AAFC RESEARCH BRANCH
Research Project Final Report
Developing Innovative Agri-Products Program (Vote 10 Funding)

Project Title:	Activity B.2.1: Test the ability of signaling compounds to help canola plants overcome temperature stresses in controlled environment and field experiments
Start Date (yyyy-mm-dd):	April 1, 2011
Expected End Date (yyyy-mm-dd):	March 31, 2013
Actual End Date (yyyy-mm-dd):	March 31, 2013
Principal Investigator (PI):	Donald L. Smith

Short Executive Summary of report:

Summary:
The objective of this sub-activity was to understand the potential for microbe-to-plant signaling compounds such as lipochitooligosaccharides (LCOc) and thuricin 17 to help canola plants deal with stressful growing conditions.
Treatment of canola seeds with micromolar (µM) LCO solution indeed accelerated the emergence of young “fastplants” (a canola genotype developed for very rapid maturity) to a very significant degree and also increased seed yield. The application of this LCO solution to seed at planting promoted the regulation of plant leaf temperatures in greenhouse experiments, resulting in less extreme leaf temperatures following treatment. LCO spray treatment produced plants with longer branches, but increased apical dominance as well; which might be manipulated under field conditions to improve crop yield. Interestingly, when stress levels were extreme the signaling compounds could have negative effects.
Field experiments were conducted in each of 2011 and 2012. Field experiments were not required as part of the grant and the 2011 experiment was, therefore, seeded after all other seeding conducted as part of our summer field research, which - in combination with a period of drought and heat - led to very difficult growth conditions and variability of results; growth values for signaling compound treated material were generally higher than controls; however, the high variability masked significance. In 2012, LCO treatment resulted in greater production of branches, pods and biomass. In general, these signaling compounds do exhibit potential to modify growth and development of canola; further experiments will clarify agronomic/commercial application of these materials.

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.

For the most part, energy enters terrestrial ecosystems at the green leaves of plants. The roots of plants exist in direct contact with soil and in a moist habitat, where there is little chance to escape direct interaction with soil microflora. Those elements of the microflora are able to co-exist closely with plants and may have direct access to photosynthetically reduced carbon and preferential access to energy. They comprise a complex and always present community associated with field-grown plants. There is a group of bacteria, living regularly and closely with plant roots and at this juncture, the rhizosphere microbiome of plants and the human bacterial microbiome, the two being similar in function, could be compared. Both of these microbiomes change with the age of the organism and play a pivotal role in

governing the condition of the organisms throughout their life cycle.
Aspects of signaling have been explored among members of the rhizosphere microflora with some interesting results. One of the most interesting plant-microbe interactions is the root nodulation symbiosis. A little over a decade ago, an initial discovery was made where the LCOs produced by rhizobia, to signal legume symbiotic partners, are able to stimulate plant growth directly (Souleimanov et al. 2002; Prithiviraj et al. 2003; Almaraz et al. 2007; Khan et al. 2008). This was subsequently confirmed by others: Oláh et al. (2005) for root growth in *Medicago truncatula*. Chen et al. (2007) showed that LCO spray on tomato accelerates flowering (a typical response to stress) and increases yield. LCO-like molecules also stimulate early somatic embryo development in Norway spruce (Dyachok et al. 2002). Enhanced germination and seedling growth and the mitogenic nature of LCOs suggest accelerated meristem activity. Products based on the presented LCO findings have been used to treat seed sown into several million hectares of crop land around the world over the last few years. However, the applicability of these materials to canola production has not yet been subject to detailed investigation. Several compounds secreted by other rhizobacteria have been discovered, that cause similar effects (e.g. Lee et al. 2009). Although chemically quite different (proteins - Gray et al. 2006a,b) from the chitin based LCOs, the similarity in plant responses suggest that these microbe-to-plant signaling compounds might act through similar mechanisms within crop plants. The Plant Growth Promoting Rhizobacterium (PGPR) *Bacillus thuringiensis* NEB17 was isolated from soybean nodules (Bai et al. 2002) and enhances nodulation when applied as a co-inoculant with *Bradyrhizobium japonicum* 532C (Bai et al. 2003). It was shown, that this bacterium produces a novel antimicrobial peptide (bacteriocin), now named thuricin 17, with a molecular weight of 3.1 kDa (Gray et al. 2006b). Thuricin 17 is not toxic to *B. japonicum* 532C (Gray et al. 2006b). The same authors noted the existence of a similar bacteriocin (bacthuricin F4) and, based on these two molecules, proposed a new class of bacteriocins: class IId. Interestingly, the effects of LCOs and thuricin 17 are much greater when stress (salt, drought, cold) is present than under optimum conditions (Subramanian, 2009; Smith et al. 2009; Subramanian et al. 2010; Smith et al. 2010; Subramanian et al. 2011). Wang et al. (2012) showed that the largest class of soybean genes activated by an LCO spray were stress-response related. These experiments represent beginnings to elucidate the plant rhizobacterial community and its activities; considerable advancements in understanding are still needed, but the potential for increased crop production through novel technologies is also considerable.

Under typical agricultural field conditions, early germination is an important factor that affects canola yield in the northern temperate area of Western Canada. Owing to a very short growing season, particularly in the prairie regions, canola is usually seeded in the early spring. At that time, temperature is below optimum, such that germination and emergence can be delayed by one to two weeks. Early spring seeding is necessary for the crop to reach maturity without experiencing reduction in yield due to summer heat stress. Furthermore, canola must be planted deeply to establish successfully under dry soil surface conditions (Harker et al. 2012). Owing to these and other factors, only 50% of planted canola seeds actually emerge (Harker et al. 2003). Reduced germination rates can increase the susceptibility of seedlings to soil borne pathogens, e.g. phoma stem canker (*Leptosphaeria maculans*), decrease the vigor of young plants, delay maturity and the depletion of red light by the crop canopy, which would otherwise inhibit weed seed germination (Harker et al. 2012).

Although science has known *Brassica napus* since the nineteenth century, systematic research into its use as a crop began only in the late 1930s (Juska and Busch 1994). At that time, many countries developed national policies to promote self-sufficiency in production of fats and oils. In the early 1970s, responding to health concerns, Canadian breeders produced higher oil varieties, with less than two percent erucic acid in the oil and less than 30μM of glucosinolates per gram of air-dried oil-free meal. In 1979, these Canadian low erucic acid varieties came to be known as canola or edible oilseed rape. The cultivated area of this crop is expanding rapidly in Canada, in part owing to the intensifying demand for biodiesel feedstock (Franzaring et al. 2008). In 2000, the average yield in Canada was 1.5 t ha^{-1} (Statistics Canada 2012). From 2008 to 2010, the average yield in Canada was approximately 1.9 t ha^{-1} (Harker et al. 2012).

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Objectives:
To understand the potential for microbe-to-plant signaling compounds such as LCOs and thuricin 17 to help canola plants deal with stressful growing conditions.

Milestones:
1. Demonstrate effects of microbe-to-plant signaling compounds on canola seed germination
2. Demonstrate effects of signaling compounds on canola plant development and architecture
3. Demonstrate effects of signaling compounds on canola plant yield

Deliverables:
An understanding of the potential for a new technology applicable to canola production under Canadian conditions.

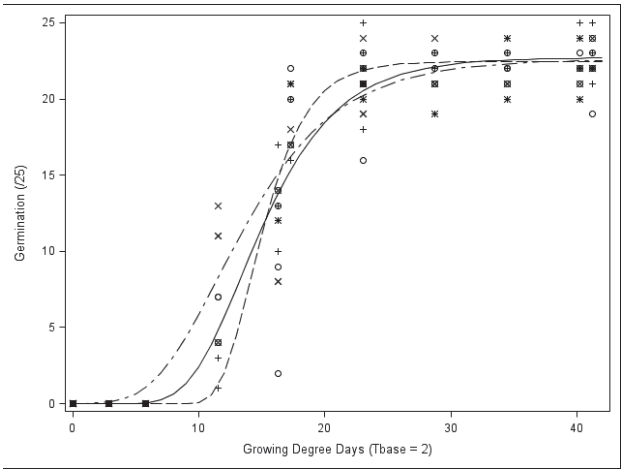
Approach:
Plants raised in pots were treated with varying concentrations of LCOs or thuricin 17 (both known to promote plant growth and aid in stress responses) to determine effects on canola plants growing under stressed and non-stressed conditions. At times similar compounds have also been included in the testing, specifically chitopentaose, which has a structure like the backbone of the LCO used in the presented work. Data were collected on variables related to growth and development, seed yield, yield components (silique number, seeds per silique, 1000-seed weight) and fatty acid composition (analyses done at the University of Manitoba lab in Winnipeg).

Materials and Methods 1

The germination assay was conducted with open pollinated canola (*Brassica napus*) lines made using conventional crosses and pedigree (Peter McVetty, University of Manitoba).
The experiment was conducted in three phases: in phase 1, it was evaluated which combination of temperature and LCO concentration enhanced canola cv. Polo germination; in phase 2, the effect of the µM LCO solution was tested on canola cvs. 04C204, Polo and Topas under mid-range (20^{o}C) and low (10^{o}C) temperature conditions; in phase 3, a set of cultivars was tested under high (30^{o}C), mid-range and low temperature conditions at the signaling compound concentrations described immediately below.
For phase 1 of the experiment, growth chambers were set to 16h at 20^{o}C, 8h at 30^{o}C, 95% humidity and zero illumination. For phase 2, growth chambers were set to 24h at either 10^{o}C or the ideal germination temperature of 25^{o}C, 70% humidity and zero illumination. For phase 3, growth chambers were set to high, mid-range and low temperature, 70% humidity and zero illumination.
For phase 1 of this experiment, Petri dishes of 25 seeds each were treated with 10ml of 10^{-5}, 10^{-6} or 10^{-9}M LCO solution; 10^{-5}, 10^{-6} or 10^{-9}M chitopentaose solution; 10^{-9}, 10^{-10}, 10^{-11} or 10^{-12}M thuricin 17 solution; or deionized water. For phases 2 and 3, Petri dishes were treated with 10ml 10^{-6}M LCO solution, 10^{-6}M chitopentaose solution or deionized water. Phase 3 also included 10ml 10^{-11}M thuricin 17 solution as a treatment.
After installation into growth chambers, germination was assessed every six hours.

Results 1
An LCO solution at µM concentration increased the early germination of our conventional canola cultivar, Polo, under standard germination conditions (Association of Official Seed Analysts, 1993; 16h at 20^{o}C followed by 8h at 30^{o}C and darkness). Before twenty Growing Degree Days (GDD), more Polo seed that was treated with the µM LCO solution germinated, compared to the other treatments and Polo seed treated with the µM concentration of LCO germinated more speedily than the controls (figure 1).

Figure 1 Gompertz curves overlaying a scatterplot showing the numbers of canola cv. Polo seeds germinated. The 95% confidence limits for Gompertz parameters, calculated for seeds treated with a 10^{-6}M LCO (broken dashed line, X symbol), did not overlap those of controls (solid line, circle symbol). The germination of seeds treated with a µM concentration of chitopentaose is shown as the dashed line (+ symbol).



The temperature-by-treatment interaction was significantly correlated. Germination of the high oil cultivar 04C111 was accelerated most strongly by the µM chitopentaose treatment at 20°C. The µM solution of chitopentaose produced quick and uniform germination for this cultivar. At 30°C, chitopentaose-treated seed had higher germination counts overall than both LCO- and water-treated seed.
Two of the experimental cultivars germinated particularly poorly. Their unsuccessful germination was even aggravated by LCO treatment. At 20°C, fewer LCO-treated or thuricin 17-treated 02C3 seeds germinated as compared to the controls and chitopentaose-treated seeds. However, under 10°C conditions fewer 02C3 seeds germinated when treated with LCO or chitopentaose than when treated with thuricin 17. Under 10°C temperature conditions, fewer LCO-treated 02C6 seeds or controls germinated than chitopentaose-treated 02C6 seeds.
In summary, if matched to environmental conditions, signaling compound treatments can improve seed germination in general, but may not at all times.

Materials and Methods 2

Peat pellets were soaked until saturated in their respective treatment solutions of either 1µM LCO or 10pM thuricin 17 or deionized water. Eight pellets were situated in each tray and three trays were allocated to each treatment per experimental replicate in time. Trays were installed into controlled environment cabinets set to 25°C/20°C, 10°C and 30°C with 14h day and 10h night. A single 04C111 seed was planted into each peat pellet. Plants were grown for 11 days and irrigated uniformly, each with 300ml of their respective treatment solution and each in a controlled environment cabinet as needed.
A second experiment was set out as a two factor centralized rotatable composite design, using Topas seeds planted individually into peat pellets that were irrigated with 10^{-12}, $10^{-11.12}$, 10^{-9}, $10^{-6.88}$ and 10^{-6}M concentrations of LCO solution. The plants grew for two weeks under a controlled 14h day and 10h night diurnal cycle under a range of controlled temperature conditions (4°C, 8°C, 17°C, 26°C and 30°C ± 2°C).

Growing conditions for the cold temperature plant culture vessel assay

A half MSB5 medium was prepared with 0.8% low-melt agarose, 1% sucrose and pH adjusted with KOH and NaCl to 5.8. Eight flasks of saline MSB5 media (0, 100, 200 or 400mM NaCl) were autoclaved and nanomolar (nM) concentrations of thuricin 17 were added using a PES filter under sterile conditions at a laminar flow bench. Thuricin 17 was not added to controls. Treatments were poured into scrubbed and autoclaved plant culture vessels (Magenta® GA 7, U.S. Pat. 4,358,908). There were four vessel

replicates per treatment. The media was left under the hood to solidify overnight. Canola cv. 04C111 seeds were disinfested in 1.5 ml microcentrifuge tube (Fisher Scientific, USA) using 20% bleach (6% sodium hypochlorite, NaOCl). Seeds were agitated using a Vortex Mixer (Fisher Scientific, USA) and rinsed with autoclave-sterilized deionized water until odorless. Using autoclaved tweezers, three seeds were planted into each vessel. While planting, the tweezers were intermittently flame sterilized and sterilized with 70% ethanol. The plant culture vessels were installed into controlled environment cabinets and randomized. Controlled environment cabinets provided a diurnal cycle of 14h illumination at 10°C and 10h darkness at 4°C. Plant culture vessels were re-randomized every two days. Plants were grown for 38 days and at the end of this time plant growth development and architectural metrics were assessed. Plants were dried in coin envelopes at 60°C for three days and weighed thereafter.

Growing conditions for the high temperature plant culture vessels assay

Canola cv. 04C111 plants were grown in both agar and low-melt agarose MSB5 media in scrubbed and autoclaved plant culture vessels. Controlled environment cabinets were set to 30°C and a diurnal cycle of 14h illumination and 10h darkness. Plants were grown for 20 days in low-melt agarose based MSB5 media that was prepared with and without nM or ten picomolar (pM) thuricin 17 concentrations and with a range of salinities (0, 0.05, 50, 100, or 200mM NaCl). After fresh weight and architectural metrics were gathered, the plants were dried in coin envelopes at 60°C for three days and weighed thereafter.

Results 2

The LCO and thuricin 17 irrigation treatments interacted with controlled temperature conditions to differently affect the architecture of 04C111 sown in peat pellets. Plants irrigated with a µM LCO solution that were grown under 30°C conditions produced more leaves than all plants grown under 25°C/20°C, a more optimal temperature condition.

Signaling compound treatment can accelerate canola development (figure 2); optimum signaling compound concentration depends on plant growth temperature. There was a quadratic effect of LCO concentration on above-ground fresh weight (figure 3). To obtain the highest above-ground fresh weights under high temperature conditions, near-nM concentrations of LCO ($10^{-8.81}$M) provided the greatest effect at the highest temperature (30°C). There was also a quadratic effect of LCO concentration on the dry weights of Topas plants. To obtain heavier dry weights, near-nM concentrations of LCO ($10^{-8.83}$M) provided the greatest effect at the highest temperature (30°C). In summary, treatment with signaling compounds increased the biomass production of canola under stressfully high temperatures.

There was a quadratic effect of LCO concentration on plant developmental stage achieved by Topas plants after two weeks of growth (figure 2). Plant development was accelerated at pM concentrations of LCO at mid-range levels of temperature (17°C).

Figure 2 A 3-D surface plot of plant developmental stages achieved by Topas plants grown for two weeks.
Stage = plant developmental stage (Sylvester-Bradley 1985),
conc = concentration of LCO (10^{x}M),
temp = temperature (^{o}C)

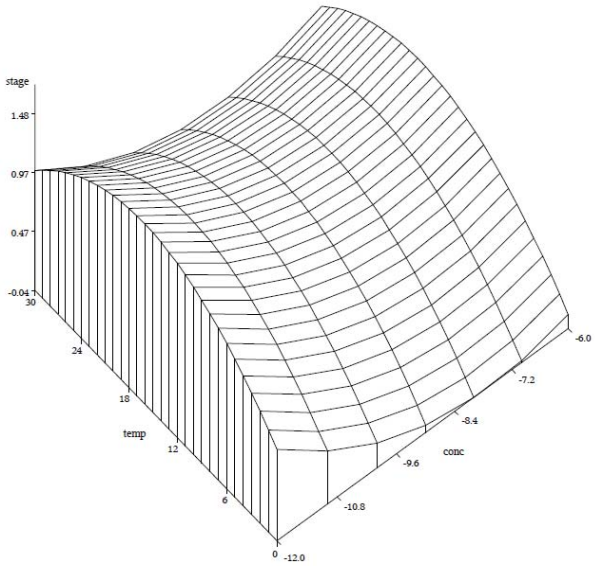
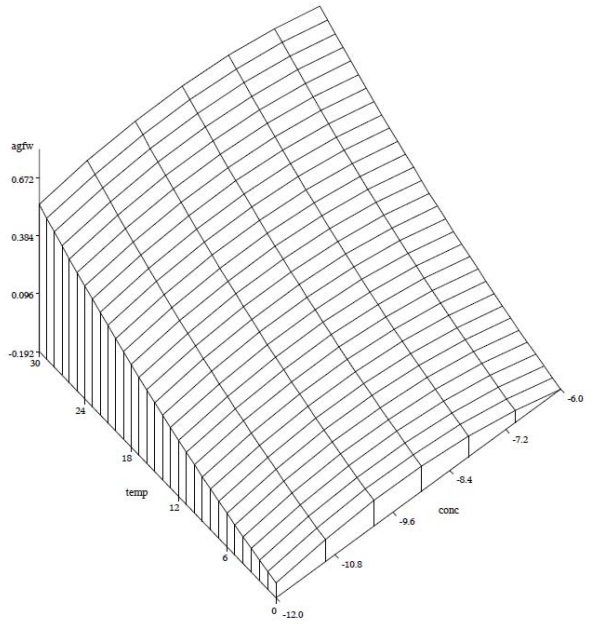


Figure 3 A 3-D surface plot of above ground fresh weight of Topas plants grown for two weeks.
Agfw = above ground fresh weight (g),
conc = concentration of LCO (10^{x}M),
temp = temperature (^{o}C).



The growth and development of young 04C111 plants were affected by chronic nano- and picomolar concentrations of thuricin 17 in agar and low-melt agarose MSB5 media in plant culture vessels (Magenta jars) and these effects interacted with salinity stress under these experimental temperature conditions. Thuricin 17 treatment increased the fresh weight of young plants under stressfully low (10°C / 4°C) temperature conditions and also prohibitively stressful salinity (200mM NaCl). This is further evidence that a signaling compound (in this case thuricin 17) can increase canola plant growth under stressful conditions.

It seems that at low temperatures, higher concentrations of LCO can be stressful themselves, so that the lower the temperature, the lower the concentration of LCO should be. At high temperatures, a higher concentration of LCO is more effective (figure 2).

In summary, signaling compound treatment can increase leaf production and biomass and accelerate canola plant growth and development under stressful conditions.

Under the doubly stressful low temperature (10°C/4°C) and saline (100mM NaCl) conditions, thuricin 17 treatment slowed plant development, i.e. plants that were grown for 38 days in low-melt agarose media with a nM concentration of thuricin 17 produced fewer leaves than plants not treated with thuricin 17. Again, it appears that when the stress conditions are severe addition of signaling compounds is detrimental.

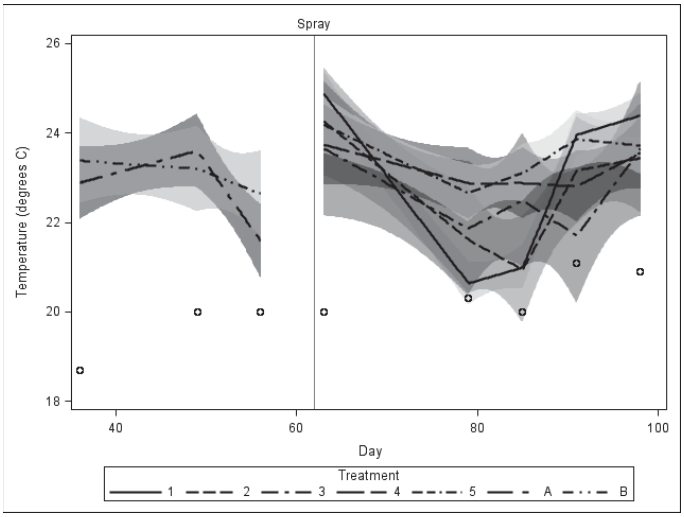
Materials and Methods 3

The experimental cultivars were grown in separate greenhouse rooms in McGill University's Plant Science Research Greenhouse in Ste Anne de Bellevue, Québec. Each greenhouse was set to specific temperature conditions (based on earlier work: 04C111 – temperature between 20°C and 25°C; for Topaz temperature for first 50 days was ~10°C, then raised at two days intervals in increments of 5°C up to the temperature of 04C111 plants, so the conditions for the two cultivars reached the same level at about day 66 after planting). The experimental methodology was similar for all three greenhouse experiments. The growth medium was a four component mix 10:5:2:10 non-mycorrhizal Promix: black earth: sand: vermiculite. The greenhouses were illuminated 800 to 1300 µmol m^{-2} s^{-1}, for 16h per day. In each pot, five seeds were planted on the surface of the growth medium to allow even application of LCO onto the seeds and then moved into the rooting medium. The surface was watered with 10ml of 1µM LCO, 10ml of 10pM thuricin 17 or 10ml deionized water. The young plants were counted as they emerged (i.e. when they reached above the level of the surface of the growth medium) and when the cotyledons were fully opened. Tepid water was provided as needed. At the third true leaf stage, each pot was thinned to two plants. Topas plants were sprayed 63 days after planting, i.e. at the flowering stage of plant development when >75% of plants bore open flowers. Similarly, 04C111 plants were sprayed 38 and 62 days after planting in the high and optimal temperature condition greenhouses, respectively. Plants were either left unsprayed or foliar-sprayed with either 0.5ml of deionized water or 0.5ml of a µM LCO solution. Plants were removed from the greenhouse for spray treatment application. Stomatal conductance and leaf temperature were measured weekly for the third true leaf (as measured from the base of each plant).

Results 3

The application of a µM LCO solution to 04C111 seed at planting promotes the regulation of leaf temperature. For example, on day 36 of growth, leaves of 04C111 plants grown from LCO-treated seed were colder than leaves of plants grown from untreated seed. However, raised leaf temperatures were observed among LCO-sprayed plants compared to the leaf temperatures of unsprayed plants on days 79 and 85 (figure 4). This ability to buffer leaf temperature could be valuable under stressful field conditions, but the full pattern of effects needs to be better understood along with the underlying mechanisms.

Figure 4 Loess curves (smooth=0.125) of canola cv. 04C111 leaf temperatures (^{o}C). Before day 62, treatment A = LCO applied to seed at planting; B = water applied to seed at planting. After day 62, treatment 1 = water applied to seed at planting; 2 = LCO applied to seed at planting; 3 = water applied to seed at planting, water foliar spray; 4 = water applied to seed at planting, LCO foliar spray; 5 = LCO applied to seed at planting, LCO foliar spray. Shaded regions indicate 95% confidence limits. Empty circles indicate average greenhouse daytime temperature for the days that leaf temperature was measured



The application of a µM LCO solution to the seed at planting increases the number and length of branches produced by the resultant plant. LCO-treated 04C111 seed produced plants with more primary branches. The application of the LCO foliar spray increased the sum of branch lengths. The LCO spray also increased the length of the fourth-longest branches, which were positively associated with yield (Harker et al 2012). Based on the comparison of stem-to-branch length ratios, unsprayed 04C111 plants were more apically dominant than LCO-sprayed 04C111 plants.
The apical racemes of Topas plants sprayed with LCO produced more siliques and more apical siliques per cm of stem than unsprayed plants. Topas plants sprayed with LCO had higher ratios of apical:lateral siliques and produced heavier total seed from apical racemes than unsprayed plants, i.e. greater yields from LCO treated plants.

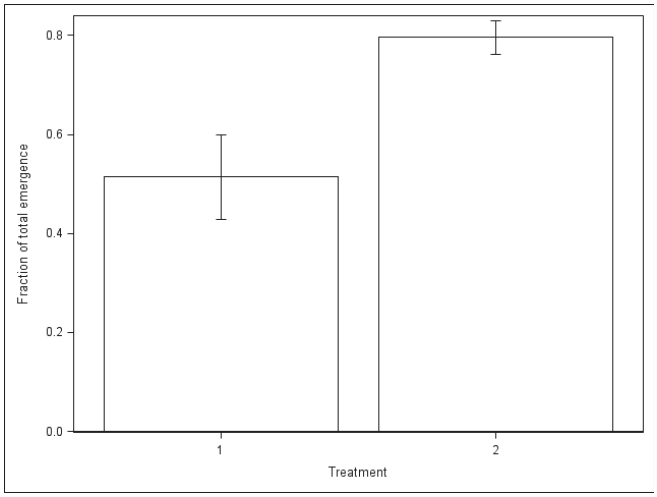
Materials and Methods 4

Rapid cycling canola (fastplant) seeds were planted into 6.4cm cube pots of Promix, 10ml of 1µM LCO solution or deionized water were applied to the seed and slow-release fertilizer (Nutricote 14-14-14) was added to the surface of the Promix. The fastplants were grown under continuous light (250 µmol m^{-2} s^{-1}) at 25^{o}C and were watered uniformly with deionized water, as necessary. Emergence data were taken after one week. Supplementary foliar sprays of µM LCO solution were applied in sufficient quantity to coat the surface of the plant at successive stages of plant development (<1 ml $plant^{-1}$). When the fastplants had senesced, pods were removed and dried in an oven at 60^{o}C for three days.

Results 4

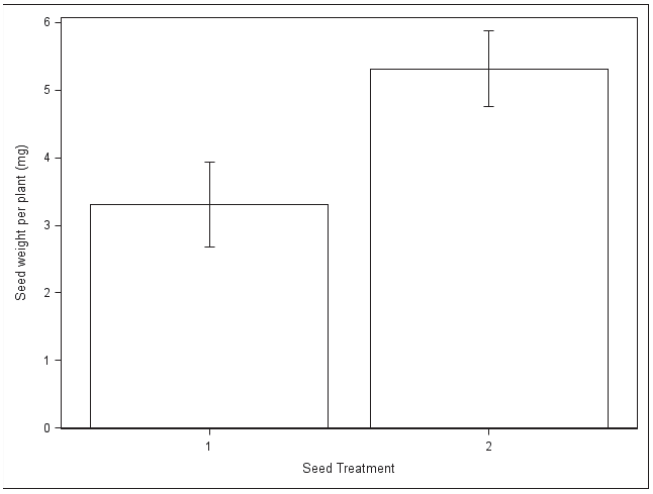
Under the controlled environment conditions that were used, the treatment of seeds with 1µM LCO solution accelerated the emergence of young fastplants, to a very highly significant degree (figure 5).

Figure 5 Effect on emergence one week after planting and treating rapid cycling canola seed with 1µM LCO solution.
1- untreated fastplants;
2- seed treated with 10ml of 1µM LCO solution.
Bars indicate standard errors (difference p = 0.0010).



The treatment of seeds with 10ml of a 1µM LCO solution increased fastplant production of seeds compared to controls (figure 6). The seed treatment contributed to increased yield of canola fastplants apparently via enhancement of factors contributing to yield.

Figure 6 Effect on seed weight per plant at maturity for rapid cycling canola seed treated with 1µM LCO solution.
1- untreated fastplants;
2- seed-treated fastplants with 10ml 1µM LCO solution.
Bars indicate standard errors.
Kruskal-Wallis tests indicated the difference between treatments 1 and 2 was significant (p = 0.0295).



Overall LCO treatment increased the seed weights. Application of LCO to the seed and as a foliar spray to fastplants and Topas plants, respectively, resulted in heavier seed weights per plant on average.

Owing to the distinction made between apical and lateral siliquae, it was observed that the apical racemes of LCO-sprayed Topas plants produced more siliquae and more apical siliquae per cm of stem than unsprayed plants. Topas plants sprayed with LCO had higher ratios of apical to lateral siliquae and produced heavier total seed from apical racemes than unsprayed plants.

General Discussion:

In Canada a canola crop frequently develops under conditions of stress, from cool soil temperatures at the time of planting to stressfully high temperatures and water limitation during development. In this work, treatment with microbe-to-plant signals such as LCOs and thuricin 17 showed to have potential as low-input management practices that could allow better canola crop production in Canada. Through a set of experiments, increases in the rate and percentage of germination of canola seed were detected. In addition, treatments with microbially produced signaling compounds could alter plant architecture leading to more branching of both the stem and the raceme. However, this has so far only been evaluated under controlled environment conditions and under the field conditions prevalent in Southwestern Quebec and only on a few canola genotypes. To know the full potential of this technology in Canada, geographically wide-spread testing is required as well as evaluation across a wide range of canola genotypes.

In the case of the fastplant work, it was shown that treatment with these signaling compounds can result in greater seed weights and increased seed production per plant. This effect was reproduced under greenhouse conditions with the Topas cultivar under simulated cool spring conditions. The initial field testing conducted showed potential for this approach under field conditions, but this requires additional investigation.

Stressfully high temperature conditions can impair plant development and result in fewer organs such as leaves (Stone 2001; Barnabás et al. 2008). The presented experiment with Topas indicated the optimum point to increase leaf number for this cultivar will be at a lower temperature (17^{o}C) and pM LCO concentration. However, when grown in peat under moderately high (30^{o}C/30^{o}C) temperature and under low (10^{o}C/4^{o}C) temperature conditions in plant culture vessels, the 10pM thuricin 17 treatment inhibited 04C111 leaf growth compared to controls. Kurepin et al. (2007) inhibited canola leaf growth by applying 10^{-4} and 10^{-5}M IAA and ABA. The observed leaf inhibition may be in part explained by the increased ABA responsiveness of brassicaceous plants under stressful conditions (Yamaguchi-Shinozaki and Shinozaki 1994).

Under 30^{o}C/30^{o}C temperature conditions, the µM LCO irrigation treatment increased the lengths of the longest, second- and third-longest petioles of 04C111 plants compared to corresponding petioles of plants grown under 25^{o}C/20^{o}C temperature conditions. Kurepin et al. (2007) subjected similarly young canola plants to low red to far red ratio treatment and produced longer petioles as expected from plants subjected to shade.

The application of a µM LCO treatment to the seeds at planting enhanced fastplant emergence. As such, these results may benefit canola production systems as a rapid and uniform emergence from the seedbed led to the production of vigorous plants with high chlorophyll content in their leaves (Ghassemi-Golezani et al. 2008). High leaf chlorophyll content, stand uniformity and fast and early crop canopy closure can provide weed-inhibiting light conditions at the soil surface to reduce herbicide inputs, reduce selection pressure for weed resistance, improve yield, improve quality and increase canola profitability (Harker et al. 2012). Rapid early light interception also improves early biomass accumulation on an area basis, and this can also contribute to higher final yields.

LCO seed-treated 04C111 and Topas plants have more branches of all types than plants grown from untreated seed. It has been noted previously in *Brassica* species, that lowering the auxin to cytokinin ratio increases the number of primordia that develop into lateral buds, e.g. exogenous cytokinin treatment has stimulated bud outgrowth. It has also been noted that plants can repress auxin signaling as a component of basal resistance. The production of larger numbers of seed-yielding branches, such as observed here among LCO-treated plants, would enable those plants to compensate for environmental stress and herbivore attacks (e.g. pollen beetle; Thomas 2003). Branch number has been identified by other groups as an agronomic trait that affects canola yield indirectly by affecting other yield components in either negative or positive ways (Diepenbrock 2000; Baradaran et al. 2007; Aytac et al. 2008; Başalma 2008). The spray treatment in the presented study could be used to encourage

productivity, if a crop was decapitated or damaged.
Topas increased apical seed weight and lower protein content from LCO-sprayed plants is a promising combination that can result in higher seed oil yields. As numerous groups have found, any condition that improves oil yield may potentially reduce the concentration of seed protein (Triboi and Triboi-Blondel 2002).

Field trials

In 2011, there was no significant effect caused by LCOs and thuricin 17 solution. One of the reasons may be improper seeding time. Seeds were sown on June 22, 2011 and average temperature went up to 28.5^{o}C in July (table 1 & figure 7).
In 2012, the plants grown from seeds primed with the LCOs solution produced more branches, pods, heavier dry biomass and seed weight than controls, and higher LCOs solution (10^{-6}M) gave a better effect. In the case of thuricin 17, quadrats where seeds had been primed with 100pM thuricin 17 solution and subsequently left unsprayed produced more plants than quadrats where seeds had been primed with nM thuricin 17 and also left unsprayed. There was no significant effect caused by LCOs or thuricin 17 treatment in yield.

Field trial 2011

The canola signaling trial in 2011 was conducted in the Emile A. Lods Agronomy Research Centre on the MacDonald Campus of McGill University in Ste-Anne-de-Bellevue, Québec (45°3' N, 74°11' W). Effect of signaling compounds (LCOs and thuricin 17) on canola plant growth was studied in the field conditions.

Materials and methods field trial 2011

The experiments were conducted as a completely randomized block design with four plot replications for each treatment. Each block had 19 treatments (table 1), which gave a total of 76 plots. Plot size was 1.5x4m, allowing seven rows of canola with 50cm spacing at the edge of each plot as buffer zones. There were 1.3m wide buffer zones between blocks and border plots at two sides of the field. Border plots received the same treatments as the neighbouring plots.
Two concentrations of LCO (10^{-6} and 10^{-7}M) and thuricin 17 (10^{-9} and 10^{-10}M) were used alone or applied with additives Organic Biolink or 0.5% carrageenan. Acting as spreader or sticker, Organic Biolink was added at the rate of one ml per four litres to the solution. A water treatment was included as a negative control. Biolink or carrageenan was added and used as a positive control in our trials. All these treatments above were applied on canola untreated seeds. Besides, pesticide coated seeds were also used, seeds being treated with solutions of LCOs or thuricin 17 in concentrations of 10^{-6} and 10^{-9}M, respectively. Correspondingly, a pesticide coated control was required. All these treatments were applied to the seeds individually in a very short time (approximately one minute) and then air dried on the same day within a few hours of sowing.
The canola hybrid Invigor 5440 was used and applied in the form of untreated or pesticide treated seeds. This trial was set up quite late on June 22, 2011. (No field trials had been planned so this was a late addition to the field work.) Stand counts of two rows per plot were conducted after canola emergence on July 16, 2011. Four months later, the plants reached maturity and ten representative plants were collected per plot on November 9, 2011. The data collected from these plants were: number of pods per plants, number of branches per plant, pods dry weight per five plants and dry biomass (no seeds and pods) per five plants.

Results field trial 2011

In 2011, the values of most measured variables were numerically greater for signalling compound treated plants and the effects seemed to be slightly larger for thuricin 17 than for the LCOs. However, the establishment of this experiment was very difficult, due to warm and dry conditions after seeding, and the resulting data were very variable. There were no statistically significant effects of the applied treatments in 2011.

Table 1. Experimental design for the 2011 field work.	
	Treatment
1	Control (water)
2	Control (water) + 0.5% carrageenan
3	Control (water) + BioLink
4	Pesticide coated control
5	LCO 10^{-6}M
6	LCO 10^{-7}M
7	LCO 10^{-6}M + Biolink
8	LCO 10^{-7}M + BioLink
9	LCO 10^{-6}M + 0.5% carrageenan
10	LCO 10^{-7}M+ 0.5% carrageenan
11	Pesticide coated seeds + LCO 10^{-6}M
12	Thurcin 10^{-9}M
13	Thuricin 10^{-10}M
14	Thuricin 10^{-9}M + BioLink
15	Thuricin 10^{-10}M + BioLink
16	Thuricin 10^{-9}M + 0.5% carrageenan
17	Thuricin 10^{-10}M + 0.5% carrageenan
18	Pesticide coated seeds + Thuricin 10^{-9}M
19	Pesticide coated seeds + Thuricin 10^{-10}M
LCO = lipochitooligosaccharide, seed = seed applied, Thuricin = thuricin 17, foliar = leaf applied.	

In summary, there was no significant effects caused by LCOs and thuricin17 solution in comparison of plant density (emergence count), number of pods per plant, number of branches per plant, pods dry weight per five plants, and dry biomass (no pods and seeds) per five plants. In visual observation, canola did not grow well in the field (figure 7), which may have been due to the very late seeding time.

Figure 7. Canola of field trial in 2011

Seeds were sown on June 22, 2011 and average temperature was then up to 28.5°C in July (table 2, figures 8 and 9). Canola plants experienced a fairly long period of fairly strong heat stress right after emergence. After growing for four months, the canola was still not fully mature: the pods were still green and very few seeds existed in each pod. Therefore, no combine harvest was conducted and no yield data is presented.

Table 2. Temperature monthly reading (June to September, 2011) Ste-Anne-de-Bellevue

Parameter	June	July	August	September
Average maximum temperature (°C)	24.3	28.5	25.7	22.3
Average minimum temperature (°C)	14.3	16.7	16.3	13.1
Temperature (extreme, °C)	31.8	34.7	30.9	29.5
Total precipitation (mm)	52.8	35.6	224.8	110.4

Figure 8. Daily temperature reading (April-August, 2011) Ste-Anne-de-Bellevue

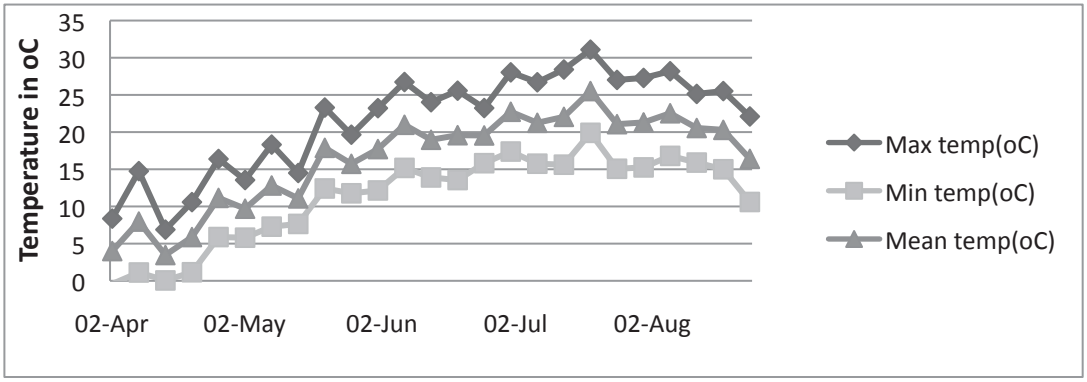
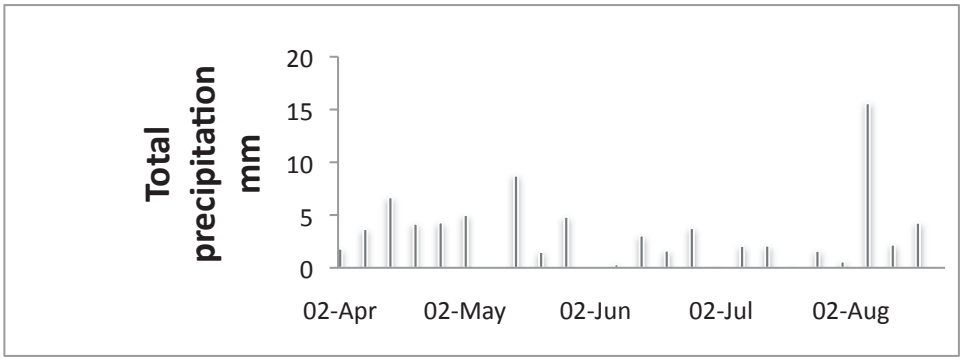


Figure 9. Daily total precipitation (April-August, 2011) Ste-Anne-de-Bellevue



Field trial 2012

The canola signaling trial in 2012 was conducted at the Emile A. Lods Agronomy Research Centre on the MacDonald Campus of McGill University in Ste-Anne-de-Bellevue, Québec (45°3' N, 74°11' W). The effects of signaling compounds (LCOs and thuricin 17) on canola plants growth was studied under field conditions.

Materials and methods field trial 2012

The experiments were conducted as a completely randomized block design with four plot replications for each treatment. Each block had 16 treatments (table 3), which gave a total of 64 plots. Plot size was 3.1x4 m, allowing 14 rows of canola (2.6m) with 50cm spacing at the edge of each plot as buffer zones. There were 1.3m wide buffer zones between blocks and border plots at two sides of the field. Border plots received the same treatments as the neighbouring plots.

In table 3 the experimental design for 2012 is presented. Two concentrations of LCO (10^{-6} and 10^{-7}M) and thuricin 17 (10^{-9} and 10^{-10} M) were used alone or applied with the additive Organic Biolink and seed film coating agent Disco AG L-323 (Incotec, USA) as seed treatment. As spreader or sticker, Organic Biolink was added at the rate of 1ml per four litres or small drop of our solution which was the same rate as seed film agent.

Table 3. Experimental design for the 2012 field work.

	Treatment
1	Seed water
2	Seed water + BioLink (1ml per four litres)
3	Seed water + foliar LCO 10^{-6}M
4	Seed water + foliar Thuricin 10^{-9}M
5	Seed LCO 10^{-6}M
6	Seed LCO 10^{-7}M
7	Seed LCO 10^{-6}M + BioLink
8	Seed LCO 10^{-7}M + BioLink
9	Seed LCO10^{-6}M + Biolink + foliar LCO 10^{-6}M
10	Seed LCO 10^{-7}M+BioLink+foliar LCO 10^{-6}M
11	Seed Thuricin 10^{-9}M
12	Seed Thuricin 10^{-10}M
13	Seed Thuricin 10^{-9}M + BioLink
14	Seed Thuricin 10^{-10}M+ BioLink
15	Seed Thuricin 10^{-9}M + BioLink + foliar Thuricin 10^{-9}M
16	Seed Thuricin 10^{-10}M + Biolink + foliar Thuricin 10^{-9}M

LCO = lipochitooligosaccharide,
seed = seed applied,
Thuricin = thuricin 17,
foliar = leaf applied.

A water treatment was included as a negative control and Biolink was added and used as a positive control in the presented trials. All the treatments above were added to untreated seeds (Invigor 5440) individually in a very short time (around one minute) and then air dried on the same day and shortly prior to sowing on May 14, 2012. One month later, at the beginning of the flowering stage (2012.6.22), these

two concentrations of LCOs and thuricin 17 were applied as foliar applications to the plants, which already had already received corresponding seed treatments. In this case, 0.125% Agral-90 was added as sticker in LCO or thuricin 17 solutions, right before foliar application. Therefore, LCOs and thuricin 17 foliar application (plus 0.125% Agral-90) without seed treatment were needed as another positive control. Canola plants were grown in the field from May to August in 2012. Meteorological data for 2012 is presented in figures 10 & 11. Stand count of two rows per plot was conducted after canola emergence on May 24, 2012. When plants reached maturity, five representative plants were randomly selected from each plot and plant height was measured on July 31, 2012. Later, plant density (number of plants per one metre of row) was recorded two times per plot (2012.8.2). On the same day, five representative plants were randomly selected from each plot. From those plants, number of pods per plant, number of branches per plant, dry biomass (no seeds) per plant and seed dry weight per plant were determined. After that, combine harvest was conducted on August 8, 2012 and yield data were collected.

Figure 10. Daily temperature reading (April-August, 2012) Ste-Anne-de-Bellevue

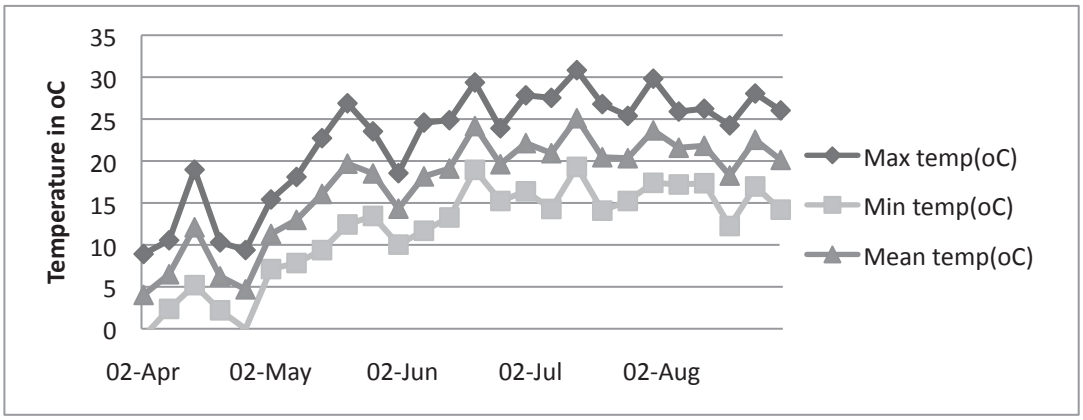
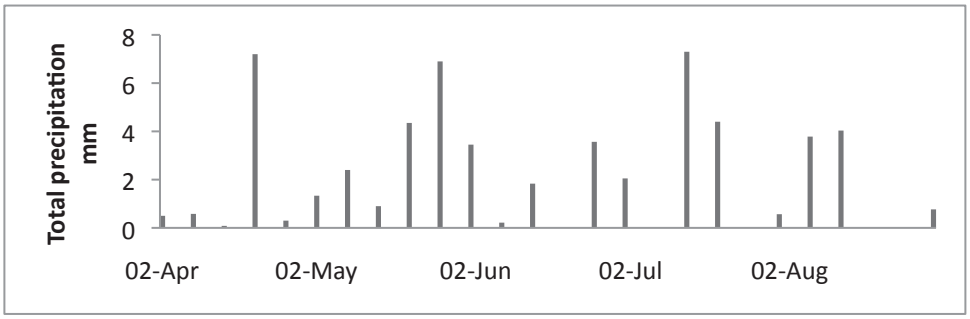


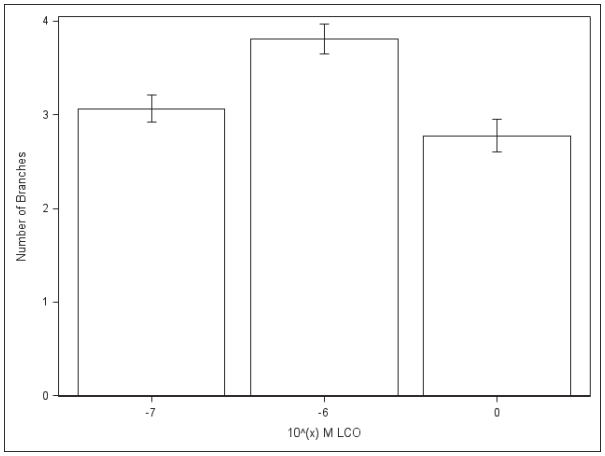
Figure 11. Daily total precipitation (April-August, 2012) Ste-Anne-de-Bellevue



Results field trial 2012

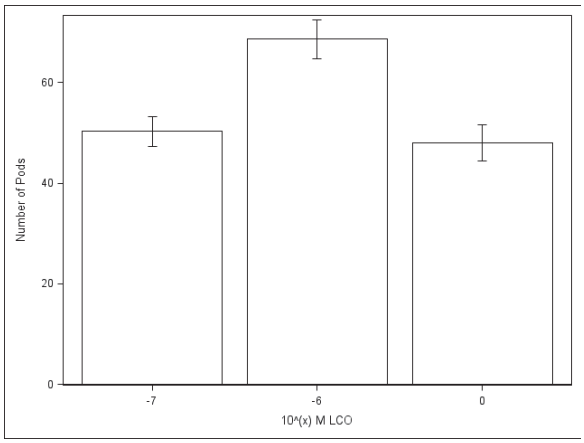
From the 2012 field trials, it was found that plants grown from seeds primed with µM LCO solution produced more branches than both the plants that were grown from seeds primed with 100nM LCO solution, and the controls, p = 0.0119 and p = 0.0016, respectively (figure 12).

Figure 12. Effect of LCO application on number of branches per plant.
0 = control,
-7 = application of 10^{-7}M LCO and
-6 = 10^{-6}M LCO.



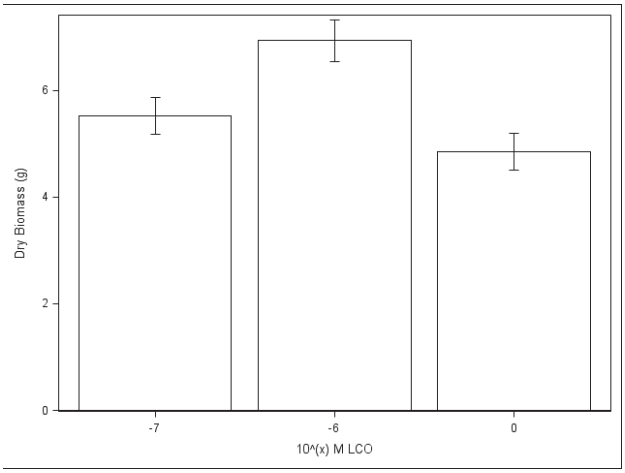
In order to meet the conventional statistical assumptions, the numbers of pods per plant were square root-transformed. Based on the transformed data, the plants grown from seeds primed with the µM LCO solution produced more pods than those that had been primed with 100nM LCO solution and controls with p = 0.0221 and p = 0.0201, respectively (figure 13).

Figure 13. Effect of LCO application on number of pods per plant.
0 = control,
-7 = application of 10^{-7}M LCO and
-6 = 10^{-6}M.



In order to meet the conventional statistical assumptions, the 2012 dry biomass data was log-transformed. Based on the transformed data, the plants grown from seeds primed with the µM LCO solution produced heavier dry biomass than those that had been primed with 100nM LCO solution and controls with p = 0.0485 and p = 0.0084, respectively (figure 14).

Figure 14. Effect of LCO application on number of plant biomass production.
0 = control,
-7 = application of 10^{-7}M LCO and
-6 = 10^{-6}M.



Field trial conclusions

In 2011, the treatment of canola with microbe-to-plant signaling compounds resulted in generally higher numerical values for many of the variables measured. However, as a result of the very difficult growth conditions for the 2011 experiment, the data was very noisy and there were no statistically significant differences in those variables. In 2012, treatment with thuricin 17 resulted in little difference. However, in 2012 treatment plants grown from seeds primed with the LCO solutions produced more branches, more pods and more dry biomass than controls and the higher LCO concentration (10^{-6}M) gave a greater result. The final seed yield was also generally numerically greater for plants treated with LCOs, but these increases were relatively smaller than those of some other the other variables and there was no statistical difference among treatments for yield. There is work to be done, but this is a hopeful start with regard to the application of these signaling compounds to canola production.

The signaling compounds evaluated here constitute a new type of plant growth regulators. While they have already been applied commercially to a number of crops, there are still some key crops, such as canola, that need to be much better understood with regard to the applicability of these compounds during crop production. It was shown here, that these compounds can have effects on a range of aspects of canola development, including, in some cases, negative effects (found in some other related work.) Like for all plant growth regulators the positive effects can be concentration, crop development and timing dependant. These compounds constitute low-input, low-cost management techniques that could be important to crop production in the near future. Given that, they can help overcome stressful conditions and that the developing increase in frequency of extreme weather events as climate continues to change.

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

- Include the name of scientist / organization.

None

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.

AAFC RESEARCH BRANCH
Research Project Final Report

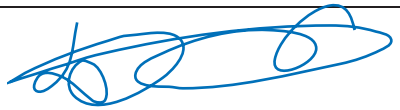
• Include name of scientist / organization.
None

C. Variance Report (if applicable, describe how the work differs from the proposed research) • Include changes to objectives and project work plan / budget, changes to the team, other constraints.
There were no major variances, except that we did manage to conduct some of the field work that had been initially included in the proposal, but was cut due to funding constraints.

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).

E. Achievements (include only those related to this project) • Include innovations, publications / conferences, technology transfer, capacity building, success stories, media, recognition and other outputs.
The work indicates that under some conditions the application of signaling compounds can alter plant architecture, cause shifts in physiology and improve yield potential.

F. Lessons learned (self-evaluation of project)
There have been some surprises in the work. Most notably that the combination of more severe stress and signaling compounds can be detrimental; in this case a combination of low temperature and high salinity. However, most plants generally experience more moderate stress on a fairly regular basis (excessively cool spring conditions or night temperatures or temporary dry conditions or periods of higher temperatures).

Donald L. Smith	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.