

genes, *SOB3/AHL29*, that over-expresses a protein with a disrupted DNA-binding domain and a normal protein/protein interaction domain. In *Arabidopsis*, this mutation confers normal adult plants that produce larger seeds and seedlings with hypocotyl stems that can be more than twice as long as the wild type. The goal of this project is to enhance camelina and canola seedling emergence when they are planted deeply in low-rainfall dryland-cropping regions (generally less than 12"/year) or in wheat stubble. This can be achieved by manipulating *AHL* gene family members to develop varieties that have long hypocotyls as seedlings yet maintain normal growth characteristics as adult. The current aims for this project are: 1) Analyze seed size of *AHL* mutations in *Arabidopsis*; 2) Identify, clone and characterize *AHL* gene family members from camelina and canola; 3) Generate transgenic camelina and canola expressing *AHL* genes; 4) Use CRISPR/Cas9-based genome editing to modify *AHL* genes. During this funding period, the Neff Lab has used a combination of molecular, genetic, biochemical, and biotechnological approaches to understand the role of *AHL* genes in plant growth and development. Our primary goal has been to characterize *AHL* genes from *Arabidopsis* and camelina, while also establishing a canola transformation system. Using transgenic *Arabidopsis* we have characterized seed size in all of the *AHL* gene dominant-negative mutations that we have identified. Surprisingly, though each mutation leads to longer hypocotyls, only the *sob3-6-like* mutants created larger seeds. We have also generated putative transgenic canola expressing *Atsob3-6*, though these still need to be verified. Because of problems with transgene silencing, we have generated additional transgenic camelina expressing *Atsob3-6*, for seed size and emergence analysis. We have also generated camelina CRISPR/Cas9 lines targeting, *sob3-like* genes, some of which may be exhibiting longer hypocotyls. Using *Arabidopsis AHL* mutants, we have now demonstrated that the long hypocotyl seedling phenotypes are regulated by plant hormones including the auxins and brassinosteroids. This work was part of David Favero's Ph.D. dissertation and was published in two peer-reviewed manuscripts, one in *Plant Journal* and the other in *Plant Physiology*. Using *Arabidopsis AHL* mutants we have shown that clade A and clade B AHLs have opposite roles in flowering time. We have also shown that clade A and clade B AHLs only interact with members of their own clade. Using CRISPR/Cas9 to target four clade B AHLs, our preliminary results suggest that mutations in this family leads to larger plants. These need to be verified by gene sequencing.

Winter Canola Nitrogen Supply and Timing Recommendations for the Pacific Northwest



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Biomass and Nitrogen (N) accumulation/requirements. Winter canola planted before late August should be managed as a two-season crop. First, Nitrogen (N) fertilization strategies are required to cover planting to winter freeze. Second, coming out of winter freezing requiring shoot regrowth, the canola N requirements will align with Unit N Requirements (UNRs) established for spring canola. Studies across eastern Oregon and Washington have shown early seeded winter canola can accumulate up to 3,000 lb dry biomass/acre and 135 lb N/acre between emergence and winter, which offers opportunities for animal grazing or silage production if mixed with high fiber straw. Late seeded winter canola may only accumulate <100 lb biomass and <5 lb plant N. If leaves don't dieback during mild winter temperatures or snow cover, the biomass N will be used during subsequent crop development and grain filling. However, if above ground biomass dies due to freezing or water stress, then perhaps plants will only recycle ~1/3 of the shoot N to support grain production. Cautionary management should consider the prospect of having too lush growth and water use stimulated by initial high N fertilization, which can lead to induced water stress and greater susceptibility to winter-kill. Coming out of the winter thaw, the N requirements are similar to spring canola. A 3,000 lb grain/acre winter canola crop will produce more than 17,000 lb/acre total dry matter and accumulate more than 225 lb N/acre. This translates to a total N supply need of 300 to 450 lb total (fertilizer + soil) N supply for a crop that is 75 to 50% efficient at accumulating the total N supply.

N timing. Davis observed that broadcast tilling all urea and ammonium phosphate fertilizer at planting of winter canola reduced yields and winter survival compared to 25% at planting with the remainder applied later as split fall: spring topdress applications. Mechanisms could include seedling damage or too lush of growth. Similarly, Wysocki also found that applying all 140 lb N/acre at winter canola planting as urea resulted in yields similar to the 0 N control, while 0 to 25% of the total N fertilizer applied at planting resulted in higher yields. Esser showed a reduction in final grain yield by placing up to 30 lb urea-N/A near the seed. Collectively, these field studies align with root studies that caution against the application of high ammonia-based fertilizers at canola planting, particularly when placed with and below the seed. Unless there is sufficient spacing between the seedling and fertilizer row, ammonia based fertilizers should be applied preplant during fallow, or as fall- and spring post-plant topdressing. Ongoing studies conducted by Dr. H. Tao will verify this hypothesis. These research results and principles were presented at three 2017 WOCS winter workshops. Fertility recommendations will be published in a forthcoming PNW nutrient management guide.

Table 1. Seed yield and survival at Moscow, ID in 2014, 2015 as affected by Nitrogen rate and timing.

Fertilizer Timing Treatment	Seed Yield			Winter Survival
	2014	2015	Mean	
	-----lbs. per acre-----			---score ¹ ---
Reduced N at Planting Only	1680 a ²	2695 a	2154 a	6.5 b
All at Planting	1978 b	2405 a	2178 a	5.4 a
None at Planting 50% in Fall, 50% in Spring	2346 c	3775 b	3038 b	6.9 b
25% at Planting, 25% in Fall, 50% in Spring	2306 c	3594 b	2929 b	6.7 b
25% at Planting, 75% in spring	2360 c	3257 b	2794 b	6.8 b

¹Scored on a scale of 1 to 9 with one equaling no survival and nine equaling complete survival.

²Means within columns with different letters are significantly ($P < 0.05$) different.

Effects of Increasing Seeding Rates on Spring Canola Yields



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Canola seed, particularly transgenic seed, is expensive. Canola is also hard-seeded, and germination of seed can be ~50%. Increased canola seed rates could offer increased crop establishment, resulting in crop and weed competitiveness, and productivity by maximizing above ground growth and yield potential. A study was established near Pullman, WA, to evaluate a range of seeding rates. Spring canola variety Hyclax 930 was planted on April 20th, 2016 using a Monosem planter calibrated to deliver seeding rate treatments detailed in Table 1, on an 10 inch row spacing. The study was conducted as a randomized complete block design with 3 replications of 10 by 75 ft plots. The entire study was fertilized with 20 lb of sulfur and 80 lb of nitrogen, and glyphosate was split applied at 0.387 lb ai A⁻¹, with 0.124 lb ai A⁻¹ of clopyralid added in the later application timing. Crop stand counts were recorded 62 days after treatment. The study was harvested using a plot combine with a 5 foot header on September 20, 2016. All data were subjected to an analysis of variance using the statistical package built into the Agricultural Research Manager software system (ARM 8.5.0, Gylling Data Management). Spring canola stand counts significantly increased as the seeding rate increased, with 10 plants m⁻¹