



**AAFC RESEARCH BRANCH
Research Project Final Report**

Developing Innovative Agri-Products Program (Vote 10 Funding)

Project Title:	Activity B.2.5, B.2.6 and B.2.7: Improving oil quality of Eastern Canadian canola
Start Date (yyyy-mm-dd):	2011-04-01
Expected End Date (yyyy-mm-dd):	2013-03-31
Actual End Date (yyyy-mm-dd):	2013-03-31
Principal Investigator (PI):	Hugh J. Earl
Short Executive Summary of report:	
<p>Elevated levels of free fatty acids (FFAs) in the seed has historically been the most important quality defect threatening marketability of the Ontario canola crop. Accordingly, variety recommendations for Ontario place a large weighting on this quality factor, and varieties that show high FFA levels in public Ontario variety trials will not be recommended for production in the province. Historical experience with the Ontario crop indicates that high FFAs are more likely to become an important quality issue in seasons where temperatures are high.</p> <p>In this project, it was proposed to develop a controlled environment stress protocol that would provide a mechanism to pre-screen candidate canola varieties for susceptibility to elevated FFAs under heat stress. However, it was found that in controlled environment growth cabinets, heat stress or combined heat / drought stress that was sufficient to reduce pod set and seed yield by up to 50% had no effect on FFA levels, which were extremely low in all cases. While disappointing, this result points out the need to reconsider current ideas about the causes of elevated FFAs and the reasons why this problem is more prevalent in Ontario than in the major canola-growing regions of Western Canada.</p> <p>This project also investigated the hypothesis that applications of foliar boron (B) to canola during early flowering could result in significant yield increases, and that these increases would be greatest when the crop was under high temperature stress during flowering. A further objective was to elucidate the physiological basis of foliar boron's effect on the canola crop.</p> <p>A series of field trials demonstrated conclusively that foliar B applications can produce a change in the pod and seed distribution of the crop (with relatively more yield on the main raceme and less on the branches), due to a reduction in abortion of pods on the main raceme. In one third of the trials a significant yield increase resulted, but the increases were generally small (approximately 5%). In two of the trials, foliar B treatment also produced a significant reduction in seed content of FFAs.</p> <p>A controlled environment study corroborated the field results, with B treatments significantly increasing main raceme pod counts and decreasing main raceme pod abortions. However, importantly, this effect was observed only when the plants were exposed to heat stress during the flowering period; plants grown under control conditions (no temperature stress) showed no response to B.</p> <p>In summary, the results indicate that foliar B applications can increase canola yields, but that these benefits are more likely to be realized when there is significant heat stress during flowering. The conventional wisdom regarding the connection between heat stress and FFAs was not supported by the controlled environment studies; additional research should be directed towards identifying the true causes of this important quality defect.</p>	



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A. Research Progress and Accomplishments (to date in relation to expected milestones and deliverables / outputs)

- Include brief summary of:
 - Introduction, literature review, objectives, milestones and deliverables / outputs.
 - Approach / methodology (summary by objectives).
- Include results and discussion (overview by objectives and milestones), next steps and references.

Introduction

In canola, high air temperatures during reproduction cause reduced fertilization and, therefore, reduced seed yield (Morrison and Stewart 2002). Exposure of *Brassica* species to hot weather for seven days during early flowering caused yield reduction due to flower abortion caused by pollen infertility (Angadi et al. 2003). Besides reducing yield, heat stress is also believed to have important effects on seed quality in canola; Ontario spring canola has occasionally suffered from higher than desirable levels of free fatty acids (FFAs), putatively caused by excessively high ambient temperatures during the seed-filling period. At grading, such “heat damaged” canola is identified by a count of seeds found to have either light brown or dark brown cotyledons when crushed. In 2005, a large fraction of the Ontario crop was graded “sample” based on brown seed counts, making it essentially unmarketable – this undermined producer confidence in both the crop and the grading system, and resulted in a decline of canola acreage in the province by two thirds the following season (it has since recovered). Because heat stress is more common and more severe in Eastern Canada than in the major canola-growing regions of the West, evaluation of canola genotypes for heat stress tolerance is essential to increasing canola cultivation in Eastern Canada. Additionally, Eastern canola production will benefit greatly from technologies or agronomic practices that can mitigate the effects of heat stress.

Two years of replicated field studies (2007, 2008) gave conflicting information regarding the benefits of foliar boron (B) applied to canola. A very large yield benefit of 0.5 t/ha was realized from foliar B applied during flowering at each of two sites in 2007, a high stress year (heat, water stress), but no benefit was seen at either site in 2008 when growing conditions were near optimal, and only very small (but statistically significant) average benefits were seen in OMAFRA strip plot trials in 2008 (H.J. Earl and B. Hall, unpublished). Since B is known to be essential to reproductive development, these field observations have led to a hypothesis that foliar B may act to mitigate the effects of heat stress on flowering, pollination and/or early pod set in canola.

Sub-Activity B.2.5

Objective

Evaluate the ability of foliar B applications to ameliorate the effects of heat stress in canola

Methods

In prior work a controlled environment reproductive period heat stress treatment that reduced main raceme pod set by 50% had been identified. Using this stress protocol, additional controlled environment experiments were conducted to determine, if foliar boron applications could reduce pod losses caused by heat stress. Plants were grown in the greenhouse, then at three days after first flower received one of six treatments:

- 1- check,
- 2- low rate of B as borax + adjuvant,
- 3- high rate of B as borax + adjuvant,



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- 4- low rate of a liquid B preparation plus adjuvant,
- 5- high rate of a liquid B preparation plus adjuvant, or
- 6- adjuvant only.

Four days after the foliar treatments, the plants were moved from the greenhouse to one of two growth cabinets: a control cabinet with day / night temperatures of 20 / 15°C, or a stress cabinet at 28 / 20°C. After 14 days in the cabinets the plants were returned to the greenhouse until physiological maturity, at which time the number of viable and aborted pods on the main raceme were counted, and pods were threshed to determine seed weight on both main racemes and on branches. The experiment was replicated six times sequentially, with two plants per temperature treatment x foliar treatment combination in each replication. Temperature treatments were randomized between the two different cabinets for each replication. Harvested seed was also analyzed for FFA content via gas chromatography.

Results

Plants in these experiments were very large and extensively branched, with over 1000 pods per plant. Pod counts were conducted on main racemes only, which bore approximately 10% of the total yield. Results are shown in table 1. No significant differences were found between any of the four foliar treatments containing B, or between the check treatment and the adjuvant only treatment, so statistical contrasts were constructed to compare the effects of the B treatments, as a group, to the control + adjuvant only treatments as a group. Under control temperature conditions, no significant effect of the B treatments were found. However, under heat stress, B was found to significantly decrease the number of main raceme pod abortions, increase the number of viable pods on the main raceme, and increase the main raceme seed yield (table 1 and figure 1). FFA content of the seed was generally low (below 0.5% of total oil) and was not affected by any of the treatments (data not shown).

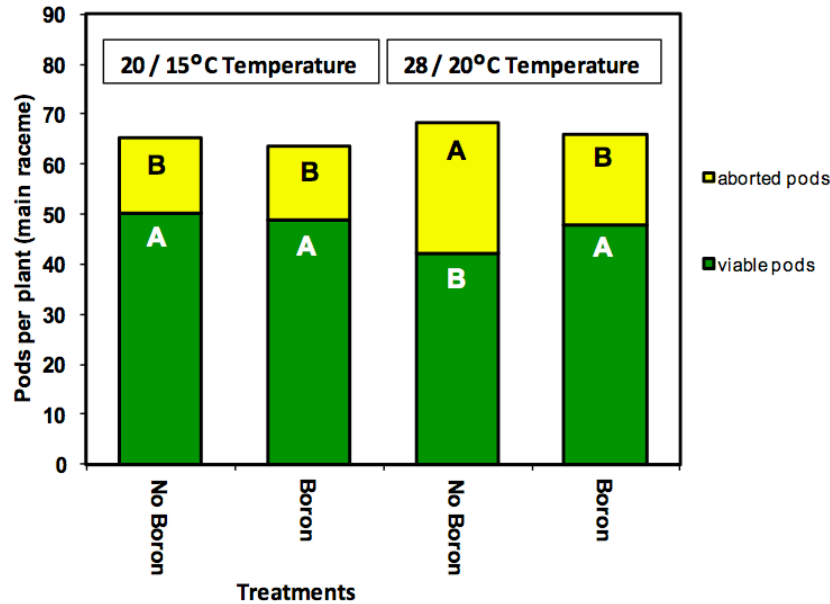


Figure 1. Aborted pods and viable (seed-bearing) pods on main racemes, as affected by temperature regime and foliar B application. Within a temperature regime, treatments followed by the same letter do not differ for aborted pods (top letters) or viable pods (bottom letters) on the main raceme, according to a protected LSD test ($P < 0.1$).



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Table 1. Effects of foliar B application on main raceme pod set and pod abortion, and on main raceme, branch and total seed yield, under both control and heat-stress conditions during pod set. All values are on a per-plant basis, and are means of 12 plants. Contrasts were considered statistically significant when the P-value was less than 0.1.

Contrasts	Means	Viable Pods	Aborted Pods	Pod Abortion (%)	Main Raceme seed weight (g)	Other Raceme seed weight (g)	Total seed weight (g)
Control							
B vs no B	B (4 treatments)	48.6	14.9	26.21	2.99	18.96	21.95
	No B (2 treatments)	50.3	14.9	25.05	2.89	19.28	22.17
	<i>P value</i>	0.62	0.99	0.64	0.55	0.69	0.79
B vs Check	B (4 treatments)	48.6	14.9	26.21	2.99	18.96	21.95
	Check (1 treatment)	51.2	13.6	23.34	2.99	18.24	21.23
	<i>P value</i>	0.54	0.53	0.38	0.98	0.49	0.51
B vs Adj only	B (4 treatments)	48.6	14.9	26.21	2.99	18.96	21.95
	Adj only (1 treatment)	49.3	16.2	26.76	2.80	20.32	23.12
	<i>P value</i>	0.87	0.52	0.86	0.37	0.19	0.28
Check vs Adj only	Check (1 treatment)	51.2	13.6	23.34	2.99	18.24	21.23
	Adj only (1 treatment)	49.3	16.2	26.76	2.80	20.32	23.12
	<i>P value</i>	0.73	0.32	0.40	0.49	0.12	0.17
Stress							
B vs no B	B (4 treatments)	47.9	17.8	27.64	2.72	17.63	20.34
	No B (2 treatments)	42.2	26.1	39.03	2.34	17.29	19.63
	<i>P value</i>	0.08	< 0001	< 0001	0.03	0.67	0.40
B vs Check	B (4 treatments)	47.9	17.8	27.64	2.72	17.63	20.34
	Check (1 treatment)	42.7	28.0	40.04	2.39	17.45	19.84
	<i>P value</i>	0.21	< 0001	0.0003	0.13	0.86	0.64
B vs Adj only	B (4 treatments)	47.9	17.8	27.64	2.72	17.63	20.34
	Adj only (1 treatment)	41.8	24.3	38.02	2.30	17.12	19.42
	<i>P value</i>	0.14	0.002	0.002	0.06	0.63	0.40
Check vs Adj only	Check (1 treatment)	42.7	28.0	40.04	2.39	17.45	19.84
	Adj only (1 treatment)	41.8	24.3	38.02	2.30	17.12	19.42
	<i>P value</i>	0.86	0.15	0.62	0.74	0.80	0.76

These results strongly support the hypothesis that foliar B applications during early flowering can enhance yield by reducing the effects of heat stress on pod set. Under non-stress conditions, no advantage of foliar B was apparent.

Sub-Activity B.2.6:

Objective

Screen spring canola varieties for variation in tolerance of heat stress.

The objective of this sub-activity was to develop a simple controlled environment method for evaluating canola varieties for FFA levels under heat stress. If successful, the method could be used to pre-screen candidate varieties for susceptibility to high FFAs prior to entering them into the Ontario variety trials.



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This would reduce field trial costs for variety sponsors, since unsuitable varieties could be efficiently identified before extensive field testing was undertaken. It would also reduce the chances that a variety susceptible to high FFAs would end up on the recommended list simply because it was tested in low-stress years that did not reveal the problem.

Methods and results

A controlled environment experiment was conducted to compare FFA levels in a single commercial spring canola variety, under low stress conditions (24°C daytime high temperature and 14°C nighttime low) or when exposed to stressful temperatures (32°C/20°C). The stress treatment was applied for two different durations (one week or two weeks) beginning at one of four different growth stages (one, two, three or four weeks after first flower), for a total of nine different stress treatments. The 32°C /20°C temperature regime proved to be a relatively mild stress, with only minor effects on pod counts. None of the stress treatments had any effect on seed FFA levels.

Therefore, a second controlled environment study was conducted, using more severe stress treatments. At two weeks after first flower, plants were either:

- i) maintained at 24°C/14°C or transferred to a growth cabinet set for
- ii) 35°C/20°C or
- iii) 38°C/22°C and maintained there until near physiological maturity (four weeks).

Also, in each of the three groups, plants were either kept well watered or else were allowed to wilt in between watering in order to induce drought stress treatment in addition to the heat stress.

These much more severe stress levels were effective in reducing pod numbers, seed size and seed yield; the most severe stress treatment reduced seed yield by approximately 50%. Despite this, none of the stress treatments had any effect on FFA levels. Indeed, FFA levels in this experiment were extremely low, averaging just 0.04% of total oil.

A third experiment was also conducted, which focused on the effects of terminal water stress events on FFA levels. Plants were grown under control temperature conditions (24 / 20°C) until two weeks after first flower, then half were transferred to 32°C/20°C. In both groups, plants were either maintained water-replete, or else watering was terminated at two, three, four, five or six weeks after first flower. In both temperature treatments, withholding water during pod set and seed fill strongly reduced seed yields (by over 50% in the most severe treatment); however, again, there was no effect on seed FFA levels, and FFAs were in general very low.

It is concluded that heat stress alone, or heat stress in combination with drought stress, applied during the seed-fill period, are not sufficient to induce high FFA levels in canola. While disappointing in terms of the original objective to develop a controlled environment pre-screening protocol for testing candidate canola varieties, these results do provide important insight into the physiological basis of high FFA levels in canola. Clearly, there is some aspect of the field environment required to produce high FFA levels that was not present in the controlled environment stress treatments; this despite the fact that the treatments were severe enough to drastically reduce seed yield. Further research is required to determine the environmental factors that induce high FFAs in canola.



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Sub-Activity B.2.7

Objective

Determine the physiological basis of yield enhancement from foliar B applications in the field

Methods

Replicated small-plot field trials were established at each of four locations in Ontario in each year of the study in 2011 and 2012. Treatments included:

- 1- check,
- 2- foliar B at 0.5kg/ha + adjuvant applied approximately seven days after first flower, and
- 3- adjuvant only, applied at the same time as treatment 2.

Prior to machine harvesting the plots, ten plants per plot were sampled by hand and pod numbers and seed weights were determined for main racemes and for branches. Seed from the machine harvest was also analyzed via gas chromatography for FFA content.

In 2011, three of the four locations (Elora, Dundalk, Shelburne) produced acceptable data with a low coefficient of variation for yield.

In 2012, only two of the locations (Elora, Meaford) produced useful data. Data from these five trials were combined with data from four other location-years from prior studies (Elora, Meaford and Shelburne in 2009; Elora in 2010) for analysis.

Yield data (but without pod counts) were also available for similar trials from both Elora and Meaford in 2007 and 2008, for a total of 13 location-years of data for yield.

Results

Across the 13 location-years, a significant yield increase in response to foliar B was observed four times: Meaford 2007 (6.1%), Elora 2010 (6.3%), Dundalk 2011 (5.2%) and Meaford 2012 (5.4%). At one extremely high-yielding site (Meaford 2009) foliar B significantly **reduced** yield (6.7%). In all other cases the foliar B treatment produced no significant effect on yield.

Averaged across the nine location-years for which yield components data were available, foliar boron treatment slightly decreased the number of pod abortions on the main raceme, and slightly increased the number of seeds per pod on the main raceme (table 2).

However, for the three location-years where there was a significant yield benefit from application of foliar B, a number of yield components were significantly affected by the B treatment. There was a reduction in the number of pods and number of seeds on the branches; an increased fraction of total pod numbers, total seed numbers and total seed weight on the main raceme, and a reduction in pod abortions on both the branches and on the main raceme (table 3). These results corroborate the findings of the controlled environment study (activity B.2.5) that foliar B application can prevent pod abortions, and tends to shift the pod load and seed mass from the branches to the main raceme.

There were two location-years where foliar B application was found to significantly reduce seed FFA content (Elora 2010; Shelburne 2011). In all other cases there was no significant treatment effect on seed FFAs.



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Table 2. Effects of foliar B treatment on yield components, yield distribution, and seed free fatty acids, averaged across nine location-years. Contrasts were considered statistically significant when the P-value was less than 0.1.

Parameter	No Boron	Boron	P-value
Main raceme pods	410	409	0.88
Other branches pods	591	583	0.84
Fraction of pods on main raceme	0.44	0.44	0.78
Number of seeds on main raceme (1000s)	8.47	8.74	0.20
Number of seeds on other branches (1000s)	13.10	13.73	0.45
Fraction of seed number on main raceme	0.42	0.44	0.22
Main raceme seed weight (g)	27.95	29.07	0.14
Other branches seed weight (g)	32.23	33.30	0.60
Fraction of seed weight on main raceme	0.49	0.50	0.18
Main raceme pod abortions	76.9	65.0	0.08
Other branches pod abortions	248.1	240.0	0.59
Percent of pod abortions on main raceme	16.4	14.2	0.14
Percent of pod abortions on other branches	30.2	29.8	0.66
Main raceme 1000-seed weight (g)	3.34	3.34	0.96
Other branches 1000-seed weight (g)	3.24	3.22	0.53
Main raceme number of seeds per pod	20.4	21.2	0.09
Other branches number of seeds per pod	22.7	22.6	0.91
Free Fatty Acids (% of Oil) ¹	1.14	1.05	0.16

¹Based on an assumed seed oil content of 45%

Table 3. Effects of foliar B treatment on yield components, yield distribution, and seed free fatty acids, averaged across the three location-years where a significant yield increase was observed in response to the B treatment. Contrasts were considered statistically significant when the P-value was less than 0.1.

Parameter	No Boron	Boron	P-value
Main raceme pods	367	351	0.29
Other branches pods	627	549	0.09
Fraction of pods on main raceme	0.39	0.42	0.03
Number of seeds on main raceme (1000s)	7.34	7.58	0.38
Number of seeds on other branches (1000s)	13.99	12.44	0.001
Fraction of seed number on main raceme	0.35	0.40	0.01
Main raceme seed weight (g)	24.68	25.45	0.47
Other branches seed weight (g)	37.35	33.20	0.14
Fraction of seed weight on main raceme	0.43	0.48	0.03
Main raceme pod abortions	78.5	59.4	0.09
Other branches pod abortions	233.1	172.2	0.01
Percent of pod abortions on main raceme	17.9	14.6	0.11
Percent of pod abortions on other branches	28.6	26.4	0.25
Main raceme 1000-seed weight (g)	3.41	3.41	0.90
Other branches 1000-seed weight (g)	3.23	3.19	0.73
Main raceme number of seeds per pod	20.1	21.7	0.32
Other branches number of seeds per pod	23.6	23.2	0.74
Free Fatty Acids (% of Oil) ¹	1.45	1.25	0.22

¹Based on an assumed oil content of 45%.



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Conclusions and next steps

The field trials investigating effects of foliar B applications significantly expanded the dataset regarding the benefits of this practice. A significant yield benefit of foliar B was found in approximately a third of valid location years and, when the benefit occurred, it was on the order of 5 to 6%. Significant yield reductions occurred only in one trial, and that was a very unusual case where yields were over three times the provincial average.

If foliar B is applied in a tank mix at early flowering when other products (insecticide and fungicide) are being applied, then the application costs can be considered negligible, and only the product cost itself, about \$5 per acre, must be considered in assessing economic benefits. At a moderate yield level of 2000 lbs/acre and a price of \$600/t, a 5% yield increase represents a \$30 revenue increase per acre against an input cost of \$5 per acre. However, since the benefit may be realized only about one third of the time, an average return of about \$10 per acre (\$5 net of input cost) is predicted. It is worth noting that the hot water soil B test was not predictive at all of yield response to foliar B application in these trials (data not shown).

The main focus of this sub-activity was the potential for foliar B to improve yields under heat stress specifically, and to uncover the physiological basis of that effect. The field trials showed conclusively that one of the effects of foliar B was to reduce pod abortions on the main raceme, thereby increasing the ratio of main raceme pods to pods on other branches. This same effect was clearly demonstrated in the controlled environment study and, moreover, it was found that B exerted its effect on pod distribution only when pod set was limited by high temperature stress. Taken together, these results suggest that foliar B applications increase yields by increasing main raceme pollination success and/or pod retention under conditions where heat stress would otherwise reduce pod numbers. These findings constitute entirely new information about the effects of B on canola – there are no previous reports of foliar B alleviating heat stress effects.

Since previous research indicates that FFAs tend to be higher in seed borne on branches than on the main raceme (May et al., 1994), one could hypothesize that B application, by shifting more of the pod load to the main raceme, would also decrease FFA levels. Such a reduction in seed FFA content in response to B application was observed in two out of nine location years.

The other main objective of this work was to develop a controlled environment stress protocol that would allow us to screen canola varieties for susceptibility to high FFA levels under stress. Given the wisdom that heat stress produces high FFA levels (May and Hume 1995), it was expected that this would be an easy task. Instead, it was found that heat stress treatments or combined heat-drought-stress treatments that reduced pod set and seed yield by as much as 50%, had no effect whatsoever on seed FFA levels. In fact, FFAs in these growth cabinet experiments were in general extremely low. While disappointing, this result clearly points to a need to revisit existing beliefs about the causes of high FFAs in canola. Given the overwhelming importance of this quality factor for the canola industry in Eastern Canada, additional research is clearly warranted. A logical starting point would be to generate hypotheses around the differences, other than temperature, that exist between the field and greenhouse environments on the one hand, and the growth cabinet environment on the other. Obvious candidates would be the quantity and quality of solar radiation impinging on the crop.

References

Angadi, S.V., H.W. Cutforth, B.G. McConkey and Y. Gan. 2003. Yield adjustment by canola grown at different plant populations under semiarid conditions. *Crop Sci.* 43:1358-1366.

May, W.E. and D.J. Hume. 1995. Free-fatty acid levels in Ontario-grown summer rape cultivars. *Can. J. Plant Sci.* 75:589-593.



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May, W.E., D.J. Hume and B.A. Hale. 1994. Effects of agronomic practices on free fatty acid levels in the oil of Ontario grown spring canola. *Can. J. Plant Sci.* 74:267-274.

Morrison, M.J. and D.W. Stewart. 2002. Heat stress during flowering in summer *Brassica*. *Crop Sci.* 42:797-803.

B (I). Funded Collaborators (Co-PI, AAFC, other federal scientists)

- Include the name of scientist / organization.

None other than the PI

B (II). Acknowledgement of non-funded collaborators (who provide support, e.g. access to other laboratory or other facilities and equipment input / advice / guidance / assistance, etc).

- For research supported by targeted funding programs (e.g. DIAP, Clusters, etc.) please list any collaborators who are receiving Contribution Vote 10 funds (e.g., university and industry collaborators). In addition, please list separately the participants who support your project but are not receiving any funding through the program.
- Include name of scientist / organization.

None

C. Variance Report (if applicable, describe how the work differs from the proposed research)

- Include changes to objectives and project work plan / budget, changes to the team, other constraints.

These activities were carried out almost exactly as originally proposed, with the exception of activity B.2.6. As explained above, it was originally proposed to conduct a single simple controlled environment study to develop a heat stress protocol that would result in elevated seed FFA levels, and then to use that protocol in a second experiment to screen a selection of commercial lines for their susceptibility to increased FFAs under heat stress. Instead, there were **three** experiments conducted in the attempt to develop the stress protocol, but ultimately without discovering a way to produce high FFA seed in growth cabinets (even when the stress was sufficient to severely reduce yield). As such it was impossible to proceed to the variety screening study, having consumed all of the time and funding in attempting to develop the heat stress protocol.



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D. Impact Assessment (if applicable, describe how the variance factors above will impact project continuation)

- Include changes to the objectives, changes to the project work plan / budget, changes to performance (i.e. meeting targets).

The failure to develop a controlled environment heat stress (or drought stress) protocol that increased FFA levels will lead to an entirely new set of hypotheses regarding the physiological causes of elevated FFAs in the field. These include radiation load or other climatic variables (especially humidity) during the seed maturation period, or possibly biotic factors (diseases and, especially, insects). Indeed, some past work in has pointed to insect damage as a significant cause of elevated FFAs (H.J. Earl and R. Hallett, unpublished) – this should be explored further.

E. Achievements (include only those related to this project)

- Include innovations, publications / conferences, technology transfer, capacity building, success stories, media, recognition and other outputs.

Results of these trials have been reported very broadly to canola growers in Ontario and elsewhere in Canada. Presentations on the effects of foliar B applications were given at the 2011 Ontario Canola Growers' Association Annual Meetings, at the 2012 Saskatoon Canola Industry Meetings, and to several smaller meetings of canola growers. Results were also presented at both the 2011 and 2012 American Society of Agronomy meetings.


F. Lessons learned (self-evaluation of project)

1. Field trials with canola have a much lower success rate than similar trials with other species. Coefficients of variation are often inflated by factors such as bird damage, late-season insect damage, and pod shattering. Achieving uniform emergence when plots are established can also be challenging. Of course, these issues become even more important when the treatment effects being investigated are small, as is this case here (e.g. 5% yield increases).

2. High seed FFAs are still the most important potential impediment to the development of a successful canola industry in Eastern Canada. The presented study demonstrates that the existing dogma regarding causes of this problem needs to be critically re-examined. Further study is needed to develop agronomic recommendations to minimize the risk. In the meantime, use of low FFA varieties (as identified in the Ontario canola variety trials; <http://www.oopsc.org/canolahome.php>) should be encouraged.



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Hugh J. Earl	29 May 13	
PI Name	Date	Signature

Note: After completion and signature, this report must be provided to the appropriate Science Director for assessment. A PDF copy of this report will be sent to Science Operations by the Science Director's office along with the project assessment.