

Evaluating Canola and Other Crucifer Cultivars for Food and Bio-diesel Fuel Production on Saline Lands

A Saskatchewan Canola Development Commission Study within the Prairie Canola Agronomy Agreement Final Report

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Overview

In 2009, the project with the Saskatchewan Canola Development Commission (SCDC), titled as above, was taken over by the Prairie Canola Agronomy Agreement (PCAA) of Agriculture and Agri-Food Canada. At the same time, the objective of the project was reformed to include two additional aspects as goals. Goals I and III were added to the original Goal II with limited funds:

- (I) Comparison of emergence, growth, grain yield, and oil production of camelina and canola crops grown from saline media;
- (II) Salinity tolerance screening of Crucifer cultivars used in food oil and biodiesel fuel production;
- (III) Comparison of the canola feedstock quality and the resulting biodiesel fuel quality from the feedstock when produced on saline soils.

Goal I. Comparing the Emergence, Height, Grain Yield and Oil Content of Camelina and Canola Crops Grown from Saline Media

Introduction

Camelina (*Camelina sativa* (L.) Crantz.) crops provided Europe with a vegetable oil for centuries until it was gradually displaced by rapeseed (*Brassica* spp.) (Agegnehu and Honermeier 1997; Crowley and Frohlich 1998). Until recently, camelina had only rarely been cultivated in North America, although it had been known, studied, recommended, and touted as a potentially valuable crop (Plessers et al. 1962; Robinson 1987; Putnam et al. 1993; Gugel and Falk 2006). Currently, considerable interest in camelina stems from its potential to serve as feedstock for biodiesel fuel production in cool, semiarid climates. Also, today's seeding implements tend to better cope with the very small seed-size (0.9-1.5 g per 1000 seed) of camelina (Gugel and Falk 2006; Urbaniak et al. 2008). With camelina's ability to germinate, grow and mature quickly, and given the shallow placement requirement for its small-size seed, agronomists recommend that the crop be seeded as early as possible in the spring to efficiently use the soil water that has accumulated over winter (McVay and Lamb 2008). Camelina facilitates this recommendation in that the crop is quite frost hardy, tolerating temperatures as low as -11°C (Marinitch 1954, reported by and also re-measured by Plessers et al. 1962).

If the argument prevails that camelina can grow and produce economically under adverse pressure gradients for water flow from soil to roots in semiarid climates, the crop might also tolerate adverse osmotic gradients in saline root zones. An on-line library search for published information on the salinity tolerance of camelina within eight different data bases failed to identify any such studies.

Officially, "canola" identifies rapeseed crops (*Brassica napus* L., *B. rapa* L. and other species) with low erucic acid ($< 20 \text{ g kg}^{-1}$) in the seed oil and low aliphatic glucosinolates ($< 30 \text{ } \mu\text{mol g}^{-1}$) in the defatted meal (Campbell 1986). The Canola Growers Manual (Thomas 1984) indicates plant emergence and resulting crop establishment as a concern in the production of canola oilseed. The Manual further describes the interference caused by near-surface salts with seed germination and crop establishment. Maximum emergence of canola plants growing in sulphate-saline media measured by Steppuhn and Raney (2005) for Hyola 401 and InVigor 2573 was reduced from 96% to 84% and from 98% to 95%, respectively, as solution salinity increased from 18 to 27 dS m^{-1} .

Seedling emergence and early survival, plant height, growth stage (data not shown herein), above-ground biomass, grain yield, and oil content were evaluated under the controlled environment of Canada's Salinity Tolerance Testing Facility at Swift Current, Saskatchewan. With these variables, the objective of this study was to compare the inherent salinity tolerance of CS15 camelina to that of InVigor 9590 canola crops subjected to a full range of sulphate-based hydroponic rooting solutions from negligibly through severely saline.

Materials and Methods

Test Seed

Bayer CropScience provided the InVigor 9590 canola seed. This cultivar falls into the Oilseed Spring Hybrid Class and contains the novel Liberty-Link gene for herbicide resistance.

The CS15 camelina seed originated from breeder supplies at the Saskatoon Research Centre of Agriculture and Agri-Food Canada. This genotype registered among the best in seed production in previous field trials (Gugel and Falk 2006). The viability of both test seed was evaluated in a commercial germinator (Convion – Controlled Environments) over 15 days. Fifty non-scarified seed from each crop were placed on filter-paper covering fine sand within each of four petri dishes. Average germination registered 95.0% for the InVigor 9590 canola and 87.0% for the CS15 camelina, respectively.

Testing

The experiment was conducted in a greenhouse featuring a controlled environment using hydroponically-nourished sand tanks. This testing facility, located at Swift Current, Saskatchewan, features automatic control over irrigation, fertility, seedbed and root-zone salinity, and ambient temperature integrated over time under an electronic, programmable logic controller (Steppuhn and Wall 1999). Plastic grow tanks (cylinders 0.85 m dia. x 1.0 m deep) were used which contain washed silica sand (99.8% pure) having an average bulk density of 1.65 Mg m^{-3} and a sand-surface area of 0.57 m^2 . At saturation, the sand uniformly holds water at a volumetric content of 31.3%.

A modified Hoagland solution consisting of 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 2.5 mM KNO_3 , 0.17 mM KH_2PO_4 , 1.0 mM MgSO_4 , 0.05 mM chelated Fe, 0.5 mM NH_4NO_3 , 0.05 mM KCl, 0.023 mM H_3BO_4 , plus trace elements including Mn, Zn, Cu, Si, and Mo provided the nutrients (Hoagland and Arnon 1950). Fortified with these nutrients, seven different treatment solutions were prepared by adding appropriate quantities of CaCl_2 , NaCl, MgSO_4 , and Na_2SO_4 to obtain solutions with electrical conductivities targeted to equal 1.4, 3, 6, 10, 15, 20, and 28 dS m^{-1} ; these solutions ranged in salinity levels from negligible (nutrients-only) to severely saline (United States Salinity Laboratory Staff 1954).

One hundred-four seed from each of the two test crops were sown 13 mm deep into the sand in rows spaced 152 mm apart within each sand tank ($182.5 \text{ seed m}^{-2}$). Upon completion of emergence (after 32 days), the remaining plants were subsequently thinned to 64 plants per tank ($112 \text{ plants m}^{-2}$).

The test was conducted with an appropriate time course for day/night sequences (adjusted every four days) mimicking an April 27th seeding date at 50° north latitude. Supplemental lighting from 475-W sodium lamps positioned 1.5 m above the sand surfaces extend day-lengths. Lamps were strategically positioned overhead in order to obtain measured radiation intensities averaging $7.9 \text{ kJ m}^{-2} \text{ min}^{-1}$ with a uniformity coefficient of 0.9 across the entire test facility. Temperature setpoints were automatically reset hourly according to a 24-hour diurnal schedule and ranged from 14 to 24°C with ambient temperatures maintained within one or two degrees of the setpoints.

Measurements and Analyses

Within each treatment, the response of the plants to root-zone salinity was determined by measuring emergence and early survival, plant height, oven-dried shoot biomass, grain yield, the oil content of the seed, and the composition of the oil. Measurements were averaged and related to electrical conductivities of the test solutions (EC_{sol}) for each test crop.

Plant Emergence and Survival Two flushes (irrigations) with the test solutions preceded seeding in order to firm the seedbed, and a template guided placement of each seed into a known position within each seedbed. This allowed assessment of the plant emergence and survival associated for each planted seed on a daily basis. Any protrusion of the plant above the sand surface counted it as emerged. Records were kept on electronic copies of the seeding template. This practice resulted in daily counts per tank of the number of newly emerged plants and their survival with time.

Plant Height and Growth Stage Plant height served to compare plant growth among the treatments and was determined from weekly measurements of the same ten plants per tank. Plant growth stage was also assessed according to a modified decimal code (Lancashire et al. 1991). The respective stages from 1 through 8 are: leaf development on main shoot, branching or tillering, stem elongation, bolting or booting, inflorescence emergence, flowering, development of fruit, seed maturity. The seed were planted on September 27th and growth measured on Oct 23, 30, Nov 6, 13, 20, 27, Dec 4, 11, 18, and at or just before harvest in January. These dates correspond with days 26, 33, 40, 47, 54, 61, 68, 75, and 82 since seeding (dss) plus at harvest. Except for the severest salt treatment, the camelina matured ahead of the canola. The final heights and stages were measured on Jan 3 or the 98th dss for the 1.4 dS m⁻¹ camelina, Jan 8 or the 103rd dss for the 3.0 through 14.7 dS m⁻¹ camelina, and Jan 18 or the 113th dss for the camelina under the severe treatments and 3 days before all the canola plants were harvested. The plant height data at harvest were analyzed with an analysis-of-variance and compared for effects of salinity treatment and crop (SAS 2007).

Shoot Biomass and Grain Yield The above-ground portion of each test plant was cut when the crop would normally have been swathed, and the harvested shoot material from each tank placed in a separate cloth bag and oven-dried at 35 °C. Any leaves which fell off the plants prior to harvest were also collected. After drying, the contents of the bags were weighed, the grain threshed and weighed, and these weights collated according to treatment. Dividing the weights per tank by 0.57 m² resulted in shoot and grain yields expressed in g m⁻². The yields from the replicate grow tanks per treatment were reported as averages. To standardize the production obtained under the salinity treatments, grain yields were also expressed on a relative basis. The usual procedure for converting absolute yield (Y) to relative yield (Y_r) employs a scaling divisor (Y_m) equal to the production where salinity has very little or no influence on the yield (Maas 1990):

$$Y_r = \frac{Y}{Y_m} \quad [1]$$

The Y_m divisor normalizes the data-set (expressed in percent) and, for non-halophytes, usually equals the maximum yield associated with each treatment.

Various empirical equations have been applied or suggested for describing Y_r as a function of a variable which reflects the average root-zone salinity (C). The measure for C in this study is EC_{sol}, where EC_{sol} equals the electrical conductivity of the test solution in dS m⁻¹. The most recent empirical analog function for determining relative product yield (Y_r) in response to increasing root zone salinity is the modified discount equation (Steppuhn et al. 2005a):

$$Y_r = \frac{1}{1+(C/C_{50})^{\exp(sC_{50})}} \quad [2]$$

where C_{50} defines C at $Y_r = 0.5$, and s represents the response curve steepness. The steepness parameter equals the average absolute value of the slope (dY_r/dC^{-1}) of the equation through C_{50} and its steepest segments on either side of C_{50} , evaluated in our study from $Y_r = 0.3$ to 0.7 . The argument sC_{50} of the exponent in Eq. 2 contributes to a symmetrical convex-concave yield response with the inflection point at C_{50} . The parameter s describes the average unit decrease in relative product yield with unit increase in root-zone salinity.

A single-value index of crop tolerance to root-zone salinity has proved useful for comparing the salinity tolerance of agricultural crops (Steppuhn et al. 2005a). If C_{50} were enhanced by a term which dictates the shape of the yield response for salinity levels approaching C_{50} , such as the argument of the exponent in Eq. 2, a comprehensive, single-value, Salinity Tolerance Index or ST Index results:

$$\text{ST Index} = C_{50} (1 + s) \quad [3]$$

where C_{50} and s can be computed as regression constants, or approximated by a visual inspection of the response data.

The grain yield measurements, scaled by the results obtained in the low-salt treatments, facilitated comparisons. The scaling divisors for the yield data were determined by substituting Y/Y_m of Eq. 1 into Eq. 2 and solving for Y_m using nonlinear regression software from SAS (2007), which is based on the maximum neighbourhood method of Marquardt (1963) and an optimum interpolation between the Taylor series method and the method of steepest descent (Bates and Watts 1988). These yield data were tested and accepted for homogeneity of variance among means using the Brown-Forsythe, Bartlett, and Welch tests (SAS 2007).

The relative grain yield determined for each test crop grown under each salinity treatment was regress-fitted to the discount response function (Eq. 2) and resulted in separate response functions for each crop. From these functions, respective C_{50} and s values were derived for each crop using the same nonlinear software as before (SAS 2007). These parameters led to salinity tolerance indices based on Eq. 3 indicating the relative salinity tolerances between the two crops.

A statistical covariance procedure utilizing paired t-tests served to compare the discount response functions for similarity and differences among the test crops. These comparisons provided the basis for assigning differences in relative salinity tolerances for the two crops.

Seed Oil Content Samples of the harvested seed (grain) from each crop were analyzed for oil content at the Oilseed Chemistry Laboratory of the Saskatoon Research Centre, Agriculture and Agri-Food Canada (AAFC). The camelina crop failed to produce any seed at the 27.02 dS m^{-1} salinity level and yielded insufficient quantities of oilseed for a complete quality evaluation at the 19.92 dS m^{-1} level. The quantity of oilseed obtained from the 27.02 dS m^{-1} canola crop also proved insufficient to conduct the glucosinolate evaluations for this salinity level. Before any analyses were conducted, the seed were further dried at 40 °C for 48 hours.

Results and Discussion

Plant Emergence and Survival

From 104 seed planted per tank, the number of plants which germinated, emerged and survived as seedlings, and established in the negligible or slight salinity environments (1.36 or 2.98 dS m^{-1}) averaged 103.0 or 99.0% and 103.3 or 99.3% for the canola and camelina, respectively (Figure 1). From these maxima, the percentages ranged downward to 76.9% and 13.9% , respectively, in the severe salinity of 27.03 dS m^{-1} . Among the seed planted in the 1.36 , 2.98 , 6.05 , and 10.00 dS m^{-1} tanks, differences in emergence between the two crops tended to be narrow with only very slight, if any, advantage to either crop. At 14.67 , 19.92 , and 27.02 dS m^{-1} , the cumulative number of plants which emerged and survived became progressively less for the camelina compared to the canola.

The lack of statistical differences in the maximum cumulated emergence of camelina and the canola seedlings when grown in saline root zones rated less than severe leads to the inference that the number of emerged plants which remain viable and grow under severe conditions might serve as a useful initial indicator for the crop salinity tolerance. Seedlings, which barely survive in controlled sand tanks, will most likely succumb to disease or insects in actual field plantings. As the growing season progressed, the number of seedlings surviving beyond the time of peak emergence was not sustained for the camelina in either of the two severely saline environments (19.92 and 27.02 dS m^{-1}); in contrast with the canola, the emerged camelina plants tended to died with time.

Plant Height and Growth Stage

The negative effect of root-zone salinity on average crop growth in height is evident from the measurements obtained over the growth period from day 26 after seeding to harvest (Figure 2). Although the height response curves for the growth of the two crops follow similar shapes, the spread between salt-level responses for the camelina tended to exceed those for the canola. These differences between crops increased with salinity until the camelina plants at 27.02 dS m^{-1} all died. The time course for the camelina height appeared proportionately congruent with that for the canola in the two lowest salinity levels, but increasingly lagged the canola trace in the five highest levels. The average height of the canola at any time and at any salinity tended to exceed that measured in the camelina. Average plant heights at the time of respective harvests for the two crops statistically differed according to salinity ($P \forall < 0.001$) and by crop ($P \forall < 0.0445$). Also, the variances for the height data were calculated to be statistically homogeneous.

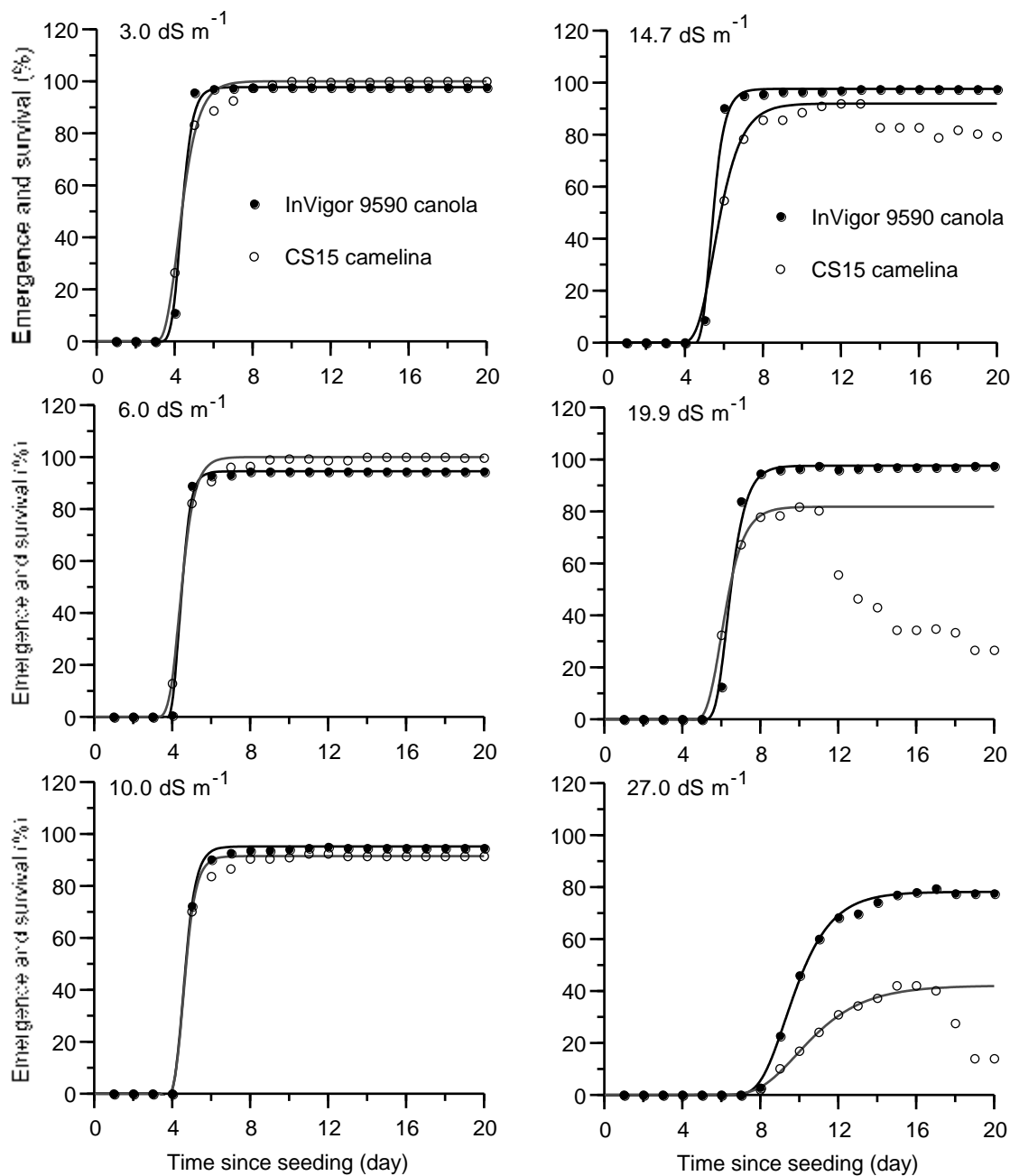


Figure 1. Gompertz equations fitted by least squares to the average cumulative emergence and survival of InVigor 9590 canola and CS15 Camelina plants (% of the best total among all salinity levels) subjected to rooting substrates averaging 3.0, 6.0, 10.0, 14.7, 19.9, and 27.0 dS m⁻¹.

The time sequences in growth stage for the two crops followed similar patterns except for the camelina at 27.03 dS m⁻¹, where the plants slowly all died (Figure 3). Although root-zone salinity initially slowed crop development especially in the canola, the rate of development from stem elongation (Stage 3) to fruit development (Stage 7) generally increased as the salinity level increased. The steepness in rates of this increase in the camelina seemed to match that for the canola. These rates in crop development contributed to completion of the final growth stage (Stage 8 when the grain seed became ready for harvest) more-or-less at the same time for all the

salinity treatments within each crop. Apparently, the genetic drive of these annual crop plants follows paths leading directly to seed production within a preset time period despite negative saline environments.

Shoot Biomass and Grain Yield

Within each crop, shoot biomass and grain yield tended to decrease as salinity increased, reflecting the negative impact of saline substrates (Table 1). The harvest indices (grain yield/shoot biomass) varied by 3.5% in the canola and 16.2% in the camelina plants within the weakest five salinity treatments of each crop (data not shown). The absolute grain yields for the InVigor canola averaged close to twice those for the CS15 camelina at the C_{50} salinity, perhaps because the crops in this study were supplied with ample water, which allowed the hybrid-genetic production-potential of the canola to be fully expressed.

Conversion of the absolute seed yields (Y) to relative yields (Y_r) indicated less salinity tolerance for the camelina than for the canola at all EC_{sol} -levels (Figure 4). Regression fits of the modified discount equation (Eq. 2) for relative oilseed yield plotted as a function of root-zone salinity resulted in respective least-square r^2 and root mean square error values of 0.944 and 0.0674 for the canola and 0.916 and 0.1112 for the camelina. The resulting C_{50} -values (EC_{sol} -based) equalled 16.9 and 6.8 $dS\ m^{-1}$ for the InVigor 9590 canola and the CS15 camelina, respectively (Table 2). With Eq. 2, the mean C_{50} -value (EC_{sol} -based) reported for dryland canola by Steppuhn et al. (2005b) is 14.2 $dS\ m^{-1}$, or a difference of 2.7 $dS\ m^{-1}$ less than that measured with the InVigor 9590 canola in the study presented herein.

The respective EC_{sol} -salinity tolerance indices (STI), derived from Eq. 3, indicate a STI difference of 10.65 between the test crops, placing the canola well over that of the camelina (Table 2). According to Steppuhn et al. (2005b), the average STI for dryland canola registers 16.00 (EC_{sol} -equivalent), some 2.02 units less than that measured for InVigor 9590 in this experiment. In a comparative trial with barley (Steppuhn and Raney 2005), the STI derived for an earlier InVigor (2573) canola equalled 16.7, or 1.3 less than the InVigor (9590) in this study. One explanation is that salinity tolerance of the InVigor breeding line has improved as the InVigor genotype improved.

The relative responses of grain yields were further compared by evaluating the statistical covariance associated with yields generated from applications of Eq. 2 (the discount response function) using both sets of C_{50} and s parameters (Table 2) in paired t-tests for each crop (Table 3). These covariance tests indicated that the InVigor 9590 canola and the CS15 camelina responses were statistically different ($P \leq 0.001$).

The differences in salinity tolerance between camelina and canola may trace to the regions where these crops developed. The CS15 camelina originated from the former Union of Soviet Socialist Republics (Gugel and Falk 2006), from stock grown in northern Europe and Russia until the mid-20th century (Knorzer 1978; Zubr 1997 and 2003: cited by Gugel and Falk 2006). In contrast, the InVigor canola was developed in North America from rapeseed originating from southern Eurasia, by way of Argentina (Thomas 1984). Soil salinity is more common in south Eurasia than in the north which likely resulted in less exposure to salinity during the camelina's development compared to that of the canola.

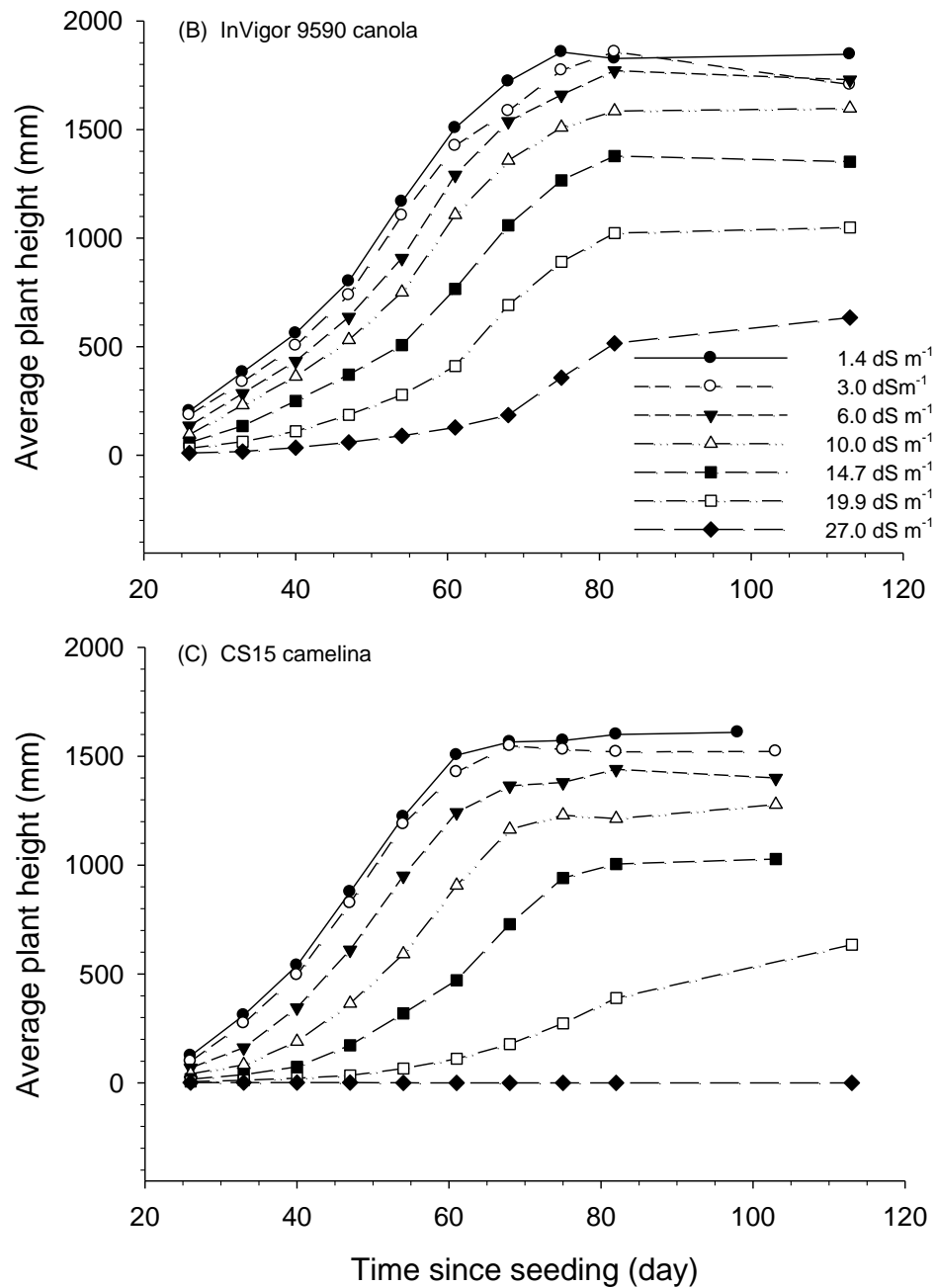


Figure 2. Average plant height of InVigor 9590 canola *Brassica* (B) and CS15 camelina *Camelina* (C) measured more-or-less weekly since seeding for each of seven salinity treatments.

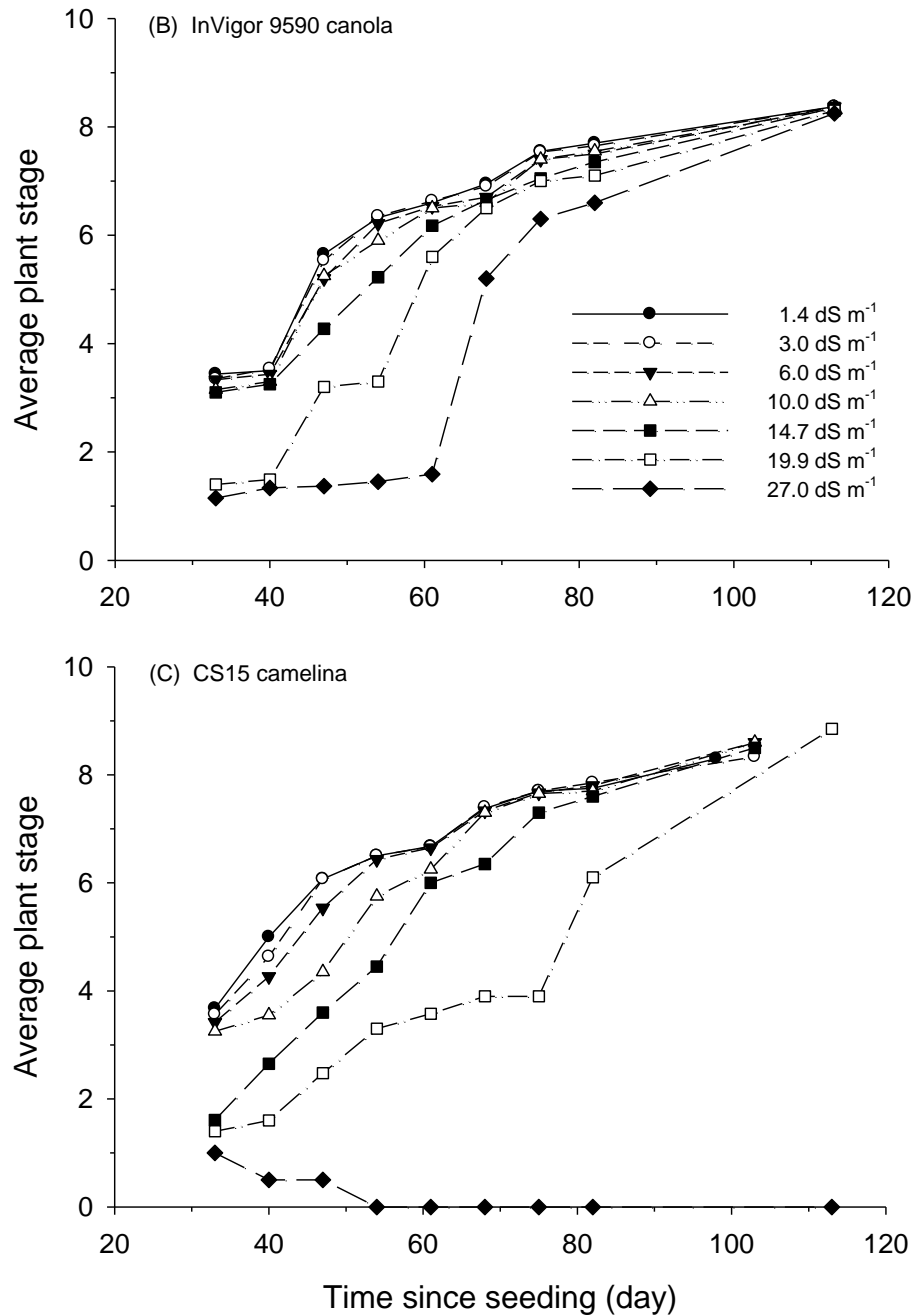


Figure 3. Average plant growth stage assessment (according to Lancashire et al. 1991) measured with time for InVigor 9590 canola *Brassica* (B) and CS15 camelina *Camelina* (C) crops for each of seven salinity treatments.

Conversion of the absolute seed yields (Y) to relative yields (Y_r) with Eq. 1 indicated less salinity tolerance for the camelina than for the canola at all EC_{sol} -levels (Figure 4). Regression fits of the modified discount equation (Eq. 2) for relative oilseed yield plotted as a function of root-zone salinity resulted in respective least-square r^2 and root mean square error values of 0.944 and 0.0674 for the canola and 0.916 and 0.1112 for the camelina.

Table 1. Mean, oven-dried, grain yield and shoot biomass from InVigor 9590 canola and CS15 camelina crops grown in respective saline rooting media listed by average electrical conductivity of the test solution.

Solution ^z	Canola		Camelina	
EC _{sol}	Grain (se) ^y	Shoot (se) ^y	Grain (se) ^y	Shoot (se) ^y
dS m ⁻¹	----- g m ⁻² -----	----- g m ⁻² -----	----- g m ⁻² -----	----- g m ⁻² -----
1.36	254.9 (14.0)	1043 (33.4)	144.5 (3.0)	572 (40.5)
2.98	241.9 (7.5)	963 (29.6)	113.2 (14.9)	507 (57.9)
6.05	240.3 (2.7)	949 (17.1)	78.3 (8.7)	370 (51.6)
10.00	199.8 (43.5)	813 (100.8)	71.9 (11.3)	303 (71.6)
14.67	144.8 (4.4)	572 (26.6)	31.4 (9.6)	129 (32.9)
19.92	121.1 (3.1)	414 (5.12)	1.44 (0.20)	9.65 (2.77)
27.02	23.78 (0.32)	162 (29.5)	0	0

^z EC_{sol} equals the average electrical conductivity of the test solution.

^y se equals the standard error.

The respective EC_{sol}-salinity tolerance indices (STI), derived from Eq. 3, indicate a STI difference of 10.65 between the test crops, placing the canola well over that of the camelina (Table 2). According to Steppuhn et al. (2005b), the average STI for dryland canola registers 16.00 (EC_{sol}-equivalent), some 2.02 units less than that measured for InVigor 9590 in this experiment. In a comparative trial with barley (Steppuhn and Raney 2005), the STI derived for an earlier InVigor (2573) equalled 16.7, or 1.3 less than the InVigor (9590) in this study. One explanation is that salinity tolerance of the InVigor breeding line has improved as the genotype improved.

The relative responses of grain yields were further compared by evaluating the statistical covariance associated with yields generated from applications of Eq. 2 (the discount function) using both sets of C₅₀ and s parameters (Table 2) in paired t-tests for each crop (Table 3). These covariance tests indicated that the InVigor 9590 canola and the CS15 camelina responses were statistically different ($P \leq 0.001$).

Seed Oil Content and Composition

In this experiment, the average oil content in percent by mass within the InVigor 9590 canola oilseed exceeded that within the CS15 camelina by 5 to 8% (Figure 5). The oil content of the canola remained at about the 40% value over the range of salinity from negligible to the 20 dS m⁻¹ salinity, beyond which the oil percentage declined. The shape of the response in camelina oil content followed that for the canola except at a plateau percentage near 35% which extended to only 10 dS m⁻¹ before declining. The canola and the camelina each registered oil contents greater than 30% for the crops grown subject to negligible through severe in root-zone salinity. In an earlier study, Steppuhn and Raney (2005) also measured a plateau in oil-content for canola oilseed grown in saline environments ranging from slightly through moderately saline.

Table 2. Response function parameters and EC_{sol} Salinity Tolerance Index (STI) for relative oil grain yield (Y_r) with standard error in parenthesis for InVigor 9590 canola and CS15 salinity. camelina crops grown in sulphate-based saline media

Crop	N^y	Parameter & Salinity Tolerance Index (STI) ^z			
		Y_m^x	$C_{50} (\pm se)^w$	$s (\pm se)^w$	STI
		$g\ m^{-2}$	$dS\ m^{-1}$	$(dS\ m^{-1})^{-1}$	
Canola	18	249	16.91 (0.76)	0.0658 (0.0089)	18.02
Camelina	18	148	6.78 (0.64)	0.0868 (0.0235)	7.37

^z The Salinity Tolerance Index = $C_{50} + sC_{50}$ which is derived from the discount response function [Eq. 2]: $Y_r = 1 / [1 + (C/C_{50})^{\exp(s C_{50})}]$, where $C = EC_{sol}$ and C_{50} defines C at $Y_r = 0.5$, and s represents the response curve steepness.

^y N equals the number of samples.

^x Y_m equals seed yield where salinity has little or no influence.

^w se equals the standard error.

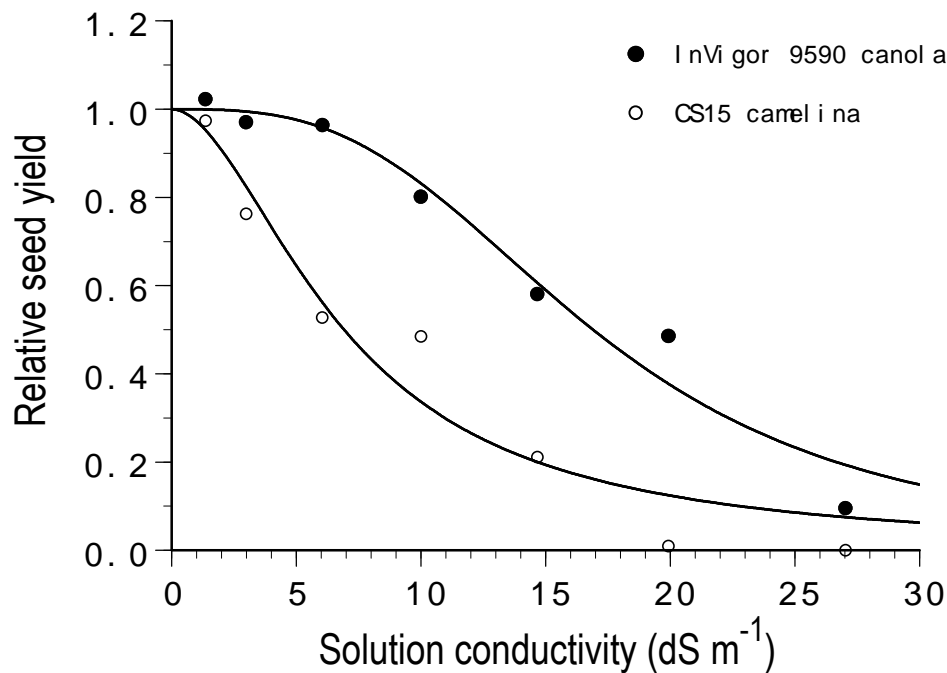


Figure 4. Mean relative seed (grain) yield for InVigor 9590 canola and CS15 camelina crops grown in increasingly saline root zones fitted to the discount function [Eq. 2].

The fatty acid composition of the harvested oilseed within each salinity level tended to differ between crops for the acids listed in Table 4. The camelina oilseed contained the omega-3 \forall -linolenic (C18:3) acid which accounted for 35% of the oil's total fatty acids by mass compared to just over 7.5% for the canola. The percentage of erucic acid found in the camelina averaged

Table 3. Statistics from four separate covariance analyses (by paired t-tests) between measured relative grain yield and respective discount response functions for comparing InVigor 9590 canola and CS15 camelina crops grown in seven saline rooting solutions from negligibly through severely saline

Crop and measured yield statistic	Mean measured relative yield	Fitted discount response function ^z	
		Canola	Camelina
<u>InVigor 9590 canola</u>	0.711		
Covariance:			
Mean difference		0.0029	-0.1601
Standard error		0.0176	0.0418
Prob.> t ^y		0.8694	<0.0001 ^{**}
Degrees of freedom		17	17
 <u>CS15 camelina</u>	 0.520		
Covariance:			
Mean difference		0.2610	0.0097
Standard error		0.0404	0.0257
Prob.> t ^y		<0.0001 ^{**}	0.7113
Degrees of freedom		17	17

^z The computed relative yield (Y_r) values and statistics from Eq. 2 ($Y_r = 1 / [1 + (C/C_{50})^{\exp(s C_{50})}]$) using seven salinity levels (C) and C_{50} & s as function parameters from statistical fits resulting from nonlinear regressions with measured data from each genotype.

^y The Prob.>|t| equals the probability for a greater absolute t-value where ^{**} signals computed and measured values which are significantly different with a Type I error probability < 0.010.

4.27% compared to 0.04% for the canola. A review of the composition percentage of each acid for each crop suggested minimal change in any of the fatty acids as the salinity increased through 14.7 dS m⁻¹ (Table 4). This further suggests a degree of stability in fatty acid composition for seed oil produced from sulphate environments in both crops over the slight through moderate salinity range. One caution in using these results, and in the results from throughout the study, relates to their dependency on sand-culture hydroponics rather than actual soil solutions.

Oilseed protein levels approached or exceeded 30% in both test crops grown from all salinity treatments (Table 5). Most of the protein likely remained with the defatted meal after oilseed crushing. A very slight tendency also appears for the saturated density of the fatty acids to increase with salinity in both crops (Table 5). This is coupled with an even slighter tendency for the iodine value (an indicator of the frequency of double C-to-C bonds and unsaturated fat content) to decrease in association with the canola and the camelina oilseed grown under increasingly saline treatments (Table 5). The camelina iodine value exceeded that of the canola by about 49% across the 1.4 through 14.7 dS m⁻¹ salinity range. Although total glucosinolate content within the oil-free meal varied somewhat over a wide salinity range, concentrations in the camelina averaged from 25% to 50% greater than for the canola (Table 5). The results listed

in Tables 4 and 5 confirm camelina's food-oil qualities; in addition, its value as a biodiesel feedstock stems from its overall oil percentage and the oil's ready conversion to acyl esters.

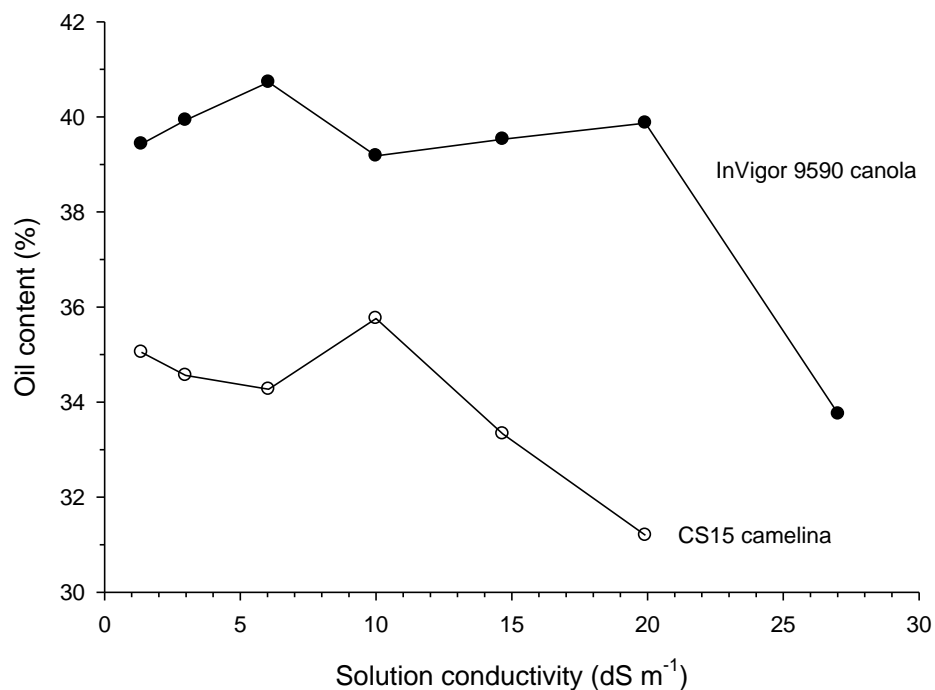


Figure 5. Mean concentration of oil in CS15 camelina and InVigor 9590 canola oilseed (% by mass) related to solution conductivity (dS m⁻¹) of the rooting medium.

Table 4. Mean concentrations of fatty acids determined from harvested InVigor 9590 canola (B) and CS15 camelina (C) oilseed crops grown in root zones arrayed by seven salinity levels (EC_{sol})²; presented as percentages of the total percent by mass.

[Please see the last page of this report for this Table 4.]

Table 5. Mean oilseed protein, saturated density, iodine value, and total glucosinolates (of the defatted meal) for InVigor 9590 canola and CS15 camelina grown subjected to respective saline rooting media

Oil content & Crop	Average actual EC_{sol}^z (dS m ⁻¹)						
	1.36	2.98	6.05	10.00	14.67	19.92 ^y	27.02 ^y
<u>Protein (%)</u>							
Canola	30.54	30.47	30.51	30.79	29.76	30.17	31.02
Camelina	31.70	31.88	32.35	30.58	31.61	33.32	---
<u>Saturated density (%)</u>							
Canola	7.94	8.12	7.83	8.12	8.26	8.46	9.74
Camelina	11.74	11.47	11.76	11.47	12.48	---	---
<u>Iodine value</u>							
Canola	105.67	105.07	104.49	105.44	104.07	101.87	104.48
Camelina	156.59	157.36	156.28	156.61	153.46	---	---
<u>Total glucosinolates (:mol g⁻¹ oil-free dry meal)</u>							
Canola	31.18	31.12	26.90	37.67	21.48	15.95	---
Camelina	48.78	46.84	49.02	46.77	53.00	---	---

^z EC_{sol} equals the average electric conductivity of the test solution.

^y The missing data in these columns reflect the limited quantity of oilseed available for analyses.

Conclusions

Root-zone salinity treatments comparing the growth of CS15 camelina and InVigor 9590 canola crops utilized seven sulphate-based concentrations in sand-culture hydroponic solutions averaging 1.4, 3.0, 6.0, 10.0, 14.7, 19.9, and 27.0 dS m⁻¹; these values mimicked salt-affected root zones classed as negligibly through severely saline. The following conclusions resulted from the test crop responses:

- ❖ Seedling emergence and early survival for the two crops remained similar until the 14.7 dS m⁻¹ salinity level beyond which the camelina emergence and survival began to lag as salinity increased (Figure 1).
- ❖ The plant heights of the camelina began to show greater negative effects of the root-zone salinity than the canola at 6.0 dS m⁻¹ including the complete loss of plants under the 27.0 dS m⁻¹ treatment (Figure 2).
- ❖ The sequences of plant growth stages with time reacted similarly in the two test crops, reaching the mature seed stage at the same time regardless of salinity (Figure 3).

- ❖ The relative grain yield from crops grown subjected to sulphate-based solutions proved considerably less for the camelina than for the canola (Figure 4). The solution-based salinity tolerance indices equalled 7.37 and 18.02 for the camelina and canola, respectively (Table 2).
- ❖ The concentration of seed oil in the camelina registered about 35% from 1.4 through the 10.0 dS m⁻¹ treatment and about 40% in the canola through the 19.9 dS m⁻¹ treatment (Figure 4). Oil percentages in both test crops decreased as salinity increased beyond these respective salinity levels.
- ❖ Protein concentrations, iodine values, and fatty acid profiles within each test crop remained relatively unaffected by the sulphate growth environments within the slight and moderate levels of salinity (Table 5).
- ❖ Root-zone salinity affected both camelina and canola grain yields at lower electrical conductivity values than those conductivities which influenced seedling emergence, plant survival, seed-oil content, and oil composition.

The primary impact of this research should impart a degree of respect for growing camelina in saline fields which previously produced adequate canola crops.

Goal II. Salinity Tolerance Screening for Food and Biodiesel Cultivars and Genotypes

Introduction

Selected canola plants tolerate root-zone salinity as well as those of Harrington barley (Steppuhn & Raney 2005). With this knowledge, producers can expand canola crop area within the seven million hectares of cultivated lands affected by slight to moderate salinity across the Canadian Prairies (Wiebe et al. 2007; Steppuhn 1996), taking advantage of production options which counter fuel-versus-food concerns. This work can also position Crucifer breeding activity for continued development of high oil-yielding cultivars to better tolerate root-zone salinity.

Salinity generally slows the rate of crop growth, resulting in plants with smaller leaves, shorter stature, and reduced economic yield (Shannon et al. 1994). The inherent ability of crop plants to withstand the effects of elevated solute concentrations of Na, Ca, Mg, SO₄, Cl, and other ions in root-zone solutions and still produce agricultural products defines salinity tolerance. A range of canola-grade oilseed feedstock cultivars were screened for salinity tolerance in Canada's Salinity Tolerance Testing Facility at Swift Current (Steppuhn and Wall 1999).

Materials and Methods

Test Cultivars

The testing facility at Swift Current can accommodate up to ten cultivars per screening set. The cultivar 'Westar' served as a common cultivar in each test. Screening was based on the sensitivity of plant tissue to root-zone salinity as measured by emergence, height growth, plant crop stage, and harvested shoot biomass. The purpose of the screening was to identify the comparative salinity tolerance for the seedlings tested and to select cultivars for further testing involving crop yield functions in response to an array of root-zone salinity levels from negligible to severe. The oilseed crops from which the test cultivars were selected for screening included:

- 1) Argentine canola open-pollinated (*Brassica napus*)
- 2) Argentine canola "Roundup-Ready" hybrid (*Brassica napus*)
- 3) Argentine canola "Liberty-Link" hybrid (*Brassica napus*)
- 4) Argentine canola "Clear-Field" hybrid (*Brassica napus*)
- 5) Polish canola (*Brassica rapa*)
- 6) Camelina (*Camelina sativa*)
- 7) Ethiopian mustard (*Brassica carinata*)
- 8) Mustard (*Sinapis alba*, *Brassica juncea*)
- 9) Canola-quality Juncea (*Brassica juncea*)

These crops included the top oil-producing cultivars of *B. napus*, *B. juncea* and other oilseed species offered by co-operating seed companies.

The first step upon obtaining the seed was to test for germination and vigour. The germination testing in an environmentally-controlled growth cabinet with petri dishes, fine sand, plotting paper, and distilled water required 200 test seeds per cultivar. This test was conducted in 70% relative humidity and under a regime of alternating 12 hour-periods with and without supplementary lighting. A radical length of 10 mm specified germination.

Screening Procedures

Three separate experiments provided the results for evaluating and screening for the comparative salinity tolerances of Crucifer cultivars and germplasm. Test plants representing

members of three cultivar groups listed in Tables 6, 7 & 8, respectively, formed three experiments each treated with one of three test solutions containing sulphate-based salts concentrated to form target salinities classed as:

- (1) negligible, nutrient-only [1.4 dS m^{-1}],
- (2) midway between slight and moderate [8 dS m^{-1}], and
- (3) midway between moderate and severe [16 dS m^{-1}].

Two experimental set-ups facilitated the sand-tank test cultures: pots and tanks.

Pots Nine-litre pots filled with 8 L pure silica (mean particle sizes from 0.1 to 0.3 mm in nominal diameter) provided seedbeds for testing two cultivars in each pot. The seedbeds were flushed four times daily with a modified, half-strength Hoagland's nutrient solution consisting of $\text{Ca}(\text{NO}_3)_2$, KNO_3 , KH_2PO_4 , MgSO_4 , chelated Fe, NH_4NO_3 , KCl, H_3BO_4 , plus trace elements which included Mn, Zn, Cu, Si, and Mo (Hoagland and Arnon 1950). Randomly selected solutions were salinized by adding CaCl_2 , MgSO_4 , NaCl, and Na_2SO_4 to obtain salinized test solutions plus the nutrient-only control and were added to the test solutions prior to seeding. This procedure duplicated the common field situation where seed must be placed directly into saline seedbeds, typical for dryland prairie conditions and practices. Solution electrical conductivities (EC_{sol}) in dS m^{-1} relate to equivalent electrical conductivity of saturated soil paste extracts (EC_e) in dS m^{-1} , as detailed in Ayers and Westcot (1985), by the approximate relationship: $\text{EC}_e = 0.5(\text{EC}_{\text{sol}})$.

Each hydroponic flushing of the root-zone continued for twelve minutes until the sand was completely saturated, after which the solutions drained into 612-litre reservoirs for the next flushing. Water lost by evapotranspiration was replenished weekly or when necessary to maintain the concentrations of salts in solution. The electrical conductivity (EC_{sol}) of each solution was checked initially and twice weekly.

The screenings were conducted with an appropriate time course for day/night time sequences (adjusted in 15-minute ephemeral increments) mimicking an April seeding at 51° north latitude. Supplemental lighting from 475-W sodium lamps positioned 1.5 m above the sand surfaces extended day-lengths. Lamps were strategically positioned overhead in order to obtain measured radiant intensities averaged $7.9 \text{ kJ m}^{-2} \text{ min}^{-1}$ with a uniformity coefficient of 0.9 within the entire test laboratory. Day/night temperatures were reset hourly according to a 24-hour diurnal schedule, and ranged from 14 to 24°C with ambient temperatures maintained within one or two degrees of the set-points.

Two irrigations with the test solutions preceded seeding in order to firm the seedbed. A template guided placement of each seed into a known position within each seedbed. This allowed assessment of emergence and survival associated with each seed on a daily basis. Any protrusion of the plant above the sand surface counted it as emerged. Records were kept on electronic copies of the seeding template. This practice resulted in daily counts per pot of the number of newly emerged plants and their survival with time.

Table 6. Third group of cultivars selected for screening.

Cultivar	Scientific Name	Type ^z
SP Force	Brassica napus L.	Argentine canola (op)(CL)
SP 621	Brassica napus L.	Argentine canola (RR hybrid)
InVigor 5440	Brassica napus L.	Argentine canola (LL hybrid)
Ace	Sinapis alba L.	Yellow mustard
Andante	Sinapis alba L.	Yellow mustard
Forge	Brassica juncea L.	Oriental mustard
AC Pennant	Sinapis alba L.	Yellow mustard
AC Parkland	Brassica rapa L.	Polish canola
Dahinda	Brassica juncea L.	Canola-quality oil
Westar	Brassica napus L.	Argentine canola (op)

CL= Clearfield; RR=Roundup ready; LL=Liberty link; op=open pollinated

^z The authors apologize for any incorrect designations in ascribing cultivar type.

Table 7. Fourth group of cultivars selected for screening.

Cultivar	Scientific Name	Type ^z
SP Banner	Brassica napus L.	Argentine canola (op)(RR)
SP Desirable	Brassica napus L.	Argentine canola (RR synthetic)
InVigor 5020	Brassica napus L.	Argentine canola (LL hybrid)
InVigor 5030	Brassica napus L.	Argentine canola (LL hybrid)
InVigor 9590	Brassica napus L.	Argentine canola (LL hybrid)
Pioneer 45H26	Brassica napus L.	Argentine canola (RR hybrid)
Proven 45P70	Brassica napus L.	Argentine canola (CL hybrid)
Pioneer 45H73	Brassica napus L.	Argentine canola (CL hybrid)
CS15-genotype	Camelina sativa (L.) Crantz	Camelina (Flax-like oil)
Westar	Brassica napus L.	Argentine canola (op)

CL= Clearfield; RR=Roundup ready; LL=Liberty link; op=open pollinated

^z The authors apologize for any incorrect designations in ascribing cultivar type.

Table 8. Fifth group of cultivars selected for screening.

Cultivar	Scientific Name	Type ^z
SW 6802	Brassica napus L.	Argentine canola (RR hybrid)
BY 997	Brassica napus L.	Argentine canola (op)(RR hybrid)
InVigor 9590	Brassica napus L.	Argentine canola (LL hybrid)
Nex 828	Brassica napus L.	Argentine canola (CL hybrid)
Nex 842	Brassica napus L.	Argentine canola (CL hybrid)
Proven 46P50	Brassica napus L.	Argentine canola (RR hybrid)
Proven 45P70	Brassica napus L.	Argentine canola (CL hybrid)
Duchess	Brassica juncea L.	Brown mustard
AC Cutlass	Brassica juncea L.	Oriental mustard
Westar	Brassica napus L.	Argentine canola (op)

CL= Clearfield; RR=Roundup ready; LL=Liberty link; op=open pollinated

^z The authors apologize for any incorrect designations in ascribing cultivar type.

Each screening utilized 60 pots laid out in groups of three forming 20 replicate blocks each containing one pot salinized according to one of the three salt levels and randomized within each block and the 60-pot layout. Each treatment was replicated four-fold. Five seed of each cultivar was position-placed 13 mm deep in either the east or west half of each test pot following a random choice. Measurements in the screening tests included: plant emergence and survival, the average number of seedlings which survived 35 days or longer after seeding, the average number of days following seeding for the seedlings to reach maximum emergence, plant heights, growth stage development (according to a code by Lancashire et al. 1991), and the harvested shoot weight. Selected results based on these measurements from three screenings (3rd, 4th and 5th Test Groups) are reported herein (Tables 6, 7 & 8). Plants from seed forming each of the cultivars in the Third and Fifth Test Groups (Tables 6 & 8) utilized the “pot” set-up.

Tanks Plastic grow tanks (cylinders 0.85 m dia. x 1.0 m deep) were used for the Fourth Test Group. These tanks contain washed silica sand (99.8% pure) having an average bulk density of 1.65 Mg m^{-3} and a sand-surface area of 0.57 m^2 . At saturation, the sand uniformly holds water at a volumetric content of 31.3%. The seedbeds and root zones were flushed four times daily (01:00, 09:00, 13:00, and 17:00 hour) with aqueous solutions containing modified Hoagland nutrients consisting of 2.0 mM $\text{Ca}(\text{NO}_3)_2$, 2.5 mM KNO_3 , 0.17 mM KH_2PO_4 , 1.0 mM MgSO_4 , 0.05 mM chelated Fe, 0.5 mM NH_4NO_3 , 0.05 mM KCl , 0.023 mM H_3BO_4 , plus trace elements including Mn, Zn, Cu, Si, and Mo (Hoagland and Arnon 1950). Fortified with these nutrients, three different treatment solutions were prepared by adding proportionate quantities of CaCl_2 , NaCl , MgSO_4 , and Na_2SO_4 sufficiently to obtain solutions with electrical conductivities targeted to equal 1.4, 8, and 16 dS m^{-1} . These test solutions represent salinity levels from negligible (nutrients-only) to severely saline (United States Salinity Laboratory Staff 1954). Gypsum (CaSO_4) precipitation was controlled by the calcium additions. This approach closely mimics the natural sulphate-based, soil-salinity-solution systems of the Great Plains and Canadian Prairies. All nutrients and salt complements were prepared and added to the test solutions prior to seeding. This procedure followed the common dryland field situation where seed must be placed directly into saline seedbeds, typical for semiarid conditions and practices. The pH-values of the test solutions averaged 7.8 across all the treatments.

Each flushing (irrigation) supplied treatment solutions to the sand tanks for five minutes, which completely saturated the sand followed by time for the sand to drain to field capacity. The drained solutions returned to 612-L supply reservoirs, where they were held ready for the next flushing. The electrical conductivities of the irrigated solutions were checked initially, weekly, and at harvest, and assumed equal to the solutions in contact with the seed and roots (EC_{sol}). Water lost by evapotranspiration was replenished weekly or when necessary to maintain the concentrations of salts in solution. Soil solution electrical conductivities (EC_{sol}) in dS m^{-1} relate to equivalent electrical conductivity of saturated soil paste extracts (EC_e) in dS m^{-1} , as detailed in Ayers and Westcot (1985), by the approximate relationship: $\text{EC}_e \approx 0.5(\text{EC}_{\text{sol}})$

The test was conducted with an appropriate time course for day/night sequences (adjusted every four days) mimicking an April 27th seeding date at 50° north latitude. Supplemental lighting from 475-W sodium lamps positioned 1.5 m above the sand surfaces extend day-lengths. Lamps were strategically positioned overhead in order to obtain measured radiation intensities

averaging $7.9 \text{ kJ m}^{-2} \text{ min}^{-1}$ with a uniformity coefficient of 0.9 across the entire test facility. Temperature setpoints were automatically reset hourly according to a 24-hour diurnal schedule and ranged from 14 to 24°C with ambient temperatures maintained within one or two degrees of the setpoints.

The test plants representing the Fourth Test Group of ten Crucifer cultivars (Table 7) were evaluated in the “tank” set-up. Camelina and InVigor 9590 plants, each seeded 104 seed per tank in each of nine tanks, also served in a related experiment (reported under the Peer-Reviewed A-base Studies). The test plants representing the remaining eight cultivars in the Fourth Test Group (Table 7) were seeded in units of four cultivars (24 seed per cultivar) per tank per salinity level (three levels) replicated three-fold within 18 tanks.

The tank arrangements followed a randomized block design with respect to the test cultivars and salinity levels, modified slightly to eliminate any bias caused by the taller plants blocking solar radiation associated with low sun angles. In these tests, full complements of salts were added to the nutrient water supplies prior to seeding. Test crops were sown 13 mm deep into the sand in rows.

Measurements

Within each treatment, the response of the plants to root-zone salinity was determined by measuring emergence, plant height, plant growth stage, and oven-dried shoot biomass for the 3rd, 4th, and 5th Groups of the cultivars screened. Measurements were averaged and related to electrical conductivities of the test solutions (EC_{sol}) for each test crop. Grain yield could only be measured among the 4th Group of screened cultivars. The harvest index (grain yield divided by the total shoot plus grain yield) was also computed for each cultivar in the 4th Group.

Plant Emergence and Survival Two flushes with the test solutions preceded seeding in order to firm the seedbed, and a template guided placement of each seed into a known position within each seedbed. This allowed assessment of the plant emergence and survival associated for each planted seed on a daily basis. Any protrusion of the plant above the sand surface counted it as emerged. Records were kept on electronic copies of the seeding template. This practice resulted in daily counts per tank of the number of newly emerged plants and their survival with time. Data were being averaged within treatments with respect to seedbed and root-zone salinity. Where appropriate, an analysis-of-variance with comparisons among the three size groups is applied using least significant difference statistics (SAS 2007).

Plant Height and Growth Stage Plant height served to compare growth among the treatments and is determined from repeated weekly measurements of a selected number of plants per tank prior to harvest. Plant growth stage was also assessed according to a decimal code (Lancashire et al. 1991). The plant height data were analyzed with statistical tests applied to salinity and seed-size treatments (SAS 2007).

Shoot Biomass Shoot biomass was harvested during the growing season. Each cut of the shoot biomass was oven-dried at 39 °C, massed in grams, and collated by treatment. The yields from harvested tank-test plants provide comparisons among the ten test cultivars.

Results and Discussion

Seedling Growth

The negative effect of root-zone salinity on canola/mustard plant height was evident as early as 14 days after seeding (data not shown). This effect intensified with time in all cultivars as shown for the Third Test Group (Figure 6). Among each test species, Ace yellow mustard, SP 621 Argentine canola, and Forge oriental mustard stand out as the best in maintaining plant height at harvest as salinity increased. All plants in this group were harvested 73 days after seeding. Canola plants, which go through a growth stage of rapid height growth (bolting) often show considerable variation in plant height between the 60th and 80th days after seeding. This can affect plant height comparisons.

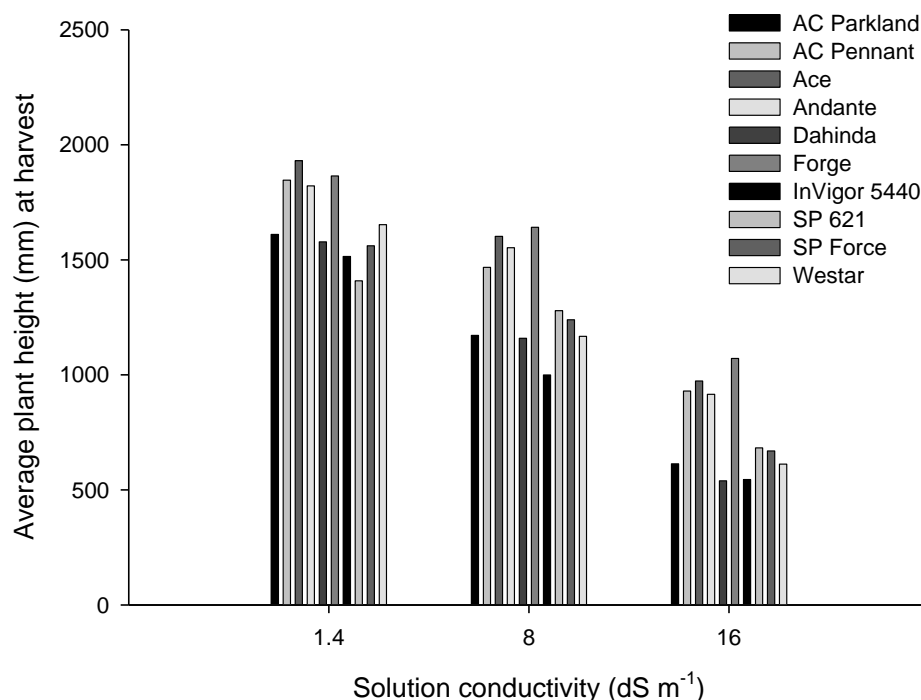


Figure 6. Average plant height (mm) of Crucifer plants in the Third Test Group at harvest for plants emerging and growing in seedbeds and root-zones solution conductivities of 1.4, 8, and 16 dS m⁻¹.

Growth Stage

A very slight but consistent retardation of growth stage in all plants of all cultivars growing in the moderate and severe salinity treatments occurred from emergence until the 28th day after seeding (data not shown). By the 28th day, mustard plant development among the 1.4 and 8 dS m⁻¹ treatments began to advance rapidly. The Polish type canola plants growing without excess salt seemed to be just entering rapid stage advancement. At that time, the Argentine type canola plants appeared to be about one week behind. By the 42nd day after seeding, the mustard and the Polish plants had reached the mid-flowering or anthesis stage, while the 8 dS m⁻¹ salinity plants were showing inflorescences. This was also about the same growth stage as the nutrient-only, Argentine type canola plants. The delay of growth caused by the root-zone salinity for all plants of all cultivars, especially the canola plants, became very evident by Day 57. At harvest, the 1.4 and 8 dS m⁻¹ plants of all cultivars were either well into or approaching inflorescence (Growth

Stage = 7.0). This also included the 16 dS m⁻¹ plants of the mustard. The rest of the plants in the severe salinity environment were still elongating and bolting.

Shoot Biomass

The salinity-free, Andante yellow mustard tended to produce more shoot biomass than any other Crucifer in the Third Test Group (Figure 8). Its non-statistical ranking decreased to number two as salinity increased to 8 dS m⁻¹. The average shoot biomass of Ace yellow mustard replaced Andante in top spot. Comparisons among all the Crucifer cultivars tested (Figures 7, 8 & 9) revealed a tendency for the negative effects of root-zone salinity on shoot biomass to decrease as the crop growth period increased. That is, as the Crucifer plants progressed toward maturity and the grain developed, the gradient of shoot biomass per dS m⁻¹ of root-zone salinity decreased.

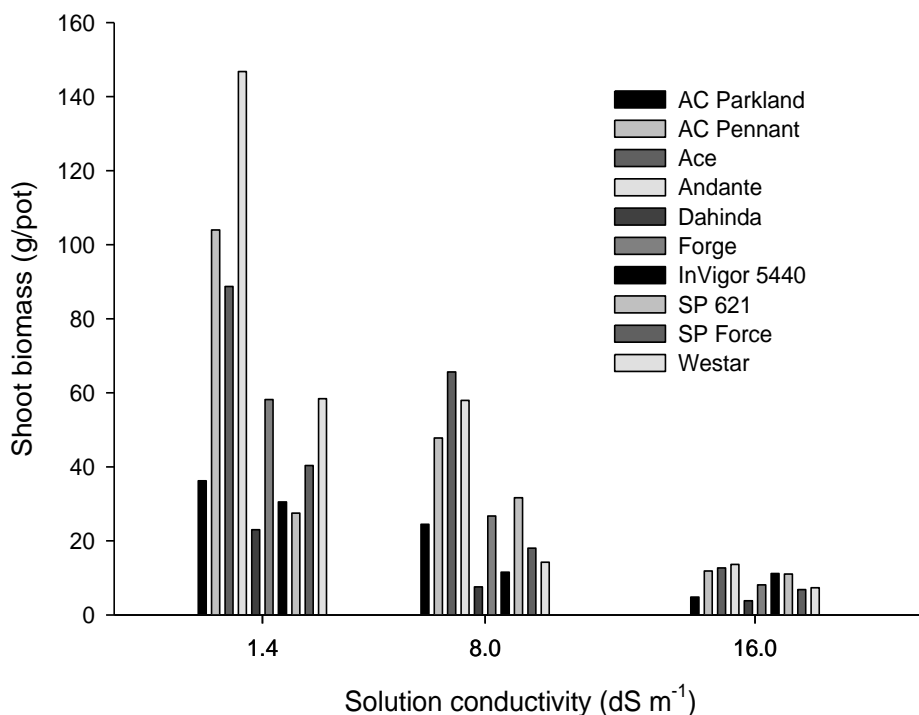


Figure 7. Average above-ground (shoot) biomass for canola plants in the Third Test Group harvested 73 days after seeding and growth while subjected to seedbed and root-zone target solution conductivities of 1.4, 8, and 16 dS m⁻¹.

Dividing the average shoot biomass grown under each salinity treatment (Figures 7, 8 & 9) by that produced in the negligible (1.4 dS m⁻¹) level of salinity resulted in relative shoot biomass values (Figures 10, 11 & 12). Based on the relative shoot biomass, the cultivars at 8 dS m⁻¹ which tended to maintain quantities of 80% or more of that grown within negligible salinity treatments (1.4 dS m⁻¹) included: InVigor 5020, SP 621, 45H73, 45H26, InVigor 5020, Banner, 46P50, BY 997, Duchess, InVigor 9590, and SW 6802. Under 16 dS m⁻¹ salinity, the cultivars

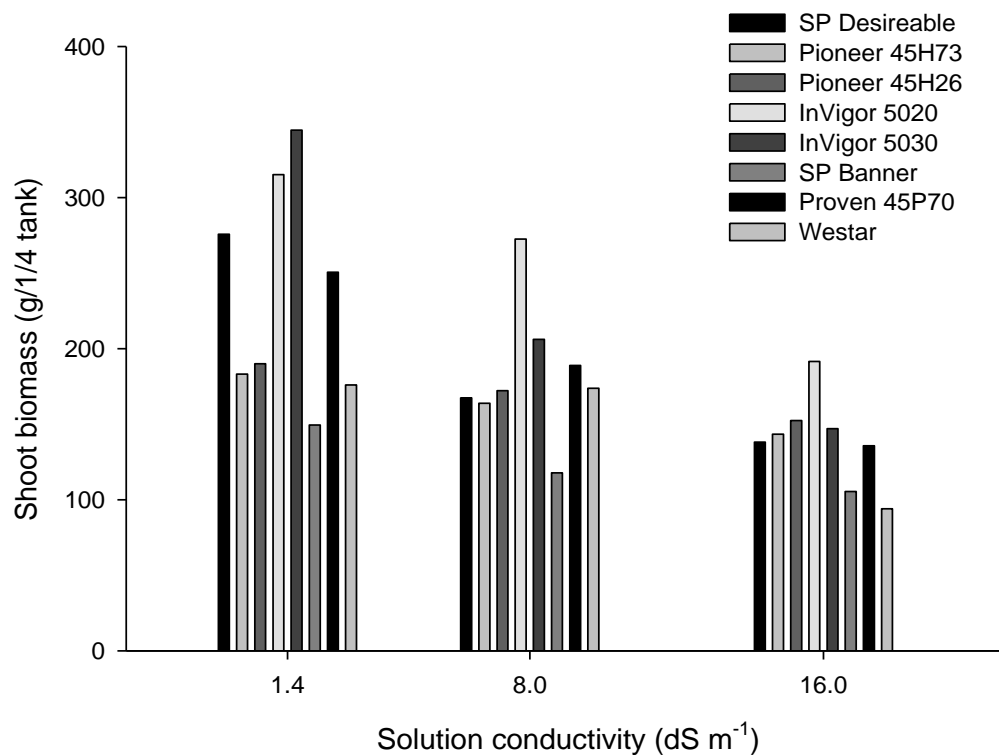


Figure 8. Average above-ground (shoot) biomass for canola plants in the Fourth Test Group harvested 116 days after seeding and growth while subjected to seedbed and root-zones solution conductivities of 1.4, 8, and 16 dS m^{-1} .

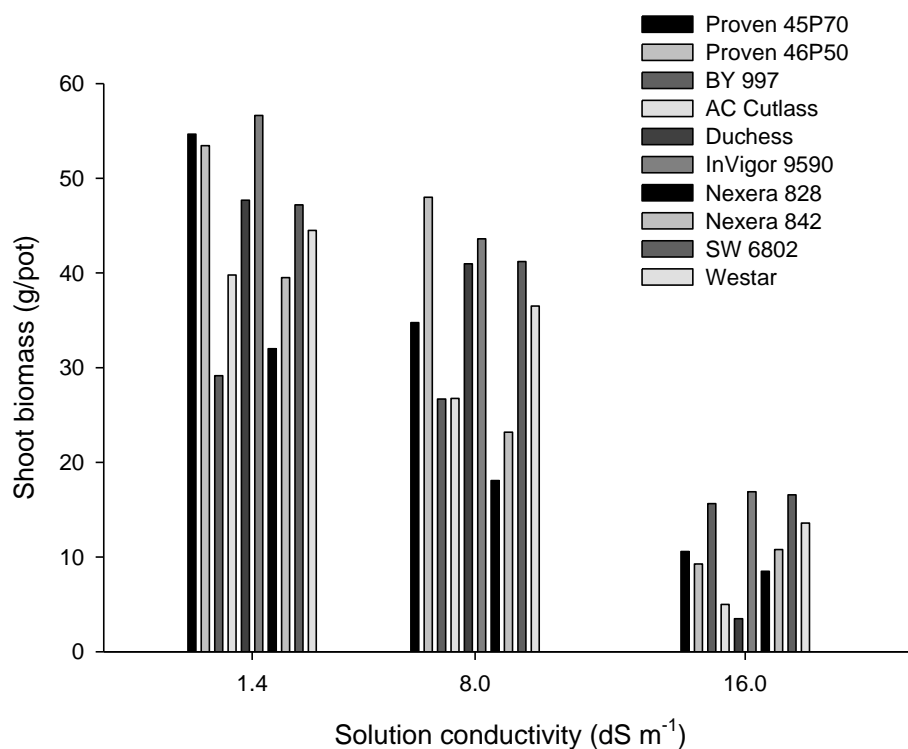


Figure 9. Average shoot biomass for canola plants in the 5th Test harvested 76 days after seeding and growth while subjected to root-zone solution conductivities of 1.4, 8, and 16 dS m^{-1} .

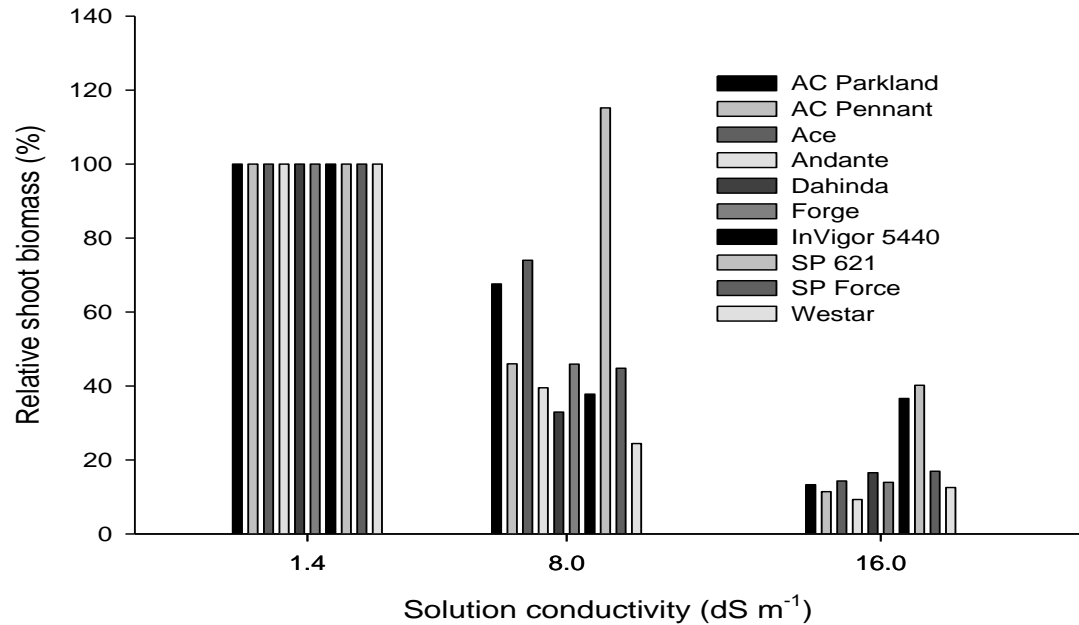


Figure 10. Average relative above-ground (shoot) biomass for canola plants in the Third Test Group harvested 73 days after seeding and growth while subjected to seedbed and root-zones solution conductivities of 1.4, 8, and 16 dS m⁻¹.

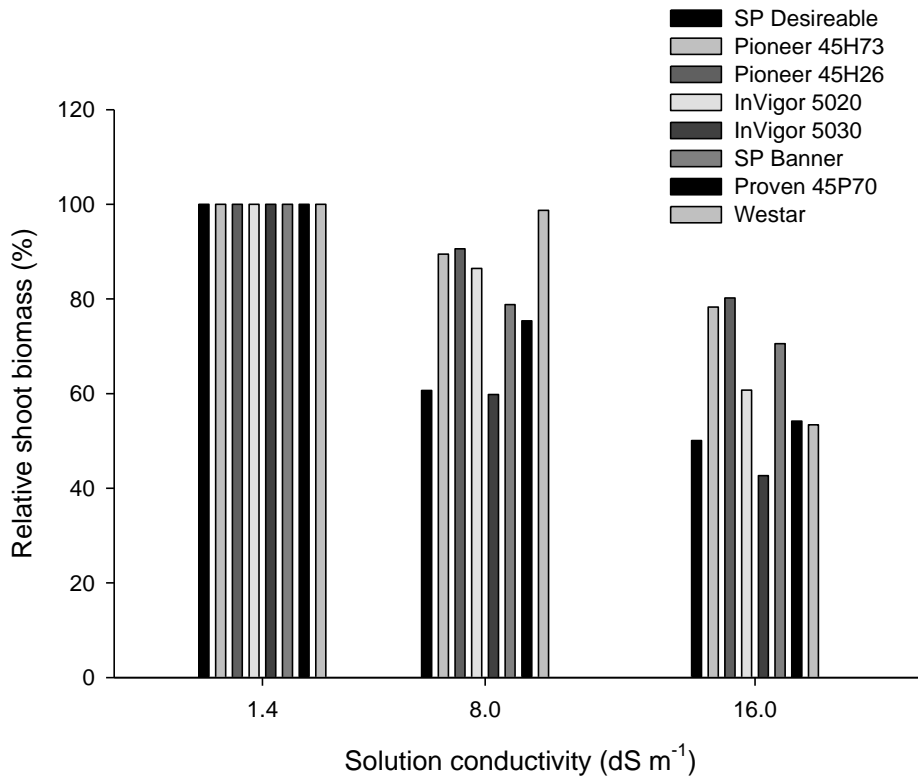


Figure 11. Average relative above-ground (shoot) biomass for canola plants in the Fourth Test Group harvested 116 days after seeding and growth while subjected to seedbed and root-zones solution conductivities of 1.4, 8, and 16 dS m⁻¹.

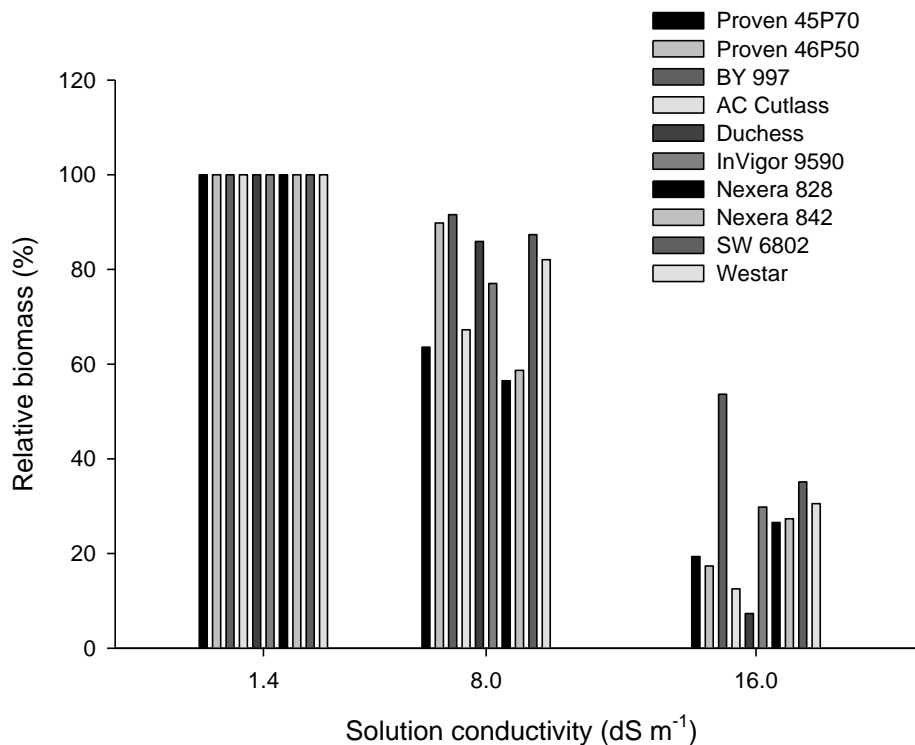


Figure 12. Average relative above-ground (shoot) biomass for canola plants in the Fifth Test Group harvested 76 days after seeding and growth while subjected to seedbed and root-zones solution conductivities of 1.4, 8, and 16 dS m⁻¹.

reaching 20% or greater of the negligible value were: InVigor 5440, SP 621, 45H73, 45H26, InVigor 4020, InVigor 5030, Banner, 45P70, 46P50, BY 997, InVigor 9590, Nex 828, Nex 842, and SW 6802.

Grain Yield

The absolute grain yield of the Fourth Test Group was evaluated for the test cultivars (Figure 13). Comparisons among the cultivars in this group showed the highest grain production resulting from the negligible salinity level belong to: InVigor 5020, InVigor 5030, 45P70, and SP Desirable. Of these cultivars, only InVigor 5020 maintained the same grain production under the 8 dS m⁻¹ salinity root zone. Under 16 dS m⁻¹, the InVigor 5020 plants retained two-thirds of their production for the negligible salinity mark; the other cultivar plants ranked noticeably less.

The range of salinity-tolerant cultivars widened when relative grain yields were compared (Figure 14). At 8 dS m⁻¹, relative yields matched those from respective 100% negligible salinity levels in 45H73, 45H26, and InVigor 5020; SP Banner retained 88%. At 16 dS m⁻¹, the same four cultivars retained 70% or more of their near-salt-free yields.

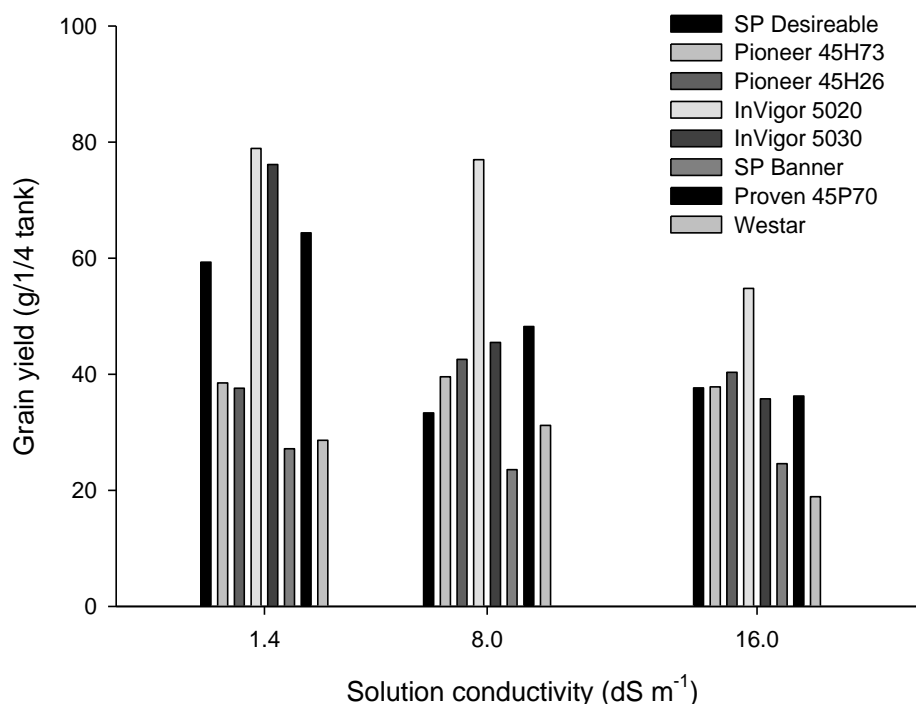


Figure 13. Average grain yield for canola in the 4th Test Group harvested 116 days after seeding and growth while subjected to root-zones solution conductivities of 1.4, 8, and 16 dS m⁻¹.

Harvest Index

The harvest indices computed for cultivar plants compared in the Fourth Test Group averaged (with \pm standard error) 0.212 ± 0.0112 under the 1.4 dS m^{-1} , 0.228 ± 0.0121 under the 8 dS m^{-1} , and 0.228 ± 0.0096 under the 16 dS m^{-1} salinity (Table 9). These averages show the usual trend of an increase in harvest index as the root-zone salinity increases. Possibly, salinity limits branching in Crucifer plants which would favour a positive increase in harvest index.

Among the cultivars in the Fourth Test Group, InVigor 5020 and Pioneer 45H73 produced the most grain per unit shoot biomass under each of the three salinity treatments (at 16 dS m^{-1} , SP Desirable was also among the most). The lowest harvest indices include those for Westar and SP Banner, with the remaining cultivars in the mid-rankings.

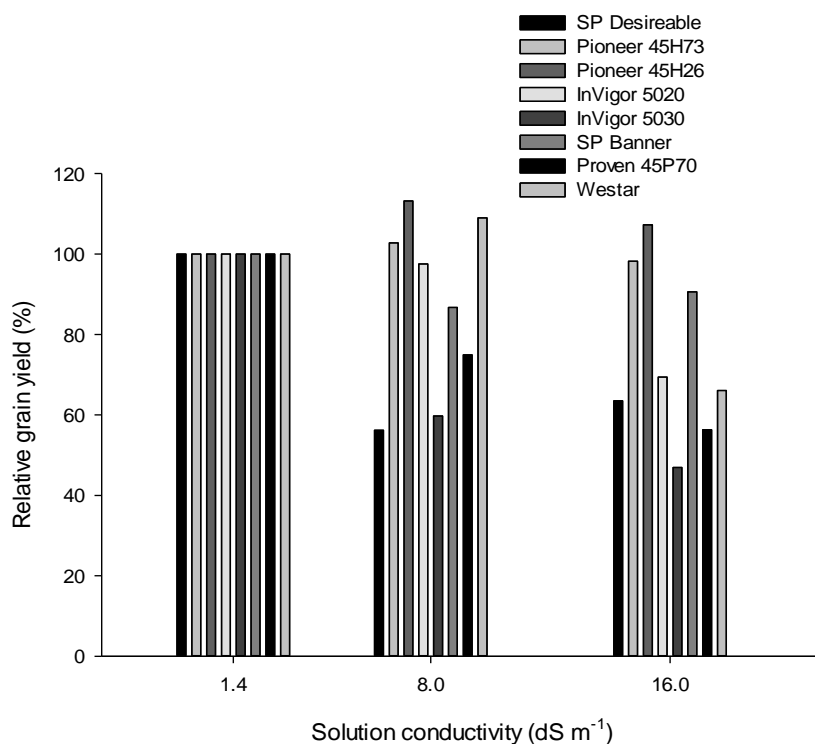


Figure 14. Average relative grain yield for canola in the 4th Test Group harvested 116 days after seeding and growth while subjected to root-zone solution conductivities of 1.4, 8, and 16 dS m⁻¹.

Table 9. Harvest indices [grain yield / (grain+shoot biomass yield)] arrayed in descending order by salinity treatment from plants representing eight Crucifer cultivars in the Fourth Test Group (cultivars listed in Table 7).

1.4 dS m ⁻¹		Target Salinity 8 dS m ⁻¹		16 dS m ⁻¹	
Cultivar	Harvest index	Cultivar	Harvest index	Cultivar	Harvest index
45P70	0.257	I5020	0.282	I5020	0.286
I5020	0.250	45P70	0.255	Desire	0.273
I5030	0.221	45H26	0.247	45P70	0.267
Desire	0.215	45H73	0.241	45H26	0.265
45H73	0.210	I5030	0.221	45H73	0.264
45H26	0.198	Banner	0.200	I5030	0.243
Banner	0.182	Desire	0.199	Banner	0.233
Westar	0.163	Westar	0.180	Westar	0.201
mean	0.212		0.228		0.254
se±	0.0112		0.0121		0.0096

se equals the standard error of the mean with N=8 cultivars

Comparative Salinity Tolerance

To compare the salinity tolerance based on shoot biomass production, the biomass yields in

the Third and Fifth Test Groups require upward adjustment because of the greater surface area in the tanks compared to that of the pots (approximately 3.5 times more). After this adjustment, the best Crucifer shoot biomasses under the 8 dS m⁻¹ treatment among all the test cultivars equalled 272 g for InVigor 5020 canola and 229 g for Ace yellow mustard. Under 16 dS m⁻¹, the best shoot biomasses reached 191 g for InVigor 5020 canola and 48 g for Andante yellow mustard.

Comparisons among the salinity treatments indicate that salinity tolerance differs among Crucifer cultivars, and many (SP Force, Pioneer 45H73, Pioneer 45H26, InVigor 5020, SW 6862, Proven 46P50, BY 997, AC Cutlass, Duchess, and Westar) maintained 80% of their salinity-free (1.4 dS m⁻¹) productivity as root-zone salinity reaches the moderate level (8 dS m⁻¹). As salinity increases to 16 dS m⁻¹, shoot production decreased in all the Crucifer cultivars tested. As salinity approached severe, only InVigor 5020, Pioneer 45H73, Pioneer 45H26, SP Banner, and BY 997 maintained 60% or more of their salinity-free productivity. Of these, under either the 8 or the 16 dS m⁻¹ treatment, the InVigor 5020 plants produced the greatest yield of shoot biomass and grain. In addition, the InVigor 5020 plants produced more absolute shoot biomass and grain than the plants representing any other Crucifer cultivar when grown subjected to nutrients only (1.4 dS m⁻¹). This trait is especially valuable for canola fields with a wide range in soil salinity ranging from negligible through severe.

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Goal III. Biodiesel Fuel Quality from Canola Feedstock Grown on Saline Land

Preview

With assistance from Alberta Agriculture, Food and Rural Development, Chinook Applied Research Association, and the Wheatland Conservation Area, producers at farms located in Alberta and Saskatchewan provided samples of canola oilseed feedstock grown in 2007 and 2008 in saline soils. Selected canola oilseed samples were processed and analyzed in an evaluation of biodiesel fuel quality resulting from salt-affected canola feedstock. The biodiesel feedstock was produced from canola crops grown across a wide range of saline soils. One aspect of this study was to determine if the Biodiesel Feedstock Quality test series outlined by the American Oil Chemists' Society (AOCS) can measure the most common aspects of feedstock quality before esterification to B100 from canola feedstock grown on saline land. This aspect was not completed. But, the percentage of oil recovered and the quality of the pure B100 biodiesel produced from Alberta-grown feedstock were evaluated. Most of the analytical and all of the biodiesel conversion research was conducted by the Biofuels Technology Centre of Olds College¹. A report, titled, "Comparing crude oil to biodiesel quality resulted from canola produced on salt-impacted soils" during 2009 and is available upon request by contacting steppuhn@agr.gc.ca.

ABSTRACT

The increased demand for biodiesel feedstock encourages producers to expand areas seeded to oilseed crops. Vegetable oil from canola-grade feedstock ranks among the best in the production of fatty acid methyl esters (FAME or biodiesel). FAME produced from canola-quality oilseed grown on salt-affected lands offer new opportunities for increased production and counter fuel-versus-food concerns provided the biodiesel product meets quality standards. The American Society for Testing and Materials (ASTM) has set the North American fuel quality standards (D6751) for 100% biodiesel (B100) to be blended with petrodiesel fuel. Canola-quality feedstock yield oil low in free fatty acids, acids which are not bonded to parent oil molecules. These free acids may negatively affect diesel engine components, especially at biodiesel oil blends greater than 20%. Also, solid and dissolved impurities, alkali/alkaline earth metals, and oxidation stability are of concern to fuel injection equipment manufacturers. Ultimately, purity, composition, and biodiesel utility depend on the quality of the feedstock supplied. Processing can improve purity, but not composition. Contaminants in biodiesel fuel may include water, sediment, S, P, K, Na, Ca, Mg, carbon residue, and various other constituents in its sulphated ash. Canterra 1818 canola feedstock grown on negligibly, slightly, moderately,

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and severely salinized soil were crushed and tested for biodiesel fuel quality. All samples yielded biofuel within the ASTM International specifications except for free glycerol in the negligibly-saline sample.

INTRODUCTION

The advancing demand for biodiesel feedstock encourages Canadian producers to increase the area seeded to canola-grade oilseed crops. Biodiesel production facilities operating and announced alone will likely require over a million tonnes of canola feedstock per year. A federal requirement for 2% biodiesel in diesel fuel by 2012 could generate a demand for 500 million additional litres of the biofuel per year (Canola Council of Canada 2007). The Council has set an annual production target of 15 million tonnes of canola by 2015 to meet this demand. Industry experts identify two major constraints to the development of the Canadian biodiesel industry: (1) assured supply of oilseed feedstock, and (2) maintenance of the quality of the fuel produced (Kemp 2006).

Oilseed supply

Selected varieties of canola (*Brassica napus* L.) tolerate root-zone salinity equally to that of Harrington barley (*Hordreum vulgatre* L.) (Steppuhn & Raney 2005). This knowledge can serve to encourage producers to introduce or increase canola cropping in the seven million hectares of cultivated lands across the Canadian Prairies identified by Wiebe et al. (2007) and Steppuhn (1996) as slightly and moderately-affected or at risk of being affected by salinity (Figure 1); these lands typically consist of many small-to-medium size areas scattered among negligibly saline areas within individual fields. By including a salinity-tolerant canola crop in rotation with barley or other forage crops in salt-affected fields, producers would gain new weed-control options, implement more efficient use of applied fertilizer, and contribute to biofuel feedstock supply without diminishing the production of food products. In addition, over three million ha of sodium-affected (solonetzic) lands might offer further canola cropping opportunities (Cairns and Bowser 1977). Together these 10 million ha of salt-affected lands represent a third of the total cultivated area across the Canadian Prairies and present opportunities to the biodiesel industry to grow fuel crops in environments detrimental to wheat and many other crops.

Salinity generally slows the rate of crop growth, resulting in plants with smaller leaves, shorter stature, and reduced economic yield (Shannon et al. 1994). The inherent ability of plant crops to withstand the effects of elevated solute concentrations of Na, Ca, Mg, SO₄, Cl, and other ions in root-zone solutions and still produce agricultural products defines salinity tolerance. The United States Salinity Laboratory Staff (1954) suggested that the electrical conductivity of water-saturated soil paste extracts (EC_e) provides the most consistent measure of root-zone salinity. They arbitrarily classified soils with EC_e<2 dS m⁻¹ as “non-saline,” 2≤EC_e<4 “slightly saline,” 4≤EC_e<8 “moderately saline,” and EC_e≥8 dS m⁻¹ as “severely saline.”

Biodiesel fuel quality

Canola oil purity, composition, and biodiesel fuel quality depend on the quality of the oilseed feedstock crushed. Processing can improve purity, but not composition. Also, blending oils from different feedstock grown under varying conditions may work to circumvent some deficits in composition. However, the best assurance for maintaining a uniform supply of biodiesel feedstock of consistently high canola-grade quality rests with knowledge of the biodiesel fuel quality expected from feedstock grown in salt-affected soils. This knowledge serves to identify production limits and contributes to cost-effectiveness and profitability.

According to Kemp (2006), oil from canola-grade feedstock ranks among the best in the production of fatty acid methyl esters (FAME or biodiesel). The American Society for Testing and Materials (ASTM International) has set the North American fuel quality standards (D6751) for 100% biodiesel content (B100). Canola-grade feedstock yield oil low in “free fatty acids,” acids which are not bonded to parent oil molecules. These free acids may negatively affect some diesel engine components, especially in blends greater than 20%. Also, solid and dissolved impurities, alkali/alkaline earth metals, and oxidation stability are of concern to the fuel injection equipment manufacturers.

The aim of this study is to evaluate canola biodiesel quality from feedstock grown in soil affected by sulphate salinity. Jia and Xu (2006) suggested that growing biodiesel feedstock on saline-alkaline lands would assist in maintaining non-saline lands for food production. But, they provided no examples of where this was currently being practiced, nor did they identify any evaluations of the biodiesel quality of the fuel produced from salt-affected soils. To our knowledge, neither the practice of growing biodiesel fuel in saline environments nor checks on the biodiesel quality of the resulting fuel have been reported. The preliminary study described herein was conducted to obtain insight into the approximate limits in root-zone salinity associated with biodiesel fuel produced from canola grown in sulphate salt-affected soil. The specific objectives of the study were to determine percent oil recovery and the standard quality of the pure B100 biodiesel fuels derived from canola grown in soils rated as negligible, slight, moderate, and severe with respect to root-zone salinity. These objectives identify feedstock production limits related to salinity with respect to biodiesel fuel quality.

MATERIALS AND METHODS

Canola feedstock test crop

In 2007, Mr. Ron Svanes grew a commercial canola crop (variety: Canterra 1818) in a salinity-affected field near Carmangay, Alberta. Using a surface electromagnetic induction propagator/sensor and a differential field geo-positioning system, Alberta Agriculture and Rural Development technical staff estimated and surveyed the root-zone salinity across the field following methods adopted from Wollenhaupt et al. (1986). According to the survey, the canola field contained saline soils classified as negligible ($EC_e < 2 \text{ dS m}^{-1}$), slight ($2 \leq EC_e < 4 \text{ dS m}^{-1}$), moderate ($4 \leq EC_e < 8 \text{ dS m}^{-1}$), and severe ($EC_e \geq 8 \text{ dS m}^{-1}$). Three replicate areas per salinity class were identified, marked, and harvested separately under guidance of the Alberta Agriculture and Rural Development technical specialists. Threshing was completed in the field, but precise grain

yields could not be obtained. Twelve oilseed samples (representing the four salinity classes each replicated from three field locations) were air-dried and kept in protected, non-heated storage first at Lethbridge, Alberta and later at Oyen, Alberta. In January of 2008, the samples were transported to Olds, Alberta, and again stored in a cool environment until they were processed.

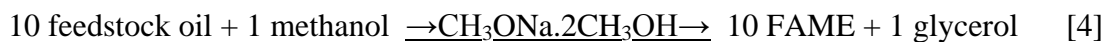
Feedstock crushing and processing

The new biodiesel pilot plant at Olds College in Alberta forms part of the School of Innovation Biofuel Technology Centre. Personnel at the Centre maintain the flexibility for processing multiple feedstock of small or large volumes. The canola oilseed feedstock samples from the test field at Carmangay were crushed and processed at this Centre. This facility features a five-tonne oilseed press, large oil filtering equipment, an appropriate oil pre-heater, a 400-L stainless steel batch reactor, a 400-L settling tank, a large capacity dry wash system, and both indoor and outdoor space for final fuel storage. However, the test samples were carefully processed and analyzed in the Centre's laboratory facilities utilizing bench-size equipment and processing. The feedstock samples representing each of the four saline conditions were obtained from three locations in the test field, weighed in equal amounts, and bulked into one sample per salinity class and crushed.

The four composite Carmangay canola oilseed samples, arrayed by field salinity levels, were measured for initial water content using a Sartorius MA100 Analyzer. The technique involved recording the change in mass with time while each sample was subjected to a temperature of 105°C. To increase crushing efficiency and make the crushed oil less viscous, each oilseed sample was tempered by adding sufficient water to reach approximately 8% in water content before crushing. Each oilseed sample was crushed using a Komet Seed Oil Extractor. The temperature of the oilseed was measured in the hopper as the samples moved into the press and again in the expressed oil leaving the extractor. Temperatures of both the oilseed and the oil were maintained below 55°C to prevent oil degradation. The crushed oil was cleansed using a Beckman Coulter J6-M1 centrifuge to remove the heavier solid particles remaining in the oil after the crushing process. The remaining oil was allowed to stand and further settle in order to remove any unwanted constituents prior to transesterification.

Transesterification, methyl ester B100 production

Transesterification and washing processes convert crushed canola oil to 100% biodiesel fuel (B100). The expressed canola oil is transformed into fatty acid methyl esters (FAME), the raw biodiesel fuel, by the addition of methanol in the presence of sodium methoxide, a catalyst (Kemp 2006). The result is a separable mixture of FAME and glycerol:



Separation of the glycerol from the FAME is accomplished by allowing the heavier glycerol to settle to the bottom of the reaction vessel and the fatty acid methyl esters to be decanted from the top. At this point, the raw biodiesel FAME contains various contaminants including methanol, catalyst reactants, glycerol, soap, gums, etc. Adding water to the raw biodiesel "washes" these contaminants from the raw FAME rendering a more refined B100 product.

Samples of FAME were produced from each Carmangay oilseed test sample over a period of three days. Each sample received the same percentage of catalyst and methanol (6 and 24% by volume of oil, respectively). All processes were completed at the Olds College Biofuel Technology Centre using bench-top hot plates and glassware. The test oils were measured and heated to 60°C in uncapped 1000L flasks with stir bars to remove any excess water prior to adding the catalyst and methanol. At 60°C, catalyst and methanol were added to the oil flask and covered with a foil cap. Temperatures were maintained above 60°C for 45 minutes and the mixture stirred vigorously during that time. Once the reaction was completed, the raw product was removed from the hot plate and the stir bar withdrawn. The mixture was then poured into a separatory flask to allow the glycerol to settle by gravity. The glycerol was removed and its volume recorded. The methyl ester FAME was gravity “washed” with de-ionised water while the flask was gently shaken and then left standing to allow the contents to settle. Four separate washings to remove excess methanol and impurities were completed. The final washed product was dried by re-heating to 60°C until all water dissipated. Lastly, vacuum filtration was applied to remove any excess soap, gums, or other impurities from the final B100 product.

Biodiesel fuel quality testing

A series of biodiesel fuel quality evaluations, following the American Society for Testing and Materials approved-protocols (ASTM International D6751-07), was employed to evaluate the merits of salinity-influenced canola oilseed feedstock:

ASTM D2500 Cloud point This is the temperature at which wax crystals begin forming as the FAME is cooled. Fuels which are operated below their cloud point are likely to cause filter plugging and subsequent fuel starvation of the engine.

ASTM D4530 Carbon residue (100% sample) A fuel sample is combusted and the remains constitute the carbon residue; excessive levels of glycerol are the likely cause of a test failure.

ASTM D5185 (EN 14538, European standard) Metals (sodium, potassium, calcium, & magnesium) Saline soil solutions commonly contain metal cations, such as Na^+ , K^+ , Ca^{++} , and Mg^{++} ; these tests indicate the degree of contamination from these ions in the biodiesel fuel produced from salt-affected soils.

ASTM D4951 Phosphorus This is a measure of contaminants resulting from the refining process for feedstock oils as well as from the use of phosphoric acid during the production process. Vegetable oil feedstock should have very low levels of phosphorous contamination.

ASTM D664A Acid number The acid number describes the free fatty acids in the FAME, which are known to lead to corrosion. Water in the fuel may be symptomatic of a high reading; the value is calculated by titrating a one-gram sample of FAME with a quantity of potassium hydroxide, measured in milligrams of the KOH base.

ASTM D130 Copper-strip corrosion Fuels which have high levels of free fatty acids will cause specially polished strips of copper to corrode when subjected to elevated temperatures; the degree of corrosion is compared to a series of reference strips to determine a pass/fail condition.

ASTM D93A Flash point FAME fuels are classified as non-flammable as their flash or ignition points (at atmospheric pressures) are above 130°C. This test is primarily a measure of residual alcohol which likely results from incomplete methanol recovery and/or washing of the

raw FAME. Methanol is highly toxic, flammable, and can be easily inhaled because of its low vapour pressure.

ASTM D6584 Free glycerol Free glycerol refers to suspended glycerol compounds that remain in the raw FAME as a result of improper washing. Excessive glycerol causes carbon deposits on fuel injection components, engine valves, valve seats, pistons, and rings, which leads to degraded engine performance and eventual engine failure. Free glycerol forms sludge in fuel storage tanks, resulting in plugged filters and engine starvation.

ASTM D6584 Total glycerol This is the sum of the free (suspended) glycerol and the bonded glycerol present in the mono-, di-, and tri-glycerides in the FAME; elevated levels of total glycerol result from incomplete reaction of the feedstock oils during transesterification and will compound the problems noted under “free glycerol.”

ASTM D445 Kinematic viscosity This is a measure of a fluid’s resistance to flow under gravitational forces. Highly viscous fluids will become less resistant to flow when heated.

ASTM D874 Sulphated ash This test indicates the quality of metallic residue left over from the catalyst used in the transesterification process. A sample of FAME is combusted and the residue is treated and massed to determine the residual non-combustible mineral ash.

ASTM D5453 Total sulphur The total sulphur test determines the amount of sulphur contained in the FAME. Reducing the sulphur content of all fuels reduces the quantity of sulphur compounds released to the lower atmosphere.

ASTM D2709 Water content (including that dissolved) Free or bonded water in FAME fuels will lead to the formation of free fatty acids and corrosion of engine and fuel storage tanks and will also promote microbial growth.

ASTM D2709 Sediment Sediment can plug fuel filters and, if the sediment is small enough to pass through filters, can abrade fuel injection and other high-tolerance engine components.

ASTM D1160 Vacuum distillation end point This is the temperature under conditions of reduced pressure at which 90% of the fuel sample will be distilled, allowing a determination of the makeup of the FAME.

Oxidation stability EN 14112 (European standard) FAME fuels are oxidized by atmospheric oxygen and therefore have a relatively limited storage life. Also, the oxidation products could damage vehicle engines. This is an accelerated oxidation test where a sample is held in a sealed reaction tube at a constant temperature of 110°C while a continuous flow of air is passed through the sample. With time, the air transports the oxidation products to a measuring vessel containing distilled water as an absorption solution. An increase in electrical conductivity of this water indicates the presence of secondary oxidation products (mainly formic and acetic acids) signalling a loss of stability. The time it takes for the conductivity to increase quantifies the oxidation stability of the fuel. The above tests were conducted by Maxxam Analytics, Inc. in Edmonton, Alberta.

The **cetane number (ASTM D6890)** test was performed by the Saskatchewan Research Council Biofuels Testing Centre in Regina, Saskatchewan. The cetane number is a direct indication of the ignitability of the fuel. A measured quantity of the test fuel powers a specially developed compression ignition engine or instrumented simulation apparatus. The fuel’s performance is compared to calibrated standards measured according to reference values or numbers.

Because of fuel quality testing costs, the procedure following oil extraction and B100 biodiesel production in this study was to array the final biodiesel products into two groups in preparation for quality tests. The B100 biodiesel produced from the negligible and severe salinity feedstock were submitted separately for analyses to which the complete series of 18 ASTM D6751-07 evaluations were applied. The B100 products from the slight and moderate salinity feedstock were submitted for only seven of the 18 evaluations; these seven were selected as those most appropriate and important for salinity-related oilseed and biofuel production: sulphated ash, sulphur content, phosphorus content, cloud point, micro-carbon residue, and metal cations: (Na + K) and (Ca + Mg).

RESULTS

Canola oil recovery

The initial water content of the four test oilseed samples from feedstock grown in soil classified as negligibly, slightly, moderately, and severely saline, ranged from 3.3 to 4.4% (Table 2). De-ionized water was added to each sample bringing the water percentages to $8\pm1\%$. The oilseed samples were then crushed to obtain canola oil and meal. The temperature of the oilseed feedstock when entering the crusher-extractor measured within 5°C ($41 - 46^{\circ}\text{C}$) for all the samples (Table 2). The average temperature of the expressed canola oil ranged from 40 through 47°C . At these temperatures, degradation of the oil would assuredly have been lessened.

The initial mass of each test oilseed sample measured 5.65 ± 0.05 g just before entering the crusher/extractor (Table 3). Upon exiting the crusher, the raw canola oil was massed and its recovery calculated as a percent of the initial seed mass (Table 3). The oil recovered from severe-salinity feedstock measured somewhat less than the other three. Based on the centrifuged oil, recovery equalled 30.5, 33.4, 34.9, and 34.8% for the severe, moderate, slight, and negligible salinity test stock, respectively. The salinity-grown oil samples required substantially more settling and centrifugation than other canola oil samples previously processed at Olds College.

Fuel quality tests

Transesterification was repeated with three separate sets of the oilseed feedstock grown on the salt-affected soils (Table 4). The sets involved either (1) different quantities of input constituents (but with the Eq. 4 proportions maintained) in the esterification and washing processes, or (2) selected repetition. The biodiesel B100 produced was bright orange in color rather than golden, and the glycerol was green, rather than dark brown.

The ASTM D6751-07 series involved some 18 tests or evaluations (Table 5). The severely and negligibly salinity-influenced B100 fuels were each evaluated in all 18 tests. No significant differences in any of the evaluations were detected between these two test fuels, except for the free glycerol content (0.058% for the negligible and 0.017% for the severe). Except for the free glycerol measured in the negligibly saline sample (0.038% above the ASTM specification), both fuels met all of the D6751-07 specifications classifying both as acceptable in B100 quality. The elevated free glycerol percentage was likely caused by incomplete washing. The moderately and

slightly salinity-influenced B100 fuel was subjected to only seven of the 18 evaluations. In these, the results neither deviated significantly nor trended differently among the fuels for the three samples from feedstock grown in salinity-affected environments; the test values all fell within the acceptable D6751-07 specifications for North American B100 biodiesel fuel (Table 5).

DISCUSSION

Assurance of biodiesel quality was achieved by testing selected properties and characteristics of the B100 product fuel. In Canada, the American Society for Testing and Materials (ASTM International) D6751-07 is the quality standard applied to biodiesel (B100) used in a blend with petrodiesel. All the B100 biodiesel FAME produced from saline soil samples conformed to the ASTM specifications in every test performed, indicating that the chemical reaction was complete and that the level of salinity in the environment where the canola feedstock was produced did not adversely impact the quality of biodiesel product derived from the raw canola oil. The importance of this finding rests with the indication that biodiesel canola fuel produced from saline soil can meet the North American standard for acceptable quality. This implies that canola grown on saline land will yield market-grade biodiesel fuel.

The results presented herein are only preliminary. Only one canola crop from one field was sampled. Furthermore, the limited funds available for this study prohibited a full-scale evaluation of all the D6751-07 evaluations in all the treatment samples. Insufficient funding also limited the number of replicate samples that could be tested. This restricted the application of statistical analyses.

The results also indicate that oil recovery for Canterra 1818 canola suffered a 13% decline when the feedstock was grown in severely saline soil. Oil quantities recovered from this feedstock grown in moderately and slightly saline soil appeared not to have declined in comparison to crops grown on negligibly-saline soils. This agrees with the earlier results obtained by Steppuhn and Raney (2005) with InVigor 2573 and Hyola 401 canola.

CONCLUSIONS

Four oilseed feedstock samples from a 2007 canola field near Carmangay, Alberta representing soil root zones rated as negligibly, slightly, moderately and severely salinized were pressed to recover oil for biodiesel production. Oil recovery was favourable (within 31-36%) for all samples. Oilseed grown under conditions of the greatest salinity had the lowest oil recovery (31%). The raw oils were successfully converted to a B100 biofuel, but the appearance of the products was different from previous extractions. The biodiesel fuel was bright orange not golden in color and the glycerol produced was green rather than brown. The reasons for the color variations are unclear. The quality of the biodiesel produced from all four oilseed samples was consistently within the ASTM International D6751-07 specifications (except for excessive free glycerol (0.058 compared to 0.02%) in the fuel produced from the negligibly-saline soil), indicating that there was no reduction in quality associated with canola feedstock grown in saline

environments. This finding suggests the acceptance of the expanded use of saline lands for biofuel production.

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Table 1. Salinity Tolerance Indices and associated parameters derived from nonlinear response functions for selected Canadian agricultural crops^[1]. (Taken from Steppuhn et al. 2003)

Crop ^[2]		Tolerance based on	Tolerance parameter ^[3]			References
Common name	Botanical name		C ₅₀ dS m ⁻¹	s steep- ness	Salinity tolerance index	
Barley ^{[4][5]} (dryland)	<i>Hordeum vulgare</i> L.	Grain yield	7.51	0.104	8.29	Steppuhn, 1993
Canola ^[5] (dryland)	<i>Brassica napus</i> L.	Seed yield	7.10	0.126	8.00	Steppuhn & Raney, 2005
Corn	<i>Zea mays</i> L.	Ear FW	5.54	0.183	6.56	Bernstein & Ayers, 1949 (p. 41-42); Kaddah & Ghowail, 1964
Flax	<i>Linum usitatissimum</i> L.	Seed yield	5.54	0.183	6.56	Hayward & Spurr, 1944
Sugar beet ^[6]	<i>Beta vulgaris</i> L.	Storage root	15.04	0.090	16.39	Bower et al., 1954
Wheat, leavened bread (irrigated)	<i>Triticum aestivum</i> L.	Grain yield	5.85	0.242	7.89	USSR ^[7] , 1979
Wheat, leavened bread ^[5] (dryland)	<i>T. aestivum</i> L.	Grain yield	2.76	0.186	3.27	Steppuhn and Wall, 1997
Wheat, flat bread ^[5]	<i>T. aestivum</i> L.	Grain yield	2.97	0.273	3.78	Steppuhn and Wall, 1997
Wheat, Durum ^[5]	<i>T. Turgidum</i> L. Desf.	Grain yield	5.36	0.243	6.66	Steppuhn and Wall, 1997
Wheat, pastry ^[5]	<i>T. aestivum</i> L.	Grain yield	6.06	0.214	7.35	Steppuhn and Wall, 1997
Alfalfa	<i>Medicago sativa</i> L.	Shoot DW	8.49	0.111	9.43	Bernstein & Francois, 1973; Bernstein & Ogata, 1966, Bower et al., 1969; Brown & Hayward, 1956; Gauch & Magistad, 1943; Hoffman et al., 1975
Alfalfa ^[5]	<i>Medicago sativa</i> L.	Shoot DW	6.20	0.095	6.79	Steppuhn et al., 1999
Fescue, tall ^[5] (dryland)	<i>Festuca arundinacea</i> Schreber	Shoot DW	7.97	0.083	8.63	Steppuhn, 1997

Table 1. Continued

Common name	Botanical name	Tolerance ^[1] based on	C ₅₀ dS m ⁻¹	s steep- ness	Salinity tolerance index	References
Ryegrass, perennial	<i>Lolium perenne</i> L.	Shoot DW	11.78	0.116	13.14	Brown & Bernstein, 1953 (p.44-46)
Wheatgrass, green ^[5]	<i>Elymus hoffmannii</i> Jensen & Asay	Shoot DW	11.80	0.095	12.92	Steppuhn & Asay 2005
Wheatgrass, intermediate ^[5]	<i>Thinopyrum intermedium</i> (Host) Bark. & Dewey	Shoot DW	7.72	0.100	8.49	Steppuhn, 1997
Wheatgrass, slender ^[5]	<i>Elymus trachycaulus</i> (Link) Bark. & Dewey	Shoot DW	7.16	0.095	7.84	Steppuhn, 1997
Wheatgrass, tall	<i>Thinopyrum ponticum</i> (Podp.) Barkworth & Dewey	Shoot DW	18.92	0.065	20.13	Bernstein & Ford, 1958 (p. 32-36)
Wildrye, beardless	<i>Elymus triticoides</i> Buckl.	Shoot DW	10.65	0.091	11.62	Brown & Bernstein, 1953

FW = fresh weight; DW = dry weight.

[1] Table based on Table 3-1, Maas and Grattan, 1999, and controlled tests of crop-yield response to increasing root-zone salinity gradually-applied to seedling plants.

[2] Botanical and common names follow the convention of Hortus Third (Liberty Hyde Bailey Hortorium Staff, 1976) where possible.

[3] In gypsiferous soils, plants will tolerate about 5-10% greater salinity than indicated.

[4] Less tolerant during seedling stage, EC_e at this stage should not exceed 4 or 5 dS/m.

[5] These data are based on tests following dryland agricultural practices, where seeds are planted directly in saline seedbeds.

[6] Sensitive during germination and emergence, EC_e should not exceed 3 dS/m.

[7] Unpublished U.S. Salinity Laboratory data.

Table 2. Water content and temperature of oilseed and raw canola oil during crushing associated with feedstock grown in soil of the indicated salinity.

Salinity	Water content		Temperature	
	Initial	Tempered [*]	Seed	Oil
	(%)	(%)	(°C)	(°C)
Severe	4.26	8.44	41-46	42-47
Moderate	4.20	7.42	42-46	40-47
Slight	4.02	7.02	42-46	39-47
Negligible	3.32	8.26	43-46	39-47

^{*} “Tempered” refers to the addition of water to the oil to increase crushing efficiency.

Table 3. Mass of the crushed oil and meal and the percent oil recovered from canola feedstock grown in soil of the indicated salinity.

Salinity	Mass				
	Initial seed	Oil	Meal	Oil recovered	Centrifuged oil
	(g)	(g)	(g)	(%)	(g)
Severe	5.70	1.78	3.91	31.3	1.74
Moderate	5.65	1.94	3.71	34.4	1.89
Slight	5.65	2.02	3.63	35.8	1.97
Negligible	5.60	1.99	3.61	36.1	1.95

Table 4. Inputs and results for the reactions and selected component volumes for three sets of salinity-influenced canola feedstock.

Set	Salinity	Raw oil	Catalyst	Methanol	Glycerol	Gums visible	Glycerol	Wash water
		(ml)	(ml)	(ml)	(ml)		(%)	(ml)
1	Severe	300	18	72	42	No	14	500
1	Moderate	300	18	72	70	Yes	23	500
1	Slight	300	18	72	37	No	12	500
1	Negligible	300	18	72	51	Yes	17	500
2	Severe	500	30	120	69	Yes	14	800
2	Moderate	500	30	120	75	Yes	15	800
2	Slight	500	30	120	73	Yes	15	800
2	Negligible	500	30	120	80	No	16	800
3	Severe A	500	30	120	71	No	14	800
3	Severe B	500	30	120	78	Yes	16	800
3	Negligible A	500	30	120	82	Yes	16	800
3	Negligible B	500	30	120	69	Yes	14	800

Table 5. Comparisons of ASTM D7651 test results to ASTM specifications for the salinity-related B100 biodiesel samples.

Test variable	ASTM method	Salinity				Units	ASTM D6751 specification
		Severe	Moderate	Slight	Neg. [*]		
Flash point	D93A	138.0	NT ^{**}	NT	138.0	°C	130 min
Acid number	D664A	0.03	NT	NT	0.03	mgKOH/g	0.5 max
Cloud point	D2500	-3	-3	-4	-4	°C	
Water & sediment	D2709	0.0080	NT	NT	0.0050	% vol	0.050 max
Free glycerol	D6584	0.017	NT	NT	0.058	% Mass	0.020
Total glycerol	D6584	0.097	NT	NT	0.13	% Mass	0.240
Sulphated ash	D874	<0.001	<0.001	<0.001	0.001	% Mass	0.020
Total sulphur	D5453	2.5	1.9	1.5	2.2	ppm	15
Copper strip corrosion	D130	1A	NT	NT	1A	No.	3A max
Cetane number	D6890	56.7	NT	NT	56.3	No.	47 min
Carbon residue	D4530	<0.010	<0.010	<0.010	<0.010	% Mass	0.050 max
Kinematic viscosity	D445	4.492	NT	NT	4.548	Mm ² /sec	1.9-6.0
Oxidation stability	EN14112	5.5	NT	NT	4.8	hours	3 hours min
Phosphorus	D4951	<0.5	<0.5	<0.5	<0.5	mg/L	10
Sodium + Potassium	D5185	<1	<1	<1	<1	mg/L	5 ppm
Calcium + Magnesium	D5185	<1	<1	<1	<1	mg/L	5 ppm
Absolute density @ 15°C	D4052	881.8	NT	NT	881.9	kg/m ³	
Distillation End Point Temp. (90% recovery)	D1160	349	NT	NT	359	°C	360 Max

* Neg. = Negligible salinity

** NT = Not tested

Risk for Soil Salinity in Prairie Landscapes According to Land Use in the 1996 Census

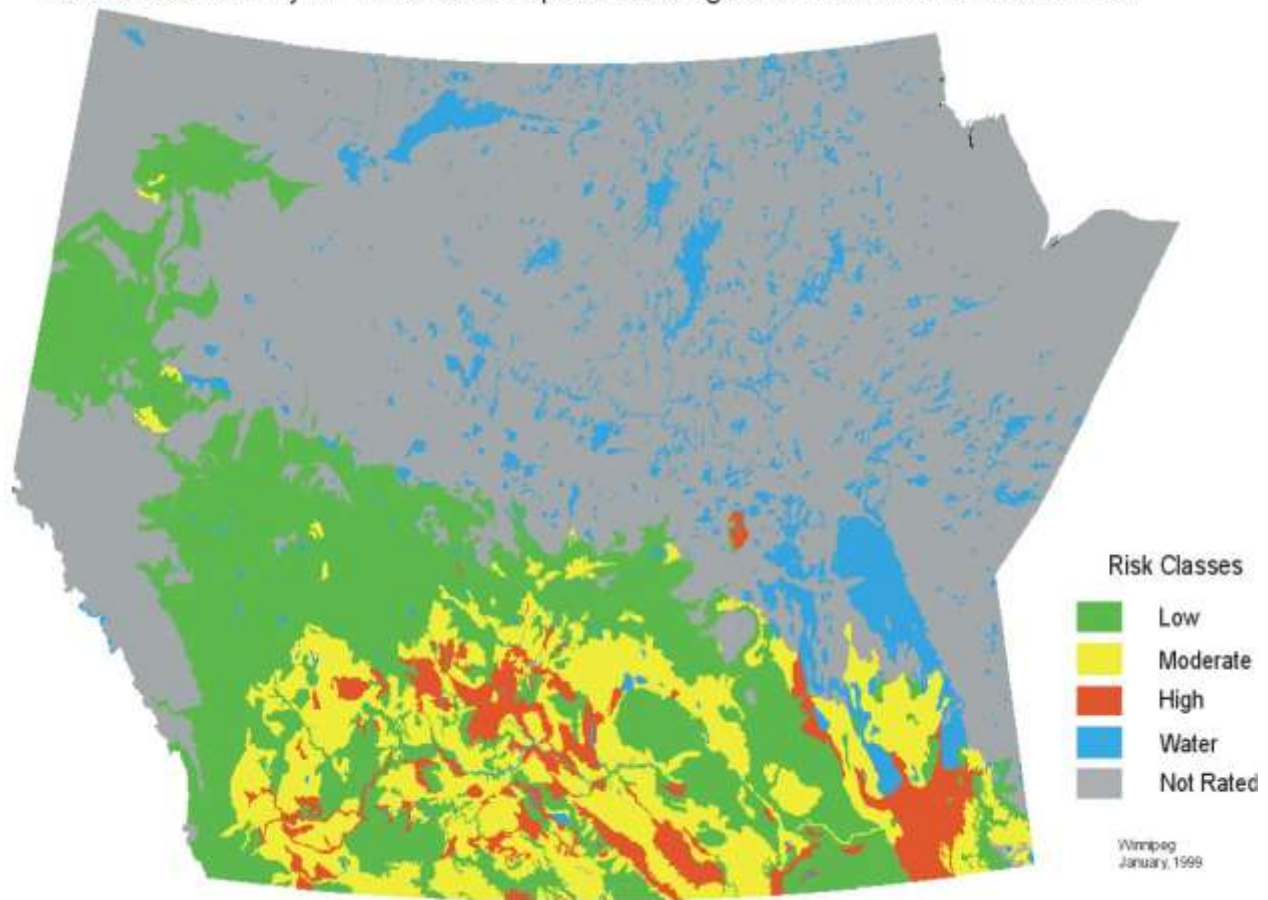


Figure 1. View of the Canadian Prairies and the 1996 soil salinity risk evaluation based on factors which include the existence of root-zone salts (Taken from Wiebe et al. 2007).

Scientific Papers, Presentations, and Technology Transfers During the Study

Scientific Papers

Steppuhn, H., Falk, K.C. and Zhou, R. 2010. Emergence, height, and yield of camelina and canola grown in saline root zones. *Can. J. Soil Sci.* 90(1): 151-164.

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Proceedings and Presentations

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Steppuhn, H., McDonald, T., Falk, K.C., Stumborg, M.A. and Marianchuk, M. 2009. Biodiesel fuel quality of canola feedstock grown on saline land. *In Proceedings, Soils and Crops Workshop, Oral presentation, CD-ROM, Univ. of Sask., Saskatoon, SK.*

Steppuhn, H., Stumborg, M.A., McDonald, T. and Dunn, R. 2009. Biodiesel fuel quality of canola feedstock grown on saline land. *In Proceedings, BioEnergy Engineering '09 Conf., (Poster), Paper No. Bio-097910, Oct. 11-14, Bellevue, WA.*

Steppuhn, H., Stumborg, M.A., McDonald, T. and Dunn, R. 2009. Fuel quality of biodiesel produced from canola feedstock grown on saline land. *Oral Presentation, Canola Science Summit, Mar. 17-19, Saskatoon, SK*

Steppuhn, H., Wall, K.G., Falk, K.C., Zhou, R. and Brandt, S.K. 2009. How well does camelina tolerate root zone salinity. *In Proceedings, Soils and Crops Workshop, Poster, CD-ROM, Univ. of Sask., Saskatoon, SK.*

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Steppuhn, H. 2010. What's new in salinity control? *In* Preprint, Outlook Workshop for Soils and Crops Specialists, Saskatchewan Ministry of Agriculture, 21 Oct 2010, Outlook, SK

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Table 4. Mean concentrations of fatty acids determined from harvested InVigor 9590 canola (B) and CS15 camelina (C) oilseed crops grown in root zones arrayed by seven salinity levels (EC_{sol})^z; presented as percentages of the total percent by mass

Fatty acid concentration with carbon chain length										
EC_{sol} ^z	Crop ^y	Palmitic C16:0	Stearic C18:0	Oleic C18:1	Linoleic C18:2	∇-Linolenic C18:3	Arachidic C20:0	11-Eico- senoicnic C20:1	11,14Eico senoicnic C20:2	Erucic C22:1
dS m ⁻¹		----- % -----								
1.36	B	3.9	2.2	66.5	15.1	7.5	0.91	1.5	0.07	0.04
1.36	C	5.8	2.8	10.2	17.5	35.0	2.32	14.7	2.21	4.25
2.98	B	3.9	2.3	66.8	14.8	7.5	0.94	1.5	0.07	0.04
2.98	C	5.8	2.7	10.4	17.3	35.4	2.16	15.6	2.20	4.23
6.05	B	3.9	2.2	67.7	14.3	7.3	0.90	1.5	0.07	0.04
6.05	C	5.9	2.8	10.3	17.8	34.7	2.25	14.5	2.20	4.36
10.00	B	4.1	2.2	66.5	14.8	7.7	0.93	1.5	0.08	0.04
10.00	C	5.9	2.7	10.5	17.6	34.8	2.13	14.8	2.24	4.23
14.67	B	4.1	2.2	67.2	14.6	7.1	0.95	1.5	0.07	0.04
14.67	C	6.2	3.0	10.0	19.1	32.9	2.41	14.6	2.22	4.43
19.92	B	4.0	2.4	68.6	13.7	6.3	1.02	1.5	0.08	0.04
19.92	C ^x	---	---	---	---	---	---	---	---	---
27.02	B	4.6	2.7	63.3	16.8	6.9	1.15	1.5	0.09	0.04
27.02	C ^x	---	---	---	---	---	---	---	---	---

^z EC_{sol} equals the average electrical conductivity of the test solution.

^y B stands for InVigor 9590 canola (*Brassica*), and C stands for CS15 camelina (*Camelina*).

^x Insufficient oilseed sample for analysis.