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Use of Essential Oils in Sprout Suppression and Disease Control in Potato Storage

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ABSTRACT

Sprout suppression and disease control are essential in assuring potato tuber quality. The increasing concerns regarding the safety and environmental impact of residues of chlorpropham (CIPC), the standard sprout suppressing compound used in potatoes, have increased interest in investigating the potential of alternate sprout inhibitors as well as disease suppressors, including essential oils. A series of studies were conducted to evaluate the suppression effect of essential oils on sprouting and fungal growth in storage. The effect of S-(+) and R-(-) carvone isomers, S-(+) containing caraway seed extracts on sprout growth in potatoes under 10°C storage was studied. Results suggested that carvone could be applied in powder, aqueous or vapour form to suppress sprout growth and sprout number without affecting tuber weight loss in 'Norland' and 'Snowdon' potatoes. Both S-(+) and R-(-)-isomers of carvone under all three methods of treatment application were effective in controlling sprout growth. Double applications of 4% dill and caraway extracts at 5-weeks apart, were able to completely suppress sprouting for at least 15 weeks, equivalent to the standard controls, CIPC and maleic hydrazide. Both isomers were effective in suppressing all diseases tested (*Fusarium solani*, *F. sambucinum*, *F. culmorum*, *F. sclerotiorum*, *Rhizoctonia solani)* with most effective control of *F. culmorum and R. solani*. Comparison between S-(+) and R-(-) carvone using *in vitro* conditions at 23°C determined R-(-)-carvone to be more effective on suppressing the growth of *F. culmorum*. R-(-)-carvone treatments almost completely suppressed the growth of *R. solani* throughout the 25 day observation period. R-(-)-carvone treatments also significantly suppressed the growth of *F. sambucinum*, *S. sclerotiorum* and *R. solani* at 4, 10 and 23°C *in vitro*. Under *in vivo* conditions, after 6 weeks at 10°C, R-(-) -carvone significantly inhibited depth of *F. sambucinum* infection in inoculated potatoes. None of the carvone treatments produced any unacceptable flavour in baked, boiled or chipped potatoes. These studies indicate the potential of using these natural plant extracts in suppressing sprouting and controlling disease under commercial storage conditions. Since caraway, dill and spearmint crops can be produced in the Canadian prairies, it represents a potential economic opportunity for essential oil extraction.

Keywords: S-(+)-carvone, R-(-)-carvone, culinary qualities, fungal disease suppression
Abbreviations: CIPC, Chlorpropham or isopropy *N*-(3-chlorophenyl) carbamate; **IPC**, isopropyl *N*-chlorophenyl carbamate; **MH**, maleic hydrazide

INTRODUCTION

Effective suppression of sprout growth during storage is critical to cost-effective production of both table and seed potatoes. At present, postharvest application(s) of the synthetic sprout inhibitor, chlorpropham (CIPC) is the standard means for sprout suppression in table and processing potatoes in North America. CIPC is presently under regulatory review due to suspected health problems associated with low-level residues of the product. In 2002, the allowable residue tolerance on fresh potatoes was reduced from 50 ppm to 30 ppm, and in Europe the residue limit is 5 to 10 ppm (Kleinkopf *et al*. 2003). There are also concerns that CIPC may be involved in depletion of atmospheric ozone.

Excessive sprout growth during storage is a common problem in potato production, and it can result in significant weight loss, reduced sugar levels and increased bruising susceptibility (Vaughn and Spencer 1993; Hartmans *et al.* 1995). The problems become more severe when growers store cultivars with a short natural dormancy period or store their crop for almost a year prior to sale in an attempt to supply off-shore markets. Standard sprout inhibitors such as maleic hydrazide (MH) and CIPC do irreversible damage to the sprouts (Meredith 1995) and are of no use for sprout control in seed potatoes (Dreger 2003). Ideally, effective sprout suppression in potatoes should rely on utilizing an inexpensive and easy to apply compound that is safe to both the consumer and the environment. No compounds are presently registered in North America for suppression of premature sprouting in seed potatoes. In seed potatoes, the effects of any sprout inhibition treatment should be reversible with no impact on seed vigour. Natural or nature-identical compounds with sprout suppression action are potentially less controversial in terms of their acceptability and environmental impact than existing methods for sprout control.

A number of aromatic oils found in herbs and spice crops appear to have significant sprout inhibitory properties (Beveridge *et al*. 1981). Studies indicate carvone is an effective inhibitor of sprout growth in potatoes (Beveridge *et al*. 1981; Oosterhaven *et al*. 1995b; Sorce *et al*. 1997). Carvone is a major component of essential oils extracts from many plants including caraway, dill and spearmint. Carvone contains two enantiomers: S-(+)-carvone and R-(-)-carvone. S-(+)-carvone is the major compound in caraway seed oil (50-70%), dill seed oil (40-60%) and dill weed oil (40%) (Hartmans *et al.* 1995; de Carvalho and Fonseca 2006). R-(-)-carvone is present in spearmint at a level greater than 51% (de Carvalho and Fonseca 2006).

Several studies have shown that, in addition to sprout suppression, carvone can effectively inhibit the growth of certain fungi and bacteria (Farag *et al.* 1989; Gorris *et al.* 1994; Oosterhaven *et al.* 1995b). A treatment of 1 to 3 mM

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of carvone was able to inhibit the growth of the plant-pathogenic fungi, *Fusarium solani* and *F. sulphureum*, as well as the growth rate of the bacteria *Streptococcus thermophilus*, *Lactococcus lactis* and *Escherichia coli* (Oosterhaven *et al.* 1995b). Other *in vitro* and *in vivo* experiments have demonstrated that carvone could also control the growth of *Phoma exigua* var. *foveata* and *Helminthosporium solani* (Hartmans *et al.* 1995; Frazier *et al.* 1998, 2004).

The majority of studies on potato sprout inhibition have focused on using the vapor form of highly purified S-(+)-carvone extracts to treat tubers (Vaughn and Spencer 1991; Hartmans *et al*. 1995; Oosterhaven *et al.* 1995a). Few investigations have been conducted to determine the efficacy of R-(-)-carvone on both sprout inhibition as well as alternative methods of application (Oosterhaven *et al.* 1995a). This series of studies initially examined the potential for using vapor, aqueous and granular forms of caraway and dill seeds extracts, (-) and (+) carvone to inhibit sprout growth in table and processing potatoes and to regulate excessive elongation of sprouts in seed potatoes. Single and repeated applications were evaluated on potatoes stored at 10°C up to 15 weeks. Since most findings on antifungal properties are based on S-(+)-carvone, we also examined the antifungal properties of S-(+)-carvone and R-(-)-carvone on five common fungal pathogens of potato. The efficacy of 4% R-(-)-carvone on *F. sambucinum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* growth *in vitro*, with treatments at 4, 10 and 23°C, under simulated storage conditions and palatability were also evaluated.

MATERIALS AND METHODS

Method of application of carvone, caraway and dill extracts for potato sprout inhibition

A series of studies were conducted to evaluate the impact of S-(+) and R-(-) isomers of carvone (99% carvone, Fluka Chemika Switzerland), caraway and dill seed oil extracts, and clorpropham (CIPC) on sprout inhibition in 'Norland' (table type) and 'Snowden' (processing type) potatoes produced in Outlook, Saskatchewan, Canada. Freshly harvested healthy potato tubers with weight ranges of 70 to 120 g/tuber, were selected for the following studies. Tubers were cured at room temperature (20 ± 2°C) for about 10 days, and then placed in growth rooms at 10°C in the dark. Two days after storing, depending upon the experiment, potatoes were treated with various concentrations of S-(+) and R-(-) pure forms of carvone as a powder, liquid or vapor; or essential oil extracts from caraway (54% carvone) and dill (47% carvone) seeds, in either liquid or vapor form. In addition, potatoes treated with CIPC as a gas in commercial storage for six weeks or treated with maleic hydrazide at 20 g/L in the field three weeks prior to harvest were included as standard treatments.

Carvone as a powder

This study was conducted using 'Norland' potatoes. After curing at room temperatures as described above, potatoes were placed in growth rooms for 6 weeks at 10°C in the dark with 80-85% relative humidity and then treated with S-(+) or R-(-) carvone as a powder at a rate 300, 400 or 500 mg/kg of potatoes. The powder formulation was prepared based on the results of a preliminary study conducted earlier, in which 1 g of finely ground perlite was found to be sufficient to cover an individual tuber surface of 1 kg of potatoes. Thus, appropriate volumes of carvone were added to glass jars containing finely ground perlite and mixed thoroughly for 10-12 minutes, using a Fisher Vortex Genie 2 shaker. The powder formulations of carvone treatments were added at appropriate amounts to each 8 kg of potatoes in double layer black plastic bags, mixed thoroughly for 10-12 minutes, left sealed in the same bag for 2 days in the 10°C growth room, and then the tubers were transferred to paper bags. Potatoes treated with ground perlite only, in the same manner as the carvone treatments, served as control. Potatoes treated with CIPC as a gas using standard practice or with MH in the field were included as standard treatments (Dreger 2003). Each treatment unit (paper bag) contained 10 tubers and had three replicates. Treatment effect on sprout growth and tuber quality was evaluated 6 weeks and 8 weeks after treatment by determining tuber weight loss, number of sprouts (>2 mm in length)/tuber and length of the longest sprout. Data were analyzed using a Randomized Complete Block Design and treatment means were compared using an LSD test (Gomez and Gomez 1984).

Carvone as an emulsion (single application)

This study was conducted using 'Norland' and 'Snowden' potatoes which were cured and stored under the same conditions as above. Potatoes were treated with two sources; essential oil extracted from dill and caraway seeds or pure form of S-(+) carvone as an aqueous emulsion solution. Based on the results of a preliminary study, 25 mL of water was sufficient to cover an individual tuber surface of one kilogram of potatoes, and 4% active ingredient (carvone) emulsion was the optimal treatment for sprout inhibition in potatoes. Thus, individual 20 kg tuber samples were treated with 4% active ingredient (carvone) emulsions prepared with essential oils of caraway and dill seeds. In addition, 4% S-(+) pure form of carvone, and the standard controls (described above) were included. The emulsions of carvone were prepared by adding required weights of essential oils or pure form of carvone to distilled water in glass jars, and mixed for 10 minutes using a Fisher Vortex Genie 2 shaker. Emulsions were applied carefully using a hand atomizer at 25 mL/kg of potatoes, to cover the surface of individual potato tubers and spread on plastic sheets. Treated potatoes were left for 2 days at room temperature (20 ± 2°C) in the dark to surface dry before transfer to storage at 10°C. As the "carvone as a powder" study, potatoes treated with CIPC and MH were included as standard treatments. In addition, potatoes treated with distilled water only, were included as control under the same treatment conditions. Data collection, analyses and treatment mean comparisons were similar to those of the study above.

Carvone as an emulsion (double applications)

Based on the observations from the single emulsion application, a separate study was conducted in the following year using 'Norland' and 'Snowden' potatoes. In this study, curing and carvone concentrations were similar to those of the single application study, except for the treatment application time and time of data collection. Based on the results of the previous study, we assumed that a single application of carvone treatment would suppress sprouts for 8 weeks after the treatment and repeated application would extend the sprout suppression period. Thus in this study, the first carvone treatment was applied 5 weeks after storage at 10°C, and 5 weeks later, one half of the treated potatoes was retreated with respective carvone treatments. Data collection was conducted 8, 12 and 15 weeks after the first treatment and analysis and mean comparisons were consistent with that of the previous studies.

Carvone as a vapor

This study was conducted using 'Norland' and 'Snowden' potatoes, which were cured and stored in a similar manner as described in the "Carvone as an emulsion" studies. The treatments consisted of: (i) a single application of the pure form of S-(+) carvone or essential oil from caraway seeds at 60 mL of active ingredient (carvone)/m^3 applied twice at 5-weeks apart, (ii) a single application of the pure form of S-(+) carvone at 100 mL/m^3 applied as a vapor, and (iii) standard treatments using CIPC and MH, as described previously. In addition, untreated potato samples were included as an untreated control. About 15 kg of each of 'Norland' and 'Snowden' potato samples were placed in 77-L sealed plastic containers, which were externally connected to a closed circulation system powered by hairdryers. This system was designed for individual treatments in such a way that liquid would evaporate from the source glass bottle to the circulation system through ventilation. Each container had its own closed system installed and after adding the required amount of the liquid to the bottles, the circulation system operated for five 15-minute periods at one hour intervals each day for two days. The first treatments were applied at 5 weeks under 10°C storage with one repeated application 5

weeks thereafter. Treatments were arranged in a randomized complete block design with three replicates. Data collection was similar to the double emulsion study. Data analysis and mean comparisons were similar to the previous studies.

Antifungal and antimicrobial properties of carvone

Five fungal species were cultured *in vitro* for several weeks before the start of treatments. *F. solani* var. *coeruleum*, *F. culmorum*, *F. sambucinum*, and *S. sclerotiorum* were taken from existing cultures, while *R. solani* was cultured from infected potato tubers. All species were placed on fresh plates with potato dextrose agar one day prior to the start of treatment except the slow growing *F. solani*. The Petri dishes were inverted so the lids were on the bottom and filter paper disks were placed in the centre of each lid. The controls were applied with 0.5 mL of distilled water or mineral oil, and the treatments consisted 0.5 mL of 4% S-(+)-carvone or 4% R-(-)-carvone (both in a mineral oil carrier). Each plate was sealed tightly and stored at 23°C. Mycelial growth (cm^2) was recorded for 25 days by taking two random measurements at 90° angles across the growth to obtain an average diameter, from which the area was then calculated. Total area at each date was obtained by subtracting the start area from the calculated area at that date. An additional *in vitro* study was done on *F. sambucinum*, *R. solani* and *S. sclerotiorum* to determine the effect of R-(-)-carvone under different storage temperatures. *In vitro* methodology was similar to the previous study, and inoculated plates were stored at 4, 10 and 23°C. Observations were taken every two days for 16 days.

In vivo potato used *F. sambucinum* grown from an existing culture on an unnamed variety 'V0865-1' (a white tuber variety). Medium sized, healthy potatoes were washed and dried before use. An approximately 3mm deep and 1mm wide hole was punctured at the two ends and the middle of each tuber. Tubers were immersed in a spore suspension of *F. sambucinum* (1.03×10^5 macroconidia per ml of distilled water) for 1 min. Potatoes were placed at room temperature (23°C) for 2 h to promote germination of *F. sambucinum* spores and establish infection. Infected potatoes were grouped as follows: one third used as an untreated control (9 mL mineral oil), one third immediately exposed to 9 mL of 40% R-(-)-carvone solution [98% R-(-)-carvone mixed with a mineral oil carrier], and the last third of the infected potatoes were left at room temperature for 24 h before exposure to 40% R-(-)-carvone solution. Treatment solutions were placed into a 50 ml beaker attached to the bottom center of a pail used to store tubers. The headspace concentration inside each pail was projected to be 1.67×10^{-5}mol/L assuming all R-(-) -carvone evaporated. All pails were tightly sealed and stored in a 10°C dark growth chamber. After six weeks of storage, potatoes were cut vertically at the wounding sites and the depth × width of the dry rot area at 90° angles were measured to obtain the average area infected.

The paired preference taste test procedures were based on techniques described by Watts *et al*. (1989). Variety 'V0865-1' was used and tubers were treated with R-(-)-carvone in the same manner as described in the *in vivo* assays against the fungi. All tubers were stored for 6.5 weeks before the taste test. Potatoes were rinsed, peeled, cut into halves, and then boiled for 22 min. Control and treated tubers were kept separate during preparation and cooking. The two potato treatments were each assigned a random code number corresponding to the specific treatment. A group of 12 untrained, in-house panelists took part in the paired preference test.

RESULTS AND DISCUSSION

Method of application of carvone, caraway and dill extracts

Both S-(+) and R-(-) isomers of carvone were active in sprout suppression with no significant difference in efficacy between these two isomers except the higher concentrations of the R-(-) isomer inhibited sprout length to a greater extent than the S-(+) isomer by the 8-week period (**Table 1**). In contrast, Oosterhaven *et al.* (1995a) found the S-(+)-carvone isomer inhibited elongation earlier than the R-(-)-carvone form. This was attributed to differential uptake of the isomers in the first 4 days. Since our first measurements occurred at 6 or 8 weeks, any initial differences would have been masked. Carvone can be applied in powder, aqueous or vapour form to suppress sprouting in all three cultivars. All three methods of application were generally effective in controlling sprout growth at all three storage temperatures compared to non-treated control tubers (**Tables 1-4**). The most effective treatment was the double application applied as a liquid, of 4% caraway or dill seed extracts which completely inhibited sprouting for at least 15 weeks at 10°C in both 'Snowden' and 'Norland' and was comparable in efficacy to both CIPC and MH (**Table 3**). Reust (2000) also reported carvone had to be re-applied to potato under prolonged storage, and this was considered to be beneficial since its low persistence translated into consumption just 15 days after treatment. However, the double application as a vapour was only effective up to 8 weeks in storage (**Table 4**). The inhibitory effects of these treatments were easily reversed by removing the vapours from the storage facilities. This supports the findings of Oosterhaven *et al*. (1995a).

Table 1 Effect of carvone (applied as a powder), caraway and dill seed extracts applied as a liquid, maleic hydrazide (applied as a liquid) and chloropropham (CIPC, applied as a gas) on tuber weight loss, sprout number and length of the longest sprout in 'Norland' potatoes over 6 and 8 weeks storage at 10°C.

Treatment	6 WAT[w]			8 WAT		
	TWL[x] (%)	SN[y]	SL[z] (mm)	TWL (%)	SN	SL (mm)
Control – powder	4.8	5.2	20.7	8.2	6.5	49.6
(-) Carvone (300 mg/kg)	5.2	0.5	3.4	6.0	1.6	5.0
(-) Carvone (400 mg/kg)	5.4	0.0	0.0	6.8	0.5	1.9
(-) Carvone (500 mg/kg)	5.6	0.0	0.0	6.7	1.4	3.4
(+) Carvone (300 mg/kg)	6.0	0.4	0.5	7.6	1.8	6.6
(+) Carvone (400 mg/kg)	4.8	0.0	0.0	5.9	2.2	5.8
(+) Carvone (500 mg/kg)	6.2	0.0	0.0	6.4	1.6	6.2
Maleic hydrazide (20 g/L) – liquid	4.5	0.0	0.0	5.5	0.3	2.8
Chloropropham – gas	5.4	0.0	0.0	5.7	0.0	0.0
Statistical significance	**	**	**	**	**	**
Lsd (P≤ 0.05)	0.4	0.4	0.6	0.9	0.5	2.1
CV (%)	6.3	16.3	6.1	7.5	15.2	11.0

WAT[w] = Weeks after treatment.
TWL[x](%) = Tuber weight loss = (Initial tuber weight, prior to treatment-tuber weight at sampling) / Initial tuber weight, prior to treatment x 100.
SN[y] = Sprout number/tuber.
SL[z] = Length of the longest sprout.
300 mg/kg = 300 mg of carvone applied to 1 kg of potatoes.
** Significant at P≤0.01.

Table 2 Effect of carvone (applied as a liquid), caraway and dill seed extracts (applied as a liquid), maleic hydrazide (applied as a liquid) and chloropropham (CIPC, applied as a gas) on tuber weight loss, sprout number and length of the longest sprout in 'Norland' potatoes over 6 and 8 weeks storage at 10°C.

Treatment	6 WAT[w]			8 WAT		
	TWL[x] (%)	SN[y]	SL[z] (mm)	TWL (%)	SN	SL (mm)
Control (Water)	5.6	6.3	25.3	9.3	5.4	41.7
(+) Carvone 4% – liquid	5.1	0.2	1.7	5.8	2.8	6.8
Dill seed extract (4% carvone)	5.5	2.0	3.9	6.5	1.3	8.3
Caraway seed extract (4% carvone)	4.5	0.0	0.0	6.0	0.7	4.2
Maleic hydrazide (20 g/L) – liquid	4.5	0.0	0.0	5.5	0.3	2.8
Chloropropham – gas	5.4	0.0	0.0	5.7	0.0	0.0
Statistical significance	**	**	**	**	**	**
LSD (P≤ 0.05)	0.7	0.2	0.7	0.5	0.3	1.7
CV (%)	7.7	12.7	15.1	4.7	10.3	10.8

WAT[w] = Weeks after treatment.
TWL[x](%) = Tuber weight loss = (Initial tuber weight, prior to treatment-tuber weight at sampling) x 100. / Initial tuber weight, prior to treatment
SN[y] = Sprout number/tuber.
SL[z] = Length of the longest sprout.
** Significant at P≤0.01.

Table 3 Effect of single (S) and double (D) applications of carvone (applied as a liquid), caraway and dill seed extract (applied as a liquid), maleic hydrazide (applied as a liquid) and chloropropham (applied as a gas) on tuber weight loss, sprout number and length of the longest sprout in 'Norland' and 'Snowden' potatoes during varying storage periods at 10°C.

Treatment	'Norland'									'Snowden'								
	TWL[x] (%)			SN[y]			SL[z] (mm)			TWL (%)			SN			SL (mm)		
	8 W[w]	12 W	15 W	8 W	12 W	15 W	8 W	12 W	15 W	8 W	12 W	15 W	8 W	12 W	15 W	8 W	12 W	15 W
Control – Water (S)	10.0	14.9	21.4	5	7	5	29	61	72	5.7	8.1	10.1	5	7	7	21	34	37
Control – Water (D)	14.6	13.3	14.1	9	7	7	29	49	72	5.2	7.9	12.2	4	7	10	12	27	28
(+) Carvone 4% (S)	10.0	11.1	16.6	13	8	7	6	40	66	4.0	5.3	9.7	5	7	7	13	19	24
(+) Carvone 4% (D)	6.8	9.8	10.6	0	0	2	0	0	10	3.9	3.9	5.3	0	0	0	0	0	0
Caraway seed extract 4% (S)	7.8	12.6	12.7	5	6	5	27	41	43	4.0	5.6	9.2	4	5	4	19	27	37
Caraway seed extract 4% (D)	8.4	9.6	11.2	0	0	0	0	0	0	3.6	4.0	4.8	0	0	0	0	0	0
Dill seed extract 4% (S)	7.2	12.5	17.4	0	6	5	21	45	70	4.8	7.4	10.5	3	7	7	11	20	40
Dill seed extract 4% (D)	7.6	14.0	10.7	0	0	0	0	0	0	4.2	5.4	6.4	0	0	0	0	0	0
Maleic hydrazide (20g/L) – (S)	5.2	10.9	11.2	0	0	0	0	0	0	5.1	6.1	8.0	0	0	0	0	0	0
Chloropropham – (S)	2.3	3.5	4.9	0	0	0	0	0	0	1.5	2.8	3.3	0	0	0	0	0	0
Statistical significance	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Lsd (P≤0.05)	1.2	1.5	1.4	1	1	1.3	2	2	2	0.6	0.9	1.0	1	1	1	1	1	1
CV%	8.7	8.1	6.3	16	17	25	12	5	4	8.0	9.4	7.6	33	19	17	15	7	4

W[w] = Weeks after treatment.
TWL[x](%) = Tuber weight loss = (Initial tuber weight, prior to treatment-tuber weight at sampling) x 100. / Initial tuber weight, prior to treatment
SN[y] = Sprout number/tuber.
SL[z] = Length of the longest sprout.
** Significant at P≤0.01.
[(S)] Single = Single treatments were applied 5 weeks after storage.
[(D)] Double= Double treatments were applied at 5 and 10 weeks after storage.

Table 4 Effect of single (S) and double (D) applications of carvone (applied as a vapor), caraway seed extracts (applied as a vapor) and chloropropham (applied as a gas) and maleic hydrazide (applied as a liquid) on tuber weight loss and sprouting in 'Norland' and 'Snowden' potatoes over varying periods of storage at 10°C.

Treatment	'Norland'						'Snowden'					
	TWL[x] (%)		SN[y]		SL[z] (mm)		TWL (%)		SN		SL (mm)	
	8 W[w]	12W	8W	12W	8W	12W	8W	12W	8W	12W	8W	12W
Untreated Control	5.9	13.2	5	6	22	71	5.5	8.7	5	6	18	32
(+) Carvone (60 mL/m^3) (D)	6.1	9.3	0	4	0	10	6.0	7.1	0	5	0	7
(+) Carvone (100 mL/m^3) (S)	6.2	9.9	0	4	0	7	6.1	8.0	0	3	0	5
Caraway seed extract (carvone 60 mL/m^3)(D)	5.6	10.0	0	3	0	5	6.2	8.1	0	4	0	3
Maleic hydrazide (20 g/L) (S)	6.9	8.3	0	0	0	0	6.9	8.2	0	0	0	0
Chlorophropham (S)	3.4	5.8	0	0	0	0	3.4	5.8	0	0	0	0
Statistical significance	**	**	**	**	**	**	**	**	**	**	**	**
LSD (P≤0.05)	0.2	1.0	1	1	1	1	1.1	1.2	1	1	1	1
CV (%)	2.0	6.0	30	18	13	5	10.8	9.0	30	17	3	9

W[w] = Weeks after treatment.
TWL[x](%) = Tuber weight loss = (Initial tuber weight, prior to treatment-tuber weight at sampling) x 100. / Initial tuber weight, prior to treatment
SN[y] = Sprout number/tuber.
SL[z] = Length of the longest sprout.
** Significant at P≤0.01.
[(S)]Single = treatments were applied 5 weeks after storage.
[(D)]Double= treatments were applied at 5 and 10 weeks after storage.

Table 5 The total area (cm^2) of *Fusarium. solani*, *F. sambucinum*, *F. culmorum*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani* growth at selected days after the start of treatment when exposed to S-(+)-carvone, R-(-)-carvone and a mineral oil control at 23°C.

Treatment	Days from start of treatment	Mineral oil	S.E.	S-(+)- Carvone	S.E.	R-(-)- Carvone	S.E.
F. solani	3	6.93 a*	0.71	0.97 b	0.17	1.16 b	0.34
	10	27.91 a	10.48	11.29 b	0.10	9.70 b	1.30
	17	46.45 a	2.85	34.38 a	15.30	34.37 a	8.71
	25	53.65 a	2.39	46.84 a	7.76	53.02 a	3.38
F. sambucinum	3	21.47 a	1.98	0.79 b	0.44	0.39 b	0.11
	10	57.54 a	0.42	7.74 b	4.93	2.04 c	1.82
	17	57.54 a	0.42	50.01 ab	11.64	42.43 ab	14.81
	25	57.54 a	0.42	55.91 a	3.48	57.65 a	0.06
F. culmorum	3	36.77 a	6.08	0.33 b	0.12	0.14 b	0.14
	10	57.46 a	0.23	0.71 b	0.25	0.35 c	0.10
	17	57.46 a	0.23	4.00 b	2.27	0.60 b	0.12
	25	57.46 a	0.23	24.85 b	15.99	1.17 c	0.20
S. sclerotium	3	57.89 a	0	0.15 b	0.13	0.11 b	0.12
	10	57.89 a	0	0.20 b	0.08	0.24 b	0.10
	17	57.89 a	0	19.00 b	25.62	19.85 b	27.50
	25	57.89 a	0	40.01 ab	24.62	23.37 b	31.52
R. solani	3	47.50 a	2.97	0.04 b	0.05	0.08 b	0.05
	10	57.89 a	0	0.03 b	0.04	0.06 b	0.04
	17	57.89 a	0	0.10 b	0.06	0.09 b	0.08
	25	57.89 a	0	4.00 b	7.08	0.14 b	0.04

*Means followed by the same letter are not significant different at P<0.05 according to the LSD test.
S.E. = Standard error

Antifungal and antimicrobial properties of carvone

Efficacy of carvone was both disease and isomer-dependent. Both forms of carvone were most effective in controlling the growth of *F. culmorum* and *R. solani* throughout the 25 day observation period (**Table 5**). Moreover, the R-(-)-carvone inhibited growth of *F. culmorum* to a greater extent, limiting growth to 1.17cm^2 compared to 24.85 cm^2 (S-(+)-carvone) and 57.46 cm^2 (control) by 25 days. *F. culmorum* commonly causes *Fusarium* head blight in grain crops. Although it is not a primary cause of *Fusarium* dry rot in potatoes, it has been implicated in the disease. Hartmans *et al.* (1995) found S-(+)-carvone to be somewhat effective in inhibiting growth of *F. culmorum*, but did not examine R-(-)-carvone. Our study showed both carvone isomers have the potential to be used as fungicide to control *F. culmorum* in potato storage, and possibly in grain production.

R-(-)-carvone treatments were all effective at 23, 10 and 4°C in controlling growth of *F. sambucinum*, *R. solani* and *S. sclerotiorum* with almost complete inhibition of growth under most treatments (**Figs. 1-3**). Even at 23°C, the R-(-)-carvone also almost completely inhibited the growth of *R. solani* which was limited to 0.14 cm^2 through 25 days (**Fig. 2, Table 5**) and appeared to kill the *R. solani* under this regime since cultures turned black and did not resume growth when the R-(-)-carvone treatment was removed (data not shown). These results support observation made by Hartmans *et al.* (1995) on the control of *R. solani* when exposed to S-(+)-carvone at 24°C.

Both S-(+)-carvone and R-(-)-carvone were equally significant in controlling the growth of *F. solani*, *F. sambucinum*, and *S. sclerotiorum* but the effect was limited to only 10 days in these diseases. However, because of its rapid growth, *S. sclerotiorum* can prove to be very challenging in storage of horticultural commodities. Presently, there is no effective control of this pathogen (Kora *et al*. 2003), and losses can be substantial. Thus, carvone has the potential to be used as an effective fungicide for preventing growth of *S. sclerotiorum* in storage.

Mycelial growth of *F. sambucinum* and *S. sclerotiorum*, in addition to *R. solani*, were monitored after removal of R-(-)-carvone treatment. Four weeks after removal of treatment, growth of *F. sambucinum* and *S. sclerotiorum* occurred, but no growth of *R. solani* was observed (data not shown). This result indicated that reapplication may be necessary to effectively control *F. sambucinum* and *S. sclerotiorum*. *R. solani* is mainly a soil-borne fungus and the infection mainly occurs in the field throughout the growing season (Tsror *et al.* 2001; Kora *et al.* 2003). Use of carvone products may suppress any further growth that may occur in storage. Future research should be done to explore the potential of applying carvone in the field to prevent *R. solani* infection.

Table 6 Effects of R-(-)- carvone on surface area of rot caused by *F. sambucinum* infection in potatoes after six weeks of storage at 10°C.

Treatment	Mean Infected Area (cm^2)
Control	3.86 a*
Carvone – No Delay	2.55 b
Carvone – 24 hrs. Delay	2.42 b

*Means followed by the same letter are not significantly different at P<0.05 according to the LSD test.

The *in vivo* study showed that the development of *F. sambucinum* on tubers was suppressed by R-(-)-carvone treatments, with no difference between the no-delay application and the 24 hr delay application (**Table 6**). The lack of difference between the 24 hour delayed application of R-(-)-carvone after inoculation and the immediate application of R-(-)-carvone after inoculation suggest that *F. sambucinum* could be inhibited at a later stage of infection. Untreated tubers not only showed a larger area of dry rot infection but also a drastic softening of tissue in the region immediately surrounding the infection site (data not shown). The carvone treated tubers had no such softening of the local tissue.

Carvone has a strong odor and is used in flavoring foods, thus potential flavor transmission to the commodity is an important consideration. Sensory tests on steamed potato treated with S-(+)-carvone showed no negative effect on the taste (Hartmans *et al.* 1995); however, reports have shown that tubers treated with spearmint [R-(-)-carvone] could have an unfavorable bitter taste (Frazier *et al.* 2000, 2004). In our taste test, of the 12 panelists, six preferred the carvone treated potatoes to the untreated potatoes (data not shown). With exactly half of the panelists preferring either treatment, the results of the two-tailed binomial test at a significance probability of 0.05 indicated that the panelists could not significantly taste the difference between samples and that the R-(-)-carvone treatments did not significantly affect the flavour of boiled potatoes. Our previous sensory studies also showed none of the (+) or (-) carvone treatments affected taste in baked, boiled or chipped potatoes at 300 mg/kg or 400 mg/kg concentrations (data not shown). This result is consistent with findings of Reust (2000) and Kalt *et al*. (1999). Previous studies have also shown neither

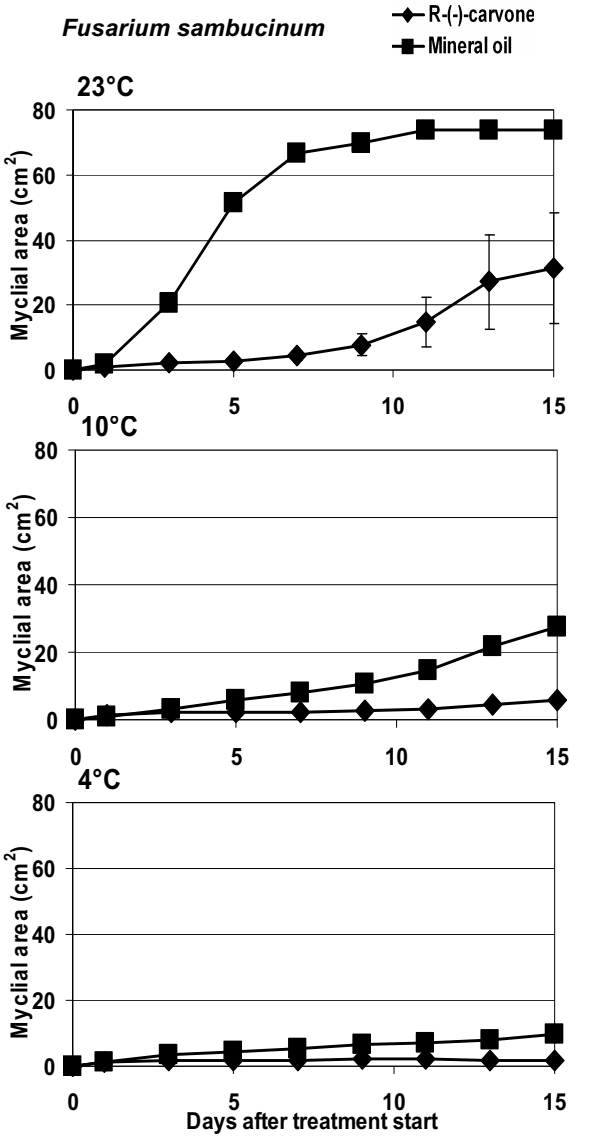

Fig. 1 Growth rate of *F. sambucinum* treated with mineral oil (control) and R-(-)-carvone at 23°C, 10°C and 4°C *in vitro*. Error bars indicate standard error between replicates in each treatment.

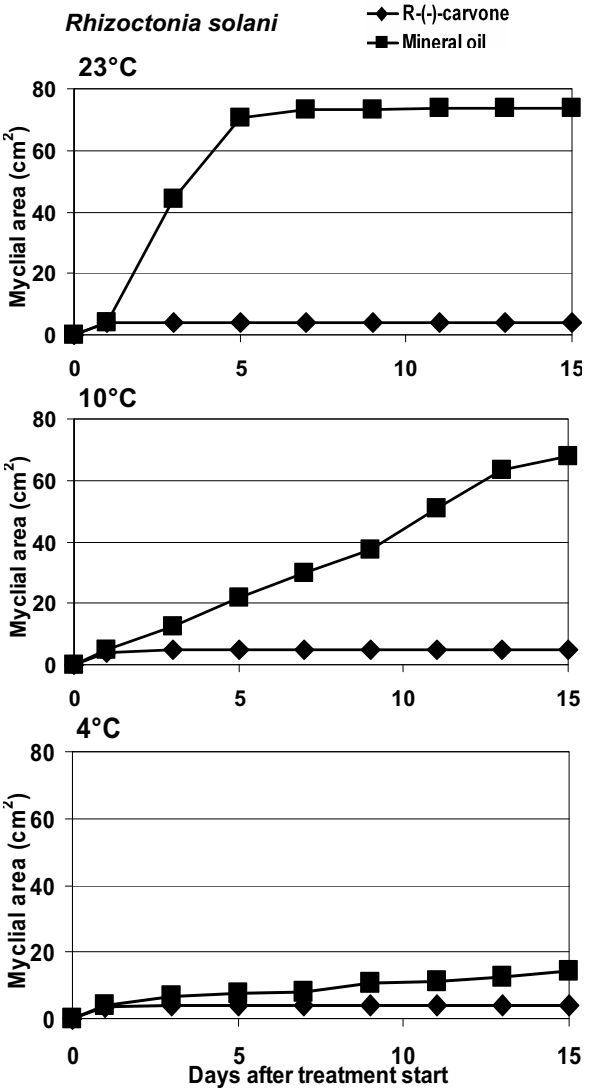

Fig. 2 Growth rate of *R. solani* treated with mineral oil (control) and R-(-)-carvone at 23°C, 10°C and 4°C *in vitro*. Error bars indicate standard error between replicates in each treatment.

(+) nor (-) carvone affected the sugar content or the fry-color of the treated tubers (Frazier *et al.* 2000, 2004).

Carvone has a low human toxicity level (Kerstholt *et al.* 1997) and has Generally Recognized As Safe (GRAS) status (Hall and Oser 1965). Hartmans *et al.* (1995) demonstrated after S-(+)-carvone treatments, the majority of the carvone residue was found in the peel, which was likely due to adsorption of the compound by the periderm layer, and less than 1% of carvone was found inside the potato. They compared average residue levels from isopropyl *N*-chlorophenyl carbamate (IPC) and CIPC, and carvone treatments and found IPC+CIPC treated tubers had over two times more residue than carvone treated tubers. Carvone is volatile and its residue eventually dissipates when the commodity is not in contact with carvone. Increased ventilation could also reduce the residual level. When unloading the tubers from the storage, Hartmans *et al.* (1995) found only very small amounts present in the tuber. This result was also confirmed by Osterhaven *et al.* (1995b).

While treatments such as carvone, caraway and dill seed extracts appear promising, practical application also depends on treatment cost. By far, CIPC is still the least expensive at $3.00/MT while pure S-(+)-carvone is $800.00/MT, if used at a rate of 400 mg/kg (Aldrich Chemical catalog) with double application. Liquid extracts of caraway or dill seed at this rate of application costs about $64.00/MT. Although lower than the pure form, costs of seed extracts still exceed CIPC. Future studies should focus on determining optimum concentrations and delivery methods to provide growers with new, reliable and cost effective options for potato sprout suppression and disease control.

CONCLUSIONS

Carvone, in either pure form or as an extract of dill or caraway, was effective in suppressing sprout growth under 10°C storage conditions in 'Norland' and 'Snowden' potato cultivars. All three methods of application tested: powder, liquid and vapour significantly reduced sprouting under these storage conditions. However, the most effective treatment consisted of double liquid applications of 4% dill or caraway extracts spaced at 5 and 10 weeks after placement into storage. This double application of extracts completely suppressed sprouting throughout the 15 week period and was equivalent to the CIPC and MH industry standards. While both S-(+)-carvone and R-(-)-carvone isomers were equally effective in sprout suppression, the R-(-)-carvone isomer was superior in disease control. Both isomers controlled growth of *Fusarium solani*, *F. sambucinum*, *F. culmorum*, *F. sclerotiorum*, and *Rhizoctonia solani* at 23°C but the R-(-)-carvone isomer was superior in suppressing *F. culmorum* and completely inhibited *R. solani* under the 4, 10 and 23°C temperature conditions tested. Since taste panels could not distinguish preference for either non-treated

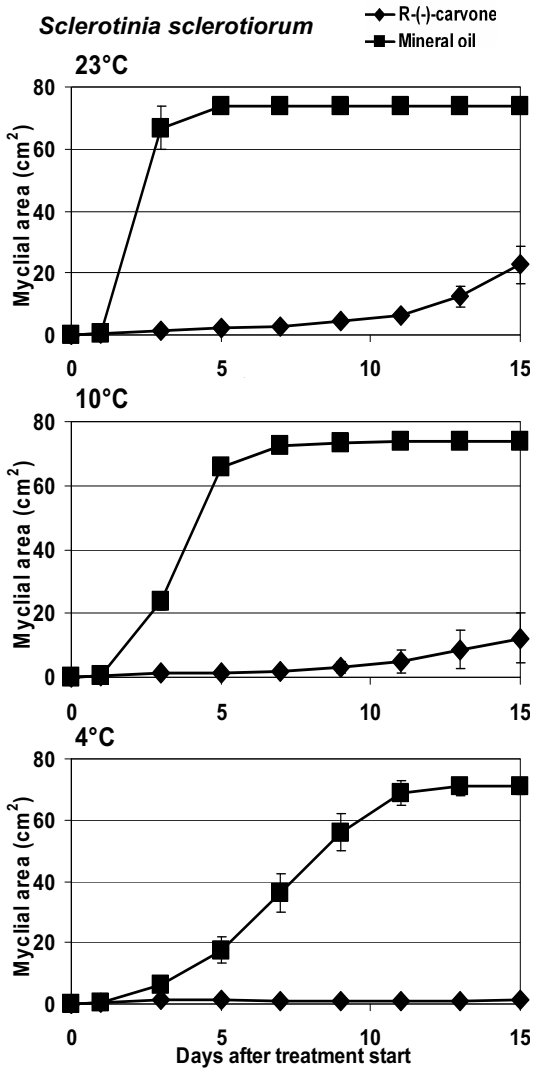

Fig. 3 Growth rate of *S. sclerotiorum* treated with mineral oil (control) and R-(-)-carvone at 23°C, 10°C and 4°C *in vitro*. Error bars indicate standard error between replicates in each treatment.

or treated potatoes, the use of caraway and dill seed extracts show promise as a replacement to CIPC in prolonging potato storage while promoting production of these locally grown crops.

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