Date Received
For Administrative Use Only

Agriculture Funding Consortium

Full Research Project Final Report Form

- All sections must be completed.
- One electronic copy and one signed original copy are to be forwarded to the lead funding agency as per the investment agreement.
- A detailed statement of expenses incurred during the course of the project must be submitted along with this report.
- For any questions regarding the preparation and submission of this report, please contact the representative of the lead funding agency.

Members of the Agriculture Funding Consortium
- Alberta Crop Industry Development Fund Ltd. (ACIDF)
- Alberta Livestock and Meat Agency Ltd. (ALMA)
- Alberta Innovates Bio Solutions (AI-Bio)
- Alberta Barley Commission (ABC)
- Alberta Beekeepers
- Alberta Canola Commission (ACPC)
- Alberta Chicken Producers (ACP)
- Egg Farmers of Alberta (EFA)
- Alberta Milk (AM)
- Alberta Pork (AP)
- Alberta Pulse Growers Commission (APGC)
- Potato Growers of Alberta (PGA)
- Alberta Wheat Commission (AWC)
- Western Grains Research Foundation (WGRF)

Section A: Project overview

1. Project number: 2012F117R
2. Project title: Proof of concept to build a nano and antibody based pathogen specific plant disease monitoring device for agricultural pest management
3. Research team leader: Xiujie (Susie) Li
4. Research team leader's organization: Alberta Innovates - Technology Futures
5. Project start date (MM/DD/YYYY): 04/01/2012
6. Project completion date (MM/DD/YYYY): 03/31/2014
7. Project final report date (MM/DD/YYYY): 06/13/2014

Section B: Non-technical summary (max 1 page)

Plant diseases are major concerns of crop producers. They cause significant crop yield loss, human and animal health and safety concern over consumption of mycotoxins contaminated food and feed, and decreased farm income due to reduced sale of the product. Current plant disease forecasting measure is based on field scouting and/or plant survey conducted in the previous year(s) which is not accurate and cannot be done in a timely fashion to prevent disease outbreak and yield lost. An accurate pathogen level detection before disease occurrence is in need.

The long term goal of this project is to develop an in-field sensor for the detection of plant disease pathogen level for disease prevention. The overall idea includes 1) selection of a plant pathogen, *Sclerotinia sclerotiorum* from Sclerotinia stem rot as antigen, 2) production of an antibody against *S. sclerotiorum*, 3) make an antibody- nanoparticle conjugate, 4) detect the conductivity of the *S. sclerotiorum*-antibody- nano-particle complex to determine the level of pathogen in the field. The hypothesis is, if the level of conductivity of the nanoparticle is correlated with the number of pathogen bind to the antibody-nanoparticle conjugate and it is measurable, a device then could be built to convert the pathogen level to conductivity and electronic signal and send the signal to farmer through an electronic device (for e.g, a cell phone). The current project is to test this hypothesis. Our results from this project indicated that there is a positive correlation, a linear relationship, between the numbers of *S. sclerotiorum* ascospores and the conductivity of their antibody-spore-gold nanoparticle complex. Our study also proved that conductivity measurement is sensitive to detect as low as 5 ascospores of *S. sclerotiorum* in the sample. A negative correlation, a linear relationship, was also obtained when *Leptosphaeria maculans* was used as a pathogen indicated that the future device could be species-specific and applied to more plant disease pathogens. A future study should include making this device and deploying it to the field for testing.

Section C: Project details

1. Project team (max ½ page)

As project lead, Dr. Xiujie Li contributed to the project coordination between AITF and University of Alberta and NINT, reporting, and presenting at conferences. She also contributed to antigen preparation and antibody production. Dr. Jian Yang contributed to the preparation of fungi, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum* spores. Dr. Jie Chen from the University of Alberta contributed to the Nano-antibody-spore complex formation and conductivity measurement.

2. Background (max 1 page)

Sclerotinia sclerotiorum (Lib.) de Bary is a fungal pathogen that infects more than 400 plant species in more than 60 families, causing significant economic losses in many crops in Canada and worldwide (Turkington et al., 2011), including stem rot of canola. The yield loss of canola infected by *S. sclerotiorum* can reach up to 100%. *Sclerotinia sclerotiorum* as a plant pathogen is becoming more important as production of canola increases. In Alberta, the production of canola increased from under 3.5 million tonnes in 2009 to 16.0 million tonnes in 2013 (Statistics Canada, 2013). *Sclerotinia* stem rot can be controlled using foliar fungicides (Kutcher and Wolf, 2006) if the disease can be accurately forecasted. Several forecasting systems have been developed, mostly for oilseed and pulse crops (Clarkson et al. 2007; Koch et al., 2007). These systems use risk-point tables based on apothecium development, soil moisture, temperature, crop canopy development, crop rotation, and crop disease levels in previous years. In Canada, additional risk assessment tools are available, including petal testing protocols (culturing of *S.*

sclerotiorum from canola petals to determine infection levels), checklists, and weather-based forecasting maps (McLaren et al., 2004), however, the acceptance of these tools is limited because:
1) Weather-based disease forecasts apply to broad regions, not individual farms,
2) Checklists require constant field assessments and cost/benefit decision points for making fungicide application decisions are rather qualitative,
3) Petal testing can be too time consuming to provide timely information.

Consequently, methods for forecasting *Sclerotinia* stem rot that require less effort and provide highly accurate data in real time would be very useful for ensuring timely and effective fungicide application, with the corollary benefit of preventing needless fungicide applications.

Nanoparticles with specific properties such as positive surface charges have the ability to bind to cells, which are negatively charged (Doiron et al., 2011). Antibodies produced against antigens such as *S. sclerotiorum* ascospores, will recognize the antigen specifically and exclusively. Therefore, if *S. sclerotiorum* ascospores encounter such an antibody, they form an *S. sclerotiorum*-antibody complex. Metal nanoparticles (e.g. gold, silver) are electrically conductive, therefore changes in configuration or dispersion of antigen-antibody-nanoparticle complexes should alter the electrical conductivity of the system if one component (e.g. the number of ascospores) is altered. This project aims to make a *S. sclerotiorum* nanoparticle complexes and then test the correlation between the formation of these complexes and electrical conductivity. Existence of such a correlation will indicate that it is possible to develop a device based on *S. sclerotiorum*-antibody-nanoparticle complexes that is capable of reporting pathogen levels. Such a device would have the advantage of providing rapid results that can be automatically analyzed and distributed.

3. Objectives and deliverables (max 1 page)

The objectives of this project include:
1. Generate and store *S. sclerotiorum* ascospores and *L. maculans* Pycnidiospores
2. Make anti-*S. sclerotiorum* and anti-*L. maculans* antibodies and antibody-nanoparticle complexes
3. Determine the conductivity of *S. sclerotiorum*-nanoparticle complex and *L. maculans*-nanoparticle complex
4. Determine the minimum detectable ascospore population with the *S. sclerotiorum*-nanoparticle complex and *L. maculans*-nanoparticle compex

The deliverables include:
1. The information of the ascospore population of *S. sclerotiorum* and their conductivity to indicate if there is correlation between them
2. Report, protectable IP and/or publications
3. Recommendations on possible commercialization pathways

4. Research design and methodology (max 4 pages)

4.1. Generate and store spores

4.1.1. Generating *S. sclerotiorum* ascospores

Ascospores of *S. sclerotiorum* were produced using the standard method that has been in place for over five years to supply the canola breeding and fungicide industries. In brief, the procedure requires generation of sclerotia of *S. sclerotiorum* (AITF accession #184) on carrot sliced roots. Sclerotia were harvested, washed and mixed in wet sand and then cold treated at 4°C until apothecial stipes form. Germinating sclerotia were transferred to a warm, lighted incubator to allow apothecium formation, and ascospores were harvested to a filter disc by a vacuum. The disc with harvested spores was stored at -20°C until antigen production and conductivity testing.

4.1.2. Generating *L. maculans* pycnidiospores

A virulent isolate of *L. maculans*, Leroy #127, was cultured on V8 agar (20% V8-juice, 0.75 g $CaCO_3$, 100 mg streptomycin sulfate, 40 mg Rose Bengal, 15 g agar) at 24±3°C for 20 days. Pycnidiospores were collected by flooding the plates with sterile distilled water, filtered through sterile nylon mesh, centrifuged at 454 ×g, resuspended in sterile water and stored at -20°C (Soledade et al. 2000).

4.2. Make anti-*S. sclerotiorum* and anti-*L. maculans* antibodies and antibody-nanoparticle complexes

4.2.1. Anti-*S. sclerotiorum* antibodies production

Polyclonal rabbit anti-*S. sclerotiorum* antibodies were produced by Cedarlane Laboratory (Burlinton ON, Canada) using the following protocol. Two rabbits were immunized with freeze dried *S. sclerotiorum* ascospores as antigen. 0.5 mg of the ascospores was emulsified with 0.5 mL of Freunds complete adjuvant for the primary immunization at day 0, three booster immunizations were conducted at day 28, 47, and 66 with 0.5 mg of ascospores emulsified with 0.5 mL of Freund's incomplete adjuvant. The antigen adjuvant mixture was injected subcutaneously at 4 sites (0.25 mL/site) in the fore and hind quarters. The anti-*S.sclerotiorum* antisera were collected at day 76 and purified with an affinity column. The activity of anti-*S.sclerotiorum* antibody was determined by indirect enzyme linked immunosorbent assay (ELISA).

4.2.2. Anti-*L. maculans* antibody production

Polyclonal rabbit-anti *L. maculans* IgG, was kindly provided by Dr. Peter Ellis of Phyto Diagnostics Company Ltd. The antibody was produced by injecting rabbits with various preparations of the virulent (V) strain (Badawy et al., 1991). Cell walls, soluble cytosol proteins, a concentrated extracellular fraction, conidia and whole mycelium were injected. Each preparation was used to immunize a young New Zealand white rabbit. For the first injection, 1 mg of immunogen emulsified with Freund's complete adjuvant was administered intramuscularly in a hind leg. The second and third injections, each containing 1 mg of immunogen emulsified with Freund's incomplete adjuvant, were administered in a hind leg at monthly intervals. Test bleeds were collected at 2-wk intervals following the last injection and titred by indirect ELISA (Stace-Smith et al., 1993).

4.2.3. Synthesis of functional gold nanoparticles

Adequate quantities of gold nanoparticles (GNPs) were synthesized using the following method: 1) 3 mL of 25 mM $HAuC_{l4}$ solution was added into 47 mL of deionized water and boiled with moderate stirring, 2) 8 mL of 34 mM sodium citrate was then added as a reductant to obtain GNPs without any capping agents. 3) polyethylene glycol (PEG) was added into the previous gold solution to obtain functional GNPs. Functional GNPs were dialysed for two days before use. The average sizes of GNPs were measured by dynamic light scattering (DLS). The surface of GNPs was characterized by X-ray photoelectron spectroscopy (XPS) (Kratos Analytical, Manchester, UK).

4.2.4. Preparation of *S. sclerotiorum* antibody-GNPs complex

One hundred microliters of 1.2 nM GNP solution was diluted to 1 mL with phosphate buffered saline (PBS, pH=7) and mixed with 30 µL antibody. Thirty milligrams of 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was then added to the solution and the mixture was shaken at room temperature for 2 hrs. After centrifugation at 10,000 rpm for 20 min, the supernatant was discarded and the pellet was washed two times with high pressure liquid chromatography (HPLC) grade water and centrifuged again. The pellet was then re-suspended into 600 µL HPLC grade water.

4.2.5. Measure the conductivity of *S.* s*clerotiorum*-nanoparticle complex

A real-time cell electronic sensing (RT-CES) system (ACEA Biosciences, San Diego, CA, USA) was used as the platform to facilitate this study. The method described previously by Xing et al. (2005) was used in this study. The sensor devices supplied with *S. sclerotionum* or *L. maculans* ascospores were mounted to a device station placed inside an incubator. Electrical cables connected the device station to the sensor analyzer. Under the control of the RT-CES software, the sensor analyzer automatically selected wells to be measured and continuously conducted measurements on wells. The electronic impedance conductivities were then transferred to a computer and plotted.

4.2.6. Cell titration test to measure the numbers of *S. sclerotiorum*-gold nanoparticle complexes, *L. maculans*-gold nanoparticle complexes and their conductivity

These tests were performed in triplicate. One hundred µl of *S. sclerotiorum*-gold nanoparticle complex suspension with approximate cell numbers of 5, 10, 20, 200, 2×10^3, 2×10^4, and 2.2×10^5 were seeded into the sensor device wells containing 100 µl phosphate buffered saline (PBS). Wells containing 200 µl of PBS only were used as blanks. The sensor device was placed into the incubator and the cell index for each well was determined.

The same procedure was also performed to measure the numbers of *L. maculans*-nanoparticle complexes. Cell numbers of 45, 450, 4.5×10^3, 4.5×10^4, 4.5×10^5, and 4.5×10^6 were used in this study.

5. Results, discussion and conclusions (max 8 pages)

5.1.Results

5.1.1. Production of spores

In this study, 2 mg of freeze dried *S. sclerotiorum* ascospores were produced for antibody production and five filter discs, approximately 2.5×10^8 *S. sclerotiorum* ascospores were produced for conductivity study. The number of *L. maculans* pycnidiospores produced was about 2.0×10^8 for conductivity study.

5.1.2. Antibody production

For the two rabbits immunized with *S. sclerotiorum*, one rabbit, No. 2038, responded well and produced high titre antibodies compare to rabbit No. 3A15. The antibody titre produced by rabbit 2038 is shown in Figure 1. The absorbance of positive serum and negative serum ratio was calculated as 2.415/0.148 = 14.493 >> 2.1 indicated a good production of polyclonal antibody against *S. sclerotiorum*.

Rabbit anti-*L. maculans* polyclonal antisera was also tested with indirect ELISA procedure, all polyclonal antisera reacted with purified antigen (1 mg/mL) at antisera dilutions of greater than 10^6 indicated that the antibody was active against *L. maculans* (Stace-Smith, et al., 1993).

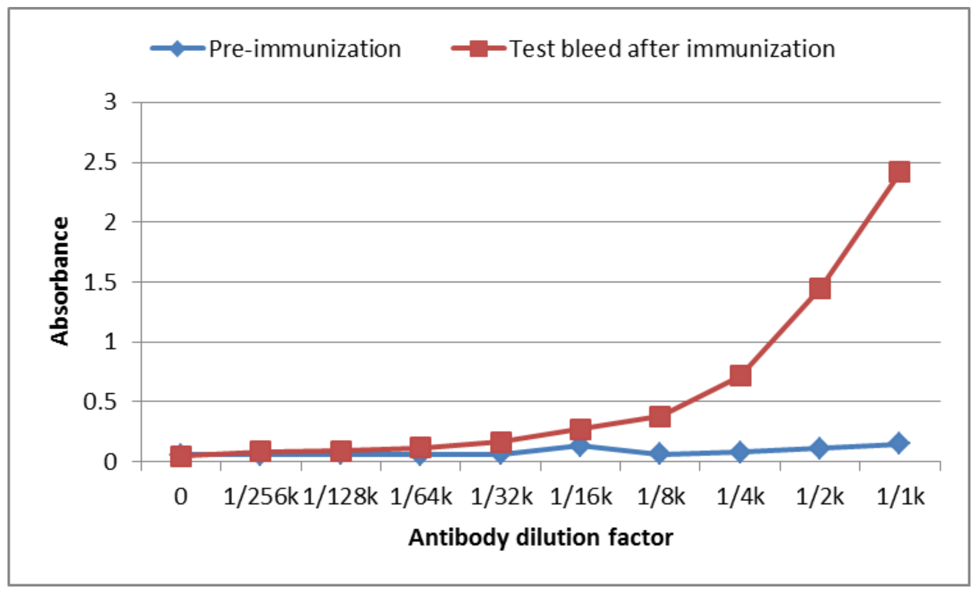

Figure 1. Rabbit anti-*S. sclerotiorum* antibody activity expressed by ELISA absorbance.

5.1.3. Production of nano particles

Gold nanoparticles (GNPs) of 20 mL with the concentration of 25 mM were successfully produced and the transmission electron microscopy (TEM) picture of GNPs produced is shown in Figure 2.

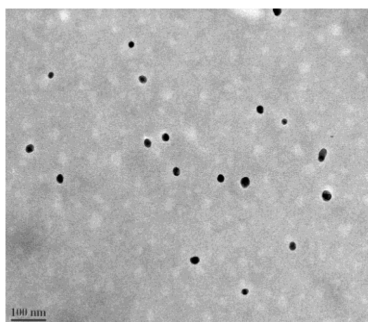

Figure 2. GNPs generated for conductivity measurement of *S. sclerotiorum* ascospores, Size of GNPs is approximately 20-30 nm, GNPs concentration is 1 nmol per well.

5.1.4. Determine the possibility of measuring the conductivity of GNP- antibody-spore complex

To allow quantitative measurements of changes in conductivity due to antibody binding, we substituted a gold electrode of a nanoelectronic sensing array (NESA). Conductivity changes across the NESA electrode were schematically represented in Figure 3. The gold electrode was first bound to specific antibody. *L maculans* or *S. sclerotiorum,* spores were then added respectively and the conductivity was measured after removal of non-bound spores by washing. Results of the conductivities expressed as sensing index in relation to the number of spores are shown in Figures 4a and 5a. Figure 4b and 5b show the correlation between the conductivity and the numbers of spores.

Design Scheme

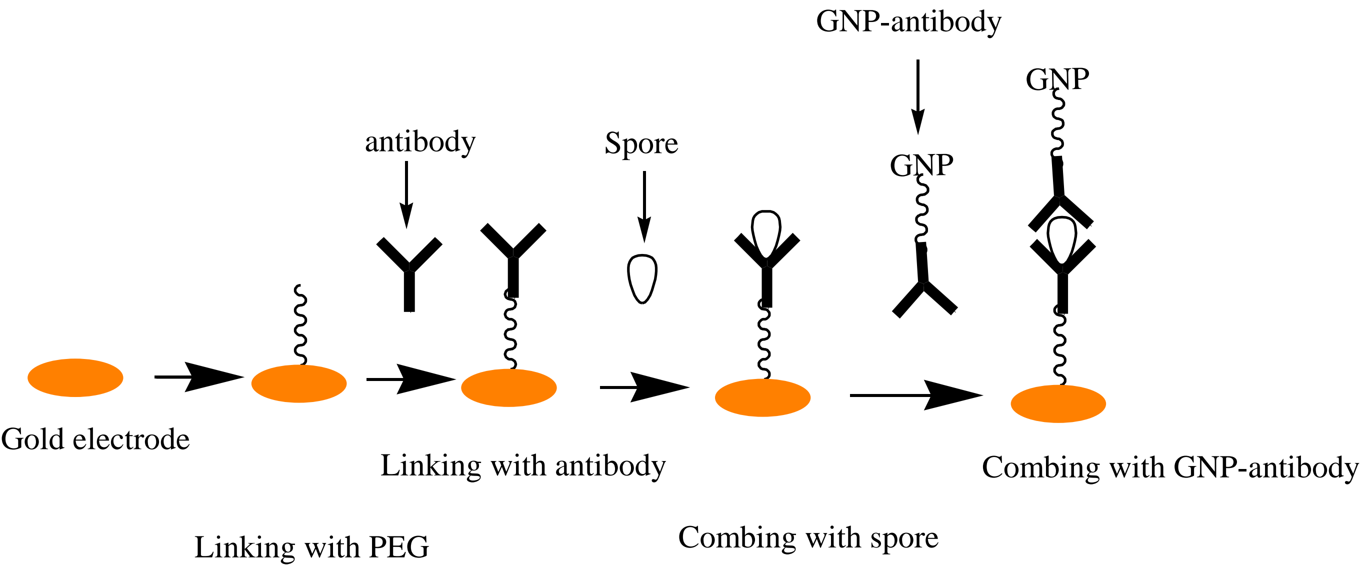

Figure 3. Schematic design of spore sensing experiment

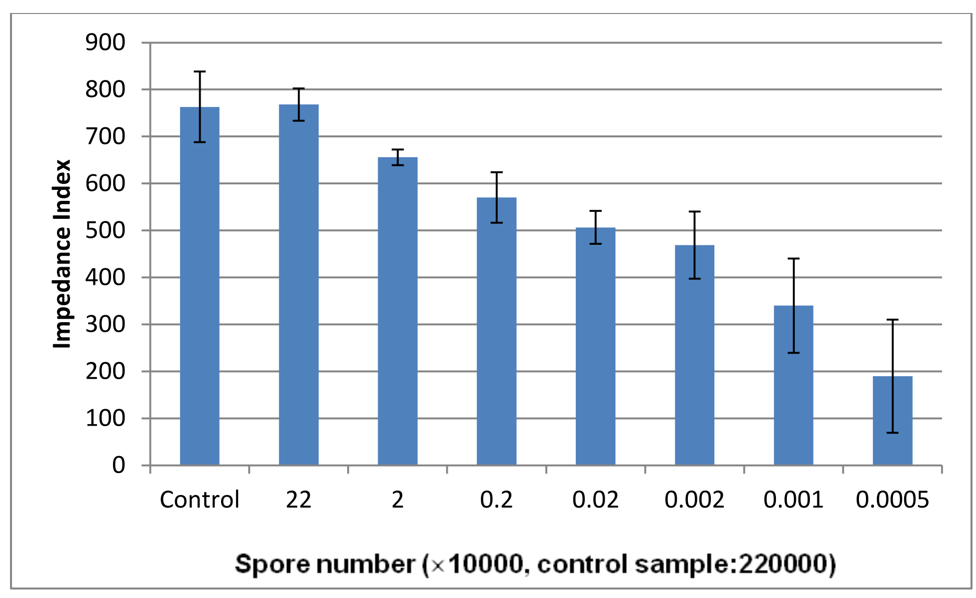

Figure 4a

Figure 4a. Measurement of *S.sclerotiorum* spore concentration using nano sensing technology, the vertical axis is a sensitivity index calculated based on electrical conductivity (higher values indicate increased conductivity, error bars indicate standard deviation of the measurement). The horizontal axis shows the number of spores added to each of replicate wells

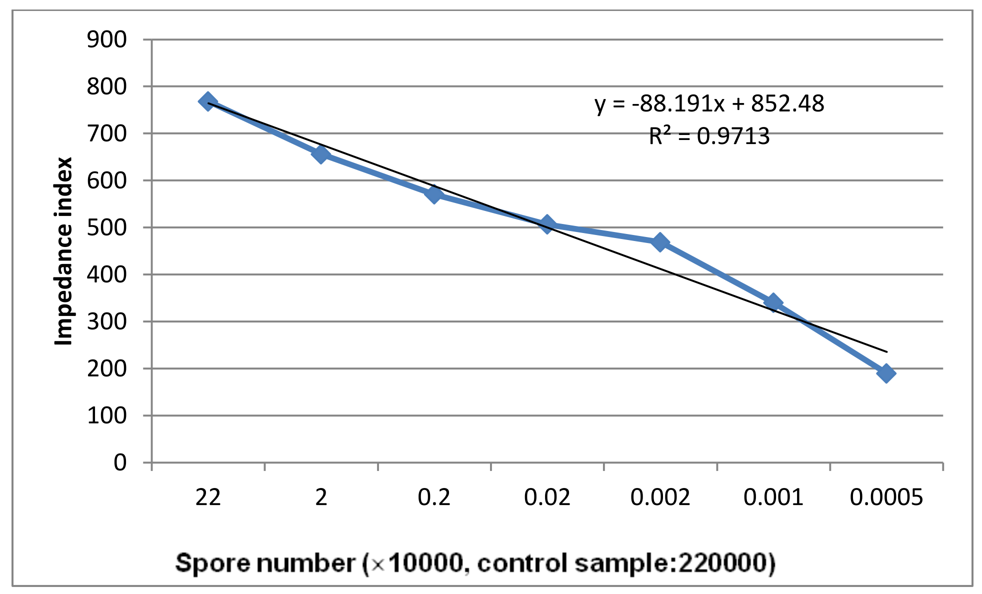

Figure 4b

Figure 4b. Correlation between the conductivity and the number of *S. sclerotiorum* spores

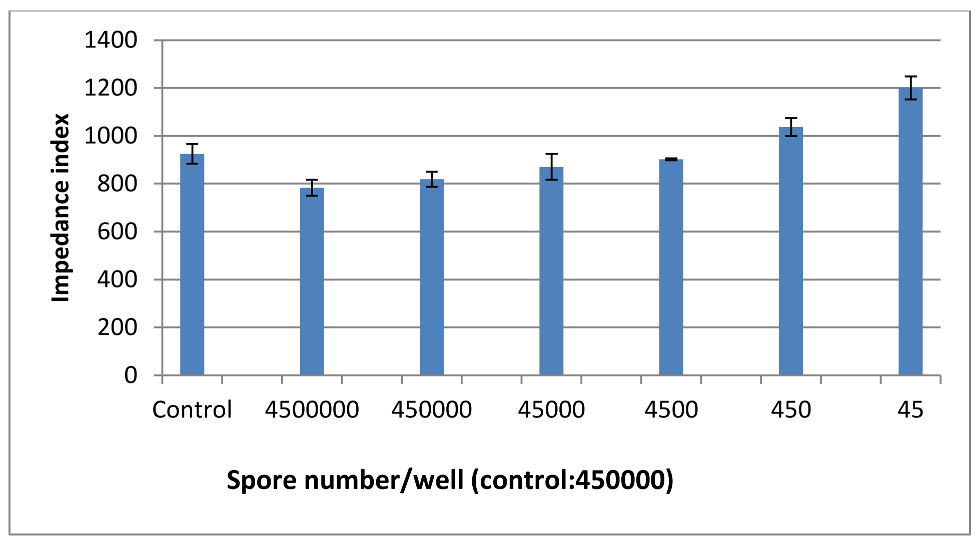

Figure 5a

Figure 5a. Measurement of *L. maculans* spore concentration using nano sensing technology, the vertical axis is a sensitivity index calculated based on electrical conductivity (higher values indicate increased conductivity, error bars indicate standard deviation of the measurement). The horizontal axis shows the number of spores added to each of replicate wells

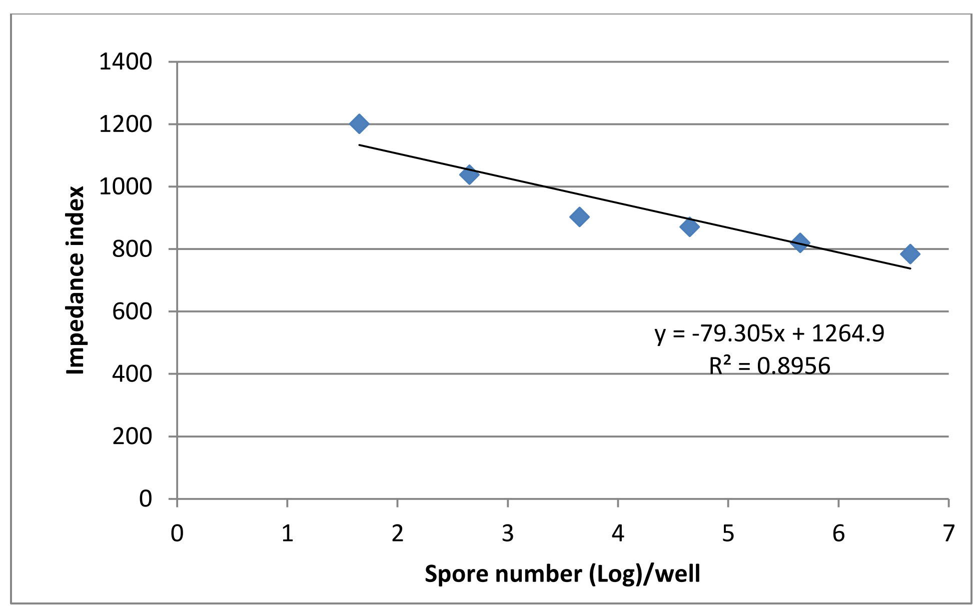

Figure 5b

Figure 5b. Correlation between conductivity and the number of *L. maculans* spores

The results indicated that there was a positive linear relationship between conductivity and ascospore concentration of *S. sclerotiorum*. However, there was a negative linear relationship for *L. maculans*, as conductivity decreased with spore number increased. The lowest numbers of ascospores of *S. sclerotiorum* that could be detected by S. sclerotiorum-nanoparticle complex in this study is five.

5.2 Discussion

We initially planned to make *S. sclerotiorum*-nanoparticle complex without antibody, this goal was modified due to the difficulty in determining whether the conductivity was generated by nanoparticle-spore complex or spore only without a specific antibody. We therefore introduced the antibody based approach and substituted "determine the possibility of measuring the conductivity of GNP -antibody-spore complex" for the existing goal. Since there was no anti-*S. sclerotiorum* antibody available in the first year of our study, we substituted *L. maculans* pycnidiospores and its antibody as a model, and this study also enable us to test whether the technology can be extended easily to other organisms. *L. maculans* is the causal agent of blackleg disease of canola. A preliminary study showed that the conductivity of *L. maculans* pycnidiospores was measurable when antibodies to this pathogen were used.

To understand the two different trends of the conductivity to numbers of spores for both *L. maculans* and *S. sclerotiorum*, the differences of these two pathogens were investigated. The only noticeable physical difference observed in this study was their spore sizes. For *L. maculans*, pycnidiospores were used whereas for *S. sclerotiorum*, ascospores were used. The size of *L. maculans* pycnidiospores and *S. sclerotiorum* ascospores were approximately 2.5-10 x 1-3.5 µm (Aveskamp et al., 2008) and 12 µm long with a ratio of length/width more than 2 (Linda 1979) respectively. The size of pycnidiospores of *L. maculans* we produced is much smaller than that of *S. sclerotiorum* ascospores. Therefore spores sizes may be a factor that influences the trend of conductivity of the sensor. On the other hand, other physical and chemical properties of the spores may also play a role in this result and further study need to be done to investigate these possibilities. It is possible that the nanosensor device developed in the future could be species-specific.

5.3.Conclusion

The objectives of this project have been successfully achieved. Conductivity measurement was sensitive to detect as low as five ascospores of *S. sclerotiorum* in the sample. This study also proved that there was a positive correlation, a linear relationship, between the numbers of *S. sclerotiorum* ascospores and the conductivity of their antibody-spore-gold nanoparticle complex. A future study should include making this device and deploying it to the field for testing.

6. Literature cited

Provide complete reference information for all literature cited throughout the report.

Aveskamp, M.M., De Gruyter, J. and Crous, P.W. 2008. Biology and recent developments in the systematics of Phoma, a complex genus of major quarantine significance. Fungal Diversity 31:1-18.

Badawy, H.M.A., H.H. Hoppe, and E. Koch. 1991. Differential reactions between the genus Brassica and aggressive single spore isolates of Leptosphaeria maculans. J. Phytophtol. 131:109-119.

Clarkson, J.P., K. Phelps, J.M. Whipps, C.S. Young, J.A. Smith, and M. Watling. 2007. Forecasting Sclerotinia disease on lettuce: a predictive model for carpogenic germination of Sclerotinia sclerotiorum sclerotia. Phytopathology. 97:621-631.

Doiron, A.L., B. Clark, and K.D. Rinker. 2011. Endothelial nanoparticle binding kinetics are matrix and size dependent. Biotechnol. Bioeng. 108(12):2988-2998.

Heaney, D. 2010. Measuring up: Assessment of diagnostic and analytical laboratory services required by Alberta's crop production and feed industries. Random Cross Consulting.

Koch, S., S. Dunker, B. Kleinhenz, M. Röhrig, and A. von Tiedemann. 2007. A crop loss-related forecasting model for Sclerotinia stem rot in winter oilseed rape. Phytopathology. 97:1186-1194.

Kutcher, H.R., and T.M. Wolf. 2006. Low-drift fungicide application technology for sclerotinia stem rot control in canola. Crop Protection 25:640-646.

Linda, M.K. 1979. Delimitation of the economically important plant pathogenic Sclerotinia species. The American Phytopathological Society. 69 (8):881-886.

McLaren, D.L., R.L. Conner, R.G. Platford, J.L. Lamb, H.A. Lamey and H.R. Kutcher. 2004. Predicting diseases caused by Sclerotinia sclerotiorum on canola and bean – a western Canadian perspective. Can. J. Plant Pathol. 26:489-497.

Soledade, M., C. Pedras, and C.J. Biesenthal. 2000 HPLC analyses of cultures of *Phoma* Spp. : differentiation among groups and species through secondary metabolite profiles. Can. J. Microbiol. 46:685-691.

Stace-Smith, R., G. Bowler, D.J.MacKenzie and P. Ellis. 1993. Monoclonal antibodies differentiate the weakly virulent from the highly virulent strain of Leptosphaeria maculans, the organism causing blackleg of canola. Canadian Journal of plant Pathology. 15(3):127-222.

Turkington, T.K., H.R. Kutcher, D. McLaren, and K.Y. Rashid. 2011. Managing Sclerotinia in oilseed and pulse crops. Insects and Diseases. 4:105-113.

Xing, J.Z, J. Zhu J.A. Jackson, S. Gabos, X.J. Sun, X.B. Wang, and X. Xu. 2005. Dynamic monitoring of cytotoxicity on microelectronic sensors. Chemical Research in Toxicology 18:154-161.

7. Benefits to the industry (max 1 page)

a) *S. sclerotiorum* is a huge problem for crop production especially oilseeds and pulse crops. It is also difficult to forecast due to the many factors that influence Sclerotinia stem rot outbreaks. Reasonably accurate prediction of the disease requires significant effort and resources for repeated monitoring of the pathogen. This project will potentially benefit farmers by enabling in-field, real-time, accurate plant pathogen forecasting that will facilitate timely fungicide applications and prevent late or ineffective use of fungicides. In turn this will maximize producer investments in fungicides, and minimize environmental impacts. The technology would also facilitate the collection of pathogen load data for disease monitoring and surveillance purposes. Outcomes would include cheaper, more timely and more extensive disease surveys, and easier and more frequent monitoring of invasive plant pathogens. Because the mature technology could be used for many other organisms than *S. sclerotiorum*, these impacts would also apply to many other diseases of crops, including cereals, fruit, forage/feed and root crops, and could be used to monitor airborne contaminants such as allergens (pollen, spores) and indoor air quality (mold contamination).

b) According to Todd Friday, Saskatoon-based Pulse and Oilseed market segment manager with DuPont, “Sclerotinia is one of the most economically damaging diseases of canola, potentially reducing yields by up to 50% in extreme cases,” “Canola produced $8.1 billion in revenue for growers last year, so in the fight against *Sclerotinia*, the stakes could not be higher.” We believe a real-time device for *Sclerotinia* forecasting will significantly reduce the yield loss as well as revenue loss and the cost benefit to canola industry is huge.

8. Contribution to training of highly qualified personnel (max ½ page)

Dr. Yuzhi Hao, a research associate of University of Alberta was involved in sensor design; Ms. Xiaoyan Yang, a technician of the University of Alberta was involved in executing the conductivity measuring.
Mr. Rodney Werezuk, technician of AITF was involved in *S.sclerotiorum* ascospores production and antigen preparation.
Ms. Wendi Dmytriw was involved in production of *L. maculans* conidia spores production.

9. Knowledge transfer/technology transfer/commercialisation (max 1 page)

Alberta Innovates - Technology Futures (AITF), University of Alberta, and National Institute of Nanotechnology (NINT) worked together to deliver this project. AITF was responsible in *S. sclerotiorum* selection and culture, University of Alberta and NINT were providing expertise in Nanotechnology, conductivity measurement and also providing nanoparticles. Consequently, three organizations have gained knowledge in nano sensor design for agricultural pathogen detection.
Each of the collaborating institutions has technology commercialization functions and capacities. These will be used to identify commercialization partners that can engineer and manufacture the envisioned instruments. The developed IP will be protected, but also published as patents and/or journal articles, in addition to the required project reporting. With the findings of the study, we are planning to file an IP disclosure to protect the technology.

Presentations included: poster presentation at the 2013 Canadian Phytopathology society annual meeting, Biomedical Circuits and Systems conference, and seminars in a joint meeting of AITF and Dow AgroScience. A manuscript is under preparation to be published in a science journal.

Section D: Project resources

1. **Statement of revenues and expenditures:**
    a) **In a separate document certified by the organization's accountant or other senior executive officer, provide a detailed listing of all cash revenues to the project and expenditures of project cash funds.** Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage; and overhead (if applicable).
    b) **Provide a justification of project expenditures and discuss any major variance (*i.e.*, ± 10%) from the budget approved by the funder(s).**

Additional objectives were added to this project based on the results of the first half year of the study which included 1) testing the effect of adding antibody against *L. maculans* on conductivity measurement and 2) obtaining antibodies for *S. sclerotiorum* which subsequently increased the project cost. Additional fund, $26,725, was provided by AITF to cover the cost.

2. **Resources:**

Provide a list of all external cash and in-kind resources which were contributed to the project.

Total resources contributed to the project		
Source	**Amount**	**Percentage of total project cost**
Agriculture Funding Consortium	$60,000	44.21%
Other government sources: Cash	$26,725	19.69%
Other government sources: In-kind	$49,000	36.10%
Industry: Cash		%

Industry: In-kind		%
Total Project Cost	$135,725	100%

External resources (additional rows may be added if necessary)		
Government sources		
Name (only approved abbreviations please)	Amount cash	Amount in-kind
Alberta Innovates - Technology Futures	$26,725	$49,000
Industry sources		
Name (only approved abbreviations please)	Amount cash	Amount in-kind

Section F: Suggested reviewers for the final report

Provide the names and contact information of four potential reviewers for this final report. The suggested reviewers should not be current collaborators. The Agriculture Funding Consortium reserves the right to choose other reviewers. Under *Section 34* of the *Freedom of Information and Protection Act (FOIP)* reviewers must be aware that their information is being collected and used for the purpose of the external review.

Reviewer #1
Name: Stephen Strelkov
Position: Associate Professor
Institution: University of Alberta
Address: 4-16A Agriculture/Forest Ctr, Edmonton AB, T6G 2P2, Canada
Phone Number: 780 492 1969
Fax Number
Email Address:stephen.strelkov@ualberta.ca

Reviewer #2
Name: David Mitlin
Position: Associate Professor
Institution: NINT / University of Alberta
Address: 9107-116 Street, Edmonton AB, T6G 2V4, Canada
Phone Number: 780 492 1542
Fax Number
Email Address: dmitlin@ualberta.ca

Reviewer #3
Name: Kelly Turkington
Position: Plant Pathologist
Institution: Agriculture and Agri-Food Canada,
Address: 6000 C and E Trail, Lacombe, Alberta T4L 1W1
Phone Number: 403-782-8138
Fax Number: 403-782-6120
Email Address: kelly.turkington@agr.gc.ca

Reviewer #4
Name: Jie Feng
Position: Research Scientist
Institution: Alberta Agriculture and Rural Development
Address: 17507 Fort Road NW, Edmonton Alberta, T5Y 6H3
Phone Number: 780 644-3436
Fax Number: 780 422-6096
Email Address: jie.feng@gov.ab.ca