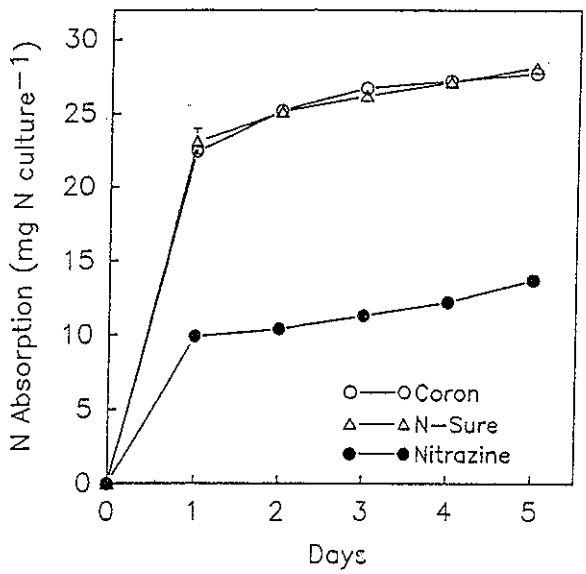

Fig. 4. Absorption of Coron, N-Sure and Nitrazine by perennial ryegrass over a five day period. Values are means of four samples ±standard deviation where larger than the symbols.

Experiment 2

Under the conditions of this experiment, a five day exposure to Melamine was visibly damaging

Table 1. Net photosynthesis of perennial ryegrass turf as a function of fertilizer treatment

Treatment	Net CO_2 Fixation
	μMol CO_2 m^{-2} s^{-1}
35 mg Urea-N	21.0 A[a]
35 mg Coron-N	18.4B
35 mg N-Sure-N	17.2BC
35 mg NO_3-N	16.8BC
10 mg Urea-N	15.5CD
10 mg NO_3-N	14.4D
35 mg Nitrazine-N	12.6E

[a]Values are means of 4 measurements. Means followed by the same letter are not significantly different by Duncan's Multiple Range Test ($P = 0.05$).

Table 2. The effect of melamine-N concentration on clipping fresh weight, dry weight, percent N, and net photosynthesis in perennial ryegrass turf

Melamine concentration	Clipping Fr. Wt.	Clipping Dry wt.	Tissue N	Net CO_2 Fixation
mg N liter^{-1}	g culture^{-1}		%	μMol m^{-2} s^{-1}
0	2.85[a]	0.54	3.98	19.8
100	3.03	0.57	4.28	21.0
200	2.49	0.51	4.50	17.4
400	2.29	0.54	5.03	9.0
800	1.43	0.51	6.02	2.8
$LSD_{0.05}$	0.30	n.s.[b]	0.37	3.7

[a]Values are means of 4 measurements.
[b]Not significant at $P = 0.05$.

to the turf at concentrations of 400 and 800 mg N liter^{-1}. The damage was severe at 800 mg N liter^{-1}, characterized by desiccation and bronzing of the leaf blades and bleaching at the tips. This damage was reflected in clipping fresh wt., although little difference was noted in clipping dry wt. (Table 2). Percent N in the clippings increased with Melamine concentration, from 3.98% in the minus-N controls to 6.02% in the 800 mg N liter^{-1} treatment (Table 2). Total five-day absorption of Melamine-N increased linearly with concentration in solution (Fig. 5), ranging from 31 to 184 mg N culture^{-1}. Photosynthesis was also severely affected at the two highest Melamine concentrations (Table 2), with an 85% inhibition at 800 mg N liter^{-1}.

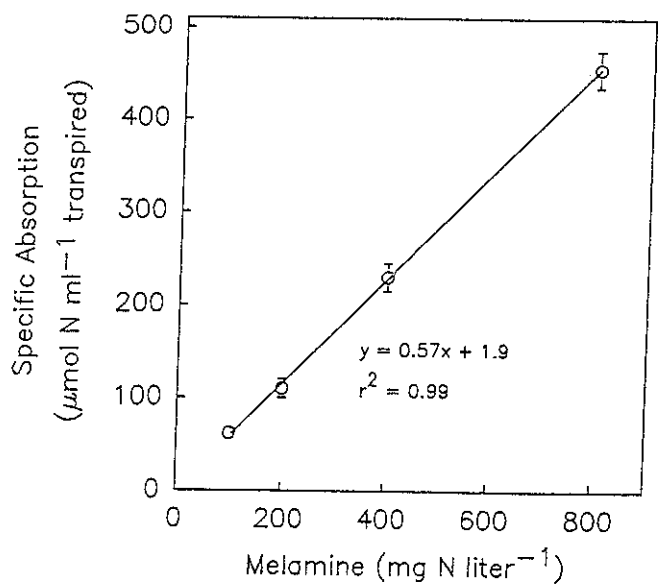

Fig. 5. Cumulative uptake of Nitrazine-N by perennial ryegrass over a five day period as a function of concentration. Values are means of four samples ±standard deviation.

Discussion

It has been reported that roots are capable of absorbing complex forms of organic N, such as amino acids [13]. The present investigation extends this information to absorption of several synthetic organic N compounds marketed as slow-release fertilizers. The combined results of the two experiments provide strong evidence that roots of perennial ryegrass are capable of absorbing, to some degree, all three of the slow-release N sources examined. Approximately 80% of the N present in Coron and N-Sure was absorbed from a nutrient solution over a five day period, the majority during the first day. Urea, which was absorbed rapidly, accounted for 10 mg of the 23 mg N of Coron and N-Sure absorbed on day 1. Thus, nearly half of the 25 mg slow-release N supplied by Coron and N-Sure was absorbed on day 1. Urea accounted for essentially all of the 10 mg N absorbed from Nitrazine on day 1, while only 4 mg melamine-N was absorbed over the remaining four days. All of the NO_3^- and urea supplied at either 10 or 35 mg N culture^{-1} was absorbed during the first 24 h period, consistent with previous reports that nitrogen deficiency greatly enhances absorption of NO_3^- and NH_4^+ by perennial ryegrass turf [4].

The pattern of uptake of Coron and N-Sure, in which a large fraction of the total N was absorbed rapidly followed by a much slower absorption over an extended period, could be due to the mixed composition of the fertilizers. The slow-release N in Coron consists of several amine-modified polymethylene urea chains ranging in length from 2 to 4 urea units, plus small amounts of methylene diurea and dimethylene

triurea. The slow-release nitrogen in N-Sure is approximately 75% triazone, with the remaining N present as substituted triazones, methylene diurea, and other organic compounds. It seems reasonable to speculate that roots may absorb these various organic compounds at different rates. The initial rapid N uptake on day 1 might then be the result of selective absorption of one or several specific slow-release compounds. In this scheme, the remaining compounds would be absorbed at a much reduced rate following the depletion of the readily-absorbed compound(s). It must also be considered that Coron and N-Sure are formulated to permit foliar applications. Absorption and metabolism could then occur simultaneously in the root and shoot. Unfortunately, no information is available on the foliar absorption of these fertilizers.

It is evident that perennial ryegrass is also capable of metabolizing at least some of the slow-release N absorbed from Coron and N-Sure. Growth on these two nitrogen sources was 60% greater than on 10 mg N supplied as NO_3^- or urea, with the difference attributable to the slow-release N. The data do not adequately resolve whether perennial ryegrass is capable of metabolizing melamine-N. Nitrazine at 35 mg N produced no more growth than NO_3^- or urea at 10 mg N, even though approximately 4 mg melamine-N was absorbed. This suggests that the melamine-N was not metabolized. However, it is also possible that the inhibition of photosynthesis by Nitrazine counteracted any potential increases in growth due to the metabolism of melamine N.

The data further indicate that Coron and N-Sure may not be metabolized as efficiently as NO_3^- or urea. This is supported by calculations of productivity per unit N absorbed. Specifically, the five-day growth increment in excess of the 10 mg N controls, averaged 190 mg dry wt. for Coron and N-Sure and 450 mg dry wt. for NO_3^- and urea supplied at 35 mg N. Approximately 18 mg N as Coron and N-Sure were absorbed, compared to 25 mg N as NO_3^- and urea, in excess of the 10 mg N control level. Consequently, Coron and N-sure had an estimated productivity, above the controls, of 10.6 mg dry wt. per mg N absorbed whereas productivity was 18 mg dry wt. per mg N for NO_3^- and urea. Another possibility is that N absorbed from Coron and N-Sure is partitioned differently within the plant than is N from NO_3^- or urea. The amount of N removed in the clippings may be used to indicate N partitioning to new leaf growth. Expressed as a percent of the N absorbed over the five day period, the partitioning of N to new leaf growth was approximately 100% for the 10 mg NO_3^--and urea-N treatments, 83% for the 35 mg NO_3^--and urea-N treatments, and only 63% for the Coron and N-Sure treatments.

It is possible that these fertilizers might bind to organic matter in the nutrient solution, such as the root system or suspended organic debris. Since organic matter is filtered out of the solution samples before Kjeldahl digestion, binding would potentially exclude some fertilizer-N from the analysis and thus overestimate N uptake. The potential for organic matter binding the fertilizer N was investigated in a preliminary trial, where filtered solution samples were obtained 30 minutes after addition of Coron, N-Sure or Melamine to turf cultures. Recovery was nearly 100% for each fertilizer (data not shown), indicating that binding of fertilizer to organic matter was not significant, at least over the short term. Additional evidence against binding was obtained in experiment 1, where essentially all the melamine-N was recovered in the filtered solution on day 1. It is also possible that over a five day period, microbial activity may have metabolized or otherwise altered the slow-release sources of N. More sophisticated analytical techniques would be necessary to confirm any changes in the compounds. Arguing against this is the relatively slow mineralization rate reported for Coron-N [11] and melamine [12] in a soil system.

The results of experiment 2 confirm that perennial ryegrass roots are capable of absorbing melamine-N. This is consistent with reports that roots readily absorb the triazine herbicides [2,3 and references therein]. The strong linear relationship between uptake and concentration suggests that absorption may occur by mass flow or simple diffusion through membranes, with a reflection coefficient of approximately 0.45. Although considerable N was absorbed, it is unclear from the data if ryegrass is able to adequately metabolize melamine to satisfy requirements for N. Given the difficulty with which

melamine is mineralized by soil microorganisms [8], it seems likely that many plants may lack the capacity to metabolize melamine.

Under the conditions of this experiment, melamine inhibited photosynthesis and produced symptoms of toxicity in perennial ryegrass leaves. As an analogue of the symmetrical triazine herbicides, which inhibit photosynthetic reactions [2], melamine might be expected to have some herbicidal activity. Recent evidence indicates that melamine may act synergistically with several herbicides, including dicamba, hexazinone and 2,4-D [J. Detrick, Pursell Industries, personal communication].

The toxicity of melamine might also be the result of precipitation of the compound in the leaf blade. Malamine has a relatively low solubility of 5000 ppm, and could, through continued xylem transport from the roots, accumulate in leaves to levels exceeding its solubility. From the data in Table 2, the average concentrations of melamine in the liquid phase of the leaf are calculated as 4900 and 17,000 ppm for the 400 and 800 ppm treatments, respectively. These values are close to or exceed the solubility of melamine. Assuming that melamine is not metabolized to more-soluble forms, it is reasonable to conclude that in this experiment, some melamine precipitated in the leaves. The specific location (apoplast vs. symplast) and possible consequences, such as vascular blockage, of melamine precipitation in leaf blades is unknown. Another obvious question raised by this study is whether Nitrazine (melamine) is toxic to plants under field conditions.

The results of this investigation indicate that plants are capable of absorbing and at least partially metabolizing a large fraction of the slow release N present in both Coron and N-Sure. Rapid absorption of these two N sources might impact their slow-release pattern by depleting the soil of mineralizable substrate. This possibility should be examined under field conditions using ^{15}N-labeled fertilizers. Melamine is also absorbed by roots, albeit at a slower rate. However, it is unclear if melamine is metabolized by the plant.

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