



**AAFC RESEARCH BRANCH
Research Project Final Report**

Developing Innovative Agri-Products Program (Vote 10 Funding)

| | |
|--|---|
| Project Title: | Activity B.2.2 & B.2.3: Evaluation of natural products on agronomic features of canola: effect of treatments on crop germination, growth, fatty acid profile and yield of canola in Eastern Canada |
| 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): | Balakrishnan Prithiviraj |
| Short Executive Summary of report: | |
| <p>Marine bio-products have been known to induce plant responses such as altered fatty acid biosynthesis, induce plant defense to pathogens, improve crop growth under unfavourable environments and increase seed yield under limiting growth conditions.</p> <p>A number of products were tested in this project. Seed germination at 10 and 15°C was not affected by treatments. However, a considerable increase in germination was observed at 25°C with treatments, particularly seaweed extract (3g/l), λ-carrageenan (3g/l) and chitosan (0.05g/l). Plants with SW, λ-carrageenan and chitosan were also taller than control plants at 25°C. To some extent, the fatty acid contents, particularly C18:1, C18:3 and C24:0 were altered in greenhouse experiment whereas C18:1 and C18:3 were altered in the field experiments, suggesting further detailed experiments are needed to determine this effect. Treatments also showed an increase in oil content that ranged from 0.5-2.0 %, indicating for a need of further scientific exploration. Seaweed extract (2g/l) lowered the percentage of sterility in canola at 25°C in the greenhouse, indicating potential of bioactive treatments to reduce sterility and increase seed yield.</p> <p>Overall, there were improvements in agronomic parameters in the field, but without statistical significance.</p> | |

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.

Sub-activity B.2.2: Test ability of marine bio-products to alleviate temperature stress in the field, greenhouse and growth chamber

Sub-activity B.2.3: Evaluate effects of marine bio-products on oil content and fatty acid composition of oil in greenhouse and field studies

Introduction:

Canola/rapeseed is a cool climate crop with low erucic acid content. It is the third largest global vegetable oil source, after soybean and palm oil. Canola is a popular oil seed crop in Western Canada. Growing demand of canola oil has emphasized the need to expand canola cultivation in the Eastern Canadian region. However, various environmental challenges in Eastern Canada such as air temperatures exceeding 27°C, particularly during flowering time, lead to poor establishment of the crop,



AAFC RESEARCH BRANCH Research Project Final Report

low seed yield and quality of oil (Angadi et al. 2000). Agronomic practices aimed to improve heat stress tolerance during sensitive crop stages are useful for maintaining or improving canola oil quality and yield. To alleviate this problem, heat-tolerant genotypes are developed via conventional plant breeding, molecular breeding and genetic engineering in other crops (Ehlers and Hall, 1998). Similarly, practices are used to modify the fatty acid composition of *Brassica* ssp. seed oil through induced mutations, genetic engineering and conventional breeding that can alter the enzymes in the fatty acid biosynthetic pathway.

Interestingly, achieving stress tolerance and high yield at the same time is challenging and often results in alterations to other plant traits. This necessitates the development of alternate strategies to mitigate environmental stresses and improve quantity and quality of oil. Induced tolerance to heat has been attributed to activation of enzymes in various biochemical pathways. Seed treatment and foliar application with inorganic salts, osmo-protectants, growth hormones and antioxidants have shown promising effects on plant stress tolerance and performance (Colclough et al., 1990; Morales et al., 2003; Xu et al., 2006). Recently, marine bio-products including extracts of some marine macroalgae and compounds isolated from marine organisms have shown effects on stress tolerance and fatty acid composition in plants (Khan et al 2009; Chouliaras et al 2009). In this project the effect of marine bio-products on growth, stress tolerance, fatty acid composition and yield was studied.

Review of literature:

Canola is a popular oil seed crop in Canada and it is the third largest global vegetable oil source, after soybean and palm oil. Rapeseed oil in *B. napus*, *B. rapa*, and *B. juncea* typically has a fatty acid composition of 5% palmitic (C16:0), 1% stearic (C18:0), 15% oleic (C18:1), 14% linoleic (C18:2), 9% linolenic (C18:3), and 45% erucic acid (C22:1), an undesirable fatty acid that has been reduced to very low levels in canola for edible uses (Ackman, 1990). *Brassica* ssp. oil has a nutritionally desirable fatty acid profile, with low saturated fatty acids and significant levels of C18:3, an omega-3 fatty acid (Eskin et al., 1996).

Heat stress due to high ambient temperatures is a serious threat to crop production worldwide. Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperatures. Higher temperatures (5–10 °C above the optimum growing temperature) increase carbon consumption through increased respiration and damage to the photosynthetic system, enzymes, cell membranes and genetic material (Georgieva, 1999). In *Brassica napus*, 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 greater yield reduction due to flower abortion caused by pollen infertility (Angadi et al. 2000). Various canola genotypes demonstrate differential heat stress tolerance and each genotype will perform differently depending on environmental conditions prevalent in the region.

The seed oil content in oilseed crops is a major selection trait for a variety and a major target of breeders during varietal development. It is also possible to manipulate the composition of seed oil through molecular engineering (Thelen & Ohlrogge 2002) such as overexpression of AtLEC1 and its orthologs in canola (*Brassica napus*), increased fatty acid level in transgenic *Arabidopsis* plants (Tan et al 2011). Genetic improvement for oil quality has also been achieved through induced mutagenesis in various oilseed crops (Savant and Kothekar, 2011) including rapeseed (Barve et al 2009). Interestingly, fatty acid composition in oilseeds crops can be altered in response to environmental stimuli such as abiotic or biotic stress, agronomic practices such as planting dates, fertilizer applications and treatment with elicitors including marine bio-products (Zheljazzkov et al., 2009).

Global climate change remains the primary reason for reduced crop productivity (Garrett et al., 2006), and mitigative measures are a priority for sustainable crop production. The use of biostimulants such as



AAFC RESEARCH BRANCH Research Project Final Report

macroalgal extracts in agriculture, to manage stress responses in crop plants, will help alleviate problems due to environmental stresses. Macroalgae have been identified as an exceptional source of biologically active compounds such as fucoidan, carrageenans and phlorotannins with potential use as plant biostimulants (Zhang et al 2003; Khan et al., 2009; Cox et al., 2010; Craigie, 2011). The application of macroalgal extracts can activate plant molecular responses linked with physiological and biochemical activities that lead to altered tolerance to a variety of stresses such as plant pathogens (Khan et al 2009; Craigie, 2011; Subramanian et al 2011), heat (Zhang et al 2003), freezing (Nair et al 2012) and can have an impact on fatty acid accumulation (Chouliaras et al., 2009; Nair et al 2012).

Extracts of the *Ascophyllum nodosum* and other seaweeds have been used as a biostimulant to promote growth and productivity in a number of agricultural production systems. The application of extracts of seaweeds *Ecklonia maxima*, *Ascophyllum nodosum*, *Laminaria hyperborea* helped plants to overcome several stress conditions. Seaweed extracts induced heat stress tolerance in creeping bent grass and this effect was attributed to cytokinin and other bioactive components in the extracts (Zhang and Ervin 2008). Seed priming with *A. nodosum* improved germination of lettuce seeds and better plant performance under high temperature stress (Moeller and Smith 1998). Recently, it was demonstrated that the lipophilic components of *A. nodosum* impart freezing stress tolerance in *Arabidopsis*, a close relative of canola, by inducing differential gene expression (Rayirath et al, 2009). Macroalgal extracts also enhanced heat stress (unpublished) and salinity stress (Wally et al, 2012) and molecular and biochemical pathways are strongly altered in plants treated with these extracts. A whole genome transcriptome analysis revealed that lipophilic components of *A. nodosum* altered the expression of a number of genes in fatty acid metabolism (Nair et al., 2012). Recent work shows that treatment of olive with *A. nodosum* extract in combination with nitrogen fertilization and Borax increased productivity and oil content and also accelerated maturation of the olive fruits (Chouliaras et al 2009). Further, the treatment also resulted in significant increase in linolenic and oleic acid concentrations, but reduced palmitoleic, stearic and linoleic acid concentrations in the oil.

The extensive literature on improved plant tolerance to various stresses suggests the need to explore use of marine bio-products to modify fatty acid profile, crop yield and impart heat stress tolerance, particularly during pollination.

The following experiments were conducted:

Experiment 1. Effect of seed treatment with marine bio-products on seed germination under different temperatures

Experiment 2. Effect of marine bio-products treatment on oilseed quality and fatty acid profile of canola

Experiment 3. Effect of marine bio-products treatment on fertility of canola under high temperature stress

Experiment 4. Effect of marine bio-products treatment on yield of canola

Plant material: Canola variety InVigor L130 was obtained from Bayer Crop Science Corporation, Lethbridge, AB. In the greenhouse, seeds were planted in 10cm diameter pots containing ProMix BX (Premier Horticulture INC. PA. USA) potting mixture and the plants were thinned to obtain one plant per pot. Plants were grown at 25°C under 300-450 $\mu\text{mol m}^{-2} \text{S}^{-1}$ and 16 h photoperiod. Field experiment was established following standard agronomy practices at two different locations in two counties in Nova Scotia.

Treatments: Powdered *Ascophyllum nodosum* seaweed extract (SW; 1-3g/l), λ -carrageenan (1-5g/l), chitosan (0.2-0.1g/l), water or LANS (nutrient solution) as control and fungicide Proline as a check for field studies.



AAFC RESEARCH BRANCH Research Project Final Report

Experimental Plan and Results.

Experiment.1. Effect of seed treatment with marine bio-products on seed germination under different temperatures

Methods: Different temperatures, particularly low temperatures, affect canola seed germination. During 2012 a greenhouse experiment was conducted to study the effect of seaweed extract, λ -carrageenan and chitosan on seed germination at different temperatures (5, 10, 15 and 25°C). Seeds were subjected to either a soaking or coating treatment. The soaking treatment consisted of seeds being soaked overnight (15 °C) in seaweed extract (1, 3 and 5g/l), λ -carrageenan (1, 3, 5g/l), chitosan (0.02, 0.05 and 0.1g/l) or water (control). The coating treatment used the same treatments as soaking, however, each treatment was mixed with talc powder facilitated with carboxymethyl cellulose (CMC) as a sticking agent. The seeds were rolled in a mixture containing talc, CMC and the treatments, and dried overnight at room temperature. The seeds were then planted in 200 well germination trays containing Pro-Mix BX (Premier Horticulture INC. PA. USA). Each treatment had 10 replicates. Each treatment was randomized in the tray and each tray was replicated three times. Trays were then placed in growth chambers set at 5, 10, 15 and 25°C and seed germination was observed at different time points (12-144h) (figure 1). The experiment was repeated three times. The same set was also used to measure plant height at seven and 14 days after planting (figure 2). Mean percent germination and plant height after treatment with marine bio-products was compared with untreated controls.

Results. Experiments performed for determining the effect of treatments on seed germination potential at lower than usual temperature conditions revealed contrasting results, both for seed soaking (figure 1a) and seed coating experiments (figure 1b). The germination was faster in seed soaking treatments compared to the seed coating. However, against expectation, the seed treatments with marine bio-products did not show significant improvement in germination rate. With seed soaking treatment, the overall seed germination was delayed at 10°C. The seedlings at 10°C started emerging only by 72h, which was also true for the coating treatment. In contrast, seed germination was observed at 24h at 15°C and 36h at 25°C. Although it appeared that treatment with SW (3g/l and 5g/l), λ -carrageenan (3g/l and 5g/l) and chitosan (0.05 and 0.1g/l) at 10°C resulted in higher percent germination after 96 h, the differences were not significant. In fact, near 100% germination was observed for all treatments by 120h after seeding (figure 1a). No clear difference in seed germination was observed between marine bio-products and control at 15 and 25°C.



AAFC RESEARCH BRANCH Research Project Final Report

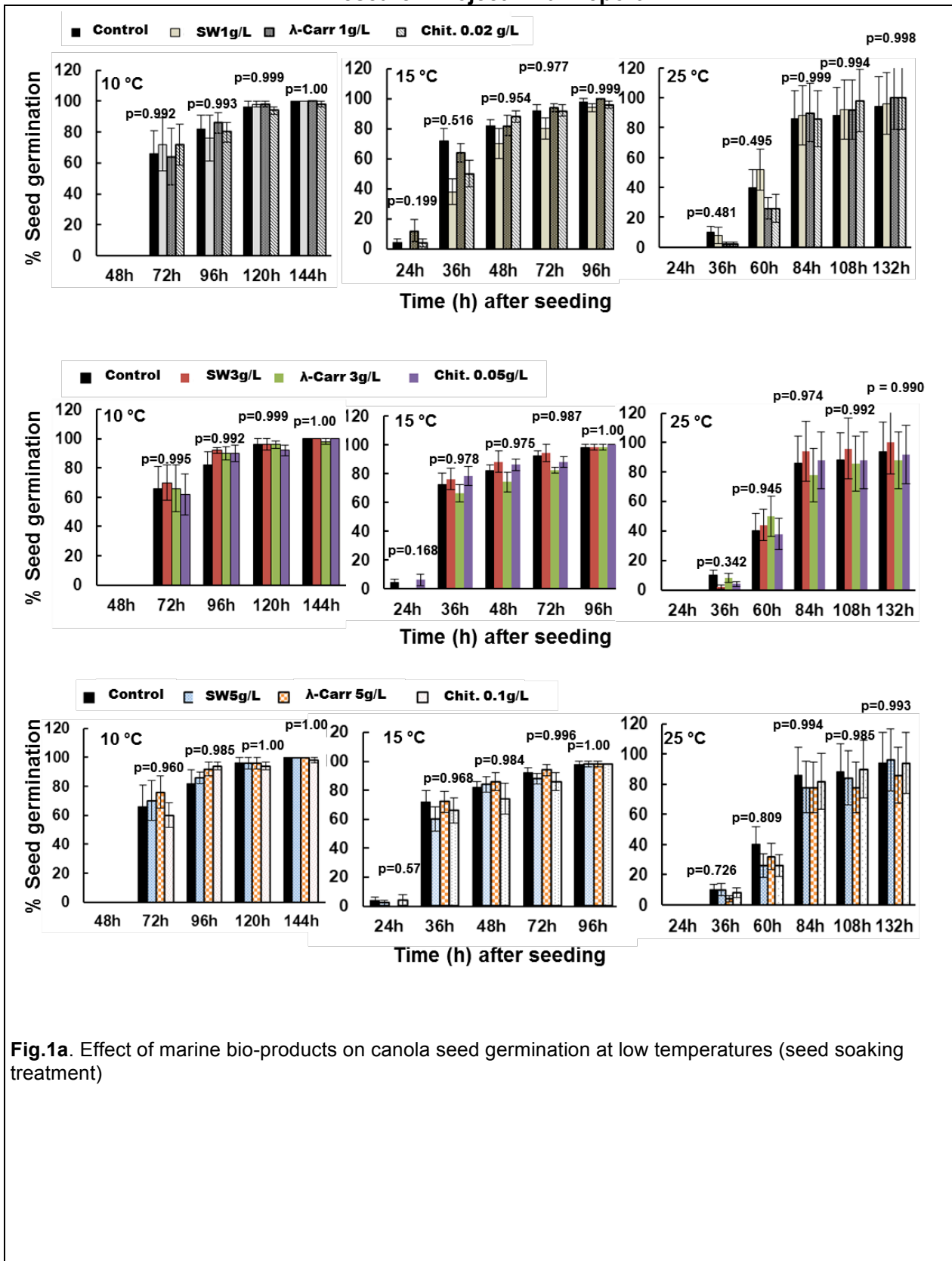


Fig.1a. Effect of marine bio-products on canola seed germination at low temperatures (seed soaking treatment)



AAFC RESEARCH BRANCH Research Project Final Report

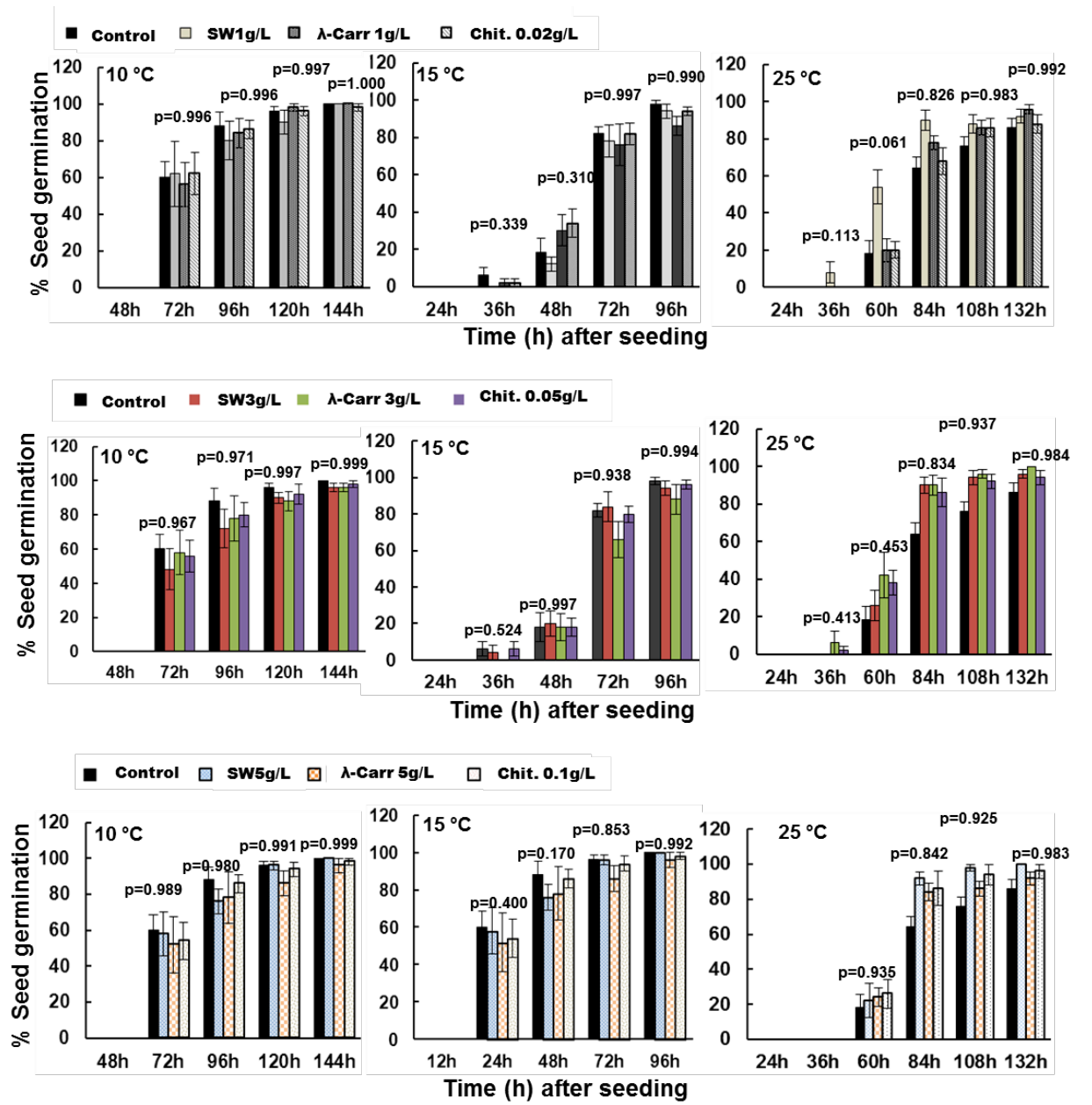


Fig.1b. Effect of marine bio-products on canola seed germination at low temperatures (seed coating treatment)

In contrast to soaking treatment, seed coating treatments at 10 and 15°C showed lower percentage of germination in all treatments as compared to control up to 96h after planting, although this difference was not significant. Surprisingly, at 25°C, control seeds either did not germinate at 36h after seeding or showed low germination whereas seeds with all three treatments (SW, λ-carrageenan and chitosan) emerged during this time.



AAFC RESEARCH BRANCH Research Project Final Report

Further, percentage of germination of control seeds was also significantly lower than after treatments with SW (3g/l), λ -carrageenan (3g/l) and chitosan (0.05g/l) at 60 to 108h after planting, indicating that seed coating treatments enhanced germination under greenhouse conditions (25°C), but no difference was observed between treatments at lower temperatures.

Plant height was also measured in different treatments one to two weeks after planting. With seed soaking treatment, the plant height at 10°C was reduced in all concentrations; however, no difference in plant height was observed two weeks after planting (figure 1c). No treatment effect was observed at 15°C. The average plant height with seed soaking treatment at 25°C was, however, altered in treatments (figure 1c). The results showed that at 25°C, plants from seeds treated with SW, λ -carrageenan and chitosan were taller than control plants.



AAFC RESEARCH BRANCH Research Project Final Report

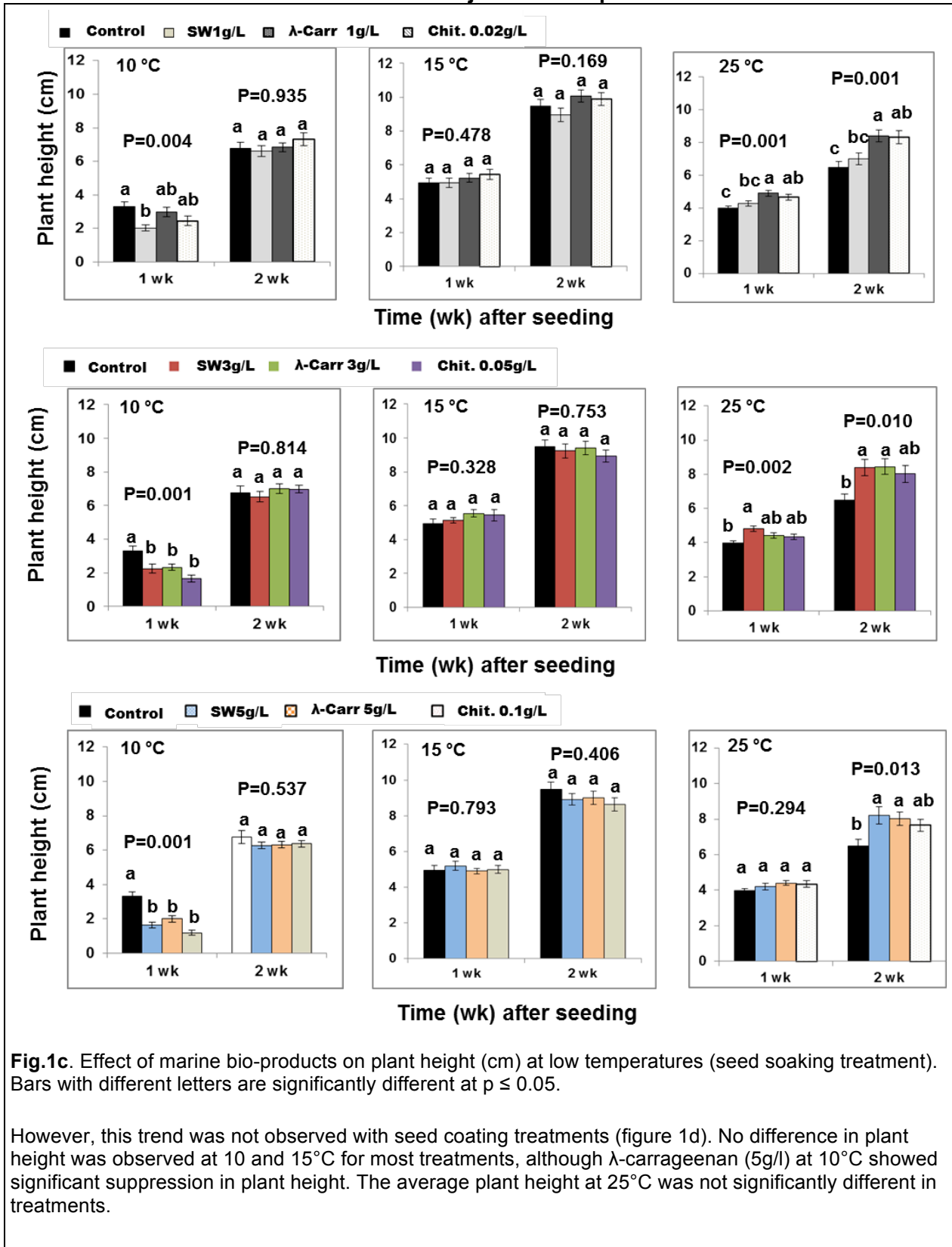


Fig.1c. Effect of marine bio-products on plant height (cm) at low temperatures (seed soaking treatment). Bars with different letters are significantly different at $p \leq 0.05$.

However, this trend was not observed with seed coating treatments (figure 1d). No difference in plant height was observed at 10 and 15°C for most treatments, although λ-carrageenan (5g/l) at 10°C showed significant suppression in plant height. The average plant height at 25°C was not significantly different in treatments.



AAFC RESEARCH BRANCH Research Project Final Report

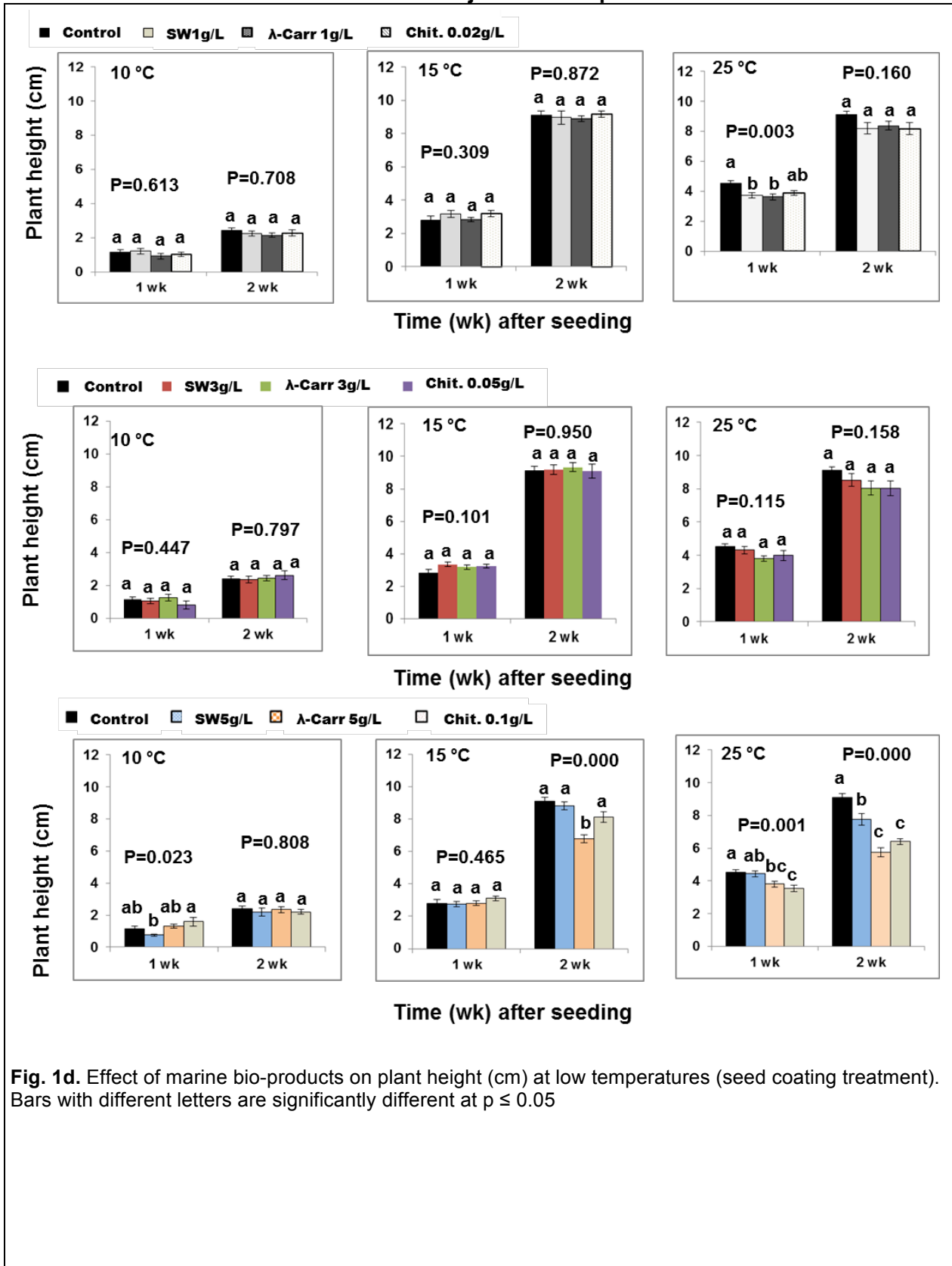


Fig. 1d. Effect of marine bio-products on plant height (cm) at low temperatures (seed coating treatment). Bars with different letters are significantly different at $p \leq 0.05$



AAFC RESEARCH BRANCH Research Project Final Report

Experiment 2: Effect of marine bio-products treatment on oilseed quality and fatty acid profile of canola

Methods: Marine bio-products have been shown to alter the fatty acid biosynthesis pathways in plants (Nair et al. 2012); this may affect the oil quality and yield in oil seed crops. To determine the effect of marine bio-products on the fatty acid profiles of canola seeds, greenhouse (2010-2011) and field experiments (2012) were conducted. For greenhouse experiments, plants were grown under greenhouse conditions until approximately 50% flowering. At this time, plants were spray treated until drip with λ -carrageenan (1g/l), seaweed extract (2g/l), chitosan (0.1g/l) and LANS control. The treatments were continued once a week for four weeks. The plants were arranged in a completely randomized design with 15 replicates per treatment and the experiment was repeated three times. Seed was harvested after maturity and fatty acid, oil content and protein dry matter were analyzed. Seed were crushed and the oil was extracted using hexane. Then, the oil was methylated (using sodium methoxide) to generate Fatty Acid Methyl Esters (FAME) for gas chromatography (GC) analysis using Agilent 7890A using standard protocol.

In the field experiments canola was planted at two different field locations; Truro, NS (Brookside Field 201, Dalhousie Agricultural Campus Farm) and Canning, NS (Lyndhurst Farms Ltd.). The Truro site was planted on May 26, 2012 while the Canning site was planted three separate times starting April 20th 2012. It was an unusually warm and dry spring and summer resulting in uneven germination and low crop density in Canning. The plots were laid out in a randomized complete block design with eight treatments and four replications per treatment. Recommended agronomic practice was followed. The crop was spray treated with λ -carrageenan (0.5, 1 and 2g/l), seaweed extract (SW; 1, 2 and 3g/l), fungicide Proline (check) and water (control). The plots were treated three times, with the first spray at the early flowering stage (approximately 35 days after planting), second and third treatments were done at weekly intervals. Treatments were applied at a rate of 400l/ha. At maturity, 50 plants per plot were harvested and used in GC analysis for oil and protein content as well as fatty acid profile.

Results. Marine bio-products SW, λ -carrageenan and chitosan did not significantly alter fatty acid profiles of canola under greenhouse conditions and the same was true for field experiments. A consistent fatty acid pattern was observed in the greenhouse and field sample (figures 2a & 2b). Under greenhouse conditions, although the difference between control and treatments [SW (2g/l), λ -carrageenan (λ -carr; 1g/l) and chitosan (0.1g/l)] appeared not significant, a minor difference was observed for fatty acids: 18:1 (oleic acid), 18:3 (alpha-linolenic acid) and 24:0 (lignoceric acid) between treatments. For example, 18:1 in the control was $69.54 \pm 0.19\%$ whereas it was $69.12 \pm 0.22\%$ in SW treatment and $68.81 \pm 0.36\%$ in chitosan, i.e. a small reduction. In contrast, the percentage of C18:3 in the control was 5.9 ± 0.08 and it was slightly higher in SW ($6.19 \pm 0.09\%$) and chitosan ($6.28 \pm 0.11\%$). The percentage of 24:0 for control was 0.25 ± 0.006 and it was 0.23 ± 0.004 in the chitosan treatment. These small differences may be an indication of treatment effect on fatty acid profile of canola that need further study.

In the field study, a lower percentage of fatty acid C18:1 (oleic acid) was observed with all three SW treatments (SW-1g/l= 63.96 ± 0.19 ; SW-2g/l= 63.78 ± 0.27 ; SW-3g/l= 63.97 ± 0.17) compared to the control ($64.03 \pm 0.19\%$), however, the differences were not significant. The % FA value of C18:3 in SW (0.2%) was 8.71 ± 0.08 and in λ -carrageenan was 8.49 ± 0.14 and the control was $8.61 \pm 0.05\%$.



AAFC RESEARCH BRANCH Research Project Final Report

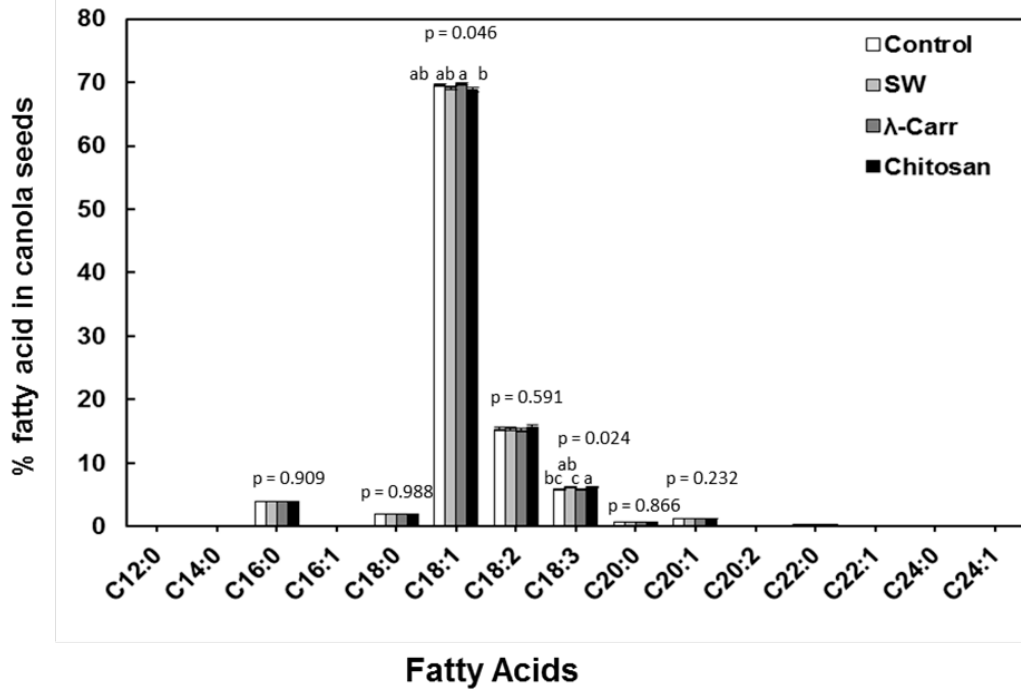


Figure 2a. Fatty acid (%) profile of canola seeds grown in greenhouse (2011).

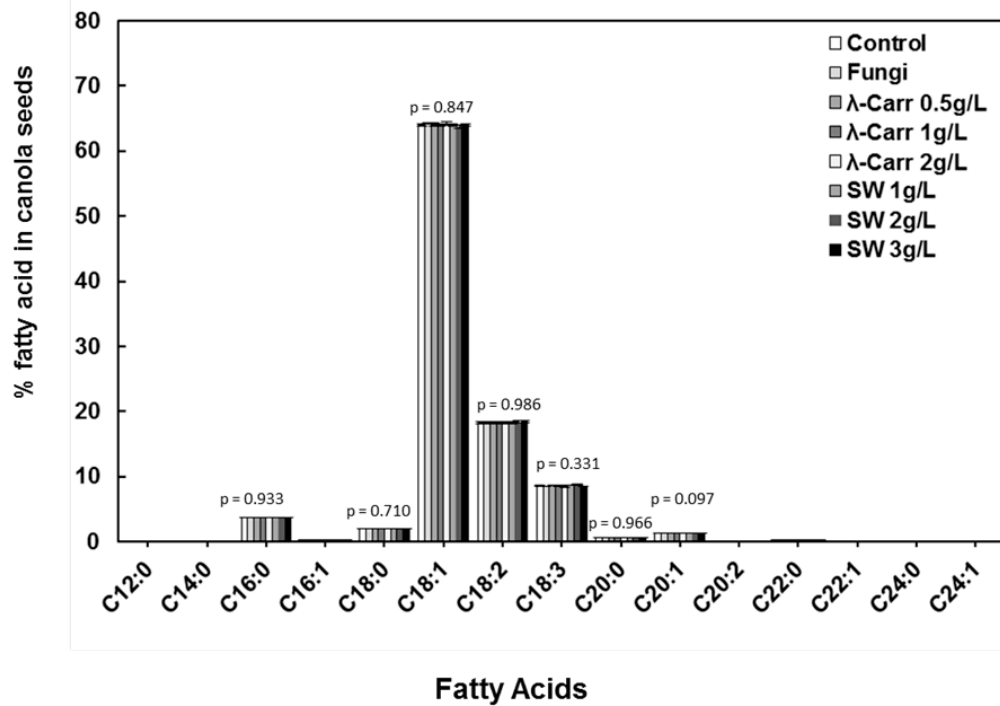


Figure 2b. Fatty acid (%) profile of field grown canola seeds (2012).



**AAFC RESEARCH BRANCH
Research Project Final Report**

A similar trend was observed with % oil content (OC) of seeds harvested from greenhouse and field. The results showed that the % OC of control plants was lower in both in the field and greenhouse, although not significant (figures 2c & 2d). When converted to relative 100 percent value of the control treatment and compared with the other treatments, the results revealed an increase in % oil content ranged from 0.5 -2.0 % (table 1).

Table 1. Oil content (%) with different treatments in greenhouse and field canola

| Oil (%) | Control | SW 1g/l | SW 2g/l | SW 3g/l | λ -Carr 0.5g/l | λ -Carr 1g/l | λ -Carr 2g/l | Fungi | Chit 0.1g/l |
|-------------------------|-------------------|----------------------|----------------------|----------------------|---------------------------|-------------------------|-------------------------|----------------------|----------------------|
| Field | 100 ± 0.90 | 100.10 ± 1.38 | 100.16 ± 1.58 | 100.01 ± 1.38 | 100.59 ± 1.48 | 101.39 ± 1.46 | 99.71 \pm 1.08 | 100.99 ± 0.70 | ---- |
| Green- house | 100 ± 0.34 | --- | 100.54 ± 0.67 | --- | --- | 101.35 ± 0.99 | --- | --- | 102.75 ± 0.59 |

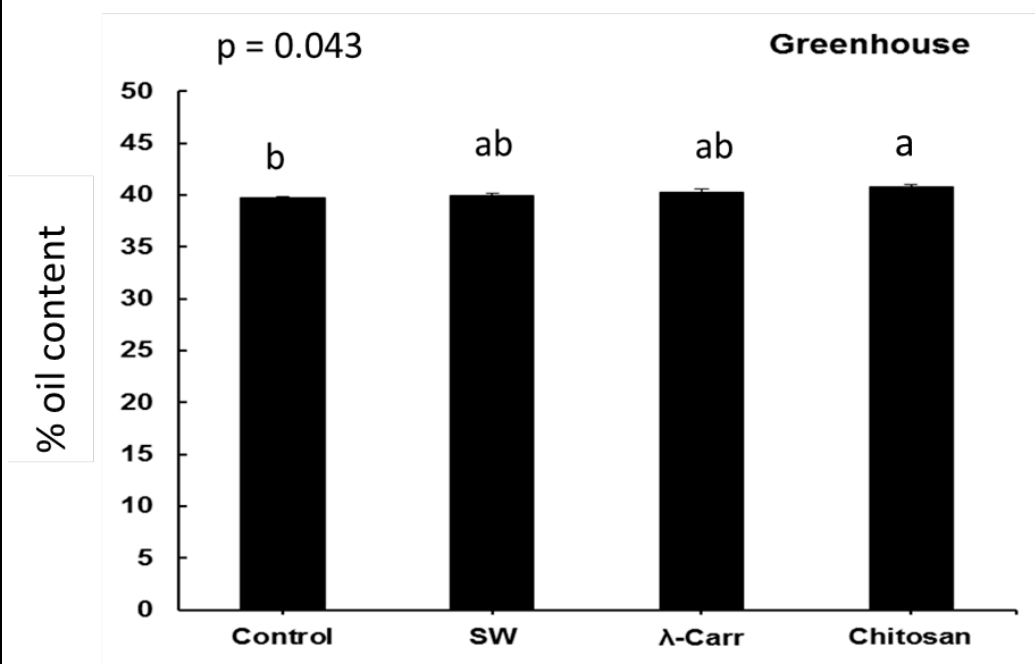


Figure 2c. Oil Content of canola seeds grown in the greenhouse (2012). Bars with different letters are significantly different at $p \leq 0.05$.

In the field samples, the SW treatment did not show significant difference in OC compared to the control whereas λ -carrageenan (1g/l) had 1.39% higher % OC, a trend very close to what was observed in the greenhouse. Under greenhouse conditions, chitosan (0.1g/l) increased OC by 2.75% compared to the control, whereas OC was 0.54% higher in SW (1g/l).



AAFC RESEARCH BRANCH
Research Project Final Report

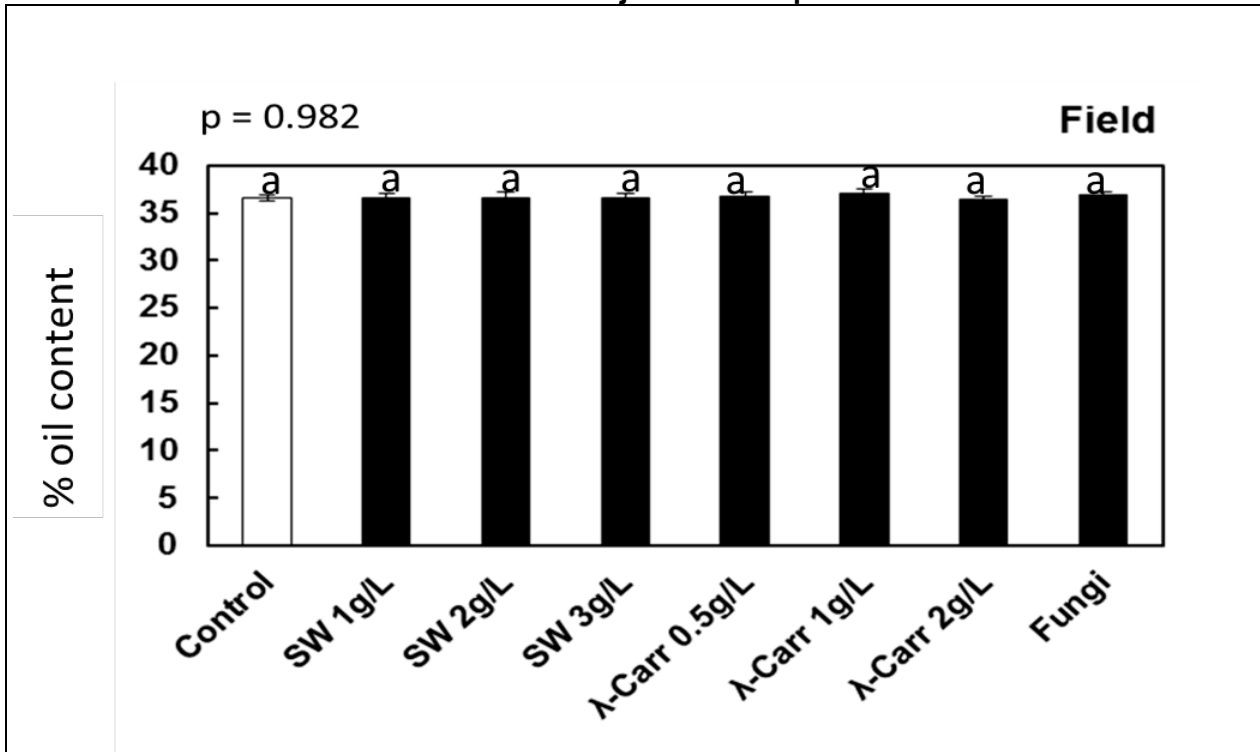


Figure 2d. Oil content of canola seeds grown in the field (2012). Bars with different letters are significantly different at $p \leq 0.05$

No significant differences were observed in % protein dry matter between treatments and the control (figures 2e & 2f).

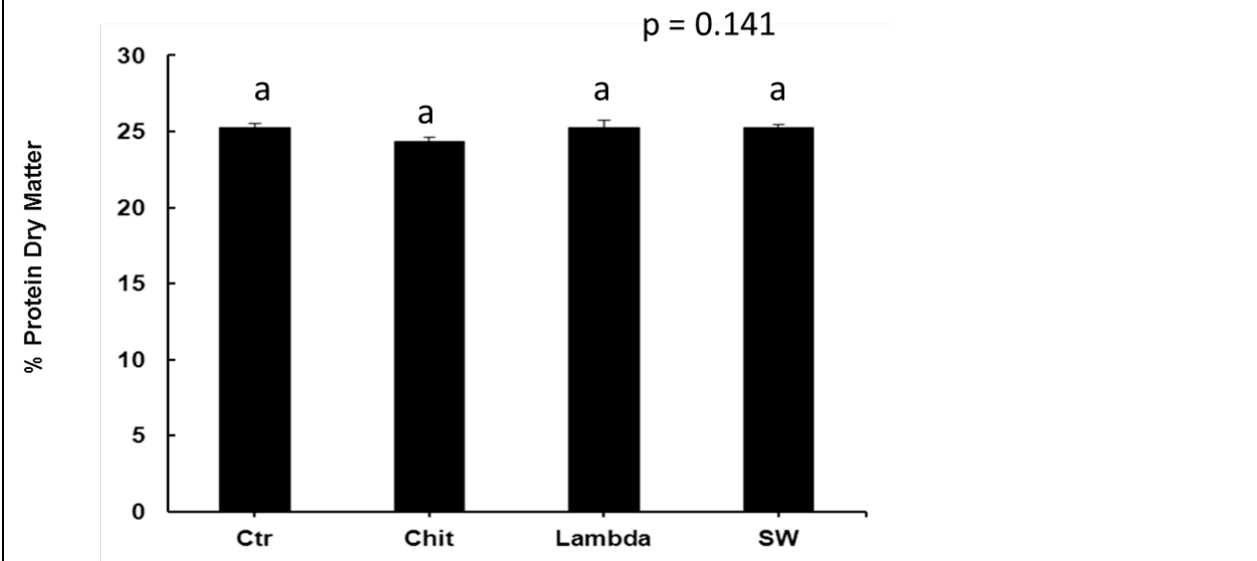


Figure 2e. Protein dry matter of canola seeds grown in greenhouse (2012). Bars with different letters are significantly different at $p \leq 0.05$



AAFC RESEARCH BRANCH
Research Project Final Report

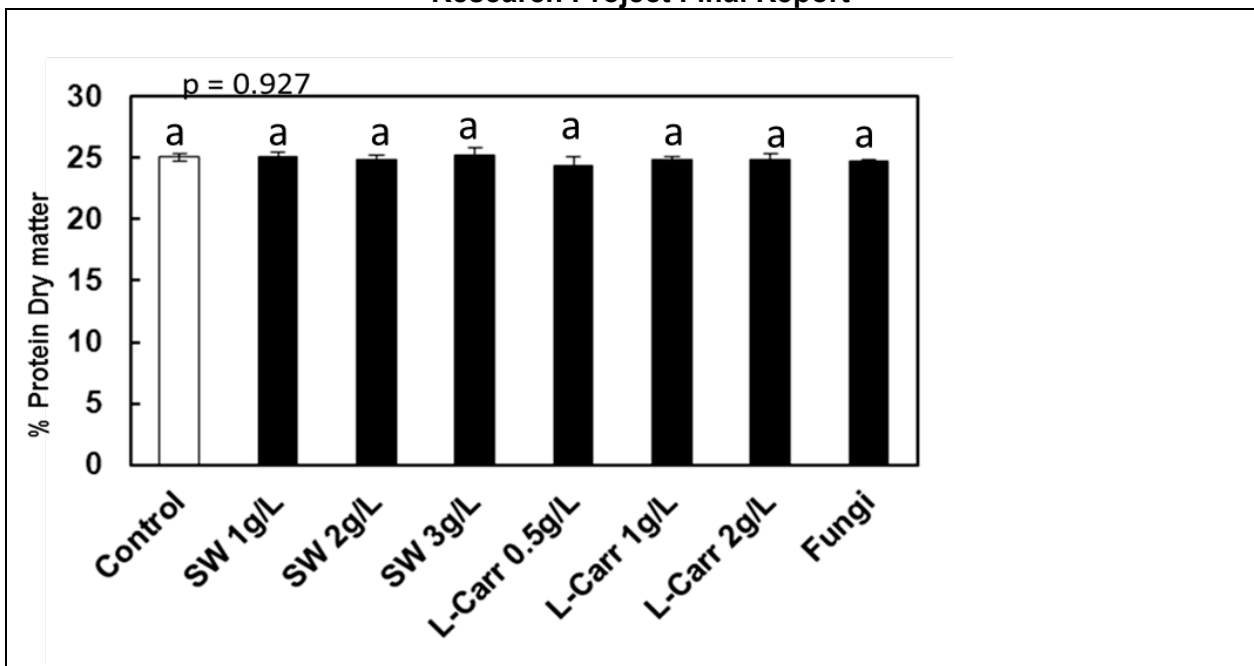


Figure 2f. Protein dry matter of canola seeds grown in the field (2012). Bars with different letters are significantly different at $p \leq 0.05$.

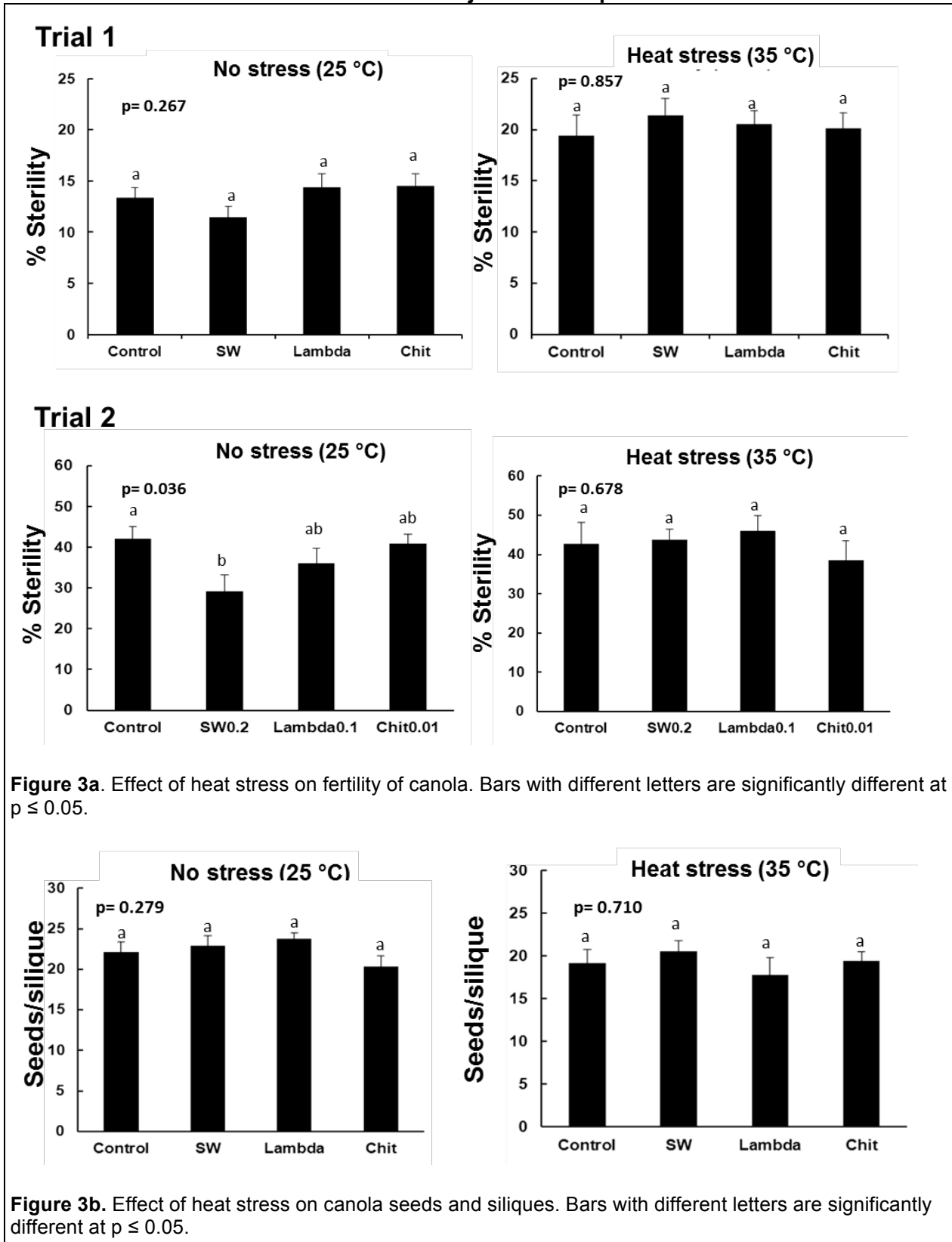
Experiment 3: Effect of marine bio-products treatment on sterility of canola at high temperature stress

Methods: Pollination, seed setting and seed maturity are affected by high temperature. Plants were grown in a greenhouse until about 50% flowering. At this time, plants were spray treated until drip with the following treatments: λ -carrageenan (1g/l), seaweed extract (SW; 2g/l), chitosan (0.1g/l) and a LANS control. The treatments were repeated at weekly intervals for four weeks. The plants were arranged in a completely randomized design with 15 replicates per treatment and the experiment was repeated three times. After first treatment, plants were placed in a growth chamber at 35°C and the subsequent treatments were carried out at this temperature. A second set of plants were given the same treatments, but were kept at greenhouse temperature (25°C). After maturity, seeds were harvested from both sets and data on the number of fertile and aborted siliques was collected. The observations were taken at six weeks after initiation of temperature stress by counting the developed siliques, undeveloped siliques and flowers, and healthy flowers. Percent sterility was determined in each treatment and compared with the control.

Results. The result showed that SW (2g/l) treatment reduced the percentage of sterility of canola at typical greenhouse conditions (25°C). Results from two trials reveal that the number of sterile flowers or dead siliques (sterility) was significantly lower in SW treatment (figure 3a). This effect was also evident with λ -carrageenan in one of the trials (figure 3a); however, the difference was not significant. When the plants were subjected to heat stress (35°C), SW or λ -carrageenan treatment did not protect against pollen abortion, although chitosan seemed to reduce sterility in second trial (figure 3a).



AAFC RESEARCH BRANCH Research Project Final Report





AAFC RESEARCH BRANCH Research Project Final Report

The results indicate that under optimal growth conditions, SW treatment reduced sterility. However, the number of seeds per silique or siliques per plant was not affected in the treatments (figure 3b).

Experiment 4: Effect of marine bio-products treatment on growth and yield of canola

Methods: To study the effect of marine bio-products on the canola seed yield, experiments were performed both in the greenhouse and in the field. Plants were grown under greenhouse conditions until approximately 50% flowering. At this time plants were spray treated until drip with λ -carrageenan (1g/l), seaweed extract (SW 2g/l), chitosan (0.1g/l) or LANS control. The treatment was repeated four times at weekly intervals. The plant was arranged in a completely randomized design with 15 replicates per treatment and the experiment was repeated three times. After the plants reached maturity they were harvested and number of siliques, total weight of siliques, seeds per silique and seed weight per plant were collected.

Field experiments were carried out at two locations. The first location was Truro, NS (Brookside Field 201, Dalhousie Agricultural Campus Farm), and the second location was in Canning, NS (Lyndhurst Farms Ltd.). The Truro site was planted on May 26, 2012, while Canning site was seeded three separate times starting April 20, 2012. It was an unusually warm and dry spring and summer resulting in uneven germination and low crop density in Canning. The plots were laid out in a randomized complete block design with eight treatments and four replications per treatment. The crop was spray treated with: λ -carrageenan (0.5, 1 and 2g/l), seaweed extract (SW; 1, 2 and 3g/l), fungicide (check) and water (control). The plots were treated three times, with the first spray at the early flowering stage (35 days after seeding), second spray followed one week later followed by the third another week after the second treatment. Treatments were applied at a rate of 400l/ha. At maturity, 50 plants per plot were harvested and the following observations were made: number of siliques, total weight of siliques, 1000 kernel weight and seed yield per plot.

Results.

Marine bio-products improved plant growth, both in the greenhouse and in the field (figures 4a & 4b). In detail, the following observations were made:

Number of siliques: Total number of siliques was higher in SW (2g/l) and chitosan (0.1g/l) treatments in the greenhouse whereas λ -carrageenan did not show any effect (figure 4a). A similar trend was observed in the field experiment: SW (2g/l) had a higher number of siliques per plant compared to the control (figure 4b). Surprisingly, this effect was not observed in SW 1g/l and 3g/l treatment. The number of siliques was higher in all three concentrations of λ -carrageenan and the difference was, however, not statistically significant.

Seeds per silique: The number of seeds per silique was higher in SW-2g/l and λ -carrageenan-1g/l treatments in the greenhouse grown plants and this effect was significantly different from the control (figure 4a). Similarly, under field conditions the number of seeds per silique was higher in SW-1g/l and 3g/l treatment, although not statistically significant (figure 4b). The number of seeds per silique with other treatments was similar to the control.

1000 kernel weight: The 1000 kernel weight was not affected by marine bio-products treatments, neither in the greenhouse (figure 4a) nor in the field (figure 4b).

Seed yield: The average seed yield per plant was not affected by treatments, although there was a higher number of siliques and seeds per siliques in the SW and λ -carrageenan treatments (figure 4a). The reason for this variation might be due to differences in the seed thrashing and cleaning process. To



AAFC RESEARCH BRANCH Research Project Final Report

rectify this, the hypothetical seed yield based on 1000 kernel weight was calculated, number of siliques per plant and number of seeds per silique and found, that seed yield could potentially increase with SW treatment (data not shown).

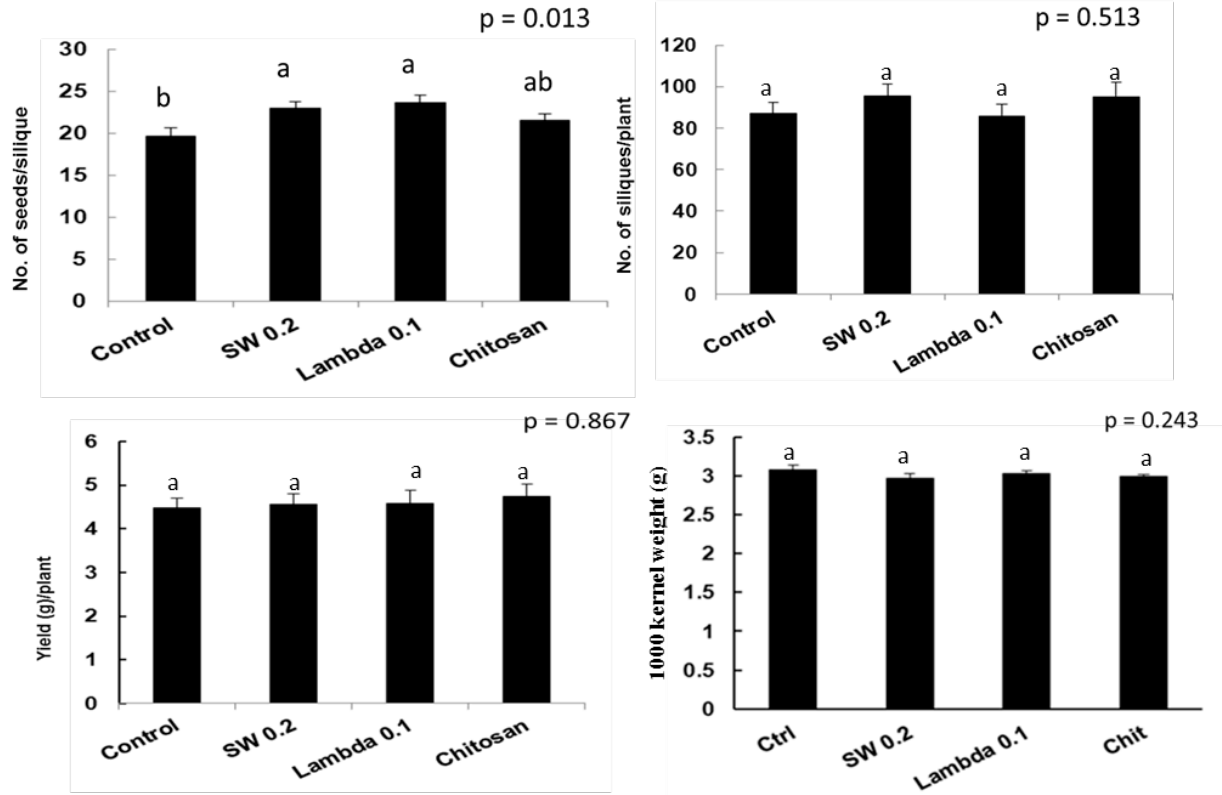


Figure 4a. Effect of marine bio-products treatment on canola yield in the greenhouse. Bars with different letters are significantly different at $p \leq 0.05$.

In the field, the average seed yields (g) per plant in the SW and λ -carrageenan treatments were higher than the control and the difference in seed yield with SW-0.2% was significant.

The yields in terms of kg/ha were:

| | | |
|-------------------------------|---|---------|
| Control | = | 2602.66 |
| SW 1g/l | = | 2695.33 |
| SW 2g/l | = | 2810.40 |
| SW 3g/l | = | 2771.62 |
| λ -carrageenan 0.5g/l | = | 2573.71 |
| λ -carrageenan 1g/l | = | 2729.27 |
| λ -carrageenan 2g/l | = | 2695.80 |
| fungicide check | = | 3022.26 |

and indicate, that marine bio-products in general increased canola yield compared to the untreated control.



**AAFC RESEARCH BRANCH
Research Project Final Report**

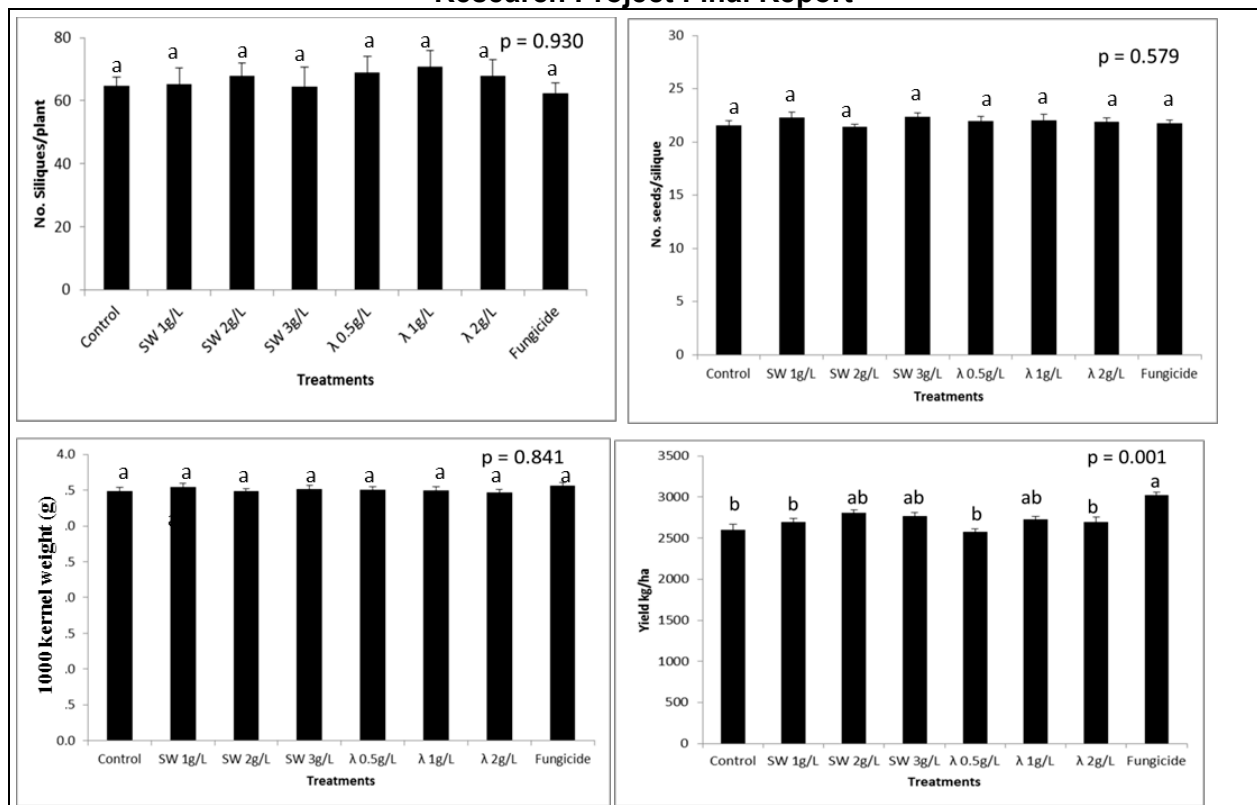


Figure 4b. Effect of marine bio-products treatment on canola yield in the field. Bars with different letters are significantly different at $p \leq 0.05$.

References:

Ackman, RG. (1990). Canola fatty acids—an ideal mixture for health, nutrition, and food use. *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*, pp 81-98.

Angadi, SV., Cutforth HW., Miller PR., McConkey BG., Entz MH, Volkmar K and S. Brandt (2000). Response of three Brassica species to high temperature injury during reproductive growth. *Can. J. Plant Sci.* 80:693–701.

Barve YY, Gupta RK, Bhaduria SS, Thakre RP and SE Pawar (2009). Induced mutations for development of *B. juncea* canola quality varieties suitable for Indian agro-climatic conditions In: *Induced Plant Mutations in Genomics Era*. Food and Agriculture Organization of the United States, pp: 373-375

Chouliaras, V., Tasioula M., Chatzissavvidis C., Therios I., and E. Tsabolidou (2009). The effects of a seaweed extract in addition to nitrogen and boron fertilization on productivity, fruit maturation, leaf nutritional status and oil quality of the olive (*Olea europaea* L.) cultivar Koroneiki. *Journal of the Science of Food and Agriculture*, 89: 984-988.

Colclough M., Blumwald E. and SJ Colombo (1990). The induction of heat tolerance in black spruce



AAFC RESEARCH BRANCH Research Project Final Report

- seedlings. In: Annual Meeting of the American Society of Plant Physiologists. Amer. Soc. Plant Physiol., Indianapolis, USA.
- Cox S., Abu-Ghannam N. and S Gupta (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*. 17: 205-220
- Craigie, JS (2011). Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* 23: 371-393.
- Ehlers JD and AE Hall (1998). Heat tolerance of contrasting cowpea lines in short and long days. *Field Crops Res.*, 55: 11–21.
- Eskin NAM., McDonald BE, Przybylski R, Malcolmson LJ, Scarth R, Mag T, Ward E and D. Adolph (1996). Canola oil. p. 1–96. In Y. H. Hui (ed.) *Edible oil and fat products: Oil and oil seeds*. John Wiley & Sons Inc., New York.
- Garrett KA., Dendy SP., Frank EE., Rouse MN. and SE Travers (2006) Climate change effects on plant disease: genomes to ecosystems. *Annu. Rev. Phytopathol.* 44: 489–509.
- Georgieva, K (1999). Some mechanisms of damage and acclimation of the photosynthetic apparatus due to high temperature. *Bulg. J. Plant Physiol.* 25:89–99.
- Khan, W., Rayirath UP., Subramanian S., Jithesh MN., Rayorath P., Hodges DM., Critchley, AT. Craigie, JS., Norrie J. and B. Prithiviraj (2009). Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* 28: 386-399.
- Moeller M and M. Smith (1998). The significance of the mineral component of seaweed suspensions on lettuce (*Lactuca sativa* L.) seedling growth. *J. Plant Physiol.* 153: 658-663.
- Morales D., Rodríguez P., Dell'amico J., Nicol'as E., Torrecillas A. and MJ S'anchez-Blanco (2003). High-temperature preconditioning and thermal shock imposition affects water relations, gas exchange and root hydraulic conductivity in tomato. *Biol. Plant.* 47: 203-208.
- Morrison MJ. and DW Stewart (2002). Heat stress during flowering in summer Brassica. *Crop Science*, 42: 797-803.
- Nair P., Kandasamy S., Zhang J., Ji X., Kirby C., Benkel B., Hodges MD., Critchley AT., Hiltz D. and B. Prithiviraj (2012). Transcriptional and metabolomic analysis of *Ascophyllum nodosum* mediated freezing tolerance in *Arabidopsis thaliana*. *BMC Genomics* 13: 643.
- Rayirath P., Benkel B., Mark Hodges D., Allan-Wojtas P., MacKinnon S., Critchley AT. and B. Prithiviraj (2009). Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in *Arabidopsis thaliana*. *Planta* 230: 135-147.
- Savant KD. And VS Kothekar (2011). Induction of variability in fatty acid profile in sesame (*Sesamum indicum* L.). *Journal of Phytology*, 3(12).
- Subramanian S., Sangha JS., Gray BA., Singh RP., Hiltz D., Critchley AT. and B. Prithiviraj (2011). Extracts of the marine brown macroalga, *Ascophyllum nodosum*, induce jasmonic acid dependent systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* pv.



**AAFC RESEARCH BRANCH
Research Project Final Report**

tomato DC3000 and *Sclerotinia sclerotiorum*. Eur. J. Plant Pathol. 131: 237-248.

Tan H., Yang X., Zhang F., Zheng X., Qu C., Mu J. and J Zuo (2011). Enhanced seed oil production in canola by conditional expression of *Brassica napus* LEAFY COTYLEDON1 and LEC1-LIKE in developing seeds. Plant physiology, 156: 1577-1588.

Thelen JJ and JB Ohlrogge (2002) Metabolic engineering of fatty acid biosynthesis in plants. Metab Eng 4: 12–21

Wally OSD., Critchley AT., Hiltz D., Craigie JS., Han X., Zaharia LI., Abrams SR. and B. Prithiviraj (2012). Regulation of phytohormone biosynthesis and accumulation in Arabidopsis following treatment with commercial extract from the marine macroalga *Ascophyllum nodosum*. J. Plant Growth Regul. 1-16.

Xu S., Li J., Zhang X., Wei H. and L Cui (2006). Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environ. Exp. Bot. 56: 274-285.

Zhang X and E Ervin (2008). Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. Crop Sci. 48: 364-370.

Zhang X., Ervin E. and R Schmidt (2003). Plant growth regulators can enhance the recovery of Kentucky Bluegrass sod from heat injury. Crop Sci. 43: 952-956.

Zheljzakov VD., Vick BA., Baldwin BS., Buehring N., Astatkie T. and B Johnson (2009). Oil content and saturated fatty acids in sunflower as a function of planting date, nitrogen rate, and hybrid. Agronomy journal, 101: 1003-1011.

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

- Include the name of scientist / organization.

Claude Caldwell, Dalhousie University

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




**AAFC RESEARCH BRANCH
Research Project Final Report**

| |
|---|
| constraints. |
| There was no deviation from the proposed research |

| |
|--|
| D. Impact Assessment (if applicable, describe how the variance factors above will impact project continuation) |
| <ul style="list-style-type: none"> • Include changes to the objectives, changes to the project work plan / budget, changes to performance (i.e. meeting targets). |
| None |

| |
|---|
| E. Achievements (include only those related to this project) |
| <ul style="list-style-type: none"> • Include innovations, publications / conferences, technology transfer, capacity building, success stories, media, recognition and other outputs. |
| Two manuscripts are under preparation for publication. |

| |
|--|
| F. Lessons learned (self-evaluation of project) |
| Marine bio-products have potential to improve seed yield and stress tolerance. The results from this project showed improved yield and stress tolerance, although non-significant. It appears that modifying the time and rate of application might yield significant results. |

| | | |
|-------------------------|--------------|---|
| Balakrishnan Prithviraj | May 30, 2013 |  |
| 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.