Activity 15
Effects of Plant Population on Stress Tolerance and Seed Quality of Spring Canola
Annual Report – March 31, 2016

Overall Objective

The overall objective of this activity is to:

1. Determine, in a series of field trials, how reduced plant populations affect physiological responses of spring canola to abiotic stresses – primarily heat stress and water stress – in the Ontario growing environment.
2. Quantify effects of canola plant population on seed quality (primarily free fatty acid content).

Audience

The audience is crop producers, specifically those who grow or who have an interest in growing canola.

Performance Measures

New/Improved Practices

This activity resulted in two new/improved practices:

1. Effects of seeding rate on Ontario spring canola yields. Despite the very high and increasing yield potential under Ontario growing conditions (exceeding 5 Mg / ha in some field trials), we found no evidence that seeding rates below those commonly used in the province are beneficial. Under low-yielding conditions, slightly higher seeding rates may be warranted. 100 viable seeds per m^2 remains a suitable target for this region.
2. Effect of seeding rate on canola abiotic stress tolerance. We found only mild evidence to support the hypothesis that the larger, more highly branched plants that develop at low seeding rates may have some enhanced tolerance to stresses such as transient soil water deficits. But any such advantage was typically small and probably not worth the risk of increasing green and brown seed counts at low populations.

Improved knowledge

An understanding of seeding rate effects on plant morphology and pod distribution under ultra-high-yielding conditions in spring canola was developed. We found that under ultra high yielding conditions, pod numbers (and seed yield) on main racemes was "saturated", and all additional yield derived from increased numbers of branch racemes and increased numbers of pods on branch racemes. Additionally, some field yields were approaching the maximum yield observed under controlled environment conditions. This raises the question of whether future yields might ultimately be limited by genetic yield potential of available varieties.

Information Items

During the activity, four presentations/information items were completed:

1. MSc Thesis, K. Meghan Griffiths. *Agronomic Practices to Reduce the Effects of Environmental Stresses on Spring Canola (Brassica napus L.) Establishment and Yield in Ontario*, August 2014
2. Poster 2015 ASA Meetings. *Exploring the Yield Potential of Spring Canola: Border Effects in a Controlled Environment Study*. Hugh J. Earl, K. Meagan Griffiths and Joshua Burrows
3. Presentation at the Canola Industry Meetings, Dec. 3 2014, Saskatoon. H.J. Earl and B. Hall. *Ontario Canola Report, 2014.*
4. Presentation to the Ontario Soybean and Canola Committee Research Meetings, Jan. 22 2015, London ON. *Seeding Rates, Stress Tolerance and Yield Potential of Spring Canola.* Hugh J. Earl.

Highlights

Spring canola yields are generally very insensitive to seeding rates, because canola plants are able to become highly branched and thereby make use of all of the available space and resources even at low plant populations. Thus, it is possible to produce similar yields either with large numbers of smaller plants, or with fewer, larger plants per unit ground area. However, there is reason to believe that plant population may affect crop stress tolerance and yield potential under certain conditions. For example, larger individual plants may also have more extensive root systems, allowing them to access soil water at deeper layers of the profile. Also, as seeding rates are reduced, plants become more highly branched and more of the total yield is borne on the branch racemes rather than the main raceme. Because branches are delayed in development relative to main stems, larger, more highly branched plants have a longer flowering period which may make them more resilient to acute stresses that occur during the critical flowering and early pod establishment stages. On the other hand, the delayed development of branches relative to main racemes may make crop maturity less synchronous at lower seeding rates, complicating harvest timing and leading to shattering losses and a higher incidence of immature (green) seed. Also, some previous reports indicate the “brown seed” (seed high in free fatty acids, an anti-quality factor) may be more abundant on branch racemes.

We carried out a series of field trials over three seasons (2013 to 2015) to test the effects of seeding rate on yield, seed quality and stress tolerance of spring canola in Ontario. The seeding rates were a low rate (50 seeds m^{-2}), a typical rate (100 m^{-2}) and a high rate (200 m^{-2}). At one location in two years, seeding rates were tested in combination with rain-fed and irrigated treatments, to test the effects of seeding rate in the presence and absence of water stress.

Yields in the six successful field trials ranged from low (1.93 Mg ha^{-1}) to extremely high (5.55 Mg ha^{-1}). Seeding rate had the expected effects on plant height (shorter plants at higher seeding rates) and seed distribution (a much greater fraction of total yield was borne on the main racemes at higher seeding rates). However, despite the hypothesized potential benefits of low plant populations, we never observed a case where the low seeding rate out-yielded the normal rate. A higher than normal seeding rate was sometimes beneficial when yield potential was generally low; under high yielding conditions, yield was generally insensitive to seeding rate, and in one high-yielding trial the high rate yielded significantly lower than the normal rate.

Seed quality was generally good in these trials, but there was one case where the low seeding rate increased brown seed, and two cases where it increased green seed, both consistent with past reports.

A greenhouse experiment designed to maximize the potential benefit of a low seeding rate failed to convincingly demonstrate that a reduced plant population could enhance tolerance to a soil water deficit. Similarly, measurement of volumetric soil water content in the field failed to provide evidence that a reduced seeding rate would enhance the crop's ability to access soil water deep in the profile. In combination, the field and greenhouse experiments provided no evidence to argue for seeding rates below those currently commonly used in Ontario.

The greenhouse experiments and one field trial in 2014 did provide the opportunity to monitor plant morphology and seed distribution of a commercial spring canola hybrid under ultra-high-yielding conditions. It was demonstrated that under these conditions pod numbers of the main raceme are "saturated" and additional yield comes entirely from increased pod numbers on the branch racemes. Yield potential of this hybrid exceeds 6.4 Mg / ha.

Outcomes

Introduction

Because of the very different growing conditions (longer season, warmer temperatures, higher precipitation and humidity, different soils and unique pest pressures), best agronomic practices do not transfer directly to eastern Canada from the major canola growing regions of western Canada.

One factor where the longer-season regions may differ strongly from the west is in the crop response to seeding rates. This issue is more complex than it may first appear, owing to canola's remarkable morphological plasticity. Maximum yields tend to occur over a broad range of seeding rates, because the number of pod-bearing racemes (branches), and the number of pods per raceme, adjust very freely to compensate for lower plant populations. Current typical seeding rates of 4 to 5 kg / ha result in approximately 120 seeds being sown per m2, producing typical final plant stands of 80 plants per m2. However, extensive research has shown that, when yield potential is high (over 2000 kg / ha), plant stands as low as 20 per m^2 can be sufficient to maximize yields (Angadi et al. 2003, McGregor 1987, Morrison et al. 1990a). In our own past work at the Elora Research Station, even 30 m^{-2} was not a low enough seeding rate to induce a yield penalty relative to the typical rate of 120 per m^2. With the introduction of herbicide-tolerant hybrid varieties, seed costs have been going up, and now represent between 20 and 25% of total variable input costs for canola production (OMAFRA, 2012). In western Canada, there are reasons for growers to continue to incur this extra expense. First, in some regions of the west, typical yields are much lower than in Ontario (where the average yield is now approaching 2300 kg / ha), and higher seeding rates are required where yield potential is lower. Second, because the branch racemes are developmentally delayed relative to the main raceme, the greater branching induced under lower seeding rates also extends the time to maturity (by up to a week in our trials; see also McGregor 1987 and Morrison et al., 1990b). This can put the crop at risk of frost damage in western Canada, resulting in high green seed counts; in central and southern Ontario this is not an issue and in fact extending the time to maturity could benefit the crop by lengthening the seed fill period and increasing late season crop growth rates (Morrison 1990b).

The increased branching and “de-synchronization” of reproductive development that occurs at lower seeding rates may have benefits with respect to crop tolerance of heat stress. Heat stress in canola does not usually prevent flowering, but can cause defects in both male and female gametophytes, as well as in post-pollination carpel development, leading to aborted flowers, aborted pods, or development of parthenocarpic (seedless) pods (Young et al., 2004). The timing of the heat stress relative to the stage of reproductive development strongly influences the effect on pod set; for example, pod set may be completely eliminated on the main raceme by a stress occurring during early flowering, but then the plant may fully compensate by setting additional pods on later-developing branch racemes once the stress has abated (Gan et al., 2004; Ramsahoi and Earl, 2010). It is for this reason that we hypothesize that, at lower plant densities, increased branching may afford the crop more flexibility to re-allocate resources amongst different racemes, thus increasing tolerance of transient stress episodes.

More obviously, there is reason to believe that the fewer, larger plants present at a lower population might also achieve deeper rooting, thus allowing the crop to better access stored soil water lower in the profile. Indeed, in 2012 field experiments which were exposed to severe water stress during early flowering, we observed much better crop growth and, especially, flowering under lower seeding rates.

A final issue relating to seeding rates, and which is specific to eastern Canadian canola, is FFA (free fatty acid) levels. FFA levels seem to be augmented by heat stress (and possibly drought stress) (May et al., 1994), and in 2005 this quality issue resulted in the majority of the Ontario crop being graded as “Canada Sample”, making it essentially unmarketable. Individual canola varieties differ significantly in their susceptibility to elevated FFAs (May and Hume 1995; OOPSCC 2011), and so this quality parameter has been measured in the Ontario Public Canola Variety Trials for over 15 years. May et al. (1994) also reported that FFA levels tended to be higher at lower seeding rates, because seed from branches was generally higher in FFAs than seed developing on the main raceme. We have recently confirmed this observation that FFAs tend to increase at lower seeding rates, making this an important consideration for any future recommendations on canola seeding rates for eastern Canada.

Objectives

To test the hypothesis that the changes in plant morphology that occur at low plant populations (more highly branched plants, extended flowering and pod set periods) can increase resilience of spring canola to transient, acute stresses. Also, to quantify the consequences of reduced seeding rates on important seed quality parameters (brown seed content, green seed content).

Approach and Results

Field Trials

In 2013 we conducted replicated field trials at both Elora, ON and Meaford, ON testing the effects of seeding rate (low, normal and high, being 50, 100 and 200 seeds m^{-2} respectively) on crop morphology and yield. Due to intense rainfall shortly after planting at Elora, stands were compromised and the trial did not produce useful data. At Meaford growing conditions were close to ideal, so it was not likely a representative site in terms of the interaction between stress conditions and seeding rates. Yields were slightly reduced at the lowest seeding rate, so it seems that under very high yielding conditions it is still possible to observe a yield reduction at 50% of a normal plant population. Also, we saw a significant increase in brown seed content at low populations, which is consistent with the findings of May et al.

(1994); apparently, this is still an issue even with a modern hybrid variety (InVigor L150 in this case). Green seed was also elevated at the low seeding rate, likely due to the delayed maturity of the branches (Table 1).

Table 1. *Effect of seeding rate on yield, crop height, seed distribution and seed quality at Meaford ON in 2013. Within a column, means followed by the same letter do not differ according to a protected LSD test at P < 0.05.*

Seeding		**Crop**	**Percent**	**-------------------Brown Seed------------------**			**-------------------Green Seed-------------------**		
Rate	**Yield**	**Height**	**Yield on**	**Total**	**Main Raceme**	**Branches**	**Total**	**Main Raceme**	**Branches**
m^{-2}	$Mg\ ha^{-1}$	cm	**Main Raceme**		**%**			**%**	
50	3.56 b	136 a	44 c	4.0 a	1.5 a	5.6 a	1.1 a	0.5 a	1.6 a
100	3.87 a	126 b	61 b	0.9 b	0.1 b	2.1 b	0.5 a	0.8 a	0.1 b
200	4.04 a	123 b	75 a	0.9 b	0.8 ab	1.4 b	0.3 a	0.3 a	0.3 b
P-value	**0.005**	**0.0003**	**<0.0001**	**0.006**	**0.02**	**0.04**	0.29	0.66	**0.04**

Additionally, in 2013, we tested the effects of seeding rate on susceptibility to Swede midge damage, also at the Meaford location. We hypothesized that the greater developmental asynchrony between flowering racemes on the more branched plants at low densities would reduce susceptibility to Swede midge infestation. The design was a split plot design with four different timings of application of the insecticide Coragen as the main plot factor (check, application at rosette stage, application 7 d after first flower, and application both at rosette and 7 d after first flower). The sub plot factor was seeding rate (same three rates as in the seeding rate trial). None of the insecticide treatments affected yield or Swede midge damage ratings. The seeding rate treatment effects on yield were similar to those reported for the above experiment, but with even stronger statistical separation. Yields were 3.87, 3.67 and 3.30 Mg / ha for the 200, 100 and 50 plants per m^2 seeding rates, respectively, and all three differed significantly from one another. Consistent with our hypothesis, Swede midge damage ratings tended to be highest at the highest seeding rate; however, overall ratings were only moderate in this experiment.

In 2014 the seeding rate experiment was performed at three locations with the same protocol as in 2013, except green and brown seed was measured on the bulk seed harvest rather than on main racemes and branch racemes separately. All three locations provided useful data, and presented a range of yield levels. Melancthon was a relatively low-yielding site. Here (only) we observed a significant positive yield response to seeding rate. Lower seeding rates also resulted in higher green seed counts, consistent with less uniform maturity on more highly branched plants. Yield distribution was not measured at this site, but at the other two sites reduced seeding rates resulted in a significantly increased fraction of the yield being borne on the branch racemes (smaller fraction of yield on main racemes), as was also seen in 2013. Also, consistent with 2013 was the observation that plant height tended to decrease as seeding rate increased.

Table 2. *Effect of seeding rate on yield, crop height, seed distribution and seed quality at three locations in 2014. Least significant difference at a 5% Type 1 error rate is given where the treatment effect was statistically significant.*

Seeding Rate	**Yield**	**Height**	**% Yield on**	**Brown seed**	**Green seed**
m^{-2}	**Mg ha^{-1}**	**cm**	**Main Raceme**	**%**	**%**
<u>Elora</u>					
50	3.43	162	28.1	3.2	1.3
100	3.57	159	37.9	3.2	0.6
200	3.47	157	46.4	3.2	0.6
LSD5%	NS	NS	6.0	NS	NS
<u>Melancthon</u>					
50	1.93	134	-	1	2.4
100	2.16	134	-	1	1.6
200	2.44	134	-	0.8	0.8
LSD5%	0.35	NS	-	NS	1
<u>Meaford</u>					
50	5.38	156	44.0	0	0.1
100	5.55	151	54.4	0	0.5
200	5.29	137	58.7	0	0.4
LSD5%	NS	12	11.6	NS	NS

In 2014 we also began the planned experiment investigating interactions of irrigation and seeding rate at the Elora location. This began one year earlier than originally proposed, in order to gain experience making the soil water measurements via TDR. The design was a split plot, with irrigation as the main plot factor (rain-fed vs. irrigated) and seeding rate (50, 100 or 200 m^{-2}) as the subplot factor. Volumetric soil water content (VSWC) was measured on eight dates between 13 and 91 days after planting, at six depths between 10 and 100 cm, using a TDR probe. Unfortunately, because of the well-distributed precipitation in 2014, it was not possible to apply a significant amount of irrigation water. A total of 21 mm was applied, all during the week of July 20 (about 40 days after planting); for the rest of the season, the ground was too wet to irrigate. Thus, the irrigation amounts were insufficient to produce a treatment response. Nevertheless, we were able to test the hypothesis that low seeding rates would produce larger individual plants with deeper rooting and better soil water extraction at depth. Consistent with our hypothesis, we observed significantly lower soil water content at the 100-cm depth (only) for the lower seeding rate compared to the high seeding rate. However, this difference was only apparent on two measuring dates around mid-season (42 and 49 DAP).

In 2015 the irrigation / seeding rate study was repeated at the Elora location. In this year, we applied 140 mm of irrigation water between late July 24 and Aug 7. As of the writing of this report we are still analyzing the results of the VSWC measurements, taken on 12 dates and with more comprehensive sampling than in 2014. Analysis of the yield, yield distribution and seed quality data from this experiment showed no main effect of irrigation treatment on any measured parameters. Effects of seeding rate are presented below, along with results from the Melancthon site. The Elora location was the only location in these trials where a significant yield penalty was observed at the high (200 m^{-2}) seeding rate. At the Melancthon location, which was lower yielding, the highest seeding rate produced the highest yields.

Table 3. *Effect of seeding rate on yield, crop height, seed distribution and seed quality at two locations in 2015. Least significant difference at a 5% Type 1 error rate is given where the treatment effect was statistically significant.*

Seeding Rate	**Yield**	**Height**	**% Yield on**	**Brown seed**	**Green seed**
m^{-2}	**Mg ha^{-1}**	**cm**	**Main Raceme**	**%**	**%**
Elora					
50	3.58	155	44.2	0.6	0.6
100	3.62	154	57.5	0.8	0.8
200	3.24	145	79.5	0.8	0.6
LSD5%	0.23	6	12.4	NS	NS
Melancthon					
50	2.53	123	39.2	0.5	0.5
100	2.59	116	46.8	0.9	0.3
200	2.81	113	61.5	0.3	0.3
LSD5%	0.17	7	12.3	NS	NS

Greenhouse Trials

In 2013 we conducted a greenhouse experiment to test the hypothesis that at low plant populations, more highly branched plants would be less susceptible to yield loss caused by an acute, transient water stress occurring during early flowering. This work was conducted as part of the MSc research of Meghan Griffiths. Plants were grown at either a high density (90 m^{-2}) or a low density (30 m^{-2}), and subjected to either control conditions (well watered) or to a drought stress treatment, for four treatment combinations in a 2 x 2 factorial. The experiment was replicated four times, sequentially. Each experimental unit consisted of four pots, completely surrounded by an additional 14 “buffer” pots with the same population. The water stress treatment was applied as seven days of declining soil water content, controlled by weighing and watering of pots daily ending at 10% of soil water holding capacity (WHC); then re-watering to 60% WHC and drying down to 10% again over another 6 days. The water stress was initiated at first flower. After the 13 d of stress treatment, all pots were maintained well watered until physiological maturity.

On an area basis, yields in the greenhouse trial were extremely high (e.g., 625 g m^{-2} = 6.25 Mg ha^{-1}) (Table 4). The seeding rate treatment strongly affected pod distribution between the main raceme and the branches, but the overall yield was not affected by seeding rate. This demonstrates the extensive ability of the crop to compensate for low plant populations under high yielding conditions.

The water stress treatment greatly reduced yields, primarily by reducing pod set on the branches; main raceme pod numbers were not strongly affected. We did not find a statistical interaction between seeding rate and water stress on yield. Thus, in this experiment there was not compelling evidence that the crop was more resilient to the stress at the lower population (Table 4).

Table 4. *Means comparisons of yield components as contributed from main racemes (MR), branches (BR) and total plots (TOT) for a spring canola (Brassica napus L.) crop as affected by density and watering treatments in a greenhouse setting at the University of Guelph, ON in 2013.*

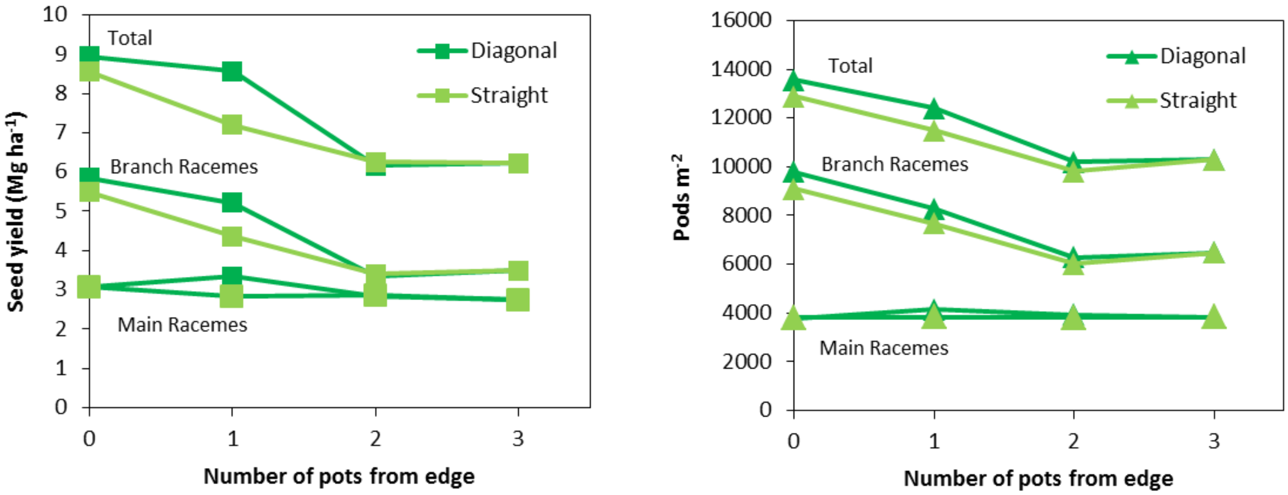
	-----------Pods (m^{-2})-----------			**----------Seeds pod^{-1}----------**			**-----1000-seed weight---------**			**--------Yield (g m^{-2})-----------**		
Treatment	**MR**	**BR**	**TOT**	**MR**	**BR**	**TOT**	**MR**	**BR**	**TOT**	**MR**	**BR**	**TOT**
Density												
90 plants m^{-2}	3650	5502	9152	18.1	16.7	17.4	3.61	3.46	3.52	237	317	553
30 plants m^{-2}	1902	6992	8894	18.1	18.4	18.3	3.74	3.39	3.47	128	427	555
p value	**< 0.0001**[1]	**0.01**	0.59	0.99	**0.002**	0.06	0.07	0.16	0.42	**0.0001**	**0.003**	0.96
Water												
Control	2907	7638	10545	17.9	18.2	18.1	3.48	3.22	3.29	180	446	625
Stress	2645	4856	7501	18.3	17.0	17.6	3.87	3.62	3.70	185	298	483
p value	**0.02**	**0.0003**	**< 0.0001**	0.59	**0.01**	0.24	**0.0003**	**< 0.0001**	**0.0001**	0.58	**0.0005**	**0.0005**

Despite the lack of a statistically significant interaction, the numerical trends in this experiment were in the direction hypothesized. Specifically, at a plant population of 90 m^{-2} the water stress treatment reduced yield by 25.4%, but at the lower plant population the stress reduced yield by just 16.4% below the highest yielding treatment (data not shown). This result suggests that the lower plant population may have afforded some protection against yield loss under water stress, but this would require additional investigation to verify.

In 2015 we undertook a second greenhouse experiment to further investigate the cause of the extremely high yields observed in the first experiment. This experiment was conducted as an undergraduate research project by Mr. Josh Burrows, who was enrolled in the BSc(Agr) program at the University of Guelph. The objective was to grow plants at increasing levels of interplant competition to identify yield levels under conditions emulating a continuous canopy. Plants were grown five per pot, with 64 24-cm pots arranged in an 8 x 8 grid (plant density = 87 m^{-2}). The seed yield in the absence of border effects was

6.2 Mg ha^{-1}, or about twice a typical field yield. Pots on the outer edge of the grid yielded 40% higher than this; the increase was attributable entirely to increased seed yield on branch racemes, as main racemes were unaffected. The increased yield on branches was due to the increased branch number and associated increased pod number, not seeds per pod or seed size. A buffer 0.48 m (two pots) wide was sufficient to fully eliminate border effects. These results demonstrated that even under full-canopy interplant light competition conditions, the yield potential of this commercial hybrid exceeds 6.2 Mg / ha; also, under these ultra-high-yielding conditions, the yield component entirely responsible for the yield increase was the pod number on branch racemes; pod numbers on main racemes were "saturated" under these conditions and could not increase further.

Figure 1. *Effect of interplant competition on pod numbers and seed yield in greenhouse-grown canola. Pots were arranged in an 8 x 8 grid so that interplant competition increased from the edge to the centre of the experiment. Seed yield per unit ground area decreased significantly (p < 0.0001) from the edge of the experiment to the center (left panel). Two border pots were sufficient to maximize this effect. The change in seed yield with pot position was due entirely to differences in seed yield on branch racemes; yield of main racemes was not affected (p = 0.47). The yield component affected by pot position was the number of pods borne on branch racemes (right panel). There was no effect on main raceme pod numbers. Other yield components (seeds per pod and 1000-seed weight, both on branches and main racemes) were also unaffected by pot position (data not shown).*



Issues

In general, these experiments were successful and provided definitive results. Not all field trials produced useful data, but we anticipated this in the original proposal, since our experience with canola small plot trials indicates that they tend to have a lower success rate that similar trials with other crops. By planning multiple locations for the field trials each year, we ensured that we produced at least some data in each year of the study.

We were disappointed not to observe a yield response to irrigation at the Elora location in 2014 and 2015. 2014 was a very wet season and so the lack of a response was not surprising. In 2015 we applied 140 mm of irrigation water around mid season, but still saw no benefit. The lack of any apparent yield reduction due to soil water deficits prevented us from testing our hypotheses around the interaction between plant population and drought stress. We were able to overcome this limitation to some extent with the 2013 greenhouse study.

Successes

In the 2013 and 2014 field trials, as well as the 2013 greenhouse study, we were able to make observations on extremely high yielding canola crops. Field yields exceeded 5.5 Mg / ha. In a follow-up study in the greenhouse in 2015, we attempted to simulate a realistic light environment by providing a complete, closed canopy (i.e., the pots on which we made measurements were completely surrounded by additional pots that were there only to create a realistic environment in terms of light competition). In that scenario, we observed final yields as high as 6.4 Mg / ha. These results are exciting in that they help to demonstrate the genetic yield potential of this crop under ideal conditions, but also suggest that some high-yielding field crops are already approaching what may be the upper yield limit of current commercial hybrids.

Lessons learned

The key lesson learned in this work is that, despite the very high yield potential and very large plant size in some Ontario canola crops, there is no demonstrated advantage, and possibly some disadvantage, to reducing seeding rates below the current typical rate of 100 viable seeds m^{-2}.

Additionally, we learned that in very high yielding canola crops, the capacity for pod formation on main racemes becomes completely saturated, and so additional yield must come from branch racemes. This may have implications for the importance of crop protection during an extended period when yield potential is high (since branch racemes are developmentally delayed relative to main racemes).

Future Work

Additional work should be focussed on the ultimate yield potential of spring canola in Ontario. Yield contest winners (and also some small plots trials in the current work) have exceeded 5 Mg / ha. The best yield we have observed under controlled environment conditions with a true continuous canopy structure is 6.2 Mg / ha. As agronomic practices improve we may soon approach the genetically-determined yield potential of current hybrids. More work should be carried out to investigate genetic variation for yield potential, as well as physiological limitations to higher yields.