Evaluating Thlaspi arvense (pennycress) and Camelina sativa (winter camelina) for the Benefits of Water Quality and Clean Energy

Our current agricultural system that is dominated by summer annual grain crops does not maintain environmental health. Since summer annual grain crops only protect the soil for a few months of each year, soils in these systems erode at much higher rates than soils under perennial grassland or woodland vegetation (Lubowski et al., 2006; Tilman et al., 2002). The United Nations Food and Agriculture Organization has sounded the alarm that 25% of the world's agricultural soils are either highly degraded or are rapidly becoming so (FAO 2011). These exposed soils also allow runoff and leaching of nutrients to occur unchecked, which is a major cause of water pollution (Randall et al., 1997). A shift in our current practice of agriculture to the use of perennial grain and cover crops or winter annual cover crops may prevent these environmental problems while keeping pace with the yield demands of our growing population (Runck et al., 2014).

My research is focused on the development of the winter annual cover crops pennycress and winter camelina, and includes five research projects. The first seeks to answer many agronomic questions related to pennycress and winter camelina, and the other four projects are on the breeding or genomics of winter camelina. This is a brief outline of each project:

1. Evaluation of **a)** the amount of N and P camelina and pennycress capture compared to other treatments, **b)** their effect on water quality, weeds, beneficial insects, and soybean yields relative to other treatments, as well as **c)** camelina and pennycress oilseed yields
2. Germplasm evaluation of camelina with the objective of identifying winter lines
3. Determination of gene action with regard to growth habit in camelina
4. Identification and validation of the gene responsible for growth habit in camelina
5. Formation of a mutagenized camelina population with the objective of discovering individuals that exhibit reduced seed shatter and earlier flowering

This is a brief background on the context that justified the investment into the agronomic project. The cover crops winter rye and forage radish have been shown to be effective in preventing the leaching of nitrates (Kladivko et al., 2014; Dean and Weil 2009; Gieske et al., 2016) and are feasible to include in Minnesota growers' rotations even considering the climate constraints (Strock et al., 2004; Gieske et al., 2016). However, they do not provide a substantial financial benefit to growers, if any at all (Strock et al., 2004; Gieske et al., 2016). This is very likely to be why only 2.1% of Minnesota cropland is cover cropped (and this does not include orchards or pasture/hay acreage, 2012 Census of Agriculture). To become more widespread on the Minnesota landscape, cover crops must be able to provide a direct financial benefit to growers without causing an adverse impact on the productivity of the primary crop. Thus, such a cover crop that is a winter annual must produce a valuable commodity quickly in the spring, so that it can be harvested without pushing back the planting date of the primary crop, which causes reduced yields in corn and soybeans (Van Roekel and Coulter 2011; Quring and Potter 2013). Pennycress and winter camelina are very early maturing – it is possible to harvest pennycress as early as May 29th in southern Minnesota (Rosemount) and winter camelina as early as June 22nd in central Minnesota (Morris) (Johnson et al., 2015; Gesch et al., 2014). Overlapping the cover crop and the primary crop for a brief period, known as relay-cropping, has been shown to have only a small adverse effect on the primary soybean crop, which was compensated for by the increased pennycress/winter camelina yield, with total oilseed yield in relay-cropped treatments being greater than for mono-cropped soybean (Johnson et al., 2015; Gesch et al., 2014).

Though research indicates that pennycress and winter camelina have potential to be viable cash cover crops in Minnesota, there has not been much research regarding their potential to improve water quality and provide other environmental benefits. My first research project seeks to address these unknowns by evaluating pennycress and winter camelina for their potential to: reduce nitrogen and phosphorus movement on Minnesota cropland, improve water quality, suppress weeds, and attract beneficial insects. In addition, there is a limited amount of data on how pennycress and winter camelina affect soybean yields in relay cropping system it is not entirely conclusive, so soybean yields will also be measured to provide more data that may further help answer this question. The yields of pennycress and camelina will also be measured which may provide more insight into the potential for entire soybean-oilseed cover crop rotation to produce a higher net income for growers than the soybean rotation that does not include an oilseed cover crop.

The objective of the second project is to apply the knowledge and methods of plant breeding to improve the agronomic potential of winter camelina. There is currently not a breeding program for winter camelina in the Upper Midwest and there are very few winter lines being used for agronomic research – the most prominent of which is Joelle, which originated in England (Gesch unpublished data). However, genetic diversity in the base breeding population is essential for realizing the genetic gains that would improve growers' incomes. More winter lines likely exist in germplasm repositories, but they must be identified. For this reason, we are screening 423 accessions of camelina of unknown biotype sent to us from germplasm repositories or breeders in Austria, Poland, Canada, Russia, and the US.

Determining how dominance operates in camelina with regard to growth habit would allow us to make more productive crosses and this is the objective of the third project. The growth habit of the F1 progeny of a cross between a spring and winter line will disclose which growth habit is dominant. Our hypothesis is that the winter growth habit is dominant in camelina as it is in *Arabidopsis thaliana*. If there are a small number of genes that determine growth habit, which is what we expect, it would be possible to introgress alleles conferring the winter growth habit into an elite spring line background using classical breeding approaches. Growing F2 plants will provide more insight into the number of genes that control growth habit, but will not identify the causal gene – this will be the basis for project four.

We will identify and validate the gene(s) governing growth habit using three approaches: bulked segregant analysis, polymerase chain reaction, and transformation using *Agrobacterium tumefaciens.* The latter approach is often challenging due to the reality that each step in the process has a success rate below 100%, so this is the reason for including two other approaches. Our hypothesis is that the flowering locus C gene, *FLC*, determines growth habit and that the *FLC* allele from winter lines is dominant. Bulked segregant analysis will identify genes that are specific to individuals grouped according to a trait – growth habit in this case. To determine if the winter allele always segregates with winter growth habit, the *FLC* gene in the F2 population will be amplified using polymerase chain reaction (PCR). In addition, we will further validate these results by transforming the winter allele of the *FLC* gene into a spring camelina biotype to test whether this would confer the winter biotype in this otherwise spring individual.

Since genes that cause seed shatter and a specific maturation date improve the fitness of undomesticated plants, yet are undesirable in terms of agricultural production, they represent targets for gene-knockout via mutation breeding (Johnson et al., 2015), which is the objective of the fifth project. Though camelina was domesticated in the late Neolithic Age in southeastern Europe and was grown widely in Europe and West Asia during the Iron Age (Dönmez and Belli 2007; Larsson 2013), it never became a crop of major importance there and still may exhibit some of these undesirable traits. For this reason we are developing populations of winter camelina for mutation breeding using ethyl methanesulfonate. This will simply be an observational study with the objective of identifying individuals that exhibit less shattering or earlier maturation compared to a control treatment.

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