PGA RESEARCH ARCHIVE

STORAGE

POTATO GROWERS OF ALBERTA

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Potato Growers of Alberta
Research Tracking

Title of Research Application: Dev. of recommendations for use of essential oils as sp inhibitors for stored potatoes

Name of Researcher: Chris Neeser

Employer: Ab Agriculture, Food and Rural Dev.

Date application was received by PGA ____

Date application was reviewed by PGA April 4, 2006

A) approved ✓ B) declined ____

Project start date: June 1, 2006 Project finish date: ____

Total amount requested: $ 2000 - Amount requested per year: $2,000 -

MOU received and signed. Once copy returned to research agency, one copy filed in current year Research Binder

Date completed June 15/06

Invoice received: # 62720061 Date funds advanced July 6/06 Cheque# 4349 $2000 -

Invoice received: # ____ Date funds advanced ____ Cheque# ____

Invoice received: # ____ Date funds advanced ____ Cheque# ____

Invoice received: # ____ Date funds advanced ____ Cheque# ____

Were reports received from the researcher? ____

What was done with the reports?

Presented at PGA meeting? ____ Put on PGA website? ____ Filed? ____

NOTES: ____

Memorandum

Crop Diversification Centre South

S.S. #4
Brooks, Alberta
Canada T1R 1E6

Main Switchboard: 403/362-1300
Phone: 403/362-1331
Fax: 403/362-1306

From Christoph Neeser
Weed Scientist

Date April 26, 2007

To Tom Switzer,
Executive Director
CARD/ACAAF
Agriculture & Food Council

Subject: Project #2006F155R "Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes"

The attached is the activity report for the completed portion of the first phase of the project entitled "Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes". It contains an accounting statement prepared by Anna Moeller, our financial administrator, a project schedule, a description of activities completed, and some preliminary results.

If this satisfies your needs, then please proceed to make effective the second instalment ($24,000) as outlined in the investment agreement #2006F155R. Please contact me if you have any questions about this report.

Also, I would like to inform you that my work title has changed from Fruit & Vegetable Scientist to Weed Scientist (effective April 1, 2007). However, I have received assurances that this change will not affect my ability to complete ongoing projects, such as this one.

Christoph Neeser

POTATO GROWERS OF ALBERTA

6008 - 46 Ave. Taber, AB, T1G 2B1
Phone 403-223-2262
Fax 403-223-2268

Memo

Date: April 27, 2007

To: Research Committee

From: Patti Lamb

Re: Updated Research Report

Attached, please find an updated report for the following research project:

"Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes" by Chris Nesser.

Any questions, please feel free to contact the office.

Thank you,

P. Lamb

Patti Lamb
Executive/Administrative Assistant
Potato Growers of Alberta

Alberta Agriculture and Food

Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes

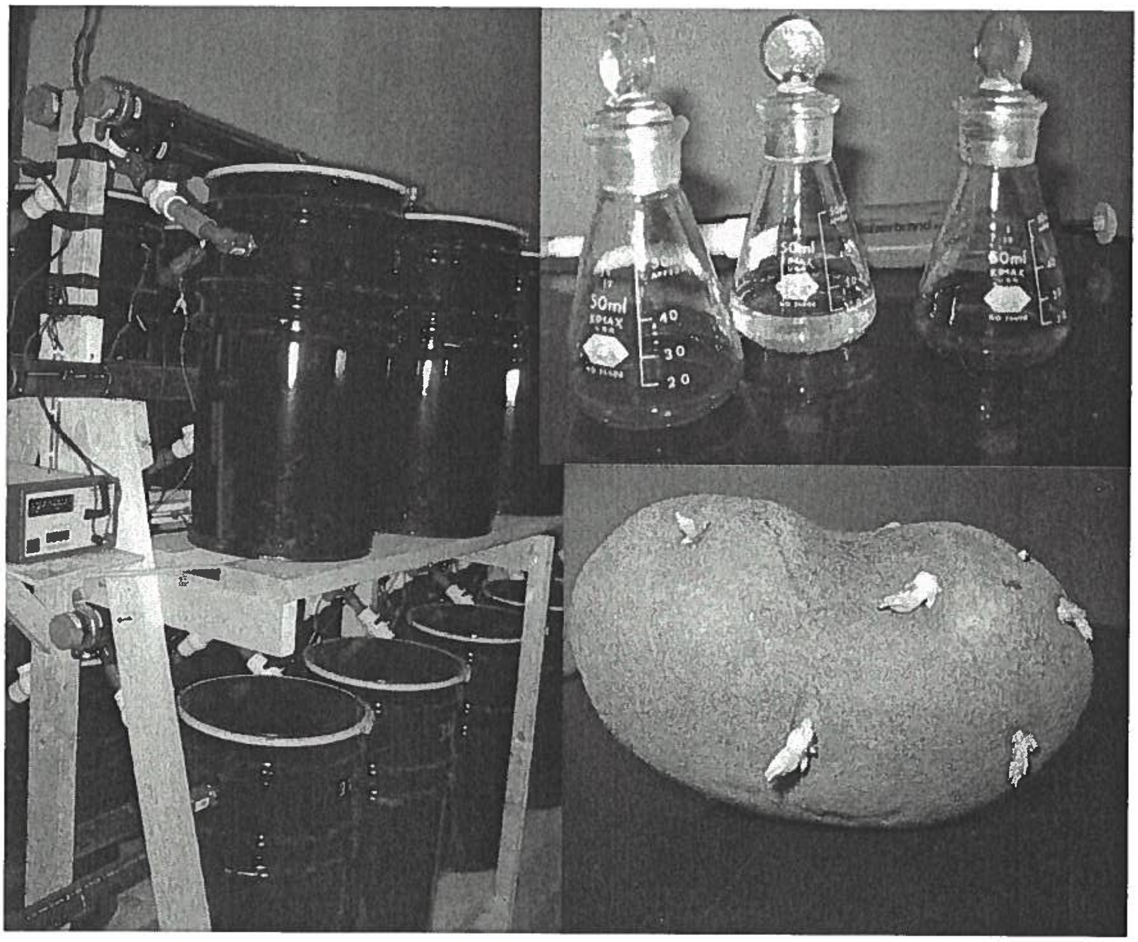
Activity Report: April 2007

File No.: FC#2006F155R

Chris Neeser[1*], Manjula Bandara[2], Michele Konschuh[2], Darcy Driedger[3]

[1]Alberta Agriculture and Food/Pest Management Systems Branch
[2]Alberta Agriculture and Food/Food Crop Development Branch
[3]Alberta Agriculture and Food/Food Processing Development

*Project leader; Tel.: 403 362-1331, email: chris.neeeser@gov.ab.ca



Summary

This project is on schedule and on budget, although several changes have been made, but they are not expected to compromise the project deliverables in any way. The most significant change has been the delay in bringing on a technical assistant. The candidate of choice was not able to start as originally envisioned and the worker hired to act as a substitute resigned within six weeks. Consequently, the project leader decided to assign a permanent staff member to this project until May, when the assistant hired in a part time capacity would be able to start work full time. This decision resulted in a larger than budgeted balance for the 2006/2007 fiscal year. Results show that all essential oils tested suppress sprouting, but the effect was not as good with clove oil, presumably because it vaporizes to slowly at the 8 C storage temperature. We also found that the response surface methodology provides the necessary data with fewer experimental units, therefore reduces the cost of the project. Finally the preliminary results obtained from Piccolo tubers treated with clove oil, indicate that the application frequency has a much greater effect on sprout inhibition than the dose at which the product is applied. This is encouraging, because it suggests that by increasing the application frequency the same effect could be achieved with less product, therefore reducing the cost.

Project background

Sprout control is an essential component of the successful management of stored potatoes. The current industry standard for sprout control is chloropropham (commonly known as CIPC). In recent years a number of countries including the US, have lowered allowable residue limits of CIPC. Since minimum residue levels vary from country to country, they are a concern for Alberta growers, who produce mostly for export markets. A number of compounds present in essential oils have been shown to inhibit sprouting in potatoes, and could be used as alternatives to CIPC. Because many of these essential oils do have "Generally Recognized as Save" (GRAS) status and are commonly used as flavor ingredients in the food industry, they are less likely to be subject to trade restrictions. However, their sprout inhibitory activity is usually reversible; therefore repeated applications are necessary to achieve the desired effect. With this project we intend to establish whether it would be technically and economically feasible to use essential oils produced in Alberta (dill and mint oil) for sprout control in stored potatoes. For the purpose of comparison a clove oil product, currently marketed in the United States, is also included.

Alberta Agriculture and Food

Project progress and budget

The project has progressed as expected and activities are on schedule (see project schedule p. 10). At this time we foresee no problems with completing the project on time. The project budget balance ($27,132) on March 31, 2007, was larger than originally estimated (Table 1). This occurred because the hiring of the technical assistant was delayed (Xin Song, a M.Sc. student at the University of Saskatchewan, will start full time on May 1, 2007), and the substitute resigned after six weeks. Because another suitable replacement wasn't available, a permanent staff member, together with the project leader provided most of the labor. This was done at the expense of less time sensitive projects. In this way we were able to push back labor costs by several months. However, as of May 1st, the amount budgeted under "Salaries & Wages" (as shown in Table 2.) will be needed for the technical assistant who will begin to work full time on this project.

Table 1. Summary of cash revenue and expenditures for the first year (April 2006 to March 2007).

Revenues	
Agriculture & Food Council	34,125.00
Potato Growers of Alberta	2,000.00
Organic Agriculture Centre of Canada	5,000.00
Little Potato Company, Inc	2,000.00
Alberta Agriculture and Food	2,500.00
Total	**45,625.00**
Expenditures	
GST on funding from industry	-244.05
Salaries & wages	-6,552.77
Employer contributions	-760.13
Materials & supplies	-9,279.55
Other services (training, software licencse)	-1,656.18
Tot. Expenditures	**-18,492.68**
Balance	**27,132.32**

Alberta Agriculture and Food

Table 2. Budget forecast to complete the remainder of this project (April 2007 to November 2008)

Expected Revenues	
Balance forwarded (04/06-03/07)	27,132.32
Agriculture & Food Council	30,450.00
Potato Growers of Alberta	2,000.00
Organic Agriculture Centre of Canada	5,000.00
Little Potato Company, Inc.	2,000.00
Alberta Agriculture and Food	2,500.00
Total	**69,082.32**

Projected Expenditures	
GST on funding from industry	-540.00
Salaries & wages	-54,000.00
Employer contributions	-6,264.00
Other services (training, software licencse)	-3,500.00
Materials & supplies	-3,000.00
Publication, travel & allowances	-1,500.00
Tot. Expenditures	**-68,804.00**
Balance	**278.32**

Research objectives

Based on discussions with industry stakeholders we defined the following research objectives:

1. Compare the ability of three essential oils (peppermint oil, dill oil, and clove oil) to suppress sprouting in stored potatoes;
2. Determine optimal application rates and frequencies;
3. Estimate treatment costs based on optimal application rates and frequencies;
4. Establish recommendation for the use of essential oils (peppermint and dill oil) as sprouting inhibitors in potatoes.

Deliverables

1. A response surface analysis of the effect of application rate and frequency on sprout inhibition for each of the essential oils and potato cultivars.
2. Comparison of application costs between CIPC (industry standard) and essential oils.
3. An interim report (April 30, 2007) and a complete final report (Nov. 30, 2008).
4. A summary report and a short article for publication (Agriculture and Food Council, Alberta Agriculture and Food, Organic Agriculture Centre of Canada, Potato Growers of Alberta)
5. An oral presentation of results at an appropriate public meeting (Potato Growers of Alberta).
6. A peer reviewed publication in an appropriate scientific journal.

Research protocol

In order to generate the data needed to achieve the proposed objectives in an efficient manner, we proposed to develop a response surface model (first year) and validate the model predictions in a scaled up storage trial during the second year of this project. The response surface approach to experimentation is particularly well suited to determine optimal levels in problems involving two or more variables (Myers and Montgomery, 2002), as is the case here, since we need to determine the best rate and application frequency.

Determining the response surfaces

For the purpose of this project we will primarily consider the following two response variables: 1. sprout weight measured in grams, and 2. duration of sprout suppression measured in weeks. We expect these values to be strongly influenced by the dose and frequency of exposure to the essential oils being tested. Furthermore, we expect that the response variables will be located on a plane that can be described by a polynomial (quadaratic) function of dose and application frequency.

Experimental design

The treatments consist of peppermint oil, dill oil and clove oil, each of which are applied to the Piccolo and Russet Norkotah potato cultivars using the following nine dose by frequency combinations: 13 mg/L (available air space) every 17 days; 25 mg/L every 10 and 24 days; 55 mg/L every 7, 17 and 27 days; 85 mg/L every 10 and 24 days; and 97mg/L every 17 days. The principal components of these essential oils are: carvone in dill oil, eugenol in clove oil, and 1-8-cineole, limonene and pulegone in peppermint oil.

Treatment chambers

The apparatus for this experiment consists of 48 steel drums, which are connected to ventilation hoses equipped with valves (Figure 1). Each of these drums has a capacity of 63 L. To insure uniform distribution of the essential oil vapors within these containers, the air is circulated through a vertically placed pipe with a 12 V fan attached on the top end. The essential oils are placed on filter paper suspended in front of the fan (Figure 2). Once the oil has been applied to the filter paper, the drums remain sealed for four days to allow sufficient time for complete evaporation of the essential oils. The valves are then reopened to insure good air exchange, which is provided by a ventilator (Figure 3) and conforms to recommendations for commercial storages (2.5 to 5.0 L/min./kg of potatoes). The entire apparatus is contained within a storage chamber that maintains the temperature at 8 °C and the relative humidity at 85 %. The exhaust from the containers is evacuated to the outside to avoid cross-contamination.

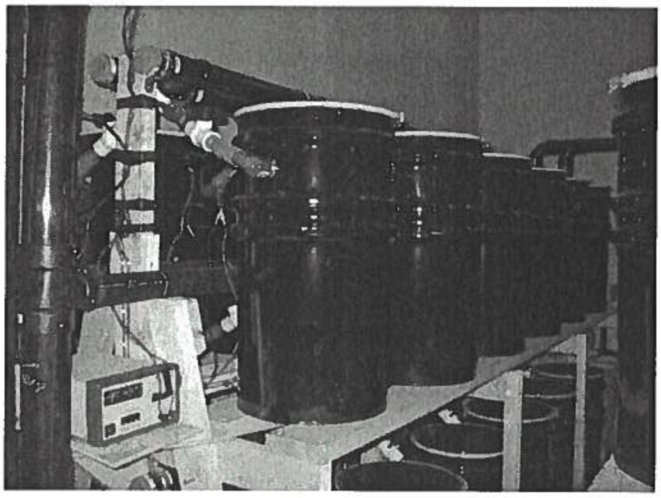


Figure 1. General view of the setup of the treatment chambers showing the ventilation system, the adjustable voltage power supply, and some of the 63 liter containers.

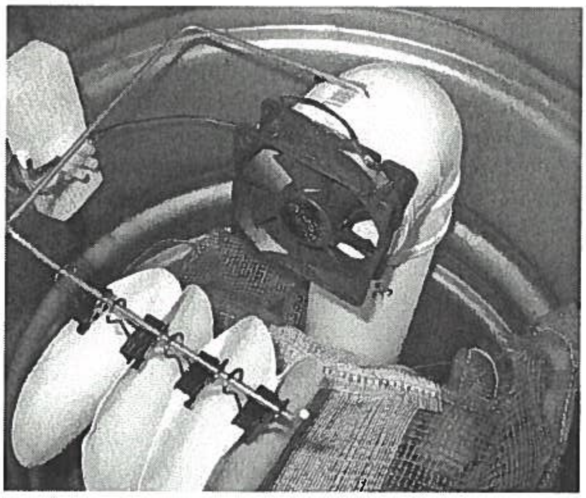


Figure 2. The evaporation system consists of suspended filter paper and a 12 V fan that circulates the air from the top to the bottom of the container. With this system the entire air volume is circulated approximately four times per minute.

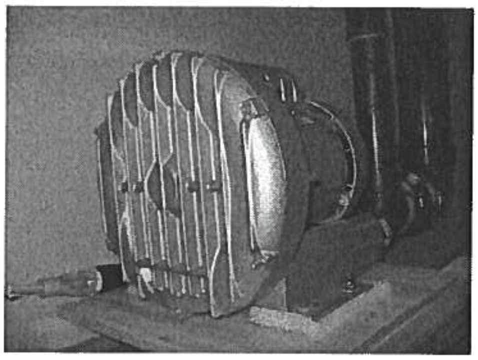


Figure 3. The GAST R6 blower used to supply fresh air to the containers. This low pressure blower supplies approximately 80 L/min. to each container.

Measurements

The essential oil treatments were initiated on December 6th and observations began the following week. Weekly observations are made on whole bags (the experimental unit) to determine the duration of the sprout inhibitory effect for the different treatments and to assess any disease problems. A bag is marked as "sprouted", once at least 50 % of the tubers in the bag have one or more visible sprout.

When all the bags for a particular set of treatments (same cultivar and essential oil) have sprouted, the set in question will be removed from the treatment containers, and the fresh weight of the sprouts (removed from tubers) and the tubers will be measured. Delaying weighing until all bags in a given set have sprouted, is necessary to insure that there are useful data points (≠ 0) for all treatment combinations. Otherwise, the reliability of inferences from the response surface would be diminished. To date, complete sprouting has only occurred in the set of treatments involving the Piccolo cultivar treated with clove oil. The results obtained in this case will be discussed below. Complete sprouting of the five remaining treatment sets (Piccolo/mint oil, Piccolo/dill oil, Norkotah/clove oil, Norkotah/dill oil, Norkotah/mint oil) is expected to take place within the next 12 weeks.

As indicated earlier, we still need to determine to what extent the target concentration of the different essential oils inside the containers were achieved. This will be done, using head space sampling protocols and gas chromatography analysis.

Finally samples will be submitted for sensory analysis to determine to what extent essential oil residues present on treated potatoes can be perceived by human olfactory sense.

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Scaled up testing

In the fall of 2007 the second phase of this project will be initiated. Here the purpose is to test whether optimal treatment combinations (dose by application frequency), determined on the basis of the response surface analysis, will give satisfactory results when applied to potatoes stored in standard storage bins (500-800 kg). The essential oils will be applied with a handheld fogger, suitable for larger storages. A key question is to determine whether the products will achieve sufficient penetration to have a uniform effect throughout the storage container, and whether the duration of sprout suppression will be consistent from the response surface model.

Measurements will include weekly sprouting and disease assessments and final sprout numbers and weight. The trial will be conducted in four chambers measuring 25 m^3 at the controlled storage facility of the Crop Diversification Centre South. The essential oil treatments will consist of concentrations and application frequencies that are expected to be effective on the basis of the response surface analysis obtained in the first phase of this project. Furthermore, the selected optimal treatments must also be economically realistic, or else the dose or application frequency will have to be adjusted. This portion of the study will be conducted on the same potato cultivars (Piccolo and Russet Norkotah).

Issues and adjustments

We found that clove oil is slow to evaporate at the 8 °C storage temperature. As a result, we didn't achieve the target vapor concentrations within the treatment chambers. Presumably this is also the reason why sprout suppression with clove oil was less than with the other two products. To resolve this issue, we designed an application chamber with a low wattage infrared heat emitter that will increase the temperature of the evaporation surface, but with only a minimal effect on the temperature of the container as a whole. The system will be tested shortly. However, we will need to repeat the set of treatments for clove oil, which we plan to do next fall.

Finally, we also eliminated the chloropropham (CIPC) treatment, because if correctly applied it will permanently inhibit sprouting, hence no useful data would be produced.

References

Montgomery, D. C. 2005. Design and Analysis of Experiments. 6th ed., Chapt. 11, John Wiley and Sons, Inc. New York, 642 pp.
Myers, R.H. and Montgomery, D.C. 2002. Response Surface Methodology: Process and Product Optimization Using Designed Experiments, 2nd ed., John Wiley and Sons, Inc. New York, 798 pp.
Vaughn, S.F. and Spencer, G.F. 1991. Volatile monoterpenes inhibit potato sprouting. American Potato Journal, 68:821-831.
Sorce, C., Lorenzi, R., Parisi, B.and Ranalli, P. 2005. Physiological Mechanisms involved in potato tuber dormancy and the control of sprouting by chemical suppressants. Acta Hort. 684:177-184.

Preliminary results

To date only the Piccolo tubers treated with clove oil have sprouted completely, and therefore are the only ones for which we do have a response surface at this time (Fig. 4). However, as mentioned above, the duration of sprout suppression, even at the highest dose and frequency, was less than expected due to poor evaporation of this oil. Nevertheless the data provided an excellent fit to the linear response surface model (adjusted r^2 = 0.8 and model parameters are highly significant). The graph shows that the time interval between application (frequency) has a stronger effect than the dose. Indeed the slope for time interval is 1.51 compared to -0.32 for the dose. Therefore the application frequency is the more sensitive variable. This is suggests that it may be possible to reduce the amount of product needed by increasing the application frequency.

For most of the remaining treatments sprouting is expected to start soon and measurements should be completed by July. The gas-chromatography work, which will confirm the calculated vapor concentrations, will also be conducted during this period.

The results obtained to date are very encouraging. They suggest that the response surface methodology is indeed an efficient design for this type of research. The more commonly used randomized complete block design with four replicates would have required 36 more experimental units, which would have increased the cost by at least 50%.

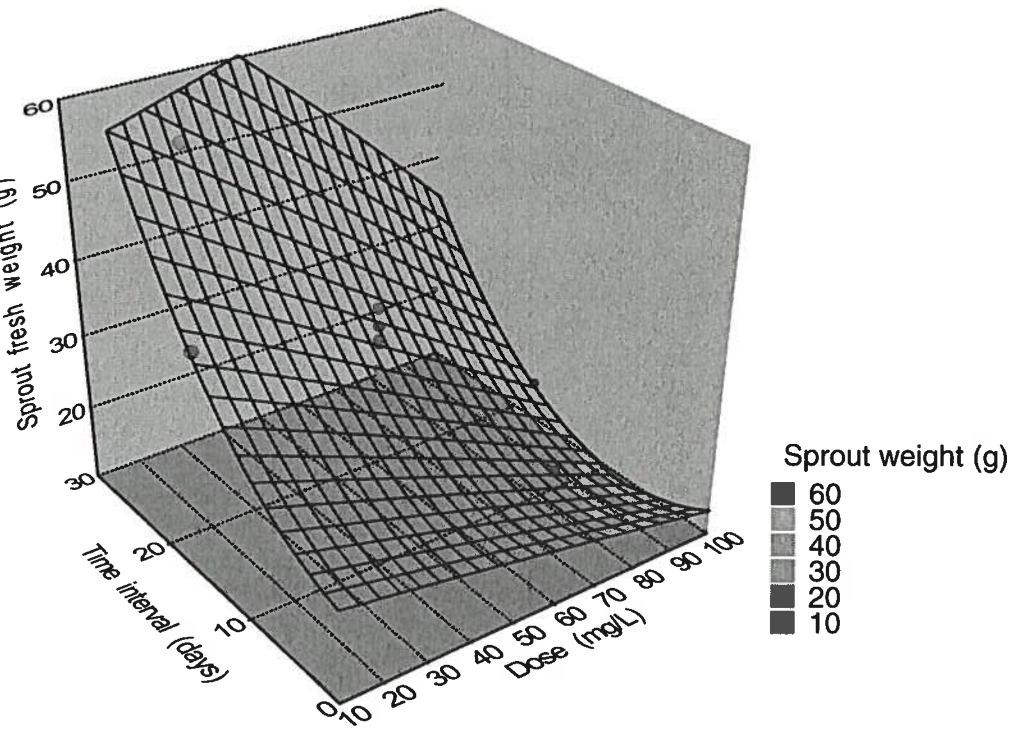
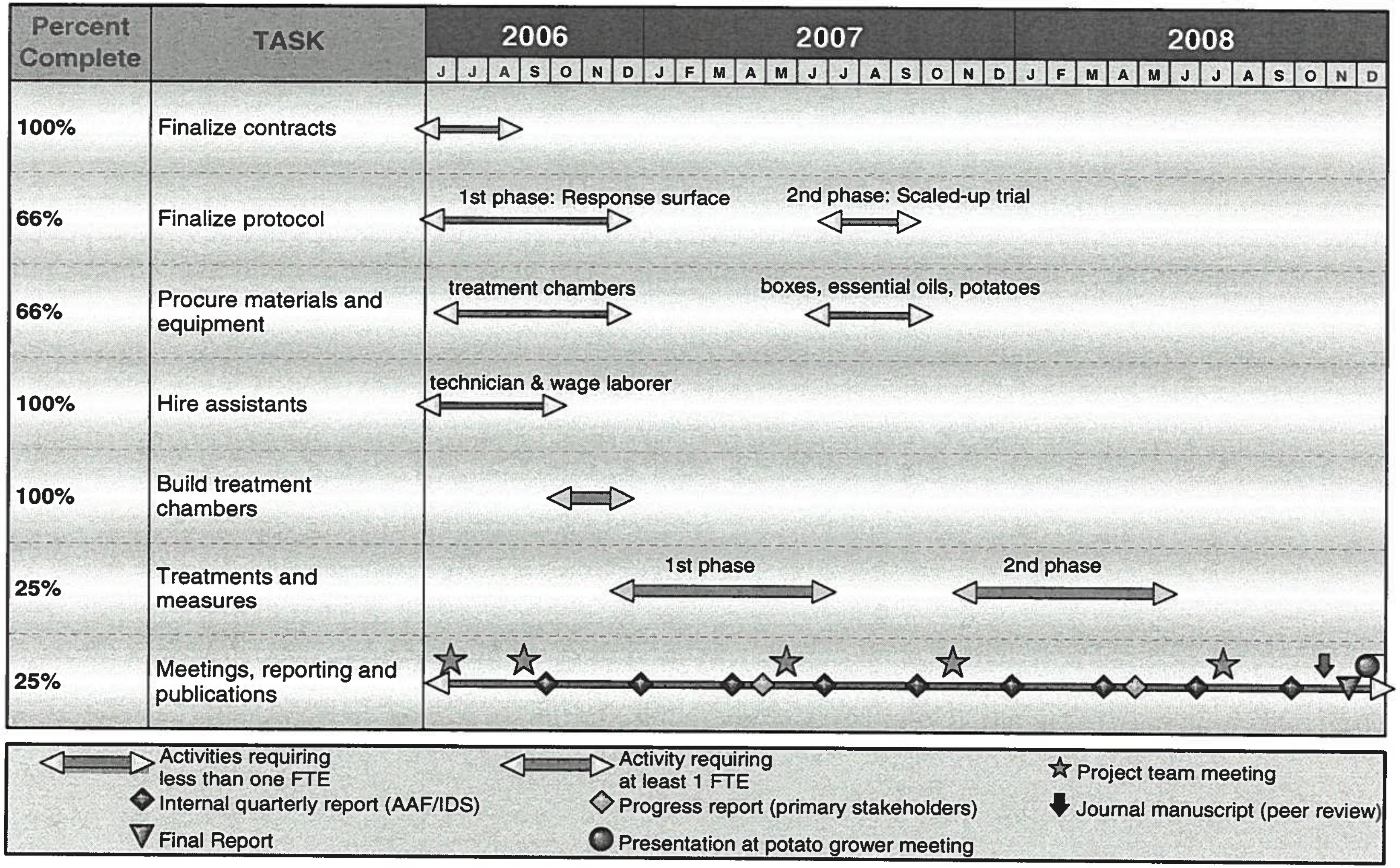


Figure 4. The effect of clove oil applications on fresh weight of sprouts produced on stored potato tubers of the cultivar Piccolo 9 weeks after initiation of the treatments. The adjusted r^2 for the fitted linear model is 0.89, the model F-ratio is 29.4 ($P<0.001$), the model constant is 21.9, the coefficient for interval is 1.51 and the coefficient for dose is –0.32. All model parameters are highly significant ($P<0.01$).

Alberta Agriculture and Food

Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes

Project Schedule 2006-2008



POTATO GROWERS OF ALBERTA

6008, 46th Avenue
Taber, Alberta T1G 2B1

Phone (403) 223-2262
Fax (403) 223-2268
e-mail: pga@albertapotatoes.ca
www.albertapotatoes.ca

April 20, 2007

Mr. Chris Nesser
Alberta Agriculture, Food & Rural Development
225 - SS #4
Brooks, AB T1R 1E6

Re: Use of Essential Oils as Sprout Inhibitors for Stored Potatoes

Dear Chris:

We are pleased to advise that the Board of Directors of The Potato Growers of Alberta has reviewed and approved continuing funding for your research project.

For the period of April 1, 2007 – March 31, 2008, the amount of $2,000 is available to meet the timelines specified in your application. When requesting the funds for the project, please provide an invoice that specifies the amount, GST and to whom payable.

We appreciate your commitment and dedication to the potato industry.

Yours truly,

Vern Warkentin
Executive Director

/pl

Potato Growers of Alberta
Research Tracking

Title of Research Application: Dev. of recommendations for use of essential oils as spr[o]
inhibitors for stored potatoes

Name of Researcher: Chris Neeser

Employer: Ab Agriculture, Food and Rural Dev.

Date application was received by PGA ____________________

Date application was reviewed by PGA April 4, 2006

A) approved ✓ B) declined ________

Project start date: June 1, 2006 Project finish date: ____________________

Total amount requested:$ 2000 - Amount requested per year: $2,000 -

MOU received and signed. Once copy returned to research agency, one copy filed in current year Research Binder

Date completed June 15/06

Invoice received: # 62720061 Date funds advanced July 6/06 Cheque# 4349 $2000 ✓

Invoice received:# ____________ Date funds advanced ____________ Cheque# ____________

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Were reports received from the researcher? ____________________

What was done with the reports?

Presented at PGA meeting? ________ Put on PGA website? ________ Filed? ________

NOTES: ____________________

POTATO GROWERS OF ALBERTA

6008, 46th Avenue
Taber, Alberta T1G 2B1

Phone (403) 223-2262
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e-mail: pga@albertapotatoes.ca
www.albertapotatoes.ca

April 4, 2006

Mr. Chris Neeser
Fruit and Vegetable Scientist
Alberta Agriculture Food and Rural Development
Crop Diversification Centre (South)
S.S. #4
Brooks, AB T1R 1E6

Re: "Development of recommendations for the use of essential oils as sprouting inhibitors for stored potatoes."

Dear Chris:

We are pleased to advise that the Board of Directors of the Potato Growers of Alberta has reviewed and approved your research funding application.

The funding will be accessible for a two year period in the amount requested of $4,000. For the period of April 1, 2006 – March 31, 2007 the amount of $2,000 is available to meet the timelines specified in your application. When requesting the funds for the project, please provide an invoice that specifies the amount, GST and to whom payable.

We appreciate your commitment and dedication to the potato industry.

Yours truly,

Vern Warkentin
Executive Director

Project # **819123**

New ✓ Renewal

Memorandum of Understanding

Between:

Potato Growers of Alberta
(hereafter referred to as "PGA")

and

Alberta Agriculture, Food & Rural Development
Crop Development-Food Branch
(hereafter referred to as "AAFRD/CDFB")

Project Title: Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes

Objectives:

1. Compare the performance of essential oils (caraway, clove, and peppermint) as an alternative to CIPC, which is the current industry standard.
2. Determine optimal application rates and frequencies.
3. Estimate treatment costs based on optimal application rates and frequencies.
4. Establish recommendation for the use of essential oils as sprouting inhibitors in potatoes.
5. Reduce current storage losses of organically grown potatoes and improve supply to the organic potato market.

Statement of Work

AAFRD/CDFB will conduct the study described in the attached proposal. As one of the co-sponsors, the PGA agrees to pay to AAFRD/CDFB a portion of the costs of conducting this study.

Period of Work

The first phase of this project, which is covered by this memorandum of understanding, will commence on June 1, 2006 and will be completed by June 3, 2007. A progress report will be provided no later than September 30, 2007.

Basis of Payment

The PGA will pay $2,000.00 to AAFRD/CDFB upon finalization of this memorandum to contribute towards the cost of casual manpower and materials pertaining to the first phase of this project. Payment is expected in the form of a cheque that must be made payable to "**Minister of Finance**" and sent directly to the project manager.

Note: If requested, AAFRD/CDFB will provide a record of revenue and expenditures upon project completion.

Responsibilities of Project Manager

- The project manager, Dr. Christoph Neeser, will provide the PGA with a technical report detailing the results obtained during the first year of this trial.

- The project manager will authorize expenses and submit them to the appropriate AAFRD/CDCS officer for processing and payment.
- The project manager is not eligible for manpower funds for himself.

Amendments or Termination

This Memorandum of Understanding may be amended by mutual consent of the parties as evidenced by an exchange of letters. Either the PGA or AAFRD/CDFB may terminate this Memorandum of Understanding by providing two weeks notice in writing to the other party.

Notices and Representatives

Notices for all purposes of or incidental to this Memorandum of Understanding shall be effectively given if delivered personally, or sent by registered or certified mail to the representatives of the parties designated as follows:

PGA	**AAFRD**
Vern Warkentin Executive Director, PGA 6008, 46th Ave. Taber, AB T1G 2B1	Henry Najda, Acting Branch Head Crop Development-Food Branch CDC South, 301 Horticultural Station Road E Brooks, AB T1R 1E6

Information generated from this project may be used by AAFRD and all co-sponsors. The co-sponsors agree that the research results may be published in a recognized scientific journal and/or in the form of an academic thesis.
As a co-sponsor, the PGA, relinquishes ownership of any materials, supplies and assets purchased with the project funds to AAFRD, which assigns control to the project manager's departmental division.

The parties affirm their acceptance of the terms of this Memorandum of Understanding by signing below. Copies bearing original signatures of this Memorandum will be kept by each party.

Signature: Date: June 15, 2006

Dr Christoph Neeser
Project Manager, Crop Development-Food Branch

Signature: Date: June 15, 2006

Henry Najda,
Branch Head, Crop Development-Food Branch

Signature: Date:

Vern Warkentin
Executive Director, PGA

Alberta
AGRICULTURE, FOOD AND
RURAL DEVELOPMENT

Crop Development-Food Branch

301 Horticultural Station Road E.
Brooks, Alberta
Canada T1R 1E6

Main Switchboard: 403/362-1300
Phone: 403/362-1302
Fax: 403/362-1306

May 29, 2006

RECEIVED JUN 0 1 2006

Mr. Vern Warkentin
Executive Director
Potato Growers Association
6008, 46th Ave.
Taber, Alberta T1G 2B1

Dear Vern,

Enclosed are two original Memorandum of Understandings for your consideration. If acceptable, please sign and date each of the two documents.

Please return **both documents** to my attention in the self-addressed envelope provided.

Upon finalization of these documents, I will send a signed original for your records.

If you have any questions, please call me at (403) 362-1302.

Thank you!

Yours truly,

Anna Moeller, Centre Administrator

/alm

Returned June 1/06

Project # **819123**

New ✓ Renewal

COPY

Memorandum of Understanding

Between:

Potato Growers of Alberta
(hereafter referred to as "PGA")

and

Alberta Agriculture, Food & Rural Development
Crop Development-Food Branch
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Signature: Date:

Dr Christoph Neeser
Project Manager, Crop Development-Food Branch

Signature: Date:

Henry Najda,
Branch Head, Crop Development-Food Branch

Signature: Date:

Vern Warkentin
Executive Director, PGA

Agriculture Funding Consortium

Application for Research Funding – 2006/2007

Pre- Proposal

Project Number: 2006F155R
Date Received: 10/1/2005

Title: Development of Recommendations for the Use of Essential Oils as Sprouting Inihibitors for Stored Potatoes

Confidentiality: yes
Primary Funding Agency: Agriculture Funding Consortium

Duration: 2 years beginning 6/1/2006
Focus Area: Agri-Food & Health - Functional Foods and Nutraceuticals

Crop Sector: Horticulture
Signatory Endorsement: Yes

Research Continuum: Applied Research
Project Specifics: Stand-Alone Project

Overall Purpose:

Sprout control is an essential component of the successful management of stored potatoes. The current industry standard for sprout control is chloropropham (commonly known as CIPC). In recent years a number of countries including the US, have lowered allowable residue limits of CIPC. Since minimum residue levels vary from country to country, they are a concern for Alberta growers, who produce mostly for export markets. A number of compounds present in essential oils have been shown to inhibit sprouting in potatoes, and could be used as alternatives to CIPC, which would be free of trade restrictions. Many of these essential oils do have "Generally Recognized as Save" (GRAS) status and are commonly used as flavour ingredients in the food industry. Their sprout inhibitory activity is usually reversible, therefore repeated applications will be necessary. The ability to inhibit sprouting for limited periods would be useful to growers of seed potatoes, who cannot use CIPC. It would reduce their storage losses and improve quality. Finally, essential oils would enable organic growers to substantially increase their ability to store potatoes.

If proven effective and economically viable, essential oils may provide an alternative to CIPC. Therefore the overall purpose of this project is to:

- Compare the performance of essential oils (caraway, clove oil as Biox-C^1, and peppermint) as an alternative to CIPC, the current industry standard;

· Determine optimal application rates and frequencies;
· Estimate treatment costs based on optimal application rates and frequencies;
· Establish recommendation for the use of essential oils as sprouting inhibitors in potatoes;
·Reduce current storage losses of organically grown potatoes and improve supply to the organic potato market.
^1 Biox-C is a commercial formulation of clove oil currently marketed in the US for sprout control in potatoes

Key Objectives / Deliverables:

Objective

· To determine the optimal application rate and frequency of essential oils from caraway, clove (Biox-C), and peppermint to minimize sprouting in two potato cultivars.

Deliverables

·A response surface analysis of the effect of application rate and frequency on sprout inhibition for each of the essential oils and potato cultivars

·Comparison of efficacy and application costs between CIPC (industry standard) and essential oils

·An interim report (Aug. 2007) and a complete final report (Nov. 2008)

·A factsheet with recommendation for use of essential oils as sprouting inhibitors in stored potatoes (distributed AAFRD/Ropin' The Web and the Organic Agriculture Centre of Canada (www.organicagcentre.ca)

·Oral presentation of results at the appropriate PGA meeting

·A peer reviewed publication in an appropriate scientific journal

Potential Benefits to Alberta:

Alberta has a very significant and successful potato industry. In order to meet changing consumer expectations Alberta potato growers need to continue to be innovative and pro-active. Reducing the use of chemicals, which may be considered unhealthy, is part of this

strategy. If proven effective essential oils may provide an alternative to CIPC, which is subject to minimum residue level restrictions in most export markets. Substitution of CIPC with any of the essential oils considered for this study, could therefore lead to new export opportunities for Alberta potatoes.
The non-permanent sprout inhibition effect of essential oils would make them useful for potato seed growers, who at this time have very limited sprout control options. Furthermore, the ability to control sprouting with materials that are acceptable under organic productions standards, will allow organic producers to better take advantage of the growing organic market, which is expected to continue to grow a rate of 12%.

This project is expected to generate the following benefits for Alberta:

·Contribute to further enhance Alberta's image as an environmentally conscious; producer of high quality potatoes that are among the world's healthiest and best tasting.
·Contribute to increase sales to European and Asian markets where CIPC minimum residue levels are set very low;
·Provide sprout control options for producers of seed potatoes;
· Improve profitability of organic potato production by 25-50 % through reduced storage loss and a longer supply window;
·Enable suppliers of gourmet potatoes (e.g. Little Potato Co.) to contract more locally grown potatoes;
· The ability to control sprouting with organically acceptable products is a key component toward the growth of an organic potato industry in Alberta, which could represent several million dollars in farm gate value within 3-5 years;
·The chemical sprout inhibitor CIPC is used on at least 650,000 t of potatoes each year. Replacement of this product with an essential oil of low toxicity could represent a significant opportunity for the Alberta essential oil industry (e.g. 50,000-80,000 L of mint oil);
·A cost benefit ratio for Canada of 0.003, calculated as the cost of the project ($130,000) divided by the value of a 5% increase in Canadian exports of potato products ($800 million/yr * 0.05 = $40 million/yr).

Research Methodology:

In order to generate the data needed to establish a reliable recommendation we will develop a response surface model and validate the model predictions in a scaled up storage trial during the second year of this project. The response surface approach to experimentation is particularly well suited to determine optimal levels in problems involving two or more variables (Box and Draper, 1987), as is the case here, since we need to determine the best rate and frequency of application.
The response surface data will be generated using a completely randomized rotatable design. The experimental unit will consist of 5 kg of potatoes in a nylon meshbag. The treatments will be applied by means of custom-made plexiglass chambers (50 L) with

perforated double floors to allow a constant and uniform airflow corresponding to commercial specifications. Airflow will be adjusted by appropriate valves on each chamber and monitored with a precision air flow meter. The temperature and relative humidity of the air intake will be adjusted to match typical storage conditions. The essential oils (dill, peppermint, and eucalyptus) will be added in gaseous form to the air intake of each chamber by passing a portion of the air through a flask whose temperature can be adjusted to control the evaporation rate to achieve the desired concentrations in the chambers, which will be monitored with a gas chromatograph following procedures described in Vaughn and Spencer 1991. There will be 9 treatment combinations consisting of three concentrations (ranging between 0.5 and 5 mg/L) of the main active compounds (carvone in caraway oil, eugenol in eucalyptus oil, and xxx in peppermint oil) multiplied by three application frequencies (continuous, bi-weekly, and monthly). There will also be a control (no treatment) and a treatment with CIPC. These treatments (11) will be applied to two potato cultivars and replicated 4 times. The experiment will be conducted over 8 months. Measurements will include: 1. monthly weight loss, 2.monthly sprouting assessments, 3. monthly disease ratings, and 4. final sprout numbers, length, and weight, and 5. a sensory evaluation with a taste panel.
The scaled up validation of the predicted optimum application frequency and concentration of the most effective essential oil will be conducted in 4 chambers measuring 25 m3 at the controlled storage facility at CDCS. The treatments will consist of concentrations that are intermediate between the ones chosen for the response surface calibration; they will be chosen to provide a complete coverage of the response surface with least amount of treatments. The experiment will be conducted on the same two varieties of potatoes contained in four 1.5 m3 wooden bins, as are typically used for storage of table potatoes. Measurements will be as described for the previous experiment.

References

Box, G. E. P. and Draper, N. R. (1987). Empirical Model-Building and Response Surfaces. John Wiley & Sons, New York, NY.
Vaughn, S.F. and Spencer, G.F. 1991. Volatile monoterpenes inhibit potato sprouting. American Potato Journal, 68:821-831.
Sorce, C., Lorenzi, R., Parisi, B.and Ranalli, P. 2005. Physiological Mechanisms involved in potato tuber dormancy and the control of sprouting by chemical suppressants. Acta Hort. 684:177-184.

Research / Development in Progress:
As far as we can tell from searching the litterature (Agricola, CAB, Highwire), reviewing major databases of listings of ongoing research projects [ICAR (Canada), CRIS (USA), ELDIS (Germany), ARAMIS (Switzerland), AGRITROP (France) CARIS (FAO)] and consulting with Canadian researchers who work on potato production and post-harvest management, there are no studies currently underway that are aimed at developing recommendations for the use of essential oils as sprouting inhibitors in potatoes.

However, this is not to say that the effect of essential oils on sprout inhibition has not been investigated. One of the co-applicants, Dr. Bandara, collaborated on a study in the mid-90's at the University of Saskatchewan (unpublished), in which carvone from dill oil

was used as a sprouting inhibitor. The present proposal draws on experience gained from this work.
The following is a list of publications that we consulted in preparation of this proposal:

Beveridge, J.L., Dalziel, J. and Duncan, H.J. 1983. Headspace analysis of laboratory samples of potato tubers treated with 1,4-dimethylnaphthalene, carvone, pulegone and citral. J. Sci. Food Agri. 34:164-168.

Frazier, M.J., Olsen, N. and Kleinkopf, G. 2004. Organic and alternative methods for potato sprout control in storage. CIS1120 University of Idaho Extension

Hartmans, K.J., Diepenhorst, P., Bakker, W. and Gorris, L.G.M. 1995. The use of carvone in agriculture: sprout suppression of potatoes and antifungal activity against potato tuber and other plant diseases. Ind. Crop. Prod. 4:3-13.

Meigh, D.F. 1969. Suppression of sprouting in stored potatoes by volatile organic compounds. J. Sci. Food Agri. 20:159-164.

Oosterhaven, K., Hartmans, K.J. and Scheffer, J.J.C. 1995. Inhibition of potato sprout growth by carvone enantiomers and their bioconversion in sprouts. Pot. Res. 38:219-230.

Vaughn, S.F. and Spencer, G.F. 1991. Volatile monoterpenes inhibit potato sprouting. American Potato Journal, 68:821-831.

The choice of essential oils and the range of concentrations is based on information from the above publications.

Project Funding:

Funds Requested	Year 1:	$ 34,000
	Year 2	$ 32,000
	Year 3	$ 0
	Year 4	$ 0
	Year 5	$ 0
Total Requested for Project		**$ 66,000**

Source	Cash./.In Kind	Year	Confirmed	Amount
Org. Agr. Centre of Can.	cash	1	No	$ 5,000
PGA	cash	1	No	$ 2,000
AAFRD/CDCS	in-kind	1	Yes	$ 25,460
Org. Agr. Centre of Can	cash	2	No	$ 5,000
PGA	cash	2	No	$ 2,000

Total Other Sources **$ 39,460**

Dr. Manjula Bandara	AAFRD/CDCS	Extensive experience with essential oils and has conducted previous work on sprout inhibition in potatoes
Dr. Michele Konschuh	AAFRD/CDCS	Potato research specialist
Dr. Darcy Driedger	AAFRD/CDCS	Expertiese in analytical methods of monoterpenes (essential oils), post-harvest technologies

Total Project Costs **$ 105,460**

Team Leader

Dr. Chris Neeser
AAFRD
SS1
Brooks, AB T1R 1E6
Phone:(403) 362-1331
Fax:(403) 362-1326
e-mail: chris.neeser@gov.ab.ca

Past Experience:
I have conducted a wide variety of agronomic research projects including several on post-harvest management of fruits and vegetables. I have extensive experience in data analysis, especially in relating environmental variables to physiological processes in plants. Recently I completed a project on herbs that involved extraction and analysis of essential oils. April 2002 – present: Fruit & Vegetable Scientist, AAFRDMay 2000 – March 2002: Weed Scientist, AAFRDMay 1997 – April 2000: Research Associate (Weed Ecology), University of Nebraska

Credentials:
1997, Ph. D. (Agricultural Production and Agroecosystems), University of Guelph 1992, M. Sc. (Plant Science), McGill University1990, B. Sc. (Agriculture, Major in Botany), McGill University

Publications / Patents
6 refereed paper, 6 conference proceedings, 3 other relevant citations

Evidence of Productivity:
Currently funded projects:- Agri-Food Council # 2005F083R, Storage Performance of Field-Crowned Processing Carrots - ACIDF #2003 A077R: Irrigation management of black currants and saskatoons (responsible for generating plant growth, yield and fruit quality data); AAFRD/New Initiatives Fund: -Evaluation of garlic, oregano, basil and parsley cultivars suitable for dehydration; Alberta Farm Fresh Producers Association: - High Tunnel Production of Strawberries – Evaluation of new raspberry and strawberry cultivars; Alberta Vegetable Growers Processing: - Control of hairy nightshade in processing peas, - carrot storage factsheet, yield and quality characterization of juicing carrot cultivars; Alberta Hort Congress Foundation: Post-harvest treatments to extent shelf-life of saskatoons; NSERC/Olds College: Evaluation of antioxidants in black currants and saskatoons, Determination of salinity tolerance in black currants.

Team Members

Name	Institution	Expertise

AFC Research Full Proposal 2006-07

ProGrid®Summary Report

Opportunity No: 2006F155R
Category: Crop Sciences
Proponent: Dr. Chris Neeser
Organization: AAFRD/CDCS
Opportunity Title: Essential Oils as Sprouting Inhibitors for Stored Potatoes

Date Received: 10/1/2005
Funding Cons.: $64,575
Other $$: $73,150
Date Start: 5/1/2006
Duration: 2 Years

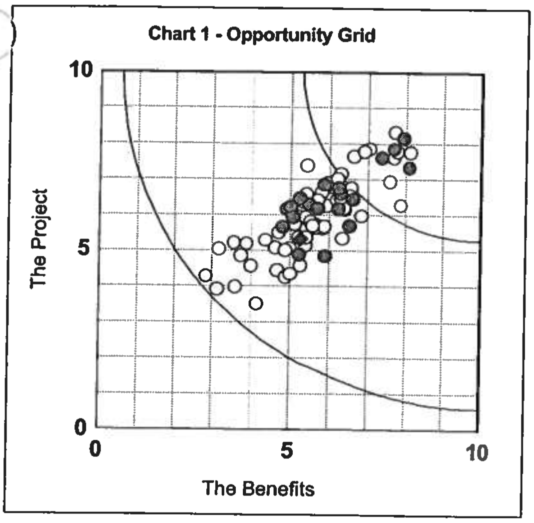
Summary:

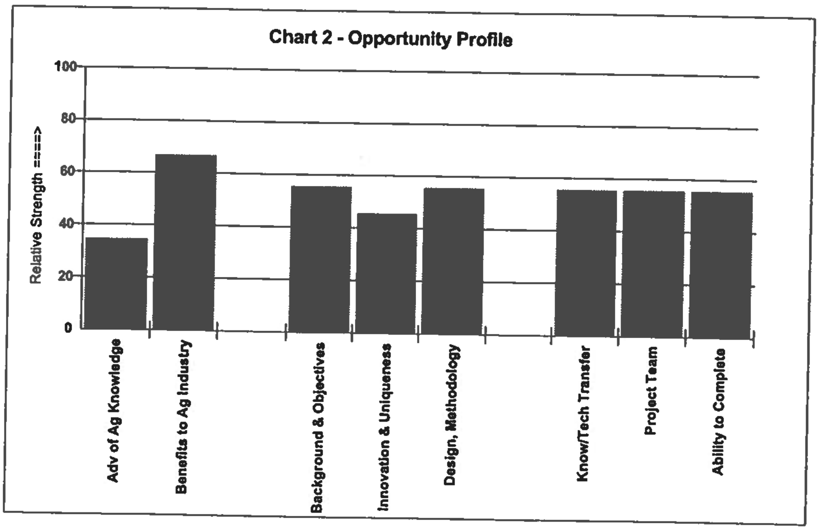
The Opportunity was evaluated according to the following Performance Criteria by: 3 Evaluators and a Committee Consensus evaluation.

The Benefits	The Project	The Connectors
Adv of Ag Knowledge	Background & Objectives	Know/Tech Transfer
Benefits to Ag Industry	Innovation & Uniqueness	Project Team
	Design, Methodology	Ability to Complete

Chart 1 - Opportunity Grid shows the grid position for the Evaluator's average (diamond) and the other Opportunities in the database (open circles) and those in the same category. (gray circles).

Chart 2 - Opportunity Profile shows the Reviewer Average ratings for each of the performance criteria. This chart is useful in identifying strengths and weaknesses.





Comments on the Opportunity:

Innovative proposal with huge potential for export market and organic market. Modest budget for benefits. Good research design. Economics vs normal practices need to be addressed. What are current maximum levels for residue?

Recommendation: Fund Conditional

Agriculture & Food Council
The Catalyst

Agriculture & Food Council
#402, 1101 - 5 Street
Nisku, Alberta T9E 7N3
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E-mail: info@agfoodcouncil.com
Web site: www.agfoodcouncil.com

March 1, 2006

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COPY

Dr. Chris Neeser
Fruit and Vegetable Scientist
Alberta Agriculture, Food and Rural Development
Crop Diversification Centre South
S.S 1
Brooks, AB T1R 1E6

Dear Dr. Neeser:

Re: Proposal #2006F155R
Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes

Thank you for your full proposal submission. The review of the 2006/07 full research proposals received by the Alberta Agriculture Research Funding Consortium[1] is now complete. Your proposal was evaluated by external (International) and internal (National) reviewers, and based on their feedback, the Funding Consortium members' Boards of Directors made their funding decisions.

The enclosed ProGrid Applicant Report provides some information about how your proposal ranked against other proposals received as well as some specific feedback from the research review process.

Your proposal has been approved in principle for a maximum of $64,575.00 over 2 years by the Agriculture & Food Council. The Agriculture & Food Council also requests clarification on the budget items listed as overhead and whether or not these are project specific costs.

The Agriculture & Food Council will be the Project Manager for this project. Please direct all correspondence in relation to this project to Tricia Huot, Project Officer, at (780) 955-3714, extension 229.

Please send a letter stating your acceptance of this funding offer to Agriculture & Food Council. We will send you an Investment Agreement that you and your organization will need to sign prior to the advancement of any funds for this project. All conditions as outlined above, including the signature of the investment agreement must be completed within 90 days of the date of offer.

Please note: Any projects for which any of the above conditions have not been met by May 31st, will NOT receive funding.

Thank you again for submitting a quality application. We look forward to working with you on this project. Congratulations on your success.

Sincerely,

Tom Switzer
Executive Director
CARD/ACAAF
Agriculture & Food Council

cc: Tricia Huot, Agri-Industry Development Officer, Agriculture & Food Council
Alfonso Parra, Potato Growers of Alberta

[1] Alberta Livestock Industry Development Fund, Alberta Agricultural Research Institute, Alberta Crop Industry Development Fund, Diversified Livestock Fund of Alberta, AVAC Ltd., Agriculture and Food Council. Climate Change Central, Alberta Barley Commission, Alberta Pork, Alberta Chicken Producers, Alberta Canola Producers Commission, Alberta Pulse Growers Commission, Alberta Egg Producers, Alberta Milk, Potato Growers of Alberta

AFC Research Full Proposal 2006-07

ProGrid® Summary Report

Opportunity No:	2006F155R	**Date Received:**	10/1/2005
Category:	Crop Sciences	**Funding Cons.:**	$64,575
Proponent:	Dr. Chris Neeser	**Other $$:**	$73,150
Organization:	AAFRD/CDCS	**Date Start:**	5/1/2006
Opportunity Title:	Essential Oils as Sprouting Inhibitors for Stored Potatoes	**Duration:**	2 Years

Summary:

The Opportunity was evaluated according to the following Performance Criteria by: 3 Evaluators and a Committee Consensus evaluation.

The Benefits	The Project	The Connectors
Adv of Ag Knowledge	Background & Objectives	Know/Tech Transfer
Benefits to Ag Industry	Innovation & Uniqueness	Project Team
	Design, Methodology	Ability to Complete

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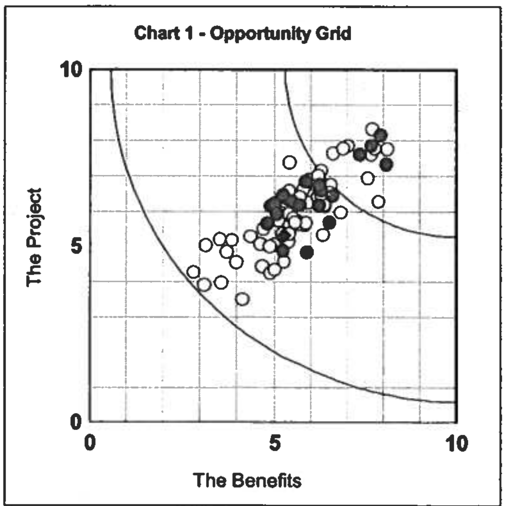

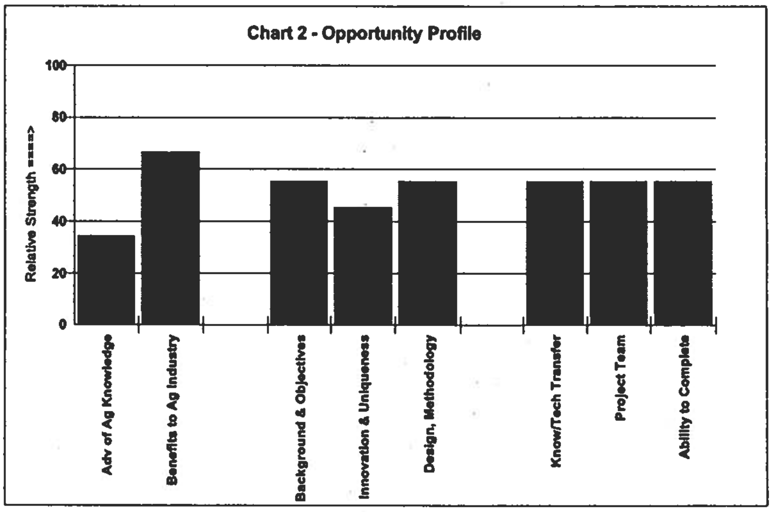

Comments on the Opportunity:

Innovative proposal with huge potential for export market and organic market. Modest budget for benefits. Good research design. Economics vs normal practices need to be addressed. What are current maximum levels for residue?

Recommendation: Fund Conditional

Project # 87925-819232

MEMORANDUM OF AGREEMENT

Between:

Potato Growers of Alberta

(Hereafter referred to as "**PGA**")

and

Her Majesty, the Queen, in right of the Province of Alberta
as represented by the
Minister of Agriculture and Food

(Hereafter referred to as "**AF**")

Project Title: Post-Harvest Management of Silver Scurf and Fusarium Dry Rot of Potatoes in Storage with Azoxystrobin and Fludioxinil

Objectives:
1. Collect potato tissue samples infected with silver scurf or dry rot from NB, AB and PEI, and isolate and identify pathogens.
2. Assess the efficacy of azoxystrobin (Quadris), fludioxinil (Scholar), and thiabendazole (Mertect), alone and in combination, against different isolates of the silver scurf and dry rot pathogens.
3. Conduct potato storage trials assessing the efficacy of azoxystrobin, fludioxinil and thiabendazole applied post harvest for the control of silver scurf and dry rot.
4. Present findings at industry and scientific meetings.

SCOPE OF WORK

1. **AF** will conduct the Research Project according to the research plan which is attached to and forms part of this Agreement.

PERIOD OF WORK

2. This Agreement will commence on September 1, 2007 and will terminate on March 31, 2008 unless extended upon agreement of both parties.

BASIS OF COSTS and PAYMENT

3. The total expense for this Research Project is $5,300 to cover the following estimated total costs:

Technologist (halftime position at $2000/month for 2.0 months)	$ 4,000
Materials and supplies	$ 1,000
GST	300
Total Cost	$ 5,300

4. **PGA** will provide to **AF**, upon execution by both parties of this Agreement, the sum of $5,300.

Cheques shall be made payable to **"Minister of Finance"** and forwarded to:

Mrs. Joan Seath
Alberta Agriculture and Food
Crop Diversification Centre North
17507 Fort Road N.W.
Edmonton AB T5Y 6H3
Phone: (780) 422-0653

5. **AF** will use the funds paid by **PGA** only for the purpose of conducting the Research Project. **AF** will provide a record of revenues and expenditures to **PGA** upon completion of the Research Project or depletion of funds.

RESPONSIBILITY OF PROJECT MANAGER

6. The project manager for this Research Project is Dr. Ron Howard of **AF** who will supervise the Research Project and provide all reports to **PGA.** The project manager will authorize expenses and submit them to the appropriate **AF** office for payment to be processed.

AMENDMENTS OR TERMINATION

7. This Agreement may only be amended upon mutual consent of the parties and evidenced in writing.

8. Either **AF** or **PGA** may terminate this Agreement in the event of a material default or breach of a substantive term, condition or provision of this Agreement, by providing two weeks notice in writing to the other party. In such event **AF** is in default then any and all amounts of the funds advanced by **PGA** hereunder that represent payment for work or services hereunder that have not been performed by **AF** up to the date of termination shall be refunded to **PGA.**

NOTICES AND REPRESENTATIVES

9. Notices for all purposes of or incidental to this Agreement shall be effectively given if delivered personally, or sent by registered or certified mail to the representatives of the parties designated as follows:

PGA Biosciences:
Mr. Vern Warkentin
Executive Director
Potato Growers of Alberta
6008 – 46th Avenue
Taber, AB T1G 2B1
Phone: 403-223-2262

Alberta Agriculture and Food:
Dr. Ron Howard
Plant Pathology Research Scientist
Crop Diversification Centre South
301 Horticultural Station Road East
Brooks, Alberta T1R 1E6
Phone : (403) 362-1328

Alberta Agriculture and Food:

Cornelia Kreplin, Director, Agriculture Research Division

20070925
Date

Potato Growers of Alberta:

Vern Warkentin, Executive Director, Potato Growers of Alberta

Sept 13/07
Date

Research Plan

The research plan for this project was briefly described in the original project proposal submitted to the PGA in 2007 (see attached copy).

Project Code: MUR07-010

Post-harvest management of silver scurf and Fusarium dry rot of potatoes in storage with azoxystrobin and fludioxinil.

2007-04-01 to 2009-03-31

Collaborators

Dr. Khalil Al-Mughrabi, Ph.D., New Brunswick Dept. of Agriculture, Fisheries and Aquaculture, NB
Role: Supervise and conduct research trials in NB; submit disease samples for testing
Dr. Ron Howard, Ph.D., Alberta Agriculture, Food and Rural Development, AB
Role: Supervise and conduct research trials in AB; submit disease samples for testing
Kelvin Lynch, New Brunswick Dept. of Agriculture, Fisheries and Aquaculture, NB
Role: Facilitate communication among project partners to optimize studies for an URMUR
Rachael Cheverie, PEI Department of Agriculture, Fisheries, and Aquaculture, PE
Role: Facilitate communication among project partners to optimize studies for an URMUR
Paul MacPhail, PEI Dept. of Agriculture, Fisheries, and Aquaculture, PE
Role: Submit disease samples for testing
Harold Wright, Syngenta Crop Protection Canada, Inc., ON
Role: Registrant; provide products and trial validation
Chris Dunbar, FoodTrust of PEI, PE
Role: Project support; submit disease samples for testing
Grower Groups: PEI Potato Board; Potatoes New Brunswick; Potato Growers of Alberta
Role: Project support

Statement of problem and brief literature review

With the increasing demand for high quality, blemish-free potatoes, storage diseases have increased in significance. One of the most chronic diseases of potatoes in storage is silver scurf, caused by *Helminthosporium solani*. Silver scurf causes metallic, silvery patches on tuber skins, which can reduce their suitability for direct sales and processing. Seed growers are also concerned about silver scurf because the pathogen can be easily spread on seed tubers. Silver scurf has been identified by stakeholders as a priority disease for which there are currently inadequate control options. Similarly, Fusarium dry rot, caused by *Fusarium* spp., results in seed tuber decay and losses in storage.

Growers have traditionally managed these diseases, at least in part, with the application of thiabendazole to tubers entering storage. However, resistance to thiabendazole in isolates of *F. sambucinum* was recorded in Europe (Hide *et al.* 1992) and became widespread in the United States (Desjardins *et al.* 1993; Hanson *et al.* 1996) and Canada (Kawchuk *et al.* 1994; Peters *et al.* 2001; Platt 1997) in the 1990s. In addition, thiabendazole-resistant strains of *H. solani* have also become commonplace (Platt 1997).

In recent years, no thiabendazole sensitive isolates of *F. sambucinum* have been recovered from stored potatoes with tuber dry rot in the Atlantic region of Canada, and thiabendazole-resistant strains of *H. solani* also predominate in this region (R.D. Peters; unpublished data). Conversely, populations of other *Fusarium* spp. causing potato tuber dry rot, including *F. coeruleum*, *F. avenaceum*, and *F. graminearum*, have generally been found to be sensitive to thiabendazole (Ali *et al.* 2005; Hide *et al.* 1992; Kawchuk *et al.* 1994; Platt 1997). No other options for post-harvest disease management have proven to be sufficiently efficacious. At present, two post-harvest treatments (Mertect and OxiDate) are registered in Canada for controlling silver scurf. Despite the availability of these products, silver scurf remains a widespread and serious problem. In recent years, the application of azoxystrobin at low rates to tubers entering storage has been found to be provide excellent control of silver scurf (personal communication, Jeff Miller, University of Idaho). Residue levels have also been found to be acceptable, and registration of this application will soon occur in the USA.

Ali, S., V.V. Rivera, and G.A. Secor. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis. 89: 105.

Desjardins, A.E., E.A. Christ-Harned, S.P. McCormick, and G.A. Secor. 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83: 164-170.

Hanson, L.E., S.J. Schwager, and R. Loria. 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86: 378-384.

Hide, G.A., P.J. Read, and S.M. Hall. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected with dry rot. Plant Pathol. 41: 745-748.

Kawchuk, L.M., J.D. Holley, D.R. Lynch, and R.M. Clear. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Amer. Potato J. 71: 185-192.

Peters, R.D., I.K. Macdonald, K.A. MacIsaac, and S. Woodworth. 2001. First report of thiabendazole-resistant isolates of *Fusarium sambucinum* infecting stored potatoes in Nova Scotia, Canada. Plant Dis. 85: 1030.

Platt, H.W. 1997. Resistance to thiabendazole in *Fusarium* species and *Helminthosporium solani* in potato tubers treated commercially in eastern Canada. Phytoprotection 78: 1-10.

Contribution of project to solving the problem

Canada produces approximately 170,000 ha of potatoes with over 70% of production in PEI, NB, Manitoba and Alberta. Much of this crop is stored for varying periods of time. This project will enhance the management of an identified priority crop (potatoes) and disease (silver scurf). The information on pesticide resistance generated will prevent the unnecessary use of pesticides, which makes no economic or environmental sense. The proposed study will supply data to support the registration of a combined azoxystrobin/fludioxinil product in Canada, should it be shown to be efficacious in the management of silver scurf and/or dry rot. This supports Canadian priorities since azoxystrobin and fludioxinil are identified as a reduced risk products and fludioxinil provides the integration of a resistance management strategy. As well, other more hazardous programs (to humans and the environment), such as ozone application via ventilation systems, should be reduced.

The objectives of our study are two-fold:
A. A survey will be undertaken in each year of the study whereby pathogens are isolated from infected tubers from PE, NB, and AB and tested for their sensitivity to fungicides.
B. Storage trials will be carried out in PE, NB, and AB to ascertain the efficacy of thiabendazole, azoxystrobin, fludioxinil and combinations thereof for control of silver scurf and dry rot.

The expected results of this work will provide data for minor use registration of a combined azoxystrobin/fludioxinil post-harvest product in Canada. Fludioxinil is included due to its observed efficacy and as part of a resistance management plan for azoxystrobin. Growers will benefit by obtaining a tool for post-harvest disease management that is badly needed. Reducing the use of an ineffective product (thiabendazole) and providing reduced risk products (azoxystrobin/fludioxinil) will also reduce environmental risk.

Methods and Experimental Design

Pathogen surveys
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Tuber disease studies
Recognizing that significant risk reduction occurs when producers have access to low risk chemicals, this project will be structured so that the data can be used to support an URMUR for azoxystrobin/fludioxinil should it prove to be effective in the management of silver scurf and/or dry rot. The registrant of this product has indicated their willingness to support an URMUR in Canada and project collaborators have been chosen who will concurrently pursue that objective. Prior to the 2007 season, a pre-submission consultation with PMRA will be requested to ensure that trial protocols will meet anticipated PMRA DACO requirements for efficacy and tolerance data.

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A source of naturally-infested tubers will be used for the studies. Tubers will be treated according to the treatment list outlined below and then stored at 10°C and 95% RH for 3-5 months. Following storage, tubers will be rated for disease incidence (number of diseased tubers) and severity (% tuber surface diseased).

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AZO = azoxystrobin 2.08 SC; FDL = fludioxonil 230 SC

All data will be statistically analyzed using standard techniques prior to presentation of data in reports or articles.

Resources

The human resources required include the experts documented in the list of collaborators (section 8). These individuals will support project planning and oversee trials in the three provinces. In addition, due to the heavy work requirements in fall/winter, a graduate student is required. Laboratory facilities at AAFC Charlottetown will be used for the isolation, storage and characterization of plant pathogens and to generate inoculum for tuber storage studies. In addition, laboratories of the New Brunswick Dept. of Agriculture, Fisheries and Aquaculture and Alberta Agriculture, Food and Rural Development will also be used to generate pathogen inoculum. A source of tubers for tuber disease studies will be generated in each province and climate-controlled potato storage facilities will also be utilized in each province. Chemicals used in the studies will be supplied by Syngenta.

Schedule and Milestones

Summer/Fall 2007
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-interim report

Winter/Spring 2008

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Presentations of findings

In addition to written outputs (reports, factsheets, papers, abstracts) various presentation options will be pursued including grower meetings across Canada (typically in winter) and scientific meetings (typically in summer) specializing in potato research (such as the Potato Association of America annual meetings).

Method of measurement of pesticide risk reduction

The project will enhance the management of an identified priority crop (potatoes). When pesticide resistance occurs, products are often applied with no net control effect, which makes no economic or environmental sense. The information on pesticide resistance generated in this study will prevent the unnecessary use of pesticides.

While the scope of this project does not include measuring pesticide reduction, significant risk reduction will occur with the registration of an effective azoxystrobin/fludioxinil product for post-harvest use on potatoes and the identification of thiabendazole resistant production areas. Ineffective thiabendazole use will be reduced. Azoxystrobin/fludioxinil could be used as a rotational product with thiabendazole to potentially extend the shelf-life of the latter chemical. The addition of fludioxinil to azoxystrobin as a combined product is part of a resistance management strategy for azoxoystrobin. Both azoxystrobin and fludioxinil are identified as a reduced risk products. Monitoring total sales of the azoxystrobin/fludioxinil product would give a good indication of grower uptake and risk reduction.

Growers participating in the resistance survey will be informed of their resistance risk. Summaries of the resistance survey will also be conveyed to producers through on-going extension and research reporting activities. Tuber disease has been identified as an issue by the national potato risk reduction group and Dr. Al-Mughrabi is the New Brunswick representative in that group which will aid in dissemination of project results.

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Dr. Khalil Al-Mughrabi, Ph.D., New Brunswick Dept. of Agriculture, Fisheries and Aquaculture, NB
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In-kind contribution: time devoted to facilitate communication among project partners to optimize studies for an URMUR
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In-kind contribution: time devoted to collection of diseased samples

Harold Wright, Syngenta Crop Protection Canada, Inc., ON
In-kind contribution: supply all (100%) of the crop protection products required for the trials; time devoted in consultation for development of experimental designs
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Budget

		Fiscal Year 2007/2008
Labour		$18,000.00
Supplies		$5,000.00
Transportation		$3,000.00
Other (specify)	storage trials in NB	$10,000.00
Other (specifiy)	storage trials in AB	$10,000.00
Total		$46,000.00

Justification for Equipment Purchase (>$2,000.00 value)

Not applicable.

Project Code: MUR07-010

Post-harvest management of silver scurf and Fusarium dry rot of potatoes in storage with azoxystrobin and fludioxinil.

2007-04-01 to 2009-03-31

Collaborators

Dr. Khalil Al-Mughrabi, Ph.D., New Brunswick Dept. of Agriculture, Fisheries and Aquaculture, NB
Role: Supervise and conduct research trials in NB; submit disease samples for testing
Dr. Ron Howard, Ph.D., Alberta Agriculture, Food and Rural Development, AB
Role: Supervise and conduct research trials in AB; submit disease samples for testing
Kelvin Lynch, New Brunswick Dept. of Agriculture, Fisheries and Aquaculture, NB
Role: Facilitate communication among project partners to optimize studies for an URMUR
Rachael Cheverie, PEI Department of Agriculture, Fisheries, and Aquaculture, PE
Role: Facilitate communication among project partners to optimize studies for an URMUR
Paul MacPhail, PEI Dept. of Agriculture, Fisheries, and Aquaculture, PE
Role: Submit disease samples for testing
Harold Wright, Syngenta Crop Protection Canada, Inc., ON
Role: Registrant; provide products and trial validation
Chris Dunbar, FoodTrust of PEI, PE
Role: Project support; submit disease samples for testing
Grower Groups: PEI Potato Board; Potatoes New Brunswick; Potato Growers of Alberta
Role: Project support

Statement of problem and brief literature review

With the increasing demand for high quality, blemish-free potatoes, storage diseases have increased in significance. One of the most chronic diseases of potatoes in storage is silver scurf, caused by *Helminthosporium solani*. Silver scurf causes metallic, silvery patches on tuber skins, which can reduce their suitability for direct sales and processing. Seed growers are also concerned about silver scurf because the pathogen can be easily spread on seed tubers. Silver scurf has been identified by stakeholders as a priority disease for which there are currently inadequate control options. Similarly, Fusarium dry rot, caused by *Fusarium* spp., results in seed tuber decay and losses in storage.

Growers have traditionally managed these diseases, at least in part, with the application of thiabendazole to tubers entering storage. However, resistance to thiabendazole in isolates of *F. sambucinum* was recorded in Europe (Hide *et al.* 1992) and became widespread in the United States (Desjardins *et al.* 1993; Hanson *et al.* 1996) and Canada (Kawchuk *et al.* 1994; Peters *et al.* 2001; Platt 1997) in the 1990s. In addition, thiabendazole-resistant strains of *H. solani* have also become commonplace (Platt 1997).

In recent years, no thiabendazole sensitive isolates of *F. sambucinum* have been recovered from stored potatoes with tuber dry rot in the Atlantic region of Canada, and thiabendazole-resistant strains of *H. solani* also predominate in this region (R.D. Peters; unpublished data). Conversely, populations of other *Fusarium* spp. causing potato tuber dry rot, including *F. coeruleum*, *F. avenaceum*, and *F. graminearum*, have generally been found to be sensitive to thiabendazole (Ali *et al.* 2005; Hide *et al.* 1992; Kawchuk *et al.* 1994; Platt 1997). No other options for post-harvest disease management have proven to be sufficiently efficacious. At present, two post-harvest treatments (Mertect and OxiDate) are registered in Canada for controlling silver scurf. Despite the availability of these products, silver scurf remains a widespread and serious problem. In recent years, the application of azoxystrobin at low rates to tubers entering storage has been found to be provide excellent control of silver scurf (personal communication, Jeff Miller, University of Idaho). Residue levels have also been found to be acceptable, and registration of this application will soon occur in the USA.

Ali, S., V.V. Rivera, and G.A. Secor. 2005. First report of *Fusarium graminearum* causing dry rot of potato in North Dakota. Plant Dis. 89: 105.
Desjardins, A.E., E.A. Christ-Harned, S.P. McCormick, and G.A. Secor. 1993. Population structure and genetic analysis of field resistance to thiabendazole in *Gibberella pulicaris* from potato tubers. Phytopathology 83: 164-170.
Hanson, L.E., S.J. Schwager, and R. Loria. 1996. Sensitivity to thiabendazole in *Fusarium* species associated with dry rot of potato. Phytopathology 86: 378-384.
Hide, G.A., P.J. Read, and S.M. Hall. 1992. Resistance to thiabendazole in *Fusarium* species isolated from potato tubers affected with dry rot. Plant Pathol. 41: 745-748.
Kawchuk, L.M., J.D. Holley, D.R. Lynch, and R.M. Clear. 1994. Resistance to thiabendazole and thiophanate-methyl in Canadian isolates of *Fusarium sambucinum* and *Helminthosporium solani*. Amer. Potato J. 71: 185-192.
Peters, R.D., I.K. Macdonald, K.A. MacIsaac, and S. Woodworth. 2001. First report of thiabendazole-resistant isolates of *Fusarium sambucinum* infecting stored potatoes in Nova Scotia, Canada. Plant Dis. 85: 1030.
Platt, H.W. 1997. Resistance to thiabendazole in *Fusarium* species and *Helminthosporium solani* in potato tubers treated commercially in eastern Canada. Phytoprotection 78: 1-10.

Contribution of project to solving the problem

Canada produces approximately 170,000 ha of potatoes with over 70% of production in PEI, NB, Manitoba and Alberta. Much of this crop is stored for varying periods of time. This project will enhance the management of an identified priority crop (potatoes) and disease (silver scurf). The information on pesticide resistance generated will prevent the unnecessary use of pesticides, which makes no economic or environmental sense. The proposed study will supply data to support the registration of a combined azoxystrobin/fludioxinil product in Canada, should it be shown to be efficacious in the management of silver scurf and/or dry rot. This supports Canadian priorities since azoxystrobin and fludioxinil are identified as a reduced risk products and fludioxinil provides the integration of a resistance management strategy. As well, other more hazardous programs (to humans and the environment), such as ozone application via ventilation systems, should be reduced.

The objectives of our study are two-fold:
A. A survey will be undertaken in each year of the study whereby pathogens are isolated from infected tubers from PE, NB, and AB and tested for their sensitivity to fungicides.
B. Storage trials will be carried out in PE, NB, and AB to ascertain the efficacy of thiabendazole, azoxystrobin, fludioxinil and combinations thereof for control of silver scurf and dry rot.

The expected results of this work will provide data for minor use registration of a combined azoxystrobin/fludioxinil post-harvest product in Canada. Fludioxinil is included due to its observed efficacy and as part of a resistance management plan for azoxystrobin. Growers will benefit by obtaining a tool for post-harvest disease management that is badly needed. Reducing the use of an ineffective product (thiabendazole) and providing reduced risk products (azoxystrobin/fludioxinil) will also reduce environmental risk.

Methods and Experimental Design

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Other (specifiy)	storage trials in AB	$10,000.00
Total		$46,000.00

Justification for Equipment Purchase (>$2,000.00 value)

Not applicable.

POTATO GROWERS OF ALBERTA

6008, 46th Avenue
Taber, Alberta T1G 2B1

Phone (403) 223-2262
Fax (403) 223-2268
e-mail: pga@albertapotatoes.ca
www.albertapotatoes.ca

May 30, 2007

Dr. Ron Howard
Alberta Agriculture, Food & Rural Development
301 – Horticultural Station Rd. E.
Brooks, AB T1R 1E6

Re: Post-harvest management of silver scurf and Fusarium dry rot of potatoes in storage with azoxystrobin and fludioxinil.

Dear Ron:

We are pleased to advise that the Board of Directors of The Potato Growers of Alberta has reviewed and approved your research funding application.

The funding will be accessible for a one year period in the total amount requested of $5000. When requesting the funds for the project, please provide an invoice that specifies the amount, GST and to whom payable.

We appreciate your commitment and dedication to the potato industry.

Yours truly,

Vern Warkentin
Executive Director

/pl

POTATO GROWERS OF ALBERTA

International Year of the POTATO 2008

HIDDEN TREASURE

April 22, 2008

Dr. Ron Howard
Alberta Agriculture, Food & Rural Development
301 – Horticultural Station Rd. E.
Brooks, AB T1R 1E6

Re: Evaluation of Biocides and Disinfection Procedures for the Effective Sanitation of Potato Storages and Equipment

Dear Ron:

We are pleased to advise that the Board of Directors of The Potato Growers of Alberta has reviewed and approved your research funding application.

The funding will be accessible for a one year period in the amount requested of $5000 plus GST.

When submitting an invoice please indicate the net amount, the total GST, and to whom the invoice is payable.

We appreciate your commitment and dedication to the potato industry.

Yours truly,

Vern Warkentin
Executive Director

/pl

6008 - 46 Avenue, Taber, AB T1G 2B1 • Office 403.223.2262 • Fax 403.223.2268
pga@albertapotatoes.ca • www.albertapotatoes.ca

Alberta Agriculture and Rural Development

Agriculture Research Division

Room 201, JG O'Donoghue Building
7000 – 113 Street NW
Edmonton, Alberta, Canada T6H 5T6

March 19, 2009

Mr. Edzo Kok
Executive Director
Potato Growers of Alberta
6008 – 46 Avenue
Taber, AB T1G 2B1

Dear Mr ~~Kuhl~~ Kok

Please find attached, two original copies of the Memorandum of Agreement between the Potato Growers of Alberta and Alberta Agriculture and Rural Development for the "Effective Sanitation of Potato Storages and Equipment" project.

Please sign both documents and return one to me for processing.

Thank you,

Joanne Phillips
Branch Administrator
Pest Management Branch

cc: Ron Howard
~~Elaine Lacroix~~

Dept ID/Program 8160/87925
Project # 819219

MEMORANDUM OF AGREEMENT

Between:

Potato Growers of Alberta

(Here after referred to as "**PGA**")

and

Her Majesty, the Queen, in right of the Province of Alberta as represented by the Minister of Agriculture and Rural Development

(Hereafter referred to as "**ARD**")

PROJECT TITLE
Evaluation of Biocides and Disinfection Procedures for the Effective Sanitation of Potato Storages and Equipment

OBJECTIVES

- Identify disinfectants effective at destroying *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*), the causal agent of bacterial ring rot of potato.
- Determine the effectiveness of disinfectants, both registered products and newly identified products, on various potato storage and equipment surfaces, e.g. urethane, galvanized metal, painted sheet metal, steel, concrete, burlap, rubber, plastic, and plywood.
- Determine if the efficacy of disinfection is enhanced by the addition of foaming agents, adjuvants, etc.

SCOPE OF WORK

1. **ARD** will conduct the Research Project according to the research plan which is attached to and forms part of this Agreement. The information generated by this project will be made available to the public.

PERIOD OF WORK

2. This Agreement will commence on April 1, 2008 and will terminate on March 31, 2010 unless extended upon agreement of both parties.

BASIS OF COSTS and PAYMENT

3. The total expense for this Research Project is **$10,000** to cover the following estimated total costs:

Manpower	$ 8,000
Materials and Supplies	$ 2,000
GST (if applicable)	n/a
Total Cost	**$10,000**

4. **PGA** will provide to **ARD**, upon execution by both parties of this Agreement, the sum of **$5,000/annum** for this Research Project. A cheque shall be made payable to **"Minister of Finance"** and sent to:

Attention: Mrs. Joanne Phillips
Alberta Agriculture and Rural Development
Pest Surveillance Branch
J.G. O'Donoghue Building
7000 – 113th Street
Edmonton, AB T6H 5T6
Phone: (780) 427-2166; Fax: (780) 427-1057

5. **ARD** will use the funds paid by **PGA** only for the purpose of conducting the Research Project. **ARD** will, if requested, provide a record of revenue and expenditure to **PGA** upon completion of the Research Project or depletion of funds.

RESPONSIBILITY OF PROJECT MANAGER

6. The project manager for this Research Project is Dr. Ron Howard of **ARD** who will supervise the Research Project and provide all reports to **PGA.** The project manager will authorize expenses and submit them to the appropriate **ARD** office for payment to be processed.

AMENDMENTS OR TERMINATION

7. This Agreement may only be amended upon mutual consent of the parties and evidenced in writing.

8. Either **ARD** or **PGA** may terminate this Agreement in the event of a material default or breach of a substantive term, condition or provision of this Agreement, by providing two weeks notice in writing to the other party. In such event **ARD** is in default then any and all amounts of the funds advanced by **PGA** hereunder that represent payment for work or services hereunder that have not been performed by **ARD** up to the date of termination shall be refunded to **PGA.**

NOTICES AND REPRESENTATIVES

9. Notices for all purposes of or incidental to this Agreement shall be effectively given if delivered personally, or sent by registered or certified mail to the representatives of the parties designated as follows:

Potato Growers of Alberta:
Mr. Edzo Kok
Executive Director
Potato Growers of Alberta
6008-46th Avenue
Taber, Alberta T1G 2B1

Alberta Agriculture and Rural Development:
Dr. Ron Howard
Plant Pathology Research Scientist
Crop Diversification Centre South
301 Horticultural Station Road East
Brooks, Alberta T1R 1E6

Alberta Agriculture and Rural Development:

Mr. Paul Laflamme, Head, Pest Surveillance Branch

Feb-24, 09
Date

Potato Growers of Alberta:

Mr. Edzo Kok, Executive Director

April 7/09
Date

Research Plan

Evaluation of Biocides and Disinfection Procedures for the Effective Sanitation of Potato Storages and Equipment

Principal Investigator: Dr. Ron Howard, Alberta Agriculture and Food

Project Coordinator: Dr. Tracy Shinners-Carnelley, Manitoba Agriculture, Food, and Rural Initiatives & National BRR Working Group

Key Words: Potato; Bacterial Ring Rot (BRR); Biofilms; Disinfectants; Microbial Contamination; Disease Management; Storage and Equipment Sanitation

Background

Bacterial ring rot (BRR) of potato, caused by *Clavibacter michiganensis* subsp. *sepedonicus,* is a very serious disease and is prevented mainly by good sanitary practices. BRR can cause direct losses to the commercial potato industry due to tuber rot and reduced yield. There is a zero tolerance for BRR on seed potato farms, and as little as one infected tuber on a farm will result in complete decertification of the farm unit.

The economic impact on the potato industry due to BRR has the potential to be significant, and nationally concern is growing over the increased prevalence of this disease in the last few years. As this concern increases, the potato industry is questioning the efficacy of the currently accepted methods for disinfection for potato storages and equipment.

In addition it has recently been shown that the bacterial ring rot pathogen forms biofilms. Biofilms are communities of microorganisms that grow attached to surfaces and bound together by a slimy exopolymeric matrix. In a biofilm, microbial cells are protected from exposure to disinfectants and antibiotics by its slimy matrix, in addition to changes in gene expression and physiology, as compared to non-attached single cell (planktonic) microbial forms. As a consequence, biofilms can be up to 10,000 times more resistant to disinfectants than single celled populations. This may have a significant impact on the effectiveness of standard disinfection procedures, which are tested for efficacy against planktonic cells under conventional methodology.

In 2006, Manitoba, Agriculture, Food, and Rural Initiatives hosted a National BRR Forum. The meeting was attended by potato industry representatives; and provincial and federal government representatives from across the country. The need to identify additional disinfectants effective against BRR was rated as a top national priority.

Bacterial ring rot must be controlled and prevented from becoming established in the Canadian potato industry. This will only be accomplished by using the most effective biocide products and sanitation procedures available. At the present time, growers are most concerned about the risk of BRR, but good sanitary practices are an important management tool for other diseases such as Fusarium dry rot, silver scurf, late blight, soft rot, and blackleg.

Economic Impact of BRR

Potato is Canada's most important horticultural crop. In 2006, 3,667 farms produced 5.5 million tons of potatoes worth $926 million, one third of all vegetable cash receipts. Overall, the financial impact on the seed potato industry across Canada from 1999 to 2005 for the 4,930 hectares that was rejected for BRR was $36.5 million. The average cost per acre was $2,998 ranging from a high of $36,599/acre for nuclear stock to a low of $1,851/acre for Foundation class seed. On a provincial basis the impact was as follows:

- Alberta loss calculation of $13.7 million for 1,197 hectares rejected during 2005, 2003, 2001 and 2000 and an average/acre cost $4,648;
- Manitoba loss calculation of $12.1 million for 1,657 hectares rejected during 2005, 2004, 2003 and 2002 and an average/acre cost $3,088;
- Quebec loss calculation of $3 million for 595 hectares rejected during 2003, 2002, 2001, 2000 and 1999 and an average/acre cost $2,065;
- New Brunswick loss calculation of $4.8 million for 848 hectares rejected during 2004, 2003, 2002, 2000 and 1999 and an average/acre cost $2,314; and
- Prince Edward Island loss calculation of $2.3 million for 634 hectares rejected during 2005, 2004, 2002 and 2001 and an average/acre cost $1,440

(Source, Canadian Horticultural Council)

If effective disinfectants are not identified and used, the seed potato industry will continue to experience significant losses and reduced customer confidence both nationally, and internationally. This would have a significant impact on all provincial potato industries.

Because the projected benefits of this project will affect the national potato industry, funds are being requested from provincial seed potato industries and provincial research granting agencies.

Objectives

1. Identify disinfectants effective at destroying *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*), the causal agent of bacterial ring rot.
2. Determine the effectiveness of disinfectants, both registered products and newly identified products, on various potato storage and equipment surfaces, e.g. urethane, galvanized metal, painted sheet metal, steel, concrete, burlap, rubber, plastic, and plywood.
3. Determine if the efficacy of disinfection is enhanced by the addition of foaming agents, adjuvants, etc.

Outline of Proposed Research

Phase 1: Susceptibility of biofilm bacteria to commercial and experimental disinfectants

The MBEC™ plate device will be used to screen potential disinfectants for efficacy against Cms. Cms will be exposed to the disinfectants at varying concentrations and times, and any remaining bacteria will be recovered to determine survival after exposure. Potential new disinfectants (e.g. Virkon, electrolyzed and ozonated water, Chemprocide, Vantocil and Menno Florades) will be compared to standard agricultural disinfectants (e.g. sodium hypochlorite (bleach), sodium peroxide (SaniDate), and the quaternary ammonia product Ag Services General Storage Disinfectant, which is the only product currently registered by the Pest Management Regulatory Agency [PMRA]). Manufacturers of disinfectants for the agriculture and/or food processing industries in Canada and the U.S.A. will be contacted to see if they are willing to provide additional promising commercial and experimental products with potential for the disinfection of bacterial ring rot.

Phase 2: Susceptibility of planktonic (free-form) bacteria to disinfectants

It is also important that the free-cell form of Cms be evaluated for susceptibility to the previously mentioned disinfectants using established assay procedures. Recovery will also be determined. Results from Phase 1 and Phase 2 will be used to select a short list of effective products for advanced testing.

Phase 3: Effectiveness of selected disinfectants on Cms-contaminated surfaces (in situ and transferred biofilms)

Various surfaces representative of those found in potato storages and on equipment (e.g. urethane, galvanized metal, painted sheet metal, steel, rubber, burlap, plastic, concrete, and plywood) will be miniaturized into test coupons and placed in BRR cell suspensions in BEST™ plates to determine if the bacterium is capable of forming "in situ" biofilms on the test surfaces. These same surfaces will also be smeared with ooze from BRR-infected potato tubers ("transferred" biofilms). The ooze will be allowed to dry ("dry slime") on half of the test coupons and kept moist ("wet slime") on the other half. Coupons with "in situ" and "transferred" biofilms will be immersed in disinfectant solutions for various time periods to simulate exposures under slow and fast drying conditions. Bacterial recovery from the surfaces will be done to determine efficacy of the biocide applied to each type of BRR-infested coupon. A study will also be performed to evaluate the potential corrosiveness of each of the disinfectants on the test surfaces. This will be done by soaking coupons in the disinfectants and examining them for visual damage and potential weight loss.

Phase 4: Evaluation of selected disinfectants in potato storages
Two or three disinfectants will be selected from the previous phases for further evaluation commercial potato storages and on potato machinery and handling equipment. Research permits will be obtained from PMRA for any non-registered products that may be selected for evaluation. Disinfectants will be applied using a mobile sanitation unit to be specially constructed for the project, or with commercial cleaning and disinfecting equipment, if and when available. Trials will also be conducted to evaluate the effect of adding "enhancers" to the disinfecting solution that may help to increase the wetting or contact time of these products on vertical surfaces. Additives to be tested may include foaming agents, surfactants and buffers.

Timetable for Completion
Year 1: Initiate Phases 1, 2 and 3
Year 2: Complete Phases 1, 2, 3; Initiate and complete Phase 4

Suitability of Principal Investigator
Dr. Ron Howard (Alberta Agriculture and Rural development [ARD], Crop Diversification Centre South, Brooks, AB) leads an active research program involving the evaluation of registered and experimental disinfectants for use in the agriculture and food industries. In collaboration with the greenhouse, potato and vegetable industries in Alberta, and with research partners in government (Food Safety Division, ARD, Edmonton) and the private sector (Innovotech, Inc., Edmonton), his research has focused on managing biofilm diseases of plants, preventing microbial spoilage of fresh and stored produce, and addressing related food safety issues. These studies have used innovative experimental techniques, such as Innovotech's MBEC™ and BEST™ plate technologies, which are high-throughput methods for the rapid evaluation susceptibility of microbial growth to agricultural and industrial biocides.

With support from the Canadian Food Inspection Agency (CFIA), Dr. Howard is currently evaluating bleach, peroxide and quaternary ammonia for efficacy against bacterial ring rot (BRR) of potato with the aim of determining their effectiveness when applied to contaminated hard surface materials typical of those found in potato storages and on field and handling equipment. The findings from these studies should lead to improvements in the sanitation programs currently used by growers and processors. With additional support, these studies could be expanded to include testing additional disinfectants (commercial and experimental), examining cleaners, foaming agents, detergents and other types of products that could be used prior to or during disinfection to improve disinfectant efficacy, and evaluating and improving the performance of commercial equipment designed for cleaning and/or disinfecting contaminated surfaces. A possible outcome of this work would be to design and build a mobile cleaning and disinfection unit that could be taken to potato storages and handling facilities to do pilot-scale evaluations and demonstrations of leading-edge sanitation equipment and practices.

Research in Progress Which Relates to this Proposal

- Efficacy of Bacterial Ring Rot Disinfection (CFIA Project #C0602): 2008-09
  Principal Investigator: Dr. Ron Howard, Alberta Agriculture and Food, Brooks, AB
  Sponsor: Dr. Solke DeBoer, Canadian Food Inspection Agency, Charlottetown, PE

Budget		
PROJECT EXPENSES	**1st year $**	**2nd year $**
a) Personnel Costs*	60,000	110,000
b) Professional Fees (*accounting and audit, technical, promotional, administrative, etc.)*	1,000	3,000
c) Facility Rental (*office, lab, processing, etc.)*	0	10,000
d) Equipment (lease/purchase) **	10,000	10,000
e) Materials, Supplies and Incidentals	8,000	20,000
f) Travel Costs	2,000	8,000
g) Other (specify) Technology Transfer	2,000	4,000
TOTAL EXPENSES	$83,000	$165,000
* Name of Employee	No. months paid (Years 1 + 2)	Monthly Rate
TBA (Professional)*	14	$3,000
TBA (Technical)*	30	$4,000
*Includes salary, benefits and overtime		

** Equipment	Justification
Year 1 – Disinfectant application equipment	Small power washer, compressed air sprayer, wands, nozzles, gas cylinders
Year 2 – Disinfection application equipment	Mobile prototype sanitation unit (washer, sprayer, tanks, pumps). Construction costs to be shared with government and industry partners.

Potential Cash Funding Sources for the Project Term (2008-09 and 2009-10)

Association or Research Fund	*Amount per year ($)*	*Status*	*Duration of funding (years)*
Potato Development Committee of British Columbia	$3000	Confirmed	1
Potato Growers of Alberta	$5000	Confirmed	2
Alberta Crop Industry Development Fund	$30,000	Applied	2
Saskatchewan Agriculture & Food Agricultural Development Fund	$30,000	Confirmed	2
Seed Potato Growers Association of Manitoba	$5000	Confirmed	2
Agri-Food Research & Development Initiative (MB)	$30,000	Confirmed	2
Federation des Producteurs de Pommes de Terres du Quebec	$5000	Applied	2
Potatoes New Brunswick	$25,000	Confirmed	1
Prince Edward Island Potato Board	$5000	Applied	2

Additional Information is available by contacting:

Dr. Tracy Shinners-Carnelley
Phone (204) 745-5640
Email: tracy.shinners-carnelley@gov.mb.ca

Alberta Agriculture and Rural Development

Development of Recommendations for the Use of Essential Oils as Sprouting Inhibitors for Stored Potatoes

Final Report: March 2009

File No.: FC#2006F155R

Chris Neeser[1*], Xin Song[1], Manjula Bandara[2], Michele Konschuh[2], Darcy Driedger[3], and Karen Tanino[4]

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Table of Content

Executive Summary

The purpose of this research project was to determine whether dill weed oil and spearmint oil, both of which are produced in Alberta, could be used to manage sprouting in stored potatoes, as this is done with clove oil in the US. Experiments were set up to determine optimal application rates and the effectiveness of these rates at a larger scale. Due to unexpected results in the large scale experiment, additional experiments were carried out to investigate some of the problems encountered. The key findings were as follows:

- Dill weed oil and spearmint oil effectively suppressed sprouting at dosages of 25 mg/L headspace or greater and application intervals of less than 25 days.
- The response to dose and application frequency was characterized by a lack of response in the effective range, i.e. robust but little room for optimization.
- Potatoes treated with dill weed oil or spearmint oil had a persistent smell that was readily detectable by the majority of the consumers tested, even after three weeks of continuous ventilation. Whereas clove oil could no longer be detected on a consistent basis after the treated potatoes had been ventilated for seven days.
- Clove oil was less effective in suppressing sprouting when applied by evaporation, because of its low evaporation rate.
- Testing of target doses failed because of excessive leakage from refrigerated chambers resulting in exposure times that were too short.
- Exposure to vapors or the aerosol of the essential oils required a minimum duration of four hours to cause any appreciable sprout suppression, but the effect continued to increase over the entire 48 hour period tested.
- Tissue concentrations of the major constituents of the three essential oils declined in a logarithmic fashion, but the rates of decline were not consistent with the evaporation rates, or the vapour pressures, of these compounds, therefore suggesting that these compounds were metabolized by the tubers.
- Sprout suppression with clove oil for long term storage is feasible but considerably more expensive then treatment with a conventional sprouting inhibitor. However, it may be economically justifiable for organic potatoes, or other specialty potatoes, which have a substantially higher market value. Sprout suppression with clove oil for periods of up to eight months can be expected cost between $34 and $65 per metric ton.

Introduction

Sprout control is an essential component of the successful management of stored potatoes. The current industry standard for sprout control is chloropropham, commonly known as CIPC (Kleinkopf et al. 2003). In recent years a number of countries including the US, have lowered allowable residue limits of CIPC (Boylston et al. 2001). Since minimum residue levels vary from country to country, they are a concern for Alberta growers, who produce mostly for export markets. A number of compounds present in essential oils have been shown to inhibit sprouting in potatoes, and could be used as alternatives to CIPC (Chowdhury et al. 2002). Because many of these essential oils do have "Generally Recognized as Safe" (GRAS) status and are commonly used as flavor ingredients in the food industry, they are less likely to be subject to trade restrictions (Brud and Gora 1990). However, their sprout inhibitory activity is usually reversible; therefore repeated applications are necessary to achieve the desired effect. With this project we intended to establish if it would be technically and economically feasible to use essential oils produced in Alberta (dill and mint oil) for sprout control in stored potatoes. For the purpose of comparison a clove oil product, currently marketed in the United States as Biox-C™, was also included.

Research objectives

Based on discussions with industry stakeholders we defined the following research objectives:

- Assess the ability of three essential oils (spearmint oil, dill weed oil, and clove oil) to suppress sprouting in stored potatoes;
- Determine optimal application rates and frequencies;
- Establish recommendation for the use of essential oils (spearmint and dill weed oil) as sprouting inhibitors in potatoes.
- Estimate treatment costs.

Research Protocol

Response to dosages applied at a range of time intervals

Oil from spearmint and dill weed supplied by Corraini Essential Oil, Ltd (Bow Island, Alberta), as well as clove oil supplied by Pace International, LLC. (Seattle, WA, USA), was evaporated inside sealed 63 L steel drums in the presence of potato tubers from the cultivar Piccolo and the cultivar Russet Norkotah. Each steel drum contained one nylon mesh bag of 100 tubers of the cultivar Piccolo (small tubers) and two such bags with 25 tubers each, of the cultivar Norkotah. The volume inside the containers not occupied by potatoes (from now on referred to as headspace), ranged from 48.7 L to 51.7 L. This was calculated on the basis of the average density of each potato variety, after each of the bags had been weighed. Treatments consisted of the following nine combinations of dose and application frequencies: 13 mg/L every 17 days; 25 mg/L every 10 and 24 days; 55 mg/L every 7, 17 and 27 days; 85 mg/L every 10 and 24 days; and 97mg/L every 17 days. The treatment levels were selected to fit a central composite design with 9 replications at the mid-point (55 mg/L every 17 days). To allow for the comparison of oil type, these treatments were randomized and blocked according to position (high or low) of the containers inside the refrigerated chamber.

The apparatus for this experiment consisted of 48 steel drums with a volume of 63 L each, which were connected to ventilation hoses equipped with valves (Figure 1). To insure uniform distribution of the essential oil vapors within these containers, the air was circulated through a vertically placed pipe with a 12 V fan attached on the top end. The essential oils were applied to filter paper (Fisherbrand P8) measuring 12.5 cm in diameter, and suspended in front of the fan (Figure 2). Once the essential oils were applied (no more then 1.0 ml per paper) the drums remained sealed for four days to allow time for the essential oils to evaporate. The valves were then reopened to insure good air exchange, which was provided by a ventilator (Figure 3) and conformed to recommendations for commercial storages (2.5 to 5.0 L/min./kg of potatoes). The entire apparatus was contained within a storage chamber that maintained the temperature between 8-12 ℃ and the relative humidity between 85-90 %. The exhaust air from each of the containers was evacuated to the outside through a separate system of pipes, to avoid cross-contamination.

Treatments were initiated on December 6, 2006 and weekly observations were made on whole bags (the experimental unit) to determine the duration of the sprout inhibitory effect for the different treatments and to assess any disease problems. Bags containing the potatoes were marked as “sprouted”, once at least 50 % of the tubers in the bag had one or more visible sprouts.

The data analysis consisted of fitting a quadratic model of the form

$$y = \beta_0 + x'\beta + x'Bx + \varepsilon = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i x_j + \varepsilon$$

The purpose of which was to obtain a response surface with the objective to identify optimal combinations of dose and time intervals between applications (Myers and Montgomery 1995).

Based on evaporation rates obtained in a separate experiment, adjustments were made to the application doses to reflect the actual amount evaporated, rather than the amount of oil applied to the filter paper. This adjustment was most significant for clove oil, where evaporation was much less than with the other oils. In addition to the response surface, summary statistics were calculated and differences between essential oils were assessed by analysis of variance.

To estimate the evaporation rate of each essential oil, 50 mg of dill weed, spearmint and clove oil were applied onto 6.0 cm^2 G6 glass filter papers to reach the targeted headspace concentration (50 mg/L). The initial weight of the filter paper and the essential oil applied were recorded. The filter paper was suspended in the jar and all jars were sealed and stored at 8 ℃. The evaporation rate was determined by measuring weight loss of the filter paper at 0, 2, 4, 8, 12, 20, 28, 44, 56, 68, 80 and 92 hours after oil application. Each measurement was taken on a separate jar, to avoid errors that would have been introduced by repeated measurements on the filter paper from a single jar. The same approach was used with the steel drums, except that in this case 5 ml of oil were applied to five Fisherbrand P8 filter papers (12.5 cm in diameter), which were suspended in front of the small fans inside the drums (Fig. 2).

Figure 1. General view of the setup of the treatment chambers showing the ventilation system, the adjustable voltage power supply, and some of the 63 liter containers.

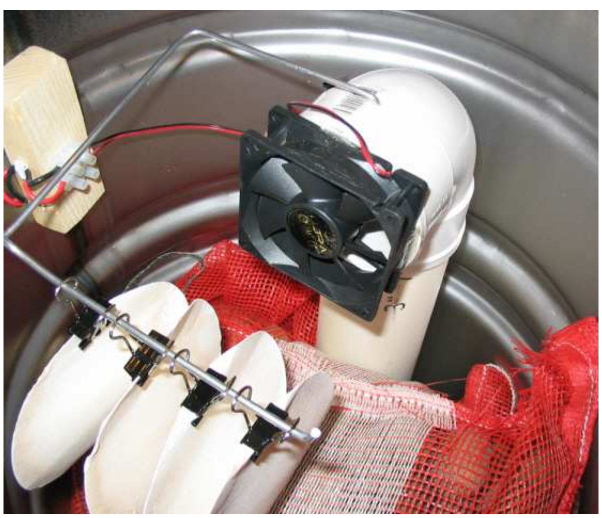

Figure 2. The evaporation system consists of suspended filter paper and a 12 V fan that circulates the air from the top to the bottom of the container. With this system the entire air volume is circulated approximately four times per minute.

Figure 3. The GAST R6 blower used to supply fresh air to the containers. This low pressure blower supplies approximately 80 L/min. to each container.

Sprout Inhibition in scaled up experiment

In order to verify the effectiveness of treatments selected on the basis of the results obtained with the steel drums, this scaled up trial was undertaken. Piccolo[1] and Russet Norkotah[2] potatoes harvested in late September and early October of 2007, were each held in 1 m^3 bins containing between 513 kg and 544 kg, and stored in 11 m^3 or 13 m^3 refrigerated chambers (Fig. 4) with the temperature set to 8 °C and relative humidity to 95 %. The treatments consisted of clove, dill weed, and spearmint oils applied every two weeks as an aerosol at 100 mg/kg and 200 mg/kg of tubers. Each oil type by dose combination was randomly assigned to one of the six refrigerated chambers and the treatments were initiated on November 9, 2007.

The experiment with the steel drums had shown that passive evaporation is slow, and therefore not likely to achieve high enough concentrations for effective sprout suppression in actual storage facilities. To address this problem we opted for a Cyclone™ Ultra-Flex[3] fogger (Fig. 4 insert), which has a small air powered turbine that can generate aerosol of varying droplet size. In this type of fogger the oil is not heated, which has the advantage to reduce potential fire or explosion hazards and the resulting aerosol is more stable, because it is at the same temperature as the ambient air. Research at the University of Idaho has found that turbine aerosol generators gave best results with the more volatile oils, such as dill and spearmint (Kleinkopf et al. 2003).

Weekly assessments were carried out on 50 tubers, which had been randomly selected from the top of each bin and labeled individually. Sprouting deeper inside the bins was assessed through transparent acrylic pipes, into which an inspection camera was inserted once per week to record the number of sprouted tubers, as well as their location within the container.

Figure 4. Left: Russet Norkotah stored in one of the storage chambers (top right: outside view); right: the aerosol fogger (Dyna-Fog® Cyclone™ Ultra-Flex "Cold Fog"/Mister) used to apply essential oil treatments to each chamber

[1] supplied by Wedge Wood Farms Ltd., Spruce Grove, AB
[2] supplied by Larry Orman, Taber, AB
[3] www.dynafog.com

Measurements included, bulk weight, total sprout weight, and the number of tubers sprouted. In addition headspace samples were drawn to measure the rate of depletion of the essential oils in the atmosphere inside the chambers following treatment. For this purpose we used a Fisher Scientific / Maxima Dry™ vacuum pump to draw air from inside the refrigerated chamber through a 1 L Erlenmeyer flask, from which 1.00 ml samples were drawn using Hamilton 81330 gas tight syringes. The content of these samples was then analysed using an Agilent 6890 gas chromatograph with a J&W DB-5MS column coated with a 0.50 µm film. The helium carrier gas flow was set to 1.8 ml/min at 19.53 psi and the sample was injected using a 35:1 splitter setting. The injection and flame ionization temperature was set to 250 ℃. Changes in the conce ntration of the constituents were calculated on the basis of the total peak area of the principal components of the oils (Vaughn and Spencer 1991).

In order to assess the amount of leakage from the refrigerated chambers during operation, we measured the pressure difference created by the cooling fans, between the inside and the outside of the chambers using a Omega[4] HHP-103 manometer. Then we turned the cooling system off and connected a variable speed blower, the speed of which was adjusted to maintain the same pressure as previously obtained with the cooling system turned on. The airflow required to maintain this pressure was then measured using a Eurotron[5] VT50 thermo-anemometer.

The data analysis consisted of compiling summary statistics, determining main effects (oil type or dose) using analysis of variance, and regression analysis to assess the significance of differences in sprouting as a function of the position of tubers within the storage bins.

Exposure time

In view of the severe leakiness found in the refrigerated chambers we set out to conduct an additional experiment to determine to what extent exposure time affected sprout inhibition. For this purpose, each of eighteen 1.0 L glass jars were filled with five randomly selected labeled tubers of the cultivar Lady Claire, and randomly placed on a table into one of the 13 m^3 refrigerated chambers with the cooling system turned off. The lids were removed from the jars and placed on a table, the door was sealed with a polyethylene film with a small hole to inject the aerosol, and two larger holes that were fitted with arm length polyethelene gloves. This allowed us to seal the jars without having to enter the chambers. The aerosol was then applied to the chamber at a rate of 16.9 µl of oil per litre of storage volume, which was equivalent to the 200 mg/kg rate used with the storage bins. The jars were tightly closed five minutes after the aerosol had been injected and the sealed jars were then taken to another storage room with the temperature set to 8 ℃ and the relative humidity to 95 %.

The treatments consisted of nine different exposure times (0, 1, 2, 4, 8, 12, 24, 32, and 48 hours), arranged in two blocks of nine jars. The treatments were applied by opening the jars at the randomly allocated predetermined intervals. The layout of the jars corresponded to a randomized complete block design. The same protocol was repeated for each of the three essential oils.

Observations on sprouting were performed every second day until no further sprouting occurred. The measurements consisted of counting the number of visible sprouts on each

[4] www.omega.com
[5] www.eurotron.com

tuber for each observation date. This was done by examining the jar from the outside to avoid repeated direct manipulation of the tubers.

Analysis of the essential oils

To determine the composition of the essential oils, samples were diluted in hexane to obtain a concentration of 2.5 µl/ml. The analysis was done on five replicates using an Agilent[6] 6890 gas chromatograph with a J&W DB-5MS column coated with a 0.50 µm film. The oven temperature was set to 80 C initially for 2 min., and ramped up to 240 C at 5 °C/min then maintained at 240 °C for 2 minutes. The oven temper ature setting was the same for all three oils, but other settings differed. For spearmint oil the column pressure was 15.62 psi with a flow rate of 1.3 ml/min. and a velocity of 32 cm/sec. The inlet temperature was 220 °C at a pressure of 15.62 psi, flow rate of 69.7 ml/min. and a splitter setting ratio of 50:1. In the case of the dill weed oil the column pressure was 17.10 psi with a flow rate of 1.5 ml/min. and a velocity of 35 cm/sec. The inlet temperature was 220 °C at a pressure of 17.10 psi, flow rate of 78.5 ml/min. and a splitter setting ratio of 50:1. For clove oil the settings were the same as for spearmint. Peaks were identified on the basis of existing libraries (Jirovetz et al. 2006).

Extraction of oils from the tubers

This experiment was undertaken to determine the persistence of the essential oils in tuber tissue. We used tubers from the cultivar Lady Claire that had been grown at the Crop Diversification Centre South in 2007, harvested in September, and stored at 8 °C for two months prior to the experiment. The tubers were washed and dried before they were exposed to treatment with the three essential oils. For this treatment approximately 100 randomly selected tubers were placed into each of three 63 L steel drums connected to a ventilation system and equipped with a small fan as described earlier. These tubers were then exposed to vapors of clove, dill, and spearmint oil by adding the oil onto filter paper at the rate of 55 mg/L of headspace, and allowing it to evaporate for 96 hours in sealed drums with the fans running. The temperature and relative humidity were set respectively at 8 °C and 95%. At the end of the treatment period the filter papers were removed and the valves were opened to insure continuous ventilation. Samples of four to five tubers were then removed at the following intervals: 0, 4, 24, 48, 168, 240, 336, and 504 hours. The tubers were kept frozen in plastic bags at -20 °C until pr ocessed.

The extraction protocol followed methods published by Oosterhaven et al. 1993b; Oosterhaven et al. 1995). The first step consisted in cutting the tubers into small pieces using an electric chopper and thoroughly mixing this material. A 50 g sample of the chopped tubers was then added to 100 ml methanol, 50 ml chloroform, 1 ml of 1 mg/ml naphthalene solution (used as internal standard) and homogenized for two minutes. Then another 50 ml of chloroform was added and homogenized for 30 seconds, followed by 50 ml of water and 30 seconds of homogenization. This was then vacuum filtered, the aqueous solution was removed and the chloroform solution was dried over anhydrous sodium sulfate, then concentrated to 5.0 ml using a rotary evaporator.

[6] www.agilent.com

The sample was analyzed in three replicates with the Agilent 6890 gas chromatograph and the same column as previously. The column temperature was set to 80 ℃ initially for 2 min., and ramped up to 240 ℃ at 5 ℃/min then main tained at 240 ℃ for 2 min. The inlet temperature was 250 ℃ at a pressure of 20.0 psi, f low rate of 64.5 ml/min. and a splitter setting ratio of 35:1 at a flow rate of 64.5 ml/min. The injection volume was 1.0 µl and helium was the carrier gas.

The response ratio was calculated for each of the principal components (s-carvone, r-carvone and eugenol) using a standard mixture of the compound with naphthalene. The following response ratio equation was used:

$$\frac{\text{peak area}}{\text{concentration}} = \text{response ratio} \times \frac{\text{internal standard peak area}}{\text{internal standard concentration}}$$

The data was analyzed by fitting a regression line on a Log transformed time scale.

Sensory evaluation

A sensory evaluation was conducted to determine if consumers would be able to detect the odor of the essential oils after ventilation periods of up to 21 days. Small to medium sized potatoes were purchased from IGA in Brooks. The tubers were from the current years harvest and produced in British Columbia[7]. One half of the tubers were used as controls, whereas the other half was divided into three equal portions and treated either with clove oil, dill oil, or spearmint. The concentration, the application method, and the storage conditions, were the same as described above in the experiment on the extraction of essential oils from tuber tissue.

The sensory test, which used the balanced triangular method, was conducted at 0, 7, 14 and 21 days of ventilation. On each test day, three tubers were randomly selected from each of the drums that had been treated with either, clove, dill, or spearmint oil, as well as from the control drum. The sensory test took place at the Farmers' Market in Brooks, where fifty people from the attending public (>16 years old) were invited to evaluate the samples. Each participant was asked to evaluate the smell of three sets of tubers corresponding to the three oils. Within each set of three paper bags there were either two bags with potatoes that had been treated with the same essential oil and one bag containing untreated tubers, or there were two bags of untreated tubers and one bag with treated tubers. The task requested from the volunteer evaluators was to identify the two bags with the same smell. When a volunteer could not identify any differences, he/she was asked to make a guess. The combination of treated and untreated tubers within each set was allocated randomly. During the test, the samples were replaced every 30 to 40 minutes. Prior to use, samples were kept in a large cooler with cold packs. The answers, demographic information (sex, age), and the temperature at the time of the evaluation were recorded on data sheets.

The data was analyzed on the basis of threshold values for correct answers for triangle tests, and the relationships between variables were examined through correlation analysis (Bi 2006).

[7] Mr. SPUD Potato, Abetkoff Farms, Grand Forks, BC.

Results and Discussion

Response to dosages applied at a range of time intervals

When potato tubers were treated by evaporation inside sealed 63 L steel drums, all three oils suppressed sprouting (Fig. 5). With dill oil sprouting of tubers from the cultivar Piccolo was suppressed for an average of 29 weeks, whereas with spearmint oil sprouting of the same cultivar was suppressed for an average of 32 weeks. In the case of the cultivar Norkotah sprout suppression lasted for an average of 26 weeks with dill oil, and 28 weeks with spearmint oil. However, sprout suppression with clove oil was much less effective, as it lasted on average only eight weeks for Piccolo, and eleven weeks for Norkotah. In the case of the first two essential oils, sprouting could certainly have been inhibited for even longer periods, had we not decided to stop the applications after 28 weeks. This was deemed necessary because differences between dose by application intervals could only be assessed if tubers actually sprouted. The much shorter sprout inhibition achieved with clove oil, was most likely due to the lower evaporation rate resulting from the low vapour pressure of this oil (Table 1).

Table 1. Physical properties of essential oils relevant in determining their suitability to application by evaporation.

Physical property	Clove	Dill	Spearmint
Density (g/ml)	1.040	0.896	0.954
Evaporation rates* (g/hour)	0.014	0.038	0.056
Vapour pressure (kPa)	0.001 (eugenol)	2.67 (limonene) 0.1 (s-carvone)	0.1 (r-carvone)

*Evaporation rates were measured using the steel drum apparatus described in the materials and methods section. The temperature was set to 8 ℃ and the oil was applied to five pieces of filter paper with a diameter of 12.5 cm, and suspended in front of a small fan that was installed inside of each drum.

In order to estimate the actual amount of oil that was evaporated inside the steel drums, we determined evaporation rates on the basis of weight loss of the filter papers to which the oil was added. The results show that under the conditions of this experiment, the evaporation rates were: 0.056 g/hr for dill oil, 0.038 g/hr for spearmint oil, and 0.14 g/hr for clove oil (Table 1). Consequently the amount of dill oil evaporated was four times higher than the amount of clove oil, which would explain the poor performance of clove oil. Therefore, at normal storage temperatures, the evaporation rate of clove oil is too low to achieve concentrations that can effectively inhibit sprouting. In the US, where clove oil is currently used for sprout inhibition, applications are typically performed with a thermo-fogger, which is a device that generates an aerosol by forcing a liquid through a heated coil. However, for technical reasons we opted to use evaporation, even if it put clove oil at a disadvantage. This was considered acceptable, because the primary objective wasn't to compare oils, but rather to find the optimal dosage for each essential oil.

The response to variations in dose and frequency of exposure to the three essential oils was less sensitive than expected. In the case of clove oil applied to the cultivar Piccolo, the model provided a satisfactory fit but the r^2 was only 0.14, which meant that a large proportion of the variation that could not be accounted for. In the case of Nokotah the model did not adequately describe the data, therefore it was not possible to define an optimal dose by application frequency combination (Fig. 6).

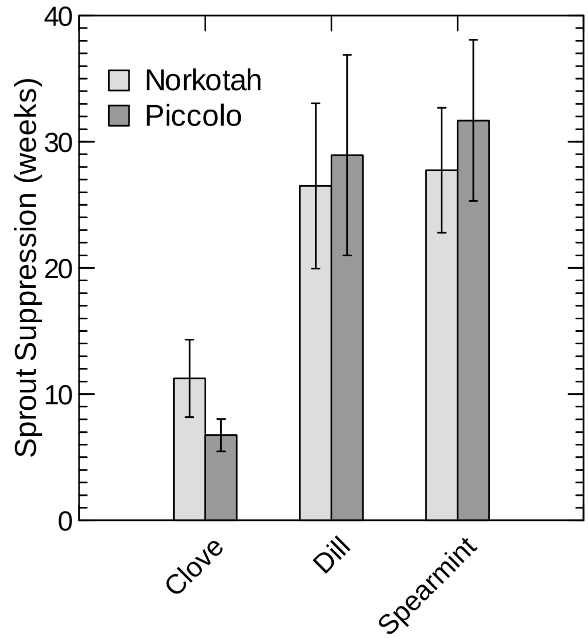

Figure 5. The overall effect of evaporated clove, dill and spearmint oil on the suppression of sprouting in Russet Norkotah and Piccolo potatoes. Sprout suppression was measured in weeks, starting with the first week of December. The error bars represent standard deviations.

The response for dill and spearmint was fairly flat, as most treatment units sprouted within three weeks of each other. Consequently, the error terms were large relative to the variation explained by the model. Therefore, in spite of high r^2 values, the quadratic models obtained with these oils exhibited significant lack of fit, which meant that systematic deviations from the model were statistically significant (Fig. 7 – 8). We tested several alternative models, but this did not provide any improvement on the lack of fit test, and in all cases resulted in lower r^2 values.

The lack of sensitivity of sprout inhibition to dose and application frequencies of essential oils had not explicitly been pointed out in previous research, but reports did show successful suppression under a variety of conditions (Duncan et al. 1992;Kalt et al. 1999;Ashiv 2002). This robustness of the response, is of course desirable from a practical point of view, because deviations from the intended dose are less likely to have an effect on the response.

Given this situation we opted to visually approximate an optimal combination of dose by application interval for each oil, and then select a common value that would be reasonably close to these estimates. The original intention was to use a formal optimization algorithm to select the best treatment combinations; however, because of the lack of sensitivity to variations in dose and application interval, this did not produce satisfactory solutions.

After carefully examining the response surface plots, we decided to use a 55 mg/L headspace (equivalent to 200 mg/kg of potatoes[8]) as a high dose and 27.5 mg/L headspace (equivalent to 100 mg/kg of potatoes) as a low dose. The application interval was set to 14 days, which was expected to be short enough to insure ongoing sprout suppression without requiring excessive amounts of oil. These two application rates were to be tested in scaled up experiment, to further validate the effectiveness of these essential oils.

[8] The 63 L drums contained on average 14 kg of potatoes and 50 L of headspace.

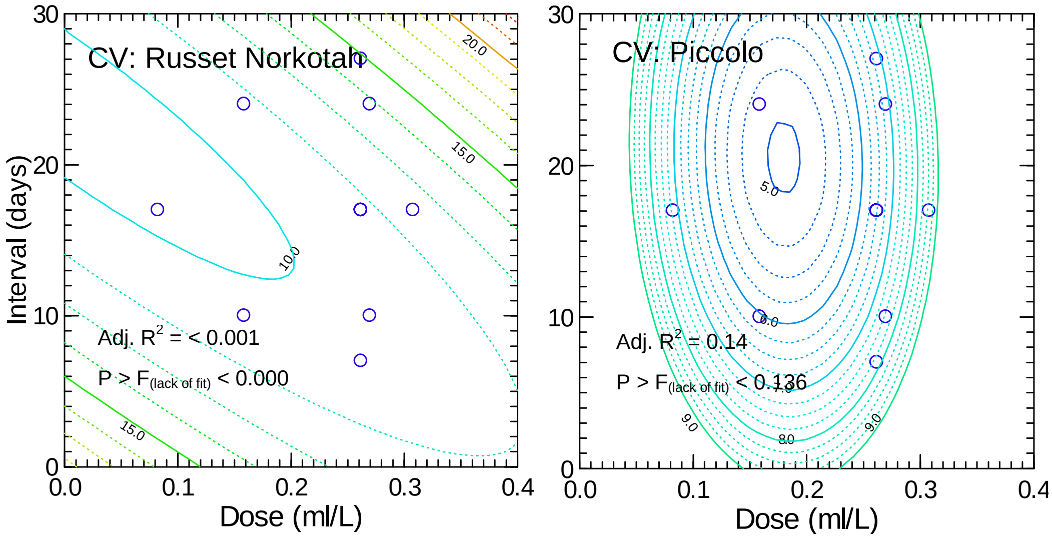

Figure 6. The effect of clove oil on sprout suppression in Russet Norkotah and Piccolo potato cultivars. The contour plots show sprout suppression in weeks as a function of treatment interval and dose as it was obtained when the oil was applied to potatoes contained in steel drums. The small circles show the location of the treatments, but overlapping centre points (10 replications) are not shown. Adjusted r^2 and lack of fit values (P = probability of a greater F-value) are given for each of the fitted quadratic response

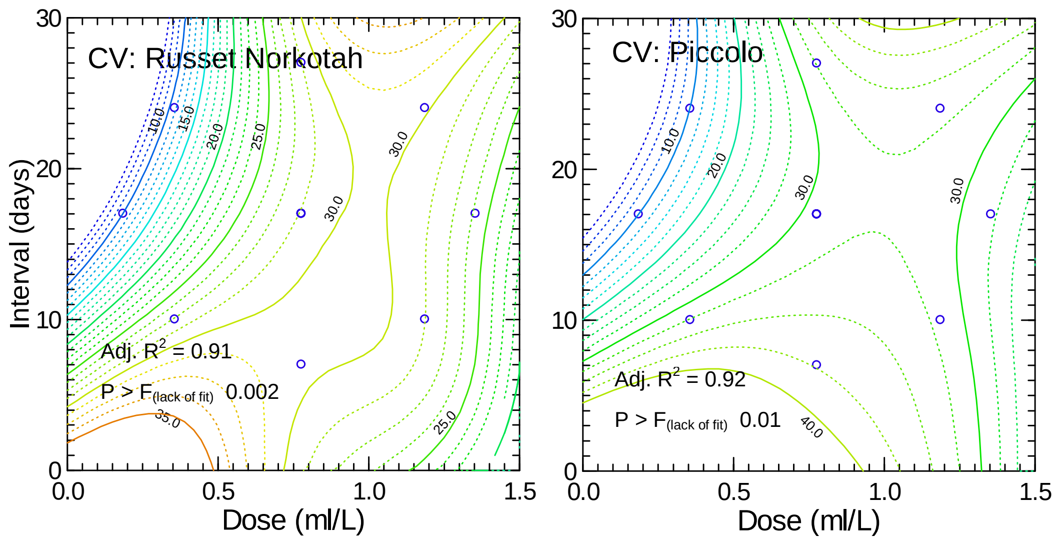

Figure 7. The effect of dill oil on sprout suppression in Russet Norkotah and Piccolo potato cultivars. The contour plots show sprout suppression in weeks as a function of treatment interval and dose, as it was obtained when the oil was applied to potatoes contained in steel drums. The small circles show the location of the treatments, but overlapping centre points (10 replications) are not shown. Adjusted r^2 and lack of fit values (P = probability of a greater F-value) are given for each of the fitted quadratic response surfaces.

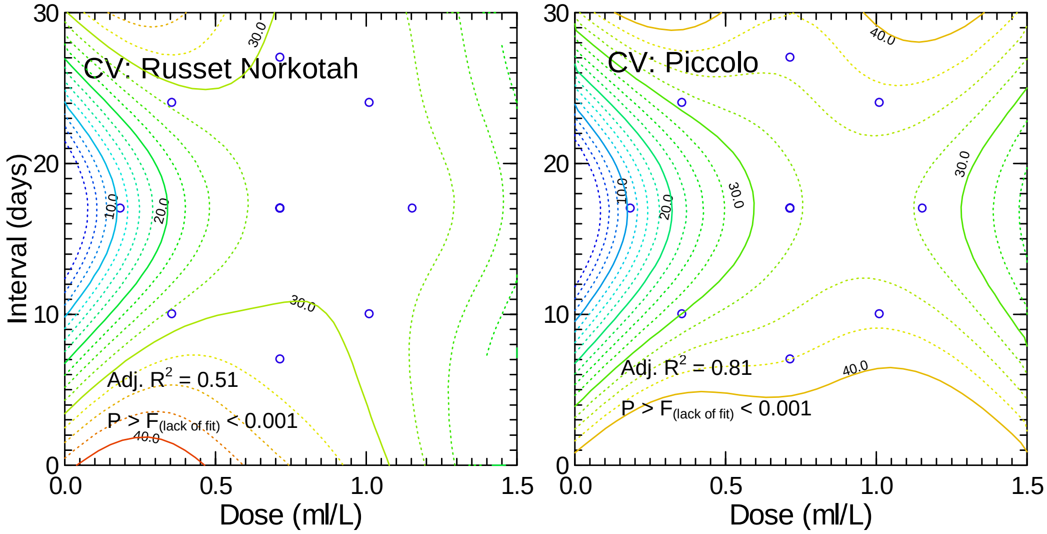

Figure 8. The effect of spearmint oil on sprout suppression in Russet Norkotah and Piccolo potato cultivars. The contour plots show sprout suppression in weeks as a function of treatment interval and dose, as it was obtained when the oil was applied to potatoes contained in steel drums. The small circles show the location of the treatments, but overlapping centre points (10 replications) are not shown. Adjusted r^2 and lack of fit values (P = probability of a greater F-value) are given for each of the fitted quadratic response surfaces.

Sprout Inhibition in scaled up experiment

Although the application equipment worked as expected none of the oils was able to inhibit sprouting. This became apparent early in the experiment, because monitoring of the labeled tubers showed no obvious effects. Irrespectively of treatments applied the median time to sprouting for the 50 tubers that were individually monitored, was 7 weeks in the case of the cultivar Piccolo and 10 weeks in the case of the cultivar Norkotah. The median sprouting time for the control was 5 weeks with the cultivar Piccolo and 10 weeks with the cultivar Norkotah. When compared to the control across treatments, the data showed that essential oils made no significant difference.

Nevertheless a more detailed analysis of variance on the means showed significant main effects for oil type in both cultivars and a significant dose effect in the case of Norkotah (Fig. 9). Also, in the case of Norkotah the average value for time to sprouting was slightly higher in the control than what was obtained with the essential oils. However from a practical standpoint these differences were far too small to be relevant. Therefore it appeared that the dosages and application intervals that gave excellent results in the steel drums didn’t work in this scaled up experiment, irrespectively of dose, application frequency, type of essential oil or even cultivar.

In order to determine whether penetration of the essential oils into the 1 m^3 storage bins was a problem, observations were made on the appearance of sprouts through a transparent pipe inserted into each container. These observations showed no difference in sprouting between the middle toward the edge of the bins, which was not unexpected since there was no significant sprout inhibitory effect due to the treatments. Upon completion of the

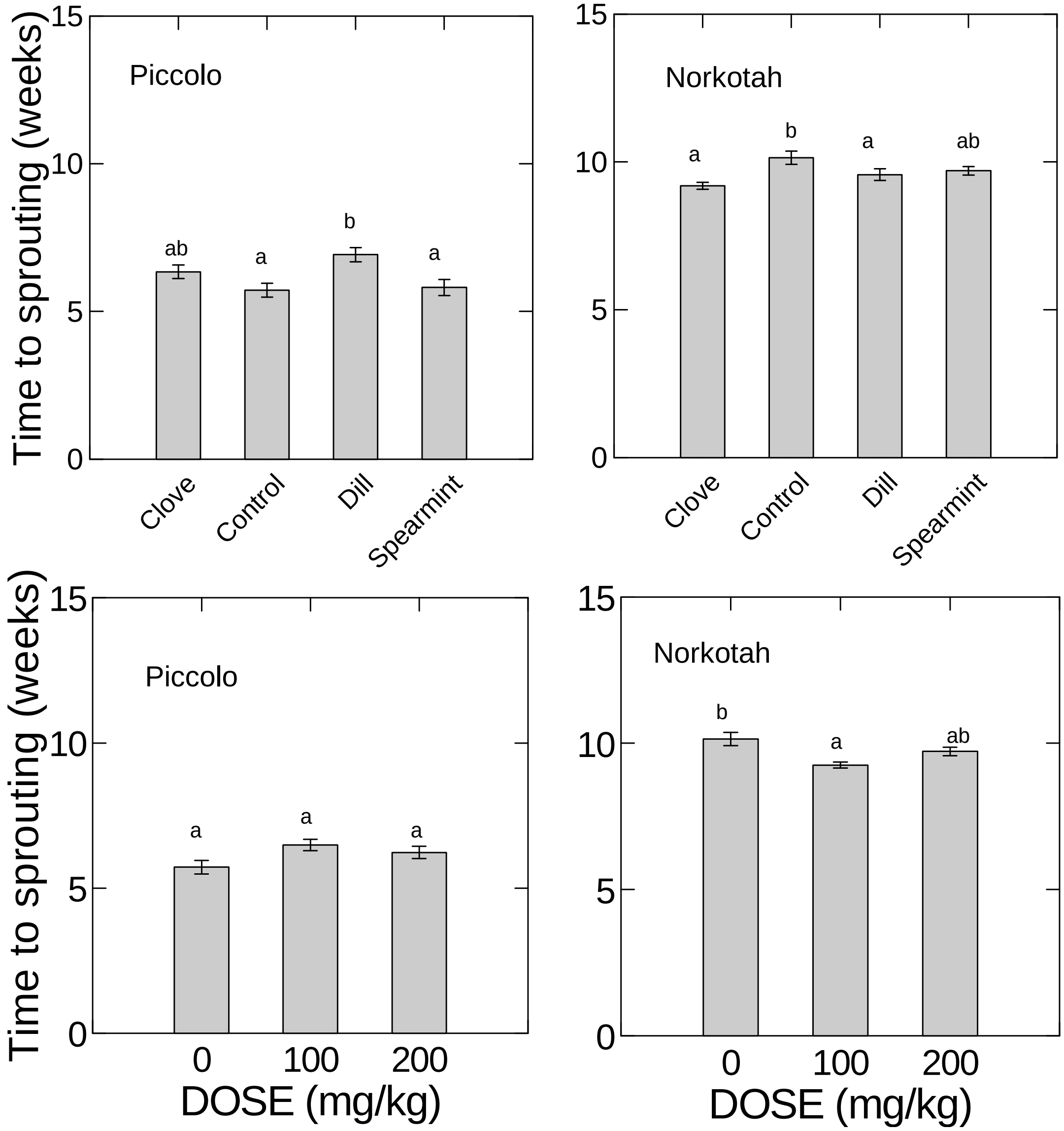

Figure 9. Sprout suppression in potato tubers of the cultivar Piccolo and Norkotha with three essential oils applied to 1 m^3 storage bins at 0, 100, and 200 mg per kg of tubers. Time to sprouting measured in weeks on a sample of 50 tubers was affected by the type of essential oil used. The overall effect of oil type was highly significant (probability of a greater F-value < 0.01) for each of the cultivars tested. In the cultivar Piccolo dill weed oil was slightly more effective than either the control or the spearmint oil, whereas in Norkotah the longest sprout inhibition was obtained with the control treatment. The dose made a difference only in the case of Norkotha, where the high dose was slightly better than the low dose. The statistical differences between means (letters in common indicate lack of significant difference) were established on the basis of a Tukey test with a 95% probability level. Vertical bars represent standard errors.

experiment we also verified if there were any differences in the number of tubers sprouted coming from different areas within the bin. In Piccolo the number of tubers sprouted ranged from 91 to 100 %, and in Norkotah it ranged between 98 and 100 %. There were generally no differences in sprouting due to the location within the bins. The only exceptions occurred with Piccolo, which had a slightly higher average sprouting on tubers taken from the top of the bin. This again shows that the essential oils did not inhibit sprouting in this experiment, given that the tubers on the top were most directly exposed to the treatments.

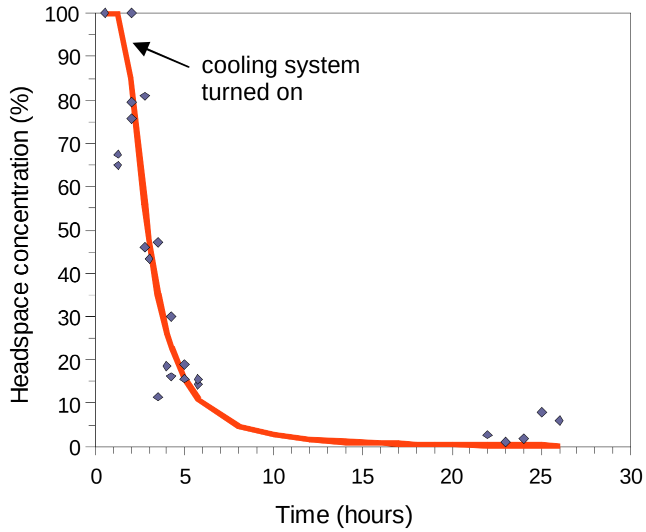

Figure 10. The rapid decline of headspace concentration of the essential oil aerosols once the cooling systems in the refrigerated chambers had been turned on. The decline could be described with an exponential model of the form y =1-exp(-exp(g-d*log(X))). The corrected r^2 was 0.84 and the estimates for g and d were 2.48 and 6.10 respectively.

The lack of any significant sprout inhibition was surprising and led us to further investigate possible causes. One such cause that appeared plausible was the loss of the aerosol through leakage. We had noticed a marked increase in the smell within the facility containing the refrigerated chambers, shortly after the cooling systems of the chambers were turned on, two hours after the application had been completed. Turning the cooling system on was necessary because of the rapidly rising temperatures inside the chambers. In order to maintain high concentrations of the essential oils inside the chambers for the targeted 48 hours, all air intakes were closed, which was expected to cause the air to recirculate with minimal addition of fresh air.

In order to understand what was happening we measured the headspace oil concentration in one of the chambers following treatment. The results showed a rapid decline of the amount of oil present in the atmosphere of the chambers once the cooling system was turned on. In fact approximately 65% of the oil disappeared within 2 hours following the turning on of the cooling system, and less than 20 % remained after 6 hours (Fig. 10). This suggested that the oil could have been lost through leakage and/or by condensation on the cooling coils and other surfaces.

Measurements of the pressure difference between the interior and exterior of the refrigerated chambers showed a positive pressure inside the chambers of 48 Pa with a standard error of 4.8 Pa. Air leakage, which was obtained by measuring the airflow required to maintain the pressure differential using a variable speed blower, was 0.149 m3/second with a standard error of 0.04 m^3/second. Considering that chamber volume ranged from 11 to 13 m^3, the air volume inside the chambers was replaced on average every 74 to 87 seconds. This high level of leakage was inherent to the design of these refrigerated chambers, and could not be fixed for the purpose of this experiment. This rapid air exchange could easily explain the quick decline of the essential oil aerosols in the chambers. Therefore it appeared that the reason we failed to obtain sprout inhibition was due to insufficient duration of the exposure to the essential oils tested.

Minimum exposure times have not been addressed in the existing literature, nor are there clear guidelines in this respect with regard to clove oil marketed as sprout inhibitors in the US (Sorce et al. 2005).

Exposure time

In view of the above finding we set out to conduct an additional experiment to determine to what extent exposure time affects sprout inhibition. Our results showed that exposure times of less than four hours were insufficient to inhibit sprouting when the tubers were treated with essential oils applied as an aerosol at a dose of 16.9 µl/L, which was approximately equivalent to the 200 mg/kg used previously. However, when the essential oils were applied by evaporation, clove and dill oil produced some sprout inhibition at exposure times of less then four hours (Fig. 11). When the oils were applied as aerosols the response was consistently more variable, suggesting that this method of application is less uniform. Also we observed that the time to achieve 50 % sprouting was almost always higher at an exposure time of 4 hours compared to an exposure time of 3 hours. the only exception to this was spearmint oil. Therefore, it appeared that under the conditions of this experiment that somewhere between 3-4 hours of exposure to sufficiently high concentrations of essential oils are required to produce a inhibitory effect on sprouting.

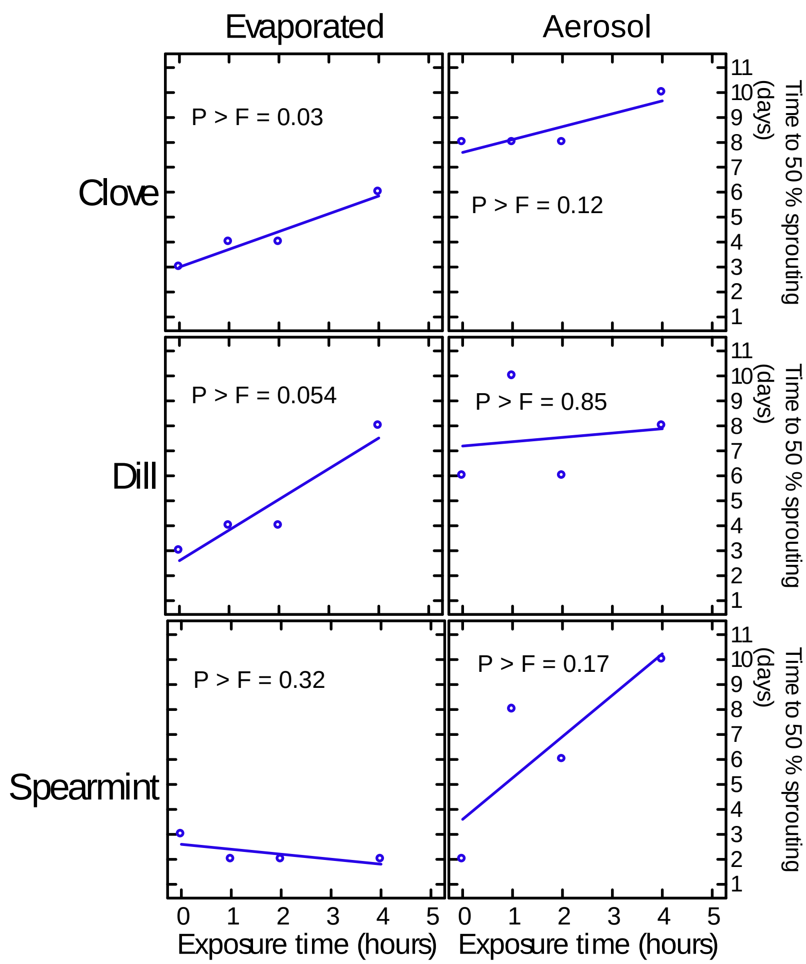

Figure 11. The effect of exposure times between 0 and 4 hours on the time required to achieve 50 % sprouting when potatoes were stored at 8 ℃. The three essential oils were applied at a rat e of 16.9 µl/L, either by evaporation or by applying the oils as an aerosol. The statistical significance of the observed trend is given as the probability of a greater F-ratio. Probabilities equal or smaller then 0.05 were considered statistically significant.

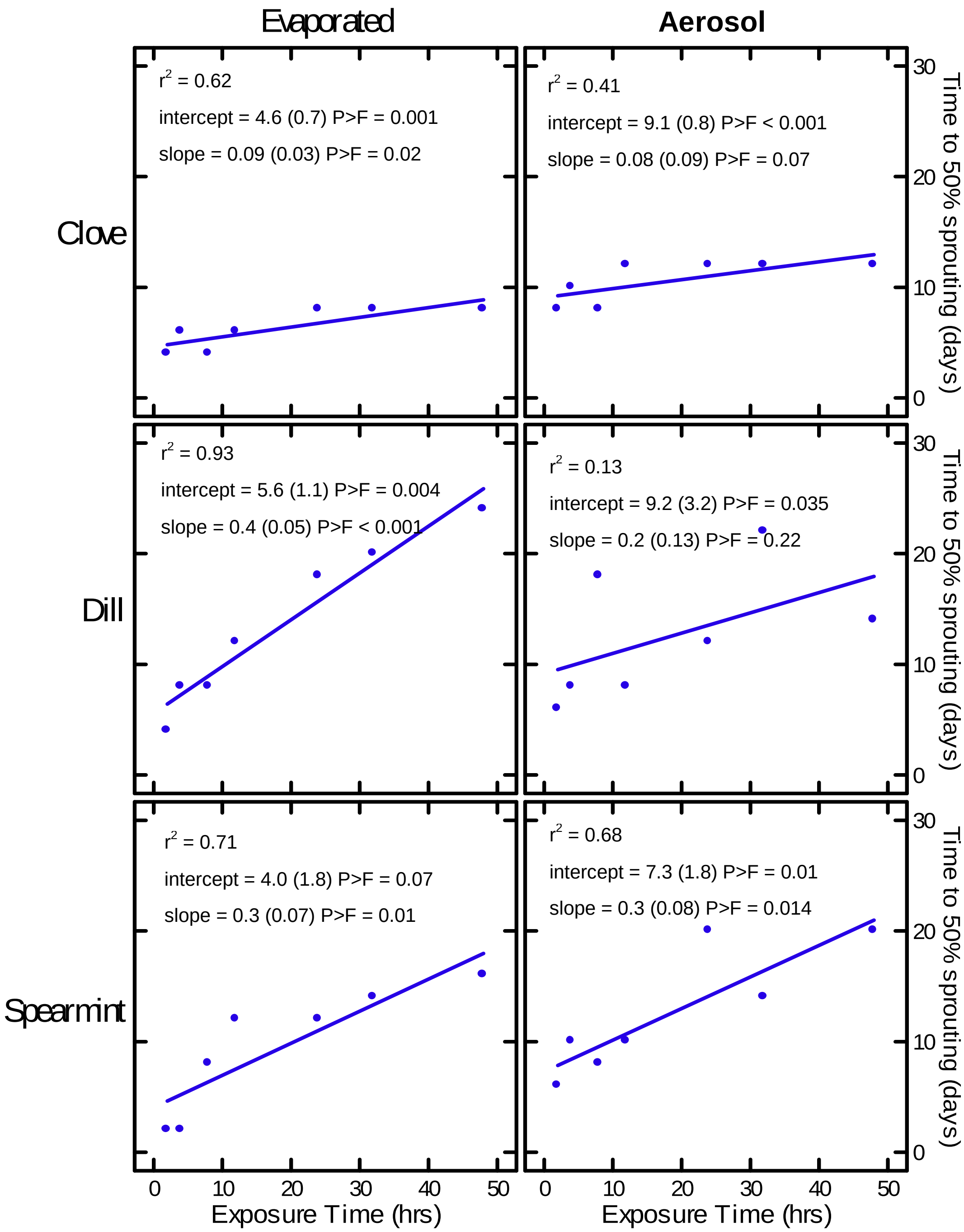

Figure 12. The effect of exposure times between 0 and 48 hours on the time required to achieve 50 % sprouting when potatoes were stored at 8 ℃. The three essential oils were applied at a rate of 16.9 μl/L, either by evaporation or by applying the oils as an aerosol. The trends were approximated by a simple linear model, with the regression parameters provided for each case.

This would explain why in our scaled up experiment, where high concentrations were only maintained for 2 to 3 hours, no significant sprout inhibition was observed.

When exposure times were between 4 to 48 hours, sprout suppression –as measured in the number of days required to achieve 50% sprouting—increased in a manner that could generally be approximated by a simple regression line (Fig. 12). The only exception was dill oil when applied as an aerosol. Although there was an increase over time, the pattern was too erratic to fit a straight line. However, vaporized dill oil gave a much better fit, and resulted in the strongest response. The weakest response was with clove oil; surprisingly there was little difference between the aerosol and the vapour applications. The expectation was that there should have been a much greater effect with the aerosol, given that clove oil is slow to evaporate due to its low vapour pressure (Table 1).

Results from this experiment showed that short exposure times in the order of just a few hours were insufficient to achieve significant sprout inhibition, especially if the oils were applied as aerosol. Over the longer time frame, sprout suppression increased for all exposure times tested. This suggests that it may be possible to achieve the same effect at a lower dose, provided that exposure time is increased (Gurdip et al. 1997;Sorce et al. 2005). It also showed that the rapid loss of essential oils in the refrigerated chambers was most likely the primary cause of the failure of the oils to inhibit sprouting in the scaled up experiment. It doesn't exclude condensation and adsorption as potential contributing factors, but the fact that excellent sprout suppression was achieved with two of the three oils when applied inside the steel drums where these mechanisms would also have been active, favors the leakage as the principal cause, since leakage in the steel drums was negligible.

Table 2. Composition of the essential oils as determined by gas chromatography.

Component	Clove	Dill	Spearmint
Eugenol (%)	82.2	--	--
trans-caryophyllene (%)	15.9	--	--
alpha-phellandrene (%)	--	17.2	--
limonene (%)	--	34.7	--
s-(+)-carvone (%)	--	41.5	--
r-(-)-carvone (%)	--	--	97.2
Other (%)	1.9	6.6	2.8

Analysis of the oils

Comparisons with appropriate standards showed that our clove oil contained 82.2 % eugenol, 15.9 % trans-caryophyllene, and 1.9 % other constituents. The dill oil contained 42.5 % s-(+)-carvone, 34.7 % limonene, 17.2 alpha-phellandrene, and 6.6 % other constituents. The composition of our spearmint oil was 97.2 % r-(-)-carvone and 2.8 % other compounds (Table 2.). The level of eugenol in the clove oil was consistent with what is commonly reported for clove oil extracted from leaves of *Syzygium aromaticum* (Gopalakrishnan and Narayanan 1988;Jirovetz et al. 2006). Clove oil extracted from the flower buds of *S. aromaticum*, has a lower eugenol content (Gopalakrishnan et al. 1982;Guan et al. 2007). The composition of the dill oil was consistent with what has been reported elsewhere for oil extracted from fresh whole plants (Ravid et al. 1992;Pino et al. 1995). Our spearmint oil was high in r-(-)-carvone, but this was most likely because it had been subjected to repeated distillation (referred to as “spearmint stripper”).

Extraction of oils from the tubers

The rate at which the essential oils disappeared from the tubers was measured on samples that had been exposed to a concentration of 55 mg/L for four days. The results from the gas chromatography analysis showed a rapid decline of the residual amount of the principal constituents of the oils as a function of time. The logarithmic model provided an excellent fit to this data (Fig. 13). Among the three components measured eugenol (clove oil) was the one with fastest rate of disappearance, followed by r-carvone (spearmint oil) and s-carvone (dill oil). This indicated that evaporation wasn’t the likely mechanism responsible for the disappearance of these compounds. If evaporation would have been the mechanism, then the carvones should have disappeared much faster, because of their much higher vapour pressure (Table 1). Consequently it appears that these compounds were metabolized by the tubers, which suggests that the inhibitory effect on sprouting may—at least in part—be caused by a physiological response, rather then strictly by the physical destruction of meristematic tissue (Oosterhaven et al. 1993a;Sorce et al. 2005).

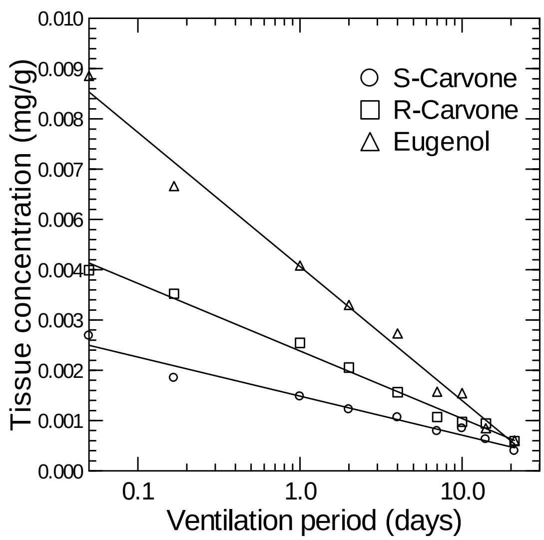

Figure 13. The decline over time in the tissue concentration of the principal constituents of the essential oils follows a logarithmic pattern. The regression parameters for eugenol (Δ)--the principal constituent in dill oil--were: intercept = 0.0045 (0.0001) P>F < 0.001, slope -0.00297 (0.00012) P>F < 0.001; for r-carvone (□)--the principal constituent in spearmint oil--were: intercept = 0.00265 (0.00005) P>F < 0.001, slope -0.00149 (0.00005) P>F < 0.001; and for s-carvone (o)--the principal constituent in dill weed oil--were: intercept = 0.00165 (0.00005) P>F < 0.001, slope -0.00086 (0.00006) P>F < 0.001.

Sensory evaluation

A sensory evaluation was conducted to determine if consumers would be able to detect the odor of the essential oils after ventilation periods of up to 21 days. Participants were asked to compare the odor of three samples and to select the two samples that had received the same treatment. If the answers had been purely random a third of the answers should have been correct. The statistical test compared the actual number of correct responses to a calculated number of correct responses needed to achieve a certain level of confidence that the observed correct responses represent real perception.

The results showed that clove oil was the only one that could not be detected consistently after a ventilation period of seven days or more (Fig. 14). However there was an increase in the proportion of correct responses over time. This increase could have been coincidental, as a result of random variations, or it could indicate that for some reason the residual odour of potatoes treated with clove oil became more pronounced over time. Temperature measurements taken during the last three tests showed a clear positive correlation (r=0.99) between temperature and the proportion of correct answers. Given that clove oil was far less volatile than the other oils, it is conceivable that evaporation of residual amounts made it easier to detect the oil even after 21 days, because of the much warmer temperatures.

The proportion of correct answers on samples treated with dill oil went from 90% without ventilation, to 72% after 7 days of ventilation, to 62% after 14 days of ventilation, and finally to 61% after 21 days of ventilation. The number of correct answers exceeded the 95% confidence limit at each assessment date. Therefore, we can be quite confident that dill oil was readily detectable by most of the participants, even if samples had been ventilated for 21 days.

In the case of mint oil we found that, with one exception, the odour persisted and was relatively easily detectable. However the testing conducted after 14 days of ventilation resulted in a very low rate of detection (26%). There was nothing

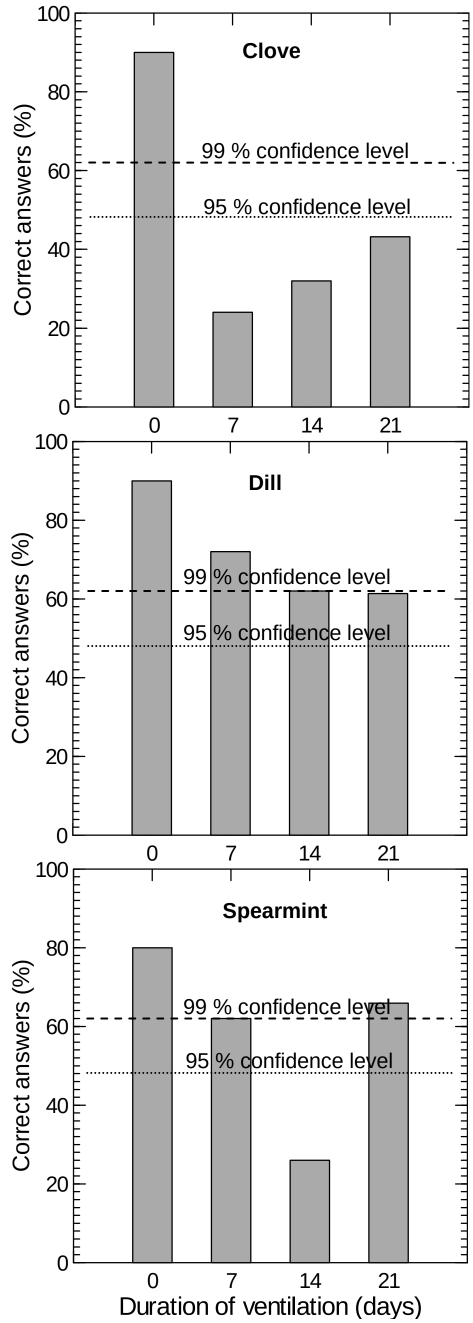

Figure 14. Percentage of potato samples, which were either treated or not treated with one of three essential oils (clove, dill weed, or spearmint), correctly identified by odour . The test was conducted on potatoes immeadiately after treatment application was complete and at 7 day intervals duing which the potatos were ventilated. Horizontal lines show the threshold values for the corresponding confidence levels.

unusual about our sample of participants that day (similar age and gender composition) that would suggest reduced competence, nor did we proceed differently in any way that could explain this result.

Whether or not the sprout inhibition treatments leave an identifiable odour on the product is of much concern for processing and table potatoes (Boylston et al. 2001;Piasecki et al. 2001). In either case the expectation is that the odour (or taste) of treated potatoes should not be distinguishable from untreated potatoes. If this criteria must be adhered to then dill and spearmint oil may not be suitable for use as sprout inhibitors on potatoes.

Economic feasibility

The general economic feasibility of using essential oils for sprout inhibition has been demonstrated in the US by companies such as Pace International Ltd and 1,4 Group Inc., both of which are marketing clove oil based products (Biox-C™ and Sprout Torch™) for sprout control in potatoes.

The application of conventional sprout inhibitors, such as CIPC (Sprout Nip™) to potato storages, is typically handled by contractors who have the trained staff and specialized equipment required for the job. This approach is also the most likely scenario for the application of essential oils in potato storages, given that the requirements in terms of expertise, labour and equipment would be very similar. The current application cost for CIPC, as quoted by Brenntag Canada Inc. for southern Alberta (February 2009), was $1.60 per metric ton. This cost should be about the same for the application of an essential oil.

The cost of Sprout Nip™ required to treat one metric ton of potatoes was $1.80. Based on current prices, and assuming an application rate of 50 ml per metric ton, the cost of clove oil for a single treatment would be in the $0.70 to $1.20 range. The total cost to treat one ton of potatoes with clove oil is therefore lower then a conventional CIPC treatment. Price quotes for clove oil based treatments obtained from contractors operating in Idaho and Washington, ranged from $2.79 to $3.90[9] per metric ton.

Clove oil would be cheaper if it would suppress sprouting as effectively as CIPC, but this is not the case since the effect of clove oil wears off after two to three weeks. Even though subsequent applications may be made at reduced rates, the cost of long term sprout control will quickly escalate. Consequently, long term sprout control with clove oil, only makes sense where CIPC cannot be used and where the value of the potatoes treated can justify the added expense. This may be the case for table potatoes intended for the premium organic market. For example, to store organic potatoes until July, between 12 to 16 applications of clove oil would be needed. Based on US price quotes, this would add anywhere from $34 to $65 to the cost per ton, which may be quite acceptable given that wholesale prices for organic table potatoes currently range anywhere from $1,500 to $3,000 per metric ton[10].

Alternatively essential oils could be used to complement a conventional sprout control program based on CIPC, to take advantage of the ability of these compounds to removes sprouts, as treated sprouts will shrivel up and eventually fall off. In this case economic considerations may have to emphasize minimizing losses, rather than reducing treatment

[9] calculated on the basis of a 1.00:1.25 US$/CA$ exchange rate.
[10] www.rodaleinstitute.org/Organic-Price-Report

costs. A possible scenario could be that an essential oil is used to burn back sprouts that have already appeared, followed by CIPC to insure that any further sprouting is kept in check. In fact products such as Sprout Torch™ are currently being marketed to US potato growers for this purpose.

Table 3. Current farm gate value of essential oils and costs of sprout inhibition treatments in Alberta and the North-Western US. All dollar values are in Canadian currency.

Description	Comments	cost*	Source
Clove (leafs)	Clove oil extracted from leafs is cheaper then clove oil extracted from buds.	$15 to $25./kg	1,4 Group Inc. (J. Forsyth)
Dill (whole plant)	Production is much lower compared to mint oils, but there is a good supply in southern Alberta. Dill oil can also be extracted from seed, but this is more expensive.	$30 to $40/kg	Cairini Essential Oils
Spearmint	The much cheaper Chinese mint oils have contributed to keep prices fairly low.	$24 to $32/kg	FAS/USDA Horticultural and Tropical Products Division
Custom application of SproutNip aerosol	This is the standard sprout inhibition treatment for stored potatoes in Alberta	$3.40/metric ton	Brenntag Canada Inc. (Feb. 2009)
Custom application of Sprout Torch	Sprout Torch is being marketed as a product to rescue potatoes that have already sprouted. It is not intended for repeated applications.	$2.75 to $3.10/metric ton	1,4 Group Inc.

Conclusion

All three oils were able to inhibit sprouting in potato tubers of either one of the cultivars tested, but clove oil was much less effective when applied by evaporation. The scaled up test with 540 kg bins did not succeed due to insufficient exposure resulting from leakage. We were not able to identify specific optimal doses for each oil, because of a lack of a differentiated response in the effective range. Therefore it appeared that the sprout inhibition effect is fairly robust, which was consistent with other published reports. A suitable dose for long term sprout inhibition, based on the data obtained in the first experiment and the experiment on exposure times, would be anywhere from 50 mg to 100 mg per kg of potatoes with no air exchange for 48 hrs, and application intervals of two to three weeks.

The problem with dill and spearmint oil was that ventilation, even as long as three weeks, was not able to reduce the odour of the residual essential oils to the point that consumers could no longer differentiate treated from untreated potatoes. We didn't test whether the dill or mint odour would persist after cooking or processing, but the fact that it persisted on the raw potatoes may be sufficient to eliminate those two oils from further consideration. This leaves us with clove oil, which is already marketed in the US for sprout inhibition in potatoes. Registration of these products in Canada will be more challenging because, unlike in the US, the registrant must demonstrate the efficacy and safety of the product.

Acknowledgements

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