THE IMPACT OF DILL WEED, SPEARMINT AND CLOVE ESSENTIAL OILS ON SPROUT SUPPRESSION IN POTATO TUBERS

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ABSTRACT

Sprout control is essential for successful management of stored potatoes. This study examined the effect of dill weed, spearmint and clove essential oils on sprouting of potatoes. Extracts of steam distilled dill weed whole plants containing 41.5-42.7% of S-(+)-carvone and spearmint foliage extracts containing 97.2-97.6% of R-(-)-carvone, were applied to tubers in a series of experiments using either 1-L glass jars or 63-L steel drums. The composition of the essential oils was consistent between years but evaporation rate varied among the oils as dill weed extract evaporated the fastest while clove oil evaporated the slowest under the same conditions. After exposure to essential oil treatments, tuber sprout number and weight were assessed and compared to untreated control and tubers treated with commercially marketed clove oil product (Biox-C^{TM}, containing 78.5-82.3% eugenol). Applications of 32.5 and 47.6 mg L^{-1} headspace of dill weed oil and 21.5 and 22.3 mg L^{-1} headspace of spearmint oil achieved 50% reduction in 'Russet Burbank' sprout weight and sprout number respectively, 30 days after the initial treatment. Tubers stored in environments with 60-240 mg L^{-1} headspace of dill weed or spearmint oils suppressed sprouting at least 5 weeks longer than that of the controls. In 63-L steel drums, repeated dill and spearmint oil vapor treatments effectively and consistently suppressed sprouting of 'Russet Norkotah' and 'Piccolo' tubers for 7-8 months when doses were 25 mg L^{-1} headspace or higher and when treatments were repeated at least every four weeks. Within this range, sprout suppression was not sensitive to treatment variations, and, therefore, an optimal treatment level could not be determined. Clove oil was less effective in suppressing sprouting, likely due to its slower vaporization compared to dill and spearmint oils. Essential oil treatment effects on seed tuber viability were evaluated on 'Piccolo'. Tubers were planted after exposure to dill or spearmint oil vapor environments ranging from 15-240 mg L^{-1} headspace for seven days. There were no adverse effects on seed viability at doses less than 120 mg L^{-1} headspace. Although environments with <120 mg L^{-1} headspace had no significant adverse impact on sprouting, sprout emergence was delayed at higher doses. Dill weed and spearmint oils could potentially be used as potato sprout inhibitors but further studies are needed to demonstrate its commercial feasibility.

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LIST OF ABBREVIATIONS

ABA: Abscisic acid

CIPC: Chloropropham, 1-methylethyl-3-chlorophenylcarbamate

cwt: Hundredweight

DMAPP: Dimethylallyl pyrophosphate

FID: Flame ionization detector

GA: Gibberellic acid

GC: Gas chromatogram

GPP: Geranyl diphosphate

GRAS: Generally Recognized As Safe

HMG-CoA reductase: 3-hydroxy-3-methylglutaryl coenzyme A reductase

IAA: Indole-3-acetic acid

IPC: Isopropyl N-phenylcarbamate

IPP: Isopentenyl pyrophosphate

MH: Maleic hydrazide

ppm: Parts per million

RCBD: Randomized complete block design

RH: Relative humidity

SE: Standard error

TM: Trademark

1.0 INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are the most important vegetable crop worldwide and play a key role in the global sustainable food system. It is one of the top four food crops in terms of annual production (Suttle 2004) and the number one non-grain food commodity in the world with global production of 320 million metric tonnes in 2007 (Potato International Centre 2008). Over one third of the total production is generated from developing countries, such as China and India, and it is an important source of income for millions of farmers (International Potato Centre 2008). In addition, potatoes play a significant role in addressing the intensifying issue of food crisis in third world countries. The crop is also favorable for the delivery of new bioproducts due to its high starch content.

In Canada, potatoes are grown in all ten provinces on over 150,000 hectares of land, and the production reached 4.7 million metric tonnes in 2008 with 0.12 million tonnes harvested in Saskatchewan and 0.79 million tonnes produced in Alberta (Statistics Canada 2009). The majority of the potatoes produced in Canada are distributed to three markets: the processing industry (45% of total production), the fresh table potato market (40%) and the seed tuber market (15%) (Parker 1994). Potatoes are a valuable crop for Canada with commercial production in every province. Every year, hundreds of millions of dollars are generated from domestic potato consumption and from export. Statistics Canada (2009) reported that in 2007 the 5.0 million metric tonnes of potatoes harvested generated approximately 966 million dollars for the Canadian potato industry. During the 2001-2002 crop year, a total of 4.65 million metric tonnes of potatoes were produced (Statistics Canada 2003), with $52 million dollars of seed potatoes, $127 million dollars of table potatoes, and $738 million dollars of frozen french fries were exported to the US. Canada is internationally recognized for supplying high quality table, processing and seed potato in particular (Agriculture and Agri-Food Canada 2003).

Depending on the usage and demand, the majority of newly harvested potatoes must be stored for several months before they reach consumers or processors. During the storage period, effective sprout control is essential to successfully maintain the tuber quality and minimize the loss. Sprouting can result in tuber weight loss mainly due to water loss through the lenticels, reducing tuber sugar level and increasing bruising susceptibility (Vaughn and Spencer 1994, Hartmans *et al.* 1995). In addition, sprouted tubers contain elevated levels of toxic glycoalkaloids compared to non-sprouted tubers (Friedman 2006). In North America, the most commonly used

sprout inhibitor is chloropropham (1-methylethyl-3-chlorophenylcarbamate or CIPC) (Kleinkopf *et al.* 2003). CIPC is applied as an aerosol fog to stored potatoes or as an emulsifiable concentrate sprayed on fresh market potatoes prior to packaging (Western Potato Council 2003). However, in recent years, there are growing concerns on the levels of CIPC residuals in tubers and their potential impacts on human health and the environment. In 2002, the allowable residue tolerance in fresh potatoes was reduced from 50 ppm to 30 ppm in the United States. In Europe the allowable residue limit ranges from 5 to10 ppm (Kleinkopf *et al.* 2003). The different regulatory requirements on minimum CIPC residue levels from country to country also create export barriers. Concerns arise from the need for a safer, healthier and more environmentally friendly alternative to suppress potato sprouting during the post-harvest storage period.

In the past decade, studies have shown that many essential oils extracted from several plant species, such as caraway, dill and mint, to be effective in suppressing potato sprouting with no adverse effects on tuber processing quality and taste (Vaughn and Spencer 1991, Hartmans *et al.* 1995, Frazier *et al.* 1998, Kleinkopf *et al.* 2003). The major components contained in these oils are generally considered to be safe to consumers and have been used as flavoring agents in the food and perfume industries for centuries (Oosterhaven 1995). With all the concerns related to chemical sprout inhibitors, treating tubers with natural compounds would be desirable as more and more consumers demand natural and chemical-free produce (Kleinkopf *et al.* 2003).

The use of essential oils as sprout inhibitors could also be beneficial to organic and seed potato growers. As a natural product, the essential oil is less prone to health and environmental concerns and is suitable for organic potato production (Plooy *et al.* 2009, Reuveni *et al.* 2009). Many essential oil components, which are reported to be effective on suppressing potato sprouting, are classified as Generally Recognized As Safe (GRAS) by the United States Food and Drug Administration. The United States Department of Agriculture, National Organic Program has approved the use of non-synthetic GRAS compounds on certified organic crops (Frazier *et al.* 2004). Unlike CIPC and other commonly used chemical sprout inhibitors which permanently inhibit sprouting, the inhibition effect caused by essential oils is often reversible (Kleinkopf *et al.* 2003). Therefore, essential oils may have potential to be used on seed potatoes to temporarily withhold sprouting during storage and transportation.

Most previous research has focused on the efficacy of the major compounds contained in certain essential oils, such as S-(+)-carvone, R-(-)-carvone, limonene, eugenol, to inhibit potato

tuber sprouting and control of fungal growth during storage. Different studies consistently reported that these compounds can suppress both sprout and fungal growth (Vaughn and Spencer 1991, Vokou *et al.* 1993, Hartmans *et al.* 1995, Oosterhaven *et al.* 1995c). However, the pure compounds are very expensive and often fall out of the affordable range of average growers and producers. Pure carvone (98%) products are sold by chemical companies such as Sigma-Aldrich for $230 per 100 mL ($2.21 g^{-1}) for S-carvone and $53.8 per 100 mL ($0.52 g^{-1}) for R-carvone (Sigma-Aldrich, 2009). In comparison, the essential oil extracts from locally grown commercial crops containing these active compounds are relatively cheaper, for example in 2009 dill weed oil produced in southern Alberta was sold to the United States at US $0.04 g^{-1} and spearmint oil was marketed for US $0.05 g^{-1} (Corraini 2009, personal communication). In addition, these essential oils are also more readily available and can be produced in large quantities in regions such as the Canadian Prairies. Nevertheless, in order to transform these essential oils into marketable products and make them available to potato growers and producers, further studies including optimum dose range, application frequency, preferred storage conditions and application methods for commercial usage of various types of essential oils are needed. In addition, the treatment effects on tuber quality, especially on seed tubers need to be investigated. Based on these current issues, the objectives of this study are:

1. To analyze the composition of dill weed, spearmint and clove oils extracted from different crop years and to evaluate the evaporation rate and the residue levels of these essential oils after treatment.
2. To evaluate the efficacy of dill weed and spearmint essential oils in suppressing potato sprouting in comparison to commercially marketed clove oil (Biox-C^{TM}) and to determine the optimal dose and treatment interval for all of these essential oils.
3. To evaluate the impact of essential oil treatment on subsequent growth and tuber yield of seed potatoes.

2.0 LITERATURE REVIEW

2.1 Potato Plant Development and Tuber Morphology

Potato (*Solanum tuberosum* L.) originates from the Lake Titicaca region in Peru and Bolivia which are the high elevation areas of South America (Hoops and Plaisted 1987). In North America, potatoes are cultivated as an annual cool-season crop in temperate regions, and are commonly propagated from 'seed' tuber pieces (Figure 2.1).

The development of potatoes can be divided into five stages: sprout development stage, vegetative stage, tuber initiation stage, tuber bulking stage and maturation stage (Western Potato Council 2003). The process begins with the sprout development stage where the developing vegetative buds located at the 'eyes' (nodes) grow outward and eventually emerge from the soil. At this stage, the seed piece is the only energy source for growth. Following emergence, all vegetative parts of the plant start to develop, including leaves, stems, roots and stolons. The vegetative stage lasts until tubers start to develop. After 30-70 days of vegetative growth, stolons, lateral stems grow horizontally underground (Figure 2.1), stop elongating and begin to develop into tubers by cell division and cell expansion which marks the beginning of the tuber initiation stage (Fernie and Willmitzer 2001). This is a crucial stage of tuber development which begins when the apical meristem of the stolons becomes dormant and apical dominance is released. The lateral portion of the stolon enlarges first due to cell elongation and longitudinal cell division in the pith and the cortex of the apical region (Xu *et al.* 1998). The timing of tuber initiation depends on planting date, soil temperature, the tuber varieties and the physiological age of the seed tubers. Tuber initiation stage lasts approximately 2 weeks. When the enlarged stolons reach the size of two to four millimeters in diameter, longitudinal cell division is replaced by random cell divisions and cell enlargement (Xu *et al.* 1998). Then newly initiated tubers start to bulk as tuber cells expand with the accumulation of water, nutrients and carbohydrates. This process can last up to three months depending on the cultivar, planting time and growing conditions. The final development stage for a potato plant is the maturation stage as the above ground stems start to turn yellow and the leaves begin to senesce. The rate of photosynthesis gradually decreases and eventually stops as the stems die down. During this time the tuber continues to expand in size, dry matter increases, free sugars are converted to starch and the tuber skin starts to thicken and harden. Due to the relatively short growing season, most potatoes

are harvested before reaching full maturity in the prairie regions in Canada. Each potato plant may initially produce approximately 20 to 30 tubers and, depending on the availability of moisture and nutrients, only 5 to 15 tubers will reach maturity by harvest (Western Potato Council 2003). The developing plant will absorb some the tubers developed initially for additional nutrients and energy.

Each potato tuber is a highly modified stem. The eyes of a potato tuber morphologically correspond to the nodes of a stem. Tuber eyes are arranged spirally on the tuber surface with more buds near the apical end and fewer near the basal end of the tuber (Figure 2.2A). Each eye contains buds which represent the apical or axillary meristems on a stem. The potato buds, from which new growth will emerge, are the most important structures for the plant. The structure of the potato tuber bud is consistent with most meristems, and it contains a distinct layer of tunica cells with a corpus region lying underneath. Studies have shown that excised buds or isolated apical meristems are capable of regenerating a complete shoot apex with sufficient supply of nutrients and hormones (van der Schoot 1996).

A tuber contains structural tissues including skin, cortex, vascular ring, outer and inner medulla (Figure 2.2B). The skin of a potato tuber is constructed with two sections of cells: a layer of epidermal cells on the outside and several layers of periderm cells under the epidermis (Barel and Ginzberg 2008). The red skin colour of varieties such as ‘Norland’ and ‘Sangre’ is due to the pigment contained in the periderm cells. When a tuber is immature, the skin can be rubbed off easily. Skin damage occurs commonly when tubers are harvested prematurely. The cortex is a narrow band of storage tissue located underneath the epidermis and above the vascular ring. Cortex contains mainly protein and starch. The vascular ring connects the tuber and the tuber buds with other parts of the plant and it plays an important role in transporting food products from the above ground portion to the tuber (Western Potato Council 2003). The outer and inner medulla functions primarily as the storage unit of the tuber. A potato tuber contains, on average, approximately 78% water, 22% dry matter and less than 1% fat. About 82% of the dry matter is carbohydrate, including mainly starch and small portions of fiber and simple sugars. Other than carbohydrate, potato also contains at least 12 essential vitamins and minerals, including vitamin C, thiamine, folic acid and iron (van der Schoot 1996).

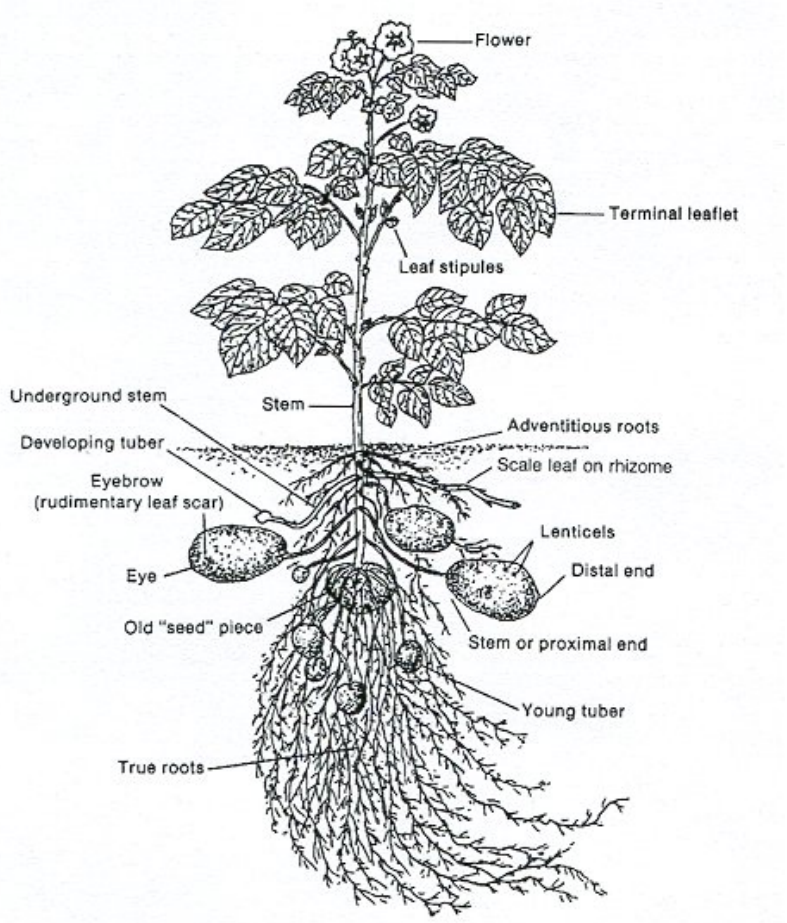


Figure 2.1 Diagram of a potato plant
(Western Potato Council 2003)

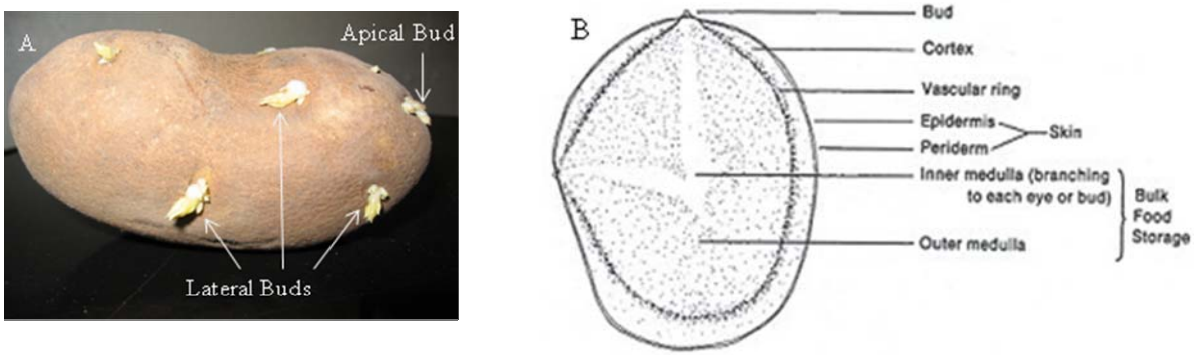


Figure 2.2 A) A sprouted 'Russet Norkotah' tuber illustrating the position of apical and lateral buds. B) Cross-section of a potato tuber
(Western Potato Council 2003)

2.2 The Regulation of Tuber Dormancy and Sprout Growth in Potato

Naturally, a tuber's life cycle starts with tuber initiation, which is followed by growth in size and a period of dormancy. After the dormancy is broken, the tuber buds start to sprout and eventually the cycle ends by generating a new plant from the tuber. However, in agricultural practice, to maintain marketable quality, sprouting is suppressed during postharvest storage, by prolonging natural dormancy or by interfering with sprout growth.

Potato tuber dormancy can be defined as "the physiological state of the tuber in which autonomous sprout growth will not occur, even when placed under favorable conditions for sprouting (darkness, temperature 15-20^{o}C, relative humidity about 90%)" (European Association for Potato Research 1985 cited by Wiltshire and Cobb 1996). Dormancy in potato tubers begins at tuber initiation and terminates when buds are capable of sprouting (Wiltshire and Cobb 1996). During the process of tuberization, the lateral buds on a tuber become dormant sequentially, and the apical bud is the last to become dormant (Fernie and Willmitzer 2001). Thus, at harvest, potato tubers are naturally at a dormant state referred to as innate dormancy or endodormancy (Jefferies and Lawson 1991, Suttle 2000). The innate dormancy is a state of dormancy that prevents the newly harvested tubers from sprouting even under favorable environments (Turnbull and Hanke 1985). After innate dormancy is released, bud growth is normally suppressed by unfavorable environmental conditions, such as low storage temperature, and tubers enter an enforced dormancy or ecodormancy (Lang 1987, Wiltshire and Cobb 1996).

The duration of innate dormancy is largely dependent on the cultivar, the environmental growing conditions, including day length, temperature and water supply, and the postharvest storage conditions, such as temperature, humidity, air circulation and concentration of oxygen and carbon dioxide (Turnbull and Hanke 1985, Claassens and Vreugdenhil 2000). For instance, long photoperiod (18-h light) during tuberization could shorten dormancy by about one week (van Ittersum 1992). Studies have also shown cool and wet growing conditions during tuber formation extend innate dormancy whereas hot (30-32^{o}C) and dry conditions shorten dormancy (van Ittersum and Scholte 1992, Suttle 2000). Moreover, when developing tubers are exposed to low temperature (<3^{o}C) in the field, the stressful conditions could result in premature sprouting due to premature breaking of dormancy (Suttle 2000). Thus, growing conditions appear to be one of the most critical factors influencing the duration of innate tuber dormancy in potatoes.

2.2.1 Mechanisms of dormancy induction and release: metabolic activities

Tuber dormancy in potatoes is triggered by environmental conditions and is regulated through physiological activities. During dormancy, the metabolic processes including respiration, transpiration and translocation are greatly suppressed to preserve energy and resources under stressful conditions. Nevertheless, potato tubers are still metabolically active during any stage of dormancy. Under low temperature storage conditions the tuber respiration rate drops quickly and respiration is maintained at a low level throughout the dormancy period. When sprouting begins, the rate of respiration increases again (Schippers 1977). Changes in respiration rate are relatively small as long as temperatures remain below 10^{o}C. When the storage temperature rises above 10^{o}C, respiration rates are typically increased (Schippers 1977, Wiltshire and Cobb 1996) Thus, potato storage is commonly maintained at constant low temperatures between 4-15^{o}C.

Sudden fluctuations in storage temperature can lead to a rapid increase in respiration (Wiltshire and Cobb 1996). Reconditioning of potato tuber, a common practice in processing tuber storage, is a process which uses this phenomenon to lower the reducing sugar level. When the temperature is increased from 10-20^{o}C, reducing sugars, such as glucose and fructose accumulated during low temperature, are metabolized by glycolysis and respiration. The lowered level of reducing sugars can enhance potato processing quality and prevent dark fry color and bitter taste caused by high levels of reducing sugars (Wiltshire and Cobb 1996).

During dormancy, the outgrowth of tuber buds is most likely restricted by the lack of resources needed for morphogenesis while excised buds obtained from dormant tubers have shown to be capable of growing into a complete shoot apex with sufficient supply of nutrients and hormones. Van der Schoot (1996) indicated tuber buds could communicate with other cells via signaling through plasma membranes or through symplasmic connections. *Vice versa*, tuber buds can also be isolated when the signaling pathways are blocked. The isolation of meristems from the rest of the tuber could limit the supply of substrates and other material required for the outgrowth of the buds. At the dormant stage, cells in the meristematic region are shown to arrest primarily in the G1 state of the cell cycle process as DNA synthesis is nearly absent and the biosynthesis of RNA and protein is highly reduced (MacDonald and Osborne 1988, Campbell *et al.* 1996). Most of the resources needed for morphogenesis are reserved in parenchyma cells and are unable to be transported to the meristems.

During tuberization, storage metabolism aids the developing tuber to accumulate carbohydrates and store as starch. Soon after the tuber is detached from the mother plant, reserve mobilization starts to occur and the tuber shifts from a sink to a source for the tuber buds (Viola *et al.* 2007). In a dormant tuber, over 70% of carbohydrate is stored as starch and sucrose (Viola *et al.* 2007). When sprouts start to develop, in order to continuously supply the building materials for cell division and cell expansion, storage carbohydrate must be converted to soluble sugars such as glucose and fructose (Claassens and Vreugdenhil 2000). Indeed, an overall decrease in starch content in non-dormant tubers was found and this decrease occurred sharply during sprouting (Davies and Viola 1988). Meanwhile, reducing sugars were reported to increase during dormancy release and prior to sprout development (Dimalla and Vanstaden 1977, Bailey *et al.* 1978). The rates of conversion and mobilization are also reflected on enzyme activities. During dormancy, starch is mainly degraded by starch phosphorylase and by amylase to a certain extent; the activities of starch phosphorylase and α-amylase increased prior to sprouting, followed by a decrease (Bailey *et al.* 1978). Davies and Viola (1988) later found that amylase activities decreased initially but α-amylase activity gradually increased during sprouting. In addition to the breakdown of carbohydrates, prior to the outgrowth of sprouts, storage proteins, such as patatin and the 22 kDa storage proteins, break down to free amino acids (Davies and Ross 1984, Suh *et al.* 1990, Brierley *et al.* 1996). This process is likely to be associated with the demand for nitrogen by sprout growth (Davies and Ross 1984, Davies and Ross 1987). Protein, RNA and DNA synthesis occurs throughout the dormancy period in tuber buds. However, the levels of synthesis were shown to increase during dormancy release (MacDonald and Osborne 1988). Alam *et al.* (1994) stated that dormancy release is likely associated with the regulation of protein synthesis, but not controlled by nucleic acid synthesis.

During dormancy release, the isolation of tuber meristematic region gradually diminish as all the metabolites including reducing sugars, amino acids and other dissolved molecules with small molecular mass move toward the tuber buds via diffusion due to chemical gradients. Therefore, the release of dormancy is likely based on the establishment of a sink-source relationship within the tuber as cells develop functional competence to mobilize and transport carbohydrates as well as other nutrients from parenchyma cells (source) to dividing cells in tuber buds (sink) (Sonnewald 2001, Viola *et al.* 2007). Once the source-sink relationships have been

established, the tuber completely releases dormancy and rapid metabolic transitions occur consistently with bud development (Viola *et al.* 2007).

2.2.2 Changes in the levels of plant growth regulators during dormancy and dormancy release

Plant growth regulators, also known as plant hormones, are substances naturally produced by plants to control plant growth and development functions, such as root and shoot growth, flowering and fruit setting and ripening. The impacts of plant growth regulators on regulating potato tuber dormancy have been extensively studied as they are generally considered to be the most important internal factors regulating tuber dormancy (Sorce *et al.* 2005). Endogenous plant hormones regulate tuber dormancy by varying the level of specific hormones or by adjusting the relative concentrations of these hormones. The sensitivity of hormone receptors also changes with physiological aging (Wiltshire and Cobb 1996, Viola *et al.* 2007).

Auxins were the first plant hormones studied as potential regulators of potato tuber dormancy (Suttle 2000). Indole-3-Acetic Acid (IAA), the most important member of the auxin family, is known to stimulate cell expansion and cell division (Goldsmith 1993, Cleland 1995). In potato tubers, auxins are necessary for sprout growth but do not influence dormancy (Wiltshire and Cobb 1996). The levels of endogenous auxins were found to only increase in tubers that had broken dormancy and sprouted (Sukhova *et al.* 1993, Sorce *et al.* 2000). Faivre-Rampant *et al.* (2004) reported a strong up-regulation of potato *ARF6*, a gene encoding a member of the auxin response factor family, in early stages of sprouting, particularly in the peripheral zones of the tunica and corpus of the apical meristem (Faivre-Rampant *et al.* 2004). Sorce *et al.* (2005) reported auxins probably regulate bud development by transporting substantial amounts of IAA from the pith to the meristem during the dormancy period.

Gibberellin Acids (GA) are involved in promoting and maintaining seed germination. Studies have shown GA-deficient mutants of tomato and *Arabidopsis* could not germinate without exogenous GA (Koornneef and Vanderveen 1980, Groot and Karssen 1987). The effect of GA on tuber dormancy was first studied by applying exogenous GA on dormant tubers which broke tuber dormancy (Brian *et al.* 1955, Hemberg 1985). Suttle (2004) later demonstrated endogenous GA levels were equivalent between tubers releasing dormancy and tubers in deep dormancy. Endogenous GA only increased after sprouts started to grow. Thus, GA appears to

play a role only in controlling subsequent sprout growth rather than breaking dormancy (Suttle 2004).

Cytokinin stimulates cell division by releasing a G1 cell cycle block (Francis and Sorrell 2001). As cells in dormant tuber buds are primarily resting in the G1 state (Campbell *et al.* 1996), cytokinins have been identified as dormancy breaking hormones. Within tubers, endogenous cytokinin levels increased at the end of dormancy (Sukhova *et al.* 1993) to stimulate cell division needed for sprout development. Zubko *et al.* (2005) developed a line of transgenic potato tubers with an elevated cytokinin level by over-expressing the *Sho* gene, which encodes an enzyme for cytokinin synthesis. Their transgenic tubers significantly reduced dormancy level. In addition to increasing cytokinin content over the dormancy period, dormant tubers also appeared to develop an increasing sensitivity to cytokinins over time (Turnbull and Hanke 1985, Suttle 2001). Newly harvested tubers are often shown to be insensitive to exogenous cytokinins. Studies have also shown exogenous cytokinins play a role in potato tuber dormancy release. In early 1970s, it was demonstrated that exogenous cytokinins were capable of breaking dormancy and inducing sprouting in dormant potato tubers (Hemberg 1970).

Abscisic Acid (ABA) plays an important role in seed dormancy (Baskin and Baskin 1998, Morris *et al.* 1991). There is evidence indicating ABA inhibits seed germination by interfering with cell wall loosening and thus inhibiting cell expansion (Schopfer and Plachy 1985). ABA is often considered to be a sprout inhibitor in potatoes. In tubers, the highest concentrations of endogenous ABA were found in dormant tubers and its content decreased during storage, which correlated with the gradual loss of dormancy (Suttle 1995). Previous and recent studies have shown that ABA is required for inducing and maintaining dormancy in potato tubers (Suttle and Hultstrand 1994, Ludford 1995, Destefano-Beltran *et al.* 2006). Sorce *et al*. (2005) studied the levels of ABA in the bud tissues and found ABA content increased throughout the dormancy period. However, the critical threshold ABA concentrations required for breaking dormancy in both buds and tubers are still to be identified. It has also been suggested that ABA is not the only factor that controls tuber dormancy (Sorce *et al.* 2005).

Ethylene is another naturally occurring plant hormone with impact on the regulation of dormancy in seeds and other tubers (Suttle 2000, 2004). Exogenous ethylene exposure can alter tuber sprout responses. Suttle (2004) indicated the response of tubers to ethylene treatments appeared to depend on concentration, duration and cultivar. Continuous ethylene treatment has

been shown to suppress sprouting (Rylski *et al.* 1974, Cvikrova *et al.* 1994, Prange *et al.* 1998). In contrast, Rylski *et al.* (1974) showed short-term ethylene treatment promoted sprouting. In plants such as poplar and Arabidopsis the accumulation of ethylene is hypothesized to be a crucial signal that controls the progress of bud dormancy development (Ruttink *et al.* 2007). Other studies have shown that during dormancy release, ethylene interferes with ABA signaling thus promote germination of Arabidopsis seed (Rohde and Bhalerao 2007). Potato tubers produce only limited amounts of ethylene and its functions in tuber dormancy regulation still remains unclear (Suttle 2004).

Several other groups of endogenous compounds were also studied in their relation to potato tuber dormancy regulation, including phenolic compounds, methyl jasmonates and volatile compounds produced by potato tubers. Potato periderm contains considerable amounts of phenolic compounds. Cvikrova *et al.* (1994) demonstrated that phenolic acids were likely participating in the endogenous regulation of tuber dormancy maintenance and release. Their study also showed that the content of free phenolic acids gradually increased during tuber dormancy and reached the peak at the end of dormancy. The loss of tuber dormancy is paired with a reduction in free phenolic acid content and an increase of phenolic conjugate content in tubers. Furthermore, tuber buds with the highest level of free phenolic acids resulted in delayed dormancy break (Cvikrova *et al.* 1994). The role of jasmonic acid derivatives such as methyl jasmonates in tuber dormancy was also studied but not clearly defined (Suttle 2004). However, studies have shown a 12-hydroxy analogue of jasmonic acid, tuberonic acid, was closely associated with tuberization (van den Berg and Ewing 1991). Since tuber dormancy is initiated concurrently with tuberization, jasmonates were suspected to play a part in dormancy induction. Furthermore, potato tubers naturally produce a variety of volatile compounds. Meigh *et al.* (1973) extracted tuber peel samples and identified a group of methylated naphthalenes with sprout-growth inhibiting activity. Individual isomers of dimethylnaphthalene and their mixtures were later shown to be effective in suppressing sprout growth when applied externally (Filmer and Rhodes 1985, Lewis *et al.* 1997). These volatile compounds extracted from potato tubers were mainly studied for sprout suppression.

Van der Schoot (1996) indicated cell-to-cell communication through plasma membranes is controlled by means of growth regulator production, signal receptor density alteration and adjustment of the cell's sensitivity to growth regulators. Some researchers suggested that rather

than being the controller of the dormancy, growth regulators in fact mediate nutrient fluxes (Trewavas 1981, Turnbull and Hanke 1985). Thus, the effects of growth regulators would be dependent on other factors, such as the propagation of secondary messengers and binding receptors. Since the system is constantly changing, the plant's responses to growth regulators are likely to be different each time signalling receptor-binding occurs. This may partially explain why the attempts of using growth regulators in sprout suppression often produce inconsistent or unsatisfactory results.

2.3 Potato Sprout Inhibition in Storage

Premature sprouting in storage can result in substantial economic losses due to weight loss and reduced tuber quality. Prevention of premature sprouting can be achieved mainly by interfering with dormancy breaking processes or by restricting the development of meristems.

Low temperature storage is commonly used to extend the storage period by prolonging the natural dormancy through an enforced dormancy (Wiltshire and Cobb 1996). Cool storage conditions can reduce metabolic activity, respiration rates, and aging in tubers, therefore prolonging natural dormancy. In most commercial storage in North America, after the curing period (a process that stimulates suberization, wound healing and reduces respiration), the tubers are stored at 4-5°C for seed, at 7-10°C for fresh market and at 10-15°C for processing (Western Potato Council 2003). While the respiration rate of potato tubers is the lowest at 2-3°C, low temperatures can cause undesirable cold-induced sweetening by degradation of starch to reducing sugars (Hartmans *et al.* 1995). Under low storage temperatures starch is converted to sucrose. Sucrose is subsequently hydrolysed to hexoses, including glucose and fructose by invertases resulting in an accumulation of these reducing sugars (Sonnewald 2001). Accumulation of reducing sugars is a particular concern for potatoes produced for fresh market and for the processing industry as it causes a browning and a bitter taste (Hartmans *et al.* 1995). Consequently, most tubers are stored between 4-15°C combined with applications of sprout suppressants to avoid sweetening and achieve good sprout inhibition.

Since the growth of the tuber bud (shoot apex) is achieved through cell division and cell expansion, the prevention of sprout growth can be accomplished through interference with cell division and cell expansion. CIPC, the most commonly used sprout suppressant in the market to date, inhibits sprouting by interfering with mitotic cell division. It interrupts the spindle

formation and permanently damages the tuber buds (Nurit *et al.* 1989, Kleinkopf *et al.* 2003). CIPC was first introduced to the market in 1951 and has been one of the most widely used sprout suppressants in commercial storage ever since. It is often applied in storage as an aerosol fog using a thermal applicator at high temperature. The fog is circulated in the storage by its ventilation system (Frazier *et al.* 2004). Other formulas such as spray, dust and delayed-released granules are also available on the market. CIPC is sometimes used as a mixture with propham (isopropyl N-phenylcarbamate or IPC). IPC has the same mode of action as CIPC, but acts faster than CIPC (Meredith 1995a). It is mixed with CIPC to achieve better initial sprout control. A single application of CIPC can achieve long-term sprout inhibition, and it is commonly applied before tubers release natural dormancy (Frazier *et al.* 2004).

Other chemical suppressants used presently are Maleic Hydrazide (MH) and Tecnazene (1,2,4,5-tetrachloro-3-nitrobenzene). MH is an isomer of uracil, a pyrimidine base in RNA (Wiltshire and Cobb 1996). Cremlyn (1978) suggested MH interferes with mitosis by incorporating into RNA. MH is applied to the crop as a foliar spray approximately 2-3 weeks before harvest or vine kill. Timing of MH application is essential for successful sprout inhibition (Wiltshire and Cobb 1996). It must be applied in the field after tuber cell division is completed during tuber developmental stages as MH inhibits cell division but not enlargement. Since it is translocated from the leaves and stems to the tubers, there must be a sufficient amount of time to allow adequate translocation. Tecnazene has been used as a sprout suppressant in UK for more than five years. Tecnazene is volatile, and is applied as a powder or as granules while loading into the storage. Its sprout suppression effect can be compromised if applied after tubers have broken dormancy. Tecnazene appears to prevent cell division and elongation but has no effect on wound healing (Meredith 1995b). However, the mode of action of this compound is not yet well understood.

In recent years, there are growing concerns on the levels of chemical residues, particularly for CIPC, in potato tubers. Most of the concerns are related to their potential adverse impacts on human health, as CIPC may potentially damage liver, kidney, spleen and erythrocytes (Nakagawa *et al.* 2004); and their environmental impacts on ozone depletion (Kerstholt *et al.* 1997). CIPC was reported to be one of the three pesticides found in highest concentrations in the diet of the average American (Gartrell *et al.* 1986). In addition, a study done in the early 1980s showed CIPC comprised over 90% of the total synthetic chemical residues found in US potatoes

(Gunderson 1988). Study has also shown that processing, i.e. extrusion, did not significantly reduce the CIPC residual level in potato peel (Camire *et al.* 1995). In 2002, the allowable residue tolerance in United States on fresh potatoes was reduced from 50 to 30 ppm, and in Europe the residue limit ranges from 5 to10 ppm (Kleinkopf *et al.* 2003). Due to health and environmental concerns, there is a need for safer alternative.

2.4 The Potential of Using Essential Oils and Their Major Components as Potato Sprout Suppressants

Due to the drawbacks of the current sprout suppression methods, naturally occurring compounds, especially essential oils extracted by steam distillation, have received considerable attention (Oosterhaven *et al.* 1995c). These water-insoluble volatile oils normally provide the distinctive scent of the plants from which they were extracted (Buchanan *et al.* 2000).

Most essential oils have low toxicities to humans and the major components are often used for their particular flavor and fragrance characteristics in cooking spices, perfumes and medicines (Vaughn and Spencer 1991). Today, essential oils are also widely used in aromatherapy to promote and maintain health and vitality.

2.4.1 Essential oil production and constituents

Essential oils are produced by a wide variety of plants and serve as a defense mechanism against insects and microorganisms and some have been shown to be involved in allelopathic interactions (Vaughn and Spencer 1991, Rajendran and Sriranjini 2008). These plant essential oils are plant secondary metabolites synthesized, stored and released by a range of epidermal and mesophyll structures such as glandular trichomes, secretory glands, glandular epidermal oil cells and resin ducts. These structures occur in leaves, stems, flowers, roots and seeds with specific attributes to plants (Buchanan *et al.* 2000, Sangwan *et al.* 2001). Essential oil production depends on the physiology of the whole plant, the metabolic state and the developmental stage of the synthesizing tissue (Sangwan *et al.* 2001). The composition of the essential oils may depend on the cultivation site, the cultivar, harvest dates and handling procedures (Hay 1993).

The composition is normally very complex for most essential oils, and the major component contained in these oils typically belong to a class of chemical compounds referred as terpenoids. However, many essential oils also contain compounds like phenylpropanoids

(Sangwan *et al.* 2001). Terpenoids and phenylpropanoids originate from different primary metabolic precursors through different pathways (Sangwan *et al.* 2001).

Terpenoids, which are the majority of components present in essential oils, are synthesized from five-carbon units of isopentenyl pyrophosphate (IPP) and its isomers, dimethylallyl pyrophosphate (DMAPP) (Sangwan *et al.* 2001). The terpenoids are classified according to the number of isoprene units present in the compound. Monoterpenes (C_{10}), containing 2 isoprene units, sesquiterpenes (C_{15}), containing 3 isoprene units, and diterpenes (C_{20}), containing 4 isoprene units, are some examples of terpenoid subclasses. Terpenoid biosynthesis is achieved through two pathways; one in the cytoplasm by the acetate/mevalonate pathway and the other in the plastid by the glyceraldehyde phosphate/pyruvate pathway. The mevalonate pathway produces sesqui- and tri-terpene carbon skeletons while the glyceraldehyde phosphate/pyruvate pathway generates mono-, di- and tetra-terpene carbon skeletons (Buchanan *et al.* 2000). These terpenoid skeletons are often subjected to secondary enzymatic modifications such as oxidation, reduction, conjugation and derivatization, which give rise to the functional properties and chemical diversities of the terpenoids (Sangwan *et al.* 2001).

The compound of interest for potato sprout inhibition, carvone, is an important member of monoterpenes. The majority of the terpenoid compounds commonly found in plant essential oils belongs be the monoterpene subfamily. The monoterpene ten-carbon skeleton is derived from the combination of the active isoprene units, IPP and DMAPP, via the mevalonate pathway which only occurs in plants (Wagner and Ibrahim 2003). There are more than one thousand naturally occurring monoterpenes which have been isolated from higher plants (Oosterhaven *et al.* 1995b). Like other monoterpenes, the carvone synthesis pathway in caraway and spearmint starts with the combination of IPP and DMAPP to form geranyl diphosphate (GPP). GPP is first cyclized to limonene and limonene is then hydroxylated to *trans*-carveol, which is in turn oxidized to carvone (Figure 2.3) (Bouwmeester *et al.* 1998).

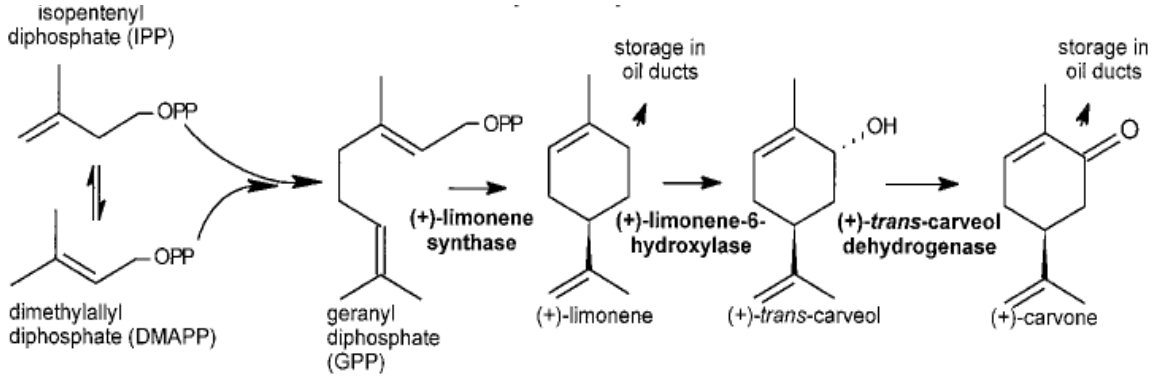


Figure 2.3 Pathway for the biosynthesis of S-(+)-carvone from the precursors IPP and DMAPP in caraway fruit.
(Bouwmeester *et al.* 1998)

Compared to terpenoids, phenylpropanoids are less common in plant essential oils. However, certain species do produce significant proportions of phenylpropanoids and, when present, these compounds normally provide the distinct and essential flavor and odour of the oil. Phenylpropanoids are produced as a defense mechanism against animals and microorganisms and as attractants for pollinators (Sangwan *et al.* 2001). Phenylpropanoids are characterized with one or more "acidic" hydroxyl groups attached to the phenylpropanoid carbon skeleton (C_6C_3) and are derived from phenylalanine and tyrosine, synthesized via the t shikimate pathway (Buchanan *et al.* 2000). The main phenylpropanoids found in certain plant essential oils include eugenol, methyl eugenols, myristicin and methyl cinnamate (Sangwan *et al.* 2001). Eugenol, the active compound in clove oil for potato sprout suppression, is synthesized from phenylalanine. Koeduka *et al.* (2006) suggested basil plants use coniferyl acetate, derived from phenylalanine, and NADPH to produce eugenol under the enzymatic reaction carried out by eugenol synthase.

2.4.2 Factors influencing oil content and composition

Differences in parameters, such as genetics, growth stage and growing conditions, can impact the accumulation and composition of essential oils obtained from natural plant materials (Rajendran and Sriranjini 2008). Barton *et al.* (1989) studied five *Eucalyptus* species and reported that both the oil content and the oil chemical compositions varied greatly among the five species evaluated. The oil content analyses of annual and biennial caraway showed the seed of annual varieties generally produce less oil than the seeds of biennial caraway and have a lower carvone to limonene ratio (Bouwmeester and Kuijpers 1993, Bouwmeester *et al.* 1995). The oil

content and composition of dill and spearmint plants were also found to depend on the seeding time, growth stage and the harvest time (Bowes *et al.* 2004, Kizil and Toncer 2006, Callan *et al.* 2007). In order to obtain the highest essential oil and carvone yield, the optimum harvest time is reported to be the early stage of seed formation before the seeds completely ripen (Yazdani *et al.* 2004). In addition, the carvone content increases from the flowering to the fruit ripening stage (Callan *et al.* 2007). Klzil and Toncer (2006) reported that the essential oil composition of *M. spicata* L. from Turkey produced the highest amount of essential oil when harvested at the full flowering stage.

Light quality and intensity can also affect the synthesis of essential oils. The study of different wavelengths and light level on dill oil synthesis reported the red light treatment resulted in the highest oil concentration and the accumulation of dill oil increased with rising light levels (Halva *et al.* 1992a, 1992b). Photoperiod was reported to directly influence the oil composition in *Mentha* species as long photoperiod increased the concentration of menthol (Voirin *et al.* 1990, Fahlen *et al.* 1997). Photoperiod was predicted to be involved in inducing the menthone-menthol pathway in young leaves (Voirin *et al.* 1990).

Water stress can substantially reduce photosynthesis, decrease plant growth and induce metabolic responses. In spearmint and peppermint water stress resulted in higher quantities of monoterpenes per unit of biomass but reduced the total monoterpene yield due to the overall reduction in plant biomass (Charles *et al.* 1990, Delfine *et al.* 2005). However, the studies showed no compositional change caused by the water stress.

2.4.3 Essential oil content and composition of dill, spearmint and clove oils

Dill (*Anethum graveolens* L.) is a hardy annual aromatic plant that belongs to the Apiaceae family (Yazdani *et al.* 2004). It is native to Asia Minor and the Mediterranean region. In Canada, dill is suited for commercial field production in the prairie regions. Dill has been used for thousands of years, and its seeds are used to flavor soups, salads, meats, cheese, potatoes and pickles (de Carvalho and Fonseca 2006). Dill seeds were also reported to have antispasmodic, carminative, emmenagogue and galactogogue properties (de Carvalho and Fonseca 2006). All parts of the dill plant produce essential oils and the major constituents include carvone, phellandren and limonene (Yazdani *et al.* 2004). Carvone content is a key factor in dill oil extract quality since dill oil trading in North America is based on carvone content in the oil. A carvone

content of at least 30% is considered the minimum acceptable level (Bowes *et al.* 2004). The oil content in dill seed ranges between 2.3 to 3.5%, and 0.4 to 0.8% in dill leaves and stems.

Dill oil can be extracted from the dill herbage, commonly referred as dill weed oil, and from mature seeds which store the oil in canals running parallel to the length of the seed (Bowes *et al.* 2004, Callan *et al.* 2007). Dill weed oil is commonly extracted from the whole plant at purple seed stage through steam distillation. Although both dill weed and seed contain essential oil, the oil content and composition differ distinctively (Santos *et al.* 2002, Bowes *et al.* 2004). The oil content of fresh dill herbage ranged from 0.09-0.93 mL per 100 g, whereas dill seeds contain 0.2-4.6 mL of oil per 100 g fresh weight (Santos *et al.* 2002). Dill weed oil contains approximately 20-40% carvone, 20-30% limonene and 20% phellandrene (Bowes *et al.* 2004, de Carvalho and Fonseca 2006) and dill seed oil was reported to contain 41-67% carvone and 23-44% of limonene (Santos *et al.* 2002). Dill oil quality in North America is based on carvone content and it has to be 30% or higher in order to qualify for trading (Bowes *et al.* 2004), and dill seed oil is generally considered to have better quality as dill seeds contain higher oil content and higher carvone content.

There are two types of spearmint oils that are commercially available on the market, one is extracted from the leaves of the native spearmint (*M. spicata* L.) and the other is extracted from the leaves of the scotch spearmint (*M. cardiaca* L.) (Chowdhury *et al.* 2007). As the name indicates, both spearmint varieties belong to the mint (Lamiaceae) family. Spearmint is a hardy perennial plant commonly known since ancient times as "the herb of hospitality" (de Carvalho and Fonseca 2006). It is native to England and now the majority of spearmint oils are supplied by the US, China and some South American countries (Chowdhury *et al.* 2007). Spearmint is suitable for growing in Canada; however the production is relatively small as it faces large competition with established US growers (Alberta Agriculture and Rural Development 2001). The world demand for spearmint oil exceeded 1500 tonnes per year since 1998. It is commonly used as a cooking ingredient and as a flavoring agent in candy, toothpaste, mouthwash and chewing gum. Spearmint oil is also known for its medical properties as being stimulant, carminative and antispasmodic.

The essential oil of spearmint is characterized by high content of R-(-)-carvone (50-70%). It also contains limonene, phellandrene and dihydrocarveol acetate (de Carvalho and Fonseca 2006). Fresh spearmint herbs contain 0.3 to 0.4% of oil (Chowdhury *et al.* 2007). The oil of

common spearmint and scotch spearmint contains 60 to 70% carvone, but they differ with regard to the remaining components. Scotch spearmint oil contains up to 2% menthone and up to 20% limonene, as opposed to 8 to 15% in native spearmint (Chowdhury *et al.* 2007). In addition, Chowdhury *et al.* (2007) identified 35 components in scotch spearmint oil but only 21 compounds in native spearmint oil.

Clove oil is obtained by steam distillation from the buds, leaves and branches of the evergreen clove tree (*Syzygium aromaticum* or *Eugenia aromatica*), which belongs to the Myrtaceae family. The clove tree is native to Indonesia, where approximately 80% of world production originates. Clove is often used in limited amounts in food, fragrances and cigarettes (Alma *et al.* 2007). Clove oil is active against bacteria and fungi and is also known for its anticarcinogenic, antiallergic and antimutagenic properties (Chaieb *et al.* 2007), and sometimes the oil is also used to eliminate bad breath (Alma *et al.* 2007). Clove oil is largely composed of eugenol (approximately 85%), a compound that has been used by dentists as an analgesic to calm the nerves within the tooth after the removal of deep decay (Burt 2004). Besides eugenol, clove oil also contains eugenyl acetate, β-caryophyllene, α-humulene and hamulene epoxide (Bainard *et al.* 2006). Similar to dill oil, the oil content and the chemical composition of clove oil are dependent on the plant part used for extraction as well as genetic, climatic and cultivation factors (Alma *et al.* 2007). Clove oils comprise more than 20 identified constituents and even though the concentration of the constituents varies based on the growing location, climate and culture practice *etc.*, a large portion of the oil is made up of eugenol, caryophyllene, humulene and eugenyl acetate. The oil quality is primarily based on the content of eugenol (Alma *et al.* 2007). Clove bud oil is generally considered the best quality product with reported oil content of over 80% of eugenol, 8-20% of eugenyl acetate and 2-12 % of β-caryopyllene (Lee and Shibamoto 2001, Alma *et al.* 2007, Chaieb *et al.* 2007). Clove leaf oil contains slightly lower content of eugenol (76.8%); and the main components in clove leaf oil also include β-caryopyllene (17.4%), α-humulene (2.1%) and eugenyl acetate (1.2%) (Jirovetz *et al.* 2006).

2.4.4 Essential oil as a alternative source for sprout inhibition in potatoes

The history of using volatile essential oil components in the inhibition of sprouting goes back many centuries. For generations, the Incas of South America have buried their potatoes in pits covered with soil and the leaves of muña (*Minthostachys glabrescens*) plants. Muña plant is

a member of the mint (Lamiaceae) family (Aliaga and Feldheim 1984). The plant, which naturally grows in the Andes region, from southern Peru to Argentina, contains a essential oil that very effectively inhibits sprouting in potatoes (Vaughn and Spencer 1993). Indeed, oil from muña plants was shown to be more effective than CIPC in reducing potato sprouting, fresh weight loss, and tuber rot over a period of 225 days (Aliaga and Feldheim 1984). Analysis of essential oil extracted from muña plants showed that it contained over 98% of monoterpenes mainly comprise α- and β-pinene, limonene, pulegone, menthone, ismenthone and trans-caryophyllene (Aliaga and Feldheim 1984).

Other than Muña plants, Vokou *et al.* (1993) showed that many aromatic plants, such as dried lavender (*Lavandula angustifolia* L.), pennyroyal (*Mentha pulegium* L.), spearmint (*Mentha spicata* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia fruticosa* L.) plants, as well as their essential oils were all effective in suppressing potato tuber sprouting. The major components in these essential oils include linalool, pulegone, carvone and 1,8-cineole. The study found that the growth and elongation of emerging sprouts were suppressed and the essential oil treatments did not delay sprouting. However, the essential oil treatments did appear to cause higher number of sprouts to emerge after essential oil treatments were removed (Vokou *et al.* 1993). Vaugh and Spencer (1991) evaluated a number of volatile monoterpenes for the sprout inhibition effect using a continuous-flow system which release the volatile compounds into the headspace surrounding the tubers for 7 days. They determined that compounds including 1,4-cineole, 1,8-cineole, fenchone and terpinene-4-ol were very effective in inhibiting sprouting. Limonene oxide and linalool were also potent inhibitors; however, the tubers treated with these two compounds were not as firm as the ones treated with the compounds mentioned above (Vaughn and Spencer 1991). Hartmans and Van Es (1987) cited in Oosterhaven (1995) confirmed the sprout inhibition effect of muña and peppermint oils, and further stated that essential oils obtained from dill (*Anethum graveolens* L.) and caraway (*Carum carvi* L.), containing mainly carvone, were also very effective in suppressing sprouting in potatoes. Beveridge *et al.* (1981) evaluated a range of natural volatile compounds including benzothiazole, 1,4-dimethylnapthalene, carvone, pulegone and borneol as potato tuber suppressants and found benzothiazole and 1,4-dimethylnapthalene were effective at 100 mg kg^{-1} and carvone, pulegone and borneol were effective in suppressing sprouting at 500 mg kg^{-1} when applied as liquids mixed with a solid carrier. Furthermore, menthone, volatile oil components of *Minthostachys*,

Satureja, *Bystropogon*, and *Mentha* species, significantly inhibited sprouting when applied at the dose of 4 μL L^{-1} to non-dormant tubers for two months at 10°C (Coleman *et al.* 2001). The mixture of menthone and neomenthol at the rate of 0.5 μL L^{-1} each also effectively suppressed sprouting of non-dormant tubers (Coleman *et al.* 2001). Other aromatic plants were also reported to be effective in suppressing potato sprouting include lemongrass (*Cymbopogon flexuosus* L., rich in citral), Japanese mint (*Mentha arvensis* L., with high content of menthol and menthone) (Farooqi *et al.* 2001) and basil (*Ocimum americanum* L., containing linalool and methyl chavicol) (Singh *et al.* 1997).

Besides inhibition effects on tuber sprouting, many essential oils and their major components were also reported to inhibit seed germination and root growth. The germination and growth of *Brassica campestris* were suppressed when exposed to cineol for 5 days. The root apical meristem mitotic index of 3-day old seedlings was reduced from 5.6% to 1.6% when exposed to 400 μmol of cineol, thus the proliferation in these root apical cells was inhibited. With the exposure to cineole, the DNA synthesis in the nuclei and the organelles were both inhibited in those cells located in the root apical meristem (Farooqi *et al.* 2000). In an earlier study, Lorber and Muller (1976) reported that exposing cucumber (*Cucumis sativus* L.) roots to purple sage (*Salvia leucophylla* L.) volatiles, mainly containing cineole and camphor, caused disruption of membrane and accumulation of lipid globules in the cytoplasm, reduction in the number of organelles and disruption of membranes surrounding nuclei and mitochondria. The germination inhibition effect of certain essential oil components, such as carvacrol, thymol, carvone and limonene, were also investigated against some common weed species including *Alcea pallid* L., *Amaranthus retroflexus* L., *Centaurea salsotitialis* L., *Raphanus raphanistrum* L., *Rumex nepalensis* Spreng, *Sinapis arvensis* L. and *Sonchus oleraceus* L. (Azirak and Karaman 2008). When applied at concentrations of 62.5 μg mL^{-1} or higher, all compounds tested significantly inhibited the germination of all weed species except for *Alcea pallid* L.

2.4.5 The additional benefits of essential oils in potato storage

In addition to the inhibitory effects on sprout growth, many of the compounds contained in these easily obtainable essential oils have reported to be bactericidal and fungicidal and are active as insect repellants. As tubers age in storage, they become more susceptible to pathogen

infections. In conventional storages, potato tubers are normally treated with both sprout inhibitors and fungicides separately.

Many of the essential oils and their major components were studied for their effects on controlling several fungal and bacterial diseases commonly occurring in potato storage. In an *in vitro* trial, essential oils of caraway, cassia, cumin, dill, and spearmint were shown to be effective in limiting the radial growth of the tuber pathogens *Fusarium solani* var. *coeruleum*, *Fusarium sulphureum*, *Phoma exigua* var. *foveata* and *Helminthosporium solani*, when applied at concentration of 160 ppm (Gorris *et al.* 1994). The essential oils obtained from lavender, peppermint, spearmint, rosemary, sage and Turkish oregano all effectively inhibited the growth of the bacteria *Erwinia carotovora* ssp. *atroseptica*, which is responsible for the black leg disease in potatoes, and *E. carotovora* ssp. *carvotovora*, which can cause potato bacterial soft rot (Vokou *et al.* 1993). The low doses of common basil (*Ocimum basilicum* L.) essential oil completely inhibited the growth of 22 different fungal species (Vaughn and Spencer 1991). Common basil oil mainly contains linalool and 1,8-cineole. Both caraway oil (mainly contains carvone) and clove oil (contains 85% of eugenol), at concentrations of 0.6 and 0.8 mg mL^{-1} respectively, were reported to be effective in inhibiting the growth of *Aspergillus parasiticus*, a mold can grow on a wide range of agricultural commodities (Farag *et al.* 1989, Song *et al.* 2008).

Many *in vitro* and *in vivo* studies have also focused on the effects of the major compounds contained in the common essential oils. Vaughn and Spencer (1994) determined that both volatile compounds cineole and menthol can inhibit the growth of *Fusarium sambucinum*, a fungus that can cause potato tuber decay in storage. A treatment of 1 to 3 mmol of carvone was able to inhibit the growth of plant-pathogenic fungi *F. solani* and *F. sulphureum*, as well as the growth rate of bacteria *Streptococcus thermophilus*, *Lactococcus lactis* and *Escherichia coli* (Oosterhaven *et al.* 1995c). In a trial carried out in a 15-tonne bulk storage, potato tubers exposed to carvone vapor had significantly reduced silver scurf index, caused by *H. solani* (Hartmans *et al.* 1995). Bång (2007) researched the antifungal effects of various essential oils for both *in vitro* and *in vivo* conditions on *H. solani*, *F. solani* var *coeruleum*, *Phoma exigua* var. *foveata* and *Rhizoctonia solani* (*in vitro* only). The results showed that in most cases the essential oil of garlic, *Allium sativum* L., displayed effective antifungal effects on all 4 species. Bång (2007) also found conflicting results between *in vitro* and *in vivo* studies caused by thyme (*Thymus vulgaris* L.) and sage (*Salvia officinalis* L.) volatiles. Thyme essential oil suppressed the

growth of all four pathogens *in vitro*, but failed to control *H. solani*, *F. solani* and *P. foveata in vivo*. In contrast, sage volatile was ineffective against *H. solani* and *P. foveata in vitro*, but was effective when tested on infected tubers. The diverging results could possibly be due to the lipophilic compounds in essential oils being converted to less toxic substances by the host as a defense mechanism (Bång 2007). The bioconversion of essential oil components were reported in studies conducted on strawberries (HamiltonKemp *et al.* 1996, Archbold *et al.* 1997) and potatoes (Oosterhaven *et al.* 1995a).

Many essential oils are naturally produced as a self-defense mechanism and contain compounds showing ovicidal, repellent and toxic effects on insects (Nawrot and Harmatha 1994, Isman 2006). There is particular interest in using essential oils and their constituents as potential fumigants for stored food commodities (Rajendran and Sriranjini 2008). A sprout inhibitor Talent^{TM}, containing mostly S-(+)-carvone as the active ingredient, was shown to be effective on preventing slug (*Deroceras reticulatum*) damage in potato storage (Ester and Trul 2000). Essential oil obtained from dill seed, which mainly contains S-carvone and R-limonene, completely suppressed the F1 generation of the Curculionid beetle, *Sitophilus oryzae*, at a concentration of 2000 ppm (Su 1989). Dill seed extract was also demonstrated high repellency against the confused flour beetle (*Tribolium confusum*) (Su 1987). Monoterpenes, such as carvone, pulegone and cineole, were effective against the rice weevil (*Sitophilus oryzae*) (Rajendran and Sriranjini 2008). Rice and Coats (1994) tested the toxic effect of a wide range of monoterpenoids and derivatives on house fly (*Musca domestica*), and concluded that monoterpenoid ketones were more insecticidal than the corresponding alcohols. There are also reports indicating that monoterpenoids can inhibit the activity of acetylcholinesterase enzyme (AchE), the key enzyme in the breakdown of acetylcholine, a neurotransmitter in both the peripheral and the central nervous system in many organisms; and thus cause insect mortality (Rajendran and Sriranjini 2008). A patent has been filed to register spearmint oil as a deer repellent. The spearmint oil was mixed with an oil extender and water and applied as foliage spray. The natural repellent can prevent deer from eating sprayed fruits, vegetables and ornamental plants (Mueller 2002).

Essential oils and their major components with inhibitory effects have low mammalian toxicity (Vaughn and Spencer 1991). For instance, the oral LD_{50} values for rats are 3.50, 2.84, and 4.41 mg kg body weight^{-1} for caraway, thyme and peppermint respectively. The LD_{50} values

for some of the active components are 0.81 mg kg body weight^{-1} for carvacrol, 2.48 mg kg body weight^{-1} for 1,8-cineole, 4.60 for limonene and 2.79 mg kg body weight^{-1} for linalool (Rajendran and Sriranjini 2008). In fact, certain compounds are commonly used in our daily life. For example, 1,8-cineole is used in expectorants and cosmetics and carvone is often used as a flavor ingredient in toothpaste, mouthwash and in certain medicines (Rajendran and Sriranjini 2008). Carvone is labeled as GRAS by the Flavoring Extract Manufacturers' Associated, and it has been approved by the Food and Drug Administration to be used in food since 1961. The daily intake level for carvone is to be 1.25 mg kg^{-1} body weight (Oosterhaven 1995). Therefore, the concern regarding human toxicity related to consuming essential oil-treated potatoes should be minimal.

2.4.6 The inhibitory mechanism of monoterpenes contained in essential oils

The investigations devoted on the mode of action of essential oils have been mainly focused on monoterpenes. A range of effects have been reported regarding the effect of monoterpenes on plants, microorganisms and insects, including inhibition of respiration, suppression of DNA synthesis and interactions on specific enzymes (Lorber and Muller 1980, Farooqi *et al.* 2000, Rajendran and Sriranjini 2008). However, due to the structural diversity of the compounds, it is unlikely that all monoterpenes have one specific mechanism and there are several targets in the cell (Oosterhaven 1995, Burt 2004). Nevertheless, several general trends were reported as potential mechanisms affecting the inhibitory effect of monoterpenes which include compound volatility, lipophilicity and the presence of certain functional groups.

Vaughn and Spencer (1991) identified several monoterpenes, including 1,4-cineole, 1,8-cineole, fenchone, limonene oxide, linalool and terpinen, with phytotoxic effects on potato sprouts. However, they did not find any single structural functional group or chemical factor specifically associated with phytotoxicity. The authors suggested that volatility of the compound may play a role in the level of phytotoxicity. In general, the more volatile \, such as 1,4-cineole, 1,8-cineole and fenchone, were more phytotoxic than those compounds, such as citral, citronellol, geraniol, pulegone and α-terpineol, with less volatility.

It was also suggested that certain monoterpenes might disrupt cell membranes by acting as a solvent. Vaughn and Spencer (1991) showed that a short-term exposure (24 hr) caused no visible injury on emerged tuber sprouts when treated with less volatile monoterpenes, but mild necrosis occurred on the tissue when exposed for 7 days. Membrane disruption was also

demonstrated on cucumber seedling roots when exposed to purple sage volatiles, primarily cineole and camphor (Lorber and Muller 1976). Knobloch *et al.* (1989) cited by Oosterhaven (1995) evaluated the antibacterial and antifungal properties of essential oil components, and concluded that the lipophilic properties of the oils are related to the membrane damage of the exposed cells. The lipophilicity of essential oils constituents enables the molecules to partition the lipids of the cell membrane and mitochondria, which causes increased membrane permeability and results in leakage of ions and other cell contents (Sikkema *et al.* 1994, Oosterhaven *et al.* 1995c, Cox *et al.* 2001, Burt 2004). Severe leakage can eventually lead to cell death (Burt 2004). As summarized in Figure 2.3, in a exposed cell the action of the monoterpenes could include degradation of cell wall (Vaughn and Spencer 1991, Helander *et al.* 1998), damage to cytoplasmic membrane (Sikkema *et al.* 1994, Oosterhaven *et al.* 1995c, Cox *et al.* 2000), damage to membrane protein (Ultee *et al.* 1999), leakage of cell contents (Oosterhaven *et al.* 1995c, Cox *et al.* 1998, Helander *et al.* 1998) and depletion of the proton motive force (Ultee *et al.* 1999).

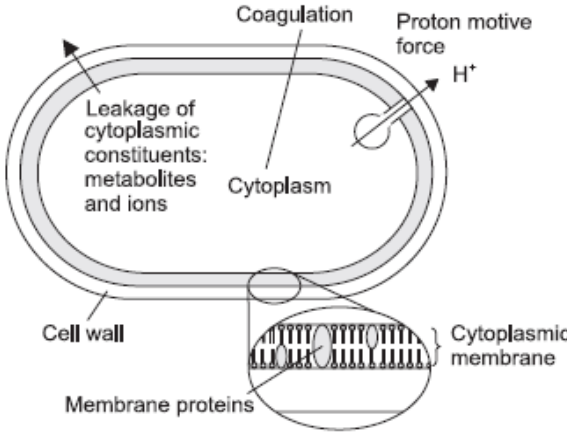


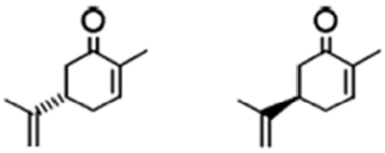
Figure 2.4 The projected activation sites and mechanisms of essential oil components in a cell expose to monoterpene compounds.
(Burt 2004)

The presence of certain specific functional groups is also believed to be associated with the phytotoxicity of the monoterpenes (Oosterhaven 1995). In general, monoterpenoids with a hydroxyl group (-OH) had the highest inhibition effect, followed by compounds containing a carbonyl group (O=CH- or O=C), whereas hydrocarbons were the least effective ones. Reynolds (1987) showed the association between the lipophilic nature of monoterpenes and their inhibitory activity on lettuce seed germination. The authors concluded that the strongest germination

inhibitors were oxygenated terpenes and that hydrocarbon monoterpenes produced the least inhibition. A similar conclusion was also presented from a study using monoterpenoids to control dried bean beetle (*Acanthoscelides obtectus*), where oxygenated monoterpenoids (such as carvacrol, linalool and terpineol) had higher toxicity against the adult beetles (Regnault and Hamraoui 1995). Capelle *et al.* (1996) also proposed that the presence of an unsaturated ketone group in compounds like carvone could play an important role in inhibiting sprout growth.

2.4.7 Carvone - the primary constituent of dill weed and spearmint oils

Carvone, 2-methyl-5-(1-methylethenyl)-2-cyclohexene-1-one, is a member of monoterpenes and it is one of the most studied monoterpene to date for its effect on sprout growth suppression (de Carvalho and Fonseca 2006). Its molecular formula is $C_{10}H_{14}O$, with a molecular weight of 150 g mol^{-1} and a specific gravity of 0.96 kg L^{-1} at 20°C. It is a colourless volatile liquid, slightly soluble in water (Capelle *et al.* 1996). It can be found in many natural plant extracts, such as caraway, dill and mint oils. Carvone contains two enantiomers: S-(+)-carvone and R-(-)-carvone (Figure 2.5). S-(+)-carvone is the major compound in caraway seed oil extract (50-70%), dill seed oil extract (40-60%) and dill weed oil extract (40%) (Hartmans *et al.* 1995, de Carvalho and Fonseca 2006). R-(-)-carvone, which smells similar to spearmint, is present in spearmint at a level greater than 50% (de Carvalho and Fonseca 2006).



S-(+)-Carvone R-(-)-Carvone

Figure 2.5 Enantiomers of carvone [S-(+)-carvone and R-(-)-carvone]. (Schlyter *et al.* 2004)

Carvone, particularly S-(+)-carvone, has been shown to be effective in suppressing sprouting both on small and large-scale studies with different apparatuses (Vaughn and Spencer 1991, Hartmans *et al.* 1995, Oosterhaven *et al.* 1995a, Oosterhaven *et al.* 1995c). Meigh (1969) first showed carvone could effectively suppress sprouting when applied as vapor at a constant concentration. Later studies supported their findings and further found the effect of carvone was

dependent on concentration and that the treatments should ensure a low, stable headspace concentration around the tubers (Hartmans *et al.* 1995, Sorce *et al.* 1997, Cizkova *et al.* 2000). As a vapor, carvone eventually disappears from storage mainly due to leakage, ventilation, absorption by the tubers and building materials, or when metabolized by tubers and microorganisms (Hartmans *et al.* 1998). Therefore, repeated applications are necessary to maintain the storage headspace concentration above a threshold level for a sufficiently long period of time (Kleinkopf *et al.* 2003). Osterhaven *et al.* (1995a), using systems consisting of sprouts growing from potato eyepieces, showed that applications of 250 μL of carvone applied to 30 eyepieces in a sealed 20 L tray (12.5 μL L^{-1} headspace), reduced sprout growth at varying rates following a 2-4 days exposure, but did not completely eliminate growth. Seven days of treatment completely inhibited sprout growth throughout the experiment. As the inhibition effect caused by carvone is not permanent, the potential of using it in seed tuber storage was also studied by Sorce *et al.* (1997). The author concluded that headspace concentrations within the range of 0.34-1.06 μmol mol^{-1} were most effective in inhibiting sprouting in seed tubers.

In 1994, a biological sprout inhibitor Talent™ was commercially marketed as a sprout inhibitor in Holland (Wiltshire and Cobb 1996). Talent™ contains 95% of the S-(+)-carvone isomer in a liquid formulation; and the recommended application rate is 600mL ton^{-1} for effective sprout inhibition (Kerstholt *et al.* 1997). In a large scale study, Hartmans *et al.* (1995) found a 100 mg kg^{-1} S-(+)-carvone treatment followed by a 42-hr ventilation free period applied every 6 weeks to 15 tonnes of tubers in a semi-large storage facility was able to successfully suppress sprouting for 6 months.

It was recommended to apply carvone treatments after curing. A study conducted on wounded tuber tissues showed the presence of S-(+)-carvone prevented the activity of suberization and cambial layer formation (Oosterhaven *et al.* 1995b). However, after the S-(+)-carvone and its bioconversion products were completely depleted from the tissue and the atmosphere, both processes were restored.

Several studies also investigated the potential of applying carvone in solid or liquid form (Beveridge *et al.* 1981, Beveridge *et al.* 1983, Cizkova *et al.* 2000, Song *et al.* 2008). Beveridge *et al.* (1981, 1983) applied carvone as a liquid mixed with aluminum solid carriers. They found carvone at a concentration of 100 mg kg^{-1} of potato did not prevent sprouting, but at 500 mg kg^{-1} sprouting was successfully suppressed. Both carvone isomers, when mixed with perlite powder,

also effectively inhibited sprout growth of 'Norland' tubers for 8 weeks at 10°C when treated at the doses ranging from 300 mg kg^{-1} to 500 mg kg^{-1} of fresh potato (Song *et al.* 2008). Liquid application of 4% aqueous solution of carvone, using caraway or dill seed extracts as sources, with a double application completely inhibited sprouting for at least 15 weeks at 10°C in both 'Snowden' and 'Norland' (Song *et al.* 2008). However, Cizkova *et al.* (2000) noted that directly spraying none-diluted carvone onto the tubers could cause necroses and rotting on the tuber surface.

Studies conducted on spearmint (*Mentha spicata* L.) showed that the primary constituent of spearmint, R-(-)-carvone, can also effectively prevent potato sprouting during storage (Frazier *et al.* 1998, Frazier *et al.* 2000, Frazier *et al.* 2004). Oosterhaven *et al.* (1995a) compared the efficacy of the two isomers and found that S-(+)-carvone is a more potent inhibitor than R-(-)-carvone, because S-(+)-carvone inhibited sprout growth after treating the non-dormant tubers for two days while it took R-(-)-carvone four days to suppress the elongation of the sprouts. The difference was likely caused by the differential uptake rates between S-(+)-carvone and R-(-)-carvone since the endogenous concentration of S-(+)-carvone and its derivatives were twice as high as R-(-)-carvone-treated sprouts after four days exposure. The same stereospecific effects were also found in apple seed germination (Reynolds 1987). Apple seed germination was reduced by 50% when treated with 0.058 mmol S-(+)-carvone and the equivalent inhibitory effect was achieved by using 0.38 mmol of R-(-)-carvone. Pathirana *et al.* (1992) investigated the chiral recognition of carvone isomers by phospholipid monolayers and found that when compressed at 30°C, monolayers with S-(+)-carvone absorbed twice as much heat and underwent a larger entropy change than monolayers with R-(-)-carvone.

Thus far, all studies have confirmed that with repeated applications at certain concentrations, both isomers of carvone can effectively inhibit sprouting for a considerable period of time. Although S-(+)-carvone appears to act upon sprout inhibition faster than its isomer, both carvone isomers may have potential to control sprouting of stored seed potatoes.

2.4.8 Mode of action of S-(+)-carvone

According to Oosterhaven *et al.* (1993, 1995c), S-(+)-carvone plays a role in enhancing the degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), a rate controlling enzyme of the mevalonate pathway, a crucial pathway for the biosynthesis of

cytokinins, gibberellic acids, abscisic acid, membrane components and photosynthetic components (Oosterhaven *et al.* 1993, Oosterhaven *et al.* 1995b).

The possible mode of action of S-(+)-carvone at a molecular level was first elucidated in animal studies. A study conducted on rats showed that cyclic monoterpenes, like cineole or menthol, reduced the activity of HMG-CoA reductase (Clegg *et al.* 1982). The enzyme catalyzes the rate limiting reaction in the mevalonate pathway (Goldstein and Brown 1990). The mevalonate pathway is important for the production of a large number of isoprenoids and their derivatives that are vital components for diverse cellular functions ranging from cholesterol biosynthesis to growth control. The blockage of the pathway in animal cell lines resulted in a loss of protein synthesis and an arrest in cell cycling (Siperstein 1984, Sinensky and Logel 1985). In plants, mevalonate pathway or HMG-CoA reductase pathway is important for the production of many important secondary metabolites including plant hormones like ABA, GA, cytokinins and membrane components (Bach 1987, Bach *et al.* 1991, Weissenborn *et al.* 1995, Bach *et al.* 1999). Therefore, HMG-CoA reductase plays a vital role in plant growth and development. When radish seedlings were treated with an HMG-CoA reductase inhibitor, mevinolin, mevalonate starvation resulted in a complete inhibition in root elongation (Bach and Lichtenthaler 1983).

In potato, S-(+)-carvone was proposed to act as an intermediate leading to enhanced degradation of HMG-CoA reductase. The impairment of HMG-CoA reductase activity was correlated with the disappearance of the enzyme (Oosterhaven *et al.* 1993, Oosterhaven *et al.* 1995b). However, it was found unlikely that the reduction in HMG-CoA reductase activity was caused by a direct effect of S-(+)-carvone on the enzyme itself, as the addition of S-(+)-carvone at concentrations ranging from 0.01 to 1μmol in the HMG-CoA reductase assay system did not decrease enzyme activity. The reduction in enzyme activity appeared to increase with time. When potato sprouts were treated with S-(+)-carvone for one day, the HMG-CoA reductase activity was partially inhibited. After a 4-day exposure, the activity was inhibited completely while the HMG-CoA reductase mRNA-level was not affected.

In addition, based on the lipophilic characteristics of S-(+)-carvone, Oosterhaven *et al.* (1993) proposed that S-(+)-carvone interacted with the membrane system of the plant cell, possibly by changing the membrane fluidity, thus the lipid micro-environment of HMG-CoA reductase was altered resulting in an enhanced degradation and/or a disturbed insertion of the

protein in the microsomal membrane. However, if this hypothesis was true, it is unlikely that enzyme HMG-CoA reductase would be the only enzyme affected by the disruption of membrane fluidity. In addition, the increasing inhibitory effect of carvone against *E. coli*, *S. thermophilus* and *L. lactis* associated with the carvone treatment concentration also indicated that carvone might be involved in disturbing the metabolic energy status of cells (Oosterhaven *et al.* 1995b).

2.4.9 Eugenol – the primary constituent of clove oil

Eugenol (hydroxyl-1-methoxy-2-allyl-4-benzene; $C_{10}H_{12}O_2$) is the major component in clove oil (Figure 2.6), and may cause physical or chemical damage to sensitive sprouting tissues according (Kleinkopf and Frazier 2002). The molecular weight of eugenol is 164 g mol^{-1} and its density is 1.06 g cm^{-3}. It is a member of phenylpropanoids. It is commonly used in dental offices as an antiseptic and analgesic and is the compound giving the characteristic odor to dental clinics. Eugenol has also demonstrated anti-inflammatory, anti-bacterial, anesthetic and neuroprotective properties (Alma *et al.* 2007, Guenette *et al.* 2007). In addition, it was reported for its antioxidant (Nassar *et al.* 2007), insecticidal (Park *et al.* 2000) and herbicidal effects (Tworkoski 2002).

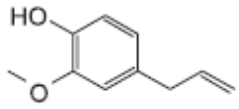


Figure 2.6 Chemical structure of eugenol.
(Burt 2004)

Limited information has been published on the sprout inhibitory effect of clove oil or eugenol on potato tubers. However, in the US, there are several products registered for sprout control based on clove oil and its primary component, including Biox-C™, Biox-A™ and Sprout Torch™. Biox-C™ contains 100% of clove oil, of which approximately 60% is eugenol (Kleinkopf and Frazier 2002). It is recommended to be used anytime after potatoes are placed into storage and can be applied through a Forced Air Distribution System at a rate between 27-87 ppm, which equals 1 US gallon Biox-C™ per 1000-3200 cwt (0.03-0.09 g kg^{-1}), with repeated applications when deemed as necessary. Biox-A™ is a new product using more purified eugenol (99%), and it has been registered in Idaho, Washington, Oregon, Colorado, California, Texas and Florida for potato sprout control (Kleinkopf and Frazier 2002). Sprout Torch™ is another product

containing 100% clove oil. It is recommended for use in pre-shipping temporary treatment of emerging sprouts and not to be used as a sprout inhibitor. Sprout Torch™ can be applied using thermo-fogging equipment at the rate of 1 US gallon per 1000-3500 cwt (0.03-0.09 g kg^{-1}).

The effects of these clove-based products have been evaluated by a group of researchers at the Kimberly GRandE Central Potato Research Facility at the University of Idaho. Kleinkopf and Frazier (2002) showed sequential Biox-A™ treatment (90 ppm application with a follow up application of 30 ppm three weeks later) could extend sprout inhibition to about 60 days without causing adverse effects on glucose/sucrose content and fry color. Frazier *et al.* (2004) showed Biox-C™ also has effective sprout control properties when applied as a thermal fog on regular basis (two to three week intervals) with an initial rate of at least 5.2 lb per 1000 cwt (0.05 g kg^{-1}). Similar to essential oil treatments containing carvone, repeated applications are required for clove oil to achieve prolonged sprout-free storage periods. However, their research results were not always consistent. The study presented in another report in which 90 ppm of fog application followed by six repeated applications of 30 ppm controlled 'Russet Burbank' sprouting at 7°C at a small scale, but resulted in increased sprout length when applied to 1-ton boxes of potatoes (Frazier *et al.* 2006). Alternate thermal aerosol treatments (Biox-C™: 67 ppm initial, then 23 ppm at 3-week interval; Biox-A™: 55 ppm initial, then 20 ppm at 3-week interval) significantly reduced the sprout weight of tubers stored in 1-tonne boxes for 8 months (Frazier *et al.* 2006). Both Biox-A™ and Biox-C™ effectively suppressed the growth of fungal pathogens including *Alternaria solani*, *R. solani* and *F. sambucinum*, *F. coeruleum*; and both clove oil products demonstrated inhibitory effect against *H. solani* (Kleinkopf and Frazier 2002, Frazier *et al.* 2004, Frazier *et al.* 2006).

The mode of action of eugenol on newly emerged potato sprouts has not been studied in great detail. However, based on bactericidal, fungicidal and herbicidal studies, the severe damage on the tissue is likely due to eugenol causing damage on cell membranes and subsequently resulting in loss of in membrane permeability (Thoroski *et al.* 1989, Cox *et al.* 2001, Tworkoski 2002). The inhibition in amylase and protease production was found in food-borne bacteria *Bacillus cereus* when exposed to sub-lethal eugenol (Thoroski *et al.* 1989). In addition, the hydroxyl group on eugenol is thought to be a key structural factor for its inhibitory properties (Wendakoon and Sakaguchi 1993). It was suggested that the hydroxyl group might bind to proteins to prevent enzyme action in the bacterium *Enterobacter aerogenes*.

2.5 Rationale of the Study

A wide variety of essential oils and their major components, primarily monoterpenes, demonstrated great potential to be used as alternative potato sprout suppressants with additional fungicidal and insecticidal effect. By far, most of the research has focused on studying the suppression effect of pure extracts of the active monoterpene compounds such as S-(+)-carvone and R-(-)-carvone (Vaughn and Spencer 1991, Vokou *et al.* 1993, Hartmans *et al.* 1995, Oosterhaven *et al.* 1995c). These purified compounds are relatively expensive and not readily available in large quantities. Dill and spearmint essential oils can be extracted via simple steam distillation and the essential oils contain significant amount (>40%) the S-(+)-carvone and R-(-)-carvone respectively. These plants are commonly growing on the prairies as herbs and these plant extracts can be easily obtained via relatively simple steam distillation process. The current study investigated three types of essential oils: dill weed, spearmint and clove oils, the first two of which were produced in Southern Alberta. Exploring the potential of using dill and spearmint oils to suppress sprouting of potatoes, particularly for organic growers, could not only provide desirable alternatives, but also provide new opportunities for the essential oil industry in Alberta. With the GRAS status, these essential oils meet the USDA organic standers (Frazier *et al.* 2004) and should be allowed to be used on potatoes. Using essential oils could also favour potato exports to Europe and other countries where CIPC residues are a concern.

Essential oils contain a mixture of compounds and the consistency in the composition of the distillated essential oils is critical in order to consistently and effectively suppress sprouting. However, the composition of essential oils may be influenced by factors such as growing conditions, method of extraction, time of extraction and plant part used (Rajendran and Sriranjini 2008). From a practical perspective, a desirable product should be able to achieve dependable and uniform sprout inhibition in storage year after year. Therefore, it is important for the essential oil suppliers to proved oils with consistent quality from year to year by adopting reliable extraction techniques. It is also important to have a good understanding of the physiochemical property of essential oils and how they would function under practical storage conditions. The current study investigated the composition of the selected essential oils obtained from different years, as well as their evaporation rate at low temperatures and residual dissipation rate under ventilation.

While the sprout suppression effect of many essential oil and their major compounds have been studied, the optimal treatment doses have not been clearly defined, especially in the case of dill and spearmint oil. This information will be important for potentially marketing these oils to commercial potato producers in the future. Therefore, the current study investigated the potential of using essential oils extracted from dill and spearmint plants, in a storage system that mimicked the actual storage conditions in terms of storage temperature, relative humidity (RH) and ventilation. The objective of this component of study was to determine the optimal combination of application dose and frequency that would minimize sprouting in two potato varieties. The results were compared with those of untreated control tubers and by tubers treated with commercially marketed clove oil product Biox-C™.

The sprout suppression caused by essential oils is reversible, and sprouts will start to grow again once the treatments were terminated or when the concentration of the treatment in the headspace is below the threshold (Kleinkopf *et al.* 2003). Therefore, essential oils may be useful as sprout inhibitors of potatoes destined to be used as seed. This would provide seed growers with an additional tool to manage sprouting. However, to be used in seed tuber storage, one must assure that the essential oil treatments will have no adverse effects on seed tubers. The current study addressed this concern by evaluating the growth and yield of potato plants generated from essential oil treated seeds.

3.0 CHARACTERIZATION OF ESSENTIAL OILS

3.1 Introduction

Essential oils contain a mixture of monoterpenes as well as phenylpropanoids. Dill weed oil mainly contains 20-40% S-(+)-carvone, 20-30% limonene and 20% phellandrene; spearmint oil contains mainly 60-70% R-(-)-carvone, 10-20% limonene and approximately 2% menthone (de Carvalho and Fonseca 2006, Chowdhury *et al.* 2007). The proportion at which each compound is present in any particular oil may vary considerably, depending on the genotype of the plant material, the harvest stage, the plant part used, the extraction method, and on the environmental conditions during plant growth (Bowes *et al.* 2004, Kizil and Toncer 2006, Callan *et al.* 2007, Rajendran and Sriranjini 2008). The essential oil-induced inhibition effect is largely dose-dependent (Hartmans *et al.* 1995, Sorce *et al.* 1997, Cizkova *et al.* 2000). Variations in the oil composition, particularly the composition of the active compounds, could have a large impact on the efficacy of the treatments. It is, therefore, imperative to analyse the composition of any essential oil to be used for the purpose of a scientific investigation.

Differences in the chemical profile of these oils may not only influence the effect on sprout inhibition, but also determine physical properties, such as evaporation and absorption rates which are important characteristics particularly when vapour form treatments are implemented. While some compounds may not be volatile enough to reach the targeted headspace concentration, other compounds may be too volatile and quickly diffuse away from the storage vessel. Beveridge *et al.* (1983) showed the headspace concentration of carvone as well as other compounds including pulegone and 1,4-dimethylnaphthalene increased with increasing initial application rate (from 100 mg kg^{-1} increase to 500 mg kg^{-1}). Under their experimental conditions, the compounds did not behave as expected. The vapor pressure and the equilibrium headspace concentration varied depending on the nature of the compound and the temperature. Also, during the course the treatment, headspace concentration fell below the constant vapor concentration, previously shown to be effective, with corresponding renewed sprout growth (Beveridge *et al.* 1983). In another sprout inhibition study, Vokou *et al.* (1993) reported that less than 50% of essential oil evaporated over a 5-week storage period at 14-23.8˚C and they also found that more hydrocarbons evaporated compared to the oxygenated compounds.

Therefore, it is important to monitor the rate of evaporation for each oil to better explain the success or failure of the vapour treatments.

In addition, the essential oil active components are predicted to suppress sprouting by reducing plasma membrane integrity which in turn causes cell death (Sikkema *et al.* 1994, Oosterhaven *et al.* 1995c). Consequently, a portion of essential oil vapor is absorbed and remains in treated tubers. The residual level would be a particular concern for growers since the oils have a strong odor and may decrease tuber market value. Thus, it is also important to determine the residual level in treated tubers and to estimate the ventilation time required to allow sufficient reduction of oil residuals.

Currently, dill and spearmint production are very limited on the prairies due to limited demand. If an additional use for these crops can be found, it provides additional value to the growers as well as another market for local extraction industries such as Corraini Essential Oil Ltd., Bow Island, Alberta, Canada.

The objectives for this study were to: a) evaluate the composition of dill weed and spearmint oils extracted from crops harvested in different years and two batches of clove oils supplied; b) determine the evaporation rates for all the essential oils applied; c) to examine the residue level of the major components of each essential oil in treated tubers under continuous ventilation.

3.2 Materials and Methods

3.2.1 Determination of the essential oil composition

3.2.1.1 Source of materials

All dill weed and spearmint oils used in this study were produced by Corraini Essential Oil, Ltd., located in Bow Island, Alberta. Clove oil, marketed as Biox-C^{TM} oil, was provided by Pace International, LLC. Seattle, US. The standards for α-phellandren, carvone, limonene, eugenol and *trans*-caryophyllene were purchased from Sigma-Aldrich, Canada.

3.2.1.2 Essential oil major compound identification

Retention time

The major compounds of each essential oil were first identified by comparing the retention times of the sample and the compound standards. The major compound elution profile was first

established by testing each standard sample. A 10 μL mL^{-1} sample of α-phellandren, carvone, limonene, eugenol and *trans*-caryophyllene standards were prepared by pipetting 10 μL of pure compounds into glass chromatographic vials containing 1 mL of hexane. Dill weed, spearmint and clove oil samples were prepared at the concentration of 10 μL mL^{-1} using the same sample preparation method. Three replications were separately prepared and analyzed for each type of oil. All samples were run in a GC Agilent 6890 system at the University of Saskatchewan. The GC system was equipped with stationary phase fused silica capillary column SP 2560 (60 m x 0.25 mm i.d., film thickness 0.25μm). Mobile phase consisted of GC grade helium. The injection volume was 1μL and the split ratio was 100:1. The oven temperature was programmed initially at 80°C for 5 min, and ramped up 8°C min^{-1} until reaching 200°C and then maintained at this temperature for 15 min. The FID detector temperature was 250°C.

Spiking

The identities of the major compounds were then confirmed by spiking the sample with each standard individually. For each essential oil, 2 μL mL^{-1} stock solutions were prepared by dissolving each essential oil sample in hexane. Three 1 mL dill weed oil samples were taken from the stock solution and separately spiked with 1 μL of the α-phellandrene, limonene or S-(+)-carvone. Three 1 mL spearmint oil samples were taken from the stock solution and were each added with 4 μL of R-(-)-carvone standard. Eugenol or *trans*-caryophyllene standard (1 μL each) were separately added into each 1 mL clove oil sample and the procedure was then repeated two more times. Standards were added to approximately double the peak area of the tested compounds in each sample without affecting the composition of other compounds. All samples were run in the GC using the same setting implemented when determining the retention time.

3.2.1.3 Essential oil composition

The essential oil composition was determined by comparing the peak area of the major compounds in relation to the total peak area integrated on the chromatogram. The same sample preparation and GC method was implemented to determine the oil composition. Dill weed, spearmint and clove oil (Biox-C™) obtained in 2006 and 2007 were all tested using samples prepared at a concentration of 2.5 μL mL^{-1}.

3.2.2 Assessment of essential oil evaporation rate

3.2.2.1 Essential oil evaporation in 1-L jars

The rate of oil evaporation was first evaluated in sealed 1-L glass jars. In this set of experiments, 55.8, 52.4 and 48.1 μL of dill weed, spearmint and clove oils were applied onto glass filter papers (Fisherbrand G6, 6 cm^2). The specific amounts of essential oil were added to reach the targeted concentration of 50 mg L^{-1} headspace. The filter paper was suspended in each jar by taping it to the underside of the lid. Fifteen jars were prepared for each essential oil. All jars were sealed and stored at 12°C in the dark in a controlled environment chamber. The weight of the filter paper before and after oil application and the application time were recorded. The amount of evaporation was measured by weighing the filter papers at 0, 2, 4, 8, 12, 20, 28, 44, 56, 68, 80, 92, 152, 200, 260 and 320 hrs after application. To ensure accuracy, at each time point of weighing, the weight was measured by opening a jar that had not been opened before.

3.2.2.2 Essential oil evaporation in steel drums

The evaporation rate of the essential oils was also measured in 63-L sealed steel drums with constant air circulation. The purpose of the experiment was to first compare the evaporation rate of high (5 mL applied in 63-L drum) and low (1 mL applied in 63-L drum) dose treatments under constant air circulation at 8°C. In each drum, 5 or 1 mL of dill weed, spearmint or clove essential oil were applied onto Fisher brand P8 filter papers with 1 mL per filter paper. All filter papers were suspended in front of a 12V fan near the top of each drum. The fan circulated the air within the sealed drum four times per minute. Eight drums were prepared for each dose and for each essential oil. The initial filter paper weight, the filter paper weight after oil was applied and the time of oil application were recorded. The amount of evaporation was determined by weighing the filter paper at 0, 4, 22, 28, 47, 52, 70, 76, 94 hrs after application. Each time, a sealed drum that had not been opened before was randomly selected for weighing.

3.2.3 Rate of disappearance of carvone and eugenol from treated tubers

3.2.3.1 Material and treatment regime

The tuber variety "Lady Claire" was used for this experiment as preferred varieties such as 'Russet Norkotah' and 'Piccolo' were not available at the time of the experiment. All tubers were

grown in the Brooks, AB area and harvested in the fall of 2007. They were stored at 8ºC with 85-95% RH. Prior to the test, all tubers were washed and dried before bagging and stored in ventilated 63-L steel drums.

Dill weed, spearmint and clove oils were applied onto Fisher brand filter papers (P8, 12.5 cm in diameter,) to reach the targeted headspace concentration of 55 mg L^{-1} headspace. After treatment application, all drums were sealed for 4 days before ventilation resumed. All filter papers were removed from the drums after a 4 day treatment exposure. Ventilation system was then turned on at a rate ranged from 2.5 to 5.0 L min^{-1} kg^{-1} of potatoes. Tubers were randomly sampled after ventilation was resumed for: 0, 0.3, 1, 2, 4, 7, 10, 14 and 21 days. At each time, 4-5 tubers (approximately 170-200 g) were removed from each treatment and sealed in Ziploc® bags then stored in a -20°C freezer before extraction.

3.2.3.2 Extraction method and GC analysis

The quantity of essential oil components was determined by using the extraction method of Oosterhaven *et al.* (1995a, b). In this procedure, 50 g of fresh tissue sample was homogenized for 2 min with 100 mL of methanol (MeOH) and 50 mL of chloroform ($CHCl_3$) and 0.5 mL of 1 mg mL^{-1} naphthalene (internal standard). Then, adding another 50 mL of $CHCl_3$ and homogenized for 30 seconds. Lastly, 50 mL distilled water was added and homogenized for 30 seconds. The homogenate was filtered through a glass-microfiber filter paper using a vacuumed filter system. The $CHCl_3$ layer was separated from the aqueous layer and dried using anhydrous sodium sulfate (Na_2SO_4). The $CHCl_3$ solution was concentrated to 5 mL using a rotary evaporator. The extracted samples were analyzed with the Agilent 6890 GC. The system was equipped with fused silica capillary column DB-5ms (30 m length x 0.25 μm i.d., 0.50 μm film thickness) and the oven program was initially set at 80°C for 2 min, increased to 240°C at 5°C min^{-1}, and finally maintained at 240°C for 2 min. The inlet temperature was 250°C. The carrier gas was GC grade helium with the flow rate of 45 mL min^{-1} and the pressure capacity was 20 psi, split ratio was 35:1, and detector temperature was 250°C. Three replications were extracted for each sample.

The concentration of carvone extracted from dill weed and spearmint oil treated tubers and the concentration of eugenol extracted from clove oil treated tubers were calculated with

Equation 3.1. Response ratio was calculated for carvone and eugenol using the standard mixture of the compound (0.2 mg mL^{-1}) with naphthalene (0.1 mg mL^{-1}) based on the Equation 3.1.

$$\frac{\text{Compound Peak Area}}{\text{Compound Concentration}} = \text{Response Ratio} \times \frac{\text{Internal Standard Peak Area}}{\text{Internal Standard Concentration}} \quad \text{(Equation 3.1)}$$

The peak area of carvone or eugenol and interval standard naphthalene from each sample were collected by integrating the chromatograms, the actual concentration of compound residual for carvone and eugenol was then calculated.

3.3 Results and Discussion

3.3.1 The composition of the essential oils produced in 2006 and 2007

Dill weed oil contained three major peaks, spearmint oil was primarily composed of one major peak, and clove oil had two major peaks (Figure 3.1). Peaks were identified by comparing retention times with known standards. For the dill weed oil, the three major peaks eluted at 15.58, 16.83 and 28.42 min. The closest matching standards were α-phellandrene, limonene and carvone, which eluted at 15.59, 16.84, and 28.45 min respectively. Spearmint oil only had one major peak eluted at 29.24 min which was around the same time as the carvone standard (29.28 min). The clove oil had two major peaks and their elution times were same as eugenol (34.55 min) and *trans*-caryopyllene (37.92 min) standards. Further verification of the peak identity was carried out by spiking oil samples with known standards and looking for peak splitting and peak shoulder. Based on the results of spiked samples, the three peaks in dill weed oil were identified as α-phellandrene, limonene and carvone and the peak in spearmint oil was carvone, and the two peaks in clove oil represented eugenol and *trans*-caryopyllene. There were no split peaks or peaks with shoulders. The GC analysis implemented in this study could not distinguish between the two carvone isomers, and both S-(+)-carvone and R-(-)-carvone eluted at the same time. However, previously published results have concluded S-(+)-carvone exist in dill weed oil and R-(-)-carvone is found in spearmint oil (de Carvalho and Fonseca 2006, Chowdhury *et al.* 2007).

The composition of each essential oil was calculated by comparing the peak area of the compound of interest to the total peak area. Total peak area was calculated by adding up all the integrated peaks present on the chromatogram with peak area greater than 1. The results showed dill weed oil contained 41-43% carvone followed by 33-35% of limonene and 12-17% of α-

phellandrene (Table 3.1). Spearmint oil used in this study contained much higher levels of carvone compared to dill weed oil. Carvone, in fact, was the single major component in the spearmint oil studied and it comprised more than 97% of total oil. Clove oil contained 78-82% eugenol and 16-19% *trans*-caryophyllene (Table 3.1).

While both dill weed and clove oil content is within, or close to previously reported concentration ranges (Bowes *et al.* 2004, de Carvalho and Fonseca 2006, Jirovetz *et al.* 2006), the spearmint oil had much higher carvone content than previously reported (50-70%) (de Carvalho and Fonseca 2006, Chowdhury *et al.* 2007). According to the supplier, the spearmint oil used in this study was the leftover portion of steam-distilled spearmint oil that has gone through the distillation process several times. Despite of the high carvone content, this type of oil is normally sold at a lower price at $13 (US) dollars per pound compared to the regular price of $23 (US) dollars per pound.

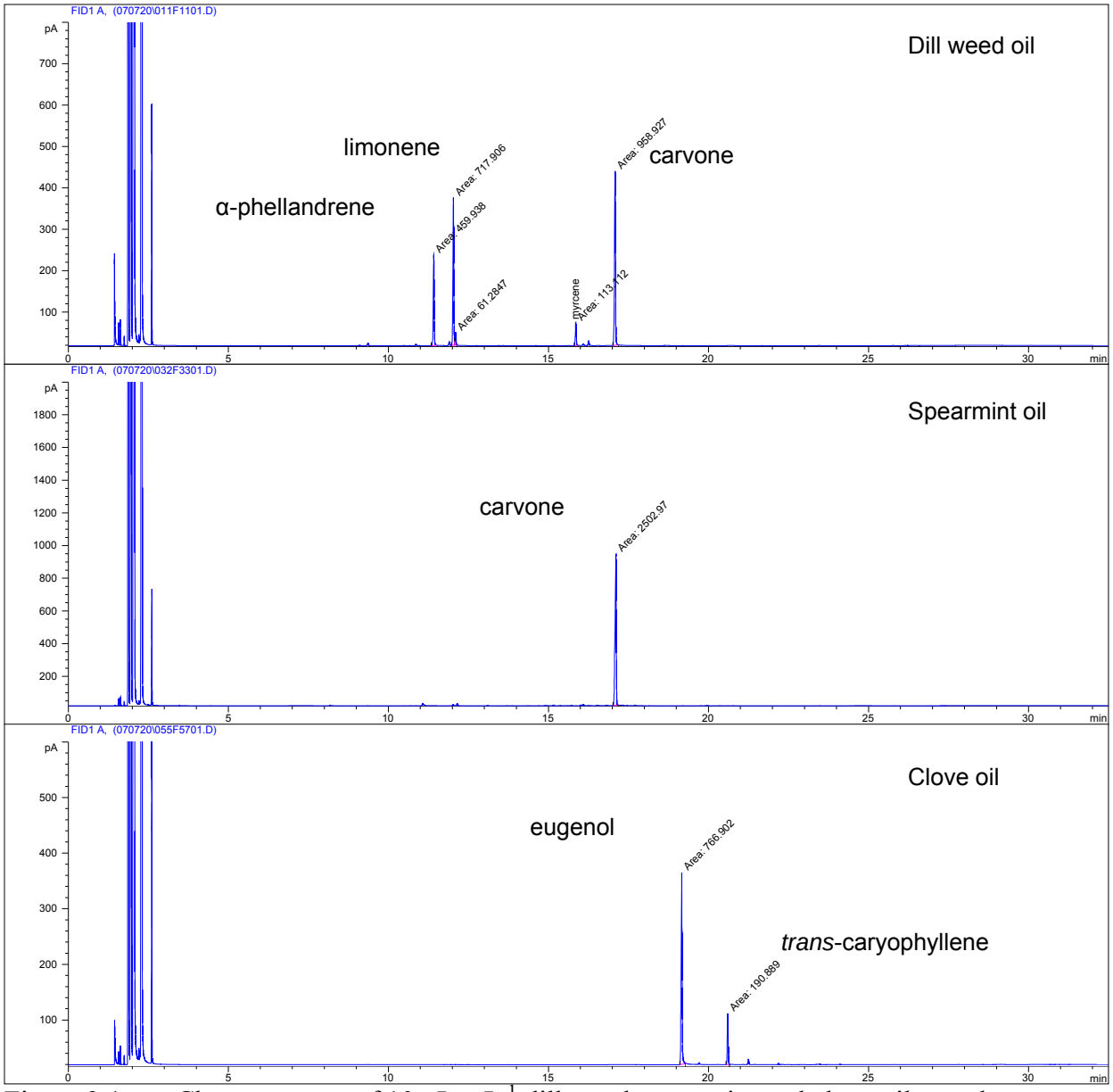


Figure 3.1 Chromatogram of 10 µL mL^{-1} dill weed, spearmint and clove oil samples.

The comparison of year-to-year composition showed that the constituents of each oil were relatively consistent between 2006 and 2007, and the concentration differences for all the major components was less than 5%. Although many genetic, environmental and cultural practices have been reported to potentially affect the composition of essential oils, the effects did not seem to cause more than 5% of variation in the concentration of the major components evaluated in this study.

Table 3.1 Composition of dill weed, spearmint and clove oils from 2006 and 2007 calculated base on the peak area of the compound to the total peak area integrated.

Dill Weed	α-phellandrene	Limonene	S-(+)-carvone	Others
2006	12.1 % ± 0.2*	33.4 % ± 0.6	42.7 % ± 0.6	11.8 % ± 0.8
2007	17.2 % ± 0.1	34.7 % ± 0.1	41.5 % ± 0.1	6.6 % ± 0.1

Spearmint	R-(-)-carvone	Others
2006	97.6 % ± 0.4	2.4 % ± 0.4
2007	97.2 % ± 0.4	2.8 % ± 0.4

Clove	Eugenol	*trans*-caryophyllene	Other
2006	78.5 % ± 0.2	18.9 % ± 0.1	2.6 % ± 0.1
2007	82.3 % ± 0.1	15.9 % ± 0.1	1.8 % ± 0.1

*± standard error

3.3.2 Essential oil evaporation

Dill weed oil evaporated the fastest, followed by spearmint oil, and clove oil evaporated much more slowly compared to the other two oils (Figure 3.2). When applying 5 mL of essential oils in each steel drum, after 76 hours of evaporation without the presence of any tubers, 84.2% of the dill weed oil and 60.7% of spearmint oil had evaporated based on the weight loss of the filter papers. By comparison, only 17.4% of clove oil evaporated. This volatility is primarily associated with vapor pressure of the major components in each essential oil. The vapor pressure of carvone is almost ten times higher than eugenol (i.e. carvone 0.053 kPa; eugenol, 0.004 kPa at 20^{o}C) (MSDS, Sigma Chemical Co.; MSDS, Sciencelab.com, Inc.). Even though all the tested essential oils are considered to be volatile, in general they are not highly volatile. In fact, these oils are much less volatile than water, which has a vapor pressure of 2.339 kPa at 20°C. The volatility of the essential oils is an important criteria when assessing its success as a volatile sprout suppressant. The relatively more volatile products, such as dill weed and spearmint oils may be more easily distributed uniformly throughout the storage via air circulation. Less volatile products, like clove oil may achieve a better inhibition effect by using a fogging/thermo-fogging system; however, such a system may cause potential problems including condensation and uneven distribution of the product throughout the potato pile.

Dill weed oil contains mainly α-phellandrene (17.2%), limonene (34.7%) and S-(+)-carvone (41.5%) and their molecular weights are 136.23 g mol^{-1}, 136.23 g mol^{-1} and 150.22 g mol^{-1}, respectively (Capelle *et al.* 1996). R-carvone, with a molecular weight of 150, represents over 95% of the total composition in spearmint oil. The heaviest oil of the three, clove oil, contains over 75% of eugenol (molecular weight: 164 g mol^{-1}) and approximately 15-20% of trans-caryophyllene (molecular weight: 204 g mol^{-1}). It appeared that compound with heavier molecular weight also had lower vapor pressure.

Besides vapor pressure and molecular weight of the major components contained in the oils, the storage conditions can also affect the evaporation rate. Air circulation near the essential oil source plays an important role in the rate of evaporation for all oils. When there was no air circulation, after 92 hours evaporation, only 30% of dill weed oil, 19% of spearmint oil and 5% of clove oil evaporated in 1-L jars. In comparison, when applied with 1 mL of each oil in 63-L drums, approximately 90% of dill weed and spearmint oils as well as 40% of clove oil evaporated after constantly circulation inside the 63-L steel drums for 94 hours (Figure 3.2). In addition, at 8°C, when applying 5 mL of dill weed oil in the steel drums, after 76 hours with air circulation, close to 90% of total applied oil evaporated and only 35% of dill weed oil evaporated when there was no fan running inside the drum (data not shown).

With 1 mL of essential oils in 63-L dreams, evaporation occurred quickly especially for dill weed and spearmint oils. However, evaporation leveled off when 90% of total oil applied had evaporated (Figure 3.2B) indicating that the filter paper retained 10% of the essential oils even when the headspace is not saturated. In addition to the filter paper, other surface areas within the storage vessel could also retain certain amounts of essential oil which needs to be taken into consideration particularly when implementing the treatment at a large scale.

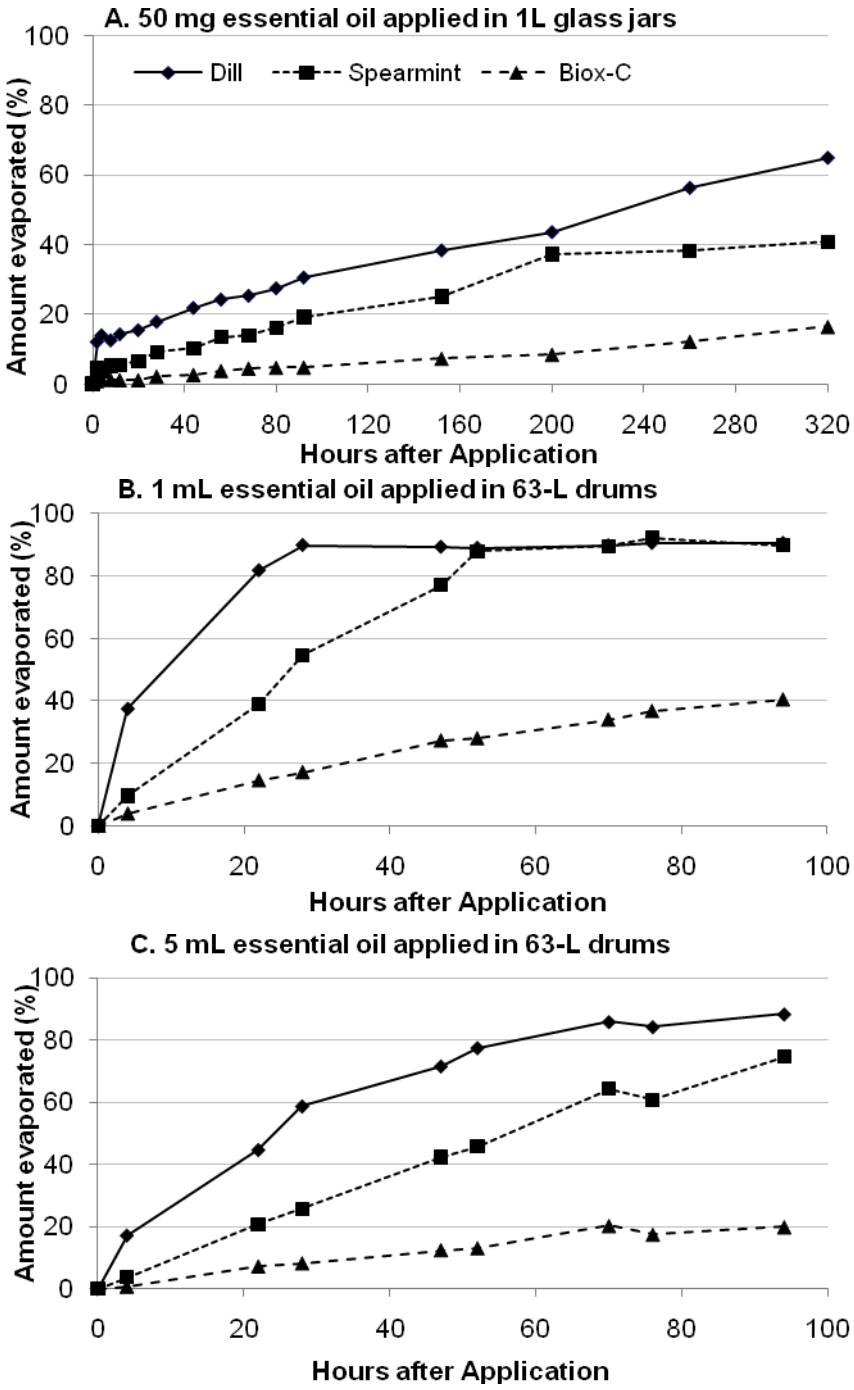


Figure 3.2 Evaporation of dill weed, spearmint and clove oils (A) in 1-L sealed glass jars with 50 mg of essential oil applied onto filter paper at 12°C, (B) in 63-L sealed steel drums with 1 mL of essential oil applied onto filter paper at 8°C and (C) in 63-L sealed steel drums with 5 mL of essential oil applied onto filter paper at 8°C.

3.3.3 Rate of disappearance of carvone and eugenol from treated tubers

The level of residue on the tubers continuously decreased during the period of ventilation particularly in the first 10 days, and by the end of 21 days, the residue level for all components was below 1 mg kg^{-1} tuber tissue (Figure 3.3). There was a significant drop in the residue level in the first 2 days of ventilation regardless of the type of oil applied and the slope became less steep in the following days. The large decrease could be due to the de-sorption of the compounds that were adsorbed on the surface of the tubers during the treatment. A large storage trial by Hartmans *et al.* (1995) showed a fast decrease in carvone residue level at the end of the storage period due to increased ventilation and the average carvone residue was determined to be approximately 1 mg kg^{-1}. The residue level showed that the majority of the carvone residue was found in the potato peel and only a small fraction was obtained in the peeled tubers. The author further suggested that higher residue retained on the potato peels could be caused by higher adsorption of carvone on the suberized potato periderm due to the lipophilic character of both substances. Similar results were also found by Oosterhaven *et al.* (1995c) who demonstrated that approximately 90% of S-carvone extracted in their trial was associated with the peel fraction.

The residue level showed that even though clove oil had lower evaporation rates than the other two oils, approximately 0.009 mg eugenol was extracted from each gram of tuber tissue after four days of treatment (Figure 3.3). In comparison, 0.004 mg of R-(-)-carvone and 0.003 mg of S-(+)-carvone per gram of tissue were extracted from tubers exposed to spearmint and dill weed oil under the same conditions. Clove oil evaporates more slowly, due to the lower vapor pressure of eugenol, and therefore is also more likely to adsorb on other surfaces rather than suspending in the headspace. S-(+)-carvone has been previously reported to have a faster uptake rate than R-(-)-carvone (Oosterhaven1995), however, since the spearmint oil used in this study had a much higher carvone content (97.2%) compared to dill weed oil (41.5%), the total amount available carvone would be likely higher in the spearmint treatment (Figure 3.3). In addition, among the three components measured, eugenol had the highest disappearance rate, even though its vapor pressure is much lower than carvone. This indicated that evaporation was not the only mechanism responsible for the disappearance of these compounds. The compounds were likely being metabolized by the tubers. Metabolism of the essential oil compounds were also reported by Oosterhaven et al. (1995a).

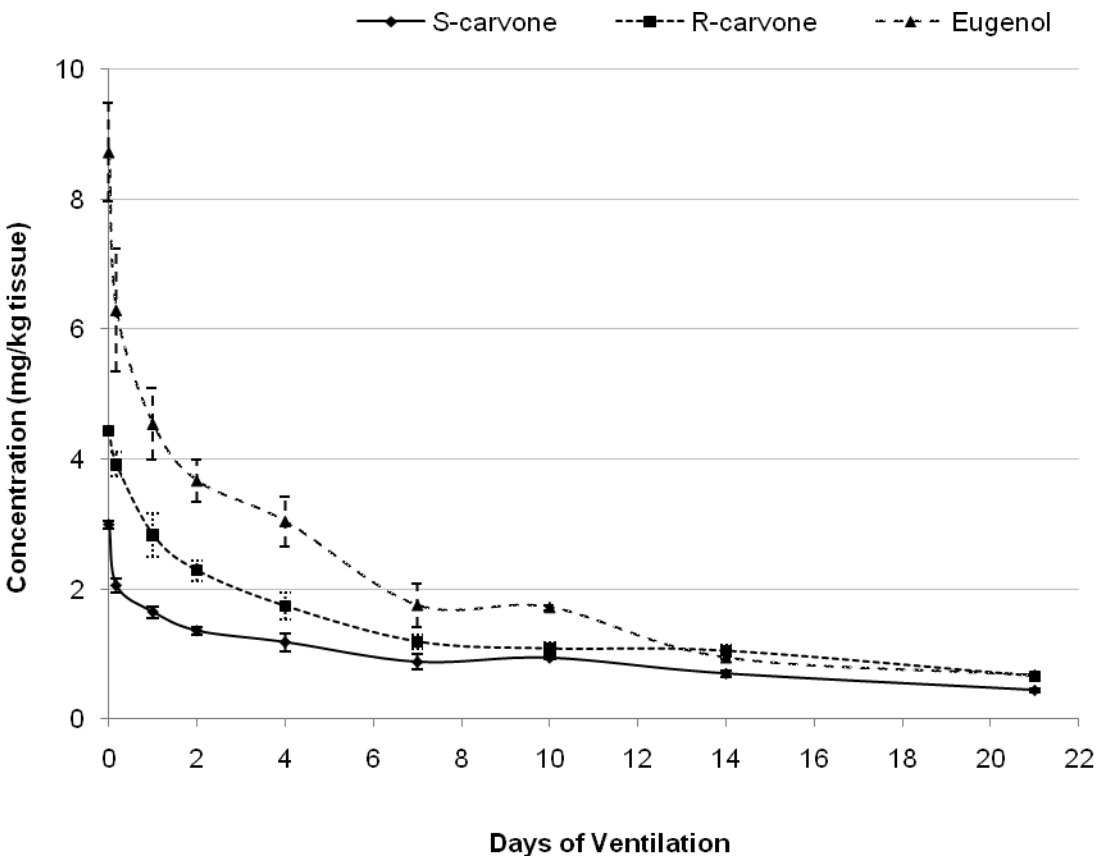


Figure 3.3 The amount of S-(+)-carvone extracted from tubers treated with dill weed oil, R-(-)-carvone extracted from tubers treated with spearmint oil and eugenol extracted from tubers treated with clove oil after the tubers were exposed to treatment for 4 days in 63-L steel drums at 8°C and then ventilated for different periods of time.

Since many essential oils, including those used in this study, have a strong odor, many growers are concerned the odor will affect the tubers. The residue level showed that after two weeks of continues ventilation, the extracted S-(+)-carvone and R(-)-carvone from the treated tubers decreased by more than 75% and close to 90% of eugenol de-sorbed from the tubers. The fast rate of reduction in essential oil residue level certainly reduces the potential of strong and undesirable odor remaining on the tubers. In addition, depending on the end use, after the tuber skin is peeled off, there will be minimal levels of residue left in the consumed portion of the tuber.

This study showed that there was little variation in the chemical composition of both the oils supplied by Corraini Essential Oils Ltd. and Biox-C supplied by Pace International in 2006 and 2007. Dill weed oil contained mainly carvone (41.5-42.7%), limonene (33.4-34.7%) and α-

phellandrene (12.1-17.2%), whereas carvone was the single major component in spearmint oil (97.2-97.6%). Clove oil contained 78.5-82.3% eugenol and 15.9-18.9% and *trans*-caryophyllene.

Among the three essential oils tested, dill weed and spearmint oils evaporated much faster than clove oil, which was consistent with the vapor pressures of the main component of these essential oils. The results also showed that the air circulation provided by a small ventilator substantially increased the evaporation rates.

The measurements of residue levels in the tuber flesh after increasing periods of ventilation found a 75-90% decline over 3 weeks. However, the rate of decline of the principal constituents was not consistent with their vapor pressures. Eugenol, the compound with the lowest vapor pressure, had the highest rate of decline. This finding suggested that, at least in this case, the loss of carvone from the tuber tissue was not determined by de-sorption or evaporation.

4.0 THE IMPACT OF DILL, SPEARMINT AND CLOVE ESSENTIAL OILS ON SPROUT SUPPRESSION IN POTATO TUBERS

4.1 Introduction

Essential oils extracted from a wide range of aromatic plants can effectively inhibit sprouting of potato tubers. This has been shown with purified constituents of essential oils, such as S-(+)-carvone and R-(-)-carvone, as well as with the unrefined steam distillates from caraway, dill, mint and a variety of other plants (Hartmans *et al.* 1995, Oosterhaven *et al.* 1995c). Beveridge *et al.* (1981) showed that carvone was effective in suppressing sprouting at the concentration of 500 mg kg^{-1}. Osterhaven *et al.* (1995a) illustrated that 250 μL of carvone applied to 30 eyepieces in a sealed 20 L tray (12.5 μL L^{-1} headspace), reduced sprout growth following a 2-4 day exposure, and growth was completely eliminated after seven days of treatment. A 100 mg kg^{-1} S-(+)-carvone treatment followed by a 42-hr ventilation free period applied every 6 weeks was able to successfully suppress sprouting for 6 months in 15 tonnes of tubers (Hartmans *et al.* 1995). In another study, Sorce *et al.* (1997) treated seed tubers with 7.15 mmol mol^{-1} of carvone and concluded that the treatment generated headspace concentrations of 0.34-1.06 μmol mol^{-1} and the treatment was effective in inhibiting sprouting in seed tubers. Although the efficacy of these essential oils and of their constituents has been demonstrated, there is little information on optimal dosages.

Dill weed and spearmint are commercially produced crops on the prairies. Potential exists to support local dill and spearmint growers by increasing the demand on a diversified use of the crop while effectively suppressing sprouting of stored potato tubers for the potato industry. Therefore, with the intention of supporting the local industry, this study was conducted using dill weed and spearmint oil extracts produced in southern Alberta. For the purpose of comparison, a clove oil product, Biox-C™, was also included in this study. Biox-C™ is currently marketed in the United States as a potato sprout suppressant. The major compounds of the selected essential oils have been previously reported to be effective in suppressing potato sprouting with no known adverse effects on potato taste and fry quality (Vaughn and Spencer 1991, Hartmans *et al.* 1995, Frazier *et al.* 1998).

To better characterize the potential of dill and spearmint essential oils as sprout inhibitors, the effective dose range was first determined. Then, the duration of sprout inhibition for a range

of doses was examined. A scaled-up study that mimicked specific commercial storage conditions was subsequently conducted to investigate the long-term efficacy of selected dose and application intervals for all three essential oils for effective sprout suppression.

4.2 Materials and Methods

4.2.1 The dose response study

4.2.1.1 Materials and storage conditions

The 'Russet Burbank' potato tubers used in this study were harvested in mid-September of 2005 at the Crop Diversification Centre South (CDCS), Brooks, Alberta. After the tubers were cured at 15°C for two weeks, they were stored in a ventilated room at 8^{o}C in the dark and the temperature was adjusted to 4^{o}C to suppress sprouting and extend the storage period. Non-dormant 'Russet Burbank' tubers, weighing approximately 200 g each, were then randomly selected.

The essential oils used in this study included dill weed oil and spearmint oils. Both oils were extracted by steam distillation from crops harvested locally in 2006 and obtained from Corraini Essential Oil Ltd. (Bow Island, Alberta). The compositions of these oils were presented in Chapter 3.3.1.

4.2.1.2 Treatment application

In order to standardize the doses between all experiments, all treatment doses were calculated based on the headspace, the volume of the storage space not occupied by the potatoes. The volumes of the tubers were calculated and then subtracted from the total storage space. Prior to the tuber volume measurement, all sprouts on each tuber were broken off. Each tuber was slowly submerged into a 500-mL beaker filled to the rim with water. The weight of the overflow water was then measured in order to estimate the volume of each tuber. All the tubers were thoroughly dried with paper towels and then placed into a one-liter glass jar.

Dill weed and spearmint oils were each applied at: 0 (control), 0.5, 5, 15, 50 and 200 mg L^{-1} headspace in a 1-L sealed glass jar. The quantity of essential oil applied to each jar was calculated based on the targeted dose and the headspace volume and pipetted onto a 9 cm Whatman #4 filter paper that was taped on the lid of each jar. The jars were sealed and arranged

in a randomized complete block design with three replications per treatment and one tuber per replication. The treatments were placed on a lab bench at room temperature (24.5 $\pm$ 3.5^{o}C). The lids were removed for air exchange four days after the beginning of essential oil treatment.

4.2.1.3 Measurements and statistical analysis

Sprout growth on each tuber was evaluated the day after treatment removal and every three days thereafter for a total of 29 days. The number and the weight of the sprouts on each tuber were measured on the 29th day.

The dose response study was analyzed using an analysis of variance in a RCBD mixed model using SAS 9.1 (2002-2003, SAS Institute Inc., Cary, NC, USA). Significance and the nature of the relationship between treatments and parameters were assessed by performing regression analysis and partitioning into linear and quadratic components (Steel and Torrie, 1980). The data were then analyzed with dose-response analysis based on the method described by Seefeldt *et al*. (1995) and the doses which resulted in 50% of sprout reduction were determined.

4.2.2 The duration response study

4.2.2.1 Materials and storage conditions

This study was conducted at the University of Saskatchewan, using the non-dormant commercial potato cultivar 'Piccolo' received in the fall of 2006 from Wedge Wood Farms Ltd., Spruce Grove, Alberta. Prior to the treatments, all the 'Piccolo' tubers were stored at CDC South, Brooks, at 6^{o}C for two months after harvest to satisfy the chilling requirement.

Dill weed, spearmint and clove oils [Biox-C^{TM}] were applied at a range of doses to determine the duration of sprout inhibition, which was then used to estimate the time interval required before applications had to be repeated to insure continued sprout suppression. Dill weed and spearmint oils were both supplied by Corraini Essential Oil Ltd. Bow Island, Alberta, and clove oil was provided by Pace International LLC., Seattle, USA. The oil compositions were presented in Chapter 3.3.1.

4.2.2.2 Treatment regime and measurements

Eight randomly selected 'Piccolo' tubers were placed in each 1-L glass jar and the treatment applications were randomly assigned to the jars. Dill weed, spearmint and clove oils were applied at the dose of 0, 15, 30, 60, 120, and 240 mg L^{-1} headspace. Based on the headspace in each jar, the calculated amount of essential oil was applied onto a 9 cm Whatman #4 filter paper taped onto the lid of each jar. After the treatment applications, all the jars were sealed and stored in a Conviron PG8 growth chamber in the dark at 10°C. All the jars were ventilated once every 7 days by opening the lids under a fume hood for 5 min to prevent CO_2 build up in sealed jars. After the first 7 days, 4 tubers were removed and planted to evaluate subsequent growth and yield (Chapter 5.2.1).

The treatments were arranged in a randomized complete block design with three replications per treatment. Visual observations were made daily to determine the impact of the treatments on sprouting. When the longest sprout was greater than 2 mm in length, the tuber was considered to be sprouted. This standard is more strict than the newly updated ¼ inch (6mm) limit defined by United States Department of Agriculture for fresh market potato grades (United States Department of Agriculture 2008). All the treatments including the untreated control were held under the same condition for 128 days. The numbers of sprouts in the apical bud region of each tuber were counted and all the apical sprouts were cut off at the base and weighed. The percentages of sprouted tubers were plotted as a function of the duration of storage to estimate the duration of sprout suppression at different doses.

4.2.3 The scaled-up sprout suppression study

4.2.3.1 Materials and storage conditions

The essential oils tested in this study included essential oils of dill weed, spearmint and clove. Dill weed and spearmint oils were supplied by Corraini Essential Oil Ltd. (Bow Island, Alberta) and the oils were extracted from crops harvested in southern Alberta in 2007. Clove oil was donated by Pace International LLC. (Seattle, USA).

Two table potato cultivars, 'Russet Norkotah' and 'Piccolo', were tested in this experiment. 'Russet Norkotah' tubers grown in southern Alberta (Taber, Alberta) and 'Piccolo' tubers from Wedge Wood Farms Ltd. (Spruce Grove, Alberta) were harvested in 2007 and stored at 10°C after the wound-healing period for approximately one month. 'Piccolo' tubers were then

moved to a storage with average temperatures maintained at 4^{o}C for approximately three weeks to hold sprout growth prior to the experiment.

4.2.3.2 Experiment setup

The setup consisted of forty-eight 63-L sealable steel drums. Within each drum, the air was circulated through a vertically placed PVC pipe with a 12-V fan attached to the top end to ensure uniform distribution of the essential oil vapors (Figure 4.1). With this system, the entire air volume is circulated approximately four times per minute. Essential oils were applied onto glass filter papers (Fisherbrand G6, 6 cm^{2}) according to the headspace and the filter papers were suspended in front of the fan. The fans were turned on after essential oil application. All drums were connected to the ventilation system, and the ventilation could be opened or closed individually for each drum. The airflow in the ventilation system was consistent with a commercial storage rate (2.5-5.0 L min^{-1} kg^{-1} of potatoes). Two nylon mesh bags of randomly selected non-dormant 'Russet Norkotah' tubers (25 tubers per bag) and one bag of randomly selected non-dormant 'Piccolo' tubers (100 tubers per bag) were placed in each steel drum. Each bag was weighed and the volume occupied by tubers was calculated based on the average density of each variety. After each treatment application, the drums remained sealed for four days before reconnecting to the ventilation system. The four-day sealing-period was expected to allow sufficient time for each essential oil to evaporate within the drums. The drum relative humidity was maintained at 85-95%. All tubers were treated continuously for 28 weeks. Storage temperature was set at 8°C for the first 20 weeks. Then, to accelerate sprouting, the temperature was increased to 12^{o}C for 8 weeks and subsequently increased to 15^{o}C to promote sprouting. After treatment termination at week 28, 'Russet Norkotah' tubers were then stored for an additional five weeks and 'Piccolo' tubers were stored for another ten weeks.

Figure 4.1 A. General view of the treatment chambers with the ventilation system, the adjustable voltage power supply, and some of the 63-L containers. B. 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.

4.2.3.3 Experimental design and statistical analysis

To determine the optimal combination of dose and treatment time interval, the experiment was set up as a response surface design. Response surface designs are used for the analysis of problems in which a response of interest is influenced by several variables, and where the objective is to optimize the response (Montgomery 2005). It examines the linear and quadratic behavior of the response within the design region. The design also allows one to estimate model parameters and carry out an analysis of variance ($P<0.05$), and tests for model adequacy by performing lack of fit test. In this study, the response variable was fit to a surface described by a polynomial (quadratic) function of dose and treatment interval within the experimental region.

The treatments consisted of three types of essential oils each applied with nine combinations of dose and application frequencies (Table 4.1). The treatment dose and time interval combinations were selected to fit a central composite design with eight replications at the centre point (55 mg L^{-1} with 17-day interval) to estimate the experimental error, assuming homogeneity of variance over the treatment range. The targeted concentrations were based on the free headspace in the drum except for clove oil treatments, which were based on the drum volume to comply with label instructions. For each drum, the headspace was calculated by subtracting the tuber volume from the total drum volume. The headspace in the 63-L drums ranged from 48.7-51.7 L.

Table 4.1 Treatment combinations for the scaled-up sprout suppression study

	Treatment Combinations								
Targeted concentration (mg L^{-1} headspace)	13	25	25	55	55*	55	85	85	97
Treatment time interval (days)	17	10	24	7	17*	27	10	24	17

*the treatment combination was replicated 8 times.

Sprout condition was visually observed weekly to determine the duration of the sprout inhibition effect. Each bag of tubers was considered an experimental unit. When over 50% of the tubers had visible sprouts (> 2 mm long) in a bag, it was considered as "sprouted". When all bags of tubers for the same variety were marked as sprouted for each treatment, sprouts were broken off and the fresh weight was measured. The sprout weight was determined to be the major response variable. The response surface analysis was conducted using SAS 9.1 (2002-2003, SAS Institute Inc., Cary, NC, USA). The sprout weight response surfaces of each tuber variety and essential oil treatment were plotted in relation to the two independent factors, the dose and treatment interval applied. Lack of fit tests were conducted as a part of the analysis. The relationship between sprout weight and the two variables was analyzed with polynomial regression.

4.3 Results and Discussion

4.3.1 The dose response study

All essential oils showed a dose-dependent inhibitory effect on the sprout growth of non-dormant 'Russet Burbank' tubers. Increasing dose resulted in a significant reduction ($P < 0.05$) in both sprout weight and number of sprouts produced on 'Russet Burbank' potatoes (Figure 4.2 and 4.3, Appendix A1 and 2). Treatment dose significantly affected sprout development in treated tubers, and the type of oil had no significant impact on both sprout weight and number of sprouts produced.

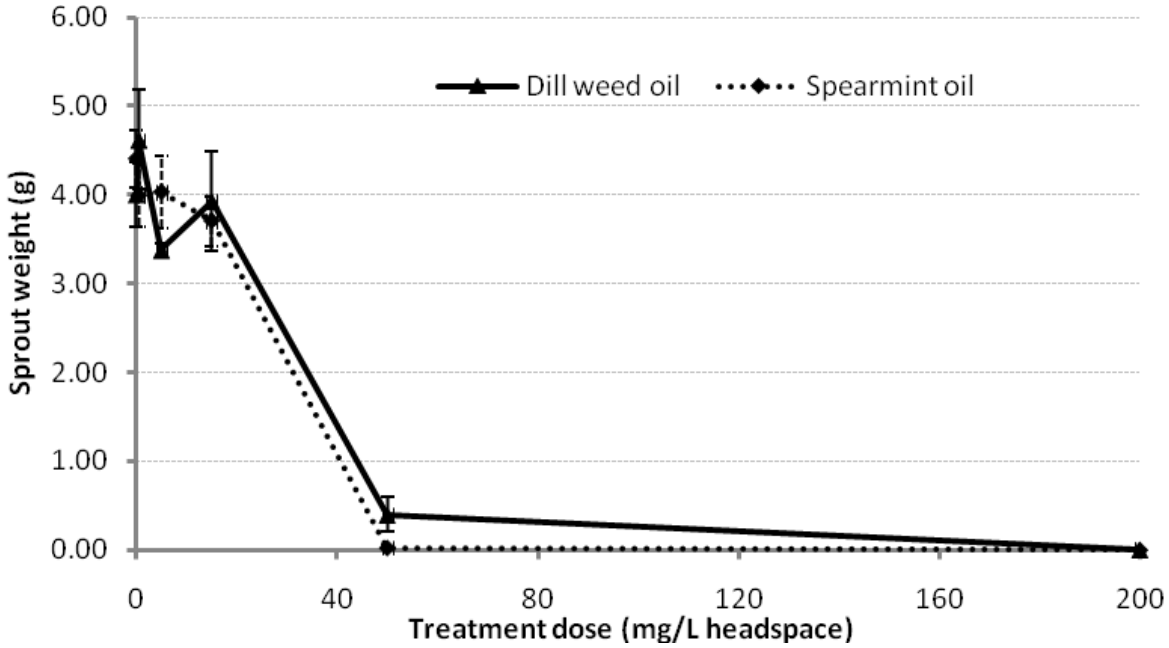


Figure 4.2 'Russet Burbank' sprout weight (g) after exposure to dill weed oil and spearmint oil treatment at different doses in 1-L glass jars at 24.5°C.
Error bars denote ± standard error. N = 3

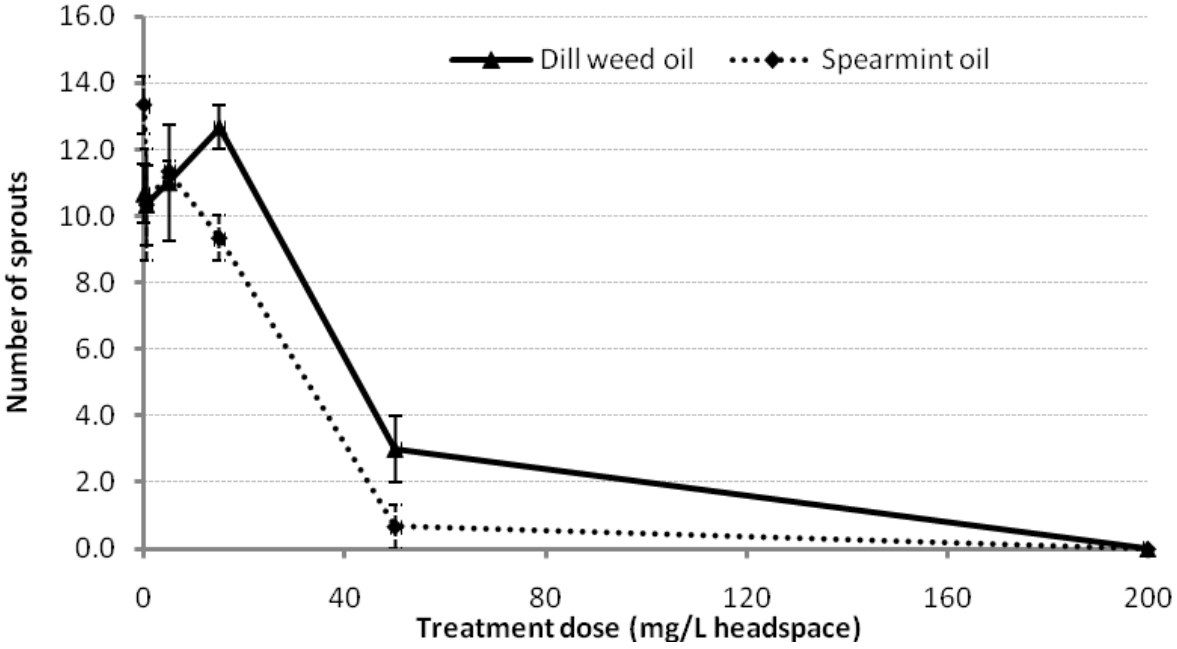


Figure 4.3 The number of sprouts produced on each 'Russet Burbank' tuber after exposed to dill weed oil and spearmint oil treatment at different doses in 1-L glass jars at 24.5°C.
Error bars denote ± standard error. N = 3

The variance of the main dose effect was mainly represented by a linear trend (76.4% for sprout weight and 73.8% for number of sprouts produced) with a small, yet significant, portion of quadratic trend (10.7% for sprout weight and 14.0% for number of sprouts produced) (Appendix A1 and 2). Thus as the treatment dose of dill weed and spearmint oil increased, sprout weight and sprout number was linearly reduced, and the reduction leveled off as the sprout growth was completely inhibited at high doses (60-200 mg/L headspace^{-1}). Between the doses 0.5 to 15 mg L^{-1} headspace, sprout development among tubers treated with dill weed and spearmint oil were similar to control tubers. As the dose increased to beyond 15 mg L^{-1} headspace, the sprout weight and number decreased drastically and when the treatment dose reached 200 mg L^{-1} headspace sprout development was completely suppressed (Figure 4.2 and 4.3).

In order to estimate the dose that resulted in 50% sprout inhibition (I_{50}) a log-logistic model was used (Seefeldt *et al.* 1995). This model was originally developed to determine the dose of herbicide treatment that achieved 50% of weed kill (I_{50}):

$$y = f(x) = C + \frac{D - C}{1 + (x / I_{50})^b} = C + \frac{D - C}{1 + \exp[b(\log(x) - \log(I_{50}))]} \qquad \text{(Equation. 4.1)}$$

where x = dose, C = lower limit; D = upper limit, b = slope, and I_{50} = dose giving 50% response.

In Equation 4.1, the upper limit D corresponds to the sprout growth mean of the control and the lower limit C corresponds to the mean response at the highest doses (200 mg L^{-1} headspace). The parameter *b* indicates the slope of the curve around I_{50}, and a larger *b* value indicates a steeper slope.

The model achieved a good fit with both $R^2 > 0.9$ (Figure 4.4 and 4.5) indicating the model was a good representation of the data. The dose of 32.5 mg L^{-1} headspace and 21.5 mg L^{-1} headspace for dill weed and spearmint oil, respectively, produced a 50% reduction in sprout weight growth within 29 days of treatment in non-dormant tubers at 24.5^{o}C (Table 4.2). In addition, 50% reduction in sprout number on each tuber was achieved with 47.6 and 22.3 mg L^{-1} headspace of dill weed and spearmint oil, respectively (Table 4.3).

The predicted curve indicated when treatment dose increased to beyond 100 mg L^{-1} headspace, sprout growth would be completely suppressed (Figures 4.4 and 4.5). This suggested that the treatment dose to completely suppress sprouting would likely be between 32.5-47.6 mg L^{-1} headspace and 100 mg L^{-1} headspace for dill weed oil and between 21.5-22.3 mg L^{-1}

headspace and 100 mg L^{-1} headspace for spearmint oil at 24.5°C. Many previous studies used treatment dose calculated based on tuber weight (Beveridge *et al.* 1981, Hartmans *et al.* 1995) and found the treatment dose ranged between 100 to 500 mg kg^{-1} effectively inhibited potato sprout growth. In the current study, the estimated headspace concentration was implemented as studies have suggested that the essential oil concentration surrounding the tubers was crucial in maintaining the suppression effect (Hartmans *et al.,* 1995, Sorce *et al.* 1997, Cizkova *et al.* 2000). Thus, we could not compare our estimated effective range directly with previous study results.

Table 4.2 Estimation of the log-logistic model parameters for the dose response analysis of 'Russet Burbank' tuber sprout weight in response to dill weed and spearmint essential oil treatments at 24.5°C.

Parameter		Estimate	Approx. Std. Error	Approximate 95% Confidence Interval	
				Lower	Upper
D (*g*)	Dill	4.0	0.2	3.6	4.5
	Spearmint	4.1	0.2	3.8	4.5
I_{50} (*mg* L^{-1} *headspace*)	Dill	32.5	13.8	3.0	62.0
	Spearmint	21.5	11.2	-2.4	45.3
b (*slope*)	Dill	5.1	4.9	-5.2	15.5
	Spearmint	5.9	8.6	-12.4	24.4

Table 4.3 Estimation of the log-logistic model parameters for the dose response analysis of 'Russet Burbank' tuber sprout number in response to dill weed and spearmint essential oil treatments at 24.5°C.

Parameter		Estimate	Approx Std. Error	Approximate 95% Confidence Interval	
				Lower	Upper
D (*# of sprouts*)	Dill	11.2	0.5	10.1	12.2
	Spearmint	11.7	0.6	10.5	12.9
I_{50} (*mg* L^{-1} *headspace*)	Dill	47.6	1.1	45.2	49.9
	Spearmint	22.3	4.0	13.8	30.8
b (*slope*)	Dill	10.8	8.2	-4.9	26.5
	Spearmint	3.4	1.3	0.7	6.2

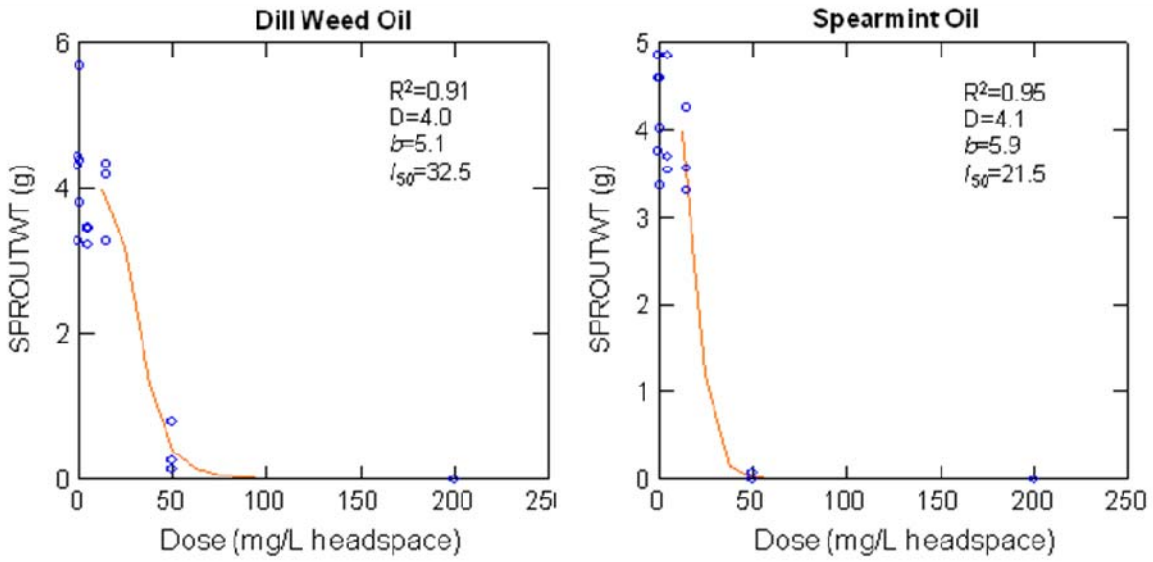


Figure 4.4 Dose response analysis curve of 'Russet Burbank' tuber sprout weight in response to dill weed and spearmint essential oil treatments at 24.5°C. The curve was described with a log-logistic model of the form y = C+(D-C)/[1+exp{*b*(log(x)-log(I_{50})}]. Sprout WT = sprout weight (g).

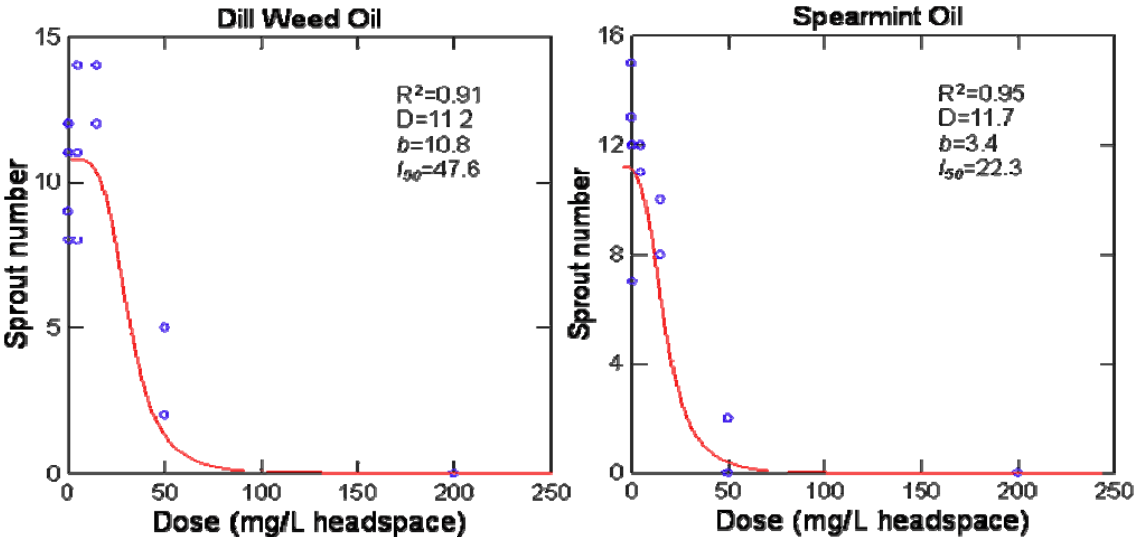


Figure 4.5 Dose response analysis curve of 'Russet Burbank' tuber sprout number in response to dill weed and spearmint essential oil treatments at 24.5°C. The curve was described with a log-logistic model of the form y = C+(D-C)/[1+exp{*b*(log(x)-log(I_{50})}]. Sprout WT = sprout weight (g).

The dose response study showed dill weed and spearmint oil treatments effectively suppressed sprouting in non-dormant 'Russet Burbank' tubers and the suppression effect largely depended on the dose of the essential oil applied (Figure 4.4 and 4.5). The dose-dependent

inhibition effect was also reported in pure carvone studies. Oosterhaven (1995) found 250 µL of S-(+)-carvone resulted in strong sprout growth inhibition in 20-L containers at 18°C, while 50 and 150 µL produced much a weaker response. In our essential oil extract study, when the treatments exceeded 15 mg L^{-1} headspace (16.7 µL L^{-1}), increases in essential oil concentration in the air space surrounding the tubers resulted in a rapid reduction of sprout growth. When the concentration reached 50 mg L^{-1} headspace (55.8 µL L^{-1}) or higher, in most cases, sprouting was completely inhibited during the course of the experiment even though all tubers were only fully exposed to the treatments for 4 days before all the jars were ventilated. The results also indicated that spearmint oil was more effective in suppressing tuber sprouting than dill weed oil. The 50% of sprout growth reduction was achieved with 21.5-22.3 mg L^{-1} headspace of spearmint oil treatment whereas dill weed oil extract required 32.5-47.6 mg L^{-1} headspace to reach the same level of sprout suppression (Table 4.2 and 4.3). While the cause for this response is not clear, the oil composition study showed that 97% of spearmint oil extract consists of R-(-)-carvone while 42% dill weed oil extract consists of S-(+)-carvone (Table 3.1). The much higher carvone content in spearmint oil likely triggered the strong treatment response.

In summary, the dose response study showed dill weed and spearmint oil treatments were effective in suppressing 'Russet Burbank' tuber sprout growth when the dose was greater than 15 mg L^{-1} headspace. In general, an increase in treatment dose resulted primarily in linear reduction in tuber sprout growth. The dose-response analysis estimated that a dose of 32.5 mg L^{-1} headspace of dill oil and 21.5 mg L^{-1} headspace of spearmint oil resulted in 50% of reduction in sprout weight, and a dose of 47.6 mg L^{-1} headspace of dill oil and 22.3 mg L^{-1} headspace of spearmint oil resulted in a 50% reduction in sprout number.

4.3.2 The duration response study

The percentage of sprouted tubers in each treatment over the storage time is illustrated in Figure 4.6. The untreated 'Piccolo' tubers started to sprout within two days and all control tubers sprouted within the first week. Tubers treated with 15-120 mg L^{-1} headspace of clove oil all started to sprout immediately after treatment began and had more than 50% of tubers sprouted by the end of the first week. When tubers were exposed to 120 and 240 mg L^{-1} headspace of clove oil, the high dose of clove oil did not immediately suppress sprouting. However, after two weeks of treatment exposure, the number of sprouted tubers started to decrease due to the treatment-

induced necrosis occurring on the sprouted buds (Figures 4.6A and Appendix C1). Overall, clove oil treatments suppressed sprouting only when the dose was greater than 120 mg L^{-1} headspace, but the suppression effect was delayed. The delayed suppression effect was likely due to the much lower evaporation rate of clove oil compared to dill weed and spearmint oils (Figure 3.2). Frazer *et al.* (2004) suggested that clove oil should be applied as a thermal aerosol and wick application was not recommended because the method depended on the natural evaporation of the oil and it was insufficient in delivering and adequate amount of compound to the atmosphere surrounding tubers.

Tubers exposed to 15 mg L^{-1} headspace of dill weed oil had a similar sprouting pattern compared to control tubers. After being treated with 30 mg L^{-1} headspace of dill weed oil, the tubers remained un-sprouted for 2 weeks but then quickly resumed sprout growth. As the treatment dose increased, the duration of sprout suppression was also extended. The 7 days exposure to 60 mg L^{-1} headspace resulted in 5 weeks of sprout suppression, and it took more than 7 weeks for 50% of tubers to sprout. Tubers treated with 120 mg L^{-1} headspace of dill weed oil did not sprout for 14 weeks, and at the dose of 240 mg L^{-1} headspace, none of the exposed tubers resumed sprout growth by the end of 19 weeks (Figure 4.6B).

Spearmint essential oil at a dose of 15 mg L^{-1} headspace did not suppress sprouting in treated tubers in that over 80% of tubers sprouted and all tubers sprouted by the end of third week. Following exposure to 30 mg L^{-1} headspace of spearmint oil, sprouting was completely suppressed for 2 weeks but once sprouting resumed, 50% of sprouting was reached in one and half weeks. At the dose of 60 L $headspace^{-1}$ unlike clove and dill weed oil, spearmint oil suppressed sprouting for 14 weeks and more than 50% treated tubers remained non-sprouted by the end of 19 weeks. None of the tubers treated with 120 or 240 mg L^{-1} headspace of spearmint oil showed any sign of sprouting within the 19 weeks (Figure 4.6C).

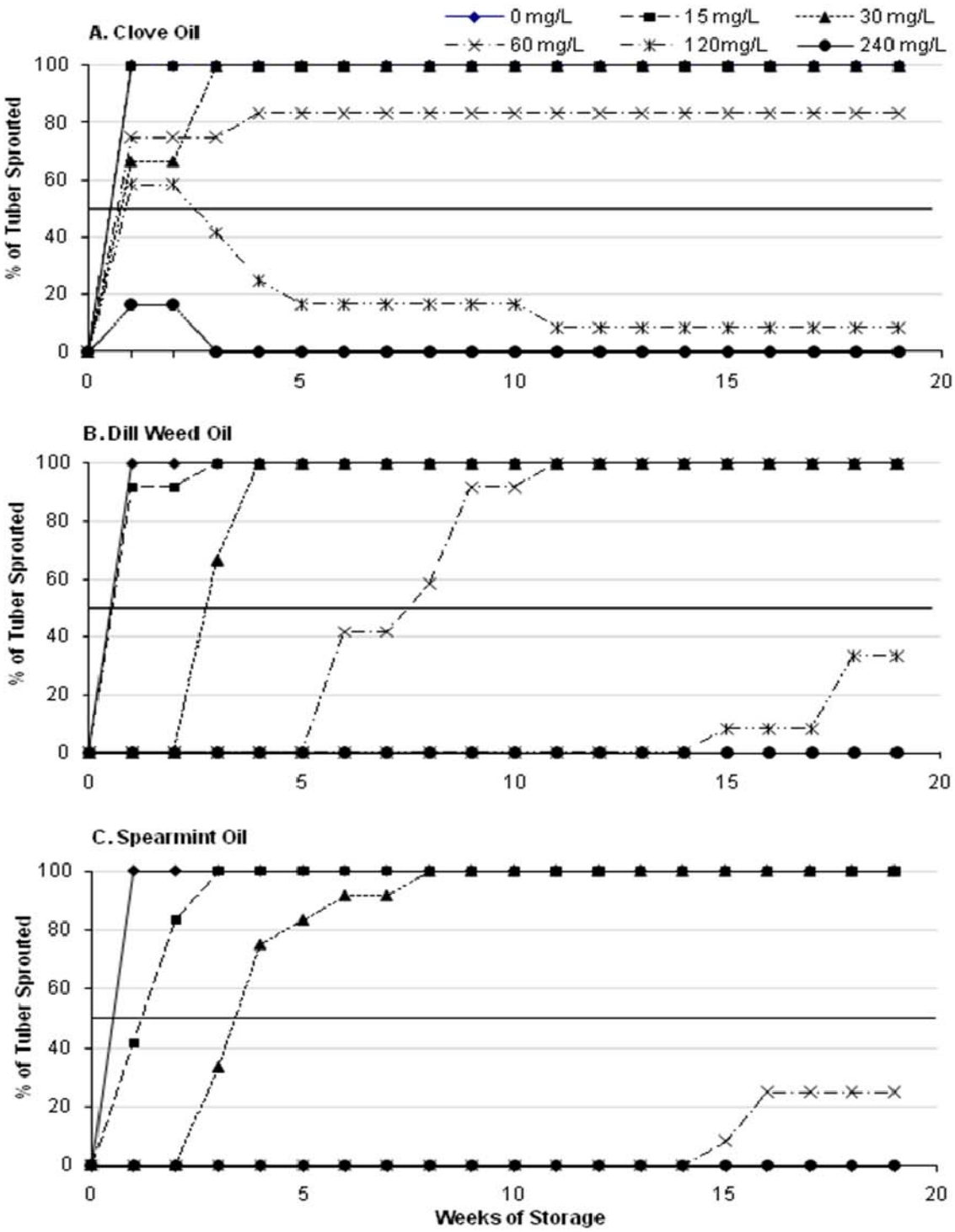


Figure 4.6 The percentage of sprouted 'Piccolo' tubers after exposed to clove (A), dill weed (B) and spearmint (C) essential oil treatments at various doses for 7 days and then stored for 19 weeks in 1-L glass jars at 10°C.
The solid horizontal line marks 50% of sprouting

Repeated essential oil applications are crucial for achieving effective sprout suppression for long-term storage (Hartmans *et al.* 1995, Frazer *et al.* 2004); however, treatment dose has been the main focus of previous studies (Beveridge *et al.* 1981, Oosterhaven 1995, Frazer *et al.* 2000). The time interval between repeated treatment applications is dependent on the dose and the type of oil applied. Meanwhile, it is important to estimate the duration of sprout suppression at specific doses in order to more effectively apply the treatments. For treatments using dill weed, spearmint and clove oil, the 15 mg L^{-1} headspace treatment was ineffective in suppressing sprouting (Figure 4.6). With 30 mg L^{-1} headspace of dill weed and spearmint oil, the time interval between repeated treatments should be at least two weeks. At the dose of 60 mg L^{-1}, the treatment should be reapplied approximately every 5 weeks for dill oil and every 14 weeks for spearmint oil treatment. High doses (120 and 240 mg L^{-1} headspace) showed an extensively long period of inhibitory control on sprouting ($\geq$ 19 weeks), under a single application for all three oils. However, tubers treated at the high doses developed necrosis (Appendix C1), which would reduce the marketability of the tubers. Therefore, doses beyond 120 mg L^{-1} headspace should not be implemented.

This portion of the study was conducted to estimate the duration of sprout suppression after exposing the tubers to a range of doses of each essential oil (dill weed, spearmint and clove oil). The results were used as a reference to determine the appropriate time interval between repeated applications for the scaled-up sprout suppression study. The results suggest that to sufficiently suppress sprouting without compromising the marketability of the treated tubers, the treatment dose should be maintained above 15 mg L^{-1} headspace and below 120 mg L^{-1} headspace. Also, within this dose range, in order to effectively suppress sprouting for long periods of time, the treatments should be repeated every 2 to 14 weeks.

The results of the duration response study also revealed that, at the same dose, spearmint oil (containing mainly R-(-)-carvone) was more effective than dill weed oil (containing mainly S-(+)-carvone). This result is consistent with the dose response study. The carvone content in dill and spearmint essential oils contained roughly 42% of S-(+)-carvone and 97% of R-(-)-carvone, respectively (Table 3.1). The higher levels of carvone in the spearmint oil treatments were likely part of the reason for its greater inhibitory effect.

In summary, when the treatment dose was greater than 30 mg L^{-1} headspace, the duration of sprout suppression increased with rising doses for both dill and spearmint essential oil. Many

previous studies have suggested repeated essential oil applications are necessary to achieve the desired sprout inhibition effect (Hartmans *et al.* 1995, Frazier *et al.* 1998, Kleinkopf *et al.* 2003). Our current study showed that when dill weed and spearmint oils were applied, the treatment should be repeated at least every two weeks at dose 30 mg L^{-1} headspace. At the dose of 60 mg L^{-1}, the treatment should be reapplied approximately every 5 weeks for dill oil and every 14 weeks for spearmint oil treatment.

While ≥120 mg L^{-1} headspace of dill and spearmint oil treatments achieved sprout inhibition effect for long period of time, these treatments also resulted in undesirable necrosis on the treated tubers (Appendix C1). The occurrence of necrosis was a warning sign of the potential side effect of high dose essential oil treatments. Tubers with necrosis would be highly undesirable and less appealing to consumers; thus, high dose treatments should be avoided. Clove oil treatments were not as effective as dill and spearmint oil treatment to suppress sprouting in treated tubers, most likely due to the low evaporation rate (Figure 3.2).

4.3.3 The scaled up sprout suppression study

Dill weed and spearmint oil treatments achieved robust inhibitory effects in the current study. Tubers response to treatment dose was less sensitive than expected. With these two essential oils, tubers treated with 13 mg L^{-1} headspace of essential oils all sprouted within 10 weeks of storage, despite the treatment interval implemented. When 25 mg L^{-1} headspace of dill weed oil was applied every 24 days, it was also insufficient to inhibit sprouting for more than 10 weeks. However the same dose of spearmint oil achieved suppression for more than 25 weeks. The remaining dill and spearmint oil treatments all effectively suppressed sprouting from 25 to over 35 weeks (Appendix B1). Sprout growth could have been suppressed by dill and spearmint oils for even longer periods, however, the treatments were stopped after 28 weeks of storage in order to access sprout inhibitory effects of the treatments based on the number of sprouts produced and the total sprout weight. After the treatment termination, 'Russet Norkotah' tubers started to sprout within 2-5 weeks. For the majority of 'Piccolo' tubers, sprouting did not begin until 4-10 weeks after treatment termination. Long term sprout suppression was also achieved in previous studies. Hartmans *et al.* (1995) demonstrated that 100 mg kg^{-1} of carvone applied every six weeks at 5-7°C suppressed sprouting in potatoes for more than 32 weeks.

When treating ‘Russet Norkotah’ and ‘Piccolo’ tubers with clove oils, the treatments did not explain majority of the variation in sprout weight (Figure 4.7 and 4.8, Appendix A3 and 4), and the adjusted R^2 were only 32.42% for treated ‘Russet Nortkotah’ tubers and 9.04% for treated ‘Piccolo’ tuber. The model was not significant (P>0.05) for both cultivars although there was no significant lack of fit ($Pr>F_{(lack\ of\ fit)}$=0.129 for 'Russet Norkotah' tubers and $Pr>F_{(lack\ of\ fit)}$=0.975 for 'Piccolo' tubers). The model could not provide a good prediction of the treatment response and the optimization could not be generated either.

When ‘Russet Norkotah’ tubers were exposed to dill weed oils, the model was significant in explaining the variation in sprout weight and model had a good fit as the adjusted R^2 was 64.10% (Figure 4.9). The analysis of variance indicated that between the dose and the treatment interval, treatment interval was the most important factor that had significant linear and quadratic effects on sprout weight produced on dill weed treated ‘Russet Norkotah’ tubers (Appendix A5). The parameter estimates showed that with the increase of treatment interval the sprout weight also increased at a rate of 0.79 times. However, when the sprout weight reached a certain level with the increase of treatment interval it began to decrease, indicated by the negative value (-1.04) of the quadratic effect parameter estimate (Table 4.4). Longer treatment intervals would allow the tubers to resume sprout growth and this resulted in higher accumulation of sprout weight. However, it was not clear why when treatment interval was extended beyond a point, it would result in lower sprout production. When treating ‘Piccolo’ tubers with dill weed oil, the model was not significant (Pr>F=0.085) and the adjusted R^2 was 40.99% (Figure 4.10). Thus, the model based on dose and treatment interval did not adequately explain the variations in sprout weight.

Large portions of the ‘Russet Norkotah’ tubers’ response to spearmint oil treatments was accounted for by the response surface model since the P value for the model was 0.004 and the adjusted R^2 was 72.69% (Figure 4.11). Between the two independent factors, dose and treatment interval, the treatment interval was the only factor that had significant effect on tuber sprout weight (Appendix A7). This response is consistent with the treatment response of dill weed oil treated ‘Russet Norkotah’ tubers. The parameter estimate indicated that extension in treatment interval had a positive linear association with accumulation in sprout weight of treated 'Russet Norkotah' tubers; however, the quadratic effect of treatment interval had a negative association (-116.38) with sprout growth (Table 4.5). The response indicated that sprout weight accumulated

when treatment interval was extended, but the accumulation leveled off when the treatment interval reached beyond certain point. The slowing down of sprout growth could be due to depletion of nutrient reserve within the tuber. The model obtained from 'Piccolo' tubers treated with spearmint oil was significant (Pr>F=0.001) with an adjusted R^2 of 81.85%. However, it also exhibited border line significant lack of fit (Pr>F=0.045) indicating that the model was inadequate in explaining all the systematic variations in the data and the response variable (sprout weight) (Figure 4.12, Appendix A8).

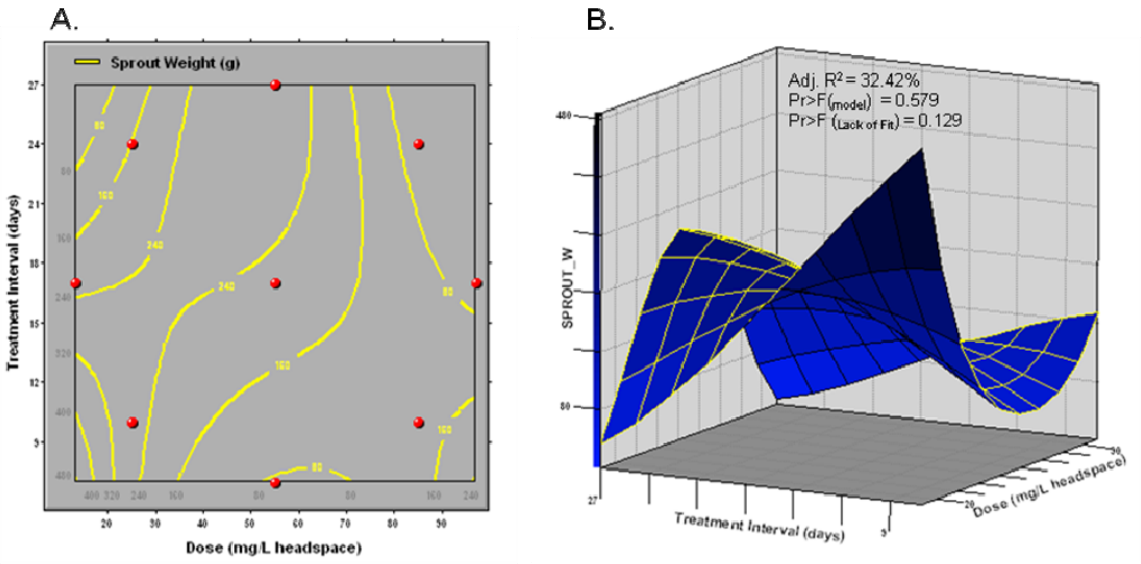


Figure 4.7 The effect of clove oil on sprout suppression in 'Russet Norkotah' potato tubers when treated in 63-L steel drums at 8-15°C. A. The contour plot shows the 2D view of sprout weight (g) as a function treatment interval (days) and dose (mg L^{-1} headspace). The dots show the location of treatments. B. The surface plot shows sprout weight (Sprout_W) as function of treatment interval and dose in 3D. Adjusted R^2 and the P value for the model and lack of fit are given for the quadratic response surface.

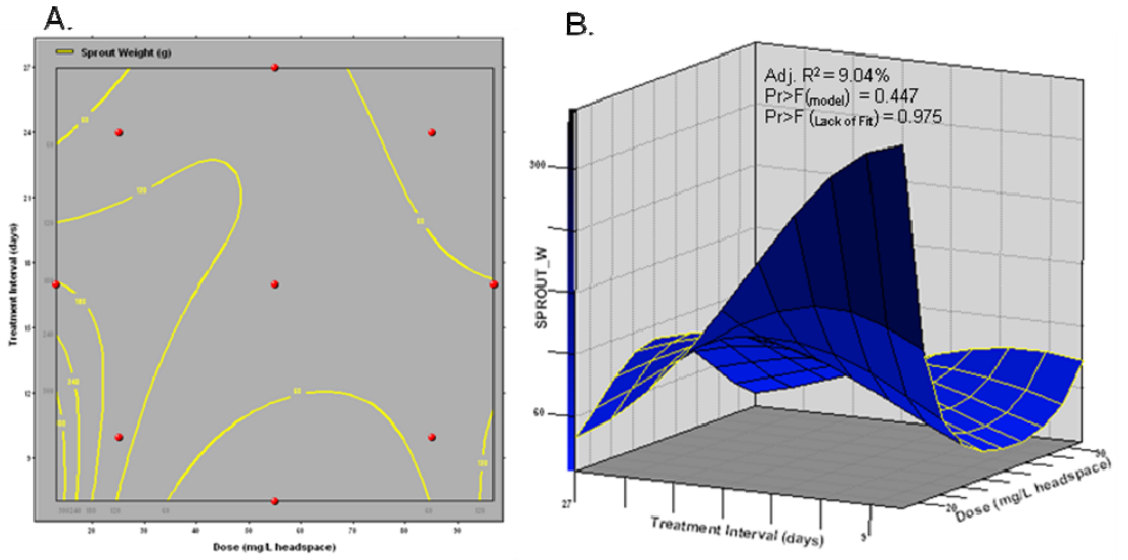


Figure 4.8 The effect of clove oil on sprout suppression in 'Piccolo' potato tubers when treated in 63-L steel drums at 8-15°C. A. The contour plot shows the 2D view of sprout weight (g) as a function treatment interval (days) and dose (mg L^{-1} headspace). The dots show the location of treatments. B. The surface plot shows sprout weight (Sprout_W) as function of treatment interval and dose in 3D. Adjusted R^2 and the P value for the model and lack of fit are given for the quadratic response surface.

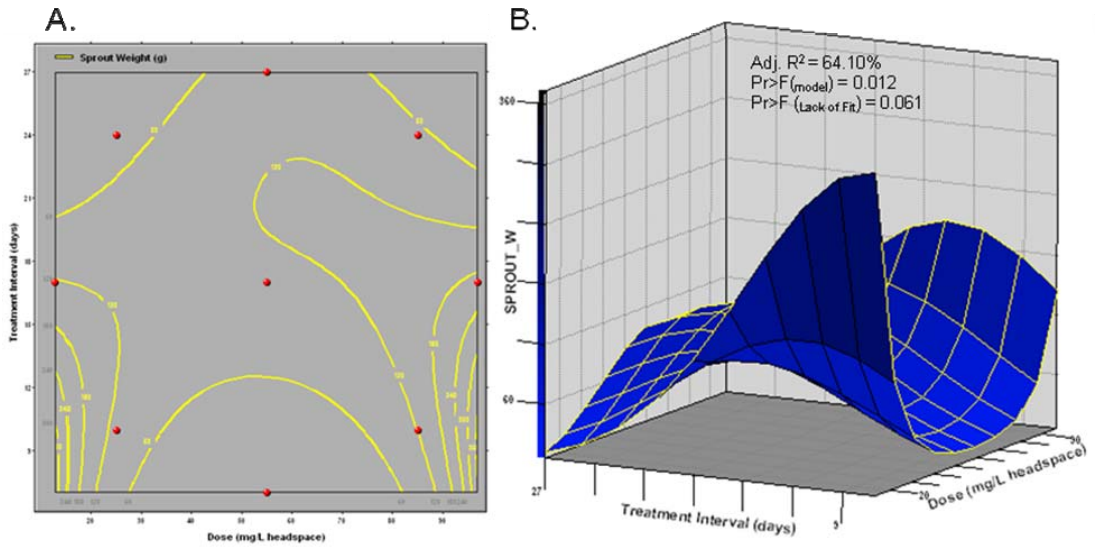


Figure 4.9 The effect of dill weed oil on sprout suppression in 'Russet Norkotah' potato tubers when treated in 63-L steel drums at 8-15°C. A. The contour plot shows the 2D view of sprout weight (g) as a function treatment interval (days) and dose (mg L^{-1} headspace). The dots show the location of treatments. B. The surface plot shows sprout weight (Sprout_W) as function of treatment interval and dose in 3D. Adjusted R^2 and the P value for the model and lack of fit are given for the quadratic response surface.

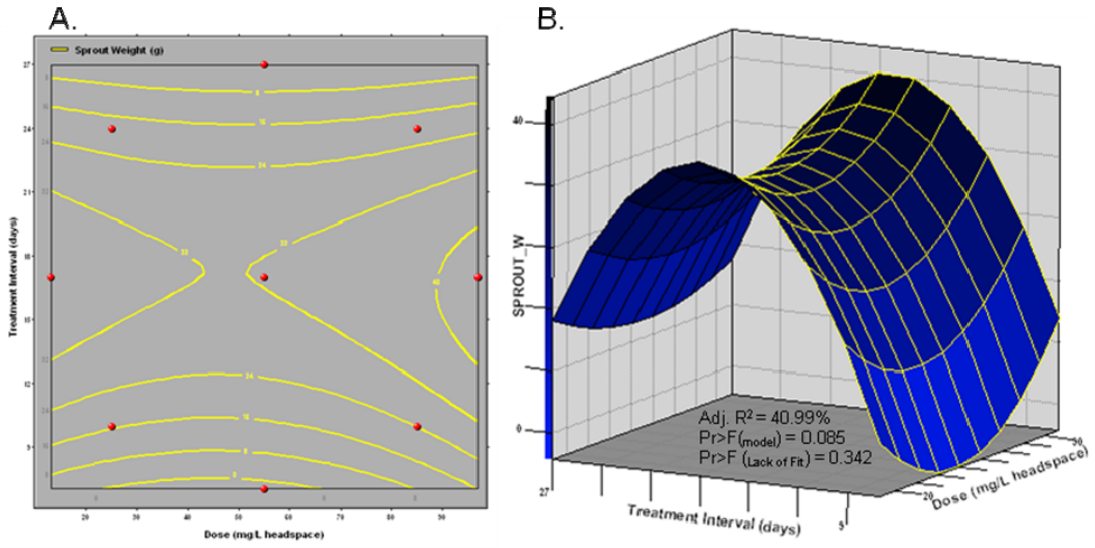


Figure 4.10 The effect of dill weed oil on sprout suppression in 'Piccolo' potato tubers when treated in 63-L steel drums at 8-15°C. A. The contour plot shows the 2D view of sprout weight (g) as a function treatment interval (days) and dose (mg L^{-1} headspace). The dots show the location of treatments. B. The surface plot shows sprout weight (Sprout_W) as function of treatment interval and dose in 3D. Adjusted R^2 and the P value for the model and lack of fit are given for the quadratic response surface.

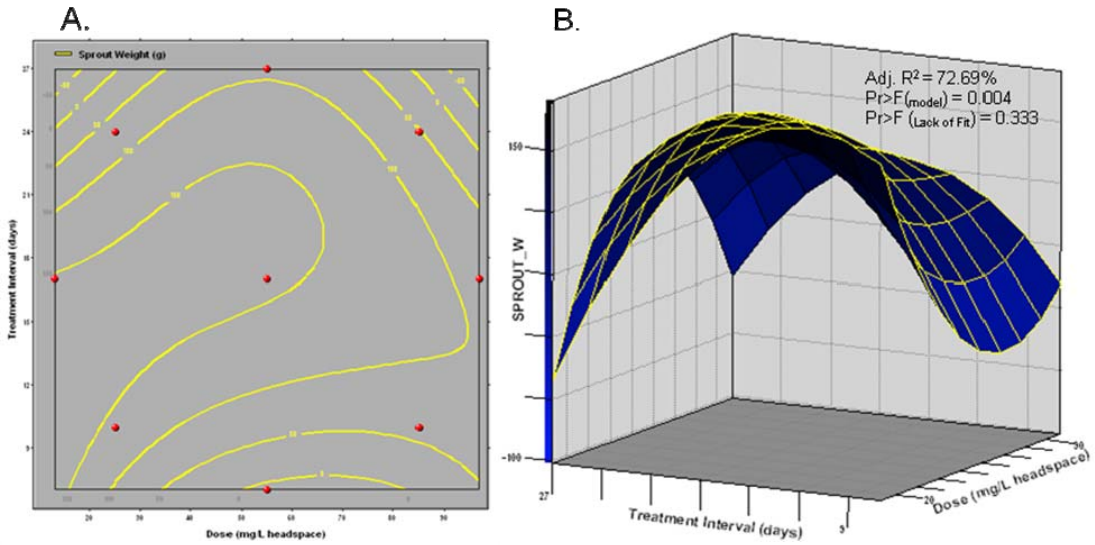


Figure 4.11 The effect of spearmint oil on sprout suppression in 'Russet Norkotah' potato tubers when treated in 63-L steel drums at 8-15°C. A. The contour plot shows the 2D view of sprout weight (g) as a function treatment interval (days) and dose (mg L^{-1} headspace). The dots show the location of treatments. B. The surface plot shows sprout weight (Sprout_W) as function of treatment interval and dose in 3D. Adjusted R^2 and the P value for the model and lack of fit are given for the quadratic response surface.

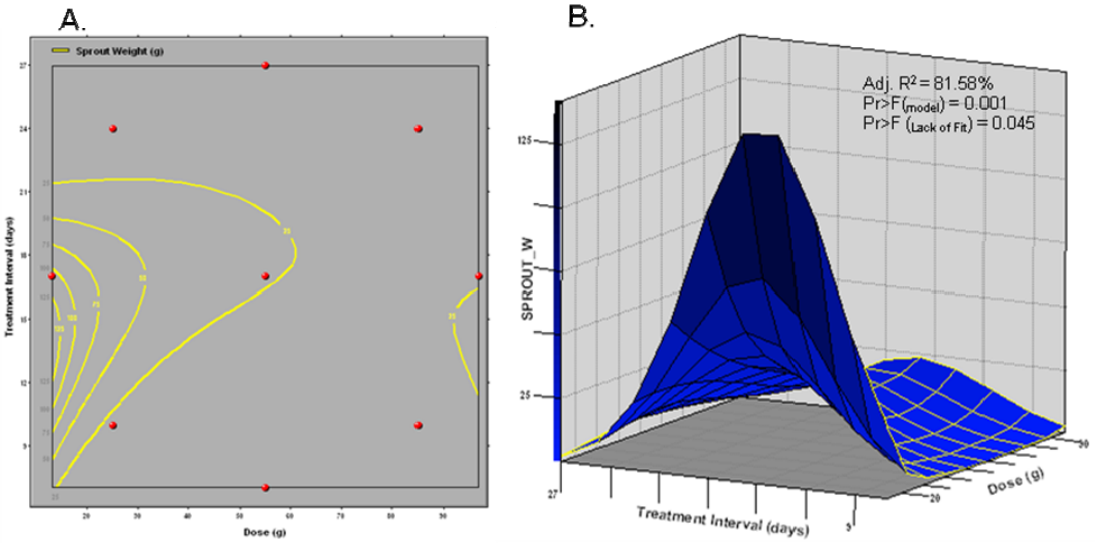


Figure 4.12 The effect of spearmint oil on sprout suppression in 'Piccolo' potato tubers when treated in 63-L steel drums at 8-15°C. A. The contour plot shows the 2D view of sprout weight (g) as a function treatment interval (days) and dose (mg L^{-1} headspace). The dots show the location of treatments. B. The surface plot shows sprout weight (Sprout_W) as function of treatment interval and dose in 3D. Adjusted R^2 and the P value for the model and lack of fit are given for the quadratic response surface.

Table 4.4 Response estimates for factors affecting the sprout weight of 'Russet Norkotah' tubers treated with dill weed oil in 63-L steel drums.

Source	Estimate	Standard Error	t	Pr > ltl
Dose	-0.98	0.45	-2.20	0.059
Trt-Int	0.79	0.20	4.04	0.004*
Dose x Dose	0.40	0.20	2.03	0.077
Dose x Trt-Int[x]	0.19	0.28	0.67	0.521
Trt-Int x Trt-Int	-1.04	0.20	-5.27	0.001*
Dose x Dose x Trt-Int	-2.61	0.55	-4.78	0.001*

[x]Trt-int = Treatment interval

Table 4.5 Response estimates for factors affecting the sprout weight of 'Russet Norkotah' tubers treated with spearmint oil in 63-L steel drums.

Source	Estimate	Standard Error	t	Pr > ltl
Dose	-52.84	50.67	-1.04	0.327
Trt-Int	50.60	22..28	2.27	0.043*
Dose x Dose	-36.08	22.17	-1.63	0.142
Dose x Trt-Int[x]	28.99	31.51	0.92	0.385
Trt-Int x Trt-Int	-116.38	22.39	-5.20	0.001*
Dose x Dose x Trt-Int	-152.08	62.08	-2.45	0.040*

[x]Trt-int = Treatment interval

The large error terms resulted in poor fit of the quadratic response surface model in most cases tested (Figure 4.7-4.12) and this was mainly due to the large variations among the replications. In all cases, the variation among the replications was high (coefficient variation > 15) (Appendix B2), which could be due to the large variation in tuber size or other physiological factor of the tubers. There were also several cases of internal sprouting, which resulted in the development of mini-tubers on the old tubers and further increased the variation of sprout weight.

Previous studies have shown that variations in treatment dose under a single application had a significant effect on sprout weight in treated tubers (Appendix A1). However, under repeated applications, the tubers became relatively insensitive to the variations in treatment doses particularly when dill weed and spearmint oil were applied (Appendix B1). The robust response was also demonstrated on the response surface plots where the shape of the models within the range of the design points have large relatively flat areas with little response variation to dose and interval changes (Figure 4.9, 4.11 and 4.12). The robustness was also reflected on the

duration of sprout suppression. When treatments were applied at ≥55 mg L^{-1} headspace for dill weed oil, ≥25 mg L^{-1} headspace for spearmint oil and the treatment interval was up to 27 days, all treatments effectively suppressed sprouting for more than 26 weeks. This vigorous response in sprout inhibition response is very desirable because these essential oils appear to be equally effective for a wide range of doses and application intervals.

The objective of this portion of the study was to use the response surface optimization algorithm to determine the best dose by treatment interval combination which would minimize the amount of sprout weight produced on treated tubers. Based on the previous results obtained from the studies conducted in 1-L glass jars, the dependent factor, sprout weight, was expected to have significant and primarily linear responses to the two independent factors, dose and treatment interval (Figure 4.2, 3 and 6). However, due to the poor fit of the model the optimum point was unable to be determined. Alternative response parameters including the sprout duration and percentage of non-sprouted tubers were also tested using the response surface model, but they did not improve the fit of the model and the lack of fit test results (data not shown). In terms of optimizing the treatment to achieve the best inhibitory effect on all tubers in storage, it appeared that treatment with 55 mg L^{-1} headspace every 7 days achieved consistent long term sprout suppression for both 'Russet Norkotah' and 'Piccolo' tubers for all essential oils tested. The 85 mg L^{-1} headspace treatment with 24-day interval also uniformly resulted in low sprout weight regardless of the essential oil used and the variety tested (Figures 4.9-4.12).

Noticeably when the model was significant, time interval was the only independent factor that caused significant variations in sprout weight (Table 4.4 and 4.5). The positive linear association between treatment interval sprout weight accumulation could be due to the reduction of available essential oil compound in the headspace near the tubers caused by leakage and ventilation and it also could be due to the compounds absorbed by the tubers were metabolized to less toxic forms over time (Bång 2007). Both factors could have allowed the tubers to resume sprout growth. However, it was not clear why the quadratic effect of treatment interval had negative association with sprout growth.

Consistent with the results from the dose response and the duration response studies, the current study indicated that clove oil treatments were not as effective as dill weed and spearmint oils in suppressing sprouting in both 'Russet Norkotah' and 'Piccolo' tubers. All 'Russet Norkotah' and 'Piccolo' tubers treated with clove oil, regardless of the treatment dose and

interval, sprouted prior to the treatment termination, much earlier than tubers under the treatments of dill weed and spearmint oils. Clove oil treated 'Russet Norkotah' tubers all sprouted within 10-20 weeks of storage (Appendix B1). When the treatment dose was ≥ 55 mg L^{-1} headspace and the treatment interval was < 27 days, 'Piccolo' tubers exposed to clove oil maintained sprout-free for more than 20 weeks, but compared to dill and spearmint oil treatments, clove oil's sprout suppression effect was not as consistent. This result was likely caused by the lower evaporation rate of clove oil compared to dill weed and spearmint oil (Figure 3.2). Clove oil contained > 75% of eugenol and carvone was the major compound in dill (>40%) and spearmint oils (>95%) (Table 3.1). The vapor pressure of eugenol (0.004 kPa at 20^{o}C) is lower than carvone (0.053 kPa at 20^{o}C) and the molecular weight of eugenol (164.2 g mol^{-1}) is higher than carvone (150.22 g mol^{-1}) which could have contributed to the lower volatility of clove oil compared to dill and spearmint oil (Figure 3.2). In this study, all treatments were applied in vapour rather than in thermal fog. The application method may place clove oil at a disadvantage due to its low volatility. However, direct contact of essential oil with the tuber through thermal fogging can also cause severe necrosis and should be avoided. Cizkova *et al.* (2000) recommended implementing the treatment as a slow and stable vapor since direct spraying caused necrosis and rotting on the tuber surface. Thus, using vapour treatment was deemed to be acceptable for this study.

Since carvone and eugenol were the major compounds contained in the essential oils evaluated, the majority of the sprout suppression response would likely be due to the effect of these two compounds. Since the chemical structure of eugenol is very different from S-(+)-carvone and R-(-)-carvone, the structural difference could also have caused eugenol and carvone to behave differently in suppressing sprouting. Studies based on monoterpenes have reported that the presence of certain specific functional groups, including hydroxyl group, carbonyl group and ketone group, were associated to the phytotoxicity the compounds (Reynolds 1987, Oosterhaven 1995, Regnault and Hamraoui 1995). Eugenol has both ether and hydroxyl functional groups, and carvone have one ketone group. It was proposed that the unsaturated carbonyl group have likely played an important role in inhibiting sprout growth (Capelle *et al.* 1996). The hydroxyl group was also reported to be an important structure for the phytotoxicity effect in monoterpenoids (Oosterhaven 1995). However, it is not clear how the presence of ether and hydroxyl groups influence the behavior of eugenol.

It appeared that, for the same oil and dose, tubers of the two varieties had different responses to the suppression effect (Appendix B1).The variety 'Piccolo' is grown for small-size tubers and thus the tubers were harvested before maturity and full development of the skin cells (epidermal and periderm). Potato skin which contains suberized phellem cells is a good barrier to the invasion of pathogens and the diffusion of toxins (Barel and Ginzberg 2008). It is also very likely to be a protective structure to minimize the uptake of essential oil active components. With a weaker barrier, more essential oil compounds could have penetrated into the 'Piccolo' tubers than the 'Russet Norkotah' tubers which may have prohibited sprouting for a longer period of time. However, the differences on inhibitory effect among the common tuber varieties need to be further studied.

In summary, in the 63-L drum sprout suppression study, the majority of the tubers treated with dill and spearmint oils at various dose and intervals remained non-sprouted for more than six months. After treatments were terminated, the residual effect continued to suppress sprouting for 2-5 weeks. The response surface model had a poor fit for most cases (Figure 4.7-4.12) due to the large variations among the replications and the insensitivity of sprout suppression in the treatment variations; therefore, it was not possible to define the optimal dose by treatment interval combinations using the response surface models. However, the robustness of the response suggested that under repeated treatments, tubers were less sensitive to variations in dose of the essential oils in the storage. It not only offers a wide window for effective sprout suppression in long term potato storage, more importantly, it provides the flexibility for the growers and producers to determine the treatment based on other important criteria such as the cost of the treatments.

5.0 IMPACT OF DILL WEED, SPEARMINT AND CLOVE OILS ON 'PICCOLO' SEED TUBER POST-TREATMENT GROWTH AND TUBER YIELD

5.1 Introduction

Well-maintained, well stored, high quality seeds are crucial for potato growers to achieve good yield. High quality potato seeds must be certified, free from seed-borne disease, free from decay, firm and physiologically young and free from stress (Western Potato Council 2003).

Seed potatoes are normally harvested in the fall and delivered between February to April of the next year. After harvest, like table and processing potatoes, the seed tubers are cured for two weeks at 13-15°C. However, seed tubers cannot be treated with sprout inhibitor CIPC because the chemical compound will jeopardize their viability (Hartmans *et al.* 1995). In fact, seed tubers should not even be stored in facilities that were exposed to CIPC in previous years, as the synthetic compound can be absorbed into the storage structure.

To overcome the challenge of pre-plant sprouting, seed potatoes are commonly stored at 3-4°C (37-39°F) and 90-95% RH to ensure their viability. Chilled or frozen tubers germinate poorly and are less vigorous, even though they may still appear to be healthy. Before planting, the storage temperature should be raised to 10-13 °C for 10 days to bring the tubers out of ecodormancy (Lang 1987) maintained by low storage temperatures. The challenge with the low temperature technique is that the method often produces inadequate suppression of sprouting (Sorce *et al.* 1997). Alternative sprout suppression methods are needed to reinforce sprout control in the seed.

Essential oils have demonstrated their potential to be used as reversible sprout suppressants (Oosterhaven *et al.* 1993), which suggested that they could be used on seed tubers (Vokou *et al.* 1993, Sorce *et al.* 1997). Sorce *et al.* (1997) evaluated sprouting of tubers exposed to six months of carvone vapour at 23°C. When carvone headspace concentration ranged from 0.34-1.06 μmol mol^{-1}, equivalent to 0.326-1.018 mg L^{-1} (calculated base on carvone molecular weight of 150 g mol^{-1} and density of 0.96 kg L^{-1}), the treatment suppressed sprouting without affecting bud viability throughout the 6 months storage period. Talent™, a registered sprout inhibitor in the Netherlands, mainly containing monoterpene S-(+)-carvone, was also evaluated for its efficacy on seed tubers (Hartmans *et al.* 1998). Tubers stored in 1-tonne boxes were treated with Talent™ for 6 months before planting. Compared to the control, treated seed tubers

produced higher numbers of stems and comparable total yield. In addition, Talent^{TM}-treated tubers produced higher numbers of tubers per plant because increased numbers of small size (<55 mm) tubers were formed over large size (>55 mm) tubers (Hartmans *et al.* 1998).

Disease control is crucial in seed tuber storage, particularly for pre-cut seeds. The wounds caused by cutting from entry points for disease pathogens (Western Potato Council 2003). Studies have shown that the fungicidal properties of the essential oils in which *Rhizoctonia, Fusarium* and *Scerotinia* were significantly suppressed (Gorris *et al.* 1994, Song *et al.* 2008). This property will be very beneficial to commercial seed tuber storages.

This study was undertaken to determine if dill weed, spearmint, and clove oil expressed any negative effect on seed potatoes. Specifically, the objective was to determine whether essential oils extracted from dill weed, spearmint crops as well as clove oil would result in any adverse effects on post-treatment sprouting, shoot development, and tuber production.

5.2 Materials and Methods

5.2.1 Materials and growing conditions

Potato tubers of the cultivar 'Piccolo', harvested in September of 2006, were supplied by Wedge Wood Farms Ltd., Spruce Grove, Alberta. Prior to treatment exposure, all 'Piccolo' tubers were stored at the Crop Diversification Centre South (CDCS), Brooks, at 6^{o}C in the dark.

The treatments were carried out in conjunction with the study on the duration of sprout inhibition in response to different doses (Chapter 4.2.2). Dill weed, spearmint and clove oils were applied at the dose of 0, 15, 30, 60, 120, and 240 mg L^{-1} headspace to randomly selected non-dormant 'Piccolo' tubers placed in 1-L glass jars. Essential oil was applied onto a 9 cm Whatman #4 filter paper taped onto the lid of each jar. The dose was calculated based on the headspace in each jar. The treatment applications were randomly assigned to the jars. After the treatment applications, all the jars were sealed and stored in a Conviron PG8 growth chamber in the dark at 10^{o}C. Seven days after imposing the treatments, four tubers out of a total of eight were randomly selected from each jar and planted in 1-L plastic pots with growth media Sunshine Mix #4 (SunGro Horticulture Inc.). Each tuber was used as a seed piece. The seed tubers were grown in a 16.5 m^2 Conviron growth chamber with 23^{o}C/18^{o}C day/night temperatures and under 16-hour photoperiod at a light intensity of approximately 240 µmol m^{-2} s^{-1} using a balanced number of fluorescent and incandescent lighting. The potato plants were

watered as required and fertilized once a week with 20:20:20 (N: P: K) at 250 mg L^{-1} starting one month after planting. All plants were harvested 100 days after seeding. This experiment was conducted at the University of Saskatchewan, in the phytotron facilities of the College of Agriculture and Bioresources.

5.2.2 Measurement and statistical analysis

The treatments were arranged in a RCBD design, with blocks placed to account for potential temperature gradients toward the door of the chamber.

There were three replications for each treatment. Treatment effects were assessed by determining date of sprout emergence, number of stems per plant at harvest, dry weight of above ground biomass, number and fresh weight of tubers produced per plant. A portion of tubers treated with 240 mg L^{-1} headspace of dill weed oil were damaged and did not sprout; therefore, no data were collected on these tubers. To be consistent among the three essential oils tested, results generated from 240 mg L^{-1} headspace treatments were omitted from the analysis.

Data were subjected to analysis of variance in a RCBD mixed model using SAS 9.1 (2002-2003, SAS Institute Inc., Cary, NC, USA). Significance and the nature of the relationship between dose and the response variables were assessed by regression analysis (Steel and Torries, 1980).

5.3 Results and Discussion

The dose response study (chapter 4.3.1), the duration response study (chapter 4.3.2) and the scaled-up sprout suppression study (chapter 4.3.3) demonstrated that the efficiency of sprout suppression was largely depending on the treatment dose applies and large variation in sprout suppression response occurred between tubers treated with different type of essential oils. Based on the observations on these studies, we hypothesized that essential oil treated seed tubers would perform differently from control tubers and the different performance was mainly caused by the treatment dose and the type of oil applies; additionally, the different response to oil type would mainly occur between tubers treated with carvone containing essential oils (dill weed and spearmint oils) and tuber treated with eugenol containing essential oil (clove oil). To examine this hypothesis, we conducted a series of analysis of variance (Appendix A9 to 13) and contrasts (Table 5.1 to 5.5).

Tuber emergence time of 'Piccolo' seed tubers was affected by both the type of essential oil applied as well as the dose. The results indicated the majority of the variation caused by oil types (81%) occurred between tubers treated with dill weed and spearmint oils and tubers treated with clove oil (Appendix A9). Tubers treated with dill weed and spearmint oils showed delayed emergence mainly in a linear trend. For dill weed oil treatment, the linear model showed that control tubers sprouted 15.4 days after planting (intercept) and the slope of 0.04 indicated that the increase of treatment dose would extend the emergence time by approximately 0.04 times (Figure 5.1B). For spearmint oil treatment, control tubers also emerged 15.4 days after planting. The linear slope of 0.14 indicated that the exposure of spearmint treatment delayed the emergence by 0.14 times as the dose increased; however, the model also showed a significant negative association between the emergence time and the quadratic effect of spearmint oil treatment indicating after the dose reached beyond certain level it started to cause a decrease in emergence time (Figure 5.1C). The effect of essential oils or their major compounds on seed tuber emergence time after planting had not been studied in previous studies (Sorce *et al.* 1997, Hartmans *et al.* 1998). However, the late emergence in dill weed and spearmint treated seeds could potentially be a concern as the late start would put the plant in disadvantage to compete with weeds when planted in the field.

In comparison to dill weed and spearmint oil treatment, the emergence time was not significantly different among tubers treated with different doses of clove oil (Figure 5.1A). The investigation on oil evaporation rate revealed that under the same conditions, clove oil evaporated much more slowly than dill weed and spearmint oil (Figure 3.2). Therefore, the amount of available active compound would likely be lower and 7 days of exposure may not have allowed the tubers to uptake sufficient quantities of eugenol to cause a delay in emergence.

Table 5.1 Effects of clove, dill weed and spearmint oil dose on sprout emergence of 'Piccolo' seed tubers.

Dose (mg L^{-1} headspace)	Number of days to sprout emergence[!]		
	Clove oil	Dill weed oil	Spearmint oil
0	14.7±0.5 [x]	14.8±0.9	15.1±1.0
15	16.8±0.9	16.5±0.7	18.5±1.0
30	17.1±1.1	16.0±0.5	17.6±0.9
60	14.9±0.8	18.5±0.7	20.6±0.7
120	15.8±1.1	19.3±0.6	17.9±1.5
Means of essential oil type [y]	15.8±0.4 a	17.0±0.4 b	17.9±0.5 b
Statistical Significance			
Dose		*	
Linear	NS	*	*
Quadratic	NS	NS	*
Essential oil		*	
Dill+Spearmint vs.Clove		*	
Dill vs. Spearmint		NS	

[!] Tubers were exposed to the treatments for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

[x] Mean value and s.e. (±)

[y] Essential oil: values followed by different letters differ significantly at P=0.05 (Student-Newman-Keuls test)

NS = not significant at P=0.05; * Significant at P<0.05.

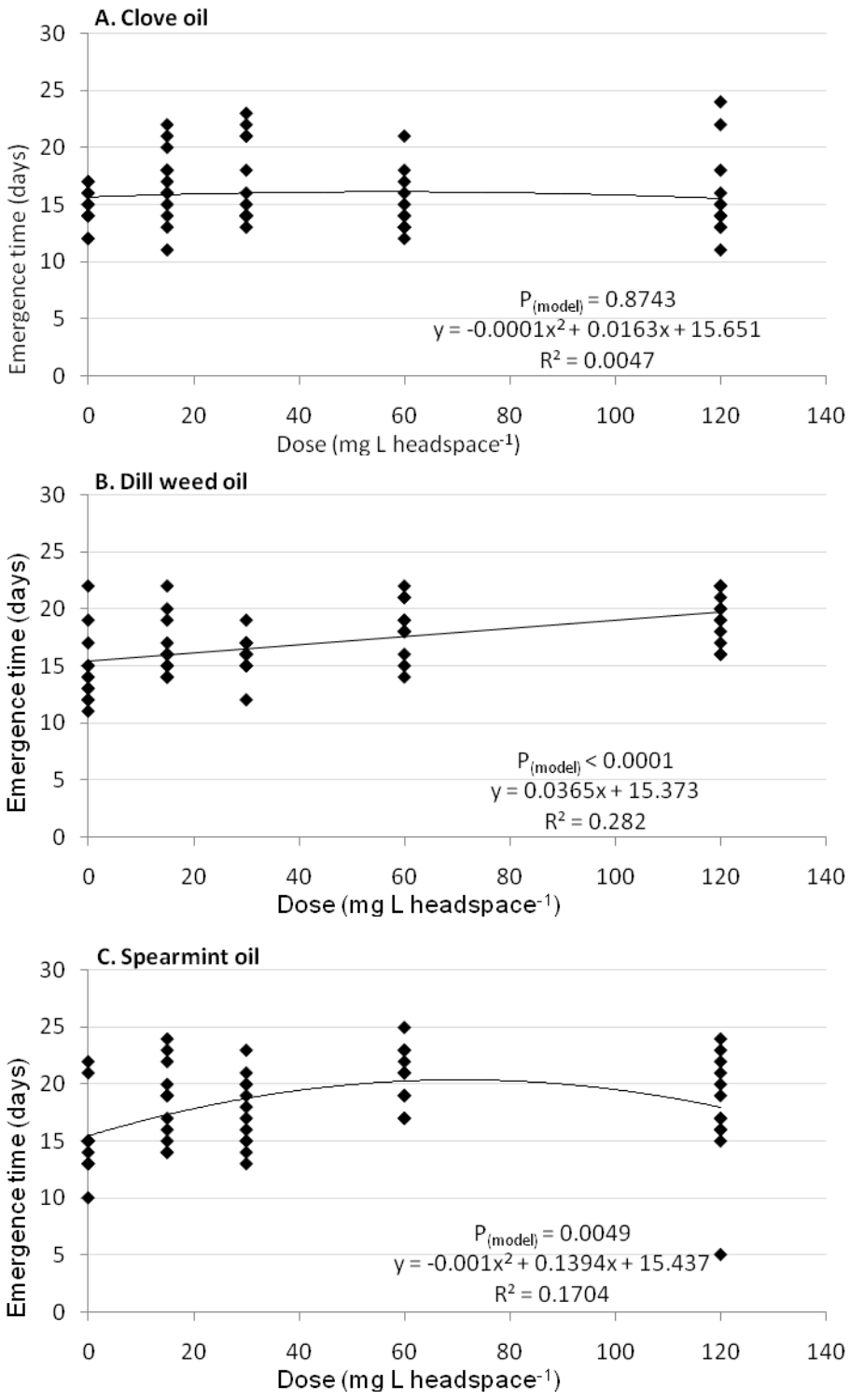


Figure 5.1 The effect of various doses of clove (A), dill weed (B) and spearmint (C) essential oil treatments on sprout emergence of 'Piccolo' seed tubers exposed to the treatments for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

The essential oils did not have significant post-treatment effect on the number of stems produced per plant despite the type of oil applied and the dose applied (Table 5.2, Figure 5.2). In our previous duration effect study, 19 weeks of dill weed oil treatment resulted in the emergence of a higher number of small sprouts in the meristematic region (data not shown). Oosterhaven *et al.* (1995a) noted S-(+)-carvone-treated tubers produced a higher percentage of branched sprouts (i.e. lateral sprouts developed on the main sprout) compared to sprouts exposed to R-(-)-carvone and control. He suggested that the treatment may have caused the loss of apical dominance. Vokou *et al.* (1993) also reported 25, 125 and 250 ppm of linalool, pulegone, carvone and 1,8-cineole treatments did not affect sprouting time but did appeared to cause higher number of sprouts to emerge. In addition, Hartmans *et al.* (1998) showed after treating seed tubers with S-(+)-carvone for six months in storage, the seeds produced more stems per plant compared to the control. Stem density is closely related to tuber yield since tuber density and yield increases with stem density and can be used as an accurate tool to predict tuber set base on the negative correlation between stem density and tuber size (Knowles and Knowles 2006, Bussan *et al.* 2007). In the same study, Hartmans *et al.* (1998) also reported the production of more small size tubers (<55 mm), likely due to the higher number of stems produced by the seed tubers. In our current study, all seed tubers were exposed to the essential oil for 7 days and the relatively short treatment exposure could have been the main limitation of the less pronounced treatment effect on the number of stems produced per plant.

Table 5.2 Effects of clove, dill weed and spearmint oil dose on the number of stems produced by 'Piccolo' seed tubers.

Dose (mg L^{-1} headspace)	Number of stems seed tubers^{-1} [!]		
	Clove oil	Dill weed oil	Spearmint oil
0	1.6±0.1 [x]	1.5±0.2	1.8±0.3
15	1.5±0.2	1.3±0.1	1.3±0.1
30	1.3±0.2	1.3±0.1	1.3±0.2
60	1.3±0.1	1.6±0.1	1.1±0.1
120	1.3±0.1	1.3±0.1	1.4±0.1
Means of essential oil type [y]	1.4±0.1 a	1.4±0.1 a	1.4±0.1 a
Statistical Significance			
Dose	NS		
Linear	NS	NS	NS
Quadratic	NS	NS	NS
Essential oil	NS		
Dill+Spearmint vs.Clove	NS		
Dill vs. Spearmint	NS		

[!] Tubers were exposed to the treatments for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

[x] Mean value and s.e. (±)

[y] Essential oil: values followed by different letters differ significantly at P=0.05 (Student-Newman-Keuls test)

NS = not significant at P=0.05; * Significant at P<0.05.

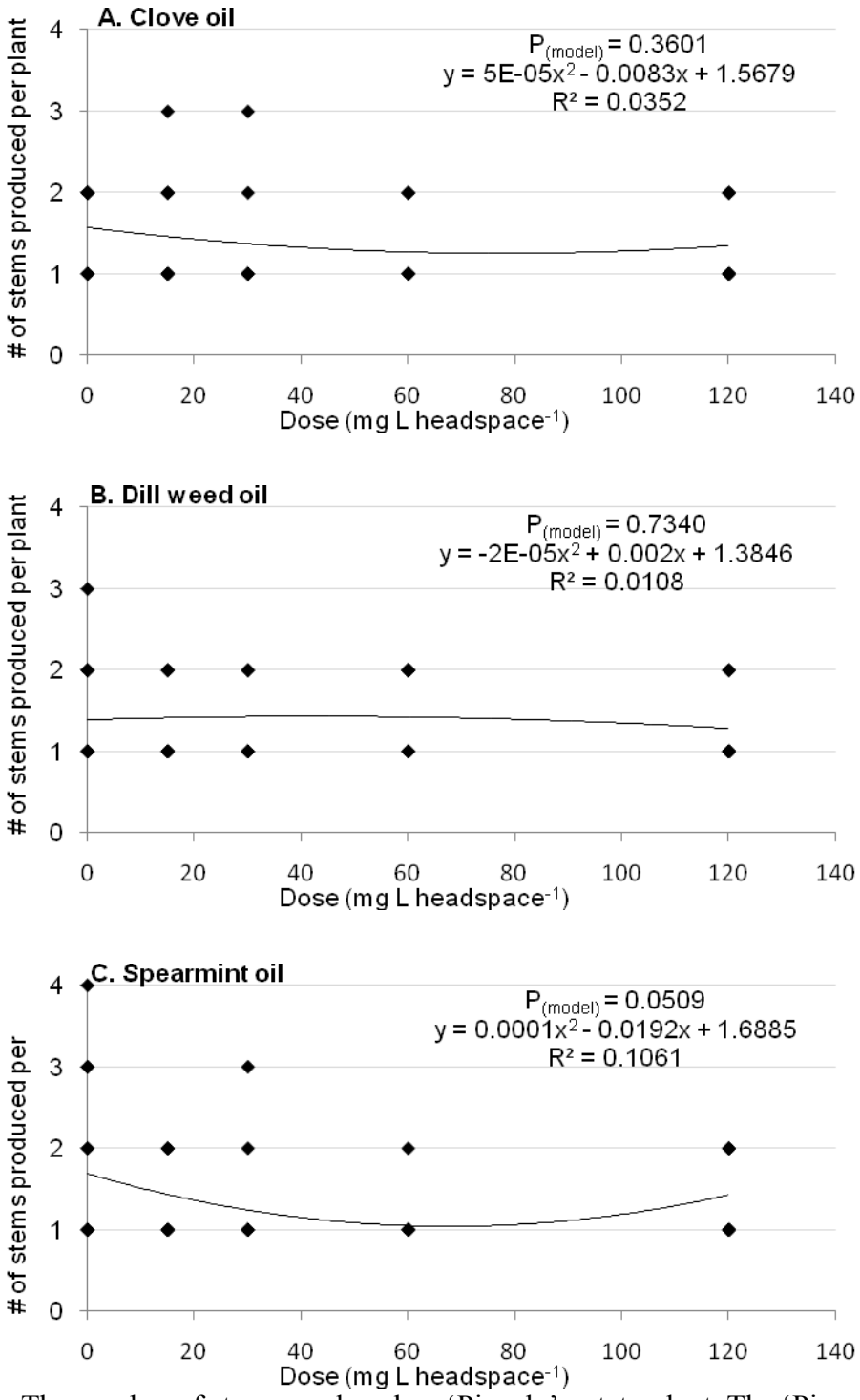


Figure 5.2 The number of stems produced on 'Piccolo' potato plant. The 'Piccolo' seed tubers were previously treated with clove (A), dill weed (B) and spearmint (C) essential oils at different doses for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

Overall, the essential oil treatment dose had no significant impact on the subsequent aboveground dry weight produced; however, the type of oil applied did have a significant effect (Table 5.3, Figure 5.3). The highly significant difference mainly occurred between tubers treated with dill weed and spearmint oils (containing carvone) versus tubers exposed to clove oil (containing eugenol) which represented 70.93% of response variations caused by the essential oils (Appendix A11). However, since clove oil clearly has a reduced evaporation rate (Figure 3.2), the difference between the oils is likely due to the resulting difference in vapor pressure.

On average, seeds treated with dill weed and spearmint oils produced higher plant dry weight compared to clove oil treated seeds. The analysis on emergence time showed that seeds exposed to dill weed and spearmint oils had delayed emergence (Table 5.1); however, according to the results collected on plant dry weight, the delay did not seem to have adverse effect on the growth of the plants. This response would be favorable from a practical perspective as establishment of an aboveground canopy, especially during the early developmental stage, is very important for the plant to be more competitive against weeds (Vangessel and Renner 1990).

The responses on plant dry weight were also significantly different between seed tubers treated with dill weed oil and seed tubers treated with spearmint oil as the latter produced higher plant dry weight (Table 5.3). In addition, although the overall dose effect was not significant, the dose variations in spearmint oil treatments did result in a significant linear and quadratic trend on plant dry weight. Between the dose of 15-60 mg L^{-1} headspace, as treatment dose increased, the plant dry weight also increased at a rate of 0.0268 (slope); however, as the dose reached 120 mg L^{-1} headspace the plant dry weight decreased as indicated by the negative association (-0.0002) between plant dry weight and the quadratic effect of treatment dose (Figure 5.3C).

Table 5.3 Effects of clove, dill weed and spearmint oil dose on the total above ground biomass dry weight of 'Piccolo' seed tubers.

	Above ground biomass dry weight (g) seed tuber^{-1} [!]		
Dose (mg L^{-1} headspace)	Clove oil	Dill weed oil	Spearmint oil
0	4.2±0.2 [x]	4.1±0.4	4.2±0.2
15	3.9±0.2	4.2±0.2	4.8±0.2
30	4.4±0.2	4.2±0.2	4.8±0.2
60	3.9±0.2	4.5±0.2	5.1±0.2
120	3.9±0.3	4.8±0.3	4.6±0.2
Means of essential oil type [y]	4.1±0.1 a	4.4±0.1 b	4.7±0.1 c
Statistical Significance			
Dose	NS		
Linear	NS	NS	*
Quadratic	NS	NS	*
Essential oil	*		
Dill+Spearmint vs.Clove	*		
Dill vs. Spearmint	*		

[!] Tubers were exposed to the treatments for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

[x] Mean value and s.e. (±)

[y] Essential oil: values followed by different letters differ significantly at P=0.05 (Student-Newman-Keuls test)

NS = not significant at P=0.05; * Significant at P<0.05.

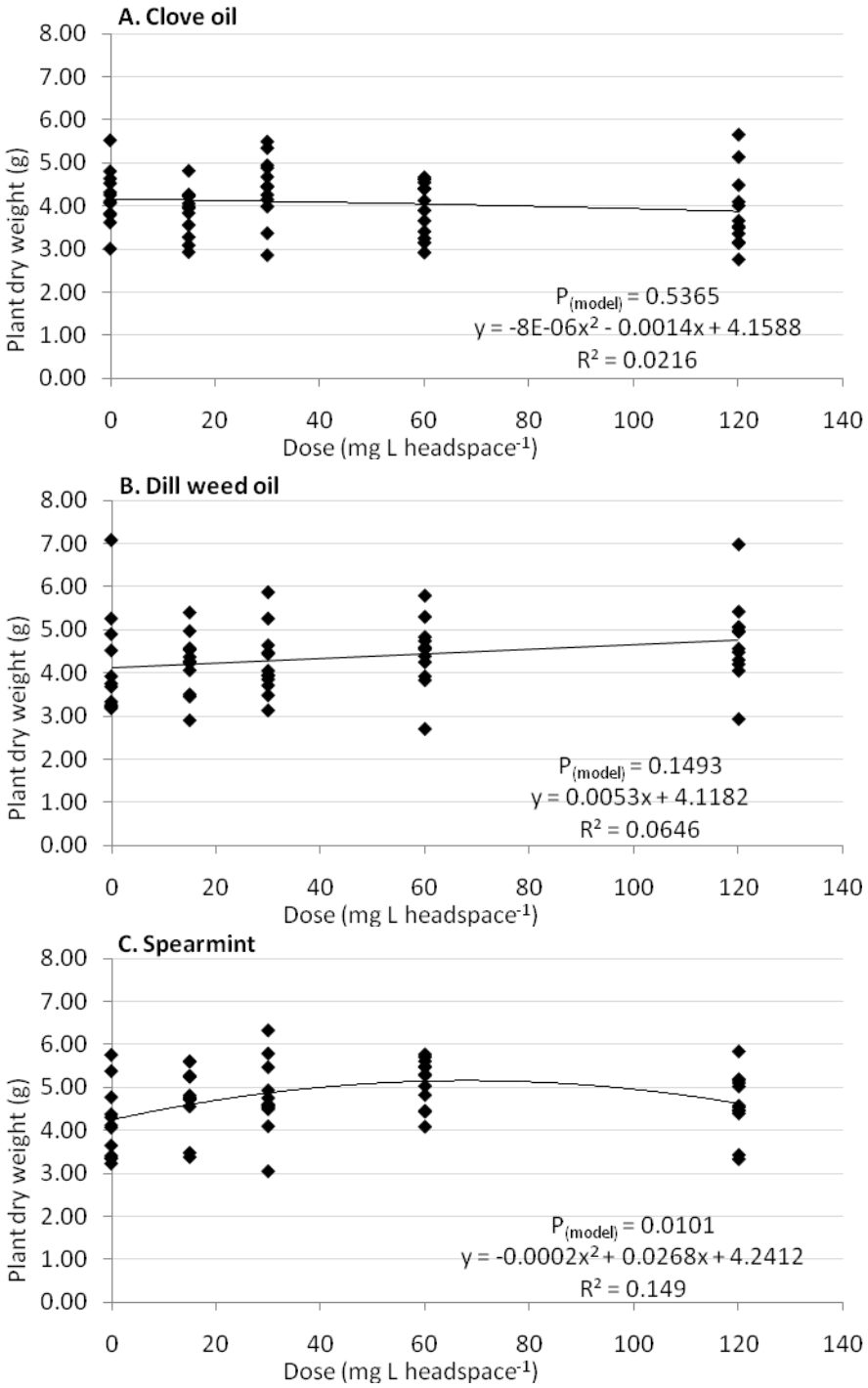


Figure 5.3 The above ground dry weight produced by seed tubers treated with clove (A), dill weed (B) and spearmint (C) essential oils at various doses for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

Despite the significant effect of dill weed and spearmint oil treatments on delaying seed tuber emergence and higher production of aboveground dry weight, all treatments applied had no significant effect on the subsequent yield of the treated seed tubers in terms of the number of tubers produced and the total tuber weight (Table 5.4 and 5.5). The non-significant linear and quadratic dose effect indicated that between the treatment doses of 15 to 120 mg L^{-1} headspace, the post-treatment seed tuber yield was at the same level as the untreated control seed tubers. The type of oil applied also had non-significant effect on the overall tuber yield. This result suggested that neither the dose nor the type of essential oil applied had any significant adverse effect on the subsequent yield of the treated 'Piccolo' seed tubers after 7 days of exposure. This outcome is particularly desirable as the tuber yielding ability would be the ultimate seed quality for the growers.

Table 5.4 Effects of clove, dill weed and spearmint oil dose on the number of tubers produced by 'Piccolo' seed tubers.

	Number of tubers produced seed tuber^{-1} [!]		
Dose (mg L^{-1} headspace)	Clove oil	Dill weed oil	Spearmint oil
0	5.4±0.5 [x]	4.9±0.3	5.0±0.5
15	5.3±0.5	6.3±0.4	5.9±0.5
30	5.8±0.4	5.6±0.2	5.1±0.4
60	5.3±0.4	4.7±0.4	5.8±0.4
120	5.3±0.3	5.3±0.6	6.4±0.5
Means of essential oil type [y]	5.4±0.2 a	5.4±0.2 a	5.7±0.2 a
Statistical Significance			
Dose	NS		
Linear	NS	NS	NS
Quadratic	NS	NS	NS
Essential oil	NS		
Dill+Spearmint vs.Clove	NS		
Dill vs. Spearmint	NS		

[!] Tubers were exposed to the treatments for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.
[x] Mean value and s.e. (±)
[y] Essential oil: values followed by different letters differ significantly at P=0.05 (Student-Newman-Keuls test)
NS = not significant at P=0.05; * Significant at P<0.05.

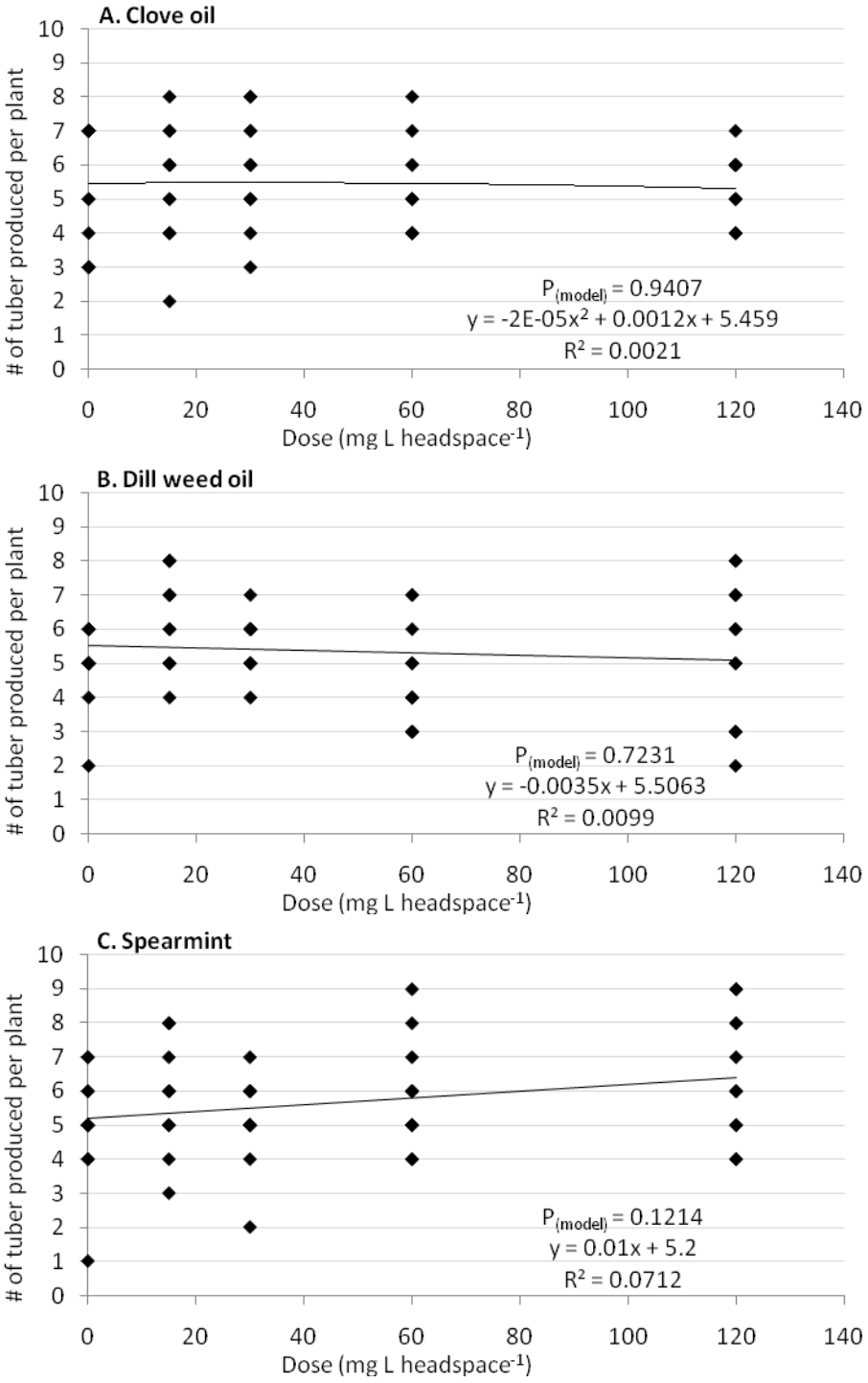


Figure 5.4 The number of tubers produced per plant by seed tubers treated with clove (A), dill weed (B) and spearmint (C) essential oils at various doses for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

Table 5.5 Effects of clove, dill weed and spearmint oil dose on tuber yield of 'Piccolo' seed tubers.

Dose (mg L^{-1} headspace)	Tuber yield (g) seed tuber $^{-1}$!		
	Clove oil	Dill weed oil	Spearmint oil
0	121.4±3.3 [x]	113.5±4.7	113.6±4.9
15	127.9±3.5	123.8±2.0	124.1±4.1
30	124.2±5.5	122.2±4.6	119.4±4.2
60	115.2±5.6	122.1±5.1	122.5±4.1
120	130.8±2.7	118.4±3.9	125.5±3.6
Means of essential oil type [y]	123.9±2.0 a	120.0±1.9 a	121.1±1.9 a
Statistical Significance			
Dose	NS		
Linear	NS	NS	NS
Quadratic	NS	NS	NS
Essential oil	NS		
Dill+Spearmint vs.Clove	NS		
Dill vs. Spearmint	NS		

! Tubers were exposed to the treatments for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

[x] Mean value and s.e. (±)

[y] Essential oil: values followed by different letters differ significantly at P=0.05 (Student-Newman-Keuls test)

NS = not significant at P=0.05; * Significant at P<0.05.

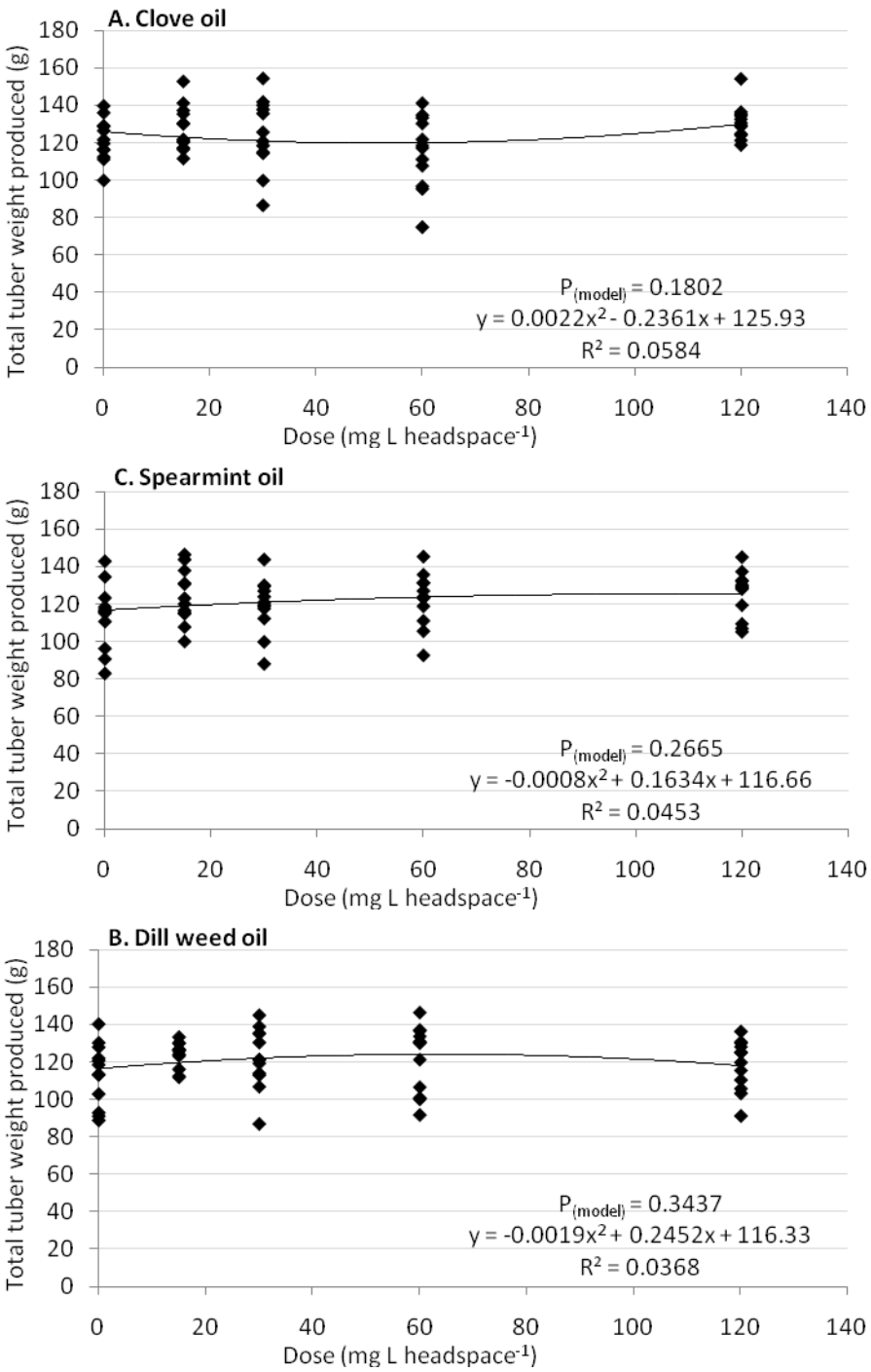


Figure 5.5 The total weight of tubers produced per plant by seed tubers treated with clove (A), dill weed (B) and spearmint (C) essential oils at various doses for 7 days at 10°C and then planted in growth chambers with 23°C/18°C day/night temperatures and under 16-hour photoperiod.

Although the essential oil treatment on 'Piccolo' seed tubers showed no adverse effect on either the number of stems produced, the plant dry weight or the tuber yield, high doses of essential oil treatments may cause damage. Among the tubers treated with 240 mg L^{-1} headspace of dill weed oil, 1/3 of seed tubers lost viability and did not sprout (data not shown). Our duration effect study also showed that high dose (>120 mg L^{-1} headspace) and prolonged treatment could cause necrosis and severe damage to the tuber buds (Appendix C1). In addition, the current study suggested that the dill weed and spearmint oil treatments extended tuber seed emergence time. Early emergence is an important factor to ensure the plant gains an early competitive advantage against weeds, avoids diseases and maximizes tuber yield. This is particularly important for the organic farming system where pesticides and fungicides are not options. Early sprouting combined with early planting may result in early bulking and higher yields earlier in the season, and thus reduces the potential loss caused by disease such as late blight (Hospers-Brands *et al.* 2008).

The current study showed when the essential oil had significant effects on the treatment responses, the majority of the variations occurred between tubers treated with clove oil and tubers treated with dill or spearmint oils (Table 5.1 and 5.3). This result was consistent with the results obtained from the scaled up sprout suppression study. This difference may be attributed to the slower evaporation rate of clove oil compared to dill and spearmint oil extracts.

Seed response differences were also observed among tubers treated with dill weed and spearmint oil extracts. For example, seeds treated with spearmint oil produced higher plant dry weight than seeds treated with dill weed oil (Table 5.3) and also 1/3 of tubers treated with 240 mg L^{-1} headspace of dill weed oil did not sprout. By comparison, tubers exposed to the same concentration of spearmint oil all sprouted. Dill weed oil contains also the S-(+)-carvone isomer while spearmint essential oil contains the R-(-)-carvone isomer. Stereospecific effects have been reported previously (Reynolds 1987, Oosterhaven *et al.* 1995a). The germination rate of apple seeds were reduced by 50% after being treated with 0.058 mM of S-(+)-carvone and the same effect was achieved with 0.38 mM of R-(-)-carvone (Reynolds 1987). Oosterhaven *et al.* (1995a) tested sprout inhibitory effects of both S-(+)-carvone and R-(-)-carvone in potato eye pieces and found S-(+)-carvone inhibited sprout elongation earlier than R-(-)-carvone. They extracted carvone from the treated sprouts and found, after four days of exposure, the concentration of S-(+)-carvone was almost twice the concentration of R-(-)-carvone in the sprouts. The authors

attributed the greater efficacy of S-(+)-carvone to the variation in uptake rate. In our current study, the 7 day treatment duration may have been insufficient to allow the tubers to absorb enough R-(-)-carvone to permanently damage the meristems. Although the physical properties of the two compounds are very similar including the molecular weight, solubility and volatility, their interactions with phospholipid monolayers and pepitides have been reported to be different (Pathirana *et al*. 1992; Nandi 2005). The chiral structure is likely to be the major difference which causes the two compounds to behave differently. Pathirana *et al*. (1992) reported that the interaction between R-(-)-carvone and the monolayer absorbed twice as much heat as S-(+)-carvone at 27.5°C, and the monolayer was more expanded with R-(-)-carvone than with S-(+)-carvone. In addition, the different tuber response to dill weed and spearmint oils may also be attributable to the faster evaporation rate of dill weed oil compared to spearmint oil (Figure 3.2), spearmint oil consists of over 97% pure carvone, more than twice the quantity of carvone than dill weed oil (42%). It is also unclear if the other components within dill weed oil (approximately 15% α-phellandrene and 34% limonene) have any negative effects. Oosterhaven (1995) had previously indicated that exposure to limonene induced necrosis in treated tubers.

In summary, dill weed, spearmint and clove oil treatments did not diminish seed tuber viability or tuber yield (total tuber weight and tuber number). However, dill weed and spearmint oil treatments did extend the sprout emergence time of the treated seed tubers but also resulted in higher plant dry weight compared to clove oil treatments. The potential problem of delayed emergency after planting might be avoided through proper management of ventilation and altering storage temperature but more research is required in this area. The current study showed short term treatment exposure (7 days) did not cause a significant negative effect on seed tuber viability. However, the scaled up sprout suppression study indicated repeated treatment application was needed to achieve long term sprout suppression. Thus, for future studies, the potential prolonged and repeated treatment effects on seed tubers should be examined preferably in a field study to further confirm the suitability of implementing the essential oil treatments in long term commercial seed potato storage.

6.0 SUMMARY, CONCLUSIONS AND FUTURE WORK

Although the composition of the essential oils is associated with many factors such as climate and growing conditions as well as cultural practices, the composition analysis showed no significant fluctuations in oil composition of crops harvested in different years. The evaporation test indicated dill and spearmint oils are more volatile than clove oil where the major compound in dill weed and spearmint oil had a much higher vapor pressure than the major compound in clove oil. Due to the volatility of the essential oils, 75-90% of the oil residues on the treated tubers evaporated within two weeks with sufficient air circulation in the storage. As the storage condition is crucial for the success of using essential oils to suppress potato sprouting, future studies should focus on defining the suitable commercial storage materials and conditions which will maximize the suppression effect of the essential oils.

Overall, the study demonstrated that the readily available, locally produced dill weed and spearmint essential oils are potent potato sprout inhibitors for short- and long-term storage conditions. Both essential oils can be applied as a vapor, and the inhibitory effect is dependent both on the dose and the application interval implemented. The time interval between applications appears to be more critical in achieving effective sprout suppression than the dose itself. However, the importance of dose should not be overlooked, as sprout inhibition will fail if the dose is too low and necrosis will occur on tubers if the dose applied is too high. The market available clove oil (Biox-C^{TM}) was implemented in the study as a comparison and the study has shown that it was less effective in suppressing sprouting compared to dill weed or spearmint oil when applied in vapor form, however, a previous study suggested it was more effective in sprout suppression when applied as a thermal fog (Frazier *et al.* 2004). To be able to implement dill and spearmint oil in large commercial storage, future studies should be focused on finding the suitable system that can ensure a continuous supply of the essential oil (Hartmans *et al*. 1995) but also at repeated intervals to optimize sprout inhibition.

The seed tuber study demonstrated that dill weed and spearmint essential oils could be used in seed tuber storage. Essential oil treated seed tubers maintained the same level of yield compared to the control. Although some treatments appeared to cause a delay in emergence when the tubers were planted immediately after 7 days of treatment exposure, due to the fast dissipation rate of the compound residue, the delayed sprout growth could be avoided by

implementing a sufficient period of ventilation prior to planting. For instance, growers might terminate essential oil treatments for a period of time prior to planting to allow the seeds to resume sprout growth and regain its vigor. In addition, growers could increase storage temperature to promote sprouting and increasing storage temperature to 10-13°C approximately 10 days before planting is a common practice in seed storage to allow the tubers to break dormancy and resume sprout growth (Lang 1987). For future studies, a field trial should be conducted after a long storage period to further investigate the potential effect of essential oil treatment after long-term exposure.

Sprout suppression efficacy of locally grown dill weed and spearmint essential oils have been demonstrated through this study. Spearmint essential oil extract appeared to have a more significant impact on sprout suppression than dill weed oil. However, it is important to determine the economic feasibility of using the dill weed and spearmint essential oils in commercial potato storage. A market analysis will be necessary for the next stage of development for the promising new potential sprouting inhibitors since profitability will be the key factor in determining its success on the market.

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APPENDIX A. AVOVA TABLES

A .1 Analysis of Variance of the effects of essential oil type and doses on sprout weight of the 'Russet Burbank' tubers 33 days after the initial exposure in 1-L glass jars at 24.5°C.

Source	DF	SS[x]	MS	F Value	Pr>F
Replication	2	0.02	0.01	0.03	0.9715
Essential oil type	1	0.01	0.01	0.03	0.8724
Essential oil dose	5	123.60	24.72	83.72	<0.0001*
Linear	(1)	97.47 (76.4%)	97.47	319.93	<0.0001*
Quadratic	(1)	13.18 (10.7%)	13.18	44.62	<0.0001*
Residual	(3)	15.96	15.96	53.2	
Essential oil type x Essential oil dose	5	1.75	0.35	1.18	0.3494
Error	22	6.50	0.30		
Corrected Total	35	131.87			

[x]SS: The percentage (76.4% and 10.7%) indicates the proportion of the variance represented by the linear and quadratic effect, respectively.
*Significant at $P < 0.05$

A .2 Analysis of Variance of the effect of essential oils and treatment dose on number of sprouts produced on 'Russet Burbank' tubers after 33 days of exposure in 1-L glass jars at 24.5°C.

Source	DF	SS[x]	MS	F Value	Pr>F
Replication	2	9.72	4.86	1.86	0.1799
Essential oil type	1	1.78	1.78	0.68	0.4188
Essential oil dose	5	852.22	170.44	65.09	<0.0001*
Linear	(1)	629.04 (73.8%)	629.04	240.21	<0.0001*
Quadratic	(1)	119.10 (14.0%)	119.10	45.48	<0.0001*
Residual	(3)	24.74	12.37	4.72	
Essential oil type x Essential oil dose	5	33.89	6.78	2.59	0.0549
Error	22	6.50	0.30		
Corrected Total	35	131.87			

[x]SS: The percentage (73.8% and 14.0%) indicates the proportion of the variance represented by the linear and quadratic effects, respectively.
*Significant at $P < 0.05$

A .3 Analysis of Variance of the effect of clove oil on sprout suppression in 'Russet Norkotah' tubers when the treatments were applied in 63-L steel drums at 8-15°C.

Source	DF	SS	MS	F Value	Pr > F
Dose	1	56.41	56.42	1.95	0.196
Trt-Int	1	33.23	33.24	1.15	0.312
Dose x Dose	1	26.86	26.68	0.92	0.362
Dose x Trt-Int[x]	1	0.33	0.33	0.01	0.917
Trt-Int x Trt-Int	1	8.34	8.34	0.29	0.605
Dose x Dose x Trt-Int	1	50.01	50.01	1.73	0.221
Model	6	143.01	23.83	0.82	0.579
Error	9	260.51	28.95		
(Lack of Fit)	2	115.15	57.57	2.77	0.129
(Pure Error)	7	145.36	20.77		
Total	15	403.52			

[x]Trt-int = Treatment interval
Significant at $P < 0.05$

A .4 Analysis of Variance of the effect of clove oil on sprout suppression in 'Piccolo' tubers when the treatments were applied in 63-L steel drums at 8-15°C.

Source	DF	SS	MS	F Value	Pr > F
Dose	1	1.19	1.19	2.45	0.152
Trt-Int	1	0.65	0.65	1.35	0.276
Dose x Dose	1	0.01	0.01	0.03	0.867
Dose x Trt-Int[x]	1	0.01	0.01	0.02	0.883
Trt-Int x Trt-Int	1	0.84	0.84	1.73	0.220
Dose x Dose x Trt-Int	1	1.02	1.02	2.11	0.180
Model	6	3.09	0.52	1.07	0.447
Error	9	4.36	0.48		
(Lack of Fit)	2	0.03	0.02	0.03	0.975
(Pure Error)	7	4.33	0.62		
Total	15	7.45			

[x]Trt-int = Treatment interval
Significant at $P < 0.05$

A .5 Analysis of Variance of the effect of dill weed oil on sprout suppression in 'Russet Norkotah' tubers when the treatments were applied in 63-L steel drums at 8-15^{o}C.

Source	DF	SS	MS	F Value	Pr > F
Dose	1	0.21	0.21	1.52	0.249
Trt-Int	1	1.26	1.25	9.09	0.015*
Dose x Dose	1	0.32	0.32	2.30	0.164
Dose x Trt-Int[x]	1	0.03	0.03	0.25	0.628
Trt-Int x Trt-Int	1	2.14	2.14	15.48	0.003*
Dose x Dose x Trt-Int	1	1.76	1.76	12.73	0.006*
Model	6	4.54	0.76	5.46	0.012*
Error	9	1.25	0.14		
(Lack of Fit)	2	0.69	0.34	4.29	0.061
(Pure Error)	7	0.56	0.08		
Total	15	5.78			

[x]Trt-int = Treatment interval
*Significant at P < 0.05

A .6 Analysis of Variance of the effect of dill weed oil on sprout suppression in 'Piccolo' tubers when the treatments were applied in 63-L steel drums at 8-15^{o}C.

Source	DF	SS	MS	F Value	Pr > F
Dose	1	38.03	38.03	0.26	0.621
Trt-Int	1	5.32	5.32	0.04	0.852
Dose x Dose	1	134.64	134.64	0.93	0.360
Dose x Trt-Int[x]	1	9.92	9.92	0.07	0.799
Trt-Int x Trt-Int	1	2191.12	2191.12	15.12	0.004*
Dose x Dose x Trt-Int	1	4.61	4.61	0.03	0.862
Model	6	2379.62	396.60	2.74	0.085
Error	9	1304.29	144.92		
(Lack of Fit)	2	344.23	172.12	1.25	0.342
(Pure Error)	7	960.06	137.15		
Total	15	3683.92			

[x]Trt-int = Treatment interval
*Significant at P < 0.05

A .7 Analysis of Variance of the effect of spearmint oil on sprout suppression in 'Russet Norkotah' tubers when the treatments were applied in 63-L steel drums at 8-15^{o}C.

Source	DF	SS	MS	F Value	Pr > F
Dose	1	4515.96	4515.96	5.01	0.052
Trt-Int	1	5120.72	5120.72	5.69	0.041*
Dose x Dose	1	2629.79	2629.79	2.92	0.122
Dose x Trt-Int[x]	1	840.42	840.42	0.93	0.359
Trt-Int x Trt-Int	1	26822.77	26822.77	29.78	0.000*
Dose x Dose x Trt-Int	1	5959.84	5959.84	6.62	0.030*
Model	6	41356.88	6892.81	7.65	0.004*
Error	9	8106.08	900.68		
(Lack of Fit)	2	2187.37	1093.69	1.29	0.333
(Pure Error)	7	5918.71	845.93		
Total	15	49462.96			

[x]Trt-int = Treatment interval
*Significant at P < 0.05

A .8 Analysis of Variance of the effect of spearmint oil on sprout suppression in 'Piccolo' tubers when the treatments were applied in 63-L steel drums at 8-15^{o}C.

Source	DF	SS	MS	F Value	Pr > F
Dose	1	2.47	2.47	6.94	0.027*
Trt-Int	1	1.30	1.30	3.64	0.089
Dose x Dose	1	0.80	0.80	2.24	0.168
Dose x Trt-Int[x]	1	0.01	0.01	0.01	0.924
Trt-Int x Trt-Int	1	20.52	20.52	57.64	0.000*
Dose x Dose x Trt-Int	1	1.94	1.94	5.44	0.045*
Model	6	25.78	4.29	12.07	0.001*
Error	9	3.20	0.36		
(Lack of Fit)	2	1.88	0.94	4.97	0.045*
(Pure Error)	7	1.32	0.19		
Total	15	28.99			

[x] Trt-int = Treatment interval
*Significant at P < 0.05

A .9 Analysis of Variance of the impact of dill weed, spearmint and clove oil treatments on sprout emergence of 'Piccolo' seed tubers planted after 7 days of treatment exposure at 10°C.

Source	DF	SS[x]	MS	F Value	Pr > F
Block	2	0.48	0.24	0.02	0.9757
Essential Oil	2	133.01	66.51	6.86	0.0014*
Dill+Spearmint vs. Clove	(1)	107.82 (81.06%)	107.82	11.12	0.0011*
Dill vs. Spearmint	1	25.21 (18.95%)	25.21	2.60	0.1088
Dose	4	222.76	55.69	5.74	0.0002*
Linear	(1)	148.23 (66.54%)	148.23	15.29	0.0001*
Quadratic	(1)	41.72 (18.73%)	41.72	4.30	0.0396*
Residual	(2)	32.81	16.41	1.69	
Essential Oil x Dose	8	187.71	23.47	2.42	0.0170*
(Dill+Spearmint)-Linear vs. Clove-Linear	(1)	66.61 (35.49%)	66.61	6.87	0.0096*
(Dill+Spearmint)-Quadratic vs. Clove-Quadratic	(1)	1.22 (0.65%)	1.22	0.13	0.7237
Dill-Linear vs. Spearmint-Linear	(1)	7.00 (3.73%)	7.00	0.72	0.3966
Dill-Quadratic vs. Spearmint-Quadratic	(1)	38.00 (20.24%)	38.00	3.92	0.0494*
Residual	(4)	74.88	18.72	1.93	
Error	163	1580.11	9.69		
Total	179	2124.06			

[x]SS: The percentage indicates the proportion of the variance represented by the factor.
*Significant at $P < 0.05$

A .10 The Analysis of Variance of the impact of dill weed, spearmint and clove oil treatments on the number of stems produced by the 'Piccolo' seed tubers planted after 7 days of treatment exposure at 10°C.

Source	DF	SS[x]	MS	F Value	Pr > F
Block	2	0.43	0.22	0.64	0.5284
Essential Oil	2	0.03	0.02	0.05	0.9519
Dill+Spearmint vs. Clove	(1)	0.03 (83.33%)	0.03	0.07	0.7861
Dill vs. Spearmint	(1)	0.01 (26.67%)	0.01	0.02	0.8755
Dose	4	2.69	0.67	1.99	0.0989
Linear	(1)	1.34 (49.81%)	1.34	3.97	0.0579
Quadratic	(1)	1.14 (42.38%)	1.14	3.38	0.0679
Residual	(2)	0.21	0.11	0.31	
Essential Oil x Dose	8	2.24	0.28	0.83	0.5780
(Dill+Spearmint)-Linear vs. Clove-Linear	(1)	0.01 (0.22%)	0.01	0.02	0.8982
(Dill+Spearmint)-Quadratic vs. Clove-Quadratic	(1)	0.04 (1.56%)	0.04	0.11	0.7457
Dill-Linear vs. Spearmint-Linear	(1)	0.27 (12.05%)	0.27	0.79	0.3760
Dill-Quadratic vs. Spearmint-Quadratic	(1)	0.76 (33.93%)	0.76	2.25	0.1354
Residual	(4)	1.17	0.29	0.86	
Error	163	55.15	0.34		
Total	179	60.55			

[x]SS: The percentage indicates the proportion of the variance represented by the factor.
*Significant at $P < 0.05$

A .11 The Analysis of Variance of the impact of dill weed, spearmint and clove oil treatments on the above ground dry biomass subsequently produced by 'Piccolo' seed tubers planted after 7 days of treatment exposure at 10°C.

Source	DF	SS[x]	MS	F Value	Pr > F
Block	2	0.84	0.42	0.69	0.5019
Essential Oil	2	11.97	5.98	9.81	<0.0001*
Dill+Spearmint vs. Clove	(1)	8.49 (70.93%)	8.49	13.92	0.0003*
Dill vs. Spearmint	(1)	3.48 (29.06%)	6.48	5.70	0.0181*
Dose	4	2.93	0.73	1.20	0.3133
Linear	(1)	1.92 (65.53%)	1.92	3.15	0.0778
Quadratic	(1)	0.89 (30.83%)	0.89	1.46	0.2288
Residual	(2)	0.12	0.06	0.10	
Essential Oil x Dose	8	9.05	1.13	1.85	0.0708
(Dill+Spearmint)-Linear vs. Clove-Linear	(1)	3.20 (35.36%)	3.20	5.24	0.0233*
(Dill+Spearmint)-Quadratic vs. Clove-Quadratic	(1)	0.04 (0.39%)	0.04	0.06	0.8101
Dill-Linear vs. Spearmint-Linear	(1)	0.02 (0.22%)	0.02	0.04	0.8475
Dill-Quadratic vs. Spearmint-Quadratic	(1)	2.61 (28.84%)	2.61	4.28	0.0402*
Residual	(4)	3.18	0.80	1.30	
Error	163	99.44	0.61		
Total	179	124.22			

[x]SS: The percentage indicates the proportion of the variance represented by the factor.
*Significant at $P < 0.05$

A .12 The Analysis of Variance of the impact of dill weed, spearmint and clove oil treatments on the number of tuber subsequently produced by 'Piccolo' seed tubers planted after 7 days of treatment exposure at 10°C.

Source	DF	SS[x]	MS	F Value	Pr > F
Block	2	0.14	0.07	0.03	0.9683
Essential Oil	2	2.88	1.44	0.64	0.5281
Dill+Spearmint vs. Clove	(1)	0.18 (6.15%)	0.18	0.08	0.7787
Dill vs. Spearmint	(1)	2.70 (93.85%)	2.70	1.20	0.2744
Dose	4	13.30	3.33	1.48	0.2102
Linear	(1)	0.90 (6.77%)	0.90	0.40	0.5275
Quadratic	(1)	0.79 (5.94%)	0.79	0.35	0.5529
Residual	(2)	11.61	5.81	2.16	
Essential Oil x Dose	8	26.73	3.34	1.49	0.1649
(Dill+Spearmint)-Linear vs. Clove-Linear	(1)	1.01 (3.78%)	1.01	0.45	0.5028
(Dill+Spearmint)-Quadratic vs. Clove-Quadratic	(1)	0.05 (0.18%)	0.05	0.02	0.8832
Dill-Linear vs. Spearmint-Linear	(1)	8.44 (31.56%)	8.44	3.76	0.0542
Dill-Quadratic vs. Spearmint-Quadratic	(1)	3.24 (12.12%)	3.24	1.44	0.2312
Residual	(4)	14.00	3.50	1.30	
Error	163	365.86	2.69		
Total	179	408.91			

[x]SS: The percentage indicates the proportion of the variance represented by the factor.
*Significant at $P < 0.05$

A .13 The Analysis of Variance of the impact of dill weed, spearmint and clove oil treatments on the total weight of tuber subsequently produced 'Piccolo' seed tubers planted after 7 days of treatment exposure at 10°C.

Source	DF	SS[x]	MS	F Value	Pr > F
Block	2	1311.23	655.62	3.13	0.046*
Essential Oil	2	491.08	245.54	1.17	0.312
Dill+Spearmint vs. Clove	(1)	455.94 (92.84%)	455.94	2.18	0.142
Dill vs. Spearmint	(1)	35.14 (7.16%)	35.14	0.17	0.683
Dose	4	2012.93	503.23	2.40	0.052
Linear	(1)	518.42 (25.75%)	518.42	2.48	0.118
Quadratic	(1)	117.32 (5.83%)	117.32	0.56	0.455
Residual	(2)	1377.19	688.60	2.04	
Essential Oil x Dose	8	1596.97	199.62	0.95	0.474
(Dill+Spearmint)-Linear vs. Clove-Linear	(1)	61.24 (3.83%)	61.24	0.29	0.589
(Dill+Spearmint)-Quadratic vs. Clove-Quadratic	(1)	505.01 (31.62%)	505.01	2.41	0.122
Dill-Linear vs. Spearmint-Linear	(1)	112.01 (7.01%)	112.01	0.54	0.466
Dill-Quadratic vs. Spearmint-Quadratic	(1)	167.71 (10.50%)	167.71	0.80	0.372
Residual	(4)	751.00	187.75	0.56	
Error	163	34123.66	338.26		
Total	179	39535.88			

[x]SS: The percentage indicates the proportion of the variance represented by the factor.
*Significant at $P < 0.05$

APPENDIX B. OTHER TABLES

B .1 Duration of sprout suppression for clove, dill and spearmint oils when various dose and treatment interval combinations were applied on 'Russet Norkotah' and 'Piccolo' tubers in 63-L steel drums at 8-15^{o}C.

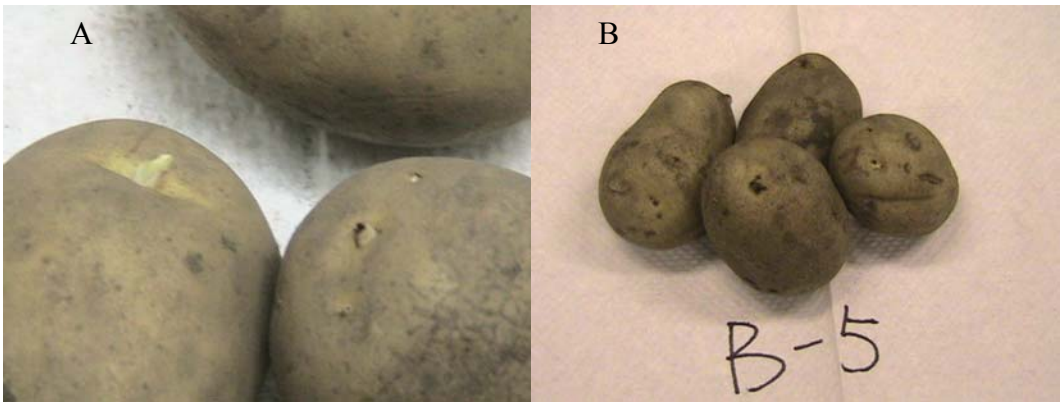
Dose (mg L^{-1} headspace) / Treatment interval (day)	Essential Oil	Time to Sprout (weeks) - Russet Norkotah	Time to Sprout (weeks) - Piccolo
13/17	Clove	10	14
	Dill	10	10
	Spearmint	10	10
25/10	Clove	10	19
	Dill	28	33
	Spearmint	29	31
25/24	Clove	11	13
	Dill	11	10
	Spearmint	29	32
55/7	Clove	10	32
	Dill	30	37
	Spearmint	31	37
55/17[x]	Clove	11	25
	Dill	29	31
	Spearmint	29	32
55/27	Clove	10	19
	Dill	29	33
	Spearmint	29	35
85/10	Clove	13	30
	Dill	29	32
	Spearmint	29	37
85/24	Clove	22	32
	Dill	30	33
	Spearmint	29	37
97/17	Clove	10	31
	Dill	26	28
	Spearmint	29	32

[x]55/17: The treatment 55 mg L^{-1} headspace of essential oil applied every 17 days was replicated 8 times.

B .2 The variation in sprout weight among the replications of 'Russet Norkotah' and 'Piccolo' tubers treated with dill weed, spearmint and clove oils at the dose of mg L^{-1} headspace every 17 days in 63-L steel drums at 8-15^{o}C.

Potato Variety	Essential Oil	Mean (g)	STDEV	CV
Russet Norkotah	Clove	236.4	99.60	42.14
	Dill weed	109.3	33.15	30.34
	Spearmint	156.7	29.08	18.56
Piccolo	Clove	123.0	78.86	64.11
	Dill weed	32.2	11.71	36.40
	Spearmint	28.6	11.96	41.83

APPENDIX C. PHOTOS



C. 1 A. The beginning of the necrosis on 'Piccolo' tubers after being treated with 120 mg L^{-1} headspace [1] of clove oil (Biox-C^{TM}) for 7 days at 10^{o}C. B. The complete necrosis of 'Piccolo' tuber buds after exposed to clove oil at the concentration of 120 mg L^{-1} headspace for 19 weeks at 10^{o}C.

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