Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year, noting the personnel involved and any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A final financial statement summary of expenses must also be submitted. In the event of major changes within the budget, supporting notes are required. The final report is a summary of activity for the final year and an overview of the entire project.

Project Title: ***Biopesticides as a Novel Management Strategy for Sclerotinia in Canola***

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Dr. Tim Dumonceaux	AAFC Saskatoon	Principal Investigator
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
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Project Start Date: April 1, 2018 **Project Completion Date:** March 31, 2024

Reporting Period: April 1, 2023 **to** March 31, 2024

CARP Project Number: **2018.01**

Instructions: This Final Project Report shall be completed and submitted on or about February 15th of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition to the Final Research Report, a *Final Research Abstract/Extension Report* is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: __March 31, 2024_____

☐ **Ahead of Schedule** ☐ **On Schedule** X☐ **Behind Schedule** ☐ **Completed**

Comments: We have received and evaluated six industrial formulations of the biocontrol agent PENSV20 for their efficacy against *S. sclerotiorum* in canola. While these have proven efficacy, the main greenhouse trial will not begin before the end of March and so will go past the project end date. Nevertheless, given the results observed so far, we anticipate that we will have a suitable industrial-grade formulation for application of the biocontrol agent in field trials, which is beyond the scope of this project but for which we have applied for funding.

2. Summary - Maximum of one page. This must include project objectives, results, and conclusions.

Our overall objectives have been met over the course of this project, with the identification of a bacterial agent (BCA) that shows remarkable efficacy in protecting canola plants from infection with the devastating fungal pathogen *Sclerotinia sclerotiorum*. This bacterium, coded PENSV20 in our culture collection, was identified taxonomically as a strain of *Bacillus atropheaus*. We sequenced the genome of this bacterium to verify its taxonomic identity and to determine the possible mechanisms that could explain it bio-protective effect. The improved and reliable BCA application and pathogen challenge regimens that were developed in the last reporting period have been helpful in allowing us to assess the effect of this BCA on both fully susceptible canola lines (Westar), and in a partially resistant winter canola line (Zhong You). Beyond identifying strains that exert a bio-protective effect and examining this effect in different canola lines, a major objective of our project was the identification of the mechanisms by which PENSV20 protects canola plants from the worst effects of *S. sclerotiorum*. To that end, we investigated two independent lines of research. In the first, we examined the effect of PENSV20 and pathogen challenge on the induction of gene synthesis in canola using RNAseq. We found three main pathways that were overexpressed in *B. napus* upon treatment; these were associated with systemic acquired resistance, induced systemic resistance, and cellular detoxification. The latter pathway was activated only in the *B. napus* cultivars treated with pathogen only, and was not up-regulated when PENSV20 was applied prior to pathogen challenge. Overall, more than 1000 differentially expressed genes were identified in *B. napus* under the experimental conditions, including many genes related to processes known to be associated with plant resistance and direct pathogen antibiosis. Our second line of investigation focused on the metabolites produced by the BCA, to determine if direct antibiosis of the pathogen could be involved in the observed phenomenon of plant protection after pathogen challenge. HPLC analysis of culture supernatants revealed that a range of metabolites are produced by PENSV20 that have been previously identified with fungal phytopathogen antibiosis, including iturin A, fengycins, and surfactins. Examination of the PENSV20 genome sequence identified 11 regions that could be involved in secondary metabolite biosynthesis, including the metabolites that were identified by HPLC. We have begun work to investigate directly the role of these metabolites in the bio-protective effect by constructing deletion mutants in key genes identified in these pathways. This work will not be completed prior to the project end date, but the groundwork has been put in place to empower us to investigate the role of these metabolites using reverse genetics approaches. Finally, a key objective of this final year of the project has been to evaluate various formulations of the BCA for efficacy, utility, and applicability. This work has been undertaken with an industrial collaborator, Montana BioAgriculture (MBA, USA)/Terra Biotechnologies (Canada). MBA has produced 10 variations on industrial formulations including spores and vegetative cells, differing carriers, drying temperatures, and other variables. Six of these have been tested on canola plants to determine their effects on plants in the absence and presence of a pathogen challenge. While

these experiments are ongoing, encouraging early results of have been obtained that suggest that there is flexibility in terms of formulation parameters, since all of the tested formulations provide good protection to pathogen-challenged plants and do not appear to damage petals upon spraying. Using the experience in formulation handling characteristics provided by this trial, we are preparing for a greenhouse-scale replicated experimental trial with a limited number of formulations to determine the efficacy of the industrial-grade formulations on canola plants challenged with pathogen. This experiment will be initiated prior to the project end date but will not be completed until later in the spring of 2024. All of this work is leading towards larger-scale field trials, and we are preparing for these trials by forming a Bioproducts Partnership with industrial collaborators, with the aim of undertaking the final stages of getting this now-proven BCA into the hands of producers.

3. Introduction – Brief project background, rationale, and objectives.

Canola is a multi-billion dollar industry in Canada and *Sclerotinia sclerotiorum*, the fungal pathogen of sclerotinia stem rot, causes annual yield losses of approximately 5-10%, where estimated losses of $15 million were reported in 1982. Annual incidence of the disease is often rated at 10-20%, but disease levels can reach as high as 94% under certain environmental conditions. In 2010, sclerotinia was widespread across the prairies in canola, with a reported 90% incidence of disease symptoms. Although this is a major disease of canola, the pathogen causes disease on over 400 plant species, particularly dicotylendonous plants. Symptoms include formation of bleached greyish lesions on the main stem, branches, or pods and eventual shredding of the canola plant, and the presence of hard, melanized black sclerotia within the infected cortex have been reported. Cultural control relies on reducing the number of sclerotia that overwinter in soil and creating environmental conditions unfavorable for disease development. Chemical fungicides of different classes effectively control the disease, but because these fungicide classes show only single-site activity, the chance for development of fungal resistance to them is quite high. But outbreaks for fungicide applications can be difficult to predict due to environmental conditions and timing of fungicide applications can be a challenge to growers. In the late 1980s and early 1990s, a forecast system on the incidence of Sclerotinia infestation of canola petals was developed but this was not useful for predicting the severity of sclerotinia stem rot. Although the search for natural host resistance through breeding is a common and preferred method for controlling this disease, strong genetic resistance remains elusive. As an alternative or complement to plant breeding, natural microbial interactions can be exploited to inhibit pathogen growth and achieve agronomic aims. The aim of biocontrol is to use microorganisms or their derived metabolites in place of synthetic chemicals to decrease the impact of plant pathogens or microorganisms associated with post-harvest spoilage on the yield and quality of agricultural products. Research at AAFC in recent years has been conducted to identify leading bacterial strains with biopesticidal activity against *Sclerotinia sclerotiorum*. Biological control with various strains of plant growth promoting bacteria (PGPR) and endophytic fungi have been studied. Several modes of action have been reported, including competition for nutrients, niche exclusion, induced resistance, and antibiosis, the production of bacterial secondary metabolites that result in an antagonistic interaction between the biocontrol microorganism and the target phytopathogen. Some of these wide spectrum antibiotics have been identified as phenazine, lipopeptides, pyoluteorin, 2,4 diacetylphloroglucinol, and many others. New alternatives to chemical fungicides for the sustainable production of canola and oilseed crops can be developed through the discovery, understanding, and exploitation of natural microbial interactions. Our overall objectives in this project were to *(i)* identify candidate bacteria displaying a phenotype of antibiosis against *S. sclerotiorum* in vitro using plate assays; *(ii)* determine the possible mechanisms of action of the best-performing strains through genome sequencing, metabolite analysis, and examination of host response; and *(iii)* perform the optimization and formulation work required to bring the identified strains to industrial application, in order to make novel disease control options available to producers.

The anticipated impacts of this clean technology, which are well on their way to being realized, include: *(i)* the development of a foliar-applied bacterial biopesticide product as a green alternative and a novel tool for integrated pest management for canola production; *(ii)* demonstrated interest by an industry partner to license the novel biopesticide for registration and eventual commercialization of the technology to deliver this novel

technology to producers; and *(iii)* improvement of the environmental performance in canola production by using biopesticide technology for sustainable canola production, which will reduce producer input costs and decrease the chemical pesticide load in the environment. As other biopesticide technologies under development at AAFC (such as for late blight on potato) is more fully developed and is licensed to an industry partner, this technology in canola also can be further extended to other oilseed Brassicas and horticultural plants affected by *Sclerotinia sclerotiorum*.

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

***In vitro* determination of antibiosis against *S. sclerotiorum*:** Bacteria were sourced from the Boyetchko Biocontrol Culture Collection, housed at the Saskatoon R&D Centre. The antibiosis of *S. sclerotiorum* by different candidate bacteria was assessed by determination of zones of inhibition, and by the assessment of fungal growth, ascospore germination, and sclerotium production on plates prepared using metabolites from cell-free culture supernatants.

Determination of zones of inhibition (ZOI): Three trials with four replicates each were done. Bacteria were streaked onto *Pseudomonas* F agar (PAF) plates, which were stored at 16°C for three days, then at 4°C for an additional two days. A single colony was used to inoculate 10 mL Yeast-Glucose medium (YGM) in a 50 mL centrifuge tube. Three trials were done at the same time and for each trial, and different colonies were used to inoculate the broth tubes. The inoculated broth tubes and three tubes for each control were incubated at 22°C for 20-24 hours. After 20-24 hours, 2 µL of inoculum, or control, were placed equidistant from the center and 1 cm from the edge of a plate of PDA. The plates were left at room temperature for at least 24 hours. The following day, a plug of *Sclerotinia sclerotiorum* clone 321 was placed in the center of each plate. After 4, 7 and 14 days, the distance between the edge of the mycelium and the bacterium was measured to determine the ZOI.

Preparation of cell-free metabolites for plate-based assays: Bacteria prepared as described above were inoculated into 10mL YGM. The inoculated broth tubes and the YGM broth control were incubated at 22°C for about 24 hours. 1 mL from each broth tube was used to inoculate a single flask. The flasks were incubated at 22°C with agitation at 150 rpm for about 48 hours. The bacterial suspensions were for 30 minutes at 6000 g, and the supernatant was filtered (Thermo Scientific, 0.2µm disposable aPES membrane filter). The metabolites were then stored at 4°C. To prepare metabolite plates, the filtered metabolites mixed at 50% (v/v) with 2x potato dextrose agar (PDA). The mixture was then poured into four plates for each isolate and experiment. Plates were inoculated with a plug of *S. sclerotiorum* clone 321 mycelium. After 4, 7 and 14 days the colony radius was measured. On day 14, the number of sclerotia produced per plate was counted.

Generation of ascospores: Conditioned *Sclerotinia sclerotiorum* (clone 321) sclerotia were transferred into sterile glass petri dishes containing sand. The sclerotia used were not sterilized. About 50 sclerotia were added per dish with just enough sterile distilled water (about 15-18 mL) to cover the sand. The dishes were covered with aluminum foil and incubated at 16°C in the dark. After 2-4 weeks, sclerotia that had developed stipes were transferred into sterile glass crystallizing dishes with sand. The glass dishes were then incubated at 15°C with a 12-hour light cycle. After about 1-2 weeks, developed apothecia were used to collect ascospores. A vacuum filter unit with a 0.22 µm Millipore filter was used to collect ascospores 2-3 times a week. Filters were aseptically transferred into a disposable petri dish and stored at -20°C until use. To harvest ascospores, pieces of the filter paper were added aseptically to a 2 mL tube with about 1.5 mL of sterile distilled water. The tube was vortexed, and the filter paper was removed. Ascospore concentration was determined using a hemocytometer (LW Scientific) and adjusted to around $1.5x10^6$ ascospores/mL. Metabolite plates were inoculated with the ascospore suspension and incubated using a 12-hour light cycle at room temperature (22-24°C).

Stem infection/*in planta* bioassays: The stem infection assays were performed to assess the level of induced resistance to Sclerotinia in plants sprayed with the biocontrol agent (BCA). Two susceptible *B. napus* cultivars (Westar and Topaz) and one partially resistant cultivar (Zhongyou) were selected for the trials. All greenhouse trials featured a completely randomized block design with five treatments and 4 to 6 replicates. The experimental unit was a pot with a single plant. The BCA was a liquid culture of PENSV20 grown in Yeast

Glucose Extract medium (YGM) for 48 hours on a rotary shaker at ambient temperature and amended with 0.02% Tween20. Plants at full flower were sprayed to run off with the BCA two days before (BCA-2), the same day (BCA-0), and two days after (BCA+2) inoculation with *S. sclerotiorum* (150-200 mL). Control plants were sprayed with 0.02% Tween20 in water. The BCA and water was applied using handheld E-Z sprayers. Plants were inoculated using toothpicks heavily colonized with mycelium of *S. sclerotiorum*. Sterile toothpicks were used for control plants. Toothpicks were inserted into the plant stem in two places, then covered with an Eppendorf tube containing 15 µl water and secured to the stem with Parafilm to maintain humidity. Lesion lengths were measured at days 7, 14, and 21 post infection.

For the *in planta* bioassays, *B. napus* cultivars Westar (susceptible) and Zhongyou (partially resistant) were used. A total of six trials were conducted, the first two of which showed poor disease severity in the pathogen control. Moreover, "burning" of the older petals was observed in the first trial when the BCA was applied full strength, so a dose response trial was conducted before proceeding further. The BCA was grown in YGM for 48 hours on a rotary shaker and used to prepare suspensions including undiluted BCA, 50% BCA/YGM, and 25% BCA in YGM. *B. napus* cv Zhongyou plants were sprayed to runoff with these suspensions prior to challenge with Sclerotinia ascospores (approximately 10^4 ascospores/mL) on the same day. Control plants were sprayed with water. For greenhouse trials of BCA efficacy, each cultivar was assessed using three independent experiments (biological replicates). Each greenhouse trial was designed as a completely randomized block with five treatments and three replicates. The experimental unit was a single pot of three plants. The BCA was grown in YGM for 48 hours on a rotary shaker, then diluted 1:2 with water (50% suspension as determined from previous experiment) for treatments. Plants were sprayed to runoff at 30-50% flowering 24 hours before (BCA-1), the same day (BCA-0) and 24 hours after (BCA+1) inoculation with a suspension of *S. sclerotiorum* ascospores (approximately 10^4/mL) in 0.02% Tween20. Control plants were sprayed with 10% YGM-0.02% Tween20. Plants were covered with clear plastic bags to maintain humidity once treatments were applied. The tops of the bags were cut open 4 days after the pathogen was applied. Plants were rated for disease severity on a percentage scale at days 7, 14, and 21 after pathogen application, and fresh weights were taken 28 days post infection.

Molecular identification and detection: Total genomic DNA was extracted from candidate biocontrol bacteria using a Wizard genomic DNA preparation kit (Promega) and taxonomic identification was performed using amplification and sequencing of taxonomic markers including chaperonin-60 [1] (cpn60; www.cpndb.ca) and 16S rRNA genes. Whole genome sequencing was performed using Illumina MiSeq, and reads were assembled using soapdenovo [2]. For the strain PENSV20, additional sequencing was performed using a nanopore platform. Reads were pooled and co-assembled using Unicycler [3] to provide a contiguous genome sequence. The genome sequence was annotated using the automated pipelines provided by the Integrated Microbial Genome database (https://img.jgi.doe.gov). The genome sequence was also analyzed for the presence of biosynthetic gene clusters related to the production of secondary metabolites using antiSMASH [4]. Primers for loop-mediated isothermal DNA amplification (LAMP) to detect *Sclerotinia sclerotiorum* were designed using LAMP Designer v1.12 (Premier Biosoft). Primers and probes for qPCR/ddPCR were designed using Beacon Designer V 8.20 (Premier Biosoft). Primers and probes for Recombinase-Polymerase Amplification (RPA) were designed manually according to the recommendations of TwistDx, the manufacturer of RPA amplification kits. RPA was performed to detect *S. sclerotiorum* according to the manufacturer's recommendations. See Table 1 for the primer and probe sequences developed for this project.

Identification of active bacterial metabolites: Metabolites from 20 different bacterial cultures were analyzed for growth antagonism against the target pathogen. Compounds produced by the bacteria that possessed the desired activities were analyzed by bio-autography to identify active metabolites. Bacterial cultures were cultured on PAF plates, and 25 mL of YGM broth was inoculated with a single colony of each culture and incubated at 15°C for 72 hours. 5 mL of each culture was then used to inoculate 500 mL of fresh YGM and left to grow for another 72 hours. To examine the cultures for the presence of metabolites, 1 mL of the final culture was diluted with 1 mL of methanol, filtered, and analyzed by UPLC. For bio-autography, metabolites from each potential biopesticide candidate culture were extracted with ethyl acetate and the solvent removed. Crude extract samples were run on Ultra Performance Liquid Chromatography ElectroSpray Ionization tandem Mass

Spectrometry (UPLC-ESI-MS), using methanol for a solvent. Extracts were spotted on a TLC plate, and developed using 2:1 methanol:dichloromethane. Mycelia from five *S. sclerotiorum* plates (taken before sclerotia formed) were suspended in 50 mL of potato dextrose broth modified with 0.1% agar and 0.1% Tween 20 and vortexed with glass beads. The mycelial suspension was sprayed over the developed TLC plate and incubated in a moist environment for 1 week at room temperature in the dark. Metabolites that inhibited the growth of the pathogen were extracted from the silica, examined using UPL-ESI-MS, and the results were compared to those of the crude extract. Tentative identities of the metabolites were determined by searching their bacterial source and their mass on Anti-base, and other databases as well as literature searches.

Formulation of BCA *B. atrophaeus* PENSV20: This work was undertaken in partnership with our industrial collaborator, Montana BioAgriculture (MBA). Six BCA formulations were initially tested for efficacy, handling characteristics, and ease of use. Formulation 1, a spore preparation using a clay carrier, was used to test the effect of the formulation on *B. napus* flower petals in the absence of pathogen, because the BCA prepared in growth medium using our typical spraying protocol sometimes results in petal "burning", which we suspected might be due to pH. Formulations 2 through 6 were used to test the efficacy of the formulated BCA on *B. napus* plants challenged with pathogen, while formulations 7-10 were examined by phase contrast microscopy (to determine if the culture had sporulated), pH determination, viable colony counts, and metabolites analysis. The formulations provided by MBA are shown in Table 4.

Application of formulations to plants challenged with pathogen: The experimental unit was a single 10 inch pot containing three *B. napus* cv. Westar plants. Each treatment was applied to two pots of plants. Another pot was sprayed with water as a negative control, and a positive control for biocontrol was the BCA prepared in YGM according to our developed protocol (48 hour culture diluted 50% with sterile reverse osmosis water prior to application). The dried formulations were suspended at 10% (w/v) in sterile reverse osmosis water, then filtered through 4 layers of sterile cheesecloth to facilitate spraying. Aliquots of the suspended formulations were removed for pH determination, phase contrast microscopy, viable plate counts, and metabolite (surfactin) quantification. Each pot was sprayed with 150-200 mL of one of the formulations (or controls) one day prior to the application of *S. sclerotiorum* ascospores (day -1), in accordance with the spraying protocols we have developed over the course of this project. After drying, the plants were covered with clear plastic bags to maintain humidity. Each pot (except negative control) was sprayed the next day with a suspension of *S. sclerotiorum* ascospores (150-200 mL, approximately 10^4 ascospores/mL). The pots were covered in plastic bags and returned to the greenhouse for weekly observations.

Examination of metabolites in the experimental formulations: Each sample was split into 2 subsamples and partitioned with ethyl acetate, or selectively partitioned by acid precipitation using the method described by Singh et al [5]. Extracted samples were dried and dissolved in 1 mL of water to concentrate the original sample by 10x. Total relative surfactin concentrations were determined by HPLC, and the total peak areas for surfactin-C15, -C14, and -C13 were determined for each formulation.

Determination of viable colony counts in the experimental formulations: Resuspended formulations (10% w/v) were serially diluted in sterile water, spread on nutrient agar plates, and incubated at room temperature for 36-48 hours until colonies were observed. Each dilution was measured in triplicate.

Molecular detection and quantification of S. sclerotiorum *in sprayed plants:* At regular intervals, leaves were harvested from the treated plants and DNA extracted using a Plant DNA mini kit (Qiagen). *S. sclerotiorum* DNA was quantified relative to *B. napus* DNA using ddPCR for each treated pot. Primers and probes used for ddPCR are shown in Table 1.

Plant defense mechanisms: For the identification of plant and bacterial genes induced upon treatment with the biocontrol agent and pathogen, a greenhouse experiment was performed using *B. napus* cultivar ZhongYou (ZY). ZY is partially resistant to Sclerotinia stem rot. Fifty plants were seeded and at the 3 leaf stage were placed at +4°C for vernalisation for 6 weeks. The plants were then transplanted to pots with two plants per pot for a total of 25 pots. The pots were randomly assigned to five treatments with five replicates per treatment, as follows: 'ZY' × sterile YGM broth (ZY), 'ZY' × Sclerotinia (SS), 'ZY '× PENSV20 (PEN), 'ZY' × PENSV20× Sclerotinia (PEN-SS), and 'ZY' × SS × PENSV20 (SS-PEN). The treatments were sprayed on the entire plants

at 30-50% flowering. The inoculated plants were evaluated at 24-hour intervals. Severity was rated as the percentage of plants infected with the disease, on 0-100% scale. Leaf tissues were collected at 24 and 48 hours post treatment (HPT) for RNA extraction. RNA extraction was done using a Qiagen kit. The RNA samples were submitted to McGill Genome Center for library preparation and RNA sequencing. RNA libraries were prepared using ribosomal RNA (rRNA) depleted library kit. rRNA depleted total RNA libraries were sequenced using NovaSeq6000 S4 v1.5, PE100, per lane. RNA sequence reads were analysed using Differential Expression Analysis of OmicsBox v1.

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

Objective 1 – Identification of candidate bacteria displaying a phenotype of antibiosis against *S. sclerotiorum in vitro* and on plants: The AAFC Boyetchko Culture Collection was used as a source of bacteria for screening for isolates featuring a phenotype of antibiosis against *S. sclerotiorum*. Fifteen strains were initially screened by evaluating the sizes of zones of inhibition on potato dextrose agar (PDA) plates inoculated with plugs of *S. sclerotiorum*, and by preparing PDA plates using cell-free metabolites from bacterial culture supernatants and inoculating these with a plug of the fungal pathogen. Metabolite plates were also used to assess the effect on ascospore germination and sclerotium formation.

The ZOI observed for each bacterial strain are shown in Table 1, and the mean *S. sclerotium* colony diameters observed on metabolite plates prepared from each strain are shown in Table 2.

Table 1. ZOI for bacterial isolates with Standard Error. The results represent three trials with four replicates each.

Isolate	ZOI (mm) Day 4/ SE	ZOI (mm) Day 7/ SE	ZOI (mm) Day 14/ SE
SASAF1	5.0±0.4 A *	4.9±0.4 A *	ND
CARAF5	4.3±0.5 A *	4.2±0.4 A *	ND
EDENAF31	3.3±0.1 B *	3.3±0.1 B *	ND
CARAF4	4.0±0.1 A *	3.3±0.1 A *	2.8±0.1 A *
PENSV20	3.6±0.4 B *	3.2±0.3 B *	ND
EDENAF12	3.1±0.1 B *	3.1±0.1 B *	ND
CARAF47	2.7±0.1 B *	2.3±0.1 B *	2.2±0.1 B *
PENSV27	1.4±0.1 C *	1.2±0.1 C *	1.1±0.1 C *
KENGFT2	0.5±0.2 D *	0.0±0.0 D	0.0±0.0 D
OXW06B1	0.0±0.0 E	0.0±0.0 D	0.0±0.0 D
WAUSV22	0.0±0.0 E	0.0±0.0 D	0.0±0.0 D
WAUSV36	0.0±0.0 E	0.0±0.0 D	0.0±0.0 D
CON BROTH	0.0±0.0 E	0.0±0.0 D	0.0±0.0 D
CON WATER	0.0±0.0 E	0.0±0.0 D	0.0±0.0 D
GW04	0.0±0.0 C	0.0±0.0 C	ND
CTB12	0.0±0.0 C	0.0±0.0 C	ND
WEYW01	0.0±0.0 C	0.0±0.0 C	ND

Means with the same letter are not significantly different, LSD

The ZOI results demonstrated some variability in the efficacy of the strains included in the screens, with some bacteria showing no inhibition (eg WEYW01; CTB12; GW04), and others showing a large zone of inhibition

(SASAF1; CARAF5; PENSV20; CARAF4). Metabolite plates prepared with cell-free culture supernatants demonstrated similar variability in the ability to inhibit the growth of *S. sclerotiorum*, and many of the same strains were inhibitory in this metabolite-based assay (Table 2).

Table 2. Growth radius (mm) of *S. sclerotiorum* on 50% bacterial metabolie/PDA plates. The results represent two independent trials with four replicates each.

isolate	Radius (mm) day 4/ SE	Radius (mm) day 7/ SE	Radius (mm) day 14/ SE	% Control day 14	taxonomic identification *(cpn60)*
CARAF5	0.0±0.0 C *	0.0±0.0 C *	0.0±0.0 B *	100	*Xanthomonas retroflexus*
EDENAF12	0.0±0.0 C *	0.0±0.0 C *	0.0±0.0 B *	100	*Bacillus atrophaeus*
PENSV20	0.0±0.0 C *	0.2±0.1 C *	0.6±0.3 C *	98.5	*Bacillus atrophaeus*
EDENAF31	0.5±0.2 B *	0.8±0.2 B *	1.4±0.2 B *	96.6	*Bacillus atrophaeus*
SASAF1	0.5±0.2 B *	1.0±0.1 B *	1.5±0.3 B *	96.3	*Bacillus subtilis*
PENSV27	0.0±0.0 E *	0.4±0.2 E *	9.6±1.5 D *	75.9	*Bacillus atrophaeus*
CARAF47	3.4±0.4 D *	5.2±0.6 D *	9.7±0.8 D *	75.7	*Bacillus atrophaeus*
WAUSV36	9.2±0.6 C *	11.7±0.5 C *	15.4±0.5 C *	61.6	*Bacillus subtilis*
CARAF4	8.8±1.1 C *	22.9±2.3 B *	31.5±1.6 B *	21.3	*Paenibacillus polymyxa*
CTB12	31.0±3.2 B *	36.5±1.2 B *	40.0±0.0 A	0	
WEYW01	40.0±0.0 A	40.0±0.0 A	40.0±0.0 A	0	
GW04	40.0±0.0 A	40.0±0.0 A	40.0±0.0 A	0	
KENGFT2	39.6±0.2 A	40.0±0.0 A	40.0±0.0 A	0	
OXW06B1	40.0±0.0 A	40.0±0.0 A	40.0±0.0 A	0	
WAUSV22	33.1±0.6 B *	40.0±0.0 A	40.0±0.0 A	0	
Control (YGM)	40.0±0.0 A	40.0±0.0 A	40.0±0.0 A	0	

* Significantly different from control (YGM), Dunnett t-test

Finally, the ability of the isolates to inhibit the germination of Sclerotinia ascospores and sclerotium generation was examined. Varying volumes of culture supernatants containing bacterial metabolites were used to prepare PDA plates onto which the fungus was inoculated. Ten bacteria out of the fifteen screened showed some inhibition against ascospores and mycelial growth. Three isolates showed high levels of inhibition (less than 5% of ascospores germinated at the lowest dose): CARAF5, WAUSV36 and EDENAF31. Five isolates showed inhibition where less than 35% of the ascospores germinated at the lowest dose; EDENAF12, PENSV20, PENSV27, WAUSV22, and CARAF4. An example of these results for isolate PENSV20 is shown in Figure 1.

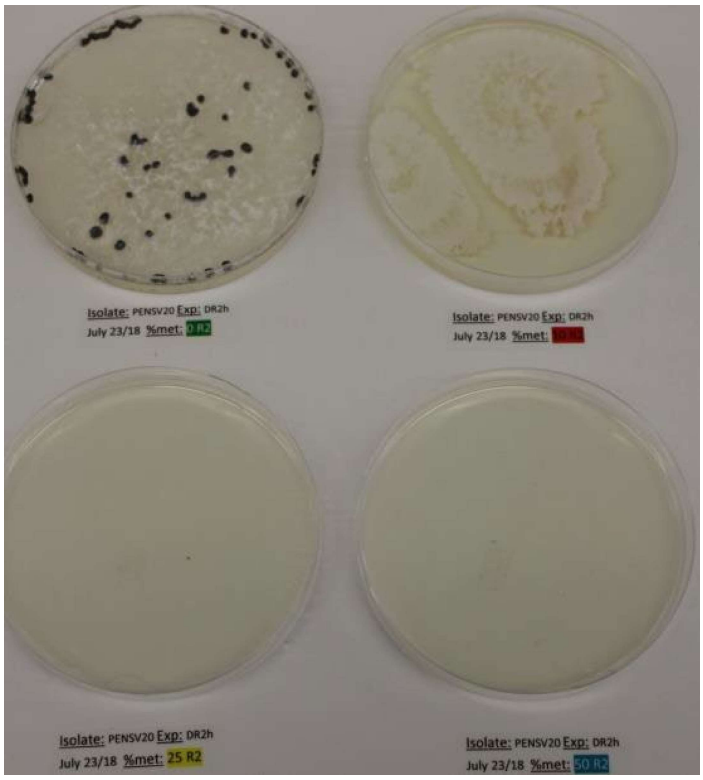


Figure 1. *S. sclerotium* ascospore germination and sclerotia production when using PDA media with PENSV20 metabolites at dosages of 0% control (top left), 10% (top right), 25% (bottom left), and 50% (bottom right). 1 of the 3 biological replicates performed is shown.

Overall, these assays demonstrated that 9 of the 15 bacterial strains displayed strong antibiosis against *S. sclerotiorum*. These isolates were selected for genome sequencing to determine their taxonomic affiliations and potential mechanisms of action. Taxonomic identification using *cpn60* gene sequences demonstrated that all of the most inhibitory strains were *Bacillus/Paenibacillus* or *Xanthomonas*, with *B. atrophaeus* the most commonly identified strain (Table 2). Genome sequencing of the most active strains yielded assemblies of variable quality, with PENSV20 being the highest quality assembly. Therefore, this highly effective strain was chosen for subsequent nanopore sequencing and assembly of a high quality, contiguous genome sequence, which was deposited to GenBank (CP050705.1).

Efficacy of B. atrophaeus *PENSV20 in protecting* B. napus *from infection with* S. sclerotiorum*:* The protective effect of PENSV20 was examined by experimentally infecting plants with the fungal pathogen, with and without application of the BCA. Using the spraying protocols developed through this project, two canola cultivars were treated with pathogen with and without BCA. *B. napus* cv. Westar is a fully susceptible line, while *B. napus* cv. Zhongyou is a winter canola line (requiring vernalization for seed germination) that is partially resistant to Sclerotinia. Despite the differences in Sclerotinia susceptibility, both lines demonstrated severe infection under our experimental conditions (Figure 2, Figure 3). This severe infection was strongly inhibited by application of PENSV20. Differences were observed in the timing of the biopesticide application, with day -1 (BCA applied one day prior to pathogen challenge) being the most effective treatment. The experimental replications have been completed for both cultivars (three biological replications) and are currently undergoing statistical analysis.

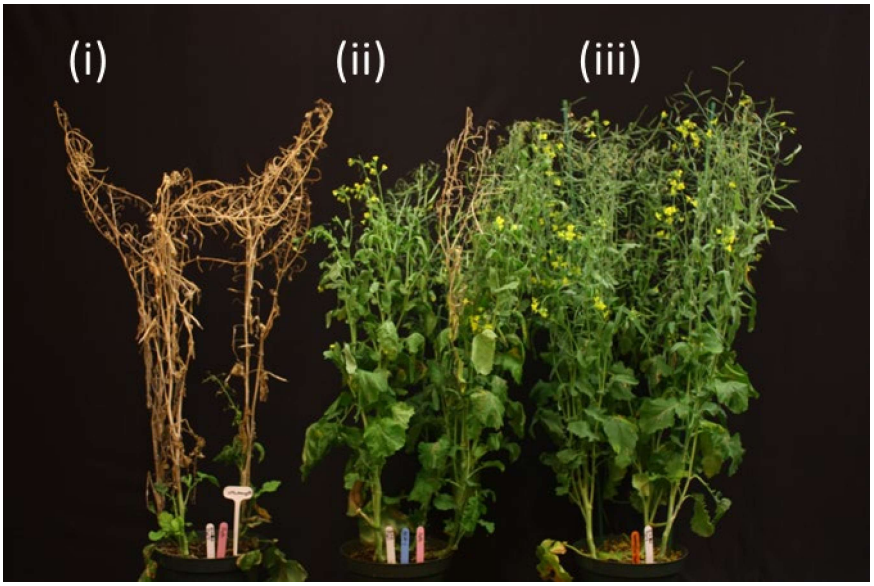


Figure 2. Pathogen challenge of *B. napus* cv. Westar (fully susceptible) and the protective effect of PENSV20. (i) pathogen only; (ii) pathogen+PENSV20 (day -1); (iii) water control. This is a representative example from three technical and biological replications.

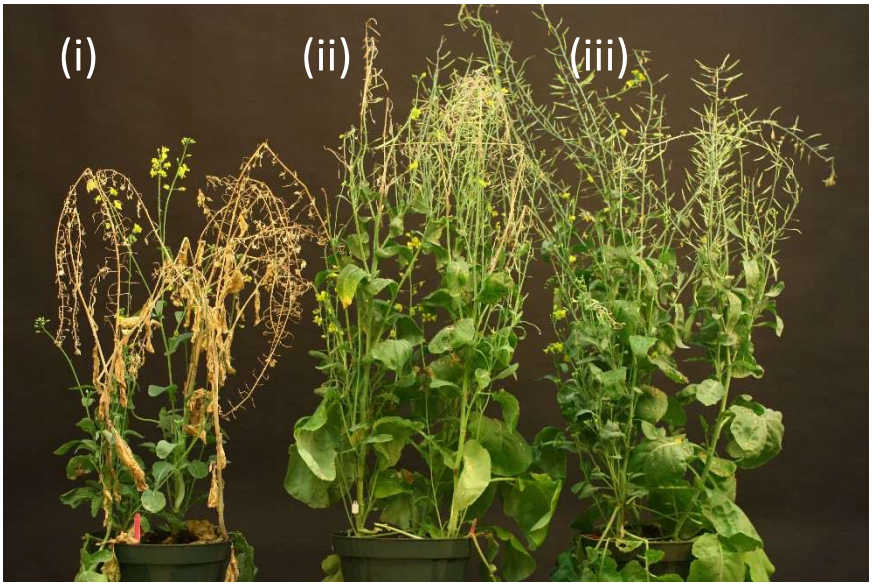


Figure 3. Pathogen challenge of *B. napus* cv. Zhongyou (partially resistant) and the protective effect of PENSV20. (i) pathogen only; (ii) pathogen+PENSV20 (day -1); (iii) water control. This is a representative example from three technical and biological replications.

The protective effect of PENSV20 in the context of Sclerotinia infection was quantified in these plants through a qualitative disease rating scale, and by determining plant weights after 28 days of infection. In addition, we implemented a quantitative assessment of pathogen levels on leaves by developing a droplet digital PCR (ddPCR) assay that simultaneously detects and quantifies *S. sclerotiorum* DNA normalized to a *B. napus* actin gene. After optimizing the amplification conditions using these primer sets for ddPCR, we applied them to experimentally infected plants. Leaves were harvested immediately after spraying (once the plants had dried), and again at day 7 after infection. Two spraying regimens were examined: application of PENSV20 the day prior to pathogen challenge (BCA-1); and application of PENSV20 the day after pathogen challenge (BCA+1).

The results of this analysis are shown in Figure 4. Plants that were not protected by the application of PENSV20 displayed very high levels of *S. sclerotiorum* DNA by day 7, with nearly all of the DNA extracted from the infected leaf corresponding to pathogen DNA. In contrast, a remarkable protective effect was observed in both PENSV20 spraying regimens (Figure 4). Application of PENSV20 the day before pathogen challenge resulted in a smaller initial pathogen load, and while the amount of pathogen increased slightly by day 7, the plants remained healthy and the levels were still lower than at day 0 of the plants not sprayed with BCA (Figure 4). Plants that were sprayed with PENSV20 the day after pathogen challenge started with levels of pathogen that were comparable to unsprayed plants, but that level decreased to be comparable to the BCA-1 plants by day 7 (Figure 4). This method was under development during the trials described above, so was not applied to all of the replicates, but we are applying this molecular pathogen quantification method to the assessment of the performance of the various formulations described under objective 3.

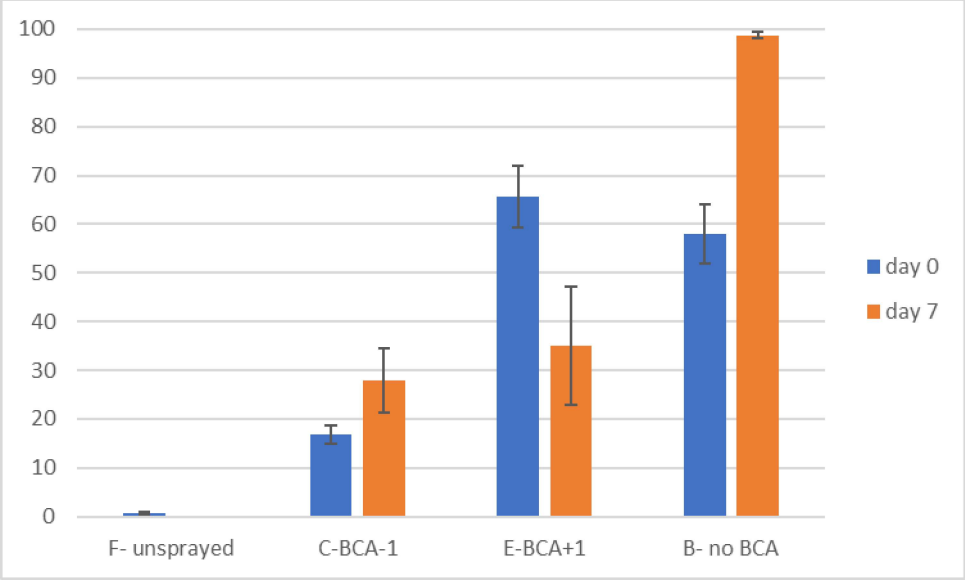


Figure 4. Quantification of pathogen load in *S. sclerotiorum*-challenged plants on the day of spraying (day 0) and at 7 days post infection. Results are expressed as the fractional abundance of *S. sclerotiorum* DNA using *B. napus* actin as a control.

Objective 2 – Determination of the possible mechanisms of action of *B. atrophaeus* PENSV20 through genome sequencing, metabolite analysis, and examination of host response: A series of complementary experiments was undertaken to investigate the question of mechanisms of action of the primary biocontrol candidate, PENSV20.

Genome sequencing and analysis: The genome of PENSV20 consisted of a single contig of 4148820 bp, and featured 3,916 genes and 8 identical 16S rRNA-encoding genes. The genome featured 11 predicted secondary metabolite anabolic pathways identified by antiSMASH (Table 3). These included several well-described biosynthetic gene clusters synthesizing secondary metabolites known to be involved in phytopathogen antibiosis, including surfactins [6] (region 2), bacillaene [7], and fengycin [8]. The gene clusters involved in secondary metabolite biosynthesis are visualized in the genome in Figure 5.

Table 3. Predicted secondary metabolite anabolic pathways of *B. atrophaeus* strain PENSV20, as identified by antiSMASH [4].

Region	Type	Most similar known cluster		Similarity
Region 1	NRPS,sactipeptide,ranthipeptide	sporulation killing factor	RiPP:Head-to-tail cyclized peptide	1
Region 2	NRPS	surfactin	NRP:Lipopeptide	0.86
Region 3	terpene			
Region 4	transAT-PKS,PKS-like,T3PKS,NRPS	bacillaene	Polyketide+NRP	1
Region 5	NRPS,betalactone,transAT-PKS	fengycin	NRP	1
Region 6	terpene			
Region 7	T3PKS	1-carbapen-2-em-3-carboxylic acid	Other	0.16
Region 8	NRPS,HR-T2PKS	pelgipeptin	NRP	0.25
Region 9	NRP-metallophore,NRPS	bacillibactin	NRP	1
Region 10	thiopeptide,LAP			
Region 11	sactipeptide	subtilosin A	RiPP:Thiopeptide	1

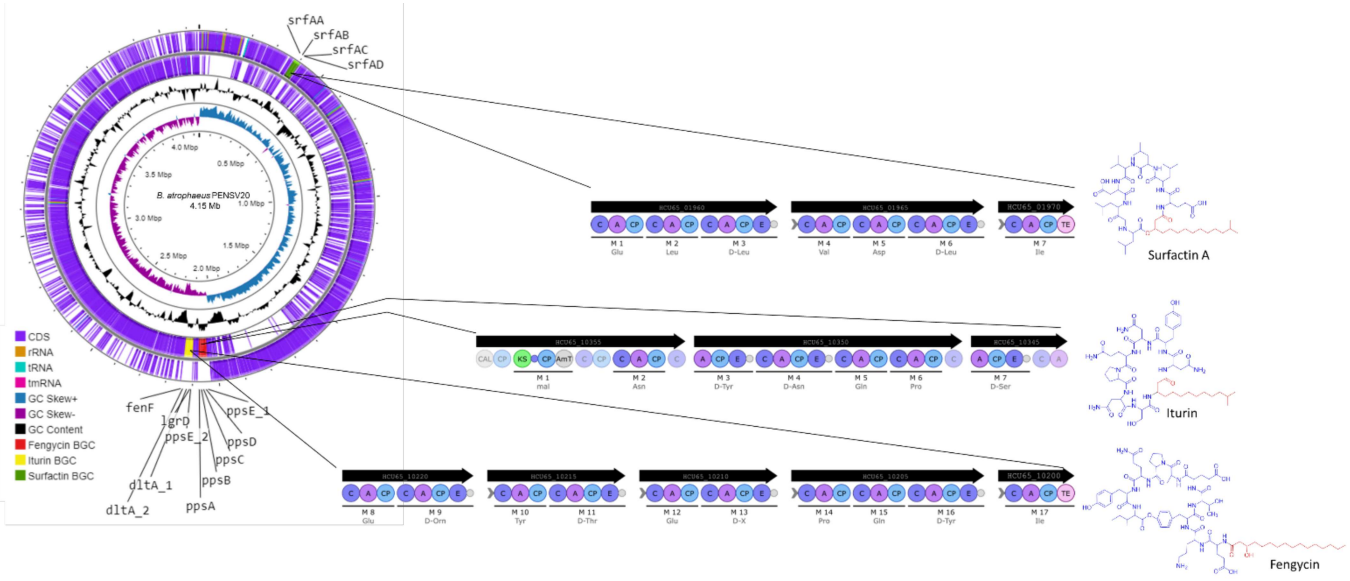


Figure 5. Schematic diagram of the PENSV20 genome showing the locations of the major secondary metabolite gene clusters identified using antiSMASH.

Metabolite analysis: Examination of the culture supernatants of the best-performing strains identified metabolites that may have growth antagonism function against *Sclerotinia*. Metabolites produced included bacillomycins, pumilocidins, mycosubtillins and iturins, which all fall within the "iturin" class of metabolites which are well known and documented anitmicrobials produced by *Bacillus* spp. In addition, surfactins were produced by many of the strains. Iturins and surfactins are lipopeptides containing a cyclic structure of amino acid units with a carbon tail. They vary in amino acid sequence and carbon chain tail length. Major metabolites produced by PENSV20, the leading candidate, included fengycins, iturins, and surfactins (Figure 6), consistent with the results of the genome sequence analysis (Figure 5). Compounds in these families have previously been identified as bioactive products produced by *Bacillus* spp [8-11]. In particular, surfactins are known to be active in directly antagonizing the growth of phytopathogens as well as activating resistance genes in the host plant [6], so these metabolites could have a dual effect in dampening the effects of Sclerotinia infection.

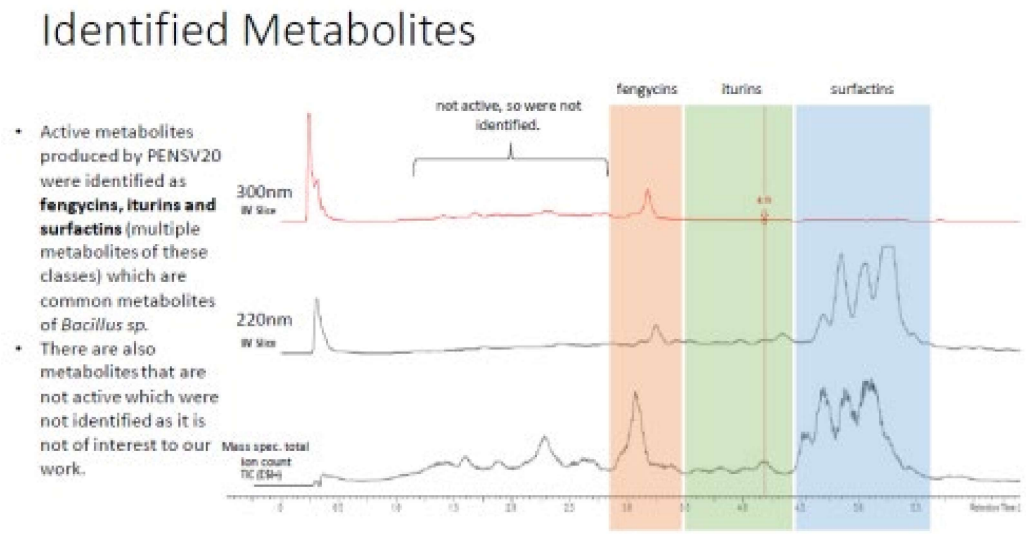
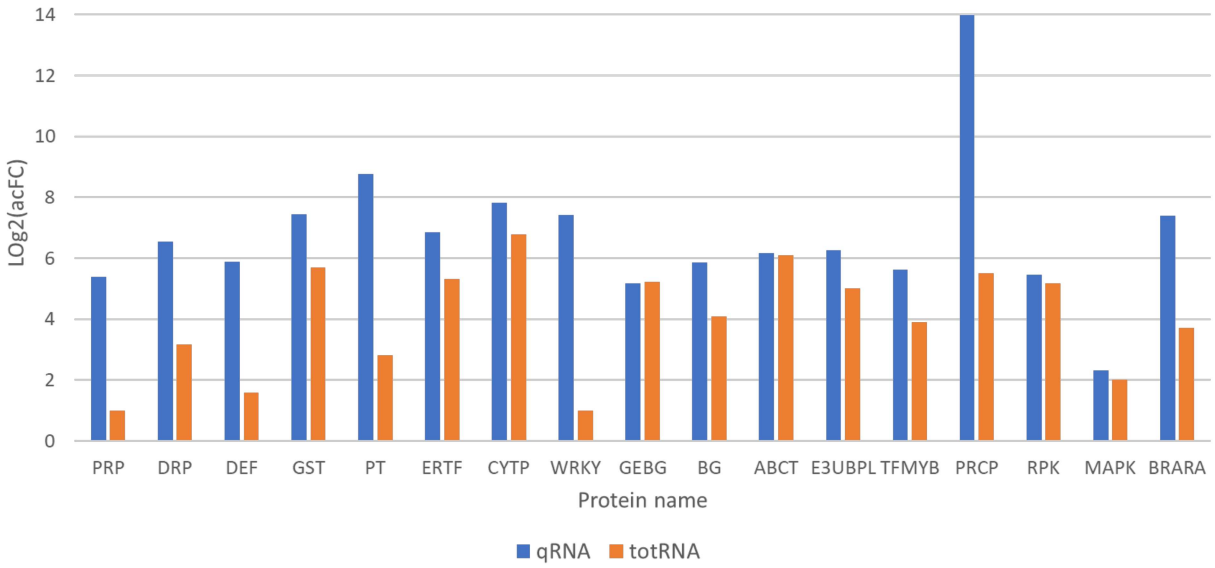


Figure 6. Metabolites produced by PENSV20 that are active in antagonizing the growth of *Sclerotinia sclerotiorum*.

Host response to pathogen challenge with PENSV20: Two canola cultivars were used to examine plant defense mechanisms against Sclerotinia stem rot in response to treatment with the potential biopesticide, PENSV20. Westar is completely susceptible to stem rot, and Zhongyou (ZY) is a partially resistant winter oilseed rape cultivar. Five treatments for each cultivar with three replicates for each treatment were investigated. The pathogen, *Sclerotinia sclerotiorum* (SS), and PENSV20 were sprayed on plants according to our previously developed application regimens. At 7 days post treatment, the phenotypic assessment showed severe infection on both cultivars treated with SS only, while no signs of infection were observed on both cultivars treated with both SS and PENSV20. RNA samples from leaf tissue were sequenced to determine the groups of genes and pathways involved in defense mechanisms in the plant globally. Three major pathways were overexpressed upon treatment, including systemic acquired resistance, induced systemic resistance, and cellular detoxification; the latter pathway was only overexpressed in plants treated with SS only. More than 1000 differentially expressed genes (DEGs) were identified, which were categories according to their putative functions (Figure 7). A subset of 9 upregulated genes strongly implicated in plant defense against pathogenic microorganisms were examined in more detail. Three genes encoding defensin-like protein 19 on chromosomes A05, C04 and C08 were highly overexpressed in PEN, SS_PEN and PEN_SS treatments compared to SS and ZY treatments. Defensins not only have antimicrobial effect but also enhance plant resistance to a diverse group of fungal pathogens and are involved in plant growth regulation and developmental processes. Two pathogenesis-related (PR) protein PR-4-like encoding genes on A03 and C03 were overexpressed in PEN, SS_PEN and PEN_SS treatments compared to SS and ZY treatments. PR genes are involved in both salicylic acid (SA) and jasmonic acid (JA) pathways during pant interactions with biotrophic and necrotrophic pathogens, respectively. Two endochitinase encoding genes on A03 were found to be overexpressed in PEN, SS_PEN and PEN_SS treatments

compared to SS and ZY treatments. Chitinase hydrolyzes the chitin polymer (a β-1,4-linked N-acetylglucosamine), a critical structural component of the fungal cell wall, in particular. Other protein-coding genes such as Chlorophyllase, and proteases were also overexpressed in various treatments. The two studies provide significant details on defense mechanisms involved in the tri-trophic interaction: canola X Sclerotinia



stem rot X PENSV20. A manuscript describing these results is currently in preparation for publication.

Figure 7. Gene categories that were up-regulated upon spraying *B. napus* (both cultivars) with SS and PENSV20. Results are shown for all genes (totRNA) and coding RNA (qRNA). The major categories of up-regulated genes included PRP (pathogenesis-related protein); DRP (disease resistance protein); DEF (defensin); GST (glutathione-S-transferase); WRKY (transcription factor); PRCP (peptidylprolylamino acid carboxypeptidase); and BRARA (hypothetical protein).

Objective 3 - Formulation of BCA *B. atrophaeus* PENSV20: Working with an industrial collaborator with decades of experience in the formulation of microorganisms for agricultural applications, Montana BioAgriculture, Inc (MBA), we have begun experiments aimed at developing effective formulations that will retain the strong biocontrol properties of PENSV20 in a format that could be directly applied by producers.

MBA has provided 10 formulations so far, with varying compositions (Table 4). The basic growth medium used to prepare these formulations was a barley flour-based broth that is more suitable for large-scale fermentations than the laboratory broth typically used for our work (yeast-glucose medium, YGM). Different carriers/stickers and drying temperatures were used, and formulations were targeted either for spores (using a longer fermentation time) or vegetative cells. Spores are anticipated to improve the stability of the formulations, but the typical application scheme we have developed (using bacterial cultures grown for 48 hours in YGM) is expected to result primarily in vegetative cells, which have proven efficacy. These dried formulations were resuspended in water at 10% (w/v) and characterized by measuring the metabolite concentrations, viable cell counts, and pH. In addition, the formulations were examined using phase contrast microscopy to determine if the cells had sporulated. Formulation 1 was applied to *B. napus* plants in the absence of pathogen, to provide data on the previously observed phenomenon of petal “burning” when using PENSV20 grown in standard growth medium. Formulations 2 through 6 have been applied to B. napus cv. Westar plants that were subsequently challenged with pathogen. While this trial is in progress, some of the interim results are provided here. A larger-scale greenhouse-based replicated experiment is planned for March, 2024. The results of these trials will provide the data required to select the most appropriate formulations to evaluate in these subsequent experiments.

Table 4. Formulations provided by MBA for evaluation.

Formulation	carrier	spores/g	Drying temperature	pH	fermentation time (hours)	CFU/mL
1	clay (25%)	$5x10^{10}$	60°C		144	
2	cellulose	$3x10^{10}$	60°C	6.5	144	3.9×10^9
3	clay (25%)	$2.5x10^{10}$	Ambient	6.9	144	5.6×10^9
4	clay (25%)	$2.5x10^{10}$	60°C	6.3	144	7.8×10^8
5	ActiGel (25%)	$2.5x10^{10}$	50°C	6.9	144	5.6×10^9
6	Kaolin (25%)	$2.5x10^{10}$	60°C	6.6	144	6.5×10^7
7	Kaolin (10%)	vegetative cells	Ambient	5.4	78	
8	Kaolin (10%)	vegetative cells	Ambient	7.2	78	
9	Kaolin (10%)	vegetative cells	Ambient	6.0	90	
10	Kaolin (10%)	vegetative cells	Ambient	7.5	78	
YGM	none	vegetative cells	none	6.7	48	3.0×10^7

The application of formulation 1 to *B. napus* plants did not result in any petal burning (not shown), suggesting that the problem we sometimes observe in applying PENSV20 prepared in laboratory medium may be less of an issue with these formulations. This also provided us with some experience in handling the formulations, and we determined that the level of carrier used interfered with application by plugging the aspirator. We therefore had to use a preliminary filtration step to be able to apply the formulation to the plant. In addition, the filtered formulation at 25% inclusion rate left a residue on the plant leaves (Figure 10), but this did not appear to impact plant growth negatively. Nevertheless, in the latest formulations the carrier level has been decreased to 10% (Table 4).

The production of surfactins C13, C-14, and C-15, which were determined to be the most abundant surfactin analogs produced by PENSV20, showed some variability among the different formulations, with formulation 2 showing the lowest level (Figure 8). PENSV20 grown in the barley flour medium that is suitable for industrial-scale fermentations showed a lower level of metabolites compared to YGM, but formulations 3, 5, and 6 had levels that were comparable to PENSV20 grown in YGM broth (Figure 8).

Examination of the formulations using phase contrast microscopy revealed that, as expected, formulations 2-6 consisted primarily of spores (Figure 9). PENSV20 grown using our standard preparation method in YGM broth was mostly vegetative cells, but some evidence that sporulation was beginning in the cultures was observed (Figure 9).

Formulations 2-6 were applied to *B. napus* cv. Westar plants with pathogen challenge. At 7 days post challenge, all of the formulations provided a significant protective effect to the plants, which was comparable to that provided by PENSV20 prepared using laboratory growth medium (Figure 10). The disease severity ratings taken at day 7 also showed no difference between the formulations and the PENSV20 culture prepared in YGM broth (Figure 11).

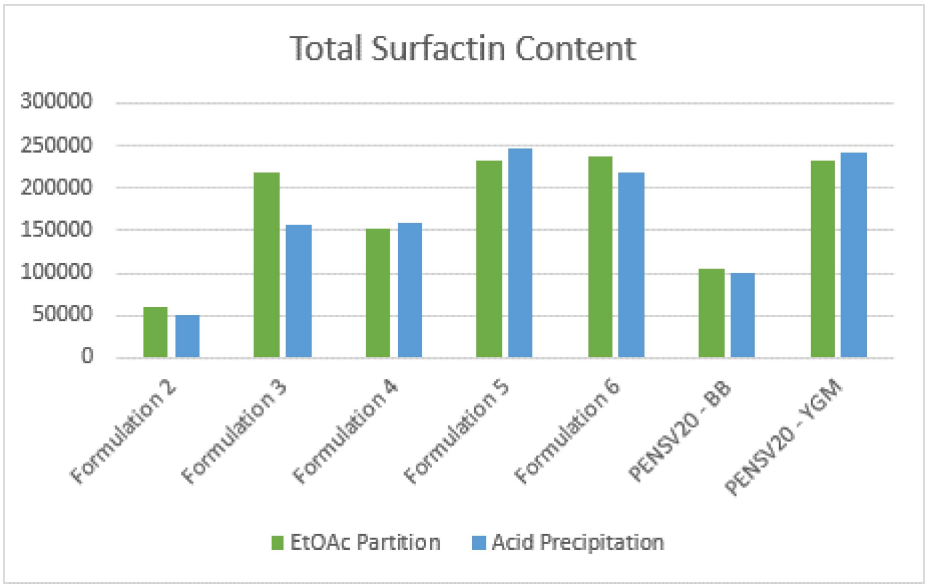


Figure 8. Total peak areas measured for surfactins C13, C14, and C15 in the different formulations and in laboratory and industrial culture media without drying. Surfactins were measured using two different preparation methods, standard ethyl acetate partitioning and acid precipitation as described by Singh et al. [5].

This ongoing trial will provide critical data on formulation performance that will be applied in our upcoming experimental evaluation of the performance of different formulations in protecting canola plants from infection with Sclerotinia. We will evaluate 2 or 3 different formulations in a fully replicated, formally randomized experiment. The results of the first of three planned experimental replications is expected near the end of April, 2024. This experiment will lead us towards our planned field trials of this biocontrol agent, for which we are currently exploring funding options.

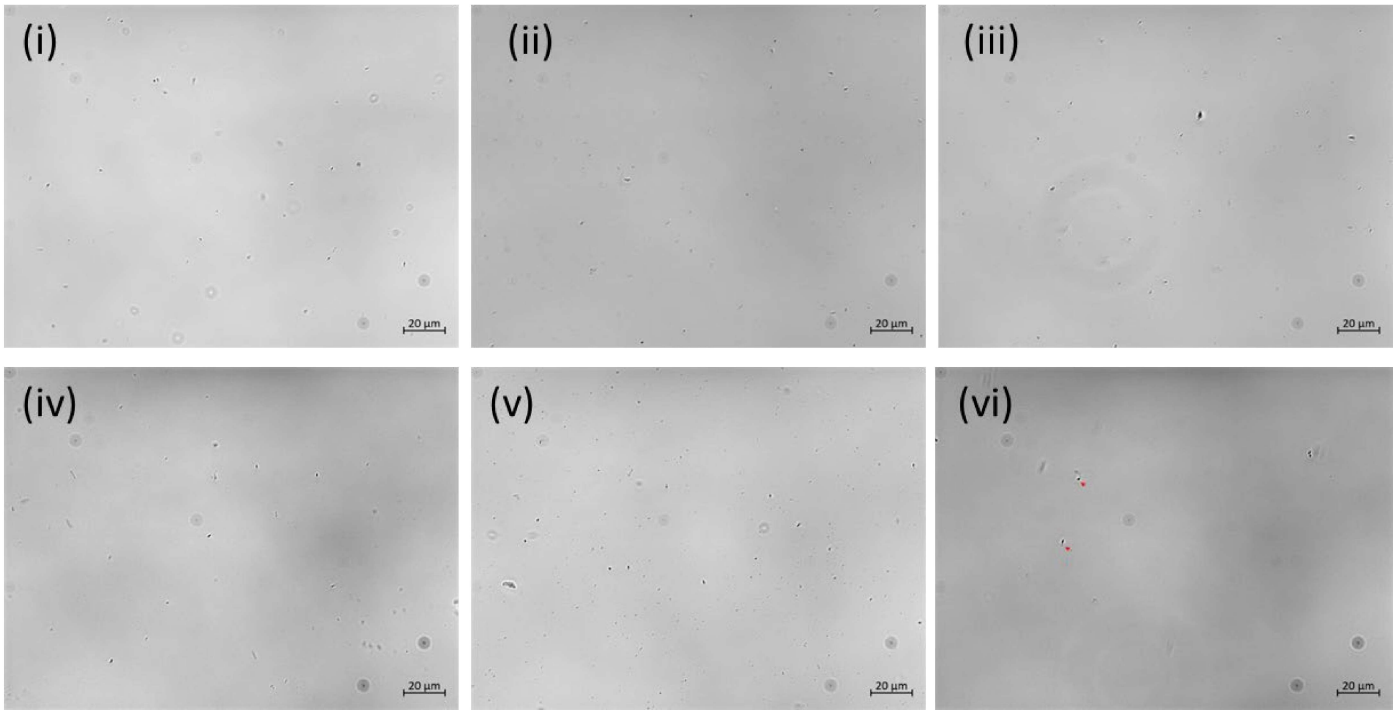


Figure 9. Phase contrast microscopy of formulations 2-6 (panels (i)-(v)) and PENSV20 grown in YGM broth for 48 hours (vi).

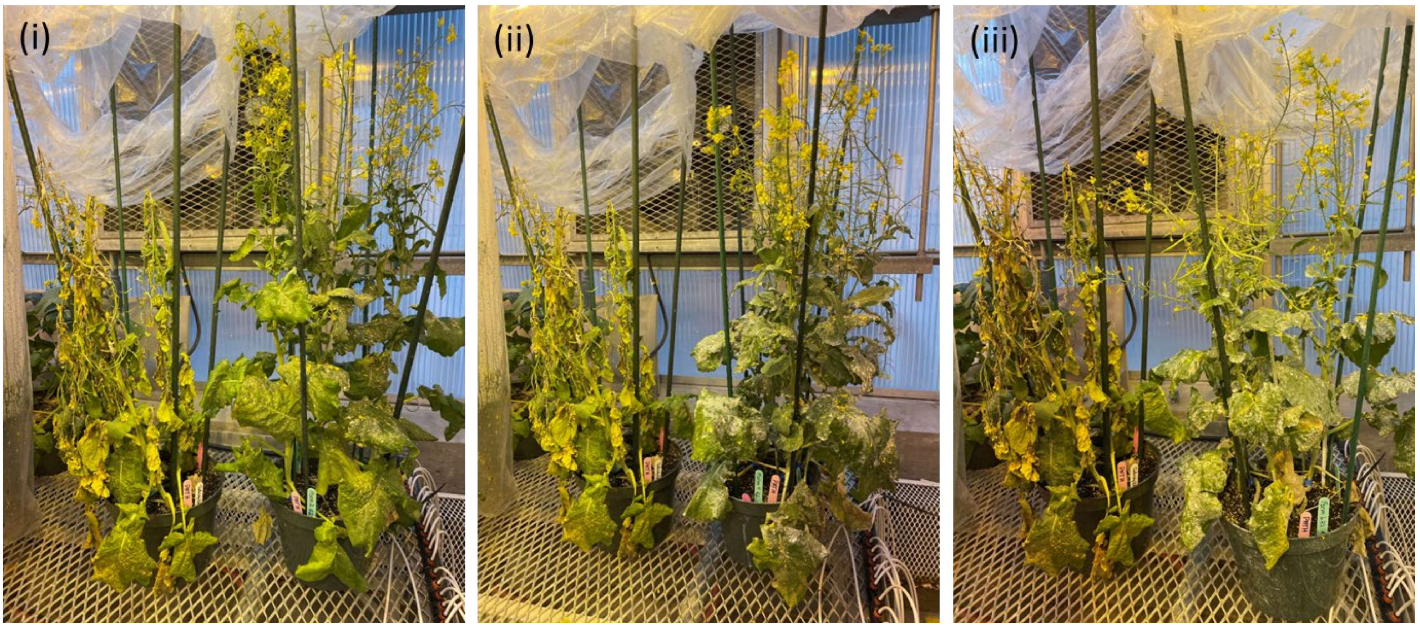


Figure 10. Examples of biocontrol provided by different formulations at 7 days post-infection with *S. sclerotiorum*. Formulations 2 (i), 4 (ii), and 6 (iii) are shown on the right of each picture. Plants challenged with pathogen in the absence of biocontrol agent are shown on the left.

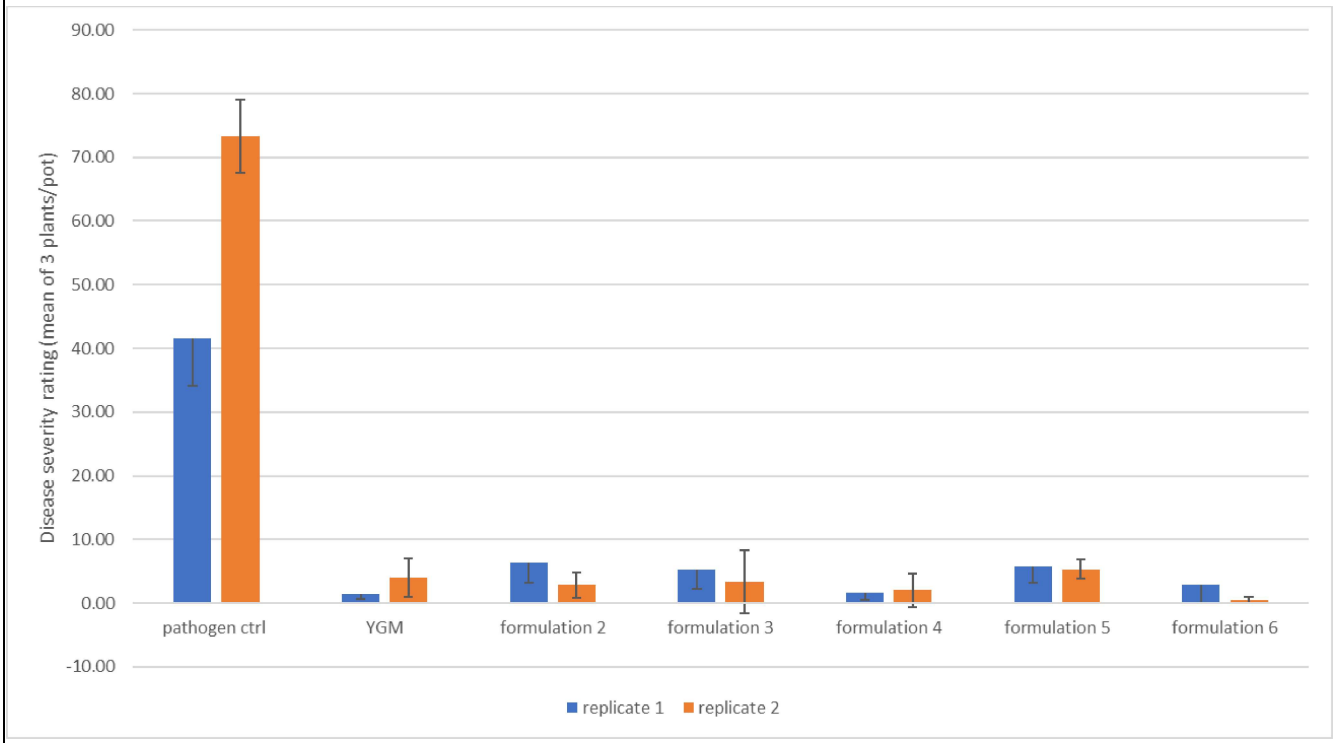


Figure 11. Disease severity ratings at 7 days after pathogen challenge. PENSV20 was applied without formulation (YGM), or as one of the indicated formulations to *B. napus* cv. Westar plants.

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

This project has resulted in the identification of many different strains of bacteria that display a phenotype of antibiosis against *S. sclerotiorum in vitro*. Many of the most effective strains were identified as *Bacillus atrophaeus*; many strains of *Bacillus* spp. have been previously identified as powerful plant growth promoting and biocontrol bacteria. Our work has identified a primary biocontrol candidate, *B. atrophaeus* strain PENSV20, and has examined potential mechanisms of action considering the totality of the tritrophic interaction (plant-bacterium-phytopathogen). The direct antibiosis resulted from the production of metabolites by PENSV20, which were identified as iturins, fengycins, and surfactins. In addition, the application of PENSV20 and pathogen has been demonstrated to result in an alteration in gene synthesis in the host plant, with many disease resistance pathways activated that could also explain the bioprotective effect. We developed effective spray challenge regimens for the biocontrol agent and determined that application of the bacterium prior to the pathogen challenge results in the best protection. In the case of Sclerotinia, infection is timed around flowering so that there is a natural window of application of the BCA. Finally, we have evaluated various industrial grade formulations of PENSV20 that, so far, are showing effective biocontrol for Sclerotinia.

Our objective in this project was always to bring an effective biological control option for Sclerotinia stem rot into the market, so that it is available as a validated option for producers to decrease their input costs and the environmental footprint associated with fungicide applications. Having identified an effective biopesticide and begun evaluating industrial-grade application formulations, our next step will be to assess these in field trials. We are currently working with our industrial partner, MBA/Terra Biotechnologies, on a proposal for a Bioproducts Partnership that will work at the distal end of the biopesticide development pipeline for this and several other AAFC-developed biological agents with agricultural application. Generally, our commercialization strategy will follow the steps we have already taken for a biocontrol agent for late blight. For that project, we filed patents in many countries for the development of a foliar biopesticide for potato late blight and have been granted patents in the USA, China, Mexico, and Brazil. These represent several of the largest agricultural producer markets worldwide. In addition, we have had interest in the technology from bioproducts companies and are in the final stages of a collaborative project with an option to license. This research serves as a prototype model for development of a foliar applied biopesticide for sclerotinia of canola as a technology in bioproducts. This research strategy showcases bioproducts as a clean technology and underscores the future of the agricultural biologicals sector. We have begun this process through our involvement with the nascent Bioproducts Partnership, and will proceed with the guidance of the AAFC Office of Intellectual Property and Commercialization. Like the late blight project, our primary focus is commercialization of the technology to make it available to producers, with publication in the open literature envisioned after patent protection has been obtained. The unflagging support of CARP in bringing this project from an idea through to a greenhouse-validated, industrially formulated, effective biocontrol option for producers is deeply appreciated.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

Presentations of the work at national and international meetings:

"Taxonomic and mechanistic analysis of plant-microbe interactions: Implications for biocontrol" – seminar with final results to be presented at AAFC (open to public) on April 4, 2024 (T. Dumonceaux)

"Taxonomic and mechanistic analysis of plant-microbe interactions: Implications for biocontrol" – seminar presented at Fustox meeting, Bordeaux France Oct 19 2023 (T. Dumonceaux)

"Understanding Plant Defense Mechanisms Against Sclerotinia Stem Rot Disease in Canola" - poster presented at PAG, San Diego, CA, USA January 2023 (A. Dakouri)

"A Bacterial Isolate Shows Promise as a Biofungicide for Sclerotinia Stem Rot Disease on Canola" - poster

presented at PAG, San Diego, CA, USA January 2019 (A. Dakouri)

Direct interactions with producers and the public to explain and promote the project:

"Biocontrol: Exploiting natural microbial interactions to provide ecologically based pest control options for Sclerotinia" – presentation at the Western Canada Crop Production Show January 9-11 2024, Saskatoon, SK. Biocontrol concepts, examples, and formulations were presented directly to producers, with many positive interactions and interest generated.

"Finding Plant Pathogens in the Field" – presentation at Biotech Blast May 4, 2023. T. Dumonceaux led an activity for elementary school students focused on the detection of Sclerotinia in infected canola, using some of the diagnostic assays developed in this project. The activity discussion dealt with the subject area of this project (Sclerotinia infections, detection, biocontrol).

"Molecular Diagnostics in Veterinary Medicine" – classroom activity/lectures. T. Dumonceaux used some of the materials developed in this project in teaching University of Saskatchewan graduate students from a range of academic departments, including Plant Science – January-April and September-December 2023.

"Techniques in Molecular Biology" – classroom activity/lectures. T. Dumonceaux led a student activity aimed at teaching graduate students detection methods for Sclerotinia in canola plants, using biocontrol as an academic framework for teaching the methods – May 2023.

Commercialization strategy:

Invention Disclosure filed as a first step for patenting PENSV20 as a biocontrol microorganism, following the successful strategy used for the recently patented BCA against *P. infestans*, which has several international patents granted. Publication of the results will follow.

Manuscript in preparation:

"Modulation of host defense mechanisms in canola-Sclerotinia- biocontrol agent tri-trophic interaction" - manuscript in preparation on the induced systemic resistance in canola by PENSV20 (A. Dakouri, Fengqun Yu, S.M. Boyetchko)

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

CARP support was acknowledged at all of the events described in section 7, including the presentations in France, USA, and at the producer-focused crop show in January 2024. The funders will also be acknowledged at the upcoming public AAFC seminar, and in the manuscripts that are in preparation.

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10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

No graduate students were trained through this project, but an undergraduate co-op student (Anika Briggs, University of British Columbia Department of Biology) completed two terms in the laboratory of Dr. Sue Boyetchko. Anika completed the in vitro characterizations of the 15 BCA that were evaluated as part of Objective 1. A Biologist was hired (Abdulsalam Dakouri) who completed the experiments aimed at characterizing the host response to pathogen and BCA challenge (Objective 2). Dr. Dakouri is now employed at Bayer Canada.

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. Invoice

13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	☐ Yes - this version can be posted X ☐ Yes - a modified version will be sent ☐ No
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	☐ Yes X ☐ No

Please send an electronic copy of this completed document to:

Ellen McNabb
Research Administrator
Canola Council of Canada
400 – 167 Lombard Ave.
Winnipeg, MB R3B 0T6
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