Original article

Fumigation of sweet cherries with thymol and acetic acid to reduce postharvest brown rot and blue mold rot

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Fumigation of sweet cherries with low levels of thymol and acetic acid to reduce postharvest brown rot and blue mold rot.

Abstract — **Introduction**. Sweet cherries are susceptible to postharvest decay. The use of synthetic fungicides is discouraged in postharvest handling because they can leave a residue and present a safety risk. Therefore, naturally occurring compounds have been considered as an alternative. Fumigation of short-chain organic acids and essential oils has shown promise in controlling fungal activities. This study reports their effects on sweet cherries. **Materials and method**. 'Hedelfingen' sweet cherries (*Prunus avium* L.) were inoculated with conidia of *Monilinia fructicola* and *Penicillium expansum*, then fumigated with three levels of thymol or acetic acid for 10 min before cold storage. **Results and discussion**. After 13 d at 10 °C, sweet cherries fumigated with 10 mg × L^{-1} of thymol significantly reduced brown rot from 21% to 12%, but had no effect on reducing blue mold rot. Fumigation with 6 or 10 mg × L^{-1} acetic acid significantly reduced blue mold rot from 16% to 2%, but had no effect on reducing brown rot. Fumigation did not have any effect on the firmness, total soluble solids and titratable acid of the sweet cherries. Fumigation with 2 or 6 mg × L^{-1} of thymol did not accelerate stem browning compared with the control, but fumigation with 10 mg × L^{-1} of thymol caused almost total stem browning. Fumigation with acetic acid showed no impact on discoloration of the stems. **Conclusion**. Thus, fumigation with acetic acid or thymol at low concentrations has a potential use for postharvest decay control without adverse effects on fruit quality.

Canada / *Prunus avium* (fruits) / keeping quality / postharvest decay / fungal diseases / fumigation / thymol / acetic acid

Fumigation de cerises avec de faibles doses de thymol et d'acide acétique pour réduire la pourriture brune et la moisissure bleue des arbres fruitiers.

Résumé — **Introduction**. Les cerises sont susceptibles de pourrir après leur récolte. L'utilisation de fongicides synthétiques est à déconseiller lors des manutentions car ceux-ci peuvent laisser des résidus et présenter un risque pour la santé. L'utilisation de composés naturels peut pallier ces désagréments. La fumigation d'acides organiques à chaîne courte et d'huiles essentielles s'est révélée prometteuse pour contrôler les activités fongiques. L'étude fait état de leurs effets sur la conservation des cerises. **Matériel et méthodes**. Des cerises « Hedelfingen » (*Prunus avium* L.) ont été inoculées avec des conidies de *Monilinia fructicola* et *Penicillium expansum*, puis ont été traitées par fumigation avec trois doses de thymol ou d'acide acétique pendant 10 min avant d'être entreposées au froid. **Résultats et discussion**. Après 13 j à 10 °C, les cerises traitées avec 10 mg × L^{-1} de thymol ont été significativement moins atteintes par la pourriture brune (12 %) que les cerises non traitées (21 %), mais le taux de cerises avec moisissure bleue n'a pas changé. La fumigation avec 6 ou 10 mg × L^{-1} d'acide acétique a diminué significativement le taux de moisissure bleue de 16 % à 2 %, mais n'a eu aucun effet sur la pourriture brune. Les divers traitements n'ont eu aucun effet sur la fermeté, le taux d'extrait sec et l'acidité titrable des cerises. La fumigation avec 2 ou 6 mg × L^{-1} de thymol n'a pas accéléré le brunissement des queues par rapport aux lots témoins, alors que le traitement avec 10 mg × L^{-1} de thymol a provoqué le brunissement de presque la totalité des queues. La fumigation à l'acide acétique n'a eu aucun effet sur le déverdissage des queues. **Conclusion**. Ainsi, la fumigation avec de faibles doses d'acide acétique ou de thymol pourrait être utilisée pour le contrôle après récoltes de la pourriture des fruits, sans que leur qualité ne soit affectée.

Canada / *Prunus avium* (fruit) / aptitude à la conservation / maladie post récolte / maladie fongique / fumigation / thymol / acide acétique

1. Introduction

In cold storage, sweet cherries (*Prunus avium*) normally have a short shelf life of about 14 d. The fruit is susceptible to postharvest decay caused by *Botrytis cinerea*, *Monilinia fructicola*, *Penicillium expansum*, *Alternaria* sp., and *Rhizopus* sp. [1, 2]. Do et al. [3] reported that a storage period of 30 d was obtained with hydrocooled 'Lambert' cherries that were treated with fungicide and placed in plastic bags. However, fungicides are discouraged in postharvest use because they can leave a residue and present a safety risk [4, 5]. The use of synthetic fungicides can also induce resistant strains in *Botrytis cinerea* and *Penicillium expansum* [6, 7]. Therefore, biological controls have been considered as an alternative to synthetic fungicides [8, 9], and two natural microbial agents were granted registration in the United States, but their success in the cherry industry remains to be demonstrated.

Mishra and Dubey [10] investigated the inhibitory action of natural substances against phytopathogenic microorganisms. Sholberg and Gaunce [11] found that vaporized acetic acid was extremely effective in inhibiting the spores of decay-causing fungi. Fumigation with 2.0 or 4.0 mg $\times$ L^{-1} of acetic acid prevented apples, grapes, kiwi fruits, pears and tomatoes inoculated with *Botrytis cinerea* and apples, oranges, and pears inoculated with *Penicillium* spp. from decaying by killing surface-borne conidia. Fumigation with short-chain organic acids including acetic, formic and propionic acids significantly reduced decay in eight cherry cultivars inoculated with spores of *M. fructicola*, *P. expansum* and *R. Stolonifer*. However, phytotoxicity, indicated by browning of the stem and pitting of the fruit surface, occurred on fumigated cherries [12]. Thymus essential oil inhibited the growth of both *B. cinerea* and *P. italicum* [13]. Fumigation with volatile fungicides appears promising in the cherry industry because they can be applied without wetting the fruit.

The potential use of essential oil to control postharvest decay has been examined for fruits, vegetables and flowers [14–16]. However, little data on the technique of fumigation is available toward its efficacy against postharvest microorganisms. Our previous study revealed that fumigation with thymol was more effective than acetic acid to prevent gray mold rot [17]. However, at 30 mg $\times$ L^{-1}, thymol-treated cherries showed more stem browning than those treated with acetic acid. The objectives of this study were to determine if lower concentrations of thymol or acetic acid could inhibit brown rot and blue mold rot on cherries with a minimum of phytotoxicity to the fruit.

2. Materials and methods

2.1. Harvest and inoculation

'Hedelfingen' sweet cherries were harvested at commercial maturity from the experimental farm of the Horticultural Research Institute of Ontario, University of Guelph (Vineland Station, Ontario), on July 9, 1998; they were stored at 0 °C soon after harvest. The average fruit weight was 7.0 ± 0.3 g. Seven days later, 10 kg sweet cherries were placed onto a laboratory counter for 6 h to warm up to 18 °C. Then 7.5 kg sweet cherries were inoculated with *Monilinia fructicola* and *Penicillium expansum*, the pathogens of brown rot and blue mold rot, by misting the fruit with 20 mL of spore suspension (1×10^5 conidia $\times$ mL^{-1}) using a mini-sprayer (Qorpak Sprayer Bottle, Fisher Scientific Ltd., Ottawa, Ontario, Canada). The pathogens in the suspension were originally isolated from infected sweet cherries. The aqueous suspension was prepared in a laminar flow hood and its concentration was determined with a hemacytometer. The misted cherries were kept at 18 °C overnight to allow spores to germinate. The other 2.5 kg cherries, which were not inoculated with the pathogens, were handled similarly, except distilled water was used to mist the fruit instead of the spore suspension. The cherries were then placed in a holding room at 2 °C before fumigation.

2.2. Fumigation and incubation

The inoculated cherries were randomly divided into seven groups. Each group was fumigated with vapors of glacial acetic acid (Caledon Laboratories Ltd., Georgetown, Ontario, Canada, HPLC grade, Minimum 99.7%) or thymol (5–methyl–2–isopropylphenol) (Sigma Chemical Co., St. Louis, MO, USA, Minimum 99.5%) at concentrations of 2, 6, and 10 mg × L^{-1} respectively in a 70-L gas-tight stainless steel tank. Inside the tank, there was a heating plate (Corning model PC-351) and a 15-cm diameter fan (32 watts) placed on the bottom. The platform of the heating plate was wrapped with a piece of aluminum sheet and the fumigant was placed directly on the platform. The chamber was sealed immediately after the cherries were placed in a colander, which was placed on a grill rack located above the fan and heating stirrer. The heating plate (at scale 4) and the fan (at low speed) were started, resulting in rapid volatilization and fast dispersal of the vapor. After 10 min of fumigation, the tank was opened and the remaining vapor was blown through an open door to the outside of the building with an air blower fan. During fumigation, the highest temperature among the cherries in the tank was 10 °C. The 'inoculated but not fumigated' cherries were treated in the same way except that no fumigant was used. After fumigation, the cherries were removed from the container and were individually placed on steel wire grilles. Fifty cherries were individually placed without touching each other on one grille, and the grille was then placed in an aluminum tray. Both the grilles and the trays were sterilized with heat before being used. The tray was loosely wrapped with a layer of VitaWrap food packaging film to keep moisture and prevent pathogen contamination. There was no difference in oxygen and carbon dioxide levels between the air inside and the air outside the tray. One tray of the cherries was used as one replicate and there were three replicates per treatment. The treatments consisted of: (1) inoculated, fumigated with 2 mg × L^{-1} acetic acid; (2) inoculated, fumigated with 6 mg × L^{-1} acetic acid; (3) inoculated, fumigated with 10 mg × L^{-1} acetic acid; (4) inoculated, fumigated with 2 mg × L^{-1} thymol; (5) inoculated, fumigated with 6 mg × L^{-1} thymol; (6) inoculated, fumigated with 10 mg × L^{-1} thymol; (7) inoculated, not fumigated. All trays were randomly placed in a storage room at 10 °C and 90–94% relative humidity for 13 d when the symptoms of decay appeared on the cherries in each treatment. Sample evaluation was taken when cherries were moved out of the storage room and placed at room temperature for 4 h.

2.3. Evaluation and analyses

The percentages of brown rot and blue mold rot were determined by counting the number of fruit showing typical symptoms caused by *M. fructicola* or *P. expansum*, respectively. All 50 cherries in each replicate were examined for decay.

Twenty uninfected cherries from each replicate were sampled for fruit quality evaluation.

Fruit firmness was determined with a Voland texture analyzer equipped with a 1.5-mm diameter probe at a speed of 2.0 mm × sec^{-1} for a 5-mm distance. Two firmness readings were taken from each cherry by puncturing two spots on opposite sides of the fruit.

Juice samples were obtained using a manual presser for the determination of soluble solids content and titratable acidity. Total soluble solids content was measured with an Abbe refractometer (AO instruments, Buffalo, NY). Titratable acidity was measured by titrating 5 mL of juice with a 0.1 N NaOH solution to a 8.20 pH endpoint using a Fisher electrometer and a Fisher dispenser (Model 380 and Model 395; Fisher Scientific, U.S.A.).

Statistical analyses were carried out with the general linear model procedure (SAS Institute, Inc., Cary, NC, USA). A completely randomized design was adopted with seven treatments and three replicates. Duncan's multiple range test was used for the multiple comparisons of means, if the F-test showed significance. To make the data for brown rot, blue mold rot and green stem

Figure 1.
Effects of fumigation with acetic acid and thymol in reducing the brown rot on 'Hedelfingen' sweet cherries. Same letters show means not significatively different according to the Duncan's multiple range test, 5% level.

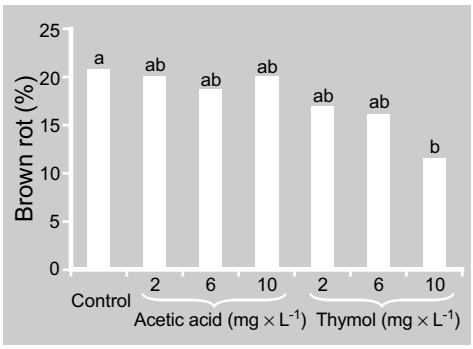


Figure 2.
Effects of fumigation with acetic acid and thymol in reducing the blue mold rot on 'Hedelfingen' sweet cherries. Same letters show means not significatively different according to the Duncan's multiple range test, 5% level.

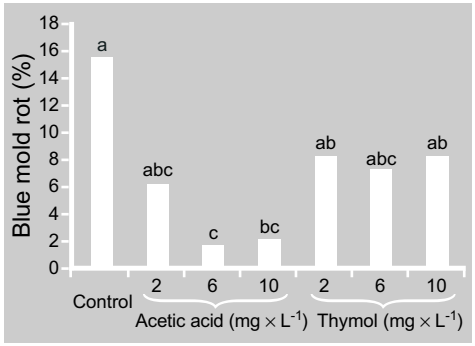
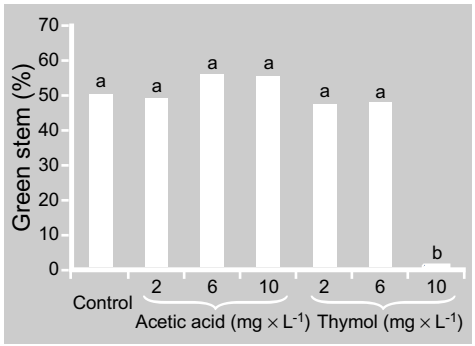


Figure 3.
Effects of fumigation with acetic acid and thymol in reducing the percent of green stem on 'Hedelfingen' sweet cherries.
Same letters show means not significatively different according to the Duncan's multiple range test, 5% level.



approximately normally distributed and account for the intrinsic binomial nature of response, the data were transformed to logit scale for statistical analysis. The formula used for the data transformation is:

logit = log [(number of cherries infected + 0.2) / (total number of cherries analyzed – number of cherries infected + 0.2)].

Then, the means were back-transformed to percentages of brown rot, blue mold rot, and green stem as shown in the result tables.

3. Result

Approximately 21% of the unfumigated sweet cherries showed brown rot. The treatment of 10 mg × L^{-1} thymol fumigation significantly reduced the incidence of brown rot to approximately 12% (*figure 1*). Other treatments might have reduced the incidence of brown rot slightly but they were not statistically significant.

Approximately 15.5% of the unfumigated sweet cherries showed blue mold rot. The treatments of 6 or 10 mg × $L^{-1}$of acetic acid fumigation significantly reduced the incidence of blue mold rot to approximately 2% (*figure 2*). Other treatments might have reduced the incidence of brown rot slightly but they were not statistically significant. Both the 6 and 10 mg × L^{-1} of acetic acid fumigation showed the same level of effect on the reduction of blue mold rot.

Approximately 54% of unfumigated sweet cherries maintained green stem when evaluated at the end of the experiment (*figure 3*). The treatment of 10 mg × L^{-1} of thymol fumigation significantly reduced the percentage of green stem on the sweet cherries to approximately 1%. Other treatments did not significantly change the percentage of green stem on the sweet cherries. Less green stem (*i.e.*, more brown stem) indicated the severity of the phytotoxicity of the treatment to cherries.

The results of this study showed that thymol and acetic acid had different effects on reducing the incidence of brown rot and blue mold rot. Thymol fumigation at the concentration of 10 mg × L^{-1} was effective in the reduction of brown rot. Acetic acid fumigation at the concentrations of between 6 and 10 mg × L^{-1} were effective in the reduction of blue mold rot. Thymol fumigation had no effect on the reduction of blue mold rot. Acetic acid fumigation had no effect on the reduction of brown rot.

Acetic acid and thymol fumigation treatment did not significantly affect cherry fruit firmness, total soluble solids and titratable acidity (*table I*).

Table I.
Quality attributes of inoculated and fumigated sweet cherries stored at 10 °C for 13 d (mean of three replications ± standard error).

Fumigation treatment	Firmness (g)	Total soluble solids (%)	Titratable acidity (%)
2 mg × L^{-1} acetic acid	24.7 ± 1.6	18.0 ± 0.1	3.4 ± 0.5
6 mg × L^{-1} acetic acid	25.5 ± 2.0	17.7 ± 0.2	3.3 ± 0.4
10 mg × L^{-1} acetic acid	24.6 ± 1.7	18.3 ± 0.2	3.6 ± 0.4
2 mg × L^{-1} thymol	24.7 ± 1.4	17.8 ± 0.2	3.5 ± 0.2
6 mg × L^{-1} thymol	25.9 ± 1.8	17.8 ± 0.1	3.6 ± 0.4
10 mg × L^{-1} thymol	25.4 ± 2.0	18.0 ± 0.2	3.9 ± 0.1
Control without fumigation	26.6 ± 1.3	17.1 ± 0.1	3.2 ± 0.2

4. Discussion

Using fumigation with acetic acid at low concentrations to control postharvest decay and extend storage life of fruits and vegetables has been well documented [11]. Their tests demonstrated that acetic acid was an effective fumigant for killing the decay-causing spores of *Botrytis cinerea* and *Penicillium* spp., and a few studies were employed to use vaporized acetic acid to kill the spores of *Monilinia fructicola*. To our knowledge, the test of effects of fumigation with thymol on postharvest decay caused by *M. fructicola* has not been reported.

Factors such as the volume of vaporized acetic acid, the number of conidia on the fruit surface, and the time of fumigation can also alter the effectiveness of the vaporized acetic acid treatment in controlling fruit decay [18]. Acetic acid was used as a fumigant against conidia of *M. fructicola* and reduced their germination to zero [19]. However, the treatment blackened the tested fruit in a few minutes. Sholberg and Gaunce [20] tested fumigation with short-chain organic acids, including acetic acid, on fruits inoculated with *M. fructicola* to reduce postharvest decay. In their study, the vapor of acetic acid (2.6 mg × L^{-1}) used for fumigation for 1 h significantly reduced decay for eight cherry cultivars, but phytotoxicity indicated by blackened stems and pitting of the fruit surface occurred on fumigated cherries. In contrast with the results cited above, our study revealed that at vapor concentrations of 2 and 6 mg × L^{-1} acetic acid and thymol for 10 min had little phytotoxicity. The different cherry cultivar chosen as the material and the different duration used in fumigation may account for the different results derived from these studies. The 10 min of fumigation used in this study, which is much shorter than the duration of 30 min or more employed in previous studies [12, 18–20], possibly is the reason why using a similar concentration of vaporized acetic acid resulted in a different efficacy of decay control on cherry fruit.

Previously, it was shown that vaporized thymol could control postharvest decay for the cherries inoculated with *Botrytis cinerea* [17], but our study is the first time it has been presented that thymol inhibited the disease-causing pathogens on cherries inoculated with *M. fructicola*. Our study also showed that thymol caused more severe phytotoxicity than acetic acid.

Stem condition is a critical component of sweet cherry quality. Maintaining a fresh, green stem is a main goal for the cherry industry to improve the techniques in storage, transportation and handling. Fumigation treatments resulting in the most effective control of decay also result in the greatest incidence of stem discoloration [21]. This would limit the commercial use of fumigation techniques for sweet cherries sold on the fresh market. A technique of fumigation to thoroughly kill disease-causing pathogens without phytotoxicity on the treated fruits has been pursued for many years, but it is still not available.

Thymol, thymol essential oil and thyme (spice) are listed by the United States Food and Drug Administration as foods for human consumption, as well as food additives. They are considered 'Generally Recognized as Safe' by the United States Environmental Protection Agency (EPA) [22]. Thymol was initially registered as a pesticide in the United States in 1964. Currently, five end-use pesticide products containing the active ingredient thymol are registered. The conditions of reregistration must be fulfilled and required data must be submitted to the EPA. At the present time, the EPA is not aware of any adverse effects of thymol to humans or the environment when it is used in a manner prescribed by product labeling. Since processed sweet cherries do not have stems attached or included, it is possible to use thymol fumigation to control postharvest decay for the cherries used for processing.

This study has shown that acetic acid and thymol are both effective for reducing postharvest decay for cherries. Each of them has particular strengths and weaknesses when applied to decay control. Acetic acid has been demonstrated to be an exceptional fumigant for reducing decay on fruits [12, 18, 20, 23, 24], but special caution should be taken when using glacial acetic acid because it has a pungent odor, produces burns on skin and causes eye irritation, and is also corrosive. Thymol has less safety problems. Exposures and health risks to people using currently registered products are expected to be relatively low [22]. In this respect, thymol is a better fumigant than acetic acid. However, thymol has its own disadvantage of an unfavorable smell, which could be sensed on the treated fruit after fumigation if a higher concentration was applied.

This study has shown that fumigation with acetic acid and thymol at lower concentrations has a potential use for postharvest decay control without adverse effects on fruit quality. Further research is required to determine if fumigation at a low temperature for a longer duration might reduce postharvest decay more effectively and minimize visual phytotoxicity on the fruit.

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Fumigación de cerezas con bajas dosis de timol y ácido acético para reducir la podredumbre parda y el moho azul de los árboles frutales.

Resumen — **Introducción**. Las cerezas pueden pudrirse tras la cosecha. No es aconsejable el empleo de fungicidas sintéticos durante el mantenimiento ya que éstos pueden dejar residuos y constituir un riesgo para la salud. La utilización de compuestos naturales puede paliar estos inconvenientes. La fumigación de ácidos orgánicos de cadena corta y de aceites esenciales se reveló prometedora para controlar las actividades fúngicas. El estudio describe sus efectos en la conservación de las cerezas. **Material y métodos**. Se inocularon cerezas 'Hedelfingen' (*Prunus avium* L.) con conidios de *Monilinia fructicola* y *Penicillium expansum*, seguidamente se trataron mediante fumigación con tres dosis de timol o de ácido acético durante 10 minutos antes de colocarlas en frío. **Resultados y discusión**. Tras 13 d a 10 °C, las cerezas tratadas con 10 mg × L^{-1} de timol fueron considerablemente menos afectadas por la podredumbre parda (12%) que las no tratadas (21%), pero la tasa de cerezas con moho azul no varió. La fumigación con 6 ó 10 mg × L^{-1} de ácido acético disminuyó significativamente la tasa de moho azul de 16% a 2% pero no tuvo ningún efecto sobre la podredumbre parda. Los diferentes tratamientos no tuvieron efecto alguno en la firmeza, tasa de materia seca y acidez titulable

de las cerezas. La fumigación con 2 ó 6 mg × L^{-1} de timol no aceleró el pardeamiento de los rabos con respecto a los lotes testigo, mientras que el tratamiento con 10 mg × L^{-1} provocó el pardeamiento de casi todos los rabos. La fumigación de ácido acético no tuvo ningún efecto en la decoloración del verde de los rabos. **Conclusión**. La fumigación con bajas dosis de ácido acético o timol podrá emplearse para el control post-cosecha de la podredumbre de los frutos sin que la calidad se vea afectada.

Canada / *Prunus avium* (fruta) / aptitud para la conservación / enfermedades postcosecha / enfermedades fungosas / fumigación / timol / acido acético