



**AAFC RESEARCH BRANCH
Research Project Final Report**

Developing Innovative Agri-Products Program (Vote 10 Funding)

Project Title:	Activity B.3: Integrated management of stem rot (<i>Sclerotinia sclerotiorum</i>) of canola in Eastern Canada including ON, QC, NB, NS and PEI
Start Date (yyyy-mm-dd):	2011-04-01
Expected End Date (yyyy-mm-dd):	
Actual End Date (yyyy-mm-dd):	2013-03-31
Principal Investigator (PI):	Balakrishnan Prithiviraj
Short Executive Summary of report:	
<p>In this project over one hundred canola genotypes were screened for stem rot resistance. However, the screening was not fruitful due to low incidence of stem rot at experimental sites. Similar issue prevailed in the field sites in which seven fungicides were screened for their efficacy against stem rot disease in Eastern Canada.</p> <p>Testing marine bioproducts for the management of stem rot disease yielded two potential candidates: <i>Ascophyllum nodosum</i> extract (=seaweed extract) and λ-carrageenan. Both the products reduced the incidence and severity of the disease. Further field evaluation of these products is recommended.</p>	

<p>A. Research Progress and Accomplishments (to date in relation to expected milestones and deliverables / outputs)</p> <ul style="list-style-type: none"> • Include brief summary of: <ul style="list-style-type: none"> - 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.
<p><u>The major objectives of this part of the project were as follows:</u></p> <p>Sub-Activity B.3.1: <i>In vitro</i> production of <i>Sclerotinia sclerotiorum</i> resistant lines from genotypes adopted to Eastern Canada</p> <p>Sub-Activity B.3.2: Greenhouse and field trial of <i>in vitro</i> selected SCR lines in Eastern Canada</p> <p>Sub-Activity 3.3.1 Testing efficacy of seed treatment for control of stem rot</p> <p>Sub-Activity 3.3.2 Testing fungicide spray for control of stem rot</p> <p>Sub-Activity 3.4.2. Selection of stem rot resistant elite germplasm</p> <p>Sub-Activity 3.5.1. Test the effect of planting density on stem rot severity</p> <p>Sub-Activity 3.5.2. Test the effect of time of planting on stem rot severity</p> <p>Sub-Activity 3.5.3 Test the effect of crop rotation on stem rot severity</p> <p>Sub-Activity 3.6 Development of marine bioproducts to reduce stem rot disease</p> <p><u>Summary of deliverables achieved:</u></p> <p>Sub-Activity B.3.1 and B.3.2: Final list of 12 canola lines developed with pre-screened resistance to <i>Sclerotinia</i> after field testing in Ontario and were field tested in 2011 in PEI and Quebec by DIAP participants.</p> <p>Sub-Activity 3.3.1 Harrington, PEI: The effect of seed treatment of canola with Prosper FL was evaluated. Seed treatment did not have an effect on the <i>Sclerotinia</i> stem rot or on yield and seed weight of canola. It should be noted that there was a low incidence of disease in the experimental plot.</p>



AAFC RESEARCH BRANCH Research Project Final Report

Sub-Activity 3.3.2

Harrington, PEI: The effect of fungicide spray was evaluated for the management of stem rot disease. Two trials were conducted. The first trial was with cultivar Invigor 5440. The following fungicides were evaluated: Tilt, Quadris, Abound, Lance, Proline, Astound and Headline. In the second trial two cultivars, Invigor 5440 and 5030, were used with fungicides: Lance, Proline and Prosaro. There was no significant effect of the treatment in both trials. This might be due to low incidence of the disease (less than 5%). Normandin, QC: Five fungicides: Lance, Proline, Prosaro, Serenade and Astound were evaluated with three cultivars (Invigor 5440, Invigor 5020 and Invigor 5030) on the incidence and severity of stem rot. There was no incidence of the disease in the 2011 and 2012 cropping seasons. The effect of fungicide spray on yield and seed weight was insignificant.

Sub-Activity 3.4.2:

Harrington PEI: Two trials were conducted on elite germplasm for resistance to *Sclerotinia* stem rot. One involved a yield scale evaluation of 12 advanced lines of canola originating from Laima Kott, University of Guelph. The second trial involved approximately 100 lines from Muhammad Tahir, University of Manitoba. Several genotypes from Manitoba did not germinate and, therefore, could not be evaluated for stem rot resistance. There was no significant difference in the disease incidence or severity between the lines obtained from Guelph.

Sub-Activity 3.5.1:

Harrington, PEI: The effect of three seeding rates (2.5, 5 and 7.5kg seed per hectare) on the incidence and severity of stem rot was evaluated. There was no significant effect of the seeding rate on stem rot disease.

Sub-Activity 3.5.2.

Harrington, PEI: The effect of three planting dates (May 23, May 30 and June 6, 2011) on the incidence of stem rot disease was evaluated. There was no significant effect of planting dates on the incidence and severity of the disease. However, the early planted crop had higher incidence of disease although the number was statistically insignificant.

Sub-Activity 3.5.3

PEI: A survey was initiated on 15 farms. There were no major foliar diseases observed in these farms. *Sclerotinia* stem rot was widespread in the farms, although at low levels. It appears that multiple observations are required to draw meaningful conclusions.

Sub-Activity 3.6 Four marine bioproducts (λ -carrageenan, ι -carrageenan, extract of the brown seaweed *Ascophyllum nodosum* (SW), and chitosan) were evaluated for their efficacy to reduce stem rot disease in the greenhouse. Three products (λ -carrageenan, SW and chitosan) showed promising results. SW and λ -carrageenan were tested under field conditions during 2012 cropping season. A significant reduction in disease incidence and severity was observed in both the treatments. SW was the most effective in reducing the disease.

Detailed description of activities:

Sub-Activity B.3.1 and Sub-Activity B.3.2: (Laima Kott)

Introduction, literature review, objective and deliverables

The University of Guelph's contribution to the project was to generate a number of *Sclerotinia* resistant/tolerant doubled haploid (DH) lines adapted to Eastern Canada that were to be distributed to DIAP collaborators for field trials. The **method used** in development of the *Sclerotinia* resistant lines was developed in the Kott lab many years ago (Kott et al. 2002). This method had since been proven in *Sclerotinia*-specific US National field trials in North Dakota where some Kott lines were entered. In official testing with a pathogen controlled environment in the North Dakota *Sclerotinia* Canola Variety Trial in Carrington, the DH canola lines performed very well. For example, in 2005, of 28 mostly commercial entries, four of the *in vitro* selected lines scored first, third, fourth and ninth for percent 'incidence of disease', where the scores were 4.5, 9.5, 11.0 and 14.5, respectively. The mean 'incidence of disease' for this trial was 27.0 and ranged from 4.5 to 68.0 percent (unpublished US Nat. *Sclerotinia*



**AAFC RESEARCH BRANCH
Research Project Final Report**

trials). For the DIAP project, the **objective** was to generate some lines adapted to Eastern Canada that carried some *Sclerotinia* tolerance or resistance. The **deliverables**, in 2011, were twelve canola lines developed with pre-screened resistance to *Sclerotinia* and they were forwarded for field testing in PEI (Richard Martin) and in QC (Denis Pageau), DIAP participants. In 2012, eleven new selected DH lines from a field of 477 lines produced for *Sclerotinia* resistance were forwarded for field testing again in PEI and in QC.

Methodology B.3.1.

Because DH production takes at least one year to complete, the lines offered for the first field season were existing lines from the Guelph canola breeding program generally adapted to Ontario conditions. All lines were generated via the microspore culture method of DH production. For *Sclerotinia* resistant DH production, during the single cell stage, spores were exposed to a mild UV treatment and subsequently screened *in vitro* with a chemical selection agent, oxalic acid. All surviving haploid plants were carried through to the three to four leaves stage in soil. In the first field season of this project the twelve DH lines were produced in this manner.

The following year, DH lines were initiated from crosses made between elite cultivars and root maggot resistant parents. All DHs were generated by the microspore culture method. This activity yielded 477 lines that were selected for *in vitro* for *Sclerotinia* resistance and subsequently were also vigorously screened for *Phoma* as well (see sub-activity B.3.2; below). After screening all remaining haploids were doubled by the colchicine method for diploid production. Diploid lines produced in this way were then planted for seed increase to be subsequently used in field trials.

Methodology B.3.2.

Haploid plants produced and selected with oxalic acid in the microspore method were then subjected to a secondary screen using the third and fourth leaf from the surviving plants. The following leaf wilt test was performed: two severed leaves from each plant were tested in concentrations of 80 and 200mM of oxalic acid in order to identify the most resistant lines. After 18 hours of exposure to the acid, resistance was determined by whether the leaf was turgid/green or flaccid/pale. Lines that scored 1 or 1.5 (out of 3) were considered resistant/tolerant and were maintained. Selected haploid plants were doubled and brought to flowering and seed set. All lines offered in 2011 had been previously field tested for agronomic traits, but not screened specifically for SCR. Seed of 12 lines was sent to Denis Pageau, and to Richard Martin, in early spring of 2011. The lines were: SCX9Y1-1, SCX9Y1-3, SCX9Y1-4, SCX9Y1-6, SCX9Y1-7, SCX9Y1-11, SCX9Y1-14, SCX9Y2-1, SCX9Y2-9, SCX9Y2-11, SCX9Y2-15 and SCX9Y2-16.

Table 1: Eleven new selected DH lines were available for field testing in 2012 (indoor scores for *Sclerotinia* (1-1.5 = best) and *Phoma* (~1 = best))

Line	SCR	PHR
SC08001	1.0	3.6
SC08016	1.0	1.0
SC08029	1.0	1.0
SC08044	1.0	0.2
SC08045	1.0	0.2
SCX9CB02A-21	1.0	0.1
SCX9CB02A-48	1.5	0.9
SCX9CB02A-56	1.5	0.7
SCX9CB02A-61	1.5	0.7
SCX9CB02A-89	1.0	0.3
SCX9CB02A-133	1.0	0.3
Scoring value range	1-3	1-9

Concurrently, new SCR lines were being produced for field tests in 2012 in a similar manner as described above. *In vitro Sclerotinia*-selection yielded 477 haploid lines and were rescreened using the



AAFC RESEARCH BRANCH Research Project Final Report

leaf wilt test. Once the level of *Sclerotinia* resistance was known, the best DHs were then screened for *Phoma*. Scoring for *Sclerotinia* was done on two haploid leaves from each plant based on a one to three value, where 1-1.5 is acceptable (2-3=worst; table 1). Scoring for *Phoma* was done on DH seed seedlings at the cotyledon stage, using *Phoma* inoculum. Damage was scored on two cotyledons of six seedlings using a one to nine value scale, where a mean value of one to three was acceptable (9 = worst; table 1).

Results

Results of the stem rot resistance field testing are reported in sub-activity 3.4.2 by the collaborators running the trials.

References

Kott, L., I. Kyrchenko and R. Fletcher. 2002. *Sclerotinia* resistance in *Brassica napus* derived from *in vitro* UV-induced mutations. In: International Conference "Biotechnology Approaches for Exploitation and Preservation of Plant Resources", 26-31 May 2002, Yalta, Ukraine. pp. 84-85.

Sub-Activity 3.3.1 (Richard Martin and Aaron Mills)

In 2011 one seed treatment was evaluated, Prosper FL. Prosper is a broad spectrum seed treatment which includes an insecticide (clothianidin) and three fungicides (carbathiin, thiram and a small rate of metalaxyl). Untreated L130 Invigor seed was treated in a small batch seed treater at a rate of 12.5ml product per kg of seed. While the treatment was relatively broad spectrum it had no impact on *Sclerotinia* stem rot or on basic agronomic characteristics of plot yield or seed weight. Results are contained in table 2.

Table 2: Influence of fungicide seed treatment on *Sclerotinia* in canola and yield components, AAFC – Harrington Research Farm, 2011.

Treatment	Aug 18			Aug 25			Plot yield (g)	TSW (g)
	Infected plants (%)	Mean severity infected plants (1-9)	Mean Severity plot (1-9)	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)		
Control	1.7	8.1	9.0	4.2	8.2	8.9	1888	2.8
Prosper FL	0.8	8.6	9.0	7.1	7.4	8.9	2041	2.8
LSD (0.05)	ns	ns	ns	ns	ns	ns	201.7*	ns

significant at p=0.094; *Sclerotinia* ratings where from 1=severe disease to 9=no disease
TSW = thousand seed weight

It should be noted that apart from a low stem rot infection (less than 10%) there was no significant foliar disease symptoms evident in this trial.

Insect dynamics were not assessed, however it was noted that the level of flea beetle just after emergence was less in the treated plots when compared to the untreated plots.

Sub-Activity 3.3.2 (Richard Martin, Aaron Mills and Denis Pageau)

Field trials 2011:

Fungicide evaluations for stem rot were in two forms. The first was the evaluation of the materials in canola. The second involved the efficacy of a number of treatments in crambe. Crambe has a similar architecture to canola and was used as a test crop because of its very high susceptibility to *Sclerotinia* infection, much higher than in canola. It was thus felt that crambe may offer the opportunity to select



AAFC RESEARCH BRANCH Research Project Final Report

materials with a higher probability of success in canola, where infection levels can be variable between years which make testing more of a challenge. Two trials were run on foliar fungicide control in canola. The first trial was on Invigor 5440. For this foliar trial the treatments were in a complete randomized block with six replicates being used for stem rot measurement, but only four replicates being actually harvested, as a result of time and staff pressures at harvest. Treatments included: Tilt (propiconazole), Quadris (azoxystrobin), Abound (azoxystrobin), Lance (boscalid), Proline (prothioconazole), Astound (cyprodinil and fludioxonil), and Headline (pyraclostrobin). The second trial was conducted on two cultivars: Invigor 5440 and Invigor 5030. In this trial Lance (boscalid), Proline (prothioconazole) and Prosaro (prothioconazole and tebuconazole) were applied. The experimental outline was similar to the single cultivar evaluation trial. Cultivar was the main effect. For both trials, crop management followed recommended production criteria. Applications for both trials were made with a small plot sprayer at a 20% flowering stage of crop development.

Canola: *Sclerotinia* levels were relatively low in the trial with an incidence of less than 5% in canola and there was no significant effect of any treatment on disease incidence or severity. From tables 3 and 4 it can be seen that there was an increase in incidence between the two sampling times but since the disease was so variable between treatments and replicates there were no significant impacts on disease or even yield ($p=0.05$) between treatments.

Table 3: Efficacy of fungicide foliar applications for the control of *Sclerotinia* in canola, AAFC – Harrington Research Farm, 2011.

Treatment	Rate (product /ha)	Aug 18			Aug 25			Yield (kg/ha)	TSW (g)
		Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)		
Control	0	0.6	8.7	9.0	3.1	7.0	9.0	2327	2.75
Tilt	500 ml	1.1	8.4	9.0	0.8	8.2	9.0	2539	2.75
Quadris	310 ml	0.0	9.0	9.0	2.2	8.2	9.0	2210	2.78
Abound Flowable	700 ml	1.7	8.5	9.0	3.1	8.2	9.0	2278	2.79
Lance	350 g	1.4	8.6	9.0	2.5	8.4	9.0	2391	2.82
Proline	350 ml	0.8	8.5	9.0	1.1	8.0	9.0	2560	2.80
Astound	775 ml	1.1	8.7	9.0	1.9	7.9	9.0	2301	2.76
Headline	600 ml	0.8	8.5	9.0	4.7	8.1	8.9	2533	2.77
Headline + Lance	600 ml + 350 g	0.3	8.8	9.0	1.4	8.3	8.9	2540	2.86
LSD (0.05)		ns	ns	ns	ns	ns	ns	281.9*	ns
SEM		0.61	0.23	0.01	1.09	0.38	0.02	96.6	0.06
Reps		6	6	6	6	6	6	4	4

Note: yield was not significant at $p=0.05$, but was at 0.094 level of probability

However, Tilt, Proline and Lance in the first trial (table 3) did hint at some potential, even though not significant. There was also no significant impact on yield at the $p=0.05$ level of probability. The lack of any significant symptoms of any foliar disease in 2011 may have been part of the reason that there was a lack of a yield benefit associated with the fungicide applications.

Crambe: The treatments in crambe were different to those in canola as this was the second year of evaluation and it was desirable to maintain the same treatments in 2011 as were used in 2010. In this case Lance, Proline, Quadris and Switch (cyprodinil and fludioxonil) were evaluated for *Sclerotinia* control. Table 5 contains the results of this trial. While there was little significant differences in infection and severity, there was a tendency for a reduction from the products with the best response being with



**AAFC RESEARCH BRANCH
Research Project Final Report**

Proline.

Table 4: Efficacy of fungicide foliar applications for the control of *Sclerotinia* in canola, AAFC – Harrington Research Farm, 2011.

	Rate (product/ha)	Aug 18			Aug 25			Plot yield (g)	TSW (g)
		Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)		
Cultivar									
Invigor 5030		2.2	8.6	9.0	4.5	8.1	8.9	2472	3.06
Invigor 5440		1.1	8.6	9.0	4.5	8.2	8.9	2566	2.79
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns
SEM		0.49	0.46	0.01	1.10	0.06	0.01	29.3	1.028
Reps		6	6	6	6	6	6	4	4
Treatment									
Control		0.1	8.9	9.0	3.2	8.3	9.0	2436	3.75
Lance	350 g	2.0	8.5	9.0	5.3	8.0	8.9	2574	4.17
Proline	315 ml	3.2	8.6	9.0	3.9	8.3	8.9	2489	3.12
Prosaro	800 ml	1.4	8.6	9.0	5.7	8.0	8.9	2575	3.96
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns
SEM		0.89	0.16	0.01	1.41	0.25	0.02	45.9	5.308
reps		6	6	6	6	6	6	4	4

Interestingly, there was actually a significant increase in the severity of disease on infected plants with Switch and Quadris. Relative to yield benefits all treatments, with the exception of Quadris, had a positive effect. The most effective component for yield benefit was with Proline at a yield increase of 121%. This may have been due to effects on stem rot infection at stages after the last rating period, on physiology of the plant unrelated to a pathogen control effect, or on other diseases (although there were no major foliar diseases other than stem rot).

Table 5: Efficacy of fungicide foliar applications for the control of *Sclerotinia* in crambe, AAFC – Harrington Research Farm, 2011.

Treatment	Rate (product/ha)	Aug 18				Aug 30	Yield (kg/ha)	TSW (g)	HI weight (g)
		Mean infected plants (#/60)	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)	Whole plot disease rating (1-9)			
Control	0 g	51.2	85.3	6.2	6.7	2.4	1149	5.48	30.62
Lance	350 g	45.8	76.3	6.3	6.9	3.8	1984	5.72	31.74
Lance (2 appl.)	350 g +350 g	49.0	81.7	6.4	6.9	4.8	1837	6.40	33.17
Proline	350 ml	42.4	70.7	6.4	7.1	6.7	2547	6.76	32.99
Quadris	700 ml	45.4	75.7	6.5	7.1	2.6	1362	5.50	30.31
Switch	775 g	46.0	76.7	6.8	7.4	5.4	2197	6.56	33.62
LSD (0.05)		(12.45)	(20.74)	0.2589	(0.5472)	2.158	579.1	0.784	1.782
SEM		4.22	7.03	0.0878	0.1855	0.723	196.3	0.266	0.604
		ns	ns		ns				

Sclerotinia scale: 1=prematurely ripened (plants dead) to 9=no symptoms
Lance (2 appl.): first application at 20-30% bloom, second at 100% bloom (7-14 days later)



AAFC RESEARCH BRANCH Research Project Final Report

Field trials 2012:

Two experiments were conducted to measure the effects of foliar fungicides for the control of *Sclerotinia* stem rot in canola. This experiment was the second year of a two year study.

The first experiment used Invigor 5440 and consisted of seven fungicide treatments and an untreated control. There were seven products that were evaluated against an untreated control. Plots were planted under a randomized complete block design with four replicates. All fertility was applied and incorporated pre-plant and consisted of 120kg/ha of total N and 50kg/ha each of total P and K. Sulfur was applied at a rate of 20kg/ha and liquid boron was applied at a rate of 2kg/ha and was delivered as part of a tank mix with the pre-emergent herbicide Bonanza. Products were selected based on registration, availability, and results from previous fungicide evaluations in other jurisdictions (table 6).

Table 6: Products used for experiment 1

Product	Rate	Active	Manufacturer
Tilt 250 EC	500 ml/ha	Propiconazole 250g/l	Syngenta
Quadris	310 ml/ha	Azoxystrobin 250g/l	Syngenta
Abound Flowable	700 ml/ha	Azoxystrobin 250g/l	Syngenta
Propel	500 ml/ha	Propiconazole 250g/l	Syngenta
Lance	350 g/ha	Boscalid 70%	BASF
Proline 480	350 ml/ha	Propiconazole 480g/l	Bayer
Astound	775 ml/ha	Cyprodinil 37.5%, Fludioxil 25%	Syngenta

The second experiment was conducted in a similar manner to the first experiment, however Invigor 5330 was also included and the number of fungicides was pared down (table 7). The second experiment was planted using all methods described above for the first experiment.

Table 7: Products used to measure fungicide x variety interaction for control against *Sclerotinia*

Product	Rate	Active	Manufacturer
Lance	350g/ha	Boscalid 70%	BASF
Proline 480 EC	350 ml/ha	Propiconazole 480g/l	Bayer
Prosaro 250 EC	800 ml/ha	Tebuconazole and prothioconazole	Bayer

Results/discussion:

Overall there was zero incidence of *Sclerotinia* in any of the plots for the duration of the season. Also, there were no significant differences between any of the products for any of the agronomic measurements. There was, however, a significant amount of *Alternaria* leaf spot in the plots. Although there were no significant differences between product treatments in terms of yield, there was a significant negative regression relationship between *Alternaria* rating and yield in both experiments (figure 1 and figure 2). As *Alternaria* tends to be a saprophytic organism, it is not likely that there was an effect of fungicide treatment on *Alternaria*. Infection happens later in the season, therefore, although there was a strong correlation between increasing disease incidence and decreasing yields, there was no clear effect of fungicide application on disease incidence either way.



**AAFC RESEARCH BRANCH
Research Project Final Report**

Table 8: Variability in mean yield values between various fungicides. There were no significant differences between yields.

Product	N	Yield (kg/ha)	Std Dev
Control	4	2289	666.79
Quadris	4	2124	701.67
Lance	4	2276	761.38
Propel	4	1792	563.57
Tilt 250 EC	4	2697	700.75
Abound Flowable	4	2351	894.26
Astound	4	2208	699.49
Proline 480	4	2374	492.23

Table 9: Variability between mean yield values between various fungicides and fungicide X variety interaction. There were no significant differences between yields.

Variety	Product	N	Yield (kg/ha)	SEM
5440	Control	4	2500	203.11
	Proline Prosa	4	2478	203.11
	Prosaro 250 EC	4	2298	203.11
	Lance	4	2364	203.11
5330	Control	4	2203	203.11
	Proline Prosa	4	2002	203.11
	Prosaro 250 EC	4	2220	203.11
	Lance	4	2513	203.11

In conclusion, due to an overall lack of natural inoculum in the present study, it was impossible to measure fungicide efficacy in the field. However, there was a clear negative relationship between increased *Alternaria* incidence and decreasing plot yield. These results suggest that *Alternaria* may be a canola disease of economic importance in Eastern Canadian canola producing regions.



AAFC RESEARCH BRANCH Research Project Final Report

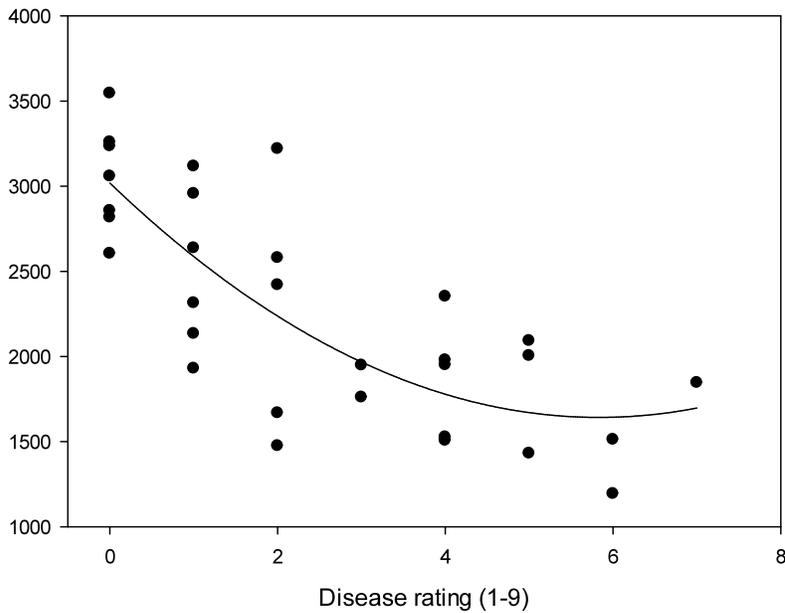


Figure 1: Regression showing relationship between increasing incidence of *Alternaria* and decreasing yield. Regression equation is $y = 3019.17 - 472.01x + 40.45x^2$ ($r^2 = 0.62$)

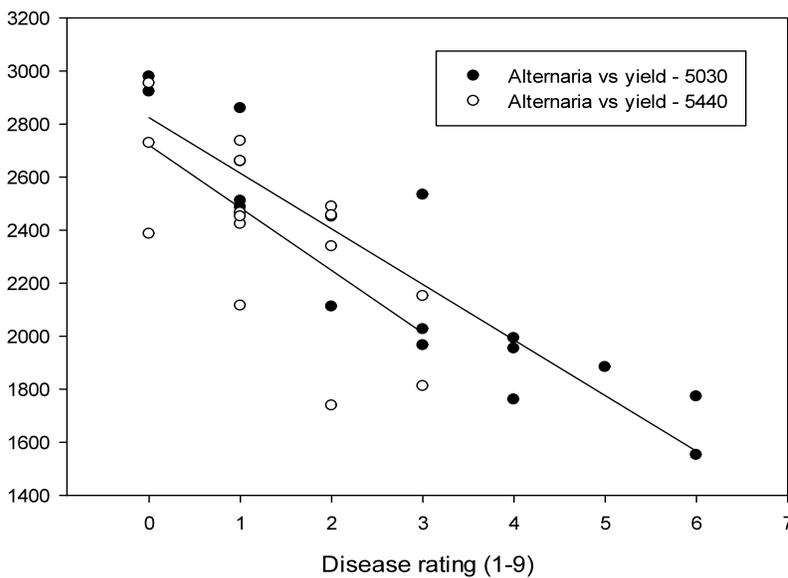


Figure 2: Regression showing a relationship between increasing incidence of *Alternaria* and decreasing canola plot yield. Regression equation for 5030 is $y = -209.62x + 2824.32$ ($r^2 = 0.83$); the equation for 5440 is $y = -236.21x + 2720.35$ ($r^2 = 0.46$).



**AAFC RESEARCH BRANCH
Research Project Final Report**

Fungicide trial at AAFC, Normandin (Denis Pageau)

The trial was seeded on June 22nd (2011) and on May 22nd (2012). Five fungicides (Lance, Proline, Prosaro, Serenade and Astound) and a check treatment were tested on the three cultivars: Invigor 5440, Invigor 5020 and Invigor 5030. All plots were fertilized with: 230 kg ha⁻¹ of 26-13-13 (N-P-K) with 0.4% B. The fungicides were applied at early bloom stage (20-30% bloom). Each plot consisted of eight rows (5.5m long with 0.18 m between the rows). The experimental design was a randomized complete block design with four replications. Plots were combined on October 24th in 2011 and on September 10th in 2012 (direct combined).

In 2011 and 2012, stem rot was totally absent: no symptoms were visible. In the region, even if it was a rainy season, the disease was also absent in the producers' fields.

The effect of fungicide on seed yield was not significant in 2011 and 2012. Seed yields for each fungicide and cultivar are presented in table 10 (2011) and table 11 (2012).

Table 10: Effect of fungicide treatment on yield – 2011

Treatment	Yield (kg/ha)		
	Invigor 5440	Invigor 5020	Invigor 5030
Check	3706	3548	3806
Lance (350 g/ha)	3670	3572	3787
Proline (315 ml/ha)	3907	3419	3867
Prosaro (0.8 l/ha)	3877	3720	3905
Serenade (4 l/ha)	3795	3493	3843
Astound (775 g/ha)	3712	3864	3645

Table 11: Effect of fungicide treatment on yield – 2012

Treatment	Yield (kg/ha)		
	Invigor 5440	Invigor 5020	Invigor 5030
Check	3751	2338	3841
Lance	3572	2476	3898
Proline	3739	2450	3622
Prosaro	3588	2521	3657
Serenade	3866	2408	3950
Astound	3881	2310	3825

Sub activity 3.4.2 (Richard Martin)

Two trials were conducted on elite germplasm for resistance to *Sclerotinia* stem rot. One involved a yield scale evaluation of 12 advanced lines of canola originating from the University of Guelph (L. Kott). The second trial involved approximately 100 lines from the University of Manitoba (M. Tahir).

The Kott lines were planted in 10 row plots with five metres in length and in a randomized complete block design with six replications. The Tahir material was planted in single five metres rows with only two



**AAFC RESEARCH BRANCH
Research Project Final Report**

replicates.

Relative to the Tahir trial, stem rot incidence and severity was determined for each line in addition to stand and flowering and maturity dates; each plot was hand harvested. No data is presented as it became apparent that there was a major issue relative to the two reps; in particular several of the lines had zero germination and the two replications did not line up for this category or match planting plans from other areas. Therefore, this trial had to be deleted (it was an additional trial to the initial workplans of the project).

With regards to the material originating from the University of Guelph there was no significant difference between the lines relative to either incidence or severity of stem rot. Specific regional checks were not included in the trial. However, the level of *Sclerotinia* appeared to be higher than perhaps with the two cultivars (Invigor 5030 and 5440) used in the foliar fungicide trials which were adjacent to this material. The mean incidence in the germplasm trial was approximately 25% while in the fungicide trial it was only between 1.7 and 4.5 percent, similarly the mean severity on the infected plants was higher at 7.4 compared to 8.6 at the first rating time (1-9 scale where 0=plant dead as a result of infection; 9= no symptoms).

Table 12: Evaluation of advanced canola germplasm originating from the University of Guelph breeding program, AAFC – Harrington Research Farm, 2011.

Line	Aug 18			Sept 1			Flowering day (July)	Yield (kg/ha)	TSW (g)
	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)			
SC7014	29.7	7.2	8.6	31.7	1.6	6.7	11	1174	2.50
SC06026	25.0	7.4	8.6	24.2	1.9	7.2	10	1365	2.46
AC C09-01	30.0	7.5	8.6	18.3	2.4	7.8	12	1204	2.52
SC 07138	20.6	7.3	8.6	34.2	1.5	6.5	9	1360	2.51
SC07152	27.0	7.4	8.6	30.0	1.8	6.8	7	1130	2.50
C B 01/07-2	28.4	7.5	8.6	24.2	2.9	7.5	11	967	2.17
SC 07076	33.1	7.5	8.5	20.9	2.4	7.6	9	1320	2.31
SC 07237	25.0	7.4	8.6	23.4	1.5	7.2	12	1166	2.23
SC 08016	27.5	7.5	8.6	25.9	2.2	7.2	13	958	2.24
SC 08029	20.0	7.6	8.7	30.0	2.2	6.9	12	1155	2.40
SC 08044	18.3	7.5	8.7	20.0	3.1	7.7	12	1197	2.61
SC 08045	24.2	7.4	8.6	22.5	1.9	7.4	12	1339	2.32
LSD (0.05)	ns	ns	ns	ns	ns	ns	1.391	ns	0.276
Reps	6	6	6	4	4	4	6	6	6

Sclerotinia ratings: 1= prematurely ripened (plant dead) through 9=no symptoms

Future:

Repeat the materials from the University of Guelph relative to the *Sclerotinia* resistance level of advanced material, but with the inclusion of several regional check cultivars, to ensure resistance is actually an improvement over currently available cultivars.

Sub-Activity 3.5.2. and Sub-Activity 3.5.3 (Richard Martin and Aaron Mills)

These two sub-activities were included in a single trial and are directly linked to the general canola agronomy **Sub-Activity 6.2 Seeding rates and dates** (research lead: Claude Caldwell, Dalhousie University). *Sclerotinia* stem rot incidence and severity is potentially one of the major issues in canola



AAFC RESEARCH BRANCH Research Project Final Report

relative to crop canopy density and as such is a criteria which should always be recorded in agronomy related projects on canola. Since stem rot is a disease of canola which can vary greatly between years and locations, being very dependent on macro and micro environmental conditions, collection of useful data can be a challenge.

The entire seeding date and rate project is presented under these two sub-activities.

Cultivar Invigor 5440 was used in the trial and seeding dates were on May 23, 30 and June 6, 2011. Earlier seeding dates were desirable, but the May 23rd date was the earliest timing that was possible relative to field readiness. Seeding rates were 2.5, 5 and 7.5 kg seed per hectare. Four replicates were run for each treatment, due to land and technical support availability limited us to four replicates. In future years increasing to six reps for the *Sclerotinia* component should be an option.

The principal research aim of the PEI site was the impact of both seeding rate and date on *Sclerotinia* stem rot. Other agronomic data was collected on stand (just prior to harvest), yield and seed weights. In addition some yield components were collected, on a limited number of plants per plot, including number of stem branches per plant, pods per plant, and seeds and seed weight on 15 pods.

Between the two evaluation dates (August 18 and August 25) there was a marked increase in average *Sclerotinia* stem rot incidence of from 2.0% to 9.0%. There was also an increase in the mean severity of the disease on infected plants of from 8.4 to 7.8 (1-9 rating scale where 9=no symptoms and 1=plant dead from infection). There was no significant difference between seeding rates and seeding dates ($p=0.05$ level of probability). However, the level of disease was low, particularly when considering severity levels. Incidence was moderate at a maximum of 10.3% from the early seeding date, but the actual severity was low on the infected plants. While not significant, there was some evidence that there was a higher level of disease in the early seeded material. However, given the difference between ratings this may be a reflection of the usual progression of the disease through the crop over time rather than a linkage to seeding date specifically. This hypothesis could be confirmed with more stem rot ratings over time, particularly later in the season. There was an indication of potential interaction relative to stem rot incidence at the first rating, but only at the $p=0.076$ level of probability. A higher number of replicates may help to identify small differences in the impact of date and or rate on *Sclerotinia* incidence and severity.

Interestingly, there was no difference in final stand count. While there was some significance of rate, where the stand counts were higher as seeding rate increased, this was only at a $p=0.096$ level of probability. What is more relevant is that the stand counts really did not match what would have been expected from the initial seed rate, where there was a three time difference between the low and high seed rates but only a mean difference of 1.45 in stand count. If consistent over sites and years, it will be relevant to investigate this phenomenon further.

Relative to yield and seed weight, there was no significant difference between seeding rates however there were some effect of seeding date on the total yield component which was significantly higher in the late seeding date followed by the early data. The lowest yield was experienced with the mid seeding time. Having said this, the difference in yields may be related to other factors; noting the lack of yield differences between the seeding dates when considering the other yield components. The same combine was used for all harvests so differences in direct combining were probably not an issue. However, differences in shattering may have been the reason behind yield differences. Shattering was an issue and it is likely that a couple of days difference may have meant significant differences in yields following direct combining.

There was some impact on number of branches per plant from seeding date, with more branching in the earlier seeding period and the lowest in the last seeding date. There was a non-significant hint at more branching from the lower seeding rate. Similarly, there was a hint of an effect of both rate of seeding and seeding date on pods per plant, but variability was such that this was non-significant ($p=0.05$). When the total yield per metre was calculated using the stand count, number of pods per plant and the mean weight of seed in the pods, it was found that there was no yield difference between dates of seeding or rate of seeding, indicating that the yield effects associated with the direct combining of the plots were perhaps not related to the actual dates of seeding but more related to crop condition at the time of harvest.



**AAFC RESEARCH BRANCH
Research Project Final Report**

Table 13: Evaluation of planting date and rate in canola (Invigor 5440) on *Sclerotinia* stem rot. AAFC – Harrington Research Farm, 2011.

Factors	Aug 18			Aug 25		
	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)	Infected plants (%)	Mean severity infected plants (1-9)	Mean severity plot (1-9)
Seeding date						
May 23	1.80	8.49	8.98	10.30	7.72	8.84
May 30	2.50	8.22	8.97	9.90	7.95	8.85
June 3	1.67	8.58	8.93	6.80	7.86	8.90
LSD (0.05)	ns	ns	ns	ns	ns	ns
SEM	0.874	0.167	0.009	1.990	0.111	0.030
Seeding rate						
2.5 kg/ha	2.08	8.51	8.97	9.20	7.88	8.86
5.0 kg/ha	2.36	8.28	8.97	8.90	7.92	8.86
7.5 kg/ha	1.53	8.50	8.89	8.90	7.72	8.86
LSD (0.05)	ns	ns	ns	ns	ns	ns
SEM	0.669	0.182	0.009	1.880	0.213	0.030
Seeding date x seeding rate						
May 23	2.5 kg/ha	0.00				
	5.0 kg/ha	3.75				
	7.5 kg/ha	1.66				
May 30	2.5 kg/ha	4.58				
	5.0 kg/ha	2.08				
	7.5 kg/ha	0.83				
June 3	2.5 kg/ha	1.67				
	5.0 kg/ha	1.25				
	7.5 kg/ha	2.08				
LSD (0.05)		3.808				
		1.288				
		P=0.076				

Sclerotinia ratings: 1= prematurely ripened, plant dead through 9=no symptoms

Sub-activity 3.5.3.

A survey was initiated in 2011 looking at approximately 15 farms in Prince Edward Island. The fields were sampled for soil analysis (chemical), nematode population dynamics/profile (Aaron Mills), and root and foliar disease levels. Work on definitive levels of nematodes under the different cropping sequences continues and are not ready as of yet, as evaluation of the large number of samples from each field continues. On each farm a transect through the field was taken with samples collected at regular intervals along that line.

There were no major foliar diseases identified in the field. *Sclerotinia* stem rot was widespread in the fields, but levels were low in most fields. It was apparent that a greater number of assessments will be required in the future for determining stem rot levels and disease development and spread within the field. It is evident from both the field survey and for the small plot trials that the disease can progress at a very rapid rate over a short period of time. As such, while levels may be low one week, by the following week significantly higher incidence may be evident and the severity definitely increases. Thus, to provide better measurement of stem rot in the field, and effects that fungicides may be having at the farm level, stem rot levels need to be measured multiple times and as closely to each other, across the farms, as possible. Thus, multiple personnel will be needed to handle the number of fields across the province in a timely manner.



AAFC RESEARCH BRANCH Research Project Final Report

From examination of roots, the principal diseases evident were *Fusarium* and *Rhizoctonia* related issues. Potential damping off was not evaluated and several early field assessments are needed. Heavy root plantings were not conducted, but if time and resources are available then incidence assessments should be undertaken on both, *Rhizoctonia* and *Fusarium*. Club root was not observed on any farm in the survey, but needs to be closely monitored. Integration of soil analysis and in particular nematode population dynamics remains to be assessed, once the population counts are completed.

Sub-Activity 3.6. (Balakrishan Prithiviraj)

Introduction

Plants are known to respond to environmental stresses by activating their defense mechanisms. Plant defense responses can be induced by bioactive elicitors of diverse chemical origin like peptides, carbohydrates, lipids etc. Among various sources of plant elicitors of natural product origin, macroalgae are reportedly known for their richness in these biologically active components that have shown the potential to activate plant defense mechanisms against a variety of phytopathogens (Khan et al., 2009; Craigie, 2011; Sangha et al 2010). Macroalgae form an integral part of coastal ecosystems of the world, providing essential ecosystem services to the inshore marine environment with about 10,000 species known so far and majority of which can be classified into the phyla Chlorophyta (green algae), Ochrophyta (brown algae) and Rhodophyta (red algae) (www.algaebase.org). The richness of macroalgal species with these bioactive compounds, minor nutrients, and the ability to produce a great variety of secondary metabolites, are implicated in a broad a spectrum of biological activities shown by the macroalgae products or the extracts (Kulik, 1995; Lordan et al., 2011). Some of these components from different macroalgae have shown protection in plants when used externally, indicating their role in plant defense elicitation that can be explored to improve agriculture sustainability by providing protection from plant pathogens (Mercier et al 2001; Sangha et al 2010).

Canola is an important oilseed crop in Canada, contributing to a major part of the Canadian economy. The canola crop is, however, vulnerable to infection by a number of pathogens infecting roots, leaves, crown or stem that can significantly reduce the yield and quality of the produce. Fungal disease such as blackleg and *Sclerotinia* stem rot cause severe damage to the crop under higher rainfall conditions that must be managed in order to get a successful crop yield and oil quality. Efficacy of disease control measures such as fungicides varies for these pathogens depending on the climate conditions, varietal resistance and crop production practices. A number of other methods of crop protection have been available as integrated management of crop diseases, and bioactive elicitors are one such approach with promising effects in disease suppression. Chitosan was used to suppress plant pathogens (Hadrami et al 2010). Extracts of brown macroalgae, *Ascophyllum nodosum*, and red seaweed polysaccharide λ -carrageenan suppressed *Sclerotinia* leaf infection in *Arabidopsis* plants, a close relative of canola. The same extracts were also effective against other plant pathogens and some insect pests. Therefore, the effect of macroalgal products along with a marine bio-product were tested against stem rot of canola under field conditions.

Literature review

Macroalgae are known for inherent bioactive components. Macroalgae are unique source of fucoidan, carrageenans and phlorotannins with potential use as plant biostimulants in agriculture (Zhang et al 2003; Khan et al., 2009; Cox et al., 2010; Craigie, 2010). It has been shown that many of these bioactive components are probably synthesized as chemical defenses to cope with the extreme environments such as microbial organisms or herbivores (Kubanek et al., 2003). This is why certain seaweeds and their extracts exhibit broad-spectrum antimicrobial activity against various microbes infecting humans, animals and plants (Paulert et al., 2007; Khan et al., 2009; Craigie, 2010). The application of macroalgal extracts can activate plant molecular responses linked with physiological and biochemical activities that



AAFC RESEARCH BRANCH Research Project Final Report

lead to altered defenses against a variety of environmental stresses such as plant pathogens (Mercier et al. 2001; Subramanian et al 2011).

Extracts of *Ascophyllum nodosum* and other seaweeds have been used as a bio-stimulant to promote growth and productivity in a number of agricultural production systems. However, they are also effective against various plant pathogens. *A. nodosum* extracts elicited defense responses in *A. thaliana* against the hemi-biotroph *Pseudomonas syringae* pv. *tomato* DC3000 and the necrotrophic pathogen *Sclerotinia sclerotiorum* (Subramanian et al 2011). It was shown that Arabidopsis resistance to these pathogens was mediated through jasmonic acid/ethylene (JA/ET) dependent response. In another study, the seaweed polysaccharide carrageenan was shown to enhance Arabidopsis resistance to *Sclerotinia* and was JA dependent (Sangha et al. 2010). Similarly, polysaccharide isolated from green alga (*Ulva* spp.), when sprayed on tomato leaves, protected the plants against the bacterium *P. syringae* (Jaulneau et al., 2010). A commercial *A. nodosum* extract was anti-fungal in activity on the bentgrass (*Agrostis stolonifera*) against dollar spot disease, caused by *Sclerotinia homoeocarpa* (Zhang et al., 2003). These studies implicate the potential application of seaweed extract to manage plant pathogens in other commercial crops, including canola.

Other elicitors are also commonly used in plant resistance to pathogens. Hadrami et al (2010) reported that bioactive chitosan molecules can be inhibitory to various fungal, viral, bacterial and other pests. Chitosan used to control plant pathogens has been extensively explored with more or less success depending on the pathosystem, the used derivatives, concentration, degree of deacylation, viscosity, and the applied formulation (i.e. soil amendment, foliar application; chitosan alone or in association with other treatments). Amendment with chitosan was reported to suppress soil-borne disease of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. It was assumed, that mechanisms of action of chitosan in reducing plant disease were through direct toxicity or chelation of nutrients and minerals from pathogens. Chitosan is known to induce plant defenses, both local and systemic, involving signaling cascades, activation and accumulation of antimicrobial compounds and proteins.

The extensive literature on improved plant tolerance to various pathogens with elicitor induced plant defense response suggests the need to explore marine bio-products to suppress canola pathogens. The use of bioactive elicitors of canola that reduce disease incidence or severity in canola, especially the stem rot pathogen, *Sclerotinia*, will help to improve crop yield and quality in Eastern Canada.

In the presented sub-activity the leaf and petiole inoculation technique of *Sclerotinia* inoculation was standardized. Using this technique the efficacy of extracts of a brown seaweed (*Ascophyllum nodosum*) extract (SW), λ -carrageenan, ι -carrageenan and chitosan were evaluated against *Sclerotinia* stem rot.

Methodology

Seeds of canola Invigor L130 were obtained from Bayer Crop Science Corporation, Lethbridge, AB. The seeds were planted in 10 cm diameter pots containing Promix potting mixture (Premier Tech Horticulture, NB) and the plants were thinned to obtain one plant per pot. Plants were grown at 24° C under 300-450 $\mu\text{mol m}^{-2} \text{S}^{-1}$ and 16h photoperiod.

Four weeks old plants (15 plants per treatment) were sprayed with: *Ascophyllum nodosum* seaweed extract (SW) (0.5-3g/l), λ -carrageenan (1g/l), ι -carrageenan (1g/l), chitosan (1g/l) and LANS (nutrient solution) treated plants were used as control.

The plants were inoculated with mycelial plugs of *Sclerotinia sclerotiorum* (5mm). To infect the stem, Petiole Inoculation Technique (PIT) was as follows: petiole of the fourth fully expanded leaf was severed with a surgical blade and the petiole was plugged into the inoculum filled 1000 μl pipette tip. The plants were observed for disease symptoms, lesion size and to score mortality. The data were recorded from 15 plants per treatment group and the experiment was performed in randomized blocks.

Plants treated with seaweed extract (SW) (2g/l) showed significant reduction in lesion size (figure 3).



AAFC RESEARCH BRANCH Research Project Final Report

λ -carrageenan (1g/l) and chitosan (1g/l) exhibited moderate tolerance to stem rot. A similar trend of resistance was also observed in petiole inoculation.

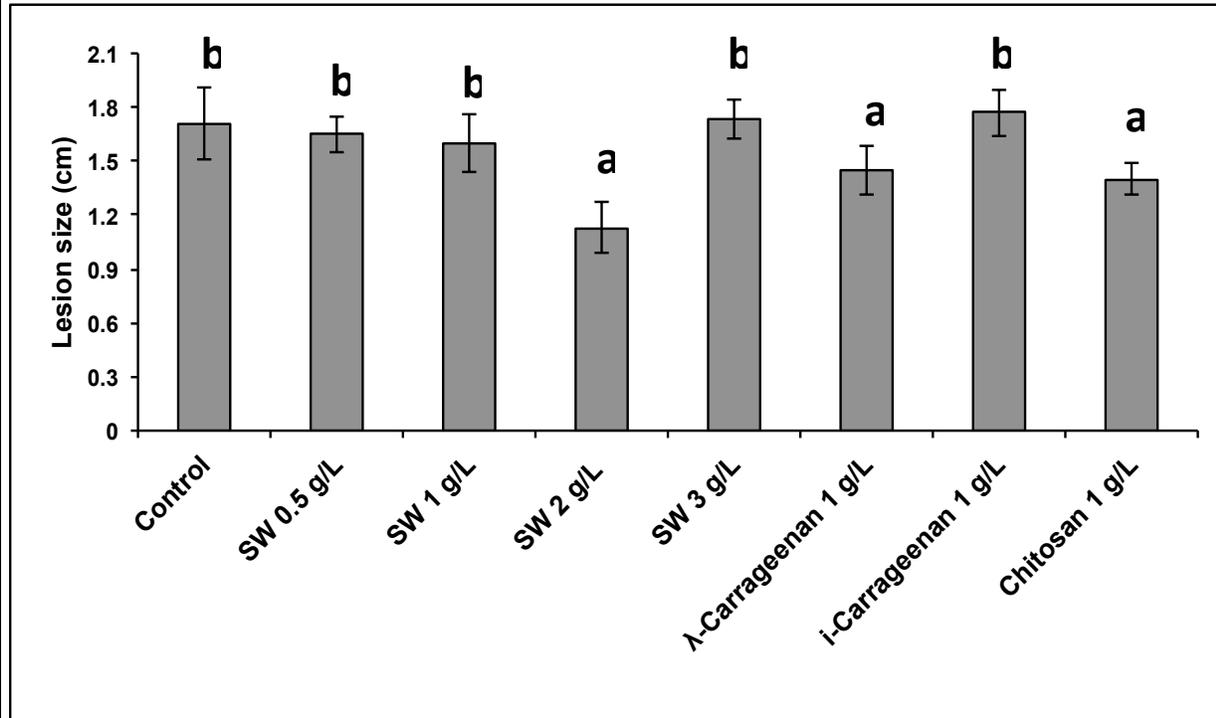


Figure 3: Effect of marine bioproducts on *Sclerotinia* stem rot of canola

2012 Cropping season

Plant material

Invigor L130 canola variety was obtained from Bayer Crop Science Corporation, Lethbridge, AB. Field plants were grown following standard farmers' practices at two different locations in two counties of Nova Scotia. **Treatments:** Seaweed *Ascophyllum nodosum* powdered extract (SW) (1-3g/l); λ -carrageenan (0.5-2g/l); water or LANS (nutrient solution) as control and fungicide (Proline) for field studies.

Experiment 1: Effect of Bioactive Treatments on Management of *Sclerotinia* stem rot of canola

Methods:

Many reports have shown that seaweed extracts can be useful in the management of plant diseases through direct or indirect effects on plant pathogens. The potential use of marine bio-products (SW and chitosan) for the management of stem rot caused by *Sclerotinia* was evaluated in two field locations. The first location was in Truro, NS (Brookside Field 201, Dalhousie Agricultural Campus Farm), and the second location was in Canning, NS (Lyndhurst Farms Ltd.). It should be noted that the weather was unusually warm and dry during 2012 summer, and that had affected seed germination, crop density and disease incidence, particularly in the Canning, NS location. The plots were laid out in a randomized complete block design with eight treatments and four replications per treatment. Fields were subjected to recommended fertilizer and maintenance practices throughout the growing season. The crop was spray treated with the following treatments: λ -carrageenan (0.5, 1 and 2g/l), seaweed extract SW (1, 2 and 3g/l), fungicide check and a 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



AAFC RESEARCH BRANCH Research Project Final Report

another week after the second. Treatments were applied at a rate of 400 l/ha. Data on disease incidence and severity was observed once every week after the first spray and continued until crop maturity.



Figure 4: *Sclerotinia* infection in the field



Figure 5: Poor growth of canola at Canning, NS due to poor rainfall and subsequent drought-like conditions in 2012

Results:

The disease survey was conducted during crop growing season 2012, particularly from pre-flowering to seed maturity stage (early July to late August). The data on disease incidence was low at first observation (July 23), a period which coincided with final spray treatment of the different bioactive elicitors. Following rains during late July and early August, the disease incidence started to increase revealing treatment effect (figure 6). The results showed considerable effect of fungicide (Proline) and a significant suppression throughout the crop season after a single spray of the chemical (figure 6).

In contrast, the disease incidence with λ -carrageenan and seaweed was lower during the first observation (July 23), which coincided with the last spray treatment and the end of the flowering stage. Following this week, the disease incidence was still low with λ -carrageenan (1g/l) and SW (1g/l) but not different with other treatments. However, the percent disease was still low with SW 2g/l and SW 3g/l during this period. In early August, a similar trend was observed (August 8th) but the reduction in disease incidence (except λ -carrageenan 5g/l) was not significant. The last observation (August 21) showed a further increase in disease incidence (about 27-30%) as weather was conducive to *Sclerotinia*. No difference was observed for bioactive treatments in terms of disease suppression, probably due to reduced effect of the spray treatments performed a month earlier.



AAFC RESEARCH BRANCH Research Project Final Report

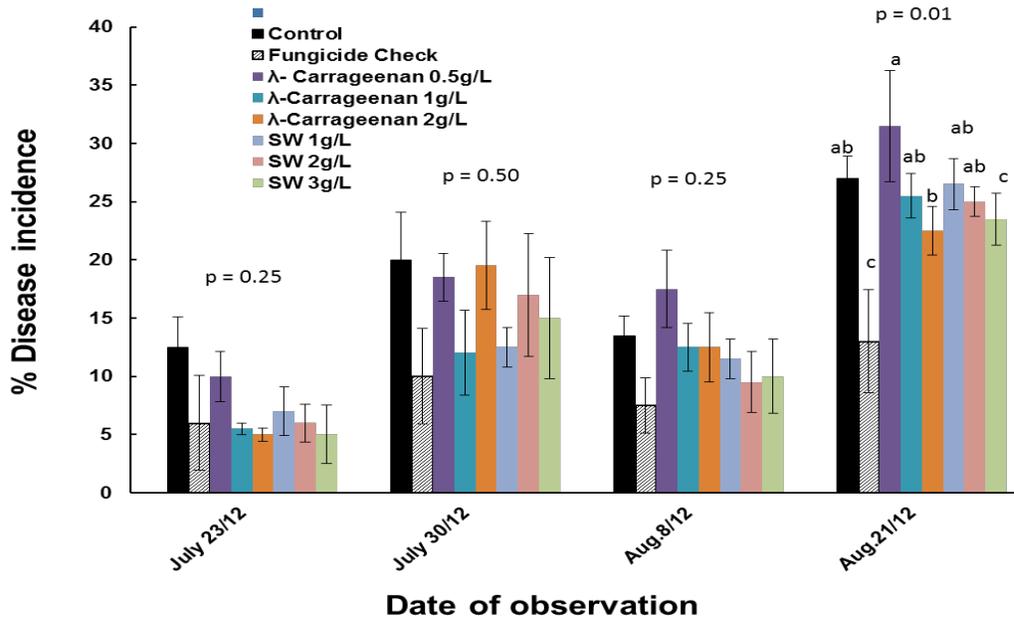


Figure 6: Effect of different treatments on *Sclerotinia* incidence on canola

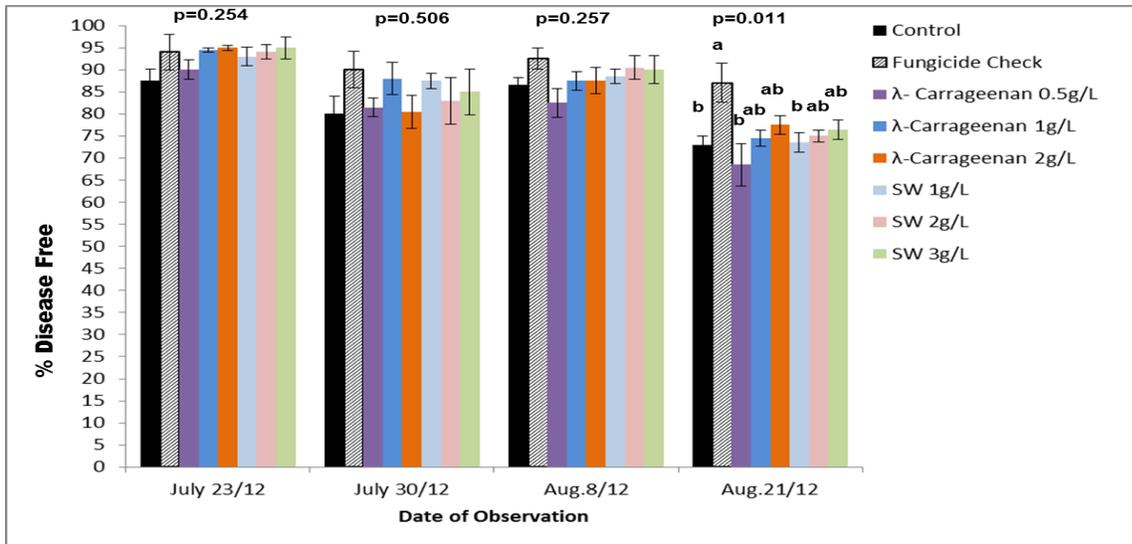


Figure 7. Effect of different treatments on the percentage of unaffected plants in the field.

A similar trend was noted for plants scored with 1, 2 and 3 disease rating (figure 8), as the disease severity did not differ significantly at all four time points. Interestingly, all treatments, except λ-carrageenan 0.5 g/l, showed lower disease severity, though not statistically significant, up to the third observation. This difference was not evident at the last observation except that fungicide treatment did show lower disease severity. However it was not significantly different than the control.

No data is presented for disease incidence at the Canning field since the crop growth was poor due to



AAFC RESEARCH BRANCH Research Project Final Report

drought-like conditions and no *Sclerotinia* infection was observed in the field (figure 5). In order to determine if disease severity was affected by treatments, a visible disease score method was used (i.e. 0=no disease healthy plants, 1=stem rot symptoms on branches only, 2=stem rot symptom on the main stem and 3=severe stem rot symptoms (plant dead)). As shown in (figure 7), no significant difference was observed for healthy plants i.e. plants scored 0 on the first three observation points. However, on the last observation, the number of healthy plants was significantly higher for the fungicide treatment as compared to the control.

Experiment 2: Effect of bioactive treatments on agronomic characteristics of canola under disease conditions

Methods

In the field experiments canola seeds were sown at two different field locations. The first location was located in Truro, NS (Brookside Field 201, Dalhousie Agricultural Campus Farm), and the second location was in Canning, NS (Lyndhurst Farms Ltd.). The Truro site was seeded May 26, 2012, while the Canning site was seeded three separate times starting April 20, 2012. It was an unusually warm and dry spring and summer resulting in very uneven germination and low crop density in the Canning, NS location. The plots were laid out in a randomized complete block design with eight treatments and four replications per treatment. Fields were subjected to recommended fertilizer and maintenance practices throughout the growing season. The crop was spray treated with the following treatments: λ -carrageenan (0.5, 1, and 2g/l), seaweed extract (SW; 1, 2 and 3g/l), fungicide check and a 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. Treatments were applied at a rate of 400 l/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

Number of siliques: Total number of siliques was higher with control plots, however SW 2g/l and λ -carrageenan (1 and 2g/l) were all very close. There was no significant difference between all treatments and the control in terms of number of siliques per plant (figure 9).

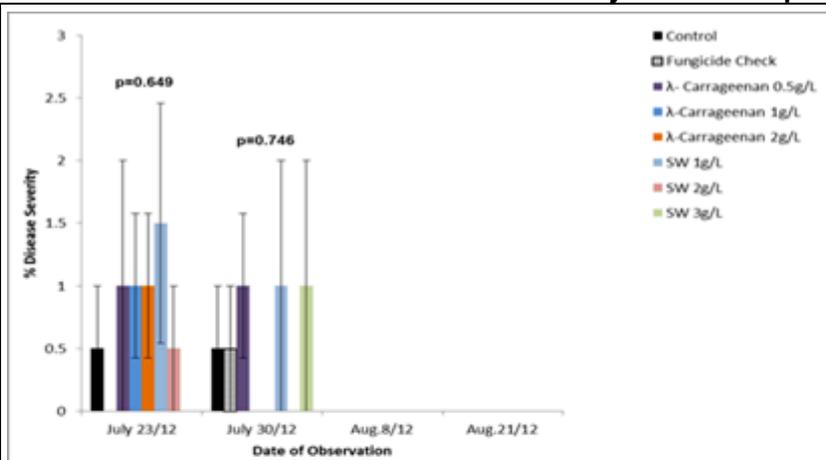
Seeds per silique: The number of seeds per silique was higher with control as well, although not significant (figure 9). The number of seeds per silique with other treatments was comparable to the control.

1000 kernel weight: In fact, the trend shows that SW treatment slightly increased the average seed weight compared to the control. However, seed weight was slightly higher, though not significant, than control plants (figure 9).

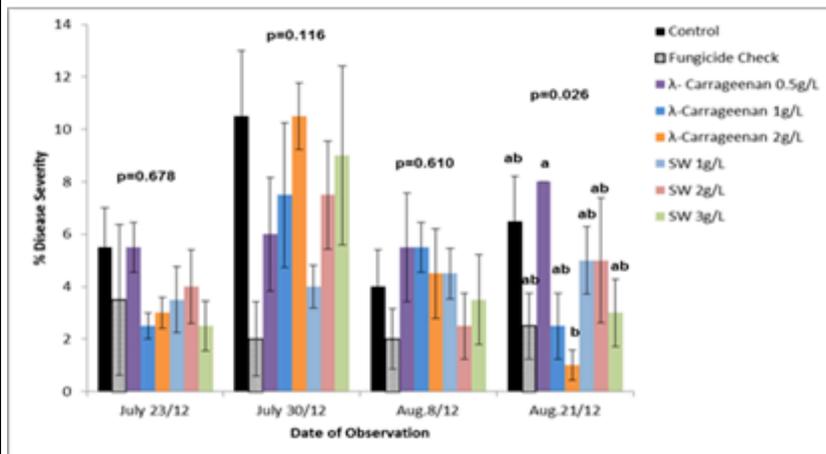
Yield: In the field, the average seed yield (kg/ha) was higher in SW 3g/l and λ -carrageenan 5g/l treatment than control. The results (control=2616.20 kg; SW 1g/l= 2654.83 kg; SW 2g/l=2699.36 kg; SW 3g/l=2844.78 kg; λ -carrageenan 0.5g/l=2803.29kg; λ -carrageenan 1g/l=2563.53 kg; λ -carrageenan 2g/l=2637.79 kg; Fungicide=2546.59 kg/ha) indicate, that canola plants sprayed with these bioactive treatments could potentially increase seed yield in the field under disease conditions (figure 9).



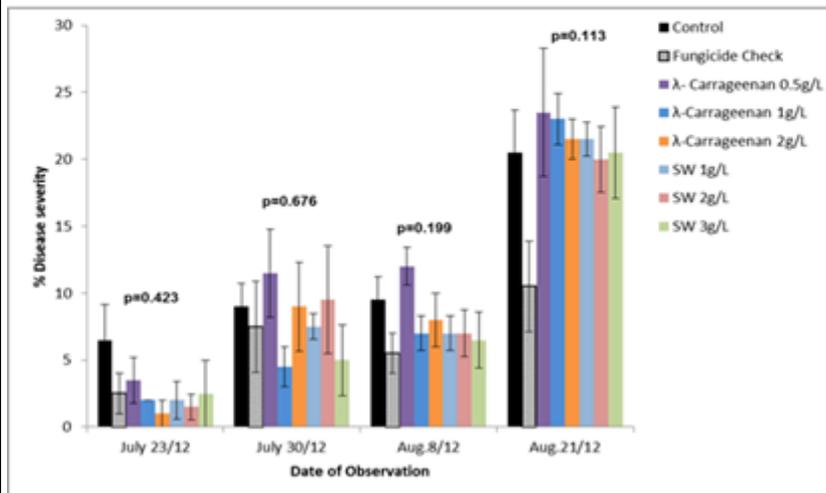
AAFC RESEARCH BRANCH Research Project Final Report



Rating # 1 - Stem rot symptoms on branches only



Rating # 2 - Stem rot symptom on the main stem



Rating # 3 - Severe stem rot symptoms plant dead

Figure 8: Effect of different treatments on disease severity in canola.



AAFC RESEARCH BRANCH Research Project Final Report

Discussion

The ability macroalgal extracts, especially brown alga *A. nodosum*, chitosan and carrageenan to suppress plant diseases is well-documented. This study shows potential evidence that *Sclerotinia* stem rot disease in canola can be suppressed, to some extent. In contrast to greenhouse studies showing consistent suppression of stem rot with marine bio-products, the weather conditions in the field were dry due to limited rainfall. Despite this, *Sclerotinia* stem rot disease incidence was observed at the Truro field. During the treatment period, the disease was lower compared to the control and this effect was significant with fungicide treatment. Further studies under disease favouring conditions will explain the exact role these treatments can play in *Sclerotinia* stem rot management in canola.

Since canola can be infected by many pathogens such as *Alternaria*, *Fusarium*, *Verticillium* etc. it would also be important to study the effect of these treatments on different pathogens of canola. The acceptance of marine bio-products in disease management tools might be accelerated with integrated disease management practices to lower the burden of chemical use in agriculture. There is a need to carefully evaluate further these products for their potential and potent roles in future control of plant pathogens.

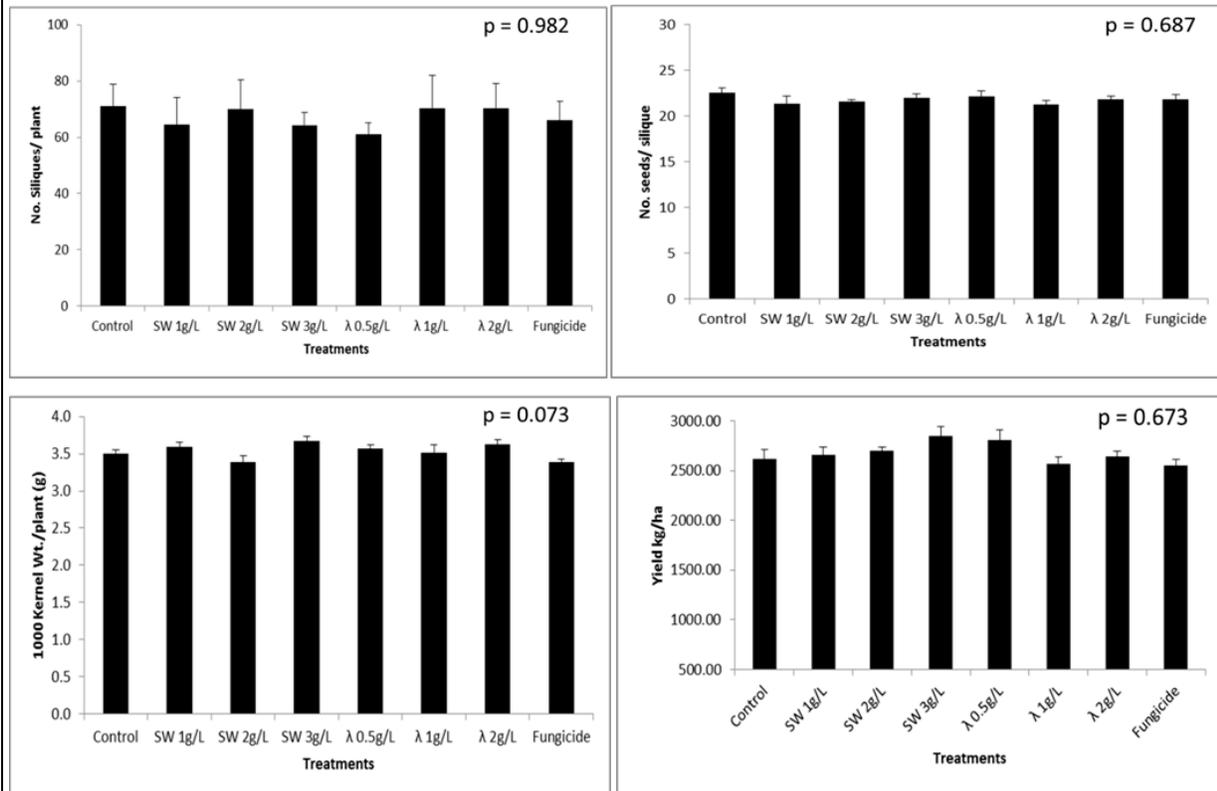


Figure 9: Effect of treatments on canola agronomy in field under disease conditions

This was true as several agronomic features were improved with the bioactive treatments and the yield was also higher in the field experiments, particularly with seaweed extract. Finally, the *Sclerotinia* stem rot incidence was low with most treatments although the effect seems to fade with time after the treatments were halted in the field. However, one single fungicide treatment was able to effectively suppress the disease for the whole season. An integrated way of using different bioactive treatments along with judicious use of fungicide should help for a broad level of *Sclerotinia* suppression and to increase yield in the Eastern Canadian region ready to adopt canola as a main cash crop. Further



AAFC RESEARCH BRANCH Research Project Final Report

extended studies will support these findings and help to support successful healthy canola cultivation in this region.

References:

Cox S., Abu-Ghannam N. & Gupta, S. (2010). An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*. 17: 205-220

Craigie, J.S. (2011). Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* 23: 371-393.

Hadrami, E.A., Adam, L.R., Hadrami, E.I., & Daayf, F. (2010). Chitosan in plant protection. *Marine drugs*, 8(4), 968-987.

Jaulneau V., Lafitte C., Jacquet C., Fournier S., Salamagne S., Briand X., Esquerré-Tugayé M.T., & Dumas B. (2010). Ulvan, a sulphated polysaccharide from green algae, activates plant immunity through the jasmonic acid signaling pathway. *Journal of Biomedicine and . Biotechnology*. 525291.

Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J. and Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* 28: 386-399.

Kubanek J., Jensen P.R., Keifer P.A., Cameron M.S., Collins D.O., & Fenical W. (2003). Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. *Proceedings of the National Academy of Sciences*. 100: 6916-6921.

Kulik M.M. (1995). The potential for using cyanobacteria (blue-green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *European of Plant Pathology*. 101: 585-Journal 599.

Lordan S., Ross R. P., & Stanton C. (2011). Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine drugs*. 9: 1056-1100.

Mercier L., Lafitte C., Borderies G., Briand X., Esquerré-Tugayé M.T., & Fournier J. (2001). The algal polysaccharide carrageenans can act as an elicitor of plant defence. *New Phytologist*. 149: 43-51.

Sangha, J.S., Ravichandran, S., Prithiviraj, K., Critchley, A.T. and Prithiviraj, B. (2010). Sulfated macroalgal polysaccharides λ-carrageenan and ι-carrageenan differentially alter *Arabidopsis thaliana* resistance to *Sclerotinia sclerotiorum*. *Physiol. Mol. Plant Pathol.* 75:38-45.

Subramanian, S., Sangha, J.S., Gray, B.A., Singh, R.P., Hiltz, D., Critchley, A.T. and Prithiviraj, B. (2011). Extracts of the marine brown macroalga, *Ascophyllum nodosum*, induce jasmonic acid dependent systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* pv. *tomato* DC3000 and *Sclerotinia sclerotiorum*. *Eur. J. Plant Pathol.* 131: 237-248.

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

Paulert R., Smânia Jr.A., Stadnik M., and Pizzolatti M. (2007). Antimicrobial properties of extracts from the green seaweed *Ulva fasciata* Delile against pathogenic bacteria and fungi. *Algological Studies*. 123: 123-130.

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

- Include the name of scientist / organization.

Claude Caldwell

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



AAFC RESEARCH BRANCH Research Project Final Report

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.

No variance from the proposed research was noted.

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).

There was no variance from the original objective.

One issue was that there was no serious incidence of *Sclerotinia* stem rot disease at two test sites (Normandin and Harrington). Therefore, efficacy of the fungicides and resistance among the elite germplasm against stem rot could not be evaluated. However, this is beyond researcher's control and closely linked to weather conditions.

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

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

1. A number of genotypes were screened for stem rot resistance.
2. Two potential marine bio-products were identified for management of stem rot of canola.
3. The project helped train a number of summer students, graduate students and a post-doctoral fellow in canola disease management.

F. Lessons learned (self-evaluation of project)

Clearly, in order for the field screening to yield results, there must be reasonable disease pressure. Although this is not news to breeders/agronomists, environmental issues always play a role in the success of any trial. In this case, in some locations there was no disease pressure and the new lines could not be assessed for level of resistance.

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.