The causal agent of blackleg is the fungal pathogen *Leptosphaeria maculans* (anamorph *Phoma lingam*). Blackleg infects rapeseed and mustard crops beginning at the seedling stage and under the proper conditions can progressively damage the crop by creating stem cankers that restrict vascular flow of water and nutrients to the upper plant. Blackleg symptoms are characterized by dull-white lesions on leaves with small dark spots (pycnidia). As the disease progresses deep brown lesions with a dark margin may be seen at the base of stems and these cankers can result in lodging. Severe blackleg infection can spread through the entire plant, creating the potential for seed infection and future transmission through planting infected seeds.

Blackleg is most severe in regions with warm, humid conditions and summer rains. While canola crops have been grown in the PNW for many years, many believe that the region's prevailing warm and dry conditions combined with little summer rainfall are not conducive to the disease blackleg. However, blackleg disease was discovered in northern Idaho near Bonners Ferry in 2011. Blackleg disease poses a major threat to canola production in the PNW region and virtually no selection has been carried out to identify resistance genes or cultivars suitable for the region.

A preliminary survey in 2015 found blackleg infected canola across several counties in northern Idaho. In 2016, leaf and stubble samples were collected from 40 locations across Latah, Nez Perce, Lewis, and Idaho counties. Included in the survey were fields of winter canola, spring canola, mustard, and a variety of Brassicaceae weeds. *Leptosphaeria maculans* was found at 14 locations. A less virulent blackleg pathogen, *L. biglobosa*, also was found at two locations. In total, 67 *L. maculans* isolates and 6 *L. biglobosa* isolates were collected. The majority of the isolates found have been confirmed to be pathogenic on blackleg susceptible canola cultivar Westar.

Isolates are currently being characterized to identify the race structure among the population already found to exist in northern Idaho. This information will be used by the University of Idaho's Canola Breeding Program to screen for resistance in current cultivars and develop new canola cultivars with superior resistance to blackleg.

Manipulating the *AT-hook Motif Nuclear Localized* (*AHL*) Gene Family for Bigger Seeds with Improved Stand Establishment

MICHAEL M. NEFF, PUSHPA KOIRALA, JESSICA BECCARI, JAZMIN MORALES-RODRIQUEZ, DAVID FAVERO, KELLY AVILA, HERNAN ROMERO, AND REUBEN TAYENGWA
DEPT. OF CROP AND SOIL SCIENCES, MOLECULAR PLANT SCIENCES GRADUATE PROGRAM, WSU

In low rainfall dryland-cropping areas of eastern Washington, stand establishment can have a major impact on yields of camelina and canola. During dry years these seeds need to be planted in deep furrows so that the developing seedling has access to water in the soil. One approach to facilitate stand establishment is to develop varieties with larger seeds and longer hypocotyls as seedlings while maintaining normal stature as adults. Few mechanisms, however, have been identified that uncouple adult stature from seedling height. The Neff lab has identified an approach to improve stand establishment by uncoupling seedling and adult phenotypes through the manipulation of members of the *AHL* family. When these genes are over-expressed, the result is seedlings with shorter hypocotyls. When the activity of multiple genes is disrupted, the result is seedlings with taller hypocotyls, demonstrating that these genes control seedling height in a redundant manner. In the Brassica *Arabidopsis thaliana,* we have identified a unique allele (*sob3-6*) for one of these

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

D. WYSOCKI[1], J.B. DAVIS[2], M. WINGERSON[2], J. BROWN[2], M. REESE[3], A. ESSER[3], B. SCHILLINGER[3], T. MAAZ[3], I. MADSEN[3], H. TAO[3], AND W. PAN[3]
[1]OREGON STATE UNIVERSITY, [2]UNIVERSITY OF IDAHO, [3]WASHINGTON STATE UNIVERSITY

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.