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Simazine and Atrazine—Suppression of Denitrification

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Abstract. **The effectiveness of the s-triazines simazine [2-chloro-6-bis (ethylamino)-s-triazine] and atrazine [2-chloro-6-(ethylamino)-6-(isopropylamino)-s-triazine and nitrapyrin [2-chloro-6-(trichloromethyl)pyridine] in suppressing denitrification was evaluated in laboratory and greenhouse studies. Parameters used to measure suppression of denitrification were N_2 and N_2O evolution, soil NO_3-N, and plant N (% N × dry weight). Simazine and atrazine significantly reduced N_2O evolution in laboratory incubation studies, but had no effect on N_2 evolution, while nitrapyrin reduced both N_2 and N_2O evolution. In greenhouse studies, 0.5 mg kg^{-1} soil of simazine and atrazine, and 2.0 mg kg^{-1} soil of nitrapyrin increased soil NO_3-N and plant N as compared to the control. It is concluded that simazine and atrazine inhibited denitrification under greenhouse and laboratory conditions and, therefore, that increased media NO_3-N availability is the most likely explanation why N concentration in the plant tissue was higher.**

Increases in plant protein with subherbicidal concentration of simazine and atrazine were reported for over 2 decades (12); however, responses obtained in field studies often have been inconsistent (1, 4). Studies have focused upon changes in NO_3-N assimilation as opposed to increased NO_3-N availability as the reason for the observed increase in plant protein content. Recent studies have shown that nitrapyrin and terrazole [5-ethoxy 3-trichloromethyl-1,2,4-thiadiazole) are inhibitory to denitrification (5, 7), with increased N levels in the plant being directly related to increased NO_3-N availability through the suppression of denitrification (6, 7). Though many plant metabolic responses were reported with simazine and atrazine (10, 11), suppression of the denitrification process could explain the N-related metabolic and physiological responses.

The objective of this study was to determine if the reported effects of simazine and atrazine on NO_3-N assimilation and plant protein content could, in part, be explained by increased NO_3-N availability due to an inhibition of denitrification.

Laboratory evaluations were performed in a liquid system containing nutrients per liter as follows: 3 g KNO_3, 0.8 g KH_2PO_4, 0.2 g K_2HPO_4, 0.2 g $MgSO_4$, 0.1 g $CaCl_2$, 1 mg Fe as Fe-EDTA, 1% dextrose, and 0.1% yeast extract. This medium supports denitrification with many bacterial species (14). Air-dried Cecil clay soil (100 g) were added to 4 liters of this medium and incubated for 48 hr at 25°C. This bacterial-enriched medium then was filtered through glass wool and used immediately as a source of inoculum. Ninety ml of fresh medium plus 10 ml of the bacterial inoculum were placed into 125-ml Erlenmeyer flasks. Flasks sealed with rubber serum caps were placed in a vacuum chamber after insertion of a hypodermic needle through the septa. Vacuum chambers were filled with He after evacuation for 30 min and the hypodermic needles were removed from the flasks under He positive pressure. Simazine, atrazine, and nitrapyrin were formulated in methanol as 1000 ppm solutions. The chemical treatments consisted of 200 μl of each stock chemical being injected into their respective flask, delivering a final concentration of 2.0 ppm (w/v)/flask. A control which received no chemical treatment was included.

Flasks were incubated at 25°C for 72 hr. Ten-ml gas samples were taken at 24-hr intervals and analyzed for N_2 and N_20. After each sampling, flasks were degassed again and refilled with He. Dinitrogen and N_2O concentration were determined by gas chromatography (5, 6). A completely randomized design with 4 replicates was used in this study.

In greenhouse studies, a 50/50 mixture of sand and Cecil clay soil was mixed thoroughly in a rotating drum. All chemical treatments were sprayed over the surface of the soil during the mixing procedure, delivering 0.5 mg simazine (Princep) or atrazine (Aatrex), or 2 mg nitrapyrin (N-Serve) per kg soil. A control with no chemical treatments was included. (These concentrations were chosen after preliminary evaluations indicated that 0.5 mg kg^{-1} soil of simazine or atrazine did not affect maize growth and that 2.0 mg kg^{-1} soil nitrapyrin was slightly above the threshold concentration required to inhibit denitrification in the medium used in this experiment.) Four kg of this medium were added to each 25-cm pot and 9 maize plants (*Zea mays* L. cv. Dekalb XL) were planted into each container. Plant populations were thinned to 3 plants/container after emergence. Nitrogen was provided as $Ca(NO_3)_2$ with other nutrients supplied as a modified Hoagland's solution minus N (3). During the first 4 weeks of the experiment, 50 mg NO_3-N kg^{-1} soil were supplied to each container at 7-day intervals. One hundred mg NO_3-N kg^{-1} soil were supplied at 7-day intervals during the last 4 weeks of the experiment. Total NO_3-N supplied to each container during the 8-week experiment was 2400 mg. Equal amounts of water were added daily to each container with the leachate captured and added back to the container to prevent NO_3-N leaching.

Maize plants were harvested after 8 weeks, dried at 70°C in a forced-air oven, and weighed. The total plant nitrogen percentage was determined then with a nitrogen auto analyzer. Nitrate nitrogen in the medium was determined with a NO_3 specific ion electrode (8). The mg of applied NO_3-N incorporated into plant N plus the mg NO_3-N remaining in the soil were summed into a value and expressed as a percentage of the applied NO_3-N recovered (plant + soil). A complete randomized block design was used in this study (4 treatments × 8 replicates).

Simazine and atrazine significantly reduced N_2O evolution below that occurring in the control (Table 1) in the laboratory study; however, N_2 evolution was unaffected by either of the s-triazine treatments. Suppression of N_2O by simazine and atrazine without a concurrent reduction in N_2 suggests that these herbicides have a more selective effect on denitrification than the generally suppressive nitrapyrin. Nitrapyrin suppressed the evolution of both N_2 and N_2O in this and previous studies (5). The generally accepted pathway of denitrification ($NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$) does not imply that all bacteria capable of denitrification reduce NO_3 to N_2, as certain bacteria complete the entire pathway while others catalyze one or more steps in the pathway (9).

Soil NO_3-N and mg total plant N were increased significantly with simazine and atrazine (Table 2) with a corresponding increase in the percentage of the applied NO_3-N recovered (soil + plant) of 16.1% and 20.4% of that obtained with the control. Ni-

Table 1. Effect of simazine, atrazine and nitrapyrin on N_2O and N_2 evolved from a liquid medium inoculated with soil (72 hr incubation).

Treatment	Concn (ppm)	N_2O evolved (μg)	N_2 evolved (mg)
Control[z]	0	380 b[y]	50 a
Simazine	2.0	100 a	51 a
Atrazine	2.0	200 a	48 a
Nitrapyrin	2.0	96 a	32 b

[z]Control received 200 μl methanol solvent.
[y]Mean separation within columns by Duncan's multiple range test, 5% level.

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Table 2. Effect of simazine, atrazine, and nitrapyrin on soil NO_3-N, plant dry weight, percentage of N, total plant N, and the accounted for percentage of applied N (N supplied as NO_3 only).

Treatment						
Chemical	Concn (mg/kg soil)	Soil NO_3-N (mg)	Total g plant dry wt	N (%)	Total[x] plant N (mg)	N recovered[y] (%)
Control	0	5 c[z]	48 b	2.49 c	1190 c	49.8
Simazine	0.5	62 a	53 ab	2.91 a	1550 a	65.1
Atrazine	0.5	57 a	56 a	2.91 a	1623 a	70.3
Nitrapyrin	2.0	42 b	51 ab	2.72 b	1390 b	59.7

[z]Mean separation within columns by Duncan's multiple range test, 5% level.
[y]Percentage of applied N recovered = soil NO_3-N + mg plant N/2400 mg NO_3-N supplied.
[x]Total plant N = (g dry wt × 1000) (percentage of N/100).

trapyrin significantly increased both soil NO_3-N and mg N absorbed over that obtained with the control, but these increases were less than those obtained with simazine or atrazine. Although nitrapyrin was less effective than either of the s-triazine herbicides evaluated in this study, use of nitrapyrin as a denitrification inhibitor in previous field studies resulted in an 18% increase in plant N content (6).

These data indicate that increased NO_3 availability may be a primary factor responsible for the increased plant N content observed with the addition of sublethal levels of s-triazines. Suppression of denitrification by these s-triazines strongly suggests that the increased protein content observed by other investigators (1, 11) occurred due to decreased denitrification rates and subsequent increases in NO_3 availability for plant utilization. Reported effects of the s-triazines on protein levels that can be explained by increased NO_3-N availability are: A) increased plant protein content occurs only when NO_3-N is the N source (13); B) observed increases in nitrate reductase activity (12) could be explained by increased substrate (NO_3) availability; and C) inconsistent responses to simazine in terms of increased protein levels (4) could be attributed to variables which affect the rate of denitrification and NO_3-N availability.

In conclusion, these data, as well as other reports (6), indicate that denitrification can be a significant factor affecting the availability of NO_3-N to plants and that increased plant N content is obtained by chemical inhibition of denitrification. However, the effect of these s-triazines on N-transformations in the soil is not limited to denitrification. Previous investigators have found that atrazine (and other chlorinated triazines asymetrically substituted at the 4 and 6 carbons) also suppress nitrification (2) and this may have a pronounced influence on studies evaluating N fertility practices in the field. Rarely are the effects of herbicides on N transformations in the soil considered with studies concerning N nutrition. If the herbicide affects both nitrification and denitrification or either of these processes, then the resulting effects on soil N transformations may have as great an influence on the data obtained as the N treatments being evaluated. Thus, results from this study indicate that the N level in the plant tissue is increased and that the protein content of horticultural and agronomic crops may be increased significantly by simazine and atrazine.

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