Alberta Agriculture and Rural Development

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

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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 mea suring 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.

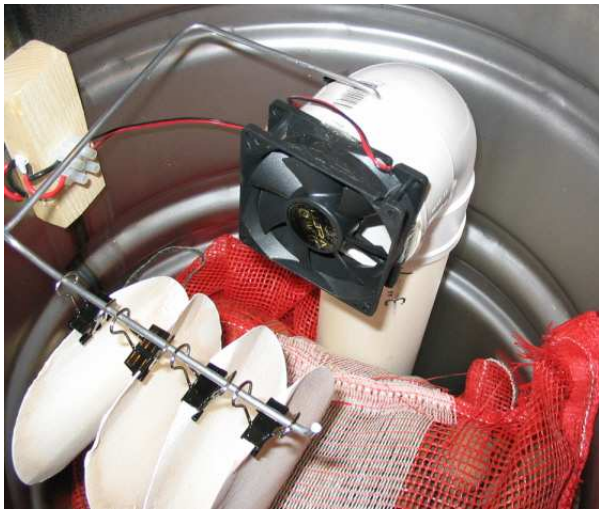

Figure 2. The evaporation system consists of suspended filter paper and a 12 V fan that circulates the air from the top to the bottom of the container. With this system the entire air volume is circulated approximately four times per minute.

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 ℃ 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 ℃/min then maintained at 240 ℃ 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 ℃ 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 ℃ 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 ℃ 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 ℃ 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 ℃ 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).

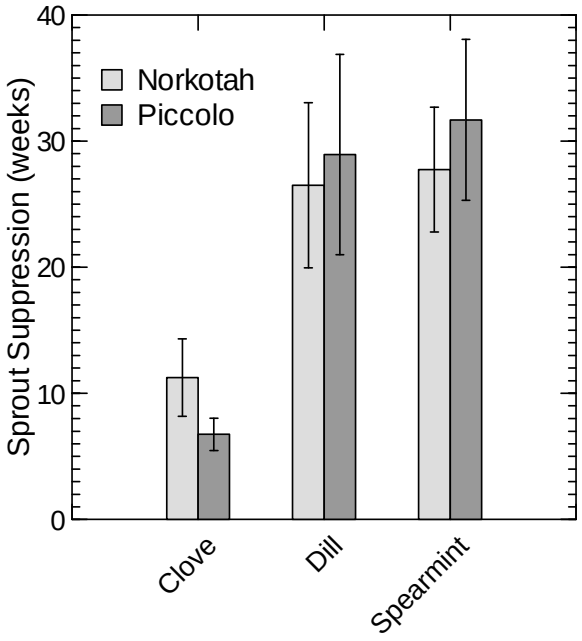

Figure 5. The overall effect of evaporated clove, dill and spearmint oil on the suppression of sprouting in Russet Norkotah and Piccolo potatoes. Sprout suppression was measured in weeks, starting with the first week of December. The error bars represent standard deviations.

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.

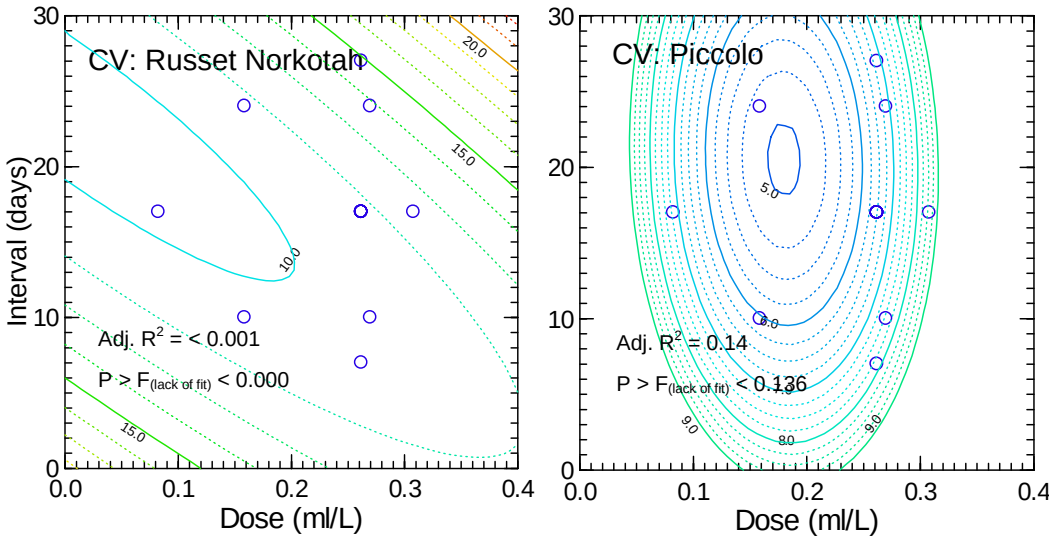

Figure 6. The effect of clove oil on sprout suppression in Russet Norkotah and Piccolo potato cultivars. The contour plots show sprout suppression in weeks as a function of treatment interval and dose as it was obtained when the oil was applied to potatoes contained in steel drums. The small circles 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

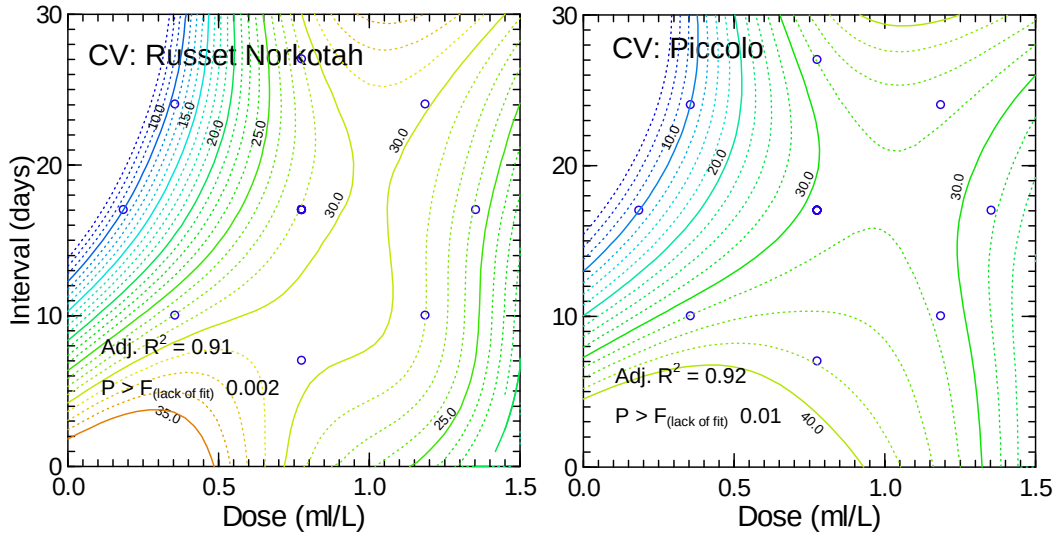

Figure 7. The effect of dill oil on sprout suppression in Russet Norkotah and Piccolo potato cultivars. The contour plots show sprout suppression in weeks as a function of treatment interval and dose, as it was obtained when the oil was applied to potatoes contained in steel drums. The small circles 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.

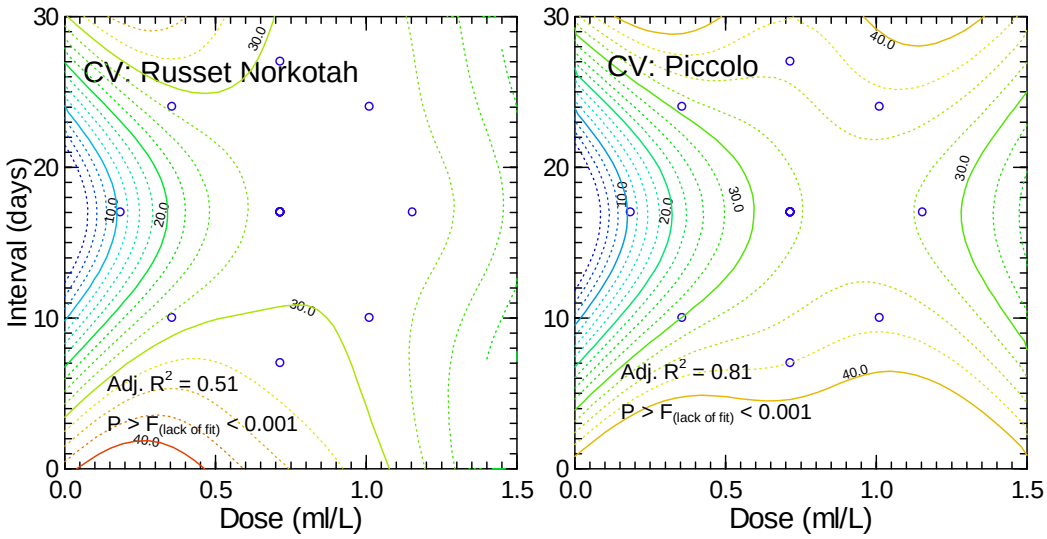

Figure 8. The effect of spearmint oil on sprout suppression in Russet Norkotah and Piccolo potato cultivars. The contour plots show sprout suppression in weeks as a function of treatment interval and dose, as it was obtained when the oil was applied to potatoes contained in steel drums. The small circles 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

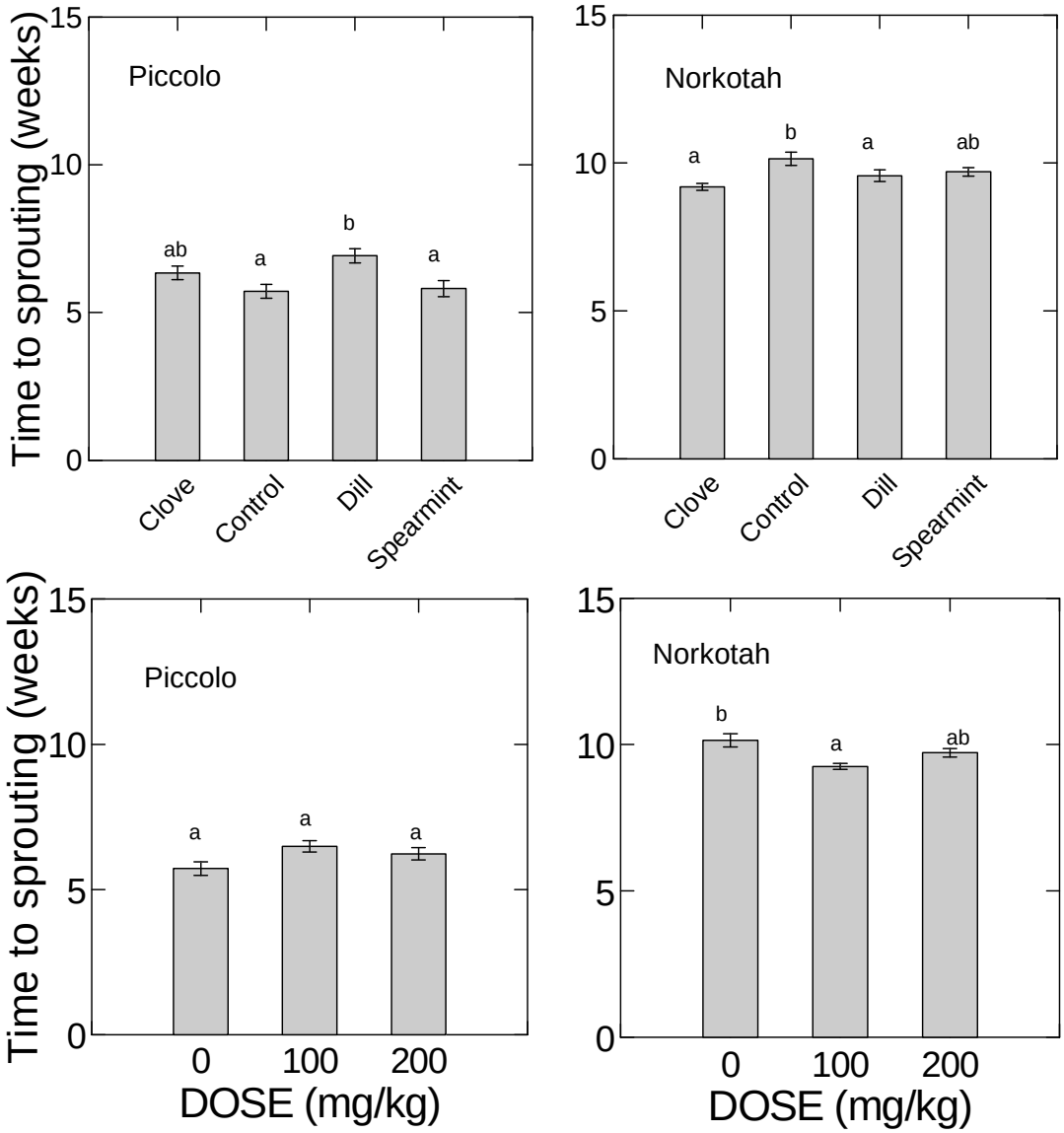

Figure 9. Sprout suppression in potato tubers of the cultivar Piccolo and Norkotha with three essential oils applied to 1 m^3 storage bins at 0, 100, and 200 mg per kg of tubers. Time to sprouting measured in weeks on a sample of 50 tubers was affected by the type of essential oil used. The overall effect 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.

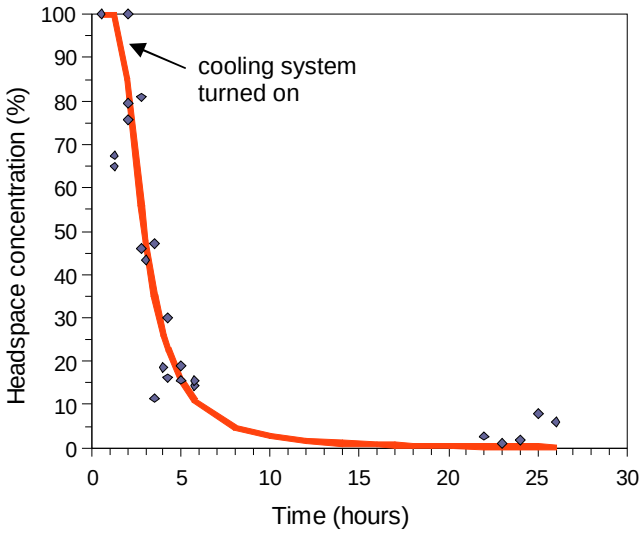

Figure 10. The rapid decline of headspace concentration of the essential oil aerosols once the cooling systems in the refrigerated chambers had been turned on. The decline could be described with an exponential model of the form y =1-exp(-exp(g-d*log(X))). The corrected r^2 was 0.84 and the estimates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

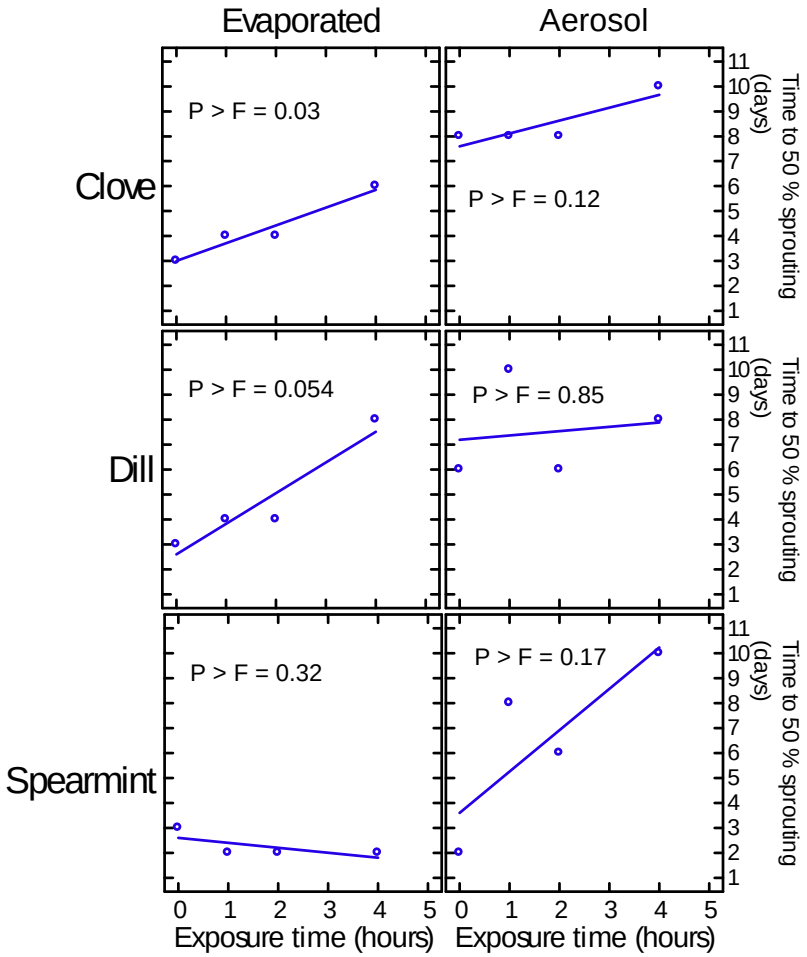

Figure 11. The effect of exposure times between 0 and 4 hours on the time required to achieve 50 % sprouting when potatoes were stored at 8 ℃. The three essential oils were applied at a rat e of 16.9 µl/L, either by evaporation or by applying the oils as an aerosol. The statistical significance of the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

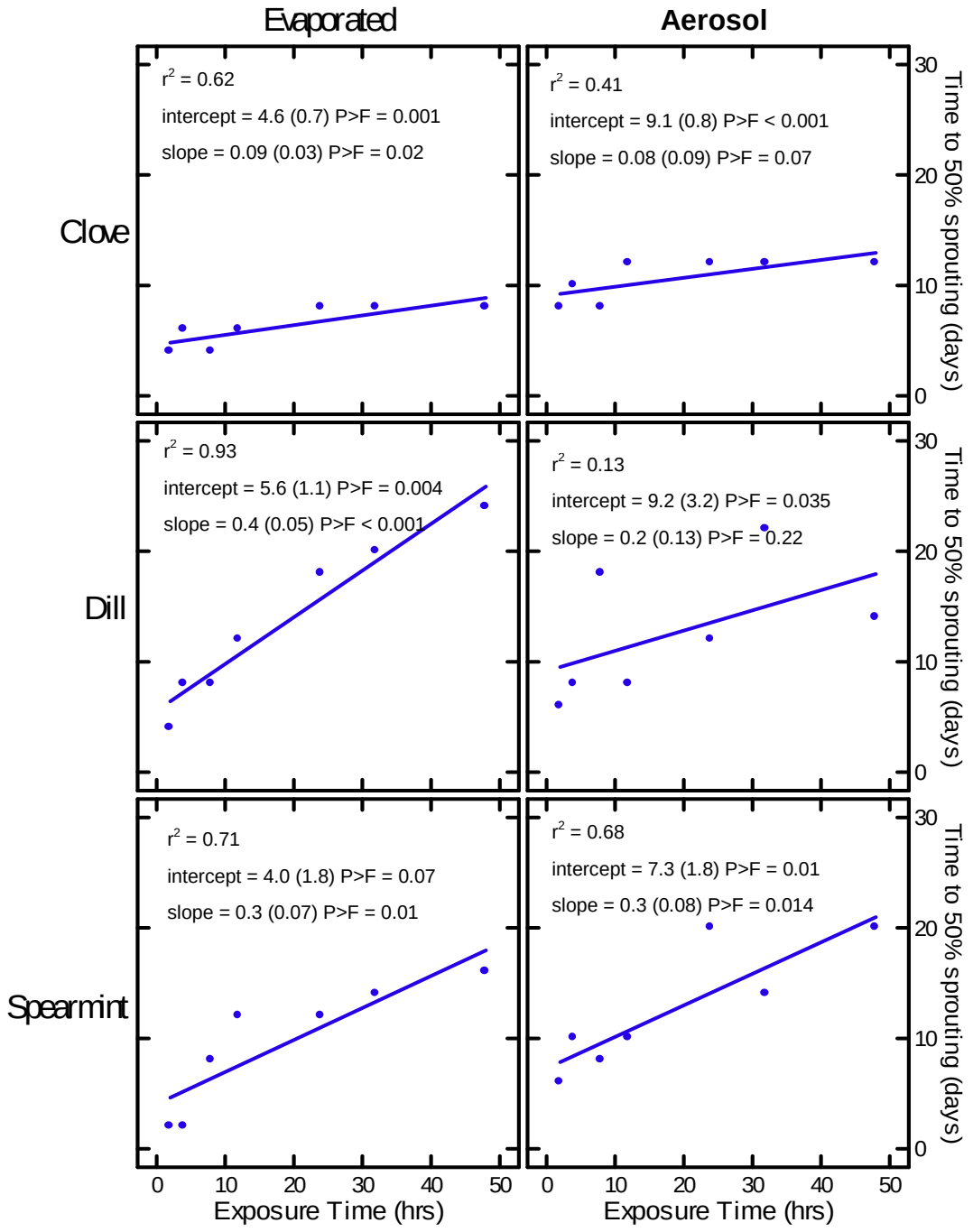

Figure 12. The effect of exposure times between 0 and 48 hours on the time required to achieve 50 % sprouting when potatoes were stored at 8 ℃. The three essential oils were applied at a rate of 16.9 µl/L, either by evaporation or by applying the oils as an aerosol. The trends were approximated by a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

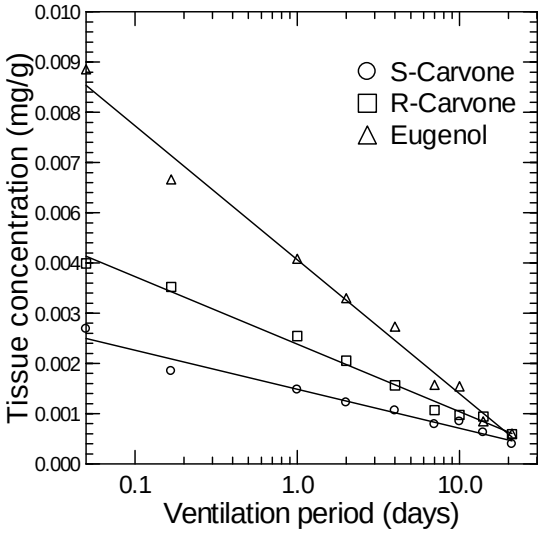

Figure 13. The decline over time in the tissue concentration of the principal constituents of the essential oils follows a logarithmic pattern. The regression parameters for eugenol (Δ)--the principal constituent in dill oil--were: intercept = 0.0045 (0.0001) P>F < 0.001, slope -0.00297 (0.00012) P>F < 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

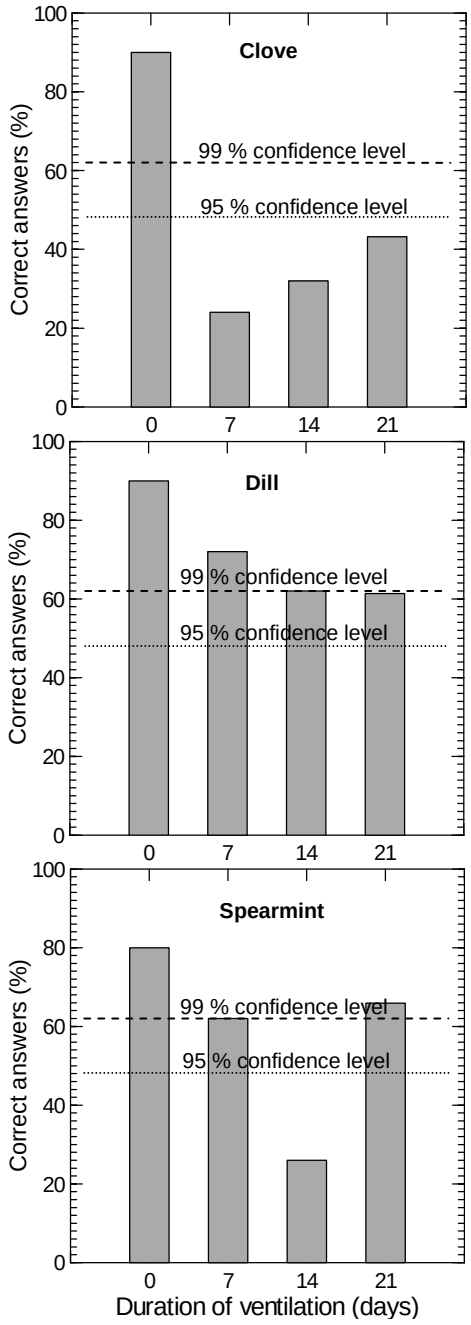

Figure 14. Percentage of potato samples, which were either treated or not treated with one of three essential oils (clove, dill weed, or spearmint), correctly identified by odour . The test was conducted on potatoes immeadiately after treatment application was complete and at 7 day intervals duing which 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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