Bromide as a Tracer for Nitrate-N Uptake in Alfalfa Herbage

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ABSTRACT

The ^{15}N isotope is a reliable way to measure nitrate-N (NO_3^-–N) uptake in N_2–fixing legumes, but is too expensive for plant breeding programs. Our goal is to develop an affordable method of selecting alfalfa (*Medicago sativa* L.) for NO_3^-–N uptake under field conditions. In this research, we tested whether the anion bromide (Br^-), which is used to trace NO_3^-–N movement in soils and is inexpensive to analyze, reflects NO_3^-–N uptake in alfalfa. 'Webfoot' (1994 and 1995), 'Agate' (1995), and 'Ineffective Agate' (1995) alfalfa were established in pots in the greenhouse. After removing herbage, daily additions of solutions containing Br^- and ^{15}N-labeled NO_3^- were made to the pots. Uptake of Br^- and N derived from NO_3^- was determined in herbage regrowth sampled after 15, 25, and 35 d and the molar ratio of NO_3^-–derived N:Br^- was calculated. In both years, when a constant molar ratio in solution was provided at variable tracer concentrations, NO_3^-–N uptake and Br^- uptake both increased with increasing applied tracer concentration, but ratios in herbage were not constant for treatment, germplasm, or days of regrowth. These results imply that selection must be carried out under well-controlled tracer supply and crop management. At a constant NO_3^-–N concentration, but increasing solution Br^- concentration, NO_3^-–N uptake remained constant and Br^- uptake increased. Molar ratios of NO_3^-–derived N:Br^- in herbage directly reflected applied solution ratios in a soil–sand mixture (1994), but were less responsive in sand (1995) above molar ratios of 100:1 in solution. Individual plant analysis showed close agreement in Br^- and NO_3^-–N uptake among plants in all germplasms across a wide range of tracer supply, and indicated that selection for NO_3^-–N uptake using Br^- uptake would result in minimal error. We conclude that Br^- is a promising alternative tracer for use in selecting alfalfa for NO_3^- uptake.

NEW ALFALFA GERMPLASMS that reduce NO_3^-–N losses to ground water and decrease fertilizer N requirements would be environmentally and economically beneficial to sustainable cropping systems. Stewart et al. (1968) suggested using alfalfa, a deeply rooted perennial, to remove NO_3^- from the soil profile and Shertz and Miller (1972) demonstrated that alfalfa effectively removes NO_3^-–N from the soil profile under field conditions. Alfalfa can remove as much as 300 kg NO_3^-–N ha^{-1} yr^{-1} from below the rooting depths of most annual crops (Mathers et al., 1975). Lamb et al. (1993) found differences in NO_3^-–N uptake among alfalfa germplasms using the ^{15}N isotope dilution technique (Legg and Sloger, 1975). Stable ^{15}N isotope has been used to measure subsoil NO_3^-–N uptake in alfalfa (Blumenthal and Russelle, 1996), but it is not economically feasible to use this isotope in a plant breeding program, where thousands of plants must be measured individually. In selecting alfalfa plants for differences in NO_3^-–N uptake, a new methodology is required that separates N derived from NO_3^-–N uptake and N derived from N_2 fixation.

Chloride (Cl^-) conceivably could serve as a tracer for NO_3^-–N uptake, but Cl^- often is found in high concentrations in alfalfa due to fertilization with KCl, and discerning uptake against a high background concentration would be difficult. Although Br^- is not essential for plant growth, it is absorbed readily by roots of several crop species, including corn (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], potato (*Solanum tuberosum* L.), and alfalfa. Chao (1966) reported a linear relationship between the amount of Br^- applied and that absorbed by sorghum. The generally noncompetitive, but related uptake of Br^- and NO_3^- in sorghum (Chao, 1966) and corn (Jemison and Fox, 1991) suggested to us that Br^- could serve as an analog for NO_3^- over a wide concentration range. Our hypothesis in this research was that Br^- can serve as an inexpensive tracer for NO_3^-–N uptake in N_2–fixing crops such as alfalfa. We conducted three experiments in the greenhouse to test this hypothesis.

MATERIALS AND METHODS

Plant Materials and Culture

1994

Five plants of Webfoot alfalfa were seeded in April 1994 in each of 144 pots (15 by 15 cm) and single plants were planted in 100 pots (10 by 10 cm) in the greenhouse. Webfoot is a dormant, branch-rooted alfalfa cultivar. Plants were potted in a mixture (by volume) of about 2/3 loamy sand soil and 1/3 sand with added P (22 g m^{-2}) and K (22 g m^{-2}), and were inoculated with a commercial mixture of *Rhizobium meliloti* (Nitragin, Liphatech, Milwaukee, WI[1]). Plants did not receive additional fertilizer nutrients and were maintained with daily watering, appropriate cutting intervals, and pesticide applications until initiation of the experiments.

1995

Five plants of Webfoot, Agate (Barnes and Frosheiser, 1973), and Ineffective Agate (Barnes et al., 1990) were seeded in each of 43 pots (15 by 15 cm), and single plants of each entry were seeded in 50 pots (10 by 10 cm) in the greenhouse in August 1995. Agate and Ineffective Agate are dormant alfalfas. Ineffective Agate is a single-gene mutant incapable of N_2 fixation, and was selected out of Agate. Plants were potted in sand with P (22 g m^{-2}), K (22 g m^{-2}), and a micronu-

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[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or the Univ. of Minnesota and does not imply its approval to the exclusion of other products that may also be suitable.

trient mixture (3.3 g m^{-2}) (Micromax Plus, Grace Sierra, Milpitas, CA), and were inoculated with *Rhizobium meliloti*. Plants were watered twice daily with a nutrient solution of $Ca(NO_3)_2$ (0.5 m*M*). Plants were maintained as described above until the experiments began.

In all experiments, herbage was harvested at different times during the regrowth period, which will be referred to in this paper as harvest dates. Harvest dates are reported as days after initiation of treatment. Herbage was oven-dried at 60°C, weighed, and ground to pass a 1-mm mesh with a Tecator mill (Cyclotec 1093 sample mill, Tecator, Des Plaines, IL). Total N and ^{15}N concentration were determined on a Carlo Erba NA 1500 analyzer (Carlo Erba Strumentatione, Milan, Italy) interfaced to a Tracermass isotope mass spectrometer (Europa Scientific, Cheshire, UK) at the Nitrogen Isotope Laboratory, Univ. of Nebraska, Lincoln. Bromide concentrations were determined by x-ray fluorescence spectrometry at the Univ. of Nebraska Dep. of Agronomy Soil Testing Laboratory. Bromide and NO_3^-–N uptake were determined for the herbage on a per-pot basis after accounting for mean background concentrations of Br^- and ^{15}N in herbage from control pots. In addition, the molar ratio of NO_3^-–N uptake (^{15}N-labeled NO_3^- × herbage dry matter yield) divided by Br^- uptake (Br^- derived from applied Br^- × herbage dry matter yield) was compared with the molar ratio of NO_3^-–N:Br^- in the solution applied.

Exp. 1: NO_3^-–N and Br^- Uptake When Supplied at a Fixed Ratio (200:1) and Increasing Concentration

Experiment 1 was conducted to study the stability of Br^- uptake relative to NO_3^-–N uptake. Increasing concentrations of both Br^- and NO_3^- were applied at a constant molar ratio of 200 NO_3^-–N:Br^-. Our hypotheses were that the uptake of Br^- and of N derived from NO_3^- would be related to the concentration supplied, and that the molar ratio of the tracers in the herbage would reflect the molar ratio in solution at all concentrations.

1994

On 7 Nov. 1994, the experiment was initiated by removing herbage and applying fertilizer treatments containing $CaBr_2$ and $Ca(^{15}NO_3)_2$ (1.036 atom % ^{15}N). The experimental design was six replicate blocks in a split plot arrangement of treatments, with tracer concentrations as whole plots and three harvest dates as subplots. Treatments were applied daily as 250 mL pot^{-1} of one of four tracer concentrations at a constant NO_3^-–N:Br^- molar ratio of 200: (i) 2 m*M* NO_3^-, 0.01 m*M* Br^-; (ii) 5 m*M* NO_3^-, 0.025 m*M* Br^-; (iii) 10 m*M* NO_3^-, 0.05 m*M* Br^-; and (iv) 20 m*M* NO_3^-, 0.10 m*M* Br^-. Six pots from each harvest date used for background measurements received daily applications of tap water at 250 mL pot^{-1}. On 21 Nov. (15 d), 1 Dec. (25 d), and 11 Dec. (35 d) 1994, herbage regrowth was harvested from six pots per treatment.

1995

On 7 Nov. 1995, the experiment was initiated by removing herbage and applying nutrient solution (250 m*M* KCl, 100 m*M* $MgSO_4$, 50 m*M* K_2HPO_4, 10 m*M* FeEDTA, 25 m*M* H_3BO_3, 0.25 m*M* $MnCl_2$, 0.2 m*M* $ZnSO_4$, 0.05 m*M* $CuSO_4$, and 0.05 m*M* $[NH_4]_4Mo_7O_{24}$) enriched with $CaBr_2$ and $Ca(^{15}NO_3)_2$ (1.057 atom % ^{15}N). The experimental design was four replicate blocks in a split-split plot arrangement with tracer concentrations applied as whole plots, germplasm entries as subplots, and harvest dates as sub-subplots. Treatments were applied twice daily as 100 mL pot^{-1} at one of four tracer concentrations with a constant NO_3^-–N:Br molar ratio of 200: (i) 1 m*M* NO_3^-, 0.005 m*M* Br^-; (ii) 2 m*M* NO_3^-, 0.01 m*M* Br^-; (iii) 5 m*M* NO_3^-, 0.025 m*M* Br^-; and (iv) 10 m*M* NO_3^-, 0.05 m*M* Br^-. Four pots from each entry and harvest date used for background measurements were watered daily with N-free and Br^--free nutrient solution. On 21 Nov. (15 d) and 6 Dec. (30 d) 1995, regrowth herbage was harvested from four pots from each treatment and entry combination.

Exp. 2: Br^- and NO_3^-–N Uptake with Increasing Br^- Concentration

Experiment 2 was conducted to determine the response of NO_3^-–N and Br^- uptake at increasing Br^- concentrations, but constant NO_3^-–N concentration in applied solutions. The hypothesis was that Br^- uptake would not be affected by the presence of a relatively high NO_3^-–N concentration (5 m*M*) in nutrient solution.

1994

The experiment was initiated on 7 Nov. 1994 by removing herbage and applying fertilizer treatments containing $CaBr_2$ and $Ca(^{15}NO_3)_2$ (1.036 atom % ^{15}N). The experimental design was six replicate blocks in a split-plot arrangement with tracer ratios as whole plots and harvest dates as subplots. Treatments were applied daily as 250 mL pot^{-1} of one of four NO_3^-–N:Br^- molar ratios (1000:1, 200:1, 40:1, and 8:1) using 5 m*M* NO_3^- and 0.005, 0.025, 0.125, and 0.625 m*M* Br^-. Six pots from each harvest date used for background measurements received daily applications of tap water at 250 mL pot^{-1}. On 21 Nov. (15 d), 1 Dec. (25 d), and 11 Dec. (35 d) 1994, regrowth herbage was harvested from six pots per treatment. Data were fit to linear response functions using regression analysis.

1995

On 7 Nov. 1995, the experiment was initiated by removing herbage and applying nutrient solution (described in Exp. 1) enriched with $CaBr_2$ and $Ca(^{15}NO_3)_2$ (1.057 atom % ^{15}N). Six treatments (1000:1, 80:1, 40:1, 20:1, 13.3:1, and 8:1) were applied twice daily as 100 mL pot^{-1} of 5 m*M* NO_3^- and 0.005, 0.0625, 0.125, 0.250, 0.375, and 0.625 m*M* Br^- to single pots (5 plants pot^{-1}) of each entry. Treatments containing 0.005, 0.125, and 0.625 m*M* Br^- were duplicated. On 21 Nov. (15 d) 1995, regrowth herbage was harvested and analyzed as before. Data from this experiment were evaluated using regression analysis.

Exp. 3: Plant-to-Plant Variation within a Population

Experiment 3 was conducted to determine the plant-to-plant variation of NO_3^-–N and Br^- uptake in a population, using the same molar ratio (200:1) as used in Exp. 1. Differences in uptake must exist for selection in a plant breeding program to be successful.

1994

On 7 Nov. 1994, the experiment was initiated by removing herbage and applying a tracer solution containing $CaBr_2$ and $Ca(^{15}NO_3)_2$ (1.036 atom % ^{15}N). Pots with individual plants received 100 mL of 5 m*M* NO_3^- and 0.025 m*M* Br^- solution daily. Five pots received tap water daily for background measurements. On 22 Dec. 1994, after 35 d of regrowth, herbage was harvested from all 92 plants that had produced sufficient herbage dry matter for chemical analysis.

Table 1. Mean squares from analyses of variance for NO_3^-–N and Br^- uptake, molar ratio of NO_3^-:Br^- uptake, and herbage yield of alfalfa for all harvest days and four tracer concentration treatments with constant NO_3^-:Br^- molar ratios in 1994 (Exp. 1).

Source of variation	df	NO_3^-–N uptake	Br^- uptake	Ratio	Herbage yield
Replication (R)	5	21.8	0.000101	45 520	8.0
Treatment (Trt)	3	271.5*	0.000782**	614 100**	4.7
Linear (L)	1	662.9**	0.002117**	30 270	—
Quadratic (Q)	1	127.1**	0.000073	1 801 000**	—
Error *a*	15	8.1	0.000045	23 480	3.0
Harvest Date (HD)	2	605.4**	0.002787**	1 186 000**	251.6**
25 d vs. 15 d, 35 d	1	6.9	0.000107	14 750	0.1
15 d vs. 35 d	1	1203**	0.005466**	2 357 000**	503.2**
Trt × HD	6	42.5**	0.000217**	58 610	1.4
L × 25 d vs. 15 & 35 d	1	0.4	0.000064	—	—
L × 15 d vs. 35 d	1	186.5**	0.001053**	—	—
Q × 25 d vs. 15 & 35 d	1	14.8*	0.000034	—	—
Q × 15 d vs. 35 d	1	33.0**	0.000066	—	—
Error *b*	40	3.4	0.000026	28 280	1.5
CV, %		20	36	20	21

*,** Significant at $P < 0.05$ and 0.01, respectively.

1995

The experiment was initiated on 7 Nov. 1995 by removing herbage and applying nutrient solution (described in Exp. 1) enriched with $CaBr_2$ and $Ca(^{15}NO_3)_2$ (0.527 atom % ^{15}N). Pots with individual plants received 100 mL of 5 m*M* NO_3^- and 0.025 m*M* Br^- solution daily. Four pots from each entry received N- and Br^--free nutrient solution for background measurements. On 21 Nov. 1995, after 15 d of regrowth, herbage was harvested from 26, 29, and 34 plants of Webfoot, Agate, and Ineffective Agate, respectively, that had produced sufficient herbage dry matter for chemical analysis.

Statistical Analysis

Analysis of variance with orthogonal single-degree-of-freedom comparisons and regression analysis were conducted using SAS (SAS Inst., 1990) and *F*-tests were used to determine significance of treatment comparisons. Nitrate-N uptake curves were fit to the rescaled Mitscherlich equation, with an added exponent to fit a sigmoidal response. This sigmoidal response curve has been used to describe uptake curves of phosphate in legumes (Barrow and Mendoza, 1990). In Exp. 2, the response of molar ratio of NO_3^-–derived N:Br^- reflected in the herbage was fit to the Mitscherlich equation.

Model building techniques were used to test the hypothesis that regression lines from different harvest dates in 1994 had the same slope for the molar ratio of NO_3^-–derived N:Br^- in Exp. 2 (Weisberg, 1985, p. 179–183; Weisberg and Cook, 1990). A general model (all harvest dates had unique intercepts and slopes) was compared with more restrictive models (some or all harvest dates had the same slope but unique intercepts). Restrictions to the model were rejected if they increased error sums of squares significantly ($P < 0.05$) (Weisberg and Cook, 1990). Finally, restrictions were applied to test for effects of harvest date on intercept coefficients ($P < 0.05$).

RESULTS AND DISCUSSION

Exp. 1: NO_3^-–N and Br^- Uptake When Supplied at a Fixed Ratio (200:1) and Increasing Concentration

1994

Nitrate-N uptake, Br^- uptake, and the molar ratio of NO_3^-–derived N:Br^- were significantly different for treatments and harvest date and there was a treatment × harvest date interaction for both NO_3^-–N uptake and Br^- uptake (Table 1). As herbage regrowth progressed, both NO_3^-–N and Br^- uptake increased, causing an interaction in magnitude, but not in direction (Fig. 1 and 2). These results were not surprising, because plants sampled at later dates had been exposed to NO_3^-–N and Br^- treatments longer and had higher herbage yield. Herbage yield differed among harvest dates, and increased at about 0.5 g d^{-1}, from 2.56 g at 15 d, to 6.05 g at 25 d, and to 12.7 g at 35 d. Spearman's rank correlation of treatments for NO_3^-–N uptake was $r = 1.0$ among all harvest dates. Rank correlation of treatments for Br^- uptake was $r = 0.8$ between regrowth at 15 d vs. 25 d and regrowth at 25 d vs. 35 d, but was only $r = 0.4$ between the regrowth 15 d vs. 35 d. Treatments that changed rank, however, were not significantly different in Br^- uptake. Thus, time allowed for regrowth would not affect selection of plants for high and low NO_3^-–N uptake based on Br^- uptake.

Nitrate-N uptake increased curvilinearly with NO_3^-–N concentration for all harvest dates in 1994. Response curves were fitted for all three harvest dates using the

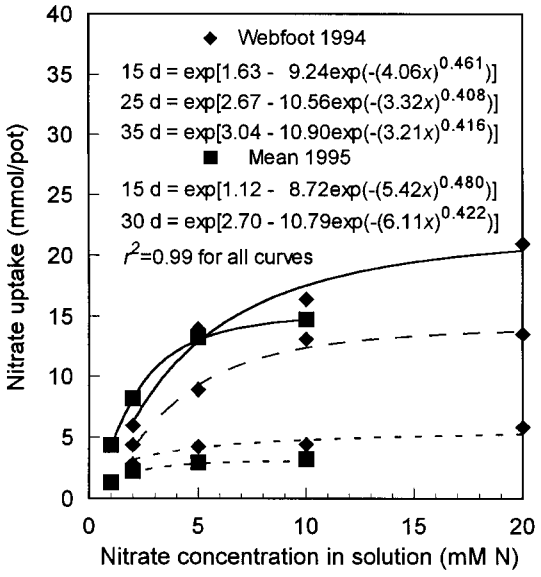

Fig. 1. Nitrate-N uptake response in alfalfa at different times after harvest to increasing NO_3^-–N and Br^- concentrations in solution treatments for 1994 and 1995 (Exp. 1).

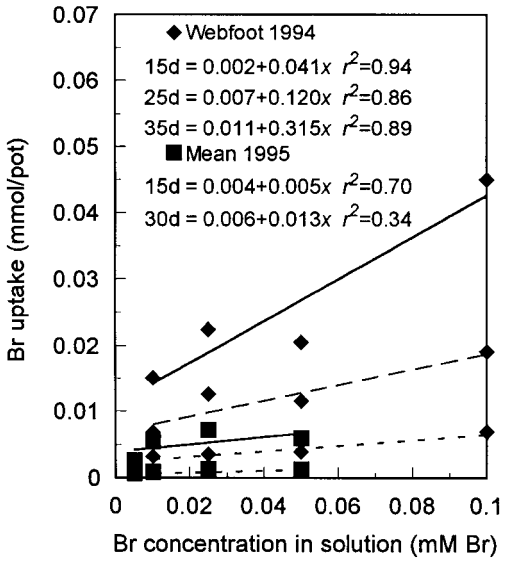

Fig. 2. Bromide uptake response in alfalfa at different times after harvest to increasing Br^- and NO_3^-–N concentrations in solution treatments for 1994 and 1995 (Exp. 1).

rescaled Mitscherlich equation (r^2 = 0.99 for all dates) (Fig. 1). As solution concentrations increased, response to added NO_3^- plateaued. This effect is consistent with the general observation that response to a limiting nutrient decreases as nutrient need is fulfilled (Barrow and Mendoza, 1990). Nitrate-N uptake rate increased as harvest date was delayed, with highest uptake at the 35-d harvest (when plants were in full bloom). Rate of NO_3^-–N uptake in other legumes, such as cowpea [*Vigna unguiculata* (L.) Walp.], green gram (*Vigna radiata* L.), and soybean [*Glycine max* (L.) Merr.], increased throughout vegetative growth, peaked during reproductive stages, and then declined during pod and seed development (Imsande and Edwards, 1988), consistent with our results.

Bromide uptake increased linearly as Br^- treatment concentration increased at all harvest dates (Table 1; Fig. 2), in contrast to the curvilinear trend in NO_3^-–N uptake. This was probably due to the much lower concentration range used for Br^- than for NO_3^-–N (200-fold lower on a molar basis). We used relatively low concentrations of Br^- to avoid possible toxicity to the alfalfa, to minimize tracer costs, and to reduce potential environmental impacts of using Br^- in the field. The linear relationship between the amount of Br^- applied and that absorbed by the plant is consistent with results of Chao (1966) in sorghum. Mechanisms of Br^- uptake are not fully understood, but studies suggest that Cl^- and Br^- use the same carrier mechanism. Epstein (1953) found that Cl^- competitively inhibited Br^- uptake, whereas NO_3^- had an uncompetitive or perhaps noncompetitive effect on Br^- uptake in excised barley (*Hordeum vulgare* L.) roots.

Because NO_3^-–N uptake was nonlinear and Br^- uptake was linear with the concentration ranges used in this experiment, the molar ratio of NO_3^-–derived N:Br^- in the herbage was not constant over treatment or harvest date, but followed a quadratic response (Table 1).

Nutrient uptake rate may be regulated by plant developmental stage (Imsande and Edwards, 1988). Our results suggest that plant selection should occur on a single harvest date when plants are uniform in maturity. As length of exposure to tracers increases and as tracer concentrations increase, plant uptake increases within the response range of the plant. In our study, plant material harvested at 15 d was vegetative and at 35 d was in full bloom and reproductive, whereas plants at 25 d were mixed in stage of development. Therefore, to ensure that differences are due to uptake and are not confounded by phenological changes, a later harvest date (before seed development) would produce more stable results and allow maximum response to the tracer. Selection for NO_3^-–N uptake using Br^- uptake will require repeated tracer (Br^-) and NO_3^-–N applications at the selected labeling levels during the regrowth period to maintain consistent NO_3^-–N:Br^- supply, because these ratios in alfalfa herbage changed with the ratios in solution.

1995

Uptake of NO_3^-–N and Br^- differed for treatments and harvest date, and there was a treatment × harvest date interaction for NO_3^-–N and Br^- uptake (Table 2). Germplasm entries also differed in NO_3^-–N uptake. The interaction of tracer concentration in solution with harvest date was again one of magnitude and not direction (Fig. 1, 2). Plants at 30 d had much higher NO_3^-–N uptake, Br^- uptake, and herbage yield than at 15 d of regrowth. As concentration of tracers applied increased, the NO_3^-–N and Br^- uptake in the plant increased, consistent with results obtained in 1994.

The ratio of NO_3^-–derived N:Br^- in herbage was significantly different for germplasm entries, harvest dates, and their interaction (Table 2). Treatment × harvest date interaction for ratio of NO_3^-–derived N:Br^- in herbage was evident, because treatments did not alter tracer ratio in alfalfa at 15 d, but the ratio increased with higher solution concentrations at 30 d (Fig. 3). Lack of stability at the 30-d harvest may be due to phenological stage, presuming that alfalfa behaves like the grain legumes studied by Imsande and Edwards (1988). The 30-d harvest in 1995 was comparable to the 25-d harvest in 1994, with plants mixed in maturity from vegetative to early reproductive stages. As with tracer uptake, the germplasm entry × harvest date interaction was one of magnitude and not direction. At 15 d, Ineffective Agate had a higher ratio of NO_3^-–derived N:Br^- in herbage and was significantly different from Webfoot and Agate, but by 30 d the entries did not differ.

Nitrate-N uptake response for both harvest dates was curvilinear (Table 2) and was fit to the rescaled Mitscherlich equation (r^2 = 0.99 for both harvests) (Fig. 1). Agate had significantly higher NO_3^-–N uptake (mean = 7.4 mmol pot^{-1}), than Webfoot (mean = 5.3 mmol pot^{-1}) or Ineffective Agate (mean = 5.9 mmol pot^{-1}). Alfalfa germplasms responded similarly in N uptake to increasing concentrations of NO_3^- in solution. Bromide uptake for both harvest dates was linear in response to Br^- supply (Table 2; Fig. 2).

Table 2. Mean squares from analyses of variance for NO_3^-–N and Br^- uptake, molar ratio of NO_3^-–N:Br^- uptake, and herbage yield of alfalfa for all harvest days and four tracer concentration treatments with constant NO_3^-–N:Br^- molar ratios in 1995 (Exp. 1).

Source of variation	df	NO_3^-–N uptake	Br^- uptake	Ratio	Herbage yield
Replication	3	4.9	0.012	313 400	0.56
Treatment (Trt)	3	187.9**	0.287**	1 322 000	7.43*
Linear (L)	1	36.3	0.031**	—	1.29
Quadratic (Q)	1	200.5**	0.001	—	4.83
Error *a*	9	10.3	0.029	616 800	1.84
Germplasm (G)	2	37.1**	0.036	14 440 000**	17.76**
Effective vs. Ineffective	1	4.2	0.002	26 780 000**	26.51**
Trt × G	6	7.4	0.018	697 200	1.43
Error *b*	24	5.1	0.032	504 900	1.11
Harvest Date (HD)	1	1433**	4.443**	31 560 000**	412.2**
Trt × HD	3	92.5**	0.157**	2 519 000**	5.18*
L × 15 d vs. 30 d	1	16.4	0.021**	1 105 000	0.93
Q × 15 d vs. 30 d	1	110.5**	0.002	6 313 000**	3.42
G × HD	2	16.7	0.022	8 942 000**	5.96*
Trt × HD × G	6	7.8	0.021	524 900	1.53
Error *c*	36	7.2	0.033	535 900	1.44
CV %		43	60	29	37

*,** Significant at $P < 0.05$ and 0.01, respectively.

Results from both 1994 and 1995 show that total uptake of NO_3^- and Br^- increased over time and as concentrations of Br^- and NO_3^-–N increased in solution. Differences observed between the two years in total uptake and tracer ratio may be due to difference in rooting media (soil–sand mixture vs. sand) and overall age of the plants during the experiment. In 1994, the plants were 28 wk old, whereas the plants in 1995 were only 14 wk old when treatments were imposed. Plants harvested at 25 d in 1994 were more comparable to plants harvested at 30 d in 1995 (average herbage yield was 6.05 g at 25 d 1994 and 5.29 g at 30 d in 1995), and in both years plants at these harvest dates were in mixed developmental stages. Response curves were curvilinear for NO_3^-–N uptake and linear for Br^- uptake; however, plant uptake increased for both NO_3^-–N and Br^- as concentrations in solution increased, which indicates that Br^- uptake may serve as an adequate surrogate for NO_3^-–N uptake in an alfalfa breeding program.

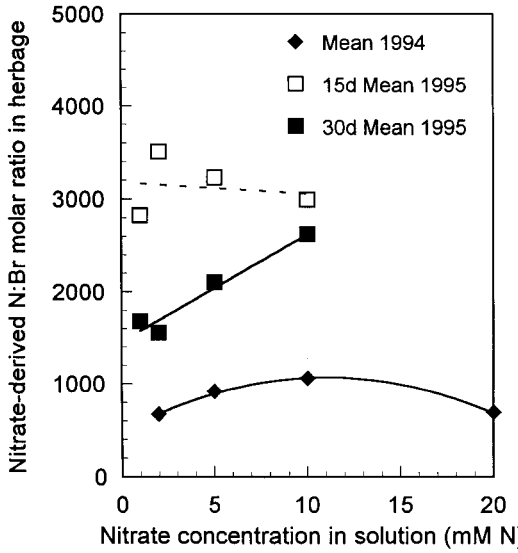

Fig. 3. Molar ratio of NO_3^-–N:Br^- uptake in response in alfalfa at different times after harvest to increasing NO_3^-–N and Br^- concentrations (provided at a constant molar ratio of 200) in solution treatments 1994 and 1995 (Exp. 1).

Exp. 2: Br^- and NO_3^-–N Uptake with Increasing Br^- Concentration

1994

At a constant solution NO_3^-–N concentration of 5 m*M*, but increasing Br^- concentration (0.005–0.625 m*M*), there was a significant effect of tracer treatment, harvest date, and their interaction for Br^- uptake and the ratio of NO_3^-–derived N:Br^- in the herbage (Table 3). The interaction was one of magnitude and not direction, as shown by Br^- uptake (Fig. 4). Bromide uptake increased linearly as Br^- concentration in the treatments and harvest date increased, which indicates that the highest Br^- concentration used (0.625 m*M*) apparently was not toxic. This linear relationship was consistent with Exp. 1. The molar ratio of NO_3^-–derived N:Br^- in the herbage (y) directly reflected the ratio in the solution applied (x) for all three harvest dates of Webfoot, although the slope decreased with plant development (15 d, $y = 4.91x - 125$; 25 d, $y = 3.19x + 84$; 35 d, $y = 2.74x + 37$, all with $r^2 = 0.99$). These positive slopes show that alfalfa absorbed relatively more NO_3^-–N than Br^- at all ratios supplied. This apparent

Table 3. Mean squares from analyses of variance for NO_3^-–N and Br^- uptake, molar ratio of NO_3^-–N:Br^- uptake, and herbage yield of alfalfa for all three harvest days over four treatments that received treatments of increasing Br^- concentrations with a constant NO_3^-–N concentration (5 m*M*) in 1994 (Exp. 2).

Source of variation	df	NO_3^-–N uptake	Br^- uptake	Ratio	Herbage yield
Replication	5	3.41	0.0065	741 200	1.70
Treatment (Trt)	3	6.41	0.4326**	48 600 000**	2.55
Error *a*	15	3.59	0.0043	888 400	1.11
Harvest Date (HD)	2	642.5**	0.1850**	3 182 000**	293.1**
Trt × HD	6	1.26	0.1082**	1 434 000**	1.16
Error *b*	38	2.77	0.0047	381 400	1.45
CV %		17	64	51	20

** Significant at $P < 0.01$.

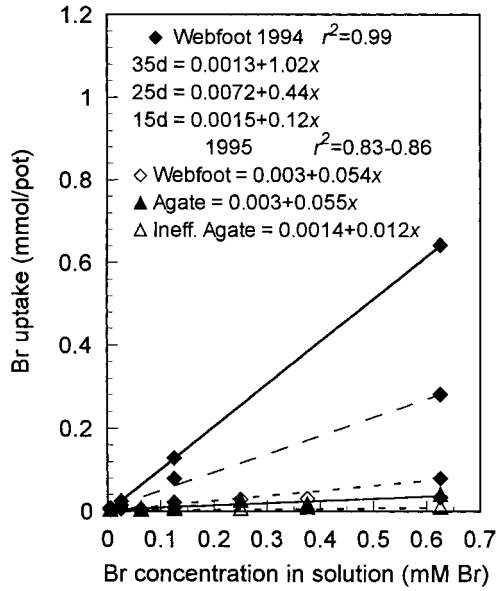

Fig. 4. Bromide uptake response in alfalfa at different times after harvest to increasing Br^- concentrations and constant NO_3^-–N concentration in solution treatments for 1994 and 1995 (Exp. 2).

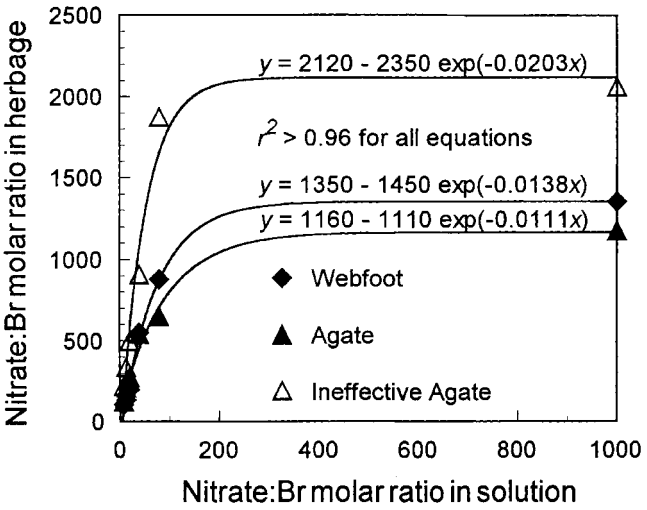

Fig. 5. Molar ratio of NO_3^-–N:Br^- response in alfalfa to increasing Br^- concentrations and constant NO_3^-–N concentrations in solution treatments for 1995 (Exp. 2).

preference for NO_3^- should not, however, affect the use of Br^- as an indicator of *relative* NO_3^- uptake.

1995

Bromide uptake increased as the solution Br^- concentration applied increased for Webfoot, Ineffective Agate, and Agate, but to a much lesser extent than in 1994 (Fig. 4). As in 1994, alfalfa absorbed considerably larger amounts of NO_3^- than Br^-, but, in contrast to 1994, the ratio of NO_3^-–derived N:Br^- in herbage was less influenced at solution ratios greater than about 100:1 (Fig. 5). Differences in growing days, rooting media conditions (soil–sand in 1994, sand in 1995), and solution composition between experiments conducted in 1994 and 1995 may have contributed to this different response. For example, concentration of other anions supplied in nutrient solution in 1995 may have reduced potential NO_3^- uptake; solutions provided to plants in the soil–sand mixture (1994) contained only $CaBr_2$ and $Ca(NO_3)_2$. To avoid this problem, selection for NO_3^- uptake based on Br^- uptake should be conducted at solution NO_3^-–N:Br^- molar ratios less than about 100:1; this recommendation should be considered preliminary, as it was developed under greenhouse conditions under only one NO_3^- concentration (5 m*M*).

The ineffective germplasm had a much higher proportion of NO_3^-–derived N:Br^- than the effective germplasms, which tends to confirm the propensity of this non-N_2–fixing germplasm to absorb more NO_3^- (Blumenthal and Russelle, 1996). However, this may also indicate that other differences in uptake mechanisms exist among germplasms, and, in addition, this response was not consistent among the current experiments, as Ineffective Agate absorbed less NO_3^-–N than Agate in Exp. 1 in 1995.

Results from this experiment show that, at a constant NO_3^-–N concentration but increasing Br^- concentrations, plants absorb Br^- in increasing amounts, but maintain the same concentration of NO_3^-–derived N in the herbage. These data support the idea that selection for NO_3^-–N uptake using Br^- uptake will not be affected by moderate concentrations of NO_3^-–N in soil solution, and support our earlier conclusion that selection should occur under uniform maturity at a later harvest date to maximize uptake.

Exp. 3: Plant-to-Plant Variation within a Population

1994 and 1995

Plant-to-plant variability was evident for NO_3^-–N uptake and Br^- uptake within each germplasm entry in both 1994 and 1995 (Fig. 6). Plants that were high in NO_3^-–N uptake were also high in Br^- uptake; therefore, individual plant selection for NO_3^-–N uptake using Br^- uptake as a selection criterion would result in minimal selection error. Hypothetical selection pressure of 5% for the population of 92 plants in 1994 is shown in Fig. 6. The overlapping boxes indicate that if Br^- uptake were used to select for NO_3^- uptake, error would be less than 2%. In 1995, both Ineffective Agate and Agate having higher mean NO_3^-–N uptake than Webfoot ($P < 0.01$), but germplasms did not differ in mean Br^- uptake. The disparity in results for NO_3^- and Br^- in this experiment may be due in part to the short period of herbage regrowth, but may also indicate a need for further method validation.

CONCLUSIONS

Our results suggest that Br^- has promise as a tracer for NO_3^-–N uptake in a field-based alfalfa plant breeding program. Responses were curvilinear for NO_3^-–N uptake and linear for Br^- uptake over the concentration ranges we used, but plant uptake increased for both NO_3^-–N and Br^- as solution concentrations increased. In other research, we have found that Br^- and NO_3^-–N uptake are linearly related under field conditions (J.M.

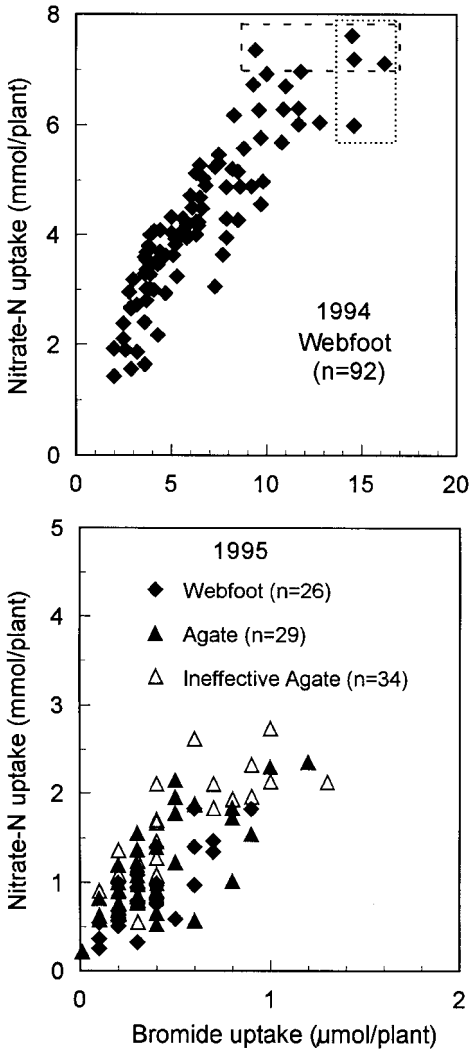

Fig. 6. Scatter plots of NO_3^-–N uptake vs. Br^- uptake for 1994 and 1995 (Exp. 3). Selection for 5% of the 1994 population using Br^- uptake compared with NO_3^-–N uptake is shown in the outlined boxes.

Blumenthal, M.P. Russelle, and J.F.S. Lamb, unpublished data, 1996). These results indicate that selection for NO_3^-–N uptake using Br^- in an alfalfa breeding program is feasible. Efficiency of this selection criterion requires relatively close linkage in these apparently independent ion uptake mechanisms.

Although Br^- could be used to indicate relative efficiency of NO_3^-–N uptake in crop management experiments, we do not recommend it for experiments on physiology of NO_3^-–N uptake, because the relationship is not sufficiently close, the uptake mechanisms probably are different, and the physiological roles of these elements differ. The differences between the two tracers raise the possibility that selection for NO_3^-–N uptake using Br^- may ultimately fail, and field testing of selections made using this procedure with direct measurement of NO_3^- uptake (using ^{15}N-labeled NO_3^-) will be needed.

Selection should take place in a population of plants that have received a relatively constant supply of Br^-. Plants should have a uniform stage of maturity (fully reproductive) and should be harvested later, rather than early, in the regrowth cycle to maximize uptake. Our data support the idea that selection for NO_3^- uptake using Br^- uptake will not be affected by moderate concentrations of NO_3^-–N in the soil. Plant selection for NO_3^-–N uptake using Br^- uptake as a selection criterion would result in minimal selection error, and plants selected for high Br^- uptake would also be high in NO_3^-–N uptake relative to the remainder of the population.

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