Herbicide Resistant Camelina: Adapting an Oilseed Crop to PNW Cropping Systems

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Introduction:

Heightened interest in Biofuel crops has increased research on camelina *(Camelina sativa*), a small seeded oil crop that is proving to be well adapted for production in the inland Pacific Northwest (Figure 1). The use of acetolactate synthase (ALS) inhibitor herbicides in the PNW has been a barrier to camelina adoption because of its extreme sensitivity to residual activity of to these herbicides in soils. The objective of this work is to develop a camelina variety with reduced sensitivity to these herbicides through mutation breeding. Other ALS inhibitor resistant crops have been developed via this method and several amino acid substitutions in the ALS enzyme have been shown to confer resistance. There are multiple chemical families that inhibit the ALS enzyme activity. Imidazolinones (e.g.imazethapyr) and sulfonylureas (e.g. sulfosulfuron) are two of the most popular chemical families. Different mutations can result in different profiles of resistance to the different chemical families.

Figure 1: Camelina field plots direct seeded at Ralston, Wa.

Approach:

Camelina seeds were mutagenized by exposure to EMS *(ethane methyl sulfonate)* which causes point mutations in the DNA. Mutagenized seeds of cultivars Calena and Cheyenne were amplified in field plots to generate bulk M2 populations. M_2 populations were then planted in the field and treated with a standard field rates of imazethapyr. The M_2 populations were also screened in the field with sulfosulfuron. Survivors from both screens were transplanted into the greenhouse to obtain M3 families.

Results:

Four mutants were found in a screen for resistance to imidazolinones and were designated IM1 to IM4. One mutant, designated SM4, was identified in a screen for resistance to sulfosulfuron. Progeny from the mutants were rescreened in greenhouse tests to verify that they had increased resistance to the herbicides (Fig. 2).

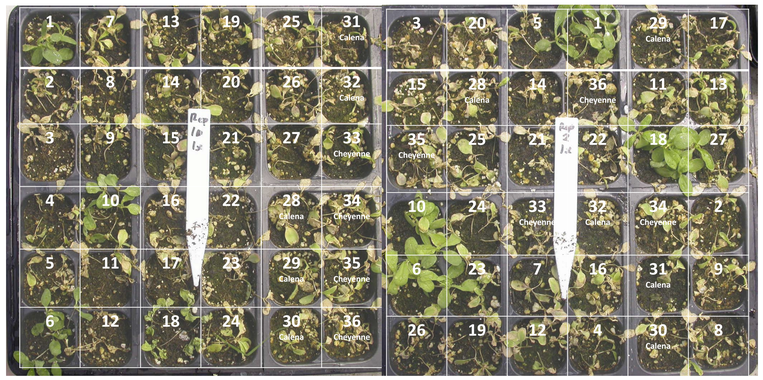

Figure 2: Testing progeny of putative mutants to verify their herbicide tolerance. Lines 1, 6, 10 and 18 are from mutants IM1, IM2, IM3 and IM4.

Each of the mutants were examined in greenhouse assays for resistance to three ALS inhibiting herbicides representing three different chemical families (Fig.3). Resistance was estimated by directly spraying seedlings with field rates of the herbicides.

Treatments		
Herbicide	**Trade Name**	**Rate**
Imazethapyr	Pursuit	52.5g/Ha (3oz/ac)
Sulfosulfuron	Maverick	17.5g/Ha (1/3oz/ac)
Flucarbizone	Everest	29.4g/Ha (.6oz/ac)

Figure 3: Herbicides used to characterize the mutants

Biomass data, based on dry weight, was used to compare the different treatments (Figure 4). Only one of the five mutants, SM4, showed increased resistance to all three chemical classes. The other four mutants were slightly more resistant to imazethapyr but not to sulfosulfuron. SM4 had nearly equal fresh weight as the untreated seedlings when treated with imazethapyr. The plants appeared slightly stunted but the biomass was similar to the untreated plants. The herbicide doses used were much higher than what would be expected from herbicide carryover in the field.

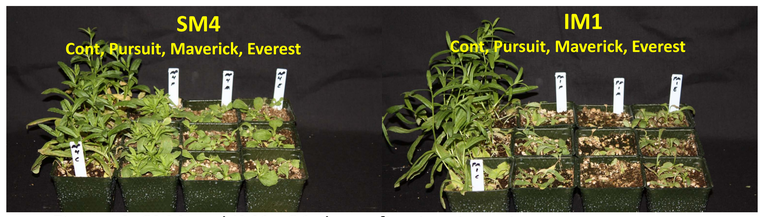

Figure 4: SM4 and IM1, 21 days after treatment.

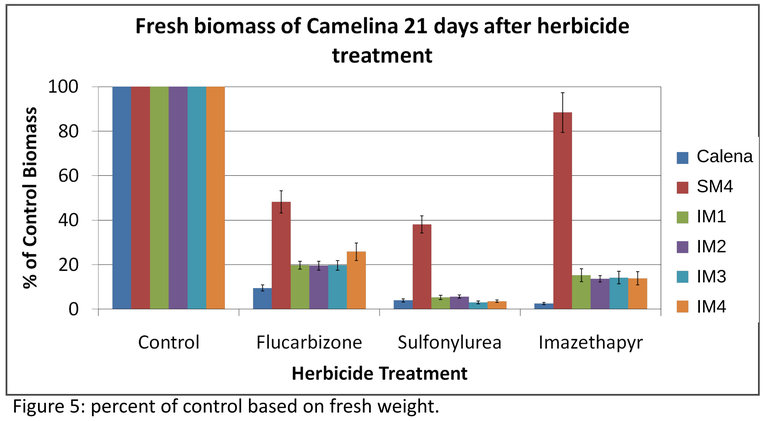

Figure 5: percent of control based on fresh weight.

Genetic analysis indicated that each of the 5 mutations were controlled by single semi-dominant genes. Sequence analysis of ALS genes from the SM4 mutant identified a gene family with at least six members. One of the genes had an base substitution changing an amino acid at a position known to affect ALS inhibitor sentitivity in yeast and is probably the cause of resistance

DNA sequence

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Cheyenne   TTGGCATGGTTATGCAATGGGAGGATCGGTTCTACAAAGCTAACCGA
SM4        TTGGCATGGTTATGCAATGGGAGGATCGGCTCTACAAAGCTAACCGA
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Amino acid sequence **578**

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Cheyenne   LATIRVENLPVKILILNNQHLGMVMQWEDRFYKANRAHTYLGNPAAE
SM4        LATIRVENLPVKILILNNQHLGMVMQWEDRLYKANRAHTYLGNPAAE
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Figure 6: DNA and amino acid sequence of an ALS gene from the SM4 mutant that may confer the herbicide resistance.

Continuing Research:

Experiments verifying that the nucleotide substitution in the ALS gene is the cause of resistance in SM4 are underway. We will also make sure the levels of resistance are adequate to prevent damage from residual levels of ALS-inhibitor herbicide carryover in the field. We have crossed the SM4 mutant to non-mutagenized camelina to clean-up its genetic background and are generating vigorous lines homozygous for the resistance gene. Seed can be requested by email: scot_hulbert@wsu.edu

Acknowledgments:

- Special thanks to the Washington State Biofuels Program and the Sun Grant Initiative for funding this research.