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Postanthesis Nitrate Assimilation in Winter Wheat[1]

IN SITU FLAG LEAF REDUCTION

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ABSTRACT

When adequate levels of soil NO_3^- are available, concurrent NO_3^- absorption and assimilation, and mobilization of vegetative N reserves accumulated prior to anthesis, may be used to supply N to developing wheat (*Triticum aestivum* L.) kernels. Vegetative wheat components (stems, leaves, spike) are known to possess NO_3^- reductase activity, but the *in situ* utilization of NO_3^- translocated to the shoot has not been studied. Assimilation and partitioning of ^{15}N was determined in winter wheat 'Doublecrop.' At 7 days after anthesis, the stem immediately above the peduncle node was heat girdled to block phloem export from the flag leaf. Control plants were not girdled. One day later, 50 micromoles of $^{15}NO_3^-$ (98 atom percent ^{15}N) was injected into the penultimate internodal lacuna, after which $^{15}NO_3^-$ utilization was determined sequentially over a 5 day period. Based on differences in spike accumulation of reduced ^{15}N excess between treatments and the amount of reduced ^{15}N excess remaining in the flag leaf, it was estimated that the flag leaf contributed 37% of the total reduced ^{15}N excess in the injected shoot. The lower shoot contribution was 18% and that of the peduncle plus spike was 45%.

Nitrogen supplied for wheat (*Triticum aestivum* L.) grain protein synthesis is derived from mobilization of vegetative N reserves accumulated before anthesis and from N absorbed during grain development. The availability and level of soil NO_3^- and the availability of C substrates that support plant transport and assimilation processes are two factors that would affect the amount of soil N accumulated after anthesis. Nitrate absorbed after anthesis can be assimilated by leaves, stem, and spike components, since each possesses NO_3^- reductase activity (5, 12). Nevertheless, the *in situ* NO_3^- assimilation role of aboveground plant parts is not well defined.

Nitrate is transported to the aboveground plant parts in the xylem transpiration stream. The transpiration rates of wheat and barley spikes range from 30 to 77% of the flag leaf transpiration rate at anthesis (3), indicating that approximately equivalent proportions of NO_3^- are delivered to the spike and flag leaf. During grain filling the transport of NO_3^- to the spike may even exceed that to leaves as the spike transpiration exceeds that of all the leaves on a stem (17). Both spike and peduncle *in situ* NO_3^- assimilation proceed rapidly immediately following anthesis, with a minimum of 15% of the total NO_3^- reduction attributed to the peduncle (8). Nitrate assimilation and export of reduced N products by flag leaves also occurs rapidly after anthesis (9), even when the flag leaf has lost as much as 50% of its N content (2).

The objective of this study was to provide an estimate of the *in situ* NO_3^- assimilation role of the flag leaf and other aboveground plant parts after anthesis. The experimental approach differed from that of previously published reports (2, 9) in that $^{15}NO_3^-$ was delivered to the flag leaf by xylem transport rather than by absorption through the leaf surface or cut leaf tip. The flag leaf was heat girdled to prevent export of assimilated $^{15}NO_3^-$. Flag leaf reduced-^{15}N excess contents and differences in spike reduced-^{15}N excess between nongirdled control stems and the heat-girdled stems gave a minimum estimate of the flag leaf $^{15}NO_3^-$ assimilation contribution.

MATERIALS AND METHODS

Winter wheat ('Doublecrop') was planted October 27, 1984 at Spindeltop Research Farm, Lexington, KY. Standard cultural and management practices were followed for planting, fertilization, and control of disease and insects. At anthesis (emergence of anthers from florets), stems were tagged. At 7 d after anthesis, half of the tagged stems were randomly selected for heat girdling using a procedure similar to that described by Patrick and Wardlaw (15) to achieve phloem blockage of the flag leaf. The stem immediately above the peduncle node swelling was heat girdled by wrapping resistance wire around the stem and supplying a regulated voltage across the wire. When the stem zone of contact by the hot wire appeared wet, indicating cell collapse and leakage of cell sap, the voltage supply circuit was opened and the resistance wire removed. The remaining nongirdled stems were used as controls.

$^{15}NO_3^-$ Injection and Plant Sampling. One d after heat girdling, 50 μl of a solution containing 50 μmol $^{15}NO_3^-$ (98 A% ^{15}N[2]) was injected into the penultimate internodal lacuna approximately 7 cm above the penultimate leaf node. The aboveground plant parts of an injected stem were harvested 0, 1, 3, and 5 DAI and separated into spikes, peduncles, flag leaves (with sheaths), and lower shoots below the peduncle node. At each harvest, three replicates of seven to eight plants were randomly selected. Plant parts were frozen, freeze-dried, weighed, and ground for analyses.

Analyses. Ground plant parts were oven dried at 50°C overnight before weighing samples for C and N analyses. Flag leaf nonstructural carbohydrate was determined by a modification of the procedure of Swank *et al.* (18). Extracts for total nonstructural carbohydrate (starch, hydrolyzed sugars, reducing sugars) were incubated for 15 min at 55°C with 1 unit of amyloglucosi-

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[2] Abbreviations: A% ^{15}N, atom percent ^{15}N; DAI, days after injection; TNSC, total nonstructural carbohydrate.

dase (Sigma No. A-7255)[3] to achieve complete starch hydrolysis before assaying for reducing sugars (13). The free carbohydrate was also determined on extracts not incubated with amyloglucosidase. Tissue N was partitioned into insoluble reduced- and soluble-N fractions by methanol-chloroform-water extraction (14, 16). Nitrate present in the methanol-water soluble-N fraction was removed by volatilization to minimize interference with soluble reduced N determinations. Aliquots from the Kjeldahl digest (10) of the insoluble- and soluble-reduced N fractions were analyzed colorimetrically for NH_4^+ by a modified Berthelot procedure (4) after adding Zn to the digest to amalgamate the Hg-Kjeldahl catalyst. Ammonium in the remaining digest was diffused from the solution in a 250-ml disposable screw cap specimen container and trapped in a 12 × 75-mm test tube containing 0.5 ml of 0.5 N HCl. The trapped ^{15}N-enriched NH_4^+ was analyzed by a freeze-layer mass spectrometer method (19). Nitrate in hot water tissue extracts was determined by a nonautomated method utilizing dissimilatory NO_3^- reduction to NO_2^- by *Escherichia coli* followed by Griess-Ilosvay colorimetric determination (7). Plant tissue containing approximately 15 μmol NO_3^- was extracted twice with 5 ml hot water for NO_3^- A% ^{15}N determination. The tissue extract was added to about 1.0 meq of anion resin (Bio Rad AG 1-X8, 50–100 mesh) that had been previously washed with 1.0 N H_2SO_4 and then rinsed with water until the eluant pH was neutral. After equilibration, the extract was removed and the resin was transferred to a 12 × 75-mm test tube for NO_3^- A% ^{15}N determination by conversion of the NO_3^- to NO and then mass spectrometric measurement (20).

RESULTS

Heat girdling just above the peduncle node resulted in an increase in flag leaf dry matter accumulation and a decrease in spike dry matter accumulation (Table I). At 0 d after injection (1 d after heat girdling), the flag leaf dry weight of girdled stems was 17% greater than control stems; this difference increased to 47% as the girdled treatment accumulated additional dry matter. Spike dry matter increased after injection for both treatments, but dry matter accumulation in the control stems was greater than in girdled stems. Neither peduncle nor lower shoot dry weight differences between treatments were significant (data not shown). Peduncle and lower shoot dry weights averaged across harvests and treatments were 288 and 1060 mg part^{-1}, respectively.

Table I. *Spike and Flag Leaf Dry Weights and Flag Leaf Total Nonstructural Carbohydrate of Doublecrop Winter Wheat Following Stem $^{15}NO_3^-$ Injection 1 Day after Heat Girdling the Stem at the Peduncle Node*

Plants were girdled 7 d after anthesis; control plants were not girdled.

Days after Injection	Spike		Flag Leaf		Flag Leaf Nonstructural Carbohydrate	
	Control	Girdled	Control	Girdled	Control	Girdled
	mg part^{-1}					
0	383 a*	385 a	216 a	257 b	28.0 a	67.8 b
1	409 ab	403 ab	212 a	277 bc	28.4 a	83.2 c
3	512 d	441 bc	221 a	286 c	28.1 a	91.5 d
5	528 d	455 c	198 a	292 c	29.7 a	99.7 d

* Treatment means within a plant part followed by the same letter are not significantly different by LSD test at a P = 0.05.

Total nonstructural carbohydrate content of the flag leaf in girdled stems was 2- to 3-fold greater than in control stems (Table I). The quantity of flag leaf TNSC remained constant for the control stems, whereas in the girdled stems it increased during the 5 d sampling period. The free carbohydrate proportion of TNSC was constant for both treatments, but relatively more free carbohydrate was present in the flag leaves of the control (89.5%) than the heat girdled (83.2%) plants.

Assimilation of injected $^{15}NO_3^-$ occurred rapidly. At 1 DAI, NO_3^- reduction was greater in the control than in the girdled treatments and reduced N accounted for 40 and 33%, respectively, of the injected NO_3^- (Fig. 1). At 5 DAI, the control had reduced at least 67% and the girdled treatment 53% of the injected NO_3^-. Of the total amount of ^{15}N-excess that was in the reduced form by 5 DAI, 61% of that was present 1 DAI and 83% was present by 3 DAI for both treatments.

At 1 DAI, about 5.3 μmol of total reduced-^{15}N excess was accumulated by the spike for both treatments (Fig. 2). During the next 4 d, the spike total reduced-^{15}N excess accumulation rate of girdled stems (1.12 μmol d^{-1}) was markedly less than the control (2.89 μmol d^{-1}). Greater than 71, 70, and 60% of the maximum total reduced-^{15}N excess content of the flag leaf, peduncle, and lower shoot, respectively, were accumulated within 1 DAI in both treatments (Fig. 2). The peduncle and lower shoot total reduced-^{15}N excess accumulation patterns of both treatments were not different, but at 1 and 3 DAI the flag leaf total reduced-^{15}N excess content of girdled stems was slightly less (significant) than that of the controls. The proportion of the total reduced-^{15}N excess, present in the soluble reduced N fraction of the spike, peduncle, and lower shoot decreased from about 54, 55, and 64% at 1 DAI to about 35, 29, and 31% at 5 DAI, respectively, for both treatments (Fig. 2, numbers adjacent to symbols). In contrast, the flag leaf soluble reduced-^{15}N excess fraction of total reduced-^{15}N excess of girdled plants averaged 52% across harvests at 1, 3, and 5 DAI, whereas the control soluble reduced-^{15}N excess fraction was only 34% at 1 DAI and decreased 4-fold over the same interval.

The total N (^{14}N + ^{15}N) contents of the peduncle, flag leaf, and lower shoot were not significantly different between girdled and control stems or across harvest dates (data not shown). But, spike total N increased from the time of injection, and the total N averaged across harvest dates of the control (541 μmol spike^{-1})

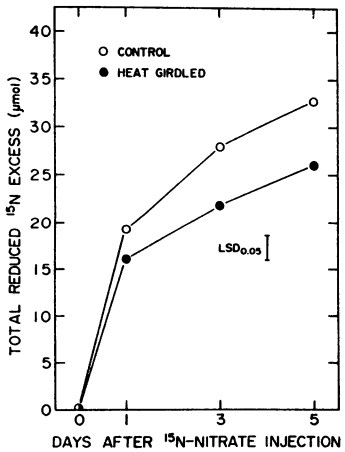

FIG. 1. Total (insoluble and soluble) reduced-^{15}N excess content in the aboveground stem of Doublecrop winter wheat injected with 50 μmol of $^{15}NO_3^-$ (98 A% ^{15}N). Injections were made into the penultimate internodal lacuna 1 d after heat girdling stems at the peduncle node. Control plants were not girdled.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or Kentucky Agricultural Experiment Station and does not imply its approval to the exclusion of other products that may also be suitable.

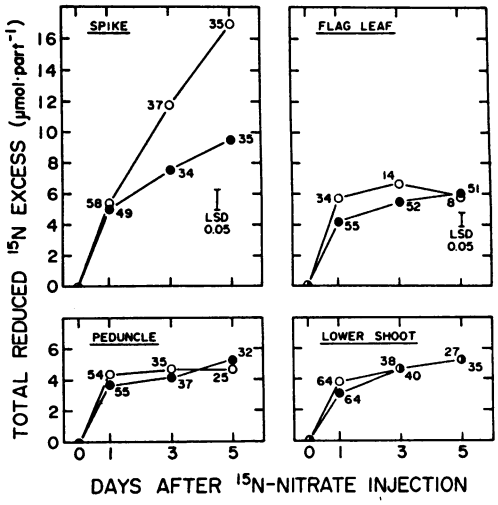

FIG. 2. Total (insoluble and soluble) reduced-^{15}N excess contents in spike, flag leaf, peduncle, and lower shoot of Doublecrop winter wheat stems injected with $^{15}NO_3^-$. Control (open symbol) and heat girdled (closed symbol) penultimate internodal lacunas were injected with 50 μmol $^{15}NO_3^-$ (98 A% ^{15}N) 1 d after heat girdling the flag leaf at the peduncle node. Numbers to the left of control symbols and to the right of heat girdled symbols indicate the percent of total reduced-^{15}N excess as soluble reduced-^{15}N excess.

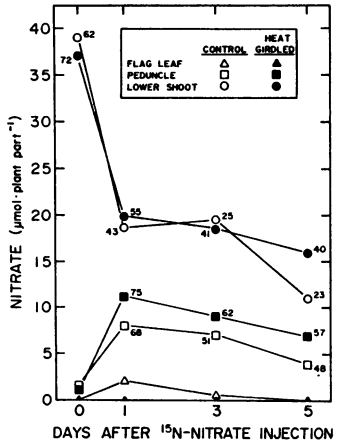

FIG. 3. Nitrate content of Doublecrop winter wheat lower shoot, peduncle, and flag leaf following injection of 50 μmol $^{15}NO_3^-$ (98 A% ^{15}N) into the penultimate internodal lacuna 1 d after heat girdling the stem at the peduncle node. Control plants were not girdled. Numbers next to the lower shoot and peduncle symbols indicate NO_3^- A% ^{15}N excess.

was significantly greater than the girdled stems (493 μmol spike^{-1}).

Nitrate in the lower shoot decreased rapidly for both control and girdled stems within 1 DAI, then decreased at a considerably slower rate over the next 4 d in the girdled and control treatments (Fig. 3). Peduncle NO_3^- markedly increased for both treatments within 1 DAI before slowly decreasing in the following 4 d. The girdled stems had significantly greater peduncle NO_3^- than control stems when averaged across all harvests. At 0 DAI, no flag leaf NO_3^- was detected, but after 1 DAI the control treatments had a flag leaf NO_3^- content of 2.07 μmol, which decreased to a just detectable level at 5 DAI (Fig. 3). No NO_3^- was detected in the flag leaf of girdled stems or the spikes of either treatment.

Nitrate isotope enrichment in the lower shoot was 62 A% ^{15}N for the control and 72 A% ^{15}N for the girdled plants at 0 DAI and decreased at each subsequent harvest (Fig. 3). Peduncle $^{15}NO_3^-$ enrichment also followed a similar pattern. The NO_3^- A% ^{15}N of the lower shoot and peduncle was consistently greater in the girdled than the control treatments (Fig. 3). It was not possible to obtain a measure of NO_3^- A% ^{15}N in the flag leaves, since the tissue NO_3^- concentration at each harvest was less than 10 μmol g dry weight^{-1} and insufficient tissue was available for extraction.

DISCUSSION

Two lines of evidence indicate that flag leaf phloem transport from the heat girdled stems was restricted. First, the increased dry weight of flag leaves from heat girdled stems was associated with a markedly greater flag leaf content of TNSC than the flag leaves of nongirdled controls (Table I). Second, the flag leaf soluble reduced-^{15}N excess fraction of the flag leaf total reduced-^{15}N excess of heat girdled stems was consistently greater than the control (Fig. 2). Our results and those of Patrick and Wardlaw (15) indicate that heat girdling the stem at the peduncle node is an effective technique for flag leaf phloem blockage, but in our experiment, $^{15}NO_3^-$ transport to the flag leaf of heat girdled stems was apparently altered. Accumulation of ^{15}N excess in the flag leaves of heat girdled stems was less than the control during the first 3 DAI, but equal at 5 DAI, indicating a slower $^{15}NO_3^-$ flux into the flag leaf. Furthermore, no NO_3^- was detected in the flag leaves of heat girdled stems, whereas, at 1 DAI NO_3^- content of the control flag leaf was about 2 μmol (Fig. 3). These results indicate that the control flag leaf NO_3^- flux rate exceeded the assimilation rate. An elevated level of nonstructural carbohydrate in wheat leaves mediates a secondary effect on net CO_2 assimilation due to a decrease in the intracellular partial pressure of CO_2 caused by stomatal closure (1). Even though stomatal conductance to water vapor was not measured, the elevated levels of nonstructural carbohydrate and decreased ^{15}N accumulation of flag leaves of heat girdled stems would be consistent with a decreased water and $^{15}NO_3^-$ flux into leaves that have been phloem blocked.

The apparent difference in xylem delivered $^{15}NO_3^-$ to the flag leaves of control and heat girdled stems makes it difficult to formulate the flag leaf contribution to *in situ* $^{15}NO_3^-$ assimilation. A simple comparison of reduced-^{15}N contents in the various plant parts will not suffice. Since both treatments had similar patterns in the dissappearance of $^{15}NO_3^-$ from the injected lower shoot, then a restricted flux of $^{15}NO_3^-$ to the heat girdled flag leaf would have either increased $^{15}NO_3^-$ assimilation in the lower shoot or increased transport to the peduncle and spike. Reduced-^{15}N excess in the lower shoot and peduncle was the same for both treatments (Fig. 2), but peduncle NO_3^- content in the heat girdled treatments was greater than the control (Fig. 3). Compared to the control, the decrease in flag leaf reduced-^{15}N excess content of heat girdled stems was equaled or exceeded by an increase in the peduncle NO_3^- in heat girdled stems (*cf.* Figs. 2 and 3). Thus, a reasonable minimum estimate of flag leaf *in situ* $^{15}NO_3^-$ assimilation would equal the difference between treatments in spike reduced-^{15}N excess, plus the amount of flag leaf reduced-^{15}N excess accumulated by the control. The flag leaf *in situ* $^{15}NO_3^-$ reduction expressed as a percent of total reduced-^{15}N excess for the control treatments averaged 37% across all harvests, except 0 DAI. This represents a minimum, because a portion of the control flag leaf reduced-^{15}N excess may have been exported in a basipetal direction (17). Nevertheless, these results indicate that the lower shoot, spike, and peduncle can also play an important role in postanthesis *in situ* assimilation of $^{15}NO_3^-$ delivered to the shoot. In this study, the lower shoot contribution to total reduced-^{15}N excess was about 18%, and that of the peduncle plus spike was 45%. In a previous study, flag leaf $^{15}NO_3^-$ assimilation was minimized by injecting into the peduncle lacuna; a minimum of about 15% of the total reduction of

$^{15}NO_3^-$ occurred in the peduncle and most of the remainder in the spike (8). The fate of the injected $^{15}NO_3^-$ in the present work represents NO_3^- utilization under a nonsteady flux of NO_3^- to the aboveground stem. We are currently analyzing an experiment with sequential $^{15}NO_3^-$ injections and harvests after anthesis, since the distribution and assimilation of NO_3^- following a single injection may differ from that of a more continuous xylem NO_3^- flux from the root.

In addition to the NO_3^- assimilation contribution of above ground plant parts, the potential contribution of the root system to reduce postanthesis absorbed NO_3^- should not be overlooked. The roots of wheat seedlings also possess the capacity to reduce NO_3^- (11). The root system of wheat during grain development may reduce a significant portion of the absorbed NO_3^- (6), particularly following anthesis, when a low soil solution NO_3^- concentration may limit maximum NO_3^- absorption rates. The role of wheat roots in the utilization of exogenous N during grain development warrants further research for understanding the N economy of winter wheat.

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