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SCIENTIFIC ARTICLE

# Extracts of wild grapevine (*Vitis* spp.) leaves reduce *Botrytis cinerea* infection during strawberry (*Fragaria* × *ananasa* Duch.) fruits postharvest

## Extractos de hojas de vid silvestre (*Vitis* spp.) reducen la infección por *Botrytis cinerea* en frutos de fresa (*Fragaria* × *ananasa* Duch.) en postcosecha

**Isela Apolonio-Rodríguez[1], Omar Franco-Mora[1]*, Jesús G. Aquino M.[2], Martha Lydia Salgado S.[2]**

[1]Laboratorio de Horticultura, Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México, Campus Universitario "El Cerrillo", 50020, Toluca, Estado de México, México.

[2]Facultad de Ciencias Agrícolas, Universidad Autónoma del Estado de México.

*Corresponding author: ofrancom@uaemex.mx

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## ABSTRACT

Worldwide, eco-friendly and innocuous techniques to reduce postharvest strawberry fruit decay are being developed. *Vitis* plants had polyphenols presenting fungistatic activity. Strawberry fruits were dipped for 3 min in *Vitis* leaves solution (7.2% w/v) of 3 accessions: resveratrol and gallic acid, 0.450 g $L^{-1}$ each, and distilled water. Then, fruits were inoculated with $1 \times 10^6$ *Botrytis cinerea* conidia and stored (19 °C/70% RH). Five days after inoculation (DAI), 75% of control fruits presented rotted peel with velvety gray mold; the other treatments showed between 14 and 30% of infection. With eight DAI, berries treated with *Vitis* extracts and gallic acid lost 2 N of firmness, while resveratrol treated berries lost less than 1 N. In contrast, control fruit lost 2.5 N in only five DAI. Extracts of Mexican wild grapevine leaves have the potential for reducing the infection and symptoms of *Botrytis cinerea* in strawberry fruits.

## KEYWORDS

Phenolics, postharvest disease, quality, softening, stilbene.

## RESUMEN

A nivel mundial se desarrollan técnicas inocuas y ambientalmente amistosas para reducir el decaimiento postcosecha de fresa. Plantas de *Vitis* presentan polifenoles fungiestáticos. Frutos de fresa se sumergieron 3 min en soluciones de extractos foliares (7.2 % p/v) de 3 accesiones de *Vitis*: resveratrol, ácido gálico (ambos a 0.450 g $L^{-1}$) y agua. Los frutos se inocularon con $1 \times 10^6$ conidios de *Botrytis cinerea* y se almacenaron (19 °C/70 % HR). A cinco días de inoculación (DDI), el daño en el control fue de 75 por ciento y de 14 a 30 por ciento en otros tratamientos. A ocho DDI, las fresas tratadas con *Vitis* y ácido gálico perdieron 2 N de firmeza y con resveratrol menos de 1 N. Los frutos control perdieron más de 2.5 N a 5 DDI. Los extractos de hojas de *Vitis* tienen potencial para reducir la infección y los síntomas de *Botrytis cinerea* en fresa.

## PALABRAS CLAVE

Fenoles, enfermedad postcosecha, calidad, ablandamiento, estilbeno.

## Introduction

*Botrytis cinerea* Pers. (Teleomorph: *Botryotinia fuckleliana*) is one of the most important pathogens responsible of strawberry fruit (*Fragaria* × *ananasa*, Rosaceae) postharvest decay (Domnez et al. 2011; Amiri et al. 2013), which, in extreme cases, causes losses over 80% of berry production (Petrasch et al. 2019). This pathogen induces gray mold, an infection which initially shows a dark circular area and softening of fruit tissue, later followed by abundant white to gray sporulation. Damaged strawberry fruits might contaminate surrounding ones (Feliziani and Romanazzi 2016). Mexico has become one of the worldwide top five strawberry producers (SAGARPA 2018) and its local industry might join the international efforts to increase the use of sustainable and innocuousness postharvest techniques over excessive use of pesticides to delay or reduce strawberry postharvest fungus incidence. To achieve this objective, cultural practices suggested including the use of resistant cultivars, correct management of weed, prevent berry-soil contact and to avoid high humidity in the field (Feliziani and Romanazzi 2016). Moreover, in planta, spray of chitosan on developing berries reduced the presence of postharvest gray mold (Reddy et al. 2010). At postharvest period, ecofriendly techniques include the application of lemon (*Citrus limon* (L.) Osbeck) and *Solidago canadensis* (L.) essential oils (Vitoratos et al. 2013; Liu et al. 2016); chitosan edible films improved with olive oil (*Olea europaea* L.) (Khalifa et al. 2016), and gelatin-based films containing cellulose nanocrystals (Fakhouri et al. 2014), among others.

It has been observed that *Vitis* plant extracts reduced the *in vitro* development of *B. cinerea*. For example, an 800 mg $L^{-1}$ *V. vinifera* (L.) tendril extract fully inhibited the *in vitro* growth of *B. cinerea* (Fraternale et al. 2015). A 12% (w/v) aqueous solution of Mexican wild grapevine (*Vitis* spp.) leaves successfully reduced, in relation to control, *in vitro* mycelial growth (72%), sporulation (75%) and spore germination (62%) of *B. cinerea* (Apolonio-Rodríguez et al. 2017). Antifungal activity of *Vitis* plant extracts over *B. cinerea* has been related to the presence of diverse polyphenols i.e. rutin, quercetin, resveratrol and p-coumaric, ellagic, cafeic and caftaric acids, among others (Mendoza et al. 2013; Fraternale et al. 2015).

On the other hand, the stilbene resveratrol (RVS) is a natural compound synthetized in several organs of the *Vitis* plants, and its synthesis has been related to an increment in the plant resistance against *B. cinerea* (Langcake and McCarthy 1979). This resistance has been related to the methyolation of hydroxyphenol groups of RVS (Adrian et al. 1997). Under *in vitro* conditions, 0.120 g $L^{-1}$ RVS inhibited 70% of mycelial growth and spore germination, and 80% sporulation of *B. cinerea* (Apolonio-Rodríguez et al. 2017). RVS as well as gallic acid, rutin and cafeic acid are present in Mexican wild grapevine leaves (Tobar-Reyes et al. 2011). Thus, present research aimed to determine the effect of aqueous extracts of wild grapevine leaves on the incidence of *B. cinerea* and fruit postharvest quality of 'Festival' strawberry fruits.

## Material and methods

### Plant material and inoculation

Berries of ‘Festival’ strawberry, at ripening scale No. 3 (Gobierno de México 2002), were harvested in the municipality of San Felipe del Progreso, State of Mexico, Mexico. Berries were then transported, for about 45 min, to the Laboratory of Horticulture of the Faculty of Agriculture, Universidad Autónoma del Estado de México.

At the laboratory, the strawberry fruits were disinfected in 1% commercial sodium hypochlorite aqueous solution for 2 min; then, fruits were rinsed with sterile distilled water, and they were allowed to dry at room temperature in aseptic conditions. The berries were randomized and divided into six homogenous groups, 600 fruits each. The six treatments were as follows: 7.2% extracts of fresh leaves of *Vitis cinerea* ((Engelm.) Engelm. ex Millardet) 'Don José' and 'E-200' and *Vitis* sp. 'TN-4'; 0.450 g $L^{-1}$ of RVS or gallic acid were used as comparatives, and distilled water was employed as control. The 600 berries of each treatment were separated in three replications, 200 berries each. Seventy berries from each replication were used to determine *Botrytis* incidence and the other 130 strawberry fruit to measure the variables.

Fresh and young leaves (around three weeks after full leaf expansion) of *V. cinerea* 'Don José', 'E-200' and *Vitis* sp. 'TN-4' were harvested in the wild grapevine germplasm bank located in Zumpahuacán, State of Mexico (Sabás-Chávez et al. 2016) as indicated by Apolonio-Rodríguez et al. (2017). At laboratory, solutions were prepared immediately before berries imbibition. After rinsing the leaves with tap water, 72 g of fresh leaves of each accession were mixed five times with distilled water in a blender (Model 6858013000, Oster®, México City, Mexico), until the plant tissue was completely mixed with the distilled water. Then, the leaf solutions were diluted to 1 L of distilled water. Resveratrol (SIGMA®, St. Louis, Missouri, USA) was dissolved in distilled water:methanol (SIGMA®) solution (1:1), whereas gallic acid (SIGMA®) was dissolved in distilled water.

Fruit imbibition, for all the treatments, was carried out in 2 L plastic containers; the berries were plenty imbibed in the corresponding solution for 3 min, one container for each replication per treatment.

Then, the berries were allowed to dry at room temperature (25 ± 2 °C) in aseptic conditions. After drying, the berries were infiltrated, in only one point in the middle of the widest flesh area, using a syringe. The suspension per fruit was of $1 \times 10^6$ conidia obtained from a one-week-old strain of *B. cinerea* (Apolonio-Rodríguez et al. 2017). After inoculation, berries were placed on plastic molds having individual containers allowing separation of 0.5 cm between continuous fruits. The room storage was maintained at 19 °C and 70% relative humidity. The plastic surfaces and their distribution in the storage room allowed a distribution in a randomized complete block design experiment.

### Postharvest evaluation

All the fruit of each replication, initially 70, were visually analyzed every day to determine the presence or absence of *B. cinerea*. If the berry showed *B. cinerea* brown pot of at least a diameter of 0.5 cm, the fruit was qualified as diseased, counted, and immediately discarded from storage. Ending each observation, the number of diseased berries was added to the accumulated number of diseased fruits. That number was used to determine the percentage of disease incidence. In each replication, specifically in the group not employed for disease incidence determination, 10 fruits were individually weighed with an analytical balance (Model JL-200®, Chyo, Õtsu, Japan) every day. Those values were used to determine the rate of fresh weight loss, expressed in percentage of the initial fruit weight. Other 10 fruits, per replication and day of storage, were used to determine flesh firmness with a texturometer (Model CT3® 1000, Brookfield, Middleboro, USA) using the cylinder P72 penetrating 5 mm of the fruit at a velocity of 5 mm $s^{-1}$ (Martínez-Mendoza et al. 2020). Then, those fruits were used to measure the content of total soluble solids (TSS) with a refractometer (Model Pal-1, Atago, Japan), and titratable acidity by titrating with sodium hydroxide (Franco-Mora et al. 2012).

### Statistical analysis

The experiment was conducted as a randomized complete block design, with three replications per treatment. For the incidence of *B. cinerea*, 70 fruits per replication were analyzed and a regression analysis was performed with the daily accumulated values. For the rest of determinations, three replications were designed, 10 fruits each, and the results were used to perform an analysis of variance. When the F value was significant, the treatments were compared with the Tukey test at 0.05 of significance.

## Results and discussion

### Incidence of *B. cinerea*

Four days after *B. cinerea* inoculation (DAI), over 50% of the control berries developed symptoms of the disease. At that time, less than 25% of the berries treated with *Vitis* leaves presented symptoms of *B. cinerea*. At 8 DAI, around 40% of the berries treated with 'Don José', 'E-200' and gallic acid presented the disease symptoms, whereas less than 25% of the berries treated with RVS developed the disease (Figure 1). The progression disease curve over postharvest period was well explained by a linear model, with the lowest value for 'Don José' (0.888, ***) and the highest for control (0.995, ***) (Figure 1).

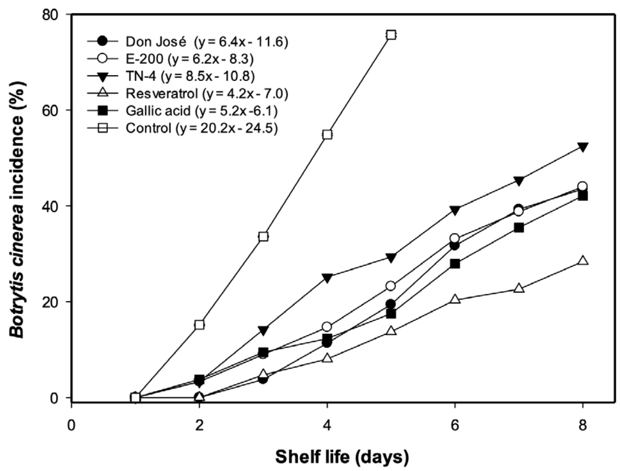


Figure 1. Accumulative incidence of inoculated *Botrytis cinerea* on strawberry fruits preventative treated with *Vitis* spp. leaves, resveratrol and gallic acid. The lineal model equation is showed for each treatment. Fruits were stored at 19 °C and 70% RH. Data are, exclusively, the average of three replications, 70 fruits each.

Under culture medium conditions, *Vitis* leaves extracts reduced, in relation to control, the *in vitro* growth of *B. cinerea*; but similarly, to present work, RVS showed higher reduction (Apolonio-Rodríguez et al. 2017). It is known that the extracts of *Vitis* leaves contain phenolic compounds i.e. gallic acic, cafeic acid, rutin and RVS (Tobar-Reyes et al. 2011), which have been suggested to act as promoters of quiescence for *B. cinerea* grow (Petrasch et al. 2019).

In the literature, it is possible to find reports with higher control of *B. cinerea* by other plant extracts, i.e., Vitoratos et al. (2013) observed 100% reduction of *B. cinerea* symptoms in strawberries with the application of 0.05 ml $L^{-1}$ lemon ((*Citrus limon* (L.) Osbeck) essential oils. However, present results suggest the reliability of applying extracts of *Vitis* leaves to reduce postharvest incidence of grey mold in strawberry. Moreover, wild grapevines are common plants in the south region of the state of Mexico (Franco-Mora et al. 2012), where around 7,800 t of strawberry are produced per year (SAGARPA 2018).

## Postharvest quality

During the initial 3 DAI, fruit weight loss was similar for all treatments; then, at 4 and 5 DAI, control fruit lost more weight than the rest of the treatments (Figure 2). Within storage at the same temperature, higher dehydration has been associated to different rates of hormone and sugar metabolism, as well as a defense mechanism (Rizzini et al. 2009), or the presence of differentiated number of injuries related to pathogens (Cheah et al. 1992). For this research, a suggested lower presence of *B. cinerea* in berries treated with *Vitis* leaves extracts, RVS and gallic acid, in relation to control, might suggest less spoilage by this pathogen and, as a consequence, reduction in fruit weight loss.

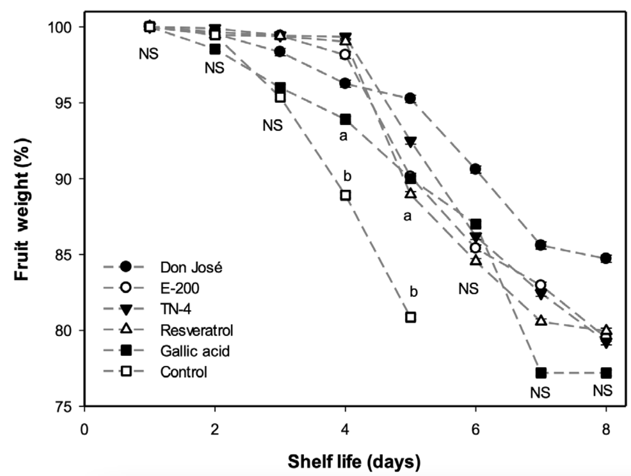


Figure 2. Effect of the extracts of fresh leaves of Mexican wild grapevine, resveratrol or gallic acid on the rate of fruit weight loss during postharvest life of strawberry fruit inoculated with *Botrytis cinerea*. Data are the average of three replications ± SD, 10 fruits each. Different literals indicate statistical difference at 0.05 of Tukey test; for better comprehension, only literals to separate statistical groups are shown.

Five DAI, the firmness in the berries treated with extracts of *Vitis* leaves and gallic acid was between 0.5 and 1 N lower than the values observed in the fruit treated with RVS, and 1.5 N higher than control fruit. Confirming, during all the storage period, it was observed that RVS highly limited berry softening, as the RVS treated berries lost less than 1 N of the initial flesh firmness in 8 days of storage (Figure 3). It is known that the presence of *B. cinerea* stimulate the activities of fungal enzymes like pectinases, laccases and proteases, as well as activities of cell wall degrading enzymes (Petrasch et al. 2019). As suggested herein, the presence of different phenolic compounds in wild grapevine extracts might, also, be related to reduction in fruit softening rate by limiting *B. cinerea* grow. Moreover, exogenous commercial RVS application reduced fruit softening and prolonged shelf life in cherimoya (*Annona cherimolla* Mill.) fruit (Salomon-Castaño et al. 2022). As in the leaves of Mexican wild *Vitis*, there is a scanty amount of RVS (Tobar-Reyes et al. 2011). The presence of this stilbene might also support, at least in part, reduction in fruit softening and prolonged strawberry shelf life.

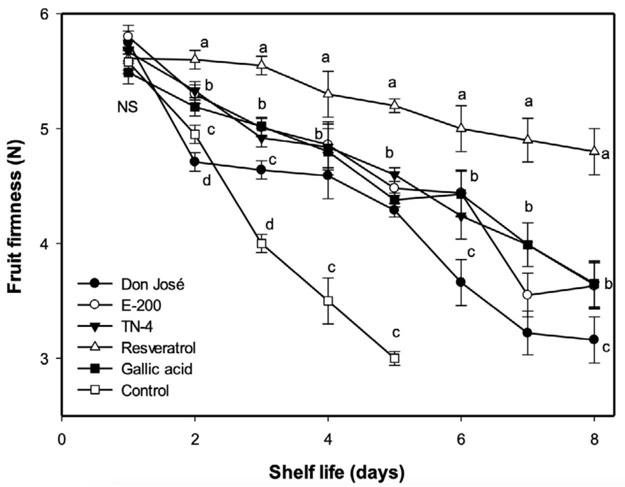


Figure 3. Effect of the extracts of fresh leaves of Mexican wild grapevine, resveratrol or gallic acid on the fruit firmness during postharvest life of strawberry fruit inoculated with *Botrytis cinerea*. Data are the average of three replications, 10 fruits each. Different literals indicate statistical difference at 0.05 of Tukey test.

RVS, as reagent, is expensive and difficult to adopt as a reliable technique to increase postharvest quality in horticultural commodities, at least in developing countries. Nevertheless, extracts of wild grapevine leaves seem to be a low-cost and efficient alternative to improve strawberry postharvest conservation, specially reducing fruit softening related to *Botrytis* presence.

Herein, all treatments reduced the incidence of *B. cinerea*, rates of fruit weight loss and fruit softening; all those factors resulted, in relation to control, in 3 more days of postharvest storage in 'Festival' strawberries. However, compared to control, application of any of the treatments resulted in lower sweetness; only control fruit reached 8 °Brix, at 5 DAI. For the rest of the treatments, 7.5 °Brix was the maximum content of TSS (Figure 4). In strawberry fruit, increase in fruit sugar content has been observed after cell wall solubilization, and it has been suggested that those sugars might serve as a nutrient for *B. cinerea* (Petrasch et al. 2019). In all treatments, reduction in fruit softening seemed to reduce cell wall solubilization. Furthermore, the content of TSS in all treated berries was commercially acceptable. 'Festival' strawberry fruit grown in the Valley of Jacona-Zamora, Michoacán, Mexico, reached 8.5 °Brix at maturity (Martínez-Bolaños et al. 2008), and in Florida, USA, an average of several seasons determined 7.5 °Brix (Jouquand et al. 2008).

There was no difference for titratable acidity among treatments. At the beginning of the storage, the berries showed 0.35% equivalents of citric acid, and that amount descended up to 0.25% (Data not shown). This acidity is considerably lower in relation to other reports on 'Festival' strawberry; i.e., in the Valley of Jacona-Zamora, Michoacán (0.46 to 1.34 %) (Martínez-Bolaños et al. 2008), and in Florida (0.75 %) (Jouquand et al. 2008).

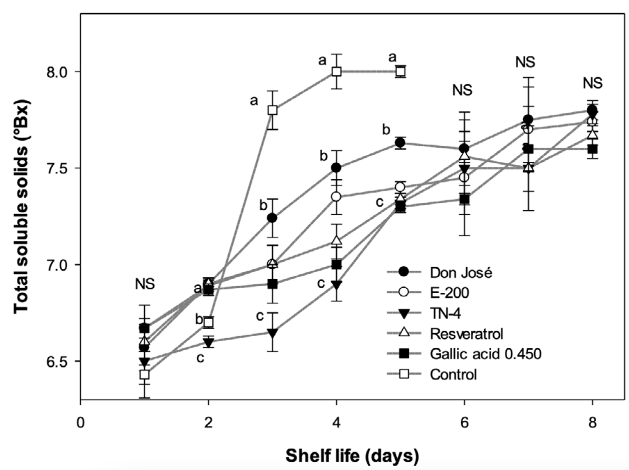


Figure 4. Effect of the extracts of fresh leaves of Mexican wild grapevines, resveratrol or gallic acid on the content of total soluble solids during postharvest life of strawberry fruit inoculated with *Botrytis cinerea*. Data are the average of three replications ± SD, 10 fruits each. Different literals indicate statistical difference at 0.05 of Tukey test.

## Conclusions

Five days after inoculation with $1 \times 10^6$ conidia of *Botrytis cinerea*, 75% of control fruit presented disease symptoms. On the contrary, 7 days after inoculation, 40% of the berries treated with extracts of *Vitis cinerea* leaves (7.2% w/v) 'Don José' and 'E-200' presented symptoms. In relation to control, all treatments resulted in lower presence of *B. cinerea* symptoms, as well as delay in fresh weight and firmness lost. Resveratrol reduced the presence of *B. cinerea* more effectively, and, it also reduced fresh weight loss and fruit softening rates significantly. On the other hand, control fruit reached 8° Brix, that is 0.5° Brix more than the rest of the treatments.

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