The Impact of Taproot N Pools on Shoot Growth of Defoliated Alfalfa (*Medicago sativa* L.) Cultivars Differing in Nodule Effectiveness

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Hypothesis

Nitrogen deprivation of alfalfa populations unable to fix N_2 alters taproot C and N metabolism, mobilization of C and N reserves to regrowing shoots post-defoliation, and impacts shoot development.

Objectives

1. To alter taproot alfalfa N pools using N fertilization of 'Saranac' alfalfa populations differing in nodule effectiveness.
2. To compare how taproot C and N pool sizes and composition impact shoot regrowth after defoliation.

Materials and Methods

Ineffective- and effective-nodulating populations of 'Saranac' alfalfa were seeded into soil-filled pots, inoculated with *Sinorhizobium melitoti*, and provided complete Hoagland's nutrient every five days for 129 days in order to establish plants. Taproot N reserves were then depleted from ineffective-nodulating plants by providing all plants minus-N Hoagland's nutrient solution for nine weeks. Contrasting N pool levels were created in taproots by replacing minus-N Hoagland's solution with complete Hoagland's solution for one-half of the plants for both populations. Plants were sampled during the three weeks of contrasting N nutrition. Plants were defoliated, roots and crowns were carefully washed free of soil, and these organs were transplanted into N-free sand. Plants were provided minus-N Hoagland's and sampled weekly for four weeks. At each sampling stems and crowns were counted, the tissues dried at 65°C and weighed. Taproots were washed free of soil or sand, fresh weights recorded, and the uppermost 2 cm of taproots placed in liquid N and stored at -80°C for RNA analysis. The remainder of the taproots were reweighed, packed in solid CO_2 and stored at -20°C until lyophilized. After lyophilization the root tissues were weighed, ground to pass a 1-mm screen, and stored at -20°C for sugar, starch, protein and amino-N analyses.

Total sugars were extracted in 80% v/v ethanol and quantitated using anthrone (Li, et al., 1996). Starch in the ethanol-extracted residue was gelatinized, hydrolyzed with α-amylase and amyloglucosidase, and the resulting glucose assayed using glucose oxidase (Li et al., 1996). Buffer-soluble proteins were extracted by suspending 60 mg of lyophilized taproot tissue in 1 mL of 100 mM $NaPO_4$ buffer (pH 6.8) containing 1 mM phenylmethylsulfonylfluoride and 10 mM 2-mercaptoethanol. Protein in the supernatant was assayed according to Bradford (1976) at 595 nm. Colorimetric analysis of amino-N in the supernatant was done with ninhydrin (Rosen, 1957). For SDS-PAGE analysis approximately 250 mg taproot tissues were extracted in 600 to 750 µL of the phosphate buffer, the supernatant mixed 1:1 with 2X sample buffer, and the samples diluted to 1 µg/µL for electrophoresis analysis. The SDS-PAGE was in 1.5-mm gels containing 15% acrylamide (Laemmli, 1970). Samples were loaded at 20 µg protein per lane for Coomassie-blue stained gels and at 5 µg per lane for Western blots (Cunningham and Volenec, 1996). Antibodies raised to the 15 and 19 kD vegetative storage proteins and to β-amylase were used in Western blot analysis (Cunningham and Volenec, 1996; Ihonor et al., 2007).

The experiment was a randomized complete-block design with four replicates. Analysis of variance was conducted by population and pre- versus post-transplanting. Variation was partitioned into N and harvest main effects and the N x harvest interaction. Where F-tests were significant, an LSD was calculated.

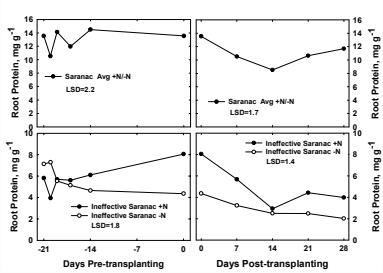

Figure 1. Taproot protein concentrations were significantly greater in Saranac compared to Ineffective Saranac throughout the experiment, and were not influenced by fertilizer application. Protein concentrations in taproots of Saranac declined 43% from Day 0 to Day 14 and increased 50% from Day 14 to Day 28. Protein concentrations in taproots of Ineffective Saranac +N plants increased 33% between Day -21 and 0. After transplanting taproot proteins of these plants declined 63% to levels found in Ineffective Saranac -N plants.

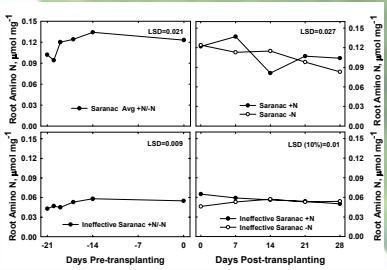

Figure 2. Amino-N concentrations in Saranac +/-N plants were 2-fold greater than in those of Ineffective Saranac plants. In Saranac –N plants amino-N concentrations declined 25% from Day 0 to Day 28, while levels in Saranac +N plants showed a cyclic pattern of depletion by Day 14 and re-accumulation on Days 21 and 28. The N x sampling date interaction was not significant for Ineffective Saranac pre-transplanting. Amino-N concentrations were significantly greater (at the 90% level) in Ineffective Saranac +N plants when compared to Ineffective Saranac -N plants at transplanting on Day 0. Amino-N concentrations did not change significantly after Ineffective Saranac -N plants were transplanted into sand, but declined gradually in taproots of Ineffective Saranac +N plants. .

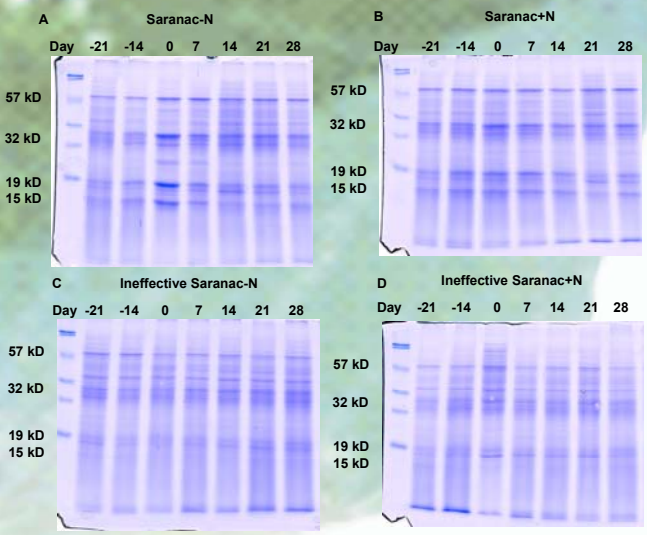

Figure 3. The 15, 19 and 32 kD vegetative storage protein (VSP) concentrations in Saranac taproots increased from Day -21 to Day 0 (day of transplanting), declined between Days 0 and 21 before increasing by Day 28 (A, B). The relative abundance of the 19 and 32 kD VSPs declines from Day -21 to Day 0 in taproots of Ineffective Saranac -N plants (C) while increasing in taproots of Ineffective Saranac +N plants (D). After transplanting (Day 0), taproot VSP levels declined in Ineffective Saranac plants irrespective of previous N nutrition. The 15 kD VSP was not detected on these gels. The molecular weights of β-amylase (57 kD) and the three VSPs (15, 19 and 32 kD) are listed to the left. Lane 1 contains molecular weight markers (19.3; 27.1; 35.8; 47.8; 81,083; 103,774 kD).

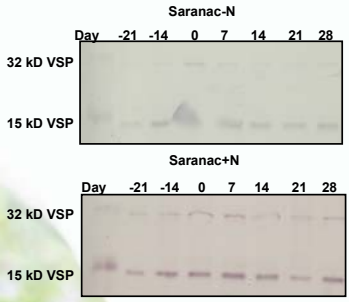

Figure 4. Antibodies raised to the 15 kD vegetative storage protein (VSP) readily detected the 15 and 32 kD VSPs in Saranac plants irrespective of N application. The 15 and 32 kD VSP concentrations increase from Day -14 to Day 0 (day of transplanting). Taproots of Saranac +N plants exhibit the typical pattern of VSP use (Days 0 to 21) followed by resynthesis (Days 21 to 28). The first lane contains the 15 and 32 kD VSPs as standards.

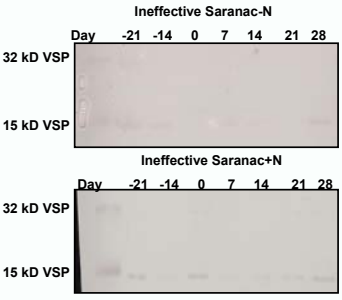

Figure 5. Antibodies raised to the 15 kD vegetative storage protein (VSP) show VSP accumulation prior to transplanting (Days -14 to 0), and utilization after transplanting (Days 0 to 14) in taproots of Ineffective Saranac +N plants. Ineffective Saranac -N plants did not accumulate the 15 kD VSP by Day 0, although low levels of the 15 kD VSP can be detected in taproots of these plants on Day 28. The first lane contains the 15 and 32 kD VSPs as standards.

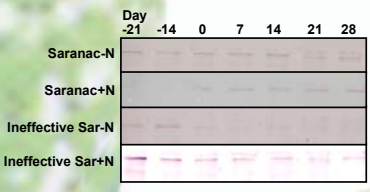

Figure 6. Antibodies raised to β-amylase show the vegetative storage protein (VSP) properties of β-amylase in taproots of Saranac plants irrespective of N application. β-amylase increases by Day 0 (day of transplanting) then is utilized and resynthesized between Days 14 and 28 (Saranac-N) and Days 7 and 21 (Saranac+N). β-amylase concentrations in taproots decline after Day -14 in Ineffective Saranac-N (Sar-N) plants. Ineffective Saranac+N (SAR+N) taproots contain β-amylase concentrations with VSP patterns similar to those found in Saranac-N plants from Days 0 to 28.

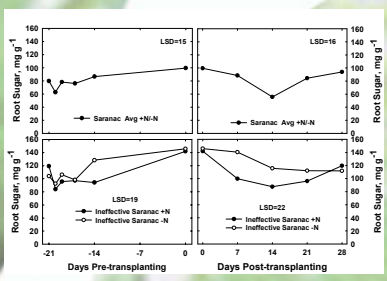

Figure 7. Prior to transplanting on Day 0, taproot sugar concentrations increased gradually in Saranac plants irrespective of N application. During herbage regrowth after transplanting, Saranac taproots exhibited a cyclic pattern of sugar depletion followed by re-accumulation. Sugar concentrations are significantly greater in taproots of Ineffective Saranac -N plants when compared to Ineffective Saranac +N plants on Day -14. After transplanting sugar concentrations in taproots of Ineffective Saranac +N plants declined 36% from Day 0 to Day 14 and then re-accumulated between Days 14 and 28 in a pattern similar to the Saranac plants.

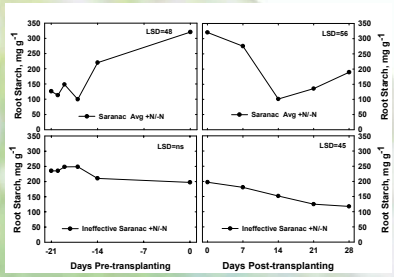

Figure 8. Starch concentrations increased significantly from Day -17 to Day 0 in Saranac taproots irrespective of N application. After transplanting on Day 0, taproot starch concentrations declined 68% by Day 14 as shoots regrew, then re-accumulated to nearly 200 mg g-1 dry wt. Nitrogen application to ineffective Saranac did not impact taproot starch concentrations. Taproot starch concentrations were higher in Ineffective Saranac plants at the start of the experiment and remained unchanged until transplanting. After transplanting taproot starch levels declined gradually between Days 0 and 28.

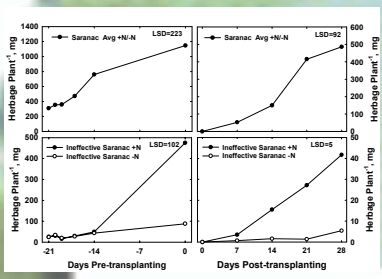

Figure 9. Between Days -21 and 0, herbage per plant increased four-fold in Saranac irrespective of N fertilizer application. Previous N application also did not influence herbage regrowth of transplanted Saranac plants. Applying N to Ineffective Saranac increased herbage accumulation by Day 0. With the additional taproot N reserves, Ineffective Saranac +N plants also had greater herbage growth rates after transplanting into N-free sand on Day 0.

Conclusions

1. Taproot protein concentrations were greater in Saranac than Ineffective Saranac and were utilized and resynthesized after transplanting to sand. Taproot protein concentrations significantly increase in Ineffective Saranac +N plants prior to transplanting (Days -14 to 0) and subsequently decline to levels measured in Ineffective Saranac-N plants once plants are transferred to N-free sand (Days 0 to 14).
2. Taproot vegetative storage proteins (VSPs) show a cyclic pattern of synthesis (Days -21 to 0), utilization (Days 0 to 21) and resynthesis (Days 21 to 28) in Saranac plants irrespective of N application. Although quantities of the 15 kD VSP are lower than found in Saranac, the 15 kD VSP shows a similar cyclic pattern of utilization and synthesis in taproots of Ineffective Saranac +N plants. β-amylase shows cyclic patterns similar to the 15 kD VSP in Saranac plants, and in Ineffective Saranac+N plants β-amylase is utilized (Days 0 to 14) and resynthesized (Days 14 to 28).
3. Application of N to all plants did not change taproot amino-N concentrations prior to transplanting. Taproot amino-N concentrations in Saranac were 2-fold greater than those measured in Ineffective Saranac. Taproot sugars increased significantly in Ineffective Saranac plants during the two weeks before transplanting and subsequently were utilized and re-accumulated during 28 days of regrowth. Application of N did not affect taproot starch concentrations. Taproot starch concentrations in Saranac showed the typical cyclic pattern of synthesis-utilization-synthesis while starch concentrations in Ineffective Saranac declined throughout the experiment.
4. Herbage accumulation is positively associated with taproot N, but not taproot C reserves. Changes in taproot protein and sugar concentrations are positively associated with increased herbage growth in Ineffective Saranac+N plants.

References

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