Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot

ARTICLE *in* POSTHARVEST BIOLOGY AND TECHNOLOGY · OCTOBER 2009

Impact Factor: 2.22 · DOI: 10.1016/j.postharvbio.2009.04.011

Dani Eshel
Agricultural Research Organization ARO

Samir Droby
Agricultural Research Organization ARO

G Model
POSTEC-9014; No. of Pages 5

ARTICLE IN PRESS

Postharvest Biology and Technology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot

Dani Eshel[a,*], Refael Regev[b], Janeta Orenstein[a], Samir Droby[a], Shmuel Gan-Mor[b]

[a] *Department of Postharvest Science, The Volcani Center, Bet Dagan 50250, Israel*
[b] *Institute of Agricultural Engineering, The Volcani Center, Bet Dagan 50250, Israel*

ARTICLE INFO

Article history:
Received 16 March 2009
Accepted 26 April 2009

Keywords:
Biological control
Carrot
Postharvest disease
Steam
Thielaviopsis basicola

ABSTRACT

Combining different control methods can improve control efficacy, increase the spectrum of controlled pathogens and reduce the possibility of resistance development. To be successful, however, the different methods need to be compatible: the first treatment should not have any deleterious effect on the succeeding one; preferably, it should contribute to its efficacy. In the last few years, carrot growers in Israel have begun to brush carrots before storage to remove the outer peel of the root. In the present study we show that this practice enhances the appearance of Black Root Rot during storage, a postharvest disease caused by the fungus *Thielaviopsis basicola*. The chemical fungicide iprodione is usually applied before storage to reduce the development of postharvest diseases. We evaluated the efficacy of combining physical, low-residue chemical and biological control agents as an alternative to the conventional chemical control approach. A technology for the precise application of steam and combined application with stabilized hydrogen peroxide (Tsunami® 100) or a yeast commercial product (Shemer™) were tested. Used alone, both the steam and Tsunami were highly effective at reducing disease decay but were phytotoxic to the roots. Application of combined treatments of sublethal steam followed by a sublethal dosage of Tsunami or Shemer improved efficacy and disease control by 80 and 86%, respectively. These combinations showed a synergistic effect as compared to each of the treatments alone. The same pattern, effecting up to 54% disease control, was observed with the non-compatible combination of applying Tsunami first, washing it off with water and then applying Shemer. Thus disease-control agents can potentially be used for a short period, then washed off, if needed, and efficiently followed by application of a biological control agent. The biological pathway and mode of action are still under investigation but to the best of our knowledge, this is the first study to mathematically demonstrate synergistic effects of sublethal treatments applied sequentially to control postharvest disease as a potential method to reduce the use of chemicals in fruit and vegetables.

1. Introduction

Adequate management of postharvest diseases is a prerequisite for the production of a stable and profitable food supply. Chemical control measures are usually used after harvest to control the development of decay-causing pathogens (Eckert and Ogawa, 1988; Vinas et al., 1991; Blasco et al., 2002). In many cases, however, growers rely on alternative methods, including disinfectants or chemicals with low-residue thresholds, physical methods, controlled atmosphere and biological control. In practice, most packinghouses rely on a single measure, applied before or during storage. Application of low-residue chemicals such as hydrogen peroxide is effective for a wide variety of postharvest fruit and vegetables (Forney et al., 1991; Fallik et al., 1994; Smilanick et al., 1995). The use of heat treatments has also been found to be effective in controlling postharvest disease, but both may damage the treated plant tissue if not used with care (Afek et al., 1999; Porat et al., 2000; Hansen et al., 2004). Biological control provides great promise for future management of postharvest diseases, because of its non-toxicity to humans and the environment. However, this type of control shows reduced efficacy, especially when pathogen inoculum density is high or it is used against pre-existing infections (Mathre et al., 1995; Hong et al., 1998; Elad and Freeman, 2002; Janisiewicz and Korsten, 2002; Droby et al., 2009). It has been suggested that different control methods be combined to improve control efficacy where a single measure is not effective enough, and/or to increase the spectrum of controlled pathogens and reduce the possibility of resistance development (Droby et al., 1998; Janisiewicz and Korsten, 2002). When two control measures are applied together, the resultant effect on the pathogen may

* Corresponding author. Tel.: +972 3 9683621; fax: +972 3 9683622.
E-mail address: dani@volcani.agri.gov.il (D. Eshel).

0925-5214/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2009.04.011

be antagonistic, additive, or synergistic (Levy et al., 1986; Kosman and Cohen, 1996). Antagonistic effects result in the efficacy of the integrated measures being lower than the sum of those of the individual components, while additive and synergistic effects are equal to or larger than, respectively, the sum of the components' separate effects (Levy et al., 1986; Kosman and Cohen, 1996). Compatibility of control methods is a prerequisite for the success of combined treatments: the first treatment should not have any deleterious effect on the succeeding one and preferably, should improve it.

In the last few years, carrot growers in Israel have been brushing carrots (removing the peel epidermis) before storage: this practice increases the appearance of Black Root Rot (BRR), a postharvest disease caused by the fungus *Thielaviopsis basicola* (Berk. & Br.) Ferraris (Syn. Chalara elegans Naj Raj W Kendrick) or *Chalaropsis thielavioides* Peyr. (Syn. Chalara thiavioides (Peyr.) Nay Raj & Kendrick (Weber and Tribe, 2004). The commercial control treatment, consisting of dipping the roots in iprodione (Rovral™, Rhone-Poulenc Agro-chemie, Lyon, France) before storage, is partially effective but leaves undesirable residue in the roots. Hence, alternative, safe control methods are needed. Shemer™, a commercial yeast-based product consisting of *Metschnikowia fructicola*, has been reported to be effective in the postharvest control of several fungal pathogens that develop on a wide variety of fruit and vegetables (Droby et al., 1998). Shemer is licensed for use in the control of the pathogenic fungus *Sclerotinia sclerotiorum* during postharvest storage of carrots, but as a stand-alone treatment, it does not provide sufficient control levels relative to fungicide treatment. In the present study, we used BRR as a postharvest disease case study to evaluate (i) synergistic efficacy by combining different control methods, and (ii) the ability to use non-compatible control methods in sequential application. A technology for the precise application of steam and the combined application of hydrogen peroxide or yeast were used as tools representing physical, low-residue chemical and biological control agents, respectively.

2. Materials and methods

2.1. Plant materials

All experiments were conducted on carrots (*Daucus carota* L.) of cultivars 'Dordogne' and 'Nairobi' grown in the Bet-Shean Valley and Sharon region of Israel. Carrots were harvested in the winter (January), spring (April) or summer (July), washed in water and brushed in a commercial machine using spiral-wound coil brushes made of 0.5-mm polyester filaments. After brushing, carrots were hydro-cooled in 4 °C water, packed in commercial 12-kg polyethylene bags and stored at 20 °C for 12 h to simulate breakdown in the cool chain and promote the infection process before the disease control treatments.

2.2. Steam treatments

A dedicated steam unit developed previously by the authors was used (Gan-Mor et al., 2008). The system is capable of applying heat from steam jets onto agricultural products on a roller conveyor which exposes each surface segment for a predetermined duration. The timing of exposure is determined by the speed at which the conveyor roller moves the carrot beneath the nozzle system. The maximal temperature on the produce surface was measured at 85 °C and its duration was recorded by a thermal infrared imaging camera using a procedure developed in the previous work (Gan-Mor et al., 2008). Exposure durations of 2, 3 and 4 s were tested using a steam pressure of 4 bar.

2.3. Spray treatments

Spray treatments were performed on a dedicated net table simulating a commercial roller conveyor. Carrots were sprayed with tap water (control), 20% stabilized hydrogen peroxide (Tsunami® 100, Ecolab, Kibbutz Dalia, Israel), iprodione (Rovral™, 0.5 g L^{-1}) or 2 g L^{-1} Shemer™, a commercial yeast product based on a *M. fructicola* isolate (Agrogreen, Minrab Group, Ashdod, Israel).

2.4. Packing and storage

Following treatments, carrots were placed in retail 1 kg packages (35 μm thick polyethylene) in 15 kg capacity export cartons. The wrappers were those commercially used, perforated with 6-mm holes at intervals of 12 cm × 12 cm. After the treatment, the products were stored for a period of one month at 0.5 °C and then transferred to a shelf-life simulation room at 20 °C for 8 d. The percentage of BRR decay and level of tissue damage were analyzed. Tissue damage from heat or Tsunami phytotoxicity was visually assessed for each carrot as "damaged" or "non-damaged". Each treatment contained 15 replicates, each consisting of 10 carrots in a retail package. Two people (the same throughout all experiments) performed the assessments on a whole-experiment basis and the scores were averaged between assessors.

2.5. Statistical analysis

After 8 d of shelf-life simulation, BRR disease severity (expressed as percentage of aerial carrot tissue affected) was visually assessed using an arbitrary percentage scale of 0% = no decay, 10, 25, 50, 75 and 100% surface decay. The percentage of decay area was arcsine-transformed before analysis.

The joint action of the control measures used was estimated by means of the Abbott formula (Levy et al., 1986; Kosman and Cohen, 1996). The expected disease-control efficacy and the joint suppressive activity against *T. basicola* were calculated as follows: $CE_{exp} = a + b - a \times b/100$ and $SF = CE_{obs}/CE_{exp}$, where a = control efficacy of one measure when applied alone, b = control efficacy of the other measure when applied alone, CE_{exp} = expected control efficacy of their combination if the two measures act additively, CE_{obs} = observed control efficacy of the combination, and SF = the synergy factor achieved by the combination: an SF of 1 indicates that the interaction between control measures is additive, SF < 1 indicates an antagonistic interaction, and SF > 1 indicates a synergistic interaction (Levy et al., 1986; Kosman and Cohen, 1996). When the control efficacy of $b = 0$, SF = infinity. Statistical analyses of the data were performed using JMP-in software (version 3 for Windows; SAS Institute, Cary, NC). Each experiment was conducted at least twice in both cultivars. Analysis of the data showed no significant interaction between treatments and the replicate experiments.

3. Results

3.1. Effect of carrot brushing on BRR disease

Postharvest tissue brushing of carrots has been introduced in most Israeli carrot packinghouses in the last three years, aimed at improving produce marketing. In all experiments performed with cultivars 'Dordogne' and 'Nairobi', brushed carrots developed BRR disease symptoms during the shelf-life simulation period following the process of simulated breakdown in the cool chain and cold storage (Fig. 1). BRR disease caused superficial symptoms over up to 80% of the carrot root area, although no soft rot developed. Correlation of symptoms to the brushing process was high and non-brushed carrots developed no symptoms in most cases.

Please cite this article in press as: Eshel, D., et al., Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot. Postharvest Biol. Technol. (2009), doi:10.1016/j.postharvbio.2009.04.011

Fig. 1. Carrot root-rot disease caused by postharvest brushing treatment. Carrots from cv. 'Dordogne' were (a) not treated or (b) brushed before storage. Carrots were stored in commercial polyethylene bags for 30 d at 0.5 °C plus 8 d under shelf-life conditions (20 °C).

3.2. Control of BRR disease by steam

Exposure of brushed carrots to 2–3 s of steam, following brushing, reduced the incidence of carrot BRR decay by 50–75% (Fig. 2). Increasing the exposure time to up to 4 s resulted in an 80% reduction in BRR decay with no significant difference relative to the 3 s exposure (Fig. 2). No significant difference was found between 3 and 4 s exposures in terms of disease control, but the 4 s exposure caused, in more than 50% of the carrots, tissue-burn damage and color change (Fig. 2).

3.3. Control of BRR disease by hydrogen peroxide

Treatment of brushed carrots with stabilized hydrogen peroxide (Tsunami) immediately after brushing showed a dose–response pattern of effectiveness in controlling the disease: at the highest dose, it eliminated the symptoms almost completely (Fig. 3). Short exposure (30 s) of the carrots to 1 mL L^{-1} of Tsunami did not affect the development of BRR decay and did not cause any phytotoxic tissue damage (Fig. 3). Spraying Tsunami without residue wash caused phytotoxic dose-responsive damage and color change, up to 100% of the carrots, at 5 mL L^{-1} (Fig. 3).

3.4. Synergistic combinations to control BRR disease

Combining sub-effective doses of steam (3 s) and then Tsunami (0.5 mL L^{-1}) showed a weak synergistic effect (SF = 1.1) and 80% reduced BRR decay (Fig. 4). These sub-phytotoxic doses did not show any additive or synergistic effect with respect to tissue damage.

Treatment of carrots with Shemer before storage did not affect BRR disease incidence during the shelf-life period after cold storage. Applying Shemer after 3 s exposure to steam resulted in a synergistic effect (SF = 1.79) and reduced BRR decay by 86% (Fig. 5). A lower synergistic effect (SF = 1.58) was achieved by combining short exposure to 1 mL L^{-1} Tsunami, washing the carrots after 30 s

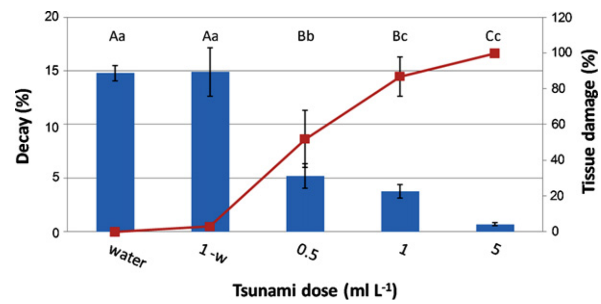

Fig. 3. Effect of carrot treatment with hydrogen peroxide (Tsunami) on postharvest Black Root Rot decay and phytotoxic tissue damage. 'Dordogne' carrots were brushed in a commercial machine, exposed to 1 mL L^{-1} of active Tsunami® 100 (commercial formulation of 20% active ingredient) for 30 s and then washed with water (1-w) or treated with several doses of Tsunami before storage in commercial polyethylene bags for 30 d at 0.5 °C plus 8 d under shelf-life conditions (20 °C). Black Root Rot decay (bars) and phytotoxic damage (curve) were assessed visually. Each treatment contained 15 replicates, each consisting of 10 carrots in a retail package. Different uppercase or lowercase letters represent significantly different ($P \leq 0.05$) percentage of decay or damage, respectively. Error bars indicate standard deviation.

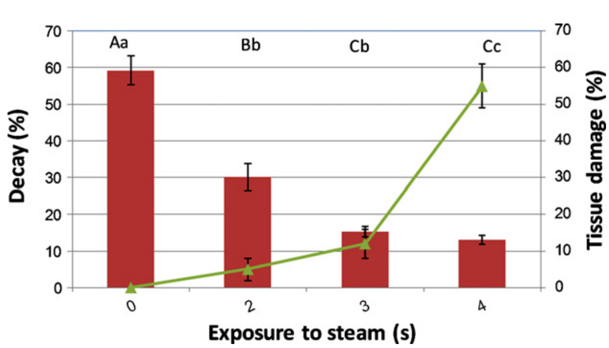

Fig. 2. Effect of exposure time of carrots to steam on postharvest Black Root Rot decay and phytotoxic tissue damage. 'Dordogne' cv. carrots were exposed to steam before storage in commercial polyethylene bags for 30 d at 0.5 °C plus 8 d under shelf-life conditions (20 °C). Black Root Rot decay (bars) and phytotoxic damage (curve) were assessed visually. Each treatment contained 15 replicates, each consisting of 10 carrots in a retail package. Different uppercase or lowercase letters represent significantly different ($P \leq 0.05$) percentage of decay or damage, respectively. Error bars indicate standard deviation.

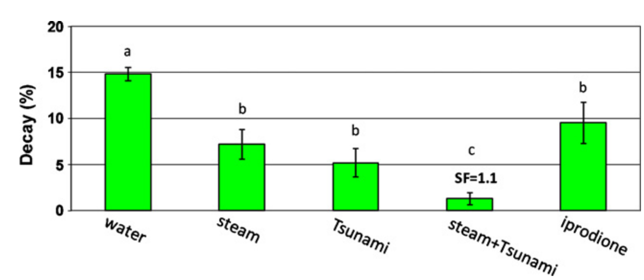

Fig. 4. Effect of sequence application of steam and stabilized hydrogen peroxide (Tsunami) in controlling postharvest Black Root Rot decay in carrots. Carrots were brushed in a commercial machine and then exposed to 3 s of steam and/or 0.5 mL L^{-1} of Tsunami® 100 (commercial formulation of 20% active ingredient) or 0.5 g L^{-1} iprodione. Carrots were stored after treatment in commercial polyethylene bags for 30 d at 0.5 °C plus 8 d under shelf-life conditions (20 °C). Each treatment contained 15 replicates, each consisting of 10 carrots in a retail package. Different lowercase letters represent significantly different ($P \leq 0.05$) decay area. "SF" is the synergy factor achieved by the treatment combination. SF > 1 indicates a significant synergistic effect in the related treatment ($P \leq 0.05$). Error bars indicate standard deviation.

Please cite this article in press as: Eshel, D., et al., Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot. Postharvest Biol. Technol. (2009), doi:10.1016/j.postharvbio.2009.04.011

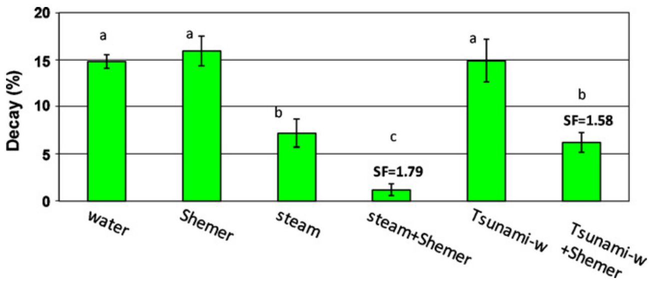

Fig. 5. Synergistic control of Black Root Rot disease by combining control methods. 'Dordogne' cv. carrots were exposed to the following treatments: 2 g L^{-1} Shemer, 3 s of steam, 1 mL L^{-1} Tsunami® 100 (commercial formulation of 20% active ingredient) for 30 s followed by a water wash (Tsunami-w), or the specified combination, before storage in commercial polyethylene bags for 30 d at 0.5 °C plus 8 d under shelf-life conditions (20 °C). Each treatment contained 15 replicates, each consisting of 10 carrots in a retail package. Different lowercase letters represent significantly different ($P \leq 0.05$) decay area. "SF" is the synergy factor achieved by the integration. SF > 1 indicates significant synergistic effect in the related treatment ($P \leq 0.05$). Error bars indicate standard deviation.

and then applying Shemer (Fig. 5). The level of control in this combination reached 54%. Both combinations (steam–Shemer and Tsunami–Shemer) gave better control of BRR than the commercial chemical treatment (iprodione) and caused only minimal phytotoxic damage.

4. Discussion

Carrot inoculation by *T. basicola* is probably related to initial inoculum density in the field and cross-contamination, especially during hydro-cooling (Punja et al., 1992; Weber and Tribe, 2004). We found that the tissue wounds caused by postharvest brushing of carrots increased disease incidence, whereas avoiding the brush process eliminates the development of BRR disease during storage and post-storage shelf-life. In brushed carrots, disinfection before packaging and cold storage was found to be effective at controlling BRR disease during shelf-life. Both steam and hydrogen peroxide have been reported to be highly effective at controlling postharvest pathogens (Forney et al., 1991; Afek et al., 1999, 2001). In brushed carrots, effective exposure time/dose to steam or Tsunami caused phytotoxic symptoms and tissue damage. The postharvest wounded tissue is probably more sensitive to physical or chemical phytotoxic effects (Barkai-Golan, 2001). Dose–response/damage curves, such as those presented in this study, for steam and Tsunami can be used to select suitable sublethal dosages for combined treatments. Exposure of the pathogen propagules to sublethal dosages of heat or methyl bromide have been shown to delay germination of *Sclerotium rolfsii* sclerotia and spores of *Fusarium oxysporum* and reduce their viability and pathogenicity (Eshel et al., 1999, 2000; Assaraf et al., 2002). Pathogen propagules that survive the first control agent, sublethal heating or metam sodium, have been shown to be more vulnerable to the sequential control of a biotic or abiotic agent (Lifshitz et al., 1983; Freeman and Katan, 1988; Fravel and Lewis, 2004). Here, application of a sublethal dosage of steam or Tsunami followed by Shemer, before storage, improved efficacy of disease control compared to each of the treatments alone. The efficacy of combined treatments was found to be high although we used high stringency conditions such as breakdown in the cooling chain and incubating the carrots, after storage, at 20 °C for 8 d. Improvement in the control efficacy of biological control agents has previously been shown by combining chemicals or other physical control methods (Mathre et al., 1995; Karabulut et al., 2002; Nunes et al., 2002; Fravel and Lewis, 2004). In semi-commercial postharvest trials with pears, the efficacy of *Candida sake* on reducing *Penicillium expansum* and *Botrytis cinerea* decay was enhanced more than 88% with the addition of 5 mM ammonium molybdate (Nunes et al., 2002). In our study, treatments were applied in sequence, simulating the use of non-compatible control methods. These experiments showed that disease-control agents can potentially be used for a short exposure period, then washed off, if needed, and followed by application of a biological control agent. The improved efficacy of Shemer might be the result of either pathogen weakening or induced resistance of carrot tissue in heat treatments (Karabulut et al., 2002). The yeast *M. fructicola*, which is the main component of Shemer, might be able to more effectively colonize the carrot wounds due to reduced competition by microflora following the first disinfectant treatment. Previous studies suggest the involvement of nutrient and space competition as well as direct parasitism in the mode of action of antagonistic yeasts (Droby et al., 1998; El Ghaouth et al., 2003). In these approaches the addition of sugars, lysozyme or generally regarded-as-safe (GRAS) chemicals in combination with yeast have been reported to improve postharvest disease control (El Ghaouth, 2000; Wilson et al., 2000).

In the present study, we found synergistic effects on the carrot-*T. basicola* system of sequential application of steam and Tsunami, or of each of these and Shemer. In a review article, Ben-Noon et al. (2003) showed that regardless of which control measures are combined, chemical, genetic or cultural, the most common effects of joint action are additive. They claimed that synergistic effects of control measures are to be expected when one control measure directly improves the efficacy of the other, or when one control measure induces host resistance or predisposes the pathogen to increased susceptibility (Ben-Noon et al., 2003). The biological pathway and mode of action of the synergistic treatments tested in this study are still under investigation. However, the synergistic effects of using sublethal treatments sequentially with a biological control agent have the potential to reduce the use of chemical control (Eshel et al., 2000).

Acknowledgements

This research was supported in part by the Israel Vegetable Growers Board. We thank Y. Canner and Kibbutz Shluhot for technical assistance in the postharvest experiments.

References

Afek, U., Orenstein, J., Nuriel, E., 1999. Steam treatment to prevent carrot decay during storage. Crop Prot. 18, 639–642.
Afek, U., Orenstein, J., Jin Kim, J., 2001. Control of silver scurf disease in stored potato by using hydrogen peroxide plus (HPP). Crop Prot. 20, 69–71.
Assaraf, M.P., Ginzburg, C., Katan, J., 2002. Weakening and delayed mortality of *Fusarium oxysporum* by heat treatment: flow cytometry and growth studies. Phytopathology 92, 956–963.
Barkai-Golan, R., 2001. Postharvest Diseases of Fruits and Vegetables: Development and Control. Elsevier Science.
Ben-Noon, E., Shtienberg, D., Shlevin, E., Dinoor, A., 2003. Joint action of disease control measures: a case study of Alternaria Leaf Blight of carrot. Phytopathology 93, 1320–1328.
Blasco, C., Picó, Y., Font, G., 2002. Monitoring of five postharvest fungicides in fruit and vegetables by matrix solid-phase dispersion and liquid chromatography/mass spectrometry. J. AOAC Int. 85, 704–711.
Droby, S., Cohen, L., Daus, A., Weiss, B., Horev, B., Chalutz, E., Katz, H., Keren-Tzur, M., Shachnai, A., 1998. Commercial testing of Aspire: a yeast preparation for the biological control of postharvest decay of citrus. Biol. Contr. 12, 97–101.
Droby, S., Wisniewski, M., Macarisin, D., Wilson, C., 2009. Twenty years of postharvest biocontrol research: is it time for a new paradigm? Postharvest Biol. Technol. 52, 137–145.
Eckert, J.W., Ogawa, J.M., 1988. The chemical control of postharvest diseases: deciduous fruits, berries, vegetables and root/tuber crops. Ann. Rev. Phytopathol. 26, 433–469.
El Ghaouth, A., 2000. Improved control of apple and citrus fruit decay with a combination of *Candida saitoana* and 2-deoxy-D-glucose. Plant Dis. 84, 249–253.
El Ghaouth, A., Wilson, C.L., Wisniewski, M., 2003. Control of postharvest decay of apple fruit with *Candida saitoana* and induction of defense responses. Phytopathology 93, 344–348.

Elad, Y., Freeman, S., 2002. Biological control of fungal plant pathogens. In: Kempken, F. (Ed.), The Mycota, A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research. XI. Agricultural Applications. Springer, Heidelberg, Germany.

Eshel, D., Gamliel, A., Katan, J., Grinstein, A., 1999. Evaluation of soil fumigants on soilborne fungal pathogens in a controlled-environment system and in soil. Crop Prot. 18, 437–443.

Eshel, D., Gamliel, A., Grinstein, A., Di Primo, P., Katan, J., 2000. Combined soil treatments and sequence of application in improving the control of soilborne pathogens. Phytopathology 90, 751–757.

Fallik, E., Aharoni, Y., Grinberg, S., Copel, A., Klein, J.D., 1994. Postharvest hydrogen peroxide treatment inhibits decay in eggplant and sweet red pepper. Crop Prot. 13, 451–454.

Forney, C.F., Rij, R.E., Denis-Arrue, R., Smilanick, J.L., 1991. Vapor phase hydrogen peroxide inhibits postharvest decay of table grapes. HortScience 26, 1512–1514.

Fravel, D.R., Lewis, J.A., 2004. Effect of label and sublabel rates of metam sodium in combination with *Trichoderma hamatum*, *T. harzianum*, *T. virens*, *T. viride* on survival and growth of *Rhizoctonia solani*. Phytoparasitica 32, 111–118.

Freeman, S., Katan, J., 1988. Weakening effect on propagules of *Fusarium* by sublethal heating. Phytopathology 78, 1656–1661.

Gan-Mor, S., Regev, R., Orenstien, J., Glik, Y., Levi, A., Eshel, D., 2008. Precise Monitoring of Produce Surface Temperature during Heat Disinfection Utilizing Thermal IR Imaging Paper Read at AgEng2008—International Conference on Agricultural Engineering, Hersonissos, Greece, 23–25 June.

Hansen, J.D., Wang, S., Tang, J., 2004. A cumulated lethal time model to evaluate efficacy of heat treatments for codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in cherries. Postharvest Biol. Technol. 33, 309–317.

Hong, C., Michailides, T.J., Holtz, B.A., 1998. Effects of wounding, inoculum density, and biological control agents on postharvest brown rot of stone fruits. Plant Dis. 82, 1210–1216.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annu. Rev. Phytopathol. 40, 411–441.

Karabulut, O.A., Cohen, L., Wiess, B.A.D., Lurie, S., Droby, S., 2002. Control of brown rot and blue mold of peach and nectarine by short hot water brushing and yeast antagonists. Postharvest Biol. Technol. 24, 103–111.

Kosman, E., Cohen, Y., 1996. Procedures for calculating and differentiating synergism and antagonism in action of fungicide mixtures. Phytopathology 86, 1263–1272.

Levy, Y., Benderly, M., Cohen, Y., Gisi, U., Bassand, D., 1986. The joint action of fungicides in mixtures: comparison of two methods for synergy calculation. EPPO Bull. 16, 651–657.

Lifshitz, R., Tabachnik, M., Katan, J., Chet, I., 1983. The effect of sublethal heating on sclerotia of *Selerotium rolfsii*. Can. J. Microbiol. 29, 1607–1610.

Mathre, D.E., Johnston, R.H., Callan, N.W., Mohan, S.K., Martin, J.M., Miller, J.B., 1995. Combined biological and chemical seed treatments for control of two seedling diseases of Sh2 sweet corn. Plant Dis. 79, 1145–1148.

Nunes, C., Usall, J., Teixido, N., Abadias, M., Vinas, I., 2002. Improved control of postharvest decay of pears by the combination of candida sake (CPA-1) and ammonium molybdate. Phytopathology 92, 281–287.

Porat, R., Daus, A., Weiss, B., Cohen, L., Fallik, E., Droby, S., 2000. Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. Postharvest Biol. Technol. 18, 151–157.

Punja, Z.K., Chittaranjan, S., Gaye, M.M., 1992. Development of black root rot caused by *Chalara elegans* on fresh market carrots. Can. J. Plant Pathol. 14, 299–1299.

Smilanick, J.L., Margosan, D.A., Henson, D.J., 1995. Evaluation of heated solutions of sulfur dioxide, ethanol, and hydrogen peroxide to control postharvest green mold of lemons. Plant Dis. 79, 742–747.

Vinas, I., Usall, J., Sanchis, V., 1991. Tolerance of *Penicillium expansum* to postharvest fungicide treatments in apple packingshouses in Lerida (Spain). Mycopathologia 113, 15–18.

Weber, R.W.S., Tribe, H.T., 2004. Moulds that should be better known: *Thielaviopsis basicola* and *T. thielavioides*, two ubiquitous moulds on carrots sold in shops. Mycologist 18, 6–10.

Wilson, C., El Ghaouth, A., Droby, S., Wisniewski, M., 2000. Non-chemical approaches to postharvest disease control. Acta Hortic. 553, 407–412.

Please cite this article in press as: Eshel, D., et al., Combining physical, chemical and biological methods for synergistic control of postharvest diseases: A case study of Black Root Rot of carrot. Postharvest Biol. Technol. (2009), doi:10.1016/j.postharvbio.2009.04.011