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Project title: *Production and evaluation of biofuel from canola and mustard irrigated with agricultural water on the Westside of central California*

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Major Objective:

Growers on the Westside of the San Joaquin Valley have tried to reduce their production of Se-laden effluent and thus sustain their agricultural land in central California. The Water Management Research Lab has studied the reuse of drainage water for producing canola and mustard; hence reducing the volume of Se-laden agricultural effluent to dispose. In order for mustard and canola to be widely accepted as potential crop, and be used as practical tools to manage the volume and Se content in effluent produced in the Westside of the SJV, it is imperative that viable economical uses for the harvested plant product be created. After growing the crops with poor quality waters, we harvested the seed, and are currently processing the seed with our "oil mill". The canola and mustard oils are to be further processed (transesterified), blended with diesel, and used as a source of B20 biofuel for operating diesel-powered engines. Alternative uses of the residual seed meal (after oil extraction) are also being evaluated.

Thus the primary objectives of this multi-year study were:

1) Grow commercially viable acreage of canola and mustard as recipient crops for disposal of Se-laden effluent.
2) Extract canola and mustard oils seed from our "oil mill" at Red Rock Ranch.
3) Process the canola and mustard oils and bring them to ASTM quality.
4) Identify alternative uses of residual seed meal after oil extraction.

Major Accomplishments:

Generally, 40 ha plots were planted to canola and mustard at Red Rock Ranch (RRR), Five Points, Ca on an annual basis (Figures 1 thru 7). The soil in the experimental area at RRR was classified as an Oxalis silty clay loam (fine montmorillonitic, thermic Pachic haploxeral with a well-developed salinity profile). Two water sources were available for irrigation; canal water from the California aqueduct, and Se-laden drainage water produced on Red Rock Ranch. The canal water had soluble Se concentrations of < 0.001 mg L^{-1}, soluble B concentrations of < 1 mg L^{-1}, and electrical conductivity for salinity EC of < 1 dS m^{-1}. The drainage water collected using subsurface tile drainage systems was pumped from a drainage sump, and had a range of soluble Se from 0.100-0.150 mg L^{-1}, soluble B from 4-7 mg L^{-1}, and a sodium sulfate-dominated salinity from 5-8 dS m^{-1}. Field experiments initially received "canal water" until plant species were deemed established (between true 2nd or 3rd leaves). Thereafter, irrigation scheduling with drainage water was based upon weather data reported from California Irrigation Management Information System (CIMIS) located at the University of California Westside Research Station (located less than 5 km from RRR field sites).

Total plant biomass and seeds were harvested and yields recorded. Plant biomass values were as high as 5.5 tons/acre, while seed yields averaged 1.5 tons/acre for both canola and mustard (Figures 8 thru 11). Both soil and all plant products were analyzed for Se content. Plant Se concentrations, including seeds, ranged from 1 to 4 mg Se/kg DM for all plant organs, while total soil Se concentrations averaged 1.5-2.0 mg Se kg^{-1} at 0-45 cm depth and 1.0-1.5 mg Se kg^{-1} at 45-90 cm depth, respectively, at the end of growing season.

Biofuel Production

Harvested canola and mustard seed were processed with our retrofitted extruder and horizontal press ("oil mill") located and housed at RRR (Figure 12 and 13). Extraction modifications to operate the "oil mill" were performed by personnel at RRR. Structural and mechanical changes were necessary for bringing the "oil mill" (for seed pressing) under compliance of the SJV Air Pollution Quality Control Board regulations (Figures 14 and 15). It is imperative for future efforts associated with oil pressing and biofuel production that manufacturers send their own personnel to initially set-up and operate "oil mill" under field conditions. We processed between 15-20 tons of seed per day with our Model 2000 RC Dry Extruder with Top Feeder and a Model 1500 Insta-Pro Continuous Horizontal Press. We realistically extracted up to 90% of the available oil in canola and mustard seed. Extracted oil was processed (i.e., transesterfied by another company; Russ Teals at Biofuel Company at no cost in Ventura, CA), and then the ASTM quality biofuel was mixed with diesel and the resultant BD 20 fuel was used to power diesel-powered stationary irrigation pumps on Red Rock Ranch under heavy irrigation load (Figure 16). We generally estimate that if we ideally achieve seed yields of canola/mustard as high as 4000 kg/ha, that we can produce a high of 7600 L of BD 20 fuel (80 % diesel and 20% canola oil) per ha, if we can extract at least 90% of the available oil (≈35 %) contained within the seed. All recovered oil was stored in air-tight tanks, allowing for natural separation of canola oil and any remaining residual particles. At this stage, the resulting cake meals from pressing and extruding canola and mustard

seeds from the 'press' and the 'extruder' were now available for other uses, e.g., animal feed, bioherbicide, as will be described later.

Not only is biofuel production important but the effects of biofuel consumption on air quality is of high importance for central California. More research is needed "under load" situations to determine if sustained reductions in NO_x production are possible when unregulated irrigation booster pumps are operating on BD 20 biofuels. Because our mobile emission laboratory was stolen at the USDA Research Facilities (police report was filed), only preliminary emission testing of the canola-based BD 20 biofuel under field conditions was performed on the Cummings 5.9 L diesel irrigation booster pumps used at Red Rock Ranch. We generally observed average reductions of CO (14%), CO_2 (13%), and THC (11%) compared to emissions emitted from the same booster pumps operating on petro diesel (Figure 17). Reductions in NO_X were not clearly observed. Interestingly, significant NO_x reductions (>100%) were measured after injectors within the diesel engine were cleaned when operating on diesel (data not shown). This observation clearly shows the importance of maintaining properly tuned engines may be more important than the type of fuel used in the irrigation booster pumps. Data obtained from the gas analyzers have shown us the limited capabilities of these analyzers even under a controlled environment to simultaneously measure NO_x, CO, CO_2, and PM (Figure 18). Hence, we must realistically analyze each component separately. Other benefits that may be just as important as the production of biofuel are the supplemental usages of the residual seed meal (after oil extraction). Outside preliminary emission analyses were also performed and comparisons were made between diesel and B20 (80% diesel and 20% biodiesel) and B50 (50% diesel and 50% biodiesel). Table 1 shows, the reductions or increases of each component analyzed; NO_x is still problematic even at B20.

Byproducts after Oil Extraction:Se-enriched feed and biofumigants

Canola grown for large scale management of Se in the SJV produces total biomass yields over 12-13 metric tons (Mg) ha^{-1} and conservatively 2 Mg ha^{-1} of seed under high salt and B growing conditions. Because the oil content of canola seed ranged from 35-40 %, the oil and residual seed meal may both ne valuable commodities. After pressing and extracting oil from seed, the residual seed meal is potentially usable as a Se-enriched animal feed meal. This meal can be used as an organic source of Se for livestock feed rations. Strict quality control on Se content is essential and this will determine the quantity to add to the daily feed ration. In this regard, canola seed meal is one of the most widely used protein supplement in the animal feed trade, and generally competes as a 2nd tier product to soy meal. Results in Table 2, show that Se, nutrient and vitamin concentrations in canola meal are adequate when compared to other reported values for grass and/or legume forage. Importantly, the Se concentration in the canola meal produced from the SJV was always less than 2 mg kg^{-1} DM, which allows its safe use as part of the daily feed ration for Holstein lactating cows; at a mixing ratio of 2.5 kg Se-biofortified canola meal per 50 kg animal feed. Other vital animal forage components within the Se-biofortified meal such as acid detergent fiber (ADF), neutral detergent fiber

(NDF), non-fibrous carbohydrates (NFC), were slightly lower for canola meal than typical values for good quality legume, grass and legume grass hays and silages, however, the crude protein (CP), crude fat (CF), soluble protein (SP), and total digestible nutrients (TDN) values were slightly better than legume hays. Importantly, the low glucosinolate content in the canola meal (21 µmol g^{-1}) is an important feed quality of the canola seed meal (Table 2), because at higher concentrations of glucosinolates (i.e., ≈ 300 µmol g^{-1} in mustard) break down into toxic aglucones and their oftentimes bitter taste results in reduced feed intake. Moreover, some forms of glucosinolates especially at higher concentrations, are considered natural toxicants and are associated with goiter and liver damage when consumed in large quantities. Typical unselenized canola seed meal cost dairy producers over $200 ton (Figure 19). With our phytoremediation system we have produced Se-biofortified canola meal that potentially has a higher cash value than most non-seleniferous canola meal because of the Se enrichment. Moreover, this Se-enriched meal typically has Se concentrations ranging from 1.75 to 2.00 mg kg^{-1} when grown at RRR, and therefore it can be of special importance as a natural source of Se to central California's livestock and dairy industries.

Indian mustard, another oil-producing *Brassica* crop, was also used for managing Se in poor quality soil and water conditions. At harvest, the mustard seed is processed for its oil, which can be used similarly to canola oil in the production of biofuel. In contrast to canola meal, the residual mustard seed meal also possesses bioactive properties, which make them useful for biofumigation. Much of the interest revolves around a constituent class of nitrogens and sulfur compounds called glucosinolates. The higher concentrations of glucosinolates (up to 345 µmol g^{-1}) are found in the meal from seeds harvested from plants grown at RRR with Se-tainted waters. They can hydrolyze into isothiocyanates (ITC) with potentially broad-spectrum biocidal activity. Isothiocyanates can be used for biofumigation and are similar to those produced by the synthetic commercial fumigants, Vapam and Basamid; albeit at much lower concentrations. While high levels of glucosinolates are not desirable in plant material used for animal feed, their presence in mustard seed meals at high concentrations has prompted research to evaluate the potential herbicidal effects of mustard seed meal when applied to soil. In this regard, we have successfully demonstrated an effective herbicidal effect (e.g., observed as emergence inhibition) on ten different weed species after incorporating the mustard seed meal into soils at values rates equivalent to 4.3-13.2 Mg ha^{-1} under field conditions (Figures 20 thru 22 and Table 3). Creating an alternative biofumigant product from mustard seed meal with its high glucosinolate content may have more use as a plant-derived herbicide in organic agriculture. In this regard, management of soil born pests can be exceedingly difficult in organic production, and the combination of weeds, nematodes, and other diseases contribute to reduced productivity over time compared to conventional fields. Moreover, factors such as soil type/texture, chemical composition, temperature and moisture content, composition of native soil microflora, cropping history, will affect the specific performance of an organic amendment like mustard seed meal used for soil disinfestations. Similarly, Banuelos is currently investigating the impact of the Se-enriched mustard meal on controlling specific insects, e.g., cabbage looper and worm in broccoli and spittlebugs, mites, lygus, beetles, slugs, and even deer in strawberry production under field conditions. Similarly, other

studies have shown that *Brassica* species that absorbed Se have also deterred insect infestation in crop species.

CONCLUSION

We have demonstrated that the coupling of Se phytomanagement (using plants as recipients of Se-laden waters) with biofuel production may provide growers with a unique opportunity to increase environmental sustainability in areas with high Se conditions and reduce some usage of petro-diesel in sensitive parts of California. However, we importantly emphasize that good quality soils and waters should not be utilized for growing oil-producing crops that will eventually be used for producing biofuels. Most important, other sources of energy, e.g., gasification, will be essential for operating larger-scale oil presses, since the costs to utilize electricity from the "grid" can be exceedingly high. Moreover, more acreage planted to *Brassica* oil crops on "non-food producing soils" is essential for operating the oil press on a sustained basis. Coupling phytomanagement of Se with the creation of new biofortified and byproducts, e.g., Se-enriched animal feed, biofumigants, and even a byproduct like biofuel, may provide California growers unique opportunities to increase the environmental and economic sustainability of a plant Se-management system, while supporting the agriculture community in central California. Successful production of canola and mustard crops may offer Westside growers an economic incentive to reuse Se-laden agricultural drainage water on a sustained basis, and hence reduce the volume of drainage water requiring treatment or discharge. If this multi-faceted phytoremediation system proves to be sustainable and is accepted by growers, there could be long term economic benefits, as well as improved water and air quality associated with agricultural production in the SJV. Moreover, sustained operation of this agronomic based system demonstrates the effectiveness of attempting to develop a holistic phytoremediation system for managing contaminants, e.g., Se, and transforming them into valuable biofortified products and byproducts.

Additional Accomplishments

Importantly, we were able to organize a special conference on the "Role of Selenium in Human Health and the Environment" in Heifei China (October 2013), and highlighted this DWR funded research, that was also sponsored by the ARI and USDA. The conference was a huge success, and attracted major attention for researchers working in Se-rich regions of China and India, especially where the production and use of biofuel is strongly advocated. Work with the *Brassica* plants has led to the future evaluation of new oil-producing plant species, e.g., camelina, jojoba, that are currently being evaluated for their salt and B tolerance, Se accumulation, and oil production capabilities in our drainage-impacted soils of the Westside of the SJV. If cultivation of biomass crops for fuel production becomes widely practiced in California, additional studies such as those described here will be needed to optimize their rotation with the high-value specialty crops which are important and unique to California agriculture. One thing is certain-if biofuel crops become a major part of the agricultural landscape in California, scientists and growers will be looking carefully at crop sequencing with the idea that employing

their residues as soil disinfectants and sources of organic Se can provide additional measures of usefulness and sustainability to their cultivation.

Table 1. Changes in measured emissions between combustion of diesel compared to B20 and B50 biodiesels

% Change versus initial test of ultra-low sulfur diesel						
Fuel type	NO_X †	PM §	THC ¥	CO α	CO_2 β	Fuel σ
20% (B20) Biodiesel	+5%	-26%	-49%	-7%	+3%	+3%
50% (B50) Biodiesel	+32%	-37%	-7%	-3%	+9%	+9%

† Nitrogen monoxide and nitrogen dioxide (NO_X)
§ Particulate matter (PM)
¥ Total hydrocarbons (THC)
α Carbon monoxide (CO)
β Carbon dioxide (CO_2)
σ Fuel consumption

Table 2 Nutritional content of canola seed meal after oil extraction

Elemental Analysis:	Se	B	Ca	Cr	Cu	Fe	K	Mg	Mn	Mo	Na	Ni	P	S	Zn
	---mg kg^{-1} DM---														
	1.85	41	4455	0.1	5	184	12601	3647	39	1	958	0.55	6661	6743	64

Feed Quality Analysis†:	ADF	Ash	CF	CP	SP	Fiber	Lignin	Moisture	NFE	NDF	Nitrogen	NFC	TDN
	---%---												
	20.6	3.5	35.7	40	51	12.2	7.2	6.8	15.6	39.1	4.2	17.4	62

Vitamin Analysis:	Vitamin A	Vitamin D	Vitamin E	Niacin	Riboflavin
	---µg kg^{-1}---			---mg kg^{-1}---	
	192	75	57	115	13

Glucosinolates: 21µ mol g^{-1}

†ADF-Acid detergent fiber; CF-Crude fat; CP-Crude protein; SP-Soluble protein; NFE-Neutral fiber efficiency; NDF- Neutral detergent fiber; NFC- Non-fibrous carbohydrates; TDN-Total digestible nutrients.

Table 3. Weed emergence counts 21 days after planting in biofumigation preliminary strawberry field trial.

Meal[†]	Rate g m^{-2}	BYG[‡]	Bluegr[‡]	Chick[‡]	Crabgr[‡]	Horse[‡]	Mallow	Pigweed	Purslane	Ryegrass	YNS[‡]
		----------Number per plot----------									
None	--	9.3	37.3	19.0	10.3	54.0	11.5	17.8	20.5	38.8	2.8
Mustard	3000	5.8	16.8	0	6.8	0	3.3	0	0.8	1.3	2.3
Canola	3000	16.3	61.5	5.8	6.5	6.8	5.3	3.5	2.0	31.3	4.3
LSD (0.05)[§]		12.7	73.9	8.4	17.2	37.6	NS	9.6	5.6	26.2	3.9

[†] Application of respective seed meal by incorporation into soil.

[‡] BYG is barnyardgrass, bluegr is annual bluegrass, chick is common chickweed, crabgr is crabgrass, horse is horseweed, and YNS is yellow nutsedge.

[§] LSD values often were fairly large due to problems in planting uniform numbers of very small seed.

ATTACHMENTS

Figure 1. Planting Brassica in poor quality soil at Red Rock Ranch (RRR) at Five Points, CA.

Figure 2. Emergence of Brassica crop at RRR.

Figure 3. More emergence of Brassica crop at RRR.

Figure 4. Developing Brassica crop at RRR.

Figure 5. Maturing Brassica plants at RRR.

Figure 6. Flowering of the Brassica crop at RRR.

Figure 7. More flowering of the Brassica crop at RRR.

Figure 8. Brassica seed pods ready for harvest at RRR.

Figure 9. Harvesting of the Brassica crop at RRR.

Figure 10. Collected Brassica seeds.

Figure 11. Unused Brassica plant material baled into hay.

Figure 12. Overview of "oil-press" at RRR.

Figure 13. Components of the "oil-press" at RRR.

Figure 14. Extraction of the oil from the oil-press.

Figure 15. Brassica-pressed oil.

Figure 16. Collected Brassica oil designated as biodiesel (B100).

Figure 17. Preliminary emission analysis of a biofuel-powered irrigation pump at RRR.

Figure 18. Some of the analytical emission components used for analyzing CO, NO_x, and THC from biofuel-powered equipment.

Figure 19. Residual Brassica seed meal after oil extraction at RRR.

Figure 20. Micro-plot appplication of residual seed meal used as a bioherbicide in sandy loam soil.

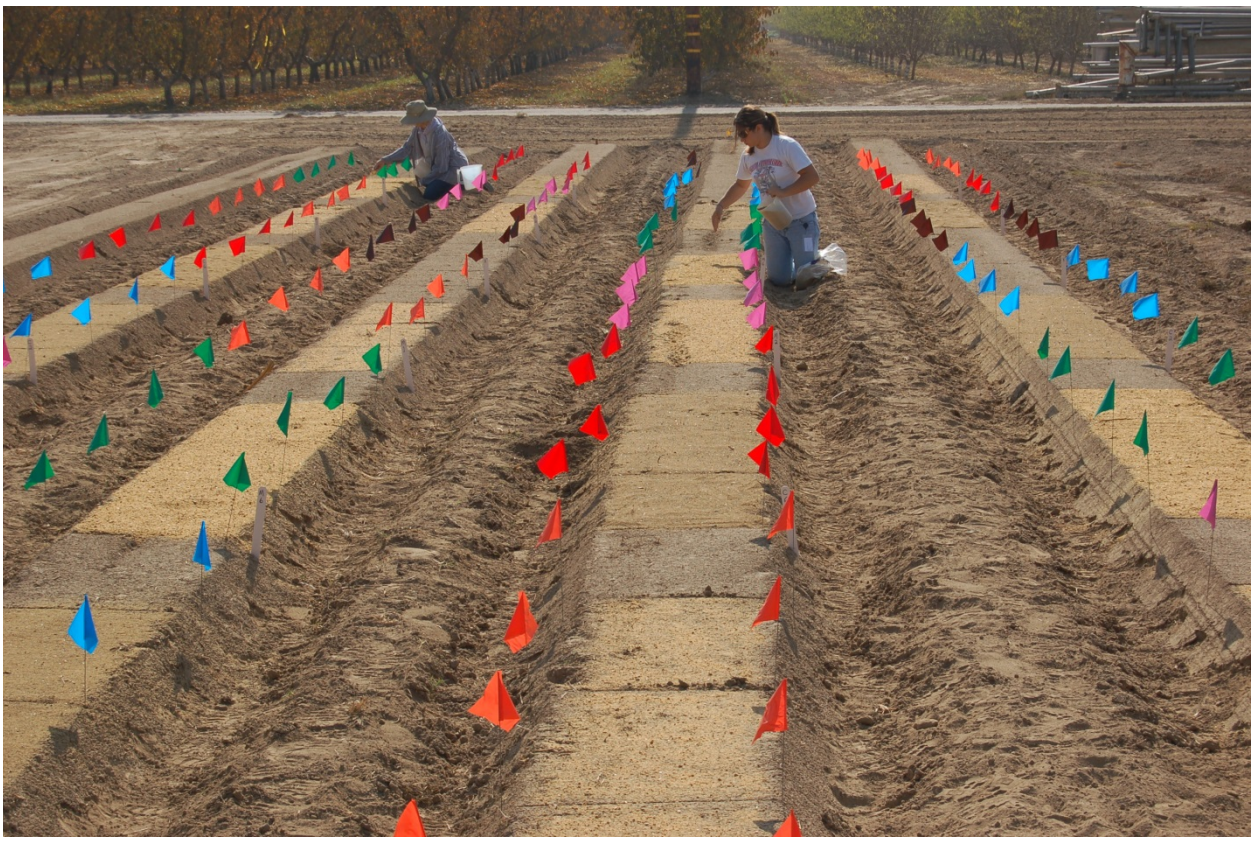

Figure 21. Close-up of applied seed meal and compared to control soil without seed meal.
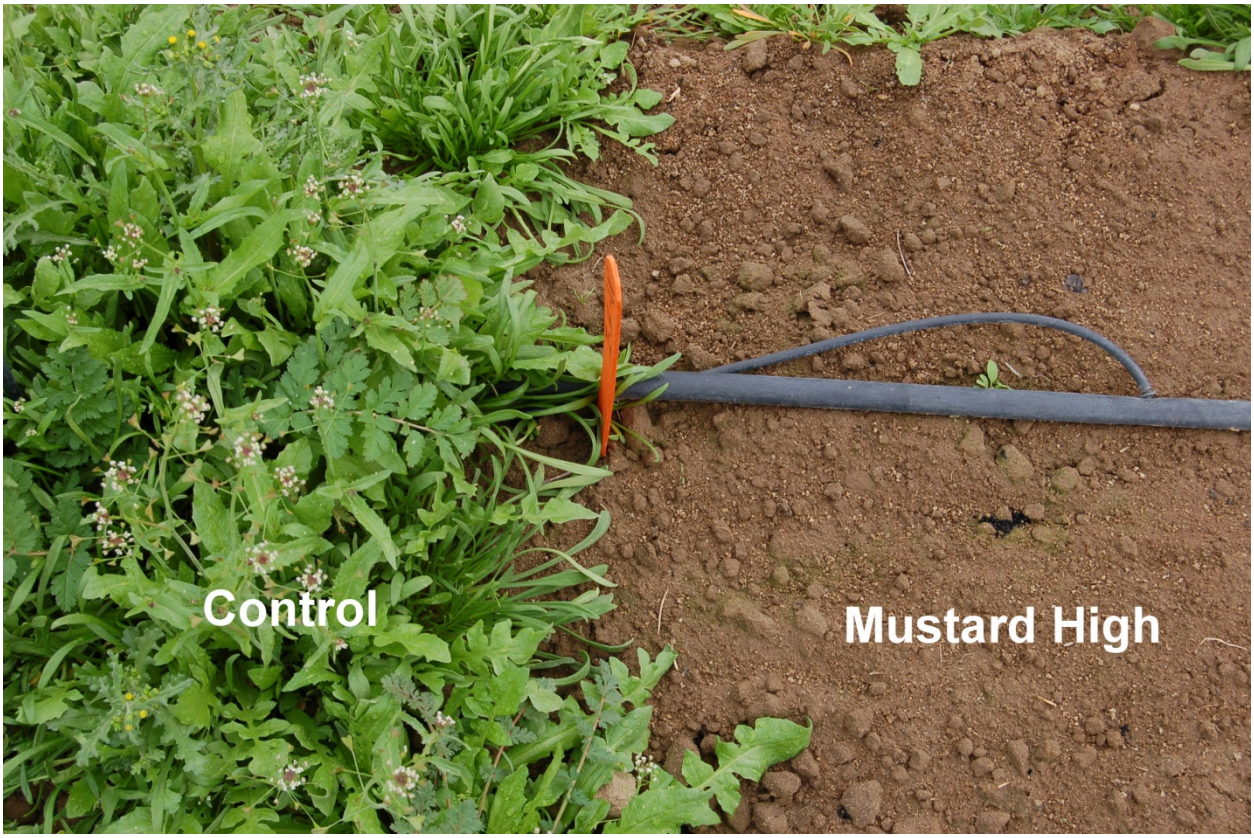
Figure 22. Effects of seed meal (mustard) on weed emergence compared to control soil without seed meal.