CARLO SORCE[1], ROBERTO LORENZI[1 *] and PAOLO RANALLI[2]

[1] Dipartimento Biologia Piante Agrarie - Sezione Fisiologia Vegetale, University of Pisa, via Mariscoglio, 34, I-56124, Pisa, Italy

[2] Istituto Sperimentale Colture Industriali, via di Corticella , 133, I-40129, Bologna, Italy

[*] Corresponding author

Running title: POTATO TUBERS SPROUT SUPPRESSION BY CARVONE

Title: THE EFFECTS OF (S)-(+)-CARVONE TREATMENTS ON *SEED* POTATO TUBER DORMANCY AND SPROUTING.

CARLO SORCE[1], *ROBERTO LORENZI*[1 *] *and PAOLO RANALLI*[2]

[1] *Dipartimento Biologia Piante Agrarie - Sezione Fisiologia Vegetale, University of Pisa, via Mariscoglio, 34, I-56124, Pisa, Italy*

[2] *Istituto Sperimentale Colture Industriali, via di Corticella , 133, I-40129, Bologna, Italy*

[*] *Corresponding author*

Running title: POTATO TUBERS SPROUT SUPPRESSION BY CARVONE

Additional keywords: concentration, gas chromatography, *SPROUT SUPPRESSION*, storage

Summary

Potato seed tubers may suffer from precocious sprouting during storage, thus limiting their suitability for cultivation. Commonly used sprout suppressant treatments negatively affect bud viability, therefore a reliable method to inhibit bud development must still be found for seed tubers. The monoterpene carvone ((S)-(+)-carvone) was tested in small scale experiments. The vapours of this compound fully inhibited bud growth of tubers cv. Monalisa stored at 23°C without affecting bud viability

throughout 6 months of treatment. The most effective range of carvone vapour concentrations was between 0.34 and 1.06 µmol · mol^{-1}. Owing to these qualities, we can expect carvone to become a suitable sprout suppressant also for seed tubers.

Introduction

The control of bud development is a key factor in potato tuber storage. Precocious sprouting, causing high weight losses, is detrimental to the nutritional quality of potatoes (Suttle & Hultstrand, 1994; Casarini & Ranalli, 1996) and to the field performance of seed tubers. The propagation material produced in Italy often starts to sprout shortly after harvest, while that cultivated at higher latitudes does not. This could be owed to the shorter photoperiod and higher temperatures that occur in our country during crop development compared to northern Europe countries (Susnoschi, 1981; Hemberg, 1985; van Ittersum,1992). Low temperatures alone are often insufficient to prevent sprouting during storage. Sprout suppression on ware potatoes is generally carried out by means of chemical treatments, but these are not suitable for seed tubers. It is well known that the most widely used compound, chlorpropham, may affect seed tubers emergence as well as its rate (Lee Kim et al., 1972; Conte et al., 1995; Hartmans et al., 1995). When searching for a suitable chemical sprout suppressant, few volatile organic compounds, especially terpenes, proved to be effective in inhibiting sprout development (Meigh, 1969; Beveridge et al., 1981; Beveridge et al., 1983). Recently the monoterpene carvone has been extensively studied and its sprout inhibiting properties have been confirmed along with the lack of

toxicological risks arising from its use (Hartmans et al., 1995; Oosterhaven et al., 1995). The present work is aimed to provide further data for a profitable use of carvone in seed tubers storage. Therefore, in a series of small scale experiments we monitored the time course of carvone concentration in the headspace of the storage containers after the addition of the liquid monoterpene. We compared such data with the degree of sprout inhibition and evaluated the effects of the treatments on subsequent bud sprouting.

Materials and methods

Tubers cv. Monalisa were planted in early April and harvested on August 22nd at the Istituto Sperimentale Colture Industriali in Bologna. The plants have been subjected to the conventional agricultural techniques. At harvesting, the tubers were placed for a week at 16°C in the dark at a relative humidity of 80%, to facilitate wound healing; then, they were graded according to diameter and tubers of uniform size (35-45 mm) were immediately sent to the laboratory in Pisa, where they were quickly washed under tap water and left to dry on blotting paper. Tubers were treated with carvone in transparent plastic boxes (cm 25 x 25 x 25). Sixty tubers, randomly collected from all those available, were placed in one box and the void box volume was measured by filling it with water. This procedure was repeated ten times, in order to determine the average volume of the tubers, which was taken into account every time that the carvone was added to the boxes. Sixty tubers were then sealed in each box, whose lids were provided with a rubber septum through which the appropriate amount of carvone

((+)-Carvone, Fluka (Switzerland) AG) was injected by a syringe immediately after sealing the box. The liquid compound dropped into a petri dish placed over the tubers; its gradual volatilization allowed it to act as vapour. Three different amounts of carvone were tested: 1.43, 7.15 and 14.31 mmol · mol^{-1}, thus meaning the liquid carvone amount · void box $volume^{-1}$ (box volume minus tubers volume) and two boxes for each treatment were utilized. The carvone was supplied again every time its headspace concentration dropped to a value near that detected 7 days after the beginning of the experiment. The control box was not supplied with the carvone. Additional control tubers (referred to as Control 1) were stored in open cardboard boxes to detect the eventual effect of box environment on sprouting behaviour. All the treatments and controls were placed in the same room, at 23°C in darkness. Every 2 months 20 tubers were withdrawn from each treatment box and placed in open cardboard boxes in the same atmosphere as Control 1, in order to assess their sprouting capacity. The remaining tubers were again sealed in a clean box and supplied with the appropriate amount of liquid carvone. The headspace concentration of carvone vapour in each box was monitored weekly. Samples (500 µl each, three per box) were taken by a gas-tight syringe provided with a side port needle (Dynatech, Baton Rouge, Louisiana) and analysed by a gas chromatograph (3865, Dani, Italy) equipped with a 1,800 x 4 mm I.D. glass column packed with 3% OV1 on 100/120 mesh Supelcoport (Supelco, Bellefonte, Pennsylvania). The carrier gas was nitrogen, with a flow rate of 20 ml · min^{-1} ; the gas was dried by flowing it through a coil bathed in liquid nitrogen. The injector and oven temperatures were set at 100°C and 145°C respectively. The gas chromatograph was equipped with a nitrogen flame

ionization detector and the chromatograms were recorded by an integrator (HP 3394A, Hewlett Packard, Avondale, Pennsylvania). The quantitation of carvone was carried out by reference to a calibration plot obtained from the gas chromatographic analyses of different amounts of standard carvone diluted in acetone. The identity of carvone was confirmed by gas chromatography/mass spectrometry, performed by a mass spectrometer (Trio 2000, VG Biotech, U.K.) coupled with a gas chromatograph (5890 Series II, Hewlett Packard). This was equipped with a 10 m x 0.25 mm I.D. capillary column with a cross-linked 0.5 μm film of methyl silicone as stationary phase (007, Quadrex, U.K.). The carrier gas was helium with a linear velocity of 60 $cm \cdot sec^{-1}$. Mass spectra were obtained by electron impact (EI^{+}) at an ionization potential of 70 eV. The acquisition was in full scan mode from 50 to 200 amu (atomic mass units), at 400 $amu \cdot sec^{-1}$ scan speed.

Each value of the headspace concentration of carvone represents the mean of six analyses (3 samples x 2 boxes) ± se. for each sampling date a chi-square test was used to compare sprouting data from each box with those of the corresponding replicate, in order to assess if there was any difference between replicates. If the values did not differ significantly, data from the two replicates of each treatment were pooled. Then, the comparison between the pooled values of control and those of treated tubers was made by a chi-square test for each sampling date.

Results

Carvone proved to be effective in inhibiting sprouting and its effect was concentration-dependent. Control and Control 1 tubers began to sprout within 20 days from harvest. Tubers from the 7.15 mmol · mol^{-1} treatment did not show any visible sign of bud development throughout 6 months of storage in the boxes (Figs. 1, 2, 3), while at the end of this period each control tuber had developed a mean of 4.36 ± 0.52 sprouts, whose average length was 56.51 ± 1.23 mm. The 1.43 mmol · mol^{-1} treatment yielded only an incomplete control, though new carvone was supplemented every 2 weeks, therefore this thesis was interrupted within 2 months from the beginning of the experiment. Tubers from the 14.31 mmol · mol^{-1} treatment did not sprout for over 4 months of storage under carvone; according to the procedure, at the end of this period twenty tubers were withdrawn from each box and after two months in open cardboard boxes (i.e. 6 months after the start of the experiment) they showed a markedly low rate of sprouting (data not shown), hence also this treatment was stopped. Thus we focused our attention on the 7.15 mmol · mol^{-1} treatment, which yielded a time course of the headspace concentration of carvone vapours in the boxes that is represented in figure 4. These data show that headspace concentrations in a range between 0.34 and 1.06 µmol · mol^{-1} were effective in inhibiting sprouting throughout the time of the experiment. It must be stressed that the time course of these values follows a pattern characterized by three different periods, each of them ending with the opening of the treatment box and the withdrawing of 20 tubers. During the first period (from day 7 to 63) carvone levels in the box headspace are lower than during the second period (from day 63 to 126) and these in turn are lower than during the third one (from day 126 to 182). This

behaviour could be a consequence of the tuber withdrawing at the end of each period: as the tuber number is reduced, the void box volumes rise and the amounts of liquid carvone must therefore be increased to restore the planned concentration, hence tuber metabolization will act on larger amounts of carvone. Nevertheless, neither sprouting inhibition nor bud viability were affected by these changes during the three periods. The resumption of bud growth in the treated tubers did not significantly differ from the controls throughout the whole storage period, as shown in table 1. For each treatment these data represent the sum of the two replicates, since the differences between them were not significant at the chi-square test ($p = 0.05$). The same test did not show significant differences between sprouting values of control and those of the treated tubers, for each sampling date. Samples were of varying sizes, owing to a bacterial disease that affected few tubers, from both control and treated material, during the first period of the experiment. The symptoms (large brown spots on the surface and rotting of the inner parenchyma of the tubers) were easily recognized, therefore the diseased material was discarded after two months, when the boxes were opened for the first time. All the tubers which did not sprout 45 days after the withdrawing from the boxes were carefully examined, in order to assess if the lack of sprout growth was due to the disease: yet, during the last two periods of storage (from day 63 to 182) the remaining tubers did not show any symptom of the disease.

Discussion

The sprout suppressing properties of carvone were confirmed in our small scale experiments, by which we could outline the range of vapour concentrations effective in seed tubers storage. Among the three levels of carvone that were tested, only the 7.15 mmol · mol^{-1} proved to be suitable for seed tubers preservation, since the 1.43 mmol · mol^{-1} was not sufficient to prevent sprouting, and the 14.31 mmol · mol^{-1} negatively affected the subsequent sprout development. The reversibility of bud growth inhibition induced by carvone has been shown by Oosterhaven et al. (1993) on potato eye pieces. Our results, obtained on intact tubers, demonstrate how this monoterpene might be suitable also for seed tubers preservation, since it did not affect bud viability. It is noteworthy that sprout suppression was complete at 23°C: if the seed tubers could be preserved at this temperature, the use of carvone would render potato storage less expensive, since the cooling of the warehouses could be avoided or limited. Yet, it is well known that storage at high temperatures can negatively affect growth vigour of seed potatoes owing to physiological ageing of the tubers: this phenomenon proceeds at a lower rate in tubers stored at low temperature (4°C) (van der Zaag & van Loon, 1987), hence cooling would remain a necessary practice for seed potatoes preservation. Another remarkable benefit arising from carvone treatments is the control of fungal diseases as shown by Hartmans et al. (1995). The results of this experiment indicate that sprouting can be suppressed by carvone for extended periods of time and this naturally occurring volatile substance provides a contribution to pressing needs to find more environmentally acceptable sprout inhibitors. Further investigations are necessary in order to assess its effectiveness and

economic value under practical storage conditions and to confirm the fully reversibility of bud growth inhibition on a wide array of cultivars.

References

Beveridge, J.L., J. Dalziel & H.J. Duncan, 1981. The assessment of some volatile organic compounds as sprout suppressants for ware and seed potatoes. Potato Research 24: 61-76.

Beveridge, J.L., J. Dalziel & H.J. Duncan, 1983. Headspace analysis of laboratory samples of potato tubers treated with 1,4-Dimethylnaphtalene, Carvone, Pulegone and Citral. Journal of the Science of Food and Agriculture 34: 164-168.

Casarini, B. & P. Ranalli, 1996. Fisiologia dei tuberi in post-raccolta e loro conservazione. In: La coltura della patata, Edagricole, Bologna, pp. 73-108.

Conte, E., G. Imbroglini, P. Bertolini & I. Camoni, 1995. Presence of sprout inhibitor residues in potatoes in relation to application techniques. Journal of Agricultural and Food Chemistry 43: 2985-2987.

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

Hemberg, T., 1985. Potato rest. In: P.H. Li (Ed.), Potato physiology, Academic Press, London, pp. 353-388.

Ittersum, M.K. van, 1992. Relation between growth conditions and dormancy of seed potatoes. 3. Effects of light. Potato Research 35: 377-387.

Lee Kim, M.S., E.E. Ewing & J.B. Sieczka, 1972. Effects of chlorpropham (CIPC) on sprouting of individual potato eyes and on plant emergence. American Potato Journal 49: 420-431.

Meigh, D.F., 1969. Suppression of sprouting in stored potatoes by volatile organic compounds. Journal of the Science of Food and Agriculture 20: 159-164.

Oosterhaven, K., K.J. Hartmans & H.J. Huizing, 1993. Inhibition of potato (Solanum tuberosum) sprout growth by the monoterpene S-carvone: reduction of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase activity without effect on its mRNA level. Journal of Plant Physiology 141: 463-469.

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

Susnoschi, M., 1981. Seed potato quality as influenced by high temperatures during the growth period. 2. Sprouting pattern in several cultivars in response to storage temperature. Potato Research 24: 381-388.

Suttle, J.C. & J.F. Hultstrand, 1994. Role of endogenous abscisic acid in potato microtuber dormancy. Plant Physiology 105: 891-896.

Zaag, D.E. van der & C.D. van Loon, 1987. Effect of physiological age on growth vigour of seed potatoes of two cultivars. 5. Review of literature and integration of some experimental results. Potato Research 30: 451-472.

Days after harvest	Total tuber number		Sprouted tubers	
	Control	carvone	Control	carvone
63	20 ± 0.0	20 ± 0.0	20 ± 0.0	18 ± 1.0
126	16 ± 2.0	18 ± 1.0	13 ± 2.0	16 ± 2.0
182	15 ± 2.0	16 ± 2.0	12 ± 1.0	15 ± 2.0

Table 1. Sprouting of tubers from the 7.15 mmol · mol^{-1} carvone treatment 45 days after the end of the storage at 23°C in darkness.

Figure 1. Control tubers withdrawn from the sealed boxes after 6 months of storage in darkness at 23°C.

Figure 2. Control 1 tubers after 6 months of storage in darkness at 23°C.

Figure 3. Carvone-treated tubers (7.15 mmol · mol^{-1}) withdrawn from the sealed boxes after 6 months of storage in darkness at 23°C.

Figure 4. Time-course of the headspace concentration of carvone vapour in the storage boxes *AT 23°C* from the 7.15 mmol · mol^{-1} treatment. Tubers were withdrawn at days 63, 126 and 182. Each point represents the mean of six values, arising from three analyses of two samples, each taken from a different box.

↑ = addition of liquid carvone.

⇑ = box opening, withdrawing of 20 tubers and addition of liquid carvone.

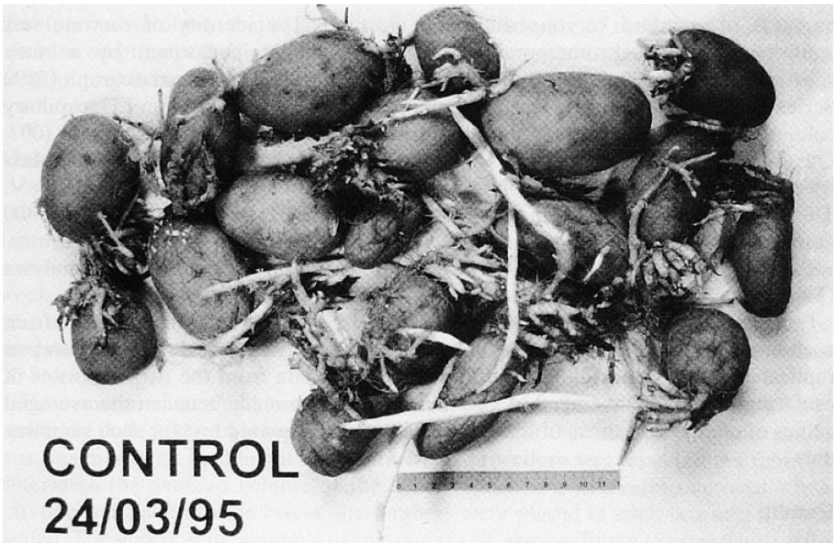

Fig. 1

Fig. 2

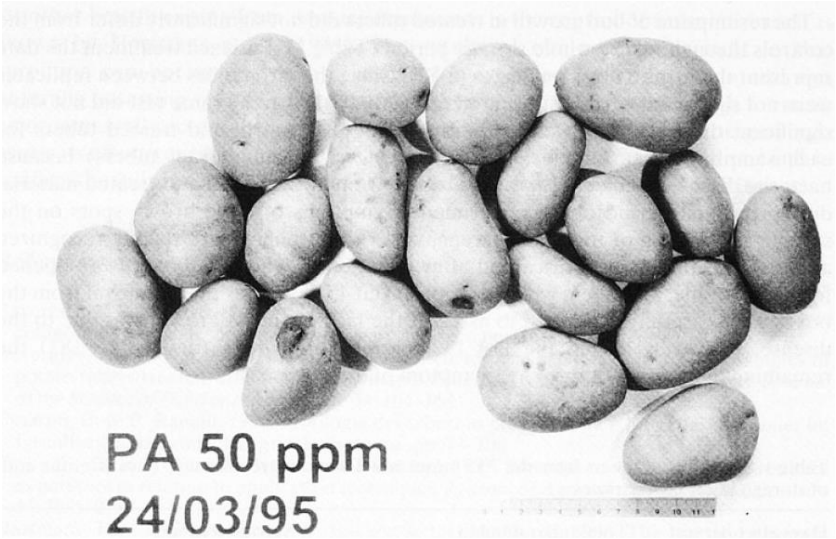
PA 50 ppm
24/03/95

Fig. 3

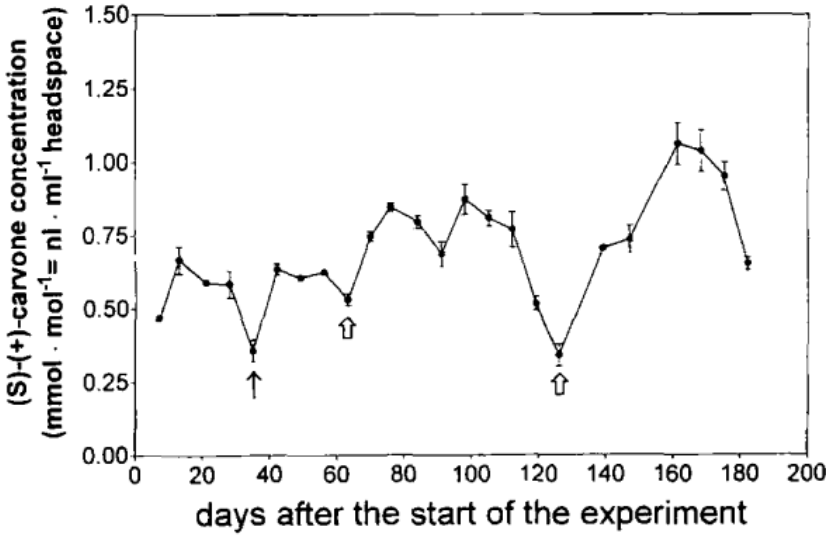
(S)-(+)-carvone concentration
(mmol · mol-1= nl · ml-1 headspace)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
20
40
60
80
100
120
140
160
180
200
days after the start of the experiment

Fig. 4